

Bigger is Better: DOE JGI's 2011 Community Sequencing Program Portfolio

Arctic algae, a cereal crop whose genetic code is nearly equivalent to sequencing two full human genomes, and microbial communities in deep-sea hydrothermal vents are among the 35 projects selected by the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) for its 2011 Community Sequencing Program (CSP) to be characterized for bioenergy and environmental applications.

Enabling scientists from universities and national laboratories around the world to probe the hidden world of microbes and plants to meet the DOE missions of bioenergy, carbon cycling and biogeochemistry, this year's CSP portfolio is composed mostly of large-scale projects, which DOE JGI Director Eddy Rubin said was in keeping with the facility's mission of large-scale genomics and analysis.

"Advances in sequencing technologies are really changing the landscape and have dramatically increased data output," he said. "This has led to enormous changes which have impacted the science we produce. In the past year alone, the JGI's sequence output has increased more than fivefold to six terabytes or six trillion nucleotides. Connected with the increased productivity, we're beginning to position the JGI to work on projects of tera- and peta- or quadrillion-base scale. Our mission hasn't changed; what is changing is the scale and complexity of the projects – which will increase. This highlights one of the directions genomics is going as data output begins to rival the output of the high-energy physics and astronomy communities."

To emphasize the directional shift, the CSP *cont. on page 4*

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Alga for biodiesel sequenced

While green algae are fairly ubiquitous, the viruses that infect them remained mostly unknown until 30 years ago, when James Van Etten, a plant pathologist at the University of Nebraska-Lincoln began working on algal viruses. One of Van Etten's projects focused on the freshwater viruses that infect the microalgae *Chlorella*, a group of double-stranded DNA viruses with genomes greater than 300 kilobases in size.

While some may associate the alga with food supplements, *Chlorella variabilis* NC64A is used as a model system for studying algal/DNA virus interactions and was selected for sequencing by the DOE JGI, in part to understand how the viruses impact the global carbon and nitrogen cycles. Photosynthetic organisms such as *Chlorella* fix more than half of the carbon dioxide in the atmosphere, and viruses can infect up to a fifth of them at any one time.

In the study published in advance online September 17, 2010 (<http://bit.ly/cE63TI>) in



Paramecium bursaria with the symbiotic chlorella NC64A

The Plant Cell, an international team of researchers including Van Etten and DOE JGI Fungal Genomics head Igor Grigoriev published the sequence of the microalga *Chlorella*.

Aside from its potential applications in understanding the global carbon cycle, the microalga's genome sequence could also prove useful in producing second-generation biodiesel.

"Like other microalgae, there is an increasing interest in using *Chlorella* in a variety of biotechnological applications, such as biofuel, sequestering CO₂ ... or removing heavy metals from *cont. on page 8*

Courtesy of Jim Van Etten, University of Nebraska-Lincoln

Barley photo by Dag Endresen



Fungal Lessons on Breaking Biomass Better

“When we go into a forest we don’t see layers of dead branches because wood decay fungi take care of them,” said Igor Grigoriev, head of the DOE JGI’s Fungal Genomics Program. “So when we think about bioenergy and degrading biomass and converting that into biofuel, we would like to learn the most efficient ways of doing that from fungi, which have invented many ways of doing that in nature.”

In a study published online July 11, 2010 in *Nature Biotechnology* (<http://bit.ly/cjkax0>), Grigoriev and colleagues from the DOE JGI and the University of Utrecht in the Netherlands published the genome of the white-rot fungus *Schizophyllum commune*.

Commonly known as the split-gill mushroom, *S. commune* is found on every continent except Antarctica and was sequenced to identify enzymes that could be used to break down cellulosic materials for biofuel production. Grigoriev, a senior author on the paper, explained that the DOE JGI had previously sequenced and published the genomes of two other wood-decaying fungi — the white-rot fungus *Phanerochaete chrysosporium* in 2004 and the brown-rot fungus *Postia placenta* in 2009.

P. chrysosporium, like other white-rot fungi, can break down the lignin but leave the cellulose untouched, and also has potential bioremediation applications, containing enzymes that can degrade contaminants such as uranium and heavy metals. *P. placenta* was found to utilize small oxidizing agents that can blast through plant cell walls to decompose cellulose into simple sugars.

