Addressing Biosecurity Concerns Related to Synthetic Biology



David Relman, M.D. Chair, NSABB Working Group on Synthetic Biology

Charge to NSABB

- Two-part charge
 - 1. Synthetic Genomics

...to address whether synthetically derived Select Agents are adequately covered by the current regulatory framework...

2. Synthetic Biology

...to identify, assess and recommend strategies to address any biosecurity or dual use research concerns that may arise from work being performed in the nascent field of synthetic biology

Synthetic Genomics

NSABB recommended:

- Development and dissemination of harmonized guidance
- Development of standards & practices for sequence providers to include nucleic acid screening
- A review of current biosafety guidelines to ensure that they are adequate for synthetically derived DNA
- Continued consultation with experts to develop a framework for predicting pathogenicity

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

> Addressing Biosecurity Concerns Related to the Synthesis of

SELECT AGENTS

DECEMBER 2006





Recent development

Federal Register/Vol. 74, No. 227/Friday, November 27, 2009/Notices



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Office of the Secretary

Screening Framework Guidance for Synthetic Double-Stranded DNA Providers

AGENCY: Department of Health and Human Services, Office of the Secretary.

ACTION: Notice.

Authority: Public Health Service Act, 42 U.S.C. 241, Section 301; HSPD–10.



NSABB Working Group on Synthetic Biology

Voting Members

- David Relman (Chair)
- Susan Ehrlich
- Claire Fraser-Liggett
- Mike Imperiale
- Harvey Rubin
- Thomas Shenk

Agency Representatives

- FBI
- OGC
- Department of State
- Department of Defense
- OSTP
- NIH
- Dept. of Homeland Security
- EPA
- USDA
- Department of HHS
- CDC
- Department of Energy
- Intelligence Community

NSABB Approach to Synthetic Biology

The Working Group considered

- The potential that information and/or technology stemming from legitimate scientific research might be misused to threaten elements of national security
- Biosecurity concerns presented by the ability to:
 - Synthesize new genes, metabolic pathways, and/or proteins
 - Design genetic systems and organisms with specified functions
- Extant oversight frameworks
 - The NSABB's proposed oversight framework for dual use research of concern
 - The NIH Guidelines for Research Involving Recombinant DNA Molecules

Scientific Roundtable Hosted by NSABB and RAC (Oct 11, 2007)

- Expertise
 - Synthetic biology
 - Microbiology, immunology, molecular biology
 - Systems biology and bioinformatics
 - Evolutionary biology
 - Engineering, computer science
 - Biosafety
 - Private sector
 - Risk assessment of emerging technologies
- Topics addressed included
 - State of the science of synthetic biology
 - Goals of research in synthetic biology
 - Predicting biological function from sequence
 - Risk assessment and management in the context of uncertainty

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

ADDRESSING BIOSECURITY CONCERNS RELATED TO SYNTHETIC BIOLOGY



DRAFT Report of the National Science Advisory Board for Biosecurity (NSABB)

What is synthetic biology?

- The design and construction of new biological parts and devices—including computational devices, and other functional nucleic acidbased structures
- The re-design of existing, natural biological systems for specific purposes, as well as
- The synthesis of self replicating entities from scratch

What is synthetic biology?

- Sometimes referred to as "engineering biology" since it often involves
 - characterizing and simplifying parts of natural biological systems and using them as components of an unnatural, engineered, biological system
 - creating novel biological structures with predictable properties and functions
 - seeking to understand the form and function of living organisms or their products and utilizing them in a predictable and controlled manner



Synthetic biology approaches

Top Down

- Involves the re-engineering of existing organisms or genomes for defined purposes
- Interweaves classical recombinant techniques with increasingly powerful methods for sequencing and synthesizing DNA
- Examples:
 - Metabolic engineering of microbes
 - Genome shuffling

Bottom Up

- Involves assembling nonliving biological components into novel systems to perform a desired function
- Predictability is based on an understanding of the fundamental nature of living organisms or biological materials
- Examples:
 - Biofabrication
 - Synthetic organism from scratch



- Highly interdisciplinary
- Researchers from diverse fields
- Practitioners who may not consider their work "biological"
- Practitioners with diverse research aims

- Life scientists
- Engineers
- Chemists
- Computer modelers
- Materials scientists
- "Re-writers"
- Students
- Non-traditional scientists, unaffiliated with universities or institutes
- Private industry

