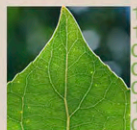


DOE Systems Biology Knowledgebase Implementation Plan



Microbes



Plants



Metacommunities

Biological Principles

Metabolism
Integration

Interactions
Data
Visualization

Proteins
Mathematics
Algorithms
Gene Expression

Computing

Predictive Understanding

DOE Systems Biology Knowledgebase Implementation Plan

As part of the U.S. Department of Energy's (DOE) Office of Science, the Office of Biological and Environmental Research (BER) supports fundamental research and technology development aimed at achieving predictive, systems-level understanding of complex biological and environmental systems to advance DOE missions in energy, climate, and environment.

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www.science.doe.gov/Program_Offices/BER.htm

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The report is available via

- www.genomicscience.energy.gov/compbio/
- www.science.doe.gov/ober/BER_workshops.html
- www.systemsbiologyknowledgebase.org

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APPENDIX A

Supporting Scientific Objective and Software Requirement Documents for Near-Term Microbial Science Needs

This appendix provides the working documents for the two selected microbial scientific objectives and their requirements. These documents were the core output from the final DOE Systems Biology Knowledgebase (Kbase) workshop held June 1–3, 2010 (see [Appendix D](#) for the report). The scientific objectives must answer the question, “What is the scientific or research goal that needs to be solved?” The related “requirements” establish workflows and provide details on the needs to accomplish these objectives. The process of identifying the scientific objective, determining its software requirements, and then developing an implementation plan from the two was described in [Chapter 1](#). These working documents are provided as backup to their implementation plans, which are described in [Chapter 2](#) and contain the final revised judgment by the community concerning the tasks needed for each objective.

In research, a scientific objective is satisfied by creating hypotheses and doing one or more experiments. For every experiment, there are rationales, protocols to be executed, a number of data inputs (data sources) and outputs (results), and analysis tools. Workflows are sequential procedures that describe the envisioned steps to answer questions. They are the bioinformatic equivalent of an experimental protocol. Detailed workflows are bridges between the experimental research and computing communities and thus are key to translating research objectives into computing requirements that will most effectively advance the science. Workflows were developed for these objectives. From these workflows and the underlying objectives, the requirements could be defined that lead to the articulation of an implementation plan with tasks and scope to achieve the scientific and technical goals.

In the microbial science area, the first objective is to improve the accuracy of metabolic network models, especially for microbes important in biofuel production and environmental remediation, so that metabolic engineering produces more predictable results. The second objective is to enable automated inference of gene regulatory networks based on data from gene expression profiling. Predicted networks then would be validated to determine their accuracy and refined to improve prediction of cellular behavior and fitness. Both objectives have tasks in developing data repositories and workflows that link into the infrastructure.

A.1 Microbial Scientific Objective 1: *Reconstruct and Predict Metabolic Networks to Manipulate Microbial Function*

The aim of the DOE Systems Biology Knowledgebase (Kbase) is to provide a set of interoperating databases and software tools to achieve the scientific objective of evaluating the metabolic potential of an organism; predicting the phenotypic outcome of specific metabolic or environmental interventions; and developing quantitative, validated metabolic models. This knowledge will lead to the informed modification of one or more specific enzymes or the introduction of entirely new enzymes and pathways. This would allow the community to better

determine strategies for manipulating biomass flow in microorganisms. More specifically, this objective is concerned with reconstructing metabolic networks, predicting the growth phenotypes of organisms from their metabolic networks, understanding the metabolic potential of an organism, and providing scientists with software tools to interrogate and interactively visualize metabolic networks and enable engineers to quickly determine the strategies necessary to remodel metabolism for specific purposes. The goal is to move beyond the current state of the art to increase the speed and automation with which metabolic networks can be reconstructed and to increase the accuracy of metabolic network predictions. Kbase should include access to a variety of data to help achieve the overall objective. Such data would include genomic-level metabolic maps, both stoichiometric and regulatory; enzyme concentration and activity levels; qualitative data on enzyme regulation and known substrate, product, and cofactor dependencies; enzyme kinetic data if available; suggested kinetic rate laws or reasonable approximations; metabolic flux maps (predicted or measured); sensitivity data (rate limitingness and control coefficients) if available; time-course data on metabolite changes and enzyme concentration changes; and finally relevant thermodynamic data (computed or measured) on individual metabolic reactions.

This objective does not currently include protein-protein and protein-DNA interactions; phosphorylation, glycosylation, or acylation states; or signaling pathways. However, part of this information (e.g., covalent modification) is currently held in resources such as MetaCyc. These processes were not within the original scope of the initial objective but could easily be included in a future revision, particularly in relation to dynamics. Gene regulatory networks also are not included in this objective because they are considered in a separate objective description. Ultimately, all three network repositories must be linked, given the close relationships among them.

Probably the three main primary sources of online metabolic data in use today are MetaCyc (www.metacyc.org), Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/), and Braunschweig Enzyme Database (BRENDA; www.brenda-enzymes.org/). MetaCyc and KEGG provide metabolic stoichiometric map data, although access for noncomputer specialists is not always easy. BRENDA has a huge range of diverse enzyme data (83,000 enzymes listed), but the data are incomplete on individual enzymes and at times tedious to access. The System for the Analysis of Biochemical Pathways–Reaction Kinetics (SABIO-RK, sabio.villa-bosch.de/) is one of the few enzyme kinetics databases. The current range of sources is therefore scattered, not always easy to use, and lacks important information. Repositories such as MetaCyc could be modified or new third-party tools developed to enhance and access the data more seamlessly.

Another source of useful data for the metabolic community is genome-based curated metabolic reconstructions. Although these data are of much higher quality, they too are scattered and stored in a number of conflicting, different, and sometimes undocumented formats. One of the primary reasons is that there is no agreed standard for storing flux balance information.

Kacser et al. (1995) provides a good review of the modern interpretation of rate limitingness in pathways.

Prioritization

Given DOE's interest in metabolic engineering for biofuel production and environmental remediation, which require detailed knowledge of metabolic dynamics, the priority for the proposed data source is high.

PRIORITY: X HIGH MEDIUM LOW

Potential Benefits

Metabolism is a critical component for many applications of interest to DOE. Naturally, DOE researchers should have access to reliable and comprehensive data sources pertaining to metabolism.

Feasibility of Success: Near, Mid, and Long Term

A timeline is presented for development of subobjectives comprising the overall goal of Microbial 1: Reconstruct and Predict Metabolic Networks to Manipulate Microbial Function.

A number of short-, mid-, and long-term goals could be achieved. Examples include the following.

Near to intermediate term (6 months)

- Subobjective 1: Generate first-generation (uncurated) metabolic reconstructions from genome annotations for all genomes within Kbase and make them available to the community via browsable and programmatic access.

Near term (1 to 3 years)

- Subobjective 2: Compare metabolic models developed within different software environments, particularly flux balance models, to facilitate combining the strengths of different environments and different models.

Near to mid term (1 to 5 years)

- Subobjective 3: A number of researchers are now measuring fluxes *in vivo* using isotopic methods. One important scientific objective is to compare flux balance model predictions to flux data generated by isotopic experiments. Databases of maps for different organisms under different conditions could be provided with flux data, both predicted and measured.

Mid term (3 to 5 years)

- Subobjective 4: One important scientific objective is to begin to map out the degree of rate limitingness in metabolic pathways (flux control coefficient, Kacser et al. 1995) and to determine how the patterns change under different conditions. For these efforts, enzyme levels and protein turnover rates should be made accessible via a publicly available database. These data also can be used to assist in building kinetic models and assessing global protein demand.

- Subobjective 5: Increase the accuracy of programs that predict growth and intracellular fluxes from metabolic models.
- Subobjective 6: Develop and make accessible, via web and programmatic interfaces, computational tools that can be used for metabolic engineering. These tools would utilize metabolic reconstructions and models developed within or outside Kbase.
- Subobjective 7: A critical scientific objective is to have validated metabolic models for predicting the metabolic potential of a cell. Five suggested levels for validating a model are proposed; each level is more demanding than the previous and requires additional model-data comparisons. The first level of validation and possibly the easiest to achieve is to compare growth and no-growth phenotypes for wildtype and mutant strains. Related to this is the comparison of flux balance analysis (FBA) predictions with isotopic flux measurements as a way to further validate the flux balance models. The next level is to compare predicted steady-state flux and metabolite levels against experimentally measured fluxes and metabolites. Level 4 validation will test the ability of the model to predict the effect of “small” perturbations in enzyme activity levels and environmental conditions. Finally, the most demanding validation test in this sequence is to compare time-course changes due to major environmental changes, such as shifts in nutrients or O₂.

Validation Levels

- Level 1: Compare growth and no-growth predictions for wildtype and mutant strains against experimental data (within 2 years).
- Level 2: Compare flux balance predictions against isotopic flux measurements (within 3 years).
- Level 3: Steady-State Validation. Compare predicted steady-state metabolite concentrations and fluxes to experimentally measured values (within 4 years).
- Level 4: Perturbation Validation. Perturb enzyme levels by specified amounts (e.g., 50% change or less) and recompute fluxes and metabolite changes that result. Compare changes with equivalent experimental perturbations (within 4 years).
- Level 5: Time-Course Validation. Apply environmental changes and significant enzyme perturbations to track time-course changes in metabolite and flux values. Validate against equivalent changes in the kinetic model (long term, 5 to 10 years).

Long term (5 to 10 years)

- Subobjective 8: To understand the role and distribution of rate-limiting reaction steps (measured using flux and concentration control coefficients) in metabolic pathways under different conditions and in relation to the activity of negative and feedback loops.

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This would complement the flux, enzyme activity, and kinetic data and would be used to engineer strains with increased flux through desired metabolic pathways.

- Subobjective 9: Integrate the spatial distribution of metabolites and enzymes with metabolic flux analysis. Data on localization of enzymes and metabolites in cells will inform flux analysis and metabolic models, and such analyses and models should be developed to be able to incorporate localization results as they become available.

Relevance to the Kbase Project

The ability to build predictive metabolic models is an important step in proposing design strategies for affecting flux through metabolic pathways. This is clearly of DOE interest, given the need to re-engineer biofuel production by microbial metabolic pathways and to stimulate microbial communities involved in environmental remediation.

Synergies and Leverages: Potential Overlap with Other Projects or Funding Agencies

The Palsson group and others have created genome-scale reconstructions of metabolism for tens of microbes (reviewed in Oberhardt, Palsson, and Papin 2009) that are available in Systems Biology Markup Language (SBML) and Excel formats. The Karp group has created two highly curated metabolic databases (MetaCyc and EcoCyc) and has generated genome-based and metabolic databases for 670 organisms (expanding on a regular basis). SEED (www.theseed.org) has recently incorporated the development and simulation of metabolic models into its database. Other DOE-relevant U.S. metabolic databases include ShewCyc (from the *Shewanella* Federation), BeoCyc (a database of 33 bioenergy-related organisms from the DOE BioEnergy Science Center), PlantCyc (metabolic databases for *Arabidopsis* and poplar from the Carnegie Institution), FungiCyc (from the Broad Institute), and YeastCyc (from Stanford). Fostering exchange of metabolic models between platforms (e.g., BioCyc, KEGG, SEED, and SBML or Excel) would be desirable to facilitate comparison of models and application of models developed under different platforms.

No concerted effort has been made to collect and curate quantitative data, enzyme levels, and time-course data. There is very little or no overlap with existing projects such as iPlant (www.iplantcollaborative.org), GenBank (www.ncbi.nlm.nih.gov/genbank/), or other efforts at the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov/). Eventually, iPlant may commit more resources to metabolic systems and modeling, but at this point, the level of interest is unknown.

Details

Scientific Discovery Process (Workflows)

No workflows are available for metabolic studies other than the recently published constraint-based model workflow (Thiele and Palsson 2010). There are, however, new experimental high-throughput procedures to measure both protein and metabolite absolute values (Bennett et al. 2008), which should prove extremely useful in helping to achieve this objective.

Inputs

- Sequenced and annotated genomes will be critical for reconstructing genome-scale metabolic networks.
- Absolute values for metabolite (Bennett et al. 2008), gene expression (RNA-Seq), and enzyme levels and activities.

A number of standards can be used in metabolic studies. Largely missing are well-established standards for storing experimental data. Standards such as SBML and BioPAX (Biological Pathway Exchange) can be used to store stoichiometric information, modifiers (e.g., inhibitors), parameter values, and rate laws, but there are very few actual data standards to store flux data, enzyme levels, time-course data and such. One possibility is to use Systems Biology Results Markup Language (SBRML) (Dada et al. 2010) for data storage, together with supporting formats and ontologies that have arisen in recent years.

- Existing standards (defined as community-agreed formats implemented in more than one software tool):
 - SBML (sbml.org): Representation of stoichiometric and regulatory models, including extension packages (e.g., FBA).
 - BioPAX (www.biopax.org): Annotation standard for biological pathway data; could complement SBML.
 - SED-ML (Simulation Experiment Description Markup Language; www.biomodels.net/sed-ml): Standard for describing simulation experiments.
 - TEDDY (Terminology for the Description of Dynamics; www.ebi.ac.uk/compneursrv/teddy): Ontology for the dynamics of biomodels.
 - KiSAO: Kinetic Simulation Algorithm Ontology (sourceforge.net/projects/kisao/).
 - SBGN: Systems Biology Graphical Notation for unambiguous visualization of pathways (www.sbgn.org).
 - SBRML: (www.comp-sys-bio.org/tiki-index.php?page=SBRML): Can be used to store data and simulation results.
 - CellML (www.cellml.org): Math-based representation of models.
- SBML extension packages conceivably could be developed to accommodate these kinds of data, using the core SBML format to anchor the metabolic network itself.
- To propose new data standards, regular focused workshops would be needed with the stakeholder community, preferably twice a year. Specification documents would need to be drawn up, test data developed, and supporting software libraries written to read and write the proposed data formats. In addition, some kind of governance structure would have to be devised to manage specifications and open-source licensing, assess

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proposals, and manage revisions. The entire process should be as open as possible and involve the community at every stage.

Outputs

- A quantitative and dynamic picture of metabolism and metabolic fluxes.
- A series of new proposed standards for data storage, perhaps as package extensions to SBML and using BioPAX as additional annotation.

Tools

Existing software tools include the following.

Noncommercial

- CellDesigner (celldesigner.org). CellDesigner is a network visualization tool that can also integrate with Systems Biology Workbench (SBW) and Complex Pathway Simulator (COPASI) for carrying out simulations.
- Constraint-based reconstruction and analysis toolbox ([COBRA](http://cobrapy.github.io)). This is a Matlab toolbox for carrying out flux balance analysis.
- COPASI (www.copasi.org). COPASI is a general purpose and SBML-compliant simulator written in C++ (free for academic use).
- FiatFlux (www.imsb.ethz.ch/researchgroup/nzamboni/Software/fiatflux) is a Matlab toolbox for carrying out metabolic flux analysis (isotopic analysis).
- JDesigner (jdesigner.sf.net). JDesigner is an open-source network visualization and simulation tool.
- Jarnac (jdesigner.sf.net). Jarnac is a script-based tool for general-purpose simulation of reaction networks.
- KEGG Tools (www.genome.jp/kegg/)
- Metatool (von Kamp and Schuster 2006). Matatool is a C-coded open-source tool for computing elementary modes.
- Model SEED (<http://seed-viewer.theseed.org/seedviewer.cgi?page=ModelView>). A resource for generating, optimizing, curating, and analyzing genome-scale metabolic models.
- OptFlux (www.optflux.org). OptFlux is an open-source tool for carrying out flux balance analysis and related optimization procedures.
- Pathway Tools (bioinformatics.ai.sri.com/ptools/)
 - Computational generation of genome-scale qualitative metabolic models.
 - Editing of metabolic models.

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- Querying and visualization of metabolic models.
- Analysis of metabolic models (reachability analysis, dead-end metabolite analysis, comparative analysis).
- PySCeS (Olivier, Rohwer, and Hofmeyr 2005). PySCeS is a Python-based SBML-compliant tool for network analysis and simulation.
- XPP (www.math.pitt.edu/~bard/xpp/xpp.html). X-Window Phase Plane (XPP) is a numerical analysis tool (C/C++/FORTRAN) used for general-purpose simulation and bifurcation analysis.
- SBW (www.sys-bio.org). SBW is an open source integrated SBML-compliant suite of tools for the numerical analysis of reaction networks (written in a variety of languages).

Commercial

- SimPheny (www.genomatica.com/). SimPheny is a commercial tool for flux balance analysis with good visualization functionality.
- SimBiology (Matlab). SimBiology is an SBML-compliant Matlab tool distributed by Mathworks for simulation and analysis.

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A.2 Software Requirements for Microbial Scientific Objective 1: *Reconstruct and Predict Metabolic Networks to Manipulate Microbial Function*

Summary of Scientific Objective

The scientific objective is to provide a knowledgebase that could be used to evaluate the metabolic potential of an organism, predict the phenotypic outcomes of specific metabolic or environmental interventions, and establish metabolic kinetics and fluxes. This knowledge will lead to the informed modification of enzymes or the introduction of entirely new enzymes and

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pathways. This would allow the community to better determine strategies for carbon flow manipulation.

This objective is concerned with reconstructing metabolic networks, predicting the growth phenotypes of organisms from their metabolic networks, understanding the metabolic potential of an organism, and providing scientists with software tools to interrogate and visualize metabolic networks. The goal is to move beyond the current state of the art in all these respects, such as increasing the speed and automation with which metabolic networks can be reconstructed and to increase the accuracy of metabolic network predictions.

Kbase should provide access to a variety of data. Such data would include metabolic maps, both stoichiometric and regulatory; enzyme concentration and activity levels; qualitative data on enzyme regulation and known substrate, product, and cofactor dependencies; enzyme kinetic data if available; suggested kinetic rate laws or reasonable approximations; metabolic flux maps (predicted or measured) and metabolite levels; sensitivity data (rate limitingness and control coefficients) if available; time-course data on metabolite changes and enzyme concentration changes; and relevant thermodynamic data (computed or measured) on individual metabolic reactions.

Resulting Requirements

IMPACT FACTOR: **HIGH** **MEDIUM** **LOW**

A variety of interoperating software tools are needed, including tools to generate metabolic reconstructions; allow scientists to query, visualize, and curate metabolic models; and predict growth phenotypes from metabolic models.

Process of the Science (Including Workflows)

A timeline for proposed requirement development follows.

Within 6 months

- Decomposition of all existing microbial SBML and CellML kinetic models (many hundreds of models exist) into individual reaction steps and rate laws. This will provide a database of published rate laws that could be used in future models. This data should be cross-referenced to metabolic maps. For example, by selecting a reaction on a metabolic map, a user will be provided with all published rate laws associated with that reaction step.

Within 1 year

- Conversion of many constraint-based models and stoichiometric maps to be stored in standard formats such as annotated SBML and SBML FBA extension format (Bergmann and Olivier 2010). Many tools already read SBML, so it would be a natural format to use. Conversion to other formats (e.g., Matlab, COBRA, and OptFlux) can be achieved easily. It is already possible, for example, to convert COBRA format to SBML (PySCeS).
- Automatically generate genome-scale metabolic reconstructions for all DOE-relevant organisms and make them available in SBML format. This could involve a combination of

existing reconstructions from BioCyc (670 models to date), Model SEED (130 reconstructions to date), and others generated from various methods (Palsson), and including aspects of model generation not currently automated.

Within 2 years

- Establish round-trip testing of metabolic models among software tools in Kbase (e.g., COBRA, OptFlux, Pathway Tools, SBW, COPASI, CellDesigner).
- Provide better access to an online metabolic regulatory map. These data would include all modifiers that affect enzymes, including both activators and inhibitors. At the simplest level, these data could be just a list of modifiers for each enzyme but could be expanded later to include mechanisms (e.g., allosteric or covalent modification) and possibly information on K_{is} , Hill coefficients, and proposed rate laws.
- Create tools to compare metabolic reconstructions generated from different sources that may rely on different genome annotations. For example, do BioCyc and Model SEED agree or disagree regarding the presence of reactions and enzymes in a given organism?

Within 3 years

- Develop a series of gold-standard manually curated metabolic reconstructions for about 20 organisms of importance to the DOE mission. These reconstructions will serve as important resources and will enable automated reconstruction systems to be calibrated and assessed for their quality.

Within 5 years

- Improve fully automated metabolic reconstruction systems, increasing their speed, comprehensiveness (meaning the types of information they can infer), and accuracy beyond current levels.
- Integrate gene functional annotations and genome-scale metabolic reconstruction and simulation capabilities within the Kbase environment enabling iterative improvement of both layers of information.
- Example workflow:
 - Genome annotations are used for automated inference of initial metabolic network reconstructions.
 - A reconstruction and simulation engine automatically generates a list of gaps (missing enzymes or transporters), potential gene candidates that may resolve them, and inconsistencies (functions without context or “dangling” compounds).
 - This list by itself is of huge scientific value, as it points scientists to open research problems and missing knowledge.

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- This list would also be used by existing and newly developed software tools to attempt gap filling and impose consistency on the annotations (e.g., negate “weak” functional assignments not supported by the functional context).
- Examine existing carbon 13 isotopic flux prediction software (e.g., FiatFlux) and improve it to enable better predictions for fluxes in all pathways in the cell, not just central metabolic fluxes. These software tools require metabolic network reconstructions, atom mappings between substrates and products, and experimental measurements (¹³C labeling distributions on metabolites, biomass composition, and cellular uptake and secretion rates) and should provide estimates for intracellular fluxes (net and exchange fluxes) and confidence intervals for the estimated fluxes.
- Develop methods for determining metabolic fluxes and their confidence intervals based on time-dependent carbon 13 isotope measurements as a function of time after carbon 13 addition (before an isotopic steady-state has been reached).
- Generate methods to integrate metabolic and regulatory models and automate refinement of such integrated models. Using the previously developed gold-standard metabolic reconstructions, develop integrated metabolic and regulatory models, which will leverage regulatory network reconstructions arising from other Kbase efforts.

Mid term (3 to 5 years)

- Create a repository of growth data for organisms of importance to DOE for use in validating metabolic prediction algorithms. The repository must also include metadata associated with the experiments (e.g., media composition, temperature, and pH).
- **Model validation.** Five suggested levels for validating a model are proposed, with each level more demanding than the previous ones. The first level of validation and possibly the easiest to achieve is to compare growth and no-growth phenotypes for wildtype and mutant strains. Related to this is to compare flux balance analysis predictions with isotopic flux measurements as a way to further validate the flux balance models. The next level is to compare predicted steady-state flux and metabolite levels against experimentally measured fluxes and metabolites. Level 4 validation will test the ability of the model to predict the effect of “small” perturbations in enzyme activity levels and environmental conditions. Finally the most demanding validation test in this sequence is to compare time-course changes due to major environmental changes such as shifts in nutrients or O₂.

Validation levels

- Level 1: Compare growth and no-growth predictions for wildtype and mutant strains against experimental data (within 2 years).

- Level 2: Compare flux balance predictions against isotopic flux measurements (within 3 years).
- Level 3: Steady-State Validation. Compare predicted steady-state metabolite concentrations and fluxes to predicted values.
- Level 4: Perturbation Validation. Perturb enzyme levels by specified amounts (e.g., a 50% change or less) and recompute fluxes and metabolite changes that result. Compare changes with equivalent experimental perturbations (within 4 years).
- Level 5: Time-Course Validation. Apply environmental changes and significant enzyme perturbations to track time-course changes in metabolite and flux values. Validate against equivalent changes in the kinetic model (long term, 5 to 10 years).
- Measure or predict computationally or theoretically the distribution of the **degree** of rate limitingness in metabolic pathways under different conditions and in relation to the activity of negative and feedback loops. This would complement the flux, enzyme activity, and kinetic data and also be related to the validation procedures (level 4).

Long term (5 to 10 years)

- Create integrated metabolic and regulatory network models, in a largely automated fashion, that pass moderate levels of validation. After additional manual curation, these models pass high levels of validation.

User Interfaces

User interfaces should be intuitive and easy to use for a broad range of users who are not bioinformatics experts. Users will be bench biologists, computational biologists, model builders, and theoreticians. Interfaces, where possible, should be interactive and cross-platform. Visualization interfaces for locally installed software should perform as well as modern computer games and, where possible, exploit graphics processing unit (GPU) technology to enhance interactivity.

Programmatic Interfaces

Interoperation of diverse software and databases is a critical part of Kbase in general and of this objective in particular. Modern computing techniques provide a diverse set of tools for accomplishing such interoperation.

One form of interoperation is the use of web services or other Internet application programming interfaces (APIs) to interconnect databases and software tools. This approach is routinely used, for example, in the systems biology modeling community to resolve ontological terms and to access models on demand from model repositories. Web services are also a suitable method for communicating between mobile devices such as the iPhone or iPad. Web services are widely used by EBI (European Bioinformatics Institute) and other bioinformatics

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resources. Modern software development environments make it relatively easy to both expose new web services and use existing ones. One reason Internet APIs are attractive is that they reduce the need to distribute software tools and to install externally developed software. Producing robustly installable software for multiple platforms can be a large burden because of incompatibility of hardware, operating system, and other system software.

In some cases, it will be appropriate to distribute algorithms and analysis methods, such as in the form of open-source reusable libraries, which can then be included in tools developed by other third-party developers. This has been an extremely useful approach used by DOE in the past for widely used numerical analysis algorithms.

Data

- **Develop gold-standard manually curated metabolic reconstructions for about 20 organisms of importance to the DOE mission** (satisfies Subobjectives 2–7). These reconstructions will serve as important resources and will enable automated reconstruction systems to be calibrated and assessed for their quality.
 - **Existing reconstructions:** *E. coli* (EcoCyc, Palsson group), ShewCyc, BeoCyc, PlantCyc, FungiCyc, YeastCyc, and Geobacter. The existing reconstructions are of variable quality; some are qualitative only, and some have received relatively little curation.
- **Develop lower-quality metabolic reconstructions for the majority of sequenced genomes** (satisfies Subobjective 1). Existing large-scale reconstruction sites such as BioCyc are likely to be extended to most genomes in the future. The BioModels.net database includes hundreds of kinetic models from a wide variety of systems; this is an important source of prebuilt curated models from the literature.

Software

Software packages needed are as follows.

- **Model management** (satisfies virtually all subobjectives). Store, query, and edit a joint metabolic, genomic, or regulatory model.
 - **Existing:** Pathway Tools has extensive capabilities in this area and should be leveraged.
- **Metabolic reconstruction** (satisfies Subobjective 1)
 - **Existing:** Pathway Tools has extensive capabilities currently focused on inference of qualitative reconstructions, although work is under way to extend its capabilities to include inference of flux balance models.
 - Model SEED has capabilities of generating metabolic reconstructions from genome annotations generated by rapid annotation using subsystem technology (RAST) and performing computational analysis of the resulting models.
- **Constraint-based analysis tools** (satisfies Subobjective 7). Predict fluxes through metabolic pathways that would lead to optimal states (e.g., maximal growth rate or

maximal ATP production), predict wildtype and mutant behaviors, generate sampling of feasible metabolic flux distributions, metabolic engineering tools, and calculation of elementary modes or extreme pathways.

- **Existing:** COBRA, OptFlux, Metatool
- **¹³C metabolic flux analysis** (satisfies Subobjectives 3 and 8). Estimates fluxes and confidence intervals from carbon labeling experiments, where cells are grown on ¹³C–labeled substrates and the abundance of ¹³C at positions in downstream metabolites is measured and used to estimate intracellular fluxes.
 - **Existing:** FiatFlux, OpenFLUX, 13CFlux
- **Network visualization** (satisfies Subobjectives 2, 3, 6, and 7). Allows visualization of metabolic networks and projection of predicted fluxes and experimental data onto these networks. Ability for users to customize the layout of networks and improvement of automated layouts would be useful.
 - Existing: Pathway Tools, Cytoscape, KEGG
- **Simulation; model analysis and data fitting; and sensitivity analysis and parameter estimation from experimental data** (satisfies Subobjectives 7–8).
 - **Existing:** Matlab, SUNDIALS (SUite of Nonlinear and Differential/ALgebraic equation Solvers)
- **Debugging of metabolic networks** (satisfies Subobjective 7). Identify dead-end metabolites, missing enzymes, reachability analysis.
 - **Existing:** Pathway Tools performs all these functions for qualitative metabolic models.
Model SEED can perform some of these functions for quantitative metabolic models.
- **Gap filling for metabolic networks** (satisfies Subobjective 7). Hypothesize which genes code for missing enzymes in metabolic networks.
 - **Existing:** Pathway Tools performs this function.
Model SEED can perform some of these functions.
A variety of context-based and inference methods also can be used to find gene candidates, such as Phylogenetic Profiles, Gene Neighbors, Gene Clusters, and Rosetta Stone.
- **Exchange and alignment of metabolic models** (satisfies Subobjective 2).
 - **Needed:** Tools for exchanging metabolic models among software platforms. Such tools should perform syntactic conversion among platforms and semantic alignment to facilitate comparisons. Semantic alignment means establishing correspondences among the identifier spaces used by different platforms, such as establishing correspondences between the compounds and reactions

Appendix A: Supporting Scientific Objective and Software Requirement Documents
for Near-Term Microbial Science Needs

produced by the Palsson platform and those produced by the Pathway Tools platform.

- **Comparison of metabolic models and simulation results** (satisfies Subobjective 2).
 - **Existing:** Pathway Tools provides extensive tools for comparison of metabolic models. More tools are needed, or capabilities should be supplemented.
 - **Needed:** A tool that reports on differences among metabolic network models, both at the reaction and gene levels.

A tool also is needed that summarizes differences among simulation results or flux states of a model, perhaps with results sortable by magnitude of difference so users can focus on important changes first and sortable by pathway to allow differences to be grouped in understandable ways.

- **Computational tools for metabolic engineering and pathway design** (satisfies Subobjective 6).
- **Computational tools for predicting rate limitingness in metabolic networks** (satisfies Subobjectives 4 and 8).

Standards

A number of standards can be used in metabolic studies. Largely missing are well-established standards for storing experimental data. Standards such as SBML and BioPAX can be used to store stoichiometric information, modifiers (e.g., inhibitors), parameter values, and rate laws, but very few data standards are available to store flux data, enzyme levels, time-course data, and such. Standards for storing experimental data need to be developed. One possibility is to use SBRML (Dada et al. 2010) for data storage together with supporting formats and ontologies that have arisen in recent years.

Existing standards (defined as community-agreed formats implemented in more than one software tool) are:

- SBML: Extensible representation of stoichiometric and regulatory models (www.sbml.org) including extension
- FBA-SBML: SBML Flux Balance Analysis extension package ([Bergmann and Olivier 2010](#))
- BioPAX: Annotation standard for biological pathway data; could be used with SBML (www.biopax.org)
- SED-ML: Standard for describing simulation experiments (www.biomodels.net/sed-ml/)
- SBO: Systems Biology Ontology used to annotate the kinetic aspects of a model (www.ebi.ac.uk/sbo/)
- TEDDY: Ontology for the dynamics of biomodels (www.ebi.ac.uk/compneur-srv/teddy)
- KiSAO: Kinetic Simulation Algorithm Ontology (sourceforge.net/projects/kisao/)

- SBGN: Systems Biology Graphical Notation for unambiguous visualization of pathway (www.sbgn.org)
- SBRML: (www.comp-sys-bio.org/tiki-index.php?page=SBRML). Can be used to store data and simulation results
- CellML: Math-based representation of models (www.cellml.org)

SBML extensions packages might be developed to accommodate these kinds of data, using the core SBML format to anchor the metabolic network itself. An absent significant standard is a method for annotating a model with the assumptions used to build it and with the data sources used to parameterize it. In addition, there is currently no easy way to create a history of a model-building process so that previous models can be easily retrieved and so that model development can be traced.

Governance

Where possible, newly developed software should be open sourced under a suitable license, preferably a Lesser General Public License, Berkeley Software Distribution, or other nonrestrictive license.

To propose new data standards and maintain existing ones would require regular focused workshops, preferably twice a year, with the stakeholder community. These meetings would serve as technical discussion groups to develop a strong community structure. Specification documents would need to be drawn up, test data developed, and supporting software libraries written to read and write the proposed data formats. A governance structure would need to be devised to manage specifications, open-source licensing, and revisions and to assess proposals. The entire process should be as open as possible and involve the community at every stage.

Governance should be handled in a mixed top-down and bottom-up process. Ideas and new proposals should originate from the grassroots community so users have a stake in development. This is critical to ensure buy-in from the community. However, a top-down process also should be in place to ensure adequate development of documentation, organization of regular meetings, maintenance of websites and wikis, quality control of any developed libraries to support standards, and a mechanism to allow the community to vote for editors on a 3-year rotation. Finally, there should also be a mechanism for organizing interoperability jamborees (or Hackathons) at least once a year. Interoperability should include loading and reading the standard correctly and, most important, ensure error-free round-tripping from one tool to the next.

Definitions and References

Definitions

Qualitative metabolic models: Metabolic models that define the reactions, enzymes, and (optionally) regulatory properties of an organism's metabolic network but do not describe its flux rates, concentrations, or kinetics.

Quantitative metabolic models: Metabolic models that define some combination of flux rates, concentrations, and kinetics of a metabolic network.

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Metabolic reconstruction: A qualitative or quantitative description of metabolic genes, enzymes, and transporters in an organism and the reactions these proteins catalyze.

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A.3 Microbial Scientific Objective 2: *Define Microbial Gene Expression Regulatory Networks*

The ready availability and evolution of genome-scale expression data (e.g., microarray, RNA-Seq, and single-molecule RNA measurement) and the rapid extension into new datatypes [e.g., transcription start site determination by high-throughput sequencing (HTS), RNA polymerase and regulator binding by ChIP-chip and ChIP-Seq, and quantitative regulator sequence-specificity determination by HTS methods) make the definition of microbial gene expression regulatory networks an attractive goal of the Kbase project (Bonneau et al. 2007). In the short term, inference of regulatory networks from just genome sequence and expression profiles under varied cellular conditions is possible and would be of very valuable general utility to researchers. In the longer term, validation and refinement of these models using various functional data types will allow robust correlation of regulatory networks to genome features. Interconnection of the regulatory networks with metabolic reconstructions and multidimensional annotations (two other high-priority objectives identified by the Kbase microbial group) would greatly facilitate development of microbial systems biology (Koide et al. 2009a).

A range of phylogenetically diverse microbes should be selected for initial efforts, ranging from well-characterized microbes for which extensive data exist to enable the most-informed analyses (e.g., *E. coli*, *S. oneidensis*, *G. sulfurreducens*, *H. salinarum*, and *D. vulgaris*), to those less well characterized (e.g., *Z. mobilis* and *C. thermocellum*), to those for which little information exists. Prioritization should be given to organisms of central relevance to the DOE mission. For the microbes chosen, the finished genome sequence would be expected to be available, together with those for a few phylogenetically related organisms. In addition, genome-wide transcriptomic data (RNA-Seq, tiling array, or spotted array) for multiple growth states would be expected. Ideally, a multilevel annotation would be advantageous. Data should focus on regulatory paradigms of greatest relevance to the microbe in question and the DOE mission. For instance, for facultative anaerobes, a focus on O_2 and C regulation would be appropriate; for *D. vulgaris*, a focus on sulfur regulation might be appropriate; for *G. sulfurreducens* and *metallireducens*, the focus could be on radiation and pollutant stress; and, for *H. salinarum*, a focus on salinity and radiative and oxidative stress would be most interesting. The remainder of this scientific objective document focuses on the case of O_2 and C regulation to describe one possible focus in greater detail, but similar descriptions could be generated for the other possible foci.

Background Information

The advent of genomics and global transcriptomics has identified an ever-increasing diversity of functions regulated by O_2 , including not only the expected metabolic functions (e.g., fermentation, respiration, and photosynthesis) but also many unexpected activities (e.g., cell surface proteins, sugar transport genes, nucleotide metabolism genes, transcription factors, and virulence factors) as well as gene products of unknown function. In *E. coli*, O_2 deprivation alters gene expression to promote a decrease in carbon flux through the citric acid cycle and a redirection of carbon and reducing equivalents from aerobic respiration to either fermentative

pathways or anaerobic respiratory pathways; these anaerobic pathways sustain ATP synthesis via substrate level phosphorylation or in the presence of alternative electron acceptors (e.g., NO_3^-) via oxidative phosphorylation. Genome-wide expression profiling by several groups has established that 5 to 10% of the open reading frames (ORFs) in *E. coli* K12 change expression more than twofold (Constantinidou et al. 2006; Kang et al. 2005; Partridge et al. 2007; Partridge et al. 2006). These data indicate that the functions regulated by O_2 are surprisingly varied and that our understanding of anaerobic growth of even a well-studied organism like *E. coli* remains quite limited.

In *E. coli* and related bacteria, multiple transcription factors collaborate to control gene expression in response to changes in O_2 . At the center of the O_2 regulatory network lies the triumvirate of FNR, ArcA, and IscR that collaborate with other regulators like NarL, NarP, DcuR, and PdhR to target a more limited set of genes. The complete set of regulators, their promoter target sites, and the patterns with which they interact (the regulatory network) remain incompletely defined. ArcA and its cognate two-component regulator ArcB independently control a large number of genes, although some genes are regulated by both FNR and ArcA. IscR activates a large number of genes when O_2 is present, represses others, and collaborates with FNR, ArcA, or both to control many of these genes. Further, the roles in O_2 regulation of small RNAs, transcriptional attenuation, and synergy with nucleoid structure are almost wholly unexplored, although involvement of small RNAs and nucleoid structure has recently been documented (Durand and Storz 2010; Oberto et al. 2009; Teramoto et al. 2010).

Of particular importance to the DOE mission due to its centrality to biofuel production, carbohydrate utilization appears to be altered in the presence or absence of O_2 . For instance, diverse sugar transporters are among the genes whose expression is controlled by the O_2 regulatory network. *E. coli* and most bacteria encode a large number of diverse transporters for various mono-, di-, and derivatized saccharides. Some of these and other transporters are downregulated during anaerobiosis, raising key questions: (1) Are transport systems that depend on the proton motive force less favored anaerobically than aerobically, which could easily be explained by the lack of electron transport-driven proton translocation?; (2) Are new transport proteins upregulated anaerobically to replace perhaps more energetically costly aerobic transporters?; (3) Is transport of substrates that can only conserve energy via respiration selectively repressed anaerobically? The known players in carbon source regulation and their binding sites, at least under aerobic conditions, are relatively well known and consist of the catabolite activator protein (CAP or CRP) that upregulates a large number of genes when glucose is low or absent as signaled by its coactivator cAMP, FruR (a regulator of central carbon metabolism), DsgA (another global regulator of carbohydrate metabolism, particularly of the PTS system), and specific activators and repressors that control catabolic genes for various sugars (e.g., LacR and AraC). Even the relatively simple paradigms that regulate specific sugar catabolism operons lead to complex individual behaviors that are incompletely understood even without the complication of O_2 regulation (Kaplan et al. 2008). The complete extent of this network is not worked out, a fact highlighted by the existence of up to 1000 CRP-binding sites detected by genome-scale ChIP-chip (Grainger et al. 2005).

Ongoing Experiments (a very incomplete list)

- Expression profiling of both coding and noncoding RNAs is at increasingly higher levels of resolution (e.g., RNA-Seq).
- Regulatory network modeling is ongoing in a variety of laboratories (Ernst 2008; Gianchandani 2009; Michoel 2009; Huttenhower 2009; Lemmens 2009; Faith 2007; Bonneau et al. 2007).
- Visualization approaches are being explored by the Karp group at SRI International (e.g., EcoCyc and Pathway Tools); RegulonDB; the NIH EcoliHub project; MicrobesOnline (Dehal et al. 2010); the Baliga group (Bare et al. 2007; Shannon et al. 2006; Gehlenborg et al. 2010); Integrated Microbial Genomes (IMG) at the DOE Joint Genome Institute (JGI); Integrated Genome Browser at the University of North Carolina, Charlotte (Nicol et al. 2009); Galaxy at Pennsylvania State University; and recently by the Wilkerson group that is part of the Great Lakes Bioenergy Research Center (GLBRC) at Michigan State University (MSU). This is an incomplete list.
- Transcription unit definition experiments (mapping the 5' and 3' ends of transcription units) to locate promoters precisely and possible attenuation sites in the absence and presence O₂ is ongoing in several laboratories [e.g., Palsson lab at University of California, San Diego (UCSD); Kiley and Landick labs at University of Wisconsin, Madison (UW Madison); Morett lab at UNAM Biotech Institute, Cuernavaca, Mexico; and the Baliga lab at the Institute for Systems Biology (ISB), Seattle]. See recent publication from Palsson lab (Cho et al. 2009) and Baliga lab (Koide et al. 2009b).
- CHIP-chip and now CHIP-Seq experiments to map regulator and RNA polymerase occupancy on promoters and genes are ongoing in several laboratories (e.g., Palsson lab at UCSD, Kiley and Landick labs at UW Madison, and the Baliga lab at ISB).
- Metabolomics and metabolic flux experiments on the flow of carbon under aerobic and anaerobic conditions are ongoing at the GLBRC. These include studies using pure sugars and sugar mixtures, as well as complex sugar mixtures present in real biomass hydrolysates that are inputs in the developing cellulosic biofuel industry. Metabolomics experiments are ongoing at UW Madison, and metabolic flux experiments are ongoing at MSU in the Schachar-Hill lab.

Prioritization

PRIORITY: X HIGH MEDIUM LOW

The broad goal of enabling creation of gene expression regulatory networks was recognized as a high priority in the microbial breakout group at the June 1–3 Kbase workshop. There were mixed opinions as to the particular microbes or networks that might make the best initial targets from the DOE perspective. These are reflected in the overview statements for both this scientific objective and the resulting requirements. As noted above, this objective concentrates on O₂ and C regulation as an illustrative example. Providing a broadly applicable tool for generating gene regulatory networks from RNA expression data is a high initial priority. The

network modeling capability should then be extended to additional data types, both to refine the models and to test their predictions against experimentally validated identification of transcription units, promoters, regulator binding sites, regulator binding specificity, protein-protein interactions, genetic interactions, metabolomics, and metabolic flux measurements.

Potential Benefits

As described above, O₂ regulation of carbon metabolism is a central issue for engineering biofuel-producing microbes. A complete understanding of the regulatory networks that mediate this regulation will allow researchers to specify the patterns and extents to which expression of different genes turns on as cells are shifted from aerobic to anaerobic growth conditions. Further, gaining complete control over gene regulation during anaerobiosis is essential to optimize the conservation of reducing equivalents into biofuels and may allow the efficient production of advanced biofuels like isopentanol or alkanes in anaerobic conditions where loss of reducing equivalents to O₂ can be avoided (at present only fermentation products like ethanol or butanol can be produced anaerobically with significant yields). Finally, elucidation of the regulatory network by which O₂ influences carbon metabolism is important to the advancement of science in general. Until we know the roles and interactions of the different regulatory modalities involved (e.g., repression, activation, small RNAs, and attenuation) and how these networks have evolved among microbial lineages, we will lack understanding of fundamental components in the evolution of life on Earth.

Feasibility of Success: Near, Mid, and Long Term

Several data sources are relatively straightforward with current technology (RNA-Seq, ChIP-Seq). Data tracking, manipulation, and visualization challenges must be overcome, but these align well with the central objectives of Kbase. The remaining annotation work for well-characterized microbes also is relatively straightforward and is mostly a matter of collecting information from experts in the field. Thus, building a regulatory network model of the effects of O₂ on carbon source utilization for well-characterized microbes should be achievable in the 1- to 3-year time. Extension to other microbes like *Zymomonas* will be more of a challenge at the annotation end because less is known about TU structure, O₂-responsive, and C source-responsive regulators. This may require the 5-year time frame. Likewise, extension to include other data types such as metabolics, metabolic flux, or quantitative definitions of regulator binding sites will require the 5-year time frame. Full extension of these regulatory models to enough microbes to build a picture of the evolution of O₂-responsive and C source-responsive regulation could be achieved in the 5-year time frame with a massive effort, but this is more likely an appropriate goal for the 4- to 10-year time frame.

Relevance to the Kbase Project

Defining the regulatory network for O₂ control of carbon metabolism in different bacterial lineages offers an outstanding match to the goals of the DOE Genomic Science program's Kbase project. From the Kbase perspective, it will require organization of genome-scale datasets for transcription regulator distributions, expression profiles, and potentially quantitative regulator binding assays, proteomics, and metabolomics. This will drive development of data manipulation and visualization tools that will aid microbial systems biology, both at the cutting

edge and in extension into everyday use in microbiology laboratories. From the perspective of the central mission of DOE Genomic Science, it will provide crucial information to aid in engineering microbes for biofuel production.

Synergies and Leverages: Potential Overlap with Other Projects or Funding Agencies

The project should synergize with tool development by the NIH Pathway Tools, EcoCyc, and EcoliHub projects as well as various DOE efforts such as MicrobesOnline and the JGI IMG project. It will also synergize with efforts at the DOE Bioenergy Research Centers, such as work from the GLBRC to accumulate, visualize, and model data related to O₂ regulation and sugar utilization in *E. coli* and other bacteria, work from the Joint BioEnergy Institute on *Zymomonas*, and work from the BioEnergy Science Center on microbial RNA-Seq. Given the scale of the problem, these overlaps are more likely to generate synergies than conflicts, provided adequate attention is given to coordinating efforts.

Specificity

The specific scientific question to be answered is definition of the mechanisms by which the presence or absence of O₂ influences C source utilization, which C metabolism and C transport genes are upregulated or downregulated by O₂ presence, and the nature of the regulatory network that controls these effects. More broadly, the long-term goal is to define how these regulatory networks have evolved among bacterial lineages. In the shorter term, the goal is to define this network fully in a model, well-characterized microbe and then extend to other bioenergy-relevant microbes like *Zymomonas*, *Cellvibrio*, and *Cellulomonas*. By initially concentrating on annotation, RNA-Seq, and ChIP-Seq and extending to metabolics, metabolic flux, and possibly proteomics only as the initial goals are achieved, a suitable level of specificity will be ensured.

Details

Scientific Discovery Process (Workflows)

An incomplete workflow to create a knowledgebase of O₂ regulation of carbon utilization is described below (for each microbe, steps must be pursued in parallel).

Data Collection

- Complete annotation of genes and regulators involved in O₂ regulation and C metabolism in the model microbes chosen. For well-characterized microbes, this objective can be achieved fairly easily by collecting information from researchers in the field to update existing knowledge in resources such as EcoCyc.
- Strand-specific “tiling” RNA-Seq profiles of RNA levels in different growth conditions (vary O₂ tension and with different sugar carbon sources). A strand-specific RNA-Seq workflow is available from the Sanger Center (Croucher et al. 2009).
- Define transcription start sites (TSS) used in different growth conditions (vary O₂ tension and with different sugar carbon sources). Workflows exist for these experiments (Mendoza-Vargas et al. 2009) but are imperfect and still in a stage of refinement and

improvement owing to the difficulty of distinguishing real TSSs from RNA degradation products and the failure of existing HT methods to detect some known TSSs.

- ChIP-Seq genome-scale binding profiles of RNA polymerase, sigma factor, and major regulators of carbon utilization of O₂ sensing in different growth conditions (vary O₂ tension and with different sugar carbon sources). ChIP-chip workflows exist (Mooney et al. 2009; Cho et al. 2008, 2009), and these can be readily adapted to Chip-Seq experiments, which are under way in several laboratories (e.g., UW Madison; Palsson group).
- (Optional depending on scale of project; for instance, this could be added in the 3- to 5-year time frame.) Metabolomic and metabolic flux measurements in different growth conditions (vary O₂ tension and with different sugar carbon sources). Workflows for metabolomics (Bennett et al. 2009) and metabolic flux (Martin 2010) analyses exist.
- (Years 3 to 5 or 5 to 10, or optional.) Generate data on binding-site affinities as a function of binding-site sequence for transcription factors (TFs) involved in O₂ and carbon utilization regulation. Workflows exist for these types of experiments (Hauschild et al. 2009; Jolma et al. 2010; Zhao et al. 2009; Zhu et al. 2009; Zykovich et al. 2009), but they are in early stages of adaptation to use of high-throughput sequencing.
- (Years 3 to 5 or 5 to 10.) Define transcription termination sites (TTS) used in different growth conditions (vary O₂ tension and with different sugar carbon sources). Workflows do not exist for these experiments. Although poly(A)-tailing followed by high-throughput sequencing should map RNA 3' ends, methods to distinguish legitimate TTSs from degradation products must be developed.

Bioinformatic Analyses

- Generate complete binding-site profiles for regulators under growth conditions tested. Algorithms exist to generate this information from ChIP-Seq data (Jin et al. 2009; Kuan et al. 2008), although they remain in developmental stages of continual improvement.
- Generate from RNA-Seq data a complete catalog of small RNAs binding-site profiles for regulators under growth conditions tested (vary O₂ tension and with different sugar carbon sources).
- Generate from RNA-Seq, TSS, and potentially TTS data a complete catalog of transcription units (TUs) used under the growth conditions tested (vary O₂ tension and with different sugar carbon sources). Workflows to identify TUs exist (Cho et al. 2009) but will need to be extended when TTS data becomes available.
- Generate from data a complete catalog of transcription units (TUs) used under the growth conditions tested (vary O₂ tension and with different sugar carbon sources). Workflows to identify TUs exist (Cho et al. 2009) but will need to be extended when TTS data become available.

Regulatory Network Modeling

- Convert data on transcription factor (TF) occupancy to a predictive model of TF and RNA polymerase occupancy on promoters under different growth conditions (vary O₂ tension and with different sugar carbon sources).
- Infer and predict regulatory roles of small RNAs in O₂ and carbon utilization regulation under different growth conditions (vary O₂ tension and with different sugar carbon sources).

Inputs

Inputs are largely noted above (for each microbe, steps must be pursued in parallel).

Outputs

The output will be a staged set of regulatory network models.

- Initially, a regulatory network model based only on expression data will be produced. This is a reasonable goal for the 1- to 2-year time frame as long as the data inputs are limited to expression data from RNA-Seq or related methods. At this stage, a basic network modeling tool for general use by microbial researchers should be validated and hosted.
- A second-stage output will be extension of these models to include a well-defined set of RNA-Seq, CHIP-Seq, DNA binding-site predictions, and possibly metabolic, proteomic, protein interaction or genetic interaction data. A corollary of this will be testing of the network predictions from the RNA expression data against experimentally determined knowledge of promoters and transcription units.
- A third-stage output (5-year time frame) will be to extend this to additional carbon sources so that the synergy of O₂ regulatory and sugar utilization can be understood. Additionally, models for other organisms can be produced.
- The fourth-stage output (10-year time frame) will be an extension of these network models to include multiple bacterial lineages and additional types of data inputs (quantitative proteomic data, complete metabolomic and metabolic flux data). Concentration on bioenergy-relevant microbes is appropriate, and thus this 10-year time frame could logically include gram-negative bacteria such as *Klebsiella*, *Zymomonas*, and *Cellvibrio*, and gram-positives such as *Clostridium thermocellum*.
- With complete regulatory models from anchor-point species in place, comparative genomics can be used to generate models that explain how O₂ regulation of carbon source utilization varies among bacterial lineages. See Hittinger et al. (2010) for a recent example of such models for yeast. These evolutionary models should enable rapid generation of new regulatory models for novel (newly isolated) bacteria with desirable bioconversion properties.

Tools

Several new types of software tools will be required.

- A sophisticated and facile genome browser able to visualize multiple types of genome-anchored data (e.g., RNA-Seq, ChIP-Seq, TSS, TU, gene annotation, and nucleic acid sequence) and to allow at least rudimentary calculations on these data (track averaging, ratioing, normalization, peak identification using multiple types of algorithms), storage and recall of workflows and notes, and generation of figures.
- A metabolic pathway viewer that illustrates quantitative expression levels for different enzymes. Importantly, such a viewer must be able to distinguish isozymes differentially used for growth with and without O₂ and to visualize sugar transport processes. Extension of this visualization tool to include metabolomic and metabolic flux data would be desirable if such data are included in the scope of the project.
- A regulatory network viewer that illustrates the relationships among different regulators, including small RNAs and small molecules.
- An extensible knowledgebase of regulator binding sites for each gene and TU in the regulatory and metabolic networks. This tool should allow continual improvement of Kbase by user editing and incorporation of improved regulatory network models. A good example of such a knowledgebase is EcoCyc (Keseler et al. 2009).

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A.4 Software Requirements for Microbial Scientific Objective 2: *Define Microbial Gene Expression Regulatory Networks*

Summary of Scientific Objective

The scientific objectives can be broadly divided into two components. The first is to enable automated inference of gene expression regulatory networks relying principally on expression profiling data. The second is to extend these inferred networks to include additional data types, both to refine the network predictions and to test them.

Enable Automated Inference of Gene Regulatory Networks. Measurements of gene expression are becoming increasingly accurate with improvements in technology. The same is true for measurement of transcriptome structure, protein interactions, and modifications. In addition to advances in technologies for making systems biology measurements, analysis of these data is rapidly progressing at multiple scales—from discovery of operons and regulons to inference of systems-scale regulatory networks. While these rapid advances in experimental and computational methodologies pose significant challenges in standardization, they present a spectacular opportunity to infer high-quality gene regulatory networks through the integration of these data and interoperation across computational tools.

Extension and Testing of Inferred Gene Expression Regulatory Networks. In the intermediate to long term, Kbase should archive a collection of diverse systems biology datasets (e.g., transcript profiles, protein interactions, precise transcriptome structures, regulator binding sites, regulator binding specificity, and small-molecule concentrations) collected using best practices and archived in a standardized manner along with meta-information on how the experiments were conducted. In the intermediate time frame, Kbase should develop algorithms

that enable refinement and validation of regulatory networks using these comprehensive genome- and organism-scale validated datasets. In addition, Kbase should be a repository for algorithms and software tools with open and standardized APIs. Access to such data and tools across DOE-relevant organisms would enable automatic inference of gene regulatory networks and would be an extremely valuable resource to advance microbial research.

Resulting Requirements

Process of the Science (Including Workflows)

Selection of microbes on which to focus modeling efforts. A variety of phylogenetically diverse microbes should be selected for initial efforts, ranging from very well characterized organisms for which extensive data exist to enable the most informed analyses (e.g., *E. coli*, *S. oneidensis*, *G. sulfurreducens*, *H. salinarum*, and *D. vulgaris*) to those less well characterized (e.g., *Z. mobilis* or *C. thermocellum*) to those for which little information exists. Prioritization should be given to organisms of central relevance to the DOE mission. For the microbes chosen, the finished genome sequence should be available, together with those for a few phylogenetically related organisms. In addition, genome-wide transcriptomic data (RNA-Seq or tiling array) for multiple growth states would be expected to be available. Ideally, a multilevel annotation would be advantageous. Data should focus on regulatory paradigms of greatest relevance to the microbe in question and the DOE mission. For facultative anaerobes, for instance, a focus on O₂ and C regulation might be appropriate; for *D. vulgaris*, sulfur regulation; for *G. sulfurreducens* and *metallireducens*, radiation and pollutant stress; and for *H. salinarum*, salinity, radiative and oxidative stress.

Short-term, intermediate-term, and long-term tasks (still a partial list).

- 3 months: Identify target list of microbes and regulatory paradigms on which project will focus. These would be based on high-priority, high-value projects.
- 6 months: Complete definition of regulatory network reconstruction workflows. This implies a modifiable workflow that would have to be re-evaluated periodically to update new understanding.
- 6 months: Identify specific network inference algorithms for top-down vs. bottom-up methods. For example, the Baliga lab uses methods of the top-down approach. Use inference based on existing or expression datasets.
- 6 months: Collate existing data from microbes of interest for which sufficient data are available. (This relates to expression data.) Improve metadata associated with them. Build and use automation tools.
- 1 year: Make available for general use a capability for inference of regulatory networks from expression data (RNA-Seq, tiling array, or possibly ORF-specific array data; if generalized as an $n \times m$ matrix, any technology that generates such data could serve as input). Evaluate network inference tools and select them. Ultimately this task is an attempt to build an environment that does all network inference in an integrated way.

Appendix A: Supporting Scientific Objective and Software Requirement Documents
for Near-Term Microbial Science Needs

- 1 year: Create and make available inferred regulatory network from existing expression datasets.
- 1 year: Create a controlled vocabulary for metainformation to capture experiment design including perturbed environmental and genetic variables, media compositions, and growth conditions. Need to work with GEO or ArrayExpress to get this information captured better to be sure they have the required controlled vocabularies.
- 1 year: Provide a user interface for import and display of existing datasets, inferred transcription regulatory networks (TRN), and predicted binding sites (e.g., Pathway Tools, Cytoscape, and BioTapestry). Import inferred networks into these tools to provide better data exchange standards to allow interoperability among these tools. This would be a short-term, quick-and-dirty standard development.
- 3 years: Standardize interfaces and APIs for interoperation across selected data repositories, algorithms, and visualization software (using middleware such as Gaggle).
- 1 year: Develop web-based tutorials and demos for user “training” and Kbase dissemination. Some existing tools are not easy to use and need tutorials to help users.
- 3 to 5 years: Generate standards for regulatory network representations. This is about description of the network itself.
- 3 to 5 years: Incorporate other data types into regulatory network models (TSS, ChIP-Seq, proteomic; genome-anchored or unbiased determinations of regulator binding site specificity) for a bottom-up definition of regulatory networks. This is experimental validation of predictions made based on expression and is for validation and reconstruction. These data measure the regulatory network and currently are difficult to integrate. We need methods for capturing evidence and quality and to use them for making further predictions.
- 3 to 5 years: Extend regulatory networks to other types of regulation (transcription elongation, translation, RNA-based regulation, 3D genome architecture).
- 5 to 10 years: Extend regulatory networks to enough organisms to build a knowledgebase of the evolution of selected regulatory networks as well as network motifs through comparative network analysis capabilities such as multiple network alignment.
- 5 to 10 years: Develop a capability for coupled regulatory network models, metabolic network models, and annotation so that information is updated and exchanged.
- Develop high-quality regulatory network models by combining inference tools and bottom-up data and data from the literature. Note: Calibrate expectations appropriately.

Specific workflow for regulatory network generation by inference (“bottom-up” approach).

For the organism of interest, the genome is assumed to have been completely sequenced and fully annotated and that RNA-Seq or tiling array data are available for a minimum of 10 growth curves with 6 time points and 3 biological replicates on biological conditions relevant to O₂ and C use regulation.

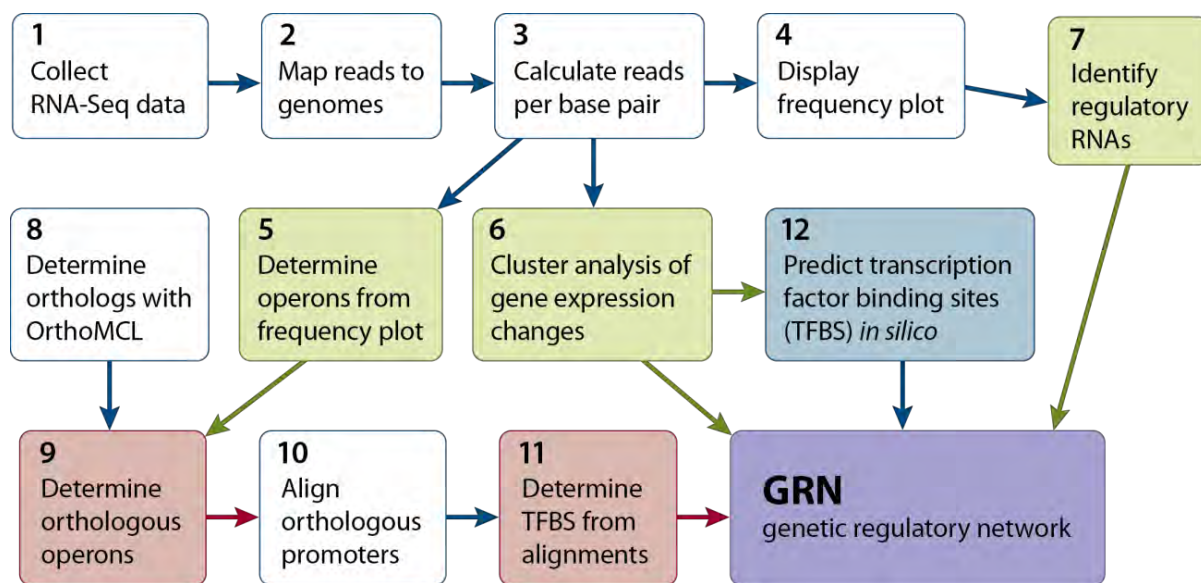


Fig. A.1. Transcriptome Analysis Pipeline for Gene Regulatory Network Prediction. White boxes are procedures we already know how to do. Green boxes are procedures that have not been determined but are expected to be fairly easy to construct (year 1). Red boxes are procedures that will be more difficult to construct (year 2). Blue boxes are optional techniques that would increase analysis accuracy. The purple box is the final product (year 2).

