Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

DOCUMENT ID:
BRS GD 2023 0001

DATE:
September 10, 2024

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

ISSUING AGENCY/OFFICE:	Animal and Plant Health Inspection Service (APHIS)/ Biotechnology Regulatory Services (BRS)
TITLE OF DOCUMENT:	Guide for Submitting Permit Applications for Microorganisms Developed using Genetic Engineering Under 7 CFR part 340
DOCUMENT ID:	BRS-GD-2023-0001
DATE OF ISSUANCE:	September 10, 2024
REPLACES:	REVISED DRAFT Guide for Submitting Permit Applications for Microorganisms Developed Using Genetic Engineering Under 7 CFR Part 340 issued on October 13, 2023
SUMMARY:	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) (85 FR 29790). For more information: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology
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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 7 CFR § 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* 7 CFR § 340.2. Developers require a permit for regulated activities involving any modified microorganism that:

- Meets the definition of a plant pest in 7 CFR § 340.3; or
- Is not a plant but has received deoxyribonucleic acid (DNA) from a plant pest, as defined in 7 CFR § 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 7 CFR § 340.3. Other important excerpts are referenced with the appropriate regulatory section. See **Appendix A** for a full list of excerpts relevant to microorganisms.

For additional information on whether a modified microbe is subject to 7 CFR part 340, please visit our questions and answers webpage and filter by "microbes".

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. 7 CFR § 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 7 CFR § 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. 7 CFR § 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. **7 CFR § 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. 7 CFR § 340.3

APHIS Plant Protection and Quarantine (PPQ) requires a permit for wild-type strains of microbes that are known plant pests, act as direct biological control organisms, or if their mode of action is unknown. When PPQ issues a permit for the wild-type strain of a microbe, BRS requires a permit for modified versions of the wild-type strain. If APHIS PPQ does not require a permit for certain activities with a wild-type strain, BRS will still require a permit for the modified type if the microbe has been modified for use as a biocontrol organism and could pose a plant pest risk.

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.

7 CFR § 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,[1] so long as the microorganism is not a plant pest as defined in 7 CFR § 340.3.

LETTERS OF NO PERMIT REQUIRED (LONPR)

To facilitate shipments of modified microbes that are exempt from BRS' permitting requirements (e.g., importation of modified disarmed *Agrobacterium* species meeting the criteria above or an EPA-registered biopesticide), developers may request a Letter of No Permit Required (LONPR) by emailing BRSNoPermitRequired@usda.gov. A 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

LETTERS OF NO JURISDICTION (LONJ)

In some instances, a developer has reason to believe their modified microbe does not meet the permitting criteria in 7 CFR § 340.2 and may wish to obtain confirmation from BRS that their modified microbe is not subject to regulation under 7 CFR part 340. If a developer has scientific information demonstrating their modified microbe does not meet the permitting criteria in 7 CFR § 340.2, they request a Letter of No Jurisdiction (LONJ). Developers may request a LONJ by emailing biotechmicrobes@usda.gov with the subject line "request for LONJ".

To request a LONJ, you must provide:

- Name
- Institution
- Full address
- Phone number
- Email
- Written explanation detailing the characteristics of your modified microbe and why the

[1] Please visit EPA's website for more information on EPA biopesticide registrations.

modified microbe does not meet the permitting criteria as supported by scientifically valid information. Explanation should include:

- The organism's genus and species.
- Construct components and donors: Genus and species of the organism(s) from which the genetic material was obtained.
- Construct components: Detailed description of functions. The phenotype or intended trait expected from the modification.

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 APHIS PPQ's 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; 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.

SUBMITTING PERMIT APPLICATIONS FOR MODIFIED MICROORGANISMS

BRS 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 an area meeting the definition of "contained facility" (that is, a "structure for the storage and/or propagation of living organisms designed with physical barriers capable of preventing the escape of the organisms"). To ensure a facility prevents the unauthorized release of a modified microbe, developers should follow containment guidelines specific to microbes.[2]

[2] 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).

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.

General information for permit applications. When completing a permit application, you will need to have the following information on hand for all types of permits related to modified microbes:

- **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.

The duration and scope of a permit are based on the type of regulated activities you plan to undertake with the modified microbe.

- Import and interstate movement permits.
    - You may apply for a multi-year permit (2-3 years) to import or move modified microbes. If your research plans change while the permit is valid (for example, if a location or

destination associated with your research changes), you must submit a permit amendment to obtain approval for a new location or activities *prior* to implementing the change in research plans.

- You may apply for an Interstate Movement permit to move multiple organisms within the same Kingdom (Multiple Bacteria, Multiple Fungi, Multiple Viruses, or Multiple Protista) to contained facilities (excluding greenhouses).
  - The permit can be a multi-year permit (2-3 years)
  - This type of permit has reporting requirements.
  - Information on how to apply can be found in **Appendix B**, along with a sample completed application and sample supplemental permit conditions.
- To otherwise import or move modified fungal and bacterial species, you may include multiple species of the same genus in a single permit application.
- The expected timeline to issue an import or movement permit is 45 days on average from receipt of an application that contains all the required information.

