1. PURPOSE

To provide a standard procedure for conducting a Good Laboratory Practice (GLP) Standards compliance inspection at biotechnology laboratories conducting studies that have been submitted and accepted by the Agency in support of applications for research or marketing permits for bioengineered pesticide products regulated by EPA [Sections 3, 4, 5, 18, and 24(c) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended] or pursuant to testing consent agreements, test rules, and pre-manufacturing requirements [issued under Sections 4 and 5 of the Toxic Substances Control Act (TSCA)].

2. SCOPE

This standard operating procedure will be used when inspecting laboratories and field sites that have conducted testing of bioengineered test substances under FIFRA and TSCA. Throughout this document, the term "bioengineered test substance" or simply "test substance" refers to the bioengineered substance under study, which may have been either a bioengineered organism (i.e., microorganism or transgenic plant) or a substance produced by the bioengineered organism. In addition, portions of this SOP apply to auditing studies that have been conducted by biotechnology laboratories. In these sections, the term "inspector" has been replaced with "auditor".

3. OUTLINE OF PROCEDURES

Organization and Personnel

Preparation of Bioengineered Test Substance

- Characterization of Donor and Recipient Microorganisms and Transgenic Plants
- Materials and Methods for Producing the Bioengineered Test SubstanceCharacterization, Production, and Handling of Bioengineered Test Substance
- Characterization of Bioengineered Test Substance
- Production of Bioengineered Test Substance
- Handling

Studies of the Bioengineered Test Substance

- Study Protocol
- Test System Care
- Efficacy
- Infectivity/Pathogenicity/Toxicity

Facilities and Equipment

- Facility Design
- Equipment

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5.0 **SPECIFIC PROCEDURES**

5.1 ORGANIZATION AND PERSONNEL

Since the biotechnology field is very specialized, it is particularly important that qualified individuals hold the key positions on the team performing the biotechnology study. Individuals contributing to a biotechnology study, particularly those in positions of responsibility such as the study director, need to have the appropriate education, training, and experience. The inspector will be concerned with many of the same general personnel issues with biotechnology studies as with other types of studies submitted under FIFRA and TSCA. For instance, regardless of the type of study, the inspector should evaluate whether a facility performing a study has a staff of sufficient size and whether their qualifications are appropriate and documented.

For guidance on personnel and organizational issues to address during the inspection, the inspector should refer to the SOP for "Conducting a Field site GLP Compliance Inspection" (SOP No. GLP-C01, Section 5.1.2) and the Good Laboratory Practices Inspection Manual, U.S. Environmental Protection Agency, September 1993.

5.2 PREPARATION OF BIOENGINEERED TEST SUBSTANCE

For studies submitted under FIFRA and TSCA, the bioengineered test substance may be a bioengineered microorganism or a transgenic plant. Methods for characterization of the donor and recipient microorganism/plant and preparation of the bioengineered test substance, and the subsequent issues of concern to the inspector, will be dependent on whether the resulting test substance is a microorganism or a plant. Specific elements the inspector should consider regarding the materials and methods used by the laboratory in the preparation of test substances are addressed below.

5.2.1 CHARACTERIZATION OF DONOR AND RECIPIENT MICROORGANISMS AND TRANSGENIC PLANTS

Microorganisms

The donor and recipient. microorganisms must have a taxonomic identification and a description of the genotypic and phenotypic characteristics. To evaluate the laboratory's characterization of the donor and recipient microorganisms, the inspector should check the storage conditions, records, and SOPs for handling the donor and recipient organisms. Questions for the characterization of the donor and recipient microorganisms include the following:

- How does the laboratory assure that it has a pure culture? What IS the frequency of this test?

- How are the organisms stored? Are storage conditions adequate to ensure the stability of the organism?

- Does the laboratory have a documented history of the bacterial strain?

- Does the laboratory have an SOP for doing the taxonomic identification?

- Does the SOP address identification of genotypic and phenotypic characteristics?

NOTE: **Genotypic** characteristics include a determination of the genetic material such as genetic maps of chromosomes and plasmids; genetic transfer capabilities such as transformation, transduction, or transfection; presence of transposable elements, insertion sequences, plasmids, phages, and phage cross-infectivity.

Phenotypic characteristics include culture requirements and characteristics; nature and degree of pathogenicity, virulence, infectivity, or toxicity to humans, other animals, plants, or microorganisms; life cycle including sexual/asexual reproduction cycle and dormant stages; appearance; antibiotic resistance; resident antibiotic production; survival/persistence; known control agent~; and biological control characteristics.

- For donor and recipient organisms from external sources (i.e., purchased or obtained from another laboratory), has the laboratory received a letter of identification (i.e., certificate) from the supplier?

  - Does the facility test the purchased organism upon arrival to certify its identity and purity?
  - What are the shipping conditions for the organisms?
  - Does the facility have records of receipt?

Plant Characterization

The selection of plant(s) to be used will depend on several factors including whether the plant can be reproductively isolated and whether or not it is likely to persist in an environment that is not cultivated (i.e., outside the test plot). The inspector will need to check the storage conditions, documentation and SOPs for characterizing and preparing the plants and/or plant cells.

- How and where are the plants and plant cells stored?
- Is there documentation of the biology of the reproductive potential of the plant (e.g., flowers, pollination requirements, and seed characteristics) being used in the study?

- **!** Does the laboratory have a documented history of controllable reproduction with lack of dissemination? Can the plant be established in an environment that is similar to the field test site?

5.2.2 MATERIALS-AND METHODS FOR PRODUCING THE BIOENGINEERED TEST SUBSTANCE

The inspector should check the storage conditions (including location) and records for materials used in producing the bioengineered test substance. The inspector should also check documentation regarding methods used in producing the bioengineered test substance.

Materials

- **!** If DNA is obtained from external sources (i.e., purchased or obtained from another laboratory), what are the shipping conditions? Was the DNA tested upon arrival? How is the DNA stored?

- **!** If a restriction endonuclease (or any other enzyme) is obtained from external sources, how does the laboratory assure that the enzyme is neither contaminated nor inactive? How frequently is it tested? How is the enzyme stored?

- **!** For other chemicals or substances used as reagents, buffers, or growth media, how does the laboratory assure the quality of the materials?

- **!** Does the laboratory use distilled, deionized water to prepare all reagents and buffer solutions?

- **!** Is the laboratory using standard methods for the preparation of reagents, buffer solutions, and media?

Methods

- **!** Does the laboratory have SOPs:

  - For isolating and identifying DNA being inserted into the recipient organism or plant cell?

  - For constructing the vector with the inserted DNA? - For verifying the DNA insertion in the vector?

- For the method being used to introduce the DNA/vector into the recipient organism?

  (1) Transformation, transduction, conjugation for microorganisms

  (2) Agrobacterium-based plant transformation, particle acceleration, electroporation, microinjection for plants

- For measuring the success of the insertion of the genetic material?

- For determining the stability of the inserted DNA in the recipient microorganism or plant?

! How does the laboratory validate the methods that it uses?

! Who constructed the vector and performed the procedures in preparing the bioengineered microorganism or transgenic plant?

! If protoplasts (for transgenic plants) are being used, are standard tissue culture procedures used?

5.3 CHARACTERIZATION, PRODUCTION, AND HANDLING OF BIOENGINEERED TEST SUBSTANCE.

5.3.1 CHARACTERIZATION OF BIOENGINEERED TEST SUBSTANCE

The GLP regulations require that the test substance be analyzed for identity, strength, purity, and composition, as appropriate for the type of study. This section addresses analysis of the bioengineered test substance for several characteristics including identity, purity, and stability. The auditor will review the identity, purity, and stability information and data that have been included in the study. Section 5.5 will address infectivity, pathogenicity, toxicity and efficacy testing.

Identity

Methods for determining the identity of the bioengineered test substance may be genotypic and phenotypic or physicochemical. The genotypic and phenotypic characterization includes the following items:

- **Genotypic**. Characterization/expression of the introduced genetic material in the organism, such as DNA fragment size, presence of coding/non-coding sequences, genetic maps of chromosomes or plasmids, type and number of repeat sequences, insertion sequences or transposable elements, and the presence of lysogenic bacteriophage;

- **Phenotypic**. Characteristics include culture requirements and characteristics; nature and degree of pathogenicity, virulence, infectivity, or toxicity to humans, other animals, plants, or microorganisms; appearance; antibiotic resistance; and survival/persistence.

The type of questions the auditor might ask with respect to the genotypic and phenotypic characterization are as follows:

- Does the laboratory have SOPs for identification of genotypic and phenotypic characteristics?

- Were appropriate methods and instruments used in the genotypic and phenotypic characterization of the bioengineered test substance?

- Was DNA sequencing conducted? If so, what method was used? Did the laboratory use a commercial kit or automated sequencer?

- If gel electrophoresis was done, was the type of electrophoresis appropriate for the size of DNA fragments? For agarose gels, was the percentage of agarose appropriate for the size of the DNA fragments to be separated? Were solutions, reagents, buffers, etc. adequately labeled (including the expiration date)? Was the person responsible for running the gels knowledgeable about the electrical parameters?

- Has the laboratory identified any unusual morphological, biochemical, or resistance characteristics that are different from the classic description of the organism? If so, have these been documented?

- Were all data available for review by the auditor?

If the desired product is a protein produced by the organism, the identity may have been determined through physicochemical tests. The type of questions the auditor might ask with respect to these analyses are as follows:

- Does the laboratory have SOPs for identifying/determining purity of the protein?

- Are the SOPs of adequate scope and detail (if reviewed by the auditor)?

- If gel electrophoresis was done, was the type of electrophoresis appropriate for the protein size? Were solutions, reagents, buffers, etc. adequately labeled (including the expiration date)?

- Were appropriate methods and instrumentation used in the identity testing?

- If ion exchange chromatography was done, how was the ion exchange gel chosen? Was the pH range where the protein is stable used? Was it appropriate for the size of the protein being separated? Was the gel equilibrated? Were any solutions, reagents, buffers, etc. adequately labeled with an expiration date?

- Were all data available for review by the auditor?

- Did the analytical data from the identity tests show any contamination of the test substance?

NOTE: Protein identity can be determined by amino acid composition analysis, partial sequence analysis, peptide mapping, polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing, or high performance liquid chromatography. These methods are described in the support document.

Purity

In many cases, the purity of the test substance will be determined concurrently with the identity testing. For example, if the laboratory is performing DNA sequencing and the resulting data show additional DNA fragments that should not be present, then the bioengineered microorganism may not be pure. Likewise, if the laboratory is performing antibiotic resistance testing and the microorganism shows sensitivity where there should be resistance, then the test substance may be contaminated. In addition to reviewing information from the identity tests, the .~auditor should also consider these questions:

- Does the laboratory assure that it has a pure culture?

- Was purity testing done on each batch of the test substance used in a study?

- Were precautions taken to prevent and control viral, bacterial, mycoplasmal, or other contamination?

Stability

Using records and data generated by the laboratory, the auditor should confirm that the test substance was analyzed for stability. The test tor stability is specific or unique to the type of bioengineered test substance and should be specified in an SOP and/or in the study protocol. The auditor should interview study personnel (as necessary) and review documentation to ensure that the tests were conducted as specified in the SOPs and/or study protocol. The auditor should ask the following questions:

- Did the laboratory test the stability of the test substance?
- Did the laboratory test to determine the reversion rate or rate of plasmid loss from the host cell? How was the rate determined? Did the laboratory resolve any problems related to this?
- Was testing done at a point in the study such that any stability problems were identified early enough to avoid an adverse effect on the study?
- Were all data available for review by the auditor?

5.3.2 PRODUCTION OF BIOENGINEERED TEST SUBSTANCE

Since biotechnology studies commonly involve large populations of bioengineered organisms, rather than individual organisms, a facility will have procedures for propagation of the organism.

Microorganisms

Once the bioengineered microorganism has been prepared, it will need to be propagated/grown into a cell population. When the cell population has reached a sufficient size, the microorganisms are harvested (i.e., extracted from the growth medium). If the desired product is a substance produced by the microorganisms (e.g., a protein), rather than the cells themselves, the desired product will need to be recovered from the microorganisms. The bioengineered test substance (either the microorganisms or the substance they produce) will then be used for testing. The facility should have SOPs in place for both propagation and harvesting and should document compliance with the SOPs.

Elements that should be considered during the inspector's evaluation are the following:

- Does the laboratory have SOPs for propagation/growth of the bioengineered microorganism?

- Do they contain information such as the growth medium, physical conditions, nutrients, and expected generation time?

- Do the SOPs set out the specific equipment to be used (e.g., type of bioreactor) and the appropriate operating parameters (e.g., pH, temperature, effective mixing, viscosity, oxygen and CO_2 concentrations)?

- Are the equipment and conditions specified in the SOP appropriate for the level of containment needed for the study? For information on containment levels, the inspector should refer to the guidelines in Biosafety in Microbiological and Biomedical Laboratories (May 1988)

- Does the laboratory have SOPs for harvesting the bioengineered microorganisms?