1. Collect RNA-Seq data and the **accompanying metadata** for each growth curve. The metadata could include optical density, substrate consumption, metabolites, temperature, and incubation condition (as comprehensive as possible). Although some data could be collected manually, Kbase would need to have the ability to store it in conjunction with RNA-Seq as an experimental project.
2. Map RNA-Seq data to genome.
3. Calculate reads per base pair (normalize and calculate expression levels of each gene and operon).
4. Display frequency plot for visual inspection and rule development for algorithms to identify the operons and regulatory RNAs in steps 5 and 7.
5. Determine operons from mapped reads (generate a separate list for each growth curve). These should include all genes, transcription initiation sites (TIS), and termination sites with the accuracy of a few base pairs for each operon.

Appendix A
Microbial 2

6. Perform cluster analysis on the calculated gene expression levels to determine co-regulated operons.
7. Identify regulatory RNAs (unknown riboswitches and small regulatory RNAs) based on analysis-derived rules identified in step 4 with expert guidance.
8. Determine orthologs from multiple related genomes using OrthoMCL or some other software tool.
9. Determine orthologous promoters from multiple related genomes.
10. Align orthologous promoters using Muscle or ClustalW.
11. Determine transcription factor binding sites (TFBS) from alignments.
12. Use *in silico* TFBS prediction tools together with co-regulated operons to predict additional TFBS (import known TFBS from a database such as RegTransBase).
13. Predict genetic regulatory network.

Specific workflow enabling inference of regulatory networks for which limited information exists. This could be implemented initially with a focus on O₂ and C source regulation, but the intent is to make this available as a tool for general use via a web portal.

Inputs. Depending on the specific type of network inference analysis a user has in mind, a different combination of the following data might be necessary. Minimally, these seven types of information cover most of what is available today.

1. Measurements of transcription (with confidence values if available) in the form of an n x m matrix with n genes and m conditions (microarray or sequencing).
2. Measurements of fitness associated with systematic gene knockouts or over-expression (maybe these last two can be condensed in measures of genome-scale gene function with confidence in the form of an n x m matrix. This could be generalized as phenotype and also have associated confidence depending on how it is measured).
3. Gene interaction networks = nodes (genes), edges (interactions and type), confidence values or weights for edges.
4. Gene locations on genome; with RNA-Seq this is becoming extremely precise with direct measurement.
5. Genome sequence or individual upstream sequences (for motif detection).
6. List of predictors (transcription factors and environmental factors including metabolites).
7. Machine-readable descriptions of conditions, specifically time series information with standardized measurements of environmental factors.

User will specify an organism and import (or broadcast) the above data items. Many of these data types are stored in existing databases and can be loaded automatically

through interoperability with these data sources. Many data types (items 2 through 4) can be obtained automatically, given the organism. Item 1 may be obtained automatically from expression databases such as GEO or MicrobesOnline. Item 3 can be obtained from STRING, and some information is also accessible in MicrobesOnline. Items 4 through 6 can be obtained from NCBI, MicrobesOnline, or other databases.

As these workflows are being developed and have increasingly precise data such as RNA-Seq and can have associated confidence measures that can be carried through the analyses, this is providing a basis for comparing the precision of results between methods and laboratories that would help to improve quality and would benefit existing systems such as GEO if applied consistently.

Apply clustering and network inference. Group the genes into putative regulatory modules whose transcription is correlated over a set of conditions (e.g., cMonkey). Select a subset of known transcription factors and environmental factors that best predict the transcription levels of each module (e.g., Inferelator, CLR). Additional inputs such as motifs or protein interactions may be statistically integrated in the clustering step or the network inference step, and shared regulatory motifs can be computed. Several algorithms have been devised for clustering and discovery of regulatory influences; some are available in R and MatLab.

Outputs

- Clusters of putatively co-regulated genes or biclusters containing genes putatively co-regulated under subsets of conditions.
- cis-regulatory motifs.
- Regulatory network mapping: Influences of predictors on genes within clusters and biclusters directly or through "and" as well as "or" operations. Confidence values for edges.

Results can be exported as raw data or presented to the user in a searchable and browsable form (e.g., in Cytoscape or BioTapestry). Users may want to start with a set of genes or a metabolic process and ask which factors are its regulators, or they may want to take a given regulator and ask what its targets are. Subnetworks can be displayed graphically along with graphical views of expression profiles and regulatory motifs and the gene content of individual clusters. Useful output also will include the ability to compute and present predictions (and confidence estimates on predictions) of the effect of TF deletions and overexpressions or environmental changes.

Scope. A user should be able to select an organism; upload, broadcast, or import expression data from public repositories or their own data; and submit a request for network inference. Metainformation on experiment design should be automatically parsed from public data, or the user should be prompted to upload this information. All other data types can be automatically parsed from public repositories. An advanced user should have privileges to change or override default settings by changing the sources of information and threshold of significance. A user should be given options for

choice of algorithms based on the amount and type of available data; the user should have access to published citations for the algorithms and basic information on workings of the algorithm in nontechnical jargon-free language. It should be possible to store a session with default or user-edited settings so the entire analysis can be recreated.

Workflow for data-driven regulatory network generation and testing and for network analysis (“bottom-up” approach). Further work after the initial implementation (years 1 to 2) would include evaluation of additional technologies and experimental verification to improve the process (5' RACE to identify additional TSSs, CHIP-Seq, microfluidic TFBS determinations, and transcription factor regulatory ligand determinations). As these data begin to accumulate from experimental efforts, possibly driven in part by solicitations from the Kbase project, the specific predictions of inferred regulatory networks should be compared to experimental data that will test TFBS predictions, transcription unit predictions, and promoter predictions. The TF-binding information can readily be combined with sigma-factor networks and genome-wide promoter architecture that are available now or currently being reconstructed by using either computational or experimental methods.

Integration of multiple datasets should be based on genome coordinates. The various types of data available will influence the confidence level of users' interpretation. Therefore any changes and interpretations need to be documented (e.g., as a comment function) with genome coordinates (base pair resolution).

The next step following regulatory network inference in a broad range of organisms implemented during years 1 to 2 of the project is to analyze and compare their topological structure and to attempt to reconstruct their evolutionary history.

The workflow for this phase of the project is as follows:

1. Implement Kbase software tools allowing users to analyze and visualize the genome-wide architecture of a regulatory network.
2. Calculate the distribution of regulon sizes and the number of regulatory inputs.
3. Perform the hierarchical layout of TRNs using a variety of algorithms (e.g., breadth-first and depth-first).
4. Minimize the number of bottom-up links. Use this layout for network visualization.
5. Identify feed-forward network motifs of different types depending on the combination of signs of regulatory interactions (activation and repression).
6. Identify and characterize cross-talk and regulatory overlap between different functional pathways.

The fraction of transcriptional regulators in bacterial genomes has been shown to systematically increase with genome size (van Nimwegen 2003). This has to be reflected in the architecture of transcription regulatory networks. To investigate these trends, one needs to analyze the topological characteristics of TRNs (e.g., average number of inputs, regulon size, and number of hierarchical layers) as a function of the organism's genome size, its lifestyle (free-living vs. parasitic), and evolutionary group. Tools need to be developed for comparing

regulatory networks in different species. These would allow users to align regulatory networks in different species using information about orthologous proteins and to trace and visualize phylogenetic profiles for network topological properties in a group of genomes selected by the user.

TFBS determination methods should be incorporated into this workflow. Examples of existing methods for this evolving technology are listed below.

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Instruments to Support Achievement of the Scientific Objective

An important caveat here is that technologies change rapidly and in unanticipated ways. It is important to separate instrumentation data (raw data) from the processed data type (gene expression). For example, technologies involving array (hybridization intensities) and sequencing (sequence reads) can both generate gene expression data as an $n \times m$ matrix, where n represents genes and m represents environmental conditions over which those measurements are made. Also, these measurements are associated with statistical significance values. Herein there might be some challenges in reconciling differences in statistical methods used to analyze different datasets. We should also make executive decisions regarding the level at which data need to be archived. Technology maturity would be a consideration. For instance, there is no sense in archiving array images, but these data were important when the technology was in its infancy.

Kbase should support data from old (legacy) as well as new technologies including arrays and RNA-Seq as well as ChIP-chip and ChIP-Seq data from Solexa, ABI Solid, and 454. For the future, additional machines such as PacBio or Ion Torrent may need to be supported. These instruments produce data of particular types and sizes that will need to be stored and managed within the context of Kbase and are further described in the Data section below.

There will be potential for use of automated or high-throughput instruments for generating phenotypic data. Metadata such as optical density may be recorded manually or in spreadsheet

output from instruments, and Kbase will need to have capabilities for manual input or upload of such electronic data, which would then be integrated within the experimental project.

User Interfaces

The anticipated users will include biologists who wish to analyze their data; bioinformaticists who want to analyze data, contribute or improve methods, and use existing methods; and scientists requiring information and visual representations for scientific publications. Users are expected to come from the academic, government, and industry communities. Users below the university level are not anticipated.

Interfaces will be needed for specifying an experimental project and locating the relevant RNA-Seq and associated experimental metadata. Users will expect to have a login space where they describe their experiment, save it, and return at a later time.

Scientific data visualization is needed that renders genome annotation, gene expression information, operons, alternative transcriptional starts, and multiple sequence alignments. User interfaces will be needed for visualizing frequency plots that show depth of coverage (relative expression levels) for genes and operons and the resulting gene regulatory network model.

Programmatic Interfaces

Kbase will need to have programmatic interfaces to support specific queries such as to return a list of all experimental conditions that an organism has been exposed to for which there are gene expression data.

Also, software that determines expression levels or predicts operons or refines their prediction will need access to genome annotation. Therefore data interfaces to NCBI's Sequence Read Archive, GEO (Gene Expression Omnibus), and GenBank (bacterial genomes) will be needed and perhaps application interfaces to IMG, RAST, or DOE JGI–Oak Ridge National Laboratory annotation systems. We will also need to import known TFBS from a database such as RegTransBase.

The results of the workflow to predict gene regulatory networks will also produce data that would be output to data interfaces such as to all systems mentioned above to supply new data, support publication submission, or update annotation.

Data

In the near term, we expect to see for a given experiment several hundred files from short read sequencing technology. These files, if based on Solexa or other next-generation sequence technology, will range in size from 100 megabytes (MB) to 100 gigabytes (GB) for the next couple of years. Current size ceiling is at about 4 GB compressed for one run. Total data storage required is based on coverage and number of replicates, conditions, and time steps and therefore would be a multiplicative factor of 4 GB (180 X minimum as proposed). For the first 1 to 3 years, 30 to 100 datasets are expected per year (each dataset corresponding to studies on one microbe); the number would grow to 100 to 300 per year in the 3- to 5-year time frame when these data will be coming from many laboratories.

Database and storage resources in the terabyte to petabyte range are needed. Data reduction will play a role in keeping storage resources manageable. Online backup capabilities are needed for disaster recovery and long-term archival.

Data types that cover high-throughput technologies to interrogate the transcriptome are required for this scientific objective.

Genome sequences and a full complement of annotation features are also required. The data representation model as characterized by a GenBank record is probably not sufficient. New data models that capture gene annotations and their relationships to other annotations will be required. Annotation can exist remotely as in the case of taxonomy information housed in the NCBI taxonomy database and other NCBI Entrez data for which stable access exists through NCBI web services.

The gene regulatory network from a data structure perspective is the collection of operons, transcription factor binding sites, sigma factor binding sites; and parameters that affect kinetics. These would benefit from representation based in semantic web technology.

Relational database technology is expected to play a limited role insofar as perhaps providing structured storage of ontologies and Resource Description Framework (RDF) tuples.

Providing easy-to-use mechanisms for data download and upload is important to meet the best practices of data collection and recording of experimental protocols as close to the instruments' raw data as possible yet usable for data processing and analysis algorithms. For example, the matrix form of gene expression data can be more suitable than the raw images. Ideally, very little or no normalization should be applied to data exposed to the user, and end users would decide how they want to preprocess or normalize data before feeding it into analysis software. The mechanisms of data access and upload also should comply with governance rules, such as ones specifying policy on how soon data should be available to the community and how data should be credited or acknowledged if being used for other publications. Similar requirements hold true for algorithms and software tools, which both should provide simple-to-use examples of how to use them. Ideally, software should be accompanied with base-level tutorials on how to use it.

Software

Software should be treated at four loosely coupled distinct levels:

- Data and metadata management level.
- Middleware level (e.g., Gaggles or workflow systems such as Kepler, DagMan, Taverna).
- Algorithm and software tools level [e.g., Context Likelihood of Relatedness (CLR) and Inferelator algorithms].
- User interface level (e.g., Cytoscape, BioTapestry, MeV, DMV). Refer to *Nature Methods'* March 2010 special issue on Biological Visualization.

Note that the elements within each layer should be pluggable and replaceable. They should be capable of addressing individual layers without being imposed to go through any specific layer.

For example, an algorithmic layer should be able to directly access the data management layer. Likewise, the data flow across multiple algorithms could be orchestrated by the middleware layer, and the output of individual algorithms could be visualized by the corresponding element in the user interface layer.

It is important that data provenance information (such as versions of algorithms and tools with algorithm parameters) has been executed on the data that must be recorded in the data- and metadata-management layer for the purpose of reproducibility and interoperability of results.

Here are some representative examples of algorithms and software tools for different elements in the workflow described above:

- Clustering software: Biclustering such as cMonkey and SAMBA (Statistical-Algorithmic Method for Bicluster Analysis) as well as R's package in Bioconductor
- TFBS prediction software

Software for performing transcriptome analysis will be needed as part of the workflow and for visualization. It will integrate existing available genome annotation and provide measures of confidence. Annotation quality will be accessed based on confidence. A specific module will focus directly on improved identification of transcription factors.

Improved annotation with confidence and evidence codes will be sent back to repositories if possible.

Clustering software will be needed to group genes and operons into clusters based on patterns of regulation. Whether a part of the clustering software or of a different package, software that focuses on fine details of the operon, such as alternative transcriptional starts and stops, will be needed.

Clustering algorithms will be compute-intensive. Other methods are manageable with mid-range servers.

Data visualization software that spans genome annotation, transcriptome analysis, and clustering also will be needed.

Microbial 2: Define Microbial Gene Expression Regulatory Networks

Table A.1 Software Requirements for Microbial 2

[Note: “Compute” means significant processor resources are required (>100 cores), and “storage” indicates significant storage resources are required (>1 Terabyte).]

Software Purpose	Availability	Improvements Needed?	Resource Impact
Maps RNA-Seq data to genome	Few	Probably not	Storage
Cluster analysis of gene expression changes	Many	Probably	Compute, Storage
Operon determination	Few	Yes	
<i>In silico</i> TFBS prediction	Many	Yes	Compute
Ortholog determination	Few	Probably not	
Orthologous operon determination	None		
Promoter alignment	Few	Yes	
Promoter prediction	Few	Yes	
Gene regulatory network prediction	Few	Yes	Compute, Storage

Standards

Standards are effective only when they are adopted widely.

- Gene regulation ontology (GRO) for terms related to gene expression.
- Gene ontology (GO) for terms related to biological processes, cellular location, and gene function.
- NCBI Sequence Read Archive xml schemas for sequence read metadata.
- Genomic Contextual Data Markup Language (GCDML) xml schema for genome metadata.
- Minimum Information about a Microarray Experiment (MIAME) regards gene expression arrays but may be relevant to RNA-Seq.
- A new schema for archiving experimental metainformation. Please note that MIAME does not capture this.

Governance

A data release policy will need to be in place, most likely the current DOE policy. Kbase is expected to enforce it, implying a private login that maps to System Architecture.

Summary and Prioritization of Requirements

In silico prediction of TBFS can be postponed until other elements of the workflow are complete (mid term). Support for microarray data was considered but has not been included for the sake of simplicity. If it were part of the requirements, it might be lower priority because we believe it is phasing out. Other requirements for possible inclusion would be various kinds of validation such as 5' RACE and TFBS verification (mid term).

System Architecture Attributes

Users will expect that data they submit will be secure in accordance with the governance model. This would be the highest priority.

There could be some performance issues resulting from the choice of clustering algorithms and the amount of input data. Performance and security are architecture issues considered of highest importance for this objective.

Kbase Key Services

- Mapping RNA sequence reads to a genome
- Identifying operons and transcriptional units
- Identifying alternative transcription starts and stops
- Identifying transcription factor binding sites
- Improvements to genome annotation based on the services above
- Data structures for representing gene regulatory networks
- Query services for retrieving gene regulatory network models
- Query services for retrieving all experimental conditions to which an organism has been exposed and for which there are gene expression data

Risk Analysis and Mitigation Strategies

- Disagreement among stakeholders over objectives and approaches could undermine the project's ability to produce tools that will find widespread use. This risk is higher because various stakeholders have been involved in different stages of the Kbase initiative's development, with not all being present at a single forum that would allow generation of consensus. This is a frequent Achilles heel in large-scale bioinformatics projects. Potential for this in the Kbase project, at least among the microbial contingent, is evident. Possible mitigation would be continued efforts to achieve consensus and careful selection of goals that will achieve the widest buy-in among stakeholders.

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- Unanticipated changes in technology (sequencing, microarray) that would significantly change the requirements or implementation plan. Mitigate by anticipating changes and adjusting requirements and implementation plan as soon as possible.
- Inadequate data or poor data quality that precludes a productive workflow as currently designed. Mitigate by testing typical datasets for adequacy and quality. Modify experimental protocol to correct and change minimum standards.
- Cluster analysis on these datasets requires more resources than currently anticipated. Mitigate by modifying algorithm to accept some additional error in return for performance speed. Allow clustering on subsets to manually find the optimum with reduced error.

APPENDIX B

Supporting Scientific Objective and Software Requirement Documents for Near-Term Plant Science Needs

This appendix provides the working documents for the two selected plant scientific objectives and requirements. These documents were the core output from the final DOE Systems Biology Knowledgebase (Kbase) workshop held June 1–3, 2010 (see [Appendix D](#) for the workshop report). The scientific objectives must answer the question, “What is the scientific or research goal that needs to be solved?” The related “requirements” establish workflows and provide details on the needs to accomplish these objectives. The process of identifying the scientific objective, determining its software requirements, and then developing an implementation plan from the two was described in [Chapter 1](#). These working documents are provided as backup to their implementation plans, which are described in [Chapter 3](#) and contain the final revised judgment by the community concerning the tasks needed for each objective.

In research, a scientific objective is satisfied by creating hypotheses and doing one or more experiments depending on the scope of the objective. For every experiment, there are rationales, protocols to be executed, a number of data inputs (data sources) and outputs (results), and analysis tools. Workflows describe this information. They are sequential procedures that describe the envisioned steps to answer questions. Workflows are the bioinformatic equivalent of an experimental protocol. Detailed workflows are bridges between the experimental research and computing communities and thus are key to translating research objectives into computing requirements that will most effectively advance the science. Workflows were developed for these objectives. From these workflows and the underlying objectives, the requirements could be defined that lead to the articulation of an implementation plan with tasks and scope to achieve the scientific and technical goals.

The first objective in the plant science area is to establish the capability to predict alterations in plant biomass properties caused by genetic or environmental changes. This capability would be based on the mining of data that reflect the complex relationships among the physical properties of plants, their genetic makeup, and the environment in which they grow. The second objective is to develop the ability to organize and analyze regulatory “omics” data to improve understanding of how plants (particularly species relevant to DOE missions) regulate gene expression. This capability will be critical for understanding genes, their action, and regulation—knowledge required to engineer plant growth and development and, in particular, biomass accumulation.

B.1 Plant Scientific Objective 1: *Integrate Phenotypic and Experimental Data and Metadata to Predict Biomass Properties from Genotype*

The ability to gain an understanding of the genetic underpinnings of desirable plant biomass properties relevant to Department of Energy (DOE) missions (e.g., biomass yield, conversion efficiencies to biofuels, and the sequestering of soil carbon or contaminants) depends in turn on the ability to conduct co-relational assessments between molecular and phenotypic data. Identification of genes underlying the expression of desired phenotypes depends upon association of multiple genotypes contributing to a trait of interest (forward genetics) or, if a candidate gene is being investigated, an understanding of the impact that a gene product's gain or loss of function has on an extended phenotype (reverse genetics). In most cases, the complexity and plasticity of plant growth and development make it difficult to predict the impact of a perturbation in one specific gene because this phenotypic impact is rarely confined to the pathway in which the gene product operates. A key objective for the DOE Systems Biology Knowledgebase (Kbase) is to facilitate understanding of genotype-phenotype relationships by providing a platform for integrating information on genotype, extended phenotypes, and the metadata associated with field and greenhouse growth conditions. The long-term goal is to support a systems biology approach for the prediction of biomass properties by new combinations of alleles through breeding strategies or by targeted modification of genes or genetic features.

Analysis of experimental data describing the chemical and physical phenotypes of plants is a complex endeavor not within the scope of any single researcher or laboratory. Acquisition, retrieval, processing, mining, and analysis of these data depend critically on robust and standardized methods to record and track key metadata used to maintain the coherence and context of observational data. Semantic technologies, such as formal ontologies, provide vocabulary control and specify the meanings of core concepts and key features of these methods. Current semantic infrastructure, however, is inadequate to support and contextualize experimental plant phenotypic data well enough to achieve the long-term goal of predicting changes in physical properties of biomass that occur as a result of a specific environmental change, genetic modification, or allelic variation. This goal will be enabled by the formal representation of community knowledge regarding relationships among phenotype, genotype, and environment as a basis for inferring the logical implications of diverse experimental datasets.

Achievement of this ambitious goal depends on the creation of a robust semantic infrastructure for collection, annotation, and storage of diverse phenotypic and environmental datasets. These data include measurements such as those from imaging and mass spectrometry technologies that capture visible phenotypes (e.g., images) and chemotypes (e.g., analytical spectra) that are fundamentally related to yield and physiological performance and sustainability. Specifically, this infrastructure will be used as a basis for software applications that extract, quantify, and catalog phenotypic features from the data for the purpose of data mining and further analysis. This involves the association of data with relevant metadata to enable querying, modeling, clustering, and comparison of data from diverse datasets generated by different platforms. For example, it will be used to identify genotypes, haplotypes, or

mutants that have a particular phenotype and to identify all phenotypes related to a specific genotype. It also will be used as the basis for tools that generate graphical representations of the data that intuitively illustrate the important relationships of data to their biological context.

The near-term goal is to establish a controlled vocabulary that allows description and representation of genotypic, phenotypic, and environmental data; associations or correlations among them; and the resulting facilitation of quantitative comparisons of spatiotemporally varied plant phenotypic data across diverse experimental contexts. One immediate implementation of such a controlled vocabulary is the development of software tools that aid the researcher in designing experimental protocols that automatically return the appropriate semantically contextualized data and metadata. Implementation of these experimental designs will be facilitated by linkage to other applications that support the actual data collection using mobile devices such as an iPhone, a laptop peripheral device, or Laboratory Information Management System (LIMS) scanner.

Background Information

The DOE Bioenergy Research Centers and laboratories funded through research activities such as the DOE and US. Department of Agriculture (USDA) Feedstock Genomics program are engaged in, for example, understanding the basis of cell-wall recalcitrance to enzymatic hydrolysis for biofuels production. Part of this effort is the generation of hundreds of transgenic lines in which genes with functional annotations related to cell-wall biogenesis are misexpressed. In the broader plant community and through the USDA, an emphasis on sustainable growth of bioenergy feedstocks will generate perhaps thousands of target genes for which functional data will be acquired in areas such as field performance, plant growth, and environmental interactions. As high-throughput phenotyping platforms develop (e.g., saccharification screens measuring release of sugars from hydrolysis of biomass genotypes or mass spectroscopy based on sample pyrolysis), these diverse datasets need to be associated with individual genotypes. In addition, some low-throughput data with high information content are related to individual genotypes, such as structural data contained in light, confocal, or electron microscopy images. No infrastructure or platform currently allows all phenotypic data to be first collated and correlated with a specific genotype and, in the long term, allows inferential learning for new knowledge.

Another limitation to associating genotypic and phenotypic variation is the expense and inconsistency among groups in phenotyping large populations. The interplay of genotype and the environment leads to extensive variation in the physical, morphological, and biochemical properties of the plant. Interpretation of phenotypic data thus requires capture of the experimental design and metadata that formally represent the experiment's controlled components and data provenance. This ultimately can enable individual datasets to be related to each other, which can be accomplished within a single species if methodologies, genetic stocks, and reagents are in common. Between-species comparisons require a formalized vocabulary that captures not only the data but how the experiment was accomplished. Therefore, a Kbase function that serves as an interface between community-established standards and a researcher's experimental plans would be essential to enabling effective

downstream cross-comparisons between diverse experimental datasets to be performed using other recommended Kbase features.

Prioritization

Since there is an immediate need for high-level integration of both high-throughput genetic datasets and different phenotypic datasets in plants across and within experiments using a variety of different analytical approaches, the development of a rich controlled language and basic experimental standards is a high priority. This is true particularly in the diverse realms of phenotypic data, experimental design components, and associated metadata. A tangible benefit to users will be a simpler infrastructure for implementing many types of statistical analysis that may be contributed by the community or that already exist for purposes such as feature extraction from raw data, application of genetic models, and drawing associations from unrelated experiments. Incorporating them into Kbase should receive high priority. This objective will benefit directly from improved genome annotation methods (see [Section 5.5](#)), genetic diversity databases, and crop-specific databases that may not even exist for target species.

PRIORITY: **HIGH** **MEDIUM** **LOW**

Potential Benefits

The process of feedstock development and redesigning feedstock properties from the level of plant architecture and yield to biomass recalcitrance would benefit from having a unifying semantic infrastructure from which to draw inferences and organize diverse datasets. These benefits may take the form of mobile applications for actually acquiring data, experimental design tools, statistical analyses that were previously inaccessible, and high-level modeling. Success would allow adoption not only within the six target species but within the larger plant science community. Tools generally will be valuable within the larger community and particularly outside the few crops that are targets of large investments in technology. For bioenergy crops and model species, integration of data from both high- and low-throughput phenotyping experiments across species and with other omics datasets, although not a short-range goal, is nonetheless critical to refining definitions of gene function, high-level model building, interpreting orthologies, and understanding the genetic architecture of traits. This goal is dependent on being able to relate diverse datasets in a broader biological context that can then be interpreted and used for inferencing.

Feasibility of Success: Near, Mid, and Long Term

This is a high-level objective that relies on development as well as adoption of standards by the plant research community.

TERM: **NEAR (1–3 years)** **MID (3–5 years)** **LONG (5–10 years)**

Implementation of assistive technologies for phenotyping applications can begin in a relatively short time frame for mobile applications, once an appropriate semantic structure has been created. Also, in the near term, the incorporation of functionality to perform a basic set of statistical analyses would provide some value to the user and would drive adoption.

For allowing feature extraction from complex biological data, appropriate analysis will depend on the type of data. Images at various scales are one of the most ubiquitous types of data, and allowing feature detection and extraction from images is feasible; some software already is available for performing these functions. Integrating these images into Kbase in a manner that can be used productively is critical for success. Such a capability is recognized as being feasible over the longer term but should begin in a time-sensitive manner.

The prospects for success in combining genotypic datasets with phenotypic datasets will depend on implementing algorithms for marker-assisted selection, association, and mapping of quantitative trait loci (QTL) that can be achieved with appropriate statistical genetic packages and rely on contributions from the community. Success will need to be tied to community-driven donation of these algorithms, integration of existing packages, and the willingness of individual groups to share data. This should be feasible with data-availability requirements of publicly funded programs and creative incentivizing of Kbase contributions (e.g., active contributors can be active users). Adoption of standards by individual communities is an issue that needs to come through consensus but may be hastened if incentives such as tools and analyses are provided. With these incentives, success is defined by adoption within the community and growth in the size of standardized experimenting. Data entry is expected to be steady but long term in nature.

Relevance to the Kbase Project

In preceding Kbase workshops and in the 2009 report ([Systems Biology Knowledgebase for a New Era in Biology](#)), significant emphasis has been placed on crops within the context of DOE's biomass research program. The activities of basic and applied plant biologists working on the six target species will amass phenotypic data from functional genomics projects, plant improvement programs, and environmental samples. In conjunction with controlled language structures, genotypic data, transcriptomics, and detailed metadata, these phenotypic data can be reused and combined in new ways to improve overall predictive value of models envisioned in the plant scientific objective described in [Section 5.3](#), Construct, Simulate, and Validate Plant Life Models.

Synergies and Leverages: Potential Overlap with Other Projects or Funding Agencies

Other programs, departments, and governmental agencies—such as the National Science Foundation (NSF), Plant Genome Initiative, and the Cooperative State Research, Education, and Extension Service within USDA's National Institute of Food and Agriculture—also should be involved in this activity because the underlying analytical software and modeling capability generally will be applicable to all crops. Other initiatives related to this area are oriented toward defining trait ontologies for individual groups of crops and developing database models to handle phenotypic, genotypic, and provenance data. Significant overlap within these groups' objectives should be resolved into individual contributions. In most cases, these activities are synergistic in that they have already laid much groundwork. No current efforts are ongoing in providing rapid analysis through integration of phenotypic and genotypic data within the context of plant improvement programs.

The following projects relate to this objective.

- **Existing LIMS systems and germplasm management systems.** Germinate and Agrobase (proprietary) and the International Crops Research Institute for the Semi-Arid Tropics. These provide some germplasm management tools, statistical analysis, and experimental design templates appropriate for self-fertilizing clonal crops; crops with cytoplasmic male sterility; cross-pollinating crops; open-pollinated crops; and polycrosses and synthetics common to some grass breeding programs.
- **Phenotyping projects incorporating smart phone technologies that engage the larger research community similar to those required for Kbase applications.** Epicollect (www.spatalepidemiology.net) and Phenomap (www.appstorehq.com/phenomap-iphone-113872/app/).
- **Trait indices, inventory management systems, and taxonomical data.** International Crop Information System (www.icis.cgiar.org/icis/index.php/ICIS_Concepts/).
- **Standards, protocols, and descriptors lists** (used by curators of germplasm stock centers). International Plant Genetic Resources Institute (IPGRI).
- **Gramene plant ontologies and trait ontologies.** (www.gramene.org/plant_ontology/).
- **Gene ontologies.** (www.geneontology.org). Gene ontology (GO); protein ontology (PO); and phenotype, attribute, and trait ontology (PATO).
- **Standard definitions, Crop Science Society of America, and others.** Specific systems for developmental staging.
- **Diversity databases.** Genomic Diversity and Phenotype Data Model (GDPDM) and Genomic Diversity and Phenotype Connection (GDPC) (www.maizegenetics.net/gdpc/).

Specificity

This is a higher-level objective that can be broken down into component parts such as incorporation of statistical models for determination of QTL or breeding value by different methods appropriate to the desired goal; integration of sequence and genotype standards; mobile applications; and structured ontologies. Adoption of these tools will be gradual as users perceive value to their research programs and produce appropriate plant populations and datasets. Thus, the necessary work also can be gradual, starting with the ability to integrate and assemble short reads derived from large numbers of individuals within a population and following with single-nucleotide polymorphism (SNP) calling.

Details

How is information stored for further related datasets in subsequent experiments or rounds of selection? What privacy policies would be acceptable to user groups to encourage data submission? The data should be leveraged for future use and should be connected to physical stocks to facilitate reuse. How are existing software tools and other groups' work best integrated into the context of bioenergy crops and models?

Scientific Discovery Process (Workflows)

Development of a robust semantic infrastructure for plant phenotyping research will streamline the acquisition, annotation, archiving, retrieving, processing, and mining of data that reflects the complex relationships among plants' physical properties, their genetic makeup, and the environment in which they grow. A typical workflow that would be supported by this infrastructure is described below.

Biologists, geneticists, and breeders set up field trials or controlled greenhouse experiments to collect data. The type of analysis to be performed is based on an interview process or similar interface to help identify the parameters to be measured (data) and key metadata based on prior assumptions, knowledge of biologically interesting features, previously collected data, and relationships among parameters inferred from this data. A formal, semantic model of the data and associated metadata is developed using semiautomatic methods that incorporate the parameters to be measured and relevant metadata. These methods involve user interfaces that allow the experiment designer to enter the parameters in response to relevant questions regarding the experimental setup, providing lists of appropriate (semantically defined) responses. Armed with such data models, applications that facilitate the collection and uploading of datasets (along with metadata) from the field and greenhouse are implemented using mobile devices such as an iPhone or laptop computer. These applications automatically record positional and temporal data and also could integrate local weatherstation data. The collected data are packaged and automatically uploaded to a server. Sequence data, including barcoding or similar methods for identifying specific plants and their treatment, is included in the uploaded data in relatively raw form. The uploaded data are collected and stored by a server for subsequent processing. For example, SNP data are generated for a specific population based on uploaded sequence data. Data are checked for consistency, for example, by identifying outliers that may arise due to human error. Using automated methods the data, along with associated metadata, are semantically annotated and archived in a database. Subsequent data processing leverages semantic annotations to populate specific models used to identify QTL and other biologically relevant information implied by the data.

Inputs

Datasets include the following.

- **Metadata.** Experimental design; plant starting source (seed, stem cutting, propagule); age; replication; field or greenhouse layout; position [global positioning system (GPS)]; climate and environmental conditions; log history of major environmental variations, date and time, laboratory, and institution; references; SNP indices; technology platforms; individual DNA sequences; and barcoding keys.
- **Population data.** Pedigrees, ploidy level, breeding type (outcrossing and inbreeding estimates), kinship estimates, population structure analysis, parents, and seed lots (more metadata).
- **Sequence datasets with associated metadata.** Genotype data from various platforms or from genetic diversity databases, nature of genetic modifications (mutation, transgene over- or underexpression, random or site specific), Illumina, and other platforms.

- **Phenotypic datasets.** Derived from individual traits; may include images and chemotypes.

Outputs

Results would be analysis dependent. For example, in genome-wide selection, the output would be the estimated breeding value of individual genotypes and haplotypic effects. A second example is the capability to perform graphical clustering of related phenotypes and phenotypic attributes when co-analyzing phenotypic datasets obtained from distinct characterization platforms.

Tools

1. **Phenote.** Software for recording some types of phenotypic ontology information (phenote.org).
- **MausDB.** Links mouse phenotypic data with genotypic data, metadata, and external data such as public web databases, a prerequisite for comprehensive data analysis and mining.
- **Mouse Phenome Database.** (phenome.jax.org).
- **Tools developed and listed above under related projects.**

References

Maier, H., et al. 2008. "MausDB: An Open Source Application for Phenotype Data and Mouse Colony Management in Large-Scale Mouse Phenotyping Projects," *BMC Bioinformatics* **9**,169.

Calder, R. B., et al. 2007. "MPHASYS: A Mouse Phenotype Analysis System," *BMC Bioinformatics* **8**, 183.

International Plant Phenomics Network (www.plantphenomics.com). Aims to develop, integrate, and provide novel technologies to analyze plant phenotypes.

B.2 Software Requirements for Plant Scientific Objective 1: *Integrate Phenotypic and Experimental Data and Metadata to Predict Biomass Properties from Genotype*

Summary of Scientific Objective

Improvements in computational infrastructure are required to support and contextualize experimental plant phenotypic data to predict changes in the physical properties of biomass that occur as a result of environmental changes and genetic diversity or manipulation. Achieving this ambitious goal depends on the creation of a robust semantic infrastructure for collection, annotation, and storage of diverse phenotypic and environmental datasets. These data include measurements such as photographic images and analytical spectra that capture visible phenotypes and chemotypes fundamentally related to yield and physiological

performance and sustainability. Specifically, this infrastructure will be used as a basis for software applications that extract, quantify, and catalog phenotypic features from data for data mining and further analysis. This involves association of data with relevant metadata to enable querying, modeling, clustering, and comparison of data from diverse datasets generated by different platforms. For example, it will be used to identify genotypes or haplotypes that have a particular phenotype and to identify all phenotypes related to a specific genotype. It also will be used as the basis for tools that generate graphical representations of data that intuitively illustrate the important relationships of data to their biological context.

In the short term, computational tools are required to aid the researcher in designing experimental protocols that provide semantically contextualized data and metadata. Implementation of these experimental designs will be facilitated by software applications that support the collection of semantically contextualized data using mobile devices such as iPhones or laptop computers. The long-term goal is the formal representation of community knowledge regarding relationships among phenotype, genotype, and environment as a basis for inferring the logical implications of diverse experimental datasets.

Resulting Requirements

IMPACT FACTOR: X HIGH MEDIUM LOW

Attaining the scientific objective requires appropriate vocabulary standards for a wide variety of data and metadata that describe phenotypes, chemotypes, genotypes, and experiments designed to collect these data. Although several such standards and ontologies exist, they require additional expressiveness to achieve the objective. To share relevant experimental data and ensure its completeness (e.g., in terms of associated metadata), a community-approved standard for the Minimum Information for A Plant Phenotyping Experiment (MIAPPHE) would be helpful. However, such a standard does not currently exist. The development of all these standards demands a long-term committed collaboration between computer scientists and plant scientists.

Appropriate standards for semantic description and exchange of primary data (physical measurements, images, and spectroscopic data) are not available. For example, standards are required to specify plant form, morphology, anatomy, coloration, development, and function. Development of such standards may involve the extension of existing standards after identification of their shortcomings. Some measurements are species specific, so customizing standards to the target plant species may be necessary in some cases. That is, currently available standards may be too species specific, not quantitative, or outdated. In other cases, entirely new standards may be required.

Initial testing of data structures and semantic annotation protocols would be facilitated by phenotypic and genomic datasets that could be analyzed retrospectively. Conclusions obtained via the newly developed Kbase infrastructure and tools would be compared to results previously obtained by manual methods.

For target species, there are no genomic databases that support specification of genetic diversity (e.g., SNPs) within the germplasm of existing stocks. These are necessary to identify

useful correlations between genetic and phenotypic variations. Populating these genomic databases requires pipelines for calling SNPs *de novo* in the absence or presence of annotated genomes. Such pipelines exist but have not been validated for the target species, leading to high false-positive rates and low rates of validation.

Methods that detect and quantify defined features from complex data (such as photographic images or spectroscopic data) are required to facilitate correlation of data within or among datasets. Comparison of vast amounts of raw data that will be generated is not practical. Furthermore, conceptualization of correlations embedded within the diverse datasets will require representation of identifiable features rather than raw data patterns. As an example, the shape of a leaf can be parameterized and represented as a value chosen from a set of possible “leaf shapes” enumerated as specific, semantically defined entities.

Statistical methods are required to assess the consistency of data, identify correlations, and provide metrics describing the confidence of any conclusions inferred from the data (e.g., genetic or environmental causality of a phenotypic variation). The general statistical framework for such analysis largely exists but is evolving. Currently, implementation of statistical methods that incorporate both phenotypic and genotypic data (e.g., for parent selection in plant breeding experiments) is extremely slow and cumbersome, and methods tailored for processing plant phenotypic data are needed. Methods to rapidly identify and implement such statistical methods are required, for example, for efficiently selecting breeding stocks within the context of a plant improvement program.

Process of the Science (Including Workflows)

A broad range of experimental protocols will be implemented, and a typical experimental workflow is described below.

Scientists will enter relevant information describing the experimental setup directly into a LIMS system or onto a PC application. This information will then be used to develop an experiment-specific data model that will be used to automatically configure an application implemented on a mobile device used for data acquisition in the field. Complementary data for the same set of plants will be generated using a broad range of instruments but will be integrated using semantic annotation and made conformant with community standards for representation and content (e.g., the proposed MIAPPHE standard). During acquisition, the experimentalist will have the opportunity to eliminate artifactual data and impute missing data using automatic, semiautomatic, and manual methods implemented on the mobile device. Data then would be uploaded along with metadata using representations that reflect the relevant experimental data model. This data model will suggest certain types of analysis that could be run automatically or prompted via an interview process. Data processing may be as simple as performing analysis of variance (ANOVA), although complex experiments might require comparing varieties or individuals whose phenotypes were recorded in different years, experimental groups, and locations, in combination with genotypic data in a genome-wide association study and archival environmental (e.g., weather) data. This will make it possible to evaluate the impacts of temperature and moisture variation across years and locations and how they affect the identification of candidate QTL or estimated breeding value.

Instruments to Support the Achievement of Scientific Objective

A wide variety of instruments is constantly evolving for this purpose, including

- Smart phones
- Barcode scanners
- GPS systems
- Plate readers
- Image recorders (cameras)
- Physiological measurement systems (e.g., water potentiometers and fluorimeters)
- Analytical chemical analysis tools (e.g., mobile pH meters, spectrophotometers, and mass spectrometers)

User Interfaces

Users will include primarily plant scientists who develop and implement traditional forward genetics experiments (e.g., plant breeding) that are performed under a broad range of environmental conditions (i.e., in the field). Users also may include experimentalists who are performing detailed studies on gene up and down regulation. Data collection in the field may be performed by field laborers with little training or relevant experience.

Programmatic Interfaces

Programmatic interfaces include

- Data-uploading protocols that interface mobile devices with Kbase servers.
- Data integration interfaces that enable association of uploaded data with relevant metadata, semantic annotation of data, and archiving annotated data in the database.
- Interfaces that allow remote applications to execute semantically enabled queries for retrieval of annotated data.

Data

A very broad range of data will be collected and processed. This includes physical measurements (e.g., size, weight), location, color, temperature, moisture, various other environmental parameters, and analytical spectroscopic data. Much of this data may be in the form of digital images that will have to be processed for feature extraction and noise reduction. In addition, genomic and genetic data—including genetic background, ancestry, and known genetic variations (e.g., SNPs or engineered genetic modifications)—also will be required. The core challenge of this scientific objective is the collection, annotation, and integration of these diverse data.

Software

Plant 1: *Integrate Phenotypic and Experimental Data and Metadata to Predict Biomass Properties from Genotype*

Table B.1 Software Requirements for Plant 1

Software Purpose	Availability	Improvement Needed?	Resource Impact
Data collection and uploading	Yes	Yes	
Experiment design and data model generation	Yes	Yes	
Graphical visualization	Yes	No	
Data feature extraction	Yes	Yes	
Statistical evaluation	Yes	Yes	Community Contributed
Data integration			
Knowledge inferencing			

Appendix B
Plant 1

Standards

Standards for maintenance of controlled vocabularies describing various data types and possible measurement values (e.g., units of measurements) are of primary importance. In addition, standards for exchanging diverse primary data would be helpful because they would minimize the amount of time required for data collation and integration at the server level. Finally, a global standard that specifies the content and form of a plant phenotyping experiment would be extremely useful. Such a standard (MIAPPHE) could be based loosely on the MIAME (Minimum Information About a Microarray Experiment) standard for microarray datasets.

Governance

Data privacy would be a concern among many users, and, in many cases, data would need to be kept until publication. Extensive community engagement is required for standards and encouraging data and algorithm contribution.

Summary and Prioritization of Requirements

Of primary importance is the development (within the first year) of the basic semantic infrastructure to support controlled vocabularies and representation of relevant concepts related to plant phenotype, chemotype, genotype, and growing environment. This effort includes the identification, extension, or *de novo* development of ontologies (or other formal

descriptions of relevant concepts and nomenclature), along with primary methods that can query these formal descriptions (e.g., to check data for proper classification or representation).

Within the first 2 years, development of prototype versions of a data collection platform that leverages the controlled vocabularies described above will be necessary. Tools for remote data collection and server-side tools for aggregation, annotation, and archiving of raw data will be needed.

The ability to collect and archive well-annotated data is a central requirement for success of the scientific objective. Support for a broad range of data types (e.g., spectroscopic and other high-volume data) will depend on the adoption or development of appropriate data-exchange standards.

Also required within the first 2 years are methods for assessing data consistency and confidence levels as well as statistical methods for correlation, clustering, multiple regression, and data dimension reduction (e.g., principle component analysis) that facilitate data mining and analysis. Methods for identifying, extracting, and quantifying features present in the raw data (e.g., spectroscopic peak ratios and morphological features in digital images) will be necessary to take full advantage of the data collection, annotation, and processing infrastructure described above.

Evaluation of experimental data and correlation of phenotypic and genetic variation will require genomic databases populated with information describing the genetic diversity (e.g., occurrence of SNPs) in breeding stocks or other sources of plant germplasm. Populating such databases requires a significant investment of resources, although pipelines for automated population are likely to become more available in the near future. Thus, acquisition of required data will be an ongoing process performed over the entire 10 years of the research project.

Ultimately, success of the scientific objective will depend on providing scientists with intuitive graphical representations of raw and processed data in the context of agronomy, plant biology, and genetics. The most powerful (i.e., contextualizing) graphical interfaces will depend on the availability of a robust semantic infrastructure. These graphical interfaces are expected to evolve over time, with prototype visualization tools being developed during the first year. These resources should improve considerably as general tools for semantic querying, browsing, and data representation are developed by computer scientists outside the Kbase team. Again, the challenge to Kbase will be implementation of the most powerful technologies and their adaptation to phenomics, genomics, and environmental data of interest.

Risk Analysis and Mitigation Strategies

Failure to implement at least prototype versions of the semantic infrastructure at an early stage will result in this project's failure, although development of ontologies and associated semantic methods will be an ongoing process aimed at increased expressiveness and robust performance. To avoid duplication of code development, the most general of methods supporting semantic annotation should be implemented as part of the Kbase global infrastructure so they can be applied in different contexts within Kbase. The risk is that parallel ontology development by groups outside the Kbase team will result in competing vocabularies

describing the same concepts. This is a well-recognized issue in ontology development, and methods (i.e., ontology alignment and mapping) are being developed continuously to maintain consistency among related ontologies and allow semantic disambiguation of data annotated using diverging ontological representations. Development of the basic infrastructure should be straightforward, but semantics currently is recognized as an area of intense study. Therefore, flexible infrastructure should be designed so that is capable of adapting to and incorporating new developments in this field.

Provided that robust mechanisms for vocabulary control (as described above) are available, development of the data acquisition platform should be a straightforward implementation of well-established technology, and the risk of failure is low. Data representation and exchange standards may evolve over time, most likely impacting the scope and efficiency of the data collection platform. That is, development of the platform's advanced functionality will be limited by our ability to efficiently and unambiguously represent raw and partially processed data using defined standards. This risk can be mitigated by allocating modest additional resources to development of data exchange standards, assuming that interest in the plant phenomics community is sufficient to support such standards.

Development of methods for assessing data consistency and confidence levels is likely to be straightforward with little risk of failure. In addition, a wide range of statistical methods is currently available, and adaptation of these methods to plant phenomics probably will be straightforward, provided that appropriately annotated raw data is generated using the methods described above.

A vast body of literature describes feature detection methods, and the major challenge will be selecting the most appropriate computational methods and their application to plant phenomics. Implementation of several of these methods is expected to be straightforward during the first 3 years, and more robust or specialized methods will be developed at later times. Failure to identify and adapt such methods is unlikely, although predicting their selectivity and sensitivity is not possible when applied to phenomics data.

The availability of appropriately indexed genomic diversity data cannot be predicted. Although a considerable amount of such data probably will be generated for biomass crops in the near future, much of it undoubtedly will remain proprietary. A considerable risk is that, although methods to populate genomic diversity databases using high-throughput sequencing data are in development, the availability of genetic diversity data is likely to remain limited without support from scientific journals, funding agencies, and the scientific community at large. For example, a requirement for depositing genetic diversity data as a condition for publication will lead to a considerable increase in the available amount of such data.

Development of intuitive visualization tools is unlikely to represent a significant risk. However, their effectiveness will depend on adequate allocation of resources for tool development.

B.3 Plant Scientific Objective 2: *Assemble Regulatory Omics Data for Target Plant Species in Common Platforms to Enable Annotation, Comparisons, and Modeling*

This objective seeks to collect several key types of regulatory omics data and associated quality metadata for six target plant species: *Brachypodium*, *Chlamydomonas*, poplar, sorghum, switchgrass, and *Miscanthus*. Such information will support other plant scientific objectives related to annotation, comparison, and modeling. RNA levels as measured by expression arrays or RNA-Seq are no longer sufficient to evaluate mechanisms and networks that regulate plant transcriptomes. Kbase also must include available small RNA and target RNA information, differential RNA processing and decay information, and epigenetic marks such as DNA methylation and histone modifications. This information is important for data integration and for filling in important missing links in gene regulatory networks within a species and facilitating their comparison across two or more species. In the short 1- to 3-year term, classical transcriptomic data (microarrays and mRNA-Seq) as well as small RNA and basic proteomic data will be assembled. Epigenetic data, small RNA target data and RNA degradome data, other types of RNA processing data, and additional proteomic data will be assembled after year one, with the most developed genomes such as *Brachypodium's* beginning first. The data will be made publicly accessible with user-friendly web interfaces and will be downloadable for power users.

Background Information

Understanding which genes are regulated during growth and development and under various conditions is critical for elucidating gene function and regulatory networks. Massive amounts of genome-wide gene expression data are accumulating in plant systems that can be used to evaluate these controls at the transcriptional and post-transcriptional levels during development and in response to stimuli such as adverse environmental conditions. RNA abundance levels have been assayed routinely using microarrays and, more recently, mRNA-Seq, which is the current state-of-the-art approach (Wang et al. 2009). Also accumulating since 2005 are small RNA data from deep sequencing that report on miRNA and siRNA abundances and gene silencing potential (reviewed in Chen 2010). Other types of emerging data and data analyses are providing insights about miRNA targets and the RNA degradome (German et al. 2008; Addo-Quaye et al. 2008) as well as other aspects of RNA processing such alternative and regulated splicing and polyadenylation (Licatalosi and Darnell 2010). Beyond RNA data, proteomic data from shotgun mass spectrometry is available for some species that allows protein levels to be evaluated to examine translational control. All these data are required to effectively evaluate gene expression, and they provide essential support for the other plant scientific objectives.

Prioritization

PRIORITY: HIGH MEDIUM LOW

Potential Benefits

To date, we have limited understanding of how plants regulate gene expression and how this is manifested in the cell. Critical to understanding and then engineering plant growth and development for DOE missions is an informed understanding of genes, their actions, and their regulation. Our early understanding of gene regulation was focused on upstream promoters and mRNA expression levels. We are now aware of entirely new pathways of regulation involving small RNAs, post-transcriptional control, the epigenome, and more. Deep research in understanding multiple types of regulation at the DNA, RNA, and protein levels is occurring in plant, mammalian, yeast, *Caenorhabditis elegans*, and fly systems. Arabidopsis is the most studied plant with respect to the regulatory pathways affecting genes and their products.

Feasibility of Success: Near, Mid, and Long Term

Achieving the foundation of this objective in the 1- to 3-year time frame is feasible.

TERM: NEAR (1–3 years) MID (3–5 years) LONG (5–10 years)

Existing and new methods for generating and analyzing large-scale omics data from experiments with differential regulation need to be applied to the target species and the results analyzed and integrated. While a portion of regulatory omics data has been generated on select target species, a comprehensive effort has not been made to characterize complete datasets of regulatory omics data. Further lacking has been a funded mechanism to collate and integrate the multiple datasets and data types. These mechanisms do exist and, if applied through a core set of funded projects and funneled into Kbase, the scientific objective will have a high likelihood of success.

Relevance the Kbase Project

This objective is essential to the DOE systems biology mission. Without key data, including the acquisition of datasets, coupled with analysis of their interactions, no informed prediction of biological systems can be attempted. Naive attempts at networks are certainly possible with co-expression data, but they are highly limited and represent neither the full spectrum of what can be accomplished with current technology nor what should be completed if the mission is to understand plant species on a systems level.

Synergies and Leverages: Potential Overlap with Other Projects or Funding Agencies

Systems biology is an immature field in plant biology (Coruzzi and Gutiérrez 2009). Certainly, large-scale datasets are being generated in an array of plant species. The focus of this objective on key species relevant to the DOE mission will deepen and expand these resources. Additional major advances relevant to this objective—such as improvements in cost and throughput funneled into genome centers—will arise from the genome technology field. Algorithmic and computational improvements in network prediction and visualization are under way in model

organisms. These typically are made available to the greater research community via publications, open-source software, and collaborations. Partnering with DOE microbial systems biology scientists who have experience in constructing regulatory networks would provide great synergy. The types of datasets may overlap with iPlant and other resources, although the focus on bioenergy crops and models is unique to DOE and USDA. Therefore, synergy is expected.

Specificity

Defining the species, datasets to be collected, and species to target as well as the timetable for generating, analyzing, and integrating data is essential. While generating data is straightforward, analysis and integration will be a challenge. Thus, we recommend that only five species be undertaken for analysis and integration during the first 1 to 3 years. Omics datasets would be limited to classical transcriptomic data (microarrays and mRNA-Seq) as well as small RNA and basic proteomic data. This would provide an appropriate dataset for resolving any bottlenecks prior to generation of omics datasets for post-transcriptional regulation and epigenetic regulation, beginning first with *Brachypodium*.

Details

Scientific Discovery Process (Workflows)

Workflows exist for large-scale regulatory omics data generation. These are straightforward and can be adapted to the target species with little effort. Analysis and integration tools can be leveraged from model organism research but will need to be adapted slightly for the specified plant species.

Inputs

For a target plant species, Kbase will need an annotated genome sequence (draft or finished) as well as omics data that are or become available in the public domain, including RNA-Seq, microarray, small RNA, and basic proteomic data initially, with methylation, RNA degradome, and RNA processing subsequently.

A genome sequence in the form of an assembly and accompanied by metadata and quality information will be downloaded from public repositories [e.g., GenBank, and the DOE Joint Genome Institute (JGI)] and stored in Kbase as a flat file and in a relational database. Genome annotations will be obtained from the same sources or from other efforts to improve plant annotations and their accessibility as described in [Section 5.5](#). Data standards exist for genome assemblies and annotation.

Omics data will be downloaded from public repositories [e.g., DOE JGI; NCBI's GenBank, Expressed Sequence Tags Database (dbEST), Short Read Archive (SRA), Gene Expression Omnibus (GEO); and others] and stored in Kbase as a flat file and in a relational database. Such data include

- Conventional expressed sequence tags (EST) and cDNA sequences (Sanger cDNAs and ESTs). Data standards exist.

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- Microarray data (Affmetrix, Agilent, NimbleGen, others). Data standards exist.
- High-throughput transcriptomic sequences (e.g., Illumina, SOLiD, 454). Data standards exist.
- Small RNA data. Data standards exist.
- Proteomic data. Data standards exist.

Subsequently:

- Epigenomic data (DNA methylation, histone modification). Data standards under development.
- RNA degradome and other RNA processing data. Data standards under development.
- Additional proteomic data.

Outputs

Functional annotation of genome and gene models with respect to the following.

- Transcript levels and differential regulation.
- Small RNA levels and differential regulation.
 - Protein coding genes.
 - Noncoding RNA genes.
- Gene and transcript associations.
- Protein levels and differential regulation.

Subsequently:

- Genome epigenetic marks.
- Transcript start sites and polyadenylation sites.
- RNA decay products, some of which identify miRNA targets.
- Alternatively spliced and alternatively polyadenylated transcripts.
- Additional protein and protein modification data.

Tools

Available software:

- Conventional transcript alignment algorithms.
- High-throughput sequencing (HTS) transcript alignment algorithms.
- Reference-guided transcript assembly algorithms.
- *De novo* transcript assembly algorithms.
- HTS gene expression analysis algorithms for transcript abundance.
- Microarray analysis algorithms.
- Within platform (e.g., microarrays) expression profile clustering algorithms.
- Proteomic data analysis algorithms.
- Algorithms for analysis of HTS-derived epigenetic data (DNA methylation, ChIP-Seq for chromatin modifications).
- Algorithms for analysis of alternative splicing and polyadenylation.
- Data visualization (Cytoscape, Genome Browser).
- Co-expression analysis software.

Software needed or needing improvement:

- Small RNA analysis algorithms.
- Algorithms for analysis of HTS-derived RNA degradome data.
- Hybrid empirical (HTS) and *de novo* gene finders.
- Between platform (microarrays vs. RNA-Seq) expression profile clustering algorithms.

B.4 Software Requirements for Plant Scientific Objective 2: *Assemble Regulatory Omics Data for Target Plant Species in Common Platforms to Enable Annotation, Comparisons, and Modeling*

Summary of Scientific Objective

This objective seeks to collect several key types of regulatory omics data and associated quality metadata for six target plant species: *Brachypodium*, *Chlamydomonas*, poplar, sorghum, switchgrass, and *Miscanthus*. This information will support other plant scientific objectives related to annotation, comparison, and modeling. RNA levels as measured by expression arrays or RNA-Seq are no longer sufficient to evaluate mechanisms and networks that regulate plant transcriptomes. Kbase must also include available small RNA and target RNA information, differential RNA processing and decay information, and epigenetic marks such as DNA methylation and histone modifications. This information is important for data integration and for filling in important missing links in gene regulatory networks within a species and facilitating

their comparison across two or more species. In the short 1- to 3-year term, classical transcriptomic data (microarrays and mRNA-Seq) as well as small RNA and basic proteomic data will be assembled. Epigenetic data, small RNA target data and RNA degradome data, other types of RNA processing data, and additional proteomic data will be assembled after year one, with the most developed genomes such as *Brachypodium*'s beginning first. Data will be made publicly accessible with user-friendly web interfaces and will be downloadable for power users.

Resulting Requirements

- **Pipeline for access to omics datasets, genome sequences, and genome annotations from external sources (see Sections 3.1, 3.2, and 5.5).** This includes traditional transcript data (cDNAs, long read ESTs); RNA-Seq data generated from high-throughput sequencing platforms; small RNA data; microarray data; epigenetic data (DNA methylation, chromatin modifications), and proteomic data. The acquired data will include sequences, quality information (e.g., Q values), and associated metadata. Sources will include NCBI (GenBank, GEO, SRA), DOE JGI, ArrayExpress, and the Plant Expression Database (PLEXdb). This must leverage other efforts to improve plant annotations and their accessibility as described in Section 5.5.
- **Analysis of data assembled by the pipeline outlined in first bullet above.** Analysis will include genome mapping; normalization (across datasets and platforms); association to annotated genome features (e.g., genes, exons, and splice junctions); *de novo* assembly of applicable HTS data; clustering of expression profiles; clustering and special analysis for small RNAs; and summarization for linkage to genome annotation pipelines. Analyses will be prioritized according to current and anticipated data availability—transcriptomic (conventional, RNA-Seq, microarray) > small RNA > RNA processing and degradome > proteomic.
- **User Interface.** A user-friendly web-based interface will enable members of the community to mine omics datasets and associated annotations. Large datasets will be available for download by power users.

IMPACT FACTOR: X HIGH MEDIUM LOW

Process of the Science (Including Workflows)

Plant biologists want to access high-quality, well-documented omics datasets associated with relevant plant gene annotations. Three main deliverables are the following:

- Consolidated platform for access to omics datasets, genome sequences, and genome annotations acquired from external sources.
- Platform for precomputed and on-the-fly analysis of plant omics datasets.
- Web-based interface enabling users to mine plant omics datasets and associated annotations.

Plant biologists want to access omics datasets in a single location (Kbase) and traverse between plant species, while being confident that the underlying data analysis and annotation methods are comparable and of consistent high quality. In addition, they will want to process new or custom omics datasets with the same tools and pipelines used to analyze the data already summarized in Kbase. To achieve these goals, Kbase will need to feature a user-friendly interface for the general user, providing summaries of gene and protein expression profiles and clusters and links to functional genomics resources (genome browser, descriptive annotations, and publications). Kbase will also need to make the analyzed and summarized data available to power users as downloadable genome-scale datasets and associated metadata. Workflows that enable the analysis of user-supplied data in Kbase will be needed. These workflows must be easy to use and made up of well-defined and -described pipeline modules.

