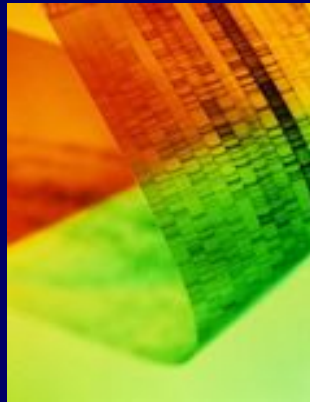
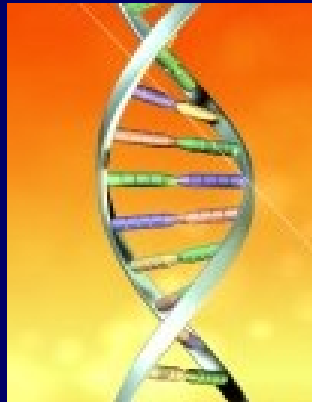


Addressing Biosecurity Concerns Related to Synthetic Biology



David Relman, M.D.

Chair, NSABB Working Group on
Synthetic Biology

Charge to NSABB

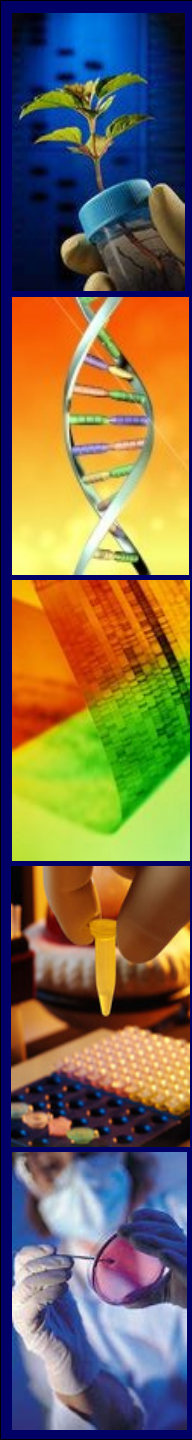
- Two-part charge

1. Synthetic Genomics

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

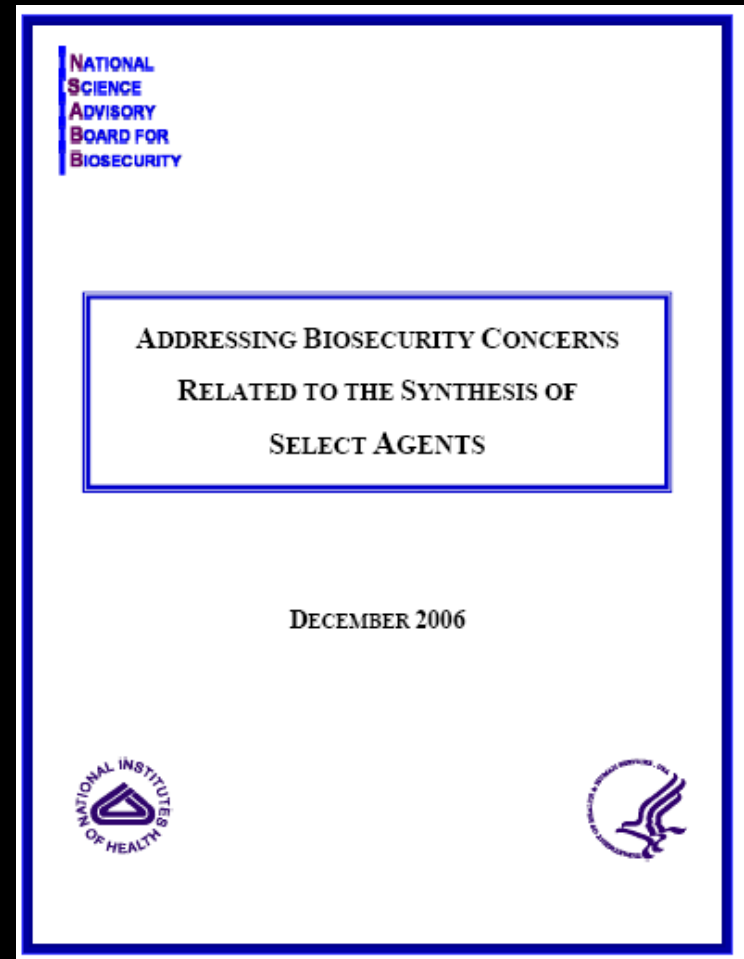
2. Synthetic Biology

...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





Recent development

al 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