- Environmental release permits.
  - You may apply for a one-year permit for the environmental release of a microbe.
  - You may only include a single species on a permit application for environmental release
  - The expected timeline to issue an environmental release permit is 120 days on average from receipt of an application that contains all the required information.

Special note on greenhouses: BRS has received inquiries regarding permit procedures for greenhouses 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 meet the definition of "contained facility" and, thus, be appropriate to identify as a location or destination on an import or interstate movement permit, or activities in a greenhouse may be more appropriately designated as a confined release and, thus, be more appropriate for an environmental release permit. For questions pertaining to greenhouse research with modified microbes, please contact biotechmicrobes@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 the facility, and any history related to previous permits, inspection BRS or PPQ inspections, SOPs, and/or photos. In the event an inspection is required, BRS has included a checklist to assist developers with preparing for a facility inspection in **Appendix C** (INSPECTION CHECKLIST) of this guide.

MOVEMENT BETWEEN CONTAINED FACILITIES

SUGGESTED INFORMATION FOR IMPORT AND INTERSTATE MOVEMENT SOPS

Your SOPs should 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 shipment. BRS has created a list of questions to assist in the development of your SOP that can be found in **Appendix D** (THINGS TO CONSIDER WHEN DEVELOPING SOPs).

- **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 suggested information 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 BRS or PPQ has previously inspected the facility where you plan to work with the modified microbe, including that information in your permit application may assist BRS staff in evaluating containment and whether any additional measures might be needed. Relevant information about prior inspection includes 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.

ENVIROMENTAL RELEASE

ADDITIONAL INFORMATION REQUIREMENTS FOR PERMIT APPLICATIONS

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.
- **Monitoring:** Describe the method you will use to monitor and how long and 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.

SUGGESTED INFORMATION FOR ENVIRONMENTAL RELEASE SOPS

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.

Including the elements below in your SOP will enable assessment of the confinement measures for your modified microbes based on its characteristics.

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.
    - Describe frequency of sampling, plus number and type of samples, or other monitoring measures. 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?
- 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.

- Describe duration and frequency of sampling, plus number and type of samples, or other monitoring method. 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

Diagnostics may be developed for detection of the modified microbe in the intended sample matrix. This means identifying the modified microbe 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 assay's ability to detect all applicable modified strains in both simple and complex matrices (*e.g.*, with and without the plant) to exclude false negatives.

SUGGESTED INFORMATION REQUIREMENTS FOR DIAGNOSTIC TESTS

- **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).

Information supplied in your permit application, including 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 biotechmicrobes@usda.gov.

VERSION HISTORY

September 10, 2024	REVISED DRAFT Guide for Submitting Permit Applications for Microorganisms Developed Using Genetic Engineering Under 7 CFR Part 340 in response to public comments
October 13, 2023	REVISED DRAFT Guide for Submitting Permit Applications for Microorganisms Developed Using Genetic Engineering Under 7 CFR Part 340 in response to public comments
March 23, 2023	Initial DRAFT Guide for Submitting Permit Applications for Microorganisms Developed Using Genetic Engineering Under 7 CFR Part 340 issued

APPENDIX A - 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.

7 CFR § 330.200(b):
Plant pests regulated by this subpart. For the purposes of this subpart, and except for an organism that has undergone genetic engineering as defined in 7 CFR § 340.3 of this chapter, APHIS will consider an organism to be a plant pest if the organism directly or indirectly injures, causes damage to, or causes disease in a plant or plant product, or if the organism is an unknown risk to plants or plant products, but is similar to an organism known to directly or indirectly injure, cause damage to, or cause disease in a plant or plant product. Plant pests that have undergone genetic engineering, as defined in 7 CFR § 340.3 of this chapter, are subject to the regulations of part 340 of this chapter.

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.

7 CFR § 340.2 Scope (specific to microbes):
Except under a permit issued by the Administrator in accordance with 7 CFR § 340.5, no person shall move any GE organism that:

(b) Meets the definition of a plant pest in 7 CFR § 340.3; or
(c) Is not a plant but has received deoxyribonucleic acid (DNA) from a plant pest, as defined in 7 CFR § 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.

7 CFR § 340.3 Definitions:
- ***Genetic engineering.*** Techniques that use recombinant, synthesized, or amplified nucleic acids to modify or create a genome.
- ***Contained facility.*** A structure for the storage and/or propagation of living organisms designed with physical barriers capable of preventing the escape of the organisms. Examples include but are not limited to laboratories, growth chambers, fermenters, and containment greenhouses.
- ***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.

APPENDIX B – PERMIT APPLICATION AID FOR SUBMITTING AN INTERSTATE MOVEMENT OF MULTIPLE SPECIES OF MODIFIED MICROBES BETWEEN CONTAINED FACILITIES (EXCLUDING GREENHOUSES)

You must meet the following requirements to submit an **interstate movement** PERMIT application for multiple microbe species within a single Kingdom in APHIS eFile. Once issued, the permit will authorize interstate movement between contained facilities. The permit will not authorize the use of:

1) Select agent organisms or organisms modified to produce a select agent or toxin; or
2) A greenhouse.
    - If a developer wishes to use a greenhouse at a later stage of the research, they must submit a new permit application for the individual species.