The promise of synthetic biology

- Synthetic biology:
 - A relatively nascent discipline
 - Rapidly evolving
 - Benefits from advances in related fields
- Numerous successes, both proofs of concept and commercial applications
 - The more ambitious goals have yet to be achieved





Significant uncertainties

- Synthetic biology is associated with several uncertainties stemming from
 - Present state of the science
 - Rapidly evolving nature of synthetic biology
 - Diverse practitioners attracted to synthetic biology

Predicting biological function

- Synthetic biology relies heavily on the ability to predict biological function from nucleic acid or protein sequence/structure
- State of the science
 - Accurately predicting biological properties from sequence or structure is very difficult
 - A better understanding of how biological context determines function is still needed

It will continue to be difficult to predict the biological risk of a synthetic entity, especially one that bears little resemblance to natural organisms.

An evolving field

- Science is evolving rapidly
 - Example: Novel genetic modules and functional RNA devices
- Cost is decreasing
 - Example: Massively parallel DNA synthesis and assembly
- Increasing rate at which information is generated
 Example: >1000 bacterial genomes sequenced

It will remain challenging to predict the new discoveries, information and technologies generated by a rapidly changing field.

An evolving field

nature

LETTERS

ARTICLES

M

Stabilized gene duplication enables long-term selection-free heterologous pathway expression

Keith E J Tyo, Parayil Kumaran Ajikumar & Gregory Stephanopoulos

Engineering robust microbes for the biotech industry typically requires high-level, genetically stable expression of heterologous Engineering robust microbes for the biotech industry typically requires high-level, genetically stable expression of heterologou genes and pathways. Although plasmids have been used for this task, fundamental issues concerning their genetic stability have not here advected a schemered. Users an describe chemically inducible chemomenous analysis of research and genes and pathways. Although plasmids have been used for this task, fundamental issues concerning their genetic stability have not been adequately addressed. Here we describe chemically inducible chromosomal evolution (CIChE), a plasmid-free. have not been adequately addressed. Here we describe chemically inducible chromosomal evolution (CIChE), a plasmid-free, high gene copy expression system for engineering Escherichia coli. CIChE uses E coli reck homologous recombination to evolve a chromosome with ...40 consecutive conise of a recombinant explorer Dathwave room number is stabilized to an A torochout high gene copy expression system for engineering Escherichia cofi. CIChE uses E coli red homologous recombination to evol a obromosome with .40 consecutive copies of a recombinant pathway. Pathway copy number is stabilized by mcA knockout. a chromosome with -40 consecutive copies of a recombinant pathway. Pathway copy number is stabilized by mcA function and the resulting engineered strain requires no selection markers and is unaffected by plasmid instabilities. Comparison of parts endowed descends markets along the control data descent instance along the stabilized by the stabilized by and the resulting engineered strain requires no selection markers and is unaffected by plasmid instabilities. Comparison of CIChE-engineered strains with equivalent plasmids revealed that CIChE improved genetic stability approximately tended and comb backgroups and the strain of CIChE-engineered strains with equivalent plasmids revealed that CIChE improved genetic stability approximately tenfold growth phase-specific productivity approximately fourfold for a strain producing the high metabolic burden-biopoymer and a strain productivity approximately to the output of the strain producing the high metabolic burden-biopoymer growth phase-specific productivity approximately fourfold for a strain producing the high metabolic burden-biopolymer poly-3-hydroxybutyrate. We also increased the yield of the nutraceutical lycopene by 60%. CIChE should be applicable in poly-3-hydroxybutyrate. We also increased the yield of the nutraceutical lycopene by ხU%. CiChi shoul many organismo, as it only requires having targeted genomic integration methods and a recA homolog.

