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U.S. Initiative: Screening Framework Guidance for Synthetic Double-Stranded DNA Providers

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Jessica Tucker, Ph.D.

Senior Policy Analyst/Contractor

Office of the Assistant Secretary for Preparedness and Response
U.S. Department of Health and Human Services

Synthetic Biology Workshop: From Science to Governance

March 18-19, 2010

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Balancing Benefits and Risks

- Synthetic biology and the underlying technologies together can provide significant scientific, health, and economic benefits.
- Nucleic acid synthesis technology is a potentially enabling technology for the *de novo* reconstruction of dangerous pathogens, either in part or in whole.
  - *De novo* synthesis of naturally-occurring pathogens
    - Access to sequences and organisms of concern
    - Evasion of current regulatory and physical access controls (e.g., U.S. Select Agents, Australia Group, pathogen security processes and procedures)
  - *De novo* synthesis of novel biological agents
    - Pathogens with unique properties
- Development of any oversight mechanism must…
  - balance the need to minimize the risk of misuse with the need to ensure that science and innovation are encouraged; and
  - involve engagement of the synthetic nucleic acid industry, the scientific community, and other stakeholders.

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Advances in *De Novo* Synthesis

2002 Polio Virus Synthesis Experiment

Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template

Jeronimo Cello, Aniko V. Paul, Eckard Wimmer*

Full-length poliovirus complementary DNA (cDNA) was synthesized by assembling oligonucleotides of plus and minus strand polarity. The synthetic poliovirus cDNA was transcribed by RNA polymerase into viral RNA, which translated and replicated in a cell-free extract, resulting in the de novo synthesis of infectious poliovirus. Experiments in tissue culture using neutralizing antibodies and CD155 receptor–specific antibodies and neurovirulence tests in *CD155* transgenic mice confirmed that the synthetic virus had biochemical and pathogenic characteristics of poliovirus. Our results show that it is possible to synthesize an infectious agent by in vitro chemical-biochemical means solely by following instructions from a written sequence.

Research on viruses is driven not only by an urgent need to understand, prevent, and cure viral disease. It is also fueled by a strong curiosity about the minute particles that we can view both as chemicals and as "living" entities. Poliovirus can be crystallized (*1*) and its empirical formula can be calculated (*2*), yet this "chemical" replicates naturally in humans with high efficiency, occasionally causing the paralyzing and lethal poliomyelitis.

Poliovirus, an enterovirus of the *Picornaviridae*, is a small, nonenveloped, icosahedral virus consisting of five different macromole-

templates for the synthesis of new viral genomes (plus-strand RNA). Newly synthesized plus-strand RNA can serve as messenger RNA for more protein synthesis, engage further in RNA replication, or be encapsidated by an increasing pool of capsid proteins (*7*, *12*). In suitable tissue culture cells (for example, HeLa cells), the entire replication cycle is complete in only 6 to 8 hours and yields 10^4 to 10^5 progeny virions per cell.

Here we describe the de novo chemical-biochemical synthesis of infectious poliovirus from basic chemical building blocks, in-

sequenced to identify either the correct DNA segments or the segments containing small numbers of errors that could be eliminated, either by combining the error-free portions of segments by an internal cleavage site or by standard site-directed mutagenesis (*13*). To ascertain the authenticity of the synthesized viral genome [sPV1(M)] and to distinguish it from the wild-type (*wt*) sequence of PV1(M) [*wt* PV1(M)] (*4*, *5*), we engineered nucleotide substitutions into the sPV1(M) cDNA as genetic markers (*13*).

We have shown previously that poliovirus cDNA carrying a phage T7 promoter for the phage RNA polymerase can be transcribed with T7 RNA polymerase into highly infectious RNA (*14*). Accordingly, the sPV1(M) cDNA and *wt* PV1(M) cDNA were transcribed (*13*) and were found to yield transcript RNAs of the same length as virion RNA (*15*). De novo synthesis of poliovirus from transcript RNA of *wt* PV1(M) cDNA in a cell-free extract of uninfected HeLa cells has been previously described by Molla *et al.* (*2*). Therefore, the incubation of transcript RNA from sPV1(M) cDNA in cytoplasmic extracts of uninfected HeLa cells should result in the generation of poliovirus. To examine this possibility, transcript RNA derived from sPV1(M) cDNA was incubated with a cytoplasmic extract of HeLa S3 cells, and the synthesis of virus-specific proteins and infectious viruses were monitored. The products of sPV1(M) cDNA–derived RNA translation and proteolytic processing were the same as those obtained with *wt* PV1(M) RNA (Fig.