Appendix B
Plant 2

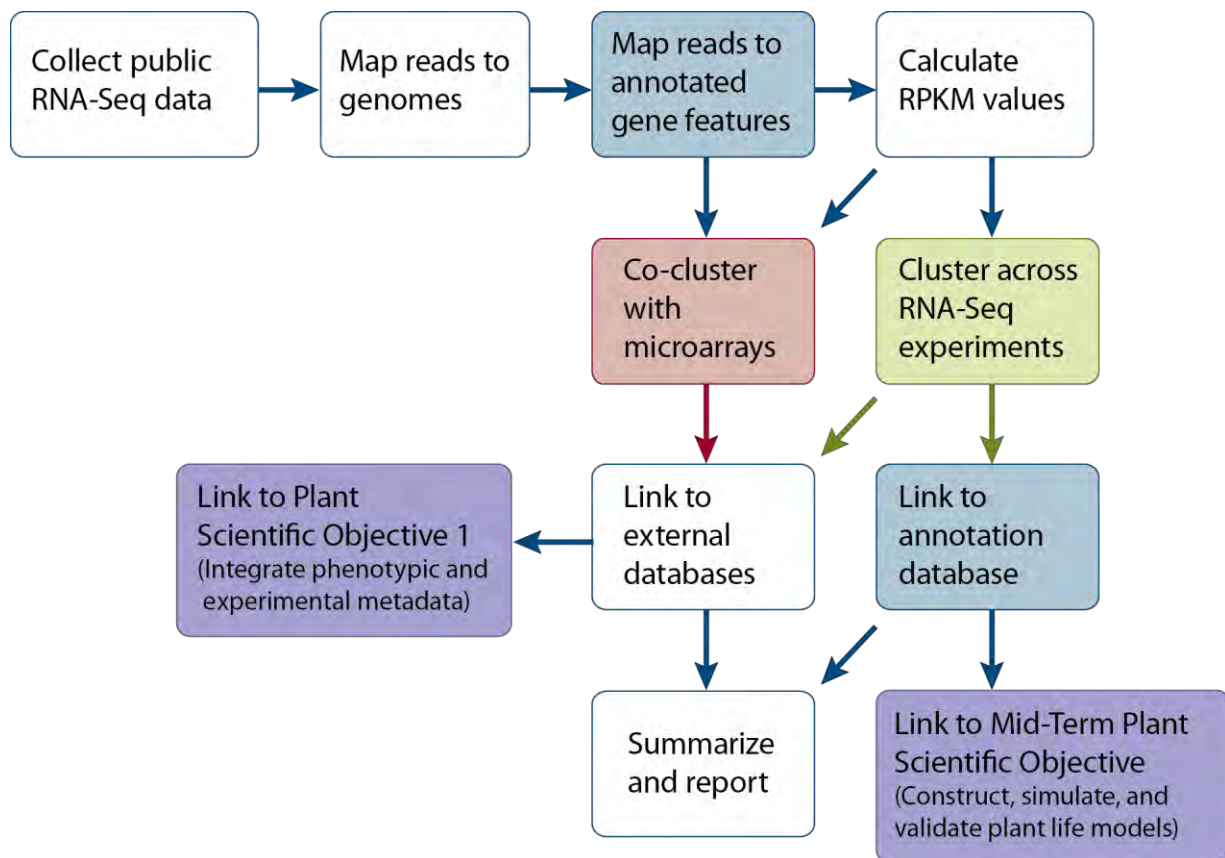


Fig. B.1. Transcriptome Analysis Pipeline for RNA-Seq Data. White boxes are established procedures. The green box is a procedure that has not been developed but is expected to be fairly easy to construct. The red box is a procedure that will require research efforts. Blue boxes depict linkages to existing and improved annotation sources (see [Section 5.5](#)). Purple boxes depict linkages to other plant scientific objectives (see [Section 3.1](#), Integrate Phenotypic and Experimental Data and Metadata to Predict Biomass Properties from Genotype, and [Section 5.3](#), Construct, Simulate, and Validate Plant Life Models).

The following workflow is an example for RNA-Seq data in particular. Other types of omics data will require similar but distinct workflows.

1. Collect RNA-Seq data from NCBI SRA.
2. Map the reads to a genome (or perform *de novo* assembly of RNA-Seq data and then map assembled contigs to a genome).
3. Use genomic coordinates to associate reads with annotated gene features.
4. Calculate RPKM (reads per kilobase of transcript target per million mapped reads) values.
5. Cluster expression profiles across experiments.
6. Cluster expression profiles across platforms (RNA-Seq vs. microarray).
7. Summarize and provide linkage to external genome annotations (see plant objective described in [Section 5.5](#)) and network modeling efforts (see plant objective described in [Section 5.3](#)).
8. Enable data mining via a public web-based interface.

Instruments to Support Achievement of Science Objectives

Currently and in the near term, transcriptomic data from the Sanger, Illumina, SOLiD, and Roche 454 platforms should be supported. Microarray platforms including Affymetrix, Agilent, and Roche NimbleGen should be supported. Technology improvements and new platforms (e.g., PacBio high-throughput sequencing) will necessitate expanded platform support in the future.

User Interfaces

Users will be plant community researchers with an interest in genetics, biochemistry, molecular biology, functional genomics, comparative genomics, evolutionary biology, plant systems biology, and ecology. Users who are biologists prefer web-based graphical interfaces such as genome browsers, report pages, and simple search tools (text and sequence based). These users require downloads of small, custom datasets as well as workflows to perform custom queries and analyses. “Power users” typically are bioinformatics-savvy scientists interested in obtaining genome-scale datasets to enable genome-scale analyses. These users require large, well-documented files in standard formats appropriate for downstream analytical pipelines.

Kbase will need a user-friendly web-based interface that will enable members of the community to mine omics datasets and associated annotations. Search options should include inputs such as locus identifiers, gene symbols, GO terms, protein domains, sequences, and free text. Datasets to be mined should be definable by the user. For example, a user should be able to choose subsets of data based on interest, and metadata associated with transcriptomic datasets should be searchable by key words.

Programmatic Interfaces

Kbase will need interfaces to external data sources for genomes, annotations, and transcriptomic and proteomic datasets (e.g., from the DOE JGI and NCBI’s SRA, GEO, GenBank; see [Section 5.5](#)). Software required for mapping omics data to annotated genomes typically is open source and can be placed into a workflow. Standard practices exist for (1) summarizing the output of such sequence mapping efforts into gene feature–level expression estimates, (2) normalizing results between datasets, and (3) summarizing results for visualization.

Data

For a target plant species, Kbase will need an annotated genome sequence (draft or finished) as well as existing omics data available in the public domain (e.g., RNA-Seq, small RNA, DNA methylations, histone modification, and proteomics).

Genome sequence in the form of an assembly accompanied by metadata and quality information will be downloaded from public repositories (e.g., GenBank and the DOE JGI) and stored in Kbase as a flat file and in a relational database. Genome annotations will be obtained from the same sources or from other efforts to leverage improved plant annotations (see Section 5.5).

Omics data will be downloaded from public repositories (e.g., NCBI GenBank, dbEST, SRA, GEO, DOE JGI, and others) and stored in Kbase as flat files and in a relational database. Such data include:

- Conventional EST and cDNA sequences (Sanger cDNAs and ESTs).
- High-throughput transcriptome sequences (e.g., Illumina, SOLiD, 454).
- Small RNA data.
- Microarray data (Affymetrix, Agilent, NimbleGen, and others).
- Epigenomic data (DNA methylation and histone modification).
- Proteomic data.

Software

Plant 2: Assemble Regulatory Omics Data for Target Plant Species in Common Platforms to Enable Analysis, Comparisons, and Modeling

Table B.2 Software Requirements for Plant 2

Software Purpose	Availability	Improvements Needed?	Resource Impact
Conventional transcript alignment algorithms	Yes	No	High
HTS transcript alignment algorithms	Yes	No	High
Reference-guided transcript assembly algorithms	Yes	Yes	High

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<i>De novo</i> transcript assembly algorithms	Yes	Yes	High
Hybrid empirical (HTS) and <i>de novo</i> gene finders	No	Yes	High
HTS gene expression analysis algorithms	Yes	Yes	High
Microarray analysis algorithms	Yes	No	High
Within platform (e.g., microarrays) expression profile clustering algorithms	Yes	No	High
Between platform (microarrays vs. RNA-Seq) expression profile clustering algorithms	No	Yes	High
Small RNA analysis algorithms	Yes	Unknown	High
Proteomic data analysis algorithms	Yes	Unknown	High
Algorithms for analysis of HTS-derived epigenetic data (DNA methylation; ChIP-Seq for chromatin modifications)	Yes	Yes	High
Algorithms for analysis of HTS-derived RNA degradome data	Yes	Yes	High

Standards

Standards are well defined for some omics data (MIAME for microarrays) and conventional EST and cDNA sequences but are either emerging, poorly defined, or nonexistent for other types of omics data. NCBI SRA and NCBI GEO standards may be acceptable surrogates for RNA-Seq and other HTS data.

Summary and Prioritization of Requirements

Support for and analysis of HTS-derived RNA degradome and epigenetic (DNA methylation and chromatin modification ChIP-Seq) datasets and proteomic datasets can be postponed until other workflows (RNA-Seq, small RNA, microarray) are complete. (There currently is comparatively little RNA degradome, epigenetic, and proteomic data available in the public domain for the target species.) RNA-Seq data simultaneously provides information about transcript expression and structure, so RNA-processing workflows should be developed along with other workflows related to RNA-Seq.

Risk Analysis and Mitigation Strategies

- Unanticipated slow adoption of one or more target species by the plant biology community and limitations or delays in the availability of genome-scale datasets for one or more target species.** Mitigate by prioritizing the target species for funding on genome-scale resource development as well as communication and collaboration with other funding agencies to ensure adoption and support of genome-scale research in these species.

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for Near-Term Plant Science Needs

- **Unanticipated changes in omics technology (namely, high-throughput sequencing and proteomics) that would significantly change the requirements or implementation plan for this scientific objective.** Mitigate by anticipating changes and adjusting requirements and implementation plan as soon as possible.
- **Inadequate omics data or poor data quality that prevents productive workflows as currently designed.** Mitigate by assessing available datasets for adequacy and quality and by modifying the platform by adjusting workflows to conform to available and projected datasets.
- **Anticipated algorithm and software improvements for several aspects of the project (reference-guided and de novo assembly of transcripts, analysis of RNA-Seq data for gene expression profiling, cross-platform expression clustering, and analysis of HTS-derived epigenetic and RNA degradome data) require more resources (software engineering) than currently anticipated.** Mitigate by anticipating improvements in open-source algorithms used as components of workflows and by adjusting requirements and the implementation plan as soon as possible.
- **Bioinformatic analysis on these datasets requires more computational resources (RAM, cores) than currently anticipated.** Mitigate by modifying algorithms or workflows to improve performance in terms of speed or hardware requirements, while possibly accepting increased error or other negative performance characteristics.

APPENDIX C

Supporting Scientific Objective and Software Requirement Documents for Near-Term Metacommunity Science Needs

This appendix provides the working documents for the two selected metacommunity scientific objectives and requirements. These documents were the core output from the final DOE Systems Biology Knowledgebase (Kbase) workshop held June 1–3, 2010 (see [Appendix D](#) for the workshop report). The scientific objectives must answer the question, “What is the scientific or research goal that needs to be solved?” The related “requirements” establish workflows and provide details on the needs to accomplish these objectives. The process of identifying the scientific objective, determining its software requirements, and then developing an implementation plan from the two was described in [Chapter 1](#). These working documents are provided as backup to their implementation plans, which are described in [Chapter 4](#) and contain the final revised judgment by the community concerning the tasks needed for each objective.

In research, a scientific objective is satisfied by creating hypotheses and doing one or more experiments depending on the scope of the objective. For every experiment, there are rationales, protocols to be executed, a number of data inputs (data sources) and outputs (results), and analysis tools. Workflows describe this information. They are sequential procedures that describe the envisioned steps to answer questions. Workflows are the bioinformatic equivalent of an experimental protocol. Detailed workflows are bridges between the experimental research and computing communities and thus are key to translating research objectives into computing requirements that will most effectively advance the science. Workflows were developed for these objectives. From these workflows and the underlying objectives, the requirements could be defined that lead to the articulation of an implementation plan with tasks and scope to achieve the scientific and technical goals.

The first objective in the metacommunities science area is to determine the metabolic role of each organism residing in a community and understand which community features provide robustness to environmental change. This will lead to improved characterizations of microbial community physiology, which is necessary to design strategies to accelerate or ameliorate microbial activity for environmental remediation.

The goal of the second objective to discover novel functions and genes within microbial communities. Data from metagenomics projects can provide the information necessary to better understand the function of poorly characterized genes. The resulting data provide actionable hypotheses about the function of many genes that have yet to be studied in detail. Additionally, scientific efforts associated with this objective will lead to the discovery of new genes that perform useful biological functions of relevance to DOE priority areas such as energy production and environmental remediation.

C.1 Metacommunities Scientific Objective 1: *Model Metabolic Processes within Microbial Communities*

Our overall scientific objective is to achieve a predictive understanding of the role of microbial communities in environments relevant to the DOE missions in bioenergy production, environmental remediation, and carbon cycling. Here, we will focus specifically on modeling the metabolic processes within the community, since this topic most directly ties into developing metagenomic workflows and single-organism systems biology tools. This predictive understanding of communities will progress in three stages. (1) Understanding: Descriptive models that provide insight into the metabolic role of the members within the community and their interactions. (2) Prediction: Predictive models that allow us to simulate the metabolic processes in the community and the response of community activity or composition to environmental conditions. (3) Manipulation: Eventually, these models will allow us to not only predict but actively drive changes in the community into desired directions (e.g., to accelerate environmental processes such as environmental remediation, cellulose degradation, or carbon sequestration in photosynthetic mats).

Background

A number of studies have been done to characterize the structure and activity of natural microbial communities (Tyson et al. 2004; Belnap et al. 2010; Ram et al. 2005; Turnbaugh et al. 2008; Wang et al. 2009; Biddle et al. 2008; Warnecke et al. 2007). Experimental technologies exist for determining the structure of microbial communities using sequencing (e.g., shotgun sequencing, pyrosequencing, and 16S rRNA sequencing reviewed in Hamady 2009) and microarray approaches (e.g., PhyloChip, DeSantis et al. 2007). However, these datasets do not always capture the complete metabolic capabilities of the microbial communities. Other experimental techniques can be used to determine the activity of the microbial community by determining the expression of genes and proteins in the community using metatranscriptomics (He et al. 2010a; Vila-Costa et al. 2010), functional gene arrays (e.g., GeoChip; He et al. 2010b), or metaproteomic methods (Belnap et al. 2010; VerBerkmoes et al. 2009; Lacerda and Reardon 2009). These datasets are useful for determining organisms and pathways that are biologically or metabolically active in the microbial community. Computational models of microbial species can be rapidly developed from annotated genomes (Thiele and Palsson 2010), and recent studies have begun to model the interactions among two and three microorganisms (Stolyar et al. 2007; Taffs et al. 2009; Miller et al. 2010). Scaling up these methods to characterize environmental microbial physiology can proceed through two broad approaches. These are (1) a bottom-up (microbes-to-communities) approach, where the microbes are isolated and cultured in the laboratory and integrated, evaluated, and modeled in a defined community and (2) a top-down (community-to-microbes) metagenome-based approach, where the DNA from environmental samples is directly sequenced for understanding the metabolic potential through bioinformatics and pathway reconstruction.

The first approach is intricately linked to the microbial systems biology workflows discussed in other breakout groups. However, such models are valuable for developing sophisticated

models of microbial communities. Hence, there is a need to develop workflows that facilitate the analysis of metagenomes as well as for the dynamics of microbial communities.

A wide variety of metagenome studies in diverse environments (e.g., acid mine drainage, enhanced biological phosphorus removal, termite gut, rumen, compost, soil, ocean, and sediment) are already under way. In many of these processes, the biotransformation activity is related to the integrated phenotype of the microbes present in the community. To enhance these biotransformation activities, characterizing the metabolic pathways of constituent members and linking individual organisms to their substrate and product profiles are important.

Prioritization

PRIORITY: HIGH MEDIUM LOW

Potential Benefits

Single microbial strains rarely, if ever, act alone, and the complex network of interactions among microbial populations drives all the major metabolic processes in the world around us. The proposed objectives will lead to improved characterization of the physiology of microbial communities, and such a characterization is necessary to design strategies to either accelerate biotransformation activity (e.g., uranium bioremediation) or ameliorate the outcome (e.g., acid mine drainage). Understanding metabolic interactions and substrate preferences of relevant organisms is anticipated to assist in developing design strategies to optimize biotransformation activity. If successful, the benefits can be valuable in providing a framework for analysis of microbial physiology in any impacted environment and can lead to lowered treatment costs as well as accelerated removal strategies.

Feasibility of Success: Near, Mid, and Long Term

TERM: NEAR (1–3 years) MID (3–5 years) LONG (5–10 years)

Clear and achievable short- and medium-term goals can be formulated both for the top-down (metagenomic) and the bottom-up (multispecies models) approaches. A “mock-up” integration of these two approaches can be achieved in the short term, although full integration into a single analysis workflow is a medium-term task. Extensions to this basic modeling paradigm to integrate additional data types, tackle spatial and temporal variation are medium to long term, while fully leveraging the predictive understanding of these communities to guide and control them is a long-term goal. At all stages of this process, the availability of relevant simplified communities (whether artificial co-cultures, low-complexity natural communities, or enrichments) should significantly speed up tool development and allow a gradual buildup to more complex communities.

Relevance to the Kbase Project

This scientific objective directly addresses the systems biology of communities and so will be able to leverage many tools and resources being developed for systems biology of single

organisms. In addition, it will provide an analysis workflow to extract highly useful knowledge from metagenomic sequence data, which probably will become one of the most voluminous data types that Kbase is likely to handle. So, on the one hand, this type of analysis will be an essential component to fulfill the overall goals of Kbase. On the other hand, data-integration aspects and systems biology tools and resources provided by the Kbase platform will be essential to the success of this scientific objective.

Synergies and Leverages: Potential Overlap with Other Projects or Funding Agencies

Existing metagenomic analysis tools such as IMG/M (Integrated Microbial Genomes with Microbiome samples), MG-RAST (Metagenome Rapid Annotation Using Subsystem Technology), or CAMERA (Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis) currently provide some initial preprocessing needed for the analysis presented here, including assembly and functional annotation. However, none currently provides satisfactory phylogenetic binning tools or, more important, the powerful systems biology analysis tools necessary to take functional analysis to the higher level. Platforms such as Pathway Tools include inference engines to predict pathways from potentially incomplete data (Dale, Popescu, and Karp 2010) or fill holes in predicted pathways (Green and Karp 2004), but they are not adapted to the noise and incompleteness inherent in metagenomic data. Databases funded by federal grants (e.g., BioCyc and KEGG) have some components necessary for the metabolic modeling parts of this workflow; however, there is no clear integrated database or simulation efforts. Leveraging existing databases to accelerate development efforts would be useful. There may be potential overlap with some human microbiome projects at the National Institutes of Health (NIH). This probably would be more on the metagenomics side than on the metabolic modeling side, where the human microbiome project will result in a large amount of data relating to the structure and activity of microbial communities that interact with their human host. Some computational and experimental methods that are developed could be applicable to some datasets and analysis being done under this program.

Specificity

The broad scientific objective of achieving a predictive understanding of the role of microbial communities will necessarily include components other than metabolic modeling. We have decided to focus specifically on the metabolic modeling aspect precisely because it integrates so well with the data, tools, and resources planned for Kbase. Other aspects of the role of microbial communities may require further development of more coarse-grained models or microbial ecology tools not specifically covered in this section, although metabolic models will clearly be of some use there as well. Some of these other requirements are covered under different scientific objectives. For example, the scientific objective focusing on comparative community analysis (see [Section 5.1](#) Analyze Understudied Microbial Phyla) covers a range of tools which that will be invaluable to ecological analysis.

Details

Scientific Discovery Process (Workflows)

Workflows for constructing metabolic models from an individual organism's genome sequences have been developed (Thiele and Palsson 2010). While many steps for generating metabolic models for microbial communities may be similar, missing information (such as missing genes) may be a more challenging problem when dealing with metagenomic datasets. Here, workflows for the microbial community modeling framework for the bottom-up and the metagenome-analysis method for the top-down approach are discussed, and the input, outputs, and tools for each are provided. Such workflows are currently available in a scattered manner, but integrating them into a common framework and database would be useful.

For the analysis of defined communities, the first step will involve incorporation of all individual metabolic models into a common environmental model. This will require information on substrates metabolized and secreted by community members as well as common nomenclature for the exchanged metabolites (Zhuang et al. 2010). In addition, kinetics of substrate uptake and secretion as well as biomass yields will be critical in developing such community models.

Reconstruction of Community Metabolic Models from Metagenomic Data

- **Metagenomic Sequencing and Assembly.** Methods in this area are fairly well established, although further development may be needed to deal with even higher-volume next-generation sequencing technologies. Improvements can be made in assembly of shorter reads and in dealing with systematic sequencing errors (e.g., 454 frameshifts).
- **Phylogenetic Binning.** Several methods have been developed, but further validation is needed. Significant advances could be achieved by combining the best features of multiple binning methods (e.g., use phylogenetic markers where available, then use k-mer frequency-based methods on contigs too short to contain useful marker genes).
- **Pathway Reconstruction from Noisy, Incomplete Data.** Most phylogenetic bins will tend to be incomplete, and the binning itself may be of differing qualities. Pathway reconstruction methods will need to take these sources of noise and uncertainty into account in a systematic manner. Knowledge of reference genome content and pathway content may help guide reconstruction.
- **Metabolic Modeling of Community Members.** Given an inventory of pathways present in each phylogenetic bin, construct a functioning metabolic simulation model for each bin. This problem is identical to metabolic network inference in single genomes, except that some of the probabilistic nature of the previous step may need to be taken into account.
- **Combine Community Metabolic Model.** Integrate component metabolic models into a whole-community model. Simulation frameworks should be capable of integrating models of individual organisms into a community model with the ability to integrate customized workflows for simulation purposes.

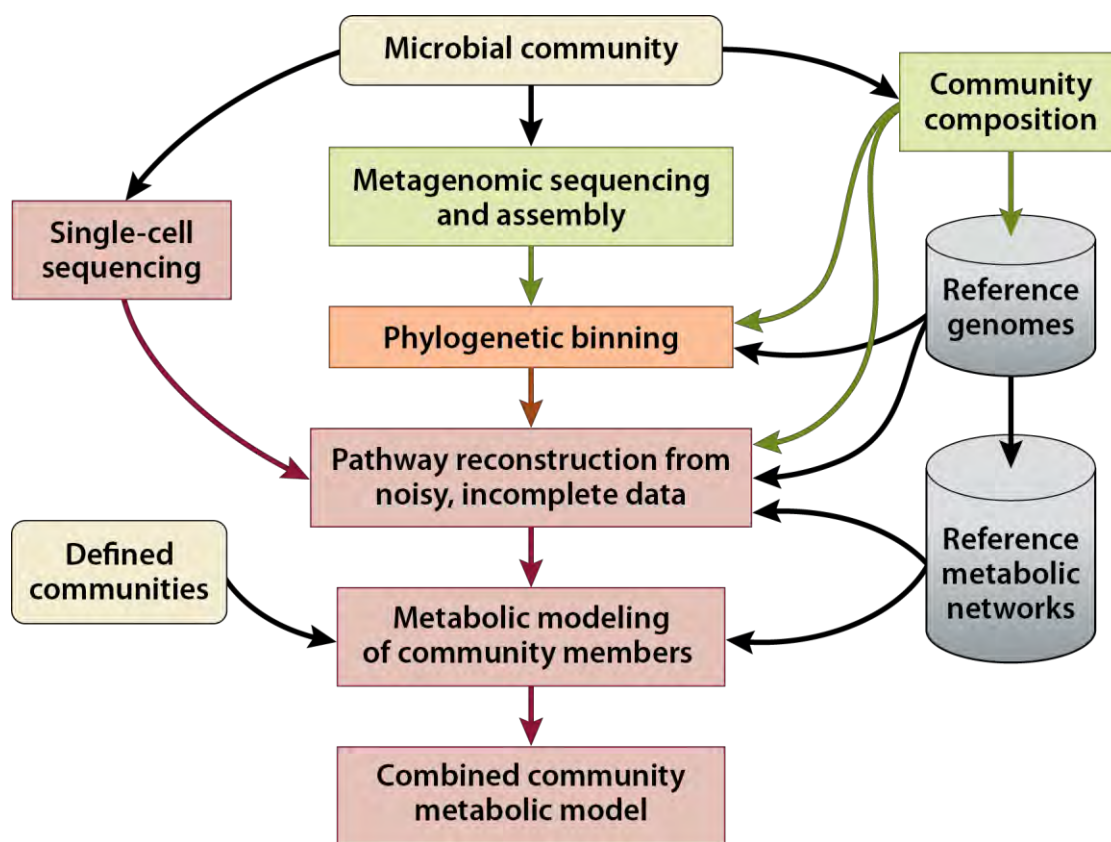


Fig. C.1. Workflow for Reconstructing Metabolic Models from Metagenomic Data. The “bottom-up” approach involves data from individual species or members, and the “top-down” approach relies on whole-metacommunity analysis. In addition to available genomic sequencing technologies, Kbase must prepare to use other data in the near future. Green modules already are reasonably well established. The orange box shows an available method that needs more development. Red boxes show capabilities that still require significant new development.

Inputs

- Metagenomic sequence data.
- Community composition (e.g., based on 16S libraries, pyrotags, or PhylChips).
- Sequenced genomes and reconstructed enzyme and pathway repertoire (e.g., BioCyc pathway or genome database) for a wide range of reference organisms from all phyla, including poorly sequenced or unsequenced phyla.
- Consistent, nonredundant database of biochemical reactions with metabolite stoichiometries and reaction names, including unique identifiers and standard naming conventions.
- Both elementally and charged-balanced biochemical reactions.

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- Thermodynamic information such as Gibbs free energy for all biochemical reactions.
- Reconstructed metabolic networks of individual organisms.
 - Standard format including Systems Biology Markup Language (SBML) file with functional information (e.g., which pathways are involved, amino acid metabolism, and glycolysis).
 - Consistent naming scheme.
- Biological data of individual organisms.
 - Kinetics of substrate uptake rates.
 - Thermodynamics.
 - Growth physiology.
- Specialized environmental data about the niche in which the community lives.
 - Geochemistry.
 - Spatial heterogeneity.
 - Surface chemistry.

Additional data that can be mapped to these models or help guide the modeling effort.

- Metatranscriptomics.
- Metaproteomics.
- Spatial localization of small molecules (e.g., nutrients, metals, and metabolites), proteins, and cells.
- Microscopy, fluorescence *in situ* hybridization (FISH), nano-secondary ion mass spectrometry (SIMS).

Outputs

- Overview of metabolic repertoire and compartmentalization into component species.
- Community predictions of
 - Dynamic shifts in community composition.
 - Metabolic interactions between the community and the environment.
 - Interspecies metabolic interactions.

Tools

- One curated, extremely well-maintained encyclopedia of biological data that includes all genomic, biochemical, physiological, experimental (transcriptomic, metabolomic) data with standard naming conventions for genes, reactions, metabolites, and pathways.

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- Open-source automated network reconstruction tools with integrated mathematical toolbox, such as COBRA (constraint-based reconstruction and analysis; Becker et al. 2007) for simulating metabolism and analyzing network properties.
- Tools or algorithms for automated prediction of localization and compartmentalization of biochemical reactions (e.g., cytosolic, periplasmic, and mitochondrial).
- Develop methods to represent uncertainties associated with the (1) reaction network inference from metagenomic data and (2) uncertainty in compartmentalization associated with binning algorithms.
- Open-source tools for integrating metabolomics and gene-expression data with model-predicted flux data so that any conflict among them can be identified.
- Tool for predicting the metabolic role of any member in the community.
- A high-quality reconstructed network in a standard format with a consistent naming scheme.
- Modeling methods and tools for coupling the network with additional biological data of individual organisms.
- A computational framework that integrates individual organism models. Must be highly adaptive, allowing current and future models to be integrated.
- Visualization tools for community composition and metabolic activities.

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C.2 Software Requirements for Metacommunities Scientific Objective 1: *Model Metabolic Processes within Microbial Communities*

Summary of Scientific Objective

The scientific objective is to be able to integrate different types of experimental measurements relating to the metabolic activity of different microbial communities in microbiomes relevant to DOE missions in bioenergy production, environmental remediation, and carbon cycling. The purpose is to (1) generate hypotheses about the nature of interactions among community members and interactions between the community and the local environment; (2) generate hypotheses about the organisms and pathways responsible for community metabolic activities; and (3) be able to predict how the community will respond to environmental changes or introduction of new microorganisms. The ability to understand and compare communities, including those that vary spatially and temporally, will also be essential to building community metabolic models and require tools for comparative community analysis.

Resulting Requirements

IMPACT FACTOR: X HIGH MEDIUM LOW

Process of the Science (Including Workflows)

Characterization of environmental microbial physiology can proceed through two broad approaches, namely, the (1) bottom-up approach, in which the microbes are isolated and cultured in the laboratory and integrated, evaluated, and modeled in a defined community; and (2) top-down metagenome-based approach, in which DNA from environmental samples is directly sequenced for understanding metabolic potential through bioinformatics and pathway reconstruction.

For analysis of defined communities, the first step will involve incorporation of all individual metabolic models into a common environmental model. This will require information on the substrates metabolized and secreted by community members, as well as common nomenclature for exchanged metabolites. In addition, kinetics of substrate uptake and secretion as well as biomass yields will be critical to develop such community models. This first step is a key requirement before more complex communities can be studied.

For the top-down approach, the metagenome sequence after assembly and annotation can proceed through the workflow described below. (See [Fig. C.1](#) for an illustration of this process.)

- **Phylogenetic Binning.** Several methods have been developed, but further validation of these methods is needed. Significant advances could be achieved by combining the best features of multiple binning methods (e.g., use phylogenetic markers where available, then use k-mer frequency-based methods on contigs too short to contain useful marker genes).
- **Pathway Reconstruction from Noisy, Incomplete Data.** Most phylogenetic bins will tend to be incomplete, and the binning itself may be of differing quality. Pathway

reconstruction methods will need to take these sources of noise and uncertainty into account in a systematic manner. Knowledge of reference genome content and pathway content may help guide reconstruction.

- **Metabolic Modeling of Community Members.** Given an inventory of pathways present in each phylogenetic bin, construct a functioning metabolic model for each bin. This problem is identical to metabolic model development for single genomes, except that some of the previous step's probabilistic nature may need to be taken into account. This step will leverage automated methods for constructing draft models for individual bins.
- **Combine Community Metabolic Model.** Integrate component metabolic models into a whole-community model. Simulation frameworks should be capable of integrating models of individual organisms into a community model with the ability to integrate customized workflows for simulation purposes. Ultimately, these models should be able to integrate different types of data (transcriptomic, proteomic, and metabolomic, as well as microbial composition).

Instruments to Support Achievement of the Scientific Objective

- Next-generation sequencing for metagenomes, including single-cell sequencing, 16S libraries, RNA-Seq, proteomics, and metabolomics.
- Functional gene arrays.
- Spatial patterning of cells in communities, FISH.
- New methods to isolate and perform single-cell measurements (transcriptome, proteome) would be beneficial.
- Need access to instruments that enable high-throughput culturing of defined communities in different environments to enable rapid physiological characterization.

User Interfaces

Interfaces are needed for uploading data, integrating and visualizing metabolic pathways, and performing simulations via web interfaces. Also needed are tools to predict, visualize, and compare community response to experimental data and to conduct queries for model and network comparison and queries across metabolic models and pathways such as reachability analysis from one metabolite to another across species boundaries. In addition, developing tools for visualizing simulation and experimental data simultaneously would be valuable, as would methods to flag conflicts among these datasets. Comparisons among community models will include clustering representations (such as trees and PCA plots) and systems representations (such as systems of metabolic maps). Other interfaces are needed to visualize predicted fluxes through metabolic networks and compare them with genome-wide "omics" data.

Programmatic Interfaces

Need the same interfaces required for modeling microbial metabolism in individual organisms, including:

- Access to genome (GenBank, Swiss Prot) and metabolic (KEGG, MetaCyc, and BRENDA) databases.
- Interfaces to automatically download models and model variations.
- Interface to inputs and outputs of binning algorithms to enable the exploration of alternative binning solutions.

Data

Needed data include metagenome sequence data; information on environmental conditions (e.g., available nutrients, extracellular metabolite profiling, carbon, nitrogen, phosphorus, oxygen, pH, temperature, and light); temporal and spatial measurements; transcriptome, proteome, metabolome, and microbial physiology data; and stable isotope probing that allows us to focus on highly active pathways and organisms. On the hardware side, we need to enable parallel computing because some of the simulation methods can be easily parallelized.

Software

Tools should work with missing and noisy data and should integrate data across multiple platforms (genomics, transcriptomics, proteomics, and metabolomics). Developed tools and databases also need to be managed and maintained over time to keep them relevant and functional. In addition, tools relating to solving differential equations with embedded linear programming problems could be needed for dynamic simulations of defined communities. These tools will overlap with microbial group requirements for the science objective.

<i>Metacommunities 1: Model Metabolic Processes within Microbial Communities</i>			
Table C.1 Software Requirements for Metacommunities 1			
Software Purpose	Availability	Improvements Needed?	Resource Impact
Assembly and gene calling and annotation pipeline			
Binning	Few	Yes	Computing
Metabolic pathway inference from noisy and incomplete data	Very few	Yes	Computing
Pathway hole filling	Few	Yes	
Network debugging for simulation	Few	Yes	
Methods for modeling incomplete networks			Computing

Appendix C: Supporting Scientific Objective and Software Requirement Documents
for Near-Term Metacommunity Science Needs

Metacommunities 1: Model Metabolic Processes within Microbial Communities

Table C.1 Software Requirements for Metacommunities 1

Software Purpose	Availability	Improvements Needed?	Resource Impact
Community diversity analysis (e.g., Mothur)	Some	Probably	
High-resolution functional annotation	Only gene-family membership and nearest homology-based (e.g., BLAST) approaches	Yes. Would prefer gene trees	High-resolution functional annotation
Comparisons of gene content and pathways	Some	Yes, including better statistical methods	Comparisons of gene content and pathways
Linkage between taxonomic profile and metabolic network reconstruction (e.g., distinguishing systems and their components in closely related subtypes. Also filling in gaps from confident core systems in reference genomes.	None (for either need?)		
Build community regulatory networks	No		
Identify interactions between organisms	Limited	Yes	
Incorporation of metadata (e.g., environmental parameters) into comparisons of community models	Limited	Yes	
Selection of communities and isolates for comparison by numerous criteria (e.g., environmental parameters, physiology, taxonomy, gene content)	Limited	Yes. Also need more complete metadata	

Standards

Models and networks need to be developed in an SBML or BioPax format that will allow visualization and simulation in a variety of software platforms.

We also need:

- Standard set of metabolites and reactions (at least the reactions that describe the transport of metabolites across cell boundaries) to effectively integrate individual models of community members.
- Thermodynamic properties of metabolites and reactions (potential overlap with microbial group).
- Metric that identifies the extent of reliance on gap filling to overcome patchy input data.

Since many tools and analyses will be used for comparative analyses, standardized data formats (input and output data) will be crucial for “plug and play” ability.

Governance

While the open-access policy that allows users to incorporate improved modules for analysis should be encouraged, some validation of computational methods should be submitted to ensure that data formats are compatible with existing modules. Methods for ranking the confidence of models, portions of models, and methods used will be important for ascertaining reliability and will be needed to highlight tools that are useful and others that need additional development. This ranking should be a requirement for developers of analysis methods submitted to Kbase.

Maintenance and updating of reference databases will require ongoing stakeholder input.

Summary and Prioritization of Requirements

Tools must account for noisy and incomplete data, with a wide range of community complexity (but usually very diverse and nonuniform), with corresponding issues including fragmentary sequence information (incomplete genes and assembly) and sparsely sampled omics data in general.

- **Short-Term Essential Components.** Essential components include the establishment of methods for modeling a defined community, a necessary first step before complex communities can be modeled. A number of phylogenetic binning methods have been proposed. They need to be more fully validated and integrated to combine the best features of each.
- **Short-Term “Nice to Have” Components.** Standards for data exchange and improved binning and modeling methods will be nice to have. We would also like to identify missing regions of taxonomic space for reference genome data, furthering efforts like GEBA (Genomic Encyclopedia of Bacteria and Archaea) but using environmental sources instead of isolates. So requirements include Kbase making linkages between metagenomic data and reference genome sources as well as tools to determine the

available reference genomes relevant to a particular environment and identify the taxonomic gaps.

- **Mid-Term Essential Components.** Another essential component is a basic genome annotation pipeline that can be integrated with the binning and network reconstruction pipeline. This workflow should be followed by a basic descriptive metabolic model of natural communities. In addition, user interfaces to import sequence data and visualize pathways in the context of multi-omic data are important. New methods that can deal with noisy and incomplete data for network reconstruction will need to be developed.
- **Mid-Term “Nice to Have” Components.** These include improved modeling methods, comparison tools for basic incomplete models at the level of ecotype and higher, and resolution comparison tools for metabolic and systems capabilities.
- **Long-Term Essential Components.** These include the ability to integrate other omic datasets with the metagenome-based metabolic network and to use such data to improve the model and develop the predictive capabilities of models. Also needed is incorporation of other networks including regulatory, signaling, and intercellular interaction networks and integration of the predictive metabolic models with models that incorporate spatial and temporal distribution of metabolic activity. Multispecies interacting metabolic models predictive of response to perturbation are required for the purpose of environmental remediation or other desirable functional behavior.

System Architecture Attributes

Need exportable formats for models that are consistent to allow model exchange

Kbase Key Services

- Methods to predict missing enzymes from sequenced datasets.
- Methods and models to predict metabolic behavior of a community.
- Models to serve as a framework to integrate other experimental datasets.

Risk Analysis and Mitigation Strategies

- Single-cell genome sequencing may render obsolete the need for metagenome sequencing and phylogenetic binning because metabolic models can be developed from such sequences, but species interaction modeling and behavior of the model in community context will continue to require community analysis.
- Relevant communities may be too complex to even permit a descriptive model due to the metabolic networks' incomplete nature. Consequences of incomplete DNA sequence datasets may block completion of tasks. Gap filling using other existing datasets may overcome this problem. Gap filling also may be addressed by additional experimentation or by obtaining additional reference genomes.

Appendix C: Supporting Scientific Objective and Software Requirement Documents
for Near-Term Metacommunity Science Needs

- Metadata associated with metacommunity (metagenomic) samples will vary widely depending on research groups and data generators, which will complicate efforts to compare samples and datasets collected by the scientific community. For example, studying the resiliency of soil communities to perturbations will require availability of metadata on perturbation conditions.

C.3 Metacommunities Scientific Objective 2: *Mine Metagenomic Data to Identify Unknown Genes and Develop Testable Hypotheses about Their Function*

Metagenomic projects have the potential to dramatically expand the number of genes known to scientists, overpowering current efforts aimed at determining the function of genes. At the same time, data generated in large-scale metagenomics projects can provide the information necessary to better understand the function of poorly characterized genes. As metagenomic data are rapidly coming online, a critical scientific objective is to develop approaches for (1) mining the data to identify previously unknown genes and (2) leveraging the wealth of metadata associated with metagenomic datasets, as well as gene and organism co-occurrence information, to identify testable hypotheses about the function of newly identified or poorly characterized genes.

Background

Roughly one-third of all genes in the *E. coli* genome have no known function (Hu et al. 2009), despite the fact that this bacterium is among the best-studied organisms. Although scientists are slowly elucidating the function of some of these genes (Weber et al. 2010), their efforts cannot keep up with the wealth of data being generated in both traditional genomics projects and through large-scale metagenomics efforts. The magnitude of the problem is perhaps best exemplified by the number of novel protein sequences identified by the Global Ocean Sampling expedition (Yooseph et al. 2007). The authors of this study identified over 1700 genes with no similarity to any known protein families. Efforts to understand the function of these genes cannot effectively be conducted without first prioritizing the genes on the basis of their importance to pressing biological questions. But how can we know which genes are important if we do not even know what they do? The key to this chicken-and-egg problem lies in the metagenomic datasets themselves. Specifically, metagenomic data are not comprised simply of DNA sequences but also contain a rich set of metadata, information linking the sequences to location (e.g., latitude and longitude, height, or depth), to physical characteristics of the environment (e.g., temperature, pH, and salinity), and to time. Also, information is available that links together multiple metagenomic datasets (e.g., multiple data generated from the same location at different points in time). Prioritization of experimental and annotation efforts as well as possible hypotheses about the function of a gene or group of genes can be derived from available metadata. For example, a particular gene might be found only in samples derived from communities known to perform a particular biological process (e.g., a gene or group of genes found only in oil-contaminated water, implying their possible role in hydrocarbon metabolism). In addition, some genes might be found only in conjunction with genes whose function is known, thereby implying their involvement in similar biological processes.

Prioritization

PRIORITY: HIGH MEDIUM LOW

Potential Benefits

The development of methods for extracting information about gene function from metagenomic datasets and associated metadata can have far-reaching impacts on biological research in general and on the DOE mission in particular. The resulting data will provide actionable hypotheses about the function of many genes that have yet to be studied in detail. Also, scientific efforts associated with this objective will lead to the discovery of new genes that perform useful biological functions of relevance to DOE priority areas such as energy production and environmental remediation. In addition, these efforts could lead to the development of sensitive markers of ecosystem health.

Feasibility of Success: Near, Mid, and Long Term

TERM: NEAR (1-3 years) MID (3-5 years) LONG (5-10 years)

Relevance to the Kbase Project

The availability of reliable functional annotations is a critical prerequisite of a successful research program in systems biology. This objective will potentially accelerate efforts aimed at characterizing the function of currently understudied genes. In addition, tools developed as part of this project will be valuable assets to scientists generating new datasets by allowing them to leverage Kbase-associated datasets in the analysis process and to generate actionable hypotheses.

Synergies and Leverages: Potential Overlap with Other Projects or Funding Agencies

Similar efforts will probably be undertaken in other research fields that are starting to apply metagenomic methods (environmental, agricultural, and health research). Potential overlap thus exists with research funded by a broad range of agencies (National Institutes of Health, National Science Foundation, National Aeronautics and Space Administration, U.S. Department of Agriculture, U.S. Food and Drug Administration). It will be necessary to maintain regular communication between DOE and these agencies and to actively and broadly disseminate the results of work performed by the Kbase effort.

Specificity

This scientific objective can be broadly broken up into two subobjectives: (1) novel gene discovery and identification and (2) functional prediction.

- **Novel Gene Discovery and Identification.** Starting with one or more metagenomic datasets, we need to reliably identify genes (metagenomic assembly and gene finding are important prerequisites to this effort) and ensure they can be tracked across datasets and databases. More precisely, if a gene G is found dataset A, we need to ensure that it is “real” (not a spurious call or possible contaminant), identify all other datasets that contain homologues of G (note: homology detection itself is a challenging

question), and determine whether homologues of G appear in public databases with or without a specific annotation.

- **Functional Prediction (or Hypothesis Generation).** Starting with a matrix containing genes or gene clusters as rows and metagenomic datasets as columns and metadata associated with or linking together these datasets, we need to develop methods for identifying genes that correlate with environmental features of interest. At the most basic level, we could perform enrichment analyses to identify genes or groups of genes significantly associated with specific environmental characteristics (e.g., genes significantly associated with datasets from oil-contaminated water). Methods for performing such analyses already exist; however, managing the above-mentioned matrix and applying these methods in the context of rich metadata are logistical nightmares. Kbase could provide the tools and infrastructure necessary for “playing” with the data as seamlessly as possible.

In the longer term, more complex analyses could be applied such as using various differential equation models to analyze longitudinal data to understand mechanistic interactions between genes, genes and organisms, and genes and environmental parameters.

Details

Scientific Discovery Process (Workflows)

- Metagenomic sequences are assembled.
- Genes are found within the assembled contigs.
- Genes are compared to other datasets registered within Kbase and to public databases, homologies are detected, and appropriate identifiers are assigned that enable tracking the same gene across datasets.
- A data matrix is constructed from user-selected (or automatically suggested) datasets.
- Statistical computations are performed on the data matrix based on user-defined criteria and column permutations (e.g., “interesting” columns are selected on the basis of a combination of metadata, and genes significantly enriched or depleted in these columns are identified using statistical software).
- The resulting data can feed into new hypotheses or predictive models of gene interactions.

Inputs

- DNA sequences.
- Data matrix as described above in Functional Prediction (or Hypothesis Generation) in the Specificity section.

Outputs

- “Universal” gene identifiers linking together homologues of a same gene across multiple datasets even if the gene is not annotated in any public database.
- Graph of connections between genes, genes and organisms, and genes and environmental parameters annotated with strength of connection and statistical significance.

Tools

- Gene “naming” tool.
- Homology detection tool.
- Tools for detection of statistical correlations within data matrix.

References

Hu, P., et al. 2009. “Global Functional Atlas of *Escherichia coli* Encompassing Previously Uncharacterized Proteins,” *PLoS Biology* **7**(4), e1000096, doi:10.1371/journal.pbio.1000096.

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Yooseph, S., et al. 2007. “The Sorcerer II Global Ocean Sampling Expedition: Expanding the Universe of Protein Families,” *PLoS Biology* **5**(3), e16.

C.4 Software Requirements for Metacommunities Scientific Objective 2: *Mine Metagenomic Data to Identify Unknown Genes and Develop Testable Hypotheses about Their Function*

Summary of Scientific Objective

To improve the characterization of microbial environments relevant to DOE missions in carbon sequestration, environmental remediation, and biofuels, the appropriate models must be constructed. To produce such comprehensive models, an extensive collection of linked data is needed allowing for the interrogation of genes, where they are found, and what the metadata are for each of the environments (e.g., co-occurrence of data, taxa, functions, and genes). To accomplish this, we need to create a digital ecosystem that allows researchers to collaboratively manage, explore, and interrogate the deluge of community-based sequence data.

Resulting Requirements

Standards Development Requirements. As has been demonstrated in many large multi-institutional projects, development of standards requires significant investment of resources. Kbase resources should be specifically designated to promote adherence, development, and utilization of standards to describe experimental, expression, genomic, and geophysical data.

These activities should be coordinated with existing international efforts as well as projects derived from other genome-related funding agencies.

Process of the Science (Including Workflows)

To begin creating such a digital ecosystem, a coordinated set of core tools (i.e., modules) will be needed for a community of researchers to generate a collection of community resources that will allow researchers to independently and collaboratively investigate metacommunities:

- Generate sequence (or import legacy data with tool for ingestion).
- Assemble sequence.
- Automate annotation of sequence.
- Generate a database (contribute to existing generated databases).
 - The generated database should allow for community contribution and interaction.
 - Tools will be associated with the database to allow for interaction (e.g., genome browsing).
- Provide a facility that will allow for the annotations and associated data to be “spidered” and made discoverable to the larger community.
- Provide the means to link and incorporate other available data (e.g., proteomic and transcriptomic data).

Instruments to Support Achievement of the Scientific Objective

Instruments related to data collection (samples, sequence, and metadata) will be involved.

User Interfaces

Users targeted by this requirement description are the bench scientists. That is, we are enabling a diverse and growing community of researchers to analyze much larger collections of data. User interfaces will fall into five categories:

- Domain-specific tools for specific analyses (e.g., assembly).
- Collection of tools to easily generate and contribute to extensible data resources.
- Interfaces that allow researchers to interrogate the data in these resources.
- Suite of tools to allow researchers to navigate through appropriate modules (allowing for selection of alternative modules) and subsequent workflows of analysis and visualization modules.
- Search-and-discovery interface allowing researchers to explore the broader collection of linked community data.

Programmatic Interfaces

To enable the pipelining of tools and modules, creation and submission to the database, appropriate data access, and data submission, application programming interfaces (APIs) need to be developed based on the standards described below. In addition, specific linked data APIs will be required to enable interlinking of resources.

These APIs should be made available as RESTful web services that can be easily incorporated into diverse environments.

Data

Data required to meet the scientific objective is standard. However, data format (to enable this modular process and the creation of linked data resources) and needed standards will be critical. Rich data formats will be needed for data interchange to ease plumbing between tools. These would not be minimal, as they would be required to create persistent digital objects with semantic consistency.

- Need for simple data exchange standards.
- Simple ways to describe and link data.
- If data have been used for search, should be able to return the search results.

Through the development of a collection of file formats to which any researcher could write, researchers would be able to upload files to their sites and allow appropriate indexing and discovery via Kbase spiders that can read these formats. This will enable a network model for the creation and publication of data (i.e., large-scale repositories will fail over time).

The system will also need access to up-to-date data resources that are utilized by various tools. For example, the assembly module will require an annotation clearinghouse.

Software

Metacommunities 2: Mine Metagenomic Data to Identify Unknown Genes and Develop Testable Hypotheses about Their Function

Table C.2 Software Requirements for Metacommunities 2

Software Purpose	Availability	Improvements Needed?	Resource Impact
Data conversion	Yes	Yes (standard interoperability)	
Data submission tools	Yes	Limited number of tools that work with comprehensive standards	
Generate sequence data	Yes		
Assembly	Yes		

Metacommunities 2: Mine Metagenomic Data to Identify Unknown Genes and Develop Testable Hypotheses about Their Function

Table C.2 Software Requirements for Metacommunities 2

Software Purpose	Availability	Improvements Needed?	Resource Impact
Automated annotation	Yes	Improvements of annotation via CAFAE	
Generate collaborative database from data	No		High
Genome browser	Yes		
Data crawling and indexing infrastructure	No		High
Workflow management system for guiding researcher through independent and configurable modules	Yes	Many such systems exist, however, such a system needs to be adapted for Kbase	
Allow linkage to other community data	No		High
Social and community tools for interaction with the published data	No		High

Standards

To be able to provide an integrated system, as described above, a core requirement is better definition and incorporation of metadata related to data and processing of data. To accomplish this, a coordinated set of standards needs to be implemented so that:

- The infrastructure can handle diverse types of metadata.
- The standards that exist are extensible.
- A governance structure ensures that people comply with standards.

A wide range of community-level standardization activities are now ongoing in the scientific community. Largely, these activities are done on a volunteer basis and their outputs include a range of checklists, ontologies (vocabularies), file formats, and tools to enable data sharing. These activities have included standards associated with complete genomes, environmental samples, metagenomes, microarrays, proteomics, and phylogeny. Typically, these activities include formation of organized consortia and societies to foster improved cross-community

interactions, with the aim of finally achieving robust data sharing and minimizing the expense of reconfiguring data described in incompatible formats.

The recent publication on “omics data sharing” (Field et al. 2009) reviews a range of data policies from major funding agencies and the kinds of content that good data policies should share. This includes active support for relevant standards and the databases that implement them. To accompany the paper, the authors have created a biosharing website with a listing of many genomic standards.

This website is dedicated to centralizing and giving a higher profile to bioscience data policies and standards. It offers a focal point for the various stakeholders in data policy by fulfilling two main roles: (1) providing a “one-stop-shop” for those seeking data policy documents and related information (including information about the standards and technologies that support them and (2) encouraging the exchange of ideas and policy components among funders and between funders and potential fundees, ultimately to harmonize policy components where feasible.

A related effort in the domain of genomics and metagenomics is the Genomic Standards Consortium (GSC, gensc.org). GSC is an initiative working toward richer descriptions of our collection of genomes and metagenomes. Established in September 2005, this international community includes representatives from a range of research institutions and major sequencing and bioinformatics centers, including the National Center for Biotechnology Information, European Molecular Biology Laboratory (EMBL), DNA Data Bank of Japan (DDBJ), J. Craig Venter Institute, DOE Joint Genome Institute, European Bioinformatics Institute, Sanger, and the Fellowship for the Interpretation of Genomes. The GSC goal is to promote mechanisms for standardizing the description of (meta)genomes, including the exchange and integration of (meta)genomic data. The number and pace of genomic and metagenomic sequencing projects will only increase as the use of ultrahigh-throughput methods becomes commonplace and standards are vital to scientific progress and data sharing.

The International Nucleotide Sequence Database Collaboration (INSDC)—which includes GenBank, European Nucleotide Archive (including EMBL-Bank) and the DDBJ—partners have recognized the Minimum Information about a (Meta)Genome Sequence (MIGS/MIMS) and the Minimum Information about an Environmental Sequence (MIENS) family of minimum standards. They have recently reserved an official keyword for compliant INSDC sequence records and are working to introduce support for submission of compliant datasets.

Reference

Field, D., et al. 2009. “Omics Data Sharing,” *Science* **326**(5950), 234–36.

APPENDIX D

Individual Reports from the 2009–2010 DOE Systems Biology Knowledgebase Workshops

DOE Workshop on Cloud Computing in Systems and Computational Biology: Workshop Report

November 16, 2009

Portland, Oregon

Convened by

The U.S. Department of Energy Office of Science
Office of Biological and Environmental Research

Organizers: Folker Meyer (Argonne National Laboratory), Susan Gregurick (U.S. Department of Energy), Peg Folta (Lawrence Livermore National Laboratory), Bob Cottingham (Oak Ridge National Laboratory), and Elizabeth Glass (Argonne National Laboratory)

Audience: 130+ people

Speakers: Folker Meyer (Argonne National Laboratory), Dawn Field (Oxford, UK), Eugene Kolker (Seattle's Children Hospital), David Haussler (University of California, Santa Cruz), Simon Twigger (Medical College of Wisconsin), Ananth Kalyanaraman (Washington State University), Michael Schatz (University of Maryland), Sam Angiuoli (University of Maryland), Narayan Desai (Argonne National Laboratory), Lavanya Ramakrishnan (National Energy Research Scientific Computing Center), Kate Keahey (Argonne National Laboratory), Bob Grossman (University of Illinois at Chicago), Judy Qiu, (Indiana University), Thomas Brettin (Oak Ridge National Laboratory), Owen White (University of Maryland), and Deepak Singh (Amazon)

Panelists: Susan Gregurick (DOE), Owen White (University of Maryland), Pete Beckman (Argonne National Laboratory), Kathy Yelick (Lawrence Berkeley National Laboratory), Dawn Field (Oxford Centre for Ecology and Hydrology), David Haussler (University of California, Santa Cruz), Jeff Grethe (University of California, San Diego), Folker Meyer (Argonne National Laboratory), Victor Markowitz (DOE Joint Genome Institute), Eugene Kolker (Seattle Children's Hospital), Bob Cottingham (Oak Ridge National Laboratory)

Introduction

According to a recent National Institute of Standards and Technology document, cloud computing is a model for enabling convenient, on-demand, network access to a shared pool of configurable computing resources (e.g., networks, servers, storage, applications, and services) that can be rapidly provisioned and released with minimal management effort or service provider interaction ("The NIST Definition of Cloud Computing," v15). This new approach is an evolving paradigm for providing and using computational services. Previously, scientists could either build local computing resources, where all aspects of the system hardware and software could be tuned to their application, or they could adapt their application to pre-existing

computing resources. There was no middle ground between these two options. Now, with cloud computing, through the use of virtual machine images (VMs), scientists can carry a full computing environment with their application to new systems, in a simple and portable fashion. A cloud platform provisions both the hardware platforms and a means to install and run a given environment stored in a virtual machine (VM).

Unlike Grid or high performance computing, cloud architectures can have little to no centralized infrastructure and up to now have mostly been characterized as a third party computing service which is rendered under a utility model or a ‘pay as you compute’ model. The virtue of this model is that the end user does not necessarily have to invest in computational hardware, software, or administration to enable their particular application or science. However, the drawbacks include latency in data transferred to and between a cloud system, potential data security issues, and system resilience. Moreover, users need to learn how to best make use of cloud interfaces and capabilities.

Nevertheless, the ‘compute as you go’ properties that are inherent to cloud computing make this an interesting platform that may be well suited for the computational needs of the systems biology research community. While other communities have shared data, the large volume of data shared within those communities typically comes from a small number of data sources. In the ‘Omics’ disciplines of systems biology such as genomics, transcriptomics, proteomics, etc., data generated using a variety of instruments (DNA sequencers, mass spectrometers, micro array readers, etc.) are shared with the community at large and are used as the basis for a variety of integrated research projects, frequently involving large computations. However, the sharing process is not yet very effective, and as more data generators and data consumers enter this arena, more efficient ways of data sharing will be required. The scale of the deployments of next-generation instruments in the biology disciplines mirrors the number of research laboratories working in the Omics disciplines. The result is a striking democratization of Omics data generation and the subsequent need for collaborative research. As a consequence, many high volume data sources exist in biology and with the data volume rising exponentially, a platform that provides low-cost, flexible computational services, like that of the cloud, could be a good match for the needs of the systems biology community.

This community is characterized by its growing creation and consumption of data as well as the inherent rise in computational demands. Two prominent features attracting solution providers in bioinformatics, computational biology, and systems biology to “the cloud” are the “seemingly endless supply of cycles” (P. Beckman) and the ability to “bring your own environment” (O. White).

Cloud Computing for the DOE Systems Biology Knowledgebase

The DOE Genomic Science program supports systems biology research to ultimately achieve a predictive understanding of microbial and plant systems for advancing DOE missions such as sustainably producing biofuels, investigating biological controls on carbon cycling, and cleaning up contaminated environments. To manage and effectively use the exponentially increasing volume and diversity of data resulting from its projects, the Genomic Science program is

developing the DOE Systems Biology Knowledgebase (genomicscience.energy.gov/compbio/). Envisioned as an open cyberinfrastructure to integrate systems biology data, analytical software, and computational modeling tools that will be freely available to the scientific community, the Knowledgebase will drive two classes of work: (1) experimental design and (2) modeling and simulation.

A cloud-based computational platform presents a promising opportunity for the DOE Systems Biology Knowledgebase (Kbase). The objective of this workshop was to elicit community input on the feasibility of using the cloud paradigm as a component of Kbase. Specifically, the workshop participants evaluated the requirements for a cloud-enabled Kbase and presented ideas for engaging the High Performance Computing (HPC) community in this effort.

The three charges for this workshop were:

1. What are the characteristics of applications that would be appropriate for effective utilization of cloud architecture?
2. What are the hardware bottlenecks that prohibit cloud architectures from being easily adopted by high-throughput biological data analytics?
3. What are specific tools that need to be developed or enhanced in order to make cloud architectures easily adopted for biological data and bioinformatics algorithms?

This workshop brought together more than 130 computer scientists, bioinformaticists, and computational biologists to discuss the feasibility of using cloud computing for DOE's systems biology Kbase. This workshop was held in conjunction with Supercomputing '09 in Portland Oregon. The one-day workshop consisted of leaders in these fields presenting work in cloud computing for biological research as well as a two-hour round table discussion centered on the charge questions.

The recommendations from this workshop are summarized below:

1. Switching to clouds will require re-engineering, at first for scalability and then for fault tolerance.
2. A healthy research community requires open source reference implementations of algorithms and pipelines. While many algorithms are open source, the pipelines tend to be closed source due to tight integration with locally existing environments. Clouds provide a unique opportunity to open up existing pipelines to more scientific scrutiny.
3. There is a need for standards in computational workflows and data, including the sharing of intermediate computational results.
4. There is no consensus for the software appliance operational model in bioinformatics (as demonstrated by an informal vote on preferences to "bring your own VM" vs. "use a provided VM").
5. Wide area data movement is still an active issue in the bioinformatics community; these problems are amplified when dealing with clouds.

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6. There is a need for a system enabling the flow of data across different VMs instantiated in a cloud, potentially for VMs from different appliance providers.

Clients for the Kbase Computing Platform

We discussed three types of potential users who would benefit from the Kbase computing platform: computational biologists, bench biologists, and integrators who aim to integrate smaller components into advanced workflows.

From an architectural perspective it is interesting to consider for whom the Kbase computing platform should be tailored. While any platform can support multiple types of users, having an understanding of which users one intends to support the most, or with highest priority, will influence the ultimate design of the hardware platform. We anticipate computational biologists and bioinformatics users being advanced users insofar as they would require more access and more control of their resources hosted by the computing platform.

Bench biologists, on the other hand, would be users of a wider variety of readily available third-party software applications, and therefore the ability to support key third-party applications would influence the architecture.

Need for Reference Data and Standards

Standards are a mechanism for capturing information in a form easily shared and integrated with other data or data types. Using data standards to capture data entities is the foundation for comparative analysis and integration of information.

Increases in biological data production are dramatic. For example, DNA sequencing throughput has grown from 100-500 megabytes per run to 30-90 gigabytes per run in a 12-18 month time frame. As an increasing number of diverse data products (e.g., genome annotation, protein families, pathways, etc.) are transformed and consumed by many different parties, there is a strong need for versioning and controlling production of reference datasets and provenance information. The choice for data transformation also is important for downstream processing (e.g., missing genes impact metabolic pathway predictions, false gene starts impact protein family curation). There is limited sharing of results—especially for compute intensive intermediate results—because data formats have been designed for different purposes. Since using a few centralized, closed pipelines can not meet the communities' dynamic data and information needs, rapidly evolving data exchange standards and data provenance descriptions will be required.

Use of Workflows

The concept of “abstract workflows” seems to be the right level of abstraction for most bioinformatics and computational biology groups. Most HPC providers are focused on parallel implementation of workflows.

In bioinformatics, large-scale computations are often encoded in scripts that wrap and automate the use of standard tools and methods, typically making the pipelines opaque and hard to port between computational platforms. The international Genome Standards

Consortium (GSC) has promoted rich, transparent descriptions of analysis pipelines, such as Standard Operating Procedures (SOPs), which can be published in academic journals and help promote best practices. While these SOPs are fine for describing a process to colleagues, they are not readily interpretable by a computer and are often not rich enough to ensure that results are reproducible at another institution. As a complement to human-readable SOPs, the GSC and its M5 metagenomics working group seek a workflow description language for describing bioinformatics pipelines that can be executed on large computational resources. The format, such as an XML, should be exchangeable between institutes, platform independent, and compatible with existing workflow systems. Of particular interest is portability across grid and cloud architectures, as groups interested in running workflows may have access to a variety of resources. An ideal format would be relatively high level and would describe the process in terms of standard tools or algorithms, such as BLAST. An ideal format would also hide certain complexity required to improve performance, such as partitioning data into batches for batch processing, as these steps may significantly impact the process flow but not the analysis algorithm (such as an readily parallelizable search like BLAST). Also, ideally, such a format already exists within the workflow community and can be adopted and promoted for use by the GSC and M5, although it is not immediately clear which, if any, existing workflow descriptions fulfill the goals outlined here. We also highlight that in cloud computing environments, there are unique opportunities to integrate analysis pipelines and data as part of a single shared resource, the “cloud.”

