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# GUIDE FOR SUBMITTING PERMIT APPLICATIONS FOR MICROORGANISMS DEVELOPED USING GENETIC ENGINEERING UNDER 7 CFR PART 340

The information contained in this document is intended solely as guidance. Except where noted, persons may choose to follow APHIS guidance or follow different procedures, practices, or protocols that meet applicable statutes and regulations.

Language implying that guidance is mandatory (e.g., “shall,” “must,” “required,” or “requirement”) should not be construed as binding unless the terms are used to refer to a statutory or regulatory requirement.

Following the guidance contained in this document should not be construed as a guarantee of compliance with applicable statutes and regulations.

Biotechnology Regulatory Services  
Animal and Plant Health Inspection Service  
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## GUIDE INFORMATION

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| <b>ISSUING AGENCY/OFFICE:</b> | Animal and Plant Health Inspection Service (APHIS)/ Biotechnology Regulatory Services (BRS)  |
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| <b>SUMMARY:</b>               | <p>This document assists developers with preparing a permit application for movement activities with modified microorganisms under 7 CFR part 340 (Movement of Organisms Modified or Produced Through Genetic Engineering). APHIS protects and enhances U.S. agricultural and natural resources using a science-based and risk-based regulatory framework to ensure the safe movement – including importation, interstate movement, and confined environmental release – of organisms developed using genetic engineering. APHIS receives its regulatory authority from the Plant Protection Act of 2000, and oversees organisms developed using genetic engineering in accordance with its regulations under 7 CFR part 340 (Movement of Organisms Modified or Produced Through Genetic Engineering) (<a href="#">85 FR 29790</a>). For more information: <a href="https://www.aphis.usda.gov/aphis/ourfocus/biotechnology">https://www.aphis.usda.gov/aphis/ourfocus/biotechnology</a></p> |
| <b>DISCLAIMER:</b>            | <p>The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to the public regarding existing requirements under the law or agency regulations.</p>   |

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## MICROORGANISMS REGULATED UNDER 7 CFR PART 340

APHIS regulates the importation, interstate movement, and environmental release of certain microorganisms developed using genetic engineering under 7 CFR part 340. Genetic engineering is defined in § 340.3 as “techniques that use recombinant, synthesized, or amplified nucleic acids to modify or create a genome.” Regulated microorganisms include plant pests and other modified microorganisms that could pose a plant pest risk. See § 340.2. Developers require a permit for regulated activities involving any modified microorganism that:

- Meets the definition of a plant pest in § 340.3; or
- Is not a plant but has received deoxyribonucleic acid (DNA) from a plant pest, as defined in § 340.3, and the DNA from the donor organism either is capable of producing an infectious agent that causes plant disease or encodes a compound that is capable of causing plant disease; or
- Is a microorganism used to control plant pests and could pose a plant pest risk.

We describe these categories of regulated microorganisms below, followed by exemptions for permitting requirements, and information about select agents. For simplicity in this document, microorganisms developed using genetic engineering are referred to as “modified microbes.” Modified microbes addressed in this guide include bacteria, fungi, oomycetes, viruses, and viroids, and the information herein could also be applicable to other taxonomic groups not specifically mentioned such as protozoa, algae, or nematodes.

**Definitions that come from 7 CFR part 340, are referenced as § 340.3. Other important excerpts are referenced with the appropriate regulatory section.** See **Appendix 1** for a full list of excerpts relevant to microorganisms.

Developers may also refer to BRS’ [Questions and Answers](#) document for additional information regarding work with modified microbes under 7 CFR part 340.

## REGULATED ORGANISMS

### Microorganisms that are Plant Pests

Modified microbes that meet the definition of a plant pest are regulated under 7 CFR part 340. (7 CFR § 340.2(b)).

The regulations define a plant pest as follows:

**Plant pest.** Any living stage of a protozoan, nonhuman animal, parasitic plant, bacterium, fungus, virus or viroid, infectious agent or other pathogen, or any article similar to or allied with any of the foregoing, that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product. **§ 340.3**

A modified microbe can meet the definition of a “plant pest” if the modified microbe itself can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product. A modified microbe could meet the definition of “plant pest” because the unmodified microbe is itself a plant pest or the modification imparts changes to a microbe such that it can directly or indirectly injure, cause damage to,

or cause disease in a plant or plant product. Plant pathogens that have been modified to reduce virulence yet remain capable of causing direct or indirect injury or damage to, or disease in, a plant or plant product also meet the definition of “plant pest.”

