**Standard Operating Procedure for Handling**

**Recombinant Plants/Plant Pathogens/Plants inoculated with Plant Pathogens**

**At BL2-P Containment**

**Introduction and Purpose of Research:** <*List organisms.* *Provide a brief description of research aims.>*

**Requirements for Inclusion in BLP-2 Practices and Containment:**

According to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules ([NIH Guidelines](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf)), BL2-P or BL1-P+ is recommended for the following experiments:

**Section III-E-2-b-(1).** Plants modified by recombinant or synthetic nucleic acid molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

**Section III-E-2-b-(2).** Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see Section V-M, *Footnotes and References of Sections I-IV*).

**Section III-E-2-b-(3).** Plants associated with recombinant or synthetic nucleic acid molecule-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-M, *Footnotes and References of Sections I-IV*).

**Section III-E-2-b-(4).** Plants associated with recombinant or synthetic nucleic acid molecule-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-M, *Footnotes and References of Sections I-IV*).

**Section III-E-2-b-(5)**. Experiments with recombinant or synthetic nucleic acid molecule-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant or synthetic nucleic acid molecule-modified microorganisms associated with them if the recombinant or synthetic nucleic acid molecule-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-M, *Footnotes and References of Sections I-IV*).

|  |  |
| --- | --- |
| PI: | Lab Location: |
| Issue Date: | Revision Date: |
| Prepared by: | OESO (Safety Office) Approval Signature: |

**Applicable Regulatory Statutes / Guidelines:**

CDC/NIH’s Biosafety in the Microbiological and Biomedical Laboratories (BMBL): (<https://www.cdc.gov/labs/BMBL.html>)

NIH rDNA Guidelines (BL2-P, Appendix K, Appendix L):

(<https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy#tab2/>)

USDA APHIS Plant and plant permitting information (<https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information>)

CDC/USDA Select Agents and Toxins Program (<https://www.selectagents.gov/sat/list.htm>)

EPA Plant Incorporated Protectants

(<https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/overview-plant-incorporated-protectants>)

**RISK ASSESSMENT:**

**<*DESCRIBE:*** *Hazard Identification, Risk/Routes of Exposure, and Consequences of Exposure, Containment of plants and pathogens to prevent release*>

**Procedural Methods and Materials**:

**Personal Protective Equipment (PPE)**: <*Describe personal protective equipment worn in the lab and Phytotron. If reusable PPE is worn, describe how lab coats are decontaminated (if applicable) before they are sent for laundry services, or if they are discarded when contaminated.>*

**Signage:**

A sign shall be posted indicating that a restricted experiment is in progress.

The sign shall indicate the following:

(i) the name of the responsible individual,

(ii) the plants in use, and

(iii) any special requirements for using the area.

If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

**Transport and removal of material(s) from the laboratory:** <*Describe how you transport plants and microorganism in a non-breakable containers, on a cart, etc*.> Materials containing experimental microorganisms, which are brought into or removed from the lab and Phytotron facility in a viable or intact state, shall be transferred in a closed non- breakable container.

**Standard methods required:** Staff will perform handwashing after removal of gloves and before leaving the work area, no mouth pipetting, no food or drink in refrigerators where material is stored, no eating or drinking in work area. If sharps are required for the work, use safe sharps practices e.g., use safer alternatives, no recapping, immediate disposal into a hard-walled puncture proof container for disposal.

<*Describe how splashes and aerosols are avoided. Describe if using absorbent material on surfaces, etc.*> Avoid splashes or aerosol by careful and deliberate handling procedures.

<*Describe decontamination process, including what is used and how this is done*.> Perform daily decontamination of all work surfaces.

**Cleaning, Devitalizing, Waste Inactivation and Disposal:** *<Describe where and how transported. Describe secondary container, cart used, etc. Describe disinfectant used, is applicable. Examples are given.>* Decontamination and devitalization of transgenic plants, inoculated plants, and plant pathogens will be performed by containing plants, soil, and plant pathogens in closed autoclavable biohazard bag, placed within a secondary autoclavable tray and autoclaved for **90 minutes at 121°C, 15 p.s.i.** and allowed to cool to room temperature before moving to the building dumpster. As above, daily decontamination of all work surfaces and other items (e.g. pipettes) when work is complete will occur using fresh made 1:10 household bleach or 70% EtOH solution.

**Spill and Accident Response Procedure:**

Pick up sharp items with mechanical device (not hands), place disposable sharp items in sharps waste container and non-sharps clean-up materials in a leak-resistant disposable bag. While wearing PPE to include, but not limited to, fully closed lab coat, gloves, and full face protection, place absorbent towels over the spill, place absorbent towels in biological waste for autoclaving. Clean the area with soap and water or disinfecting cleaning spray (e.g. Clorox Multi-Surface Cleaner + Bleach).

To ensure devitalization of any remaining material, freshly prepared 1:10 bleach*\** solution *(stock is at least 5.25% sodium hypochlorite. Working solution is at least 0.525% sodium hypochlorite. Greater concentrations may be used. Rinsing may be required to prevent corrosion.) is applied* to the entire area of spill starting on the outer edges and working inward. Allow contact time of 20 minutes and clean up using absorbent towels. Place towels in biological waste for autoclaving. *\*Large amounts of household bleach will not be autoclaved.*

**Environmental Release**

Release/spills are required by the NIH to be reported to the OESO-Biological Safety Division as soon as spill/release is known or suspected. Call 919-684-8822 or email: [biosafety@duke.edu](mailto:biosafety@duke.edu).

**TRAINING:**

**Training Requirements**: *Workers conducting research under this procedure must comply with the following training requirements:*

* Complete online Laboratory Safety-General training provided by Duke’s Occupational & Environmental Safety Office ([www.safety.duke.edu](http://www.safety.duke.edu)). This training is required annually and is documented by OESO.
* All personnel shall read and fully adhere to this SOP.
* ***P.I. will keep documentation of personnel reading and understanding this lab-specific SOP using a signature page.***

PLANT BIOSAFETY LEVEL 2 (BL2-P)



**Principal Investigator:**

**Building: Chamber/Room #**:

**Organisms:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Special Instructions/Requirements Prior to Entry, Working, or Exit (*i.e. personal protective equipment, etc.*):**  **REMOVE PPE AND WASH HANDS BEFORE LEAVING THE WORK AREA.** | | | |
| **EMERGENCY CONTACT/ADVICE** | **CONTACT** | **WORK PHONE** | **AFTER HOURS PHONE** |
| PRIMARY |  |  |  |
| SECONDARY |  |  |  |
| Departmental Contact (if necessary) |  |  |  |

*Staff Signature Page:*

**Standard Operating Procedure for Handling**

**Plant Pathogens and Plants inoculated with Plant Pathogens**

**At BL2-P Containment**

**“I have read and understand this SOP. I agree to fully adhere to its requirements.”**

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