“*Schizophyllum commune* is the second white rot fungus and third wood degrader we’ve sequenced,” Grigoriev said, noting that the DOE JGI has sequenced and annotated 40 fungal genomes, and 40 more are in progress. The fungus breaks down cellulose and lignin by invading xylem



Schizophyllum commune by Carla Wick

tissue, and researchers hope that studying its 38.5-million nucleotide genome (containing about 13,200 genes) will help them harness the most relevant set of enzymes for specific biofuel production strategies.

Han Wosten, a microbiologist at the University of Utrecht (and the paper’s co-senior author with Grigoriev) who is studying *S. commune*’s mushroom-forming capabilities, said that one additional advantage is that the fungus can be easily grown in the lab. Additionally, he said, researchers can inactivate the genes in *S. commune* by deleting them. “This is the only mushroom-forming fungus in which gene deletions have been made,” he said, “allowing us to study the roles of genes in wood degradation and mushroom formation.” Wosten also suggested that there are opportunities for inserting genes and modulating expression levels to drive target protein production pathways.

“The surprise is how significantly larger is the variety of enzymes in *S. commune* that

are involved in plant biomass degradation compared to *P. chrysosporium*,” Grigoriev said. “In fact, *S. commune* has among the most extensive enzymatic machinery for degrading cellulose, hemicellulose, and lignin of the fungi we examined.”

Grigoriev said the DOE JGI is in the process of sequencing over a dozen more wood-decaying fungi. According to the Genomes OnLine Database (GOLD) the DOE JGI is responsible for more than a third of all fungal genomes sequenced or in the queue to be sequenced worldwide, and with two white-rot fungi and a brown-rot fungus done, he added, “we think we’re only touching the surface and we need to look at more genomes in order to understand the whole scope of diversity and mechanisms applied to degrading cellulose.”

Watch Grigoriev and Wosten discuss the *S. commune* sequencing project on the DOE JGI’s YouTube channel at <http://bit.ly/9bcGbe>.



Igor Grigoriev was interviewed by *Genome Technology* magazine regarding the role of fungal genomics in the global move to replace fossil fuels with renewable and clean energy sources. Read the article online at <http://bit.ly/9jOpQS> (Note: Registration to access the site is required but free of charge.)

Igor Grigoriev, head of DOE JGI’s Fungal Genomics Program

Turning up the heat for cleaner water

More than 100 wastewater treatment facilities worldwide are devoted to degrading terephthalate (TA) wastewater resulting from the production of a raw chemical used to make many plastic products. These facilities in turn rely on interactions between microbial communities of bacteria and archaea to help break down the organic matter while the methanogens remove the hydrogen released so that the degradation process can continue.

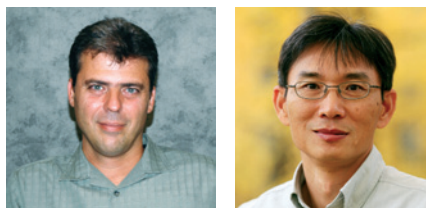
"In anaerobic conditions this is energetically unfavorable," explained DOE JGI research scientist and study first author Thanos Lykidis. "Only in the presence of [hydrogen-consuming] methanogens can this process proceed and that's why in these communities you have bacteria and archaea in very close proximity."

To better understand the syntrophic processes by which certain organisms in the microbial community live off the byproducts of others, Lykidis and his colleagues studied a thermophilic TA-degrading community in a lab-scale bioreactor originally developed by DOE JGI collaborator Wen-Tso Liu when he was still in Singapore. This first metagenome analysis of a microbial community grown in an anaerobic methanogenic (methane producing) bioreactor was published August 5, 2010 in the advanced online version of the *International Society for Microbial Ecology (ISME)'s Journal* (<http://bit.ly/bMOR27>).

Lykidis noted that the bioreactor analysis offers researchers a picture of all the populations that participate in the processes and provides a model to explain the presence of the various bacterial populations.

"The whole process is more complicated than just having two populations," he said. "It reveals there are additional players in this phenomenon besides hydrogen-producing bacteria and hydrogen-consuming methanogens."

