



TEXAS A&M UNIVERSITY SAN ANTONIO

Guidelines for Transgenic Plant Containment

The *National Institutes of Health Guidelines for Research Involving Recombinant of Synthetic Nucleic Acid Molecules (NIH Guidelines)* outlines practices for work involving recombinant/synthetic nucleic acid (e.g., rDNA) molecules, synthetic nucleic acids, and organisms and viruses that contain recombinant/synthetic nucleic acid. The *NIH Guidelines* also provides information about determining risk assessment, containment, work practices, and facility design. The *NIH Guidelines* indicate that the ultimate responsibility for handling of transgenic or genetically modified plants and plant-associated organisms lies with the PI and other persons who manage any aspect of the research. Compliance with the *NIH Guidelines* is a condition of funding for the entire institution.

This document must be read in conjunction with Guidelines on Transgenic Organisms.

Specific regulations for research involving modified whole plants can be found in sections III-D-5 and III-E-2 of the *NIH Guidelines*. Appendix L of the *NIH Guidelines* “Physical and Biological Containment for Recombinant/synthetic nucleic acid Research Involving Plants” outlines the physical and biological containment practices that must be employed for greenhouse, laboratories, or growth chambers research at the four plant biosafety levels (BL-P), BL1-P through BL4-P.

The use of genetic manipulation and other techniques to produce transgenic or genetically modified plants, recombinant plant pathogens, and transgenic arthropods is common in plant science research.

Plant Biosafety Levels (BL-P)

A plant biosafety level designation will dictate the physical and biological containment practices aimed at reducing the public health threat and avoid an unintentional transmission or release of regulated plant material into the environment. The BL-P levels of containment were specifically devised to describe containment for transgenic plants. There are four BL-P, and as the level increases, the level of protection and physical and/or biological containment practices also increase.

There are several issues to consider when determining the appropriate BL-P:

- What is the source and nature of the introduced genetic material?
 - Is it from an exotic infectious agent or pathogenic organism?
 - Is it a fragment of DNA or a complete genome?

- What is the nature of the host organism?
 - Can the host readily disseminate the genetic material? By what mechanism(s)?
 - Is the recipient likely to be invasive to local ecosystems?
 - Is the recipient a USDA APHIS-listed noxious weed or capable of interbreeding with noxious weeds?
 - What is the potential for outcrossing between the recipient organism and related species?
 - What is the potential for detrimental impact on natural or managed ecosystems?

- Are bioactive proteins expressed?
 - What is the nature of expressed proteins?
 - Are the proteins vertebrate toxins or potential/known allergens?
 - Are the proteins toxic to other organisms in the local environment?

- What is the profile of the local environment?
 - Are potentially affected important crops located nearby?
 - Are sexually compatible wild plant or weed species capable of sustaining and/or spreading the genetic modification(s)?

- What experimental procedures may impact containment?
 - Will it be necessary to transport sensitive materials to/from the greenhouse?
 - Will arthropods or other potential vectors be used during the course of the project? How will these be contained to prevent or minimize the release of genetically modified materials?

BL1-P

BL1-P designation is for experiments that are deemed a low risk to the environment. This designation also applies to plant associated microorganisms that are considered to have a minimal impact on the environment. Some examples include plants that are not noxious weeds, plants with no potential for out-crossing with related species, and *Agrobacterium*-mediated transfer of innocuous (not harmful) genetic material.

Work involving other organisms that require a containment level of BL1-P or lower may be conducted concurrently in a greenhouse bay as long as all work is conducted using BL1-P practices. See **Appendix A** for specific BL1-P requirements.

BL2-P

BL2-P applies to experiments with transgenic plants and plants associated organisms that have the potential for rapid and widespread dissemination, and the capability of interbreeding with weeds or related species. However, these materials are not likely to have a serious detrimental impact on natural ecosystems.

Work involving other organisms that require a containment level of BL2-P or lower may be conducted concurrently in a greenhouse bay as long as all work is conducted using BL2-P practices. See **Appendix A** for specific BL2-P requirements.