- Do the SOPs contain guidelines for separation and purification?

- Has the separation procedure been validated?

- Has the purification system been validated?

- How are parameters for the separation and purification systems monitored?

- Do the systems yield a uniform product? Are tests conducted to determine uniformity?

- Does the laboratory have SOPs for waste recovery and decontamination of any wastes remaining after separation and purification?

- If the bioengineered microorganism is produced in more than one batch (i.e., consecutive batches, or concurrent batches in more than one bioreactor), what are the criteria for pooling these batches for subsequent portions of the study (i.e., application and testing)?.

5.3.3 HANDLING

The inspector should ensure that the facility has procedures in place for handling the bioengineered test substance. (Note: The bioengineered test substance refers to the bioengineered organism, the product generated by the bioengineered organism, or a

transgenic plant.) These procedures should address proper identification, storage, and distribution of each batch/aliquot of the test substance. To evaluate the laboratory's handling procedures, the inspector may interview laboratory personnel, examine SOPs, observe laboratory practices, and review substance control logbooks. The following are issues to guide the inspector in evaluating the facility's compliance for handling of the bioengineered microorganism or transgenic plant:

- Does the laboratory have SOPs for handling the bioengineered test substance?
- Are these SOPs adhered to and any deviations properly authorized and documented?
- How is the test substance stored?
- Do storage conditions appear to be adequate for preserving the test substance?
- Are storage procedures designed to limit the potential for contamination or degradation of the substance?
- How are samples/batches distributed?
- How is the test substance transported to the test site?
- Are appropriate containers used so as to preserve the characteristics (especially purity) of the test substance?
- Does the laboratory document the receipt and distribution of the test substance?
- Does the documentation contain complete information, including who received each sample/batch, when, and in what quantity?
- Are all samples/batches of the test substance properly identified throughout storage and distribution?
- Are handling conditions specific to the test substance clearly identified, such as temperature conditions or length of storage time before reversion of transformation?
- Does the laboratory retain reserve populations of microorganisms or plants?

5.4 STUDIES OF THE BIOENGINEERED TEST SUBSTANCE

The TSCA and FIFRA GLP Regulations include specific requirements for the protocol for and conduct of a study submitted under TSCA and FIFRA. The GLP Regulations require that each study have an approved study protocol in place and that all changes to the protocol be explained, documented, and signed and dated. The regulations also require that the study be conducted in accordance with the approved protocol and that data generated during the study be recorded and maintained properly. This section addresses the items the auditor should evaluate with regard to the study protocol, conduct of the study, field sites for testing, test system care, and the efficacy and infectivity, pathogenicity, toxicity of the bioengineered test substance. For general questions regarding the study and test system, the auditor should refer to SOP GLP-DA-06 "Auditing Efficacy Studies."

5.4.1 STUDY PROTOCOL

The studies of bioengineered test substances may also be subject to FDA or USDA guidelines. In these cases, the auditor should determine if the study has been conducted in accordance with the applicable FDA or USDA guidelines (see Section 4.0, References, for complete titles).

Field Tests

- Does the protocol describe the experimental design, including methods for the control of bias? Are the objective for the field test clearly set out?
- Does the field test protocol describe test plot preparation, the spacing of plants, and the growing cycle?
- Does the protocol specify the method of application/route of administration of the test substance and the reason for its selection? Are the application/dosage level and the frequency of application specified? Are there provisions for controlling the unintended release or spreading of the test substance during application?
- Does the protocol specify sampling locations?
- Does the protocol describe the procedures for packaging and transporting samples from the field site to the laboratory for processing? Were these procedures adequate for ensuring that samples remained segregated and uniquely identified (e.g., labeling of sample containers)? Are procedures specified for sample preservation?

- Does the protocol describe how unused test substance, field test samples and materials are to be disposed of after analysis and/or use?

Field Sites

- Were the conditions of the field test site documented, including a description of the site (e.g., size, proximity to other test sites), its location, soil characteristics (e.g., texture, pH, cation exchange capacity), and site history (i.e., past uses)? Did the site conditions meet the requirements of the protocol? Was the historical agricultural practice for the test site documented?

- Were the test sites maintained in accordance with the specifications of the protocol (e.g., application of fertilizers/pesticides other than the test substance, tilling, weeding)?

- Before use in the study, was the test system acclimatized to the environmental conditions specified for the tests (e.g.~ gradual reduction in humidity from culture conditions to testing conditions)? Did the acclimatization period appear to be adequate?

- Was the field equipment (e.g., balances, application equipment, and field analytical equipment) inspected and calibrated before use? Were the precision and accuracy of the test equipment verified? Was the equipment decontaminated between sampling events?

- Do the study records contain a description of the meteorological conditions (e.g., air and soil temperatures, relative humidity, wind direction and velocity, rainfall) that existed during the study?

- Were containment measures taken to prevent contamination? Was the reproductive isolation of plants ensured? For microorganisms, was the gene transfer capability monitored?

- Were sample locations recorded with the analytical results or on a site map?

5.4.2 TEST SYSTEM CARE

The GLP Regulations define the test system as “any animal, plant, microorganism, chemical or physical matrix, including but not limited to, soil or water, or components thereof, to which the test, control, or reference substance is administered or added for study." In accordance with the Regulations, the test system should be cared for to ensure that the integrity of the study is not compromised by unhealthy or diseased test subjects, contamination from other species or test systems or from the feed, soil, water, or bedding. The auditor should review study notes and observations, interview study personnel, and visit test sites to evaluate whether the study implemented proper procedures for test system care. For animal and plant test systems, the questions the auditor would evaluate are found in other relevant SOPs. Specific elements the auditor would evaluate related to microorganism test systems are as follows:

- If the test system was a cell culture (i.e., microorganisms) and was purchased, from an outside source, were the microorganisms characterized? Was a letter of identification (regarding the characterization) received from the supplier? Were records of the characterization in the study files?

- How were the microorganisms stored? Were the storage conditions appropriate to ensure stability of the microorganisms?

- Was the cell culture tested for contamination before being used in the study?

5.4.3 EFFICACY

Efficacy, or product performance, is a measure of all aspects of a product's effectiveness and usefulness. Efficacy of the test substance is affected by the activity of the test substance itself, as well as by application methods, the type of target pest organisms, dosage rate and frequency, duration of use, other environmental conditions, and the use of other pest control substances or methods. Initial efficacy testing is generally conducted in the laboratory, in greenhouses, or on small field plots. If the results of the small-scale testing indicate sufficient product effectiveness, large-scale testing in the laboratory, field, and through simulated-use tests is subsequently done. The auditor should consider the following elements in evaluating the efficacy testing:

- Were efficacy tests done to confirm support for any proposed product labeling claims?

- Was the testing done at various dosage levels? Did testing include the dosage levels corresponding with the proposed use?

- Did the testing program determine the effective exposure range and the minimum effective dosage for the uses involved?

- Did the testing program evaluate the effectiveness of the test-substance under different application methods?

- Did the testing program consider the effects of using different application schedules and the time of application relative to the time of planting, stage of growth, and time Of harvest?

- If the test substance was formulated into a mixture and is intended to be used as such, was the efficacy of any other active ingredients also determined (from either existing data or through the testing program)?

- Were the number and type of samples taken described? Did samples allow for analysis of all the characteristics of the test population that are to be evaluated?

- Did testing procedures include the evaluation of control or reference substances? Were the results of the product tests compared against the results from similar measurements done on the control or reference substances so that product performance could be evaluated against a baseline? Were containment practices used to keep control or reference substances free from contamination with the test substance or other substances?

- If testing indicated poor or inconsistent product efficacy, were subsequent tests and statistical analysis done to further evaluate the same responses?

- Were tests done under multiple meteorological conditions (e.g., dry, rainfall, humidity, temperature, sunlight) to determine product effectiveness under climatic conditions expected to occur during product use?

- Were tests done under various soil conditions (e.g., moisture, pH, texture, fertility) and soil treatments (e.g., fertilizers, irrigation) so that effects of these variables on product efficacy could be determined?

5.4.4 INFECTIVITY/PATHOGENICITY/TOXICITY

Through examination of records and data generated by the laboratory, the auditor should confirm that the bioengineered test substance was tested for infectivity/pathogenicity/toxicity. All notebooks, worksheets, scratch sheets, notes, computer printouts, calculations, graphs and tables pertaining to the test should be considered raw data and subject to examination. If possible, the auditor should also interview the scientists, and, if appropriate, the technicians who performed the tests . The following series of questions are based on the Pesticide Testing Guidelines Subdivision M: Microbial and Biochemical Pest Control Agents (July 1989) and are only guidelines for laboratories. However, the questions should help the auditor focus on specific points or aspects of the tests under review.

- Was the test substance tested for infectivity, pathogenicity, and toxicity?
- Did the laboratory identify the test methods by name or by reference?
- How did the laboratory select the animal that was tested? Was the animal determined to be disease free?
- Was the technique used to quantify the dose described?
- Was the technique used for application, treatment or dosing described?
- Were the appropriate dose or strength, vehicle, and volume used?
- Was a control group used?
- What was the observation period for the test? How was it determined?
- How often did the laboratory examine the animals? Was the examination comprehensive (e.g., skin and fur, eyes and mucous membranes, respiratory system, circulatory system, diarrhea, lethargy, salivation, etc.)? Were the observations adequately documented?

- Was the technique used to determine the clearance of the test substance described?

- Was the technique used to enumerate the test substance in body parts (i.e., tissues, organs, and body fluids) described? What body parts were used for the analyses? Were recovery values, detection limits, and sensitivity limits determined for each technique used?

- Was a gross necropsy of all animals done?

Cell culture tests

- How was the cell culture selected? Was the cell line adequately identified?

- Was the most permissive host system used?

- Were the appropriate controls used?

- Was a cell transformation assay done?

- Were the procedures used for toxicity evaluation described?

5.5 FACILITIES AND EQUIPMENT

5.5.1 FACILITY DESIGN

In accordance with the FIFRA and TSCA GLP Regulations, the facilities used for biotechnology studies must be sufficient to enable the proper conduct of the study. The facilities must provide appropriate space, environmental conditions, containment, decontamination areas, and support systems (e.g., air, water) for the study being conducted. Facility design should take into account the Biosafety controls required for the organisms used in the study, the need for maintaining a controlled environment for the development of the bioengineered organism and should have testing facilities that adequately provide for a controlled environment and separation of test systems. The facility also needs to have adequate areas for receipt and storage of the host Organism, any stocks of plants and soils used in the study, and the bioengineered test substance, as well as facilities for waste disposal. Additionally, the facility needs to include sufficient space for archives that allow for the storage and retrieval of raw data and cell cultures by approved personnel.

The inspector needs to evaluate both the laboratory facilities and any separate facilities, such as greenhouses and field sites, that are used for testing the bioengineered test substance. This

evaluation requires a walk through inspection of all areas of the facility and the field plots so the inspector can make a direct assessment of the adequacy of the facilities. Specific elements that should be considered during the inspector's evaluation are the following:

- Are the laboratory and testing facilities appropriate for work with infectious agents or potentially infectious materials?

- Are any outdoor testing facilities, such as field sites, of sufficient design (layout, size, and location) to support the testing conditions of the study?

- Are these facilities sufficiently segregated from other testing areas to prevent any cross-contamination?

- Are indoor testing facilities designed to provide adequate separation of test systems?

- Do the facilities, particularly those used for testing, allow for adequate control of environmental conditions, such as temperature, humidity, ventilation, and lighting? For instance, does the facility have proper ventilation so that air flows from areas of low contamination to areas of higher contamination and is complete air containment and decontamination provided?

- Are environmental conditions monitored using appropriate instruments? Are these conditions as specified in the protocol for the ongoing study?

- Is an ongoing record kept of the environmental conditions, noting any deviations from those intended for the study?

- Are there appropriate and sufficient facilities for the receipt and storage of both the host organism and test substance, as well as for the other materials (e.g., soil, feed) used in the study? Are these areas separated from one another and from other areas of the facility?

- Do the storage facilities provide environmental conditions (e.g., temperature, moisture) to maintain the purity, strength, and stability of the bioengineered test substance?

- Does the facility provide the necessary containment for the appropriate biosafety level to protect persons and the environment both in and around the study facility?

- Are rooms and testing facilities designed to provide a barrier to the unintended release, particularly through the atmosphere, of any microorganisms if a spill or application accident were to occur?

- Are decontamination facilities separated from the other areas of the facility?

- Does the laboratory have procedures for collecting and disposing of contaminated plants, soils, and other

- Does the laboratory have decontamination procedures for containing or killing bioengineered organisms and host organisms?

- What types of support systems are available? For example, what is the source of water? Are water supplies sufficient? How are water conditions regulated?