Science, vol. 297, issue 5583 (9 August 2002): 1016-1018

"Our results show that it is possible to synthesize an infectious agent by in vitro chemical-biochemical means solely by following instructions from a written sequence."

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Genesis of the Synthetic DNA and Security Interagency Process

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NSABB

- The purpose of the National Science Advisory Board for Biosecurity (NSABB) is to provide advice, guidance, and leadership regarding biosecurity oversight of dual use research, defined as biological research with legitimate scientific purpose that may be misused to pose a biologic threat to public health and/or national security.
- NSABB Charge: Examine the potential biosecurity concerns raised by the synthesis of Select Agents and recommend strategies for addressing these concerns.
- In the 2007 report, *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents*[1], NSABB recommended the U.S. Government, in consultation with outside experts, develop a screening process to be used by providers of synthetic DNA.

[1]*Report can be accessed at http://oba.od.nih.gov/biosecurity/pdf/Final_NSABB_Report_on_Synthetic_Genomics.pdf*

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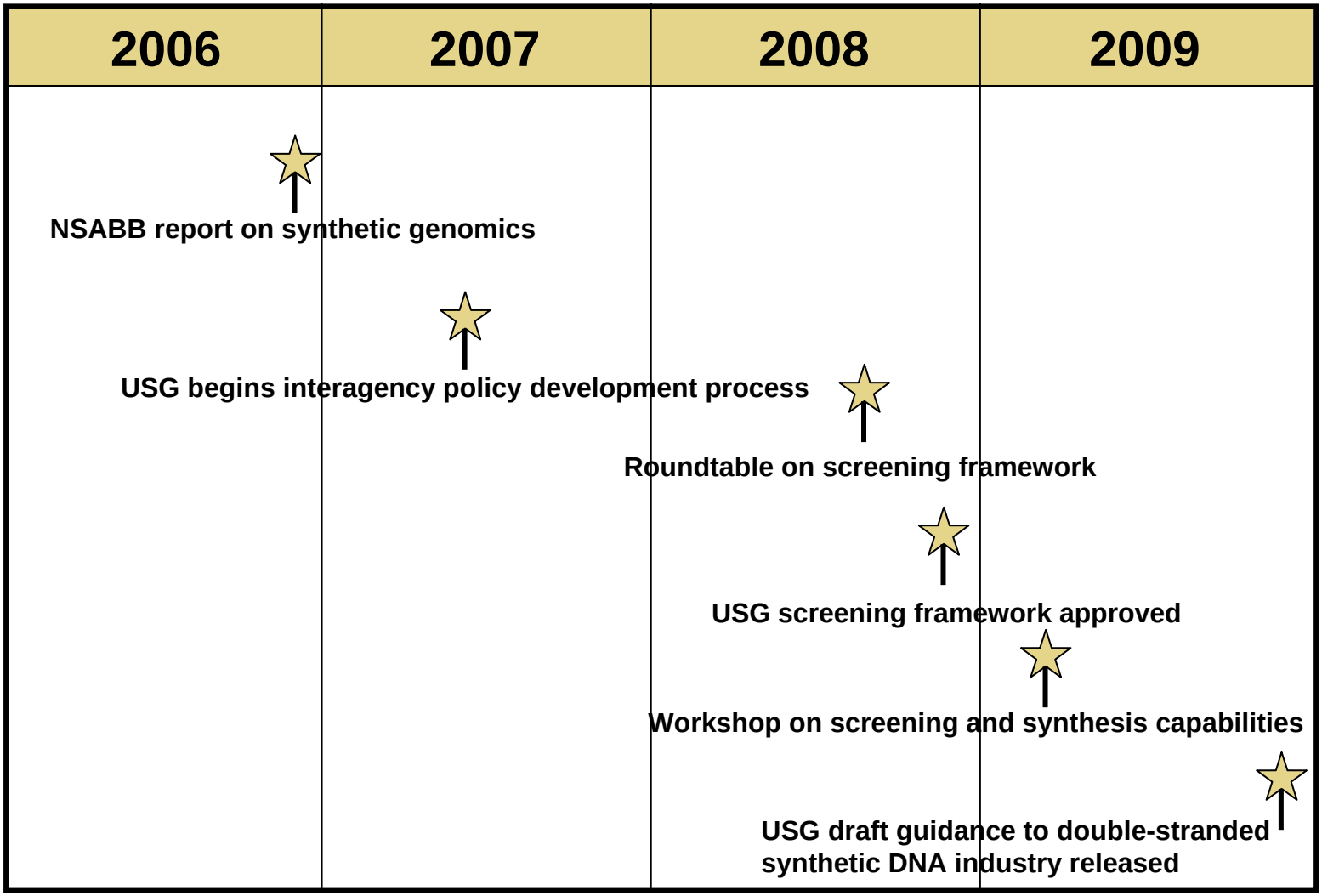
Key Milestones for USG Efforts on Synthetic DNA and Security