The project was selected for sequencing



Left: Thanos Lykidis, DOE JGI research scientist and first author of the paper on terephthalate-degrading syntrophic communities. Right: DOE JGI collaborator Wen-Tso Liu of the University of Illinois at Urbana-Champaign. Courtesy of Wen-Tso Liu

under the DOE JGI's 2006 Community Sequencing Program for its bioremediation applications. The volume of the TA-containing wastewater generated is equivalent to the amount of wastewater generated by (equivalent to a volume of about 20 billion gallons). Currently, while the wastewater is commonly discharged at temperatures of 50-54°C, the treatment is done by anaerobic microbes that thrive at moderate temperatures of 35-37°C. The sampled anaerobic bioreactor operates at higher than normal temperatures to help degrade TA wastewater,

"This is a groundbreaking finding," said study senior author and DOE JGI collaborator Wen-Tso Liu, now at the University of Illinois at Urbana-Champaign. "The findings from this study clearly show that both primary and secondary syntrophic interactions are needed to make the degradation of chemicals to methane stable. The finding will further improve the future research on microbial fuel cells, bioremediation of toxic chemicals and radionuclides, and biofuel production."

"High carbon concentration-containing wastewater should be treated to a certain level by regulation for both domestic wastewater and for industrial wastewater," Liu said. "Even though TA is not a 'toxic' chemical in the wastewater, other compounds like benzoate, methylbenzoate, and unknown and possible toxic chemicals are also present at

high concentrations. Thus, TA-containing wastewater must be treated biologically."

According to Liu, the process is already running at mesophilic temperatures but people are pushing to run it at higher temperatures of, say, 50-55°C because then they increase the degradation rate," he said. "It'll be better because once you have a better degradation rate you don't have to operate such a big facility. This is one advantage. Since water is coming out at around 50°C, if we can have another microbial community that grows at 45-50°C and do the same job at the same degradation rate, why not do that? That's what this paper is about."

Liu added that a TA-degrading bioreactor that could work at higher than normal temperatures could be useful in reducing the footprint of TA-degrading wastewater treatment plants, as well as in developing other bioreactors that can break down other organic compounds.

Yoichi Kamagata, Director of the Bioproduction Research Institute at the National Institute of Advanced Industrial Science and Technology of Japan (AIST), noted that the syntrophic bacterial community "is indispensable in anaerobic environments. His paper has shed new light on the degradation mechanism based on a comprehensive metagenomic approach. This is the most important and significant point of the paper."

Dr. Kamagata also remarked that the research on anaerobic degradation could benefit not just environmental engineering companies interested in cleaning up organic contaminants but the oil, chemical and pharmaceutical industries as well. "Surprisingly, despite the long history of anaerobic methane fermenting process studies, the pathways and biochemistry of degradation of simple chemicals such as hydrocarbons and phenolic compounds are not known at all, let alone

Community Sequencing Program Portfolio cont. from page 1

2011 call urged researchers to submit proposals for projects that advance capabilities in fields such as large-scale resequencing, single-cell genomics and metatranscriptomics.

Of the 35 approved proposals, two involve plant genomes and two involve algal genomes; 10 are fungal projects; nine are microbial projects, six of which involve single-cell genomics; and 12 are metagenome (microbial communities) or metatranscriptome projects. The projects use most of the DOE JGI's increased sequencing capacity, allocating 10 trillion bases (TB or terabases), a 30-fold increase compared with last year's one-third of a trillion nucleotides.

One of the microbial projects approved builds upon a previous CSP project to probe the evolutionary diversity of bacteria and archaea in nature. Since its inception, more than 100 microbes have been sequenced as part of the Genome Encyclopedia of Bacteria and Archaea (GEBA) to provide information on unrepresented branches of the Tree of Life. An analysis of the first 56 genomes from the GEBA project was published in the December 24, 2009 edition of the journal *Nature* (<http://go.usa.gov/iOr>). Now the team, led by the DOE JGI and UC Davis researcher Jonathan Eisen, plans to sequence another 61 bacteria and archaea not just to increase the phylogenetic reference genomes but to also identify new proteins and subfamilies that could be useful to the DOE missions.

The single largest project, and the Institute's largest to date, involves sequencing barley, which has an anticipated five-billion base genome. Proposed by Gary Muehlbauer of the University of Minnesota, barley ranks fifth in the world among all crops cultivated and is grown on four million acres in the United States alone. The crop can be used to produce ethanol from the grain or for cellulosic



Image by Jason Hollinger



Kakouris Spyridon/Spirorsk photography, www.flickr.com/photos/spirorsk



Top left: Cleft-foot *Amanita*; top right: Etoliko Lagoon, Greece; bottom left: A. Sea ice near Svalbard, Norway; B. *Aureobasidium pullulans* var. *subglaciale* on MEA medium; C. Secovlje solar salterns, Slovenia; D. Cells of *Aureobasidium pullulans* var. *pullulans*.