neutra necessario egos a necessario e oparen negaste canze o const so oresproduce biochemical products from tenevable resources. Such overprovance onociennosi provancis inom renevanor i nouvices nasio advances include fabricating large synthetic pathways (*de novo* synthesiovances monuce surricumg large symmetric paravays use non-optimized stred DNA sequences)^{1,2} and optimizing pathway expression through naro Lova sequences – ana optimizing panway capacision univego transcription, or translation-level engineering^{1,4} which is essential to avoid buildup of toxic products. This progress has relied mainly on avou ouncup or user: produces, a new progress can react another of plasmid-based gene expression or single-copy genemic integration. saamin-oused gene expression or single-copy genomic immeration. Although plasmids are easy to insert into a cell and allow strong Annotago puannos are casy uo maen uno a cen ano anow strong gene expression, they suffer from genetic instability due to three procgene expression, user surret none general instatoury due to three proc-eases that reduce the number of active recombinant alleles in a cul-2009

ones that require the minutes of active recomminant anters in a car-ture". (i) segregational instability, in which unequal distribution of our: us segregational instatutty, in works unequal carriegnon or plasmids to daughter cells results in plasmid-free cells. (ii) structural presentes or usagener i cons resume in puentino -ree consi (11) structura Instability, in which some plasmids contain an altered DNA sequence unscaonity, in which some plasmids contain an ancred U-NA sequence that causes incorrect expression of the desired proteins: and (iii) allele is which productive plasmids are displaced by noninductive cells that are resistant

Becent breaktnoughs in metabolic engineering have made it easter to advance microbial overgroduction using heterologous pathways. Here conservatives biochemical conducts from researching conservation over research with technicous engineering for his conservation actives and we present such a sentimeter, totation unoprinted parameter ange-neering in microbial bosts (Fig. 1) to circumvent allele segregation, a neering to neerootat const (reg. 1) to chamerent aner expression, a fundamental flaw in plasmid-based gene expression (Fig. 2). We use unuamentai tawi ni pianina-uasea grati capitanni vrupie, i vec use a mathematical model to explain that random plasmid inheritance. a uncontinuous anoun su explain cost tanaon parsone universal. rather than mutation rates, drives productivity loss, whereas ordered ration management rates, univers procession by most encouragement of the international states of the state of insertion ce, sout as withing, can searching providence of ennou-longer. We also demonstrate that CIChE allows cells with heavy metasonget, we also uninsonate and cartain shows only white servery incase bolic bardens to remain productive and maintain or increase yield tone partness to remain productive and maintain or interest yand for many more generations than do analogous plasmid construct These results open the possibility for the broad use of CIChE-enj neered microbes in large-scale industrial production.

Random distribution, not mutation rates, limit the genetiv

Although structural and segregational instability have been unde stability of plasmids unitacilitat and segregational managements have very unit store², strategies devised to mitigate these instability

The breadth of genomic diversity found among organians in nature allows populations to adapt to diverse environments1.^{1,2} However, stores populations to scape to strene environments - normanic genanic diversity is difficult to generate in the laboratory and new genues, urrang o aracter to generate of the montainer sector of the phenotypes do not easily wise on practical timescales'. Although in pnenotypes do not easily arise on practical timescaler. Ambough in vitro and directed evolution methods+ have created genetic variants with usefully attend phenotypes, these methods are limited variants in the to uncertairy an error plannory peo, trener matinum servirum to to lab or out a and servid manipulation of single egenes and are not used to ano ar out o anna se raw m ann punataon or sange e genno a nara se mor unon for paral lei and c en finu ous directe d'evolu fion of gen e net works or roo para na tata este una una outra outro outro enormano ou gente met moreas or gen annes. Here, w e de scribe multi plex a utiona ied genome engines tgenumes, more, we consume an another a statement of genome suggestion ing (MAGE) for large-scale programming and evolution of cells. ring converse range were programming unit evolution to MAGE simultaneously targets many locations on the chrom for modification in a single cell or across a population of cells, thus retransmission and the suggestion of account preprior another of extent of the process in producing combinatorial genomic diversity. Recause the process is producing communications in contrast, in case the protocol of cyclical and acadeble, we constructed protocype devices that autocyatoa ana nantao, we onto a so prove protectade and continuous mate the MAGE technology to facilitate rapid and continuous terms are obtained volumenting of to independent regressions communities generation of a diverse set of genetic changes (misma chas, inter-tions, deletions). We applied MAGE to optimize the 1-decay-to volume e absorber (TWW) interventions for external to the topology.