5.5.2 EQUIPMENT

The types of equipment commonly used in a biotechnology laboratory will vary based not only on the types of biotechnology processes and organisms used, but also on whether the equipment is used during the development and processing of the microorganism or plant, during application of the test substance to the test system, or during testing. Types of equipment commonly used include bioreactors, air compressors, sterilization equipment, product recovery systems (e.g., centrifuges, cell disrupters), waste recovery and decontamination equipment, sampling and analysis instruments, safety equipment (e.g., biosafety cabinets, protective clothing), equipment for transporting biological materials (e.g., sealed containers), and environmental control equipment.

Since many of the questions will be similar to those asked in other laboratories submitting studies under TSCA or FIFRA, the inspector should refer to "Conducting a Field Site GLP Compliance Inspection" (SOP No. GLP-C-01, Section 5.1.2).

/s/_____ 01/04/99
Reviewed by: Daniel Myers Date
Chemist/Inspector

/s/_____ 01/04/99
Approved by: Francisca E. Liem Date
Chief, Laboratory Data Integrity Branch

/s/_____ 01/04/99
Approved by: Rick Colbert Date
Director, Agriculture and Ecosystems Division
U.S. Environmental Protection Agency
Office of Enforcement and Compliance Assurance
Office of Compliance

GLOSSARY

Bacteriophage: a virus that lives in and kills bacteria: also called "phage"

Biochemical: the product of a chemical reaction in a living organism

Chemoheterotroph: an organism that derives energy and carbon from the oxidation of preformed organic compounds

Chemolithotroph: an organism that uses carbon dioxide as its principal source of carbon for growth and obtains its energy by the oxidation of inorganic compounds

Chemoorganotroph: an organism that obtains its energy by the oxidation of organic compounds, which are also its principal sources of carbon

Dicotyledon: any plant of the class Magnoliopsida, all having two cotyledons

Dioecious: having the male and female reproductive organs on different individuals

Explant: to transfer living tissue for culture in an artificial medium

Flagellate: having flagella (relatively long, whiplike parts of certain bacteria or protozoans that provide locomotion and produce a current in the surrounding fluid)

Fermentations: an anaerobic process of growing microorganisms to produce various chemical or pharmaceutical compounds

Genotype: genetic make-up of an organism

Gram-Negative/Gram-Positive Cell: cells distinguished from each other by the composition of cell wall. Gram-negative cell has a multilayered, complex cell wall; gram-positive ¢ell has a cell wall consisting of a single layer that is often much thicker than the wall of a gram-negative cell.

Immunology: study of all phenomena related to the body's response to antigenic challenge (i.e., immunity, sensitivity, and allergy)

Lysis: rupture of a cell that results in loss of the cell's contents

Medium: a substance containing nutrients needed for cell growth

Morphology: a branch of biology that deals with the structure and of an organism at any stage of its life history

Motility: capable of or exhibiting spontaneous motion

Oxidation: a chemical reaction that increases the oxygen content of a compound

Peritrichous: of bacteria, having a uniform distribution of flagella on the body surface

Phage: see "bacteriophage"

Phenotype: observable characteristics resulting from interaction between an organism's genetic make-up and the environment

Phototropism: movement of a part of a plant toward or away from light

Physiology: the study of the basic activities that occur in cells and tissues of living organisms by using physical and chemical methods

Plasmid: a small circular form of DNA that carries certain genes and is capable of replicating independently in a host cell

Sporangium: a case or envelope in which spores are formed

Spore: a single-celled or multi-celled, asexual reproductive or resting body that is resistant to unfavorable environmental conditions and produces a new vegetative organism when the environment is favorable

Striated: marked with minute lines, bands, grooves, or channels

Transposable Elements: mobile DNA sequences that change positions on chromosomes; also called "transposable genetic elements" or simply “transposons"

Vector: the agent: (e.g., plasmid or virus) used to carry new DNA into a cell

Appendix A

Standard Operating Procedure for Conducting GLP Compliance Inspections of Biotechnology Facilities Inspection Checklist

PART I -INSPECTOR CHECKLIST (2)

PART 11-AUDITOR CHECKLIST (8)

(Suggested for use with the GLP FIFRA/TSCA compliance checklist)

Facility:_____Insp. Init.:_____Date_____

PART I - INSPECTOR CHECKLIST

General Instructions/Information

1. For any "No" answers, provide an explanation in Remarks column.
2. Remarks can be continued in the "Comments" section on the back of each page.
3. Places a line through and missing item. For example, "...name/signature".
4. Section numbers refer to the corresponding sections in the SOP.

Section 5.2 Preparation of Bioengineered Test Substance	**Yes**	**No**	**N/A**	**Remarks**
Section 5.2.1 Characterization of Donor and Recipient Microorganisms and Transgenic Plants - *Microorganism* How does the laboratory assure tat it has a pure culture? What is the frequency of this test?				
How are the organisms stored? Are storage conditions adequate to ensure the stability of the organism?				
Does the laboratory have a documented history of the bacterial strain?				
Does the laboratory have an SOP for doing the taxonomic identification?				
Does the SOP address identification of genotypic and phenotypic characteristics?				
For donor and recipient organisms from external sources (i.e., purchased or obtained from another laboratory), has the laboratory received a letter of identifications (i.e., certificate) from the supplier?				
- Does the facility test the purchased organism upon arrival to certify its identity and purity?				
- What are the shipping conditions for the organisms?				
- Does the facility have records of receipt?				

Facility:_____Insp.Init.:_____Date_____

Section 5.2.1 Characterization of Donor and Recipient **Microorganisms and Transgenic Plants - *Plant Characterization***	**Yes**	**No**	**N/A**	**Remarks**
How and where are the plants and plant cells stored?				
Is there documentation of the biology of the reproductive potential of the plant (e.g., flowers, pollination requirements and seed characteristics) being used in the study?				
Does the laboratory have a documented history of controllable reproduction with lack of dissemination? Can the plant be established in an environment that is similar to the field test site?				
Section 5.2.2 Materials and Methods for Producing the Bioengineered Test Substance - *Materials*				
If DNA is obtained from external sources (i.e., purchased or obtained from another laboratory), what are the shipping conditions? Was the DNA tested upon arrival? How is the DNA stored?				
If a restriction endonuclease (or any other enzyme) is obtained from external sources, how does the laboratory assure that the enzyme is neither contaminated nor inactive? How frequently is it tested? How is the enzyme stored?				
For other chemicals or substances used as reagents, buffers, or growth media, how does the laboratory assure the quality of the materials?				
Does the laboratory use distilled, deionized water to prepare all reagents and buffer solutions?				
Is the laboratory using standard methods for the preparation of reagents, buffer solutions, and media?				
Section 5.2.2 Materials and Methods for Producing the Bioengineered Test Substance - *Methods*				
Does the laboratory have SOPs: - For isolating and identifying DNA being inserted into the recipient organism or plant cell? - For constructing the vector with the inserted DNA? - For verifying the DNA insertion in the vector?				

Facility:_____Insp.Init.:_____Date_____

Section 5.2.2 Materials and Methods for Producing the Bioengineered Test Substance - *Methods* (continued) - For the method being used to introduce the DNA/vector into the recipient organism? (1) Transformation, transduction, conjugation for microorganisms (2) *Agrobacterium*-based plant transformation, particle acceleration, electroporation, microinjection for plants				
- For measuring the success of the insertion of the genetic material?				
- For determining the stability of the inserted DNA in the recipient microorganism or plant?				
How does the laboratory validate the methods that it uses?				
Who constructed the vector and performed the procedures in preparing the bioengineered microorganism or transgenic plant?				
If protoplasts (for transgenic plants) are being used, are standard tissue culture procedures used?				
Section 5.3 Characterization, Production and Handling of Bioengineered Test Substance	**YES**	**NO**	**N/A**	**REMARKS**
Section 5.3.2 Production of Bioengineered Test Substances - *Microorganism* Does the laboratory have SOPs for the propagation/growth of the bioengineered microorganism?				
Do they contain information such as the growth medium, physical conditions, nutrients, and expected generation time?				
Do the SOPs set out the specific equipment to be used (e.g., type of bioreactor) and the appropriate operating parameters (e.g., pH, temperature, effective mixing, viscosity, oxygen and CO_2 concentrations)?				
Are the equipment and conditions specified in the SOP appropriated for the level of containment needed for the study? For information on containment levels, the inspector should refer to the guidelines in *Biosafety in Microbiological and Biomedical Laboratories* (May 1988)				
Does the laboratory have SOPs for harvesting the bioengineered microorganisms?				

Facility:_____Insp.Init.:_____Date_____

Section 5.3.2 Production of Bioengineered Test Substance - *Microorganisms* (continued)				
Do the SOPs contain guidelines for separation and purification?				
Has the separation procedure been validated?				
Has the purification system been validated?				
How are the parameters for the separation and purification systems monitored?				
Do the systems yield a uniform product? Are tests conducted to determine uniformity?				
Does the laboratory have SOPs for waste recovery and decontamination of any wastes remaining after separation and purification?				
If the bioengineered microorganism is produced in more that one batch (i.e., consecutive batches, or concurrent batches in more than one bioreactor), what are the criteria for pooling these batches for subsequent portions of the study (i.e., application and testing)?				
Section 5.3.3 Handling	**YES**	**NO**	**N/A**	**REMARKS**
Does the laboratory have SOPs for the handling the bioengineered test substance?				
Are these SOPs adhered to and any deviations properly authorized and documented?				
How is the test substance stored?				
Do storage conditions appear to be adequate for preserving the test substance?				
How are samples/batches distributed?				
Are storage procedures designed to limit the potential for contamination or degradation of the substance?				
How is the test substance transported to the test site?				
Are appropriated containers used so as to preserve the characteristics (especially purity) of the test substance?				
Does the laboratory document the receipt and distribution of the test substance?				
Does the documentation contain complete information, including who received each sample/batch, when, and in what quantity?				
Section 5.3.3 Handling (continued)				

Are all samples/batches of the test substance properly identified throughout storage and distribution?				
Are handling conditions specific to the test substance clearly identified, such as temperature conditions or length of storage time before reversion of transformation?				
Does the laboratory retain reserve populations of microorganisms or plants?				
Section 5.5 Facilities and Equipment	YES	NO	N/A	REMARKS
Section 5.5.1 Facility Design Are the laboratory and testing facilities appropriate for work with infectious agents or potentially infectious materials?				
Are any outdoor testing facilities, such as field sites, of sufficient design (layout, size, and location) to support the testing conditions of the study?				
Are these facilities sufficiently segregated from other testing areas to prevent any cross-contamination?				
Are indoor testing facilities designed to provide adequate separation of test systems?				
Do the facilities, particularly those used for testing, allow for adequate control of environmental conditions, such as temperature, humidity, ventilation, and lighting? For instance, does the facility have proper ventilation so that air flows from areas of low contamination to areas of higher contamination and is complete air containment and decontamination provided?				
Are environmental conditions monitored using appropriate instruments? Are these conditions as specified in the protocol for the ongoing study?				
Is an ongoing record kept of the environmental conditions, noting any deviation from those intended for the study?				
Are there appropriate and sufficient facilities for the receipt and storage of both the host organism and test substance, as well as for the other materials (e.g., soil, feed) used in the study? Are these areas separated from one another and from other areas of the facility?				
Do the storage facilities provide environmental conditions (e.g., temperature, moisture) to maintain the purity, strength, and stability of the bioengineered test substance?				

Does the facility provide the necessary containment for the appropriate biosafety level to protect persons and the environment both in and around the study facility?				
Are rooms and testing facilities designed to provide a barrier to the unintended release, particularly though the atmosphere, or any microorganisms if a spill or application accident were to occur?				
Are decontamination facilities separated from the other areas of the facility?				
Does the laboratory have procedures for collecting and disposing of contaminated plants, soils and other materials?				
Does the laboratory have decontamination procedures for containing or killing bioengineered organisms and host organisms?				
What types of support systems are available? For example, what is the source of water? Are water supplies sufficient? How are water conditions regulated?				

Facility:_____Insp.Init.:_____Date_____

PART II - AUDITOR CHECKLIST

General Instructions/Information

1. For any "No" answers, provide an explanation in Remarks column.
2. Remarks can be continued in the "Comments" section on the back of each page.
3. Places a line through and missing item. For example, "...name/signature".
4. Section numbers refer to the corresponding sections in the SOP.

Section 5.3 Characterization, Production, and Handling of Bioengineered Test Substance	**Yes**	**No**	**N/A**	**Remarks**
Section 5.3.1 Characterization of Bioengineered Test Substance - ***Identity*** Does the laboratory have SOPs for identification of genotypic and phenotypic characteristics?				
Were appropriate methods and instruments used in the genotypic and phenotypic characterization of the bioengineered tests substance?				
Was DNA sequencing conducted? If so, what method was used? Did the laboratory use a commercial kit or automated sequencer?				
If gel electrophoresis was done, was the type of electrophoresis appropriate for the size of DNA fragraments? For agarose gels, was the percentage of agarose appropriate for the size of the DNA fragments to be separated? Were solutions, reagents, buffers, etc. adequately labeled (including the expiration data)? Was the person responsible for running the gels knowledgeable about the electrical parameters?				
Has the laboratory identified any unusual morphological, biochemical, or resistance characteristics that are different from the classic description of the organism? If so, have these been documented?				
Were all data available for review by the auditor?				
Does the laboratory have SOPs for identifying/determining purity of the protein?				
Are the SOPs of adequate scope and detail (if reviewed by the auditor?)				
If gel electrophoresis was done, was the type of electrophoresis appropriate for the protein size? Were solutions, reagents, buffers, etc. adequately labeled (including the expiration date)?				