Photo D: Nina Gunde-Cimerman and Cene Gostinčar

ethanol from the straw.

One of the projects approved for CSP 2011 involves resequencing the wild grass *Brachypodium distachyon*. Resequencing, as the name suggests, revisits previously sequenced genomes but focuses on comparing new data with a reference model. Earlier this year, the genome sequence of this plant was published in the journal *Nature* (<http://go.usa.gov/iOY>) to help researchers develop grasses tailored to serve as feedstocks for biofuel production. Led by John Vogel of the US Department of Agriculture Agricultural Research Service, the project involves sequencing 50 inbred lines of the grass to compare to the previously sequenced reference, develop genomic resources for *Brachypodium* and then carry out comparisons of their gene sets to enable identification of candidate genes involved in traits such as drought tolerance that are of interest to the DOE.

Researchers at the DOE JGI have been

among the pioneers of single-cell genomics, in which the DNA isolated from a lone cell is amplified, allowing researchers to study organisms that have not or cannot be cultured in a laboratory setting, a category that microbiologists hold to include 99 percent of the microbial world.

Two-thirds of the approved CSP 2011 microbial projects involve the use of single cell genomics to learn more about uncultured microbes. One such project is led by Stefan Sievert from the Woods Hole Oceanographic Institute, who is interested in using single-cell genomics to sequence nearly a dozen genomes of uncultivated bacteria that are found in deep-sea hydrothermal vents to learn about their roles in the global carbon, sulfur and nitrogen cycles. Additionally, the genomic information could be used to reduce sulfur emissions and resulting smog and acid rain.

Another project that involves the application of single cell genomics focuses on

Susannah Tringe and Shaomei He, DOE JGI



Left: Sampling wetlands in the Sacramento-San Joaquin Delta area; right: *L. pedicellatus* shipworm

the microbial diversity in the methane-rich environment of western Greece's Etoliko lagoon (left), which essentially supports two ecosystems by having both an oxygen-rich area and a distinct oxygen-poor zone. The anoxic zone is noted for having increased levels of sulfides and salinity. Led by George Tsiamis of Greece's University of Ioannina, the proposal calls for sequencing nearly two-dozen microbes to learn more about the microbial diversity in the lagoon, and the genomic information collected will also enrich the DOE JGI's ongoing GEBA project.

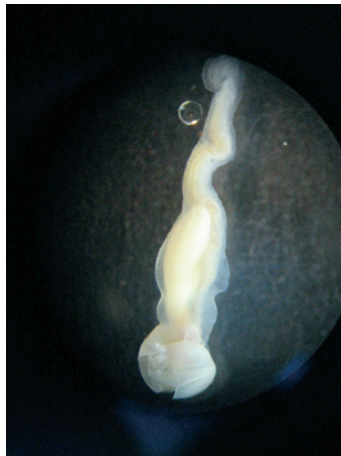
Metatranscriptomics focuses on the complex region of the complete genetic code that is transcribed into RNA molecules and provides information on gene expression and gene function. Half of the projects that look at the genomes of microbial communities (metagenomes) also involve plans to study their transcriptomes. One such project comes from Donald Bryant of Penn State University, who is interested in the microbial mats found in Yellowstone National Park that can capture light energy without producing oxygen, and then convert the light energy into chemical energy.

Among the other projects selected, one of the algal projects is noteworthy partly due to the stated intent of involving student participation to boost undergraduate education in genomics and informatics. A

team including DOE JGI's Education head Cheryl Kerfeld, who was recently honored by the American Society for Biochemistry and Molecular Biology (see page 6), plans to study algal strains from isolated acidic waters to understand their role in the carbon cycle, specifically how they can fix carbon under these conditions under these conditions.

"While integrating educational opportunities into the data generated by the proposal is not a stipulation of the CSP program, this is truly a commendable effort and it serves as a further metric of the dedication these authors obviously have for the work," noted one reviewer regarding the proposal submitted.