#### **Microorganisms Modified with DNA Capable of Causing Plant Disease**

Modified microbes that have received DNA from plant pests meeting the criteria below are regulated under 7 CFR part 340:

Is not a plant but has received deoxyribonucleic acid (DNA) from a plant pest, as defined in § 340.3, and the DNA from the donor organism either is capable of producing an infectious agent that causes plant disease or encodes a compound that is capable of causing plant disease.

#### **§ 340.2(c)**

In these cases, the microbial species in its unmodified state may not necessarily be a plant pest. However, because the modified microbe contains DNA that is capable of producing an infectious agent that causes plant disease or contains DNA that encodes a compound that is capable of causing plant disease, the modified microbe can directly or indirectly injure, cause damage to, or cause disease in a plant or plant product and therefore meets the definition of “plant pest.” An example of a modified microbe in this category would be a bacterium engineered to express infectious clones of plant viruses, for purposes of basic research, or for developing diagnostics or therapeutics.

#### **Microorganisms Used for Biocontrol of Plant Pests**

Modified microbes used to control plant pests and that could pose a plant pest risk are regulated under 7 CFR part 340:

Is a microorganism used to control plant pests...and could pose a plant pest risk

#### **§ 340.2(d)**

**Plant pest risk.** The potential for direct or indirect injury to, damage to, or disease in any plant or plant product resulting from introducing or disseminating a plant pest, or the potential for exacerbating the impact of a plant pest. **§ 340.3**

In these cases, if APHIS Plant Protection and Quarantine (PPQ) requires a permit for a wild-type strain because it could pose a direct or indirect plant pest risk, APHIS BRS requires a permit for modified versions of the wild-type strain. If APHIS PPQ does not require a permit for a wild-type strain, APHIS BRS will require a permit for a modified version if the unmodified version has been used as a biocontrol organism, or if the modified version is being investigated or proposed for use as a biocontrol organism.

### **EXEMPTIONS FROM PERMITTING REQUIREMENTS**

The following modified microorganisms are exempt from permitting requirements in 7 CFR part 340.

**Exemption for GE disarmed Agrobacterium species.** A permit for importation or interstate movement is not required for any GE disarmed Agrobacterium species, provided that it is moved as a secure shipment, the modified genetic material is stably integrated into the genome, and the modified material does not include the complete infectious genome of a plant pest.

#### **§ 340.5(d)**

**Exemption for certain microbial pesticides.** A permit is not required for the movement of any GE microorganism product that is currently registered with the Environmental Protection Agency (EPA) as a microbial pesticide, so long as the microorganism is not a plant pest as defined in § 340.3. **§ 340.5(f)**

Information regarding EPA biopesticide registrations can be found here:

<https://www.epa.gov/ingredients-used-pesticide-products/biopesticide-active-ingredients>.

To facilitate shipments of modified microbes that are exempt from BRS' permitting requirements (e.g., importation of GE disarmed *Agrobacterium* species meeting the criteria above), developers may request a Letter of No Permit Required (LONPR) by emailing [BRSNoPermitRequired@usda.gov](mailto:BRSNoPermitRequired@usda.gov). An LONPR does not expire, and it can be used indefinitely. For this reason, developers may wish to have a permanent employee with oversight of the laboratory to which the material will be shipped request the LONPR.

**To request a LONPR, you must provide:**

- Name
- Institution
- Full address
- Phone number
- Email

## SELECT AGENTS

The Agricultural Bioterrorism Protection Act of 2002 and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 require entities that possess, use, or transfer biological agents or toxins deemed a severe threat to animal or plant health or products to notify and register with the Federal Select Agent Program. If the modified organism is a select agent or is capable of producing a toxin listed in the Federal Select Agent Program website, APHIS BRS cannot issue a permit to authorize its use. Select agents that are plant pathogens are regulated specifically by the APHIS PPQ branch of the Federal Select Agent Program.

A current list of all select agents and toxins is available at: <http://www.selectagents.gov/>.