Starting a Multiple Species Interstate Movement Permit Application

- Navigate to https://efile.aphis.usda.gov/s/ using Google Chrome (APHIS recommends accessing APHIS eFile using Chrome).
- Log in to eFile using your login.gov credentials.
- If you do not have a login.gov account, select Create Account and follow the steps to register for a login.gov account. **You must have a login.gov account to access and submit applications in APHIS eFile.** If you have any questions about login.gov, please see this Frequently Asked Questions page.
- Once you are logged into the APHIS eFile portal, select 'APHIS 2000 (Web)' from the 'Ready to Apply?' dropdown menu and click 'Get Started'. This will bring you to the BRS Permitting Assistant. Follow the steps below to start an application for a multiple species microbial movement permit (for general information about using the BRS Permitting Assistant, see the BRS Permitting Assistant User Guide.)

Step 1: Enter your Organism Details & Search

A. In the 'Organism' search bar, enter one of these four options and select the result that appears directly below the search bar.
    - "Multiple Bacteria" – e.g., Archaebacteria and Eubacteria
    - "Multiple Fungi" – e.g., Ascomycetes and Basidiomycetes
    - "Multiple Viruses" – e.g., viroids, viruses
    - "Multiple Protista" – e.g., Oomycetes, Brown Algae, Green Algae, Red Algae, Amoeboid Protozoa, Ciliated Protozoa, Flagellated Protozoa
B. In the 'Intended Use' dropdown, select "Traditional".
C. In the 'What are you applying for' dropdown, select "Permit".
D. In the 'Movement Type' dropdown, select "Interstate Movement".

After A – D are complete, click the blue 'Search' button in the Step 1 box.

Step 2: Select Attributes

There are no selectable options in this step. Continue to Step 3.

Step 3: Select Your Outcome

A. Click the blue '+ Add Outcome' button.
B. Click the blue 'Next Step' button.
C. On the BRS Permitting Assistant summary page, click the blue 'Proceed to Applications' button to make the 'Select Account and Responsible Person' popup appear.
D. Select the appropriate Team Sharing Account and Responsible Person, then click the 'I Understand & Continue' button.

After clicking the 'I Understand & Continue' button, you will automatically be brought to the 'My Activity' page and your draft permit application will be the topmost activity card. Click the 'View Details' button within this activity card to complete and submit your BRS permit application.

Completing and Submitting your Multiple Species Interstate Movement Permit Application

After clicking the 'View Details' button, you will be brought into your BRS permit application. For general information about navigating the BRS permit application user interface, see the APHIS 2000 Permit Application and Compliance Reporting Job Aid.

Application Details Chevron

A. In the Related Activity section, indicate if the Organism was originally collected from OUTSIDE OF THE UNITED STATES (if country is known, specify) within the 'Additional Information' field. If you wish to provide additional information not captured in one of the categories above, you can also provide such information in this field.

Organisms Chevron

A. Click the '+ Add Organism' button.
B. In the popup that appears, select each individual microbial organism this multiple species movement permit application will encompass.
C. If one or more of your microbial organisms are not in this list, contact BRS.efile@usda.gov to have the organism(s) added.

Constructs Chevron

A. Click the '+' Add Construct button.
B. At this point, you will enter ONLY A SINGLE generalized construct for the organisms listed in your multiple species permit application. You will provide more specific information about the modification(s) you make to your microorganism as part of an annual reporting requirement.
    - For an example of what a completed construct will look like in a completed application, see the Multiple Bacteria Authorization PDF example for the Generalized Construct.
    - In the '**Construct Details**' section of the popup that appears, select or enter the following in the displayed fields:
        - **Construct Name** – Enter the name that you want to call your construct
        - **Organism** – Select the Multiple organism that was entered i.e., Multiple Bacteria
        - **Modification Method** – Enter how organisms will be modified
            - In the event of multiple modification methods, select "**Other**" in the drop-down menu and list the multiple modification methods in the adjacent '**Transformation Events/Construct Desc**.' field.

Transformation Event/Construct Description: Leave this field blank.

A. After saving your Construct Details, click the '**Add Intended Trait'** button to enter all possible Intended Trait(s) your generalized construct will cover. Intended Trait(s) consists of Trait and Phenotype.
   - **Trait** – This is a general and observable (able to be seen or otherwise identified) characteristic of a modified organism. Traits can be classified into one of 10 distinct categories using the dropdown menu.
   - **Phenotype** – Your statement should capture the primary purpose of the trait, e.g., "Altered metabolism of a desirable compound production"

B. After saving your Intended Trait(s), click the '**Add Genotype**' button to enter a generalized genotype and construct component to the authorization. This is where you will be linking the construct to the multiple organisms listed in the Organism Chevron. Constructs do not need to be entered for the individual genus and species. See the example PDF for an illustration.
   - Enter a single genotype and a single construct component under "**Genotype**".
     - Select "**Gene of Interest(s)**" for "**Genotype Category**"
     - Select "**Gene**" for "**Construct Component**" under "**Genotype**"
     - Provide a "**Construct Component Name**" for your gene, e.g., "multiple genes and edits," "multiple edits," or other description
     - In "**Donor**" enter "**multiple organisms**;" this applies even if all you are doing is making genome edits to the multiple organisms in your permit application.
     - In "**Construct Component Description**", provide the function of the Gene of Interest, e.g. "Research on the listed phenotypes and traits"

   - When you are finished, the genotype in the application will look like this:
     - Gene(s) of Interest
     - Gene: Multiple genes and edits **from** multiple organisms – Research on the listed phenotypes and traits

     Note: Generalized construct description as shown is acceptable. Additional construct information will be submitted as reports to BRS.