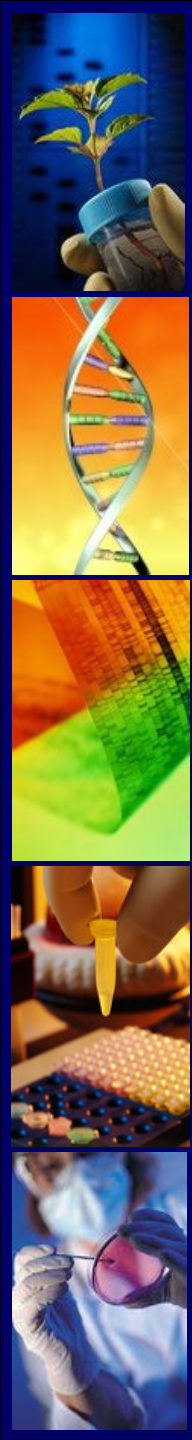
- David Relman (Chair)
- Susan Ehrlich
- Claire Fraser-Liggett
- Mike Imperiale
- Harvey Rubin
- Thomas Shenk

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

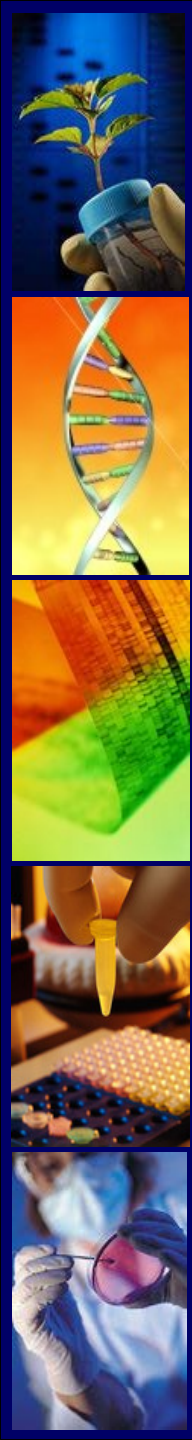
NSABB Approach to Synthetic Biology

- 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*



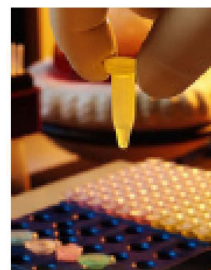
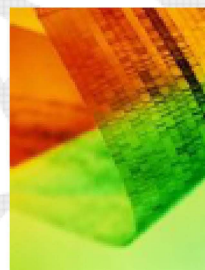
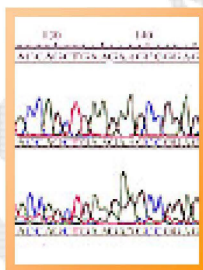
Scientific Roundtable Hosted by NSABB and RAC (Oct 11, 2007)

