**NOTICE PERTINENT TO THE APRIL 2002 REVISIONS OF THE *NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)***

## ROLES AND RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR (PI)

Under the amendment to Section IV-B-7, which was published in the *Federal Register* on November 19, 2001 (66 FR 57970, *specifically*: 57975) and became effective on December 19, 2001, a PI may delegate the reporting responsibilities set forth in Appendix M-I-C, *Reporting Requirements*, to another party, with written notification of the delegation to OBA. A letter from each PI indicating to whom they have delegated the reporting requirements set forth in Appendix M-I-C must be on file with OBA. This delegation of reporting responsibility may, if appropriate, be extended to include the material submitted under Appendix M-I-A, *Requirements for Protocol Submission*, of the *NIH Guidelines*. To that end, a letter from the PI should be submitted to OBA, either directly by the investigator or as part of the material submitted under Appendix M-I-A.

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**Summary of Amendments [Major Actions]**

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|---|---|
| Page 10 | Section I-E: Additions to General Definitions. New sections -- I-E-8, I-E-9, I-E-10 |
| Page 36 | Appendix B-1 [Lines 5-8]: New – General definition of an *E. coli* strain as a RG1 agent |
| Page 97-98 | Appendix M-1-C-3: Annual Reports. New – (Harmonized submission requirements) |
| Page 98 | Appendix M-1-C-4: Safety Reporting. New appendix – (Harmonized reporting requirements) |
| Page 99 | Appendix M-1-C-5: Confidentiality. New appendix |
| Page 99 | Appendix M-1-D: Safety Assessment. New appendix |
| Page 106 | Appendix M-IV: Privacy; deleted "…and Confidentiality" from heading; clarification of protection measures. |

Effective June 24, 1994, Published in Federal Register, July 5, 1994 (59 FR 34496)
Amendment Effective July 28, 1994, Federal Register, August 5, 1994 (59 FR 40170)
Amendment Effective April 17, 1995, Federal Register, April 27, 1995 (60 FR 20726)
Amendment Effective December 14, 1995, Federal Register, January 19, 1996 (61 FR 1482)
Amendment Effective March 1, 1996, Federal Register, March 12, 1996 (61 FR 10004)
Amendment Effective January 23, 1997, Federal Register, January 31, 1997 (62 FR 4782)
Amendment Effective September 30, 1997, Federal Register, October 14, 1997 (62 FR 53335)
Amendment Effective October 20, 1997, Federal Register, October 29, 1997 (62 FR 56196)
Amendment Effective October 22, 1997, Federal Register, October 31, 1997 (62 FR 59032)
Amendment Effective February 4, 1998, Federal Register, February 17, 1998 (63 FR 8052)
Amendment Effective April 30, 1998, Federal Register, May 11, 1998 (63 FR 26018)
Amendment Effective April 29, 1999, Federal Register, May 11, 1999 (64 FR 25361)
Amendment Effective October 2, 2000, Federal Register, October 10, 2000 (65 FR 60328)
Amendment Effective December 28, 2000 Federal Register, January 5, 2001 (66 FR 1146)
Amendment Effective December 11, 2001 Federal Register, December 11, 2001 (66 FR 64051)
Amendment Effective December 19, 2001 Federal Register, November 19, 2001 (66 FR 57970)
Amendment Effective January 10, 2002 Federal Register, December 11, 2001 (66 FR 64052)
Amendment Effective January 24, 2002 Federal Register, November 19, 2001 (66 FR 57970)

# NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)

# April 2002

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**Visit the OBA Web site at:**
**http://www4.od.nih.gov/oba**
**For current information on Guidelines, Protocols, Principal Investigators, Meetings, and information about upcoming Gene Therapy Policy Conferences**

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**National Institutes of Health**
**Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)**

These *NIH Guidelines* supersede all earlier versions and shall be in effect until further notice.

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## SECTION I. SCOPE OF THE *NIH GUIDELINES*

### Section I-A. Purpose

The purpose of the *NIH Guidelines* is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

**Section I-A-1.** Any recombinant DNA experiment, which according to the *NIH Guidelines* requires approval by NIH, must be submitted to NIH or to another Federal agency that has jurisdiction for review and approval. Once approvals, or other applicable clearances, have been obtained from a Federal agency other than NIH (whether the experiment is referred to that agency by NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval. (See exception in Section I-A-1-a regarding requirement for human gene transfer protocol registration.)

**Section I-A-1-a.** For experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in Section I-E-7) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; and (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

### Section I-B. Definition of Recombinant DNA Molecules

In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

### Section I-C. General Applicability

**Section I-C-1.** The *NIH Guidelines* are applicable to:

**Section I-C-1-a.** All recombinant DNA research within the United States (U.S.) or its territories that is within the category of research described in either Section I-C-1-a-(1) or Section I-C-1-a-(2).

**Section I-C-1-a-(1).** Research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines*.

**Section I-C-1-a-(2).** Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

**Section I-C-1-b.** All recombinant DNA research performed abroad that is within the category of research described in either Section I-C-1-b-(1) or Section I-C-1-b-(2).

**Section I-C-1-b-(1).** Research supported by NIH funds.

**Section I-C-1-b-(2).** Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

**Section I-C-1-b-(3).** If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the *NIH Guidelines*.

**Section I-D. Compliance with the *NIH Guidelines***

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*.

Information concerning noncompliance with the *NIH Guidelines* may be brought forward by any person. It should be delivered to both NIH/OBA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985, 301-496-9838/301-496-9839 (fax) (for non-USPS mail, use zip code 20817).

In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the *NIH Guidelines*, applicable DHHS and Public Health Service procedures shall govern.

The policies on compliance are as follows:

**Section I-D-1.** All NIH-funded projects involving recombinant DNA techniques must comply with the *NIH Guidelines*. Non-compliance may result in: (i) suspension, limitation, or termination of financial assistance for the noncompliant NIH-funded research project and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

**Section I-D-2.** All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the *NIH Guidelines*. Noncompliance may result in: (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

**Section I-E. General Definitions**

The following terms, which are used throughout the *NIH Guidelines*, are defined as follows:

**Section I-E-1.** An "institution" is any public or private entity (including Federal, state, and local government agencies).

**Section I-E-2.** An "Institutional Biosafety Committee" is a committee that: (i) meets the requirements for membership specified in Section IV-B-2, *Institutional Biosafety Committee (IBC)*, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-B-2, *Institutional Biosafety Committee (IBC)*.

**Section I-E-3.** The "Office of Biotechnology Activities (OBA)" is the office within the NIH that is responsible for: (i) reviewing and coordinating all activities relating to the *NIH Guidelines*, and (ii) performing other duties as defined in Section IV-C-3, *Office of Biotechnology Activities (OBA)*.

**Section I-E-4.** The "Recombinant DNA Advisory Committee" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-C-2, *Recombinant DNA Advisory Committee (RAC)*.

**Section I-E-5.** The "NIH Director" is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

**Section I-E-6.** "Deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

**Section I-E-7.** “Enrollment” is the process of obtaining informed consent from a potential research participant, or a designated legal guardian of the participant, to undergo a test or procedure associated with the gene transfer experiment.

**Section I-E-8.** A "serious adverse event" is any event occurring at any dose that results in any of the following outcomes: death, a life-threatening event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization also may be considered a serious adverse event when, upon the basis of appropriate medical judgment, they may jeopardize the human gene transfer research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

**Section I-E-9.** An adverse event is "associated with the use of a gene transfer product" when there is a reasonable possibility that the event may have been caused by the use of that product.

**Section I-E-10.** An "unexpected serious adverse event" is any serious adverse event for which the specificity or severity is not consistent with the risk information available in the current investigator's brochure.

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## SECTION II. SAFETY CONSIDERATIONS

### Section II-A. Risk Assessment

#### Section II-A-1. Risk Groups

Risk assessment is ultimately a subjective process. The investigator must make an initial risk assessment based on the Risk Group (RG) of an agent (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*). Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria: (1) Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans. (2) Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available. (3) Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available. (4) Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

#### Section II-A-2. Criteria for Risk Groups

Classification of agents in Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, is based on the potential effect of a biological agent on a healthy human adult and does not account for instances in which an individual may have increased susceptibility to such agents, e.g., preexisting diseases, medications, compromised immunity, pregnancy or breast feeding (which may increase exposure of infants to some agents).

Personnel may need periodic medical surveillance to ascertain fitness to perform certain activities; they may also need to be offered prophylactic vaccines and boosters (see Section IV-B-1-f, *Responsibilities of the Institution, General Information*).

#### Section II-A-3. Comprehensive Risk Assessment

In deciding on the appropriate containment for an experiment, the initial risk assessment from Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, should be followed by a thorough consideration of the agent itself and how it is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V-B, *Footnotes and References of Sections I-IV*).

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II-B, *Containment*). The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant DNA experiments described in Sections III-A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation*; III-B, *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation*; III-C, *Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and NIH/OBA Registration Before Initiation*; III-D, *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*.

Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level (BL) 2 containment (see Section II-B); however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.

Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult the applicable Occupational Safety and Health Administration (OSHA) regulation, 29 CFR 1910.1030, and OSHA publication 3127 (1996 revised). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV- or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features not addressed in *Biosafety in Microbiological and Biomedical Laboratories* (see Section V-C, *Footnotes and References of Sections I through IV*). For information regarding the importation, possession, or use of these agents see Sections V-G and V-H, *Footnotes and References of Sections I through IV*.

### Section II-B. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see Section V-B, *Footnotes and References of Sections I-IV*). The existing programs rely upon mechanisms that can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in Appendix G, *Physical Containment*. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see Appendix I, *Biological Containment*).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the *NIH Guidelines*.

Physical containment conditions within laboratories, described in Appendix G, *Physical Containment*, may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I, *Biological Containment*, may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1-P through BL4-P) are described in Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*. BL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3-P and BL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2-P and BL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4-P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*. BL1-N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2-N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3-N and BL4-N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the *NIH Guidelines*, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the *NIH Guidelines* to permit the use of these procedures.

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## SECTION III. EXPERIMENTS COVERED BY THE *NIH GUIDELINES*

This section describes six categories of experiments involving recombinant DNA: (i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see Section III-A), (ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see Section III-B), (iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see Section III-C), (iv) those that require Institutional Biosafety Committee approval before initiation (see Section III-D), (v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-E), and (vi) those that are exempt from the *NIH Guidelines* (see Section III-F).

**Note:** *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed*. If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.

Any change in containment level, which is different from those specified in the *NIH Guidelines*, may not be initiated without the express approval of NIH/OBA (see Section IV-C-1-b-(2) and its subsections, *Minor Actions*).

### Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation (See Section IV-C-1-b-(1), Major Actions).

### Section III-A-1. Major Actions under the *NIH Guidelines*

Experiments considered as *Major Actions* under the *NIH Guidelines* cannot be initiated without submission of relevant information on the proposed experiment to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), the publication of the proposal in the *Federal Register* for 15 days of comment, review by RAC, and specific approval by NIH. The containment conditions or stipulation requirements for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D, *Major Actions Taken under the NIH Guidelines*, which may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

**Section III-A-1-a.** The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B, *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.

### Section III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/OBA. The containment conditions for such experiments will be determined by NIH/OBA in consultation with *ad hoc* experts. Such experiments require Institutional Biosafety Committee approval before initiation (see Section IV-B-2-b-(1), *Institutional Biosafety Committee*).

### Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with $LD_{50}$ of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an $LD_{50}$ of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

### Section III-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment

### Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants

For an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in Section I-E-7) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*).

In its evaluation of human gene transfer proposals, the RAC will consider whether a proposed human gene transfer experiment presents characteristics that warrant public RAC review and discussion (See Appendix M-I-B-2). The process of public RAC review and discussion is intended to foster the safe and ethical conduct of human gene transfer experiments. Public review and discussion of a human gene transfer experiment (and access to relevant information) also serves to inform the public about the technical aspects of the proposal, meaning and significance of the research, and any significant safety, social, and ethical implications of the research.

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. After a human gene transfer experiment is reviewed by the RAC at a regularly scheduled meeting, NIH OBA will send a letter, unless NIH OBA determines that there are exceptional circumstances, within 10 working days to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate, summarizing the RAC recommendations.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

In order to maintain public access to information regarding human gene transfer protocols (including protocols that are not publicly reviewed by the RAC), NIH OBA will maintain the documentation described in Appendices M-I through M-V. The information provided in response to Appendix M should not contain any confidential commercial information or trade secrets, enabling all aspects of RAC review to be open to the public.

**Note:** For specific directives concerning the use of retroviral vectors for gene delivery, consult Appendix B-V-1, *Murine Retroviral Vectors.*

**Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation**

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c), *Minor Actions*).

**Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems** (See Section II-A, Risk Assessment)

**Section III-D-1-a.** Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

**Section III-D-1-b.** Experiments involving the introduction of recombinant DNA into Risk Group 3 agents will usually be conducted at BL3 containment. Experiments with such agents will usually be conducted with whole animals at BL3 or BL3-N containment.

**Section III-D-1-c.** Experiments involving the introduction of recombinant DNA into Risk Group 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

**Section III-D-1-d.** Containment conditions for experiments involving the introduction of recombinant DNA into restricted agents shall be set on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G and V-M, *Footnotes and References of Sections I-IV*). Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

**Section III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems**

**Section III-D-2-a.** Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see Section II-A, *Risk Assessment)* is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the *NIH Guidelines* (see Section III-F, *Exempt Experiments*). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with $LD_{50}$ of Less than 100 Nanograms Per Kilogram Body Weight*) or shall be conducted under NIH specified conditions as described in Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*.

**Section III-D-2-b.** Containment conditions for experiments in which DNA from restricted agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/OBA following a case-by-case review (see Section V-L, *Footnotes and References of Sections I-IV*). A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

**Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems**

**Caution:** Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

**Note:** Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-J, *Footnotes and References of Sections I-IV*) being considered identical (see Section V-K, *Footnotes and References of Sections I-IV*), are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1, *Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus*.

**Section III-D-3-a.** Experiments involving the use of infectious or defective Risk Group 2 viruses (see Appendix B-II, *Risk Group 2 Agents*) in the presence of helper virus may be conducted at BL2.

**Section III-D-3-b.** Experiments involving the use of infectious or defective Risk Group 3 viruses (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) in the presence of helper virus may be conducted at BL3.

**Section III-D-3-c.** Experiments involving the use of infectious or defective Risk Group 4 viruses (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*) in the presence of helper virus may be conducted at BL4.

**Section III-D-3-d.** Experiments involving the use of infectious or defective restricted poxviruses (see Sections V-A and V-L, *Footnotes and References of Sections I-IV*) in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

**Section III-D-3-e.** Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BL1.

**Section III-D-4. Experiments Involving Whole Animals**

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

**Caution** - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

**Section III-D-4-a.** Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, *Footnotes and References of Sections I-IV*). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, *Experiments Involving Whole Animals*. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, *Footnotes and References of Sections I-IV*. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

**Section III-D-4-b.** For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III-D-1, *Experiments Using Human or Animal Pathogens (Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems*, or III-D-4-a, *Experiments Involving Whole Animals*, the appropriate containment shall be determined by the Institutional Biosafety Committee.

**Section III-D-4-c.** Exceptions under Section III-D-4, *Experiments Involving Whole Animals*

**Section III-D-4-c-(1).** Experiments involving the generation of transgenic rodents that require BL1 containment are described under Section III-E-3, *Experiments Involving Transgenic Rodents*.

**Section III-D-4-c-(2).** The purchase or transfer of transgenic rodents is exempt from the *NIH Guidelines* under Section III-F, *Exempt Experiments* (see Appendix C-VI, *The Purchase or Transfer of Transgenic Rodents*).

**Section III-D-5. Experiments Involving Whole Plants**

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in Sections III-D-5-a through III-D-5-e. If experiments involving whole plants are not described in Section III-D-5 and do not fall under Sections III-A, III-B, III-D or III-F, they are included in Section III-E.

**NOTE -** For recombinant DNA experiments falling under Sections III-D-5-a through III-D-5-d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

**Section III-D-5-a.** BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic (see Section V-M, *Footnotes and References of Sections I-IV*) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

**Section III-D-5-b.** BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V-M, *Footnotes and References of Sections I-IV*) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta.*

**Section III-D-5-c.** BL4-P containment is recommended for experiments with a small number of readily transmissible exotic (see Section V-M, *Footnotes and References of Sections I-IV*) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

**Section III-D-5-d.** BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an $LD_{50}$ of <100 nanograms per kilogram body weight fall under Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with $LD_{50}$ of Less than 100 Nanograms Per Kilogram Body Weight*, and require NIH/OBA and Institutional Biosafety Committee approval before initiation.

**Section III-D-5-e.** BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

**Section III-D-6. Experiments Involving More than 10 Liters of Culture**

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, *Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*, shall be used. Appendix K describes containment conditions Good Large Scale Practice through BL3-Large Scale.

**Section III-E. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation**

Experiments not included in Sections III-A, III-B, III-C, III-D, III-F, and their subsections are considered in Section III-E. All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III-D, *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV-A, *Policy*). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-E and may be conducted at BL1 containment.

**Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus**

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J, *Footnotes and References of Sections I-IV*]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems*, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

**Section III-E-2. Experiments Involving Whole Plants**

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-D, or III-F. It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

**Section III-E-2-a.** BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in Section III-E-2-b or other sections of the *NIH Guidelines*. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see Section V-M, *Footnotes and References of Sections I-IV*) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

**Section III-E-2-b.** BL2-P or BL1-P + biological containment is recommended for the following experiments:

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

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

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

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

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

### Section III-E-3. Experiments Involving Transgenic Rodents

This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4, *Experiments Involving Whole Animals*.

### Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required:

**Section III-F-1.** Those that are not in organisms or viruses.

**Section III-F-2.** Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

**Section III-F-3.** Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

**Section III-F-4.** Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

**Section III-F-5.** Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), *Major Actions*). See Appendices A-I through A-VI, *Exemptions Under Section III-F-5--Sublists of Natural Exchangers*, for a list of natural exchangers that are exempt from the *NIH Guidelines*.

**Section III-F-6.** Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Section III-F-6* for other classes of experiments which are exempt from the *NIH Guidelines*.

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## SECTION IV. ROLES AND RESPONSIBILITIES

### Section IV-A. Policy

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The *NIH Guidelines* cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The *NIH Guidelines* are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. The *NIH Guidelines* will never be complete or final since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, *it is the responsibility of the institution and those associated with it to adhere to the intent of the NIH Guidelines as well as to their specifics.* Each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all recombinant DNA research conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines*. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

### Section IV-B. Responsibilities of the Institution

#### Section IV-B-1. General Information

Each institution conducting or sponsoring recombinant DNA research which is covered by the *NIH Guidelines* is responsible for ensuring that the research is conducted in full conformity with the provisions of the *NIH Guidelines*. In order to fulfill this responsibility, the institution shall:

**Section IV-B-1-a.** Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the *NIH Guidelines*. As part of its general responsibilities for implementing the *NIH Guidelines*, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the *NIH Guidelines*. Such procedures may include: (i) statements formulated by the institution for the general implementation of the *NIH Guidelines*, and (ii) any additional precautionary steps the institution deems appropriate.

**Section IV-B-1-b.** Establish an Institutional Biosafety Committee that meets the requirements set forth in Section IV-B-2-a and carries out the functions detailed in Section IV-B-2-b.

**Section IV-B-1-c.** Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant DNA research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large-scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in Section IV-B-3.

**Section IV-B-1-d.** Appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*.

**Section IV-B-1-e.** Appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*.

**Section IV-B-1-f.** Ensure that when the institution participates in or sponsors recombinant DNA research involving human subjects: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary), (ii) all aspects of Appendix M have been appropriately addressed by the Principal Investigator; and (iii) no research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*), Institutional Biosafety Committee approval has been obtained,

Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained. Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

**Section IV-B-1-g.** Assist and ensure compliance with the *NIH Guidelines* by Principal Investigators conducting research at the institution as specified in Section IV-B-7.

**Section IV-B-1-h.** Ensure appropriate training for the Institutional Biosafety Committee Chair and members, Biological Safety Officer and other containment experts (when applicable), Principal Investigators, and laboratory staff regarding laboratory safety and implementation of the *NIH Guidelines*. The Institutional Biosafety Committee Chair is responsible for ensuring that Institutional Biosafety Committee members are appropriately trained. The Principal Investigator is responsible for ensuring that laboratory staff are appropriately trained. The institution is responsible for ensuring that the Principal Investigator has sufficient training; however, this responsibility may be delegated to the Institutional Biosafety Committee.

**Section IV-B-1-i.** Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory. The *Laboratory Safety Monograph* discusses various components of such a program (e.g., records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience). Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples cited in the *Laboratory Safety Monograph* include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the *Laboratory Safety Monograph* are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

**Section IV-B-1-j.** Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to NIH/OBA within thirty days, unless the institution determines that a report has already been filed by the Principal Investigator or Institutional Biosafety Committee. Reports shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

### Section IV-B-2. Institutional Biosafety Committee (IBC)

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant DNA. The Institutional Biosafety Committee shall meet the following requirements:

### Section IV-B-2-a. Membership and Procedures

**Section IV-B-2-a-(1).** The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P, *Physical and Biological Containment for Recombinant DNA Research*

*Involving Plants*, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, *Biological Safety Officer*). When the institution participates in or sponsors recombinant DNA research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary); (ii) all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); and (iv) final IBC approval is granted only after the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*). Institutional Biosafety Committee approval must be obtained from the institution at which recombinant DNA material will be administered to human research participants (rather than the site involved in manufacturing gene transfer products).

**Note:** Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines*, are encouraged to adhere to the standards and procedures set forth in Sections I through IV (see Section IV-D, *Voluntary Compliance*. The policy and procedures for establishing an Institutional Biosafety Committee under *Voluntary Compliance*, are specified in Section IV-D-2, *Institutional Biosafety Committee Approval*).

**Section IV-B-2-a-(2).** In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

**Section IV-B-2-a-(3).** The institution shall file an annual report with NIH/OBA which includes: (i) a roster of all Institutional Biosafety Committee members clearly indicating the Chair, contact person, Biological Safety Officer (if applicable), plant expert (if applicable), animal expert (if applicable), human gene therapy expertise or *ad hoc* consultant(if applicable); and (ii) biographical sketches of all Institutional Biosafety Committee members (including community members).