Reference Virtual Machine Images

First introduced in the 1960s on IBM’s 370 platform, virtual machines (VMs) enable running multiple operating systems on the same hardware platform using a hardware abstraction layer (nowadays referred to as “hypervisor”). Using VM technology, the process of loading an operating system environment becomes similar to loading an application in today’s desktop environments. The VM-image is a single file not dissimilar to existing application programs (e.g., GNU Emacs).

The DOE FastOS program (run out of the DOE Office of Advanced Scientific Computing Research) has clearly demonstrated the value of OS customization for application performance. Clouds (VM instances in particular) provide an unprecedented opportunity to tailor runtime systems to bioinformatics applications. Thus, it seems clear that the ability to run arbitrary VMs is going to be a key component of the Kbase systems architecture.

The creation and maintenance of a set of reference VMs can add significant value, enabling a large number of bioinformatics scientists. A “VM marketplace” with open, maintained reference images and the ability for individual developers to add functions and/or programs to those images will be an important component.

The team providing the computational platform (cloud) should be charged with providing a set of reference VMs and maintaining them over time. Outside developers should be enabled to generate “appliances” using the VMs provided (or using user-provided VMs). The use of a configuration system (e.g., BCFG) should be encouraged to separate images (provided by the resource provider) and the semantics of value provided by third parties.

The provision of reference VMs as an open-maintenance model and a configuration management tool to enhance the VMs was addressed in a discussion about infrastructure as a service (IAAS) vs. software as a service (SAAS). It seems clear that a core team should provide infrastructure as a service enabling a distributed team to provide software services.

Evaluation of Use Paradigms for Clouds

Despite the availability of cloud computing IAAS and SAAS offerings over the last few years, adoption in the computational science community in general and bioinformatics community in particular has been slow. Recently, groups (several of whom presented at the workshop) have begun to evaluate and use cloud resources as a part of their computational platform. Cloud resources provide a set of capabilities and operational properties that are distinct from traditional computational resources, both in terms of computation as well as data storage. Effective use of these resources will likely require explicit adaptation of applications, use policies, and operational practices in order to accommodate these differences.

The key challenges posed by cloud resources are scalability, fault tolerance, lack of locality, and pricing. One of the primary promises of clouds is elastic, on demand scaling. In order to take advantage of this capability, pipelines need to eliminate scalability bottlenecks and support the dynamic addition and release of resources. With the addition of scalable computational resources, fault tolerance quickly becomes an issue. Pipelines must be able to cope with frequent resource failures in a transparent and robust fashion.

Bioinformatics applications tend to be quite I/O intensive. This will be a key challenge with cloud systems, where locality and network topology are not well exposed. Traditional network file systems are not well positioned to solve this problem, as they are usually deployed in fixed configurations and cannot easily be migrated to follow compute resources as they move around inside the cloud. Data aware programming models (like MapReduce/Hadoop) are capable of solving this problem, but require substantial reworking of analysis pipelines.

The final challenge is pricing. Commercial clouds provide compelling services, but are not optimized for computationally bound workloads. Cloud pricing models include raw node hours consumed, as well as data transfer, storage, and use forecasting. These must be taken into consideration when deciding which tasks to run on commercial clouds instead of local resources. This issue may also drive adoption of cloud approaches (such as Eucalyptus, Nimbus, or Hadoop) on local resources.

Several approaches to adapt pipelines to cloud resources were presented at the workshop. Each of these demonstrated pros and cons of particular approaches, and none is likely to represent eventual production cloud architectures. Several groups demonstrated architectures that extended the local computing infrastructure directly into clouds without modification. This has the benefit of being relatively straightforward to implement, but suffers from a number of potential security issues, when exposing local infrastructure to cloud resources. The lack of clear locality information makes this approach suboptimal for highly I/O intensive analysis techniques. Another approach was to build a work management system (AWE) that has knowledge of the semantics of work units. This allows the re-use of intermediate results (that have already been computed), as well as the optimization of task placement based on data

requirements. This approach has been more labor intensive than the previous approach, however, it should provide better scalability, security, and a better fault tolerance model. A third approach was to completely adapt applications to the Hadoop/MapReduce programming model. This programming model has explicit support for data parallel operations, hence it supports very aggressive data locality optimizations. This approach is the most labor intensive of the three discussed here, however, it supports the most aggressive data locality optimizations of the three.

Applications Suitable for Cloud Technology

Applications Suitable for Porting to “the Cloud”

As was demonstrated by a number of presentations during the workshop, not all computations are equally well suited for running on a distributed cloud platform. A cloud-based approach is only one of many conceivable technical choices. For specific tasks, using a large shared-memory machine might be more appropriate. In some instances, a local cluster might offer benefits over a cloud machine (e.g., when the amount of computation is small, and the amount of data is large, as is the case for the image analysis step of DNA sequencing pipelines).

Currently there is no fixed, well understood model for when an existing application is “a good fit” for a cloud-based solution. The weakly defined nature of “cloud computing” is an important contributing factor. Assuming that “cloud” is synonymous with “distributed VM image-based computing,” the following factors seem to play a role in determining whether a cloud platform is a good target for a given application:

- What are the communication patterns of the application (client-server vs. intense client-client communication)? And in this context another important consideration is available network bandwidth.
- Does the application rely on a central/global high-performance file system with specific semantics or performance?

Example Applications

Various groups in bioinformatics have already gathered experiences with the use of cloud (or cloud like) platforms. Here we highlight some of the use-cases presented during the workshop.

While the scales of their computational requirements are different, several groups found aspects of cloud computing to be enabling for their data analysis needs. A group from the Medical College of Wisconsin used Amazon’s EC2 product to make an existing internal pipeline available to more users (overcoming internal resource limitations that were throttling use of an instrument pipeline). This can be taken as a typical example where relatively small groups are enabled to provide their pipeline to outsiders, without the need for the group to invest in local hardware and/or charging users for computational services. Both the amount of data and the computational resources required for this type of approach are modest.

Examples two and three both showcase metagenomics applications that consume large quantities of resources with “larger” gigabyte sized datasets. Both examples are pipelines that are in constant use and are currently resource limited.

Example four from the University of Maryland is showcasing how the novel computational metaphors coming available within the cloud context alter application development.

Example 1: A Cloud-Enabled Proteomics Workflow at Medical College of Wisconsin. Modern mass spectrometers are capable of generating data many times faster than a typical single desktop computer is able to analyze it. We have brought together two recent developments, open source proteomics search programs and distributed on-demand or “cloud” computing, to allow for the construction of a highly flexible, scalable, and very low cost solution to proteomics data analysis: the Virtual Proteomics Data Analysis Cluster (ViPDAC). On boot, the application sets up the databases, links launch scripts, executes worker daemons, and starts monitoring the running processes. Access to the application is via a web browser to a server name provided by EC2 on startup. Users create a new search job and upload their datafile, which is split into independent chunks that are stored on S3 and distributed to waiting worker nodes. Each worker searches the datafile against a database specified in the job, storing the search results back on S3. When the job is complete, the head node downloads and assembles the result files into an archive suitable for use with other analysis tools.

Example 2: Argonne’s MG-RAST Server. Metagenomics applications were among the first to explore the use of cloud computing. These large resource consumers are traditionally implemented as distributed applications, requiring a complex software stack and a central file system. They are also very similar to many of the existing genome analysis pipelines.

Argonne National Laboratory’s metagenomics RAST server (MG-RAST) is one example for a recent development in that type of application. More than 120 gigabases of DNA have been analyzed via MG-RAST using a local cluster, TeraGrid, and cloud like resources. While the integration of TeraGrid happened by manually moving datasets and computations to TeraGrid, the integration of cloud resources was facilitated by using a novel workflow system: AWE. AWE (Argonne Workflow Engine) was initially used to run the similarity computation step of the pipeline on a variety of cloud-like resources.

AWE relies on a set of appliances that connect to a scalable fault tolerant server infrastructure for coordination. Both client and servers are lightweight and highly scalable. The server assigns work to clients based on the current workload and client capabilities. Work units are typically a small fraction of the full similarity comparison. AWE understands the structure and semantics of the work that is to be done, and hence can reuse intermediate results as well as scale the size of the work units depending on the speed and capabilities of the client execution environment. Similarly, AWE can use work unit data requirements to route work to locations where needed data is already present. Finally, AWE uses a lease mechanism in work assignment that allows automatic detection and re-routing of failure work units.

AWE provides a lightweight mechanism for distributing work across heterogeneous resources, including HPC clusters, clouds, Blue Gene systems, and systems with accelerators (GPUs or FPGAs). Effectively harnessing these resources is a key challenge in order to maximize the analysis progress we can make.

Example 3: JGI’s IMG/M. The DOE Joint Genome Institute (JGI) is one of the major sources of microbial genome and metagenome sequence data, currently conducting about 21% of the

reported bacterial genome projects worldwide. Genome and metagenome sequence datasets from JGI and other centers are processed using annotation pipelines and then included in the Integrated Microbial Genome (IMG) system and its metagenome counterpart, IMG/M for comparative analysis. The JGI annotation pipelines support the annotation of genome and metagenome datasets sequenced using any kind of sequencing technology (Sanger, 454 GS0 – 454 Titanium, Illumina). Thus, in the past two years, the metagenome pipeline has processed more than 330 datasets, with variable sizes and distribution of sequence length. This pipeline employs a cluster of 280 CPUs with a processing rate of 24 hours for an average 454Titanium dataset of approximately .5 M reads. For datasets generated by new sequencing platforms (e.g., Illumina) the processing time is estimated to increase several fold with the current computing infrastructure. The integration of such datasets into IMG and IMG/M is also expected to require substantially larger computing capabilities. In order to determine the best solution for meeting this increasing demand for computing resources, a collaboration of researchers from the JGI's Genome Biology Program, Lawrence Berkeley National Laboratory's Biological Data Management and Technology Center, Advanced Computing for Science Department, and the National Energy Research Scientific Computing Center (NERSC) explored the performance and scalability of BLAST on a variety of platforms including a traditional HPC Platform (NERSC's Cray XT4 "Franklin" system), a commercial "Infrastructure as a Service" Cloud (Amazon's EC2), and a shared research "Platform as a Service" Cloud (Yahoo's M45).

The pricing model for cloud services rewards long-term subscription to resources, as shown by the JGI/NERSC group and Wilkening et al. (IEEE Cluster 2009). This aspect of the pricing model limits the ability to dynamically scale their computation in resource limited environments. Additional overhead costs of scientific computing on a commercial cloud include boot up time, data transfer, and loading time.

Hadoop provides an alternative programming model for data intensive computing. It has a number of interesting capabilities including moving computation to the data in distributed environments as well as fault tolerance capabilities not present in traditional HPC systems. While Hadoop can be used with pre-existing applications, replacing workflow systems, its real potential is in new fault tolerant, scalable bioinformatics algorithms. An example of this is shown in the next section.

Example 4: Using Hadoop for Genome Assembly. Michael Schatz from the University of Maryland presented Crossbow, a novel Hadoop-enabled pipeline for quick and accurate analysis of resequencing data for large eukaryotic genomes using clouds.

It combines one of the fastest sequence alignment algorithms, Bowtie, with a very accurate genotyping algorithm, SoapSNP, within Hadoop to distribute and accelerate the computation. The pipeline can accurately analyze an entire genome in one day on a 10-node local cluster or in about three hours for less than \$100 using a 40-node, 320-core cluster rented from Amazon's EC2 cloud computing service.

In addition, Schatz presented a new assembly program Contrail (contrail-bio.sf.net), which uses Hadoop for de novo assembly of large genomes from short sequencing reads. Contrail relies on the graph-theoretic framework of deBruijn graphs, similar to other leading short read assemblers (Velvet, Euler-USR, and ABySS). Preliminary results show Contrail's contigs are

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similar to those generated by other leading assemblers when applied to small bacterial genomes, but provides superior scaling capabilities when applied to large genomes.

Architecting the Cloud Machine

A number of preferences were clearly visible in the audience comments and in the presentations during the workshop regarding the layout of a possible cloud machine to support Kbase work.

VMs. A cloud machine for the Kbase should support both predefined virtual machine images (VMs) and the capability to run user-provided VMs. This feature will enable easy porting of existing software environments adapted to pre-existing local installations. In addition, however, a number of groups will require help with creating the runtime environments required for their work and will benefit greatly from a set of predefined images. The model suggested by Kate Keahey (Argonne/University of Chicago) was to provide a VM marketplace for the machine, with user-provided (non-supported) and supported VMs.

File System. Since a lot of the computation in genomics will be data driven, a fast parallel file system providing storage for all active datasets is another requirement that was implicit in many of the presentations and became abundantly clear in the discussions during the breaks in the workshop. Because of the large amount of existing code and data, this file system needs to support the typical Linux file system semantics. Hadoop Distributed File System (HDFS) is not an option because it does not integrate with existing code and binaries used in bioinformatics and genomics.

Nodes. As application requirements are vastly different between among machine types, the cloud machine should support a variety of node types, similar to EC2. A user performing BLAST analysis will need many (16) cores with a modest amount of memory (8-16 GB), whereas a single-core sequence assembly program (velvet) benefits from maximizing the available memory (e.g., 256 GB RAM).

Node Interconnect. While many HPC machines use MPI and rely on fast internal interconnects, the vast majority of bioinformatics applications do not benefit from fast interconnects. Instead, most communication is between a node and one or more data servers, not between nodes. For this reason a fast parallel file system will add more value to a Kbase cloud machine than any fast interconnect.

Support Model. An operational model like that for EC2 is well suited for a Kbase cloud machine, as its primary technical customers will be bioinformatics groups providing solutions.

Governance Model for Kbase

Governance. The Kbase cloud infrastructure must include management and oversight as appropriate to serve the needs of the scientific community. The Kbase administrators will be expected to be aware of the user communities accessing the system. They also will continuously address issues of user satisfaction, engage in frequent use-case development, and provide resource allocation mechanisms. Several methods should be considered to ensure appropriate utilization and governance of the system.

Usage Advisory Committee. A usage advisory committee should be established to ensure fair use of the Kbase cloud system. The committee should comprise Kbase IT staff, senior Kbase personnel, and possibly experienced service providers from the external community. The advisory committee should be prepared to meet on a rapid ad hoc basis to resolve contention issues and to review the scientific merit of projects creating a high demand for the system. Other committee issues may include verification of the user's credentials, appropriateness of applications run on the system, general availability of cloud resources, and rapid development of hardware or software solutions. Mechanisms for resource requests for the general scientific community should also be developed and reviewed by the usage committee. The Kbase cloud should establish robust configuration mechanisms for all hardware, software and storage utilization. Resource allocation of cluster size, performance, and scheduling will also be reviewed by the usage committee. This committee will address issues such as the need for service level agreements and will provide recommendations on the type of service provided by the Kbase cloud.

Performance metrics. The Kbase administrators will establish performance metrics for the cloud resource. The metrics will monitor reliability, usage, and general utilization of the system. The Kbase cloud resource should also consider the use of surveys to monitor its utilization by the research and bioinformatics community. Surveys should address whether researchers are aware of the cloud resource, whether users utilize the system for their own research, whether any publications have resulted from the resource, and whether use of the system has contributed to the DOE mission. Usability studies should also be performed during the course of the cloud project. Users and workflow developers should be contacted to address the quality of services, documentation, and APIs (Application Programming Interface) of the cloud system.

Scientific advisory board. The Kbase cloud resource should include a scientific advisory board (SAB) to ensure the successful deployment of this facility and help achieve the scientific goals of the users. The SAB will meet on a regular basis and establish overall policies for resource allocation, accounting, and monitoring. The SAB should include representation from the general scientific community, individuals with technical expertise in cloud systems, Kbase staff, and IT administrators. All SAB meetings should be attended by DOE Program Officers and possibly other funding agency representatives. All survey results, performance metrics, usability studies, and newly developed procedures will be reviewed by the SAB and DOE Program Officers. The SAB will also review long-term strategic objectives of the cloud resource and specify new objectives when necessary during the course of the project.

Outreach and communication. The Kbase cloud should also dedicate resources to education, training, and outreach. Staff should provide training materials, on-line presentations, and

documentation so users can fully utilize all aspects of the system. Training should be directed to users and developers with a broad range of experience levels. Topics on how to perform genome annotation, assembly, and expression analysis as well as general workflow development techniques should be addressed. Incorporation of the Kbase cloud into existing class curricula in university courses should also be considered. The Kbase should also establish multiple modes of communication such as an online wiki, error-reporting systems, electronic newsletters, and an email contact list. Kbase staff should regularly present on the Kbase cloud at workshops and scientific conferences. Other forms of outreach such as posters, promotional materials, and advertising should be used.

Glossary*

Appliance (or Software appliance)

A software appliance is a software application that might be combined with just enough operating system (JeOS) for it to run optimally on industry standard hardware (typically a server) or in a virtual machine.

IAAS

Infrastructure as a Service (IaaS) is the delivery of computer infrastructure (typically a platform virtualization environment) as a service.

Omics

Informally refers to genomics, transcriptomics, proteomics, metabolomics, and other global molecular analyses that identify and measure the abundance and fluxes of key molecular species indicative of organism or community activity under defined environmental conditions at specific points in time.

SAAS

Software as a service (SaaS, typically pronounced 'sass') is a model of software deployment whereby a provider licenses an application to customers for use as a service on demand. SaaS software vendors may host the application on their own web servers or download the application to the consumer device, disabling it after use or after the on-demand contract expires.

In the Kbase context SAAS is often used to refer to a setup where the cloud service provider provides a fixed set of VM images that users can choose to run on the machine. The other option (open set of VM images) is often referred to as IAAS.

VM (virtual machine)

System virtual machines (sometimes called hardware virtual machines) allow the sharing of the underlying physical machine resources between different virtual machines, each running its own operating system. The software layer providing the virtualization is called a virtual machine monitor or hypervisor.

*sources include Wikipedia

Joint USDA-DOE Plant Genomics Knowledgebase Workshop Report

Setting the Stage for the Plant Knowledgebase Workshop: Bioinformatics Use in Advancing Plant Genomics, Genetics, and Breeding

Friday, January 8, 2010, 10:30 a.m. – 4:00 p.m.

Plant and Animal Genome XVIII

Town & Country Hotel

San Diego, California

Convened by the

U.S. Department of Energy (DOE)

Office of Science

Office of Biological and Environmental Research

U.S. Department of Agriculture (USDA)

National Institute of Food and Agriculture

Workshop Organizers: Catherine Ronning (DOE), Susan Gregurick (DOE), Ed Kaleikau (USDA), Gera Jochum (USDA), and Bob Cottingham (Oak Ridge National Laboratory)

Audience: 100 plant scientists, geneticists, breeders, and bioinformatics specialists

Speakers: Catherine Ronning (DOE Office of Biological and Environmental Research), Bob Cottingham (Oak Ridge National Laboratory), David Francis (Ohio State University), Steve Rounsley (University of Arizona), Eva Huala (The Arabidopsis Information Resource), Doreen Ware (Cold Spring Harbor Laboratory), and Dan Rokhsar (DOE Joint Genome Institute)

Introduction

The Department of Energy (DOE) Genomic Science program supports systems biology research to ultimately achieve a predictive understanding of microbial and plant systems for advancing DOE missions such as sustainably producing biofuels, investigating biological controls on carbon cycling, and cleaning up contaminated environments. To manage and effectively use the exponentially increasing volume and diversity of data resulting from its projects, the Genomic Science program is developing the DOE Systems Biology Knowledgebase (genomicscience.energy.gov/compbio/).

A DOE workshop held in May 2008 defined the vision for the Knowledgebase—an open cyberinfrastructure to integrate systems biology data, analytical software, and computational modeling tools that will drive two classes of work: (1) experimental design and (2) modeling and simulation. This community-driven Knowledgebase will need to be understandable and accessible to the entire research community and must have an intuitive design that facilitates sharing and contribution among all users. To provide computational capabilities that support DOE systems biology research and other application areas, the Knowledgebase will need to serve multiple roles, including a flexible, adaptable repository of data and results from high-throughput experiments; a collection of tools to derive new insights through data synthesis, analysis, and comparison; a framework to test scientific understanding; a heuristic capability to

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improve the value and sophistication of further inquiry; and a foundation for prediction, design, manipulation, and, ultimately, engineering of biological systems.

For the USDA National Institute of Food and Agriculture, a grand challenge in plant genomics, genetics, and breeding is to identify gene combinations that lead to significant innovation in agriculture and production of raw materials for food, feed, fiber, and fuel. An interdisciplinary approach such as molecular plant breeding may be able to meet this challenge and revolutionize 21st century plant improvement. Molecular plant breeding is founded on the integration of advances in biotechnology, genomic research, and molecular marker applications with conventional plant breeding practices. This integration would require a combination of molecular markers and high-throughput genome sequencing efforts, new knowledge of genome structure and function, statistical approaches to estimate genetic effects, experience in both laboratory molecular methods and field-based breeding practices, and the ability to manage large datasets with diverse data types. This workshop also was intended to assist in developing strategies to expand bioinformatic tools to enable the breeder-centric, high-throughput data management and visualization tools and platforms necessary for integrating genome sequence information with other data types and to provide the breeder-centric views of map and trait data that best serve plant breeders' needs. Implementing such strategies will require (1) broadly training a new generation of plant researchers to fully master key areas such as bioinformatics and quantitative genetics and breeding; (2) establishing partnerships with universities, federal laboratories, industry, and international centers to take advantage of the best training opportunities; and (3) developing a new cohort of researchers able to translate and integrate basic research endeavors with applied plant improvement and value added outcomes for sustainable bioenergy production systems.

Workshop Description

The Plant Genomics Knowledgebase Workshop—held in conjunction with the Plant and Animal Genome XVIII conference in San Diego, California—brought together 100 plant scientists, geneticists, breeders, and bioinformatic specialists to discuss current issues facing plant breeders in light of ever-increasing amounts of genomic data. The workshop featured lectures by leaders in the plant breeding, genomics, and bioinformatics communities. These presentations set the stage for afternoon breakout discussions by addressing the data needs of more-applied breeding programs and describing resources emanating from more-fundamental plant genomics and bioinformatics research. This event is part of a series of DOE-supported workshops to engage the scientific community in discussing scientific objectives the Knowledgebase could serve and determining which endpoints could be achieved in the near, mid-, and long term.

The overarching goal of the workshop was to address the following question:

How can we best design the Knowledgebase to have the flexibility to grow with and adapt to new data and information challenges in the future?

A key objective was to specifically identify the requirements for effectively developing data capabilities for systems biology as applied to plants, particularly the research and development of plant feedstocks for biofuels. The current state of plant informatics is represented by many

disparate databases primarily focusing on specific taxonomic groups or processes. To enable a systems biology approach to plant research, integrating all types of data (including molecular, morphological, and “-omics”) for bioenergy-relevant plant species is important. Thus, the challenge will be to develop uniformity of data format and database architectures to effectively integrate diverse data types and enable user-friendly acquisition and analysis.

Charge Questions

All participants were asked to address two charge questions:

1. What types of experimental data are currently available, and of these, which format(s) are most useful and valuable? Can data from various sources and of various types be standardized into this “ideal” format and then be organized and integrated into one common, searchable application?

For example, a researcher studying cell wall biosynthesis in grasses may benefit from work being performed in poplar. How can we best facilitate cross-species comparisons? How can we use these tools to leverage and apply knowledge gained from model species (e.g., *Arabidopsis* and rice) to crop plants?

2. What are the challenges for plant bioinformatics in a 2- to 3-year time frame? Given the development of an integrated, uniform system (Question 1), what types of analyses do you foresee, and what types of analysis tools will maximize the system’s utility?

How do we best organize, for example, pathways and processes, and how can we organize and distinguish common processes from those that are taxon-specific? How can these informatics resources best be used to enhance plant breeding (i.e., “genotype to phenotype”)? Will these resources be effective in designing decision support tools for plant breeders in the field?

Summary of Workshop Recommendations

Three recommendations from the workshop are:

- 1. Establish community–agreed upon data formats and storage protocols for environmental and experimental metadata and workflows.**

This includes gene annotations; gene product functions; protein interactions; expression and methylation data; natural variation data; and phenotypic data such as geographical coordinates of a field, sampling dates, weather conditions, experimental designs, scoring methods, and images. Although some of these data types and metadata informatics have well-established formats and protocols, others do not and are not well linked to upstream genomic data. Standards development endorsed by the research community needs to be a collaborative and iterative effort between data generators and developers of cyberinfrastructure such as the Knowledgebase. Active, community-driven development of standards will require resource commitments in the form of coordination workshops; new tools to facilitate annotation and data deposition; curation; and compliance through journals, agencies, and peers.

2. Develop the ability for comparative analyses of gene sequences, transcript and protein abundance, phenotypes, and the relationships among these components across multiple species.

Because the plant community comprises both systems biologists and plant breeders, the Knowledgebase must be adaptive to different user needs. Developing comparative analyses across species will require different levels of community support for different research needs. Moreover, coordination is needed among the various plant bioinformatic efforts sponsored by different agencies to avoid duplication of effort and to identify opportunities for collaboration.

3. Establish long-term support for maintaining repositories of a variety of genomic and phenotypic data types.

This will be key to success of a knowledgebase that tries to integrate information from these resources.

Data and Analytical Challenges for Bioenergy Feedstocks

Although this workshop focused on data capabilities relevant to developing plant feedstocks for biofuels, many of the tools, approaches, and issues discussed are applicable to non-biofuel plant species, including well-studied model organisms such as *Arabidopsis*. Thus, Knowledgebase efforts can be leveraged to other plant bioinformatics systems and biological research areas and vice versa. Workshop participants identified several data and analytical issues for plant genomics, including the diversity of data types available, the challenges of dealing with phenotypic data, cross-species analyses, data integration, and standards for interoperability among data and information resources.

Available Data Types for Plant Genomics

The range and quality of available data depend on the extent to which a particular genome has been studied. For a well-studied model organism like *Arabidopsis*, a broad range of data types supported by a rich history of published research helps researchers move from gene sequence to molecular function, associated phenotype, relevant metabolic or regulatory pathways, and interaction partners. As the types of data being generated for different species of bioenergy feedstocks continue to grow, a top priority will be developing appropriate repositories for handling each data type.

What Kinds of Data are Available from Arabidopsis Research?

- **High-quality genome annotation.** The annotated genome forms the basis of all other “-omics” data. The *Arabidopsis* genome has been revised nine times since the initial sequence was completed in 2000, and its annotation continues to evolve. Current revisions to the annotation include adding splice variants and untranslated regions (i.e., 5' and 3' UTRs) as transcript data improves, correcting sequencing errors, and adding features that are more difficult to annotate such as noncoding RNAs and genes that encode small proteins. In the last 5 years, The Arabidopsis Information Resource (TAIR)

has added or updated about half of the genes in the current release, and large, new datasets continue to be generated. Revising genome annotation is a continuous process.

- **Experimental gene function data.** In addition to refining gene structures, TAIR curators have been adding gene function annotations based on experimental data from research articles. To date, 8,622 genes have been annotated with results from published experiments—a total that continues to increase rapidly. Most data used in this manual annotation process were not from high-throughput experiments but from those focusing on a single gene. Gene Ontology annotations describe biological process, molecular function, and cellular compartment. Plant ontology annotations describe the anatomical part and developmental stage associated with expression patterns. This is a rich dataset to consider transferring to other plant species.
- **Phenotypic data** for *Arabidopsis* have largely been qualitative. Currently, these data are in a free-text form, and efforts are needed to use a plant ontology to describe these phenotypes. *Arabidopsis* phenotypic data also include about 5,000 images in a form that is not yet readily transferable to other plants.
- **Protein interaction data** build on existing foundational datasets to generate networks of interactions.
- **Natural variation data** include more quantitative data than some other kinds of data.
- **Expression, methylation, pathways, and networks data** provide more of a genome-wide view of how this plant functions.

Next-Generation Sequencing Data. With the expanding use of next-generation sequencing technologies such as Illumina and 454, an important challenge will be dealing with the vast volume and variable quality of short read sequences generated by many different sources. Needed are resources for assembling and curating these massive amounts of data and tools for using the data to identify and develop single nucleotide polymorphism (SNP) markers, such as the current iPlant effort.

Environmental Metadata. One of the more difficult data challenges identified by workshop participants will be defining appropriate data formats and storage protocols for environmental and experimental metadata. Such data include geographical field coordinates, sampling dates, weather conditions, experimental design, scoring methods, and images. Metadata collection systems will need to be standardized and automated (e.g., using bar codes).

Phenotypic Data Challenges

Enabling large-scale generation of useful phenotypic data and ensuring easy access to it are some of the most important challenges for the bioenergy feedstock research community. Many bioinformatic efforts for plant biology have emphasized non-phenotypic data (e.g., DNA sequence, SNP markers, gene expression, and epigenetics). Phenotypic data—an extremely broad category of data—are subject to considerable noise, have few or no uniform standards, and are highly dependent on genetic context (e.g., particular individuals that have a specific genotype) and environmental context (e.g., timescales, locations, and precipitation). Some

critical challenges identified by workshop participants include developing standards and more efficient methods for generating and managing phenotypic data, improving the ability to link specific genes to phenotypes on a quantitative scale, and establishing central repositories for storing phenotypic data and genetic material. One organization that will address these issues is the International Plant Phenomics Initiative (www.plantphenomics.com), which is being organized by European, Canadian, and Australian researchers to promote international collaboration for plant phenomics.

Limited Availability of Phenotypic Data. The availability of phenotypic data is key to identifying quantitative trait loci and genes associated with important bioenergy-related traits. However, phenotypic data is currently limited. At present, for example, 33% of the protein-coding genes in the *Arabidopsis* genome have experimental annotations, and only 9% have phenotypic descriptions (including “no visible phenotype”). Understanding of genes in biofuel species is even less developed. The lack of robust phenotypic data and functional annotation will result in the continued extensive use of transitive annotation based on sequence similarity from generic databases such as Pfam and UniProt. This is a primitive approach to improving our understanding of plant biology with respect to biofuels.

The amount of meaningful phenotypic data available in public databases is very small compared to the amount of genomic data available. Moreover, the limited resources handling phenotypic data do not address all phenotypes and often do not include lines used for breeding. They thus are not providing breeders with needed information. Participants suggested the need to develop a system of quality scores that could provide a measure of confidence for the heritability and/or measurement of a particular phenotype.

For many applied breeding objectives, a greater focus is needed on generating more phenotypic information in more populations of a given species and, importantly, generating data in actual elite breeding populations. Collecting phenotypic data for complex traits in plants is time consuming. Many potential bioenergy crops are perennial, so successive-year data are needed for individual plants or accessions—information difficult and expensive to obtain. In addition, measuring environmental effects on phenotypes, which requires quantitative data, is as important as defining genotype.

More Objective and Quantitative Phenotypic Data. Descriptors supported by the Union for the Protection of New Varieties of Plants (UPOV) or USDA’s National Plant Germplasm System (NPGS) Germplasm Resources Information Network (GRIN) are used by breeders to classify traits into defined categories. For example, in GRIN, a trait such as color is assigned a numerical color category like “1” for green or “2” for yellow. Although these descriptor systems attempt to make all trait data more uniform, they fail to account for inherent variation within an accession (e.g., how “green” is it?). They also are disassociated from contemporary systems of measurement and disconnected from data scales used by expert practitioners.

Trait data should be quantitative and objective whenever possible. For example, there are very objective systems for measuring color, such as the RGB system for computers. High-throughput systems are needed that can extract quantitative phenotypic data from images. The advantages of such objective measures for phenotype are clear: the ability to interconvert systems of

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measurement and the ability to easily obtain estimates of variation (and therefore estimates of heritability). Tools that enable the mapping of one system to another (e.g., a descriptor to an ontology and a scale to quantitative data) also are needed. The Australian Plant Phenomics Facility (www.plantphenomics.org.au/) is developing high-throughput phenotyping platforms for reproducibly capturing quantitative phenotypic data in parallel with environmental conditions.

Organizational Systems for Phenotypic Data. One organizational system pioneered through GRAMENE and other “-omics”-related projects (www.plantontology.org) uses hierarchical ontology for trait data, with vocabularies derived from published sources and terms appropriately defined. Input from user communities outside the basic researcher vary within trait ontology efforts. Within the international Solanaceae sequencing effort and the Solanaceae Genome Network, interaction with applied research communities is growing, and the system is proving flexible enough to account for diverse traits. Efforts are under way to ensure that ontologies are consistent with existing descriptors and have quantitative definitions. However, use of these ontologies by the community is lagging, an issue that needs to be addressed. Other initiatives include the development of Phenom-Networks (phnserver.phenome-networks.com/icis/), a web-based system to import raw data and facilitate analysis across experiments. Phenom-Networks draws its standards from the International Crop Information System (ICIS), a framework for integrated management of crop-improvement data for both individual crops and farming systems. The ICIS framework is being developed by the Consultative Group on International Agriculture (CGIAR) and has established guidelines for germplasm and data management (www.icis.cgiar.org/icis/index.php/ICIS_Concepts).

Support for Germplasm Stock Centers. The availability of germplasm (plant genetic material) linked to genetic information presented in bioinformatic resources will strongly influence both the value and audience of these resources. Germplasm housed within the National Plant Germplasm System (NPGS) is of historical interest but often does not meet the needs of breeding programs today. In contrast, immortal mapping populations (e.g., recombinant inbred lines and segmental substitution populations) may be too limited for broad inferences or may be based on accessions that are more interesting to basic scientists than those actively engaged in crop improvement. Databases designed to foster crop improvement will need to accommodate mapping populations, breeding populations and pedigrees, and germplasm accessions as defined by the user community. Permanent, long-term support to maintain a germplasm stock center for bioenergy-related species is critical.

Cross-Species Analyses

An important goal is developing databases that permit comparative analyses of gene sequences, transcript and protein abundance, phenotypes, and the relationship among these components across multiple species so that the value of genomic information can be expanded. However, the challenge of developing databases designed to be useful across species begins prior to data collection or formatting. It is critical that gene orthologs, experimental conditions, and genotypes be considered before any meaningful comparison can be achieved between any two genomic datasets. Also highly valuable would be resources for connecting gene or protein

expression data and other information available in the database for one species to the most likely ortholog in other species.

A few critical data integration requirements must be considered when developing the standards and tools needed to connect data across species. These requirements include defining common terms for gene function among different species, having high-quality genome annotations with accurate depictions of gene structures, and obtaining standardized ortholog sets for navigating between genomes. In addition to comparing across species, analytical tools are needed that permit meta-analysis across experimental studies.

Defining “Data Integration”

Workshop speaker Eva Huala noted in her presentation that although “data integration” is a widely used expression, it can have different meanings depending on audience and context. For example, “data integration” can mean:

- Integration of data from many experiments of a similar type in a single species (e.g., many different microarray experiments on *Arabidopsis*).
- Integration of data from experiments of different types in a single species (e.g., gene expression, protein expression, metabolic pathways to generate a network diagram or create a summary of all data for one gene).
- Integration of data from two or more species.
- Use of an integrated dataset to extract new knowledge.

Each type of “data integration” involves different sets of problems and bottlenecks. Determining whether or not data have been integrated appropriately entails much more than simply combining data; it also involves determining whether or not useful information can be extracted from the combined data.

Standards for Interoperability

There is a perception that funding practices and cultural pressures for attaining professional recognition within research communities often encourage the development of more new tools and bioinformatic resources rather than support maintenance and improvement of existing resources. With this push to build isolated, project-specific bioinformatic resources, there is little incentive to set the standards needed to promote interoperability among these resources. User metrics, such as web statistics and literature citations, are useful for evaluating the impacts and quality of tools, databases, or datasets.

When summarizing recommendations from the Workshop on Plant Bioinformatics and Databases sponsored by the European Commission-United States (EC-US) Task Force on Plant Biotechnology Research, Doreen Ware noted several efforts for which standards development could help create a unified platform for plant genome biology:

- **Assessments of genomic tools and datasets.** Establishing periodic assessments of important genomic tools and datasets, similar to CASP (Critical Assessment of Techniques for Protein Structure Prediction), will be important for monitoring the

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quality of datasets and selecting the best tools for data analysis and integration. This effort will be essential for ensuring best practice and quality for reference data.

- **Genome sequence assemblies.** There is a life cycle associated with sequence datasets whereby additional improvement in the annotation for a reference genome sequence is needed even after the reference genome has been completed. With recent developments in next-generation sequencing, a standardized system should be established for evaluating the quality of sequence assemblies. Mechanisms are needed to describe the range of genome sequence models and assemblies that can now be produced, and researchers need to understand the status and quality of the genome annotations with which they are working.
- **Plant-specific ontologies.** Multiple plant-specific databases have ontologies, but there are no consistent standards among them. Coordinated efforts are needed with respect to controlled vocabularies for data collection and submission across databases, such as those used by the Plant Ontology Consortium (PO; www.plantontology.org), as well as leadership-driven efforts to generate phenotype ontologies. For phenotypes relevant to plant breeding activities, a system is needed for linking the terms used in genomic functional annotations to the phenotype terms used by breeders.
- **Curation.** For community-based curation and the curation of legacy data, there currently are no agreed-upon standards.

Knowledgebase Usability and Data Availability Issues

Long-term Sustainability of Data and Databases

Workshop participants were concerned that expiration of funding for existing databases could be problematic for sustaining the availability of important data types. This issue applies to both small boutique databases and larger community databases. Transfer of data from small, project-based databases into larger, more permanent data repositories can be difficult because of differences in schema design and scope. An additional challenge for small project-scale databases is frequent periods of unavailability due to server or network problems. Although a standard database schema (Chado) exists, it is not ideal for all purposes and has performance issues for high data volumes and usage levels. Participants also noted that getting funding for new databases currently is easier than securing continued funding for existing databases, compounding the problem of data longevity. Some participants believe the creation of new resources should continue to be the funding priority, since development of something new ensures that it will be tailored to the needs of the project. Others think that funding support needs to shift toward promoting reuse of existing resources and tools to encourage emergence of standards. Promoting reuse would require that money be made available for adapting existing tools to fit new projects, as there is always some work to be done before an existing tool or standard can be used.

Cultural Differences within the Potential User Community

The diversity of the potential Knowledgebase user community suggests that a one-size-fits-all solution may be difficult to achieve. A user's scientific culture influences how he or she views

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data and asks questions about the data, so different users within the plant-science community have different needs and expectations from a knowledgebase. A systems biologist, for example, needs tools to discover how a plant works. A plant breeder, however, is simply interested in predicting the phenotype that results when a particular genotype is grown in a certain environment—without really needing to know how and why the observed phenotype is produced. In this case, black box methods for predicting phenotypes may suffice. A knowledgebase therefore needs to be adaptable to the different needs of diverse users. Although many existing bioinformatics resources have focused on engaging molecular biologists, genome scientists, computational biologists, and bioinformatics specialists, more effort is needed to bridge the gap and explore the information needs of users in more-applied fields such as plant breeders and crop scientists.

User-Dependent Data Formats

Users whose daily work is focused on plant breeding or laboratory experiments want to access bulk data in relatively simple formats (e.g., CSV flat files or GFF) for further manipulation on their own computers. Some interest was expressed in portability of data or databases so that work could be performed offline (e.g., while traveling or in remote areas where internet access is slow or unavailable). Other users with a more computational focus preferred more complex data formats such as XML. Nexus format for phylogenetic data also was suggested as a good standard. Participants pointed out that certain data types (e.g., sequence and microarray data) already have well-defined standards. In general, many scientists do not want to spend time addressing format issues; they want data presented to them in an intuitive way that does not require them to become programming experts.

Education, Training, and Communication

In the life sciences, adopting informatic resources requires a user community that is educated in bioinformatics concepts, methods, and tools and is equipped with skills in computational and quantitative analytical approaches from the fields of computer sciences, statistics, and mathematics. A key problem is a lack of people with sufficient training to fully exploit the genomic information and resources available. Training the current and next generations of biologists in computational and statistical methods is a major challenge.

In the physical sciences, the computational skills required to manipulate large datasets are considered indispensable and are taught to every undergraduate and graduate student in these disciplines. Similar training in computational approaches to biology is needed at all levels, especially the undergraduate. Workshop participants specifically proposed pre- and postdoctoral cross-training fellowships in quantitative genetics, bioinformatics, and computational biology of biofuel species. For maximum impact, these fellowships should not be tied to standard research grants, where typical 3-year cycles would impede recruitment of fellows, as the hire needs to be coordinated with the duration of the grant.

Existing databases can play an important role through tools that assist self-learning (e.g., online tutorials). Although there is a need to provide tools simple and intuitive enough for those without computational training, these resources should be designed to gradually enhance

understanding of underlying concepts and progressively lead the user to use the tools in more sophisticated ways. An example is a query tool that provides canned statements (in Structured Query Language or other appropriate formats) that can be altered easily by users to fit their particular needs. Eventually a user should be able to write new queries based on the knowledge gained from using and modifying the examples.

Plant Bioinformatic Efforts Relevant to Knowledgebase Development

Two ongoing bioinformatics efforts for plant biology were featured in the presentations at this workshop: the iPlant collaborative funded by the National Science Foundation (NSF) and presented by Steve Rounsley (University of Arizona) and the DOE Joint Genome Institute's Phytosome, presented by Dan Rokhsar (JGI).

The iPlant Collaborative: Cyberinfrastructure for the Plant Sciences

The NSF-supported iPlant Collaborative is an effort to develop a cyberinfrastructure that is nimble enough to address an evolving array of plant science grand challenges. According to NSF, the cyberinfrastructure is a combination of High Performance Computing (HPC), data, data analysis capabilities, and virtual organizations that also can serve as a resource for training and workforce development. The collaborative establishing iPlant includes more than 25 institutions and 45 additional researchers and continues to grow. Once the research community identifies the major problems in plant sciences, iPlant's mission is to provide the cyberinfrastructure that brings together the information needed for researchers to address these grand challenges.

iPlant's community-driven process identified two grand challenge projects that will be the focus over the next 2 years:

1. **Plant Tree of Life (iPToL).** The iPToL goal is to "build the cyberinfrastructure needed to scale up phylogenetic methods by 100-fold or more, to enable the dissemination of data associated with such large trees, and to implement scalable 'post-tree' analysis tools to foster integration of the plant tree of life with the rest of the botanical science." The largest phylogenetic tree that currently can be built is about 100-fold smaller than the number of green plants that exist. For this grand challenge, iPlant aims to design the computational approach that can be used to build a tree with 500,000 taxa in it. Using algorithms available today, the largest trees that can be built contain about 55 taxa and take about 3,000 CPU hours to construct. Some of the significant computational bottlenecks that iPToL will address will require redesigning algorithms. In addition to providing needed cyberinfrastructure, this project involves building, visualizing, and extracting data from the trees.
2. **Genotype to Phenotype (iPG2P).** The goal of the Genotype to Phenotype grand challenge is to elucidate "the relationship between plant genotypes and the resultant phenotypes in complex (e.g., non-constant) environments, one of the foremost challenges in plant biology." Although solving this grand challenge is not possible in a 2-year time frame, the project aims to help overcome the current computational and data management bottlenecks preventing researchers from even attempting to address this challenge today. Much of this effort concerns handling the different data generated

from genomics experiments (e.g., sequence, expression, metabolic, whole-plant, environmental), integrating these data, bringing in the modeling and statistical inference tools to analyze the data, visualizing the results, and providing the interfaces that researchers can use to work with their own results.

Phytozome: A DOE JGI Resource for Green Plant Comparative Genomics

The DOE Joint Genome Institute (JGI) has developed a central hub (www.phytozome.net) to provide all researchers with an interface to interact with plant genomic data in a unified way. About 20 plant genomes are included in version 5 of Phytozome, and a year from now JGI is expected to have 50 genomes of similar quality. All the genomes in Phytozome are reasonably high quality drafts, with enough data available to provide an approximation of the gene set. The genomes at Phytozome range from *Arabidopsis*, which is a highly developed, well-annotated genome, to cassava, which is a 454 draft genome that has just recently become available.

Genomes can serve as an organizing principle for much of the information emanating from modern biological studies. Looking across a phylogenetic tree of angiosperms, the timescale for their radiation is comparable to diversification of mammals (~150 million years), so the extent of diversity seen in angiosperms parallels what is seen among mammals (ranging from bats to elephants to humans). Thus, the work that has been done to compare mammal and other animal genomes indicates where comparisons of plant genomes could be in a few years. Genomes are a central axis for moving from organism to organism and seeing how different species have evolved, and certain comparisons between two different species can be useful in identifying particular kinds of candidate functional elements.

Principles Guiding Future Development of Phytozome

- Adopt open-source, community standards where possible, pulling from advanced comparative genomics already under way in vertebrates.
- Provide standardized datasets to the community. Although several versions of annotation for a genome may exist, the research community needs to agree that one version serves as the reference set at any given time.
- Take advantage of the handful of reference genomes (e.g., *Arabidopsis* and maize) that have benefited from a richer history of past research to help develop resources for the numerous new genomes that will be generated from Illumina and 454 sequencing.
- Continue to develop genome annotation assistance and browsers [e.g., JGI plant pipeline and GMOD (Generic Model Organism Database project)] using open-source community standards so that any researcher can locally set up a customized GBrowse for a particular species.
- Improve an array of features by building on existing resources:
 - “Phylogenomic” gene families (calibrated molecular divergence, synteny, molecular phylogenetic methods).
 - Comparative genomics taking advantage of VISTA and comparative tools for animal genomes.

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- Genomic diversity that builds on resources developed for human HapMap.
 - Complex queries. A guiding principle is to be able to download data in a standardized format that researchers can use in a customized way.
 - Customized analysis. GALAXY and other tool kits are built to hold data in a standardized format. Once a tool is brought into GALAXY, anyone can use it on any genome.
 - Links to TAIR, DOE Bioenergy Research Center knowledgebase efforts, iPlant, and other resources.
- Support workshops to systematically annotate the gene complement across plants.

Appendix 1: Agenda

USDA National Institute of Food and Agriculture Plant Genome, Genetics, and Breeding Project Directors' Meeting

and

Joint USDA-DOE Plant Knowledgebase Workshop

Town and Country Resort and Convention Center

San Diego, California

Friday, January 8, 2010

- | | |
|---------------------------|---|
| 7:30 a.m. | Light refreshments available |
| 7:45 – 10:00 a.m. | Morning Session I: Plant Genome, Genetics, and Breeding
<i>(Pacific Salon 3)</i> |
| 7:45 a.m. | Ed Kaleikau, USDA NIFA
"AFRI Plant Genome, Genetics and Breeding Program" |
| 8:00 a.m. | Phil McClean, North Dakota State University
"BeanCAP – A NIFA Coordinated Agricultural Project" |
| 8:20 a.m. | Scott Jackson, Purdue University
"Genome Sequence for Common Bean" |
| 8:30 a.m. | Gary Muehlbauer, University of Minnesota
"Barley Coordinated Agricultural Project: Leveraging Genomics, Genetics, and Breeding for Gene Discovery and Barley Improvement" |
| 8:50 a.m. | Tim Close, University of California, Riverside
"Advancing the Barley Genome" |
| 9:00 a.m. | Jeff Bennetzen, University of Georgia
"Development of Genomic and Genetic Tools for Foxtail Millet: Use of these Tools in the Improvement of Biomass Production for Bioenergy Crops" |
| 9:20 a.m. | John Vogel, USDA ARS
" <i>Brachypodium distachyon</i> : A New Model for the Grasses" |
| 9:40 a.m. | Peter Bretting, USDA ARS
"GRIN-Global: An International Project to Develop a Global Plant Genebank Information Management System" |
| 10:00 – 10:30 a.m. | Break |
| 10:30 – 12:30 p.m. | Morning Session II: Setting the Stage for the Plant Knowledgebase Workshop: Bioinformatics Use in Advancing Plant Genomics, Genetics, and Breeding
<i>(Pacific Salon 3)</i> |

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- 10:30 a.m. Cathy Ronning, DOE BER
“Introduction to the Workshop”
- 10:35 a.m. Bob Cottingham, Oak Ridge National Laboratory
“DOE Systems Biology Knowledgebase”
- 10:50 a.m. David Francis, Ohio State University
“A Plant Breeding Perspective”
- 11:10 a.m. Steve Rounsley, University of Arizona
“The iPlant Collaborative”
- 11:30 a.m. Eva Huala, TAIR
“Leveraging *Arabidopsis* Data for Research on Other Plant Species”
- 11:50 a.m. Doreen Ware, Cold Spring Harbor Laboratory
“US-EC Plant Bioinformatics”
- 12:00 p.m. Dan Rokhsar, Joint Genome Institute
“Genomes as an ‘Organizing Principle’ for the Knowledgebase”
- 12:20 p.m. Instructions and Move to Breakout Rooms
- 12:30 – 2:30 p.m. **Working Lunch: Plant Knowledgebase Breakout Sessions and Discussion**
5 Groups; Facilitators: Rex Bernardo, Steve Knapp, Robin Buell, Lukas Mueller, and Todd Mockler
(*Pacific Salons 2, 4, 5, 6, 7*)
- 2:30 – 2:45 p.m. Coffee Break
- 2:45 – 4:00 p.m. Report out (15 minutes for each group)
(*Pacific Salon 3*)
- 4:00 – 4:30 p.m. Facilitators gather to summarize and wrap up
(*Pacific Salon 3*)
- 4:00 – 6:00 p.m. **Poster Session**
(*Golden Ballroom*)

Appendix 2: Participants and Observers

Participants

Eduard Akhunov (Kansas State U.)	Matias Kirst (U. Florida)
Steve Baenziger (U. Nebraska)	Steve Knapp (U. Georgia)
Ali Barakat (Pennsylvania State U.)	Jan Leach (Colorado State U.)
William Barbazuk (U. Florida)	Thomas Lubberstedt (Iowa State U.)
Eric Beers (Virginia Tech U.)	Laura Marek (Iowa State U.)
Jeffrey Bennetzen (U. Georgia)	Michael Mazourek (Cornell U.)
Rex Bernardo (U. Minnesota)	Phil McClean (North Dakota State U.)
William Berzonsky (S. Dakota State U.)	Susan McCouch (Cornell U.)
Jim Bradeen (U. Minnesota)	Richard Michelmore (U. California - Davis)
Charles Brummer (U. Georgia)	Amit Mitra (U. Nebraska)
Marcia Buanafina (Pennsylvania State U.)	Todd Mockler (Oregon State U.)
Robin Buell (Michigan State U.)	Gary Muehlbauer (U. Minnesota)
John Burke (U. Georgia)	Lukas Mueller (Cornell U.)
Victor Busov (Michigan Technological U.)	Seth Murray (Texas A & M U.)
Patrick Byrne (Colorado State U.)	David Neale (U. California - Davis)
John Carlson (Pennsylvania State U.)	Joseph Onyilagha (U. Arkansas - Pine Bluff)
Tim Close (U. California - Riverside)	Jiwan Palta (U. Wisconsin)
Luca Comai (U. California - Davis)	Cameron Peace (Washington State U.)
Carlos Crisosto (U. California - Kearney)	Zhaohua Peng (Mississippi State U.)
Richard Cronn (USDA FS)	Andy Pereira (Virginia Tech U.)
Thomas Davis (U. New Hampshire)	Dan Rokshar (JGI)
Katrien Devos (U. Georgia)	Pam Ronald (U. California - Davis)
Amit Dhingra (Washington State U.)	Jeffrey Ross-Ibarra (U. California - Davis)
David Douches (Michigan State U.)	Steve Rounsley (U. Arizona)
Andrew Doust (Oklahoma State U.)	John Warner Scott (U. Florida)
Jorge Dubcovsky (U. California - Davis)	Kevin Smith (U. Minnesota)
Ismail Dweikat (U. Nebraska)	Carol Soderlund (U. Arizona)
David Francis (Ohio State U.)	David Spooner (U. Wisconsin)
Bikram Gill (Kansas State U.)	Dina St. Clair (U. California - Davis)
Jim Giovannoni (Cornell U.)	Steve Strauss (Oregon State U.)
Jose Gonzalez (S. Dakota State U.)	Christian Tobias (USDA-ARS)
Pam Green (U. Delaware)	Jerry Tuskan (ORNL)
Maria Harrison (Cornell U.)	Allen Van Deynze (U. California - Davis)
Patrick Hayes (Oregon State U.)	Richard Veilleux (Virginia Tech U.)
Sam Hazen (U. Massachusetts)	Wilfred Vermerris (U. Florida)
Eva Huala (TAIR)	John Vogel (USDA-ARS, Albany CA)
Amy Iezzoni (Michigan State U.)	Dong Wang (U. Nebraska)
Eric Jackson (USDA ARS)	Shizhong Xu (U. California - Riverside)
Scott Jackson (Purdue U.)	Janice Zale (U. Tennessee)
James Kelly (Michigan State U.)	Hongyan Zhu (U. Kentucky)

Observers

Peter Bretting (USDA)
 Randy Johnson (USFS)
 Ed Kaleikau (USDA)
 Shing Kwok (USDA)
 Liang-Shiou Lin (USDA)
 Gail McLean (DOE)
 Jack Okamura (USDA)
 Jane Silverthorne (NSF)
 Sharlene Weatherwax (DOE)

DOE Genomic Science Microbial Systems Biology Knowledgebase Workshop

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Section I: Knowledgebase Concept and Workshop

The Department of Energy (DOE) Genomic Science program within the Office of Biological and Environmental Research (BER) supports science that seeks to achieve a predictive understanding of biological systems. By revealing the genetic blueprint and fundamental principles that control plant and microbial systems relevant to DOE missions, the Genomic Science program (genomicscience.energy.gov) is providing the foundational knowledge that underlies biological approaches to producing biofuels, sequestering carbon in terrestrial ecosystems, and cleaning up contaminated environments.

Knowledgebase Vision and Background

The emergence of systems biology as a research paradigm and approach for DOE missions has resulted in dramatic increases in data flow from new generations of experimental technologies in areas such as genomics and imaging. While some resource centers are generating large datasets with workflows designed to answer specific scientific questions, there is also a great increase in data production, generally from individual laboratories. New scientific questions arise and can be answered by combining and analyzing such data across laboratories and projects. Great value has derived from the ability to combine sequence and structure data across producers, and in some research communities, such as the yeast field, general access to functional genomic data has greatly accelerated discovery and technology development. Over the last decade, BER—through its Genomic Science program—has sought to solve bioenergy, environmental remediation, and carbon sequestration challenges that demand understanding biological activities exhibited by complex populations and the individuals within them. Since we seek to understand the molecular basis of these dynamics and activities on scales from individual genomes through cellular networks to community function and evolution, these projects are generating multiscale information that could be organized more effectively to aid

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the science of individual projects and to synergize data across projects with related missions. Perhaps even more important, the data from multiple, possibly unrelated programs could be flexibly reorganized and analyzed to aid new scientific discoveries and provide insight to researchers in environmental microbiology and biotechnology generally.

Enabling the community to serve, query, combine, and analyze these diverse data types is therefore imperative, as is building a blueprint and system to enable the design, implementation, and use of new analytical tools and frameworks for working with such data. To manage and effectively use this ever-increasing volume and diversity of data, the Genomic Science program is developing the DOE Systems Biology Knowledgebase—an open, community-driven cyberinfrastructure for sharing and integrating data, analytical software, and computational modeling tools. Historically, most bioinformatics efforts have been developed in isolation by people working on individual projects, resulting in isolated data and methods. An integrated, community-oriented informatics resource such as the Knowledgebase would provide a broader and more powerful tool for conducting systems biology research relevant to BER's complex, multidisciplinary challenges in energy and environment. It also would be easily and widely applicable to all systems biology research.

In general, a knowledgebase is an organized collection of data, organizational methods, standards, analysis tools, and interfaces representing a body of knowledge. For the DOE Systems Biology Knowledgebase, these interoperable components would be contributed and integrated into the system over time, resulting in an increasingly advanced and comprehensive resource. Other elements of the Knowledgebase vision are defined in a March 2009 report (genomicscience.energy.gov/compbio/) based on a DOE workshop that brought together researchers with many different areas of expertise, ranging from environmental science to bioenergy. The report highlights several roles the Knowledgebase will need to serve, including:

- An adaptable repository of data and results from high-throughput experiments;
- A collection of tools to derive new insights through data synthesis, analysis, and comparison;
- A framework to test scientific understanding;
- A heuristic capability to improve the value and sophistication of further inquiry; and
- A foundation for prediction, design, manipulation, and, ultimately, engineering of biological systems.

Beyond these perspectives from the last report, the Knowledgebase is now envisioned as a robust, flexible, and well-documented open architecture. This architecture would allow for both organized and distributed community development, facilitate the sharing of data and tools for data transfer, integration, query, analysis, and visualization, and be committed to interoperating with community resources and standards.

The Knowledgebase would differ from current informatics efforts by integrating data and information across projects and laboratories—tracking diverse, multiscale biological data from the genome through molecular networks, to cellular populations and communities, to environmental function, and combining data centralization with distributed data. Integration

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implies that the Knowledgebase be a community effort rather than a monolithic project overseen and contributed to by only a few people. The Knowledgebase also will need to be more standardized than today's informatics resources. Although standardized components may not always be "cutting edge," they will be more interoperable, enabling comparisons among different laboratories and thus yielding important new insights. Standardization will involve not only data but also experimental protocols.

Another fundamental feature is that Knowledgebase development will have a more mature software engineering approach. In the past, biologists not necessarily trained in state-of-the-art computational techniques reasonably applied the computational tools of the day to their research. However, the dramatic increase in the amount of sequencing and other data being generated requires the support of a more robust computational infrastructure, with analyses that no longer are carried out in an *ad hoc* manner. Many current development efforts are based on computational technologies created 10 to 15 years ago. More modern analytical technologies are needed. To be useful, these new techniques must be developed by the entire research community rather than by informatics specialists working in isolation.

To establish the Knowledgebase as a community effort, several basic principles need to be considered. One is *open access*—the concept that data and methods contributed to the system will be available for anyone to use. Another is *open source* or *open contribution*, meaning that source code is managed in an open environment and is freely available to access, modify, and redistribute under the same terms. Perhaps the most important concept is *open development*, which would allow anyone to contribute to Knowledgebase development under organizational guidelines. Analogous to submitting a publication, this would involve a review process by an authoritative group that would determine if a particular contribution meets established criteria. In such an environment, different groups would work together on a common piece of software to meet common needs, the review process would facilitate integration into the Knowledgebase and quality control, and the product would be better than what an individual alone could create.

Several existing systems and applications can serve as reference models for thinking about Knowledgebase development. Exemplifying the concept of an open-source development is the computer operating system Linux, which is being built by a community of software developers working collaboratively to create a sophisticated and fairly successful system. Other familiar examples include iPhone or Google apps that enable users to pick and choose the kinds of features and capabilities they want and integrate them into a phone or other device. We are familiar with user interfaces that show layering of data from Google maps and Google Earth annotations (e.g., locations of landmarks and restaurants). Experimental design and research in the future will be conducted in the context of a user model similar to these successful systems. As research users gain new insights in systems biology from experiments and analyses, their interaction with the Knowledgebase populates new detail in the biological systems, forming the basis for new referential insight.

Wikipedia development also is open source and open development, allowing individuals or groups to contribute content. It has an editorial model, and, over time, the quality of its content evolves and improves. For the Knowledgebase, such an open-development environment

conceivably would enable noncomputing experts to play a role in the project's development and evolution.

Although these historical examples are approximations of the Knowledgebase vision, they provide a notion of possibilities in their commonly understood characteristics of flexible community development, data layering, editorial control, and peer review integration. The take-away lesson is that we see the initial Knowledgebase development like an operating system kernel that provides a platform on which open contribution of new applications can occur while the Knowledgebase simultaneously is managed to provide core functions like protection of legacy data and development of the underlying access and sharing model and architectural methods.

Workshop Description, Goals, Inputs, and Outputs

Although the 2009 Knowledgebase report describes a vision and long-term objectives for the Knowledgebase, it does not provide details about a plan to implement the system. To that end, DOE has launched an R&D project to establish the requirements for the Knowledgebase and to outline a plan for implementing them. As part of this project, DOE is sponsoring a series of community workshops. The first—held in conjunction with the November 2009 Supercomputing conference in Portland, Oregon—explored the potential for applying the cloud computing approach to systems biology research. The second workshop—held prior to the January 2010 Plant and Animal Genome meeting—addressed the Knowledgebase requirements necessary for developing data capabilities for plants. The output for these and subsequent workshops is now or will soon be posted online at www.systemsbiologyknowledgebase.org/workshops. As the third event in this series, the DOE Genomic Science Microbial Systems Biology Knowledgebase workshop was held Feb. 9–10, 2010, during the DOE Genomic Science Contractor-Grantee meeting in Crystal City, Virginia.