Bacilli strains are frequently used in biotechnology applications, and taxonomic designations have been challenging. For example, note that *Bacillus cereus* biovar *anthracis* appears on the list of the U.S. Department of Health and Human Services' (HHS) Select Agents and Toxins, and *B. anthracis* and *B. anthracis* Pasteur strain appear on the HHS and USDA Overlap List. If the identity of a *B.cereus s.l.* strain in a BRS permit application is not yet determined, then, at a minimum, applicants must be able to exclude strains that are designated as select agents.

For questions related to microorganisms that produce select agents and toxins that can cause disease in humans, contact Centers for Disease Control and Prevention Division of Select Agents and Toxins at 404-718-2000; email: [LRSAT@cdc.gov](mailto:LRSAT@cdc.gov); and for those that can cause disease in animals and plants contact Animal and Plant Health Inspection Service Division of Agricultural Select Agents and Toxins at 301-851-2070; email: [DASAT@usda.gov](mailto:DASAT@usda.gov).

## SUBMITTING PERMIT APPLICATIONS FOR MODIFIED MICROORGANISMS

APHIS regulates and issues permits for the importation, interstate movement, and environmental release of certain modified microbes under 7 CFR part 340. A BRS permit is not required if a developer is creating a modified microbe and conducting research activities involving that modified microbe in a contained facility designed to prevent the modified microbe's unauthorized releases into the environment and is not moving the microbe across state lines or releasing it into the environment. To ensure a facility prevents the unauthorized release of a modified microbe, developers should follow containment guidelines specific to microbes. For examples of containment guidelines, see [NIH Guidelines](#); [CDC Biosafety in Microbiological and Biomedical Laboratories](#); [APHIS PPQ Containment Facility Guidance](#); and [Practical Guide to Containment: Plant Biosafety in Research Greenhouses, Adair & Irwin \(2008\)](#). Unauthorized, accidental, or inadvertent release of a modified microbe that would otherwise be subject to 7 CFR part 340 is a violation of the regulations. If you are unsure whether your contained facility is adequate to prevent an unauthorized release of a modified microbe, please apply for a permit following the information below.

To apply for a BRS permit, the applicant creates a permit application and an associated "Standard Operating Procedures" (SOP) using the APHIS [eFile](#) electronic permitting system. The APHIS eFile system will not accept a BRS permit application unless at least one document with the attachment type of "SOP" is uploaded to the SOP/Attachments section of APHIS eFile. The purpose of the SOP is to provide a description of how the modified microbe will be:

- contained during movement and at the points of origin and destination, including intermediate destinations, and
- confined during release into the environment "in a manner so as to prevent its unauthorized release, spread, dispersal and/or persistence in the environment" (7 CFR § 340.5(i)(1)).

Details on submitting permit applications can be found on the [BRS webpage](#) and on the [APHIS eFile training](#) page. To minimize redundancy, information that is required for **all** BRS permits in the APHIS eFile electronic permitting system may not necessarily be repeated in this document. For example, confidential business information (CBI) must be appropriately designated if applicable (see [CBI Submission Guidance](#)). Additionally, when submitting a permit application for a modified microbe, use the "Traditional" permit application regardless of the intended use. The other permit designation, Pharmaceutical, Phytoremediation, and Industrial permits ("PMPI"), is specific to plants, only.

When completing a permit application, you will need to have the following information on hand describing the modified microbe:

- **Scientific name:** The species name, as well as the strain, isolate, race, and/or pathovar, as applicable. For importation or interstate movement of foreign isolates, provide the location where the organism was originally collected or isolated.
- **Construct elements:** All genetic elements used in imparting the modification, including the name, donor (source) organism, and a brief description of the function. If applicable, describe targeted deletions.
- **Phenotype:** Brief description of the intended phenotype that the modification(s) are expected to confer.

You will also need to have information related to all activities involving the modified microbe at all intermediate and final destinations. As a practical matter, you will describe these activities and associated containment and confinement measures in your “Standard Operating Procedures” (SOPs), which is a required attachment in the APHIS eFile system that is discussed more fully below.

Multiple species belonging to the same genus may be submitted on a single permit application for importation and/or interstate movement if containment protocols are appropriate and all species are securely contained during shipment. For release permits, only a single species may be submitted on a given permit application.