Locations Chevron

A. Provide the **Location Type**(s) and **Location Name**(s) and address(es), along with County, State, and Zip Code.
   - Multiple **Origin**, **Destinations**, or **Origin and Destination** locations can be added.
     - You may enter up to 250 **Origin**, **Destinations**, or **Origin and Destination** locations**.**
   - If you intend to ship and receive regulated material to and from a location, select **Origin and Destination** for that location type.

B. For **Location Description,** provide the APHIS Inspection number(s) for your location(s), if available.

C. For **Quantity, Material Type,** and **Unit of Measure:** Enter the required information. Enter the highest expected amount of material to be moved over the lifetime of the permit. BRS recommends overestimating the amount of material to be moved.

D. Provide the **Agent**(s) Name, Day Telephone, and Email

Sample Permit – Multiple Bacteria Interstate Movement Permit

Authorization No. AUTH - 0000523071
No CBI Copy

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICES
APPLICATIONS FOR PERMIT UNDER 7 CFR 340
(Genetically Engineered Organisms or Products)

1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

Name: FreshBRS ApplicantTest
Title: Professor
Organization: BRS Test Org 3
Address: 555 Cloudy City,
Green City, Maryland, 12345
Day Telephone: (444) 444-4444
FAX:
Email: email@email.com

2. PURPOSE OF PERMIT

☐ Industrial Product
☐ Pharmaceutical Product
☐ Phytoremediation
☒ Traditional

3. MOVEMENT TYPE

☐ Importation
☒ Interstate Movement
☐ Interstate Movement and Release
☐ Release

Proposed Effective Date: 10-01-2024

Proposed Expiration Date: 10-01-2027

4. APPLICANT REFERENCE NUMBER:

5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)
Does this application contain CBI? ☐ Yes ☒ No
CBI Justification:

6. REQUEST TYPE
☒ New ☐ Amendment

7. MEANS OF MOVEMENT
Fedex, Private Courier

8. ORGANISM

Scientific Name: Xylella fastidiosa
Common Name: Pierce's Disease, phoney peach disease, bacterial leaf scorch, oleander leaf scorch, citrus variegated chlorosis disease
Cultivar and/or Breeding Line:

Scientific Name: Xanthomonas oryzae
Common Name: Rice bacterial blight
Cultivar and/or Breeding Line:

Scientific Name: Streptomyces turgidiscabies
Common Name: Streptomyces turgidiscabies
Cultivar and/or Breeding Line:

Scientific Name: Streptococcus lividans
Common Name: Streptococcus lividans
Cultivar and/or Breeding Line:

Scientific Name: Paenibacillus borealis
Common Name: Paenibacillus borealis
Cultivar and/or Breeding Line:

SAMPLE

WARNING: Any use of eFile to make materially false, fictitious, or fraudulent statements or representations is subject to civil penalties of up to $250,000 (7 U.S.C. § 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. §1001).

Scientific Name: Lactobacillus paracasei
Common Name: Lactobacillus paracasei
Cultivar and/or Breeding Line:

Scientific Name: Bacillus amyloliquefaciens
Common Name: Bacillus amyloliquefaciens
Cultivar and/or Breeding Line:

Scientific Name: Escherichia coli
Common Name: E. coli
Cultivar and/or Breeding Line:

Scientific Name: Agrobacterium rhizogenes
Common Name: Agrobacterium rhizogenes
Cultivar and/or Breeding Line:

Scientific Name: Multiple Bacteria
Common Name: Bacteria
Cultivar and/or Breeding Line: See other listed organisms.

9. ORGANISM SUPPLIERS OR DEVELOPERS

Name	Location	Contact Information

SAMPLE

Authorization No. AUTH - 0000523071
No CBI Copy

10. CONSTRUCTS

1) Construct Name:	Generalized Construct Example
Organism:	Multiple Bacteria
Transformation Events/Construct Desc.:	
Modification Method:	Electroporation

Intended Trait(s)
OO-Other -
Altered nitrogen metabolism

OO-Other -
Altered carbon metabolism

OO-Other -
Altered pathogenicity

AP-Agronomic Properties -
Altered plant architecture

MG-Marker Gene -
Increased detectability

Genotype(s)
Gene(s) of Interest
Gene: Multiple genes and edits from Multiple organisms -
Research on the listed phenotypes and traits.

