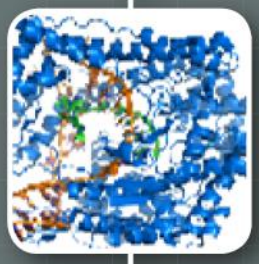

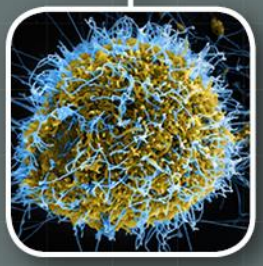

GUIDANCE ON THE REGULATION OF SELECT AGENT AND TOXIN NUCLEIC ACIDS

February 2020

Centers for Disease Control and Prevention
Division of Select Agents and Toxins

Animal and Plant Health Inspection Service (APHIS)
Agricultural Select Agent Program

Table of Contents

Changes/Highlights

Revisions: This is a living document subject to ongoing improvement. Feedback or suggestions for improvement from registered Select Agent entities or the public are welcomed. Submit comments directly to the Federal Select Agent Program at:

CDC: LRSAT@cdc.gov
APHIS: AgSAS@usda.gov

Revision History:

May 2017 – Initial posting
September 2019 – Created PDF of document; revised correct editorial errors from previous version.
February 2020 – Revised correct editorial errors from previous version, improve clarity and update the information related to a policy on our website that was also updated.

Introduction

The Federal Select Agent Program (FSAP) is providing guidance to entities that create or use nucleic acids that are capable of producing infectious forms of select agent viruses or encode for the toxic form(s) of any 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*, including complete genomes as well as recombinant and/or synthetic nucleic acids. The following genetic elements, recombinant and/or synthetic nucleic acids, and recombinant and/or synthetic organisms are regulated as select agents (See sections 3(c) and 4(c) of 42 CFR Part 73, 9 CFR Part 121, and section 3(c) of 7 CFR Part 331):

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

The purpose of this guidance is to address advances in molecular biology that may influence the production of infectious forms of select agent viruses, or the toxic forms of select toxins from recombinant and/or synthetic nucleic acids. For example, positive strand RNA viruses and certain double strand DNA viruses that utilize host polymerases contain nucleic acids that can produce infectious forms of the viruses. Such nucleic acids are subject to the select agent regulations.

Tier 1 Nucleic Acids

To ensure that select agents and toxins are secured according to the appropriate level of risk, a subset (Tier 1) of the select agent list has been identified that presents the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence. Currently, only nucleic acids that encode for the toxic forms of Botulinum neurotoxins, or infectious forms of Foot-and-Mouth Disease virus, are regulated as Tier 1 agents and therefore all Tier 1 regulatory requirements apply to these nucleic acids.

Genetic Elements

Genetic elements are sequences of nucleic acids. Genetic elements from select agents are regulated if:

- They encode for a toxic form of a select toxin and can be expressed *in vivo* or *in vitro* or they are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*, or
- The nucleic acids are inherently infectious, as discussed above.

Organisms containing regulated recombinant and/or synthetic nucleic acids

Any organisms that contain regulated recombinant or synthetic nucleic acids are subject to the regulations unless the organisms or nucleic acids are excluded as:

- A non-viable select agent or nontoxic toxin
- An excluded attenuated strain
- An excluded select toxin modified to be less potent or toxic
- Regulated nucleic acids that can produce infectious forms of any select agent virus that has been subjected to a validated inactivation procedure that is confirmed through a viability testing protocol. Surrogate strains that are known to possess equivalent properties with respect to inactivation can be used to validate an inactivation procedure; however, if there are known strain-to-strain variations in the resistance of a select agent to an inactivation procedure, then an inactivation procedure validated on a lesser resistant strain must also be validated on the more resistant strains.
- Regulated nucleic acids that can produce infectious forms of any select agent virus not subjected to a validated inactivation procedure if the material is determined by the HHS Secretary to be effectively inactivated. To apply for a determination an individual or entity must submit a written request and supporting scientific information to CDC. A written decision granting or denying the request will be issued.

Please see the Exclusions Guidance Document for more information.

If currently regulated select agent recombinant and/or synthetic nucleic acids (see above) are introduced into either a select agent organism or an organism used for molecular cloning (e.g., E. coli), the resulting recombinant and/or synthetic organism would be subject to the select agent regulations, including Tier 1 requirements for Tier 1 agents and toxins.