and accelerated evolution

raena, energana), vre appaea antere to operation tai estericità xylulose 5-phosphate (DXP) biosyndiesis pathway in Escherichia aynnoneo-phonpunte (1887) nuovymatena patriway in tacentrama coli to overproduce the industrially i important isoprenoid lycop ene. Twenty four genetic components in the DXP pathway were modii wenny-tour generae componenna in use DAF paunay were mon-fied simultaneously using a complex pool of synthetic DNA, Teed summaneously using a compass pain or synthesis to two, creating over 4.3 billion combinatorial genomic variants per day. We isolated variants with more than fivefold increase in lycopene we assuance variants with more than investing increase in sycope ne production with in 3 days, a significant improvement over existing promotion manager and a second entraces engineering in the context of colution by expediting the design and evolution of organisms with new and improved

with the advent of next-generation fluorescent DNA = our ability to sequence genom

Harris H. Wang 123, Farren J. Isaacs¹*, Peter A. Carr^{4,5}, Zachary Z. Sun⁴, George Xu⁴, Craig R. Forest⁷

Vol 460/13 August 2009/doi:10.1038/nature08187

Programming cells by multiplex genome engineering

of the replication fork during DNA replication**. We optimized a number of parameters (see Supplementary Information, Supplement numeer or parameters (see supplementary renormalised, suppose a tay Fig. 2 and Supplementary Table 1) to maximize efficiency of uny rg. a una compromonany state a) en manana constant so oligo-mediated a le le replacement. To generate sequence diversity ougo-mean and store representative to generate superior surveying in any region of the chromosome by sitelic replacement, a pool of na any region or me caronnosome by anene repracement, a pool of targeting eligos is repeated y in troduced into a cell. Under optimized angeung engen in operant y introduce new genetic modifications conditions, we can saccessfully introduce new genetic modifications in >30% of the cell population (Supplementary Fig. 2d) every Oligo-mediated alcic replacement is capable of introducing a

ungeneration areas represented a capable of introducing a watery of genetic modifications at high efficiency. The efficiency of gen en ting a misma tch or intertion modification is correlated to the gen caung a statuteur or statuteur meter statuteur a contractor to terrate statuteur a st amount or homosogous sequence per ween me origo a no me national sound target (Fig. 2a, b); the efficiency of producing a deletion modi-fication is correlated to the size of the deletion (Fig. 2c); Figure 2d nections is non-measure as the bine to the non-more than $V(\Phi, e_{\Phi})$, equive an above that the predicted two-state hybridi ration free energy ΔG (ref. atoms and any product of the same introductation into the gy into par-(5) between the oligo and target chromosomal sequence is a pre-(2) remean one ongo and ranges uncommonstant mappings on a pro-dictor of the allelic replacement efficiency. Thus, in a pool of oligon with degenerate equences objectively more homology to the taget wan organizer as acquirence a couper was more nonninogy to use a gen will be incorporated in the chromosome at a higher frequency than those with less homology. This forture of MAGE enables tunable generation of divergent sequences along favourable evolutionary

Lines

Increasingly powerful methods significantly accelerate reengineering capabilities rigars 1) Multiplex automated genome engineering enables the repid and

sion of genetic constructs through de novo chromosomal engineer. regardless of mutation rate. The way-ing, rather than artificial plasmid-based systems, is much needed to binant pathways typically place heavy metabolic tr is ubiquitous in industrial biotechin endeavors to produce bioproducts using minimal-ge Department d'Ohenical Engineering, Massachunetts Institute of Technology, Cambridge, Massachunetts, USA. Conespondence should be addre Instrument de Ohenical Engineering, Massachunetts Institute of Technology, Cambridge, Massachunetts, USA. Conespondence should be addre

Received 31 March, accepted 29 June, published online 26 July 2009, doi:10.1038/idx1555 VOLUME 27 NUMBER 5 AUGUST 2005 NAT Efficiency of the MAGE process was characterized using a modiaross different length scales, a cara a continuous generation of sequence diversity at many largened

Evaluates you are interesting transmission was constructed as a single evaluation of the end of train (EcNR2). Mediated by the bacteriophage λ -Red Too E. ant Ream (ECNR2). Monaroo by the Doctorophage A-not stDNA-binding protein fl, allelic replacement is achieved in ECNR2 by directing sdDNA or oligonadoxidos (oligos) to the lagging strand constantions generated or insponents drive any sensitivity sequence chromosomial boations across a large population of each through the Contentionen a science access a sarge population of each strongen the populated introduction of synthesic DNA. Each all contains a different set of

ministeinen, producing a haden genome offen i kan om contensa a det brendsteitet by dialast deremonenna is different alle). Degenomi e eligi prasis dia