- 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**

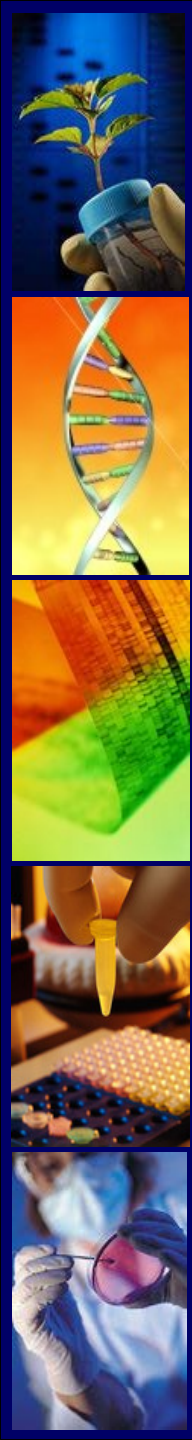
**ADDRESSING BIOSECURITY CONCERNS RELATED
TO SYNTHETIC BIOLOGY**



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

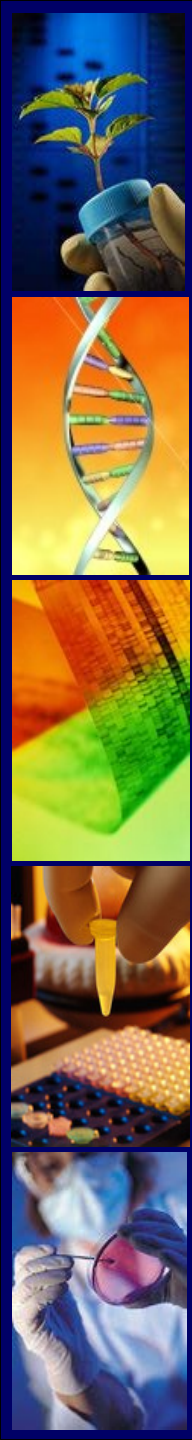
What is synthetic biology?

- The design and construction of new biological parts and devices—including computational devices, and other functional nucleic acid-based 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 non-living 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

Who are synthetic biologists?

- 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

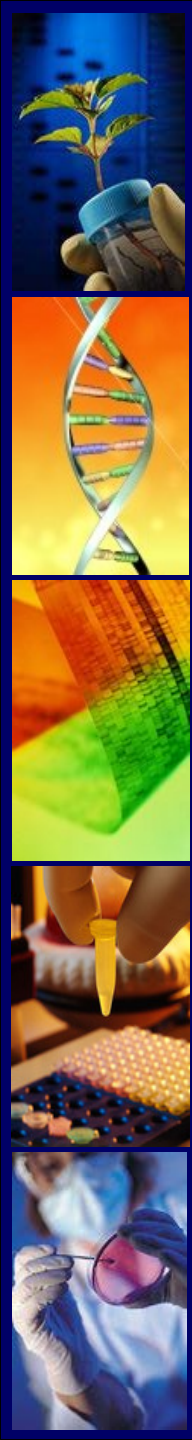
The promise of synthetic biology

- 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

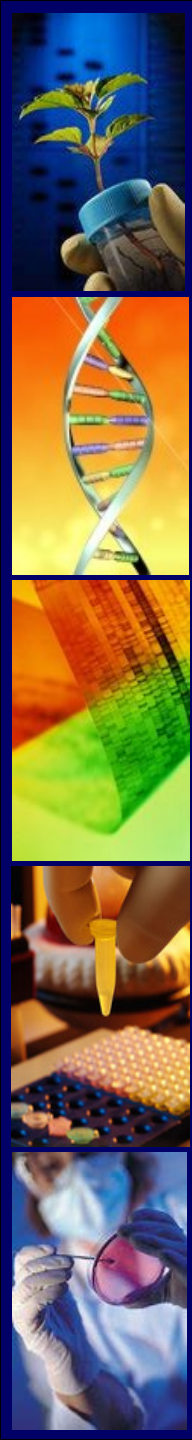
- Synthetic biology is associated with several uncertainties stemming from
 - Present state of the science
 - Rapidly evolving nature of synthetic biology
 - Diverse practitioners attracted to synthetic biology



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

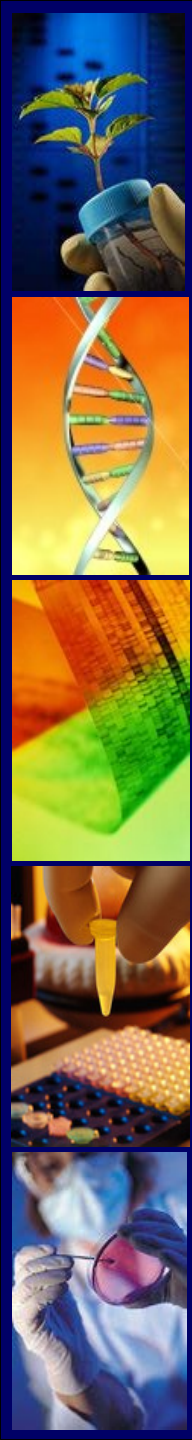
It will continue to be difficult to predict the biological risk of a synthetic entity, especially one that bears little resemblance to natural organisms.