Facility:_____Insp.Init.:_____Date_____

Section 5.3.1 Characterization of Bioengineered Test Substance - *Identity* (continued)				
Were appropriate methods and instrumentation used in the identity testing?				
If ion exchange chromatography was done, how was the ion exchange gel chosen? Was the pH range where the protein is stable used? Was it appropriate for the size of the protein being separated? Was the gel equilibrated? Were any solutions, reagents, buffers, etc. adequately labeled with an expiration date?				
Were all data available for review by the auditor?				
Did the analytical data from the identity tests show any contamination of the test substance?				
5.3.1 Characterization of Bioengineered Test Substance - *Purity*				
Does the laboratory assure that it has a pure culture?				
Was purity testing done on each batch of the test substance used in the study?				
Were precautions taken to prevent and control viral, bacterial, mycoplasmal, or other contamination?				
5.3.1 Characterization of Bioengineered Test Substance - *Stability*				
Did the laboratory test the stability of the test substance?				
Did the laboratory test to determine the reversion rate or rate of plasmid loss from the host cell? How was the rate determined? Did the laboratory resolve any problems related to this?				
Was testing done at a point in the study such that any stability problems were identified early enough to avoid an adverse effect on the study?				
Were all data available for review by the auditor?				

Facility:_____Insp.Init.:_____Date_____

5.4 Studies of the Bioengineered Test Substance	YES	NO	N/A	REMARKS
5.4.1 Study Protocol - *Field Tests*				
Does the protocol describe the experimental design, including methods for the control of bias? Are the objective for the field test clearly set out?				
Do the field test protocol describe test plot preparation, the spacing of plants, and the growing cycle?				
Does the protocol specify the method of application/route of administration of the test substance and the reason for its selection? Are the application/dosage level and the frequency of the application specified? Are there provision for controlling the unintended release of spreading of the test substance during application?				
Does the protocol specify sampling locations?				
Does the protocol describe the procedures for packaging and transporting samples from the field site to the laboratory for processing? Were these procedures adequate for ensuring that samples remain segregated and uniquely identified (e.g., labeling of sample containers)? Are procedure specified for sample preservation?				
Does the protocol describe how unused test substance, field test samples and materials were deposed of after analysis and/or use?				
5.5.1 Study Protocol - *Field Sites*				
Were the conditions of the field test site documented, including a description of the size (e.g., size, proximity to other test sites), its location, soil characteristics (texture, pH, cation exchange capacity), and site history (i.e., past uses)? Did the site conditions meet the requirements of the protocol? Was the historical agricultural practice for the test site documented?				
Were the test sites maintained in accordance with the specifications of the protocol (e.g., application of fertilizers/pesticides other than the test substance, tilling, weeding)?				
Before use in the study, was the test system acclimatized to the environmental conditions specified for the tests (e.g., gradual reduction in humidity from culture conditions to testing conditions)? Did the acclimatization period appear to be adequate?				
5.4.1 Study Protocol - Field Sites (continued)				

Was the field equipment (e.g., balances, application equipment, and field analytical equipment) inspected and calibrated before use? Were the precision and accuracy of the test equipment verified? Was the equipment decontaminated between sampling events?				
Do the study records contain a description of the meteorological conditions (e.g., air and soil temperatures, relative humidity, wind direction and velocity, rainfall) that existed during the study?				
Were containment measures taken to prevent contamination? Was the reproductive isolation of plants ensured? For microorganisms, was the gene transfer capability monitored?				
Were sample locations recorded with the analytical results or on a site map?				
5.4.2 Test System Care				
If the test system was a cell culture (i.e., microorganisms) and was purchased from an outside source, were the microorganisms characterized? Was a letter of identification (regarding the characterization) received from the supplier? Were records of the characterization in the study files?				
How were the microorganisms stored? Were the storage conditions appropriate to ensure stability of the microorganisms?				
Was the cell culture tested for contamination before being used in the study?				
5.4.3 Efficacy				
Were efficacy tests done to confirm support for any proposed product labeling claims?				
Was the testing done at various dosage levels? Did testing include the dosage levels corresponding with the proposed use?				
Did the testing program determine the effective exposure range and the minimum effective dosage for the uses involved?				
Did the testing program evaluate the effectiveness of the test substance under different application methods?				
Did the testing program consider the effects of using different application schedules and the time of application relative to the time of planting, stage of growth, and time of harvest?				

If the test substance was formulated into a mixture and is intended to be used as such, was the efficacy of any other active ingredients all determined (from either existing data or through the testing program)?				
Were the number and type of samples taken described? Did samples allow for analysis of all the characteristics of the test population that are to be evaluated?				
Did testing procedures include the evaluation of control or reference substance? Were the results of the product tests compared against the results from similar measurements done on the control or reference substances so that product performance could be evaluated against a baseline? Were containment practices used to keep control or reference substance free from contamination with the test substance or other substances?				
If testing indicated poor or inconsistent product efficacy, were subsequent tests and statistical analysis done to further evaluate the same responses?				
Were tests done under multiple meteorological conditions (e.g., dry, rainfall, humidity, temperature, sunlight) to determine product effectiveness under climatic conditions expected to occur during product use?				
Were test done under various soil conditions (e.g., moisture. PH. Texture, fertility) and soil treatments (e.g., fertilizers, irrigation) so that effects of these variables on product efficacy could be determined?				
5.4.4 Infectivity/Pathogenicity/Toxicity				
Was the test substance tested for infectivity, pathogenicity/toxicity?				
Did the laboratory identify the test methods by name or by reference?				
How did the laboratory select the animal that was tested? Was the animal determined to be disease free?				
Was the technique used to quantify the dose described?				
Was the technique used for application, treatment or dosing described?				
Were the appropriate dose or strength, vehicle and volume used?				
Was a control group used?				

What was the observation period for the test? How was it determined?				
5.4.4 Infectivity/Pathogenicity/Toxicity (continued)				
How often did the laboratory examine the animals? Was the examination comprehensive (e.g., skin and fur, eyes and mucous membranes, respiratory system, circulatory system, diarrhea, lethargy, salivation, etc.)? Were the observations adequately documented?				
Was the technique used to determine the clearance of the test substance described?				
Was a gross necropsy of all animals done?				
Was the technique used to enumerate the test substance in body parts, (i.e., tissues, organs, and body fluids) described? What body parts were used for the analyses? Were recovery values, detection limits, and sensitivity limits determined for each technique used?				
5.4.4 Infectivity/Pathogenicity/Toxicity ***- Cell culture test***				
How was the cell culture selected? Was the cell line adequately identified?				
Was the most permissive host system used?				
Were the appropriate controls used?				
Was a cell transformation assay done?				
Were the procedures used for toxicity evaluation described?				

for Conducting GLP Compliance Review of Biotechnology Facilities

Biotechnology Facilities Standard Operating Procedures Support Document

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5.1 ORGANIZATION AND PERSONNEL

Since the biotechnology field is particularly specialized and involves the application of many emerging technologies, individuals conducting a biotechnology study need to have specific qualifications. The education, training and experience of personnel in key positions should include a significant amount of work with bioengineered organisms or in closely related scientific disciplines, such as organic chemistry or toxicology. Standard positions found in biotechnology laboratories and facilities and the appropriate qualifications for individuals in these positions are described in the document Biotechnology Compensation and Benefits Survey, 1992, which may be obtained from the Scientific Support Branch on request. This information should assist the inspector in determining if a biotechnology study is led and staffed by adequately trained individuals.

The positions discussed above require degrees in scientific disciplines related to biotechnology. A listing of these disciplines and their definitions are provided in the Glossary of Disciplines. Chemists in a biotechnology laboratory usually have experience in one or more of the following areas: biochemistry, synthetic chemistry, organic chemistry, formulation chemistry, analytical chemistry, physical chemistry, polymer chemistry, peptide chemistry, and toxicology. Biologists in the Principal Scientist position are generally knowledgeable in one or more of the following fields: agronomy, botany, entomology, forestry, cell biology, microbiology, immunology, membrane biology, biochemistry, histology, and genetics.

5.2 PREPARATION OF BIOENGINEERED TEST SUBSTANCE

5.2.1 Taxonomic Identification

Taxonomy is the study concerning the appearance and structure of a plant or animal, its proper classification and name in the plant or animal kingdom, how it differs from other organisms, and a description of its surroundings. Traits to verify, taxonomic classification include morphological, biochemical, immunological, and physiological characteristics.

The taxonomic identification of a microorganism involves the use of standard tests to determine the individual characteristics of the microorganism which collectively lead to the exact identification. A complete taxonomic identification can be done in a stepwise fashion for an unknown bacterial culture. A summary of the steps are as follows:

1. Start with a pure, uncontaminated culture.

2. Determine the energy requirements. These are defined through isolation and culture methods that will indicate whether the organism is phototrophic, chemolithotrophic, or chemoheterotrophic.

Biotechnology Facilities Standard Operating Procedures Support Document

3. Examine living cells by phase contrast microscopy and describe the morphological characteristics (i.e., rod., coccus, vibrio, spiral, spirochete, filament, sheath, etc.) Examine Gram-stained cells by bright-field microscopy and determine whether the organism is Gram positive or negative.

4. Examine cells for spores, stalks, prosthecae, or other identifying characteristics. The examination for spores should be done carefully using different culture media to induce sporulation.

5. Examine for motility in wet mounts and determine whether the organism is polarly or peritrichously flagellated.

6. Examine colonies or mass growth for pigments or other unique characteristics.

7. Test for oxygen requirements.

8. Test the dissimilation of glucose or another simple sugar to determine whether the microorganism is oxidative or fermentative.

9. Complete any additional tests necessary based on the results of the previous tests.

The SOP used by the lab for taxonomic identification would contain some or all of these tests to confirm the type of organism being used. For example, since the laboratory should know the identity of the organism (e.g., if from external sources, through the letter of identification), then only specific tests would need to be done to verify the identification.

Characteristics of Some Commonly Used Microorganisms

Three microorganisms that are frequently used in the preparation of bioengineered microorganism or transgenic plants are Agrobacterium tumefaciens, Bacillus thuringiensis, and Escherichia coli. The common and unique qualities of these organisms are discussed below.

Agrobacterium

This genus includes organisms of similar morphology that cause tumor like growths of plant tissues in dicotyledonous plants, resulting in the called crown gall and hairy root. The disease results from infection by the bacteria that enter the plant through a wound or break in the plant's outer protective layer.

The organisms are small, short rods, motile by means of flagella arranged either peritrichously or subpolarly . *Agrobacterium* is related to Rhizobium morphologically, in its ability to infect plants, and in its genomes. *Agrobacterium* contains a plasmid called the Ti (for Tumor inducing) plasmid that contains the trait for tumor formation. ritrichously or subpolarly. The percentage of DNA base composition ranges from 58 to 63.5 G-C.

Characteristics. Non-sporing; Gram-negative; growth on carbohydrate-containing media usually accompanied by copious extracellular, polysaccharide slime; colonies non-pigmented and usually smooth, tending to become striated with age; chemoorganotrophic; aerobic, but able to grow under reduced oxygen tensions in plant tissue; optimum temperature 25-30 degrees Centigrade; optimum pH range 6.0-9.0.

Bacillus

This genus is a heterogeneous group that can be considered an assemblage of closely related organisms. The organisms are rod-shaped, that are usually motile, and possess peritrichous flagella. The percentage of DNA base composition varies from 30 to 50 G-C and studies on nucleic acid homologies by hybridization and genetic transformation also support considerable genetic heterogeneity. Members of the genus are easy to isolate from soil or air and are among the most common organisms to appear when soil samples are streaked on agar plates containing various nutrient media. Many Bacilli produce extracellular hydrolytic proteins that break down polysaccharides, nucleic acids, and lipids, permitting the organisms to use these products as carbon and energy sources. In addition, many Bacilli produce antibiotics, including bacitracin, polymyxin, tyrocidin, gramicidin, and circulin.

A number of *Bacilli* are insect pathogens. They work by forming a crystalline protein during sporulation that is deposited within the sporangium, but outside the spore. The action of this toxin (i.e., the crystalline protein) causes fatal diseases of moth larvae such as the silkworm, cabbage worm, tent caterpillar, and gypsy moth.

Characteristics. Rod-shaped 0.3-2.2 by 1.2-7.0 um; heat-resistant endospores not repressed by exposure to air; majority Gram-positive; chemoorganotrophic; metabolism strictly respiratory, strictly fermentative or both respiratory and fermentative, using various substrates; catalase formed by most species.