BL3-P/BL4-P

Texas A&M University-San Antonio (A&M-SA) does not have containment facilities to conduct BL3-P or BL4-P designated research, and thus BL3-P/BL4-P containment procedures will not be presented in this document. For more information on these levels of containment, please see [Appendix L of the NIH guidelines](#).

Containment

Containment is essential in preventing the accidental release of transgenic research materials into the environment. When planning an experiment, all ways that an organism can breach containment must be considered. Traffic flow of personnel, air flow within the facility, prevention of cross-contamination, proper labeling, and permit requirements are important elements in this process.

Access

A&M-SA laboratories or greenhouse, growth chambers, or storage spaces containing transgenic plants, plant material or seeds must be kept locked with access restricted to individuals directly involved with the experiments. The Greenhouse Manager will authorize the use of secure bay or growth chamber space upon obtaining an IBC approved protocol. Additionally, all individuals with access to the transgenic plants, plant material or seeds must be knowledgeable of any special containment strategies, required personal protective equipment, and entry/exit procedures. All support staff and external contractors must be approved to have access.

Records

Individuals working with or having access to transgenic plants, plant material or seeds in a A&M-SA laboratory or at the Greenhouse must maintain current and accurate record logs of all in-process experiments (IBC registrations or a simple list of experiments are suitable records for BL1-P).

It is the responsibility of the PI to provide the Greenhouse Manager and Biosafety Officer access to the record logs at the time of decommissioning or in the event of an audit by regulatory authorities. Additionally, any specific federal/state permits required for greenhouse projects must be on file with the Biosafety Officer to ensure compliance with all procedural and containment expectations indicated in the permit(s).

Structural Containment

Regular inspections of the physical condition of the Greenhouse are performed by the Biosafety Officer and/or Greenhouse Manager. All authorized users are required to be vigilant for structural damage due to age related wear and tear, seasonal influences, extreme weather, vandalism, and other causes. Observations must be reported to the Greenhouse Manager, PI, and/or the Biosafety Officer.

Items include, but are not limited to, the following:

- Doors that do not properly close;
- Damaged door sweeps;
- Cracks, breaks to glass;
- Damage to screens or vents;
- Evidence of insects in the greenhouse; and/or
- Damaged or missing seals between structural components, around pipes and conduit.
- Damaged or missing screens on floor drains.

*If it is suspected that structural damage has resulted in a loss in containment of transgenic plants, plant material, or seeds the Biosafety Officer must be notified since this is reportable to the NIH Office of Biotechnology Activities, USDA APHIS, and/or other designated authorities.

Transgenic and Non-Transgenic Plants Cultivated in the Same Space

Non-transgenic plants housed on the same bench space with transgenic plants must be treated in the same manner as transgenic plants. Non-transgenic plants housed in the same bay as transgenic plants but not on the same bench may be treated separately, which will be decided on a case-by-case basis by the Greenhouse Manager and the Institutional Biosafety Committee.

Proper Hygiene/Housekeeping

Good basic hygiene/housekeeping practices are important in preventing the accidental release and/or unintentional spread of plant pests and pathogens. Basic practices and procedures include:

- Keep greenhouse bay(s) clean and uncluttered;
- Do not eat or drink in Greenhouse bays;

- Wash hands before leaving the Greenhouse;
- Wear facility-dedicated lab coats when working with transgenic plants, plant material, or seeds;
- Thoroughly inspect street clothes/shoes for transgenic material (especially for seeds and/or pollen) prior to leaving the bay and the Greenhouse;
- Eliminate any unnecessary equipment in Greenhouse bays.

Pest Control

The NIH Guidelines specify that a weed and pest control program must be in place for all levels of greenhouse containment. In the Greenhouse, the Greenhouse staff is responsible for pest management. Plants must be regularly inspected for signs of insect infestation. Plants with heavy infestations must be removed and disposed from the greenhouse in the interest of greenhouse integrity.

Transporting Transgenic Material

Transgenic plants, plant material or seeds that are transported to and from the Greenhouse must be transported in a leak-proof, shatterproof container. The outside of the container must be sanitized prior to transport to ensure that transgenic pollen and seed are removed.