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

ARTICLES

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 genes and pathways. 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* recA homologous recombination to evolve a chromosome with ~40 consecutive copies of a recombinant pathway. Pathway copy number is stabilized by recA knockout 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 tenfold and growth phase-specific productivity approximately fourfold for a strain producing lycopene by 60%. CICHE should be applicable in many organisms, as it only requires having targeted genomic integration methods and a recA homolog.

Recent breakthroughs in metabolic engineering have made it easier to overproduce biochemical products from renewable resources. Such advances include fabricating large synthetic pathways (*de novo* synthesis of DNA sequences)^{1,2} and optimizing pathway expression through transcription- or translation-level engineering^{3,4}, which is essential to avoid buildup of toxic products. This progress has relied mainly on plasmid-based gene expression or single-copy genomic integration. Although plasmids are easy to insert into a cell and allow strong gene expression, they suffer from genetic instability due to three processes that reduce the number of active recombinant alleles in a culture: (i) segregational instability, in which unequal distribution of plasmids to daughter cells results in plasmid-free cells; (ii) structural instability, in which some plasmids contain an altered DNA sequence that causes incorrect expression of the desired proteins; and (iii) allele segregation, in which productive plasmids are displaced by non-productive plasmids, leading to nonproductive cells that are resistant to selection pressure.

Alternative strategies have been implemented to reduce genetic instability, including the use of chromosomal integration, which is not mitigated by segregational instability. However, chromosomal integration (PCI) has not been considered widely in biotechnology. In this work, we describe a new method for stabilizing gene expression in *E. coli* by using a high-copy, non-replicating plasmid that is integrated into the chromosome by homologous recombination (HR). This method, called CICHE, allows cells to maintain active plasmids for only ~35 generations (an evolutionary timescale) before the plasmid is lost. The loss of the plasmid alone because of instability is not a problem, as the plasmid can be reintroduced into the cell by transformation. The loss of the plasmid would not be explained by the loss of the plasmid alone because of instability is not a problem, as the plasmid can be reintroduced into the cell by transformation.

Decreasing genetic instability is a key challenge in metabolic engineering. This is particularly true for the production of high-value products in batch or continuous fermentation. This instability can be addressed by using a high-copy, non-replicating plasmid that is integrated into the chromosome by homologous recombination (HR). This method, called CICHE, allows cells to maintain active plasmids for only ~35 generations (an evolutionary timescale) before the plasmid is lost. The loss of the plasmid alone because of instability is not a problem, as the plasmid can be reintroduced into the cell by transformation. The loss of the plasmid would not be explained by the loss of the plasmid alone because of instability is not a problem, as the plasmid can be reintroduced into the cell by transformation.

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. Correspondence should be addressed to gregstep@mit.edu.
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VOLUME 27 NUMBER 8 AUGUST 2009 NATI

nature biotechnology

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LETTERS

Programming cells by multiplex genome engineering and accelerated evolution

Harris H. Wang^{1,2,3*}, Farren J. Isaacs^{1*}, Peter A. Carr^{4,5}, Zachary Z. Sun⁶, George Xu⁶, Craig R. Forest⁷ & George M. Church¹

