

**ADDRESSING BIOSECURITY CONCERNS
RELATED TO THE SYNTHESIS OF
SELECT AGENTS**

DECEMBER 2006



Introduction:

Background

DNA synthesis technology, in combination with other rapidly-evolving capabilities in the life sciences, such as directed molecular evolution and viral reverse genetics, has galvanized segments of the scientific community.¹ It also has captured the attention of the general public and policymakers, and prompted far-reaching questions about the potential uses of these techniques—including the synthesis of novel forms of life. These techniques promise to accelerate scientific discovery and provide access to previously-unexplored biological and molecular diversity.. This technology also is employed in the field of Synthetic Genomics² to create sophisticated live vaccines and to discover new therapeutics for infectious diseases. However, this same technology can be misused to generate dangerous pathogens *de novo* that are subject to oversight, thus circumventing the extant regulatory framework for controlling the possession and use of such organisms. This dichotomy illustrates the dual use nature of synthetic genomics and underscores the need to develop strategies to address the possibility that knowledge and technologies emanating from vitally important biological research will be misused to threaten public health or national security.

In this regard, rapid advances in DNA synthesis technology and the open availability of pathogen genome sequence data have raised concerns in the scientific community and general public regarding the possible use of this technology and information to generate biological agents that could threaten public health, agriculture, plants, animals, the environment, or materiel. Special concern has been voiced about the use of this technology to generate Select Agents³ *de novo*. While traditional recombinant DNA technology has raised similar or related concerns, approaches based on *de novo* synthesis avoid any need for access to the naturally occurring agents or naturally occurring nucleic acids from these agents, and greatly expand the potential availability of these agents. The National Science Advisory Board for Biosecurity (NSABB) has been charged with identifying the potential biosecurity concerns raised by the ability to synthesize Select Agents and providing advice on whether current United States Government (USG) policies and regulations adequately cover the *de novo* synthesis of Select Agents or whether additional biosecurity measures are necessary.

This report describes the biosecurity concerns identified by the NSABB Working Group on Synthetic Genomics that are raised by the ability to reconstruct Select Agents *de novo*, the Working Group's assessment of the adequacy of the current regulatory framework to safeguard against the misuse of this science and its recommendations for addressing these concerns. These

¹ This array of technologies can be used to create any specified DNA sequence by synthesizing and combining fragments of DNA (oligonucleotides). Encoded product(s) can be expressed and subjected to selection for desired properties.

² "Synthetic Genomics" generally refers to the design and production of viral genomic DNA or RNA for the purpose of expressing the encoded viral product. Because of technical challenges, expression of bacteria and other larger life forms from synthetic genomes is not currently feasible, but efforts are underway to achieve these goals.

³ Select Agents are biological agents and toxins regulated by the Select Agent Rules (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73) that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products. A list of Select Agents and Toxins can be found at <http://www.cdc.gov/od/sap/docs/salist.pdf>.

recommendations were approved by the NSABB on October 25, 2006 for submission to the USG.

Issue

Viral genomes can be reconstructed using time-intensive recombinant DNA methods and reverse engineering techniques. Such methods typically require access to a natural source of the virus of interest or its genomic material. Recent advances in DNA synthesis technologies enable the *de novo* construction of viral genomes and have dramatically increased both the ease and accuracy with which large fragments of genomic sequences can be constructed. This has greatly enhanced the ability of researchers to acquire, with a short turnaround time, accurate and specific gene- and multi-gene length sequences from an increasing number of commercial suppliers, both nationally and internationally. These technological developments are significantly advancing life sciences research.

DNA synthesis technologies also can and have been used to generate the viral genomes of dangerous pathogens, which can then be expressed as infectious agents that can be used for both beneficial and harmful purposes. It is also possible to use this technology to create genomes that express toxins or virulence factors. Furthermore, the scientific community anticipates that scientific and technological advances will expand this capability to include bacterial and fungal pathogen genomes. These developments have raised questions about the adequacy of extant regulations to safeguard against the use of these technologies to threaten public health, agriculture, plants, animals, the environment, or materiel.

NSABB Charge

The NSABB was charged with examining the potential biosecurity concerns raised by the synthesis of Select Agents, and by synthetic biology⁴ in general, and with recommending strategies for addressing these concerns.

As a first step in addressing this charge, the NSABB formed the Working Group on Synthetic Genomics to assess whether synthetically derived Select Agents are adequately covered by the current regulatory framework for Select Agents i.e., whether Select Agents synthesized *de novo* escape the purview of the extant oversight system, and, what are strategies and mechanisms that might prevent or mitigate potential misuse of synthetic genomics while minimizing restrictions on the beneficial uses of this important field of science.

Following that phase of the charge, the Working Group (WG) will identify, assess and recommend strategies to address potential dual use concerns that may arise from work being performed in the nascent field of synthetic biology. This will be the subject of a later report.

⁴ The goal of synthetic biology is to extend or modify the behavior of organisms and engineer them to perform new tasks. Andrianantoandro et al. "Synthetic biology: new engineering rules for an emerging discipline" *Molecular Systems Biology* 2: 1 – 14, 2006.

Summary of Findings:

Approach

Assessing the adequacy of the current regulatory framework requires the identification and assessment of pertinent laws, regulations and policies in addition to gauging the current state of the science. Therefore, in carrying out the first phase of its charge, the WG examined the state of the science and technology⁵ that can be used to synthesize a Select Agent *de novo* and the pertinent oversight framework. Specifically, the WG received presentations from and held discussions with:

- industry experts about the current technological capabilities for synthesizing nucleic acids and the resources needed to do so;
- eminent researchers on the state of the science, in a few key application areas, for deriving infectious agents from synthetic nucleic acids;
- USG officials from the Department of Health and Human Services (HHS) Centers for Disease Control and Prevention (CDC), Department of Commerce (DOC), and Department of Agriculture (USDA) on the extant legal/regulatory framework for controlling Select Agents; and
- key stakeholders regarding their perspectives about biosecurity concerns related to the ability to synthesize Select Agents.