The expected timeline for issuance of a permit that contains all required information is 45 days for importation and interstate movement, and 120 days for environmental release.

**Special note on greenhouses:** BRS has received inquiries regarding permit procedures for greenhouses and other semi-contained structures in terms of whether the developer should apply for an interstate movement permit, environmental release permit, or both. We recognize that structures referred to as a “greenhouse” could represent a range of containment levels. Depending on the measures in place to prevent escape into the environment, proposed research in a greenhouse could either be considered as a contained destination on a movement permit, or activities in a greenhouse may be more appropriately designated as a confined field release. For questions pertaining to greenhouse research with modified microbes, please contact [BiotechQuery@usda.gov](mailto:BiotechQuery@usda.gov). After the initial inquiry, a consultation may be scheduled with BRS subject matter experts. When preparing for such a consultation, it is helpful to gather information about the proposed research activities and greenhouse, including transport to/from a contained facility, and any history related to previous permits, inspection BRS or PPQ inspections, SOPs, and/or photos.

## MOVEMENT BETWEEN CONTAINED FACILITIES

### Summary of Requirements for SOPs

Your SOPs must describe the procedures you will use to contain the modified microbe during shipment and at the destination. In other words, describe how you will prevent the release and dissemination of the modified microbe into the environment.

- **During shipping:** You must secure all shipments of modified microbes. Please refer to the document “[Suggestions for SOPs Submitted for APHIS BRS permits](#)” (in Section 2.1 Packaging and Shipping) for information on what to include in your SOP, such as descriptions of packaging materials, any additional biological material that may be present, and devitalization/sterilization procedures upon receipt.
- **At the destination:** To receive regulated modified microbes at a contained facility (*e.g.*, laboratory, contained greenhouse, or other contained structure), BRS requires information about the destination in the permit application. Containment considerations are case-by-case, but some useful resources are provided in the following section.

**Note:** If the laboratory/facility proposed for work with the modified microbe has been previously inspected, either by BRS or PPQ, that information may assist BRS staff in evaluating containment and whether any additional measures might be needed. It is recommended for applicants to provide



facility numbers, dates of inspection, any BRS or PPQ permits associated with the inspection, and/or other pertinent information in the application, if it is known.

### Considerations for Containment

Considerations include the following:

- Where will you use the modified microbe (*e.g.*, laboratory, walk-in growth chamber, greenhouse)?
- How will you use the modified microbe (*e.g.*, DNA extraction for genetic analysis, pathogenicity testing, vector transmission studies, etc.)?
- How will you securely store and maintain the modified microbe in all areas of the facility for all activities?
- How will you devitalize or sterilize the modified microbe and all items in contact with it? This includes all plants, growth media, soil, storage containers, laboratory surfaces, etc. Consider the biology of the microorganism, such as cell wall structure, and types of plants and media. The choice of disinfectant, concentration, and exposure time will be based on each microorganism.
- Will you inoculate plants with the modified microbe? If so, what plants will you use and how will they be inoculated?
- How will you prevent dispersal and transmission of the microbe?
  - If air-dispersed, what measures will you take to prevent dissemination in the facility?
  - If spore-producing, how will you contain the spores?
  - If vectored by insects, how will you control insects and to prevent unintended transmission?
  - If water or splash dispersed, how will you prevent splash dispersal, and/or what measures will you take to treat the water?

## ENVIRONMENTAL RELEASE

### Summary of Requirements and Considerations

#### Summary of Requirements

In addition to the general requirements discussed above, you will need the following information to complete a permit application for an environmental release:

- **Release site:** Provide information such as land area (size), GPS coordinates, and land use history at the site and adjacent areas.
- **Experimental procedures for confinement (SOPs):** Describe how you will maintain the modified microbe at the release site and prevent its spread and persistence after the termination of a field trial.
- **Diagnostics:** Describe how you will detect the modified microbe and distinguish it from the wild type for monitoring purposes.
- **Monitoring:** Describe how long and how often you will monitor to ensure modified microbes have not spread and will persist in the environment.
- **Final disposition for release:** Describe how you will devitalize the modified microbes at the end of the field trial.