The breadth of genomic diversity found among organisms in nature allows populations to adapt to diverse environments^{1,2}. However, genomic diversity is difficult to generate in the laboratory and new variants with useful alterations^{3,4} have created genetic for parallel and continuous directed evolution of gene networks or genomes. Here, we describe multiplex automated genome engineering (MAGE) for large-scale programming and evolution of cells. MAGE simultaneously targets many loci on the chromosome for modification in a single cell or across a population of cells, thus producing combinatorial genomic diversity. Because the process is cyclical and scalable, we constructed prototype devices that automate the MAGE technology to facilitate rapid and continuous generation of a diverse set of genetic changes (insertions, deletions, substitutions). We applied MAGE to optimize the 1-deoxy-xylose-5-phosphate (DXP) biosynthesis pathway in *Escherichia coli* to overproduce the industrially important isoprenoid byproduct. Twenty-four genetic components in the DXP pathway were modified simultaneously using a complex pool of synthetic DNA, creating over 4.3 billion combinatorial genomic variants per day. We isolated variants with more than a fivefold increase in lycopene production within 3 days, a significant improvement over existing metabolic engineering techniques. Our multiplex approach accelerates the design and evolution of organisms with new and improved properties.

With the advent of next-generation fluorescent DNA sequencing⁵, our ability to sequence genomes has greatly outpaced our ability to modify genomes. Existing cloning-based techniques are confounded by serial and inefficient introduction of synthetic DNA into cells, requiring thousands of clones to achieve a single modification. Large-scale genetic targets using single-stranded DNA (ssDNA)⁶⁻¹⁰ or parallel ssDNA (pssDNA)¹¹ have been made to modify genomes on a large and automated solution to simultaneously modify many genomic loci (for example, genes, regulatory regions) across different length scales from the nucleotide to the genome level (Fig. 1).

Efficiency of the MAGE process was characterized using a modified *E. coli* strain (BGR2). Mediated by the bacteriophage λ -Red by directing ssDNA or oligonucleotides (oligos) to the lagging strand of the replication fork during DNA replication¹². We optimized a number of parameters (see Supplementary Information, Supplementary Fig. 2 and Supplementary Table 1) to maximize efficiency of oligo-mediated allelic replacement. To maximize sequence diversity in any region of the chromosome by allelic replacement, a pool of targeting oligos is repeatedly introduced into a cell. Under optimized conditions, we can successfully introduce new genetic modifications in >30% of the cell population (Supplementary Fig. 2d) every 2–2.5 h.

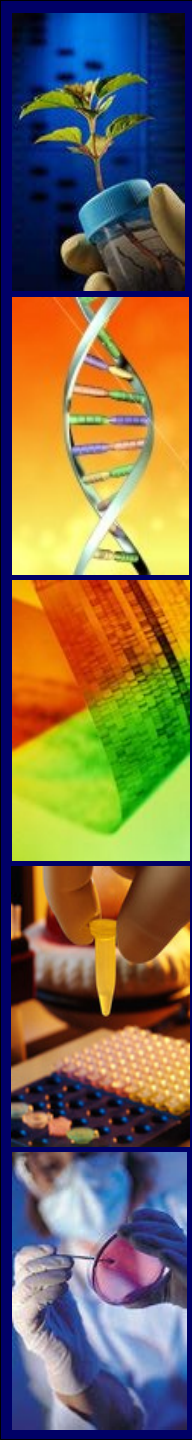
Oligo-mediated allelic replacement is capable of introducing a variety of genetic modifications at high efficiency. The efficiency of generating a mismatch or insertion/modification is correlated to the amount of homologous sequence between the oligo and its chromosomal target (Fig. 2a,b); the efficiency of producing a deletion/modification is correlated to the size of the deletion (Fig. 2c). Figure 2d shows that the predicted two-state hybridization free energy ΔG (ref. 13) between the oligo and target chromosomal sequence is a predictor of the allelic replacement efficiency. Thus, in a pool of oligos with degenerate sequence, oligos with more homology to the target will be incorporated in the chromosome at a higher frequency than those with less homology. This feature of MAGE enables tunable generation of divergent sequences along favourable evolutionary paths.