The goals of this workshop were to outline the near-, mid-, and long-term trajectory of microbial sciences for energy and environment and to map the associated workflows and data integration methods that can inform Knowledgebase specifications and requirements.

Participants were asked to provide responses to six charge questions:

1. For systems biology of interest to genomic sciences, what are the scientific objectives that a knowledgebase could address in both a 5-year and longer time frame?
2. What are the key workflows that could be developed to accomplish these goals? Provide comprehensive usage examples that lead to scientific objectives.
3. What types of data are required to accomplish these objectives?
4. What bottlenecks to data integration and data usability need to be addressed to accomplish these goals?
5. What bottlenecks in bioinformatic and computational algorithms need to be addressed to accomplish these goals?
6. What would success look like? What would the benefit be?

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The workshop featured presentations discussing the current, near-, and long-term prospects for microbial systems biology research in the context of the Knowledgebase. Formal presentations were given by Robert Cottingham (Oak Ridge National Laboratory) describing Knowledgebase background and objectives, by Robert Kelly (North Carolina State University) on the “Near-Term Prospects for Functional Microbial Genomics: Moving Beyond the Monoculture Paradigm,” and by Adam Arkin (University of California, Berkeley, and Lawrence Berkeley National Laboratory) on “From Pathways to Populations and Back Again: Long-Term Prospects for the Microbial Systems Biology Knowledgebase.”

Kelly indicated the rapidity with which new genome sequence information appears in public databases is presenting a growing challenge for the data storage, analysis, and utilization necessary to foster scientific and technological advances. The systems biology framework has arisen in response to this challenge, but new computing strategies are needed to take advantage of this new context for examining microbial biology.

Kelly also pointed out that most of what is now known about microbial biology was learned from the study of pure laboratory cultures. The “monoculture” paradigm has been quite productive and will continue to be at the heart of microbiology. However, monocultures are not representative of how microbial systems exist in nature. To this end, metagenomics has provided a means for examining microbial complexity, but complementary functional information is still needed to understand the “metaphenotype.”

Illustrating the need for microbial community studies is the hypothesis that a significant portion of every microbial genome encodes elements designed to regulate and mediate intercellular interactions. These elements may not be responsive in laboratory monocultures and may be triggered only by certain environmental and ecological stimuli. Do these genomic elements exist? What are the studies needed to make this determination? If these genomic elements exist, how can they be identified, characterized, and manipulated? If multispecies systems are to be examined via systems biology, what are the consequences in terms of experimental design and analysis? What is the best way to construct a systems biology knowledgebase for multispecies (multiphenotype) investigations?

Over the next several years, efforts are needed to link the complexity reflected in metagenomes to what is already known from monoculture studies. Kelly indicated this learning curve will necessarily start with relatively simple systems because even co-cultures can exhibit phenotypes not easily predicted from pure culture information. Extending functional microbial genomics beyond monocultures was discussed with a view toward the integration of experimental design, experimental methods, and data analysis strategies. Kelly used hyperthermophile communities to illustrate some challenges that arise when moving beyond monocultures.

In his presentation, Arkin indicated the grand challenge to predict phenotype from genotype is particularly difficult in the microbial world. At its core, this challenge seeks to understand the principles of biological architecture and function sufficient for predicting behavior and, of course, for changing it. A systems biology knowledgebase should grow into an indispensable tool for molecular, environmental, evolutionary, medical, and epidemiological microbiologists

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and for biotechnologists to understand and engineer their systems. However, there are challenges in accomplishing this that are found in few other systems.

Microbes rarely work alone but operate in complex communities that form spatial and temporal webs of mutual support, parasitism, and predation. Perhaps unique to microbes and their communities are the astonishingly rapid mechanisms for evolution and the deeply intertwined ecology of mobile genetic elements that aid in the preservation, diversification, and dissemination of function and may be central drivers themselves of the architecture of microbial networks.

The Knowledgebase, in the long term, will be faced with capturing and interrelating data about all these processes at scales from molecules to meters. Sequencing technologies reveal information on the identities of microbial players in these communities and can hone in on some aspects of gene expression. Structural techniques can provide key information on molecular identity and sometimes function. New imaging technologies can give us information on the arrangements and interactions among molecules, cells, and their environment. However, the complexity of the data increases greatly when moving beyond the sequence of single genomes and crystal structures of single proteins. The data also become far more conditional on unmeasured conditions and interactions and less precise and accurate metrologically, all of which present challenges for organizing and navigating this information. Arkin presented an example process outlining how such information could be assembled, navigated, and used in a knowledgebase. At each level, the challenges and acuteness of need for the community were described.

In ensuing discussions at the workshop, emphasis was placed on establishing agreed-upon scientific objectives that will result in a successful, community-driven Knowledgebase. To build a system that helps achieve important scientific goals, informatics experts need input from and frequent dialogue with the research community on what these goals are, including how the research technologies, data types and quantities, and goals change over time (see Fig. 1.1. Knowledgebase R&D Project).

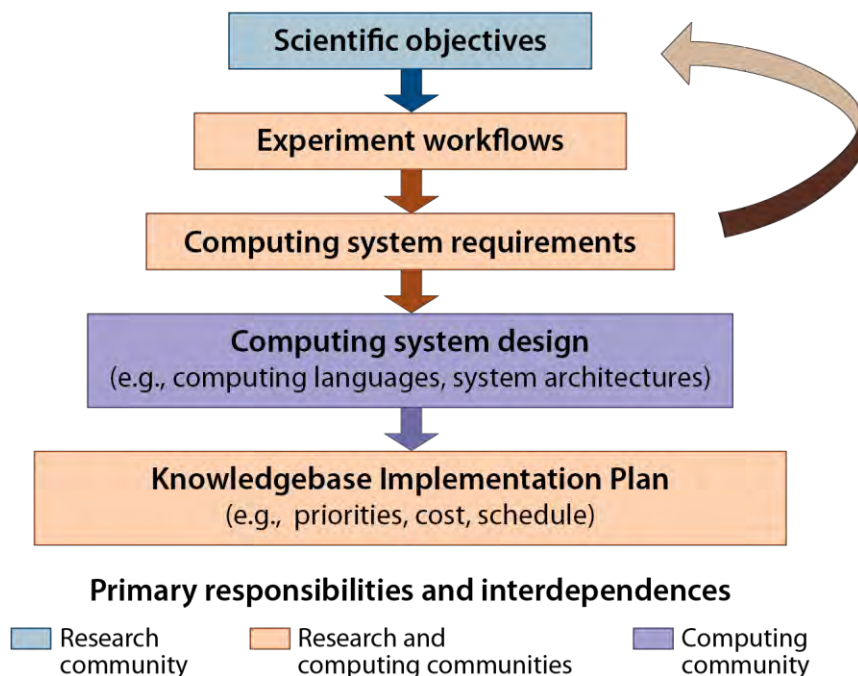


Fig. 1.1. Knowledgebase R&D Project: Scientific Objectives, Intense Collaboration Critical to Successful Knowledgebase Implementation Plan. The final product of this project, the Knowledgebase Implementation Plan, is being developed to incorporate the components and functionality necessary for the systems biology research community to meet its defined scientific objectives. To do this, the research and computing communities must work closely together to define—realistically and at a significant level of detail—the scientific objectives and experiment workflows (protocols) necessary for defining computing system requirements and design and for completing the implementation plan for a robust, durable Knowledgebase.

Workshops, such as this one, provide opportunities to discuss and identify appropriate and community-generated scientific objectives. Any and all input was welcomed, and participants were encouraged to contribute to the final R&D report at www.systemsbiologyknowledgebase.org. To be effective, scientific objectives must be credible, impactful, and achievable in a few years. Participants were asked to discuss objectives based on current research activities and consider candidates and priorities to recommend.

Several examples of potential scientific objectives related to microbes were presented to stimulate discussion. The first was improved prediction of gene regulatory networks based on integrating genomic sequences from phylogenetically related organisms with high-resolution expression (RNA-Seq) data from multiple biological states. Suppose the goal was to predict gene regulation in a particular situation. What are the Knowledgebase capabilities necessary for predicting gene regulation in a subsystem? One need would be the ability to upload raw RNA-Seq sequence data or provide access it. Another need would be tools to process raw sequence into standard formats. A third involves data visualization capabilities.

The limit in the future might be how many biological samples are available to be assayed by RNA-Seq and not the availability or cost of the technique. As cost rapidly declines, it is conceivable that thousands of states could be measured. From plots of expression profiles, genes that are statistically represented in a particular biological state can be readily visualized.

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Which genes are in probable states or pathways that are of particular research interest? This should all be readily available to any researcher. Even small regulatory sequences are visible. Imagine doing RNA-Seq analysis on a set of phylogenetically related organisms and comparing them based on genomic structure. This new data will reinforce past experiments in these same organisms. Based on the alignments, we can find promoters and make predictions about gene regulatory binding sites. This illustrates the type of understanding achievable with a knowledgebase characterized by good algorithms and data integration technologies that have been built up over time. When using Google maps to find the nearest Starbucks location, users rely on a series of technologies that have been developed in such a way. A set of standards allows this information to be mapped together, enabling the system to generate the appropriate directions. The data integration underlying Google maps is analogous to many of the current challenges associated with integrating biological data.

A second example of a scientific objective would be integrating phenotypic response with specific genotypes or pathways so that regulatory or genetic changes could be predictably associated with microbial behavior and response. The idea of relating phenotypes to genotypes and putting that information in context is of wide interest. What are the sources of data? How do we transform them? What are the analytical steps, and what tools are currently available?

As with any scientific objective considered for the Knowledgebase, these two examples would be evaluated to determine if they are credible, impactful, and achievable. If a particular objective meets these three criteria, then community input would help set priorities for the development and implementation timeline of the Knowledgebase.

Section II: Workflows—Knowledgebase Use Cases

Workflows as a Bridge from Bench to Computer

The focus of this workshop, particularly on the second day, was on creating workflows. In research, a scientific objective is satisfied by creating hypotheses and doing one or more experiments depending on the scope of the objective. For every experiment, there are rationales, protocols to be executed, a number of data inputs (data sources) and outputs (results), and analysis tools. Workflows describe this information. Detailed workflows are bridges between the research and computing communities and thus are key to translating research into computing requirements that will most effectively advance the science.

Workflows provide important details for Knowledgebase design, both in terms of the underlying data as well as the experimental or analytical objective. Knowledgebase architecture will have layers such as data repositories, workflow management, and output visualization, all of which relate to workflows developed by the scientific community participating in this Knowledgebase development process. Workflows are essentially communication mechanisms that exchange ideas and information between the researchers and those who actually build the computing system. Included in this report are six workflows drafted to satisfy research objectives important in DOE systems biology. These workflows encompass diverse problem-solving methodologies representative of the broad scientific community and are works in progress—presented here to stimulate discussions between the research and bioinformatics

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communities so that robust computing system requirements and an implementation plan can be developed.

Developing an executable Knowledgebase Implementation Plan must be a community effort—involving both the experimental and computing research communities—where we integrate across projects and research laboratories. Fully developed, robust workflows will foster this integration and lead to a more standardized approach. To handle a new level of biological complexity, we need to embrace more strategic software engineering approaches; we can no longer afford to create isolated and ad hoc systems.

As the key products of this workshop, workflows are critical inputs to the participants of the final workshop (June 1–2, 2010, Crystal City, Virginia). Prior to and during the final workshop, representatives from the computing and biological research communities will work closely together to refine the scientific objectives and workflows and to translate the workflows into computing system requirements. These requirements will form the basis of the Knowledgebase design—a prerequisite to the Knowledgebase Implementation Plan, which is the final product of the DOE Systems Biology Knowledgebase Research and Development Project.

The workflows described in this section are critical to the success of systems biology research and reflect the data inputs, outputs, and experiments being carried out in the DOE-sponsored research community. Over the next several months, assessments will be made to ensure that the highest priority workflows, as identified by community consensus, will be included in the Knowledgebase Implementation Plan. The workflows generated in this workshop are:

1. Metabolic Network Reconstruction (Ines Thiele)
2. Metabolic Flux Analysis via Isotope Labeling (Hector Garcia Martin)
3. Inference of Gene Regulatory Networks (Adam Arkin and Nitin Baliga)
4. Signaling (Aindrila Mukhopadhyay and Loren Hauser)
5. Structural Biology (Paul Adams)
6. Imaging Bioinformatics (Bahram Parvin)

To foster further interactions among the biology research communities, both experimental and computational, most of these have been included as originally submitted as a snapshot in time showing the current range of thought on what a workflow is and how the concept relates to various researchers and areas of research. [Note: An additional workflow on microbial community science is under development and will be available in May. This workflow is based on discussions from the Knowledgebase workshop held in conjunction with the DOE Joint Genome Institute's annual user meeting (March 23, 2010). Workflows associated with the microbial community scientific objectives will be discussed by the interdisciplinary participants at the June Knowledgebase workshop where Knowledgebase system requirements will be discussed and drafted.]

To facilitate workflow development, participants in this workshop were instructed to focus on describing several workflow components: data and sources (inputs), process steps (transformation rules or algorithms), and results or output. They also were asked to explain why

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the workflow is important to the research endeavor and how it might be improved. As an example, consider the first workflow on Metabolic Network Reconstruction starting on p. 11. This example lists input data and even outlines how to obtain it. The process diagram associated with this workflow identifies each process step (see Figure 1. Detailed Workflow for Metabolic Network Reconstructions, p. 12). Many of these steps are common bioinformatic transformations that could readily be included in a future Knowledgebase. As the authors note, many steps are not precise and require some type of manual intervention such as curation. This identifies areas for improvement in either the underlying data or a need for better standards. Some of the process steps are experimental and produce specific results. Again, issues of data quality and accuracy can be important. Although not entirely automatable, this process is of wide utility and interest. This presents an excellent example of a workflow that the research community could prioritize to focus on in the Knowledgebase. By having a range of researchers focused on the bottlenecks, there would likely be improvements not only for metabolic reconstruction, but for other areas of research that depend on similar process steps.

Workflow 1: Metabolic Network Reconstruction

Summary

The metabolism workflow consists of two parts:

1. The metabolic network reconstruction protocol [1] and required data and
2. The protocol to obtain fluxomic data required by the metabolic network reconstruction protocol.

Genome-scale metabolic network reconstructions are biochemically, genetically, and genomically (BiGG) structured knowledgebases, the goal of which is to formally represent the metabolic activities of a specific organism. Genome-scale metabolic networks have been published for more than 30 organisms to date, though they are of varying quality and completeness. Reconstructions are useful because they can be mathematically converted into constraint-based models, allowing important predictive calculations like flux balance analysis to be performed. This comprehensive workflow details nearly 100 iterative steps in the following categories:

1. Draft reconstruction
2. Refinement of reconstruction
3. Conversion of reconstruction into computable formats
4. Network evaluation
5. Data assembly and dissemination

The output of this workflow is a highly curated, accurate, and comprehensive representation of biochemical transformation taking place in the organism of interest. It is not yet possible to automate all steps within the process without loss of accuracy or correctness.

We also attached the comprehensive standard operating procedure (SOP) for biochemical network reconstruction [1] to this workflow.

Input

Required organism-specific data

- Gene information (ID, coordinates, function)
- Protein information (function, location, complex formation)
- Enzymatic reaction (stoichiometry at cellular pH, substrate specificity, cofactor specificity, location, directionality)
- Biomass composition (fraction of macromolecule, molecular composition of macromolecules)
- Phenotyping data (growth medium composition, other growth conditions – e.g. temperature, pH, etc)
- Knock-out strain information (growth phenotypes, other characteristics)

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- P/O ratio

How to obtain this information*Online resources:*

- Genome database containing genome annotation (i.e., locus ID, gene coordinates, (putative) annotation) – e.g., GOLD, TIGR, SEED, etc.
- Biochemical reaction database for metabolic reactions – e.g., KEGG, BRENDA
- Transport database for transport reaction mechanisms – e.g., Transport DB
- Organism-specific databases – e.g., EcoCy, PyloriGene, GeneCards
- Protein location prediction – e.g., PSORT, PA-SUB
- Thermodynamic information (estimation of standard Gibbs free energy of formation ($\Delta_f G^\circ$) and of reaction ($\Delta_r G^\circ$)) – e.g., Web GCM
- CMR database (estimation of DNA, RNA and protein composition)

Tools:

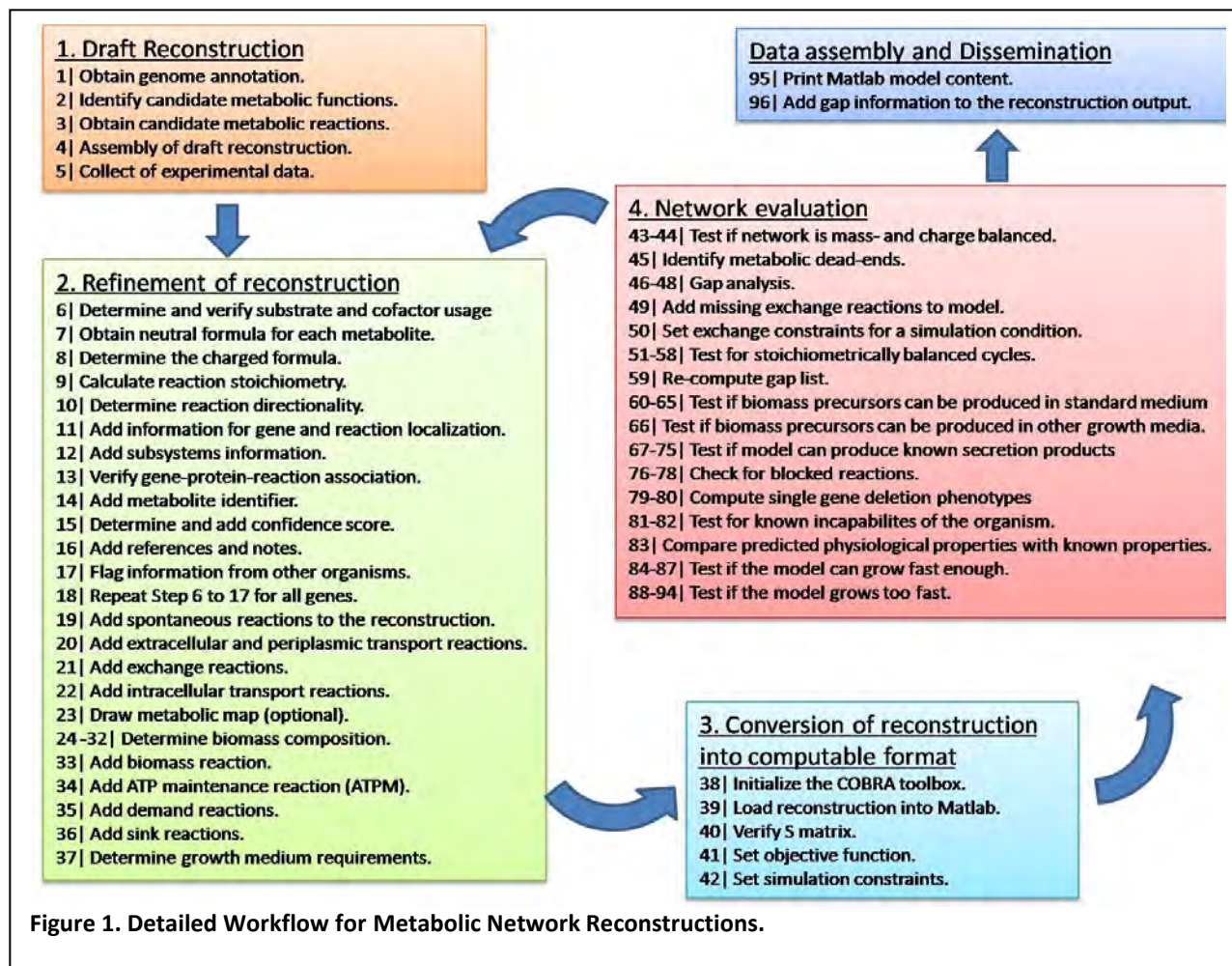
- Blast (if not or insufficient genome annotation is available), other gene function annotation tools

Bibliome:

- Primary and review literature about organism, its metabolic characteristics and its components (proteins, genes)
- Biochemical textbooks
- Organism-specific books

Experiments:

- Measurement of biomass composition (lipids, amino acids, nucleotides, cofactors, etc.)
- Measurement of growth environments (e.g., biologi)
- Measurement of single and double knockout mutants
- Measurement of possible secretion products (and ratios) at different growth environments
- Omics data: Metabolomics, fluxomics, proteomics, transcriptomics
- Transcriptional regulatory information – which pathways are active under which conditions



Workflow Process to Metabolic Network Reconstruction

The biochemical network reconstruction process is well established for metabolism and has been applied to many model organisms. The same approach can also be applied for other cellular functions, such as signaling [2, 3] and macromolecular synthesis [4]. The reconstruction process has been reviewed by numerous groups [5–8]. More recently, it has been formulated in the form of a standard operating procedure (SOP), or protocol, which explains the necessary stages and steps in great details [1]. Readers interested in reconstruction are advised to also refer to the SOP.

The metabolic reconstruction process can be grouped into 5 major stages (see Figure 1):

1. **Generation of a draft reconstruction based on genome annotation and biochemical databases.** Generally, the genome annotation is downloaded from a repository (e.g., NCBI) or the sequencing center (e.g., TIGR), and it should list at least a unique identifier, genome coordinates, and potential gene product function. Many of genome resources have also enzyme commission (EC) numbers for the genome encoded enzymes. These EC numbers along with key words can be used to compile a sublist of potential metabolic functions in the target organism. This list can be then used to obtain from

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biochemical databases (e.g., KEGG [9]. BRENDA [10]) the metabolic reactions catalyzed out by the enzymes. This list represents the draft reconstruction. The characteristics of this draft reconstruction are that it is incomplete (missing or wrong annotations) and it has an organism-independent reaction list: KEGG, as well as partially BRENDA, list all possible metabolic transformation catalyzed by a particular enzyme. However, the enzyme of the target organism may be able to bind to a subset of the listed substrates, or only one of the listed coenzymes can participate in the reaction in the target organism. This substrate and coenzyme pluripotency is one of the main reasons why manual curation is necessary.

2. **Refinement and expansion of the draft reconstruction through manual curation and extensive use of biochemical literature specific for the target organism.** Starting from the draft reconstruction, every entry will be evaluated for the following criteria:
 - a. Is the assigned function of the gene product correct? Use of biochemical literature, enzyme purification studies, a more detailed, phylogeny based annotation are helpful to answer this question.
 - b. What is the substrate and coenzyme specificity of the target organism's enzyme? Use of biochemical data, enzyme assays and protein structure will be helpful for answering this question. Finding evidence for this issue can be difficult. The use of closed relative organisms can be helpful.
 - c. Is the biochemical reaction(s) mass- and charge balanced? Therefore, the neutral formula of each metabolite in the reaction has to be obtained (e.g., from KEGG or PubChem [11]). The charged formula has to be determined for each metabolite for a set pH value (e.g., pH 7.2) by determining the protonation state of each functional group within the metabolite. Software tools are available to assist this step (see Thiele and Palsson for details [1]). Once the charged formula has been determined for each metabolite, the occurrences of each element (e.g., C, H, N, S, O, P), as well as the charge, on the left- and right-hand side has to be counted. Stoichiometric coefficients may need to be adjusted such that the same amount of each element appears on both sides of the reaction. In some cases, protons (H⁺) or water may be added to the reactions to obtain a mass- and charge balanced reaction.
 - d. The reaction directionality needs to be determined using thermodynamic information (refer for details to Thiele and Palsson [1], Feist et al [12], and Fleming et al [13]).
 - e. Localization of reaction needs to be determined, especially, if multiple compartments are considered (e.g., human metabolic network accounts for eight cellular compartments, while many bacterial reconstructions account for two or three compartments, which are extracellular space, periplasm, and cytosol). Information about reaction location may be obtained from the genome sequence if it encodes for a signal peptide (for protein export) or by targeted experiments (e.g., using GFP tagging and fluorescence microscopy).

- f. Gene-protein-reaction (GPR) association needs to be determined: while the genome annotation indicates that the gene product has a particular function, one should investigate if further gene products are needed for function, as is the case for protein complexes, or if alternate gene products exist that can carry out the function, i.e., isozymes. The reconstruction contains these GPR associations in form of Boolean rules: for example, a protein complex is encoded as 'gene_A & gene_B', while isozymes are encoded as 'gene_A or gene_B'. Any combination of these rules is possible. Beside genome annotation, biochemical data, protein purification, and/or structural genomics can provide information regarding the GPR association.
 - g. Confidence score, references, and notes: The steps listed above collect valuable information for a particular enzyme or function in the target organism. This information should be associated with the network reaction (e.g., in special columns in the spreadsheet). This information is thought to increase the traceability of reaction/gene evidence as well as highlight/summarize the amount of knowledge currently available. Often, a confidence scoring system is employed, which allows easy identification of high-confidence/low confidence reactions in the network. This is of particular value during the network debugging and evaluation stage (see below). The highest confidence score (4) is given to reactions that have biochemical evidence (e.g., protein purification, protein assays, protein structure information). A score of 3 is given if genetic data is available (e.g., knock-out mutant characterization, knock-in experiments, over-expression of a protein). A score of 2 is given if either physiological data (e.g., secretion products, growth capability on substrate) or (high confidence) sequence annotation is available. A low confidence score of 1 is given if reactions are included for modeling purposes without any of the aforementioned evidence. In some cases, a confidence score of zero is also employed, which highlights reactions that have not yet been evaluated for supporting evidence.
 - h. Finally, different information should be collected in this stage of the reconstruction process to facilitate the following stages. This information includes the biomass precursors, necessary to produce a new cell (target organism) which is ideally derived from experimental data (see Thiele and Palsson for a detailed description on how to compile this information). Furthermore, information about enzyme reaction rates (v_{max}) should be collected, as many biochemical publications contain this information. Information about growth media should be also collected.
3. **Conversion of the manual curated metabolic reconstruction into a mathematical model.** The reconstruction process is an iterative process as shown in Figure 1, where the initial reconstruction is converted into a mathematical format, the so called stoichiometric (S) matrix. This model conversion also includes the addition of balances and bounds. Balances in biochemical networks can be, for example, mass- and energy conservation. For instance, the majority of modeling applications of metabolic models assume the system to be in quasi steady state. This assumption implies that the sum of producing reactions for a particular metabolite is equal to the sum of consuming reactions. Bounds on metabolic reactions can include maximal reaction rates based on

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the catalyzing enzyme's properties, thermodynamic information (e.g., reaction directionalities), etc. Often, the mathematical models are stored and computed in Matlab (Mathwork, Inc). Commonly, metabolic reconstructions and models are stored in the systems biology markup-language (SBML) format [14], which is platform independent and can be loaded in numerous systems biology applications.

- 4. Network debugging and evaluation to ensure that the metabolic model has similar phenotypic properties as the target organism.** Once the metabolic reconstruction is converted into a mathematical format and balances and bounds are applied, a comprehensive investigation of the model's properties begins. Most reconstructions contain initially numerous dead-end metabolites (i.e., metabolites that are only produced or consumed in the network). Due to the balance constraints, reactions which contain such dead-end metabolites cannot carry any reaction flux in any simulation conditions. A detailed evaluation of these dead-end metabolites is necessary to identify whether these metabolites can be connected to the remaining network by adding one or more reactions to the reconstruction. However, one has to be careful, as arbitrary filling of the so-called gaps will alter significantly the model's properties. All added reactions should have experimental, genome and/or physiological data as supporting evidence. Some dead-end metabolites may remain in the network, as current knowledge does not support any filling of gaps they are causing. In addition to these 'knowledge gaps' the reconstruction can contain 'scope gaps.' In the case of scope gaps, reactions are known, which could connect the dead-end metabolite, but they are either non-metabolic or not within a previously defined scope of the reconstruction (e.g., tRNA charging with amino acids).

Once all dead-end metabolites have been characterized and partially connected to the network by repeating part of the second and third stage, the model's capability to produce biomass precursor is evaluated. This process will lead to further identification of network gaps, which need to be filled. This step can be quite time-consuming, and detailed evaluation of dead-end metabolites in the earlier step will directly pay off. When the model can produce all biomass precursors, one can compile them into one reaction (the biomass reaction) by considering their fractional contributions to cell composition. This stage also includes further (i) quality tests, such as the model's capability to grow on known carbon, nitrogen, phosphor and sulfur sources; (ii) the capability to reproduce accurately measured growth rates and to secrete known by-products. The list of tests depends on the properties of the target organism as well as the availability of experimental data (e.g., phenotyping data, knock-out mutant growth phenotype data, etc.). Note that this stage is iterative, in which network reactions will be added (by repeating partially, or in full, stage 2 and 3) or in some cases reactions will be removed from the metabolic reconstruction. This stage is deemed to be finished if the model reproduces accurately the target organism's phenotypic characteristics and/or experimental data is exhausted.

- 5. Prospective use of the reconstruction and the metabolic models. This stage is certainly the most exciting part of the reconstruction process.** Numerous applications have been developed over last decade or so, including biological discovery [15], metabolic

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engineering [16-17], prediction of outcome of adaptive evolution [18], network topology [19], and assessment of phenotypic behavior [20-22]. Some of these applications have been summarized in a recent review [23-24].

Output

The output of this workflow is a highly curated, accurate and comprehensive representation of biochemical transformation taking place in the organism of interest (Figure 2). Note that to date, it is not possible to automate all steps within the 5 stages without loss of accuracy or correctness.

A

Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7
Rxn name	Rxn description	Formula	GPR	Genes	Subsystem	Reversible
PFK	phosphofru ctokinase	atp[c] + f6p[c] -> adp[c] + fdp[c] + h[c]	(b3916 or b1723)	b1723 b3916	Glycolysis/Gl uconeogenesi s	0

Col 8	Col 9	Col 10	Col 11	Col 12	Col 13	Col 14	Col 15
LB	UB	Objective	CS	E.C. number	rxnKeggID	Notes	References
0	1000	0	4	2.7.1.11	R00756	E. coli has to genes for PFK (pfkA and pfkB) where pfkA is the major form.	PMID: 63101 20;149128 ;6310120

B

Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
Metabo- lite name	Descrip- tion	Formula	Charge	Compa- rtment	KeggID	Pub- ChemID	CheBI ID	Smiles	InChi
glc-D[c]	D- Glucose	C6H12O6	0	Cyto- plasm	C00031	3333	17634	OC[C@H]1OC(O)[C@H](O)[C@H](O)[C@H]1O	1/C6H12O6/c7-1-3(8)4(9)5(10)6(11)12-2/h2-C@H](O)[C@H]1H,1H2/t2-,3-(O)[C@H]1O

Figure 13:
Figure 2. Data contained in the metabolic network reconstruction after completion of the presented workflow.

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Workflow 2: Metabolic Flux Analysis via Isotope Labeling

Summary: Metabolic fluxes are a key determinant of cellular physiology, representing the final functional output of the interaction of all the molecular machinery (genes, proteins, metabolites) studied by the other “omics” fields. This workflow (a schematic of which is presented in Figure 1) describes the input data required for measuring fluxes using an isotope labeled feed, along with the expected output and the processes needed to obtain it. The main input data are metabolite labeling patterns after a carbon labeling experiment, a metabolic reconstruction, and measured extracellular and biomass fluxes. The desired output is the rate (i.e., number of molecules through the reaction) for each of the reactions considered in the model, along with confidence intervals. Here, we will focus on the most common and well-established form of flux analysis through isotope labeling: ^{13}C Metabolic Flux Analysis (^{13}C MFA) from proteogenic amino acids in the exponential phase. Nonetheless, the modular nature of the workflow presented here will allow for other varieties of ^{13}C MFA in development to be easily incorporated.



Figure 1. Workflow for ^{13}C metabolic flux analysis. Blue blocks indicate processes (e.g., experiments or algorithms), and the green blocks indicate datasets or physical objects. See text on next page for callout to this figure.

The following workflow for metabolic flux analysis though isotope labeling will focus on its most common and established form: ^{13}C Metabolic Flux Analysis (^{13}C MFA) from proteogenic amino acids in the exponential phase. This is not to say that it is the most important, but rather the most mature and where agreement on a common workflow is most likely. That having been said, the modular nature of the workflow presented here allows for other varieties to be easily incorporated, some of which are still in development. For example, if intracellular metabolite labeling were to be used instead of amino acid labeling, this data (and the necessary metabolite concentrations) could be easily inserted at the same point in the diagram as amino acid labeling

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(along with a connection to the metabolomics workflow). Other such variations such as flux analysis in a non-steady state [1], labeling of atoms other than carbon [2], or usage of NMR data [3] can be added in a similar fashion.

The workflow described here is an important tool for us as researchers for several reasons: 1) it explains the process to new members of the group as well as collaborators, 2) it helps define the standards for stored data in order to replicate and compare results in the future 3) it defines the steps used to track project completion and to help plan and develop high-throughput experiments.

The first step we include in the workflow is the characterization of the strain growth, a process not exclusive to ¹³C MFA. This characterization produces two sets of data that will be useful for planning the isotope labeled experiment: the growth curve and the concentration of extracellular metabolites. The growth curve provides the mid-log point used for sampling, and the extracellular metabolite concentration provides a rough idea of which metabolic pathways are important in addition to measured transport fluxes for later use. An example of a possible data input of extracellular metabolite concentration is shown in Figure 2. Useful metadata involves strain details, including plasmid and genetic modifications, along with materials and methods for OD and extracellular metabolite measurements.

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Compound Summary										
Sequence start:	2/20/2009 11:22:46 AM									
Operator:										
Method file name:	C:\CHEM32\1\DATA\FEB-20-2009 2009-02-20 11-22-22\YEAST_FERMENTATION_JENNIFER.M									
Sample Name	Sample Amt [g/L]	Multip. * Dilution	FileName .D	RetTime [min]	Amount [g/L]	Compound				
Blank	0.00000	1.0000	001-0801	9.222	-	-				
				9.325	-	-				
				9.876	-	-				
				12.810	-	-				
				13.083	-	-				
				13.769	-	-				
				14.072	-	-				
				14.345	-	-				
				15.418	-	-				
				15.693	-	-				
				22.392	-	-				
				1-1	0.00000	1.0000	011-0901	9.217	0.032	Pyruvate
								9.230	16.379	Glucose
								9.876	-	-
12.809	5.33320e-1	Lactate								
13.085	5.23185e-1	Lactate								
13.777	9.03251e-3	Glycerol								
14.087	6.99238e-2	Formate								
14.344	6.28119e-2	Formate								
15.423	3.43505e-1	Acetate								
15.701	3.34890e-1	Acetate								
22.410	1.87606e-1	EtOH								
1-2	0.00000	1.0000	012-1001					9.215	0.027	Pyruvate
								9.229	16.774	Glucose
								9.876	-	-
				12.807	4.39012e-1	Lactate				
				13.083	4.06618e-1	Lactate				
				13.769	-	-				
				14.087	5.33141e-2	Formate				
				14.343	3.99210e-2	Formate				
				15.420	2.81520e-1	Acetate				
				15.699	2.65478e-1	Acetate				
				22.408	1.16859e-1	EtOH				
				1-3	0.00000	1.0000	013-1101	9.215	0.030	Pyruvate
								9.225	16.606	Glucose
								9.876	-	-
12.807	4.11458e-1	Lactate								
13.079	3.89868e-1	Lactate								
13.769	-	-								
14.087	5.74127e-2	Formate								
14.342	4.45182e-2	Formate								
15.419	3.00310e-1	Acetate								
15.694	2.99122e-1	Acetate								
22.401	1.36531e-1	EtOH								
2-1	0.00000	1.0000	014-1201					9.215	0.028	Pyruvate
								9.226	16.606	Glucose

Figure 2. Example of output of HP-LC analysis used as input of extracellular metabolite concentration. A standard format for this information would be useful.

The main experimental process in the workflow is the performance of the labeling experiment, the workflow for which has been described by Zamboni et al [4] (see Figure 3). The necessary input for planning and performing the experiment includes the growth curve and extracellular metabolite concentrations, which has been discussed above, and the feed labeling, which affects the range of fluxes that can reliably be determined [5] [6]. The output includes the main piece of data needed to constrain the metabolic fluxes: the amino acid labeling pattern. The labeling information should include as metadata details of the experiment including sampling points, initial feed labeling and materials, and methods for labeling measurement. Examples of amino acid labeling data in terms of the derivatized fragments [7] or amino acid backbone labeling can be seen in Figs. 4 and 5 [8] [9].

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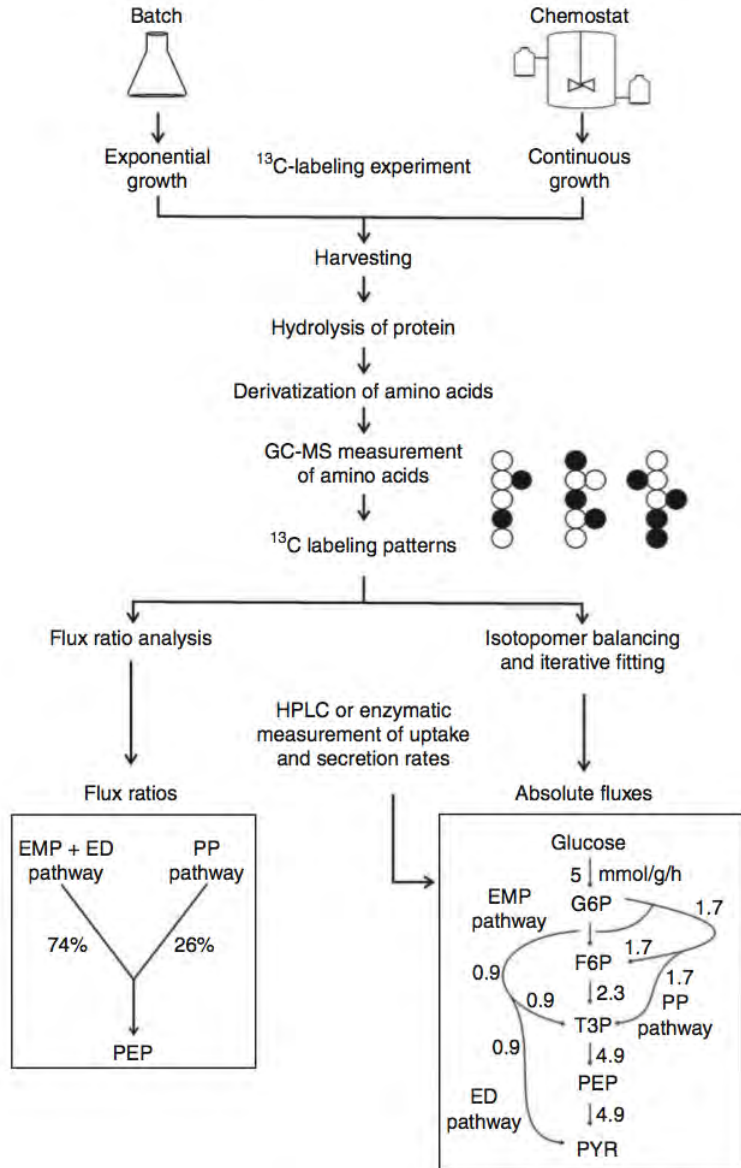


Figure 3. Workflow for carbon labeling experiment as per Zamboni et al [3] showing the two types of methods to obtain flux profiles: through flux ratio analysis or isotopomer balancing and iterative fitting.

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SAMPLE:	WT+MET	WT+MET	WT+MET	MET28	MET28	CBF1	CBF1
REPLICATE	A	B	C	A	B	A	B
TBDMS							
FRAGMENT							
Ala232 (M0)	0.5666	0.5638	0.5633	0.5757	0.5755	0.5632	0.5699
Ala233 (M1)	0.3151	0.3174	0.3170	0.3102	0.3135	0.3173	0.3133
Ala234 (M2)	0.0907	0.0912	0.0916	0.0876	0.0857	0.0912	0.0898
Ala235 (M3)	0.0238	0.0235	0.0240	0.0227	0.0214	0.0242	0.0234
Ala236 (M4)	0.0034	0.0035	0.0036	0.0035	0.0037	0.0035	0.0032
Ala237 (M5)	0.0004	0.0005	0.0005	0.0003	0.0002	0.0005	0.0004
Ala260 (M0)	0.5603	0.5570	0.5542	0.5678	0.5626	0.5582	0.5595
Ala261 (M1)	0.3171	0.3192	0.3197	0.3115	0.3155	0.3174	0.3161
Ala262 (M2)	0.0930	0.0934	0.0944	0.0913	0.0898	0.0941	0.0939
Ala263 (M3)	0.0250	0.0255	0.0269	0.0247	0.0269	0.0258	0.0258
Ala264 (M4)	0.0040	0.0040	0.0040	0.0041	0.0045	0.0040	0.0041
Ala265 (M5)	0.0007	0.0008	0.0007	0.0007	0.0006	0.0005	0.0006
Ala266 (M6)	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Gly218 (M0)	0.7614	0.7592	0.7579	0.7612	0.7621	0.7585	0.7605
Gly219 (M1)	0.1603	0.1605	0.1613	0.1589	0.1575	0.1606	0.1596
Gly220 (M2)	0.0664	0.0685	0.0682	0.0664	0.0643	0.0687	0.0671
Gly221 (M3)	0.0098	0.0100	0.0104	0.0109	0.0128	0.0102	0.0107
Gly222 (M4)	0.0019	0.0017	0.0019	0.0021	0.0025	0.0019	0.0020
Gly223 (M5)	0.0002	0.0001	0.0003	0.0005	0.0008	0.0001	0.0002
Gly246 (M0)	0.7479	0.7491	0.7479	0.7529	0.7581	0.7511	0.7529
Gly247 (M1)	0.1694	0.1683	0.1696	0.1673	0.1640	0.1667	0.1664
Gly248 (M2)	0.0706	0.0710	0.0708	0.0687	0.0667	0.0703	0.0693
Gly249 (M3)	0.0103	0.0099	0.0100	0.0093	0.0094	0.0103	0.0096
Gly250 (M4)	0.0018	0.0016	0.0018	0.0018	0.0019	0.0017	0.0017
Val260 (M0)	0.4119	0.4087	0.4130	0.4269	0.4181	0.5802	0.6023
Val261 (M1)	0.3806	0.3828	0.3795	0.3737	0.3797	0.2760	0.2637
Val262 (M2)	0.1532	0.1531	0.1525	0.1482	0.1475	0.1105	0.1039
Val263 (M3)	0.0451	0.0447	0.0436	0.0430	0.0447	0.0273	0.0248
Val264 (M4)	0.0086	0.0094	0.0098	0.0082	0.0099	0.0056	0.0046
Val265 (M5)	0.0006	0.0013	0.0015	0.0000	0.0000	0.0003	0.0006
Val288 (M0)	0.4098	0.4054	0.4083	0.4247	0.4139	0.5779	0.5984
Val289 (M1)	0.3812	0.3839	0.3802	0.3729	0.3776	0.2771	0.2638
Val290 (M2)	0.1545	0.1552	0.1559	0.1497	0.1550	0.1120	0.1062
Val291 (M3)	0.0440	0.0448	0.0448	0.0428	0.0441	0.0272	0.0255
Val292 (M4)	0.0091	0.0093	0.0095	0.0087	0.0088	0.0052	0.0055
Val293 (M5)	0.0013	0.0014	0.0013	0.0012	0.0005	0.0007	0.0007
Leu274 (M0)	0.3110	0.3088	0.3100	0.3312	0.3190	0.7240	0.7293
Leu275 (M1)	0.3885	0.3898	0.3890	0.3845	0.3895	0.1899	0.1854
Leu276 (M2)	0.2111	0.2114	0.2100	0.2035	0.2063	0.0748	0.0723
Leu277 (M3)	0.0689	0.0688	0.0699	0.0624	0.0662	0.0098	0.0113
Leu278 (M4)	0.0170	0.0175	0.0174	0.0157	0.0161	0.0015	0.0017
Leu279 (M5)	0.0031	0.0032	0.0032	0.0025	0.0029	0.0000	0.0000
Leu280 (M6)	0.0005	0.0005	0.0004	0.0002	0.0000	0.0000	0.0000

Figure 4. Amino acid labeling for different derivatized fragments, taken from [7]. The name on the left column corresponds to the amino acid and the fragment type [6]. Each of the following columns corresponds to the fraction of molecules with 0,1,2... extra mass units incorporated due to isotopic variation (from carbon or other atoms).

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Table 1. Relative Intensity and Error Associated with the Measurement of Each Amino Acid Isotopomer^a

amino acid	rel intens (%)	error (ppm)	amino acid	rel intens (%)	error (ppm)	amino acid	rel intens (%)	error (ppm)	amino acid	rel intens (%)	error (ppm)
Gly			Val			Lys			Phe		
M0	1.30	0.21	M0	11.60	0.07	M0	0.90	0.15	M0	0.65	0.17
M1	4.20	0.22	M1	42.40	0.01	M1	4.20	0.09	M1	2.60	0.21
			M2	1.80	0.00	M2	8.00	0.10	M2	5.60	0.20
						M3	0.30	0.09	M3	3.70	0.21
Ala			Thr			Glu			Arg		
M0	4.70	0.00	M0	1.20	0.02	M0	8.30	0.16	M0	0.30	0.27
M1	19.50	0.01	M1	6.00	0.02	M1	23.30	0.08	M1	1.00	0.27
M2	0.40	0.00	M2	11.50	0.03	M2	1.00	0.09	M2	1.80	0.37
			M3	0.20	0.03				M3	0.07	0.21
Ser			Leu (and Ile)			Met			Tyr		
M0	2.30	0.03	M0	100	0.06	M0	0.15	0.17	M0	nd ^b	
M1	7.30	0.05	M1	71.70	0.01	M1	1.00	0.18	M1	0.10	0.25
M2	0.20	0.00	M2	4.20	0.01	M2	4.40	0.14	M2	0.20	0.42
			M3	0.10	0.04	M4	8.20	0.13	M3	0.15	0.15
Pro			Asp			His					
M0	19.00	0.03	M0	1.45	0.01	M0	2.40	0.12			
M1	55.20	0.02	M1	7.30	0.03	M1	3.80	0.12			
M2	2.50	0.02	M2	15.10	0.03	M2	0.90	0.09			
M3	0.06	0.40	M3	0.30	0.07	M4	0.30	0.08			

^a M0, M1, M2, etc., refers to isotopomers with 0, 1, 2, etc., ¹³C incorporated in the backbone of the amino acid. A RSD of $\leq 2\%$ is associated with each relative intensity measurement. Errors refer to one single measurement. A variation of 10% is associated with it. ^b Not detected.

Figure 5. Amino acid labeling for carbon backbone. M0, M1, M2... indicate the fraction of molecules with 0,1,2.... labeled carbons incorporated [8].

Extracellular metabolite concentrations from the growth characterization experiment are used to derive the transport fluxes, (i.e. uptake and secretion rates). The calculation from extracellular metabolites is straightforward, and it involves calculating the change in metabolite concentration in the media. Another important set of known fluxes is the fluxes to biomass production, obtained from the change in OD and the cell composition.

Fitting the fluxes to the labeling data is the main computational process in the workflow. A variety of methods are available to do this [7] [10] [11]. Some involve determination of local flux ratios, and some are based on iterative fittings for the whole metabolic network under consideration (see Figure 3). Among the latter, the fit can either be performed in a search space involving fluxes and labeling, with the labeling pattern included as a constraint [12], or in a search space involving only fluxes, with the labeling determined for each flux profile. Labeling corresponding to each flux profile can be produced using several methods, including isotopomer mapping matrices [13], cumomers [14] or elementary metabolic units [15], to name a few. The search through the flux phase space can, as well, be carried over via a variety of techniques, including genetic algorithms, sequential quadratic programming and simulated annealing [7]. Software for flux calculations include 13CFLUX [16] [4] and FIATFLUX [17], none of which are available in open source format, and openFLUX [18], a recent application based on elementary metabolic units available in open source format. For the purpose of designing a workflow, what is important is not the differences among these methods but the fact that they all require the same input: 1) transport and biomass fluxes, 2) amino acid labeling, 3) initial feed labeling, and 4) the carbon transitions included in a metabolic reconstruction. Amino acid and initial labeling patterns, and measured fluxes have all been discussed above. The metabolic reconstruction has been discussed at length above; the only required condition is that it includes atomic transitions (see example in Figure 6 [19]). This metabolic model may be a coarse grained version of the models considered above. See, for example, Figs. 6 and 7, where

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some reactions have been clumped together to ease calculations. Recently, a new tool for sharing, storing, and constructing these atomic transitions embedded in a metabolic reconstruction has become available [20]. Standard formats used in this program are the 13CFLUX format and SBML.

Supplementary Table S-2 Reaction lists:

```
% Lactate to pyruvate
1: ABC(1) -> ABC(2)

% Pyruvate to Acetyl-CoA
2: ABC(2) -> BC(3) + A(17)

% Oxaloacetate to citrate
3: AB(3) + abcd(11) -> dcbBAa(5)

% Citrate to isocitrate
4: ABCDEF(5) -> ABCDEF(6)

% Isocitrate to 2-oxoglutarate
5: ABCDEF(6) -> ABCDE(7) + F(17)

% 2-oxoglutarate to succinyl-CoA
6: ABCDE(7) -> BCDE(8) + A(17) :: ABCDE(7) -> EDCB(8) + A(17)

% Succinate to Malate
7: ABCD(8) -> ABCD(9)

% Fumarate -> malate
8: ABCD(9) -> ABCD(10)
```

Figure 6. Example of atomic transitions input needed form 13C MFA [18]. The first number indicates the reaction number as per Figure 7, and the numbers in parenthesis indicate the metabolite numbers. Carbon transitions are indicated as strings of letters: e.g., ABC -> AB + C indicates that the first two carbon in the reactant end up as the two carbons in the first product and the last carbon goes to the second product.

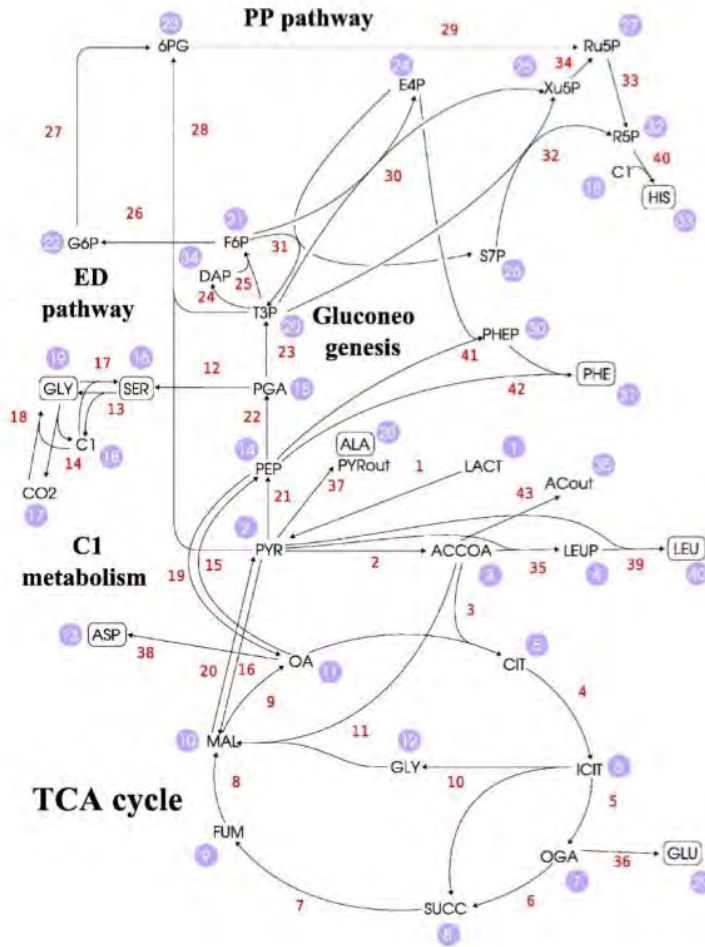


Figure 7. Reaction network for *Shewanella* central carbon metabolism [18]. Notice how some of the reactions (e.g., g6p to Ru5P in the pentose-phosphate pathway) have been clumped together to ease calculations).

The output should include the flux profile giving the best fit for the experimental data, a confidence interval, and the computed labeling patterns. The metabolic flux profile gives the best guess (compatible with the data) of the rate for each of the metabolic reactions considered in the metabolic model of the cell. This information is useful *per se* as a widely recognized highly relevant characteristic of the phenotype [21], and has numerous applications in (e.g.) metabolic engineering [11]. As with every experimental measurement, it is also desirable to assign confidence intervals to flux estimates, and a variety of algorithms are available for this purpose [7].

A simple list of Fluxes with their corresponding confidence intervals for each metabolic reaction can be very difficult to make productive use of, particularly for large models. Hence, visualization is an important part of the workflow and several possibilities are available [22] [23] [24] [25], although not all of them allow flux visualization for models with clumped reactions.

Finally, a useful visual check that the fit is appropriate is to compare computational predictions with experimental data, as shown in Figure 8.

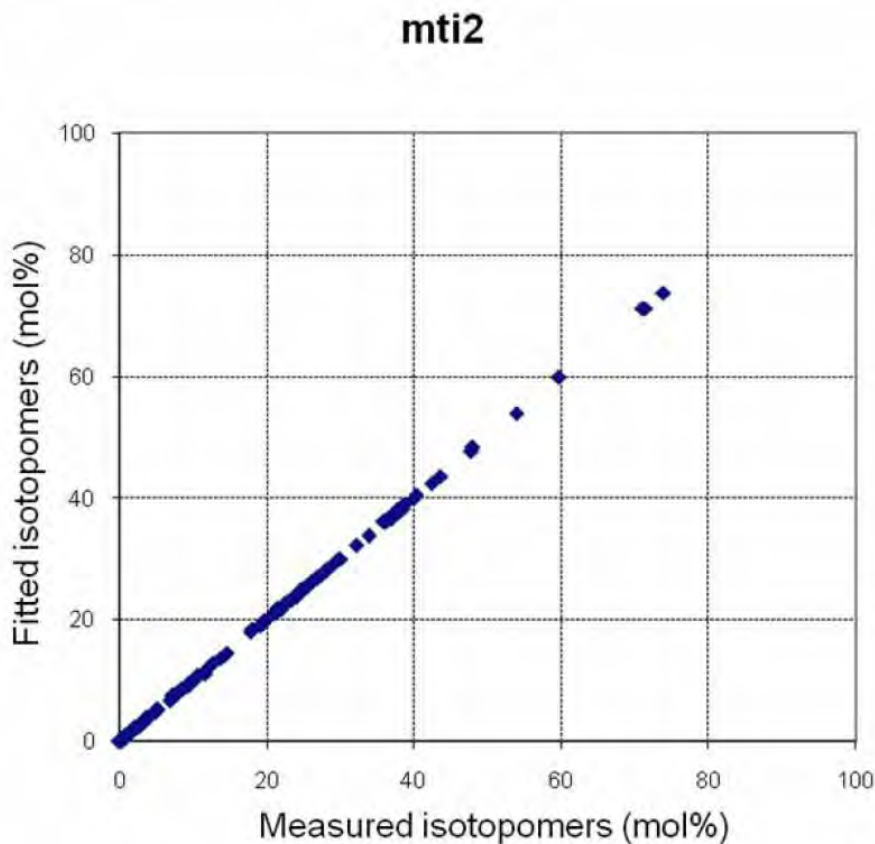


Figure 8. Comparison between computed and measured labeling data [7]. A good fit does not deviate from the diagonal.

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Workflow 3: Inference of Gene Regulatory Networks

Summary

Gene regulatory networks (GRNs) are the “on-off” switches and rheostats of cells that operate at the gene level. They dynamically orchestrate the level of expression for each gene in the genome by controlling whether and how vigorously that gene will be transcribed into mRNA. Understanding how GRNs work is key to systems biology and its successful applications. An array of input data types exists. Knowledgebase users should be able to select an organism, upload, broadcast, or import expression data from public repositories, and submit a request for gene regulatory network inference. Meta-information on experiment design should be automatically parsed from public data, or the user should be prompted to upload this information. Users may want to start with a set of genes or a metabolic process and ask which factors are its regulators. Another use scenario is that researchers may want to know the gene targets of regulatory elements.

Genes will be grouped into putative regulatory modules whose transcription is correlated under specific conditions. For each module, the user selects a subset of known transcription factors and environmental factors that best predict the transcription levels. Additional inputs, such as motifs or protein interactions, may be statistically integrated in the clustering step or the network inference step, and shared regulatory motifs can be computed. Several algorithms have been devised for clustering and discovery of regulatory influences. Results can be exported as raw data or presented to the user in a searchable and browsable form. Subnetworks can be graphically displayed along with views of expression profiles and regulatory motifs and the gene content of individual clusters. Useful output will also include the ability to compute and present predictions (and confidence estimates on predictions) of effect of transcription factor deletions/overexpressions and/or environmental changes.

Inputs

1. Depending on the specific type of network inference analysis a user has in mind, a different combination of the following data might be necessary; but minimally, these seven types of information cover most of what is available today.
2. Measurements of transcription (with confidence values [if avail.]) in the form of an $n \times m$ matrix with n genes and m conditions (microarray or sequencing)
3. Measurements of fitness associated with systematic gene knockouts or over-expression (maybe these last two can be condensed in measures of genome-scale gene function with confidence in the form of an $n \times m$ matrix.... This could be generalized as phenotype and also have associated confidence depending on how it's measured.
4. Gene interaction network(s) = [nodes (genes), edges (interactions/type), confidence values or weights for edges]
5. Gene locations on genome—with RNA-Seq this is becoming extremely precise with direct measurement.
6. Genome sequence or individual upstream sequences (for motif detection)

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7. A list of predictors (transcription factors, environmental factors including metabolites)
8. Machine-readable descriptions of conditions, specifically time series info with standardized measurements of environmental factors

User will specify an organism and import (or broadcast) the above data items. Many of these data types are stored in existing databases and can be loaded automatically through interoperability with these data sources. Many of these data types (items 2-4) can be obtained automatically given the organism. Item 1 may be obtained automatically from expression databases such as GEO or MicrobesOnline. Item 3 can be obtained from STRING, and some information is also accessible in MicrobesOnline. Items 4-6 can be obtained from NCBI or MicrobesOnline or other databases.

However, these data are not available for all organisms. One addendum to this work is that many of these types of measurements follow a standard experimental workflow. Once a genome of a cultivated organism gets sequenced, it might be useful to develop a minimal set of functional measurements to aid in this.

As these workflows are being developed and have increasingly precise data such as RNA-Seq and can have associated confidence measures that can be carried through the analyses, this is providing a basis for comparing the precision of results between methods and laboratories that would help to improve quality and would benefit existing systems such as GEO if applied consistently.

Apply clustering and network inference

Group the genes into putative regulatory modules whose transcription is correlated over a set of conditions. Select a subset of known transcription factors and environmental factors that best predict the transcription levels of each module. Additional inputs, such as motifs or protein interactions, may be statistically integrated in the clustering step or the network inference step, and shared regulatory motifs can be computed. Several algorithms have been devised for clustering and discovery of regulatory influences; some are available in R and MatLab.

Outputs

- Clusters of putatively coregulated genes or biclusters containing genes putatively coregulated under subsets of conditions
- Cis-regulatory motifs
- Regulatory network mapping: influences of predictors on genes within clusters/biclusters directly or through *and* and *or* operations. Confidence values for edges.

Results can be exported as raw data or presented to the user in a searchable and browsable form. Users may want to start with a set of genes or a metabolic process and ask which factors are its regulators. Or, users may want to take a given regulator and ask what are its targets. Subnetworks can be graphically displayed along with graphical views of expression profiles and regulatory motifs and the gene content of individual clusters. Useful output will also include the

ability to compute and present predictions (and confidence estimates on predictions) of effect of TF deletions/overexpressions and/or environmental changes.

Scope

A user should be able to select an organism, upload, broadcast, or import expression data from public repositories, and submit a request for network inference; meta-information on experiment design should be automatically parsed from public data, or a user should be prompted to upload this information. All other data types can be automatically parsed from public repositories—an advanced user should have privileges to change or override default settings by changing source of information, threshold of significance, etc. A user should be given options for choice of algorithms based on the amount and type of available data; the user should have access to published citations for the algorithms and basic information on workings of the algorithm in non-technical jargon-free language. It should be possible to store a session with the default or user-edited settings so the entire analysis can be recreated.