Several fungal projects were also selected. One of them focuses on *Aureobasidium pullulan* (image on previous page), the genetic information from which could lead to the development of drought-tolerant and salt-tolerant crops. Proposed by Martina Turk and Nina Gunde-Cimerman of the University of Ljubljana in Slovenia, the fungus thrives in a variety of environments and has been found on plants, PVC pipes and even on the walls of the Chernobyl nuclear power plant. The genomic information could help researchers studying ionizing radiation to develop new methods of assessing radiation effects. Additionally, as recent studies indicate, a strain of this fungus has been



Damon Tighe, DOE JGI

found in Arctic glaciers, and researchers hope to learn more about its role in the carbon cycle, especially as these glacial habitats are being affected by climate change. From a bioenergy perspective, the yeast-like fungus may hold enzymes that can help break down biomass, underscoring the metabolic versatility of fungi and relevance to and the DOE mission.

Another fungal project is focused on developing a comparative transcriptomics pipeline and is led by Antonio Pisabarro of Spain's Public University of Navarre. Using several brown rot and white rot fungi including *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Postia placenta*, all fungi whose genomes either have been or are in the process of being sequenced by the DOE JGI for their relevance to bioenergy and carbon cycling, Pisabarro and his collaborators want to improve the process by which gene expressions and gene functions of these fungi are compared.

Finally, one of the metagenome projects involves the so-called termite of the sea, the shipworm. As a wood-boring bivalve and the only marine animal known to sustain normal growth and reproduction by feasting solely on wood, the shipworm has two bacterial populations that can break down this material – one in the gut and the other in a specialized organ in the gills. The DOE JGI sequenced the only shipworm species adapted to cold water – *Bankia setacea* – as part of the CSP 2009 portfolio (<http://go.usa.gov/aaT>) to identify the enzymes in these microbes involved in breaking down wood for cellulosic biofuel production. Now DOE JGI collaborator Daniel Distel of the Ocean Genome Legacy Foundation has proposed studying both microbial communities in different species of shipworm, including *Lyrodus pedicellatus* (above), a shipworm species adapted to warm water.

For the complete list of CSP 2011 sequencing projects, see: <http://go.usa.gov/aab>.

Marsupial microbiome helps break down biomass

Meligan Murphy, Smithsonian's National Zoo



Australia and New Zealand were separated from other landmasses for millennia, and the unique marsupials found there such as kangaroos and wallabies have forestomachs adapted to effi-

ciently break down lignocellulosic plant mass to extract nutrients.

These forestomachs may contain unique microbes that could be useful in breaking down plant biomass for the production of cellulosic biofuels. To answer this question, in 2007 the DOE JGI selected sequencing the foregut microbiome of the Tammar wallaby as one of the Community Sequencing Program projects.

In a metagenomic analysis published online July 28, 2010 in the *Proceedings of the National Academy of Sciences*, a team including DOE JGI's Susannah Tringe, Kerrie Barry, Jan-Fang Cheng, and Phil Hugenholtz, now director of the Australian Center for Ecogenomics at the University of Queensland, looked at the plant biomass conversion process of the Tammar wallaby's foregut microbiome.

The wallaby microbiome DNA was sequenced using Sanger and 454 pyrosequencing, and the data was annotated using the DOE JGI's Integrated Microbial Genomes with Microbiome (IMG/M) system. Among their findings, the team identified unique bacterial lineages that break down drought-tolerant plants, which is common in Australia's native species. They said the data indicate that the enzymes in the Tammar wallaby and other Australian animals are unique and, in a nod to other DOE JGI metagenomic sequencing projects, added that "their repertoire ... is distinct from those of the

microbiomes of higher termites (<http://go.usa.gov/aaj>) and the bovine rumen (<http://go.usa.gov/aaD>)."

Practical Genomics and Bioinformatics Training for Undergraduates

In response to the National Research Council of the National Academies' call to "involve students in working with real data and tools that reflect the nature of life sciences research in the 21st century," the DOE JGI's Education Program, under the leadership of structural biologist Cheryl Kerfeld, collaborated with faculty members from several universities around the country to develop bioinformatics curricula that can be incorporated into life sciences coursework for undergraduates.

The result, published online in *PLoS Biology* on August 10, 2010 by Kerfeld, DOE JGI's Seth Axen and Edwin Kim, and other collaborators, is a platform called Integrated Microbial Genomes Annotation Collaboration Toolkit (IMG-ACT).

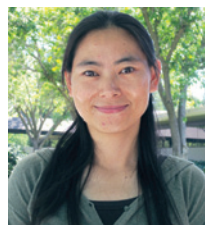
IMG-ACT offers access to genomes sequenced by the DOE JGI, including those done as part of the Genome Encyclopedia of Bacteria and Archaea,

offering students "virtually endless" research possibilities, bioinformatics databases, instructor course management and student notebooks.