Authorization No. AUTH - 0000523071
No CBI Copy

11. LOCATIONS

Point of Origin

Location Name & Description	Location Address	Agent(s)

Origin and Destination

Location Name & Description	Location Address	Agent(s)
1) HufflePuff Analytics	**Address:** 58A Willow Avenue **City:** Davis **County:** Yolo **State:** California (CA) **Country:** United States of America **Zip Code:** 65422	1) Scientist 02 Andrea Smith **Primary:** ☒ **Organization:** The Institute **Day Telephone:** 5-555-5555 Ext 55 **Email:** asmith@theinstitute.org
Material Types: 1000.000 Individual of Petri Dishes		
2) Gryffindor Labs	**Address:** 2 Quidditch Court **City:** Surrey **County:** Middlesex **State:** Massachusetts (MA) **Country:** United States of America **Zip Code:** 65412	2) Scientist 01 Robert Jones **Primary:** ☒ **Organization:** The Institute **Day Telephone:** 3-333-3333 **Email:** rjones@theinstitute.org
Material Types: 1000.000 Individual of Petri Dishes		

Destination

Location Name & Description	Location Address	Agent(s)

SAMPLE

Authorization No. AUTH - 0000523071
No CBI Copy

12. ATTACHMENTS

CBI	CBI-Deleted/Non-CBI
	SOP_2023.docx

13. ADDITIONAL INFORMATION

I, FreshBRS ApplicantTest, hereby certify that the information in this application and all attachments is complete and accurate to the best of my knowledge and belief.
I acknowledge this is not an application to move or import select agents, the genes expressing select agents, or the toxins made by the select agents, as described in 9 CFR 121.
I will not introduce the Organisms described in this application until APHIS has deemed the application complete and has granted the permit. By signing this permit, I agree to comply with any and all state, local, and tribal laws and regulations that may apply to the introduction of the articles described in this applications.

14. SIGNATURE OF RESPONSIBLE PERSON	15. DATE
FreshBRS ApplicantTest	08-16-2024

Supplemental Permit Conditions for Interstate Movement of Multiple Microorganisms

1. **Authorized Activities.** You are authorized for **interstate movement** of the organism under permit. This permit requires compliance with standard conditions (7 CFR § 340.5(i)) and supplemental permit conditions described below (collectively, "permit conditions").
This permit does not authorize: the **importation or environmental release** of the organism under permit; use of the regulated organism in a **greenhouse**; and production or use of any **select agent or toxin** as listed on the Federal Select agent and Toxins list.

2. **The Conditions in this Permit are Controlling.** You must comply with the permit conditions described in this permit. You should ensure any Standard Operating Procedures (SOP) meets the conditions described in this permit. If an associated SOP conflicts with or does not address permit conditions, you must follow these permit conditions.

3. **Duration.** This permit is valid for a period of **{one (1) year}** as identified by the effective and expiration dates of this permit.

4. **Authorized Locations.** The permit is valid at locations described in this permit, and intermediate locations described in any associated SOP provided:
    - You must use and/or store the regulated organism and any inoculated plant parts (such as pollen and/or insect vectors) solely in a contained area within the facility that has physical barriers to prevent the escape of the modified organism during all life stages (including air and/or water borne spores) into the environment or non- contained areas of the facility. The regulated organism includes material and objects with which it comes in contact (e.g., any media or cultures, inoculated plants or soil, other organisms harboring or that may harbor the regulated organism, containers, implements, and equipment). This means you must treat anything that comes into contact with the regulated organism to ensure the regulated organism is devitalized in accordance with the permit conditions.
    - If at any time you lose containment of the regulated organism, you must devitalize the regulated organism.
    - You must maintain a record of any breach of containment, the method you used to devitalize the regulated organism, and notify BRS as described below (see Reporting a Possible or Actual Unauthorized Release).

5. **The Responsible Person and Designated Agents.** If you designate an agent to act in whole or in part on your behalf, they must maintain control over the regulated organism described in this permit during its movement and ensure compliance with all applicable permit conditions and the requirements in 7 CFR part 340. The act, omission, or failure of any agent may also be deemed your act, omission, or failure.

6. **Responsibilities After Permit Expiration.** After the expiration date on this permit, organisms (including any plant material and any associated media that may harbor the regulated organism under permit) remain regulated and you must continue to prevent their unauthorized release, spread, dispersal, and/or persistence in the environment until they are devitalized, or APHIS has determined that they are not subject to the regulations under 7 CFR part 340.

SAMPLE

7. **Proposed Changes to an Issued Valid Permit.** To make changes to an issued permit, you must submit a permit amendment. You must contact APHIS Permitting staff via phone (301-851-3886) or email (BRSPermits@usda.gov) and obtain an amended permit before deviating from the issued permit. Changes may include: additional constructs (including any other identifying information, e.g., strains/lines) or new locations (including different laboratories in the same building).

8. **Record Maintenance.** You must maintain, and make available upon request, records of sufficient accuracy, quality, and completeness to demonstrate compliance with all permit conditions and that include information related to:
   - Locations: Your records must include the addresses and any other information necessary to identify all intermediate and final destinations where you use or store the regulated organism(s), including room numbers and growth chambers;
     - for each location, the type of regulated organism(s) used or stored, use and disposition of the regulated organism(s), and strain or line identification;
     - the location and method of final disposition.

9. **Containment in Transit.** You must ensure containment and secure shipment of the regulated organism as specified in § 340.5(m). At a minimum, you must use two adequately sealed layers of containment that are each independently sufficient to prevent release of the regulated organism into the environment.