**Section IV-B-2-a-(4).** No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

**Section IV-B-2-a-(5).** The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

**Section IV-B-2-a-(6).** When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

**Section IV-B-2-a-(7).** Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

**Section IV-B-2-b. Functions**

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

**Section IV-B-2-b-(1).** Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* as specified in Section III, *Experiments Covered by the NIH Guidelines*, and approving those research projects that are found to conform with the *NIH Guidelines*. This review shall include: (i) independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research; (iii) ensuring that all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iv) ensuring that no research participant is enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*), Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained; (v) for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principal Investigator's response to the RAC recommendations; (vi) ensuring that final IBC approval is granted only after the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); and (vii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*.

**Section IV-B-2-b-(2).** Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

**Section IV-B-2-b-(3).** Lowering containment levels for certain experiments as specified in Section III-D-2-a, *Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems*.

**Section IV-B-2-b-(4).** Setting containment levels as specified in Sections III-D-4-b, *Experiments Involving Whole Animals*, and III-D-5, *Experiments Involving Whole Plants*.

**Section IV-B-2-b-(5).** Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the *NIH Guidelines*.

**Section IV-B-2-b-(6).** Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

**Note:** The *Laboratory Safety Monograph* describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the *Laboratory Safety Monograph*. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

**Section IV-B-2-b-(7).** Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

**Section IV-B-2-b-(8).** The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the *NIH Guidelines* until NIH (with the advice of the RAC when required) establishes the containment requirement.

**Section IV-B-2-b-(9).** Performing such other functions as may be delegated to the Institutional Biosafety Committee under Section IV-B-2, *Institutional Biosafety Committee*.

### Section IV-B-3. Biological Safety Officer (BSO)

**Section IV-B-3-a.** The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules.

**Section IV-B-3-b.** The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

**Section IV-B-3-c.** The Biological Safety Officer's duties include, but are not be limited to:

**Section IV-B-3-c-(1).** Periodic inspections to ensure that laboratory standards are rigorously followed;

**Section IV-B-3-c-(2).** Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator;

**Section IV-B-3-c-(3).** Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;

**Section IV-B-3-c-(4).** Providing advice on laboratory security;

**Section IV-B-3-c-(5).** Providing technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

**Note:** See the *Laboratory Safety Monograph* for additional information on the duties of the Biological Safety Officer.

### Section IV-B-4. Plant, Plant Pathogen, or Plant Pest Containment Expert

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, the institution shall appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee).

### Section IV-B-5. Animal Containment Expert

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, the institution shall appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee).

### Section IV-B-6. Human Gene Therapy Expertise

When the institution participates in or sponsors recombinant DNA research involving human subjects, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary) and (ii) all aspects of Appendix M, *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)* , have been appropriately addressed by the Principal Investigator prior to submission to NIH/OBA.

### Section IV-B-7. Principal Investigator (PI)

On behalf of the institution, the Principal Investigator is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research. A Principal Investigator engaged in human gene transfer research may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone, and fax numbers of the contact. The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

**Section IV-B-7-a. General Responsibilities**

As part of this general responsibility, the Principal Investigator shall:

**Section IV-B-7-a-(1).** Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections III-A, III-B, III-C, III-D, and III-E, *Experiments Covered by the NIH Guidelines*) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;

**Section IV-B-7-a-(2).** Determine whether experiments are covered by Section III-E, *Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation*, and ensure that the appropriate procedures are followed;

**Section IV-B-7-a-(3).** Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) within 30 days. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

**Section IV-B-7-a-(4).** Report any new information bearing on the *NIH Guidelines* to the Institutional Biosafety Committee and to NIH/OBA (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

**Section IV-B-7-a-(5).** Be adequately trained in good microbiological techniques;

**Section IV-B-7-a-(6).** Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination; and

**Section IV-B-7-a-(7).** Comply with shipping requirements for recombinant DNA molecules (see Appendix H, *Shipment*, for shipping requirements and the *Laboratory Safety Monograph* for technical recommendations).

**Section IV-B-7-b. Information to Be Submitted by the Principal Investigator to NIH OBA**

The Principal Investigator shall:

**Section IV-B-7-b-(1).** Submit information to NIH/OBA for certification of new host-vector systems;

**Section IV-B-7-b-(2).** Petition NIH/OBA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the *NIH Guidelines*;

**Section IV-B-7-b-(3).** Petition NIH/OBA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in Sections III-A-1, *Major Actions Under the NIH Guidelines*, and III-B, *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation*;

**Section IV-B-7-b-(4).** Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and

**Section IV-B-7-b-(5).** Petition NIH/OBA for determination of containment for experiments not covered by the *NIH Guidelines*.

**Section IV-B-7-b-(6).** Ensure that all aspects of Appendix M have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA, and provide a letter signed by the Principal Investigator(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OBA complies with the requirements set forth in Appendix M. No research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been

completed (see Appendix M-I-B, *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; Institutional Review Board (IRB) approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

**Section IV-B-7-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee**

The Principal Investigator shall:

**Section IV-B-7-c-(1).** Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;

**Section IV-B-7-c-(2).** Select appropriate microbiological practices and laboratory techniques to be used for the research;

**Section IV-B-7-c-(3).** Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, III-B, III-C, III-D, or III-E (*Experiments Covered by the NIH Guidelines*), to the Institutional Biosafety Committee for review and approval or disapproval; and

**Section IV-B-7-c-(4).** Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

**Section IV-B-7-d. Responsibilities of the Principal Investigator Prior to Initiating Research**

The Principal Investigator shall:

**Section IV-B-7-d-(1).** Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

**Section IV-B-7-d-(2).** Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents; and

**Section IV-B-7-d-(3).** Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

**Section IV-B-7-e. Responsibilities of the Principal Investigator During the Conduct of the Research**

The Principal Investigator shall:

**Section IV-B-7-e-(1).** Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

**Section IV-B-7-e-(2).** Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

**Section IV-B-7-e-(3).** Correct work errors and conditions that may result in the release of recombinant DNA materials; and

**Section IV-B-7-e-(4).** Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

**Section IV-B-7-e-(5).** Comply with reporting requirements for human gene transfer experiments conducted in compliance with the *NIH Guidelines* (see Appendix M-I-C, *Reporting Requirements*).

### Section IV-C. Responsibilities of the National Institutes of Health (NIH)

### Section IV-C-1. NIH Director

The NIH Director is responsible for: (i) establishing the *NIH Guidelines*, (ii) overseeing their implementation, and (iii) their final interpretation. The NIH Director has responsibilities under the *NIH Guidelines* that involve OBA and RAC. OBA's responsibilities under the *NIH Guidelines* are administrative. Advice from RAC is primarily scientific, technical, and ethical. In certain circumstances, there is specific opportunity for public comment with published response prior to final action.

### Section IV-C-1-a. General Responsibilities

The NIH Director is responsible for:

**Section IV-C-1-a-(1).** Promulgating requirements as necessary to implement the *NIH Guidelines*;

**Section IV-C-1-a-(2).** Establishing and maintaining RAC to carry out the responsibilities set forth in Section IV-C-2, *Recombinant DNA Advisory Committee* (RAC membership is specified in its charter and in Section IV-C-2);

**Section IV-C-1-a-(3).** Establishing and maintaining NIH/OBA to carry out the responsibilities defined in Section IV-C-3, *Office of Biotechnology Activities*;

**Section IV-C-1-a-(4).** Conducting and supporting training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers and other institutional experts (if applicable), Principal Investigators, and laboratory staff.

**Section IV-C-1-a-(5).** Establishing and convening Gene Therapy Policy Conferences as described in Appendix L, *Gene Therapy Policy Conferences.*

### Section IV-C-1-b. Specific Responsibilities

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the *NIH Guidelines* and presents no significant risk to health or the environment.

### Section IV-C-1-b-(1). Major Actions

To execute *Major Actions*, the NIH Director shall seek the advice of RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and *Proposed Actions* shall be published in the *Federal Register* at least 15 days before the RAC meeting. The NIH Director's decision/recommendation (at his/her discretion) may be published in the *Federal Register* for 15 days of comment before final action is taken. The NIH Director's final decision/recommendation, along with responses to public comments, shall be published in the *Federal Register*. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

**Section IV-C-1-b-(1)-(a).** Changing containment levels for types of experiments that are specified in the *NIH Guidelines* when a *Major Action* is involved;

**Section IV-C-1-b-(1)-(b).** Assigning containment levels for types of experiments that are not explicitly considered in the *NIH Guidelines* when a *Major Action* is involved;

**Section IV-C-1-b-(1)-(c).** Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

**Section IV-C-1-b-(1)-(d).** Permitting experiments specified by Section III-A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation*;

**Section IV-C-1-b-(1)-(e).** Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I-II, *Certification of Host-Vector Systems*). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

**Section IV-C-1-b-(1)-(f).** Adopting other changes in the *NIH Guidelines*.

**Section IV-C-1-b-(2). Minor Actions**

NIH/OBA shall carry out certain functions as delegated to it by the NIH Director (see Section IV-C-3, *Office of Biotechnology Activities*). *Minor Actions* (as determined by NIH/OBA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to RAC and Institutional Biosafety Committee Chairs:

**Section IV-C-1-b-(2)-(a).** Changing containment levels for experiments that are specified in Section III, *Experiments Covered by the NIH Guidelines* (except when a *Major Action* is involved);

**Section IV-C-1-b-(2)-(b).** Assigning containment levels for experiments not explicitly considered in the *NIH Guidelines*;

**Section IV-C-1-b-(2)-(c).** Revising the *Classification of Etiologic Agents* for the purpose of these *NIH Guidelines* (see Section V-A, *Footnotes and References of Sections I-IV*).

**Section IV-C-1-b-(2)-(d).** Interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels;

**Section IV-C-1-b-(2)-(e).** Setting containment under Sections III-D-1-d, *Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems*, and III-D-2-b, *Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems*;

**Section IV-C-1-b-(2)-(f).** Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I-II, *Certification of Host-Vector Systems*);

**Section IV-C-1-b-(2)-(g).** Decertifying already certified host-vector systems;

**Section IV-C-1-b-(2)-(h).** Adding new entries to the list of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*); and

**Section IV-C-1-b-(2)-(i).** Determining appropriate containment conditions for experiments according to case precedents developed under Section IV-C-1-b-(2)-(c).

**Section IV-C-2. Recombinant DNA Advisory Committee (RAC)**

The RAC is responsible for carrying out the functions specified in the *NIH Guidelines*, as well as others specified in its charter or assigned by the Secretary of Health and Human Services or the NIH Director. The RAC membership and procedures, in addition to those set forth in the *NIH Guidelines*, are specified in the charter for the RAC which is filed as provided in the General Services Administration Federal Advisory Committee Management regulations, 41 CFR part 101-6, and is available on the OBA web site, http://www4.od.nih.gov/oba/rac/RACcharter2002.pdf. In the event of a conflict between the *NIH Guidelines* and the charter, the charter shall control.

The RAC will consist of not less than 15 voting members, including the Chair, appointed under the procedures of the NIH and the Department of Health and Human Services. The maximum number of voting members will be established in the charter of the RAC. At least a majority of the voting members must be knowledgeable in relevant scientific fields, e.g., molecular genetics, molecular biology, recombinant DNA research, including clinical gene transfer research. At least 4 members of the RAC must be knowledgeable in fields such as public health, laboratory safety, occupational health, protection of human subjects of research, the environment, ethics, law, public attitudes or related fields. Representatives of the Federal agencies listed in the charter shall serve as non-voting members. Nominations for RAC members may be submitted to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

All meetings of the RAC shall be announced in the *Federal Register*, including tentative agenda items, 15 days before the meeting. Final agendas, if modified, shall be available at least 72 hours before the meeting. No item defined as a *Major Action* under Section IV-C-1-b-(1) may be added to an agenda following *Federal Register* publication.

RAC shall be responsible for:

**Section IV-C-2-a.** Advising the NIH Director on the following actions: (1) Adopting changes in the *NIH Guidelines*. (2) Assigning containment levels, changing containment levels, and approving experiments considered as *Major Actions* under the *NIH Guidelines*, i.e., the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. (3) Promulgating and amending lists of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment. (4) Certifying new host-vector systems.

**Section IV-C-2-b.** Identifying novel human gene transfer experiments deserving of public discussion by the full RAC;

**Section IV-C-2-c.** Transmitting to the NIH Director specific comments/ recommendations about: (i) a specific human gene transfer experiment, or (ii) a category of human gene transfer experiments;

**Section IV-C-2-d.** Publicly reviewing human gene transfer clinical trial data and relevant information evaluated and summarized by NIH/OBA in accordance with the annual data reporting requirements;

**Section IV-C-2-e.** Identifying broad scientific, safety, social, and ethical issues relevant to gene therapy research as potential Gene Therapy Policy Conference topics;

**Section IV-C-2-f.** Identifying novel social and ethical issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the preparation of relevant Informed Consent documents; and

**Section IV-C-2-g.** Identifying novel scientific and safety issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the design and submission of human gene transfer clinical trials.

**Section IV-C-3. Office of Biotechnology Activities (OBA)**

OBA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. OBA shall carry out such other functions as may be delegated to it by the NIH Director. OBA's responsibilities include (but are not limited to) the following:

**Section IV-C-3-a.** Serving as the focal point for public access to summary information pertaining to human gene transfer experiments;

**Section IV-C-3-b.** Serving as the focal point for data management of human gene transfer experiments;

**Section IV-C-3-c.** Administering the annual data reporting requirements (and subsequent review) for human gene transfer experiments (see Appendix M-I-C, *Reporting Requirements*);

**Section IV-C-3-d.** Transmitting comments/recommendations arising from public RAC discussion of a novel human gene transfer experiment to the NIH Director. RAC recommendations shall be forwarded to the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate.

**Section IV-C-3-e.** Collaborating with Principal Investigators, Institutional Biosafety Committees, Institutional Review Boards, and other DHHS components (including FDA and the Office for Human Research Protections), to ensure human gene transfer experiment registration compliance in accordance with Appendix M-I, *Requirements for Protocol Submission, Review, and Reporting-Human Gene Transfer Experiments* of the *NIH Guidelines*.

**Section IV-C-3-f.** Administering Gene Therapy Policy Conferences as deemed appropriate by the NIH Director (see Appendix L, *Gene Therapy Policy Conferences*).

**Section IV-C-3-g.** Reviewing and approving experiments in conjunction with *ad hoc* experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an $LD_{50}$ of less than or equal to 100 nanograms per kilogram body weight in organisms other than *Escherichia coli* K-12 (see Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with $LD_{50}$ of Less than 100 Nanograms Per Kilogram Body Weight*, Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*);

**Section IV-C-3-h.** Serving as the executive secretary of RAC;

**Section IV-C-3-i.** Publishing in the *Federal Register*:

**Section IV-C-3-i-(1).** Announcements of RAC meetings and tentative agendas at least 15 days in advance (Note: If the agenda for a RAC meeting is modified, OBA shall make the revised agenda available to anyone upon request in advance of the meeting);

**Section IV-C-3-i-(2).** Announcements of Gene Therapy Policy Conferences and tentative agendas at least 15 days in advance;

**Section IV-C-3-i-(3).** Proposed *Major Actions* (see Section IV-C-1-b-(1), *Major Actions*) at least 15 days prior to the RAC meeting; and

**Section IV-C-3-j.** Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in Section IV-B-2, *Institutional Biosafety Committee (IBC)*, giving its approval to the Institutional Biosafety Committee membership.

**Section IV-C-4. Other NIH Components**

Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

## Section IV-D. Voluntary Compliance

### Section IV-D-1. Basic Policy - Voluntary Compliance

Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines* are encouraged to follow the standards and procedures set forth in Sections I through IV. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations and individuals who have no organizational affiliation. For purposes of complying with the *NIH Guidelines*, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the *NIH Guidelines*.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures are provided in Sections IV-D-2 through IV-D-5-b, *Voluntary Compliance*, in order to address these concerns.

### Section IV-D-2. Institutional Biosafety Committee Approval - Voluntary Compliance

It should be emphasized that employment of an Institutional Biosafety Committee member solely for purposes of membership on the Institutional Biosafety Committee does not itself make the member an institutionally affiliated member. Except for the unaffiliated members, a member of an Institutional Biosafety Committee for an institution not otherwise covered by the *NIH Guidelines* may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. Section IV-B-2-a-(4), *Institutional Biosafety Committee*, is modified to that extent for purposes of these institutions.

### Section IV-D-3. Certification of Host-Vector Systems - Voluntary Compliance

A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II, *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section IV-D, *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

### Section IV-D-4. Requests for Exemptions and Approvals - Voluntary Compliance

Requests for exemptions or other approvals as required by the *NIH Guidelines* should be submitted based on the procedures set forth in Sections I through IV. In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under Section IV-D-5-a, *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

### Section IV-D-5. Protection of Proprietary Data - Voluntary Compliance

#### Section IV-D-5-a. General

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial or financial information that is obtained from a person and that is privileged or confidential." Under 18 U.S.C. 1905, it is a criminal offense for an officer or employee of the U.S. or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or) processes...of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of RAC are "special Government employees."

In submitting to NIH for purposes of voluntary compliance with the *NIH Guidelines*, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential, commercial, or financial information. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released. If NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised and the actual release will be delayed in accordance with 45 Code of Federal Regulations, Section 5.65(d) and (e).

**Section IV-D-5-b. Pre-submission Review**

Any institution not otherwise covered by the *NIH Guidelines*, which is considering submission of data or information voluntarily to NIH, may request pre-submission review of the records involved to determine if NIH will make all or part of the records available upon request under the Freedom of Information Act.

A request for pre-submission review should be submitted to NIH/OBA along with the records involved to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). These records shall be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom on Information Act. NIH/OBA will seek a determination from the responsible official under DHHS regulations (45 CFR Part 5) as to whether the records involved, (or some portion) will be made available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from NIH/OBA files, will be considered records of the institution and not NIH/OBA, and will not be received as part of NIH/OBA files. No copies will be made of such records.

NIH/OBA will inform the institution of the NIH Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of NIH/OBA files. If the institution instructs NIH/OBA to return the records, no copies or summaries of the records will be made or retained by DHHS, NIH, or OBA. The NIH Freedom of Information Officer's determination will represent that official's judgment at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom on Information Act if at the time of the determination the records were in NIH/OBA files and a request was received for such files under the Freedom of Information Act.

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## SECTION V. FOOTNOTES AND REFERENCES OF SECTIONS I THROUGH IV

**Section V-A.** The NIH Director, with advice of the RAC, may revise the classification for the purposes of the *NIH Guidelines* (see Section IV-C-1-b-(2)-(e), *Minor Actions*). The revised list of organisms in each risk group is reprinted in Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*.

**Section V-B.** Section III, *Experiments Covered by the NIH Guidelines*, describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*" (see Section IV-B-7-c-(1)). For cases falling under Sections III-A through III-E, *Experiments Covered by the NIH Guidelines*, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research" (see Section IV-B-2-b-(1), *Institutional Biosafety Committee*). The Institutional Biosafety Committee may refer specific cases to NIH/OBA as part of NIH/OBA's functions to "provide advice to all within and outside NIH" (see Section IV-C-3, ). NIH/OBA may request advice from the RAC as part of the RAC's responsibility for "interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels" (see Section IV-C-1-b-(2)-(f), *Minor Actions*).

**Section V-C.** U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and the National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition, 1999. Copies are available from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402-9371 (stock # 017-040-00547-4), Phone (202) 512-1800.

**Section V-D.** *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

**Section V-E.** Chin, James ed., *Control of Communicable Diseases Manual*, 17th Edition, 2000. ISBN: 087553-242-X, American Public Health Association, 800 I Street, N.W., Washington, D.C. Phone: (202) 777-2742.

**Section V-F.** *World Health Organization Laboratory Biosafety Manual*, 2nd edition. 1993. WHO Albany, NY. Copies are available from: WHO Publication Centre, USA, (Q Corp) 49 Sheridan Avenue, Albany, New York 12210; Phone: (518) 436-9686 (Order # 1152213).

**Section V-G.** A U.S. Department of Agriculture permit, required for import and interstate transport of plant and animal pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

**Section V-H.** American Type Culture Collection Catalogues of plant viruses, animal viruses, cells, bacteria, fungi, etc. are available from American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Phone: (703) 365-2700.

**Section V-I.** U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR 1910.1030, *Bloodborne Pathogens*. See also*, Exposure to Bloodborne Pathogens,* OSHA 3127, 1996 (Revised).

**Section V-J.** As classified in the *Virus Taxonomy: The Classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, 2000 (0123702003) San Diego, CA.

**Section V-K.** *i.e.*, the total of all genomes within a family shall not exceed two-thirds of the genome.

**Section V-L.** Organisms including alastrim, smallpox (variola) and whitepox may not be studied in the United States except at specified facilities. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

**Section V-M.** In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V-G, *Footnotes and References of Sections I-IV*). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

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## APPENDIX A. EXEMPTIONS UNDER SECTION III-F-5--SUBLISTS OF NATURAL EXCHANGERS

Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent are exempt from these *NIH Guidelines* (see Section III-F-5, *Exempt Experiments*). Institutional Biosafety Committee registration is not required for these exempt experiments. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice from the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*). For a list of natural exchangers that are exempt from the *NIH Guidelines*, see Appendices A-I through A-VI, *Exemptions Under Section III-F-5 Sublists of Natural Exchangers*. Section III-F-5, *Exempt Experiments*, describes recombinant DNA molecules that are: (1) composed entirely of DNA segments from one or more of the organisms within a sublist, and (2) to be propagated in any of the organisms within a sublist (see *Classification of Bergey's Manual of Determinative Bacteriology*; 8th edition, R. E. Buchanan and N. E. Gibbons, editors, Williams and Wilkins Company; Baltimore, Maryland 1984). Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (see *Biosafety in Microbiological and Biomedical Laboratories*, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH Office of Biosafety, Bethesda, Maryland).