Kerfeld and her colleagues noted that since IMG-ACT was launched in 2008, more than 100 faculty members and 1,600 students nationwide have participated in the program.

One of the results of incorporating IMG-ACT into college coursework has been a change in teaching methods at the University of California, Los Angeles, where all life science majors take part in an interdisciplinary laboratory curriculum that involves the IMG-ACT platform to develop a community of peer experts in bioinformatics.

Beyond Genomics to Metatranscriptomics



Ultra-high throughput DNA sequencing platforms are enabling more focused genomic studies, such as those involving the trans-

criptome, a tiny but complex fraction of the complete genetic sequence that is transcribed into different types of RNA molecules. These molecules provide researchers with information about what genes are turned on and off under various conditions, and what the functions of these genes are.

Researchers focus on the messenger RNA (mRNA), which transfer the genetic information from the DNA to functional proteins. However, a significant portion of cellular RNA is composed of ribosomal RNA (rRNA), which most researchers prefer to remove before sequencing, and choosing a technique for rRNA removal can be tricky.

To help researchers interested in submitting metatranscriptomic projects to the DOE JGI, Shaomei He (above), an Energy Biosciences Institute-funded postdoctoral researcher working at the DOE JGI and

Kerfeld honored for Outstanding Work in Education



Cheryl Kerfeld was named the recipient of this year's American Society for Biochemistry and Molecular Biology (ASBMB) Award for Exemplary Contributions to Education. Kerfeld was recognized for "encouraging effective teaching and learning of biochemistry and molecular biology through her own teaching, leadership in education, writing, educational research, mentoring and public enlightenment."

Learn more about the award and other ASBMB recipients at <http://bit.ly/actTJG>.

her colleagues validated two popular rRNA removal methods in use. The methods were tested using commercially available kits: Ambion's MICROExpress™ Bacterial mRNA Enrichment Kit and Epicentre's mRNA-ONLY™ Prokaryotic mRNA Isolation Kit. The Ambion kit uses a technique called subtractive hybridization, which involves oligonucleotide probes that target rRNA sequences, while the Epicentre kit preferentially degrades processed rRNAs using enzymes called exonucleases.

He, first author of the study published online September 19, 2010 in *Nature Methods*, said the validation study was prompted in part by researchers interested in having the DOE JGI conduct meta-transcriptomic analyses and asking for recommendations on how to enrich the mRNA molecules. "When people use these methods there's no systemic analysis to show if there's an associated bias," she said. "The two questions we wanted to answer in this project were: 1) how efficient are these methods at rRNA removal? and; 2) do they introduce any biases in mRNA?"

He and her colleagues tested both rRNA removal methods using two artificial communities made by mixing RNA from multiple microbial species whose genomes had been sequenced, some of them at the DOE JGI. One of the team's findings was that though some researchers favor combining both techniques, a process that can produce higher levels of rRNA removal, it also introduces a large skew in mRNA relative abundance.

Both rRNA removal techniques have limitations, reported the team, and rRNA removal efficiency depends on a combination of microbial community composition and RNA integrity. For example, said He, the hybridization kit uses generic probes that miss some bacteria and all archaea while the exonuclease kit also removes partially degraded mRNA. "Ultimately what you use depends on samples of interest and the biological question being asked,"

she said. The final outcome favors the use of the subtractive hybridization method by itself, which adequately preserved mRNA relative abundance for quantitative analyses, and He noted that researchers are already working on ways to improve the subtractive hybridization method.

Cataloguing DNA enhancer sequences



Primer Elements by Eve Stockton www.evestockton.com/

The genetic bases of many defects that cause heart disease are still unknown, and one of the challenges has been identifying variants in gene regulatory sequences that

could play a role in heart disease because their locations remain a mystery.

"DNA enhancer sequences act as switches that control when and where genes are expressed during development," said DOE JGI's Matt Blow, first author of the study that appeared on the September 2010 cover of *Nature Genetics*. "We would like to catalog these enhancer sequences because they are important for the normal development of an organism, and because they may play a role in disease. However, they are typically hard to identify."