10. **Containment and Storage at Destinations.** You must ensure the regulated organism is in a locked container or room or in a building or room with access restricted to authorized personnel only.

11. **Receiving of Shipment:** You must open secure shipments of the regulated organism in a contained location that is authorized in this permit. The contained area must be appropriate for the organism and its dispersal method (e.g., spore producing material must be opened in a biosafety cabinet with appropriate filtration) and designed to prevent the escape of the organism.

12. **Disposal of Packaging/Shipping Container:** You must treat any material in contact with the regulated organism to ensure the regulated organism is devitalized before disposing of any packaging material, shipping containers, and other material accompanying the secure shipment.

13. **Devitalization and Disposition.** After any use, and upon expiration of this permit, you must render the regulated organism (except any retained for future use) non-viable before disposal. **This includes the organism under permit, any media or cultures, any inoculated plants or soil, and any other organisms harboring, or that may harbor the regulated organism.** You must treat waste materials, containers, implements, and equipment that have come in contact with the regulated organism to render the regulated organism non-viable before re-use, disposal, or nonregulated use. Appropriate means of devitalization include autoclaving and incineration. If you use an alternative method to devitalize the regulated organism, you must maintain, and make available upon request, a scientifically justified reference, documentation, or testing information that indicates the method is appropriate to devitalize all life stages of the regulated organism.

SAMPLE

14. **Reporting Requirements.** You must submit reports as described in reporting requirements below.

    You must submit the required reports by email or postal mail. If you are claiming Confidential Business Information (CBI) within reports, you must submit both CBI and non-CBI copies following the regulations of 7 CFR 340.7. Email reports to BRSCompliance@usda.gov. Postal mail reports to:

    Animal and Plant Health Inspection Services
    Biotechnology Regulatory Services (BRS)
    Document Control Officer
    4700 River Rd. Unit 146
    Riverdale MD 20737

15. **Multiple Species Interstate Movement Reports.** You must submit a report to BRS no later than 6 months after the permit's effective date, and, thereafter, every 12 months, with the last report due no later than 30 calendar days after the permit's expiration date.

    Your reports must include the following:
    a. Authorization number
    b. Organism under permit (e.g., "Multiple Bacteria", "Multiple Fungi")
    c. Location name, county, and state of the responsible person
    d. For each regulated microorganism moved under the permit, the genus, species, any relevant subspecies, and common name of the microorganism, each gene(s) that was inserted or targeted for editing in the microorganism, and the associated trait and putative phenotype for each inserted or edited gene in the microorganism
    e. Description of interstate movement of the organism(s) under permit, including each authorized location from which the organism(s) was moved, the starting date for each movement, the authorized receiving location(s), and the receipt date

16. **Reporting a Possible or Actual Unauthorized Release.** In the event of a possible or actual Unauthorized Release, you must report the discovery to BRS Compliance staff as follows:
    - Contact BRS Compliance staff via phone (301-851-3935) or email (BRSCompliance@usda.gov) within 24 hours of discovery. If your call advances to voicemail, you must leave a message describing the discovery.
    - Subsequently, you must submit a written statement of facts describing the possible or actual Unauthorized Release no later than 5 business days after the discovery.

    7 CFR 340.3 defines an Unauthorized Release. Examples of Unauthorized Releases include: loss of a package during interstate movement/ importation; dispersal of the regulated organism (loss of containment) due to damaged packaging materials; inadvertent or intentional movement of the regulated organism outside of any location authorized in this permit (including rooms, labs, growth chambers, freezers, refrigerators, storage areas, etc.); release into the environment due to damage incurred to the facility where the regulated material is located (e.g., vandalism, break-in, tornado, or earthquake damage); and movement of a regulated organism with an unauthorized construct.

SAMPLE

APPENDIX C – INSPECTION CHECKLIST

Below is a general list of considerations for conducting contained research activities for modified microbes. Please note that not all items may apply, as it depends on the biology, the modification, and ecology of the microbes and the level of risk involved.

Greenhouse Facility Inspection Checklist for Research Involving Microorganisms Developed using Genetic Engineering (Modified Microbes)

Biotechnology Regulatory Services

Organization: _____ Inspection Date: _____

Greenhouse Responsible Party: _____ Authorization Number: _____

Site Location: _____ Inspector Info: _____

Purpose of Inspection:

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
1. Was the facility previously inspected by BRS and/or PPQ? If yes, include the inspection number in the comments box below.	☐	☐	☐
Comments:			
2. Is the greenhouse managed by personnel other than the responsible person or an agent? Is the greenhouse manager aware that this research involves material regulated under 7 CFR part 340?	☐	☐	☐
Comments:			
3. Does the responsible person or agent have a training program developed for applicable SOPs for personnel authorized to work in the greenhouse? Is there a plan to keep a record of training dates for authorized personnel who work in the greenhouse?	☐	☐	☐
Comments:			
4. Will visitors be allowed in the greenhouse? If so, what protocols are in place to assure that they are aware of and adhere to applicable containment requirements?	☐	☐	☐
Comments:			
5. Is the staff present during inspection knowledgeable about monitoring for breaches in containment? Is a plan in place if one were to occur?	☐	☐	☐
Comments:			