Biological Containment Techniques

Unless integral to the research project, the production/dissemination of transgenic pollen and seed should be eliminated. There are several special practices that can be used to prevent the spread of transgenic material that include, but are not limited to, the following:

- Removing flower heads or bagging plants prior to flowering;
- Harvesting material before the reproductive stage;
- Using male sterile lines;
- Localizing engineered genes in the non-reproductive parts of the plant by expressing the transgene transiently rather than in stably transformed plants; or
- Conducting the experiment when pollination will not occur outside (e.g. winter months).

Transgenic or unmodified insects or mites that are associated with transgenic plants should be housed in appropriate containment caging systems (e.g. BugDorms) to minimize escape from the greenhouse bay. A cost-effective alternative can be constructed using plastic sheeting. Additional biocontainment techniques to be used when working with insects and mites include:

- Treatment or evaporation of runoff water to kill eggs and larvae;
- Destruction of pollinating insects in cages after pollen transfer.

Recombinant microbes such as bacteria, fungi, protozoa, viruses, and nematodes may be used during experiments. Additionally, unmodified microbes may be used in association with transgenic plants. In these cases, the goal of containment is to minimize dissemination of pollen and the microorganisms. Containment techniques that can be used when working with microorganisms include:

- Elimination of potential vectors;
- Genetic attenuation of the microorganism;
- Limiting production of aerosols during inoculation;
- Ensuring adequate distance between infected and susceptible hosts;
- Chemically treating runoff water to kill microorganisms;
- Using microorganisms that have an obligate association with the plant host.

Disposal of Materials

Transgenic plants, plant material, and seeds must be rendered biologically inactive by autoclaving before final disposal. Transportation of viable material to the autoclave must occur using a leak-proof, shatterproof container. If transportation in a leak-proof, shatterproof container is not achievable, transportation may be conducted in double walled containers (such as an

autoclave bag in an autoclave tub) with prior approval from the IBC. After plant materials are inactivated, they may be disposed of in the regular trash.

Decontamination of transgenic plant soil will be decided on a case-by-case basis by the Greenhouse Manager and the Institutional Biosafety Committee. In addition, decontamination of irrigation water is not necessary, but an appropriately sized filter or screen must be installed in each greenhouse bay or growth chamber to ensure transgenic plant material does not exit the Greenhouse.

Containment Breach

Weather related incidents, vandalism, or human error can result in a containment breach. Seeds can become attached to clothing and/or shoes, especially if containment practices are not rigorously followed. These seeds can be easily spread by the wind and could grow in the surrounding area, causing environmental contamination and resulting in transgenic plants growing outside of containment (volunteers). There are several steps that should be taken if an accident results in the inadvertent release or spill of transgenic plants, transgenic plant material, or transgenic seeds from physical containment:

- If known, seed/pollen dissemination distances should be considered when determining the monitoring area. If a known breach of containment has occurred, volunteer monitoring should be enhanced by increasing the monitoring zone and/or frequency of monitoring.
- Determine if any transgenic material has been removed from the Greenhouse bay or growth chamber or is otherwise unaccounted for.
- Contain and recover all transgenic materials as best as possible.
- The PI must report the containment breach to the Greenhouse Manager, Biosafety Officer, and other appropriate agencies within 24 hours, or as indicated by permit.

Appendix A: Physical and Biological Containment for Recombinant/synthetic nucleic acid Research Involving Plants ([Appendix L of the NIH Guidelines](#))

Appendix L specifies physical and biological containment conditions and practices suitable to carrying out experiments involving recombinant/synthetic nucleic acid-containing plants, plant-associated microorganisms, and small animals. All provisions of the *NIH Guidelines* apply to plant research activities with the following modifications:

Appendix L shall supersede Appendix G (*Physical Containment*) when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix L include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species, and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant,

plant pathogen, or plant pest containment principles when experiments utilizing Appendix L require prior approval by the Institutional Biosafety Committee.

Appendix L-I. General Plant Biosafety Levels

Appendix L-I-A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants.