Data requirements and computational complexity

It seems that these might be important numbers both from the user's perspective and from the planning perspective, but can these be coherently calculated? We can give some perspectives to the user, for instance, to infer a network with causal influences time series data are a must; for better coverage of regulons one needs to probe responses to at least half a dozen or a dozen environmental perturbations with different dosages and over the time scale of the response; to incorporate mechanisms we need to have physical interactions (P-D, P-P), or information on TF-cis-regulatory motif relationships. However, in principle, one could learn a network based on correlations and cis-regulatory motifs with a relatively small dataset (30-50 experiments - see Gardner's CLR algorithm or Bar-Joseph's DREM). Such a network will give a very limited view of transcriptional control but could be deemed extremely valuable for an organism for which absolutely nothing was known previously. Given the diverse variations in use cases, while we could consider very simple to very sophisticated cases, I would argue that we should focus on use-cases of simple to mid-scale complexity. I say this because advanced users with sophisticated needs are likely to have the capability to do it themselves (without a knowledgebase).

We could have minimal requirements imposed on algorithm developers when they submit their work. This would include a listing of requirements (number of experiments, interaction data etc.). It might be instructive to have the following information as well; I am not sure if we can generalize this to other use cases.

- Estimates of number and diversity of experiments necessary for clustering
- Estimates of quality needed—issues of quality, compendium biases, etc.
- Estimates of computational complexity of biclustering/bayes nets/etc.

Notes

Are there other players we'd like to incorporate, like RNA regulatory elements, for instance? Would we want to get more out? Network motifs? How about Lee's fusion of kinetics and GRNs? Does that require additional input or generate additional output?

Certainly, moving beyond the inference of regulatory structure and gross dynamics would require different experiments. Inferring metabolism requires both different sorts of functional assays and genome-scale experiments; inferring signaling pathways has its own troubles (see Aindrila Mukhopadhyay's document); inferring complex regulation like that implemented in control of sporulation requires more detailed microscopic measurement and mechanistic modeling. However, here we have the opportunity for something that could almost become a standard after analysis of any sequenced genome.

Inference and Measurement

Is it possible to describe the situations where it is better to try to infer genetic regulatory network topology, rather than try to measure the regulatory interactions directly? There have been remarkable experimental strides made in determining the sequences to which transcription factors bind (e.g., Hesselberth et al, "Global mapping of protein-DNA interactions *in vivo* by digital genomic footprinting," *Nature Methods* 6, 283 - 289 (2009) doi:10.1038/nmeth.1313).

Workflow 4: Signaling

Summary

Due to the focus on microbes and microbial communities, the workflow pertains to signaling in bacteria. Bacterial signaling forms a subset of the Genetic Regulatory Network discussion (see Workflow 3) and therefore contains overlap in experimental design, analysis and workflow.

Microbial genomes present signaling systems to sense and respond to both external and internal stimuli^{1,2}. Signals include numerous factors considered to be stresses, intracellular cues, and environmental changes. In bacteria, two component signal transduction systems, typically comprised of a sensor histidine kinase and a response regulator, provide the primary mechanism of signal sensing and response^{3,4}. Signal transduction occurs via phosphotransfer or phosphorelay and results in an activated response regulator. The best-studied response mechanisms include either the direct modulation of chemotaxis by the activated response regulator, or in a large number of studies, response regulator modulated differential expression of target genes. New classes of response regulators that modulate function via alternate mechanisms such as c-diGMP cyclase or phosphodiesterase domains have also been described^{1,2}. Available sequenced genomes from environmental organisms encode numerous sensor and response regulator proteins containing domains of unknown function indicating that additional mechanisms for effector function have yet to be discovered. Environmental bacteria such as *Geobacter metallireducens*, *Desulfovibrio vulgaris*, and the cyanobacterium *Nostoc spp.* have upward of 60, to more than 150, sensor kinases⁵. The responses regulated by the corresponding two component systems are no doubt at the core of environmental process of key significance. These systems also provide the parts for developing valuable sensory modules to build sophisticated engineered systems (using synthetic biology methods).

Definition of a signal: With regard to the type of research being conducted by the Genomic Science groups, signals can vary widely. In environmentally relevant microbes, a signal could be a change in environmental cue (e.g., the lack/abundance of resources such as carbon source, electron acceptors, electron donors, amino acids, vitamins, etc.); stresses (e.g., salt, pH, heat, cold, metals, toxins, oxygen, a variety of small molecules); or variability in other organisms in the microenvironment. The responses to these signals, including the triggering of altered physiological states (e.g., biofilm formation, sporulation, virulence, swarming, etc.) are all initiated via signal sensing and corresponding response. In microbes that are being engineered for industrial uses (e.g., biofuel production), perturbation from toxins present in carbon feed, intracellular triggers due to imbalance in metabolic intermediates, and accumulation of final (often toxic) products serve as signals.

A vast body of knowledge exists for these systems from individually studied systems. Efforts to compile and integrate information on regulatory modules from such studies have only recently begun to emerge as described in Workflow 3. However, the impact of multiple stimuli on a given organism or comprehensive understanding of all signal sensing for a single organism is still rare. In the few cases where such studies have been undertaken, valuable and interesting phenomenon have been discovered^{6,7}. The tremendous increase in sequenced organisms and

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corresponding computational^{2,5,8} and experimental tools^{9,10} now makes it more possible to undertake such efforts.

Metadata: This documents what signals are being studied, under what defined conditions, on which organisms and by whom? What media and growth phases are being used? What methods are being used (sequencing, arrays, analytical)? What analysis tools, *in silico* prediction algorithms, and validation experiments are to be used?

Aspects of studying signaling

- *What are the genomes in question?* For a given organism, there may be more than one genome sequence if there are modified, engineered, evolved, adapted or shuffled versions.
- *Sensing and responding to signals:* Study of two-component, cAMP, and c-diGMP systems, transcriptional factors, global regulators, sigma factors; cell-wide studies (transcriptomics, proteomics, and metabolomic studies), and mapping ligand (signal) binding, phospho-transfer, and other post-translation modification.
- *Information gathered:* Ligand binding and transport, two component phosphorylation, other assays (chemotaxis, binding to cyclic-diGMP, DNA gel shifts), ChIP-chip arrays, ChIP-seq, microarrays, RNA-Seq, mapping post translational modifications (phosphoproteome, methylations etc), mapping protein interactions and localization.

Data types

This will form the core of the database for this topic

- Genome sequences
- Knockout and expression libraries: corresponding phenotypic data (e.g. from omniglogs or other such HT strategies)
- Transcript level data: microarray, RNA-Seq, absolute mRNA quants (e.g. nCOUNTER)
- DNA binding: ChIP-chip, ChIP-seq, microfluidics
- Mass Spec data: Protein levels, Post translational modifications, metabolites
- (data from different types of mass spectrometers)
- Ligand binding mapping: Semi HT
- Regulator-DNA binding: Semi HT
- Regulatory motifs and maps generated using computational methods

Resources

- Common sensory proteins include histidine kinases, methyl-accepting chemotaxis receptors, Ser/Thr/Tyr protein kinases, adenylate and diguanylate cyclases and c-di-GMP phosphodiesterases. A webpage maintained Galperin and coworkers contains a

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fairly comprehensive repository of signal transduction systems from over 500 bacteria and archaea¹¹.

- Predictive tools and databases (e.g. Regtransbase¹², MicrobesOnline¹³, and MiST¹⁶).
- Specific tools for predicting cognate partners of sensor histidine kinases and response regulators such as that developed by Burger and van Nimwegen⁸.
- Methods developed for mapping two component phosphotransfer and relay⁹, rewiring sensor kinases¹⁴.
- Classification system developed for categorizing bacterial signaling proteins^{1,2,15}.

Illustration using one concrete problem

BESC is studying a number of *Caldicellulosirupter* species, which are non-sporulating, anaerobic, gram positive, thermophilic bacteria that can facilitate the direct conversion of cellulosic biomass (e.g. from switchgrass) to ethanol, H₂ and other products.

A study of this organism will utilize the features afforded by other knowledgebases: Annotated genomes and their use in generating arrays, predictions for regulatory networks and motifs, predicted two component systems, transcriptional factors (including those that work with transporters), sigma factors, small RNA regulators.

A given experiment would entail growth of *Caldicellulosirupter* on ground plant material and monitor production of waste products including ethanol, H₂, acetate etc. Genetic engineering could create the production of alternative end products in different proportions.

A range of factors (signals) would be examined in this context. Beneficial factors include C source, cell density, etc. Harmful factors include exposure to O₂, cold shock, inhibitors from lignocellulosic biomass, acetate, non-optimal pH, salt, and the accumulation of other final products.

Current studies include: Log phase growth using cellobiose and switchgrass (pretreated), stationary phase growth in switchgrass (pretreated), log phase growth under ethanol stress with either cellobiose or switchgrass.

A systematic examination of any of the above factors could be conducted using the following:

1. Transcript level measurements: arrays, RNA-Seq, other targeted measurements.
2. CHIP-chip, CHIP-seq
3. Analytical assays:
 - a. HT: Mass spec based analysis (protein levels, protein complexes, PTMs)
 - b. MT: ligand-docking, transport,
 - c. LT: Mapping HK-RR phosphotransfer, RR-DNA gel shifts
 - d. LLT: Imaging for morphological changes or cellular localization of complexes.
4. Study of knockout or expression strain libraries (transposon, targeted, site specific). Corresponding phenotypic data and iterative (1), (2) and (3)

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5. Collecting and integrating the above data types into previous or initial regulatory network prediction (see Workflow 3: Inference of Gene Regulatory Networks).

HT: High throughput; **MT:** Medium throughput; **LT:** Low throughput; **LLT:** Low Low throughput; **PMT:** Post translational modifications.

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Workflow 5: Structural Biology

Summary

The nucleic acid sequence of a protein gene encodes an amino acid sequence that typically folds to generate a specific three-dimensional shape. This structure is often vital for the protein's function. In enzymes, the structure serves to keep key catalytic residues in a unique geometry, poised to act on substrate molecules. As such, the relationship between primary sequence and tertiary shape is central to our understanding of molecular biology. There is a wealth of information that is ripe for analysis in the context of the Knowledgebase. For example: the relationship between sequence and fold (for proteins and nucleic acids), the assembly of single molecules to form larger complexes, and the evolutionary relationships within and between protein families. Rapid progress can be made in providing functionality to researchers via the Knowledgebase. Initial workflows can focus on visualizing the linkage between sequence and structure (see first and second workflows below) and dissection and visualization of cellular compartments (see third workflow below). These will provide users with powerful tools to probe sequence/structure relationships, which otherwise are limited to experts.

Three structural biology workflows were submitted:

1. Locating and visualizing an enzyme active site
 - a. Goal: Assign, then visualize the amino acid residues in a protein sequence involved in enzymatic activity
2. Determine and visualize the oligomeric state of molecular complexes
 - a. Goal: Determine and then visualize the oligomeric state of a protein complex
3. Locating and visualizing a cellular compartment
 - a. Goal: Locate (segment) and visualize one or more cellular compartments in a microbe.

For each research goal, the Inputs, Analysis process, Outputs, Tools, and Knowledgebase context were provided.

1. Locating and visualizing an enzyme active site

Goal: To assign and then visualize the amino acid residues in a protein sequence involved in enzymatic activity.

Inputs

- Sequence of a protein
- High resolution protein structure (from X-ray crystallography or NMR) or high fidelity homology model
- One or more related sequences/structures with known active site residues

Process

- Perform alignment (most likely using multiple proteins) of sequence with unknown active site residues (may also include multiple family members) against known residues
- In cases of high sequence similarity, the active site residues in the unknown can be identified by sequence conservation
- In cases of remote similarity, more complex models (e.g. hidden Markov, sequence motifs, combined sequence/structure alignment) may need to be generated to infer the likely equivalent residues in the unknown
- Predictions of active site residues can be validated against any prior biochemical data and/or phylogenetic information

Outputs

- Protein sequence with active site residues highlighted
- Visual representation in standard molecular viewing software with active site residues highlighted

Tools required

- Parsing protein structure and sequence
- Single and multiple sequence alignment
- Combined sequence/structure alignment
- Sequence display
- 3D structure display

Knowledgebase context

- Provides linkage to and automatic retrieval of related structures in the Protein Data Bank
- Performs complex sequence and sequence/structure analysis without detailed user learning
- Cross validates against other experimental data within the Knowledgebase and in other outside resources
- Displays results in easy to understand visual forms and for download and subsequent analysis

2. Determining and visualizing the oligomeric state of molecular complex

Goal: To determine and then visualize the oligomeric state of a protein complex.

Inputs

- Sequence of a protein
- One or more related sequences/structures with known oligomeric state
- Optionally experimental data to define oligomeric state, such as small angle X-ray scattering (SAXS)
- Optionally high resolution protein structure (from X-ray crystallography or NMR) or homology model

Process

- Perform alignment (most likely using multiple proteins) of sequence with unknown oligomeric state (may also include multiple family members) against sequences of known state
- In cases of high sequence similarity, the likely oligomeric state can be identified from the nearest similar sequence
- In cases of remote similarity, more complex models (e.g. combined sequence/structure alignment) may need to be used to determine if structural features involved in oligomerization interfaces are likely to be conserved
- Predictions of oligomeric state can be validated against any prior experimental data (e.g. SAXS), biochemical data and/or phylogenetic information

Outputs

- Three-dimensional model of oligomer
- Protein sequence with residues involved in oligomerization highlighted
- Visual representation in standard molecular viewing software with interface residues highlighted

Tools required

- Parsing protein structure and sequence
- Single and multiple sequence alignment
- Combined sequence/structure alignment
- SAXS data analysis
 - o Calculation of standard distributions
 - o Comparison of distributions to those calculated from 3D models
 - o Searching of known structures for similar SAXS curves

- Protein structure writing
- Sequence display
- 3D structure display

Knowledgebase context

- Provides linkage to and automatic retrieval of related structures in the Protein Data Bank
- Performs complex sequence and sequence/structure analysis without detailed user learning
- Cross validates against other experimental data within the Knowledgebase and in other outside resources
- Displays results in easy to understand visual forms and for download and subsequent analysis

3. Locating and visualizing a cellular compartment

Goal: To locate (segment) and visualize one or more cellular compartments (e.g. mitochondria) in a microbe.

Inputs

- Three-dimensional reconstruction of one of microbes of interest (e.g. from EM-tomography or soft X-ray tomography)
- Characteristics describing the compartment of interest (e.g. shape, density, proximity to other features), or a human-generated training set
- Optionally a visual label identifying the compartment of interest

Process

- Read 3D data
- Perform pattern matching analysis to identify likely compartments on the basis of input data
- Segment volume data to assign the identity of compartments (note that for some data, it is possible to *a priori* segment on the basis of density, but the problem of identifying compartments still remains)
- Calculate statistics (e.g. volume of cell occupied by compartment, standard deviations between samples)
- Cross validate against any other relevant biochemical data

Outputs

- Statistics of compartments segmented
- Visual representation in volume rendering viewing software with compartments highlighted

Tools required

- Parsing large 3D volume datasets
- Pattern matching algorithms to identify compartments
- 3D volumetric data display

Knowledgebase context

- Performs segmentation analysis without detailed user learning
- Cross validates against other experimental data within the Knowledgebase and in other outside resources
- Displays results in easy to understand visual forms and for download and subsequent analysis

Workflow 6: Imaging Bioinformatics

Summary

One of the major advantages of phenotypic characterization through microscopy is the ability to visualize cellular organization, morphology and ultrastructure, and localization. More importantly, microscopic imaging allows cell-by-cell measurements, revealing a cellular heterogeneity that is often lost when using OMIC data only. For example, *Desulfovibrio vulgaris* (Dv) is known to form micro-colonies at certain stages of development due to cell-cell communication, a complex mechanism that remains largely unknown. Such population phenotypes can then be interrogated at multiple scales through multiplexed imaging probes to identify changes in structure, morphology, and localization on a cell-by-cell basis. These morphometric features can then be linked to omic data to query molecular predictors of a specific phenotypic subset. The main challenge in managing image-based data is identifying a quantitative view for each assay, which can be integrated with omic data. These quantitative views are often represented as vectors and relationships between vectors. Figure 1 is an example of a typical workflow in *Imaging Bioinformatics*.

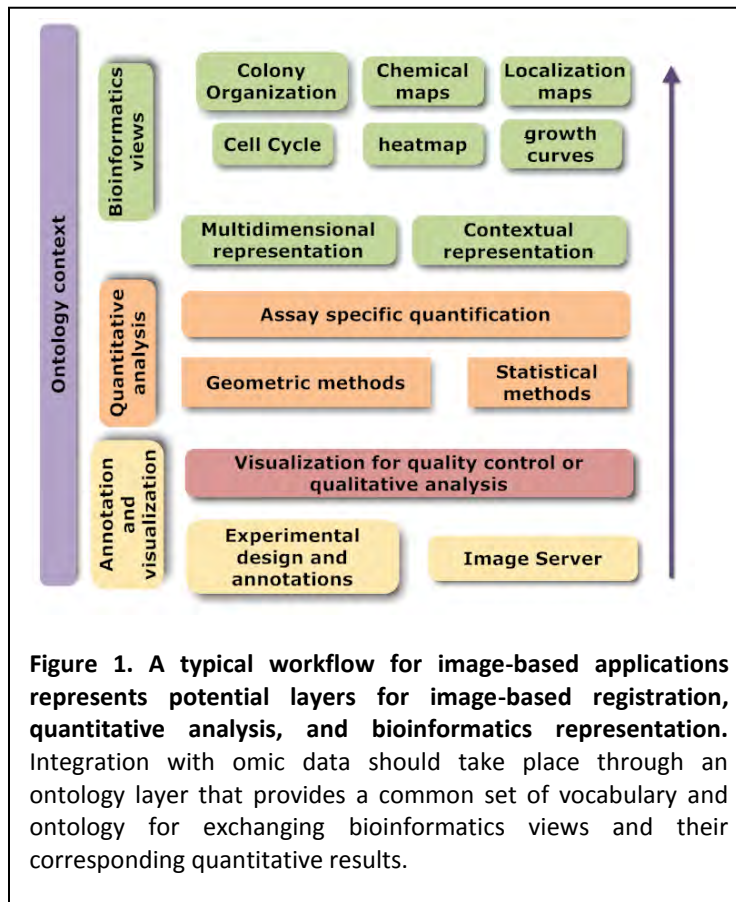


Figure 1. A typical workflow for image-based applications represents potential layers for image-based registration, quantitative analysis, and bioinformatics representation. Integration with omic data should take place through an ontology layer that provides a common set of vocabulary and ontology for exchanging bioinformatics views and their corresponding quantitative results.

Input Data

The input data consists of four types of information: (i) experimental design variables, (ii) imaging system parameters, (iii) raw image files, and (iv) queries used to target specific endpoints. (i) Experimental design refers to the model system, stress conditions, harvest time, imaging assay (e.g., labeling), etc. The main challenge has been reducing the number of user interactions needed to specify experimental design variables, since one rarely enters metadata at the granularity level that is often needed. There are no standards for capturing experimental variables; however, the microarray community has defined a complete protocol that can be leveraged. (ii) Most modern microscopes capture instrument setup information (e.g., optical path, illumination source) and store it as a header (e.g., in the form of a TIFF header) with raw data. Nevertheless, the Open Microscopy Environment (OME) has defined a schema for

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specifying instrument configurations, and some vendors plan to support the proposed schema. (iii) Raw data are usually stored in a binary form, and the format varies between different vendors. However, LOCI and OME developed a transformer that can read any image format, parse it, and store it in a five-dimensional format. The end result is a homogenized representation of a diverse file format. (iv) The endpoints or biological queries have to provide a series of templates for guiding quantitative analyses. One can design a taxonomy that allows users to select from multiple templates.

Quantitative Analysis

Analytical requirements for image-based data are quite heterogeneous, and any computational pipeline must be extensible for new application software programs. However, common computational modules can be defined, integrated, and enhanced for a specific application. Nevertheless, there has to be a balance between excessive generalization versus specificity, as too much generalization adds to the complexity of a system and thus increases the learning curve required to use it efficiently. In general, image-based data analysis needs to incorporate a model to recover objects of interest in a robust fashion. Such a model can be expressed either geometrically or statistically. In some cases, model-free methods can be used, at a low level, to aggregate rich tokens for higher-level analysis. Once the images have been quantified, information can be composed and aggregated to form bioinformatics views (see “Output Data” below for more information). With respect to image analysis, the ITK image library provides a rich set of software and an extensible framework for adding new applications. However, it requires expertise in advanced software engineering, which may not be readily available at every institution.

Output Data

One of the characteristics of image-based assays is that a large number of data are often transformed into a very small amount of data. This is referred to as “bioinformatics views,” which are often constructed by downloading computed information, and then processed further by using one of many statistical or data analysis stand-alone software packages. However, it is possible to integrate some basic capabilities into the bioinformatics platform. Examples include a dose-response curve, a growth curve, and co-localization frequencies. One of the advantages of imaging is that it maps cellular localization (or co-localization), chemical composition, and morphometric properties.

Current State of the Art

BioSig (ribo.lbl.gov:8080/biosig/home.do) is an example of an imaging bioinformatics system, which is being used for mammalian systems. BioSig builds on OME for image harmonization, leverages MIAMI (www.mged.org) standards for specifying experimental design variables, and has defined a number of tagged templates for assay-specific quantitative analysis. It also supports a schema for multidimensional profiling of cell-based assays for high-content screening, as shown in Figure 2.

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The Next Generation

Current imaging bioinformatics platforms lack (i) an ontology and controlled vocabulary for microorganisms of interest to DOE, (ii) an integrated pipeline for bioinformatics and image analysis, (iii) an interface for integrating omic data, and (iv) the necessary analysis tools for mapping at multiple scales of different imaging modalities. The latter is quite important since it enables chemical mapping (e.g., Raman microscopy), localization mapping (e.g., electron or optical microscopy), and mass spectrometry imaging (e.g., MALDI imaging). Furthermore, having created these maps at multiple scales, one is also interested in correlative analysis between these imaging modalities for the model systems of interest under specific environment conditions. A potential correlative query would be how the chemical composition of the plant cell wall, visualized and quantified with Raman, is altered as a result of increase in biomass that is imaged with electron microscopy. In short, the next generation of breakthroughs in quantitative image analysis and imaging bioinformatics resides at the interface of different imaging modalities, and their integration with omic data.

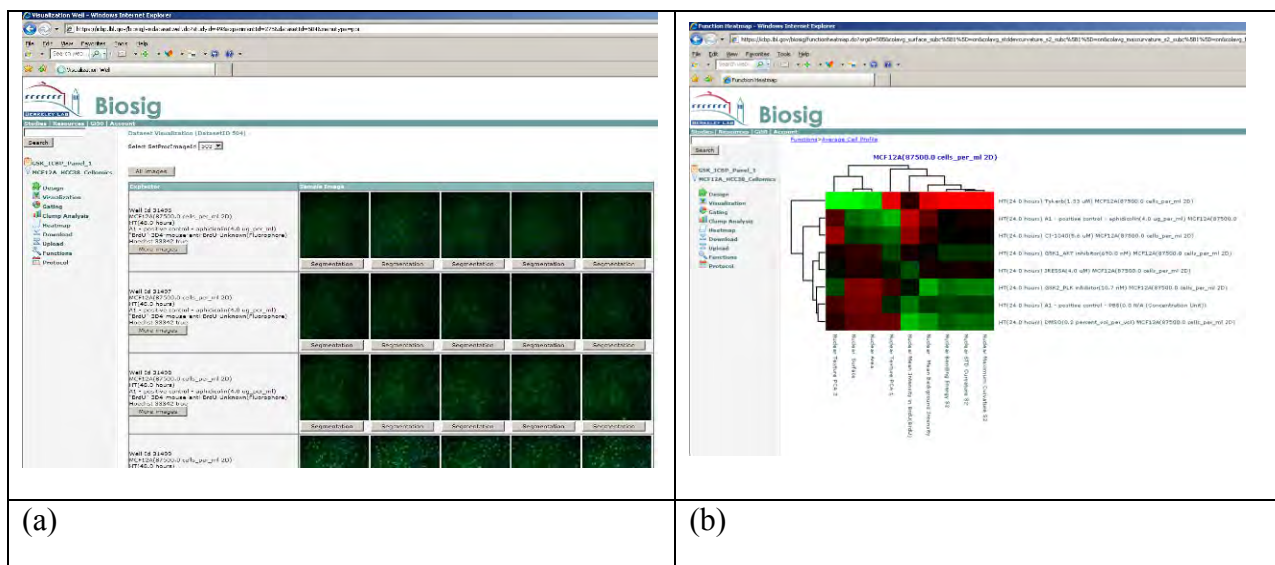


Figure 2. Imaging bioinformatics views. (a) Thumbnail visualization enables comparison of biological replicates (columns) for each set of experimental variables (rows). Each thumbnail is a hyperlink to a full-resolution version, where quantitative results can be overlaid on top of it. (b) Images in (a) are processed, and each cell is represented by multidimensional features. The user can select a subset of computed features, put them in a particular order, and view them through a heatmap. As a result, multiple phenotypic representations can be viewed simultaneously and compared in the context of experimental variables.

Section III: Strawman Knowledgebase Architecture

The preliminary diagram below was developed as a result of discussions held in conjunction with this workshop. Though this schematic will be refined in upcoming discussions, it is included in this report to indicate how the workflows (research protocols) relate to the ultimate system architecture. The workflows being developed by experimentalists to satisfy scientific objectives are critical to the development of many Knowledgebase architecture layers, such as data repositories (red), computing workflow management, and output visualization design.

The workflows provide information on data sources and types that must be accommodated by the Knowledgebase architecture. In-depth discussions will result in refinement of the workflows by the research and computing communities.

Schematic Diagram of Knowledgebase Architectural Components

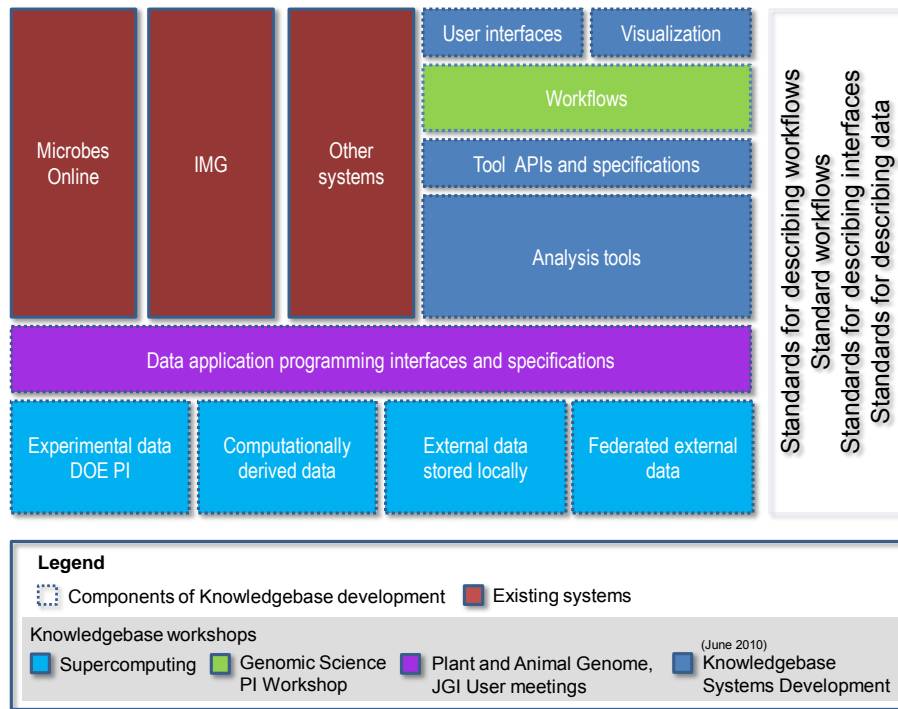


Fig. 3.1. Example Schematic of Knowledgebase Architectural Components.

Data

The data layer represented in the bottom of Fig. 2 illustrates several data components that will be important to achieving the goals of the Knowledgebase project. These data components will utilize several technologies. Relational database technology such as Oracle or MySQL will be used to manage data that is well structured and suited to relational technologies. Examples include the storage of account information, user configurations, and certain data and tool-related metadata. More recently developed technologies for representing data, such as Semantic Web, will be used for biological data with complex data model characteristics.

An important component of the data layer is data available from other sites that are remotely accessed and used singularly or in a federated manner. Federating external data sources will make heavy use of web services technologies. Web services are newer technologies allowing interoperability between software systems located at distinct sites. Federation will allow us to leave stable data at remote sites (i.e., NCBI Taxonomy) when a façade (wrapper, adaptor, bridge, etc.) can be constructed around the access routines provided by the remote site. The façade will serve to standardize access to data provided by multiple, distinct remote data sources.

Experimental data derived from DOE-funded work that is not available in other data sources in a suitable format will be structured and shared appropriately as part of the data layer. This data generally is thought of as the results of experiments funded by DOE. The data should not be limited to DOE-funded work; if others outside DOE wish to contribute, all the better.

The data layer also will contain data that exists remotely but is aggregated locally. Local aggregation can enhance data usefulness by putting the data in a modified format that corrects for missing metadata, incompatible formatting, or because internal computation integrates additional data. Pathway data, genome data, transcriptome data, and regulatory network data all stored in a suitable form for mash-ups are examples of data that likely will be found in the data layer component that represents locally aggregated external data. Another example of why external data is aggregated locally is because there will be external published data of use and specific data derived from DOE-funded work that is not available in the public domain. Other examples can be driven simply by the fact that computations such as similarity searching require local data sources for performance reasons.

Computationally derived data should represent another component of the data layer. Computations often can produce entirely new datasets rather than just adding value to existing ones. These computations may operate on existing datasets but generally produce a new type of data. For example, a computation on RNA sequencing–based gene expression data might produce a histogram of coverage statistics. This histogram is a new data type linked to the RNA sequencing data through descriptive metadata technology.

Analysis

The analysis component of the architecture will allow for development of both libraries and interfaces that promote the integration of analytical tools into the recognizable Knowledgebase. This component also will provide the facilities needed by the community to develop new algorithms and applications enabled by Knowledgebase infrastructure and data

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access layers. Goals of the Knowledgebase project are to build and promote an environment where analysis tools are primarily derived from open contributions.

In direct support of the data layer, semantic-enabled search algorithms will allow metadata—information about the data stored in the data layer—to be more than just an attached publication or protocol list. Metadata is extremely valuable when making new scientific discoveries. Representing, integrating, searching, and performing logic on metadata can be challenging enough when the object being measured is sequence or crystal structure. Complex and conditional data derived from functional measurement of molecules, cells, and communities will make this an exceptional challenge. The rapid development of new technologies for making such measurements further increases the need to track the key information about experimental and analytical protocols for producing data and the processing applied to data before it is stored in accessible formats. A key goal for this effort will be developing tools that attach such information to data as easily as possible, identify the most important pieces of this data for scientific purposes and searching, capture experimental design and goals, and allow queries of this information.

Existing and New Systems and Projects not Developed by the Knowledgebase

Existing systems such as MicrobesOnline, the collection of IMG systems, the RAST systems, and others are expected to continue and benefit from the centralized or virtually centralized (federated) data stores and from direct programmatic access to the open methods developed as part of the Knowledgebase. We also anticipate that new systems will emerge.

It is expected that existing system developers can and will create application programming interfaces (APIs) to their systems and publish the specifications of those interfaces as part of Knowledgebase API specifications. These API specifications are analogous to the Sun Java Docs for the Java APIs. These interfaces may be used by other existing system developers or by the Knowledgebase development community.

As new systems emerge, embracing and nurturing them will be important. Guiding such projects so that they become important components of the Knowledgebase also will be necessary.

Workflows

Scientific workflows can help scientists, analysts, and computer programmers create, execute, and share experimental and analytical processes. These workflows can be captured as free text use cases or more formally represented using workflow languages. Regardless of whether a workflow is captured in a structured or unstructured manner, an important part of the Knowledgebase system architecture will be a graphical user interface that is available to the community so that anyone can access existing workflows and develop new ones.

User Interactions

The user experience will primarily take place through what is known as a horizontal web portal. These portals deliver an integrated front end to what is commonly thought of as several independent websites that allow users to easily search, visualize, and run analytical software on Knowledgebase information. Standard browsers, plugins, and web portal technology will enhance the user experience when command line or other existing user interfaces are not

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suitable. This will allow members of the research community to have a customizable entrance to the Knowledgebase.

User interactions can be thought of in two parts—user interfaces and visualization—because these two components use markedly different technologies. For an effective Knowledgebase user experience, we will need to focus on both the user interfaces and the more challenging aspects of scientific data visualization.

Relying heavily on web technologies such as HTML, Javascript, and their derivatives, user interfaces allow a user to navigate the system, configure analysis environments, input data into the system, and retrieve results from it. These user interfaces can leverage the latest advancements in social networking to provide tools to the community for shared annotation and quality assessment and provide forums that easily reference Knowledgebase information.

Visualization (referred to as scientific visualization in some communities) relies heavily on graphics packages. The goal is to present data in a form that is useful for scientific discovery. There may be no interaction required when a visual representation of data is generated and presented.

Standards

Standards will be instrumental in achieving many aspects of the Knowledgebase project. These aspects range from scientific to engineering. From a scientific perspective, describing biological data and the relationships between data in a standardized way is critical to advancing our ability to interpret it. In relation to engineering, standards will enable healthy, continued evolution and growth of the system.

Several objectives will provide efficient and necessary utilization of standards. These objectives include embracing community standards when they are adequate, engaging in the community-development process of a particular standard when there is an existing standard that might be considered inadequate in its current form, and helping the community by initiating standards development where gaps exist.

Although standards for describing data and workflows will be critical, other types of standards will be important as well. Having community standards for data sharing is just one example of what will have to be supported in the Knowledgebase project. Another such example is developing standard workflows and benchmarking data that can be used by the community to facilitate a higher level of exchange among scientists.

In support of an open environment, standards for describing analytical tools, software libraries, data schemas, and other technical artifacts used to build the Knowledgebase will be essential for broad acceptance and use. Software tools and libraries implemented in the Java programming language benefit from a Java community–accepted standard on how to describe APIs. Requiring the use of these standards in code libraries will result in a solid documentation base that is needed for general acceptance and further use of the library by the community. Other programming languages such as Perl have similar standards.

Section IV: Workshop Summary and Conclusions

Workshop participants discussed the need for some level of individual research privacy, which could be achieved with user accounts. Data and code could be held in private, and analyses conducted in a nonpublic environment. The Knowledgebase also will need to allow users to track version history and provenance so that new analyses can be usefully compared with previous ones. Other important capabilities workshop participants discussed include:

- Curation not only of data, but also of models and representation of scientific concepts
- Comparison and analysis of methods and results over time
- Simulation, including the ability to modify and improve models
- Predictions based on simulation and analysis to form new hypotheses
- Comparison of predictions and results to guide experimental design

Only a few researchers today have comprehensive access to such computational capabilities, yet these tools are necessary to conduct research that will lead to important scientific innovations in energy and environment.

Also envisioned for the Knowledgebase are high standards for usability, understandability, discovery, and contribution. System design should be intuitive so that researchers can use it with minimal training. Knowledgebase components also need to be understandable. Although able to use a given software package, many people often do not understand the process by which the software derived its results (e.g., BLAST). Understandability implies that there is a good foundational basis for knowing that results returned to a user are based on robust scientific knowledge or assumptions. If results are not understandable, system features should allow the user to drill down to acquire information about how results were obtained. The Knowledgebase also should promote an environment of discovery, leading to new rounds of experiments or lines of research. Finally, engaging the entire research community in Knowledgebase contribution is critical. Any system being used by scientists ultimately should be measured on how well it accomplishes these concepts, advances research, and supports the scientific method.

Future Considerations for Workflow Definitions. Here we see a range of styles and level of content in the workflows. For the future final report of the Knowledgebase R&D Project, we will need to settle on a style. The Structural Biology workflow is very terse when compared with the others, but it is also very clear. In developing a standard for future workflows, this should be considered. An important question to raise: Do these workflows provide sufficient detail to allow requirements to be established that can drive the Knowledgebase Implementation Plan, and if not, how much more detail is needed?

Appendix 1: Agenda

DOE Genomic Science Microbial Systems Biology Knowledgebase Workshop

Crystal City, Virginia

Tuesday, February 9, and Wednesday, February 10, 2010

February 9

- 2:00 – 2:30 p.m. Robert Cottingham, Oak Ridge National Laboratory
“Microbial Systems Biology Knowledgebase: Scientific Objectives and Current Prospects”
- Focus on examples of scientific objectives, benefits, and outcomes*
- 2:30 – 3:00 p.m. Discussion
- 3:00 – 3:30 p.m. Robert Kelly, North Carolina State University
“Near-Term Prospects for Functional Microbial Genomics: Moving Beyond the Monoculture Paradigm”
- One organism to two organisms, adding complexity*
- 3:30 – 4:00 p.m. Discussion
- 4:00 – 4:30 p.m. Adam Arkin, Lawrence Berkeley National Laboratory
“From Pathways to Populations and Back Again: Long-Term Prospects for the Microbial Systems Biology Knowledgebase”
- Much larger complexity of systems, data, models, and impacts*
- 4:30 – 5:00 p.m. Discussion
- 5:30 p.m. Adjourn

February 10

- 1:00 – 3:00 p.m. Impromptu follow-up session focusing on workflows

Appendix 2: Participants and Observers

Adam Arkin (LBNL)	Kristen Munch (NREL)
Nitin Baliga (Institute for Systems Biology)	Ambarish Nag (NREL)
Jill Banfield (University of California, Berkeley)	Chongle Pan (ORNL)
Chris Bare (Institute for Systems Biology)	Nicolai Panikov (Northeastern University)
Ben Bowen (LBNL)	Morey Parang (ORNL)
Tom Brettin (ORNL)	Charles Parker (Names for Life, LLC)
William Cannon (PNNL)	Bahram Parvin (University of California)
James Cole (Michigan State University)	Amanda Petrus (University of Connecticut)
Robert Cottingham (ORNL)	Madeleine Pincu (University of California, Irvine)
Brian Davison (ORNL)	David Pletcher (JBEI/LBNL)
Mitch Doktycz (ORNL)	Iris Porat (ORNL)
Ronan Fleming (University of Iceland)	David Reiss (Institute for Systems Biology)
Cheri Foust (ORNL)	Dmitry Rodionov (Burnham Institute)
Hector Garcia Martin (LBNL/JBEI)	Blake Simmons (LBNL)
George Garrity (Michigan State University)	Steve Singer (LBNL)
Adam Godzik (Burnham Institute)	Marvin Stodolsky (DOE)
Susan Gregurick (DOE)	Ines Thiele (University of Iceland)
Loren Hauser (ORNL)	Judy Wall (University of Missouri)
Alyssa Henning (Cornell University)	Sharlene Weatherwax (DOE)
Kimberly Keller (University of Missouri)	Steven Wiley (PNNL)
Robert Kelly (North Carolina State University)	Jian Yin (PNNL)
Julia Krushkal (University of Tennessee, Memphis)	
Libbie Linton (Utah State University)	
Yukari Maezato (University of Nebraska)	
Betty Mansfield (ORNL)	
Victor Markowitz (LBNL)	
Lee Ann McCue (PNNL)	
Folker Meyer (ANL)	
Jonathan Millen (University of Rochester)	
Aindrila Mukhopadhyay (LBNL)	

Acronyms

ANL	Argonne National Laboratory
DOE	U.S. Department of Energy
JBEI	Joint BioEnergy Institute
LBNL	Lawrence Berkeley National Laboratory
NREL	National Renewable Energy Laboratory
ORNL	Oak Ridge National Laboratory
PNNL	Pacific Northwest National Laboratory

DOE Systems Biology Knowledgebase Workshop Report from the 5th Annual JGI User Meeting

Tuesday, March 23, 2010, 8:30 a.m. – 5:00 p.m.

Convened by

The U.S. Department of Energy Office of Science as part of the DOE Joint Genome Institute's (JGI) Genomics of Energy and Environment 5th Annual User Meeting, Walnut Creek, California

Workshop Organizers: Susan Gregurick (DOE) and Bob Cottingham (Oak Ridge National Laboratory)

Workshop Cochairs: Victor Markowitz (JGI, Lawrence Berkeley National Laboratory) and Jill Banfield (University of California–Berkeley)

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[Appendix 1: Agenda](#)

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Introduction

This report covers discussion and material from the Department of Energy (DOE) Systems Biology Knowledgebase workshop held on March 23, 2010, prior to the 5th Annual DOE Joint Genome Institute (JGI) User meeting. The focus of this knowledgebase workshop was to discuss scientific objectives and challenges for data handling and knowledge integration specific to the study of microbial communities or metagenomes. The topics also included some discussions and items pertinent to all development and initial implementation of knowledgebases for the broader biological community.

A brief table of contents for this report is provided above. First, there is a background summary of the purpose of the DOE Systems Biology Knowledgebase planning project. Next is a summary of several topics presented and discussed during the workshop. Many of these topics require more discourse than could be fully covered during the meeting itself. Several groups and individuals were assigned to elaborate on these topics for inclusion within the report. These expanded topics are in the next section but directly refer to topics in the preceding section. For example, in the discussion of science objectives, having illustrative examples of workflows for the study of microbial communities was desired. Finally, there are appendices containing the participants list and agenda.

Prior to the workshop, participants were asked to consider the following **charge questions**:

1. What are key experimental and computational next steps that build on the sequencing data and information provided by JGI and that are feasible for an initial Knowledgebase implementation associated with research in microbial communities?
2. What types of data and information are currently available or required to accomplish these objectives?
3. How are these research goals hindered by an inability to access and integrate data from various sources or of other types?
4. What are the bottlenecks in bioinformatics and computational algorithms that need to be addressed to accomplish these goals? Specifically, is there a benefit to closer collaboration between sequencing analysis and downstream analysis?

As part of the Knowledgebase planning project, DOE is sponsoring a series of community workshops to establish the requirements for the Knowledgebase and to outline a plan for implementing them. Previous meetings include the following, and the output from each is available online at www.systemsbiologyknowledgebase.org/workshops.

1. **Knowledgebase workshop at the Supercomputing conference** in Portland, Oregon (November 2009). Explored the potential for applying the cloud computing approach to systems biology research.
2. **Joint USDA-DOE Plant Genomics Knowledgebase workshop** at the Plant and Animal Genome meeting (January 2010). Addressed the Knowledgebase requirements necessary for developing data capabilities for plants.

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DOE Systems Biology Knowledgebase Workshop Report from the 5th Annual JGI User Meeting, March 23, 2010

3. **DOE Genomic Science Microbial Systems Biology Knowledgebase workshop** at the DOE Genomic Science Contractor-Grantee (PI) meeting in Crystal City, Virginia (February 2010). Outlined workflows and data integration methods pertaining to microbial sciences that can inform Knowledgebase specifications and requirements.

Since the goal of the Knowledgebase planning project is to develop an initial prioritized plan for a useful systems biology knowledgebase, there is a continued consensus that these initial efforts cannot be all things for all users. It is better to show strong success in a few areas than minimal progress in many areas. That this needs to move forward is also reflected in the standards discussion below. Having too broad an approach has stymied and slowed past efforts.

2. Background

The Department of Energy Genomic Science program, within the Office of Biological and Environmental Research (BER), supports science that seeks to achieve a predictive understanding of biological systems. By revealing the genetic blueprint and fundamental principles that control plant and microbial systems relevant to DOE missions, the Genomic Science program (genomicscience.energy.gov/) is providing the foundational knowledge that underlies biological approaches to producing biofuels, sequestering carbon in terrestrial ecosystems, and cleaning up contaminated environments.

Knowledgebase Vision and Background

The emergence of systems biology as a research paradigm and approach for DOE missions has resulted in dramatic increases in data flow from a new generation of genomics-based technologies. To manage and effectively use this ever-increasing volume and diversity of data, the Genomic Science program is developing the DOE Systems Biology Knowledgebase—an open, community-driven cyberinfrastructure for sharing and integrating data, analytical software, and computational modeling tools. Historically, most bioinformatics efforts have been developed in isolation by people working on individual projects, resulting in isolated databases and methods. An integrated, community-oriented informatics resource, such as the Knowledgebase, would provide a broader and more powerful tool for conducting systems biology research relevant to BER's complex, multidisciplinary challenges in energy and environment. It also would be easily and widely applicable to all systems biology research.

In general, a knowledgebase is an organized collection of data, organizational methods, standards, analysis tools, and interfaces representing a body of knowledge. For the DOE Systems Biology Knowledgebase, these interoperable components would be contributed and integrated into the system over time, resulting in an increasingly advanced and comprehensive resource. Other elements of the Knowledgebase vision are defined in a March 2009 report (genomicscience.energy.gov/compbio/) based on a DOE workshop that brought together researchers with many different areas of expertise, ranging from environmental science to bioenergy. The report highlights several roles the Knowledgebase will need to serve.

3. Topics Discussed at the Workshop

This section attempts to briefly summarize the wide-ranging discussion during the meeting. Where there appeared to be a general consensus, this is indicated. The level of discussion detail was not the same for all topics, and thus the level of detail in this report is uneven. Many of the topics were assigned to participants to develop further details after the workshop for inclusion in this report. Discussion of science objectives and the resulting workflows (Section 3a and related Sections 4a and 4b) was the primary focus of the meeting.

3a. Proposed Science Objectives for Microbial Community Analyses

The earlier 2009 report summarized needs and visions for knowledgebases. Here we are challenged to define precise science objectives: What do we want to accomplish in the science now? These prioritized science objectives will be a mix of priorities for importance and for current feasibility. Most of these objectives will require the exchange of data and insights (i.e., knowledge). To drive this interoperability, the Knowledgebase must have challenge problems that require cooperation and integration. A number of science objectives were described and discussed at the workshop. More potential objectives were gathered from online input to the charge questions. It will be obvious that there are common themes within objectives articulated in the report and in the earlier workshops. However, there are some unique aspects with respect to metaomics, or microbial community studies.

Some of these unique aspects with respect to microbial community studies are:

- Massive amounts of data. There will be terabytes of data resulting from genomic sequencing and increasingly from other techniques.
- Datasets that never “close.” Unlike a genome for a microbial isolate, one can never finish—more data will just provide deeper details and resolution without reaching an inherent endpoint.
- Experimental protocols will continue to develop and rapidly change. An example is the increased application and development of RNA sequencing technologies.
- All studies are studies of populations. Even a species within a natural community must be considered ultimately as a population of genetic individuals that will change and evolve.
- Natural communities are closely linked to their environmental context. Unlike a laboratory study, this environment will not be controlled and must be observed. Despite the best available knowledge to capture the most important measurements, these observations will be incomplete. This provides a serious metadata challenge.
 - Note: Metadata is the associated data and information that provides context for the primary dataset. For example, a microbial community is analyzed for its metagenome by 16SRNA (the genomic sequences are the primary dataset). The metadata would be, for example, the location, time, environmental conditions, method of genomic isolation, 16SRNA.

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DOE Systems Biology Knowledgebase Workshop Report from the 5th Annual JGI User Meeting, March 23, 2010

Four science objectives for initial study of microbial communities were proposed and discussed during the workshop. These were broadly affirmed as valuable by workshop participants. However, these and the expanded list were not prioritized during this meeting. The prioritization of these and other objectives will be a primary goal of the final Knowledgebase workshop in June 2010. The objectives discussed were:

- Metagenome analysis workflows
- Genome-based prediction of culture conditions
- Linkage and feedback from transcriptomic and proteomic data to gene calls
- Expanding metabolic pathways from metabolomic data and linking to other datasets

Metagenomic analysis workflows were seen as important in both this workshop and the one held in conjunction with the Genomic Science PI meeting. This workflow discussion has been given its own section below (Section 4a). One example of this challenge problem is that the first phase in analyzing a metagenome is done at one site, export to the binning into analysis of organisms at another site, exporting for pathways analysis at another site, followed by regulatory analysis at another site. This would drive interoperability and connections between the different groups, resulting in great science. More participants liked this collaborative model, but some preferred an approach where analysis tool needs are identified *a priori*, the tools are developed and distributed via the Knowledgebase, data is analyzed using those tools, and feedback is provided to the developers.

The need to develop expanded workflows relevant to the science community studying the microbial communities was recognized at the PI meeting and at this meeting. A small group was assigned to work offline on describing such workflows—both present and needed. Their effort is almost a stand-alone report and is presented in Section 4b and briefly summarized below. *The recommendations from this sub-report should be expanded upon to create a more detailed initial guidance in the final workshop.*

From the perspective of the metagenomics community, the DOE Systems Biology Knowledgebase will need to fulfill a range of requirements to achieve the research community's envisaged goals. These include:

- Providing a common mechanism for collecting, organizing, annotating, analyzing, and distributing data that enables easy data sharing and comparative analyses.
- Facilitating **dynamic** interconnection of data types, data sources, applications, and workflows to allow **data integration** for biological insight.
- Enabling researchers to identify, assess, and access all relevant **datasets** worldwide.
- Allowing scientists and facilities to “publish” their data, applications, and workflows into the “live data network.”
- Providing space for larger-scale data integration, analysis, and publishing.

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- Providing scientifically accepted rewards for researchers who “publish” well-annotated, good quality data, applications, and workflows.

We suggest that this could be achieved through the development of:

- A set of community-accepted semantic description formats (ontologies)
- A peer-to-peer based system of data, metadata, ontology, analysis tools, and workflow registration repositories that are integrated in discovery, access, and utilization through common semantics.
- Guidelines and software libraries that allow scientists and facilities to “publish” their data, applications, and workflows into the Knowledgebase in a set of agreed forms.
- A mechanism that allows scientists and facilities to easily and rapidly annotate, change, and correct research results and annotations in the Knowledgebase, capturing source, reason, quality, and proof for changes.
- User-friendly interfaces (APIs and people) to access data and application modules, **as well as** derived data products, enabling other users to build novel solutions with the data.
- A framework of citable, unique identifiers for data, applications, workflows, and researchers.
- Guidelines, training, and workshops for all new products and concepts provided by the Knowledgebase.

Genome-based prediction of culture conditions. Here the challenge is: Using a partial single microbial genome found within microbial communities, can we predict how to cultivate (and isolate) this target species? Put another way, can we predict culture conditions from genomic information? This Knowledgebase tool will be very valuable in rapidly culturing currently “unculturable” isolates from microbial communities. This would expand the study of difficult-to-culture or new microbes with interesting properties. This could lead to better integration or new experiments where one could envision testing 500 isolates a day to achieve a goal of studying newly discovered organisms with unique properties faster and cheaper.

For example, if the genome identifies heterotrophic metabolism features, will this organism grow on lactate? Is it an auxotroph, or will it require some amino acid supplement? This tool would tell you what experiments are necessary to test the proposed metabolism hypotheses. Further development of this concept would be needed including: What aspects of this tool could be automated? After the success or failure of the initial experimental cultivation tests, what information should come back to you? How do you incorporate knockout data, and can you predict the effects of knockouts? This becomes a capability tools and challenge for both the informatics and experimental communities.

The prediction of culture condition is the initial goal, but this scientific objective can be seen as the first step to a broader scientific goal in the area of genome-based functional prediction. This high-level goal would move knowledge from genetic information (which is more and more easily available compared to other data) into molecular or protein function, then to organismal function, and on to community function. It is complexity across scales. These studies are a prerequisite for investigating the function of both microbes and microbial communities. At a higher level, this would also provide potential data to feed back into improved annotation and validation. However, as stated in many other objectives, the consensus among workshop participants was that this initial effort will move most rapidly if used to address a specific problem. Each of these objectives would require detailed workflows to be developed.

Linkage and feedback from transcriptomic and proteomic data to gene calls. This scientific objective is a subset of the broader need to improve gene calls or annotation. The higher-level needs to move annotation beyond simple homology inferences were well described throughout the 2009 Knowledgebase report. The challenge here is using the massive amounts of data from transcriptomic and proteomic measurements to improve gene calls. This data is already used in the most straightforward manner—to promote gene calls from hypothetical to putative when a transcript or protein signature is observed. However, even this use does not often extend beyond the specific metagenome or genome under study. We need to find ways to draw further functional confirmations to improve gene calls, to invalidate and correct false calls, and to provide better descriptions for use in further homology searches.

With the rapid improvement of techniques such as RNA sequencing, it is clear that transcriptomic data for metagenomic communities soon will not be limited by the current requirement for an *a priori*—determined metagenome for that community. This will also enable better proteomic data analysis. This will require improved cluster analysis and the inference of pathways and function. Localization data from parts of the community (such as using laser dissection to gather small samples) will be needed to create estimates of community structure and function.

Expanding metabolic pathways from metabolomic data and linking to other datasets. There is a clear, if sometimes difficult, path from genomic to transcriptomic and proteomic datasets. Each is linked by the underlying gene. There is a different challenge in taking metabolomic data and validating and expanding metabolic pathways, as well as linking these pathways to the proteins and regulation. Since metabolites are pathway oriented, not genome oriented, the challenges of metabolomics will be largely similar, whether dealing with single microbes, communities, or plants. A related issue and challenge is extending metabolite concentration data into flux estimates. Due to tightly controlled multistep pathways, key intermediates can be present at very small levels, while the flux through that intermediate is large. With metabolomics, thousands of metabolites might be detected. However, there may be no final answer, and the dynamic range issue can confound the depth of analysis (concentrations can range from mM to single molecules). On the positive side, while there are thousands of metabolites potentially present, most experimental research targets, particular processes, or pathways (with the identification and quantification of tens of metabolites) are all that is

needed. Still most metabolomic techniques are untargeted (i.e., they try to measure everything).

Challenges here include the positive identification of detected metabolites. For example, in the synthesis of lignocellulosic biomass, there are many similar compounds such as sugar isomers. The gold standard is the purification of synthesis of a compound for use as a standard for identification. As identification libraries continue to expand, do we need to save raw data to allow later identification of metabolites from saved data?

Another challenge is to link confirmed metabolites with the measured proteins that catalyze that reaction. (Note that this requires the correct functional identification of the protein.)

Clustering, visualization, and other tools are needed to extract insights from metabolomic data. We need to have these both for microbes and for observing the change of function within a community. This is needed to determine how the rest of the microbial community environment influences the pathways of member organisms and how they utilize their genetic potential. These tools should also highlight apparent “gaps” in pathways where either metabolites or enzymes do not appear to be present. This can help identify needed experiments to fill in important pathways.

Other potential science objectives have been proposed from several other sources. Workshop participants were reminded to return to the broad objectives in the 2009 Knowledgebase roadmap. There were also a number of potential science objectives suggested in response to the charge questions and posted by participants on the Knowledgebase wiki site (www.systemsbiologyknowledgebase.org). We are continuing to extract these objectives and will place them in the final report in Section 4f.

3b: Standards: The Role of Standards-Setting in the Knowledgebase

Standards to expedite data and file sharing are important. Gene sequence data is relatively established as a standard. mRNA expression (MIAME) and other standards are being developed. However, participants had a range of opinions on the priority of standards (i.e., when do we focus on the standards?). Historically, standards development committees by community consensus have taken a very long time, and there is a need for this effort to move faster. Part of this long duration is driven by the desire to make the standards do all things for all people and uses. For example, required metadata lists quickly become wish lists of all possible information. There have also been “dictatorial” attempts at setting standards. These can lead to frustration as they are outgrown, such as in the file formats used for annotation for the last decade. Nevertheless, at a minimum, there was agreement in the need to have some standards for file-sharing formats to expedite transfer (I/O protocols). On the other side, there is the sense that if we do the needed work, the standards will sort themselves out. If the data exists, and there is a need to share, “someone” will create a protocol for sharing, which in effect is a small *de facto* standard. The challenge here is that this leads to duplication and balkanized tools. Within the context of this workshop, the range of consensus was narrower after the discussion. *Standards are important, but standards-setting is not the first task or top priority of building a*

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Knowledgebase community. This workshop, the developed workflows and the final workshop report need to focus on science needs and what the initial Knowledgebase version 1 will do. If some standard setting is required as part of this implementation, it can be addressed at that time. There was an agreement that this group not be distracted into spending time in the actual standards discussions. Beyond the need for I/O, it was not clear that major effort was required in standards-setting in the first year or two. Broadly, the first two years of the Knowledgebase should focus on implementation data and tools to enable specific science.

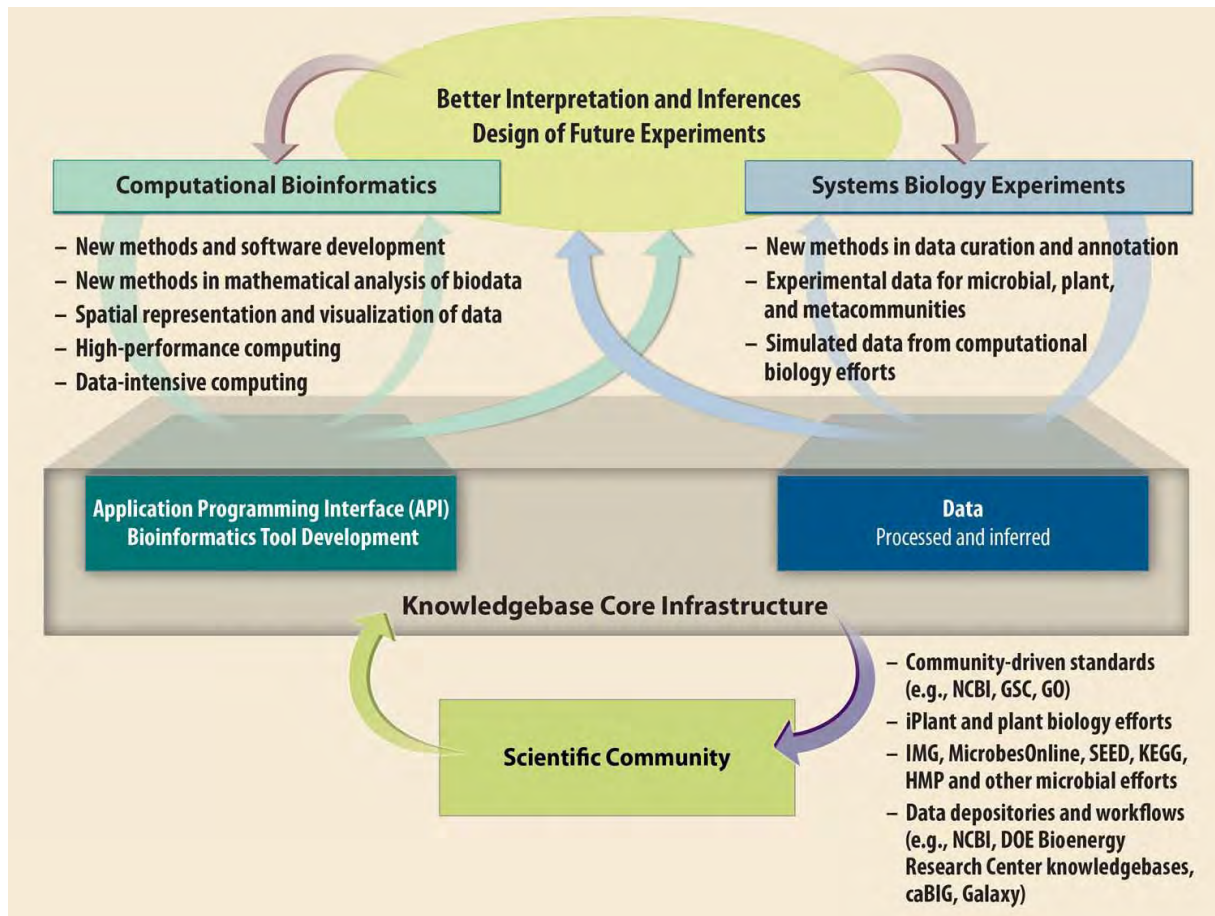
This I/O-focused approach is re-emphasized below in the API interface discussions and workflows. There is a minimalist view that standards are actually formalized file formats, but the discussions of required metadata move beyond that interpretation.

3c. Tool Builders and Data Generators: The Need to Engage the Various Scientific Communities

Another consensus was on the goal of knowledgebases. The Knowledgebase will enable better understanding and interpretation by the “experimental” biologist and will enable testing and development of new analysis tools by the computational biologist. This reaffirms the goals stated in the 2009 report and showcases two critical science communities essential for the Knowledgebase: (1) the computational biologist or bioinformaticians who build the tools and (2) the systems biology data generators who design and run the experiments and usually provide initial interpretations. Both need to provide insights and inferred knowledge to each other through the Knowledgebase then out to the broader scientific community. This concept is presented at a high level in the Fig. 1. A challenge for both groups is the need for confidence versus just information. This was well articulated as: “I’d rather have less data but be more confident that the data is “real. I’d rather see less data with higher quality.” This data would be used to create processed interpretations, like the calling of a gene. This is a challenge in assessing quality and confidence in the sea of data. For example, it is hard to assess and utilize negative experimental data because publications release only what worked. Elsewhere, frustration was expressed at the loss of underlying information when the data is processed. For example, more information goes into the calling of a gene than is saved in BLAST (i.e., intermediate analysis is lost). Also, the identification of a protein from three peptide fragment hits will lose the possible post-translational modification data hidden in an MS spectra from a “missing” peptide fragment. The combination and cross-correlation of multiple datasets from different sources into a synthesizing computational analysis struggle with different qualities of data and unreported conditions. An example recent work shows that errors in genome annotation are propagating.¹

¹ Schnoes, A. M., S. D. Brown, I. Dodevski, and P. C. Babbitt. 2009. “Annotation Error in Public Databases: Misannotation of Molecular Function in Enzyme Superfamilies,” *PLoS Computational Biology* 5(12), e1000605. doi:10.1371/journal.pcbi.1000605.