Synthetic nucleic acids and genomes

The Department of Health and Human Services' *Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA* "sets forth recommended baseline standards for the gene and genome synthesis industry and other providers of synthetic double-stranded DNA products regarding the screening of orders so that they are filled in compliance with current U.S. regulations . . . ."[1][1] The guidance also encourages "best practices in addressing biosecurity concerns associated with the potential misuse of their products to bypass existing regulatory controls."[2] Even when the double-stranded DNA sequence being processed does not fall under the current select agent regulations, providers are advised to refer to this policy for additional guidance on screening such orders.

[1] Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA (Summary) p. 1, October 13, 2010.
[2] Id.

Regulated Materials

FSAP regulates select agent and toxin nucleic acids that are:

1. Inherently infectious and are immediate precursors to virus production (i.e., the nucleic acids are capable of generating infectious forms of a regulated virus by utilizing host polymerases but without the need for any additional exogenous factors [proteins, nucleic acids, etc.])
2. Encode for the toxic form(s) of any of the select toxins if the nucleic acids can be expressed *in vivo* or *in vitro*, or are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*.

For regulated genetic elements and recombinant and/or synthetic nucleic acids, the select agent regulations (security, biosafety requirements, etc.) are the same as those applied to the respective select agents and toxins.

Examples of Nucleic Acid Materials of Select Agents and Toxins That May be Regulated

Inherently infectious materials that are immediate precursors to virus production

Positive strand RNA virus genomes are regulated. For the viruses listed below, regulation is limited to positive strand RNA forms of the viral genome which can be translated into protein precursors for virus production. A cDNA copy of the viral genomes listed below would not be regulated because they would first need to be transcribed into RNA then translated into protein and therefore would not be an immediate precursor to virus. Positive strand RNA forms subjected to regulation include:

- Classical swine fever virus
- Eastern equine encephalitis virus (North American genotypes)
- Foot-and-mouth disease virus (FMDV)
- Kyasanur Forest disease virus
- Omsk hemorrhagic fever virus
- SARS-associated coronavirus (SARS-CoV)
- Swine vesicular disease virus
- Tick-borne encephalitis complex (flavi) viruses:
    - Far Eastern subtype
    - Siberian subtype
- Venezuelan equine encephalitis virus subtypes IAB and IC

Recombinant and/or synthetic nucleic acids that encode for the toxic form(s) of the regulated toxins

Recombinant and/or synthetic nucleic acids that encode for the toxic form(s) of the regulated toxins are regulated if the nucleic acids:

- Can be expressed *in vivo* or *in vitro*, or
- Are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*.

Genetically modified select agents and toxins

Depending upon the extent of the modification, genetically modified select agents or toxins may be regulated. Genetic modifications include but are not limited to deletion mutants, insertion mutants, point mutants, and chimeric select agents. If the genetic modification renders the select agent or toxin attenuated or less potent or toxic, then the select agent or toxin may be excluded.

Chimeric viruses whose genomes contain the backbone and replication machinery of a select agent virus or contain genes from different select agent viruses are regulated. Regulated chimeric viruses have to be evaluated on a case-by-case basis to determine if the viruses exhibit sufficient attenuation to be excluded. The select agent regulations do not apply to nucleic acids encoding for genetically modified select agents unless they can produce infectious forms of any of the select agent viruses which is currently limited to positive strand RNA virus genomes (see above) or encode for the toxic form(s) of any of the select toxins if the nucleic acids can be expressed *in vivo* or *in vitro*, or are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*. For example, the nucleic acids encoding for a chimeric monkeypox virus would not be regulated while nucleic acids encoding for a chimeric EEE virus would be regulated.

Chimeras that are comprised of select agent and non-select agent genes

Chimeras that are comprised of select agent and non-select agent genes from the same virus family require careful review to determine select agent status. It is the entity's responsibility to determine if the resultant chimera is a select agent; however, FSAP encourages entities to submit these types of chimeras for review.

FSAP also regulates select agent bacteria that are genetically modified. Regulated genetically modified bacteria have to be evaluated on a case-by-case basis to determine if the bacteria exhibit sufficient attenuation to be excluded. The select agent regulations do not apply to nucleic acids that encode for genetically modified bacteria as these nucleic acids are not considered infectious.