### Appendix A-I. Sublist A

Genus Escherichia
Genus *Shigella*
Genus *Salmonella* - including *Arizona*
Genus *Enterobacter*
Genus *Citrobacter* - including *Levinea*
Genus *Klebsiella* - including *oxytoca*
Genus *Erwinia*
*Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, and Pseudomonas mendocina*
*Serratia marcescens*
*Yersinia enterocolitica*

### Appendix A-II. Sublist B

Bacillus subtilis
*Bacillus licheniformis*
*Bacillus pumilus*
*Bacillus globigii*
*Bacillus niger*
*Bacillus nato*
*Bacillus amyloliquefaciens*
*Bacillus aterrimus*

### Appendix A-III. Sublist C

Streptomyces aureofaciens
*Streptomyces rimosus*
*Streptomyces coelicolor*

### Appendix A-IV. Sublist D

Streptomyces griseus
*Streptomyces cyaneus*
*Streptomyces venezuelae*

### Appendix A-V. Sublist E

One way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

### Appendix A-VI. Sublist F

*Streptococcus sanguis*
*Streptococcus pneumoniae*
*Streptococcus faecalis*
*Streptococcus pyogenes*
*Streptococcus mutans*

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## APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (see Sections V-C, V-D, V-E, and V-F, *Footnotes and References of Sections I through IV*. Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the *NIH Guidelines*.

### Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

| | |
|---|---|
| Risk Group 1 (RG1) | Agents that are not associated with disease in healthy adult humans |
| Risk Group 2 (RG2) | Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available |
| Risk Group 3 (RG3) | Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk) |
| Risk Group 4 (RG4) | Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk) |

### Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions); adeno- associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen); and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

### Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

### Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3))
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
--*Clostridium botulinum*, *Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani*
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoeae*, *N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*

### Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents

*--Blastomyces dermatitidis*
*--Cladosporium bantianum, C. (Xylohypha) trichoides*
*--Cryptococcus neoformans*
*--Dactylaria galopava (Ochroconis gallopavum)*
*--Epidermophyton*
*--Exophiala (Wangiella) dermatitidis*
*--Fonsecaea pedrosoi*
*--Microsporum*
*--Paracoccidioides braziliensis*
*--Penicillium marneffei*
*--Sporothrix schenckii*
*--Trichophyton*

### Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents

*--Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
*--Ascaris* including *Ascaris lumbricoides suum*
*--Babesia* including *B. divergens, B. microti*
*--Brugia* filaria worms including *B. malayi, B. timori*
*--Coccidia*
*--Cryptosporidium* including *C. parvum*
*--Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
*--Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
*--Entamoeba histolytica*
*--Enterobius*
*--Fasciola* including *F. gigantica, F. hepatica*
*--Giardia* including *G. lamblia*
*--Heterophyes*
*--Hymenolepis* including *H. diminuta, H. nana*
*--Isospora*
*--Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
*--Loa loa* filaria worms
*--Microsporidium*
*--Naegleria fowleri*
*--Necator* human hookworms including *N. americanus*
*--Onchocerca* filaria worms including, *O. volvulus*
*--Plasmodium* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
*--Sarcocystis* including *S. sui hominis*
*--Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
*--Strongyloides* including *S. stercoralis*
*--Taenia solium*
*--Toxocara* including *T. canis*
*--Toxoplasma* including *T. gondii*
*--Trichinella spiralis*
*--Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
*--Wuchereria bancrofti* filaria worms

### Appendix B-II-D. Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strain TC-83
--Western equine encephalomyelitis virus

Arenaviruses
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Caliciviruses

Coronaviruses

Flaviviruses (Togaviruses) - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Yellow fever virus vaccine strain 17D
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)
--Cytomegalovirus
--Epstein Barr virus
--*Herpes simplex* types 1 and 2
--*Herpes zoster*
--Human herpesvirus types 6 and 7
Orthomyxoviruses
--Influenza viruses types A, B, and C
--Other tick-borne orthomyxoviruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Papovaviruses
--All human papilloma viruses

Paramyxoviruses
--Newcastle disease virus
--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus

Parvoviruses
--Human parvovirus (B19)

Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types
--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses
--Rabies virus - all strains
--Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)
--Rubivirus (rubella)

**Appendix B-III. Risk Group 3 (RG3) Agents**

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

**Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia**

--*Bartonella*
--*Brucella* including *B. abortus, B. canis*, *B. suis*
--*Burkholderia (Pseudomonas) mallei, B. pseudomallei*
--*Coxiella burnetii*
--*Francisella tularensis*
--*Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
--*Pasteurella multocida* type B -"buffalo" and other virulent strains
--Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri)
--*Yersinia pestis*

**Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents**

--*Coccidioides immitis* (sporulating cultures; contaminated soil)
--*Histoplasma capsulatum, H. capsulatum var.. duboisii*

**Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents**

None

**Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions**

Alphaviruses (Togaviruses) - Group A Arboviruses
--Semliki Forest virus
--St. Louis encephalitis virus
--Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Arenaviruses
--Flexal
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus

Flaviviruses (Togaviruses) - Group B Arboviruses
--Japanese encephalitis virus
--Yellow fever virus
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Poxviruses
--Monkeypox virus

Prions
--Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, *Footnotes and References of Sections I through IV*, for containment instruction)

Retroviruses
--Human immunodeficiency virus (HIV) types 1 and 2
--Human T cell lymphotropic virus (HTLV) types 1 and 2
--Simian immunodeficiency virus (SIV)

Rhabdoviruses
--Vesicular stomatitis virus

**Appendix B-IV. Risk Group 4 (RG4) Agents**

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

**Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents**

None

**Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents**

None

**Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents**

None

**Appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents**

Arenaviruses
--Guanarito virus
--Lassa virus

--Junin virus
--Machupo virus
--Sabia

Bunyaviruses (Nairovirus)
--Crimean-Congo hemorrhagic fever virus

Filoviruses
--Ebola virus
--Marburg virus

Flaviruses (Togaviruses) - Group B Arboviruses
--Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
--Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
--Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

### Appendix B-V. Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses
--Herpesvirus ateles
--Herpesvirus saimiri
--Marek's disease virus
--Murine cytomegalovirus

Papovaviruses
--Bovine papilloma virus
--Polyoma virus
--Shope papilloma virus
--Simian virus 40 (SV40)

Retroviruses
--Avian leukosis virus
--Avian sarcoma virus
--Bovine leukemia virus
--Feline leukemia virus
--Feline sarcoma virus
--Gibbon leukemia virus
--Mason-Pfizer monkey virus
--Mouse mammary tumor virus
--Murine leukemia virus
--Murine sarcoma virus
--Rat leukemia virus

### Appendix B-V-1. Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

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## APPENDIX C. EXEMPTIONS UNDER SECTION III-F-6

Section III-F-6 states that exempt from these *NIH Guidelines* are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Sections III-F-6*, for other classes of experiments which are exempt from the *NIH Guidelines*." The following classes of experiments are exempt under Section III-F-6:

### Appendix C-I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see Appendix C-VII-E, *Footnotes and References of Appendix C*),

that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed in Appendix C-I-A.

**Appendix C-I-A. Exceptions**

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents, (iv) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*), and (v) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

**Appendix C-II.** *Escherichia coli K-12* **Host-Vector Systems**

Experiments which use *Escherichia coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the *NIH Guidelines* provided that: (i) the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-VII-B, *Footnotes and References of Appendix C*) shall be used as vectors. However, experiments involving the insertion into *Escherichia coli* K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-VII-C, *Footnotes and References of Appendix C*) with *Escherichia coli* may be performed with any *Escherichia coli* K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *Escherichia coli* K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

**Appendix C-II-A. Exceptions**

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

**Appendix C-III.** *Saccharomyces* **Host-Vector Systems**

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the *NIH Guidelines*. For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

### Appendix C-III-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

### Appendix C-IV. Bacillus subtilis or Bacillus licheniformis Host-Vector Systems

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than $10^{-7}$ may be used for cloning DNA with the exception of those experiments listed in Appendix C-IV-A, *Exceptions*. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

### Appendix C-IV-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

### Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these *NIH Guidelines*.

*Bacillus amyloliquefaciens*
*Bacillus amylosacchariticus*
*Bacillus anthracis*
*Bacillus aterrimus*
*Bacillus brevis*
*Bacillus cereus*
*Bacillus globigii*
*Bacillus licheniformis*
*Bacillus megaterium*
*Bacillus natto*
*Bacillus niger*
*Bacillus pumilus*
*Bacillus sphaericus*
*Bacillus stearothermophilis*
*Bacillus subtilis*
*Bacillus thuringiensis*

*Clostridium acetobutylicum*
*Lactobacillus casei*
*Listeria grayi*
*Listeria monocytogenes*
*Listeria murrayi*
*Pediococcus acidilactici*
*Pediococcus damnosus*
*Pediococcus pentosaceus*
*Staphylococcus aureus*
*Staphylcoccus carnosus*
*Staphylococcus epidermidis*
*Streptococcus agalactiae*
*Streptococcus anginosus*
*Streptococcus avium*
*Streptococcus cremoris*
*Streptococcus dorans*
*Streptococcus equisimilis*
*Streptococcus faecalis*
*Streptococcus ferus*
*Streptococcus lactis*
*Streptococcus ferns*
*Streptococcus mitior*
*Streptococcus mutans*
*Streptococcus pneumoniae*
*Streptococcus pyogenes*
*Streptococcus salivarious*
*Streptococcus sanguis*
*Streptococcus sobrinus*
*Streptococcus thermophylus*

### Appendix C-V-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

### Appendix C-VI. The Purchase or Transfer of Transgenic Rodents

The purchase or transfer of transgenic rodents for experiments that require BL1 containment (See Appendix G-III-M, *Footnotes and References of Appendix G*) are exempt from the *NIH Guidelines*.

### Appendix C-VII. Footnotes and References of Appendix C

**Appendix C-VII-A.** The NIH Director, with advice of the RAC, may revise the classification for the purposes of these *NIH Guidelines* (see Section IV-C-1-b-(2)-(b), *Minor Actions*). The revised list of organisms in each Risk Group is located in Appendix B.

**Appendix C-VII-B.** A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

**Appendix C-VII-C.** Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III-F-5, *Exempt Experiments*.

**Appendix C-VII-D.** As classified in the *Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses*, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

**Appendix C-VII-E.** i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

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## APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE *NIH GUIDELINES*

As noted in the subsections of Section IV-C-1-b-(1), the Director, NIH, may take certain actions with regard to the *NIH Guidelines* after the issues have been considered by the RAC. Some of the actions taken to date include the following:

**Appendix D-1.** Permission is granted to clone foot and mouth disease virus in the EK1 host-vector system consisting of *E. coli* K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

**Appendix D-2.** Certain specified clones derived from segments of the foot and mouth disease virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech, Inc., has been approved under BL1 + EK1 conditions.

**Appendix D-3.** The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K-12 employing the non-conjugative *Hemophilus* plasmid, pRSF0885, under BL1 conditions.

**Appendix D-4.** Permission is granted to clone certain subgenomic segments of foot and mouth disease virus in HV1 *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems under BL1 conditions at Genentech, Inc., South San Francisco, California.

**Appendix D-5.** Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

**Appendix D-6.** Permission is granted to clone in *E. coli* K-12 under BL1 physical containment conditions subgenomic segments of rift valley fever virus subject to conditions which have been set forth by the RAC.

**Appendix D-7.** Attenuated laboratory strains of *Salmonella typhimurium* may be used under BL1 physical containment conditions to screen for the *Saccharomyces cerevisiae* pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

**Appendix D-8.** Permission is granted to transfer certain clones of subgenomic segments of foot and mouth disease virus from Plum Island Animal Disease Center to the laboratories of Molecular Genetics, Inc., Minnetonka, Minnesota, and to work with these clones under BL1 containment conditions. Approval is contingent upon review of data on infectivity testing of the clones by a working group of the RAC.

**Appendix D-9.** Permission is granted to Dr. John Sanford of Cornell University to field test tomato and tobacco plants transformed with bacterial (*E.coli* K-12) and yeast DNA using pollen as a vector.

**Appendix D-10.** Permission is granted to Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to release under specified conditions *Pseudomonas syringae*, pathovars (pv.) *syringae,* and *Erwinia herbicola* carrying *in vitro* generated deletions of all or part of the genes involved in ice nucleation.

**Appendix D-11.** Agracetus of Middleton, Wisconsin, may field test under specified conditions disease resistant tobacco plants prepared by recombinant DNA techniques.

**Appendix D-12.** Eli Lilly and Company of Indianapolis, Indiana, may conduct large-scale experiments and production involving *Cephalosporium acremonium* strain LU4-79-6 under less than Biosafety Level 1 - Large Scale (BL1-LS) conditions.

**Appendix D-13.** Drs. W. French Anderson, R. Michael Blaese, and Steven Rosenberg of the NIH, Bethesda, Maryland, can conduct experiments in which a bacterial gene coding for neomycin phosphotransferase will be inserted into a portion of the tumor infiltrating lymphocytes (TIL) of cancer patients using a retroviral vector, N2. The marked TIL then will be combined with unmarked TIL, and reinfused into the patients. This experiment is an addition to an ongoing adoptive immunotherapy protocol in which TIL are isolated from a patient's tumor, grown in culture in the presence of interleukin-2, and reinfused into the patient. The marker gene will be used to detect TIL at various time intervals following reinfusion.

Approval is based on the following four stipulations: (I) there will be no limitation of the number of patients in the continuing trial; (ii) the patients selected will have a life expectancy of about 90 days; (iii) the patients give fully informed consent to participate in the trial; and (iv) the investigators will provide additional data before inserting a gene for therapeutic purposes. (Protocol #8810-001)

**Appendix D-14.** U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) may conduct certain experiments involving products of a yellow fever virus originating from the 17-D yellow fever clone at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

In addition, USAMRIID may conduct certain experiments involving vaccine studies of Venezuelan equine encephalitis virus at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

**Appendix D-15.** Drs. R. Michael Blaese and W. French Anderson of the NIH, Bethesda, Maryland, can conduct experiments in which a gene coding for adenosine deaminase (ADA) will be inserted into T lymphocytes of patients with severe combined immunodeficiency disease, using a retroviral vector, LASN. Following insertion of the gene, these T lymphocytes will be reinfused into the patients. The patients will then be followed for evidence of clinical improvement in the disease state, and measurement of multiple parameters of immune function by laboratory testing.

Approval is based on the following two stipulations: (I) that intraperitoneal administration of transduced T lymphocytes not be used before clearance by the Chair of the Recombinant DNA Advisory Committee; and (ii) that the number of research patients be limited to 10 at this time.

In addition to the conditions outlined in the initial approval, patients may be given a supplement of a CD-34+-enriched peripheral blood lymphocytes (PBL) which have been placed in culture conditions that favor progenitor cell growth. This enriched population of cells will be transduced with the retroviral vector, G1NaSvAd. G1NaSvAd is similar to LASN, yet distinguishable by PCR. LASN has been used to transduce peripheral blood T lymphocytes with the ADA gene. Lymphocytes and myeloid cells will be isolated from patients over time and assayed for the presence of the LASN or G1NaSvAd vectors. The primary objectives of this protocol are to transduce CD 34+ peripheral blood cells with the adenosine deaminase gene, administer these cells to patients, and determine if such cells can differentiate into lymphoid and myeloid cells *in vivo*. There is a potential for benefit to the patients in that these hematopoietic progenitor cells may survive longer, and divide to yield a broader range of gene-corrected cells. (Protocol #9007-002)

**Appendix D-16.** Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma who have failed all effective therapy. These patients will be treated with escalating doses of autologous tumor infiltrating lymphocytes (TIL) transduced with a gene coding for tumor necrosis factor (TNF). Escalating numbers of transduced TIL will be administered at three weekly intervals along with the administration of interleukin-2 (IL-2). The objective is to evaluate the toxicity and possible therapeutic efficacy of the administration of tumor infiltrating lymphocytes (TIL) transduced with the gene coding for TNF. (Protocol #9007-003)

**Appendix D-17.** Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on patients with acute myelogenous leukemia (AML). Using the LNL6 retroviral vector, the autologous bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of this gene marking experiment is to determine whether the source of relapse after autologous bone marrow transplantation for acute myelogenous leukemia is residual malignant cells in the harvested marrow or reoccurrence of tumor in the patient. Determining the source of relapse should indicate whether or not purging of the bone marrow is a necessary procedure. (Protocol #9102-004)

**Appendix D-18.** Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on pediatric patients with Stage D (disseminated) neuroblastoma who are being treated with high-dose carboplatin and etoposide in either phase I/II or phase II trials. All the patients in these studies will be subjected to bone marrow transplantation since it will allow them to be exposed to chemoradiation that would be lethal were it not for the availability of stored autologous marrow for rescue. The bone marrow cells of these patients will be transduced with the gene coding for neomycin resistance using the LNL6 vector. The purpose of this gene marking study is to determine whether the source of relapse after autologous bone marrow transplantation is residual malignant cells in the harvested marrow or residual disease in the patient. Secondly, it is hoped to determine the contribution of marrow autographs to autologous reconstitution. (Protocol #9105-005/9105-006)

**Appendix D-19.** Dr. Albert B. Deisseroth of the MD Anderson Cancer Center of Houston, Texas, can conduct experiments on patients with chronic myelogenous leukemia who have been reinduced into a second chronic phase or blast cells. The patients in these studies will receive autologous bone marrow transplantation. Using the LNL6 vector, the bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of these marking studies is to determine if the origin of relapse arises from residual leukemic cells in the patients or from viable leukemic cells remaining in the bone marrow used for autologous transplantation. (Protocol #9105-007)

**Appendix D-20.** Drs. Fred D. Ledley and Savio L. C. Woo of Baylor College of Medicine of Houston, Texas, can conduct experiments on pediatric patients with acute hepatic failure who are identified as candidates for hepatocellular transplantation. Using the LNL6 vector, the hepatocytes will be transduced with the gene coding for neomycin resistance. The purpose of using a genetic marker is to demonstrate the pattern of engraftment of transplanted hepatocytes and to help determine the success or failure of engraftment. (Protocol #9105-008)

**Appendix D-21.** Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma, renal cell cancer, and colon carcinoma who have failed all effective therapy. In an attempt to increase these patients' immune responses to the tumor, the tumor necrosis factor gene or the interleukin-2 gene will be introduced into a tumor cell line established from the patient. These gene-modified autologous tumor cells will then be injected into the thigh of the patient. To further utilize the immune system of the patient to fight the tumor, stimulated lymphocytes will be cultured from either the draining regional lymph nodes or the injected tumor itself. The patients will be evaluated for antitumor effects engendered by the injection of the gene modified tumor cells themselves as well as after the infusion of the cultured lymphocytes. (Protocol #9110-010/9110-011)

**Appendix D-22.** Dr. James M. Wilson of the University of Michigan Medical Center of Ann Arbor, Michigan, can conduct experiments on three patients with the homozygous form of familial hypercholesterolemia. Both children and adults will be eligible for this therapy. In an attempt to correct the basic genetic defect in this disease, the gene coding for the low-density lipoprotein (LDL) receptor will be introduced into liver cells taken from the patient. The gene-corrected hepatocytes will then be infused into the portal circulation of the patient through an indwelling catheter. The patients will be evaluated for engraftment of the these treated hepatocytes through a series of metabolic studies; three months after gene therapy, a liver biopsy will be taken and analyzed for the presence of recombinant derived RNA and DNA to document the presence of the gene coding for the normal LDL receptor. (Protocol #9110-012)

**Appendix D-23.** Dr. Michael T. Lotze of the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, can conduct experiments on 20 patients with metastatic melanoma who have failed conventional therapy. A gene transfer experiment will be performed, transducing the patients' tumor infiltrating lymphocytes (TILs) with the gene for neomycin resistance. Through the use of this gene marking technique, it is proposed to determine how long TIL cells can be detected *in vivo* in the peripheral blood of the patients, and how the administration of interleukin-2 and interleukin-4 affects localization and survival of TIL cells in tumor sites. (Protocol #9105-009)

**Appendix D-24.** Dr. Gary J. Nabel of the University of Michigan Medical School, Ann Arbor, Michigan, can conduct gene therapy experiments on twelve patients with melanoma or adenocarcinoma. Patient population will be limited to adults over the age of 18 and female patients must be postmenopausal or have undergone tubal ligation or orchiectomy. The patient's immune response will be stimulated by the introduction of a gene encoding for a Class I MHC protein, HLA-B7, in order to enhance tumor regression. DNA/liposome-mediated transfection techniques will be used to directly transfer this foreign gene into tumor cells. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. These experiments will be analyzed for their efficacy in treating cancer. (Protocol #9202-013)

**Appendix D-25.** Kenneth Cornetta of Indiana University, Indianapolis, Indiana, can conduct gene transfer experiments on up to 10 patients with acute myelogenous leukemia (AML) and up to 10 patients with acute lymphocytic leukemia (ALL). The patient population will be limited to persons between 18 and 65 years of age. Using the LNL-6 vector, autologous bone marrow cells will be marked with the neomycin resistance gene. Gene marked and untreated bone marrow cells will be reinfused at the time of bone marrow transplantation. Patients will then be monitored for evidence of the neomycin resistance gene in peripheral blood and bone marrow cells in order to determine whether relapse of their disease is a result of residual malignant cells remaining in the harvested marrow or inadequate ablation of the tumor cells by chemotherapeutic agents. Determining the source of relapse may indicate whether or not purging of the bone marrow is a necessary procedure for these leukemia patients. Further studies will be performed in order to determine the percentage of leukemic cells that contain the LNL-6 vector and the clonality of the marked cells. (Protocol #9202-014)

**Appendix D-26.** Dr. James S. Economou of the University of California, Los Angeles, can conduct gene transfer experiments on 20 patients with metastatic melanoma and 20 patients with renal cell carcinoma. These patients will be treated with various combinations of tumor-infiltrating lymphocytes and peripheral blood leukocytes, including CD8 and CD4 subsets of both types of cells. These effector cell populations will be given in combination with interleukin-2 (IL-2) in the melanoma patients and IL-2 plus alpha interferon in the renal cell carcinoma patients. The effector cells will be transduced with the neomycin resistance gene using either the LNL6 or G1N retroviral vectors. This "genetic marking" of the tumor-infiltrating lymphocytes and peripheral blood lymphocytes is designed to answer questions about the trafficking of these cells, their localization to tumors, and their *in vivo* life span. (Protocol #9202-015)

**Appendix D-27.** Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 15 human immunodeficiency virus (HIV) seropositive patients (18-45 years old) undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma and 15 HIV-seropositive patients (18-50 years old) who do not have acquired immunodeficiency syndrome (AIDS)-related lymphoma and who are not undergoing bone marrow transplantation to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV-TK) gene. This vector will deliver both a marker gene and an ablatable gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of *in vivo* survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells. (Protocol #9202-017)

**Appendix D-28.** Dr. Malcolm Brenner of St. Jude Children's Research Hospital, Memphis, Tennessee, can conduct gene therapy experiments on twelve patients with relapsed/refractory neuroblastoma who have relapsed after receiving autologous bone marrow transplant. In an attempt to stimulate the patient's immune response, the gene coding for Interleukin-2 (IL-2) will be used to transduce tumor cells, and these gene-modified cells will be injected subcutaneously in a Phase 1 dose escalation trial. Patients will be evaluated for an anti-tumor response. (Protocol #9206-018)

**Appendix D-29.** Drs. Edward Oldfield, Kenneth Culver, Zvi Ram, and R. Michael Blaese of the National Institutes of Health, Bethesda, Maryland, can conduct gene therapy experiments on ten patients with primary malignant brain tumors and ten patients with lung cancer, breast cancer, malignant melanoma, or renal cell carcinoma who have brain metastases. The patient population will be limited to adults over the age of 18.