Escherichia

This genus almost universally inhabits the intestinal tract of humans and warm-blooded animals. The microorganisms in this group are generally non-sporulating rods, nonmotile or motile by peritrichously occurring flagella, and are facultative anaerobes.

They have relatively simple nutritional requirements and they ferment sugars to a variety of end products. The percentage of DNA base composition is about 50 G-C.

Characteristics. Straight rod-shaped cells 1.1-1.5 by 2.0-6.0 um; Gram-negative; chemoorganotrophic; metabolism respiratory and fermentative; motile by peritrichous flagella or nonmotile; colonies on nutrient agar may be smooth (S), low convex, moist, shiny surface, entire edge, gray and easily emulsified in saline solution or rough (R), dry and not well emulsified.

5.2.2 Materials and Methods for Preparing the Bioengineered Products

Materials

The materials used in biotechnology laboratories are very diverse ranging from simple neutralization buffers to the Northern hybridization solution. A listing of the various reagents and buffer solutions used in molecular biology protocols can be found in Short Protocols in Molecular Biology Second Edition - A Compendium of Methods from Current Protocols in Molecular Biology published by Greene Publishing Associates and John Wiley & Sons, 199'. For example, the technique for minipreps of plasmid DNA (as listed in this reference) requires the following materials:

- LB medium (tryptone, yeast extract, NaCI, NaOH) containing the appropriate antibiotic
- Glucose/Tris/EDTA (GTE) solution
- NaOH/SDS solution
- Potassium acetate solution, pH 4.8
- 95% and 70% ethanol.

Methods

Bioengineered Microorganisms

There are several methods that can be used to introduce DNA from one organism into another. These methods include:

- Transformation. The process in which free DNA is inserted directly into a competent recipient cell

- Transduction. The transfer of bacterial DNA from one bacterium to another through a temperate or defective virus

- Conjugation. Transfer of genetic information from one cell to another by cell-to-cell contact.

For example, one commonly used process is genetic transfer through the transformation process. In this process, recombination involves (1) the insertion of a fragment of genetically different DNA derived from a donor microorganism into a small self-replicating chromosome such as a plasmid (i.e., the vector) and (2) the introduction of the recombinant plasmid into a recipient or host microorganisms where the vector will replicate. An example is the in vitro synthesis of an E. coli plasmid containing one gene of Drosophila. The recombinant DNA (or recombinant plasmid) is transferred into a host E. coli cell where it is replicated to produce many identical copies for subsequent biochemical analyses. The various processes for construction of recombinant

DNA including transformation and transduction are summarized in the Principles of Genetics Eighth Edition by Gardner, simmons, and Snustad, 1991.

The specific molecular biology methods used in preparing and analyzing bioengineered organisms are described in Short Protocols in Molecular Biology Second Edition - A Compendium of Methods from Current Protocols in Molecular Biology published by Greene Publishing Associates and John Wiley & Sons, 1992. The methods compiled in this book cover the following areas:

- Escherichia Coli, Plasmids, and Bacteriophages
- Preparation and analysis of DNA
- Enzymatic manipulation of DNA and RNA
- Preparation and Analysis of DNA
- Construction of recombinant DNA libraries
- Screening recombinant DNA libraries
- DNA sequencing
- Mutagenesis of Cloned DNA
- Introduction of DNA into mammalian cells
- Analysis of proteins.

These are all important techniques that may be used in biotechnology laboratories. For example, DNA sequencing is an

important tool in verifying the insertion of the DNA gene into the vector, (DNA sequencing and protein analysis are discussed in more detail in Section 5.4.) The inspector is referred to the resources listed above for information if s/he finds that more detailed information is necessary for a particular inspection

Transgenic plants

There are several methods that can be used to produce transgenic plants. These methods are described below.

Agrobacterium-based Plant Transformation

Using this method, the transfer of genetic material into the plant occurs through Agrobacterium Ti plasmid-mediated transformation. The Ti plasmid which is in the cytoplasm of

the bacterium is transferred into the cytoplasm of the plant's cells in an area infected with Agrobacterium. After the transfer, the new genes overproduce a plant hormone known as cytokinin. The overproduction upsets the plant's normal metabolism and results in the formation of galls (i.e., tumors) that support the proliferation of bacteria in the plant walls.

This transformation method is done either with tissue explants or protoplasts co-cultivated with A. tumefaciens cells harboring the Ti plasmid. The easiest procedure involves co-cultivating sterile leaf discs or root sections with A. tumefaciens for a few days, and then transferring the inoculated explants into selection/regeneration medium. The selection/regeneration media contains (1) an antibiotic that kills the Agrobacterium and (2) the appropriate antibiotic (e.g., kanamycin, hygromycin) to select for the transformed plant cells.

The Ti plasmid can be used to transport other genes inserted into the plasmid DNA through recombinant DNA techniques. This technique has been successfully used in transforming dicotyledonous plants such as tobacco, tomato, potato, petunia, and sunflower. The Ti plasma mediated system has been used to produce insect resistance in tobacco using the Bacillus thuringiensis crystal protein toxin gene. One disadvantage of this method is limited range of plants to which this method can be applied.

Particle Acceleration

This method, which is also known as particle bombardment involves coating the DNA onto tiny particles that are then accelerated into intact plant cells with the intent of integrating

the DNA into targeted plant cells. An example of the method is the Use of an electric discharge apparatus to propel DNA-coated gold particles at rapidly growing soybean tissue taken from immature seeds. The transformation is stable and the foreign genes introduced into the soybean tissue are expressed in progeny plants. This system has several advantages:

- **!** It only uses the DNA that the scientist wants to incorporate into the plant
- **!** It can be used for any plant species
- **!** It is not dependent on many parts of tissue culture regeneration that are time and labor intensive.

Electroporation

This method uses a short pulse of high-intensity electric current to disrupt cell membranes and render them temporarily permeable to DNA molecules. The DNA molecules cross into the plant cell's cytoplasm and become part of the cell's genetic code. Electroporation requires the use of protoplasts which are single plant cells with their walls removed through the use of enzymes. In this method, once the cells have been prepared, hundreds of thousands of cells can be treated at the same time. This system has some disadvantages compared to the other methods:

- **!** Lengthy tissue culture manipulations are necessary and many cells are lost in the regeneration steps
- **!** In many cases, the transformation is short-lasting (i.e., the gene alteration only persists for a short time).

Microinjection

This method involves the direct physical injection of DNA into the cytoplasm or nucleus of the target cell using a special micromanipulator and fine glass micropipettes. This process must be carried out under a microscope and involves the use of protoplasts. This method requires considerable expertise and is labor intensive, since the DNA must be injected into each plant. cell individually. In addition, it involves considerable effort in tissue culture manipulations.

5.3 CHARACTERIZATION, PRODUCTION, AND HANDLING OF BIOENGINEERED MICROORGANISMS

5.3.1 CHARACTERIZATION OF BIOENGINEERED ORGANISM

Methods

Techniques commonly used in characterizing the bioengineered microorganism (DNA sequencing) and the test substance protein (protein analysis) are described in the next sections.

DNA Sequencing

Genotypic characterization of bioengineered genetic material may require DNA sequencing. Most DNA sequencing techniques are based on polyacrylamide gel electrophoresis, which allows the resolution of oligonucleotide that differ in length by a single base. This level of resolution may be obtained between oligonucleotide of up to 300 to 500 bases.

Two widely used DNA sequencing techniques are dideoxy (Sanger) sequencing and chemical sequencing. These differ primarily in the mechanism used to generate a "ladder" of oligonucleotide of varying length for use on the gel.

Dideoxy sequencing uses a DNA polymerase enzyme to synthesize a complementary copy of a single strand of the DNA template being sequenced. A short oligonucleotide is attached to the DNA template to serve as a primer for annealing the synthesized DNA to the template. The oligonucleotide is then labeled with a radioactively-labeled base. At this stage, one protocol calls for a round of DNA synthesis before sequencing (the labeling-termination protocol) while the other protocol does not (the Sanger protocol). The Sanger protocol is most reliable for short oligonucleotide and the first few bases of longer oligonucleotide. The labeling-termination protocol is best for generating sequence information for longer oligonucleotide. At this point, the reaction mixture is divided into four aliquots, one each for the A, G, T, and C reactions. Each

aliquot receives a mixture of the bases and a single type of dideoxy base (e.g., dATP). The DNA polymerase synthesizes a complement until it is halted by the addition of the dideoxy base. In this way, synthesized oligonucleotide of varying length are produced, each ending with a single type of base. Each reaction mixture is loaded on a separate lane on the polyacrylamide gel. Electrophoresis then resolves the various complementary oligonucleotide based on length. The DNA sequence can then be determined by finding the lane in which each successively longer oligonucleotide appears.

Dideoxy sequencing can be done with a number of radiolabels. If radiolabeled dATP is used in the reaction mixture, ^{35}S or ^{33}P may

be substituted for the standard ^{32}P label. ^{35}S has the advantages of sharper autoradiographic bands, lower radiation energy, and longer storage life of labeled DNA. ^{33}P combines some of the advantages of each radiolabel. Alternatively, labeling may be done through a 5'-end-labeled primer. The label may be ^{32}P or ^{35}S. This approach has been found particularly effective with large, double-stranded DNA templates.

Unlike dideoxy sequencing, which is achieved through DNA synthesis, chemical sequencing (or Maxam-Gilbert) is achieved through cleavage of the oligonucleotide being studied. Four aliquots of a 3'- or S'-end-labeled oligonucleotide are subjected to four separate chemical reagents that cleave the DNA at one or two specific bases. The four reaction mixtures are loaded and resolved via electrophoresis in the same manner as the dideoxy sequencing technique.

Dideoxy sequencing is more rapid than chemical sequencing and can achieve excellent electrophoresis band resolution. Chemical sequencing eliminates problems that DNA polymerases encounter with certain DNA compositions and structures. Chemical sequencing is best for small oligonucleotide.

Other related techniques include: substituting chemiluminescent detection for autoradiographic detection, multiplex sequencing through the use of probes specialized for a particular sequence (such as an insertion sequence, transposon, or possible repeat sequence), automated sequencers, and thermal cycle sequencing (involving repeated rounds of denaturation, annealing, and synthesis to generate a sequencing ladder).

Protein Analysis

Protein identity may be determined through a number of analytical techniques. Among the useful techniques are amino acid composition analysis, partial sequence analysis, peptide mapping, polyacrylamide gd electrophoresis (PAGE), isoelectric focusing, and high performance liquid chromatography (HPLC).

Amino acid composition analysis involves the determination of the number of each amino acid present in a peptide. This type of analysis provides no information as to the sequence of amino acids in the peptide. A standard procedure is to hydrolyze the peptide into its constituent amino acids. The amino acids may then be separated by ion-exchange chromatography or HPLC.

Reaction of the amino acids with a compound such as ninhydrin, fluorescamine, or orthophthalaldehyde yields a colored or fluorescent derivative that may be quantified via a spectrophotometer.

The standard method for sequence analysis of peptides is the Edman degradation. This method uses sequential drivitization and identification to determine the sequence of amino acids in the peptide. Phenyl isothiocyanate derivitized the amino terminal of the peptide. The derivitized amino acid is then liberated, leaving a shortened peptide. The derivitized amino acid may be identified chromatographically; alternatively, the shortened peptide may be analyzed for amino acid composition, revealing the identity of the removed amino acid by comparison to prior composition. The entire procedure is then repeated on the shortened peptide. Several rounds of Edman degradation will elucidate the sequence of the entire peptide.

Since sequence analysis may be difficult on entire proteins or lengthy peptides, peptide mapping may be necessary. Cleaving the protein into smaller peptides allows sequencing of the shorter segments. The next step is to construct a map showing how the shorter peptides fit together to form the protein. Cleavage is achieved at specific sites in the protein by chemical or enzymatic methods. The resulting peptides are separated chromatographically. To determine the order of peptide segments, a different chemical or enzyme is used on the protein to create overlap peptides. These peptides are cleaved at different sites, and their sequences can be used to establish the order of the first set of peptides.

Polyacrylamide gel electrophoresis may be used to confirm the identity of a protein. The protein may be isolated through this technique and then compared to a standard protein. Several approaches are available to achieve this. One-dimensional gel electrophoresis simply separates proteins based on their mobility through the voltage applied across the gel. Gradient electrophoresis adds the additional separator of a gradient of increasing concentration of polyacrylamide in the gel, causing larger molecules to move slower than small ones. Isoelectricfocusing is based on the fact that proteins contain both positively and negatively charged functional groups. since a protein's charge depends on the pH, a protein moving across a gel that has a pH gradient will cease moving when it reaches the pH where it is uncharged.