State of the Science

The presentations from industry experts focused on the capabilities of current synthesis technologies and oversight procedures that are being employed by commercial entities to ensure compliance with the Select Agent Rules (SAR) and other pertinent regulations. Much of the industry is composed of suppliers of small oligonucleotides used in routine experiments, whereas providers of gene-length sequences are a smaller fraction of the sector, and the generation of genome-length sequences is left to a handful of companies servicing a boutique market. Participants differed in their interpretation of what is required of them under the current regulatory system; they also noted that not all providers felt legally obligated to know what sequences they were making and providing to clients. Furthermore, they observed that complying with U.S. requirements is complicated by the global distribution and multi-national nature of DNA synthesis providers, their clients and suppliers of key ingredients and equipment.

The briefings by researchers described methodologies used to recover infectious virus from the DNA of Select Agent viruses. Through these presentations it became apparent that it is possible to construct infectious agents from synthetic or naturally derived DNA. The technology for synthesizing DNA is readily accessible, straightforward and a fundamental tool used in current biological research. In contrast, the science of constructing and expressing viruses in the laboratory is more complex and somewhat of an art. It is the laboratory procedures downstream from the actual synthesis of DNA that are the limiting steps in recovering viruses from genetic material.

⁵ Such technologies are referred to as synthetic genomics in the remainder of this report since the term generally refers to an array of technologies that can be used to create virtually any specified DNA sequence by synthesizing and then combining fragments of DNA (oligonucleotides).

Based upon these briefings and the review of key scientific literature, the WG found that:

- reagents and equipment for synthesizing DNA are readily available around the world;
- synthesizing oligonucleotides accurately up to 120 base pairs (bp) in length is routine and common although synthesizing oligonucleotides of more than 180 bp remains somewhat of an art;
- complete genomes of some viruses can be synthesized at the present time, but not all DNA synthesis providers have this capability;
- it is possible, and routine in some laboratories, to recover/reconstruct infectious virus from DNA for certain Select Agents; however, successful use of such reverse genetic systems currently requires that one be “skilled in the art”; and
- some researchers create infectious chimeric viruses on a routine basis using combinations of genomic material from various select agents; these novel organisms do not fit into traditional classification schemes.

Pertinent Legal Authorities

Understanding the scientific landscape allowed the WG to determine possible biosecurity concerns for which oversight might be necessary. Before recommending any strategies to address such concerns, however, the WG assessed the current oversight framework to determine if it adequately addresses recent advances in synthetic genomics that allow for the creation of Select Agents *de novo*.

Using information provided via briefings from federal agencies responsible for the implementation and enforcement of controls for Select Agents, the WG identified those components of the oversight system for Select Agents that are most relevant to synthetic genomics. These are the SAR (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121), the section of the U.S. Code that addresses variola virus (18 U.S.C. 175c), the Export Administration Regulations (15 CFR Part 7), and the biosafety guidelines for working with DNA (National Institutes of Health - NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) and Centers for Disease Control and Prevention and National Institutes of Health - Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL Manual 4th ed.)

The SAR, the central component of this framework, aim to monitor and track the possession and use of certain dangerous pathogens (deemed Select Agents) and provide a legal basis to hold liable individuals who violate the SAR by possessing, using or transferring these pathogens without proper authorization. The SAR address both genetic material that encodes for Select Agent toxins and Select Agent genomic material that is inherently capable of producing a Select Agent virus. Such genomes include RNA viruses that are in message sense, DNA viruses that do not require a special viral enzyme to replicate and nucleic acids that, if inserted in the appropriate host system, can create a fully functional toxin (Attachment 2). Accordingly, synthesized genomes and toxin expression systems of these Select Agents are also regulated.

Specifically, the SAR describe regulated nucleic acids and genetically modified entities as:

Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms:

- (1) Nucleic acids that can produce infectious forms of any of the select agent viruses
- (2) Recombinant nucleic acids that encode for the functional form(s) of any of the select toxins if the nucleic acids:
 - (i) Can be expressed in vivo or in vitro, or
 - (ii) Are in a vector or recombinant host genome and can be expressed in vivo or in vitro
- (3) Select agents and toxins that have been genetically modified

Title 18 of the U.S. Code includes federal criminal statutes applicable to biological agents and toxins. Section 175c of Title 18 specifically applies to the synthesis of the variola virus. This statute deems it unlawful to knowingly produce, synthesize or engineer variola virus, which is defined in the Code to include “any derivative of the variola major virus that contains more than 85% of the gene sequence of the variola major virus or the variola minor virus.”

The Export Administration Regulations implemented and enforced by the DOC are another key regulation of the genetic material of Select Agents because they control export of such material from the U.S. The DOC Bureau of Industry and Security classifies items or types of items through the use of a specific Export Control Classification Number (ECCN). All ECCNs are listed in the Commerce Control List (CCL), which includes genetic elements (defined to include, among other matter, chromosomes, genomes, plasmids, transposons and vectors, whether genetically modified or unmodified) and genetically modified organisms.

The definition of recombinant DNA molecules covered by the NIH Guidelines includes molecules constructed outside of living cells by joining both natural and synthetic DNA segments. The NIH Guidelines detail safety practices and containment procedures for basic and clinical research involving recombinant DNA, including the creation and use of organisms and viruses containing recombinant DNA. An institution must follow the NIH Guidelines if it is conducting or sponsoring any recombinant DNA research that is funded by the NIH. Also, adherence to the NIH Guidelines may be a condition of support from other federal agencies or privately funded research. Regardless of NIH funding, institutions may be subject to local ordinances, federal or state regulations, or agency guidelines that require compliance with the NIH Guidelines.