Patients will be divided into two groups based on the surgical accessibility of their lesions. Both surgically accessible and surgically inaccessible lesions will receive intra-tumoral injections of the retroviral Herpes simplex thymidine kinase (HS-tk) vector-producer cell line, G1TkSvNa, using a guided stereotaxic approach. Surgically accessible lesions will be excised seven days after stereotaxic injection, and the tumor bed will be infiltrated with the HS-tk producer cells. The removed tumor will be evaluated for the efficiency of transduction. Ganciclovir (GCV) will be administered beginning on the fifth postoperative day. In the case of surgically inaccessible lesions, the patients will receive intravenous therapy with GCV seven days after receiving the intra-tumoral injections of the retroviral HS-tk vector-producer cells. (Protocol #9206-019)

**Appendix D-30.** Dr. Albert D. Deisseroth of MD Anderson Cancer Center, Houston, Texas, can conduct gene transfer experiments on ten patients who have developed blast crisis or accelerated phase chronic myelogenous leukemia (CML). The retroviral vectors G1N and LNL6 which code for neomycin resistance will be used to transduce autologous peripheral blood and bone marrow cells that have been removed and stored at the time of cytogenetic remission or re-induction of chronic phase in Philadelphia chromosome positive CML patients. Following reinduction of the chronic phase of CML and preparative chemotherapy, patients will be infused with the transduced autologous cells.

This protocol is designed to determine the cause of relapse of CML. If polyclonal CML neomycin marked blastic cells appear at the time of relapse, their presence will indicate that relapse arises from the leukemic CML blast cells present in the autologous cells infused following chemotherapy. If residual systemic disease contributes to relapse, the neomycin resistance gene will not be detected in the CML leukemic blasts at the time of relapse.

This study will compare the relative contributions of the peripheral blood and bone marrow to generate hematopoietic recovery after bone marrow transplantation and evaluate purging and selection of peripheral blood or bone marrow as a source of stem cells for transplant. The percentage of neomycin resistant CML cells which are leukemic will be determined by PCR analysis and detection of bcr-abl mRNA. (Protocol #9206-020)

**Appendix D-31.** Dr. Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, can conduct gene transfer experiments on up to 48 patients with multiple myeloma, breast cancer, or chronic myelogenous leukemia. The retroviral vectors G1N and LNL6 will be used to transfer the neomycin resistance marker gene into autologous bone marrow and peripheral blood stem cells in the presence of growth factors to examine hematopoietic reconstitution after bone marrow transplantation. The efficiency of transduction of both short and long term autologous bone marrow reconstituting cells will be examined.

Autologous bone marrow and CD34+ peripheral blood stem cells will be enriched prior to transduction. Myeloma and CML patients will receive both autologous bone marrow and peripheral blood stem cell transplantation. These separate populations will be marked with both the G1N and LNL6 retroviral vectors. If short and long term marking experiments are successful, important information may be obtained regarding the biology of autologous reconstitution, the feasibility of retroviral gene transfer into hematopoietic cells, and the contribution of viable tumor cells within the autograft to disease relapse. (Protocol #9206-023/9206-024/9206-025)

**Appendix D-32.** Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with metastatic melanoma who are HLA-A2 positive and who have failed conventional therapy. This is a phase I study to examine whether allogeneic HLA-A2 matched melanoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By

allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-021)

**Appendix D-33.** Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with renal cell carcinoma who are HLA-A2 positive and who have failed conventional therapy. This Phase I study will examine whether allogeneic HLA-A2 matched renal cell carcinoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-022)

**Appendix D-34.** Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, can conduct experiments on twenty patients with metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer, or colon cancer who have failed standard therapy. Patients will receive multiple subcutaneous injections of autologous tumor cells combined with an autologous fibroblast cell line that has been transduced *in vitro* with the gene coding for Interleukin-4 (IL-4) to augment the *in vivo* antitumor effect. Patients will be monitored for antitumor effect by PCR analysis and multiple biopsy of the injection site. (Protocol #9209-033)

**Appendix D-35.** Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 10 patients per year will be enrolled in the studies over a period of four years. Patients will undergo autologous bone marrow transplantation with a selected population of Interleukin-3 (IL-3) or granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) peripheral blood repopulating cells (PBRC) that have been transduced with the gene coding for neomycin resistance ($neo^R$) using the retroviral vector, LN. Patients will be continuously monitored for $neo^R$ to determine the relative contribution of autologous PBRCs to long-term hematopoietic reconstitution. Demonstration of long-term contribution of autologous PBRC to hematopoiesis will enable the use of PBRC alone for autologous transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-027/9209-028)

**Appendix D-36.** Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 5 patients per year will be enrolled in the study over a period of four years. Patients will undergo allogeneic bone marrow transplant with granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) PBRC harvested from an identical twin that have been transduced with $neo^R$ using the retroviral vector, LN. Patients will be continuously monitored for $neo^R$ to determine the relative contribution of G-CSF stimulated allogeneic PBRCs to long-term bone marrow engraftment. Demonstration of long-term contribution of allogeneic PBRC to hematopoiesis will enable the use of PBRC alone for allogeneic transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-029)

**Appendix D-37.** Dr. Malcolm K. Brenner of St. Jude Children's Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, can conduct a multicenter uncontrolled human gene transfer experiment on 12 patients ≤ 21 years of age with Stage D Neuroblastoma in first or second marrow remission. Autologous bone marrow cells will be separated into two fractions, purged and unpurged. Each fraction will be transduced with the $neo^R$ gene by either LNL6 or G1Na. Patients will be monitored by the polymerase chain reaction (PCR) for the presence of $neo^R$. The protocol is designed to evaluate the safety and efficacy of the Neuroblastoma Bone Marrow Purging System following high dose chemotherapy. (Protocol #9209-032)

**Appendix D-38.** Drs. Carolyn Keierleber and Ann Progulske-Fox of the University of Florida, Gainesville, Florida, can conduct experiments involving the introduction of a gene coding for tetracycline resistance into *Porphyromonas gingivalis* at a physical containment level of Biosafety Level-2 (BL-2).

**Appendix D-39.** Dr. Scott M. Freeman of Tulane University Medical Center, New Orleans, Louisiana, can conduct experiments on patients with epithelial ovarian carcinoma who have clinical evidence of recurrent, progressive, or residual disease who have no other therapy available to prolong survival. Patients will be injected intraperitoneally with the irradiated PA-1 ovarian carcinoma cell line which has been transduced with

the herpes simplex thymidine kinase (HSV-TK) gene. The patients will then receive ganciclovir therapy. Previous, data indicates that HSV-TK+ tumor cells exhibit a killing effect on HSV-TK- cells when exposed to ganciclovir therapy. Patients will be evaluated for safety and side effects of this treatment. (Protocol #9206-016)

**Appendix D-40.** Dr. Michael J. Welsh, Howard Hughes Medical Institute Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, may conduct experiments on 3 cystic fibrosis (CF) patients ≥ 18 years of age with mild to moderate disease. This Phase I study will determine the: (1) *in vivo* safety and efficacy of the administration of the replication-deficient type 2 adenovirus vector, Ad2/CFTR-1, to the nasal epithelium; (2) efficacy in correcting the CF chloride transport defect *in vivo*; and (3) effect of adenovirus vector dosage on safety and efficacy. (Protocol #9212-036)

**Appendix D-41.** Dr. Ronald G. Crystal, National Institutes of Health, Bethesda, Maryland, may conduct experiments on 10 cystic fibrosis (CF) patients ≥ 21 years of age. Patients will receive an initial administration of the replication-deficient type 5 adenovirus vector, AdCFTR, to their left nares. If no toxicity is observed from intranasal administration, patients will receive a single administration of AdCFTR to the respiratory epithelium of their left large bronchi. Five groups of patients (2 patients per group) will be studied based on increased dosage administration of AdCFTR. This study will determine the: (1) *in vivo* safety and efficacy of the administration of AdCFTR into the respiratory epithelium; (2) efficacy of the correction of the biologic abnormalities of CF in the respiratory epithelium; (3) duration of the biologic correction; (4) efficacy of the correction of the abnormal electrical potential difference of the airway epithelial sheet; (5) clinical parameters relevant to the disease process; and (6) if humoral immunity develops against AdCFTR sufficient to prevent repeat administration. (Protocol #9212-034)

**Appendix D-42.** Dr. Kenneth Culver, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John Van Gilder, University of Iowa, Iowa City, Iowa, may conduct experiments on 15 patients ≥ 18 years of age with recurrent malignant primary brain tumors or lung, melanoma, renal cell carcinoma, or breast carcinoma brain metastases who have failed standard therapy for their disease. Patient eligibility will be limited to those patients who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies. The number of patients treated will be equally divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

Following surgical debulking, patients will receive a maximum of 3 intralesional injections of the G1TkSvNa vector- producing cell line (VPC) to induce regression of residual tumor cells by ganciclovir (GCV) therapy. Patients who demonstrate stable disease for a minimum of 6 months following this treatment will be eligible for additional VPC injections and subsequent GCV therapy. (Protocol #9303-037)

**Appendix D-43.** Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients ≥ 1 year and ≤ 21 years of age at the time of initial diagnosis of acute myelogenous leukemia (AML). The investigators will use the two retroviral vectors, LNL6 and G1Na, to determine the efficacy of the bone marrow purging techniques: 4-hydroxyperoxicyclophosphamide and interleukin-2 (IL-2) activation of endogenous cytotoxic effector cells, in preventing relapse from the reinfusion of autologous bone marrow cells. (Protocol #9303-039)

**Appendix D-44.** Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney, St Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments of 35 patients ≤ 21 years of age who will be recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts for leukemia. In this Phase I dose escalation study, spontaneous lymphoblastoid cell lines will be established that express the same range of Epstein-Barr Virus (EBV) encoded proteins as the recipient. These EBV-specific cell lines will be transduced with LNL6 or G1Na and readministered at the time of bone marrow transplant. This study will determine: (1) survival and expansion of these EBV-specific cell lines *in vivo*, (2) the ability of these adoptively transferred cells to confer protection against EBV infection, and (3) appropriate dosage and administration schedules. (Protocol #9303-038)

**Appendix D-45.** Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., Gaithersburg, Maryland, may conduct experiments on 15 cystic fibrosis (CF) patients who have mild to moderate disease ≥ 21 years of age. The replication-deficient type 5 adenovirus vector, Av1CF2, will be administered to the nasal and lobar bronchial respiratory tract of patients. This study will demonstrate the: (1) expression of normal cystic fibrosis transmembrane conductance regulator

(CFTR) mRNA *in vivo*, (2) synthesis of CFTR protein, and (3) correction of epithelial cell cAMP dependent $Cl^-$ permeability. The pharmacokinetics of CFTR expression and ability to re-infect the respiratory tract with AvCF2 will be determined. Systemic and local immunologic consequences of Av1CF2 infection, the time of viral survival, and potential for recombination or complementation of the virus will be monitored. (Protocol #9303-041)

**Appendix D-46.** Dr. James M. Wilson of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct experiments on 20 adult patients with advanced cystic fibrosis lung disease. An isolated segment of the patients' lung will be transduced with the E1 deleted, replication-incompetent adenovirus vector, Ad.CB-CFTR using a bronchoscope for gene delivery. Ad.CB-CFTR contains the human gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Pulmonary biopsies will be obtained by bronchoscopy at 4 days, 6 weeks, and 3 months following treatment. Patients will be monitored for evidence of CFTR gene transfer and expression, immunological responses to CFTR or adenovirus proteins, and toxicity. (Protocol #9212-035)

**Appendix D-47.** Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina, may conduct experiments on 20 patients with disseminated malignant melanoma. Autologous tumor cells will be transduced with a retroviral vector, pHuγ-IFN, that contains the gene encoding human γ-IFN. Following lethal irradiation, the transduced cells will be readministered to patients for the purpose of generating cytotoxic T cells that are tumor specific along with the up-regulation of Class I major histocompatibility antigens. Patients will be monitored for clinical regression of tumors and generation of tumor-specific cytotoxic T lymphocytes. (Protocol #9306-043)

**Appendix D-48.** Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of the Children's Hospital of Los Angeles, Los Angeles, California, may conduct experiments on 10 patients with Gaucher disease. CD34(+) hematopoietic stem cells will be isolated from bone marrow or from peripheral blood treated with granulocyte-colony stimulating factor. CD34(+) cells will be transduced with a retrovirus vector, G1Gc, containing cDNA encoding human glucocerebrosidase and administered intravenously. Patients will be monitored for toxicity and glucocerebrosidase expression. (Protocol #9306-047)

**Appendix D-49.** Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 12 patients with AIDS to be divided into 4 experimental groups. CD4(+) lymphocytes will be isolated from peripheral blood and transduced with Rev M10, a transdominant inhibitory mutant of the *rev* gene of the human immunodeficiency virus (HIV). Transduction of the *rev* mutant will be mediated either by the retrovirus vector, PLJ-cREV M10, or by particle-mediated gene transfer of plasmid DNA. Patients will be monitored for survival of the transduced CD4(+) cells by polymerase chain reaction and whether Rev M10 can confer protection against HIV infection to CD4(+) cells. (Protocol #9306-049)

**Appendix D-50.** Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 24 patients with advanced cancer. Patients will undergo *in vivo* transduction with DNA/liposome complexes containing genes encoding the HLA-B7 histocompatibility antigen and beta-2 microglobulin in a non-viral plasmid. These DNA/liposome complexes will be administered either by intratumoral injection or catheter delivery. Patients will be monitored for enhanced immune responses against tumor cells, and safe and effective doses will be determined. (Protocol #9306-045)

**Appendix D-51.** Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct experiments on 5 patients with Gaucher disease. The CD34(+) hematopoietic stem cells will be isolated from peripheral blood and transduced *in vitro* with the retrovirus vector, N2-Sv-GC, encoding the glucocerebrosidase (GC) enzyme. Following reinfusion of the transduced cells, patients will be monitored by PCR analysis for GC expression in peripheral blood leukocytes. Patients currently receiving GC replacement therapy and who demonstrate clinical responsiveness will be withdrawn from exogenous GC therapy. Patients not previously treated with exogenous GC, will be monitored for clinical reversal of lipid storage symptoms. (Protocol #9306-046)

**Appendix D-52.** Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 12 HIV-infected patients who have a seronegative identical twin. CD4(+) and CD8(+) cells will be isolated from the seronegative twin and induced to polyclonal proliferation with anti-CD3 and interleukin-2.

The enriched population of cells will be transduced with either LNL6 or G1Na, which contain the $neo^R$ gene. The transduced cells will be expanded in tissue culture and administered to the HIV-infected twin. Patients will be monitored for immune function and the presence of marked cells. (Protocol #9209-026)

**Appendix D-53.** Dr. Corey Raffel of the Children's Hospital Los Angeles, Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, may conduct experiments on 30 patients between 2 and 18 years of age with recurrent malignant astrocytoma. Fifteen patients will be accrued into this study initially. If at least one patient responds to therapy, an additional 14 patients will be treated. Patients with either surgically accessible or non-accessible tumors will be treated with the vector producing cell line (PA317) carrying the retrovirus vector, G1TkSvNa. This vector will transduce tumor cells *in vivo* with the *Herpes simplex* thymidine kinase (HS-tk) gene that renders the cells sensitive to killing by ganciclovir. Surgically accessible patients will undergo surgical debulking of their tumor followed by repeated administration of the HS-tk vector producer cells into the tumor bed. Children with unresectable tumors will undergo stereotaxic injection of vector producer cells into tumors. (Protocol #9306-050)

**Appendix D-54.** Dr. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, may conduct experiments on 15 HIV(+) asymptomatic patients. Patients will receive 3 monthly intramuscular injections of the retrovirus vector (N2IIIBenv) encoding the HIV-1 IIIB envelope protein. Patients will be monitored for acute toxicity, CD4 levels, HIV-specific CTL responses, and viral burdens. (Protocol #9306-048)

**Appendix D-55.** Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York, may conduct experiments on 20 patients with advanced breast, ovary, and brain cancer. CD34(+) hematopoietic stem cells will be isolated from bone marrow, transduced with the retrovirus vector, PHaMDR1/A, and readministered to patients. Patients will be monitored for the presence and expression of the MDR-1 gene. The investigators will determine whether MDR-1 expression increases following chemotherapy. (Protocol #9306-051)

**Appendix D-56.** Dr. Enzo Paoletti of Virogenetics Corporation, Troy, New York, may conduct experiments with poxvirus vectors NYVAC, ALVAC, and TROVAC at Biosafety Level 1.

**Appendix D-57.** Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, may conduct experiments on 9 patients (18 years old or greater) with cystic fibrosis to test for the safety and efficacy of an E1-deleted recombinant adenovirus containing the cystic fibrosis transmembrane conductance regulator (CFTR) cDNA, Ad.CB-CFTR. A single dose of $10^8$, $3 \times 10^9$ or $10^{11}$ pfu/ml will be administered to the nasal cavity of 3 patients in each dose group. Patients will be monitored by nasal lavage and biopsy to assess safety and restoration of normal epithelial function. (Protocol #9303-042)

**Appendix D-58.** Dr. Joyce A. O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 18 patients (18-60 years old) with Stage IV breast cancer who have achieved a partial or complete response to induction chemotherapy. This study will determine the feasibility of obtaining engraftment of CD34(+) hematopoietic stem cells transduced by a retroviral vector, G1MD, and expressing a cDNA for the human multi-drug resistance-1 (MDR-1) gene following high dose chemotherapy, and whether the transduced MDR-1 gene confers drug resistance to hematopoietic cells and functions as an *in vivo* dominant selectable marker. Patients will be monitored for evidence of myeloprotection and presence of the transduced MDR-1 gene." (Protocol #9309-054)

**Appendix D-59.** Drs. Larry E. Kun, R. A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients (3-21 years old) with progressive or recurrent malignant supratentorial tumors resistant to standard therapies. Mouse cells producing the retroviral vector containing the herpes simplex thymidine kinase gene (G1TKSVNa) will be instilled into the tumor areas via multiple stereotactically placed cannulas. Patients will be treated with ganciclovir to eliminate cells expressing the transduced gene. Patients will be monitored for central nervous system, hematologic, renal or other toxicities, and for anti-tumor responses by magnetic resonance imaging studies. (Protocol #9309-055)

**Appendix D-60.** The physical containment level may be reduced from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system of Life Technologies, Inc., Gaithersburg, Maryland.