Proteins may also be identified through High Performance Liquid Chromatography. The retention time are elution volume of the unknown protein may be compared to that of a known standard, thus identifying the unknown. Reversed-phase, ion-exchange, size-exclusion, or hydrophobic-interaction chromatography columns may be used to separate proteins, depending on the type of proteins to be analyzed.

5.3.2 PRODUCTION OF BIOENGINEERED ORGANISMS

Bioengineered Microorganisms

Propagation/Growth of the Organism

Growth of microorganisms occurs in two ways: by the growth of individual cells in the absence of cell division, which basically is an increase in the size and weight of the individual cell, and by the growth of a population of cells, which is an increase in the number of cells as a result of cell growth and division. since microorganisms are so small and the products they generate (e.g., proteins) are generally produced in very small quantities, studies involving these substances are performed using populations of cells. The Master Cell Bank (MCB) is a collection of the cells of uniform composition stored in aliquots under defined conditions, from which all the subsequent cell banks are made. In many cases, a single host cell containing the expression vector is cloned to give rise to the MCB.

To achieve the desired population of organisms for the study (either to be used themselves or to be used to produce the test substance if it is a protein), a suitable medium for growth is necessary. The medium for growth is chosen based on the nutrients it provides, the generation time achieved (i.e., the time it takes for the population to double), and the controls for purity and uniformity of product. The culture medium must be properly prepared and sterilized, and conditions during growth need to be carefully monitored and controlled.

Bioprocess technology is used to propagate the microorganisms to produce the desired populations for the study. The term fermentation is often used interchangeably with bioprocessing, although fermentation is actually the subset of bioprocessing technology. that is conducted without oxygen. Such anaerobic bioprocessing uses yeasts and bacteria to yield products in the absence of oxygen. Aerobic bioprocessing requires oxygen and bacteria to produce the desired products which are usually more complex than those produced by anaerobic bioprocessing. The results of bioprocessing are an increase in the size of the organism population, the generation of proteins (including enzymes), and the production of secondary products such as ethanol and methane.

The first step in bioprocess technology or fermentation is to prepare a seed culture so that the organism can be propagated. To increase the volume of the seed culture so that a sufficient cell mass is achieved, the seed culture is incubated and transferred to successively larger containers in a sterile manner. Parameters such

as pH, oxygen, and the level of nutrients in the growth medium should be monitored to ensure successful growth. The seed culture is grown in this manner until a sufficient population is produced for introduction to a bioreactor.

A bioreactor provides a durable, controlled, aseptic environment for growth of the bioengineered microorganism. In order to support such growth, a bioreactor must contain sufficient nutrients and oxygen to fulfill the metabolic needs of the microorganism. A substrate serves as the growth medium for the bioengineered microorganism in the bioreactor. Substrates contain carbohydrates in the form of sugars and refined feedstocks, starches, or cellulosic materials. Substrates are pretreated and sterilized before entry into the bioreactor and prior to inoculation with a bioengineered microorganism. Sterilization of the substrate can be accomplished in several ways, including batch sterilization and continuous sterilization. Continuous sterilization can consist of heat treatment, filtration, irradiation, or chemical sterilization. To meet the oxygen needs of the bioengineered microorganism, sterilized air is supplied to the bioreactor. The air

supply is filtered to remove other microorganisms and contaminants. If high volumes or concentrations of air are necessary, such as for acetic acid formation, the air supply is also compressed before entry into the bioreactor.

The laboratory should have specific protocol addressing the type of bioreactor used [e.g., mechanical or non\mechanical (which both provide agitation), plug flow (which may provide agitation), and immobilization], the operating parameters (e.g., pH, temperature, mixing, oxygen concentration), and nutrients to achieve a desired generation time for microorganism growth. Operating parameters should be carefully monitored and controlled since changes in temperature, pH, pressure, agitation, oxygen and CO2 concentration, moisture, liquid flow rate, exhaust gases, foam, and nutrients all impact the growth of the microorganism.

Growth of microorganisms occurs in four phases, identified as the lag phase, exponential phase, stationary phase, and the death phase. Upon inoculation of the growth medium, a period of time, the lag phase, passes before growth begins. The length of the lag phase depends on the growth history of the inoculum, the composition of the medium, and the size of the inoculum. A period of exponential growth follows the lag phase. During the exponential phase, the number resulting in no net growth. During the stationary phase, growth has slowed due to the exhaustion of an essential nutrient in the medium and/or accumulation of a toxic metabolic product. The

stationary phase is eventually followed by the death phase at which point cell lysis or another cause of loss of cell viability surpasses growth.

Separation and Purification Techniques

Once the desired amount of product is generated (either a substance produced by the organisms, such as a protein, or the organisms themselves), harvesting is done and the product is recovered and purified. If the desired product is a protein or other constituent of the microorganism, the organisms may be killed by heat, chemicals, or mechanical disruption. Processing may also involve solid-liquid separation, centrifugation, filtration, cell disruption, precipitation, liquid-liquid extraction, or chromatography to concentrate or purify the desired products and freezing or drying to facilitate handling and storage. [f the microorganisms themselves are the desired product, filtration and centrifugation are generally used to separate and concentrate the organisms. Cell disruption is avoided as it negatively impacts microbial viability. Brief descriptions of each of these techniques are included below. Several of these techniques may be used in succession to achieve product recovery.

- ***Solid-liquid Separation***. This technique may be used at several points during product recovery, including to separate the biological material from the broth in the bioreactor and to remove the cell debris after cell disruption. Separation may be accomplished using chemical, physical, or biological methods. Methods include coagulation, flocculation, and pH adjustment (chemical); heating, freezing and thawing in succession, and freezing and stirring (physical); and the use of enzymes to achieve aggregation, and cell aging or gene insertion to increase floccillation (biological).

- ***Liquid-liquid extraction***. Use of this technique is generally limited to the purification of intracellular and extracellular products. The method consists of selective removal of the desired substance from a liquid mixture through the addition of a solvent (e.g.. benzene, methyl ethyl ketone) in which the desired product is soluble. The mixture is then agitated, the solvent/product portion is removed, and the solvent is separated from the product through precipitation, solvent evaporation, or steam stripping.

- ***Cell disruption***. In some cases, the desired product is intracellular (i.e., it is produced within the bioengineered microorganism and is not subsequently excreted into the surrounding environment). To recover the intracellular material, the cell walls must be disintegrated by physical, chemical, or enzymatic methods to release the material. Cell disruption can be achieved through both mechanical (e.g., high speed bead/ball mills. high pressure,

homogenizers) and non-mechanical (e.g., enzymatic lysis, chemical lysis, heat treatment) methods; Cell disruption techniques are generally conducted at temperature below 15°F (-9°C) to prevent destruction of the desired products.

- ***Precipitation***. Precipitation is often used as an early purification step for proteins. Precipitation converts a soluble protein to an insoluble form, through techniques such as pH variation and salting-out. Although precipitation is not as effective as many other purification methods, it has continued to be used after the development of other more effective techniques because precipitation methods can handle large quantities of crude material in continuous operation.

- ***Centrifugation***. This solid-liquid separation method is generally used to isolate viable cells from the bioreactor broth. Intracellular and extracellular products (e.g., proteins) are usually not heavy enough to be separated from liquid broth through centrifugation. Separation by centrifugation is relatively quick and free of operational problems compared
to filtration methods, but can lead to cell denaturation and the production of aerosols that must then be contained.

- ***Filtration***. Filtration can be used to segregate microorganisms and intracellular and extracellular products from bioreactor broth. There are two general types of filtration systems, membrane filtration and open air filtration systems that use plate and frame presses and rotary drum filters. Rotary drum filters (either vacuum or pressure-operated) provide the advantage of continuous operation, but since they are open-air systems they are only useful when containment requirements are not strict. Membrane filtration relies on the use of a porous membrane to filter media of different sizes and is also referred to as microfiltration or ultrafiltration depending on the size of the pores. Membrane filtration can be used for cell harvesting, removal of cell debris, and concentration of intracellular and extracellular products.

- ***Chromatography***. This method can be used to separate low concentrations of intracellular and extracellular products, generally proteins. Types of chromatography used are affinity, hydrophobic, ion-exchange, and gel filtration. ton-exchange is often used for protein purification and affinity for biologically active substances. All types of chromatography rely on physicochemical interactions between the dissolved components of a mixture and a stationary phase (a solid or a liquid supported on a solid that is contained in a packed column).

After product recovery, freezing and drying are used for preservation of the product during storage and for ease of transportation and handling. Freezing is generally preferred since it does not encourage the formation of aerosols that must then be contained. To prevent ice crystals from destroying cells during thawing, either glycerol is added when viable cells are frozen or ice crystals are sublimed during slight warming without thawing. Spray dryers and drum dryers are used to remove water from the product and operate at temperatures low enough to avoid product damage. Freeze drying and vacuum-tray drying are often used for small volumes of materials that are in liquid or paste form.

Plants

The propagation/growth of transgenic plants and bloengineered microorganisms are similar since in both cases the desired result may be production of the organism itself or the generation of compounds of interest produced by the organism. However, if the test substance is the transgenic plant itself, production of the test substance involves regeneration of the whole plant, as opposed to production of single-cell microorganisms. Compounds of interest may be produced by a cell culture similar to that used for microorganisms, or may be produced by regenerated cell tissue. The regeneration of cell tissue involves some of the complexities. since plant regeneration requires extensive growth and subsequent tissue variation, it is more complex than microorganism production.

Propagation and Harvesting of Test Substance from Plant Cells

lf the desired product is a compound of interest (e.g., a protein) generated by bioengineered plant cells, the techniques used to attain the product are similar to those employed with microorganisms. For propagation/growth of the plant cells, various types of bioreactors have proven effective. The most commonly used bioreactor is the conventional stirred tank reactor, which is a type of mechanical bioreactor. Bioreactor technology has been successfully applied for growing large volumes of plant cells. Growth can be achieved using either a single-stage or two-stage process. The single stage (or first stage of the two-stage process) is used for the production of biomass. In the second stage of the two stage process, growth conditions are set to encourage generation of the desired product. The single stage process is applicable to the production of biomass, primary metabolites, or some enzymes. The two stage process is generally used when generation of the desired product results from tissue or organ development rather than from cell growth and division.

Another technique used to generate desired products from plant cell growth is immobilized cell technology. With immobilized cell systems, plant cells are entrapped within a gel matrix. The technology

immobilized plant cells continue to generate products while spent matrix is removed for product extraction. However, because of difficulties in achieving release of the product into the medium, low productivity, and genetic instability, immobilized cell technology has not been used for commercial applications. A third technique for generating desired products from plant cells 15 the growth of regenerated plant tissue. ' This growth may be in the form of plant callus, hairy roots, or other types of tissue. All or parts of the tissue are then harvested for extraction of the product. In general, recovery of the desired product usually involves partial harvesting of a continuously growing culture, followed by water removal and concentration. After concentration is achieved, absorption and partition/extraction are generally used to complete product recovery. Product recovery &m regenerated plant tissue encounters some of the problems associated with regeneration of whole plants (discussed below) in that a small amount of the desired product is contained in a large amount of biomass or water. However, product recovery from plant cell biomass is less difficult than recovery from whole plant biomass since rigorous treatments to break down tissues and woody, waxy structures are not needed.

Regeneration of Whole Plants

In many cases, the desired products are plants regenerated from bioengineered plant cells. New plants develop in two ways: embryogenesis and organogenesis. Organogenesis involves the successive formation of shoots and then roots from plant cell and tissue cultures. In embryogenesis, shoot production and root formation occur simultaneously in a coordinated manner. Both methods can bring about plantlet formation.

Organogenesis can be used to generate whole plants from roots or shoots through clonal propagation. A cell mass or callus can be induced to undergo organogenesis through alteration of the ratio of two hormones, auxin and cytokinin. A high auxin/cytokinin ratio induces production of roots, while a low ratio induces production of shoots. Within two to three weeks, transformed callus tissue grows on the cut surfaces of leaf disks or root sections and begins to differentiate into shoots. The shoots are excised and placed into root-inducing medium. After root formation, the roots or shoots can be transferred to growth media (e.g., soil) for regeneration of whole plants. The resulting transgenic plant is usually obtained within five to six weeks of the co-cultivating process.

Somatic embryogenesis involves replicating the process of reproductive embryogenesis by using media to cause somatic tissue to acquire totipotency, or the ability to regenerate an entire plant. Generally, the proliferation of proembryogenic masses (a stage leading to embryo formation) can be induced by the presence of auxin. A culture of recurrent embryogenesis may be produced, where embryos do not mature but instead produce successive cycles of embryos, through the use of a very high initial auxin concentration followed by maintenance on low levels of auxin. Active embryos may be obtained by placing these

cultures on an auxin-free medium. Growth is then induced in a sucrose culture. Desiccation of the plantlets before growth may be necessary to create vigorous plants after growth. Following this growth period, plantlets are acclimatized to climatic conditions by slow, progressive reduction in humidity from their water-saturated cultures.