The BMBL Manual describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. The recommendations are advisory. They are intended to provide a voluntary guide or code of practice as well as goals for upgrading operations. They also are offered as a guide and reference in the construction of new laboratory facilities and in the renovation of existing facilities. This is a commonly used reference within the scientific community.

Although the WG noted that synthetically derived DNA is addressed in the legal framework, there are points where clarification is needed to ensure compliance with these U.S. requirements. Furthermore, the speed of advances in this technology will require governance options that are capable of keeping pace with rapidly evolving science.

Biosecurity Concerns

The biosecurity concerns identified by the WG stem from the finding that synthetic genomics enable the synthesis and production of a Select Agent by nontraditional means, perhaps without HHS/USDA review. Additionally, it is possible to develop and produce agents that resemble and have the attributes of specific Select Agent(s) without being clearly identifiable as a Select Agent(s) based on its sequence and are therefore not covered by extant regulatory framework for Select Agents. These concerns arise from scientific advances and current industry practices, and highlight several associated issues (Figures 1 and 2), which include:

- ease of acquisition of synthetic Select Agent nucleic acids;
- need for additional regulatory clarity in specific areas; and
- difficulty in developing a suitable regulatory framework due to the lack of consensus among scientists regarding preferred approaches and methods for identifying/defining Select Agents and for screening sequences and due to current capabilities for constructing new pathogens.

Ease of Acquisition: The SAR regulate the use, possession and transfer of certain Select Agent nucleic acids; however, even when requests to genome providers are screened for sequences covered under the SAR, interpreting the results of such screens is complex and difficult. The behavior and properties of the expressed product of any synthetic genome that varies at all in its sequence from the exact sequence of a Select Agent “type strain” may be difficult to predict. Because it may even be difficult to make a taxonomic assignment for this putative agent, the request for synthesis may not trigger current regulatory oversight mechanisms, and as a result, an agent with properties equivalent to those of a Select Agent may be synthesized and distributed. Currently there are no highly effective standardized procedure(s) for screening sequences. Moreover, synthetic genomics technology is globally distributed and used by scientists worldwide. It is increasingly feasible for small groups or individual scientists to synthesize and assemble gene-length or longer DNA constructs. Thus, synthetically-derived Select Agent nucleic acids could be acquired from many sources.

Need for Additional Regulatory Clarity: Responsible agencies, affected scientists and commercial providers differ in their interpretation of the laws, regulations and policies. The WG identified specific concerns pertaining to the SAR, the CCL and 18 U.S.C. 175c.

Under the SAR, regulated viral nucleic acids are defined as “[n]ucleic acids that can produce infectious forms of any of the select agent viruses.” Proper interpretation of this definition requires an understanding of what is meant by “*can produce*” and what constitutes a “*select agent virus*.” Despite the description in the SAR of what is regulated, gene synthesis providers remain uncertain about what they are allowed to manufacture and ship without prior authorization from the CDC or the USDA Animal and Plant Health Inspection Service (APHIS).

The CCL and the SAR differ in their description of genetic material subject to these regulations such that it might be interpreted that the transfer of certain genetic material is allowed by one regulation while restricted by the other. Yet, the effectiveness of an oversight system relies upon the consistency coordination of activities across agencies sharing the oversight responsibility.

Also of concern is the statutory language pertaining to the synthesis of variola virus, 18 U.S.C. 175c. The statute allows for multiple interpretations of what is actually covered, and the sequence homology stipulation is arbitrary. Furthermore, other regulations laws and regulations⁶ already provide protection against unauthorized conduct with respect to this agent.

Difficulty Developing Suitable Regulatory Framework: It is now feasible to produce synthetic genomes that encode novel and taxonomically unclassified agents with properties equivalent to, or potentially more harmful than, current Select Agents. The rapid rate of scientific and technological advancements outpaces the development of the current list-based regulations. Furthermore, not all countries recognize the dissemination of synthetic genomics research and technology as an issue of global biosecurity concern to the same degree. The development of a complementary or alternative oversight framework will require broad scientific consensus and may require an ongoing process for its effective maintenance. Although some have suggested that a list of virulence-associated genetic sequences be created for use in screening synthetic constructs and for identifying constructs of concern, virulence is a complex multigenic trait that is not easily defined on the basis of sequence, nor well-understood. Rather, it results from an intricate dynamic between pathogen and host. Considerable study will be required before the traits of a synthetic virus or other organism can be predicted from its sequence.

Policy Options Considered

The WG recognized that various other groups and organizations have been grappling with issues pertaining to the potential misapplication of synthetic genomics. Therefore, the NSABB sought outside input regarding the biosecurity concerns and possible solutions. This was accomplished via consultations with stakeholders and by considering strategies proposed by scientists and policy analysts in workshops and conferences not associated with the NSABB.

The stakeholders consulted included practicing synthetic biologists, representatives from the intelligence community, organizations that have conducted or are conducting policy studies on the implications of synthetic genomics or synthetic biology, and federal agencies responsible for implementing and enforcing the SAR. These discussions provided the WG with points to consider in developing its recommendations.

A general sentiment was that biosecurity concerns stem from advances in synthesis technology that make the manipulation and creation of DNA sequences more simple, faster and more accessible. The WG was also advised to recognize in its recommendations that synthetic genomics is an international technology. Because major primary sources of key material are located outside the United States, it is not feasible to control or monitor access to this material. Also, the WG was told that any policy options adopted should take into account that the primary investment in synthesis technology is from private sources and was reminded that the strongest argument for investing in synthetic genomics is to increase research efficiency, which could be undermined by ill-conceived regulations.