**Appendix D-61.** Dr. Albert B. Deisseroth of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 10 patients (≥ 16 to ≤ 60 years of age) with chronic lymphocytic leukemia. Autologous peripheral blood and bone marrow cells will be removed from patients following chemotherapy and marked by transduction with two distinguishable retroviral vectors, G1Na and LNL6, containing the neomycin resistance gene. The gene marked cells will be reinfused into patients to determine the efficiency of bone marrow purging and the origin of relapse following autologous bone marrow transplantation. (Protocol #9209-030)

**Appendix D-62.** Dr. Jonathan Simons of the Johns Hopkins Oncology Center, Baltimore, Maryland, may conduct experiments on 26 patients (≥ 18 years of age) with metastatic renal cell carcinoma to evaluate the safety and tolerability of intradermally injected autologous irradiated tumor cells transduced with the retrovirus vector, MFG, containing the human granulocyte-macrophage colony stimulating factor gene. Acute and long-term clinical toxicities and *in vitro* and *in vivo* induction of specific anti-tumor immune responses will be monitored. (Protocol #9303-040)

**Appendix D-63.** Dr. Albert B. Deisseroth of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 20 patients (≥ 18 and ≤ 60 years old) with ovarian cancer. A murine viral vector was constructed from the third generation of L series retroviruses with the insert of the human multi-drug resistance-1 (MDR-1) transduced gene. The investigators will assess the safety and feasibility of administering CD34 (+) autologous peripheral blood and bone marrow cells. Patients will be monitored for the presence of the MDR-1 gene and for the effect of gene transfer on hematopoietic function following the transplantation. (Protocol #9306-044)

**Appendix D-64.** Dr. Joseph Ilan of the Case Western Reserve University School of Medicine and University Hospital of Cleveland, Cleveland, Ohio, may conduct experiments on 12 patients (≥ 18 years of age) with advanced brain cancer. Human malignant glioma tumor cells will be cultured, transfected with Epstein-Barr virus (EBV)-based vector, anti-Insulin growth factor-I, lethally irradiated, and injected subcutaneously into patients in an attempt to express antisense Insulin growth factor-1. Patients will be monitored for toxicity and immunologic responses to the vector. (Protocol #9306-052)

**Appendix D-65.** Drs. James S. Economou and John Glaspy of the University of California, Los Angeles, California, may conduct experiments on 30 patients (≥ 18 to ≤ 70 years of age) with metastatic melanoma. A human melanoma cell line (M-24) will be transduced with the retroviral vector, G1NaCvi2, expressing the human interleukin-2 (IL-2) gene. The IL-2 producing cells will be mixed with the patient's autologous tumor cells, irradiated, and injected subcutaneously in an attempt to enhance the tumor-specific immunologic response. Patients will be monitored for toxicity, *in vitro* and *in vivo* immunologic responses, and clinical anti-tumor effects. (Protocol #9309-058)

**Appendix D-66.** Drs. Peter Cassileth, Eckhard R. Podack, and Kasi Sridhar of the University of Miami, and Niramol Savaraj of the Miami Veterans Administration Hospital, Miami, Florida, may conduct experiments on 12 patients (≥ 18 years of age) with limited stage small cell lung cancer. Autologous tumor cells will be removed, expanded in culture, and transduced by lipofection with the BMG-Neo-hIL2 vector (derived from bovine papilloma virus). The objective of this protocol is to demonstrate the safety and efficacy of administering IL-2 transduced autologous tumor cells in an attempt to stimulate a tumor-specific cytotoxic T lymphocyte (CTL) response, and to determine the quantity and characteristics of the CTL that have been generated. (Protocol #9309-053)

**Appendix D-67.** Drs. Edward H. Oldfield and Zvi Ram of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 20 patients (≥ 18 years of age) with leptomeningeal carcinomatosis. The patients will receive intraventricular or subarachnoid injection of murine vector producing cells containing the retroviral vector, G1Tk1SvNa. Tumor cells expressing the herpes simplex thymidine kinase gene will be rendered sensitive to killing by subsequent administration of ganciclovir. Patients will be monitored for safety and anti-tumor response by magnetic resonance imaging (MRI) and cerebral spinal fluid cytological analysis. (Protocol #9312-059)

**Appendix D-68.** Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Chicago, Illinois, may conduct experiments on 12 subjects who differ in at least 3 out of 6 alleles at the Class I histocompatibility locus (≥ 18 years of age) with Stage IV malignant melanoma. The subjects will be immunized with a lethally irradiated allogeneic human melanoma cell line transduced with the human

interleukin-2 expressing retroviral vector, pZipNeoSv-IL-2. Subjects will be monitored for toxicity, induction of B and T cell antitumor responses *in vitro* and *in vivo,* and any clinical evidence of antitumor effect. (Protocol #9309-056)

**Appendix D-69A.** Dr. Michael J. Welsh of the Howard Hughes Medical Institute, Iowa City, Iowa, may conduct experiments on 20 patients (≥ 18 years of age) with cystic fibrosis. The investigator will administer increasing doses of either of the two adenovirus vectors, AD2/CFTR-1 or AD2-ORF6/PGK-CFTR, to the nasal epithelium of 10 patients (1 nostril) or maxillary sinus epithelium of 10 patients (1 maxillary sinus). Patients will be isolated for a period of 24 hours following vector administration; however, if 1 patient demonstrates secreted virus at 24 hours, the investigator will notify the Recombinant DNA Advisory Committee for reconsideration of the isolated period. Patients will be assessed for the safety and efficacy of multiply administration of adenovirus vectors encoding the cystic fibrosis transmembrane conductance regulator (CFTR) gene. (Protocol #9312-067)

**Appendix D-69B.** Dr. Richard Haubrich of the University of California at San Diego Treatment Center, San Diego, California, may conduct experiments on 25 human immunodeficiency virus (HIV)-infected, seropositive, asymptomatic subjects (≥ 18 to ≤ 65 years of age). Subjects will receive 3 monthly intramuscular injections of the retroviral vector, N2/IIIB *env/rev*, which encodes for HIV-1 IIIB *env/rev* proteins. The objective of the study is to induce HIV-1- specific CD8(+) cytotoxic T lymphocyte and antibody responses in order to eliminate HIV-infected cells and residual virus. This Phase I/II study will evaluate acute toxicity, identify long-term treatment effects, and evaluate the disease progression. (Protocol #9312-062)

**Appendix D-70.** Dr. Mario Sznol of the National Institutes of Health, Frederick, Maryland, may conduct experiments on 50 subjects (≥ 18 years of age) with advanced stage melanoma. Subjects will receive subcutaneous injections of lethally irradiated allogeneic melanoma cells that have been transduced by lipofection with the plasmid DNA vector, CMV-B7, derived from bovine papilloma virus to express the human B7 antigen. The B7 antigen, which binds to the CD28 receptor of T cells, will serve as a co-stimulatory signal to elicit an antitumor immune response. Subjects will be monitored for induction of cytotoxic T lymphocyte, antitumor responses *in vitro* and *in vivo* and any clinical evidence of antitumor effect. (Protocol #9312-063)

**Appendix D-71.** Dr. Joseph Rubin of the Mayo Clinic, Rochester, Minnesota, may conduct experiments on 15 subjects with hepatic metastases from advanced colorectal cancer (≥ 18 years of age). Subjects will receive intratumoral hepatic injections of the plasmid DNA/lipid complex, pHLA-B7/β-2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and β-2 microglobulin. Subjects must be HLA-B7 negative. The objective of this study is to determine a safe and effective dose of the DNA/lipid complex. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9312-064)

**Appendix D-72.** Dr. Nicholas J. Vogelzang of the University of Chicago Medical Center, Chicago, Illinois, may conduct experiments on 15 subjects with metastatic renal cell carcinoma ≥ 18 years of age. Subjects will receive intratumoral injections of the plasmid DNA/liposome vector pHLA-B7/β-2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and β-2 microglobulin. Subjects must be HLA-B7 negative. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9403-071)

**Appendix D-73.** Dr. Evan M. Hersh of the Arizona Cancer Center and Drs. Emmanuel Akporiaye, David Harris, Alison T. Stopeck, Evan C. Unger, and James A. Warneke of the University of Arizona, Tucson, Arizona, may conduct experiments on 15 subjects with metastatic malignant melanoma ≥ 18 years of age. Subjects will receive intratumoral injections of the plasmid DNA/liposome vector, pHLA-B7/β-2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and β-2 microglobulin. Subjects must be HLA-B7 negative. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9403-072)

**Appendix D-74.** Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas, may conduct gene marking experiments on 9 subjects with ovarian carcinoma or peritoneal carcinomatosis (≥ 16 years of age). Autologous CD3(+)/CD8(+) tumor infiltrating lymphocyte derived T cells will be transduced with the retroviral vector G1Na that encodes for $neo^R$. Subjects will receive intraperitoneal administration of bulk expanded transduced and nontransduced T cells and recombinant interleukin-2. Previously documented tumor sites and normal tissues will be monitored for $neo^R$ and the proportion of CD3(+)/CD8(+) T cells will be determined. (Protocol #9406-075)

**Appendix D-75.** Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct gene marking experiments on 20 subjects undergoing autologous bone marrow transplantation for therapy of leukemia or solid tumor (< 21 years of age). The distinguishable retroviral vectors, LNL6 and G1Na (both encoding $neo^R$), will be used to determine the rate of reconstitution of untreated versus cytokine expanded CD34(+) selected autologous bone marrow cells. (Protocol #9406-076)

**Appendix D-76.** Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 10 fully evaluable subjects (maximum of 20 entered) with Stage III or IV breast cancer (≥ 18 and ≤ 60 years of age). Subjects will receive autologous CD34(+) peripheral blood cells that have been transduced with the retroviral vector, pVMDR-1, which encodes the multi-drug resistance gene. The objective of this study is to evaluate the safety and feasibility of transducing early hematopoietic progenitor cells with pVMDR-1 and to determine *in vivo* selection of chemotherapy resistant hematopoietic cells. (Protocol #9406-077)

**Appendix D-77.** Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients with Fanconi anemia (≥ 5 years of age). Subjects will receive autologous CD34(+) cells that have been transduced with the retroviral vector, FACC, which encodes the normal Fanconi anemia complementation group C gene. The objective of this study is to determine whether autologous FACC transduced hematopoietic progenitor cells can be safely administered to subjects, the extent of engraftment, and correction of cell phenotype. (Protocol #9406-078)

**Appendix D-78.** Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, may conduct experiments on 15 subjects with recurrent residual glioblastoma multi-forme (≥ 18 years of age). Subjects will receive subcutaneous injections of autologous tumor cells that have been lethally irradiated and transduced with the retroviral vector, G1NaCvi2.23, which encodes for interleukin-2. Subjects will be monitored *in vitro* for cellular and humoral antitumor responses and *in vivo* for antitumor activity. (Protocol #9406-080)

**Appendix D-79.** Dr. Alfred E. Chang of the University of Michigan Medical Center, Ann Arbor Michigan, may conduct gene marking experiments on 15 subjects with metastatic melanoma (≥ 18 years of age). Subjects will undergo adoptive immunotherapy of anti-CD3/interleukin-2 activated lymph node cells that have been primed *in vivo* with tumor cells that have been transduced with the retrovirus vector, GBAH4, encoding the gene for interleukin-4. The investigator will evaluate the antitumor efficacy and *in vivo* immunological reactivity in subjects receiving adoptively transferred T cells, and the *in vitro* immunological reactivities of the activated T cells that might correlate with their *in vivo* antitumor function. (Protocol #9312-065)

**Appendix D-80.** Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct gene marking experiments on 40 HIV(+) subjects (≥ 18 years of age). The investigator may also enter an additional number of subjects (to be determined by the investigator) who will receive a single administration of $1 \times 10^7$ HIV-specific CD8(+) cells. The investigator will: (1) Assess the safety and tolerance of the adoptive transfer of anti-HIV cytotoxic, syngeneic, CD8(+) peripheral blood lymphocytes that have been transduced with the retrovirus vector, r*kat*4svgF3e-, that encodes for a universal chimeric T cell receptor. (2) Determine the longevity of the genetically marked CD8(+) lymphocytes in the subject's peripheral blood. (Protocol #9403-069)

**Appendix D-81.** Dr. Joseph Rosenblatt of the University of California, Los Angeles, California, and Dr. Robert Seeger of Children's Hospital, Los Angeles, California, may conduct gene transfer experiments on 18 subjects with neuroblastoma (≤ 21 years of age). Patients at high risk of relapse with minimal or no detectable disease following myeloablative therapy and autologous bone marrow transplantation, or patients with progressive or persistent disease despite conventional therapy will be serially immunized with autologous or allogeneic neuroblastoma cells transduced to express γ interferon. Cells will be transduced with the retroviral vector, pHuγ-IFN, encoding the human gene for γ interferon and lethally irradiated prior to use as an immunogen. The objectives of the study are: (1) to determine the maximum tolerable dose of transduced cells; (2) to determine the local, regional, and systemic toxicities of injected cells; and (3) to determine the antitumor response *in vivo* as measured by standard clinical tests and immunocytologic evaluation of marrow metastases. (Protocol #9403-068)

**Appendix D-82.** Dr. Kenneth L. Brigham of Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 subjects (≤ 21 years of age) in two different patient protocols (5 for each protocol). Both protocols will use the same DNA/liposome preparations to deliver a plasmid DNA construct, pCMV4-AAT, encoding human alpha-1 antitrypsin gene driven by a cytomegalovirus promoter. In patients scheduled for elective pulmonary resection, the DNA/liposome complexes will be instilled through a fiber optic bronchoscope into a subsegment of the lung. Tissues of the lung will be obtained at the time of surgery. Transgene expression will be assessed by immunohistochemistry, *in situ* hybridization, and Western and Northern blot analyses. The effect of DNA/liposome complex administration on the histological appearance of the lung will also be evaluated. In patients with alpha-1 antitrypsin deficiency, the DNA/liposome complexes will be instilled into the nostril. Transgene expression will be determined in cells obtained by nasal lavage and nasal scraping, and the time course of transgene expression will be measured. The secretion of the alpha-1 antitrypsin protein in nasal fluid will be determined. Histological appearance of nasal mucosa will also be examined. The study will assess safety and feasibility of gene delivery to the human respiratory tract. (Protocol #9403-070)

**Appendix D-83.** Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 20 subjects with refractory or recurrent metastatic breast cancer (≥ 18 years of age). Autologous breast cancer cells will be transduced with the DNA/liposome complex, pMP6-IL2, containing a plasmid DNA vector derived from adeno-associated virus (AAV) that expresses the gene for human interleukin-2. Subjects will receive 4 subcutaneous injections of lethally irradiated tumor cells transduced with the DNA/liposome complex prior to injection. The objective of this study is to: (1) evaluate the safety and toxicity of the treatment, (2) determine the immunological effects, (3) determine the duration of clinical responses, and (4) measure patient survival. (Protocol #9409-086)

**Appendix D-84.** Drs. Flossie Wong-Staal, Eric Poeschla, and David Looney of the University of California at San Diego, La Jolla, California, may conduct gene transfer experiments on 6 subjects (≥ 18 and ≤ 65 years of age) infected with human immunodeficiency virus-1 (HIV-1). Autologous CD4(+) T lymphocytes will be transduced *ex vivo* with the retroviral vector, pMJT, expressing a hairpin ribozyme that cleaves the HIV-1 RNA in the 5' leader sequence. The transduced cells will be expanded and reinfused into the patients. The objectives of the study are: (1) to evaluate safety of reinfusing the transduced lymphocytes, (2) to compare (*in vivo*) the kinetics and survival of ribozyme-transduced cells with that of cells transduced with a control vector, (3) to determine *in vivo* expression of the ribozyme sequences in transduced lymphocytes, (4) to determine whether host immune responses directed against the transduced cells will occur *in vivo*, and (5) to obtain preliminary data on the effects of ribozyme gene therapy on *in vivo* HIV mRNA expression, viral burden and CD4(+) lymphocyte levels. (Protocol #9309-057)

**Appendix D-85.** Dr. Friedrich Schuening of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 10 subjects (≥ 18 years of age) with Type I Gaucher's disease. The peripheral blood repopulating cells (mobilized by patient pretreatment with recombinant granulocyte colony-stimulating factor) will be harvested and CD34(+) cells selected. CD34(+) cells will be transduced *ex vivo* with the retroviral vector, LgGC, that encodes human glucocerebrosidase cDNA. Following transduction, the transduced cells will be infused into the patient without myeloablative treatment. The primary endpoint of this study is to examine the safety of infusing CD34(+) cells transduced with the human glucocerebrosidase cDNA. Patients will be monitored for persistence and expression of the glucocerebrosidase gene in hematopoietic cells. (Protocol #9312-061)

**Appendix D-86.** Dr. Terence R. Flotte of the Johns Hopkins Children's Center, Baltimore, Maryland, may conduct gene transfer experiments on 16 subjects (≥ 18 years of age) with mild cystic fibrosis (CF). An adeno-associated virus (AAV) derived vector, encoding cystic fibrosis transmembrane conductance regulator (CFTR) gene, (tgAAVCF), will be administered to nasal (direct) and airway (bronchoscope) epithelial cells. This is a dose escalation study involving 8 cohorts. Each subject will receive both intranasal and bronchial administration of the adenoviral vector at 4 escalating doses. Nasal doses will range between $1 \times 10^6$ and $1 \times 10^9$ pfu. Lung administration will range between $1 \times 10^7$ and $1 \times 10^{10}$ pfu. The primary goal of the study is to assess the safety of vector administration. Respiratory and nasal epithelial cells will be evaluated for gene transfer, expression, and physiologic correction. (Protocol #9409-083)

**Appendix D-87.** Drs. Jeffrey M. Isner and Kenneth Walsh of St. Elizabeth's Medical Center, Tufts University, Boston, Massachusetts, may conduct gene transfer experiments on 12 subjects (≥ 40 years of age) with peripheral artery disease (PAD). A plasmid DNA vector, phVEGF165, encoding the human gene for vascular endothelial growth factor (VEGF) will be used to express VEGF to induce collateral neovascularization.

Percutaneous arterial gene transfer will be achieved using an angioplasty catheter with a hydrogel coated balloon to deliver the plasmid DNA vector to the artery. The objectives of the study are: (1) to determine the efficacy of arterial gene therapy to relieve rest pain and/or heal ischemic ulcers of the lower extremities in patients with PAD; and (2) to document the safety of the phVEGF arterial gene therapy for therapeutic angiogenesis. Subjects will undergo anatomic and physiologic examination to determine the extent of collateral artery development following phVEGF arterial gene therapy. (Protocol #9409-088)

**Appendix D-88A.** Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 26 patients (≥ 15 years of age) with cystic fibrosis (CF). A replication deficient recombinant adenovirus vector will be used to transduce epithelial cells of the large bronchi with the E1/E3 deleted type 5 adenovirus vector, $Ad_{GV}$CFTR.10, which encodes the human cystic fibrosis transmembrane conductance regulator (CFTR) gene. The objective of this study is to define the safety and pharmacodynamics of CFTR gene expression in airway epithelial cells following single administration of escalating doses to the vector. If single administration is determined to be safe, subjects will undergo repeat administration to localized areas of the bronchi. (Protocol #9409-085)

**Appendix D-88B.** Drs. Eric J. Sorscher and James L. Logan of the University of Alabama, Birmingham, Alabama, may conduct gene transfer experiments on 9 subjects (≥18 years of age) with cystic fibrosis (CF). The normal human cystic fibrosis transmembrane conductance regulator (CFTR) gene will be expressed by a plasmid DNA vector, pKCTR, driven by the simian virus-40 (SV40) early gene promoter. The CFTR DNA construct will be delivered by cationic liposome-based gene transfer to nasal epithelial cells. The objectives of the study are to: (1) evaluate the safety of lipid-mediated gene transfer to nasal epithelial cells (including local inflammation and mucosal tissue); and (2) evaluate efficacy as determined by correction of the chloride ion transport defect, and wild-type CFTR mRNA and protein expression. (Protocol #9312-066)

**Appendix D-89.** Dr. Steven M. Albelda of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 12 subjects with advanced mesothelioma. The adenovirus vector encoding the *Herpes simplex* virus thymidine kinase (HSV-TK) gene, H5.020RSVTK, will be administered through a chest tube to the pleural cavity. Tumor biopsies will be assayed for gene transfer and expression. Subjects will be monitored for immunological responses to the adenovirus vector. Ganciclovir will be administered intravenously 14 days following vector administration. The primary objective of this Phase I study is to evaluate the safety of direct adenovirus vector gene delivery to the pleural cavity of patients with malignant melanoma. (Protocol #9409-090)

**Appendix D-90.** Drs. Jeffrey Holt and Carlos B. Arteaga of the Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 female patients (over 18 years of age) with metastatic breast cancer. Patient effusions from pleura or peritoneum will be drained and the fluid will be replaced with supernatant containing the retroviral vectors, XM6:antimyc or XM6:antifos, which express c-*myc* and c-*fos* antisense sequences, respectively, under the control of a mouse mammary tumor virus promoter. The objectives of this study are to: (1) assess uptake and expression of the vector sequences in breast cancer cells present in pleural and peritoneal fluids, and determine if this expression is tumor specific, (2) assess the safety of localized administration of antisense retroviruses, and (3) monitor subjects for clinical evidence of antitumor response. (Protocol #9409-084)

**Appendix D-91.** Dr. Jack A. Roth of MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 14 non-small cell lung cancer subjects (≥ 18 and ≤ 80 years of age) who have failed conventional therapy and who have bronchial obstruction. LNSX-based retroviral vectors containing the β-actin promoter will be used to express: (1) the antisense RNA of the K-*ras* oncogene (LN-K-*ras*B), and (2) the wildtype *p53* tumor suppressor gene (LN*p53*B). Tumor biopsies will be obtained to characterized K-*ras* and *p53* mutations. Relative to their specific mutation, subjects will undergo partial endoscopic resection of the tumor bed followed by bronchoscopic administration of the appropriate retrovirus construct. The objective of this study is to evaluate the safety and efficacy of intralesional administration of LN-K-*ras*B and LN*p53* retrovirus constructs. (Protocol #9403-031)

**Appendix D-92.** Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, may conduct gene transfer experiments on 12 subjects (≥ 18 years of age) with metastatic colon carcinoma. The autologous skin fibroblasts will be transduced with the retroviral vector, LNCX/IL-2, which encodes the gene for human interleukin-2 (IL-2). In this dose-escalation study, subjects will receive

subcutaneous injections of lethally irradiated autologous tumor cells. The objectives of the study are to: (1) evaluate the safety of subcutaneous administration of LNCX/IL-2 transduced fibroblasts, (2) determine *in vivo* antitumor activity, and (3) monitor cellular and humoral antitumor responses. (Protocol #9312-060)

**Appendix D-93.** Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct gene transfer experiments on 18 subjects (≥ 18 years of age) with advanced melanoma, 6 with T-cell lymphoma, breast cancer, or head and neck cancer. Subjects should have accessible cutaneous tumors, and have failed standard therapy. Over 4 weeks, subjects will receive a total of 4 intratumoral injections of autologous fibroblasts transduced with the retrovirus vector, TFG-hIL-12-Neo. This vector, which consists of the murine MFG backbone, expresses both the p35 and p40 subunits of interleukin-12 (IL-12) and the *neo*$^{R}$ selection marker. The objectives of the study are to: (1) define the local and systemic toxicity associated with peritumoral injections of gene-modified fibroblasts, (2) examine the local and systemic immunomodulatory effects of these injections, and (3) evaluate clinical antitumor efficacy. (Protocol #9406-081)

**Appendix D-94.** Drs. Evan Hersh, Emmanuel Akporiaye, David Harris, Alison Stopeck, Evan Unger, James Warneke, of the Arizona Cancer Center, Tucson, Arizona, may conduct gene transfer experiments on 25 subjects (≥ 18 years of age) with solid malignant tumors or lymphomas. A plasmid DNA/lipid complex designated as VCL-1102 (IL-2 Plasmid DNA/DMRIE/DOPE) will be used to transduce the human gene for interleukin-2 (IL-2). Patients with advanced cancer who have failed conventional therapy will undergo a procedure in which VCL-1102 is injected directly into the tumor mass to induce tumor-specific immunity. The objectives of the study are to: (1) determine safety and toxicity associated with escalating doses of VCL-1102; (2) confirm IL-2 expression in target cells; (3) determine biological activity and pharmacokinetics; and (4) determine whether IL-2 expression stimulates tumor regression in subjects with metastatic malignancies. (Protocol #9412-095)

**Appendix D-95.** Drs. Richard Morgan and Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiments on 48 human immunodeficiency virus (HIV) seropositive subjects (≥ 18 years of age). This Phase I/II study involves identical twins (one HIV seropositive and the other HIV seronegative). CD4(+) T cells will be enriched following apheresis of the HIV seronegative twin, induced to polyclonal proliferation with anti-CD3 and recombinant IL-2, transduced with either the LNL6/Neo$^{R}$ or G1Na/Neo$^{R}$, and transduced with up to 2 additional retroviral vectors (G1RevTdSN and/or GCRTdSN(TAR)) containing potentially therapeutic genes (antisense TAR and/or transdominant Rev). These T cell populations will be expanded 10 to 1,000 fold in culture for 1 to 2 weeks and reinfused into the HIV seropositive twin. Subjects will receive up to 4 cycles of treatment using identical or different combinations of control and anti-HIV retrovirus vectors. The relative survival of these transduced T cell populations will be monitored by vector-specific polymerase chain reaction, while the subjects' functional immune status is monitored by standard *in vitro* and *in vivo* assays. (Protocol #9503-103)