To identify regulatory DNA sequences such as enhancers and promoters, a team of researchers including Blow and led by Len Pennacchio, head of the Genome Technologies group at DOE JGI, used an alternative approach to comparing homologous DNA sequences to as many sequences have been evolutionarily conserved over time. Not all enhancers can be identified through this technique, including enhancer sequences that work in the heart, which are considered among the most challenging to identify because they aren't as conserved as other regulatory sequences.

Using a technique known as ChIP-Seq, which involves massively parallel sequenc-

ing, Blow and his colleagues searched specifically for heart enhancers by surveying the binding of an enhancer associated protein across the genome in mouse embryonic heart tissue.

"We identified more than 3,000 potential heart enhancers, and by testing over 100 of these predictions in transgenic mice were able to confirm that these predictions were highly accurate," Blow said. "Intriguingly, most of these candidate heart enhancers were found to show less sequence conservation in than enhancers from other tissues. This finding is surprising given that the heart is one of the most deeply evolutionary conserved organs, and suggests that depending on the tissue, some sets of enhancers will be hard to predict on the basis of sequence conservation. Nevertheless, using this alternative strategy we were able to successfully identify thousands of heart enhancers that will be a valuable resource for future investigations into the genetic bases of heart development and disease."

Cultivating a cellulose-degradative fungal garden



Jarrod J. Scott, University of Wisconsin-Madison

Leaf-cutter ants are community gardeners on a very large scale. Living in colonies composed of several millions, the ants harvest hundreds of kilograms of leaves annually and use them to cultivate spongy fungal gardens that serve as their primary food source.

In a paper published September 23, 2010 on *PLoS Genetics*, DOE JGI's Susannah Tringe, Kerrie Barry and Lynne Goodwin teamed with longtime DOE JGI collaborator Cameron Currie and his colleague Garret Suen, both from the University of Wisconsin-Madison and the Great Lakes Bioenergy Research Center to study the microbiome of the leaf-cutter ant *Atta colombica*. *cont. on page 8*

Photo: Betty Mansfield



Benjamin Tulley (left) and Rosalynn Lee (center) from the University of Southern California and Kathryn Eilers (right) from the University of Colorado, Boulder had heard that the DOE JGI had put together a collection of 52 sequencing projects representing the four Program areas (Plant, Fungi, Microbes and Metagenomes) and the three DOE mission-relevant areas of study (bioenergy, carbon cycling and biogeochemistry). They visited the GTL booth at the International Society for Microbial Ecology meeting held in Seattle, Washington August 22-27, 2010, in search of these educational flash cards and learned about the DOE JGI, the DOE's Office of Biological and Environmental Research and the Genomic science program.

Those interested in receiving genomics flash cards should contact David Gilbert (degilbert@lbl.gov).

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Chlorella *cont. from page 1*

wastewaters. The sequence of the NC64A genome will help in the optimization of these various processes," wrote the team in their paper.

Chlorella has a very high lipid content, which is why biodiesel researchers are interested in its potential as a feedstock. The 46-million base genome, sequenced using the whole genome shotgun Sanger approach, revealed that the alga, long thought to be asexual,

might engage in sexual reproduction after all.

Another interesting finding involves the alga's very rigid cell walls, which are built with chitin, the same material used by crustaceans for their hard exoskeletons. The team's findings suggest that the genes for chitin are the result of "the capture of metabolic genes by horizontal gene transfer from either algal viruses, prokaryotes or fungi."

Turning up the heat *cont. from page 3*

more complex chemicals such as PAH (polycyclic aromatic hydrocarbon), lignin, or crude oil," he said. "From this point of view, there are a number of

industries who would be interested in knowing more, and taking advantage of the knowledge on anaerobic degradation of chemicals."

Fungal Garden *cont. from page 7*

Currie's team took samples from the top and bottom layers of ant fungal gardens in Panama to better understand how the biomass is broken down at each stage. These fungal gardens, noted the researchers in their paper, serve as the ants' external digestive system and have five separate layers to break down the plant biomass into nutrients. They knew that ants and the fungi they tend enjoy a mutualistic relationship, but that the fungi aren't capable of breaking down cellulose, which was

found to decrease by 30 percent on average from the top to bottom layers in these gardens.

The researchers identified a previously unknown microbial community that is involved in breaking down the cellulose in these fungal gardens, and said the ants' ability to also cultivate these microbes "likely represents a key step in the establishment of these ants as widespread, dominant insect herbivores" in the temperate zone that encompasses South America.

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