An additional issue raised was that current biosafety guidelines are implemented in institutional settings which might complicate the oversight of synthetic genomics: Not only is this technology

⁶ 42 CFR Part 73, 42 U.S.C. 262a, 18 U.S.C. 175, 49 CFR Part 100-185, 18 U.S.C. 175b and 15 CFR Chapter 7

being embraced by groups, such as high school and undergraduate students, that are not always closely associated with academic institutions with Institutional Biosafety Committees (IBCs), but many practitioners of synthetic genomics are generally educated in disciplines that do not routinely include formal training in biosafety, such as engineering.

Although each of the stakeholders recognized the value of requiring the screening of requested sequences for homology with the known sequences of pathogens, they also emphasized the need for guidance in identifying the specific sequences for which current regulations require prior authorization for use, possession or transfer. Nevertheless, these stakeholders suggested how screening could be employed to guard against misuse of synthesis technologies. It was noted that the USG could provide incentives to encourage providers to screen by 1) requiring grantees to acquire synthetic DNA only from entities that screen and 2) investing in improved screening software and in an enhanced understanding of sequences associated with virulence.

The WG was also advised to consider the spirit of the regulations in assessing their adequacy. In the case of the SAR, the Rules intentionally do not apply until the functional infectious agent or toxin is generated. Thus, the language pertaining to nucleic acids and genetically modified entities aims to regulate the penultimate step to possessing an active and functional Select Agent. The aim is to avoid both the regulation of many key research reagents/products necessary for scientific advancement and unnecessarily hampering the pace of research while managing risk.

The WG considered additional options proposed by scientists and policy analysts for addressing biosecurity concerns including, but not limited to:

- restricting access to new sequence information about Select Agents;
- monitoring the sale of chemicals and lab equipment used to synthesize DNA;
- voluntary/involuntary surveillance/tracking of researchers/students using or trained to use synthetic genomics;
- modifying the SAR so that all select agent genomes are covered; and
- modifying the SAR or issuing new regulations defining Select Agents in terms of their sequence.

The NSABB chose not to adopt such recommendations because they are either not feasible, likely to be ineffective, and/or would unduly hinder scientific research. In certain instances, science has not advanced to the point that such recommendations could be implemented.

Recommendations:

The following NSABB recommendations are based upon the current state of the science as well as anticipated scientific advances enabled by synthetic genomics (Attachment 3). Nevertheless, the NSABB recognizes that this technology is rapidly changing; thus, there is a need for continued oversight and review of this topic.

Some issues surrounding the biosecurity concerns are more complex than others. Consequently, they will require different lengths of time to resolve. The NSABB was charged with to assess the adequacy of the current oversight framework for Select Agents, given advances in synthetic genomics. However, it is apparent that an agent generated from a synthetic genome that includes

fragments from multiple genomes, including that of a Select Agent(s), might not be classified as a Select Agent despite the fact that such an agent may be just as dangerous as a Select Agent. Therefore the NSABB concluded that there is a need, not only to provide recommendations related to the extant framework for Select Agents, but also to recommend longer term strategies for addressing biosecurity concerns related to the evolving field of synthetic genomics.

The recommendations below are listed in the order in which the NSABB suggests they might be fully addressed and executed. Certain aspects of the longer term strategies rely on the execution of other shorter term recommendations; however, the initiation of an effort to develop longer term strategies need not wait for the completion of these shorter term tasks, and can begin in the near term.

Recommendation 1: The NSABB recommends that HHS and USDA collaboratively develop and disseminate harmonized guidance to investigators and nucleic acid/gene/genome providers concerning the SAR with respect to synthetically-derived DNA. Specifically,

- 1.1. increase awareness among investigators and nucleic acid/gene/genome providers about their responsibilities to know what they possess, manufacture and/or transfer in order to comply with the SAR; and
- 1.2. provide clarification of what genetic elements or genomes are covered by 42 CFR 73.3c and 73.4c including:
 - 1.2.1. a list of the organisms whose genomes are explicitly covered and where the reference sequence can be found; and
 - 1.2.2. instructions for whom to contact if an investigator or provider has questions about covered genetic material.

The NSABB recognizes that the language of the SAR is structured such that its coverage of nucleic acids will evolve as science advances. In other words, any nucleic acids capable of producing a Select Agent are subject to the SAR; the identity of such nucleic acids is based upon the current state of science and will expand as scientific understanding grows. Currently, such nucleic acids are understood to include the intact genomes of the positive-sense, single-stranded RNA viruses and of the herpesviruses on the Select Agent List meaning that the intact genomes of the following viruses are subject to the SAR: Tick-borne encephalitis complex viruses (European subtype, i.e. Central European encephalitis virus; Siberian subtype, i.e. Russian Spring and Summer encephalitis virus; and Far Eastern subtype, i.e. Far Eastern Tick-borne encephalitis virus), *Kyasanur Forest disease virus*, *Omsk hemorrhagic fever virus*, *Eastern equine encephalitis virus*, *Venezuelan equine encephalitis virus*, *Classic swine fever virus*, *Foot-and-mouth disease virus*, *Japanese encephalitis virus*, *Swine vesicular disease virus*, *Cercopithecine herpesvirus 1* (Herpes B virus), and *Alcelaphine herpesvirus 1* (Malignant catarrhal fever virus).⁷ In addition, the SAR govern the use, possession and transfer of the “reconstructed replication competent forms of the 1918 pandemic influenza virus containing any

⁷ Species names are in italics whereas strain and colloquial names are not.

portion of the coding regions of all eight gene segments.”⁸ The SAR also apply to nucleic acids capable of expressing functional SA toxins. However, this does not mean that other nucleic acids are not subject to the SAR.