**Appendix D-96.** Dr. Harry L. Malech of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiments on 2 subjects ≥ 18 years of age (with or without concurrent serious infection), and 3 subjects ≥ 18 years of age (with or without concurrent serious infection) or minors 13-17 years of age who have concurrent serious infection who have chronic granulomatous disease (CGD). CGD is an inherited immune deficiency disorder in which blood neutrophils and monocytes fail to produce antimicrobial oxidants (p47$^{phox}$ mutation) resulting in recurrent life-threatening infections. Subjects will undergo CD34(+) mobilization with granulocyte colony stimulating factor (G-CSF). These CD34(+) cells will be transduced with the retrovirus vector, MFG-S-p47$^{phox}$, which encodes the gene for normal p47$^{phox}$. The objectives of this study are to: (1) determine the safety of administering MFG-S-p47$^{phox}$ transduced CD34(+) cells, and (2) demonstrate increased functional oxidase activity in circulating neutrophils. (Protocol #9503-104)

**Appendix D-97.** Drs. Chris Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct gene transfer experiments on 6 subjects (≥ 18 and ≤ 76 years of age) with rheumatoid arthritis. Rheumatoid arthritis is a chronic, progressive disease thought to be of autoimmune origin. A gene encoding an interleukin-1 receptor antagonist protein (IRAP) will be delivered to the rheumatoid metacarpal-phalangeal joints to determine the autoimmune reactions can be interrupted. The vector construct, DFG-IRAP, is based on the MFG murine retrovirus vector backbone, and encodes the human IRAP gene. Synovial fibroblasts will be generated from the rheumatoid arthritic joint tissue obtained from patients who are scheduled to undergo surgery. The fibroblasts will be transduced with the DFG-IRAP vector, and the transduced cells injected into the synovial space. The synovial fluid and joint material will be collected 7 days later to determine the presence and location of the transduced synovial fibroblasts and the level of IRAP in the joint fluid. (Protocol 9406-074)

**Appendix D-98.** Dr. R. Scott McIvor of the University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on 2 children with purine nucleoside phosphorylase (PNP) deficiency. PNP deficiency results in severe T-cell immunodeficiency, an autosomal recessive inherited disease which is usually fatal in the first decade of life. Autologous peripheral blood lymphocytes will be cultured in an artificial capillary cartridge in the presence of anti-CD3 monoclonal antibody and interleukin-2 and transduced with the retroviral vector, LPNSN-2, encoding human PNP. Subjects will undergo bimonthly intravenous administration of transduced T cells for a maximum of 1 year. The objectives of the study are to determine: (1) the safety of intravenous administration of transduced T cells in children with PNP deficiency, (2) the efficiency of PNP gene transfer and duration of gene expression *in vivo*, and (3) the effect of PNP gene transfer on immune function. (Protocol #9506-110)

**Appendix D-99.** Drs. Nikhil C. Munshi and Bart Barlogie of the University of Arkansas School for Medical Sciences, Little Rock, Arkansas, may conduct gene transfer experiments on 21 subjects (>18 and <65 years of age) with relapsed or persistent multiple myeloma who are undergoing T cell depleted allogeneic bone marrow transplantation. Donor peripheral blood lymphocytes will be cultured in vitro with interleukin-2 and anti-CD3 monoclonal antibody. T cell depleted lymphocytes will be transduced with the retroviral construct, G1Tk1SvNa.7, which encodes the Herpes simplex virus thymidine kinase (HSV-TK) gene. The transduced cells will be reinfused. In this dose escalation study, 3 subjects will undergo cell-mediated gene transfer per cohort (maximum of 5 cohorts) until Grade III or IV Graft versus Host Disease (GVHD) is observed. A maximum of 6 additional patients may be entered at that maximum tolerated dose. The objectives of this study are to determine the: (1) safety of transduced donor cell infusions, (2) effectiveness of donor cell infusions in decreasing the effects of severe GVHD, (3) effectiveness of donor cell infusions in prolonging multiple myeloma remission, and (4) effectiveness of ganciclovir in eliminating donor cells for the purpose of preventing the depletion of erythrocytes. (Protocol #9506-107)

**Appendix D-100.** Dr. Wayne A. Marasco of Dana-Farber Cancer Institute, Boston, Massachusetts, may conduct gene transfer experiments on 6 subjects (≥ 18 and ≤ 65 years of age) with human immunodeficiency virus type-1 (HIV-1). Autologous lymphocytes from asymptomatic subjects will be transduced *ex vivo* with a retroviral vector, LNCs105, encoding the sFv105 antibody specific for the HIV-1 envelope protein. An identical aliquot will be simultaneously transduced with a control retroviral vector lacking the sFv105 cassette. Transduced cells will be reinfused into patients and the differential survival of both populations of CD4+ lymphocytes compared. The objective of the study is to determine whether the intracellular expression of a human single chain antibody against HIV-1 envelope glycoprotein gp160 that blocks gp160 processing and the production of infectious virions can safely prolong the survival of CD4(+) lymphocytes in HIV-1-infected subjects. (Protocol #9506-111)

**Appendix D-101.** Dr. Henry Dorkin of the New England Medical Center, Boston, Massachusetts, and Dr. Allen Lapey of Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, propose to conduct gene transfer experiments on 16 subjects (≥ 18 years of age). An E1/partial E4-deleted, replication-deficient, type 2 adenovirus vector, AD2/CFTR-2, will be used to deliver the human cystic fibrosis transmembrane conductance regulator (CFTR) gene by aerosol administration (nebulization) to the lung of CF patients. Aerosol administration will be initiated only after initial safety data has been obtained from the lobar administration protocol (#9409-091). This is a single administration dose-escalation study in which subjects will receive between $8 \times 10^6$ and $2.5 \times 10^{10}$ pfu. Subjects will be assessed for evidence of adverse, systemic, immune, inflammatory, or respiratory effects in response to AD2/CFTR-2. Subjects will be monitored for virus shedding and transgene expression. Health care workers present in the facility will be required to sign an Informed Consent document regarding the possibility of virus transmission. (Protocol #9412-074)

**Appendix D-102.** Drs. Charles J. Link and Donald Moorman of the Human Gene Therapy Research Institute, Des Moines, Iowa, may conduct gene transfer experiments on 24 female subjects (≥ 18 years of age) with refractory or recurrent ovarian cancer. Subjects will undergo intraperitoneal delivery (via Tenkhoff catheter) of the vector producing cells (VPC), PA317/LTKOSN.2. These VPC express the *Herpes simplex* virus thymidine kinase (HSV-TK) gene which confers sensitivity to killing by the antiviral drug, ganciclovir (GCV). The LTKOSN.2 retrovirus vector is based on the LXSN backbone. Two weeks following intraperitoneal delivery of the VPC, subjects will receive 5 mg/kg intravenous GCV twice daily for 14 days. Subjects will receive between $1 \times 10^5$ and $1 \times 10^8$ VPC/kg in this dose escalation study. Subjects will be evaluated by X-ray and peritoneoscopy of the abdomen for evidence of clinical response. The objectives of this study are to determine the safety of intraperitoneal VPC administration. (Protocol #9503-100)

**Appendix D-103.** Dr. David T. Curiel of the University of Alabama, Birmingham, Alabama, may conduct gene transfer experiment of 15 subjects (≥ 18 years of age) with metastatic colorectal cancer. Subjects will receive intramuscular injection of the polynucleotide vaccine, pGT63, which is a plasmid DNA vector expressing carcinoembryonic antigen (CEA) and hepatitis B surface antigen (HBsAg). The objectives of the study are to: (1) characterize the immune response to CEA and HBsAg following a single intramuscular injection and following 3 consecutive intramuscular injections, and (2) determine the safety of intramuscular injection of the plasmid DNA vector at doses ranging between 0.1 to 1.0 milligrams (single dose) and 0.9 to 3.0 milligrams (total multidose). (Protocol #9506-073)

**Appendix D-104.** Dr. Chester B. Whitley of the University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on two adult subjects (18 years of age or older) with mild Hunter syndrome (Mucopolysaccharidosis Type II). The autologous peripheral blood lymphocytes will be transduced *ex vivo* with the retroviral vector, L2SN, encoding the human cDNA for iduronate-2-sulfatase (IDS). The transduced lymphocytes will be reinfused into the patients on a monthly basis. The study will determine the frequency of peripheral blood lymphocyte transduction and the half-life of the infused cells. Evaluation of patients will include measurement of blood levels of IDS enzyme, assessment of metabolic correction by urinary glycosaminoglycan levels, clinical response of the disease, and monitoring for potential toxicity. This Phase I study is to demonstrate the safety of the L2SN-mediated gene therapy and to provide a preliminary evaluation of clinical efficacy. (Protocol #9409-087)

**Appendix D-105.** Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California, may conduct gene transfer experiments on 25 subjects (≥ 18 years of age) with metastatic melanoma. The protocol is an open label, Phase I trial to evaluate the safety and immunological effects of administering lethally irradiated allogeneic and autologous melanoma cells transduced with the retroviral vector, IL-7/HyTK, which encodes the gene for human interleukin-7 (IL-7). Subjects will receive 1 x $10^7$ irradiated unmodified autologous tumor cells in combination with escalating doses of IL-7/HyTK transduced allogeneic melanoma cells (M24 cell line). The number of M24 cells administered will be adjusted based on the level of IL-7 expression. Subjects will receive 3 biweekly subcutaneous injections of M24 cells expressing 10, 100, or 1000 nanograms of IL-7/hour *in vivo*. A final cohort of 5 subjects will receive IL-7/HyTK transduced autologous cells. Subjects will be monitored for antitumor activity by skin tests, biopsy analysis, tumor-specific antibody activity, and cytotoxic T lymphocyte precursor evaluation. Non-immunologic parameters will also be monitored. (Protocol #9503-101)

**Appendix D-106.** Dr. Jack A. Roth, MD Anderson Cancer Center, may conduct gene transfer experiments on 42 subjects (≥ 18 years of age) with refractory non-small cell lung cancer (NSCLC). Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human *p53* tumor suppressor gene. The E1 region of AD5CMV-p53 has been replaced with a *p53* expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) 21 subjects will receive Ad5CMV-p53 alone, and (2) 21 subjects will receive Ad5CMV-p53 in combination with cisplatin. Following vector administration, subjects will be isolated for 96 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of this study are determine: (1) the maximum tolerated dose of AD5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutants using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer > 5 x $10^{10}$) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc (control adenovirus vector containing the luciferase gene) to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9406-079)

**Appendix D-107A.** Dr. Gary Clayman. M.D. Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 21 subjects (≥ 18 years of age) with refractory squamous cell carcinoma of the head and neck. Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human *p53* tumor suppressor gene. The E1 region of AD5CMV-p53 has been replaced with a *p53* expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) those with non-resectable tumors, and (2) those with surgically accessible tumors. Subjects will receive multiple injections of vector in each dose-escalation cohort. Following vector administration, subjects will be isolated for 48 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of the study are to determine: (1) the maximum tolerated dose of AD5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutants using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer > $5x10^{10}$) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9412-096)

**Appendix D-107B.** Drs. Bernard A. Fox and Walter J. Urba of Earle A. Chiles Research Institute, Providence Medical Center, Portland, Oregon, may conduct gene transfer experiments on 18 subjects (≥ 18 years of age) with metastatic renal cell carcinoma or melanoma. Autologous tumor cells will be surgically removed, transduced *in vitro* with the cationic liposome plasmid vector, VCL-1005, which encodes human leukocyte antigen (HLA)-B7 and beta-2 microglobulin. Subjects will receive subcutaneous injection of lethally irradiated transduced cells in one limb. The contralateral limb will be injected with lethally irradiated untransduced tumor cells in combination with Bacille Calmette-Guerin (BCG). Approximately 21 days following tumor cell injection, subjects will undergo lymphadenectomy for subsequent in vitro expansion of anti-CD3 activated lymphocytes. Activated lymphocytes will be adoptively transferred on approximately day 35 in combination with a 5-day course of interleukin-2 (IL-2). On approximately day 45, subjects will receive a second cycle of IL-2. The objectives of this study are to determine: (1) the safety of administering anti-CD3 activated antitumor effector T cells in draining lymph nodes, and (2) whether HLA-B7/β-2 gene transfer augments the sensitization of anti-tumor effector T-cells in draining lymph nodes. (Protocol 9506-108)

**Appendix D-108.** Dr. Mitchell S. Steiner, University of Tennessee, Memphis, Tennessee, and Dr. Jeffrey T. Holt, Vanderbilt University School of Medicine, Nashville, Tennessee, may conduct gene transfer experiments on 15 male subjects (35 to 75 years of age) with metastatic prostate cancer. Malignant cells obtained from advanced prostate cancer subjects have been demonstrated to express high levels of the protooncogene c-myc *in vivo*. The mouse mammary tumor virus (MMTV) long terminal repeat (LTR) is expressed at high levels in prostate tissue. Following removal of malignant cells via biopsy, subjects will receive a single transrectal ultrasound-guided intraprostate quadrant injection of the retrovirus vector, XM6:MMTV-antisense c-myc, for 4 consecutive days at the site of the original biopsy. The objectives of this Phase I study are to: (1) quantitatively assess the uptake and expression of XM6:MMTV-antisense c-myc by prostate cancer cells *in vivo*, (2) determine whether c-myc gene expression is prostate tumor-specific, (3) assess safety of intraprostate injection of XM6:MMTV-antisense c-myc, and (4) biologic efficacy (antisense inhibition of tumor growth). (Protocol #9509-123)

**Appendix D-109.** Drs. Ronald G. Crystal, Edward Hershowitz, and Michael Lieberman, New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 18 subjects (18 to 70 years of age) with metastatic colon carcinoma with liver metastases. In this Phase I dose-escalation study, subjects will receive computed tomography (CT)-guided intratumoral injections of the adenovirus vector, $Ad_{GV}$CD.10, into the same hepatic metastasis in 4 equal volumes (100 microliters), each with a separate entry into the liver. This dosage schedule will be performed on Days 1 and 7. 5-fluorocytosine (200 milligrams/ kilogram/24 hours) will be administered orally in 4 equal doses starting on day 2 and continuing through the time of laparotomy. The objectives of this study are to: (1) determine the dose-dependent toxicity of direct

administration of $Ad_{GV}CD.10$ to hepatic metastases combined with oral administration of 5-fluorocytosine, (2) quantitatively assess transfer and expression of the cytosine deaminase gene in target cells, and (3) determine the biologic effects of direct $AD_{GV}CD.10$ administration to hepatic metastases. (Protocol #9509-125)

**Appendix D-110.** Drs. Andres Berchuck and H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 18 subjects (≥ 18 years of age) with refractory metastatic ovarian cancer. Autologous tumor cells obtained from ascites or surgically removed tumor will be transduced with the cationic liposome vector, PMP6A-IL2, that contains an adeno-associated virus derived plasmid DNA, a cytomegalovirus (CMV) promoter, and interleukin-2 (IL-2) complementary DNA (cDNA). In this dose-escalation study, subjects will undergo 4 cycles of intradermal injections (thigh or abdomen) of *ex vivo* transduced, lethally irradiated tumor cells in an attempt to induce an antitumor response. The objectives of the study are to evaluate: (1) the safety of intradermally injected transduced cells, and (2) antitumor response following therapy. (Protocol #9506-110)

**Appendix D-111.** Drs. Stephen L. Eck and Jane B. Alavi of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 18 subjects (>18 years of age) with malignant glioma. The adenovirus vector encoding the *Herpes simplex* virus thymidine kinase (HSV-TK) gene, H5.020RSVTK, will be injected by a stereotactic guided technique into brain tumors. Afterwards, the patients will receive systemic ganciclovir (GCV) treatment. Patients eligible to undergo a palliative debulking procedure will receive the same treatment followed by resection on day 7, and a second dose of the vector intra-operatively. Brain tissues removed by resection will be analyzed for adenovirus infection, transgene expression, and signs of inflammation. The size and metabolic activity of tumors will be monitored by scanning with magnetic resonance imaging and positron emission tomography. The objective of the study is to evaluate the overall safety of this treatment and to gain insight into the parameters that may limit the general applicability of this approach. (Protocol #9409-089)

**Appendix D-112.** Drs. Robert Grossman and Savio Woo of the Baylor College of Medicine & Methodist Hospital, Houston, Texas, may conduct gene transfer experiments on 20 subjects (≥ 18 years of age) with refractive central nervous system malignancies. Subjects will receive stereotaxic injections of a replication-defective, type 5 E1/E3-deleted adenovirus vector, ADV/RSV-tk, to deliver the *Herpes simplex* virus thymidine kinase (HSV-TK) gene to tumor cells. Expression of the HSV-TK gene is driven by a Rous sarcoma virus long terminal repeat (RSV-LTR). Subjects will receive a single time-course of intravenous ganciclovir (GCV) (14 consecutive days) following vector administration. Following demonstration of safety with the initial starting dose of $1 \times 10^8$ particles in 5 subjects, additional cohorts will receive between $5 \times 10^8$ and $1.5 \times 10^9$ particles. Each cohort will be monitored for toxicity for one month before administration of the next higher dose to subsequent cohorts. Subjects will be monitored for evidence of clinical efficacy by magnetic resonance imaging and/or computer tomography scans. The primary objective of this Phase I study is to determine the safety of vector administration. (Protocol #9412-098)

**Appendix D-113.** Drs. Gabriel N. Hortobagyi, Gabriel Lopez-Berestein, and Mien-Chie Hung, of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on a maximum of 24 adult patients (12 for each cancer) with metastatic breast or ovarian carcinoma. Overexpression of the HER-2/*neu* oncogene occurs in 30% of ovarian and breast cancers, and it is associated with enhanced metastatic potential, drug resistance, and poor survival. The E1A gene of the adenovirus type 5 functions as a tumor suppressor gene when transfected into cancer cells which overexpress the HER-2/*neu* oncogene. E1A expression induces down regulation of the level of the HER-2/*neu* oncoprotein by a transcriptional control mechanism. A plasmid, pE1A, encoding the adenovirus E1A gene with its own promoter will be administered as a DNA/lipid complex via the intraperitoneal or intrapleural route. The objectives of the study are: (1) to determine E1A gene transduction into malignant cells after the administration of E1A/lipid complex by intrapleural or intraperitoneal administration, (2) to determine whether E1A gene therapy can down-regulate HER-2/*neu* expression after intrapleural or intraperitoneal administration, (3) to determine the maximum biologically active dose (MBAD), or the maximum tolerated dose (MTD) of E1A/lipid complex, (4) to determine the toxicity and tolerance of E1A/lipid complex administered into the pleural or peritoneal space, and to assess the reversibility of such toxicity, and (5) to evaluate tumor response. (Protocol #9512-137)

**Appendix D-114.** Drs. Keith L. Black and Habib Fakhrai of the University of California, Los Angeles, California, may conduct gene transfer experiments on 12 subjects (≥ 18 years of age) with glioblastoma multiform. An Epstein-Barr virus (EBV) based plasmid vector, pCEP-4/TGF- β2 antisense, encoding antisense RNA will be used to inhibit TGF- β2 production. Tumor samples obtained from the patients at the time of clinically indicated

surgery will be grown in culture to establish a cell line for each patient. The patients' tumor cells will be genetically altered with the pCEP-4/TGF-β2 vector to inhibit their secretion of TGF-β. Following completion of the traditional post surgical radiation therapy, the first cohort of patients will receive, at 3 week intervals, 4 injections of 5 x $10^6$ irradiated gene modified autologous tumor cells. Subsequently, in dose escalation studies, the second cohort will receive 1 x $10^7$ cells, and the third cohort, 2 x $10^7$ cells. The results of this Phase I trial will be used to assess the safety of this form of gene therapy and may provide preliminary data to evaluate the potential utility of TGF- β2 antisense gene therapy in the management of gliomas. (Protocol #9512-138)

**Appendix D-115.** Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on a total of 21 (with an option for an additional 5) normal males and female subjects, age ≥ 18 years. Replication-deficient adenovirus (Ad) vector previously has been used in a number of human gene therapy strategies to transfer genes *in vivo* for therapeutic purposes. The purpose of this protocol is to characterize the local (skin), systemic (blood), and distant compartment (lung) immunity in normal individuals after intradermal administration of a replication deficient Ad5-based vector, named $Ad_{GV}$CD.10, carrying the gene coding for the *E. coli* enzyme, cytosine deaminase (CD). Following intradermal administration of the vector to normal individuals, the skin, blood, and lung immune responses to the Ad vector and CD transgene will be evaluated over time. This vector has been safety administered intrahepatically ten times to five individuals with colon carcinoma. No adverse effects in Protocol #9509-125 have been observed. The present protocol will yield insights into normal human immune responses to both the Ad vector, as well as to a heterologous (i.e., non-human) gene product (CD). Note: This study is designed to answer basic biological questions regarding characterization of the immune responses to such vectors that have been previously documented. (Protocol #9701-171)

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## APPENDIX E. CERTIFIED HOST-VECTOR SYSTEMS (See Appendix I, Biological Containment)

While many experiments using *Escherichia coli* K-12, *Saccharomyces cerevisiae*, and *Bacillus subtilis* are currently exempt from the *NIH Guidelines* under Section III-F, *Exempt Experiments*, some derivatives of these host-vector systems were previously classified as Host-Vector 1 Systems or Host-Vector 2 Systems. A listing of those systems follows:

### Appendix E-I. *Bacillus subtilis*

### Appendix E-I-A. *Bacillus subtilis* Host-Vector 1 Systems

The following plasmids are accepted as the vector components of certified *B. subtilis* systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of Host-Vector 1 systems based on these plasmids.