Fig. 1. Relationship between the DOE Systems Biology Knowledgebase and the Larger Scientific Community



There are two communities that must be both served and enabled by the Knowledgebase. One focus needs to be on the biologists sitting at their computers having done an experiment, who want to understand their results. Another focus is enabling tool builders. All agreed that this is not an ever-growing but static archive. *It is a combination of new* experimental data and tools that accesses a growing reference data. By having common access to quality data, tool-builders will also have the transformations of the data products in one place. This should accelerate the evolution of transformations and provide a better process for designing new data products. Some innovative ideas in this arena were suggested. These included tools registries, challenges and challenge grants to answer “tools needs,” and Facebook-style entries of “my experiment” to advertise. There is a more detailed section on how a tools registry might function (see Section 4c). A vision is to improve data analysis sufficiently that experimental sample generation becomes a bottleneck, despite the massive amounts of data generated per experimental sample.

This can start with well-defined workflows leading to a mapped interface with access to data and tools. Then we can string tools together to do powerful operations without having to worry about the data formats and come up with an answer. This answer may be a better

interpretation or a better designed experiment. This may link into web services and other models. This interface or API should be practical, with not much investment early on.

An early priority should be to develop initial APIs to get to the data with an interface to the tools. The API should make it easier to develop and test new tools for biologists to use and to add them into the interface. There was broad consensus on the importance of the API—that it should be modular and interchangeable. An open question for the architecture and the data transfer challenges relates to how much analysis is done where the data resides versus at the viewer's site (download and I/O concerns).

There is also the need to consider architecture that relates to the massive data transfers that we are potentially considering, especially in a federated or distributed approach. This can be considered as the datasets increase; it is more and more difficult to perform “all versus all” comparisons. A number of web-based applications for metagenomics exist that do not currently support large-scale sequence analysis, including, but not limited to, on-demand clustering of user-provided datasets. Thus, the evolution of centralized data repositories and analytical services in metagenomics is currently not in sync with the accumulation of next-generation sequence data as it relates to end-user capabilities. To put things in perspective, consider the initial ScalaBLAST calculation in which comparing 1.6 million proteins in the IMG 1.6 database against the 3.2 million proteins in the nonredundent database consumed approximately 5 years of CPU time. Individual investigators simply cannot achieve these calculations due to technical or infrastructure limitations, and even if they could, the visualization tools needed to interpret and compare next-generation metagenomic and metaproteomic datasets do not scale with data volume and complexity. While good progress has been made in developing tools to inventory and, to a lesser extent, to compare microbial community structure and function, there is no comprehensive tool that allows integrating and comparing multimolecular datasets (e.g., DNA, RNA, protein, and metabolites), which are needed to fully realize the vision of microbial systems ecology.

There is continued consensus for a federated model. However, this federation cannot be the current system of separate unconnected sites. Here, federated means distributed resources, data, and tools but integrated and coordinated in a manner to be apparently seamless to an outside user. Some very mature examples are the current genome data repositories (e.g., GenBank), which actually are distributed in three sites (the United States, Europe, and Japan) but appear as one to the science community. Of course, reaching this level of integration will take a long time and effort and is beyond an initial plan. The use of a federated model brings with it the underlying challenge of how much centralization is required in deposition, curation, or “advertising.” (Note: “advertising” was discussed as a possible mechanism to draw attention to new datasets or tools in the ongoing development of the Knowledgebase.) A possible consensus in this group was that this does not matter as long as the access and the goals can be accomplished.

The development of an API allows the potential of an “open-source” system. The potential and challenges of “open” systems are discussed in more detail in Section 4d.

This use and development and data deposition in knowledgebases must be balanced with the need for some level of public/private embargo and the need to further the careers of

bioinformaticists and experimentalists. This was deemed important and is covered in more detail in Section 4e.

3d. NIH Interactions: Data Resources and Leverage

There was discussion about the need for awareness, linkage, and leverage with NIH-led efforts, in particular NCBI. Current and planned NCBI efforts are described elsewhere. Workshop consensus was that we should leverage resources as much as possible. In particular, we should use both existing and under-development NCBI capacities as an archive and repository as much as possible. But there will always be a gap in filling current BER Genome Science needs and challenges, therefore we will also need our own efforts and to link them with other projects.

4. Expanded Discussion from Workshop: Assignments

4a. Workflows and the Systems Biology Knowledgebase

In bioinformatics, complex biological analyses frequently require large-scale computations that compose standard tools and methods into a pipeline, or workflow, that runs a series of tasks to achieve a specific outcome. There are two major types of workflows, namely:

1. **Ad hoc Interactive:** In ad hoc interactive workflows, the biologist is fundamental in driving the steps involved in the workflow. Interactive tools (e.g., Cytoscape, DMV, R scripts) are used to analyze and visualize data, and the results from one tool become the inputs to the next tool in the workflow. The biologist typically drives the transition between tools based on his or her observations of the state of the analyses, and data is moved between tools either manually (e.g., saving files in specific formats) or by using a lightweight data transfer tool like Gaggle (www.systemsbiology.org/Technology/Data_Management/Gaggle).
2. **Automated:** Automated workflows, also called pipelines, take a set of input data and apply a series of analyses to the data to produce outputs. No human intervention is necessary to invoke the next step in the workflow and to transfer data between computations. Automated workflows can take anything from seconds to weeks to execute, and the steps in the workflow are commonly controlled by scripts or workflow tools like Taverna (www.taverna.org.uk/).

While the precise software mechanisms used to coordinate the steps in a workflow vary between the interactive and automated cases, the ease of construction of workflows in both cases is hampered by two fundamental technical issues:

- **Tool heterogeneity:** Standard tools and algorithms are not created using a common software framework so that they can be readily “plugged together” to form a workflow.
- **Data heterogeneity:** Standard tools and algorithms consume and produce data in a variety of different data formats. Feeding the outputs from one tool into another commonly requires data transformations to produce inputs in a format that a given tool is expecting.

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For these reasons, creating effective bioinformatics workflows is non-trivial and requires considerable effort from biologists and software engineers alike in order to meet scientific objectives.

The Systems Biology Knowledgebase is an opportunity to address the current complexity of creating both interactive and automated workflows. The Knowledgebase can create a lightweight, flexible software infrastructure that enables tool developers to “componentize” their existing and new algorithms, providing standard interfaces that can be used to compose tools into workflows. In addition, the Knowledgebase infrastructure can support the flexible, discoverable definition of data formats that tools produce and consume. By describing a given tool’s data requirements using metadata, converting data from one tool to another becomes simpler, and potentially automatable.

We therefore recommend the Knowledgebase implements a set of simple programming interfaces that enable much more effective workflow construction and reliable execution. By reducing the “levels of pain” experienced by biologists and software engineers in creating workflows, we envisage the creation of a software ecosystem in which useful workflows can be rapidly built, deployed, and shared with the community through the Knowledgebase infrastructure. This would be analogous to social networking sites such as Facebook, which encourage development and sharing of new applications based on the software infrastructure and programming tools that Facebook makes available. This model, which is expanded upon in the next section, is designed to (1) encourage development of many tools that provide multiple approaches to solving a particular problem and (2) enable the end-users to determine which approaches survive. Applications that accurately solve a problem in a particularly elegant or succinct manner will become highly adopted, and others will slip into oblivion.

A primary issue to be addressed under the Knowledgebase plan is the motivation of the developer. Platforms like Facebook, Twitter, or the Apple App Store can provide a financial incentive for *de novo* application development. Knowledgebase infrastructure and early applications will need to be developed under more conventional models. But as the programming platforms become established, the project will need to consider funding models designed to maintain and expand innovation over the long term. This may include a combination of standard funding models and models designed to reward *de novo* application development. Failure to address this basic issue will almost certainly result in stagnation of the development cycle.

4b. Metagenomics Systems Biology Knowledgebase: Workflows, Background, Design Goals, and Recommendations

Integrating Metagenomic-Enabled Workflows

Most metagenomic data come from microbial ecosystems. Data derive from a broad range of environment types—from the deep subsurface to the human gut—motivating many questions such as how are microbial communities structured, and how do they function? Do genetic profiles vary across environment types? Using metagenomics to answer such questions will

require the effective integration of information about metabolic potential (genomic sequence); metrics for function (proteomics, transcriptomics, metabolomics); contextual information; data that define the physical and chemical environment (metadata); and methods to consistently and accurately update annotation as new evidence becomes available (see Fig. 2).

Metagenomic data may be collected from one or many samples, whereas proteomic, metabolomic, and transcriptomic data typically stem from a diversity of experiments such as time series, environmental perturbation, and genetic manipulation.

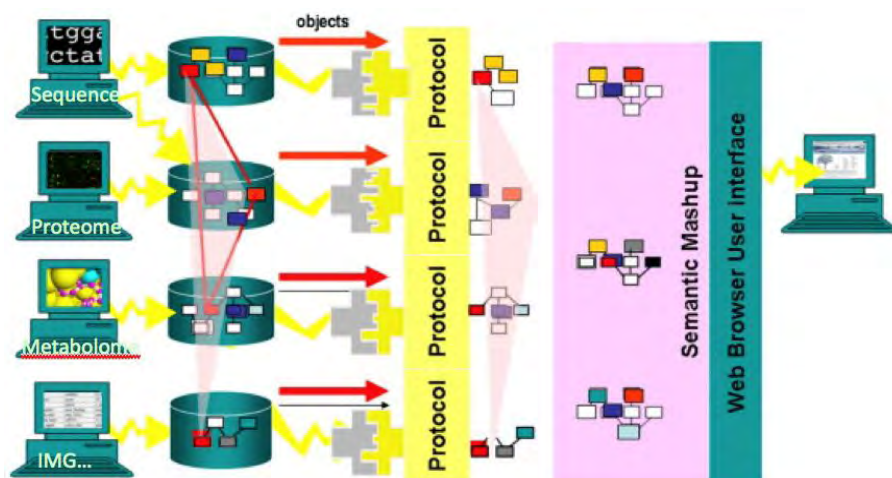


Fig. 2. Data Warehouse. Data are linked by common or discovered identities and shared annotations (tags) drawn from controlled vocabularies, and managed by identity and ontology authorities. The data are accessed through simple application programming interfaces (APIs) and aggregated through browser scripting based on common identities and tags. [Slightly modified from Goble and Stevens 2008.]

One of the most important aspects of metagenomic investigations is that sequence information is (or will be) intimately linked with proteomic, metabolomic, and transcriptomic data.

Commonly, metagenomic workflows begin with sequence information, but a Knowledgebase—an artificially intelligent tool that provides a mechanism for collecting, organizing, analyzing, and distributing data—must be designed to facilitate dynamic interconnection of these data types to allow data integration for biological insight (see Fig. 3). The need for dynamic interconnection is underlined by the observation that data can exist in many states: “there is live data, living data (more live than live), stale data (archived?), dead data (archived?), lost data, vandalized data (valid data overwritten by non-valid data).”² For example, when data consumers download a specific dataset from a resource and put it into a new form for their own purposes, the data become disconnected from the original source in the absence of dynamic linking or a provenance system. It therefore cannot benefit from changes or upgrades in the source (i.e., it becomes “stale” or “dead”). Examples of changes at the source include reassignment of a gene function, re-searching of a proteomic spectra database with new genomic sequence, and changed identification of a metabolite due to the addition of new, standard metabolite profiles to reference databases. These types of data insertions, deletions, and mergers represent problems for all subsequent users of a resource.

² Web commentary, www.ted.com/talks/tim_berniers_lee_on_the_next_web.html

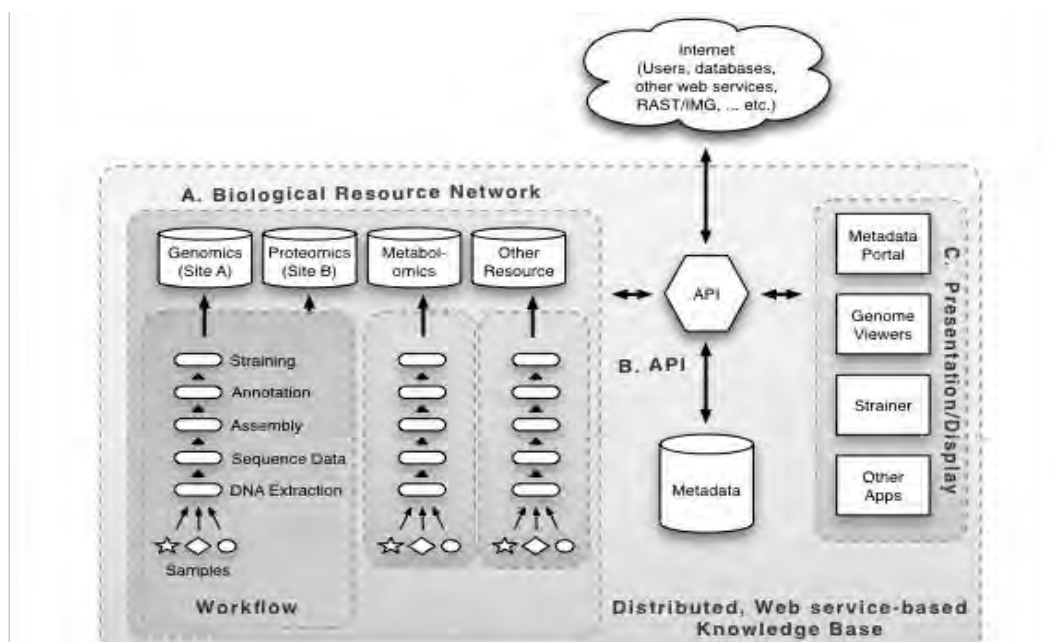


Fig. 3. Overview of Metagenomic-Enabled Workflows Feeding into a Knowledgebase System. This figure provides examples of data-generating experiments, data types, file formats, and processing steps. Note that not all studies include other “omics” methodologies. Data derive from a common source (a natural sample or series of samples, an experiment or series of experiments), and data are integrated via tools to answer specific questions. Lines with arrowheads represent data flow, cylinders indicate a data resource, and rectangles indicate an application (stand-alone or web-based). Data analyses can draw on a wide variety of existing and newly developed tools (e.g., assembly programs, gene prediction, functional annotation, analysis of regulatory structure), as well as tools developed specifically for metagenomics (e.g., example methods to visualize and analyze strain variation).

Metagenomic Data Challenges

Five key attributes associated with metagenomic data pose challenges that require special consideration.

(1) Volume of Data Generated. Sequencing of metagenomes generates somewhat to highly fragmentary datasets, often with low redundancy levels and potentially high error rates (due to low genomic coverage with error-prone sequencing).

The growth in sequencing capabilities has led to a flurry of metagenome sequencing and analysis projects in recent years. Interestingly, computational analysis costs are now quickly outpacing data generation costs (Goble and Stevens 2008). As shown in Fig. 4, running similarity searches (BLASTX) for data generated by one run on an Illumina GS-FLX instrument (costing approximately \$15,000) will take 60,000 hours of compute time on a recent machine (or cost approximately \$120,000 if run on Amazon’s EC2 service).

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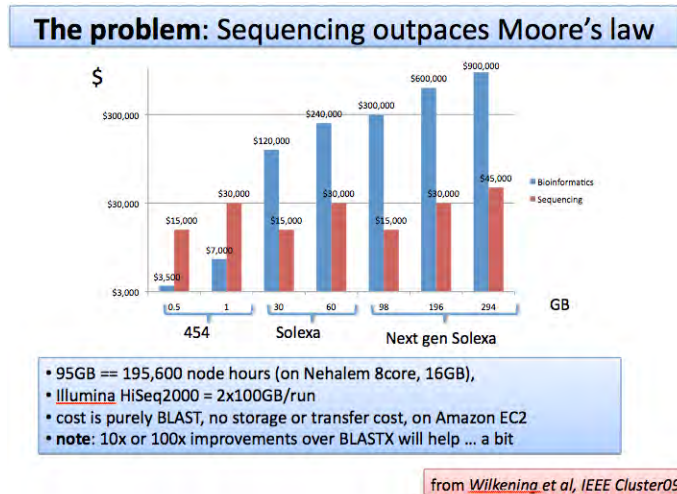


Fig. 4. Cost Comparison. The computational costs (blue) are rising significantly with growing sequencer yield. Already they can be up to 10 times the sequencing cost (red) on some instruments. Costs shown are for running BLASTX against the current National Center for Biotechnology Information (NCBI) non-redundant protein database. [From Goble and Stevens 2008.]

An associated problem is that while hundreds of metagenome datasets are publicly available, these are widely distributed across the world, and it is at times challenging to identify and access all relevant datasets. Many more experimental data sources are not publicly discoverable. Therefore, few high-profile studies have emerged that attempt the systematic comparison of available public data because this task is difficult (and, in part, because currently the primary focus of most investigators is on their own datasets). One way of enabling both general discovery and access (e.g., via web services, as well as “local” access to the data) is via a peer-to-peer based framework of dataset registries. Unique identifiers such as Digital Object Identifiers (DOI) known from publications, URLs, or persistent URLs (PURLs) could enable the citation of high-quality, well-annotated datasets, and offer identification and linkage between datasets. Any such framework also would encourage metadata and semantic enrichment, enabling queries such as “Display all soil metagenomes from the Midwest” or even “What is the number of short-read metagenome datasets currently available from a specific sequencing platform.” However, without accompanying metadata, the sequences may be next to useless for some of the broader purposes.

(2) Never-Ending Analysis. With metagenomic data investigations, the analysis is never finished. Unlike an isolate genome where a final set of open reading frames and relatively stable functional annotation is generated, metagenomic datasets are prone to continual revision resulting from new sequence data, additional manual curation, new reference genomes, and more. Gene numbers, for example, may change multiple times, and so can organism assignments of genome fragments. Although curated metagenomic datasets share many features with isolate datasets, there is a major difference: there is no single answer in almost all cases because populations are not clonal cultures. In many current studies, the extent of metagenomic sequence curation is minimal, but this must change. A Knowledgebase system must be *dynamic* in order to be able to deal with this key attribute.

(3) Sequence Variation. One of the most important features of a metagenomic dataset is the sequence variation that is captured via the sequencing reads. Typically, each sequencing read derives from a different individual, and thus a metagenomic dataset provides information about sequence heterogeneity. Although it is arguable whether or not the primary sequence data must be retained (or just the base-calling scores), ***a system to tie read-based data to the composite, assembled sequence is essential.*** In addition to providing access to such data, an annotated and permanent record of research steps taken during curation will be essential to establishing a powerful, relevant, and long-lasting scientific Knowledgebase. To our knowledge, this capacity is not available via any shared resource. Sequence-variation analyses will be critical to population genetics and evolutionary studies, for efforts to identify the reasons for fine-scale variation in functional attributes, and to locate potentially interesting gene variation for targeted bioengineering applications.

(4) Tools and Computational Infrastructure Are Required for Data Sharing and Comparative Analyses. As the number of sequencing and “omics” instruments available for metagenomic research grows, ever increasing the volume of available data, the community needs both the computational infrastructure to analyze and compare the data as well as the tools to analyze the results.

An important approach to tool development is the generation of a series of modular components (as opposed to large, integrated pipelines suited to run on a centralized computational facility). In addition to portability, an advantage of the “components”-based approach is that the user retains considerably more flexibility with regard to the way in which the data are processed. An example of current interest involves software for correcting homopolymer errors in 454 sequences. Currently, this capability is contained within a complex package within a pipeline. For practical reasons, it is undesirable to send the entire dataset to a staff member at the centralized facility for homopolymer correction, and, more importantly, data reprocessing in a new pipeline will unlink information already associated with the sequence.

(5) Diversity of Data Types, File Formats, and Processing Steps. Scientific research itself has become more specialized on the individual level and more collaborative and international on the community level, making it desirable and necessary to relate one’s own local research results meaningfully to the geographically distributed, multifaceted results being compiled elsewhere in the world. Systems biology has a long tradition of utilizing diverse research results from experimental and computational methods that stem from varied and distributed sources. Commonly, these research results are locally integrated and synthesized with the scientist’s own findings, then published as yet another valuable source of information. Over the years, the community has created a wealth of outstanding data sources and tools for access, integration, and analysis. Unfortunately, these ***sources of scientific knowledge and analysis are mostly characterized by a diversity of data formats, data representation, metadata, and access methods,*** making it difficult to identify all of the relevant data sources for a given topic, assess their quality, and integrate them into the scientific research process.

Architecture Design Goals and Knowledgebase Adoption Strategies

As experiences in other scientific communities have shown, it could take many years to change working practices and move to a central deposition system based on community-agreed, standard data formats, metadata, and semantic data descriptions, with associated discovery and analysis tools. This type of integration has been most successful in slower-moving fields with less diversity in their research methods than metaomics, fewer data sources, and more standard analysis software. In these fields, such integrations have been very successful in terms of making data more widely known, used, and effectively analyzed by the community. Despite the larger challenges faced by our community, such central deposition systems have their place in the Knowledgebase, but it appears unlikely that the more rigid structures of a central data and applications repository could effectively meet all systems biology needs. Instead, a more flexible approach is needed. We advocate combining the benefits of the more rigid, standardized frameworks with a “live data network” of shared experimental results.

Ideally, the live data network component of the Knowledgebase would be compatible with current, more distributed working practices, while at the same time assisting with greater integration of resources. A peer-to-peer based system of data, metadata, analysis tools, and workflow registration repositories, integrated through common semantics would seem desirable. In this scenario, users could “publish” their data, applications, and workflows into the live data network by describing their provenance, content, location, access, and usage methodologies (both for other users and computer applications) in one of a set of community-agreed semantic description formats (ontologies). In designing these semantic descriptions and underlying metadata, it will be important to focus on the data content and ontologies. Important concepts that must be included, rather than the particular local implementation, need to be identified. Semantic ontologies will allow the mapping between different expressions of the same concepts (within reason), as well as linking concepts where their expressions do not overlap but are related.

If a “local” format contains desired information, it is relatively simple to map these data to the shared semantic description and make the local knowledge available in a community format. These conversion interfaces are not difficult, but they can be time consuming to establish. Therefore, offering different levels of community participation would be desirable. Initially, one might submit only enough information to allow others to discover one’s resource. More functionality can be added over time to support full integration. Alternatively, central data centers might offer services to smaller groups to integrate their data into the archive and “publish” it for them (including data analysis, such as normalization and filtering, annotation, and more). This type of tiered approach to Knowledgebase participation is critical because it would help to bring about adoption by the wider community by supporting the twin (and sometimes competing) goals of (1) defining relevant data standards and formats for participation in a more centralized repository and (2) the necessity of allowing dynamic integration to be done at a smaller, local, and scientific inquiry-driven level.

Similar to the underlying resources (see Fig. 5), links between people, data, applications, and workflows could be explicitly recorded and published, or discovered following the semantic description “trail.” This approach is analogous to the LinkedData Web proposed by Tim Berners Lee but is extended with more domain-specific information about the data, especially the

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applications and workflows, to aid directed scientific discovery and experimentation. An added benefit could result if the Knowledgebase provided space for larger-scale data integration, analysis, and publishing—following the same format described above—to aid smaller institutes and preserve important results after funding for their further curation elapses.



Fig. 5. Linking Open Data. This cloud diagram gives an overview of published datasets and their interlinkage relationships.

When designing the community-agreed metadata and semantic formats to be utilized, advice from the data curation community should be considered. Their aim is the development and provision of methodologies and tools that allow the perpetual reuse of data by its designated user communities, by keeping it “living” (i.e., adapting its representation to changing community trends without affecting its integrity). Data policy examples, data life cycle models, archival reference models, and more will help to define critical components of the data, analysis software, and workflow descriptions.

The Knowledgebase’s success will depend strongly on the quality of the data, tools, and workflows that are available through it, as well as the ability of researchers to identify, assess, and integrate resources quickly. Much of the latter will depend crucially on the quality and extent of the metadata and semantic information about the resources. Herein lies a potential problem. Although good data or tools will usually lead to direct scientific rewards in the form of publications and resulting citations, the time-consuming annotation and preparation of sharable datasets is not directly rewarded by the community in similar fashion. This often results in a lack of motivation to provide this vital information, or to provide it with the required due diligence. One way to resolve this issue might be to enable scientists to “publish” their datasets, following similar style, content, and peer-review standards that they are required to follow throughout the publishing world (e.g., see DataCite at www.datacite.org/). This way, datasets would be citable and earn the owner well-deserved recognition and rewards.

Similarly, to encourage participation in the Knowledgebase, it will be critical to ensure that utilized data, analysis tools, and workflows are correctly attributed to their owner, an often difficult task due to similar names and changing names and affiliations. Recently, the research

community has initiated more concerted efforts to develop unique identifier systems for researchers, although most of them are focused on publications (e.g., International Standard Name Identifier (ISNI) being developed by the International Organization for Standardization (ISO) as Draft International Standard 27729, and Open Researcher Contributor ID (ORCID) being organized by the ORCID Initiative) and not data elements. Additionally, protocols for maintaining permanent data identifiers are being developed and increasingly deployed in the biological domain. The combination of Uniform Resource Names (URNs) and Uniform Resource Locators (URLs) are one example. URNs represent a persistent, location-independent identifier, and they promote mapping to other namespaces. A URL is the specific location of a URN. For example, a specific protein name and its annotation from the Kyoto Encyclopedia of Genes and Genomes (KEGG) can be represented by both a unique identifier and a location. Using the Life Science Identifiers system (lsids.sourceforge.net/), a URN in the Knowledgebase might be “urn:lsid:doekb.gov:eco:b1743”, which uniquely names a specific gene in *E. coli*. The location of the data element is accessed using the URL www.genome.jp/dbget-bin/www_bget?eco:b1743. This combination of URN and URL provide an unchanging name (the URN) and a location (URL) of data about the URN. Using a common naming system allows for data linkages and for the development of rich semantic descriptions. Another identifier system is the persistent URL system, or PURL. This system achieves the same goal of specifically naming and locating data, but it does not directly describe the location. Instead, it references an intermediate location that redirects the request to the proper location. PURLs depend on a master system for redirection and on contributors to maintain their links. As an example, the same *E. coli* protein described previously can be accessed from the UniRef Database using a PURL system from this link: purl.uniprot.org/uniprot/P77754.

Using unique identifiers (DOI, URN/URL, or PURLs) for data, and potentially applications and workflows, will make it possible to utilize the services the library and web technology communities offer for information discovery and access. Furthermore, different data publications and publishers could be easily linked through citations, as could the history and connectivity of data elements in the Knowledgebase.

Some Data Sharing and Analysis Needs

As data sharing is becoming more common, data storage is essential, but as with the World Wide Web, it need not be centralized. As noted previously, repositories must be dynamically linked to information sources; otherwise, we run the risk of “stale data.” To enhance research, each repository should consist of user-friendly interfaces (APIs and people) to access data and application modules, as well as derived data products, enabling other users to build novel solutions. One such product provides genomic neighborhood views across multiple genomes. Both the DOE Joint Genome Institute’s Integrated Microbial Genomes (IMG) system and Argonne National Laboratory’s Rapid Annotation Subsystem Technology (RAST) offer these views (see Fig. 6). However, such foundational capabilities need to be extended to meet real systems biology needs. For example, a user may need to integrate proteomic, metabolomic, or transcriptomic data in a display such as that shown in Fig. 6. Semantic data interfaces could provide vital support in this endeavor, as they insulate tool developers (including those of user-friendly interfaces and data products) from differences and changes in local data formats, data organization, and access mechanisms. Based on the community-agreed semantic descriptions

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(ontologies), any software can search and request data using semantic concepts; for example, “Give me the first protein of the second metabolic pathway that the system identified in KEGG for *Shewanella*.” This type of request would still continue to work even when the underlying data sources change, as long as the data sources maintain their semantic interfaces to the Knowledgebase. Therefore, any tool development effort can focus on new functionality, rather than redundantly expending effort on data search and access methods. Similarly, it will be much easier to combine development efforts that previously would have only benefitted selected data sources. Application maintenance will again require reduced resources, as changes to underlying data sources must not affect the usability of any tools, unless they want to benefit from any additional information (new concepts, not more data in the same structure).

In general, we anticipate building upon tools such as those shown in Fig. 6 to address new needs and provide additional capabilities. For example, a user may need to answer a specific question such as the temporal distribution of strain genotypes. Thus, the “display” tool may need the ability to output specific data characteristics as inputs to other programs (e.g., the library of origin of a set of sequencing reads, or the environment type from which specific gene contexts derive). A benefit of a web service—based system is that it allows for the development of tools for such specific questions. In general, continually capturing **innovation** by the broader community for purpose-specific application development provides a way to address the unavoidable limitation that no program can ever meet all possible user needs.

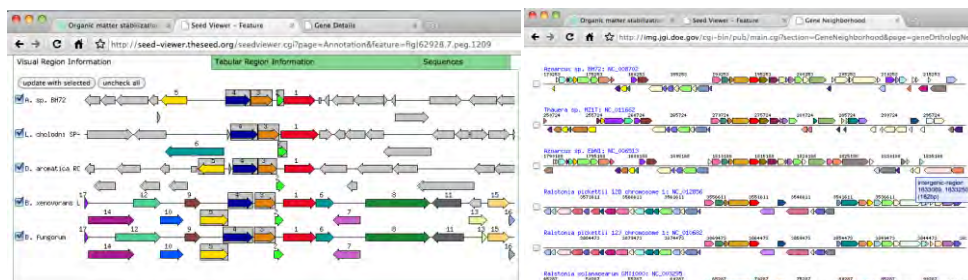


Fig. 6. Data Displays. The chromosomal context of a protein in *Azoarcus sp.* BH72 as shown by the SEED-Viewer (left) and IMG (right). Both systems provide a similar view of data that are computationally expensive.

With the growing volume of data, there is an opportunity to introduce “lightweight standards” to allow the exchange of many sequence datasets and to reduce the cost of computational analysis. The Genomics Standards Consortium (GSC) has presented the Minimum Information about a Metagenomic Sequence/Sample (MIMS) standard, which allows the exchange of contextual data for metagenomes (e.g., location, sampling method, and biome description). This provides an initial description of the sample, but only minimal information about computational sample processing is included. The GSC’s M5 working group has presented a draft metagenome interchange standard (MTF) that includes computational results and MIMS metadata (see Fig. 7). However, as important as standards are, they can have a downside. It is also important to ensure that standards are flexible enough to adapt to cutting-edge advances in the field, allowing specific users to add additional information.

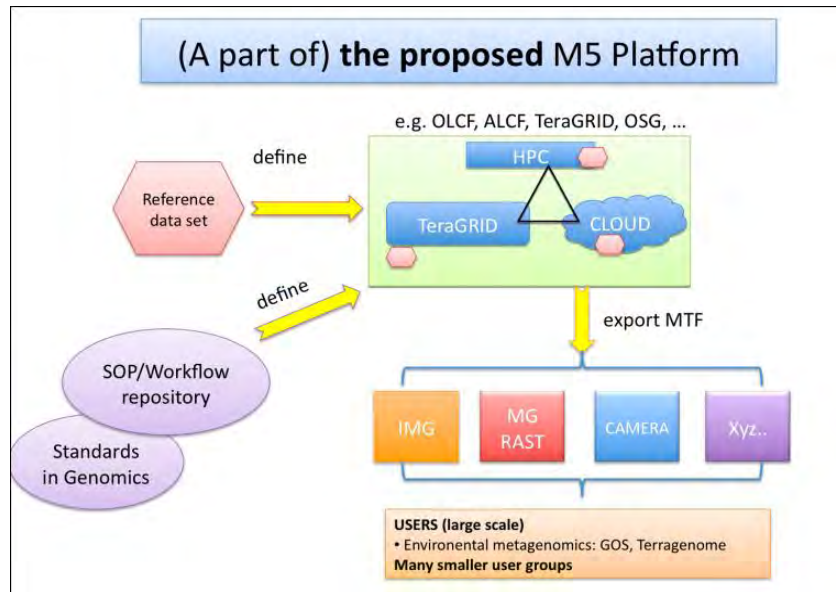


Fig. 7. Standard Development. The proposed M5 platform will include a standardized processing pipeline that can be executed by third parties (e.g., large supercomputer facilities), and, via a reference non-redundant protein database, it will enable many groups to use the results. JGI’s IMG/M and MG-RAST, as well as the Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis (CAMERA), are working on the M5 standard.

Integrating data across multiple disciplinary Knowledgebase subcomponents. An important concept for a DOE-wide Knowledgebase is that it will interlink components tailored to specific areas of systems biology investigations that have been or will be designed by those with special expertise with the various data types and needs. A possible solution is a Metagenomics Knowledgebase (MKb) developed on a database structure flexible enough to be ported to other laboratories and populated with data from any of a wide diversity of systems.

Such an MKb must enable free flow of data into and out of a larger DOE-wide Knowledgebase structure, as well as into and out of many other publically available databases (e.g., KEGG and Pride). One component will be data repositories (e.g., from a specific research group or team) with one or more data resources. Many studies will include both metagenomic sequence and other data forms (e.g., proteomic, metabolomic, or microarray data). Each data repository will have specialized tools, so that one might visualize links from an MKb to, for example, a project-specific transcriptomic dataset in the Transcriptomics Knowledgebase (TKb), or the Proteomics Knowledgebase (PKb). Developing guidelines for assisting local groups in creating resources such as these is an important goal for the DOE Knowledgebase. A system that describes and publishes data dynamically will greatly aid in this step, and incorporating the tiered participation option mentioned previously will ensure that all stakeholders are represented.

One of the most important obstacles to overcome is the lack of data integration in a form that enables data mining by groups not previously involved in specific kinds of experiments. To some extent, web-services type interfaces to the data and a comprehensive dataset registry will allow only a certain subset of queries and satisfy just a subset of researchers. “Web services” typically means an API that can be accessed over a network and executed on a remote system hosting

the service. It is usually in one of two forms: “big web services” (e.g., KEGG) and representational state transfer services (RESTful services; e.g., UniRef and GO).

Currently, “the integration requirements of biologists working with unpublished data are not being widely addressed by the community.”³ To illustrate the opportunities associated with data integration across experiment types, the different data types generated in different laboratories by different methods (e.g., metabolomic data and proteomic data) must be considered. Another researcher might be interested in a specific protein of unknown function suspected to play a role in a certain metabolism. So long as the data are accessible, patterns may emerge through the integration of this information with information from other research groups. This can be accomplished as long as the experimental groups use a consistent “resource description framework” (RDF), a data description document that describes and links the data (e.g., gene A is translated into protein B).

Both centralized, high-performance computing (HPC) services and dynamic, “local” web services can be envisioned as important coexisting and linked parts of the MKb. For example, each of the experimental platforms (e.g., metagenomic sequencing, proteomics, and transcriptomics) requires extensive HPC, but the calculations and analysis only need to be done once, and the results then are shared (e.g., by using a common data description and a RESTful service). In fact, the raw data could be made publically available upon generation, and as the local system converts raw data to processed data, the broadcast version is continually updated (“live data”). This should be feasible within the framework of the laboratories that participate in data sharing, although it is likely necessary that central data warehouses will create new releases on a regular basis.

In the specific case of metagenomics, raw sequence is generated in vast quantities. Over time, these sequences are assembled and annotated. In parallel, comparisons among reads and assembled fragments reveal within populations variation in gene sequence and gene content. A whole suite of computational tools is required to collect and present the data as part of the in-house analysis, but this work product (the added value) currently is never distributed. For mass spectral datasets, a resource description could be generated that enables a researcher to access the up-to-date analysis and download components (e.g., reads, contig sequence, and single-nucleotide polymorphism (SNP) concentrations). In this way, long-running, CPU-intensive analyses can be part of a Knowledgebase approach that allows biological data integration via web service protocols. This will accomplish the important goal of keeping those responsible for data generation and data upkeep (“live data”) connected to widely distributed data analysis tasks. Furthermore, the data are continuously enhanced as the network of links to new experiments expands.

³ Anwar, N. and E. Hunt. 2009. “*Francisella tularensis novicida* Proteomic and Transcriptomic Data Integration and Annotation Based on Semantic Web Technologies,” *BMC Bioinformatics* **10**(Suppl 10:S3), PMID: 2755824. Electronic resource.

Annotations change as our understanding of protein families and complete genomes improves over time. Work on specific genes or proteins builds a body of functional evidence around each of these entities and their families. An optimal annotation system should permit easy and rapid updating of specific sequence annotations in response to new experimental data, and provide a straightforward method to record the **source and quality** of the updates. Automated annotation systems should be able to use the quality data to inform new annotations and update overlapping datasets. Such a system would dramatically reduce the time from discovery to annotation and enable very richly annotated descriptions of genes, pathways, and organism function. It also would facilitate collaborative research by disparate laboratories focused on a common genetic system and reduce propagation of erroneous annotations into new datasets.

The primary problem with such a system is ensuring the accuracy of changes made by the broad research community. In the 1980s, GenBank dealt with this problem by restricting annotation changes to the individual that submitted the sequence. However, the MKb must move beyond this static model to embrace systems used by organizations such as Wikipedia to validate changes. Therefore, in addition to recommending a MKb that focuses on sharing of “live” experimental and associated data, our working group recommends finding a mechanism in which new knowledge deposited within the data warehouses (e.g., NCBI) can be updated easily.

Role of grand challenges in linking community, shaping Knowledgebase development.

Transition to a scientific framework in which data sharing and data integration is facile will present many challenges. At this time, small groups are beginning to address parts of the problem (e.g., metabolomic-metagenomic-proteomic data integration on a small scale), but the effort at the “omics” community level has a long way to go. A recommendation of our working group is to motivate the formation of linkages and overall architecture of a Knowledgebase with this goal via “grand challenges” that require data integration and sharing. We find this approach preferable to tasking a group of bioinformatics experts with establishing a system that will be later used by the community.

As one example of a grand challenge, consider the potential for data integration to improve protein annotation [g1]. Currently, most genomes encode a significant number of lineage-specific proteins that have not been studied biochemically. These proteins may hold considerable significance for DOE efforts in environmental remediation and bioenergy, as they may be involved in novel pathways for metal redox transformations or degradation of complex organic compounds. Similarly, there are probably many small non-coding RNAs coded on genomes and genome fragments for which annotations are lacking in public databases. Consequently, many gene predictions are uncertain, and a significant number of predicted proteins discovered via short-read sequencing may be corrupted by frameshifts. A single confident identification of a hypothetical protein via proteomics (or transcriptomics) converts a “hypothetical” to a “protein of unknown function” (an annotation that could be amended with the words “validated by proteomics”). If all the curated proteomic data from all samples worldwide could be integrated in a single analysis, many annotations in public databases could be updated. In addition, detection of the first peptide in a protein can confirm either the start site or the truncation status of the mature protein (e.g., due to cleavage of signal peptides). High-throughput improvement of start-site information from either proteomics (or transcript

sequencing) will provide important constraints for better gene prediction and regulatory models. Such tools and models are essential for confident systems biology studies.

Recommendations

The Systems Biology Knowledgebase will need to fulfill a range of requirements to achieve the research community's envisaged goals. These include:

- Providing a common mechanism for collecting, organizing, annotating, analyzing, and distributing data that enables easy data sharing and comparative analyses.
- Facilitating **dynamic** interconnection of data types, data sources, applications, and workflows to allow **data integration** for biological insight.
- Enabling researchers to identify, assess, and access all relevant **datasets** worldwide.
- Allowing scientists and facilities to “publish” their data, applications, and workflows into the “live data network.”
- Providing space for larger-scale data integration, analysis, and publishing.
- Providing scientifically accepted rewards for researchers who “publish” well-annotated, good quality data, applications, and workflows.

We suggest that this could be achieved through the development of:

- A set of community-accepted semantic description formats (ontologies).
- A peer-to-peer based system of data, metadata, ontology, analysis tools, and workflow registration repositories that are integrated in discovery, access, and utilization through common semantics.
- Guidelines and software libraries that allow scientists and facilities to “publish” their data, applications, and workflows into the Knowledgebase in a set of agreed forms.
- A mechanism that allows scientists and facilities to easily and rapidly annotate, change, and correct research results and annotations in the Knowledgebase, capturing source, reason, quality, and proof for changes.
- User-friendly interfaces (APIs and people) to access data and application modules, **as well as** derived data products, enabling other users to build novel solutions with the data
- A framework of citable, unique identifiers for data, applications, workflows, and researchers
- Guidelines, training, and workshops for all new products and concepts provided by the Knowledgebase

References for Section 4b

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4c. Toolkit Registry Development

The development and management of a Systems Biology Knowledgebase will provide a unique resource to integrate experimentation, modeling, and bioinformatics across disparate levels of biological inquiry. Foremost, the successful implementation of a modular cyberinfrastructure to service the informatic needs of the systems biology community must actively recognize the broad potential user base of the resource. This recognition is critical to populate the resource with the appropriate data, tools, workflows, and corresponding literature consistent with the expectations of the user community and commensurate with the varying expertise of the Knowledgebase clientele.

Fundamental to the success of the Knowledgebase will be the bioinformatic services and resource sharing potential of the portal. At present, non-computational biologists seeking to incorporate high-level informatic investigations into their research program do not have access to a resource analogous to that which is envisioned in the development and deployment of the Knowledgebase. Most often direct consultation with expert bioinformaticians is required to initiate such investigations, effectively dissociating experimentalists with their data in the pipeline of biological discovery. To improve the efficiency of bioinformatic investigations, a centralized resource announcing the availability and utility of the various tools, applications, and algorithms available will stand as a tremendous advance toward minimizing the opacity of "–omics"-based data analysis and interpretation. To meet this need, it is recommended that a Knowledgebase-hosted toolkit registry be developed to provide a comprehensive inventory of software available to the user community. The construction of such a registry will also assist in defining the architectural strategy of the Knowledgebase engine, including compute resources, need for portage, development of APIs for web-based analysis, and demarcation of analysis subsystems specific to particular tasks and objectives.

Below is a brief list of thematic applications and additional resources that could initiate inventory within the Knowledgebase resource:

- | | |
|----------------------|---|
| Sequence assembly | Statistical analysis |
| Sequence annotation | Metadata integration |
| Comparative genomics | Geographic Information System (GISP deployment) |
| Phylogenetics | Data QA/QC |
| Cluster analyses | Transcriptomic resources |

Proteomic resources

Genetic database resources

Metabolomic resources

Metabolic subsystem database

Strategically, each of these resources should contain a brief description of the type of data or analysis provided, its product, and rationale for why a user might be interested in using a given tool or database. Importantly, the Knowledgebase must also exist as a central forum where researchers and developers can exchange ideas to nucleate new tool development. Curation and expansion of the toolkit registry should be guided through an interactive Wiki environment where users can post comments and suggestions for including new resources into the Knowledgebase. Additionally, resource examples and workflows should be included to assist users. An exquisite example of a tool registry has been developed as part of the Neuroscience Information Framework accessible at neuinfo.org/nif_tools/nif_registry.shtm.

Ultimately, content within the Knowledgebase must be adequately indexed to allow integration across the multi-dimensions of available data. Key drivers of this need include incorporating genetic, genomic, transcriptomic, proteomic, and metabolic datasets with phylogenetic, metabolic, imaging, ecological, and geospatial information. Although such considerations are beyond the scope of the toolkit registry described here, it is critical that a composite inventory of analysis tools be available to augment discovery and seed the data integration process.

4d. The Knowledgebase as an Open Development Platform

Enable tool development and integration, by providing an open developer platform inspired by the Facebook Platform / Google Apps API. Allow outside developers to produce novel analysis and visualization tools that can query the database directly (with appropriate access controls) and display and exchange results through a common UI. There will always be disagreement between research communities on which analysis is the best for any particular data type. DOE should not be in the position of enshrining one type of analysis over another. It should provide the platform, let the individual researchers develop the tools, and let the community reach a consensus.

Platform Infrastructure. The foremost task for the knowledgebase platform is to provide the user to the underlying knowledgebase data, if necessary shielding the user from how that access is achieved (e.g. federated versus centralized, cloud-based versus central server, etc.). It should also provide the user with elementary analysis and visualization tools to apply to that data, a way to store intermediate results, data standards to allow data to be exchanged between tools, and ways to chain analysis tools together to create ad-hoc interactive workflows. In addition, it should provide a low-threshold infrastructure for tool development, reuse, and dissemination.

User Empowerment and Community Collaboration. Regardless of the size and quality of the Knowledgebase development team, there will inevitably be more developers, talent, and ideas (not to mention time to implement) “outside” than “inside.” We should aim to leverage the talent within the Knowledgebase user community to develop and choose the best tools. Many novel bioinformatics tools suffer from a “failure to launch,” never reaching beyond the initial

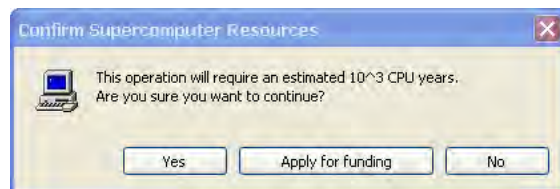
journal publication, due to a lack of a web-based implementation or lack of marketing skills of the developer. By enabling tool developers to integrate their tools with the Knowledgebase platform and tie directly into its user interface, we can expose a wider variety of tools to a wider variety of users and enable more users to become tool developers themselves.

Components, Scripts, and Open-Source Development. Individual tools may be as simple as calculating the GC content of a DNA sequence or displaying a matrix of numbers as a heatmap. More complex tools can then be constructed by combining these elementary components, piping data from one tool to another—similar to unix shell scripts that are composed of elementary data manipulations such as “grep” and “sort,” with some control logic to pipe data between them. At the extreme end, entire processing pipelines could be encapsulated into a single tool, calling upon dozens of other analysis and visualization tools. By making these tools open source, we can enable even relatively novice programmers to tinker with and improve upon them (e.g. swapping out one statistical test for an improved one or adding a novel visualization tool).

Reputation, Attribution, and Credit. If we open up tool development to the world, some mechanisms are needed to enable the community to disseminate, vote, and prioritize the highest quality tools. The reputation of a tool may be based on various factors, including usage statistics (how many other tools incorporate this tool and how frequently is it actually called), direct votes by users, and the reputation of its developer. Developer reputation in turn would depend primarily on the reputation of the tools they have contributed. Community reputation may be a powerful incentive for contribution, especially for junior members. A credible mechanism for attribution and credit could potentially also be used to drive funding and even tenure decision for tool developers, on par with journal impact factors.

Some important issues which need to be resolved:

Computing Resources. Some tools can easily be run on the user’s own computer, some should be run on the server side because of higher CPU or storage requirements (e.g., BLAST against NR), others may require substantial high-performance (or cloud) computing capabilities. How do we throttle processes to achieve an equitable distribution of resources? How do we keep users from making an expensive mistake? How should we deal with a “poorly behaved” tool? Can we estimate a priori (e.g., based on previous usage statistics) how much computing power a specific tool will require? How do we fund additional computing time on this system? Can users simply buy more compute power on the cloud?



Incentives for Contribution. How do we encourage an active and vibrant developer ecosystem? Some of the larger components such as the Knowledgebase platform and early applications will need to be developed under more conventional funding models. But as the programming platforms become established, the project will need to consider funding models designed to maintain and expand innovation over the long term. Significant attention should be paid during the design phase of the Knowledgebase platform to social engineering and design of interactions between tool developers and scientists. How do scientists share and evaluate

tools? How can we leverage existing networks of interaction to enhance community buy-in and involvement?

A suitable pilot project for an open Knowledgebase development platform might be to mirror part of an existing genome database (e.g. IMG, MicrobesOnline, SEED, etc.), implement rudimentary tool development infrastructure, make this platform accessible online, and then invite an external user to write a novel analysis or visualization tool not already found in the original database.

Sidebar: the Facebook Platform

Facebook released its “Facebook Platform” in May 2007, enabling users to “build the next generation of applications with deep integration into Facebook, mass distribution through the social graph, and a new business opportunity.” The Facebook experience has shown that this is an excellent way to involve the community in the development of the platform. Users immediately jumped on the opportunity and started generating little tools and widgets—sometimes in direct competition with tools Facebook had already implemented. The Platform provides multiple integration points for apps to integrate seamlessly into the existing Facebook user interface. Many Facebook apps turn out to be useless or poorly designed and disappear into obscurity, but some are absolute hits and propagate rapidly throughout the community, resulting in far more high-quality tools than the Facebook developers could ever have implemented themselves. As of June 2009, two years after introduction of Facebook Platform, Facebook reported 350,000 active applications from over 950,000 developers. A significant part of the Platform infrastructure itself was open-sourced in 2008, and it is possible that some pieces of this could be leveraged, although the needs for a Knowledgebase platform are likely to be very different than for a social networking site like Facebook. Note, however, that the underlying Facebook database is much larger than existing genomic databases and has orders of magnitude more users and hits.

4e. Institutional and Career Considerations Surrounding Open-Source Development

This section describes some considerations with regard to institutional technology transfer philosophy and the career impact of open-source software development and use in the DOE BER Knowledgebase program.

Science assumes a clear account of methodology that is repeatable by others. Open source provides a definitive account of methods where software was used in analysis. Open source has become widely practiced in federally funded research. An Open Source Policy would be at least in keeping with the spirit of recently proposed legislation to provide free open access to all federally funded research within 6 months of publication. Open source does not have to mean immediate release. The Open Source Policy could be similar to the Data Release Policy where there is some allowance for limited access before being made public. At a glance, open source seems like an obvious choice for the Knowledgebase, but there are real issues associated with making all software immediately open source. Key issues are discussed below:

(1) Experience has shown that conflict arises between the open-source concept and the desire by home institutions to license IP in the process of facilitating technology transfer. This has in the past involved situations where the home institution licensed software to companies and

earned revenue from software developed under DOE grants. This issue can arise within universities, national laboratories, and private industry. While resolvable, this will need to be addressed. In the open-source scenario, where software is publicly available, licensing efforts are defeated, and the contractors performance and thus award fee depends partly on their success with technology transfer. This will depend in part as to whether the software has unique value or is merely routine in nature. While an open-source policy might seem in conflict with an expectation of technology transfer placed on institutions, such policy is intended to encourage rapid transfer of IP to provide a basis for new business development and benefit society. An open-source policy would accomplish that objective.

(2) The DOE Genomic Science program has a strong history of doing bioinformatics research—that is, developing new algorithms or solving the hardest computational problems in new ways. Examples include gene finders, transcription binding predictions, protein structure prediction, protein dynamics and function, metabolic pathway simulations, and large-scale cellular process modeling. To a researcher in bioinformatics, such products represent the publishable results of research, and such investigators have a right to publish their algorithms and their performance in the literature prior to open release in a manner similar to experimental scientists. As we work toward large infrastructure and in many current national laboratory SFAs (Science Focus Areas), we see the role of bioinformatics changing in part from research to that of programming and operational support, for example in building databases or websites for projects. It is clear that we have or perhaps should have two classes of bioinformatics tasks: (i) publishable research, which develops new algorithms or methods for key problems, and (ii) infrastructure support and development, which is likely to be much less publishable and where methods are more likely to be mature. The infrastructural element is analogous to core facilities for major experimental capabilities such as sequencers.

[Fig. 1](#) illustrates the potential collaborative nature and continuum of interests and capabilities across the scientific community between the pure experimentalists and pure computationalists that become evident in the context of the Knowledgebase as a platform for future research that brings different groups and communities together. Publishable tools development is an aspect of research, while the infrastructure development is more linked with the development of the Knowledgebase itself. Infrastructure development and deployment are much more amenable to immediate open-source standards, with rewards to such individuals much less likely to be publications or novel results. A concern is that in large projects and in the push toward open source, we forget that the research mission in bioinformatics (tools development) is very important and the types of individuals that do such research are vital to the DOE Genomic Science program. There needs to be a strong research activity to generate solutions for next-generation problems in bioinformatics. We need to identify proper incentives for both paths and encourage top people in both for sustained careers. Ultimately both are required, working together, to attain the ambitious scientific objectives of the future.

4f. Other Potential Science Objectives and Knowledgebase Features Drawn from Responses to Preworkshop Charge Questions

These potential science objectives were drawn from the online responses to the charge questions. This section needs further expansion and revision for the final report. The development of these lists from this and prior workshops will speed the community's process of identification and then prioritization of the set of scientific objectives that will be developed in much greater detail needed for the final workshop and ultimately for the final report—the Knowledgebase Implementation Plan.

Regarding data quality and annotation:

- Use data quality indicators.
- Use experimentally verified data only.
- Create a clean computing system using some model organisms with only experimentally verified data.
- We need the ability to update annotations in genome sequence repositories.
- Need statistical correlations of datasets.
- We need standard quantitative approaches for dealing with the different data types for normalization and assessment of statistical significance.

Regarding microbial community omics data integration, we need the following capabilities:

- To integrate short read sequence data (Illumina data) and proteome data.
- To routinely integrate all omics data for every newly sequenced organism to minimally include: RNA sequences, proteomics, metabolic phenotype (Biolog) profile. Move beyond gene-based annotations to pathways.
- Comprehensive tools that allow integrating and comparing multimolecular datasets, which are needed to fully realize the vision of microbial systems ecology.
- To integrate sequencing data and downstream analysis into a common analytic pipeline that enables end users at different skill levels to interrogate their data in interactive ways in real time. Controlled vocabularies or ontologies to leverage metadata across different organisms or samples.
- To link genotype with biogeochemistry or biogeography.
- Under defined conditions, to compare proteins expressed in related strains of bacteria to predict metabolic potential of microbial communities and resolve physiological differences; use this information to identify biomarkers diagnostic for specific biogeochemical processes. Intercompare spectral libraries to identify unique peak profiles as new gene models are added to the protein database.

Appendix D

DOE Systems Biology Knowledgebase Workshop Report from the 5th Annual JGI User Meeting, March 23, 2010

Appendix 1: Agenda

DOE Systems Biology Knowledgebase Workshop

Walnut Creek, California

Tuesday, March 23, 2010

8:30 a.m. – 8:40 a.m.	Welcome, Susan Gregurick
8:40 a.m. – 9:00 a.m.	“Introduction to Knowledgebase Initiative and Workshop Objectives” Bob Cottingham
9:00 a.m. – 9:30 a.m.	“Science Presentation on Metagenomics: Current Experience and Future Expectations for the Knowledgebase” Phil Hugenholtz
9:30 a.m. – 10:00 a.m.	“Science Presentation on Metagenomics: Current Experience and Future Expectations for the Knowledgebase” Jill Banfield
10:00 a.m. - 10:30 a.m.	Panel Summary and Audience Questions
10:30 a.m. – 11:00 a.m.	Break
11:00 a.m. – 12:30 p.m.	“Informatics Perspectives and Roundtable: How to Transition from the Present Towards an Open, Shared, Integrated Knowledgebase” Discussion Leads: Adam Arkin, Folker Meyer, Ed Uberbacher, Nikos Kyrpides, Peter Karp, Tatiana Tatusova, Victor Markowitz, Bob Cottingham
12:30 p.m. – 1:00 p.m.	Working Lunch
1:00 p.m. – 1:30 p.m.	“Presentation of Metagenomic Workflow Example” Jill Banfield
1:30 p.m. – 3:00 p.m.	Panel Discussion, Jill Banfield
3:00 p.m. – 3:30 p.m.	Break
3:30 p.m. – 4:30 p.m.	Panel Discussion, Jill Banfield
4:30 p.m. – 5:00 p.m.	Conclusions and Adjourn, Bob Cottingham

Knowledgebase Wiki: sites.google.com/a/systemsbiologyknowledgebase.org/kbase/

Appendix 2: Participants and Observers

Participants

Adams, Paul (LBNL)

Allen, Eric (University of California, San Diego)

Allgaier, Martin (LBNL, JBEI)

Anderson, Gordon (PNNL)

Arkin, Adam (LBNL)

Baker, Scott (PNNL, JGI)

Banfield, Jill (University of California, Berkeley)

Benton, David (University of Wisconsin)

Bhaya, Devaki (Stanford University)

Bowen, Ben (LBNL)

Bownas, Jennifer (ORNL)

Brettin, Tom (ORNL)

Bristow, Jim (LBNL, JGI)

Broughton, Jeff (LBNL)

Canon, Shane (LBNL)

Chen, Amy (LBNL, JGI)

Chivian, Dylan (LBNL, JBEI)

Collart, Frank (ANL)

Cottingham, Bob (ORNL)

Davison, Brian (ORNL)

D'haeseleer, Patrik (LLNL)

Drell, Daniel (DOE BER)

Foust, Cheri (ORNL)

Gorton, Ian (PNNL)

Gregurick, Susan (DOE BER)

Grossman, Arthur (Stanford University)

Hallam, Steven (University of British Columbia)

Heidelberg, Karla (University of Southern California)

Hess, Matthias (JGI)

Hugenholtz, Phil (JGI)

Jansson, Janet (LBNL)

Karp, Peter (SRI International)

Kleese van Dam, Kerstin (PNNL)

Kodner, Robin (University of Washington)

Konstantinos, Mavrommatis (JGI)

Kosky, Anthony (JGI)

Kuske, Cheryl (LANL)

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Swingley, Wesley (University of California, Merced)

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Thomas, Brian (University of California, Berkeley)

Tringe, Susannah (JGI)

Uberbacher, Edward (ORNL)

Wang, Zhong (LBNL)

Woyke, Tanja (JGI)

Acronyms

ANL	Argonne National Laboratory	LBNL	Lawrence Berkeley National Laboratory
DOE	U.S. Department of Energy	NIH	National Institutes of Health
GLBRC	Great Lakes Bioenergy Research Center	NREL	National Renewable Energy Laboratory
JBEI	Joint BioEnergy Institute	ORNL	Oak Ridge National Laboratory
JGI	Joint Genome Institute	PNNL	Pacific Northwest National Laboratory

DOE Knowledgebase System Development Workshop Report

June 1–3, 2010, Crystal City, Virginia

Workshop Organizers: Susan Gregurick (DOE) and Bob Cottingham and Brian Davison (Oak Ridge National Laboratory)

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1. Introduction

This report reviews discussion and material associated with the Department of Energy (DOE) Knowledgebase System Development workshop held June 1 - 3, 2010. The goal of this workshop was to establish initial actionable plans to create the Knowledgebase. The first day focused on the prioritization of clear scientific objectives and specific requirements for the Knowledgebase derived from these objectives. The second day focused on the development of an implementation plan, system architecture, and governance for the initial system. The last day focused on finishing writing assignments leading to the Final Report, which will be the plan for creating the Knowledgebase.

First, a background summary is given below describing the purpose of the DOE Systems Biology Knowledgebase (Kbase) planning project. Next is a summary of pre-workshop activities, topics presented and discussed during the workshop, and post-workshop activities.

Since the goal of the Knowledgebase planning project is to develop an initial prioritized plan for a useful systems biology knowledgebase, there is a continued consensus that these initial efforts cannot be all things for all users. It is better to show strong success in a few areas than minimal progress in many areas. There was also continued consensus on the principles from past workshops on which Kbase is being founded that (1) science drives Knowledgebase development, (2) the project be a community effort, (3) that it be open access and open contribution, and (4) that it be distributed.

2. Background

The Department of Energy Genomic Science program, within the Office of Biological and Environmental Research (BER), supports science that seeks to achieve a predictive understanding of biological systems. By revealing the genetic blueprint and fundamental principles that control plant and microbial systems relevant to DOE missions, the Genomic Science program (genomicscience.energy.gov/) is providing the foundational knowledge that underlies biological approaches to producing biofuels, sequestering carbon in terrestrial ecosystems, and cleaning up contaminated environments.

Knowledgebase Vision and Background

The emergence of systems biology as a research paradigm and approach for DOE missions has resulted in dramatic increases in data flow from a new generation of genomics-based technologies. To manage and effectively use this ever-increasing volume and diversity of data, the Genomic Science program is developing the DOE Systems Biology Knowledgebase—an open, community-driven cyberinfrastructure for sharing and integrating data, analytical software, and computational modeling tools. Historically, most bioinformatics efforts have been developed in isolation by people working on individual projects, resulting in isolated databases and methods. An integrated, community-oriented informatics resource, such as the Knowledgebase, would provide a broader and more powerful tool for conducting systems biology research relevant to BER’s complex, multidisciplinary challenges in energy and environment. It also would be easily and widely applicable to all systems biology research.

In general, a knowledgebase is an organized collection of data, organizational methods, standards, analysis tools, and interfaces representing a body of knowledge. For the DOE Systems Biology Knowledgebase, these interoperable components would be contributed and integrated into the system over time, resulting in an increasingly advanced and comprehensive resource. Other elements of the Knowledgebase vision are defined in a March 2009 report (genomicscience.energy.gov/compbio/) based on a DOE workshop that brought together researchers with many different areas of expertise, ranging from environmental science to bioenergy. The report highlights several roles the Knowledgebase will need to serve.