Recommendation 2: The NSABB recommends that the USG should:

- 2.1. charge relevant federal agencies, in consultation with outside experts, to:
 - 2.1.1. develop a process to be used by providers of synthetic DNA for determining the sequences for which to screen (Select Agents or otherwise);
 - 2.1.2. develop and promote standards and preferred practices for screening orders and interpreting the results, and require that orders be screened by providers;
 - 2.1.3. draft Points to Consider for determining whether genomic material that does not exactly match the genomes referenced in 1.1.1 should be considered covered under the SAR; and
 - 2.1.4. develop standards and practices to be used by providers for retaining records of orders for gene-length or genome-length nucleic acids, and require that records be retained by providers;
- 2.2. require federal grantees and contractors to order from providers that screen and retain information about requests for Select Agent sequences following standards and practices developed by relevant federal agencies (See 2.1.1 – 2.1.4); and
- 2.3. foster an international dialogue and collaboration with the goal of developing and implementing universal standards and preferred practices for screening sequences and related matters.

The NSABB believes that establishing uniform and standardized screening practices among providers of synthetic DNA would help safeguard against the intentional or unintentional circumvention of the SAR. The NSABB recognizes the magnitude of the effort involved and that establishing such practices requires the USG to fund the development of improved sequence databases and software tools, enhanced understanding of virulence, and improved framework for interpreting sequence screening results. While private initiatives to create such databases and software are currently underway, it is important that such efforts be harmonized with public efforts, that the products be standardized and that they be vetted by a broad range of experts to ensure scientific consensus. Furthermore, as best practices are defined and demonstrated to work for gene- and genome-length DNA segments, the application of such methods and standards of practice should be considered for shorter oligonucleotides. Such a strategy will become even more important as methods for assembly of DNA improve.

If there is to be a review or use of the results of the screening effort, records will need to be retained. The least intrusive way to accomplish effective implementation and compliance would be for record-keeping to be standardized, and for the providers to maintain and retain records;

⁸ “Possession, Use, and Transfer of Select Agents and Toxins—Reconstructed Replication Competent Forms of the 1918 Pandemic Influenza Virus Containing Any Portion of the Coding Regions of All Eight Gene Segments,” **Fed. Reg.** Vol. 70, No. 202 (October 20, 2005)

accordingly, record-keeping will need to be standardized for effective implementation and compliance. Effective compliance requires provider acceptance and may also require audits, fines and/or other legal actions.

To best achieve these goals, the USG should work with recognized experts from the gene-synthesis industry and research communities, and integrate international expertise into the process. The NSABB can provide a forum for convening such experts and facilitating collaboration among these experts and the federal agencies responsible for implementing and enforcing the SAR.

Recommendation 3: The NSABB recommends that the USG:

- 3.1 repeal 18 U.S.C. 175c⁹ because current scientific insight precludes meaningful definition of an agent based solely on sequence homology;
- 3.2 examine the language and implementation of current biosafety guidelines to ensure that such guidelines and regulations provide adequate guidance for working with synthetically derived DNA and are understood by all those working in areas addressed by the guidelines; and
- 3.3 continue to reconcile the genetic elements language in the CCL¹⁰ with that in the SAR.

At the present time, arriving at a meaningful definition of variola virus or any other agent on the sole basis of genome sequence similarity is a profoundly difficult, unsolved scientific challenge, yet the definition of “variola virus” in 18 U.S.C. 175c is based on genome sequence similarity which the NSABB found to be problematic. Because current scientific understanding does not permit an adequate correlation of sequence with function, the definition allows for multiple interpretations of what is covered, and thus, the sequence homology stipulation is arbitrary. There are many regions of the variola major virus genome and variola minor genomes that are significantly greater than 85% similar to sequences found in related but relatively harmless viruses. The current definition of variola virus, as provided in the statute, could be interpreted to include other less harmful naturally occurring poxviruses such as vaccinia virus that are vital to beneficial research, thereby inadvertently restricting and potentially making criminal many types of beneficial research such as the development and production of smallpox vaccine. For these reasons, the NSABB recommends that 18 USC 175c be repealed, particularly because the misuse of variola virus is adequately covered by other criminal laws already in place.⁶

Discussions with stakeholders revealed that some practitioners of synthetic genomics are educated in disciplines that do not routinely entail formal training in biosafety. In addition, scientists employing synthetic genomics are unclear as to the circumstances under which they should consult an IBC. Thus, providers of synthetic DNA may not be using appropriate

⁹ This statute deems it unlawful, unless explicitly so authorized, to knowingly produce, synthesize or engineer variola virus, which is defined in the Code to include “any derivative of the variola major virus that contains more than 85% of the gene sequence of the variola major virus or the variola minor virus.”

¹⁰ Genetic elements are defined to include, among other matter, chromosomes, genomes, plasmids, transposons and vectors, whether genetically modified or unmodified, and genetically modified organisms.

laboratory procedures that ensure biosafety. Therefore, there is a need for the USG to work with the scientific research community to ensure that the current biosafety guidelines and regulations are appropriate, adequate and easily understood.

The NSABB recognizes that the effectiveness of any oversight system relies upon activities across USG agencies that share the oversight responsibility. The DOC should continue efforts to reconcile the SAR and the CCL such that there is consistency between the Select Agent genetic material that can be imported and used domestically, and the genetic material that can be exported.