Figure 1 Multiplex automated genome engineering enables the rapid and continuous generation of sequence diversity at nearly targeted chromosomal locations across a large population of cells through the repeated introduction of synthetic DNA. Each cell contains a different set of target-specific genomic positions enable the generation of a diverse set of sequences at each chromosomal location.

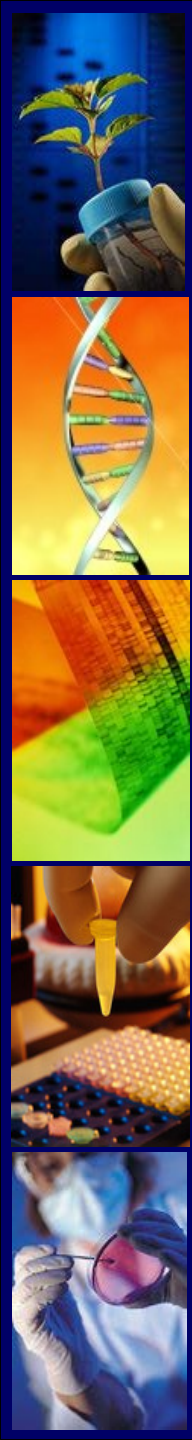
Diverse practitioners, diverse applications

- 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



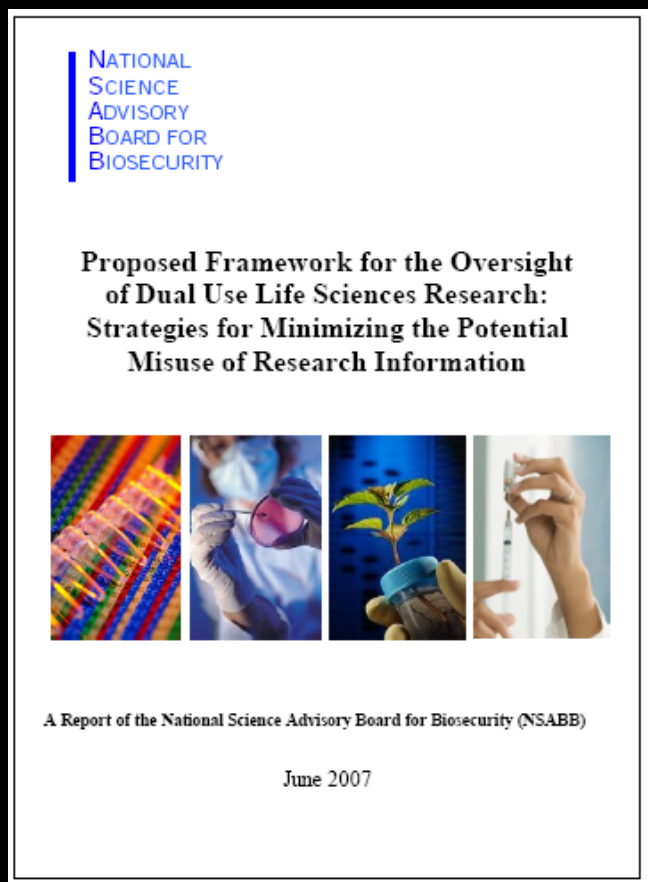
Current oversight paradigms

- *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

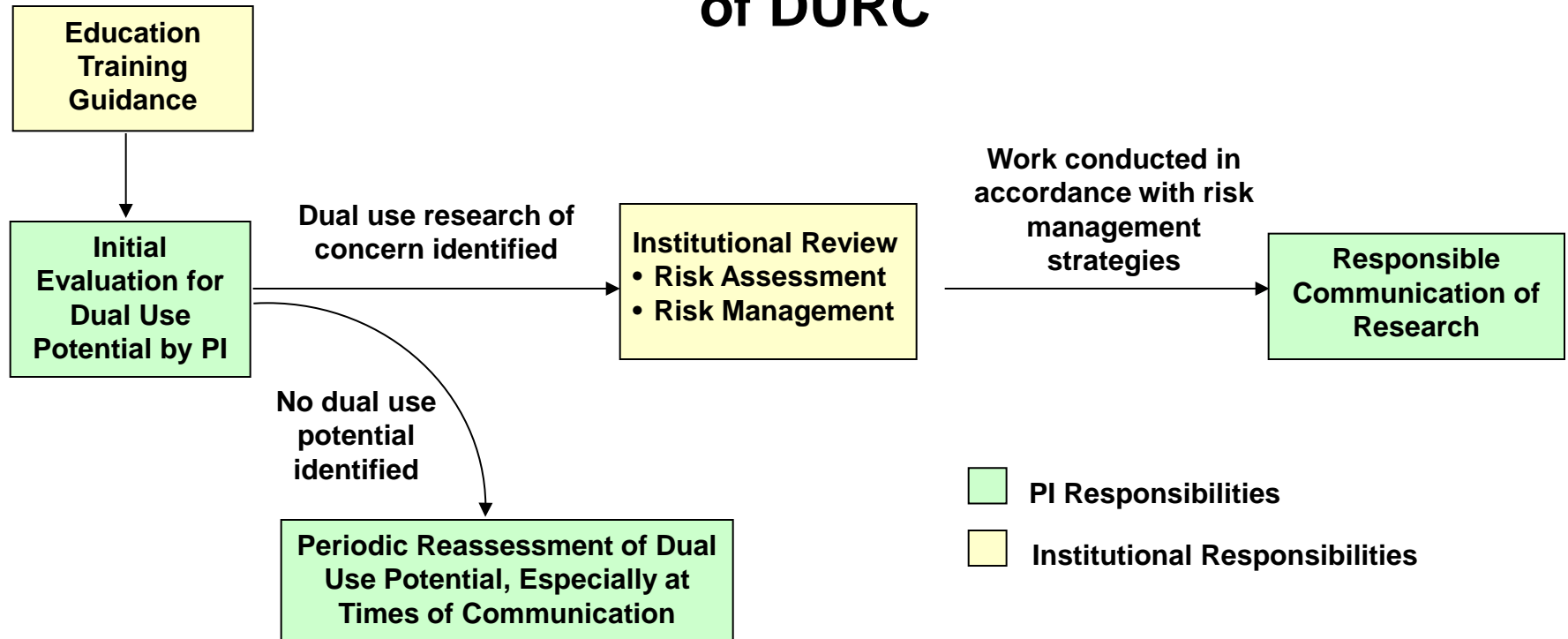
Current oversight paradigms



- 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