Non-Regulated Materials

Under the current select agent regulations the following are examples of materials that would **not be regulated** as a select agent:

- **Nucleic acids or genetic elements that cannot produce infectious forms of any of the regulated select agent viruses.** These include:
    - Nucleic acids encoding complete genomes of single-stranded negative strand RNA viruses,
    - Double stranded RNA viruses, and
    - Double-stranded DNA viruses that require a unique polymerase (Variola virus*****, monkeypox virus [except West African clade], African swine fever virus, goat pox virus, Lumpy skin disease virus, and sheep pox virus).

These genomes are incapable of producing infectious virus when introduced by itself into an animal or permissive cell without the introduction of rescue plasmids or other exogenous factors:

- **PCR products and primers or DNA fragments of select agents or toxins** (unless they encode for a toxic form of a select toxin and can be expressed)
- **Complementary DNA made from regulated select agent nucleic acids (**only the positive strand RNA form of the viral genome is regulated). Complementary DNA copies of select agent viral genomes, including reverse genetics systems, are not regulated because they would first need to be transcribed into RNA, then translated into protein, and therefore would not be an immediate precursor to the virus
- **Nucleic acids that encode for the genomes of select agent bacteria or fungi, including chromosomal, recombinant, or synthetic DNA**
- **Nucleic acids that encode for toxins not subject to the select agent regulations but derived from regulated select agents**
- **Nucleic acids that encode for toxins and the genomes of select agents that have been excluded from regulation under section 3(e) of the select agent regulations.** Please see the FSAP website for a list of excluded select agents. If the entity possesses an attenuated strain that is not included in the list of excluded agents, the entity can request that the strain be excluded. Please see the Exclusions Guidance Document for additional information on how to request exclusion
- **Select agent nucleic acid sequence information**

*With the exception of activities conducted by or under the authority of the HHS Secretary, under federal criminal law it is unlawful for any person to knowingly produce, engineer, synthesize, acquire, transfer directly or indirectly, receive, possess, import, export, or use, or possess and threaten to use, variola virus. See 18 U.S.C. § 175c. For purposes of 18 U.S.C. § 175c, “variola virus” is defined as “a virus that can cause human smallpox or any derivative of the variola major virus that contains more than 85 percent of the gene sequence of the variola major or the variola minor virus.”

Working with FSAP Regulated Genomes at Lower Containment Policy

Policy Statement (https://www.selectagents.gov/policystatement.html):
The FSAP received recommendations from a group of Federal subject matter experts (SME) regarding working safely with regulated genomes at lower containment if certain conditions are met. Based upon the SME recommendations it is the policy of the FSAP that a registered entity can perform laboratory work with the full-length genomes of regulated Risk Group 3 and 4[2] (RG3[3] and RG4[4]) agents and VS select agents at one containment level lower than the infectious virus without RNA inactivation.

Note: Regardless of the biosafety level used, the full-length genomes of any of the select agent viruses capable of producing infectious virus are regulated and must be handled in registered space.

If working with genomic material from a Tier 1 positive strand RNA virus (currently only Foot-and-mouth disease virus), lower containment laboratories would still have to maintain the Tier 1 requirements. To work with RG3, RG4 and VS (+) ssRNA genomic material in laboratories one containment level lower than the level required for the infectious virus, the following additional safety practices must be in place:

- The genomic material must be free of infectious virus, as required in 42 C.F.R. 73.3(d)(4) and 73.4(d)(4), and 9 C.F.R. 121.3(d)(4) and 121.4(d)(4), before removing the genomic material from the laboratory designated to work with the live virus.
- For RG4 and FMDv genomic work in BSL-3 laboratories, work must be performed inside a biosafety cabinet (BSC).
- For RG3 and VS agents (other than FMDv) genomic work in BSL-2 laboratories, work should be based on a risk assessment by the entity, although work in the BSC is preferred.
- No concurrent work with mammalian cell culture or in vitro translation experiments are conducted in this laboratory
- No concurrent transfection work or in vitro translation reagents are used or stored in this laboratory
- Personal protective equipment (PPE) must afford adequate mucosal membrane protection to avoid the risk of auto-inoculation and include the following:
    - Disposable or suite-dedicated lab coats
    - Protective eyewear or face shield as required by procedure
    - Gloves (latex, vinyl, nitrile etc.) are chosen to resist those chemicals and or solvents used in cloning procedures
- Avoid glassware – plastic ware is recommended
- Avoid sharps, including needles and syringes

[2] NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, Appendix B.
[3] Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
[4] Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)