#### Considerations

Permit conditions and procedures carried out by the applicant should prevent the unauthorized release, spread, dispersal and/or persistence of the modified microbe in the environment. Additionally, the

information supplied in the application, including experimental procedures for confinement (SOPs), will help BRS determine the appropriate level of National Environmental Policy Act and Endangered Species Act analyses. We encourage developers to consult with BRS early in the planning process to discuss confinement procedures, diagnostic testing, and other possible background studies. If you are interested in a consult, please write to [BiotechQuery@usda.gov](mailto:BiotechQuery@usda.gov).

Additional considerations as applicable may include the following biological properties of the modified microbe:

### Organism

- **Geographic origin:** Is the same species/strain of the organism found in the locale of the proposed release? Documentation could include external references, the molecular basis for the determination, and biological properties of the modified microbe.
- **Environment:** What is the habitat and environmental conditions that are favorable and/or where the microbe is typically found? Are there dormant stages that need to be considered? What environmental conditions, habitat, and soil types, for example, are favorable or unfavorable for persistence of the microbe?
- **Host range (both primary and alternative hosts):** Consider all possible host plants of the microbe, including, for example, groups of plants typically affected, as well as wild or weed species that may act as reservoirs. Consider whether any other primary or alternative host plants may be present in the area of the proposed release. If the organism is used for biocontrol of invertebrate plant pests, what is the host range of the microbe?
- **Dissemination and spread:** How does the modified microbe spread naturally in the environment? Is it vectored by another organism, or can it spread via water, wind, or adherence to animals or objects such as tools used for cultivation? Can the microbe be secondarily spread via seeds or pollen from inoculated plants? Consider all possible routes of transmission and dispersal.
- **Does/would application of the modified microbe result in changes to the microbial community (rhizosphere, phyllosphere, and/or endosphere) that enhance plant pest risk?**
- **Are there interactions with other microbes that may need to be considered?**

### Modification

- **Does the modification to the microbe alter any of the aspects described above? For example:**
  - Does the modification involve a change in host range, or allow the microbe to survive under different environmental conditions?
  - Does the modification affect how the microbe is spread?
- **Effects on other organisms**
  - What is the effect on host plants that may or may not be part of the proposed trial?
  - If the modified microbe is a plant pathogen, is there any change in virulence from the wild-type?
  - Does the modified microbe produce antagonistic compounds such as toxins or antibiotics? For example, chitinases, siderophores, proteases, or mycotoxins.
  - Is the modified microbe antagonistic to other microbes that benefit plant health?
  - Does the modified microbe synergistically interact with a plant pathogen to enhance disease severity?

- Does the modified microbe benefit plant growth? How?
- Does the modified microbe affect invertebrate plant pests? How?
- Does the modified microbe affect invertebrates that benefit plant health?
- **Has horizontal gene transfer been considered?** For example, on the frequency of occurrence and species of organisms that could be potential recipients? For example, among strains of *Bacillus subtilis*, or, for genetic elements that may confer a fitness advantage?
- **Does the modification involve a conditional switch or other mechanism for reducing spread or persistence?**

*Supporting information can be provided in studies conducted by the applicant in controlled environments and/or in peer-reviewed publications.*

### **Experimental Procedures for Confinement (SOPs)**

SOPs are required for all permit applications; the SOP for an environmental release should describe procedures to ensure a confined field trial. Procedures should be appropriate to prevent spread beyond the trial site (confinement in physical space) and prevent persistence beyond the duration of the trial (confinement in time). Readers are referred to Section 3.0 of "[Suggestions for SOPs Submitted for APHIS BRS permits](#)" for general information about protocols for confined field trials.

You should include the elements described below in an SOP for confinement of modified microbes in a field trial. Your SOP should account for your particular trial with the particular modified microbe.