Proposed Steps in Local Oversight of DURC



Biosafety and biosecurity concerns

- Biosafety and biosecurity are distinct but related concepts
- Biosafety – refers to the prevention of accidental exposure to hazardous materials
- Biosecurity – 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

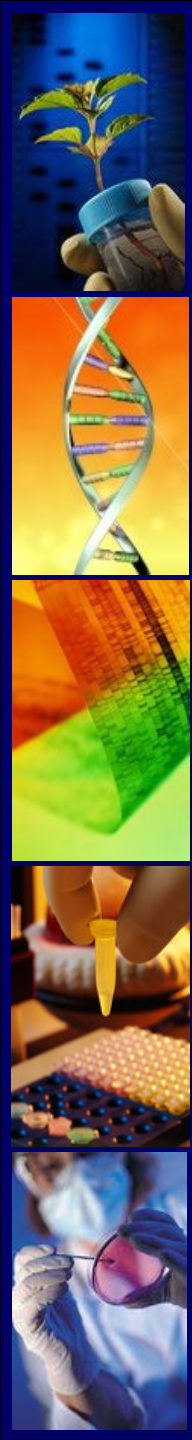
- Biosafety concerns: recombinant techniques typically utilized in synthetic biology would be adequately covered by the *NIH Guidelines*
- Biosecurity concerns: should be adequately addressed by PI and institutional review in NSABB's oversight framework for dual use research

Current oversight addresses individuals conducting life sciences research within universities or institutional settings 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