Recommendation 4: The NSABB recommends that the USG, after taking into account the results of implementing Recommendation 2,

- 4.1 convene a group of experts from the scientific community to conduct an open and in-depth examination of the Select Agent classification system to determine if it is possible to reconcile the current controls for Select Agents with the anticipated scientific advances enabled by synthetic genomics;
- 4.2 assemble a group of experts from the scientific community to determine if an alternative framework based on predicted features and properties encoded by nucleic acids, such as virulence or pathogenicity, can be developed and utilized in lieu of the current finite list of specific agents and taxonomic definitions; and
- 4.3 consider the potential international implications of any proposed changes to the current oversight framework for synthetic DNA and synthetic genomes, and foster an international dialogue and collaboration on these issues.

Current studies of human pathogens using genomics-based approaches have revealed an enormous level of strain diversity that has challenged our notion of microbial species as discrete entities with well-defined properties. This diversity in large part reflects the fact that microbial genomes are dynamic entities shaped by multiple forces, including acquisition of new functions via lateral gene transfer. One implication of these observations is that in some instances the assignment of a genus/species name to an organism may be difficult, and of limited utility, in predicting the phenotypic properties of a particular isolate, in particular with regard to virulence, infectivity and pathogenicity. Therefore, the genus/species based approach that is currently used in Select Agent classification is imperfect since it does not take into account the great degree of genetic variability that can exist within species as they are currently defined.

Advances in the science of synthetic genomics and synthetic biology will further confound this murky situation. It is increasingly easy to produce synthetic genomes that encode novel and taxonomically unclassified agents with pathogenic properties equivalent to, or possibly more harmful than, current Select Agents. Reliance on taxonomic definitions for Select Agents becomes increasingly irrelevant in an age of synthetic or engineered genomes that can produce biological agents with novel features and properties that might render them as harmful as Select Agents. The development of a new oversight framework should be supplemented through the issuance of guidance as information becomes available with which to define the genetic

sequences that form the basis for an organism's harmful properties. Current scientific understanding reveals that it is often the combination or interaction of genetic elements that underlie these properties rather than one specific gene sequence. Furthermore, the harmful consequences of biological agents are dependent upon the coordination multiple factors, including host susceptibility, the agent's infectivity, transmissibility and virulence, and the availability of prophylactic or therapeutic interventions.¹¹

Given recent scientific advances in recombinant DNA technology and synthetic genomics and the wide spectrum of potential agents with the possibility for causing harm, future standards for oversight of work with agents should be based on presumed/predicted functionality rather than sequence homology or taxonomy. Recent efforts by the USG to assess and quantify risks associated with a wide variety of naturally occurring agents have revealed significant degrees of uncertainty in the estimates of risks and difficulty in distinguishing among a large subset of these agents based on their associated risks. This subset of agents includes some that are currently on the Select Agent List and some that are not. In order to facilitate the development of an improved oversight framework for natural, engineered and synthetic agents, the USG should continue to fund research in a variety of relevant disciplines, including pathogenesis, genomics and bioinformatics, so as to understand and recognize sequences that are responsible for properties such as virulence, tropism and transmissibility. Future oversight of infectious agents and toxins should consist of a tiered system of levels of control and associated burdens, based on upon scientifically based projections of risk. Such controls will be less onerous and more effective since they would be titrated to the projected degree of risk.

The group assembled to identify the attributes of an alternative framework should include researchers with expertise in microbial pathogenesis, genomics, computational molecular biology, structural biology, evolutionary biology and risk analysis. While a broad range of expertise is represented on the NSABB, additional types of expertise would be required if this group were to assume this charge. Nevertheless, the NSABB is appropriately composed so as to identify and convene such experts, as well as facilitate the development of options to guide the USG in developing standards, practices and an alternate framework.

Next Steps in Addressing Biosecurity Issues:

Synthetic genomics and synthetic biology are already widespread; biosecurity issues now known and those not yet known will be increasingly global in scope. International cooperation encourages standard practices across the industry worldwide. In turn, harmonized international standards will encourage organizations, industry and researchers to conform their research activities to the applicable guidelines. Furthermore, international guidelines that are scientifically or technically based will be more readily implemented than those that are more conjectural and/or political.

While these recommendations focus on Select Agents, they touch on some of the broader potential biosecurity issues related to synthetic biology. The WG, on behalf of NSABB, will build upon these findings in carrying out the second phase of its charge--which is to identify,

¹¹ Refer to the NSABB's "Criteria for Identifying Dual Use Research of Concern" for further explanation.

assess, and recommend strategies that address potential dual use concerns that may arise from work in the nascent field of synthetic biology.

Salient points the NSABB will address include: How can possible risks associated with the generation of novel organisms be addressed? What strategies can be employed to safeguard against the misuse of synthetic biology and associated technologies? How can public concerns be assuaged? How can global cooperation in addressing related biosecurity concerns be encouraged?

ATTACHMENT 1

Current U. S. Authorities

Current USG laws, regulations, and policies related to oversight of Select Agents:

- The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and Agricultural Bioterrorism Protection Act of 2002, which are implemented by the Select Agent Rules (42 CFR part 73 (threats to human health), 7 CFR part 331 (threats to plant health and plant products) and 9 CFR part 121 (threats to animal health and animal products)). These Acts and Regulations govern the possession, use and transfer of “select agents,” which are biological agents and toxins that have been determined to have the potential to pose a severe threat to human, plant and/or animal health. The Acts provide for penalties for violations of the Rules.
- The USG Policy on Biosecurity in Life Sciences Research, which was announced in Spring 2004 and 1) established a biosecurity advisory body (the NSABB) to advise NIH, HHS and other federal agencies on specific strategies for the effective oversight of federally conducted or supported dual use biological research, taking into consideration both national security concerns and the needs of the research community and 2) mandated several actions to promote the development and implementation of biosecurity principles throughout the national and international scientific communities.
- 18 U.S.C 175, which mandates fines and/or imprisonment for 1) individuals who knowingly develop, produce, stockpile, transfer, acquire, retain or possess any biological agent, toxin or delivery system for use as a weapon and 2) individuals who knowingly possess any biological agent, toxin or delivery system of a type or in a quantity that, under the circumstances, is not reasonably justified by a prophylactic, protective, bona fide research or other peaceful purpose.
- 18 USC 175b, which mandates fines and/or imprisonment for 1) a restricted person shipping, possessing or receiving a Select Agent in or otherwise affecting interstate or foreign commerce through the use of a Select Agent or 2) transferring a Select Agent to a person who the transferor knows or has reasonable cause to believe is an unregistered person or 3) knowingly possessing a Select Agent for which the person has not obtained registration.
- 18 U.S.C. 175c, which includes federal criminal statutes applicable to biological agents and toxins. Section 175c specifically applies to the synthesis of the variola virus. This statute deems it unlawful to knowingly produce, synthesize or engineer variola virus, which is defined in the Code (18 U.S.C. 175c (d)) to include “any derivative of the variola major virus that contains more than 85% of the gene sequence of the variola major virus or the “variola minor virus.”

ATTACHMENT 2

Classification of Select Agent Viruses

	Single-stranded positive RNA ¹²	Single-stranded negative RNA	Double-stranded RNA	Double-stranded DNA
CDC Select Agent Viruses	<ul style="list-style-type: none"> ▪ Tick-borne encephalitis complex (flavi) viruses: <ul style="list-style-type: none"> - Central European Tick-borne encephalitis - Far Easter Tickborne encephalitis - Russian Spring and Summer encephalitis - Kyasanur Forest Disease - Omsk Hemorrhagic Fever 	<ul style="list-style-type: none"> ▪ Crimean-Congo haemorrhagic fever virus ▪ Ebola viruses ▪ Lassa fever virus ▪ Marburg virus ▪ South American Haemorrhagic fever viruses: <ul style="list-style-type: none"> - Junin, - Machupo, - Sabia, - Flexal, - Guanarito 		<ul style="list-style-type: none"> ▪ Variola major virus (Smallpox virus) and Variola minor virus (Alastrim) ▪ Cercopithecine herpesvirus 1 (Herpes B virus)¹² ▪ Monkeypox virus
CDC/USDA Overlap Select Agent Viruses	<ul style="list-style-type: none"> ▪ Eastern Equine Encephalitis ▪ Venezuelan Equine Encephalitis virus 	<ul style="list-style-type: none"> ▪ Nipah virus ▪ Hendra virus ▪ Rift Valley fever virus 		
USDA Select Agent Viruses	<ul style="list-style-type: none"> ▪ Classical swine fever virus ▪ Foot-and-mouth disease virus ▪ Japanese encephalitis virus ▪ Swine vesicular disease virus 	<ul style="list-style-type: none"> ▪ Akabane virus ▪ Avian influenza virus (highly pathogenic) ▪ Newcastle Disease virus (velogenic) ▪ Peste des petits ruminants virus ▪ Rinderpest virus ▪ Menangle virus ▪ Vesicular stomatitis virus (exotic) 	<ul style="list-style-type: none"> ▪ African horse sickness virus ▪ Blue tongue virus 	<ul style="list-style-type: none"> ▪ African swine fever virus ▪ Camel pox virus ▪ Goat pox virus ▪ Lumpy skin disease virus ▪ Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1)¹² ▪ Sheep pox virus

¹² The intact genomes of these viruses are subject to the SAR

ATTACHMENT 3

Recommendations of the NSABB on Addressing Biosecurity Concerns Related to the Synthesis of Select Agents

Recommendation 1: The NSABB recommends that HHS and USDA collaboratively develop and disseminate harmonized guidance to investigators and nucleic acid/gene/genome providers concerning the SAR with respect to synthetically-derived DNA. Specifically, the Departments should provide clarification of what genetic elements or genomes are covered by 42 CFR 73.3c and 73.4c. Such clarification should include a list of the organisms whose genomes are explicitly covered and where the reference sequence can be found, and instructions for whom to contact if an investigator or provider has questions about covered genetic material. There is also a need for HHS and USDA to increase awareness among investigators and nucleic acid/gene/genome providers about their responsibilities to know what they possess, manufacture and/or transfer in order to comply with the SAR.

Recommendation 2: The NSABB recommends that the USG should charge relevant federal agencies, in consultation with outside experts to 1) develop a process to be used by providers of synthetic DNA for determining the sequences for which to screen (Select Agents or otherwise); 2) develop and promote standards and preferred practices for screening orders and interpreting the results, and require that orders be screened by providers; 3) draft Points to Consider for determining whether genomic material that does not exactly match the genomes referenced in Recommendation 1 should be considered covered under the SAR; and 4) develop standards and practices to be used by providers for retaining records of orders for gene-length or genome-length nucleic acids, and require that orders be screened by providers. The NSABB also recommends that the USG require federal grantees and contractors to order from providers that screen and retain information about requests for Select Agent sequences following standards and practices developed by relevant federal agencies, and foster an international dialogue and collaboration with the goal of developing and implementing universal standards and preferred practices for screening sequences and related matters.

Recommendation 3: The NSABB recommends that the USG repeal 18 U.S.C. 175c¹³ because current scientific insight precludes meaningful definition of an agent based solely on sequence homology; examine the language and implementation of current biosafety guidelines to ensure that such guidelines and regulations provide adequate guidance for working with synthetically-derived DNA and are understood by all those doing work in areas covered by the guidelines; and continue to reconcile the genetic elements language in the CCL¹⁴ with that in the SAR.