Waste Recovery and Decontamination Techniques

Bioengineered Organisms

Wastes generated during the production of the bioengineered test substance must be decontaminated before disposal. These wastes include contaminated air and gases, as well as solid and liquid wastes. Air and gaseous waste streams are generally treated through one of the following methods:

- Filtration. Filtration is accomplished through either high efficiency particulate air (HEPA) filters or membrane filters used in series to decontaminate vent or exhaust gases.

- Incineration. Incineration may be used independently or as a supplement to filtration and is generally used for small volume gas streams. Automatic safety devices should be used with incinerators to protect against problems resulting from power failures and overheating.

- Irradiation. Irradiation involves exposing the waste materials to x-rays, ultraviolet rays, or other ionizing radiation to decontaminate them.

For liquid wastes, treatment is either chemical or thermal. When liquid wastes are of limited volume, chemical treatment is often used. However, since proteins present in liquid wastes can deactivate the sterilant used in chemical treatment, thermal sterilization may be more appropriate for wastes involving bioengineered microorganisms. Solid wastes are usually sterilized by autoclaving and may then be incinerated.

5.4 STUDIES OF THE BIOENGINEERED TEST SUBSTANCE

Tests with microorganisms generally involve large populations, rather than a single organism. Therefore, during field tests, the bioengineered microorganisms will come in contact with one another and with microorganisms from the surroundings. The test system design should incorporate containment measures to prevent contamination and horizontal gene transfer, but complete containment cannot always be attained. Certain characteristics of the microorganism (e.g., dispersal, survival, and multiplication; interactions with other microorganisms; and potential for gene transfer), test site characteristics (e.g., meteorological conditions, physical layout), and experimental practices used at the test site will all impact containment of the microorganism.

Plants

Plants used in biotechnology field studies are generally domesticated, can be reproductively isolated, and are not likely to persist in a non-cultivated environment. Containment is affected by:

- Certain characteristics of the plant [e.g., the biology of the reproductive potential of the plant, history of controllable reproduction of the plant
- Nature of biological vectors used in transferring DNA to the plants (if this application technique is used)]
- Test site characteristics (e.g., meteorological conditions, physical layout-proximity of plants to one another) and
- Experimental practices used at the test site.

Vector Considerations

When biological vectors are used to create transgenic plants, measures should be taken to ensure that the vector does not remain capable of acting as an infectious agent. The vector should either become biologically inactive or be eliminated from the transgenic plant. If the vector does not naturally become biologically inactive, it is necessary to either eliminate the vector from the plant or inactivate it after the transformation of the plant has occurred. To assist with this process, the DNA used in developing the transgenic plant should possess the following characteristics:

- thoroughly characterized and unlikely to be transmitted after entering the plant
- Its donor plant should be of the same or a closely related species as the recipient plant
- Transferred from non-pathogenic prokaryotes or non-pathogenic lower eukaryotic plants
- Come from plant pathogens only if the DNA sequence for production of disease or damage in plants has been removed.

Field site Considerations

Field experiments should be designed so that plants are reproductively isolated from sexually compatible plants outside the test site, bioengineered microorganisms are not released into the environment outside the test site, and the plants used will not cause unintended, uncontrolled adverse effects.

Some current experimental practices that are used to maintain reproductive isolation in plants are the following:

- **!** Spatial Separation. The distance the field should be from any field containing plants of the same species (i.e., sexually compatible plant populations) depends on the biology of the plant species involved. Self-pollinated species with fragile pollen do not need to be widely-separated to achieve reproductive isolation. However, open pollinated species that have hardy pollen may need to separated by more than several miles to achieve reproductive isolation.
- **!** Removal of Reproductive structures. For some dioecious plants, removing the male or female reproductive structures may preserve reproductive isolation in plants grown in close proximity to compatible plants. For example, mechanical detasseling in seed corn production removes the pollen-producing male flowers and prevents genetic transfer.
- **!** Use of Cytoplasmic Male Sterility Trait. By incorporating this trait into a plant, production of viable pollen is prevented and the plant is biologically and reproductively isolated.

Examples of Methods for Reducing Population Levels of Microorganisms in the Environment[4]

Habitat	Immediate[1]	Short-term[2]	Long-term[3]
Free-Living	Fumigation Flooding Chemicals	Fumigation Flooding Chemicals Erosion Control Soil Amendments	Fumigation Flooding Erosion Control Soil Amendments
Plants	Burning (Eradication) Quarantine Tillage Chemicals Biological Control Irrigation/flooding Insect vector control Machinery sanitation Run-off water control Solarization (cover with plastic	Breeding for resistance Biological control Quarantine Chemicals Crop rotation Cultivar rotation Irrigation/flooding Heat treatment Soil solarization Induced resistance Meristem/tissue culture Insect vector control Weed/natural host control Erosion control	Breeding for resistance Biological Control Crop rotation Cultivar rotation Soil amendments Weed/natural host control Erosion control

1 Treatment effective within hours to several days.
2 Treatment effective within weeks to 3 years.
3 Treatment effective after more than 3 years.
4 Table is from *Good Developmental Practices for Small Scale Field Research with Genetically-modified Plants and Micro-organisms,* Organization for Economic Co-operation and Development, March 1990, as taken from the Annual Review of Phytopathology. 27:551-581.

- ***Temporal Isolation***. By growing the plants at a time that will produce flowers either earlier or later than those for compatible plants in close proximity, genetic transfer and pollen dissemination is limited or eliminated. Dispersion of pollen can also be eliminated by placing bags or other physical barriers over plant flowers.

- ***Early Harvesting***. If seed is not required for the field test, plants might be harvested before flowering. This method can achieve reproductive isolation in plants that are difficult to isolate (e.g., plants that are insect-pollinated).

Application Issues

The equipment used in applying the test substance for the study should be similar to equipment that would be used in real world applications so that effects related to application can be simulated. Methods of application include aerial, irrigation system, directed sprays, and subsurface soil application. Some specific considerations for each application method are discussed below:

- *Aerial Application*. Many variables can affect the efficacy of a test substance or increase damage to crops/plants in the target area when aerial application is used. These variables include dosage, spray volume, maximum height from nozzles to target, type of aircraft (fixed wing or helicopter)~ ground speed, nozzle type and pressure, wind velocity and direction, and relative humidity. Therefore, field efficacy and crop/plant phytotoxicity data should be generated to support claims to be made on the proposed label.

- *Irrigation System Application*. When an irrigation system is used, data on efficacy and crop/plant phytotoxicity, yield, and quality should be developed for multiple plots within a treated field so that pesticide distribution can be evaluated. In order to make such evaluations, data taken for the individual plots should be reported separately rather than averaged together. Data should also include soil texture, percent soil organic matter, relative soil moisture condition at application, acre-inches of water applied, and precipitation quantities in the week after application. If an overhead sprinkler irrigation system is used, test plots should be situated at several nozzle positions, at the extreme ends of the spray area, and in areas where sprinkler patterns overlap. If a surface irrigation system (flood and furrow) is used, test plots should be located both where the treated water enters the field and at the lower end of the field.

- *Directed Sprays*. In a directed spray system, sprays are directed toward or away from certain sections of the test plot. When this type of application method is used, the study

should include an indication of nozzle arrangements and orientations, and the extent of spray contact with soil or plants.

- ***Subsurfase Soil Application***. Subsurface soil application involves the application of the test substance directly beneath the soil surface. If this application is used, study data should include detailed information on equipment. For example, for injection equipment, the nozzle spacing, depth of operation, nozzle pressure, speed of operation, and volume of liquid or gas applied per unit area should be recorded.

Analysis of Study Control Plots

To make efficacy determinations, the results derived from test plots treated with the test substance should be compared with the data obtained from evaluating untreated control plots/plants. The control plot(s) should receive the same treatments as the test plots, except for application of the test substance. Similar treatments should include equal application of pesticides (other than the test substance), irrigation, tilling, and meteorological conditions. The data collected and analysis for both the test plots and the control plots should be the same. If the test substance is a herbicide, both test and control plots should be evaluated for weed species present, density of weed populations, and weed vigor.

5.5 FACILITIES AND EQUIPMENT

5.5.1 FACILITY DESIGN

The design of a facility that handles infectious agents provides secondary containment to protect persons and the environment outside the laboratory from exposure to infectious materials. There are three types of laboratory designs that provide different levels of containment. The type of laboratory that is appropriate for a particular biotechnology study is generally determined by the nature of the study and the degree of biosafety necessary. The inspector should review the study protocol to determine what level of biosafety and type of laboratory were intended for the study. To assess the appropriateness of these elements, the inspector should use the information below (developed from the CDC-NIH guidelines) and further refer to the information supporting the guidelines in Biosafety in Microbiological and Biomedical Laboratories (May 1988). The three types of laboratories are described below:

- Basic Laboratory. These facilities are generally appropriate for Biosafety Levels I and 2 (described below). Basic labs are also used for biotechnology studies where there is a minimal level of hazard and study personnel can achieve sufficient protection from

the implementation of standard laboratory practices. The organisms used in the study are not associated with disease in healthy adults.

- **!** Containment Laboratory. Containment labs qualify as Biosafety Level 3 facilities. These facilities are designed with protective features to allow for the handling of hazardous materials in a way that prevents harm to the study personnel and the surrounding persons and environment. Containment labs may be freestanding buildings or segregated portions of larger buildings, as long as the laboratory is separated from public areas by a controlled access zone. Containment labs also have a specialized ventilation system to regulate air flow.

- **!** Maximum Containment Laboratory. These laboratories are Biosafety Level 4 facilities. Maximum containment labs are designed to provide a safe environment for carrying out studies involving infectious agents that pose an extreme hazard to laboratory personnel or may caus¢,serious epidemic disease. These facilities have secondary barriers, including sealed openings into the lab, air locks, a double door autoclave,- a separate ventilation* system, a biowaste treatment system, and a room for clothing change and showers that adjoins the lab. Maximum containment labs are usually located in independent buildings, but may also be in a separate, isolated portion of a larger building.

The four bio-safety levels referred to in the above laboratory descriptions consist of three elements: laboratory practices and techniques, safety equipment, and laboratory facilities. The first two elements are considered primary containment since they provide protection within the laboratory to personnel and the immediate environment. The third element, the design of the facility itself, is considered secondary containment since it gives protection to persons and the environment outside of the facility. Important characteristics of each of the biosafety levels are summarized below. Unless superseded by a more stringent criterion, the characteristics of a biosafety level apply to all successive levels (from I to 4). These characteristics, along with the training and experience of the study personnel and any special conditions in the operation of the laboratory or the organisms involved, should be considered in determining an appropriate biosafety level. For more detailed descriptions of the biosafety levels, the inspector is referred to the CDC-NIH guidelines, which can be obtained from the Scientific Support Branch on request.

- **!** Bio-safety Level 1. The organisms involved in the study are defined and characterized strains of microorganisms that are of minimal hazard and are not known to cause disease in healthy human adults. Although access to the

lab may be restricted by the lab director, the facility is generally not closed off from the rest of the building. Most work is conducted on open bench tops and special containment equipment is usually not needed. The lab is designed to facilitate cleaning, with space between equipment and cabinets and bench tops that are impervious to water and resistant to solutions. Each laboratory has a hand washing sink and fly screens for any windows that open. Lab personnel should be knowledgeable in lab procedures and supervised by a scientist trained in microbiology or a related science. Decontamination of work surfaces should be done daily and after spills, and all contaminated wastes should be decontaminated before disposal. Personal safety equipment, such as lab coats or uniforms, should be worn and hands washed before and after handling viable materials. Procedures are performed in a manner that limits the creation of aerosols. Any contaminated materials that will be decontaminated at another location are transported in a durable leak proof container that is sealed before removal from the lab.

! Bio-safety Level 2. Work done under Biosafety Level 2 involves organisms of moderate potential hazard. Many of the characteristics of this level are the same as those for Bio-safety Level 1; the differences and additional guidelines are indicated here. For Bio-safety Level 2, laboratory access is limited while work is being conducted and only persons informed of the potential hazards of the lab environment and meeting any other entry restrictions developed by the lab director are allowed entry. Biological safety cabinets (Class I or II) ,are used for containment when procedures with a high potential for treating infectious aerosols (e. g., centrifuging, blending) are conducted and when h I e h concentrations or large volumes of infectious agents are used. (The inspector is referred to Appendix A of the CDC-NIH guidance for a description of bio-safety cabinet classifications.) Lab personnel are trained in handling pathogenic agents and are under the direction of skilled scientists. An autoclave is available for Use in decontaminating infectious wastes. Before leaving the lab, personnel either remove any protective clothing and leave it in the lab or cover it with a clean coat. Skin contamination with infectious materials is avoided and gloves worn when such contact is unavoidable. Spills and accidents causing overt exposure to infectious materials are reported promptly to the lab director/Safety Officer and appropriate treatment provided and records of the incident maintained. If warranted by the organisms at Use in the lab, baseline serum samples for all at-risk personnel are collected and stored. A bio-safety manual

is developed and personnel are required to be familiar with it and to follow its instructions.