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
6. Has a copy of the greenhouse floor plan been provided to BRS with the permit application? Has the responsible researcher provided the inspecting official with a copy of floor plans indicating which areas will be used for this research?	☐	☐	☐
Comments:			
7. Is the greenhouse a separate building or is it connected to other buildings/greenhouses?	☐	☐	☐
Comments:			
8. Is the greenhouse located in an agricultural area?	☐	☐	☐
Comments:			
9. Are the tops and sides of the greenhouse made of impervious materials to prevent escape of regulated material?	☐	☐	☐
Comments:			
10. Are appropriate collection/disposal bins located in the greenhouse? (Please make a note of this method). If the regulated material is moved to another area in the facility for devitalization, how will it be contained and/or stored until devitalized?	☐	☐	☐
Comments:			
11. Will soil or potting medium used in this research be re-used? Please list the method of soil treatment/devitalization or disposal proposed in the comments box below.	☐	☐	☐
Comments:			
12. Will any non-regulated sexually compatible species to the inoculated material or other non-regulated plant species be present in the same greenhouse at any point during the length of the authorized research?	☐	☐	☐
Comments:			
13. Do SOPs specify methods for clean-up/disposal of spilled regulated material, including inoculated plants, plant parts and/or inoculated soil? Are appropriate spill/clean-up materials available in the greenhouse?	☐	☐	☐
Comments:			
14. Will wastewater be collected and disinfected, OR do drains have filters, screens, or another method to contain/devitalize the wastewater appropriate for the regulated material?	☐	☐	☐
Comments:			

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
15. Is there an effective pest scouting and control program in place to eliminate undesired pests (e.g., aphids, whiteflies, scale insects) in the greenhouse? Will pest treatment records be kept on file?	☐	☐	☐
Comments:			
16. If the regulated microbe can be transported/transmitted via pollen, plant parts, or seeds, what procedures will be used to mitigate/trap/or kill potential dispersal agents such as rodents?	☐	☐	☐
Comments:			
17. Are all surfaces in contact with regulated material (including the floor) easy to inspect, non-porous, and able to withstand repeated decontamination? Is the decontamination method kept in the greenhouse for easy access?	☐	☐	☐
Comments:			
18. Is there a specific area designated for cleaning equipment that comes into contact with the regulated material inside the greenhouse?	☐	☐	☐
Comments:			
19. Will plants be inoculated with the regulated material in the greenhouse? Please describe the inoculation method in the comment box below.	☐	☐	☐
Comments:			
20. Will the research include any study on insect (e.g., transmission study)?	☐	☐	☐
Comments:			
21. If applicable, is a contingency plan in place for a potential breach in containment caused by loss of power? Do automatic doors, air filtration, air pressure fans, and water collection have a backed-up power source?	☐	☐	☐
Comments:			
22. Is there a system to detect broken glazing panels or other containment breaches?	☐	☐	☐
Comments:			
23. Does the electrical system maintain containment under emergency situations?	☐	☐	☐
Comments:			
24. Are there entry and re-entry protocols for the greenhouse?	☐	☐	☐
Comments:			
25. Are the greenhouse doors self-closing? Do thresholds/doors have seals that securely seal doors upon closing?	☐	☐	☐

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
Comments:			
26. Do greenhouse doors, windows and all alternate exits have locks? Is the greenhouse always locked or are there open hours? Are there provisions for lost, extra, or returned keys (if a staff member no longer needs access)?	☐	☐	☐
Comments:			
27. Does the greenhouse have a double-door entry system or a head-house/vestibule to help prevent the escape of regulated material into the surrounding environment? If not, please briefly describe how they plan to maintain containment upon exiting the greenhouse.	☐	☐	☐
Comments:			
28. If applicable: Will filters and screens from this greenhouse be decontaminated within the facility prior to disposal? If so, by what method?	☐	☐	☐
Comments:			
29. When exterior doors are opened, does air move out of the greenhouse?	☐	☐	☐
Comments:			
30. If applicable: Do greenhouse controls have an override to prevent vents from opening automatically, and is it working?	☐	☐	☐
Comments:			
31. If screening is used in the greenhouse: Are roof and/or side vents screened with an appropriate screen size to prevent insect movement? Is the screen size adequate to prevent the movement of insects that can vector this microorganism?	☐	☐	☐
Comments:			
32. Are there any noticeable holes or gaps in the greenhouse building?	☐	☐	☐
Comments:			
33. Is the internal or external exhaust/air exchange method filtered or screened? If so, what is the mesh size (if known)? Add filter types if known.	☐	☐	☐
Comments:			
34. Are evaporative coolers (swamp coolers) used in the greenhouse? If so, do they have an insect barrier/ fine-mesh screen covering to prevent entry or escape of pests?	☐	☐	☐
Comments:			
35. Will APHIS be notified if there are structural or functional changes that may affect containment security?	☐	☐	☐