Workshop Background

To develop a successful open informatics endeavor for systems biology (the Kbase effort), a series of workshops have been held to include key stakeholders (plant and microbial genomic researchers, bioinformaticians, computer scientists, database developers, and software engineers) and to elicit their goals, challenges, and expectations for the development and management of the Kbase. This final workshop was a culmination of these previous workshops to provide clear prioritization and tasks to allow the final design and implementation of the Kbase to be developed. The workshop was held June 1–3 and involved 80 participants representing university, national laboratory, and international researchers. In addition, the workshop had representation from DOE's Joint Genome Institute; DOE's Bioenergy Research Centers; NSF's iPLANT; and NIH's NCI and NCBI. The goal of the workshop is to develop a robust design and implementation plan for the Systems Biology Knowledgebase. The participants were charged with developing and prioritizing 3 to 5 scientific objectives in each of the areas of microbial, meta-communities, and plant research. From these scientific objectives, two days were spent developing scientific requirements, time frames, and effort for each of the scientific objectives. An additional half day was spent discussing detailed plans for architecture, implementation, and governance. Extensive pre-meeting conference calls helped to lay the groundwork of the science objectives. Participants were not charged to define funding or contractual structures, and they are continuing to finalize requirements based on the discussed objectives and transfer these into an implementation plan.

Outlined below are the prioritized scientific objectives and rough time frames for the implementation of these objectives. The details of the requirements of each objective as well as the architecture and governance plans will be developed over the summer, culminating in a final implementation plan report by September 30, 2010.

3. Pre-Workshop Activities

3a. Conference Calls

A series of conference calls were scheduled in May before the workshop. The first of these were to organize the three science area breakout leads. Each science area (Plant, Microbial, and Meta-Communities) had two leads for Scientific Objectives and two leads for Requirements. Then calls were held with all members of each breakout. The first call was to introduce the workshop and define what is meant by a Scientific Objective, and the second call focused on reviewing the Scientific Objective template and beginning discussion on what would be the recommended Strawman List of Scientific Objectives for each breakout. At the workshop, the Strawman List would be reviewed and finalized on the first day, and participants would establish the consensus priority (High, Medium, Low) and feasibility (Near: 1-3 years; Mid: 3-5 years; Long 5-10 years). Based on this, the top 3 to 5 Scientific Objectives would be the focus of the initial Kbase.

A third call introduced the template for Requirements and how these would be derived from a Scientific Objective. The most detailed and complete Requirements write-ups are needed for the top Scientific Objectives, with decreasing detail needed for mid- and long-term Objectives.

3b. Templates

The Scientific Objectives and Requirements templates—along with filled-out examples that were given to the participants—are included in Appendix 3 and 4, respectively. These provide focused guidance toward establishing the most important objectives and detailed requirements that guide Kbase development.

3c. Google Docs

In order to begin rapid development of the Scientific Objectives, a Google Docs folder was established for each breakout group. Writing teams then formed around each proposed Scientific Objective, and a significant amount of preliminary writing was accomplished in advance of the meeting. During the conference calls, multiple participants would edit the draft documents as they were being discussed. Initially, these areas were accessible only by members of the breakout. At the workshop all areas were made accessible to all participants.

3d. Goal to Establish Scientific Objectives and Requirements

For most attendees, this was a different kind of workshop. Its primary focus was on establishing the best Scientific Objectives and Requirements for the DOE Systems Biology Knowledgebase, especially the high-priority requirements for the first 1-3 years. The Requirements are the most important result of the workshop, as these define what the initial Kbase will be. The Science Area breakouts first focused on the Scientific Objectives, and then in the second half of the first day, the Breakout Leads switched and the focus was on Requirements with the same Breakout group. This process allows an easier transition from objectives to requirements and encourages feedback so the objectives are tractable.

4. Topics Discussed at Workshop

4a. Microbial Science Objectives

1. Integrated Description of Genomic Features

Summary: This objective will create the ability to represent and update experimental data and inferred knowledge about genes and genomes so that the experimental and computational results drive progressively richer and more accurate gene models and predictions. This ability would allow users to access existing genomic sequence information, upload new experimental data in order to define and refine models, and test consistency between the two. This objective was given high priority, as many other objectives require this ability to build on. This objective requires integration with JGI/IMG and NCBI and will require some standards development for data and access to large-scale computing resources. This objective will take 1-3 years.

2. Reconstruction, Prediction, and Manipulation of Metabolic Networks

Summary: The scientific objective is to provide a method to evaluate the metabolic potential of an organism, predict the phenotypic outcome of specific metabolic or environmental interventions and perturbations, and establish metabolic kinetics capabilities and fluxes for short-term dynamic responses. This knowledge will lead to the informed

modification of one or more specific enzymes or the introduction of entirely new enzymes and/or pathways for metabolic engineering purposes. This objective would allow the community to better determine strategies for carbon flow manipulation and for understanding microbial communities. This objective requires integrating new experimental data with known data and models on metabolic pathways, as well as developing methods to automatically create new metabolic reconstructions from newly sequence organisms. This objective requires linking together known metabolic models with databases such as chEBI, UniPROT, KEGG, and GO with experimental data. This objective is given medium priority (3-5 years), and it is suggested to apply this objective to a selected set of organisms relevant to DOE's research efforts.

3. Microbial Gene Expression Regulatory Networks

Summary: The scientific objectives can be broadly divided into two components. The first is to enable automated inference of gene expression regulatory networks relying principally on expression profiling data. The second is to extend these inferred networks to include additional data types, both to refine the network predictions and to test them. The availability and evolution of genome-scale expression data and the rapid extension into new data types makes the definition of microbial gene expression regulatory networks an attractive goal of the Kbase project. In the short-term, inference of regulatory networks from just genome sequence and expression profiles under varied cellular conditions is possible and could be of general utility to researchers in constructing and understanding of carbon and nitrogen processes. Interconnection of the regulatory networks with metabolic reconstructions and multidimensional annotations (two other high-priority objectives identified by the Kbase microbial group) would greatly facilitate development of microbial systems biology. This objective could work synergistically with NIH pathway tools, EcoCYC, and DOE efforts such as MicrobesOnline and JGI. Much of the experimental work would come from the Bioenergy Research Centers and the larger DOE science-focused work on microbial systems. This project was given high priority. This objective can achieve some near-term goals but may take 2-10 years to complete. It was suggested to work on DOE-related organisms and in coordination with the second scientific objective.

4b. Metagenomics/Meta-Communities Science Objectives

1. Metabolic Modeling of Microbial Communities

Summary: This objective focuses specifically on modeling the metabolic processes within a microbial community, since this topic most directly ties into developing metagenomics workflows and the single microbial organisms systems biology tools (above). This predictive understanding of communities will progress in three stages: **(1) Understanding:** Descriptive models that provide insight into the metabolic role of the members within the community and their interactions. **(2) Prediction:** Predictive models that allow us to simulate the metabolic processes in the community and the response of community activity or composition to environmental conditions. **(3) Manipulation:** Eventually, these models will allow us to not only predict, but actively drive changes in the community into desired directions (e.g., to accelerate environmental processes such as bioremediation, cellulose

degradation, or carbon sequestration). This objective outlined as a first step the Knowledgebase need to develop workflows for analyzing metagenomes of a microbial community and to leverage existing data to create community metabolic models. This objective was seen as a medium priority (3-5 years) and would require leveraging existing tools (IMG, MG-RAST, CAMERA) and databases (BioCyc, KEGG) as well as developing analysis tools.

2. Expand Our Understanding of Poorly Studied Genes

Summary: Data generated in large-scale metagenomics projects can provide the information necessary to better understand the function of poorly characterized genes. This scientific objective is to develop approaches for (1) mining the data in order to identify previously unknown genes and (2) leveraging the wealth of metadata associated with metagenomic datasets, as well as gene/organism co-occurrence information in order to identify testable hypothesis about the function of newly identified or poorly characterized genes. This objective was given high priority and could leverage all of the metagenomic sequencing efforts from DOE and NIH.

3. Analysis of Understudied Microbial Phyla

Summary: The goal of this objective is to understand the role of unclassifiable members of a microbial community in terms of genetic and phenotypic comparison. To achieve this scientific objective, a specific requirement will be linking physiologic and metabolic datasets to metagenome annotations in order to provide context and evidence. This will create a product that is more informative and flexible. The specific datasets that will be utilized are the genomes and accompanying physiologic and metabolic data of understudied microbial phyla. Questions that this objective would address are: (1) where are members of a novel phylum found, (2) how do we facilitate phylogenetic binning to preclude assignment as orphan genes, and (3) what are the emerging concepts of their metabolomes? This objective was given medium priority (3-5 years) and requires the development of infrastructure and tools to accomplish the goals. This will likely be merged into Objective 2.

4. Metagenomic Interpretation to Identify Conditions Required for Growth by Key Microbial Communities Relevant to DOE Missions.

Summary: Using a partial single microbial genome found within microbial communities, can we predict how to cultivate (and isolate) this target species? Put another way, can we predict culture conditions from genomic information? This will require metagenomic sequence, assembly into species genomes, and pathway analysis of these partially assembled genomes. While workflows do exist to perform some of these tasks, they will need to be developed much further and altered to make use of supercomputing facilities to handle gap-finding exercises. It is not clear if relevant tools exist, and this was given medium priority, as it will take years to develop (5-10 years).

4c. Plant Science Objectives

1. Integration of Phenotypic and Experimental Metadata to Enable Prediction of Biomass Properties based on Genotype

Summary: Improvements in computational infrastructure are required to support and contextualize experimental plant phenotype data to an extent that will enable one to predict the changes in the physical properties of biomass properties that occur as a result of environmental changes and genetic diversity or manipulation. Achievement of this ambitious goal depends on the creation of robust semantic infrastructure for collection, annotation, and storage of diverse phenotypic and environmental datasets. These data include measurements such as photographic images and analytical spectra that capture visible phenotypes and chemotypes that are fundamentally related to yield and physiological performance and sustainability. Specifically, this infrastructure will be used as a basis for software applications that extract, quantify, and catalogue phenotypic features from the data for the purpose of data mining and further analysis. This involves association of the data with relevant metadata to enable querying, modeling, clustering, and comparison of the data from diverse datasets generated by different platforms. Attainment of the scientific objective requires appropriate vocabulary standards for wide variety of data and metadata that describe phenotypes, chemotypes, genotypes, and the experiments designed to collect this data. Although several such standards and ontologies exist, they require additional expressiveness to achieve the objective. In order to share the relevant experimental data and ensure its completeness (in terms of associated metadata, etc), a community approved standard for the Minimum Information for A Plant Phenotyping Experiment (MIAPPHE) would be helpful. However, such a standard does not currently exist. The development of all of these standards demands a long-term, committed collaboration between computer scientists and plant scientists. This objective was seen as high priority and could be carried out in 3 to 5 years. This would require a community of scientists to agree to standards of data to describe phenotypes and needs to be coordinated with iPLANT.

2. Assemble Regulatory Omics Data in Common Platforms to Enable Annotation, Comparisons, and Modeling

Summary: This objective will integrate several key types of regulatory omic data and associated quality and metadata for six target plant species: *Brachypodium*, *Chlamydomonas*, poplar, sorghum, switchgrass, and *Miscanthus*. This information will support the other objectives, including annotation, comparison, and modeling. RNA levels as measured by expression arrays or RNA-Seq are no longer sufficient to evaluate mechanisms and networks that regulate plant transcriptomes. The Kbase must also include available small RNA and target RNA information, differential RNA processing and decay information, and epigenetic marks such as DNA methylation and histone modifications. This information is important for data integration and to fill in important missing links in gene regulatory networks within a species and facilitate their comparison across two or more species. In the short term (1-3 years), classical transcriptome data (microarrays and mRNA

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DOE Knowledgebase System Development Workshop Report, June 1–3, 2010

seq) as well as small RNA and basic proteomic data will be assembled. Epigenetics data, small RNA target data/RNA degradome data, other types of RNA processing data, and additional proteomic data will be assembled after year one, with the most developed genomes such as *Brachypodium* beginning first. The data will be made publicly accessible with user-friendly web interfaces and downloadable for power users. The acquired data will include sequences, quality information (e.g., Q values) and associated metadata. Sources will include NCBI (GenBank, GEO, SRA), the DOE JGI, ArrayExpress, and PLEXdb. This was given high priority and could be accomplished in 1-3 years. This requires collaboration with iPANT and USDA for selection of relevant species.

3. Improvement and Availability of Plant Genome Annotation Datasets

Summary: Currently, plant genomes are typically annotated in isolation and with varying methods. Even more problematic is that the annotation is rarely, if ever, updated. As a consequence, annotation across genomes is not comparable, becomes stale rapidly, and frequently is of undocumented quality. Without confidence in the gene model annotations, biological interpretations will be greatly hampered, if not erroneous. The research goal is to generate high-quality, documented, uniform, and integrated annotation for plant genomes. Six target genomes have been identified (*Brachypodium*, *Chlamydomonas*, sorghum, poplar, switchgrass, and *Miscanthus*). The goal is to develop a platform that results in higher-quality annotation than what has been provided to date rather than to annotate more genomes. In the initial phase, only two genomes that are phylogenetically diverse will be annotated in years 1-2. Subsequently, in years 2-3—with refinement of the platform—another two genomes will be annotated, and the platform will be further refined. In years 3-10, all genomes will be iteratively annotated to capture newly available empirical data and algorithmic improvements. This scientific objective would need to be coordinated with the 'omics data integration objectives and with DOE JGI, NCBI, iPLANT, and the plant communities. This was given high priority and would be accomplished in 1-3 years.

4. Modeling, Simulation, and Validation

Summary: Enable semi-automated inference, construction, simulation, validation, and query of complex multilevel (gene, protein, metabolite, small RNA, organelle, cell, and tissue) models of plant life, with a focus on models useful for integration and exploration of experimental data types collected during study of biomass recalcitrance, the carbon cycle, and bioremediation. Four sub-objectives proposed herein are automation and streamlining of model construction; development of a semi-automated model validation process; development of advanced semantic querying capability targeted to biological models and representations; and phylogenetic inference of functional networks (itself a model construction exercise). Model construction and validation are very closely aligned with Kbase objectives. Exploratory model construction is completely dependent on a conceptual framework, together with multiple datasets (annotated genome, proteomic, metabolomic, transcriptomics) to populate instances of this framework. Validation depends on well-structured and -annotated experimental data. At the same time, the dependencies are modular, which facilitates separate development of software for specific or more

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DOE Knowledgebase System Development Workshop Report, June 1–3, 2010

generalized tasks. Semantic query will enable scientists to more rapidly and precisely develop hypotheses and conclusions from the complex metabolic and regulatory models that arise from genome-scale studies. This science objective requires interfacing with existing plant genomic databases as well as KEGG, GO, Metacyc, PMN. This was given high priority but was also noted to take up to 10 years in stages.

4d. Computational Area Breakouts: System Architecture, Implementation Plan, and Governance

On the second day of the workshop, a follow-up set of breakouts was held to address the major topics associated with constructing the Knowledgebase computation system. The System Architecture group is working to establish the technical principles and basis for recommending specific System Architecture, considering specific architectural attributes and their relative priorities. The Implementation Plan group is evaluating each Scientific Objective and associated Requirements to assess the major tasks and recommended plan for implementation. The Governance group is considering and will recommend a governance model and principles that will guide the development, management, and operation of the Knowledgebase for the research community. Based on the Kbase vision, principles, and scientific objectives, each of these groups is working toward writing up recommendations for the associated sections of the Final Report.

5. Post-Workshop Plan

Each breakout topic group is finalizing its write-ups with a June 30 deadline. The focus has been on completing the Scientific Objectives and Requirements and then on integrating these into a Science Area report that will become part of the Final Report. In parallel, work is under way to create the Implementation Plan section for each of the Scientific Objectives that typically focuses on the 3-4 major required tasks and then the associated effort and expertise recommended to accomplish the tasks.

A follow on writing meeting will be held in July that will focus on finalizing the Implementation Plan for each Scientific Objective.

Appendix 1: Agenda

DOE Knowledgebase System Development Workshop

Crystal City, Virginia
Tuesday, June 1, 2010

June 1, 2010

9:00 a.m. – 9:10 a.m.	Welcome, Susan Gregurick
9:10 a.m. – 10:00 a.m.	Workshop Objectives and Expectations, Bob Cottingham
10:00 a.m. – 12:00 p.m.	Divide into Three Breakout Groups Microbial Communities — Scientific Objectives Breakout Leaders — Jim Liao and Wim Vermaas Plant Communities — Scientific Objectives Breakout Leaders — Maureen McCann and Pam Green Meta-Communities — Scientific Objectives Breakout Leaders — Jack Gilbert and Jared Leadbetter
10:30 a.m. – 10:45 a.m.	Break
12:00 p.m. – 12:30 p.m.	Working Lunch
12:30 p.m. – 2:00 p.m.	Breakout Groups report back on Scientific Objectives and Priorities
2:00 p.m. – 4:00 p.m.	Divide into Three Breakout Groups Microbial Communities — Requirements Breakout Leaders — Bernhard Palsson and Bob Landick Plant Communities — Requirements Breakout Leaders — Robin Buell and Will York Meta-Communities — Requirements Breakout Leaders — Steve Slater and Jeff Grethe
3:00 p.m. – 3:15 p.m.	Break
4:00 p.m. – 5:30 p.m.	Breakout Groups report back on Requirements and Priorities
5:30 p.m. – 5:45 p.m.	Conclusions and Adjourn, Bob Cottingham
6:30 p.m.	Working Dinner for Chairs and Breakout Leaders

June 2, 2010

8:00 a.m. – 8:15 a.m.	Recap of June 1, Bob Cottingham
8:15 a.m. – 10:00 a.m.	Divide into Three Breakout Groups

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Microbial Communities — Requirements

Breakout Leaders — Bernhard Palsson and Bob Landick

Plant Communities — Requirements

Breakout Leaders — Robin Buell and Will York

Meta-Communities — Requirements

Breakout Leaders — Steve Slater and Jeff Grethe

10:00 a.m. – 10:15 a.m.	Break
10:15 a.m. – 12:00 a.m.	Breakout Groups report back on Final Requirements
12:00 p.m. – 12:30 p.m.	Working Lunch
12:30 a.m. – 4:00 p.m.	Divide into Three Breakout Groups

System Architecture

Breakout Leaders — Ian Gorton and Dan Stanzione

Implementation Plan

Breakout Leaders — Peter Karp and Ed Uberbacher

Governance

Breakout Leaders — Miron Livny and Steve Goff

3:00 p.m. – 3:15 p.m.	Break
4:00 p.m. – 5:30 p.m.	Breakout Groups report back on System Architecture, Implementation Plan, and Governance
5:30 p.m. – 5:45 p.m.	Conclusions and Adjourn, Bob Cottingham

June 3, 2010

9:00 a.m. – 9:40 a.m.	Recap of June 1st and 2nd, Bob Cottingham
9:40 a.m. – 10:30 a.m.	Writing Assignments
10:30 a.m. – 11:00 a.m.	Break
11:00 a.m. – 12:30 p.m.	Group Recap, Bob Cottingham <ul style="list-style-type: none"> • Where we are? • Missing pieces • Assignments
12:30 p.m. – 1:00 p.m.	Working Lunch
1:00 p.m. – 4:45 p.m.	Continue work
3:00 p.m. – 3:30 p.m.	Break
4:45 p.m. – 5:00 p.m.	Conclusions and Adjourn, Bob Cottingham

Knowledgebase Wiki: sites.google.com/a/systemsbiologyknowledgebase.org/kbase/

Appendix 2: Participants and Observers

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Acronyms

ANL	Argonne National Laboratory	LBNL	Lawrence Berkeley National Laboratory
BESC	BioEnergy Science Center	LLNL	Lawrence Livermore National Laboratory
BNL	Brookhaven National Laboratory	NIH	National Institutes of Health
DOE	U.S. Department of Energy	NREL	National Renewable Energy Laboratory
BER	Biological and Environmental Research	ORNL	Oak Ridge National Laboratory
GLBRC	Great Lakes Bioenergy Research Center	PNNL	Pacific Northwest National Laboratory
JBEI	Joint BioEnergy Institute	USDA	U.S. Department of Agriculture
JGI	Joint Genome Institute		

Appendix 3: Scientific Objectives Template

Scientific Objective: <title – Note: 1 Objective per each filled in template>

Breakout Group: <group>

Contributing Authors: <authors>

Date: <date>

1. Scientific Objective

Brief statement of Scientific objective

[What is the scientific or research goal? What is written here will usually be derived after filling in the remainder of the template. Responses to sections below will help to refine this statement. Sometimes it is easier to think of an objective in terms of a problem that exists that needs to be solved.]

Background information

[Include ongoing experiments, future planned experiments, historical results, literature references, relevant past impediments to research progress, etc.]

2. Prioritization

[This is meant to help prioritize this scientific objective in the context of other scientific objectives. There are several axes of consideration. One is the need or benefit to the research community. Another is the level of difficulty or feasibility.]

PRIORITY (check one): HIGH MEDIUM LOW

Potential Benefits

[Why is this objective important? What is its level of impact? What would the benefit be? Who would benefit?]

Feasibility of success Near, Mid and Long term

[What is the level of difficulty? How likely is it that this objective can be achieved in a 1-3 year time frame? What would be the measure of success? Consider and rate feasibility in the near term (1-3 years, midterm (3-5 years) and long term (5-10 years). The most important objectives, those that are high priority and most feasible in the near term must have the most detail. Mid and long term can be provided in decreasing levels of detail.]

TERM (check one): NEAR (1-3 years) MID (3-5 years) LONG (5-10 years)

Relevance to DOE systems biology knowledgebase project

[The DOE Genomic Science program's ultimate goal of achieving a predictive understanding of biological systems is a daunting challenge and will require the integration of immense amounts of diverse information. The DOE Systems Biology Knowledgebase is envisioned as an open cyber-infrastructure to integrate systems biology data, analytical software, and computational modeling tools that will be freely available to the scientific community. Briefly explain how the proposed objective is relevant to what is envisioned for Kbase.]

Synergies/Leverage: Potential overlap with other projects or funding agencies

[Are there existing systems that relate to this objective such as NCBI, GenBank, BioCyc, iPlant, etc. Is there a potential for synergy that would benefit both efforts? Is there a potential overlap that needs to be resolved?]

3. Specificity

[This section pertains to finding the right level of objective, especially avoiding objectives that are specified at too high a level. Start with a high level objective and refine. What is the specific science question to be answered?]

4. Details

[The intent here is to begin to capture elements that form the basis for continuity between the science objective and the software requirements that are derived from this objective. We start to articulate high level requirements here that are further refined in the requirements document.]

Scientific discovery process (workflows)

[Have workflows already been developed or can they be derived from existing work?]

Inputs

[What datasets would be required? Are there data standards? Are there available data sources or examples? Are there publications that use or describe an associated analysis process?]

Outputs

[What would the results be?]

Tools

[Existing or new analysis software]

REFERENCES

[Use as needed]

APPENDICES

[Use as needed]

- **Figures**
- **Tables**

EXAMPLE Scientific Objective: Improve Prediction of Microbial Gene Regulatory Networks

Breakout Group: Microbial

Date: May 12, 2010

5. Scientific Objective

Brief statement of Scientific objective

[What is the scientific or research goal? What is written here will usually be derived after filling in the remainder of the template. Responses to sections below will help to refine this statement. Sometimes it is easier to think of an objective in terms of a problem that exists that needs to be solved.]

Informative Example: Next generation sequencing technology will provide high quality RNA-Seq data at low cost. This presents an opportunity to substantially improve the quality of predicted gene regulatory networks compared with what has been possible with expression microarrays. This data together with transcription factor binding site predictions or determinations will provide the necessary data to built genetic regulatory networks for microbial genomes. High quality genetic regulatory networks of experimentally tractable organisms would increase the efficiency of experimental designs and genetic engineering. In the long term, having a collection of transcript profiles collected in a high quality, standardized manner across DOE relevant organisms such that genetic regulatory networks could be automatically determined in the context of the Kbase would provide an extremely valuable resource to advance microbial research.

Background information

[Include ongoing experiments, future planned experiments, historical results, literature references, relevant past impediments to research progress, etc.]

Informative Example: Next generation sequencing technology provides high quality RNA-Seq data at low cost. When acquired in sufficient quantity RNA-Seq data has dramatically better dynamic range and sensitivity than gene expression arrays and will probably replace them in 3-5 years. Transcriptome data can be used to define operons including transcription initiation and termination sites. Cluster analysis over multiple conditions will identify co-regulated operons and therefore defines co-regulated promoters. This data together with transcription factor binding site predictions or determinations will provide the necessary data to built genetic regulatory networks for microbial genomes.

6. Prioritization

[This is meant to help prioritize this scientific objective in the context of other scientific objectives. There are several axes of consideration. One is the need or benefit to the research community. Another is the level of difficulty or feasibility.]

Informative Example: Since genetic regulatory networks will facilitate efficient genetic engineering and other experimental designs (Cho et al., 2009), the priority of this objective should be high. (This statement of priority can be written at the workshop based on discussion.)

PRIORITY (check one): HIGH MEDIUM LOW

Potential Benefits

[Why is this objective important? What is its level of impact? What would the benefit be? Who would benefit?]

Informative Example: Genetic regulatory networks of experimentally tractable organisms would increase the efficiency of experimental designs and genetic engineering. Microbes will be increasingly more important in manipulating a variety of organic molecules for biofuels, alternative plastics, other biochemical feedstocks, carbon sequestration and environmental remediation. Having the ability to efficiently manipulate and engineer these organisms will be absolutely crucial for cost effective design and large scale production of useful biochemicals.

Feasibility of success Near, Mid and Long term

[What is the level of difficulty? How likely is it that this objective can be achieved in a 1-3 year time frame? What would be the measure of success? Consider and rate feasibility in the near term (1-3 years, midterm (3-5 years) and long term (5-10 years). The most important objectives, those that are high priority and most feasible in the near term must have the most detail. Mid and long term can be provided in decreasing levels of detail.]

TERM (check one): NEAR (1-3 years) MID (3-5 years) LONG (5-10 years)

Informative Example: Collecting RNA-Seq data is already feasible and will only become more cost effective as third generation sequencing technologies are available in the next year. The community is already engaged in the development of analytical tools capable of integrating genomic DNA sequence and RNA-Seq data. The methods for predicting operons and their structure, cluster analysis of transcriptomic data to predict co-regulation of operons, predicting transcription factor binding sites and regulatory elements are already available but need to be streamlined and integrated. Implementing these kinds of analytical capabilities within the Kbase would be feasible in the first 1-2 years. RNA-Seq data is expected

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to be widely available in the midterm 3-5 years and will need to be standardized to avoid some of the problems seen with GEO. Producing a functional genetic regulatory network for one or more bacterial organisms important to the Bioenergy centers appears to be achievable in 2-3 years if sufficient resources are applied.

Relevance to DOE systems biology knowledgebase project

[The DOE Genomic Science program's ultimate goal of achieving a predictive understanding of biological systems is a daunting challenge and will require the integration of immense amounts of diverse information. The DOE Systems Biology Knowledgebase is envisioned as an open cyber-infrastructure to integrate systems biology data, analytical software, and computational modeling tools that will be freely available to the scientific community. Briefly explain how the proposed objective is relevant to what is envisioned for Kbase.]

Informative Example: Predicting genetic regulatory networks requires integration of standardized sets of data and associated analysis methods along with the ability to test and improve the methods as envisioned for the Kbase. In the long term, having a collection of transcript profiles collected in a high quality, standardized manner across DOE relevant organisms such that genetic regulatory networks could be automatically determined in the context of the Kbase would provide an extremely valuable resource to advance microbial research.

Synergies/Leverage: Potential overlap with other projects or funding agencies

[Are there existing systems that relate to this objective such as NCBI, GenBank, BioCyc, iPlant, etc. Is there a potential for synergy that would benefit both efforts? Is there a potential overlap that needs to be resolved?]

Informative Example: Generating the necessary RNA-Seq data would leverage JGI's production sequencing capabilities and could be synchronized with the genomic sequencing, while developing the analysis pipeline could be accomplished by ORNL's annotation group and incorporated into its' annotation pipeline. Individual PIs and smaller projects already pursue such analysis based on microarray data and the decreasing cost of RNA-Seq will eventually make RNA-Seq transcriptomics routine. Having a community of data integrated based on standards will provide a powerful resource. A natural byproduct will be better gene models and operon structures. This information will augment what is available in GenBank. An ancillary objective would be to update the associated annotation in GenBank.

7. Specificity

[This section pertains to finding the right level of objective, especially avoiding objectives that are specified at too high a level. Start with a high level objective and refine. What is the specific science question to be answered?]

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Informative Example: Integration of 'omics data especially in complex systems such as plant microbe interfaces is an ambitious challenge that is too high level for the purposes of establishing version 1 of the Kbase, and not feasible in the near term (1-3 years) although it would be appropriate for the long term with a suitable scientific focus. However this high level aim could be made more tractable by simplifying in several ways. First, focus in on a simpler system such as a specific microbe. Second, instead of integrating all 'omics, take just two types of 'omics data, say genomic and transcriptomic as in this example.

In this example we started by considering 'omics integration and the large number of possible scientific objectives might derive from that such as a substantial model of major subsystems of a cell which would clearly be overly ambitious. By considering various combinations of 'omics data the level can be refined. In this example we recognized that by integrating just two kinds of 'omics data, genomics and transcriptomic using RNA-Seq, we would be able to have a science objective of improved prediction of gene regulatory networks that would be something tractable to accomplish in the relative near term within the Kbase and something useful to the microbial research community.

8. Details

[The intent here is to begin to capture elements that form the basis for continuity between the science objective and the software requirements that are derived from this objective. We start to articulate high level requirements here that are further refined in the requirements document.]

Scientific discovery process (workflows)

[Have workflows already been developed or can they be derived from existing work?]

Informative Example: Genetic regulatory networks have been created for *E. coli* (Cho et al., 2009) and *Halobacteria salinarum* NRC-1 (Bonneau et al., 2007). These papers describe workflows.

Inputs

[What datasets would be required? Are there data standards? Are there available data sources or examples? Are there publications that use or describe an associated analysis process?]

Informative Example: For a particular microbe of interest it would be expected that a finished genome sequence is available and for a few phylogenetically related organisms. In addition it would be expected that RNA-Seq of multiple growth states would have been obtained to a high level of coverage.

Outputs

[What would the results be?]

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Informative Example: The results would be genetic regulatory network predictions for all microbes studied.

Tools

[Existing or new analysis software]

Informative Example: Numerous independent tools that have been developed. It will be necessary to develop analytical pipelines based on agreed workflows that integrate the RNA-Seq data, genomic sequence data, gene expression array data (if available), transcription factor binding site predictions and experimental verification (if available) in order to generate the genetic regulatory network predictions for a particular microbe.

REFERENCES

[Use as needed]

Bonneau, R., Facciotti, M.T., Reiss, D.J., Schmid, A.K., Pan, M., Kaur, A., Thorsson, V., Shannon, P., Johnson, M.H., Bare, J.C., *et al.* (2007). A predictive model for transcriptional control of physiology in a free living cell. *Cell* 131, 1354-1365.

Cho, B.K., Zengler, K., Qiu, Y., Park, Y.S., Knight, E.M., Barrett, C.L., Gao, Y., and Palsson, B.O. (2009). The transcription unit architecture of the Escherichia coli genome. *Nat Biotechnol* 27, 1043-1049.

APPENDICES

[Use as needed]

- **Figures**
- **Tables**

Appendix 4: Requirements Template

Software Requirements for Scientific Objective: *Improve Prediction of Microbial Gene Regulatory Networks*

Breakout Group: Microbial

Reference Scientific Objective Number in Group: _____

Date: May 18, 2010

1 Scientific objective

[Describe the scientific objective that this software system requirements document supports. Description can be derived from the Scientific Objective template]

2 Resulting Requirements

[In the following sections list the requirements resulting directly from the identified scientific objective. Provide information for each requirement stating whether there are technologies available today to fulfill all or part of it that you are aware of, or if you expect that new development would be required. All requirements should indicate whether they are near, medium or long term requirements. The following Impact Factor is your group's assessment of the impact that addressing these requirements would have toward improving research productivity.]

IMPACT FACTOR (check one): HIGH MEDIUM LOW

2.1 Process of the science (incl. workflow)

[Describe the process by which scientists use or want to use the data, software, and instruments for knowledge discover such as a scientific workflow. Identify both required and optional components. Indicate the state of the art of the different parts of the workflow.]

2.2 Instruments to support the achievement of the science objectives.

[List or describe instruments that generate relevant data connected to the scientific workflow above.]

2.3 User interfaces

[Describe generally who the users will be, and the user interfaces that play a role in achieving the scientific objective in the context of the workflows outlined above. Not all user interfaces will be directly involved in a workflow, and these if they exist should be captured here as well.]

2.4 Programmatic interfaces

[Describe the interfaces that will provide programmatic access to data or functionality that allow for automated data access, analyses and workflows in the context of the workflows outlined above. Not all programmatic interfaces will be directly involved in a workflow, and if these exist, capture them here as well.]

2.5 Data

[Describe the data and data types required to meet the scientific objectives. Include publicly available data, reference data, and new experimentally derived data. Discuss how the data is obtained such as is the data to reside locally on Kbase or would it exist remotely, outside of Kbase. Data representations including semantic web technologies or references can be included here, as well as references to existing data standards or relational tables. If known, include computer hardware resource requirements – such as the size of the data collection, and type and size of compute resources (processors, memory, temporary storage) required to manage and process the data.]

2.6 Software

[Describe which software algorithms, services and packages will be needed, if they exist or not, to achieve the scientific objective, and what computer hardware or other resources and data these would utilize.]

Software purpose	Availability	Does it need improvement	Resource impact

2.7 Standards

[Specify requirements that are derived from existing standards and/or regulations. While we don't expect much in the form of regulation, we should list those existing standards that we will use and areas where new standards need to be developed.]

2.8 Governance

[Related governance issues (usage policy, data policy, overall governance structure, community engagement for usage and development) should be described here. Some governance issues map to components of the system and these mappings should be called out in the System Architecture. How can governance help the implementation of standards?]

2.9 Summary and prioritization of requirements

[Summarize and prioritize your requirements, which ones are essential and which one are nice to have or could wait. Which requirements are near term, midterm and long term?]

3 System Architecture Attributes

[The common attributes are performance, reliability, availability, security, portability, interoperability, and usability (usually speaks to the importance of user interfaces with which humans interact as compared to a fully automated system that users just depend on). Important attributes from the list above should be discussed in the context of the scientific objective. For example, does achieving the science objective require a system that runs 24/7 with a yearly downtime of less than 8 minutes (this reflects the system's availability attribute). Will it perform calculations that require thousands of cores in order to complete in a reasonable time. Prioritize the relative importance of each architecture attribute and provide explanations of why, for example, why would security be more or less or equal in importance to performance.]

4 Kbase Key Services

[Optional – do this if able: Provide a list and description of the major functions/services that the Kbase system will need to provide to meet the scientific objective(s). This could include a mapping of existing functions onto existing systems such as MicrobesOnline, IMG, etc., or new services such as a central resource for temporary storage of data from different sources to be jointly analyzed. Here we can get into the finer details of what the system will do in order to meet the scientific objectives. Each function should be called out as a sub heading in this section]

4.1.1

4.1.2

4.1.3

5 Risk Analysis and Mitigation strategies

[Compile the list of potential risks in meeting the requirements of the scientific objective.]

-
-
-

6 Acryonyms, definitions and abbreviations

7 References

EXAMPLE Software Requirements for Scientific Objective: *Improve Prediction of Microbial Gene Regulatory Networks*

Breakout Group: Microbial

Reference Scientific Objective Number in Group: _____

Date: May 18, 2010

8 Scientific objective

[Describe the scientific objective that this software system requirements document supports. Description can be derived from the Scientific Objective template]

Improve Prediction of Microbial Gene Regulatory Networks

Next generation sequencing technology will provide high quality RNA-Seq data at low cost. This presents an opportunity to substantially improve the quality of predicted gene regulatory networks compared with what has been possible with expression microarrays. This data together with transcription factor binding site predictions or determinations will provide the necessary data to built genetic regulatory networks for microbial genomes. High quality genetic regulatory networks of experimentally tractable organisms would increase the efficiency of experimental designs and genetic engineering. In the long term, having a collection of transcript profiles collected in a high quality, standardized manner across DOE relevant organisms such that genetic regulatory networks could be automatically determined in the context of the Kbase would provide an extremely valuable resource to advance microbial research.

When acquired in sufficient quantity RNA-Seq data has dramatically better dynamic range and sensitivity than gene expression arrays and will probably replace them in 3-5 years. Transcriptome data can be used to define operons including transcription initiation and termination sites. Cluster analysis over multiple conditions will identify co-regulated operons and therefore defines co-regulated promoters. This data together with transcription factor binding site predictions or determinations will provide the necessary data to built genetic regulatory networks for microbial genomes.

9 Resulting Requirements

[In the following sections list the requirements resulting directly from the identified scientific objective. Provide information for each requirement stating whether there are technologies available today to fulfill all or part of it that you are aware of, or if you expect that new development would be required. All requirements should indicate whether they are near, medium or long term requirements. The following Impact Factor is your group's assessment of the impact that addressing these requirements would have toward improving research productivity.]

IMPACT FACTOR (check one): HIGH MEDIUM LOW

9.1 Process of the science (incl. workflow)

[Describe the process by which scientists use or want to use the data, software, and instruments for knowledge discover such as a scientific workflow. Identify both required and optional components. Indicate the state of the art of the different parts of the workflow.]

(NOTE: This is an example that has been intentionally simplified and therefore extensions such as validation with computational or experimental methods such as 5' RACE to identify additional transcription initiation sites, or transcription factor regulatory ligand determinations have been removed. Others are welcome to take this as a starting point and expand for a specific "real" Scientific Objective.)

Taken from Scientific Objective section 4.2 Inputs: For a particular microbe of interest it would be expected that a finished genome sequence is available and for a few phylogenetically related organisms. In addition it would be expected that RNA-Seq of multiple growth states would have been obtained to a high level of coverage.

For the organism of interest it is assumed that the genome has been completely sequenced, fully annotated, and that RNA-Seq data is available for a minimum of 10 growth curves with 6 time points and 3 biological replicates on biological conditions relevant to the functional network(s) of interest.

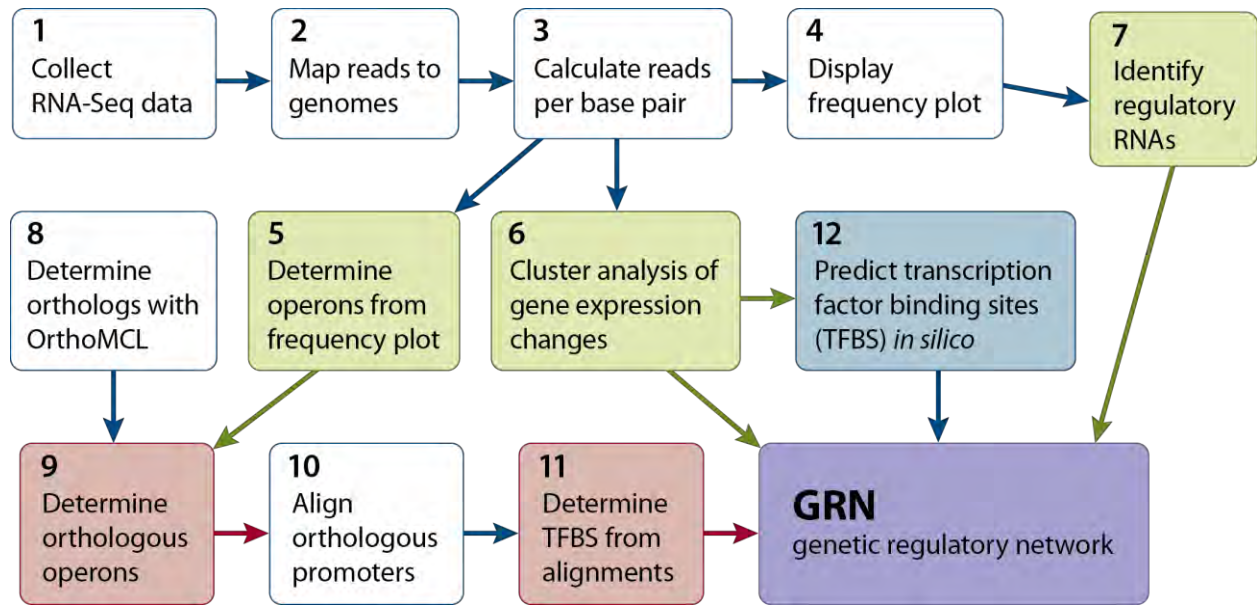


Figure 1. Transcriptome Analysis Pipeline for Gene Regulatory Network Prediction. White boxes are procedures we already know how to do. Green boxes are procedures that have not been determined but expected to be fairly easy to construct (year 1). Red boxes are procedures that will be more difficult to construct (year 2). Blue boxes are techniques that are optional, but would increase the accuracy of the analysis. The purple box is the final product (year 2).

1. Collect RNA-Seq data and the accompanying metadata for each growth curve. The metadata could include optical density, substrate consumption, metabolites, temperature, and stirring condition. Although some of this data could be manually collected the Kbase would need to have the ability to store it in conjunction with the RNA-Seq as an experimental project.
2. Map RNA-Seq data to genome.
3. Calculate reads/bp (normalize and calculate expression levels of each gene and/or operon).
4. Display frequency plot for visual inspection and rule development for algorithms to identify the operons and regulatory RNAs in steps 5 and 7.
5. Determine operons from mapped reads (generate a separate list for each growth curve). These should include all the genes, the transcription initiation sites (TIS) and terminations sites with accuracy of a few bp for each operon.
6. Perform cluster analysis on the calculated gene expression levels to determine co-regulated operons.
7. Identify regulatory RNAs (unknown riboswitches and small regulatory RNAs) based on analysis derived rules identified in step 4 with expert guidance.
8. Determine orthologs from multiple related genomes using OrthoMCL or some other software tool.

9. Determine orthologous promoters from multiple related genomes.
10. Align orthologous promoters using Muscle or ClustalW.
11. Determine Sigma Factor and other Transcription Factor Binding Sites (TFBS) from alignments.
12. Use in silico TFBS prediction tools together with co-regulated operons to predict additional TFBS (import known TFBS from a database such as RegTransBase).
13. Predict Genetic Regulatory Network.

Further work after the initial implementation (years 1-2) would include evaluation of additional technologies and experimental verification to improve the process (5' RACE to identify additional TISs, microfluidic TFBS determinations and transcription factor regulatory ligand determinations). As the quality of the gene regulatory network predictions improves and the models are validated the workflow will be increasingly automated (years 3-5).

9.2 Instruments to support the achievement of the science objectives.

[List or describe instruments that generate relevant data connected to the scientific workflow above.]

Kbase should support RNA-Seq data from Solexa, ABI Solid, and 454. For the future there may be additional machines that will need to be supported such as PacBio. These instruments produce data of particular types and sizes that will need to be stored and/or managed within the context of the Kbase and are further described in the Data section below.

There will be potential for use of automated or multi-well instruments for generating growth curve data. Metadata such as optical density may be recorded manually or in spreadsheets output from instruments and Kbase will need to have capabilities for manual input or upload of such electronic data that would then be integrated within the experimental project.

9.3 User interfaces

[Describe generally who the users will be, and the user interfaces that play a role in achieving the scientific objective in the context of the workflows outlined above. Not all user interfaces will be directly involved in a workflow, and these if they exist should be captured here as well.]

The anticipated users will include biologists who wish to analyze their data, bioinformaticists who want to analyze data, contribute or improve methods and use existing methods, and scientists requiring information and visual representations for scientific publications. It is

anticipated that users will come from the academic, government and industry communities. It is not anticipated that there will be users at a level below the university level.

Interfaces will be needed for specifying an experimental project and locating the relevant RNA-Seq and associated experimental metadata. Users will expect to have a login space where they describe their experiment that they will be able to save and return to at a later time.

Scientific data visualization is needed that renders genome annotation, gene expression information, operons, alternative transcriptional starts, and multiple sequence alignments. User interfaces for visualizing frequency plots that show depth of coverage (relative expression levels) for genes and operons will be needed. Additionally, an interface that allows users to visualize the resulting gene regulatory network model will be needed.

9.4 Programmatic interfaces

[Describe the interfaces that will provide programmatic access to data or functionality that allow for automated data access, analyses and workflows in the context of the workflows outlined above. Not all programmatic interfaces will be directly involved in a workflow, and if these exist, capture them here as well.]

Kbase will need to have programmatic interfaces to support specific queries such as to return a list of all experimental conditions that an organism has been exposed to for which there is gene expression data.

Also, software that determines expression levels, or predicts or refines operon predictions will need access to genome annotation. Therefore data interfaces to NCBI SRA (Sequence Read Archive), GEO (Gene Expression Omnibus) and GenBank (bacterial genomes) will be needed or perhaps application interfaces to IMG, RAST or JGI-ORNL annotation systems. Will also need to import known TFBS from a database such as RegTransBase

The results of the workflow to predict gene regulatory networks will also produce data that would be output to data interfaces such as to all of the systems mentioned above in order to supply new data, support publication submission or update annotation.

9.5 Data

[Describe the data and data types required to meet the scientific objectives. Include publicly available data, reference data, and new experimentally derived data. Discuss how the data is obtained such as is the data to reside locally on Kbase or would it exist remotely, outside of Kbase. Data representations including semantic web technologies or references can be included here, as well as references to existing data standards or relational tables. If known, include computer hardware resource requirements – such as the size of the data collection, and type and size of compute resources (processors, memory, temporary storage) required to manage and process the data.]

In the near term, we expect to see for a given experiment several hundred files from short read sequencing technology. These files, if based on Solexa technology, will range in size from 100Mbytes to 100GBytes for the next couple of years. Current size ceiling is at about 4GBytes compressed for one run. Total data storage required is based on coverage and number of replicates, conditions and time steps, and therefore would be a multiplicative factor of 4GB (180X minimum as proposed). For the first 1-3 years it is expected that there would be 30-100 datasets per year (each dataset corresponding to studies on one microbe), and then grow to 100-300 per year in the 3-5year time frame when this data will be coming from many laboratories.

Database and storage resources – terabyte to petabytes range storage are needed. Data reduction will play a role in keeping storage resources manageable. Online backup capabilities needed for disaster recovery and long term archival.

Data types that cover high-throughput technologies to interrogate the transcriptome, are required for this scientific objective.

Genome sequences and a full complement of annotation features are also required. The data representation model as characterized by a GenBank record is probably not sufficient. New data models that capture gene annotations and their relationships to other annotations will be required. Annotation can exist remotely as in the case of taxonomy information housed in the NCBI taxonomy database and other NCBI Entrez data for which stable access exists through NCBI web services.

The gene regulatory network from a data structure perspective is the collection of operons, transcription factor binding sites, sigma factor binding sites; and those parameters that affect kinetics. These would benefit from representation that is based in semantic web technology.

It is expected that relational database technology will play a limited role in so far as perhaps providing structured storage of ontologies and RDF tuples.

9.6 Software

[Describe which software algorithms, services and packages will be needed, if they exist or not, to achieve the scientific objective, and what computer hardware or other resources and data these would utilize.]

Software for performing transcriptome analysis will be needed as part of the workflow and for visualization. It will integrate existing available genome annotation and provide measures of confidence. Annotation quality will be accessed based on confidence. A specific module will focus directly on improved identification of transcription factors.

Improved annotation with confidence and evidence codes will be sent back to repositories if possible.

Clustering software will be needed to group genes and operons into clusters based on patterns of regulation. Whether a part of the clustering software or part of a different package, it is anticipated that software which focuses on the fine details of the operon such as alternative transcriptional starts and stops will be needed.

Clustering algorithms will be compute intensive. Other methods are manageable with mid-range servers.

Data visualization software that spans genome annotation, transcriptome analysis and clustering will also be needed.

Software purpose	Availability	Does it need improvement	Resource impact
Maps rna-Seq data to genome	Few	Probably not	Storage
Cluster analysis of gene expression changes	Many	Probably	Compute, Storage
Operon determination	Few	Yes	
In silico TFBS prediction	Many	Yes	Compute
Ortholog determination	Few	Probably not	
Orthologous operon determination	None		
Promoter alignment	Few	Yes	
Promoter prediction	Few	Yes	
Gene regulatory network prediction	Few	Yes	Compute, Storage

Table 1: Types of software required for this scientific objective. Column-Resource impact: Compute means requires significant processor resource (>100 cores), and Storage means requires significant storage resource (>1 TB).

9.7 Standards

[Specify requirements that are derived from existing standards and/or regulations. While we don't expect much in the form of regulation, we should list those existing standards that we will use and areas where new standards need to be developed.]

- Gene regulation ontology (GRO) for terms related to gene expression
- Gene ontology (GO) for terms related to biological processes, cellular location and gene function
- NCBI sequence read archive xml schemas for sequence read metadata

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- GCDML xml schema for genome metadata
- MIAME regards gene expression arrays but may be relevant to RNA-Seq.

9.8 Governance

[Related governance issues (usage policy, data policy, overall governance structure, community engagement for usage and development) should be described here. Some governance issues map to components of the system and these mappings should be called out in the System Architecture. How can governance help the implementation of standards?]

A data release policy will need to be in place. This would most likely be the current DOE policy and it is assumed that the Kbase will enforce this. This implies a private login which maps to System Architecture.

9.9 Summary and prioritization of requirements

[Summarize and prioritize your requirements, which ones are essential and which one are nice to have or could wait. Which requirements are near term, midterm and long term?]

In silico prediction of TBFS can be postponed until other elements of the workflow are complete (midterm). Support for microarray data was considered but has not been included for the sake of simplicity. If it would part of the requirements it might be lower priority because we believe it is phasing out. Other requirements for possible inclusion would be various kinds of validation such as 5' RACE and TFBS verification (midterm).

10 System Architecture Attributes

[The common attributes are performance, reliability, availability, security, portability, interoperability, and usability (usually speaks to the importance of user interfaces with which humans interact as compared to a fully automated system that users just depend on). Important attributes from the list above should be discussed in the context of the scientific objective. For example, does achieving the science objective require a system that runs 24/7 with a yearly downtime of less than 8 minutes (this reflects the system's availability attribute). Will it perform calculations that require thousands of cores in order to complete in a

reasonable time. Prioritize the relative importance of each architecture attribute and provide explanations of why, for example, why would security be more or less or equal in importance to performance.]

Users will be expecting that the data they submit will be secure in accordance with the governance model. This would be the highest priority.

It is anticipated that there could be some performance issues resulting from the choice of clustering algorithms and the amount of input data. Performance and security are architecture issues considered of highest importance for this objective.

11 Kbase Key Services

[Optional – do this if able: Provide a list and description of the major functions/services that the Kbase system will need to provide to meet the scientific objective(s). This could include a mapping of existing functions onto existing systems such as MicrobesOnline, IMG, etc., or new services such as a central resource for temporary storage of data from different sources to be jointly analyzed. Here we can get into the finer details of what the system will do in order to meet the scientific objectives. Each function should be called out as a sub heading in this section]

- 11.1.1 Mapping RNA sequence reads to a genome
- 11.1.2 Identifying operons
- 11.1.3 Identifying alternative transcription starts and stops
- 11.1.4 Identifying transcription factor binding sites
- 11.1.5 Improvements to genome annotation based on services 4.1.1 – 4.1.4
- 11.1.6 Data structures for representing gene regulatory networks
- 11.1.7 Query services for retrieving gene regulatory network models
- 11.1.8 Query services for retrieving all experimental conditions that an organism has been exposed to for which there is gene expression data

12 Risk Analysis and Mitigation strategies

[Compile the list of potential risks in meeting the requirements of the scientific objective.]

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- Unanticipated changes in technology (sequencing, microarray) that would significantly change the requirements or implementation plan. Mitigated by anticipating changes and adjusting requirements and implementation plan as soon as possible.
- Inadequate data or poor data quality that precludes a productive workflow as currently designed. Mitigate by testing typical datasets for adequacy and quality. Modify experimental protocol to correct and change minimum standards.
- Cluster analysis on these datasets requires more resources than currently anticipated. Mitigate by modifying algorithm accept some additional error in return for performance speed. Allow clustering on subsets to manually find the optimum with reduced error.

13 Acronyms, definitions and abbreviations

14 References

APPENDIX E

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APPENDIX F

Acronyms

AFRI Agriculture and Food Initiative	CellML XML-based open standard language for describing mathematical models
AJAX Asynchronous Javascript and XML	CGIAR Consultative Group on International Agricultural Research
ANI average nucleotide identity	ChEBI Chemical Entities of Biological Interest
ANOVA analysis of variance	CHIP-Seq used to analyze protein interaction with DNA
API application programming interface	CLR Context Likelihood of Relatedness algorithm
ARB/Silva database of aligned small and large RNA sequences	COBRA constraint-based reconstruction and analysis toolbox
ASCR DOE Office of Advanced Scientific Computing Research	COG clusters of orthologous groups
B biology (used in Staffing Resources tables)	COPASI Complex Pathway Simulator
BER DOE Office of Biological and Environmental Research	CPU central processing unit
Bfx, BFX software or file extension regarding bioinformatics (used in Staffing Resources tables)	CS Computer science, computer scientist (used in Staffing Resources tables)
BioCyc collection of 673 pathway and genome databases	CSREES Cooperative State Research, Education, and Extension Service
BioPAX Biological Pathway Exchange (a language for biological pathway data)	DOE U.S. Department of Energy
Bio2RDF atlas of post-genomic knowledge	DM data management
BIRN Biomedical Informatics Research Network	dhIP-Seq chip sequencing used to analyze protein interactions with DNA
BRC DOE Bioenergy Research Centers (includes the Joint BioEnergy Institute, Great Lakes Bioenergy Research Center, and BioEnergy Science Center)	dbEST Expressed Sequence Tag Database
BRENDA Braunschweig Enzyme Database	dbGaP Database of Genotypes and Phenotypes
caBIG cancer Biomedical Informatics Grid	DOTUR software program for community diversity analysis
CAMERA Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis	EBI European Bioinformatics Institute
cDNA complementary DNA	EcoCyc bioinformatics database that describes the genome and biochemical machinery of <i>Escherichia coli</i>

Appendix F: Acronyms

EPA Environmental Protection Agency	GRO gene regulation ontology
ESnet Energy Sciences Network	GSC Genomic Standards Consortium
EST expressed sequence tag	GWAS genome-wide association studies
FBA flux balance analysis	HMP Human Microbiome Project
FTP file transfer protocol	HPC high-performance computing
FIGfams protein families generated by the Fellowship for Interpretation of Genomes	hPDL Hadoop Process Definition Language (an XML-based workflow definition language)
FISH fluorescence <i>in situ</i> hybridization	HTS high-throughput sequencing or high-throughput screening
FTE full-time equivalent	IBP Integrated Breeding Platform
GB gigabyte	ICIS International Crop Information System
GC gas chromatography	ICRISAT International Crop Research Institute for the Semi-Arid Tropics
GCDML Genomic Contextual Data Markup Language, xml schema for genome metadata	IMG Integrated Microbial Genomes
GDCP Genomic Diversity and Phenotype Connection	IMG/M Integrated Microbial Genomes with Microbiome samples (a data management and analysis system for metagenomes)
GDPDM Genomic Diversity and Phenotype Data Model	INSDC International Nucleotide Sequence Database Collaboration
GenBank sequence database	I/O input/output
GEBA Genomic Encyclopedia of Bacteria and Archaea	iPlant NSF-funded plant science research collaborative
GEO Gene Expression Omnibus, database repository of high-throughput gene expression data and hybridization arrays, chips, and microarrays	IPGRI International Plant Genetic Resources Institute
GForge open-source system for software project management and collaboration	IPPN International Plant Phenomics Networks
GMOD Generic Model Organisms Database	IT information technology
GO gene ontology	IUPAC/ASTM International Union of Pure and Applied Chemistry/ASTM International
GOLD Genomes OnLine Database	JGI DOE Joint Genome Institute
GPS global positioning system	JGIMP DOE Joint Genome Institute Metagenomics Program
GPU graphics processing unit	
GRN genetic regulatory network	

Appendix F: Acronyms

JGIMGP DOE Joint Genome Institute
Microbial Genomics Program

JGIPGP DOE Joint Genome Institute Plant
Genomics Program

KiSAO Kinetic Simulation Algorithm
Ontology

KPI key performance indicator

KEGG Kyoto Encyclopedia of Genes and
Genomes

KO KEGG Orthology Database

LIMS laboratory information management
system

M5 Metagenomics, Metadata and
MetaAnalysis, Models, and
MetaInfrastructure initiative funded by
DOE

Matlab short for matrix laboratory, Matlab
is programming language and
interactive numerical computing
environment

MetaCyc a database of metabolic pathways

MGA MetaGeneAnnotator

MG-RAST Metagenome Rapid Annotation
using Subsystem Technology

MIAME Minimum Information About A
Microarray Experiment

MIAPPHE Minimum Information About a
Plant Phenotyping Experiment

MIENS Minimum Information about an
Environmental Sequence

MIGS Minimum Information about a
Genome Sequence

MIMS Minimum Information about a
Metagenome Sequence

miRNA microRNA

Mothur open-source bioinformatics
software package

MPD Mouse Phenome Database

MPHASYS Mouse Phenotype Analysis
System

mRNA messenger RNA

mzXML open data format for storage and
exchange of mass spectrometry data

nano-SIMS nano-secondary ion mass
spectrometry

NOAA National Oceanic and Atmospheric
Administration

NCBI National Center for Biotechnology
Information

NCBO National Center for Biomedical
Ontology

NIH National Institutes of Health

NIR near infrared

NISO National Information Standards
Organization

NISO MIX NISO metadata for images in XML

NSF National Science Foundation

NINJA neighbor joining (algorithms)

NITRC Neuroimaging Informatics Tools and
Resources Clearinghouse

OBI Ontology for Biomedical Investigations

OptFlux: an open-source software platform
for *in silico* metabolic engineering

ORF open reading frame

OrthoMCL method for constructing
orthologous groups across multiple
eukaryotic taxa using a Markov Cluster
algorithm

OTU operational taxonomic unit

OWL Web Ontology Language

Appendix F: Acronyms

PATO phenotype, attribute, and trait ontology	RPKM reads per kilobase of transcript target per million mapped reads
PB petabyte	SAMBA Statistical-Algorithmic Method for Bicluster Analysis
PCA principal component analysis	siRNA small interfering RNAs
PDB Protein Data Bank	S statistics (used in Staffing Resources tables)
PLEXdb Plant Expression Database	SABIO-RK System for the Analysis of Biochemical Pathway–Reaction Kinetics
PMN Plant Metabolic Pathway Database, of which PlantCyc is a central feature	SBGN: Systems Biology Graphical Notation
PO protein ontology	SBML Systems Biology Markup Language
PySCes python simulator for cellular systems	SBO Systems Biology Ontology
Q values, quality information	SBRML Systems Biology Results Markup Language
QC quality control	SBW Systems Biology Workbench
qPCR quantitative polymerase chain reaction	SDK software development kit
QTL quantitative trait locus	SE software engineering (used in Staffing Resources tables)
RAST rapid annotation using subsystem technology	SED-ML Simulation Experiment Description Markup Language
RAxML software to determine phylogenetic relationships through maximum likelihood	SEED open-source tool for genome annotation
RAM random-access computer memory	SO sequence ontology
RDBMS relational database management system	SOAP Simple Object Access Protocol; lets applications exchange information over HTTP
RDF Resource Description Framework	SOLID next-generation sequencing platform that supports a wide range of applications
RDP Ribosomal Database Project	SRA Short Read Archive, an NCBI database
REST Representational State Transfer; software architectural style on which the web is built	SQL Structured Query Language
RNA ribonucleic acid	SNP single-nucleotide polymorphism
rRNA ribosomal RNA	SPARQL query language for Resource Description Framework
RNA-Seq high-throughput sequencing to determine a sample’s RNA content, also called whole-transcriptome shotgun sequencing	

SUNDIALS SUite of Nonlinear and
Differenial/ALgebraic equation Solvers

SVD singular value decomposition

TB terabyte

TEDDY Terminology for the Description of
Dynamics

TFBS transcription factor binding site

TF transcription factor

TIGRFAMS collection of protein families

TRN transcriptional regulatory network

TSS transcription start site

TTS transcription termination site

TU transcription unit

UAL user access layer (Kbase)

UCSB University of California, Santa Barbara

UDP User Datagram Protocol

UI user interface

UniFrac online tool for comparing microbial
diversity in a phylogenetic context

UniProt Universal Protein Resource

USDA United States Department of
Agriculture

VISTA comprehensive suite of programs
and databases for comparative analysis
of genomic sequences

XML extensible markup language

XPP X-Windows Phase Plane

5' RACE rapid amplification of 5' cDNA ends

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