- Bio-safety Level 3. Work done under Biosafety Level 3 conditions generally occurs in clinical, diagnostic, teaching, research or production facilities and involves organisms that may cause serious or potentially lethal disease following exposure through inhalation. The lab is segregated from general access areas of the building and two sets of self closing doors must be passed through to enter the lab from access hallways. Lab access is limited to persons who must be present for program or support functions and lab doors remain closed during experiments. All work with infectious materials is conducted in a bio-safety cabinet (Class I, II or III) or other physical containment device or by personnel wearing the necessary personal protection clothing. Upon completing work with infectious materials, all work surfaces are decontaminated. Protective clothing is worn in the laboratory and is removed before exiting the facility. All such clothing is decontaminated before laundering. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps. The HEPA-filtered exhaust air from Class I or II bio-safety cabinets is discharged directly to the outside or through the building exhaust system, but may be recirculated within the lab if the cabinet is appropriately certified and tested. A ducted exhaust air ventilation system that draws air into the lab through the entry areas is in place. Walls, ceilings, and floors are water resistant to facilitate cleaning and windows are closed and sealed. The lab sinks are operable by foot, elbow or automation and are located near the exit of each lab room.

- Bio-safety Level 4. This safety level is necessary for work with organisms that present a high individual risk of life-threatening disease. These facilities are located in a separate building or in a completely segregated, controlled area of a building. Access to the facility is controlled by the use of locked doors and all personnel entering must sign a logbook. All personnel must enter and leave the facility through the clothing change and shower rooms and must shower before exiting. Any supplies or materials that do not enter through the shower and change rooms must enter through a double-door autoclave, fumigation chamber, or airlock that is decontaminated between each use. All organisms classified as Biosafety Level 4 are handled in Class III bio-safety cabinets or in Class I or II bio-safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system. All biological

materials removed from a Class III cabinet or maximum containment lab in a viable condition are placed in an nonbreakable, sealed primary container and then enclosed in a secondary container that is removed through a disinfectant dunk tank, fumigation chamber, or an airlock. All other materials must be autoclaved or decontaminated before removal from the facility. Walls, floors, and ceilings of the facility together form a sealed internal shell and any windows are resistant to breakage. A facility is available for the quarantine isolation. and treatment of personnel with potential or known lab-related illnesses.

5.5.2 EQUIPMENT

Studies involving bioengineered microorganisms will use many types of equipment and instruments in the development, application, testing, transportation, and decontamination procedures that are part of a biotechnology study. While some of this equipment is common to other types of scientific studies, the specialization of biotechnology necessitates the use of specific equipment. A description of many of the special equipment and instruments is included below to provide the inspector with the additional expertise necessary to conduct a thorough biotechnology inspection.

! Biosafety Cabinets. These cabinets are a commonly used primary containment device for work involving infectious organisms. Their primary function is to protect the lab worker and the immediate environment by containing any infectious aerosols produced during manipulation of organisms within the cabinet. Biosafety cabinets are classified into three types (I, II and III) based on their performance characteristics. Class I and II cabinets are appropriate for use with moderate and high-risk microorganisms. These cabinets have an inward face velocity of 75 linear feet per minute and their exhaust air is filtered by HEPA filters. The Class I cabinet can be-used with either a full-width open front, an installed front closure panel, or an installed front closure panel equipped with arm-length rubber gloves. The Class II cabinet is a vertical laminar-flow cabinet with an open front. In addition to the protection provided by the Class 1 cabinet, the Class II cabinet protects materials inside the cabinet from extraneous airborne contaminants since the HEPA filtered air is recirculated within the work space. The Class III cabinet is a totally enclosed ventilated cabinet that is gas tight. Class III cabinets are used for work with infectious organisms. Work in a Class III cabinet is conducted through connected rubber gloves. The cabinet is maintained under negative pressure with supply air drawn in through HEPA filters and exhaust air filtered by two HEPA filters. The exhaust air is discharged to outside the facility using an exhaust fan that is generally separate from the facility's overall exhaust fan. Each of the cabinet types is only protective if it is operated and maintained properly by trained personnel.

- Organism Preparation. In developing the bioengineered test substance, commonly used laboratory equipment include culture plates, roller bottles, shake flasks, and seed fermenter These devices are used to bring the organism from its origination in the master cell bank through its preparation for growth/propagation. For work with bioengineered microorganisms, the organism preparation system is generally contained in a biosafety cabinet.

- Bioreactors. The main purpose of a bioreactor is to grow/propagate a microorganism in a controlled, aseptic environment. Bioreactors come in various sizes and four basic designs (mechanical, non-mechanical, plug flow, and immobilization). The most popular type is the mechanical fermenter which uses mechanical stirrers to agitate the organism. One of the most commonly used mechanical fermenters is the stirred tank fermenter (STF). Agitation is provided by compressed gas or pumped liquid in non-mechanical fermenters. Plug flow reactors are usually tubular reactors that may Use direct agitation. Immobilization reactors foster grow propagation on a permanent solid substrate. In order to satisfy the metabolic requirements of the microorganism, aeration must be adequate to provide sufficient oxygen. Those bioreactors using agitation need to be designed to maintain a uniform environment within the bioreactor.

- Bioreactor Control. To control the metabolic processes within the bioreactor, facilities should have adequate monitoring and control equipment. There are three classes of such monitoring systems, off-line, on-line, and in-line. These systems track parameters such as pH, temperature, and agitation and aeration rates within the bioreactor. For off-line systems, a sample is taken from the bioreactor at specified intervals and chemically analyzed using automated laboratory instruments. since off-line systems can have a lengthy turnaround time for analytical results and do not provide a high level of containment, they are not recommended for work with bioengineered microorganisms. For on-line systems, sampling and analysis are done continuously and often provide additional secondary containment. In-line systems, through the use of probes, sensors, and sampling devices that directly contact the material, provide a continuous, non-invasive indication of bioreactor conditions.

- Air Compressors and Sterilizers. Since most bioprocessing is aerobic; proper growth and production of the organism requires that oxygen be drawn into the bioreactor. To prevent contamination of the organism, the air needs to be compressed and sterilized before it enters the bioreactor. Compressors generally used are either dynamic/centrifugal or positive displacement in design. To remove undesirable organisms and

particles from the air, the compressed air is circulated through filters before entering the bioreactor

- Product Recovery. To separate and concentrate the desired final product from the contents of the bioreactor, a product recovery or purification system is required. Such systems include centrifugation, cell disruption, broth conditioning, filtration, extraction. chromatography, and drying/freezing techniques. The type of equipment at a particular study facility depends on the type(s) of product recovery system(s) employed at the study facility. Types of centrifuges that are used to separate viable cells from liquid broth include batch-operated solid bowl machines, semi-continuous solids-discharging disc separators, and continuous decanters (decanter centrifuges). Types of batch centrifuges include the solid-bowl disc centrifuge, one-chamber centrifuges (used for protein fractionation from blood plasma), zonal centrifuges (used to separate intracellular and extracelluar products such as virus purification or cell constituent isolation), and tube centrifuges (used to separate liquid phases). Safety cabinets must be used during solids removal from batch centrifuges. Generally, semi-continuous solids-discharging machina provide the best containment and are the most widely used type for biotechnology applications. Cell disruption is used to recover intracellular products and can be performed using mechanical or non-mechanical methods. Mechanical methods include ball mills and high speed homogenizers, non-mechanical methods include chemical or enzymatic Iysis, heat treatment, freeze-thaw, and osmotic shock. Non mechanical methods are easily contained and are most often used in laboratories. Broth conditioning can take place within the bioreactor (when an STF is used) or in a separate vessel (if bioprocessing is performed in an unstirred vessel). Filtration units are used to separate cellular, intracellular or extracellular solids from broth. Types of filtration units include continuous rotary drums, continuous rotary vacuum filters, and tangential flow filtration systems using either microporous or ultrafiltration membrane filters). The type of filtration unit used is dependent on the type of product being recovered. Affinity and gel filtration chromatography processes are used to purify intracellular or extracellular products. Products are purified using an eluting solvent in a packed column and are collected in a fraction collector. If adequate containment is provided (e.g., biological safety cabinet), product recovery using chromatography can be used to purify hazardous organisms. Types of liquid-liquid extraction equipment includes centrifugal extractors (used to extract biotechnology products), spray and packed towers, mechanically agitated columns, pulse columns, or mixer-settler units. Either freezing or drying may be used to facilitate handling and storage of products. Organisms to be frozen are placed in vials (performed in a biosafety cabinet) and frozen. The most

common types of dryers used are freeze dryers and vacuum tray dryers. since freezing provides primary containment and produces less aerosols than dryers, it is more appropriate for product storage. If drying is performed, proper filtration and ventilation systems must be provided.

! Waste Recovery and Decontamination. The primary method of decontaminating exhaust gases mixed with liquid broth, is through the use of filters. Before filtration, the mixture may be passed through a condenser, a coalescing filter, and a heat exchanger. Filtration is then done using pairs of HEPA or membrane filters. Another method of decontaminating air and gaseous waste streams is thermal destruction or incineration. Incineration may be used alone or in addition to filtration. Liquid wastes can be decontaminated through chemical or heat treatment. However, for large volumes of liquid wastes and for wastes containing genetically engineered microorganisms, heat treatment is generally preferred. Solid wastes (e.g., microbial cultures, cell debris, glassware, protective clothing) is generally decontaminated by autoclaving, and if necessary, followed by incineration. To decontaminate laboratory devices exposed to bioengineered substances, the most common practice is the use of pressurized steam that contains an appropriate chemical. For heat-sensitive equipment, such as electronic instruments, decontamination is generally achieved through chemical sterilization or irradiation. Gaseous sterilants are applied by a steam ejector that sprays down from overhead. If decontamination by steam, liquid, or gas sterilization is not possible, ionizing or ultraviolet radiation is used. However, since irradiation methods do not always inactivate all types of microbes, steam or gaseous chemical sterilization should be used for devices contaminated with bioengineered organisms.

! Other Common Types of Equipment. Other common types of equipment used in laboratories performing biotechnology studies are as follows:

- Autoclave to sterilize equipment, wastes, etc.

- Freezers, refrigerators, water baths and incubators for incubation and storage. The operating temperature of this equipment 15 dependent on the type of organism being grown

- Heating blocks for conducting enzymatic reactions in test tubes or micro-centrifuge

- Geiger counter and/or scintillation counter for measuring radioactivity

- Magnetic stirrers with heaters

- Microwave oven for agar and agarose melting
- Visible and UV spectrophotometer
- Water purification equipment
- Vacuum desiccator/lyophilizer.

Glossary of Disciplines

Agricultural Science	Discipline dealing with the selection, breeding, and management of crops and domestic animals
Agronomy	The principles and procedures of soil management and of crop improvement, management, and production
Analytical Chemistry	Branch of chemistry dealing with techniques that yield any type of information about chemical systems
Biochemistry	The study of chemical substances occurring in living organisms and the reactions and methods for identifying these substances
Bioengineering	The application of engineering knowledge to the fields of medicine and biology
Biology	A division of the natural sciences concerned with life and living organisms
Botany	A branch of the biological sciences that studies plants and plant life
Chemical Engineering	The branch of engineering dealing with chemically converting basic raw materials into products
Chemistry	The scientific study of the properties, composition, and structure of matter, the changes in structure and composition of matter, and accompanying energy changes
Entomology	The scientific study of insects
Enzymology	Branch of science dealing with the chemical nature, biological activity, and biological significance of enzymes
Forestry	The science that deals with the development, maintenance, and management of forests
Formulation Chemistry	Branch of chemistry dealing with the particular mixtures of base chemicals and additives required for products

Genetic Engineering	The intentional production of new genes and alteration of genomes by the substitution or addition of new genetic material
Genetics	The science concerned with the study of biological inheritance
Histology	The study of the structure and chemical composition of animal and plant tissues as related to their function
Immunology	Branch of biology concerned with the native or acquired resistance of higher animal forms and humans to infection With microorganisms
Microbiology	The science and study of microorganisms, including protozoans, algae, fungi, bacteria, viruses, and rickettsiae
Molecular Biology	Part of biology that attempts to interpret biological events in terms of the physicochemical properties of molecules in a cell
Mycology	The branch of botany that deals with the study of fungi
Organic Chemistry	The study of the composition, reactions, and properties of carbon-chain or carbon-ring compounds and mixtures
Pathology	The study of the causes, nature, and effects of diseases and other abnormalities
Pharmacology	The science dealing with the nature and properties of drugs, particularly their actions
Physical Chemistry	The branch of chemistry that deals with the interpretation of chemical phenomena and properties in terms of the underlying physical processes, and with the development of techniques for their investigation
Taxonomy	The study of the classification of organisms that best reflects the totality of similarities and differences

Toxicology	The study of poisons, including their nature, effects, and detection, and methods of treatment
Virology	The branch of biology dealing with the study of viruses
Zoology	The science that deals with knowledge of animal life