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USG Development of a Screening Framework

- Overarching goal:
  - Minimize the risk that unauthorized individuals or individuals with malicious intent will gain access to toxins and organisms of concern through the use of nucleic acid synthesis technologies; simultaneously minimize any negative impacts on the conduct of research and business operations
- Key elements:
  1. Appropriate sectors of the synthetic nucleic acid industry
     - dsDNA gene and genome synthesis sector
  2. Mechanism(s) by which a screening framework should be pursued
     - Voluntary- Greater efficacy and reduced negative economic impact
  3. Principles and objectives of screening
     - Providers should know customers and whether products they are selling pose a hazard
  4. Process for enabling timely response to orders of concern
     - Scenarios for contacting USG
  5. Enabling development of tools to facilitate implementation
  6. Evaluating implementation and impact(s)

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Summary of Guidance Recommendations

- The U.S. Government recommends that all orders for synthetic double-stranded DNA 200 base pairs (bps) in length or greater be subject to a screening framework that incorporates both sequence screening and customer screening.
- *Customer Screening*
  - The U.S. Government recommends that, for every order, synthetic nucleic acid providers:
    - Verify the customer's identity.
    - Screen customers against several lists of proscribed entities.
    - Check for 'red flags.'
  - In any case where *customer screening* raises a concern, providers should conduct *follow-up screening.*

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Summary of Recommendations, Continued

- *Sequence Screening*
  - The U.S. Government recommends that:
    - Nucleic acid sequences be screened against GenBank using a ‘‘Best Match’’ approach to identify nucleic acids that are unique to Select Agents and Toxins.
    - For foreign orders, nucleic acids be screened using a ‘‘Best Match’’ approach to identify nucleic acids that are unique to pathogens and toxins on the Commerce Control List and nucleic acids that are unique to Select Agents and Toxins.
    - Sequence screening be performed for both DNA strands and the resultant polypeptides derived from translations using the three alternative reading frames on each DNA strand (or six-frame translation).
    - Sequence alignment methods should permit the detection of hidden ‘‘sequences of concern’’ as small as 200 bps.
    - In any case where *sequence screening* raises a concern, providers should conduct *follow-up screening*.

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Summary of Recommendations, Continued

- *Follow-up Screening*
  - When customer screening reveals any 'red flags' or sequence screening identifies a sequence of concern, the U.S. Government recommends that
    - Providers ask for information regarding the customer's proposed end-use of the order to assess their need and the scientific legitimacy of their work.
    - Providers take additional steps to verify the customer's identity and need.
- *Domestic and Foreign Orders*
  - The U.S. Government reminds providers to check against various lists of restricted entities before filling every order; these lists vary for domestic and foreign customers.
- *Contacting the U.S. Government*
  - In cases where *follow-up screening* cannot resolve concerns raised by *customer screening* or *sequence screening, or when providers are otherwise unsure about whether to fill an order*, the U.S. Government recommends that providers contact relevant agencies.

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Summary of Recommendations, Continued

- *Sequence Screening Software and Expertise*
  - The U.S. Government recommends that:
    - Providers select a sequence screening software tool that utilizes both a global and local sequence alignment technique.
    - Providers have the necessary expertise in-house to perform the sequence screenings, analyze the results, and conduct the appropriate follow-up research to evaluate the significance of dubious sequence matches.
- *Records Retention*
  - The U.S. Government recommends that providers retain electronic copies of customer orders for at least eight years.

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Process Summary and Next Steps

- Draft Guidance was posted for public comment in the Federal Register on November 27, 2009 and was open for public comment for a period of 60 days.
- A public meeting was hosted by the American Association for the Advancement of Science on January 11, 2010 to solicit additional feedback from scientists on the draft Guidance.
- Please see http://edocket.access.gpo.gov/2009/E9-28328.htm for a copy of the draft Guidance.
- The U.S. Government is in the process of reviewing and considering public comments for possible incorporation into the Guidance.
- The final Guidance will be publicly released at the conclusion of the process.
- An interagency group has been established to develop plans to monitor the implementation and to evaluate the effectiveness of the Guidance.

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Thank you.