Appendix L-I-B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Appendix L-I-C. Four biosafety levels, referred to as Biosafety Level (BL) 1 - Plants (P), BL2-P, BL3-P, and BL4-P, are established in Appendix L-II, Physical Containment Levels. The selection of containment levels required for research involving recombinant or synthetic nucleic acid molecules in plants or associated with plants is specified in Appendix L-III, Biological Containment Practices. These biosafety levels are described in Appendix L-II, Physical Containment Levels. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix L-I-D. BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant or synthetic nucleic acid molecules. These biosafety levels, in conjunction with biological containment conditions described in

Appendix L-III, Biological Containment Practices, provide flexible approaches to ensure the safe conduct of research.

Appendix L-I-E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in Appendix G, Physical Containment. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see Appendix L-III, Biological Containment Practices), if botanical reproductive structures are produced that have the potential of being released.

Appendix L-II. Physical Containment Levels

Appendix L-II-A. Biosafety Level 1 - Plants (BL1-P)

Appendix L-II-A-1. Standard Practices (BL1-P)

Appendix L-II-A-1-a. Greenhouse Access (BL1-P)

Appendix L-II-A-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix L-II-A-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix L-II-A-1-b. Records (BL1-P)

Appendix L-II-A-1-b-(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix L-II-A-1-c. Decontamination and Inactivation (BL1-P)

Appendix L-II-A-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix L-II-A-1-d. Control of Undesired Species and Motile Macroorganisms (BL1-P)

Appendix L-II-A-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix L-II-A-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix L-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P)

Appendix L-II-A-1-e-(1). Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

Appendix L-II-A-2. Facilities (BL1-P)

Appendix L-II-A-2-a. Definitions (BL1-P)

Appendix L-II-A-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix L-II-A-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix L-II-A-2-b. Greenhouse Design (BL1-P)

Appendix L-II-A-2-b-(1). The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Appendix L-II-A-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Appendix L-II-B. Biosafety Level 2 - Plants (BL2-P)

Appendix L-II-B-1. Standard Practices (BL2-P)

Appendix L-II-B-1-a. Greenhouse Access (BL2-P)

Appendix L-II-B-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

Appendix L-II-B-1-a-(2). Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix L-II-B-1-b. Records (BL2-P)

Appendix L-II-B-1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix L-II-B-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix L-II-B-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH OSP and other appropriate authorities immediately (if applicable). Reports to the NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on the OSP website (www.osp.od.nih.gov). Documentation of any such accident shall be prepared and maintained.

Appendix L-II-B-1-c. Decontamination and Inactivation (BL2-P)

Appendix L-II-B-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix L-II-B-1-c-(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix L-II-B-1-d. Control of Undesired Species and Motile Macroorganisms (BL2-P)

Appendix L-II-B-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix L-II-B-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix L-II-B-1-e. Concurrent Experiments Conducted in the Greenhouse (BL2-P)

Appendix L-II-B-1-e-(1). Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

Appendix L-II-B-1-f. Signs (BL2-P)

Appendix L-II-B-1-f-(1). 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.

Appendix L-II-B-1-f-(2). 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.

Appendix L-II-B-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix L-II-B-1-g. Transfer of Materials (BL2-P)

Appendix L-II-B-1-g-(1). Materials containing experimental microorganisms, which are brought

into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

Appendix L-II-B-1-h. Greenhouse Practices Manual (BL2-P)

Appendix L-II-B-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix L-II-B-2. Facilities (BL2-P)

Appendix L-II-B-2-a. Definitions (BL2-P)

Appendix L-II-B-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix L-II-B-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

Appendix L-II-B-2-b. Greenhouse Design (BL2-P)

Appendix L-II-B-2-b-(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix L-II-B-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix L-II-B-2-c. Autoclaves (BL2-P)

Appendix L-II-B-2-c-(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix L-II-B-2-d. Supply and Exhaust Air Ventilation Systems (BL2-P)

Appendix L-II-B-2-d-(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix L-II-B-2-e. Other (BL2-P)

Appendix L-II-B-2-e-(1). BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

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