### Appendix E-I-B. *Bacillus subtilis* Host-Vector 2 Systems

The asporogenic mutant derivative of *Bacillus subtilis*, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

### Appendix E-II. *Saccharomyces cerevisiae*

### Appendix E-II-A. *Saccharomyces cerevisiae* Host-Vector 2 Systems

The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: Ylp1, YEp2, YEp4, Ylp5, YEp6, YRp7, YEp20, YEp21, YEp24, Ylp25, Ylp26, Ylp27, Ylp28, Ylp29, Ylp30, Ylp31, Ylp32, and Ylp33.

## Appendix E-III. *Escherichia coli*

### Appendix E-III-A. *Escherichia coli* (EK2) Plasmid Systems

The *Escherichia coli* K-12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, and pHB1. The following *Escherichia coli*/S. cerevisiae hybrid plasmids are certified as EK2 vectors when used in *Escherichia coli* chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3, and SHY4: YIpI, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEP24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

### Appendix E-III-B. *Escherichia coli* (EK2) Bacteriophage Systems

The following are certified EK2 systems based on bacteriophage lambda:

| Vector | Host |
|---|---|
| λgt *WES*λB' | DP50*sup*F |
| λgt *WES*λB* | DP50*sup*F |
| λgt Z*J vir*λB' | *Escherichia coli* K-12 |
| λgtALO·λB | DP50*sup*F |
| Charon 3A | DP50 or DP50*sup*F |
| Charon 4A | DP50 or DP50*sup*F |
| Charon 16A | DP50 or DP50*sup*F |
| Charon 21A | DP50*sup*F |
| Charon 23A | DP50 or DP50*sup*F |
| Charon 24A | DP50 or DP50*sup*F |

*Escherichia coli* K-12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50*sup*F provided that the *su*-strain not be used as a propagation host.

## Appendix E-IV. *Neurospora crassa*

### Appendix E-IV-A. *Neurospora crassa* Host-Vector 1 Systems

The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

In1 (inositol-less) strains 37102, 37401, 46316, 64001, and 89601. Csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

## Appendix E-V. *Streptomyces*

### Appendix E-V-A. *Streptomyces* Host-Vector 1 Systems

The following *Streptomyces* species: *Streptomyces coelicolor, S. lividans, S. parvulus*, and *S. griseus*. The following are accepted as vector components of certified *Streptomyces* Host-Vector 1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

## Appendix E-VI. *Pseudomonas putida*

### Appendix E-VI-A. *Pseudomonas putida* Host-Vector 1 Systems

*Pseudomonas putida* strains KT2440 with plasmid vectors pKT262, pKT263, and pKT264.

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## APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

### Appendix F-I. General Information

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an $LD_{50}$ of < 100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under Section III-B-1 (*Experiments Involving the Cloning of Toxin Molecules with $LD_{50}$ of Less than 100 Nanograms Per Kilogram Body Weight*) and require Institutional Biosafety Committee and NIH/OBA approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an $LD_{50}$ ≥ 100 micrograms per kilograms of body weight. Experiments involving genes coding for toxin molecules with an $LD_{50}$ of < 100 micrograms per kilograms and > 100 nanograms per kilograms body weight require Institutional Biosafety Committee approval and registration with NIH/OBA prior to initiating the experiments. A list of toxin molecules classified as to $LD_{50}$ is available from NIH/OBA. Testing procedures for determining toxicity of toxin molecules not on the list are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). The results of such tests shall be forwarded to NIH/OBA, which will consult with *ad hoc* experts, prior to inclusion of the molecules on the list (see Section IV-C-1-b-(2)-(c), *Minor Actions*).

### Appendix F-II. Cloning of Toxin Molecule Genes in *Escherichia coli* K-12

**Appendix F-II-A.** Cloning of genes coding for molecules toxic for vertebrates that have an $LD_{50}$ of >100 nanograms per kilograms and <1000 nanograms per kilograms body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under Biosafety Level (BL) 2 + EK2 or BL3 + EK1 containment conditions.

**Appendix F-II-B.** Cloning of genes for the biosynthesis of molecules toxic for vertebrates that have an $LD_{50}$ of >1 microgram per kilogram and <100 microgram per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordetella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

**Appendix F-II-C.** Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *Escherichia coli*, *Klebsiella*, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *Escherichia coli* and of *Yersinia enterocolitica*.

### Appendix F-III. Cloning of Toxic Molecule Genes in Organisms Other Than *Escherichia coli* K-12

Requests involving the cloning of genes coding for toxin molecules for vertebrates at an $LD_{50}$ of <100 nanograms per kilogram body weight in host-vector systems other than *Escherichia coli* K-12 will be evaluated by NIH/OBA in consultation with *ad hoc* toxin experts (see Sections III-B-1, *Experiments Involving the Cloning of Toxin Molecules with $LD_{50}$ of Less than 100 Nanograms Per Kilogram Body Weight*, and IV-C-1-b-(2)-(c), *Minor Actions*).

### Appendix F-IV. Specific Approvals

An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an $LD_{50}$ of <100 nanograms per kilogram body weight is available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

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## APPENDIX G. PHYSICAL CONTAINMENT

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large-scale (over 10 liters) research or production, Appendix K (*Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*) supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P (*Physical and Biological Containment for Recombinant DNA Research Involving Plants*) supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q (*Physical and Biological Containment for Recombinant DNA Research Involving Animals*) supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4 - Animals.

### Appendix G-I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see Appendices G-III-A through G-III-J, *Footnotes and References of Appendix G*). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV-B-1-h, *Responsibilities of the Institution--General Information*, and IV-B-7-d, *Responsibilities of the Principal Investigator Prior to Initiating Research*). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV-B-7-d, *Responsibilities of the Principal Investigator Prior to Initiating Research* and IV-B-7-e, *Responsibilities of the Principal Investigator During the Conduct of the Research*). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see Section IV-B-1-f, *Responsibilities of the Institution--General Information*).

The *Laboratory Safety Monograph* (see Appendix G-III-O, *Footnotes and References of Appendix G*) and *Biosafety in Microbiological and Biomedical Laboratories* (see Appendix G-III-B, *Footnotes and References of Appendix G*) describe practices, equipment, and facilities in detail.

### Appendix G-II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see Appendix G-III-B, *Footnotes and References of Appendix G*). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see Appendix G-III-C, *Footnotes and References of Appendix G*).

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The *NIH Guidelines*, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of

primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given to other combinations which achieve an equivalent level of containment (see Sections IV-C-1-b-(1), *Major Actions* and IV-C-1-b-(2), *Minor Actions*).

**Appendix G-II-A. Biosafety Level 1 (BL1)** (See Appendix G-III-M, *Footnotes and References of Appendix G*)

**Appendix G-II-A-1. Standard Microbiological Practices (BL1)**

**Appendix G-II-A-1-a.** Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

**Appendix G-II-A-1-b.** Work surfaces are decontaminated once a day and after any spill of viable material.

**Appendix G-II-A-1-c.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-A-1-d.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-A-1-e.** Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

**Appendix G-II-A-1-f.** Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.

**Appendix G-II-A-1-g.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-A-1-h.** In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

**Appendix G-II-A-2. Special Practices (BL1)**

**Appendix G-II-A-2-a.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-A-2-b.** An insect and rodent control program is in effect.

**Appendix G-II-A-3. Containment Equipment (BL1)**

**Appendix G-II-A-3-a.** Special containment equipment is generally not required for manipulations of agents assigned to BL1.

**Appendix G-II-A-4. Laboratory Facilities (BL1)**

**Appendix G-II-A-4-a.** The laboratory is designed so that it can be easily cleaned.

**Appendix G-II-A-4-b.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-A-4-c.** Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-A-4-d.** Each laboratory contains a sink for hand washing.

**Appendix G-II-A-4-e.** If the laboratory has windows that open, they are fitted with fly screens.

**Appendix G-II-B. Biosafety Level 2 (BL2)** (See Appendix G-III-N, *Footnotes and References of Appendix G*)

**Appendix G-II-B-1. Standard Microbiological Practices (BL2)**

**Appendix G-II-B-1-a.** Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

**Appendix G-II-B-1-b.** Work surfaces are decontaminated at least once a day and after any spill of viable material.

**Appendix G-II-B-1-c.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-B-1-d.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-B-1-e.** Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

**Appendix G-II-B-1-f.** Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

**Appendix G-II-B-1-g.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-B-1-h.** Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

**Appendix G-II-B-2. Special Practices (BL2)**

**Appendix G-II-B-2-a.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-B-2-b.** The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

**Appendix G-II-B-2-c.** The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

**Appendix G-II-B-2-d.** When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

**Appendix G-II-B-2-e.** An insect and rodent control program is in effect.

**Appendix G-II-B-2-f.** Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

**Appendix G-II-B-2-g.** Animals not involved in the work being performed are not permitted in the laboratory.

**Appendix G-II-B-2-h.** Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

**Appendix G-II-B-2-i.** All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

**Appendix G-II-B-2-j.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

**Appendix G-II-B-2-k.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix G-II-B-2-l.** When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

**Appendix G-II-B-2-m.** A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

**Appendix G-II-B-3. Containment Equipment (BL2)**

**Appendix G-II-B-3-a.** Biological safety cabinets (Class I or II) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

**Appendix G-II-B-3-a-(1).** Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O, *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

**Appendix G-II-B-3-a-(2).** High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

**Appendix G-II-B-4. Laboratory Facilities (BL2)**

**Appendix G-II-B-4-a.** The laboratory is designed so that it can be easily cleaned.

**Appendix G-II-B-4-b.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-B-4-c.** Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-B-4-d.** Each laboratory contains a sink for hand washing.

**Appendix G-II-B-4-e.** If the laboratory has windows that open, they are fitted with fly screens.

**Appendix G-II-B-4-f.** An autoclave for decontaminating laboratory wastes is available.

**Appendix G-II-C. Biosafety Level 3 (BL3)** (See Appendix G-III-P, *Footnotes and References of Appendix G*)

**Appendix G-II-C-1. Standard Microbiological Practices (BL3)**

**Appendix G-II-C-1-a.** Work surfaces are decontaminated at least once a day and after any spill of viable material.

**Appendix G-II-C-1-b.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-C-1-c.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-C-1-d.** Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

**Appendix G-II-C-1-e.** Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

**Appendix G-II-C-1-f.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-C-1-g.** Persons under 16 years of age shall not enter the laboratory.

**Appendix G-II-C-1-h.** If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

**Appendix G-II-C-2. Special Practices (BL3)**

**Appendix G-II-C-2-a.** Laboratory doors are kept closed when experiments are in progress.

**Appendix G-II-C-2-b.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-C-2-c.** The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

**Appendix G-II-C-2-d.** The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

**Appendix G-II-C-2-e.** When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

**Appendix G-II-C-2-f.** All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

**Appendix G-II-C-2-g.** The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

**Appendix G-II-C-2-h.** An insect and rodent program is in effect.

**Appendix G-II-C-2-i.** Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

**Appendix G-II-C-2-j.** Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

**Appendix G-II-C-2-k.** Molded surgical masks or respirators are worn in rooms containing experimental animals.

**Appendix G-II-C-2-l.** Animals and plants not related to the work being conducted are not permitted in the laboratory.

**Appendix G-II-C-2-m.** Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see Appendix G-III-K, *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

**Note:** Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

**Appendix G-II-C-2-n.** All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

**Appendix G-II-C-2-o.** Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix G-II-C-2-p.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix G-II-C-2-q.** Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

**Appendix G-II-C-2-r.** Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

**Appendix G-II-C-2-s.** A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

**Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)**

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

### Appendix G-II-C-3. Containment Equipment (BL3)

**Appendix G-II-C-3-a.** Biological safety cabinets (Class I, II, or III) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

### Appendix G-II-C-4. Laboratory Facilities (BL3)

**Appendix G-II-C-4-a.** The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

**Appendix G-II-C-4-b.** The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

**Appendix G-II-C-4-c.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-C-4-d.** Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-C-4-e.** Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

**Appendix G-II-C-4-f.** Windows in the laboratory are closed and sealed.

**Appendix G-II-C-4-g.** Access doors to the laboratory or containment module are self-closing.

**Appendix G-II-C-4-h.** An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

**Appendix G-II-C-4-i.** A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

**Appendix G-II-C-4-j.** The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G-III-L, *Footnotes and References of Appendix G*)) that avoids any interference with the air balance of the cabinets or building exhaust system.

## Appendix G-II-D. Biosafety Level 4 (BL4)

### Appendix G-II-D-1. Standard Microbiological Practices (BL4)

**Appendix G-II-D-1-a.** Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

**Appendix G-II-D-1-b.** Only mechanical pipetting devices are used.

**Appendix G-II-D-1-c.** Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

**Appendix G-II-D-1-d.** All procedures are performed carefully to minimize the creation of aerosols.

### Appendix G-II-D-2. Special Practices (BL4)

**Appendix G-II-D-2-a.** Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

**Appendix G-II-D-2-b.** No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before exiting the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix G-II-D-2-c.** Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the Principal Investigator, Biological Safety Officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

**Appendix G-II-D-2-d.** Personnel enter and exit the facility only through the clothing change and shower rooms. Personnel shower each time they exit the facility. Personnel use the air locks to enter or exit the laboratory only in an emergency.

**Appendix G-II-D-2-e.** Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing (may be disposable), including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When exiting the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. Protective clothing shall be decontaminated prior to laundering or disposal.

**Appendix G-II-D-2-f.** When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biosafety symbol is posted on all access doors. The sign identifies the agent, lists the name of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

**Appendix G-II-D-2-g.** Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

**Appendix G-II-D-2-h.** An insect and rodent control program is in effect.

**Appendix G-II-D-2-i.** Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

**Appendix G-II-D-2-j.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

**Appendix G-II-D-2-k.** A system is set up for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

**Appendix G-II-D-2-l.** Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems, such as Horsfall units (see Appendix G-III-K, *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

### Appendix G-II-D-2-m. Alternative Selection of Containment Equipment (BL4)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

### Appendix G-II-D-3. Containment Equipment (BL4)

**Appendix G-II-D-3-a.** All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

### Appendix G-II-D-4. Laboratory Facilities (BL4)

**Appendix G-II-D-4-a.** The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

**Appendix G-II-D-4-b.** Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain high efficiency particulate air/HEPA filters.

**Appendix G-II-D-4-c.** Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

**Appendix G-II-D-4-d.** Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-D-4-e.** Laboratory furniture is simple and of sturdy construction; and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-D-4-f.** A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

**Appendix G-II-D-4-g.** If there is a central vacuum system, it does not serve areas outside the facility. In-line high efficiency particulate air/HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent back-flow.

**Appendix G-II-D-4-h.** If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the back-flow protected distribution system supplying water to the laboratory areas.

**Appendix G-II-D-4-i.** Access doors to the laboratory are self-closing and locking.

**Appendix G-II-D-4-j.** Any windows are breakage resistant.

**Appendix G-II-D-4-k.** A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

**Appendix G-II-D-4-l.** A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

**Appendix G-II-D-4-m.** Liquid effluent from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

**Appendix G-II-D-4-n.** An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

**Appendix G-II-D-4-o.** The exhaust air from the facility is filtered through high efficiency particulate air/HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

**Appendix G-II-D-4-p.** The treated exhaust air from Class I and II biological safety cabinets may be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at six-month intervals. *The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of high efficiency particulate air/HEPA filters in series, via the facility exhaust air system*. If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G-III-L, *Footnotes and References of Appendix G*)) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

**Appendix G-II-D-4-q.** A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area. The exhaust air from the suit area is filtered by two sets of high efficiency particulate air/HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is greater than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit areas.

**Appendix G - Table 1. Possible Alternate Combinations Of Physical And Biological Containment Safeguards**

| Classification of Physical & Biological Containment | Alternate Physical Containment | | | Alternate Biological Containment |
|---|---|---|---|---|
| | Laboratory Facilities | Laboratory Practices | Laboratory Equipment | |
| BL3/HV2 | BL3<br>BL3 | BL3<br>BL3 | BL3<br>BL4 | HV2<br>HV1 |
| BL3/HV1 | BL3<br>BL3 | BL3<br>BL3 | BL3<br>BL2 | HV1<br>HV2 |
| BL4/HV1 | BL4<br>BL4 | BL4<br>BL4 | BL4<br>BL3 | HV1<br>HV2 |

BL - Biosafety Level
HV - Host-Vector System

**Appendix G-III. Footnotes and References of Appendix G**

**Appendix G-III-A.** *Biosafety in Microbiological and Biomedical Laboratories,* 4th edition, May, 1999, DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland.

**Appendix G-III-B.** *Biosafety in Microbiological and Biomedical Laboratories*, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

**Appendix G-III-C.** *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

**Appendix G-III-D.** *National Institutes of Health Biohazards Safety Guide*, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740-00383, 1974.

**Appendix G-III-E.** A. Hellman, M. N. Oxman, and R. Pollack (eds.), *Biohazards in Biological Research*, Cold Spring Harbor Laboratory 1973.

**Appendix G-III-F.** N. V. Steere (ed.), *Handbook of Laboratory Safety*, 2nd edition, The Chemical Rubber Co., Cleveland, Ohio, 1971.

**Appendix G-III-G.** Bodily, J. L, "General Administration of the Laboratory," H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), *Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections*, American Public Health Association, New York, 1970, pp. 11-28.

**Appendix G-III-H.** Darlow, H. M. (1969). "Safety in the Microbiological Laboratory," in J. R. Norris and D. W. Robbins (eds.), *Methods in Microbiology*, Academic Press, Inc., New York, pp. 169-204.

**Appendix G-III-I.** *The Prevention of Laboratory Acquired Infection*, C. H. Collins, E. G. Hartley, and R. Pilsworth, Public Health Laboratory Service, Monograph Series No. 6, 1974.

**Appendix G-III-J.** Chatigny, M. A., "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W. W. Umbreit (ed.), *Advances in Applied Microbiology*, Academic Press, New York, New York, 1961, 3:131-192.

**Appendix G-III-K.** Horsfall, F. L. Jr., and J. H. Baner, *Individual Isolation of Infected Animals in a Single Room*, J. Bact., 1940, 40, 569-580.

**Appendix G-III-L.** Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry, Ann Arbor, Michigan.

**Appendix G-III-M.** Biosafety Level 1 is suitable for work involving agents of unknown or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see Appendix G-III-B, *Footnotes and References of Appendix G*).

**Appendix G-III-N.** Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see Appendix G-III-B, *Footnotes and References of Appendix G*).

**Appendix G-III-O.** Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, *Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research*, NIH, Bethesda, Maryland 1978.

**Appendix G-III-P.** Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the Principal Investigator.

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## APPENDIX H. SHIPMENT

Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the U.S. Postal Service (39 Code of Federal Regulations, Part 3); the Public Health Service (42 Code of Federal Regulations, Part 72); the U.S. Department of Agriculture (9 Code of Federal Regulations, Subchapters D and E; 7 CFR, Part 340); and/or the U.S. Department of Transportation (49 Code of Federal Regulations, Parts 171-179).

**Note.** A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II, *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section IV-D, *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice (see Section IV-D-3, *Certification of Host-Vector Systems - Voluntary Compliance)*.

**Appendix H-I.** Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant DNA, if they are regulated as human pathogens by the Public Health Service (42 Code of Federal Regulations, Part 72) or as animal pathogens or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

**Appendix H-II.** Host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA when: (i) the recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest; or (ii) the recombinant DNA codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or (iii) the recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

### Appendix H-III. Footnotes and References of Appendix H

For further information on shipping etiologic agents contact: (i) The Centers for Disease Control and Prevention, ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883; (ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or (iii) U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

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## APPENDIX I. BIOLOGICAL CONTAINMENT (See Appendix E, *Certified Host-Vector Systems*)

### Appendix I-I. Levels of Biological Containment

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established. Appendices I-I-A through I-II-B describe levels of biological containment (host-vector systems) for prokaryotes. Specific criteria will depend on the organisms to be used.

### Appendix I-I-A. Host-Vector 1 Systems

Host-Vector 1 systems provide a moderate level of containment. Specific Host-Vector 1 systems are:

#### Appendix I-I-A-1. *Escherichia coli* K-12 Host-Vector 1 Systems (EK1)

The host is always *Escherichia coli* K-12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof (see Appendices I-III-A through G, *Footnotes and References of Appendix I*) and variants of bacteriophage, such as lambda (see Appendices I-III-H through O, *Footnotes and References of Appendix I*). The *Escherichia coli* K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

#### Appendix I-I-A-2. Other Host-Vector 1 Systems

At a minimum, hosts and vectors shall be comparable in containment to *Escherichia coli* K-12 with a non-conjugative plasmid or bacteriophage vector. Appendix I-II, *Certification of Host-Vector Systems*, describes the data to be considered and mechanism for approval of Host-Vector 1 systems.

### Appendix I-I-B. Host-Vector 2 Systems (EK2)

Host-Vector 2 Systems provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be $< 1/10^8$ under specified conditions. Specific Host-Vector 2 systems are:

**Appendix I-I-B-1.** For *Escherichia coli* K-12 Host-Vector 2 systems (EK2) in which the vector is a plasmid, no more than $1/10^8$ host cells shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

**Appendix I-I-B-2.** For *Escherichia coli* K-12 Host-Vector 2 systems (EK2) in which the vector is a phage, no more than $1/10^8$ phage particles shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation, or survival in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

## Appendix I-II. Certification of Host-Vector Systems

### Appendix I-II-A. Responsibility

Host-Vector 1 systems (other than *Escherichia coli* K-12) and Host-Vector 2 systems may not be designated as such until they have been certified by the NIH Director. Requests for certification of host-vector systems may be submitted to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Proposed host-vector systems will be reviewed by the RAC (see Section IV-C-1-b-(1)-(f), *Major Actions*). Initial review will based on the construction, properties, and testing of the proposed host-vector system by a subcommittee composed of one or more RAC members and/or *ad hoc* experts. The RAC will evaluate the subcommittee's report and any other available information at the next scheduled RAC meeting. The NIH Director is responsible for certification of host-vector systems, following advice of the RAC. Minor modifications to existing host-vector systems (i.e., those that are of minimal or no consequence to the properties relevant to containment) may be certified by the NIH Director without prior RAC review (see Section IV-C-1-b-(2)-(f), *Minor Actions*). Once a host-vector system has been certified by the NIH Director, a notice of certification will be sent by NIH/OBA to the applicant and to the Institutional Biosafety Committee Chairs. A list of all currently certified host-vector systems is available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). The NIH Director may rescind the certification of a host-vector system (see Section IV-C-1-b-(2)-(g), *Minor Actions*). If certification is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher level of physical containment level, unless NIH determines that the already constructed clones incorporate adequate biological containment. Certification of an host-vector system does not extend to modifications of either the host or vector component of that system. Such modified systems shall be independently certified by the NIH Director. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications to a certified host-vector system requires submission of complete testing data.