#### **Trial design and execution**

- Procedures for application or inoculation of plants or soil, including for example, whether plants and/or soil will be inoculated in a greenhouse and then moved to the field.
- Inoculum: Amount and concentration of the modified microbe and how it will be applied, *e.g.* foliar spray, soil drench, or seed treatment.
- Frequency of applications, *i.e.*, number of environmental releases.
- Duration of the trial.
- Scale (*e.g.* approximate number of plants, size of the field plot).
- A detailed map and/or diagram of the proposed trial, including GPS coordinates.
- If applicable, describe procedures for packaging and transportation of organism and inoculated material to/from the field site, and include these facility locations in the permit application.
- Procedures to prevent dissemination of the modified microbe via air, water, plant parts, or vector organisms, as appropriate. For example:
  - Physical isolation from primary and alternative host plants that are not part of the trial
  - Prevention of unintended transmission via vector organisms.
  - Water and irrigation management (*e.g.* drip or sprinkler irrigation, berms or ditches around the release site to manage water runoff).
  - Other example methods could include cages, plastic sheeting, bare ground, or inoculation of potted plants.
- In-trial monitoring procedures to ensure that the modified microbe is not disseminated beyond the designated trial site.
  - Should describe frequency of sampling, plus number and type of samples.

- Samples could be from soil (*e.g.* within trial site, adjacent to trial site, or outside of trial site) or from plants (*e.g.* treatments, controls, or buffer plants).
- Further information on diagnostics is provided below.

### **Trial termination**

To prevent persistence in the environment, procedures should describe how plants, soil, and other materials as relevant will be treated to devitalize the modified microbe at the field site. Describe methods to devitalize the modified microbe after use (*e.g.*, autoclaving, chemical treatment, or burning), or how the regulated material will be returned to and maintained in a contained facility.

- Termination and devitalization of inoculated plants.
- Treatment of soil in the trial zone and buffer zones.
- If the microbe requires the host plant to survive, how will the plot be kept clear of applicable vegetation to prevent persistence of the microbe? For example, will bare ground be maintained at the plot for some defined period of time?
- If inoculated plants are not destroyed at the end of the trial, such as trees, how will devitalization of the modified microbe be ensured?
- Consider all stages of the life cycle of the modified microbe, including vegetative, reproductive, and dormant/overwintering structures as applicable.
- Trial termination must occur on or before the expiration date of the permit unless the permit has been renewed.

### **Post-trial monitoring**

Your post-trial monitoring procedures should describe how you will determine that the modified microbe does not persist and spread in the environment after conclusion of the field trial. Refer above to “Additional Considerations” for some aspects that are relevant to evaluating the potential for persistence.

- Should describe duration and frequency of sampling, plus number and type of samples.
- Samples could be from soil, plants (*e.g.* sentinel plants, or post-treatment trees), water, insects, or any other matrix depending on the trial.
- Further information on diagnostics is provided below.
- Documentation of post-trial monitoring for all BRS release permits is submitted as a “Volunteer Monitoring” Report in APHIS eFile.
- Monitoring must be conducted until the modified microbe is no longer detectable for consecutive time points. Again, the sampling regime and associated diagnostic test(s) must be appropriate for the trial.

### **Diagnostics for In-Trial and Post-Trial Monitoring**

*Diagnostic assays must be developed for detection of the modified microbe in the intended sample matrix.* This means distinguishing the modified microbe from the unmodified strain, in field-relevant samples (*e.g.*, soil where the microbe is to be released) appropriate for the method of application (*e.g.*, seed treatment, soil drench, or foliar spray).

The limit of detection (LOD) of the assay(s) can be determined in controlled settings, but the diagnostic method must be able to detect the modified microbe in field-derived samples. At a minimum, validation

data should support the specificity (true positives) and selectivity (true negatives) of the assay for the taxon of host and microorganism for which it is developed. Selectivity includes healthy host tissue (e.g. from control plants) and unrelated microorganisms that may be found with the host (within intended purpose). Specificity should demonstrate the assays' ability to detect all applicable modified strains in both simple and complex matrices (e.g., with and without the plant) to exclude false negatives.