Recommendation 4: The NSABB recommends that the USG, after taking into account the results of implementing Recommendation 2, 1) convene a group of experts from the scientific community to conduct an open and in-depth examination of the Select Agent classification system to determine if it is possible to reconcile the current controls for Select Agents with the anticipated scientific advances enabled by synthetic genomics; 2) assemble a group of experts from the scientific community to determine if an alternative framework based on predicted features and properties encoded by nucleic acids, such as virulence or pathogenicity, can be developed and utilized in lieu of the current finite list of specific agents and taxonomic definitions; and 3) consider the potential international implications of any proposed changes to the current oversight framework for synthetic DNA and synthetic genomes, and foster an international dialogue and collaboration on these issues.

¹³ This statute deems it unlawful, unless explicitly so authorized, to knowingly produce, synthesize or engineer variola virus, which is defined in the Code to include “any derivative of the variola major virus that contains more than 85% of the gene sequence of the variola major virus or the variola minor virus.”

¹⁴ Includes genetic elements (defined to include, among other matter, chromosomes, genomes, plasmids, transposons and vectors, whether genetically modified or unmodified) and genetically modified organisms.

FIGURE 1

Process for Deriving Select Agents De Novo Using Mail-Ordered DNA

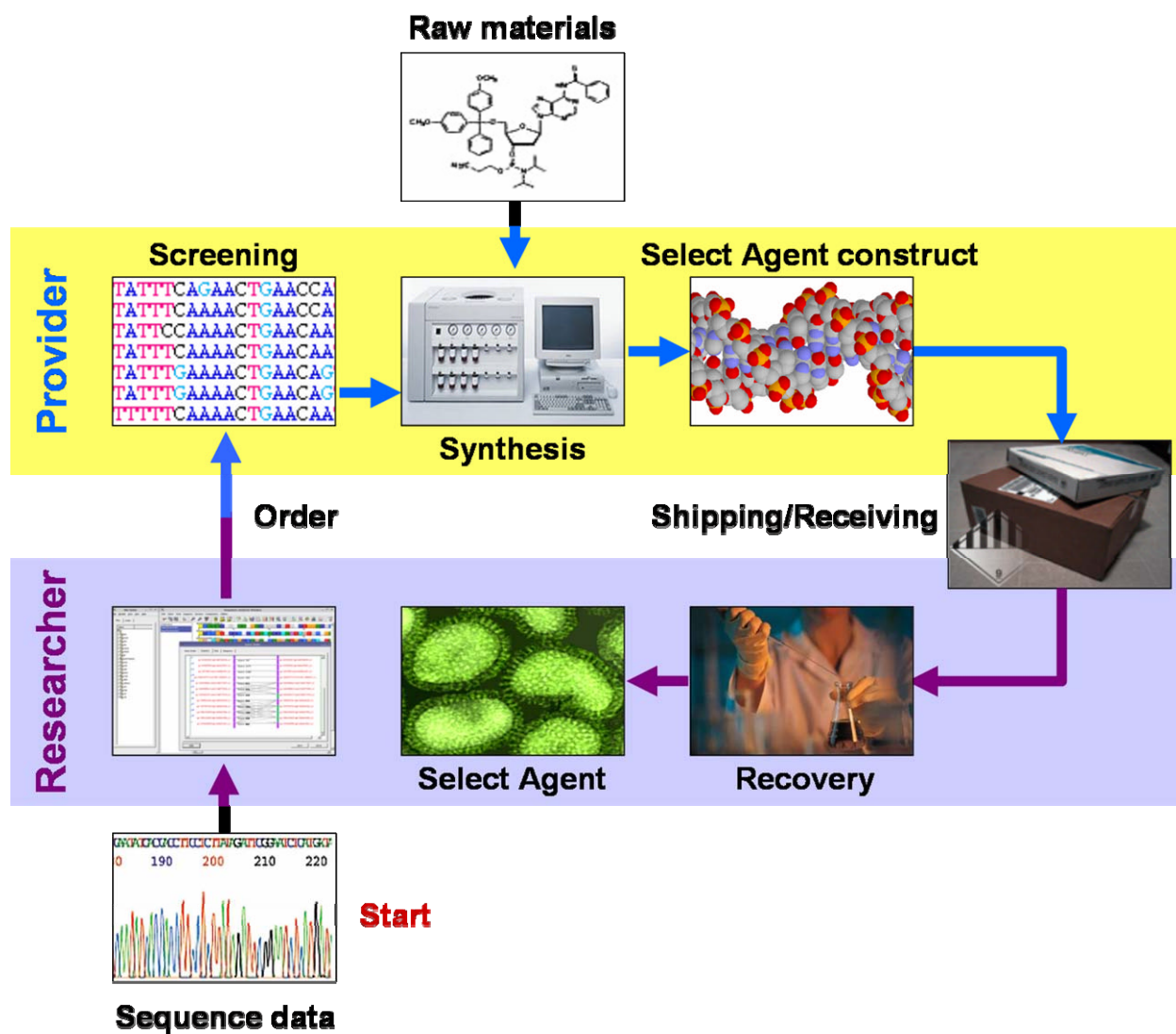


FIGURE 2

Biosecurity Concerns Mapped to Process

Synthesis of DNA						
Access Sequence Data	Screen Orders	Use Raw Material	Use Equipment	Derive Genetic Material	Transfer Material	Recover/ Reconstruct
	Need for additional regulatory clarity			Difficulty developing a suitable regulatory framework	Non- compliance with SAR	Ease of acquisition of synthetic SA nucleic acids Construction of new pathogens