G2009 Macmillan Publishers Limited, All rights reserved

Diverse practitioners, diverse applications

- Synthetic biology is attracting a growing number of diverse practitioners
 - Diverse disciplines and interdisciplinary collaborations
 - Different research interests and goals
 - Discovery-based
 - Application-driven
 - Technology optimization and development
- Diversity is good for the scientific enterprise as it leads to the convergence of expertise and leads to new findings

Significant uncertainties

It is impossible to predict the information, technologies, and new applications that will be developed by, or applied to this relatively new field

Calls for

- Greater awareness of biosecurity (and biosafety) risks
- Pursuit of methods for predicting functions associated with DNA constructs and engineered proteins and organisms

Current oversight paradigms

- NIH Guidelines for Research Involving Recombinant DNA Molecules
 - Outline principles for safe research with recombinant DNA molecules
 - Detail procedures for handling and containment of genetically modified microorganisms, plants, and animals
 - Institutional Biosafety Committees (IBCs) review research involving rDNA
 - Recombinant DNA Advisory Committee (RAC):
 - Provides in-depth review of scientific, safety, and ethical dimensions of human gene transfer experiments
 - Advises NIH Director on content and implementation of NIH Guidelines

Current oversight paradigms

 Proposed updates to the NIH Guidelines address synthetic biology by including nucleic acid molecules made by synthetic means

The RAC has found that

- In most cases, research with synthetic nucleic acids presents biosafety risks that are comparable to recombinant DNA research
- Current risk assessment framework can be used to evaluate synthetically produced nucleic acids
- Safety issues surrounding synthetic nucleic acids will likely need to be revisited in the near future since the field is evolving so rapidly

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

Current oversight paradigms

Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information

A Report of the National Science Advisory Board for Biosecurity (NSABB)

June 2007

- NSABB has recommended a framework for the oversight of dual use life sciences research including
 - Steps in the local oversight of DURC
 - Criterion and guidance for identifying DURC
 - Tools to assess and manage the dual use risk associated with certain research
 - Tools for the responsible communication of research
 - Responsibilities of those conducting life sciences research

NSABB's Recommended Oversight Framework for DURC

Biosafety and biosecurity concerns

- Biosafety and biosecurity are distinct but related concepts
- <u>Biosafety</u> refers to the prevention of accidental exposure to hazardous materials
- <u>Biosecurity</u> refers to the prevention of unauthorized possession, loss, theft, misuse or diversion of hazardous agents; and the misuse of scientific information to threaten elements of national security
- NSABB's focus is biosecurity, but the two concepts converge since they both require the assessment and management of laboratory risks

Overarching biosafety and biosecurity concerns

- Biosafety concerns: recombinant techniques typically utilized in synthetic biology would be adequately covered by the NIH Guidelines
- Biosecurity concerns: should be adequately addressed by PI and institutional review in NSABB's oversight framework for dual use research

Current oversight addresses individuals conducting <u>life</u> <u>sciences research</u> <u>within universities or institutional settings</u> but...

- Not all synthetic biologists operate within these settings
- Many practitioners have backgrounds that are not rooted in the life sciences
- Not all practitioners consider their work "biological" in nature and may not regularly consider the biological or public, plant and animal health implications of their work

- Synthetic biology should be subject to institutional review and oversight since some aspects of this field pose biosecurity risks
 - NSABB has proposed an oversight paradigm that should adequately address dual use research issues associated with synthetic biology and strongly urges the federal government to develop and implement such policy

- Oversight of dual use research should extend beyond the boundaries of life sciences and academia
 - Gaps in oversight remain, primarily due to the large numbers of synthetic biology practitioners who come from backgrounds that are not traditionally considered life sciences or who lack formal institutional affiliations

- Outreach and education strategies should be developed that address dual use research issues and engage the research communities that are most likely to undertake work under the umbrella of synthetic biology
 - Education efforts should be developed that target synthetic biology researchers who are
 - a) not subject to federal biosafety and biosecurity requirements,
 - b) not formally affiliated with universities or research institutions, and
 - c) students (at all levels)

- The US Government should include advances in synthetic biology and advances in our understanding of virulence/pathogenicity in "tech-watch" or "science-watch" endeavors
 - It is appropriate for tech-watch or science-watch activities to identify emerging dual use technologies and new knowledge that could change the calculus about dual use risks and biosecurity concerns

More information

www.biosecurityboard.gov

nsabb@od.nih.gov