#### List of Information to be Submitted

- **Material and sample preparation:**
  - Sample collection:
    - From relevant soil and/or plant tissues.
    - From areas including, for example, from initial inoculation site to other areas of the plant.
    - At time points from Time 0 (baseline), for a duration to include the trial and post-trial monitoring.
  - Nucleic acid extraction protocol(s), adapted for sample types: From pure cultures, potting media, soil, plant material, and/or vector insects, as applicable.
- **Description of the strategy/methodology used, e.g.,** PCR, Sanger sequencing, or High-throughput sequencing.
- **Description of the procedures required to perform the diagnostic test from sampling to results reporting.**
- **Specificity:** Describe how the diagnostic method specifically detects only the modified microbe. Validation of the diagnostic test involves distinguishing:
  - the modified microbe from the unmodified (wild-type) strain, and
  - the modified microbe from closely related species, as well as other species expected to be commonly found at the release site.
 Information regarding test specificity includes:
  - *In silico*: For example, the description of primer design.
  - *In vivo*: Provide the list of species that were tested in a laboratory and/or greenhouse setting to confirm specificity.
- **Sensitivity:** Describe how the limit of detection is determined and verified. Diagnostic tests should be able to detect the modified microbe in field-derived samples and be appropriate for monitoring after the field trial is ended.
- **Robustness:** sample size and types of samples assessed (e.g. simple or complex matrix, and with or without plants used to validate).

## APPENDIX I - EXCERPTS PERTINENT TO MODIFIED MICROBES

### From Title IV: Plant Protection Act. 7 USC 7702. SEC. 403. Definitions.

(2) Biological Control Organism.—The term “biological control organism” means any enemy, antagonist, or competitor used to control a plant pest or noxious weed.

### From 7 CFR 330.200(d):

*Biological control organisms not regulated by this subpart.* Paragraph (c) of this section notwithstanding, biological control organisms that have undergone genetic engineering, as defined in § 340.3 of this chapter, as well as products that are currently under an EPA experimental use permit, a Federal Insecticide Fungicide and Rodenticide Act (FIFRA) section 18 emergency exemption, or products that are currently registered with EPA as a microbial pesticide product, are not regulated under this subpart. Additionally, biological control organisms that are pesticides that are not registered with EPA, but are being transferred, sold, or distributed in accordance with EPA’s regulations in 40 CFR 152.30, are not regulated under this subpart for their interstate movement or importation.

### From 7 CFR 340.2 Scope:

Except under a permit issued by the Administrator in accordance with § 340.5, no person shall move any GE organism that: ...

- (b) Meets the definition of a plant pest in § 340.3; or
- (c) Is not a plant but has received deoxyribonucleic acid (DNA) from a plant pest, as defined in § 340.3, and the DNA from the donor organism either is capable of producing an infectious agent that causes plant disease or encodes a compound that is capable of causing plant disease; or
- (d) Is a microorganism used to control plant pests, or an invertebrate predator or parasite (parasitoid) used to control invertebrate plant pests and could pose a plant pest risk.

### From 7 CFR 340.3 Definitions:

- **Genetic engineering.** Techniques that use recombinant, synthesized, or amplified nucleic acids to modify or create a genome.
- **Move (moving, movement).** To carry, enter, import, mail, ship, or transport; aid, abet, cause, or induce the carrying, entering, importing, mailing, shipping, or transporting; to offer to carry, enter, import, mail, ship, or transport; to receive to carry, enter, import, mail, ship, or transport; to release into the environment; or to allow any of the above activities to occur.
- **Plant pest.** Any living stage of a protozoan, nonhuman animal, parasitic plant, bacterium, fungus, virus or viroid, infectious agent or other pathogen, or any article similar to or allied with any of the foregoing, that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product.
- **Plant pest risk.** The potential for direct or indirect injury to, damage to, or disease in any plant or plant product resulting from introducing or disseminating a plant pest, or the potential for exacerbating the impact of a plant pest.
- **Release into the environment (environmental release).** The use of an organism outside the physical constraints of a contained facility.
- **Secure shipment.** Shipment in a container or a means of conveyance of sufficient strength and integrity to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

 **RESOURCES**

- **Q&As on Working with Modified Microorganisms:**  
<https://www.aphis.usda.gov/biotechnology/downloads/faq-modified-microbes.pdf>
- **Guidance for Submitting Confidential Business Information:**  
[https://www.aphis.usda.gov/brs/pdf/CBI\\_Submission\\_Guidance.pdf](https://www.aphis.usda.gov/brs/pdf/CBI_Submission_Guidance.pdf)
- **Suggestions for SOPs:** <https://www.aphis.usda.gov/help/eFile/sop-suggestion-submissions.pdf>
- **APHIS eFile Training Resources:** <https://www.aphis.usda.gov/aphis/banner/help/efile/efile-training>