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
Comments:			
36. Are any insects, rodents, standing water, loose soil, leaf litter or plant waste present during the time of inspection?	☐	☐	☐
Comments:			
37. Is the researcher conducting the experiments in the greenhouse the same person listed as the agent on the permit application?	☐	☐	☐
Comments:			
38. Is access to the greenhouse controlled to ensure only authorized personnel are entering and working in the facility?	☐	☐	☐
Comments:			
39. Does the party conducting the research have a copy of the permit, including SOP and permit conditions? Does the SOP match the one with the authorized permit?	☐	☐	☐
Comments:			
40. Are records (log or inventory) maintained regarding modified microorganism quantities received, amounts used with dates, amounts stored in facility, and devitalization of regulated material?	☐	☐	☐
Comments:			
41. Are procedures for working with modified microorganisms in the greenhouse (e.g., sample collection, inoculation, movement, devitalization) being followed as described in the SOP and SPCs?	☐	☐	☐
Comments:			
42. Does the facility have a sign that states “Authorized Personnel Only”? Are there signs on the walls or doors of individual rooms for research stating that USDA Regulated Material is present?	☐	☐	☐
Comments:			
43. Are security measures, such as badge access, locks and/or alarms, in place and in use as described in the SOP?	☐	☐	☐
Comments:			
44. Is the devitalization of regulated material performed according to authorized methods listed in the SOP and SPCs?	☐	☐	☐
Comments:			
45. Is a devitalization method for waste liquid in contact with the regulated material used as written in the SOP and SPCs?	☐	☐	☐

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
Comments:			
46. If regulated material will be removed from the greenhouse, are regulated materials contained during interbuilding movement in accordance with the SOP and SPCs?	☐	☐	☐
Comments:			
47. Are all regulated materials labeled as described in the SOP? Are markings or labeling clear and durable?	☐	☐	☐
Comments:			
48. If any non-regulated sexually compatible or other non-regulated plant species to the inoculated material are present in the same greenhouse during the length of the research, are isolation practices within the greenhouse being followed in accordance with the SOP? Are inoculated plants in the greenhouse labeled to distinguish regulated material from material that is not regulated?	☐	☐	☐
Comments:			
49. If plants are being inoculated with the regulated material in the greenhouse, is the inoculation method in accordance with the SOP?	☐	☐	☐
Comments:			
50. Are procedures for clean-up/disposal of spilled regulated material, including plants, plant parts and/or inoculated soil, followed in the greenhouse? If no: what process is used? Are the materials and/or equipment for devitalization present within the greenhouse?	☐	☐	☐
Comments:			
51. Do authorized staff use the decontamination method(s) approved in the SOP for workstations, benches, storage areas tools, and equipment (including PPE if used) that comes in contact with the regulated material?	☐	☐	☐
Comments:			
52. Are screens/filters over air vents/windows/water system (as applicable) in place and functional for purpose?	☐	☐	☐
Comments:			
53. If any holes or gaps were identified in the greenhouse building during pre-inspection, did the responsible party make repairs? (Proof of repairs must be submitted to BRS prior to permit issuance with associated documents.) Is the integrity of the greenhouse walls, floors, roof and doors adequate to exclude rodent/varmint or insects?	☐	☐	☐
Comments:			

CONSIDERATIONS FOR GREENHOUSE ACTIVITIES	YES	NO	N/A
54. Are any insects, rodents, standing water, loose soil, leaf litter or plant waste present during the time of inspection?	☐	☐	☐
Comments:			
55. Is a pest management plan described in the SOP? If so, are pest management practices in use the same as were approved in the SOP? If not described in the SOP, please note generally what their pest management practices are in comments.	☐	☐	☐
Comments:			
56. Are all microbe events (constructs) including the inoculated plant species planted in the greenhouse covered under the permit?	☐	☐	☐
Comments:			
57. Are there additional locations within the facility where the regulated material is authorized, and if so, are these locations listed in the SOP? Is the regulated material confined to approved locations?	☐	☐	☐
Comments:			
58. If the research includes insect work, is the containment and devitalization of insect vectors done in accordance with SOP requirements?	☐	☐	☐
Comments:			
59. Inspecting official—please list any other concerns about the capability of this greenhouse to contain the regulated organisms in the "potential compliance concerns" section below. (Please include photos as needed of labs, growth chambers, greenhouses, and storage areas.)	☐	☐	☐
Comments:			
60. Are there any identifiable breaches of containment? Is there any evidence that may be indicative of a breach of containment that has been repaired and not reported (such as cracked windows or torn screens with tape, hole/crack/tear repairs in wall/roof materials, etc.)? If yes, please explain in the comments box below.	☐	☐	☐
Comments:			

APPENDIX D – THINGS TO CONSIDER WHEN DEVELOPING SOPS

BRS has included a list of probing questions to assist developers in evaluating their planned research and developing complete SOPs to support research activities and containment and/or confinement of modified microbes. Specific answers to each of these probing questions are not required to apply for a permit; rather, these probing questions are intended to aid in developing the SOP that developers submit with their permit application.

Things to consider when importing or moving modified microbes interstate:

- 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 (like 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. Selecting a disinfectant, concentration, and exposure time is often based on each microorganism.

Things to consider when assessing whether a facility meets the definition of a "contained facility":

- 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?

Things to consider when planning an environmental release to prevent the unauthorized release, spread, dispersal and/or persistence of the modified microbe in the environment.

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.

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
- **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