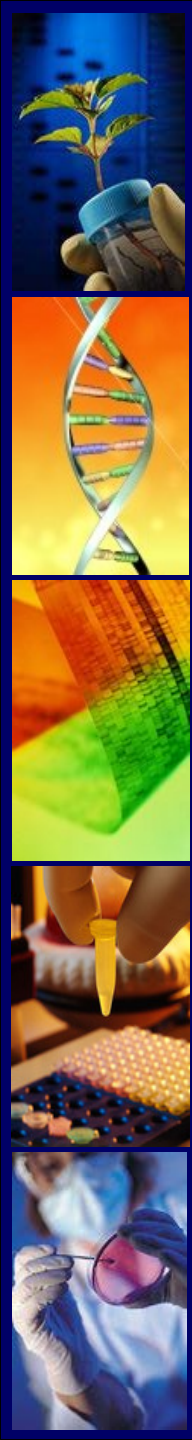
Recommendation 1

- **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



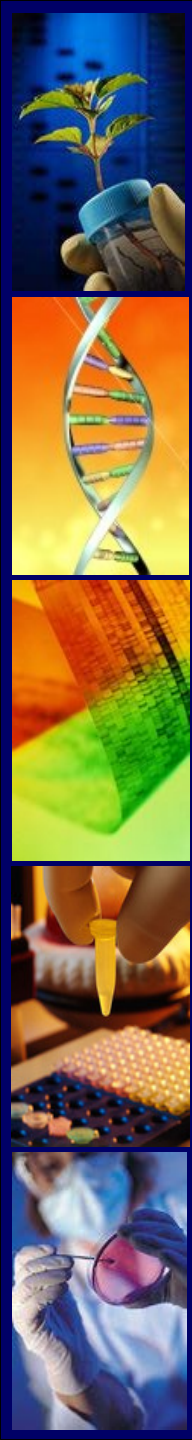
Recommendation 2

- **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



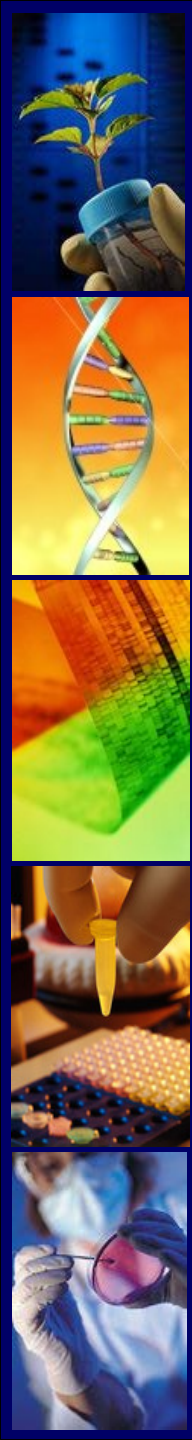
Recommendation 3

- 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)



Recommendation 4

- 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



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News and Events
NSABB Meetings
Frequently Asked Questions
NSABB Documents
Participating Agencies

NSABB - Past Meetings

April 2009 Meeting - April 3

Agenda and Webcasts

- [Agenda](#) [Webcast](#)
- Minutes will be posted once they are available.

Presentation Materials

Background and Introduction to the Personnel Reliability Issue

- [Understanding and Improving Laboratory Security, Personnel Reliability, and Safety](#) 
Diane DiEulis, Ph.D.
Office of Science and Technology Policy

Panel 1 - Extant Models of Personnel Reliability Programs

- [The Army Biological Personnel Reliability Program \(BPRP\)](#) 
John Humpton
Combating WMD and Proliferation Policy Division G-3/5/7, Headquarters, Department of the Army
- [LLNL Select Agent Human Reliability Program](#) 
Eric Gard, Ph.D.
Global Security Directorate, Lawrence Livermore National Laboratory
- [Bioterrorism Risk Assessment Group](#) 
John Stovers
Bioterrorism Risk Assessments, Criminal Justice Information Services, FBI
- [NSABB Briefing: Security Risk Assessments for Possession, Use, and Transfer of Select Agent](#)

nsabb@od.nih.gov