### Appendix I-II-B. Data to be Submitted for Certification

#### Appendix I-II-B-1. Host-Vector 1 Systems Other than *Escherichia coli* K-12

The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) a description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction, survival, and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and the type of information is exchanged; and any relevant information about its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an Host-Vector 1 system.

#### Appendix I-II-B-2. Host-Vector 2 Systems

Investigators planning to request Host-Vector 2 systems certification may obtain instructions from NIH/OBA concerning data to be submitted (see Appendices I-III-N and O, *Footnotes and References of Appendix I*). In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including

information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation; and (vi) in some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals or one or more human subjects. Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments. Data shall be submitted 12 weeks prior to the RAC meeting at which such data will be considered by the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Investigators are encouraged to publish their data on the construction, properties, and testing of proposed Host Vector 2 systems prior to consideration of the system by the RAC and its subcommittee. Specific instructions concerning the submission of data for proposed *Escherichia coli* K-12 Host-Vector 2 system (EK2) involving either plasmids or bacteriophage in *Escherichia coli* K-12, are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

**Appendix I-III. Footnotes and References of Appendix I**

**Appendix I-III-A.** Hersfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski, *Plasmid Co1E1 as a Molecular Vehicle for Cloning and Amplification of DNA*. Proc. Nat. Acad. Sci., 1974, 71, pp. 3455-3459.

**Appendix I-III-B.** Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness, *A System for Mapping DNA Sequences in the Chromosomes of Drosophila Melanogaster*. Cell, 1974, 3, pp. 315-335.

**Appendix I-III-C.** Tanaka, T., and B. Weisblum, *Construction of a Colicin El-R Factor Composite Plasmid in Vitro: Means for Amplification of Deoxyribonucleic Acid*. J. Bacteriol., 1975, *121*, pp. 354-362.

**Appendix I-III-D.** Armstrong, K. A., V. Hershfield, and D. R. Helinski, *Gene Cloning and Containment Properties of Plasmid Col E1 and Its Derivatives*, Science, 1977, *196*, pp. 172-174.

**Appendix I-III-E.** Bolivar, F., R. L. Rodriguez, M. C. Betlack, and H. W. Boyer, *Construction and Characterization of New Cloning Vehicles: I. Ampicillin-Resistant Derivative of PMB9*, Gene, 1977, 2, pp. 75-93.

**Appendix I-III-F.** Cohen, S. N., A. C. W. Chang, H. Boyer, and R. Helling. *Construction of Biologically Functional Bacterial Plasmids in Vitro*. Proc. Natl. Acad, Sci., 1973, 70, pp. 3240-3244.

**Appendix I-III-G.** Bolivar, F., R. L. Rodriguez, R. J. Greene, M. C.Batlack, H. L. Reyneker, H. W. Boyer, J. H. Cross, and S. Falkow, 1977, *Construction and Characterization of New Cloning Vehicles II. A Multi-Purpose Cloning System*, Gene, 1977, 2, pp. 95-113.

**Appendix I-III-H.** Thomas, M., J. R. Cameron, and R. W. Davis (1974). *Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA*. Proc. Nat. Acad. Sci., 1974, 71, pp. 4579-4583.

**Appendix I-III-I.** Murray, N. E., and K. Murray, *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments*. Nature, 1974, 51, pp. 476-481.

**Appendix I-III-J.** Ramback, A., and P. Tiollais (1974). *Bacteriophage Having EcoRI Endonuclease Sites Only in the Non-Essential Region of the Genome*. Proc. Nat. Acad. Sci., 1974, 71, pp. 3927-3820.

**Appendix I-III-K.** Blattner, F. R., B. G. Williams, A. E. Bleche, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Gunwald, D. O. Kiefer, D. D. Moore, J. W. Shumm, E. L. Sheldon, and O. Smithies, *Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning*, Science 1977, 196, pp. 163-169.

**Appendix I-III-L.** Donoghue, D. J., and P. A. Sharp, *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance*, Gene, 1977, 1, pp. 209-227.

**Appendix I-III-M.** Leder, P., D. Tiemeier and L. Enquist (1977), *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The λgt WES System*, Science, 1977, 196, pp. 175-177.

**Appendix I-III-N.** Skalka, A., *Current Status of Coliphage AEK2 Vectors*, Gene, 1978, 3, pp. 29-35.

**Appendix I-III-O.** Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein, *Standardized Laboratory Tests for EK2 Certification*, Gene, 1978, 3, pp. 36-38.

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## APPENDIX J. BIOTECHNOLOGY RESEARCH SUBCOMMITTEE

The National Science and Technology Council's Committee on Fundamental Science determined that a subcommittee should be continued to identify and coordinate Federal research efforts, identify research needs, stimulating international cooperation, and assess national and international policy issues concerning biotechnology sciences. The primary emphasis will be on scientific issues to increase the overall effectiveness and productivity of the Federal investment in biotechnology sciences, especially regarding issues which cut across agency boundaries. This subcommittee is called the Biotechnology Research Subcommittee.

Membership of the Biotechnology Research Subcommittee will include Federal agencies that support biotechnology research. Agencies represented are: U.S. Department of Agriculture, Department of Commerce, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Interior, Department of Justice, Department of State, Department of Veterans Affairs, Agency for International Development, Environmental Protection Agency, National Aeronautics and Space Administration, and National Science Foundation. The Biotechnology Research Subcommittee will function in an advisory capacity to the Committee on Fundamental Science, the Director of the Office of Science and Technology Policy, and the Executive Office of the President. The Biotechnology Research Subcommittee will review the scientific aspects of proposed regulations and guidelines as they are developed.

The primary responsibilities of the Biotechnology Research Subcommittee are to: (i) describe and review current Federal efforts in biotechnology research; (ii) identify and define the priority areas for future Federal biotechnology research, including areas needing greater emphasis, describing the role of each agency in those areas, and delineate where interagency cooperation would enhance progress in the biotechnology sciences, with an emphasis on integrated research efforts, where appropriate; (iii) assess major international efforts in the biotechnology sciences and develop mechanisms for international collaboration. For example, activities of the U.S.-European Community Task Force on Biotechnology have been coordinated through the Biotechnology Research Subcommittee; (iv) identify and review national and international policy issues (such as public education) associated with biotechnology; and (v) provide reviews, analyses, and recommendations to the Chairs of the Committee on Fundamental Science on scientific issues related to regulations and the applications of biotechnology research and biotechnology policies and issues.

In 1990, the Biotechnology Research Subcommittee replaced the Biotechnology Sciences Coordinating Committee. Both the Biotechnology Research Subcommittee and the Biotechnology Sciences Coordinating Committee previously functioned under the Federal Coordinating Council on Science, Engineering, and Technology (FCCSET). While regulatory issues became the primary focus of the Biotechnology Sciences Coordinating Committee, the Biotechnology Research Subcommittee focuses on scientific issues, although it will still provide scientific support for regulatory responsibilities.

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## APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

Appendix K specifies physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to large-scale research or production activities as specified in Section III-D-6, *Experiments Involving More than 10 Liters of Culture*. It is important to note that this appendix addresses only the biological hazard associated with organisms containing recombinant DNA. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this appendix.

All provisions shall apply to large-scale research or production activities with the following modifications: (i) Appendix K shall supersede Appendix G, *Physical Containment*, when quantities in excess of 10 liters of culture are involved in research or production. Appendix K-II applies to Good Large Scale Practice; (ii) the institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the Biological Safety Officer shall include those specified in Section IV-B-3, *Biological Safety Officer*; (iii) the institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require Biosafety Level (BL) 3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance, and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual, or extended illnesses of employees to determine possible occupational origin.

### Appendix K-I. Selection of Physical Containment Levels

The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in Section III, *Experiments Covered by the NIH Guidelines*. For purposes of large-scale research or production, four physical containment levels are established. The four levels set containment conditions at those appropriate for the degree of hazard to health or the environment posed by the organism, judged by experience with similar organisms unmodified by recombinant DNA techniques and consistent with Good Large Scale Practice. The four biosafety levels of large-scale physical containment are referred to as Good Large Scale Practice, BL1-Large Scale, BL2-Large Scale, and BL3-Large Scale. Good Large Scale Practice is recommended for large-scale research or production involving viable, non-pathogenic, and non-toxigenic recombinant strains derived from host organisms that have an extended history of safe large-scale use. Good Large Scale Practice is recommended for organisms such as those included in Appendix C, *Exemptions under Section III-F-6*, which have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment. BL1-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale and that do not qualify for Good Large Scale Practice. BL2-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL2 containment at the laboratory scale. BL3-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL3 containment at the laboratory scale. No provisions are made for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

### Appendix K-II. Good Large Scale Practice (GLSP)

**Appendix K-II-A.** Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters.

**Appendix K-II-B.** Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant DNA molecules are handled prudently and that the work place is kept clean and orderly.

**Appendix K-II-C.** In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules. Eating, drinking, smoking, applying cosmetics, and mouth pipetting shall be prohibited in the work area.

**Appendix K-II-D.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment.

**Appendix K-II-E.** Discharges containing viable recombinant organisms shall be handled in accordance with applicable governmental environmental regulations.

**Appendix K-II-F.** Addition of materials to a system, sample collection, transfer of culture fluids within/between systems, and processing of culture fluids shall be conducted in a manner that maintains employee's exposure to viable organisms containing recombinant DNA molecules at a level that does not adversely affect the health and safety of employees.

**Appendix K-II-G.** The facility's emergency response plan shall include provisions for handling spills.

**Appendix K-III. Biosafety Level 1 (BL1) - Large Scale**

**Appendix K-III-A.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Laboratory Director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix K-III-B.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G-II-A, *Physical Containment Levels--Biosafety Level 1*, are met.

**Appendix K-III-C.** Culture fluids (except as allowed in Appendix K-III-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

**Appendix K-III-D.** Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

**Appendix K-III-E.** Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing recombinant DNA molecules to the environment.

**Appendix K-III-F.** A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in Appendix K-III-C above. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

**Appendix K-III-G.** Emergency plans required by Sections IV-B-2-b-(6), *Institutional Biosafety Committee*, and IV-B-3-c-(3), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

**Appendix K-IV. Biosafety Level 2 (BL2) - Large Scale**

**Appendix K-IV-A.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix K-IV-B.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G-II-B, *Physical Containment Levels--Biosafety Level 2*, are met.

**Appendix K-IV-C.** Culture fluids (except as allowed in Appendix K-IV-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

**Appendix K-IV-D.** Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

**Appendix K-IV-E.** Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

**Appendix K-IV-F.** A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in Appendix K-IV-C above. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

**Appendix K-IV-G.** Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

**Appendix K-IV-H.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing sensing devices that monitor the integrity of containment during operations.

**Appendix K-IV-I.** A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

**Appendix K-IV-J.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

**Appendix K-IV-K.** The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

**Appendix K-IV-L.** Emergency plans required by Sections IV-B-2-b-(6), *Institutional Biosafety Committee*, and IV-B-3-c-(3), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

### Appendix K-V. Biosafety Level 3 (BL3) - Large Scale

**Appendix K-V-A.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix K-V-B.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in Appendix G-II-C, *Physical Containment Levels--Biosafety Level 3*, are met.

**Appendix K-V-C.** Culture fluids (except as allowed in Appendix K-V-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

**Appendix K-V-D.** Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

**Appendix K-V-E.** Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

**Appendix K-V-F.** A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in Appendix K-V-C above. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

**Appendix K-V-G.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.

**Appendix K-V-H.** Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

**Appendix K-V-I.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

**Appendix K-V-J.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

**Appendix K-V-K.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, maintenance, and use of this equipment for research production activities involving viable organisms containing recombinant DNA molecules.

**Appendix K-V-L.** The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

**Appendix K-V-M.** Emergency plans required by Sections IV-B-2-b-(6), *Institutional Biosafety Committee*, and IV-B-3-c-(3), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

**Appendix K-V-N.** Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:

**Appendix K-V-N-1.** The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.

**Appendix K-V-N-2.** The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

**Appendix K-V-N-3.** Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

**Appendix K-V-N-4.** All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

**Appendix K-V-N-5.** Hand washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.

**Appendix K-V-N-6.** A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

**Appendix K-V-N-7.** The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.

**Appendix K-V-N-8.** The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may not be discharged to the outdoors without being high efficiency particulate air/HEPA filtered, subjected to thermal oxidation, or otherwise treated to prevent the release of viable organisms.

**Appendix K-V-O.** The following personnel and operational practices shall be required:

**Appendix K-V-O-1.** Personnel entry into the controlled area shall be through the entry area specified in Appendix K-V-N-1.

**Appendix K-V-O-2.** Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

**Appendix K-V-O-3.** Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

**Appendix K-V-O-4.** Persons under 18 years of age shall not be permitted to enter the controlled area.

**Appendix K-V-O-5.** The universal biosafety sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

**Appendix K-V-O-6.** The controlled area shall be kept neat and clean.

**Appendix K-V-O-7.** Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

**Appendix K-V-O-8.** Animals and plants shall be excluded from the controlled area.

**Appendix K-V-O-9.** An effective insect and rodent control program shall be maintained.

**Appendix K-V-O-10.** Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Serve doors leading directly outdoors shall be sealed and locked while work is in progress.

**Appendix K-V-O-11.** Persons shall wash their hands when exiting the controlled area.

**Appendix K-V-O-12.** Persons working in the controlled area shall be trained in emergency procedures.

**Appendix K-V-O-13.** Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

**Appendix K-V-O-14.** The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

**Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL) - Large Scale (LS) Practice** (See Appendix K-VI-A, *Footnotes Of Appendix K*)

| CRITERION [See Appendix K-VI-B, *Footnotes of Appendix K*] | | GLSP | BL1-LS | BL2-LS | BL3-LS |
|---|---|---|---|---|---|
| **1.** | Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures. | K-II-A | G-I | | |
| **2.** | Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees. | K-II-B | G-I | | |
| **3.** | Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work. | K-II-C | G-II-A-1-h | G-II-B-2-f | G-II-C-2-i |
| **4.** | Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place. | K-II-C | G-II-A-1-d<br>G-II-A-1-e | G-II-B-1-d<br>G-II-B-1-e | G-II-C-1-c<br>G-II-C-1-d |
| **5.** | Internal accident reporting. | K-II-G | K-III-A | K-IV-A | K-V-A |
| **6.** | Medical surveillance. | NR | NR | | |
| **7.** | Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment). | NR | K-III-B | K-IV-B | K-V-B |
| **8.** | Culture fluids not removed from a system until organisms are inactivated. | NR | K-III-C | K-IV-C | K-V-C |
| **9.** | Inactivation of waste solutions and materials with respect to their biohazard potential. | K-II-E | K-III-C | K-IV-C | K-V-C |
| **10.** | Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system. | Minimize<br>*Procedure*<br>K-II-F | Minimize<br>*Engineer*<br>K-III-B<br>K-III-D | Prevent<br>*Engineer*<br>K-IV-B<br>K-IV-D | Prevent<br>*Engineer*<br>K-V-B<br>K-V-D |
| **11.** | Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms. | NR | Minimize<br>K-III-E | Prevent<br>K-IV-E | Prevent<br>K-V-E |
| **12.** | Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure. | NR | K-III-F | K-IV-F | K-V-F |
| **13.** | Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features. | NR | NR | NR | K-V-G |
| **14.** | Rotating seals and other penetrations into closed system designed to prevent or minimize leakage. | NR | NR | Prevent<br>K-IV-G | Prevent<br>K-V-H |
| **15.** | Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment. | NR | NR | K-IV-H | K-V-I |
| **16.** | Validated integrity testing of closed containment system. | NR | NR | K-IV-I | K-V-J |
| **17.** | Closed system to be permanently identified for record keeping purposes. | NR | NR | K-IV-J | K-V-K |
| **18.** | Universal biosafety sign to be posted on each closed system. | NR | NR | K-IV-K | K-V-L |
| **19.** | Emergency plans required for handling large losses of cultures. | K-II-G | K-III-G | K-IV-L | K-V-M |
| **20.** | Access to the work place. | NR | G-II-A-1-a | G-II-B-1-a | K-V-N |
| **21.** | Requirements for controlled access area. | NR | NR | NR | K-V-N&O |

*NR = not required*

**Appendix K-VI. Footnotes of Appendix K**

**Appendix K-VI-A.** This table is derived from the text in Appendices G (*Physical Containment*) and K and is not to be used in lieu of Appendices G and K.

**Appendix K-VI-B.** The criteria in this grid address only the biological hazards associated with organisms containing recombinant DNA. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this grid.

**Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K**

**Appendix K-VII-A. Accidental Release.** An accidental release is the unintentional discharge of a microbiological agent (i.e., microorganism or virus) or eukaryotic cell due to a failure in the containment system.

**Appendix K-VII-B. Biological Barrier.** A biological barrier is an impediment (naturally occurring or introduced) to the infectivity and/or survival of a microbiological agent or eukaryotic cell once it has been released into the environment.

**Appendix K-VII-C. Closed System.** A closed system is one in which by its design and proper operation, prevents release of a microbiological agent or eukaryotic cell contained therein.

**Appendix K-VII-D. Containment.** Containment is the confinement of a microbiological agent or eukaryotic cell that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include: physical and biological barriers and inactivation using physical or chemical means.

**Appendix K-VII-E. *De minimis* Release.** *De minimis* release is the release of: (i) viable microbiological agents or eukaryotic cells that does not result in the establishment of disease in healthy people, plants, or animals; or (ii) in uncontrolled proliferation of any microbiological agents or eukaryotic cells.

**Appendix K-VII-F. Disinfection.** Disinfection is a process by which viable microbiological agents or eukaryotic cells are reduced to a level unlikely to produce disease in healthy people, plants, or animals.

**Appendix K-VII-G. Good Large Scale Practice Organism.** For an organism to qualify for Good Large Scale Practice consideration, it must meet the following criteria [Reference: Organization for Economic Cooperation and Development, *Recombinant DNA Safety Considerations*, 1987, p. 34-35]: (i) the host organism should be non-pathogenic, should not contain adventitious agents and should have an extended history of safe large-scale use or have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment; (ii) the recombinant DNA-engineered organism should be non-pathogenic, should be as safe in the large-scale setting as the host organism, and without adverse consequences in the environment; and (iii) the vector/insert should be well characterized and free from known harmful sequences; should be limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the construct in the environment unless that is a requirement of the intended function; should be poorly mobilizable; and should not transfer any resistance markers to microorganisms unknown to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

**Appendix K-VII-H. Inactivation.** Inactivation is any process that destroys the ability of a specific microbiological agent or eukaryotic cell to self-replicate.

**Appendix K-VII-I. Incidental Release.** An incidental release is the discharge of a microbiological agent or eukaryotic cell from a containment system that is expected when the system is appropriately designed and properly operated and maintained.

**Appendix K-VII-J. Minimization.** Minimization is the design and operation of containment systems in order that any incidental release is a *de minimis* release.

**Appendix K-VII-K.** Pathogen. A pathogen is any microbiological agent or eukaryotic cell containing sufficient genetic information, which upon expression of such information, is capable of producing disease in healthy people, plants, or animals.

**Appendix K-VII-L. Physical Barrier.** A physical barrier is considered any equipment, facilities, or devices (e.g., fermentors, factories, filters, thermal oxidizers) which are designed to achieve containment.

**Appendix K-VII-M. Release.** Release is the discharge of a microbiological agent or eukaryotic cell from a containment system. Discharges can be incidental or accidental. Incidental releases are *de minimis* in nature; accidental releases may be *de minimis* in nature.

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## APPENDIX L. GENE THERAPY POLICY CONFERENCES (GTPCS)

In order to enhance the depth and value of public discussion relevant to scientific, safety, social, and ethical implications of gene therapy research, the NIH Director will convene GTPCs at regular intervals. As appropriate, the NIH Director may convene a GTPC in conjunction with a RAC meeting. GTPCs will be administered by NIH/OBA. Conference participation will not involve a standing committee membership but rather will offer the unique advantage of assembling numerous participants who possess significant scientific, ethical, and legal expertise and/or interest that is directly applicable to a specific gene therapy research issue. At least one member of RAC will serve as Co-chair of each GTPC and report the findings of each GTPC to RAC at its next scheduled meeting. The RAC representative for each GTPC will be chosen based on the participant's area of expertise relative to the specific gene therapy research issue to be discussed. All RAC members will be invited to attend GTPCs. GTPCs will have representation from other Federal agencies, including FDA and OHRP. GTPCs will focus on broad overarching policy and scientific issues related to gene therapy research. Proposals for GTPC topics may be submitted by members of RAC, representatives of academia, industry, patient and consumer advocacy organizations, other Federal agencies, professional scientific societies, and the general public. GTPC topics will not be limited to discussion of human applications of gene therapy research, i.e., they may include basic research on the use of novel gene delivery vehicles, or novel applications of human gene transfer. The RAC, with the Director's approval, will have the primary responsibility for planning GTPC agendas. GTPC findings will be transmitted to the NIH Director and will be made publicly available. The NIH Director anticipates that this public policy forum will serve as a model for interagency communication and collaboration, concentrated expert discussion of novel scientific issues and their potential societal implications, and enhanced opportunity for public discussion of specific issues and potential impact of such applications on human health and the environment.

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## APPENDIX M. POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO ONE OR MORE HUMAN RESEARCH PARTICIPANTS (POINTS TO CONSIDER)

Appendix M applies to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH. Researchers not covered by the *NIH Guidelines* are encouraged to use Appendix M (see Section I-C, *General Applicability*).

The acceptability of human somatic cell gene transfer has been addressed in several public documents as well as in numerous academic studies. In November 1982, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research published a report, *Splicing Life*, which resulted from a two-year process of public deliberation and hearings. Upon release of that report, a U.S. House of Representatives subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, *Human Gene Therapy*, which concluded that civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene transfer of somatic cells in humans for specific genetic diseases. Somatic cell gene transfer is seen as an extension of present methods that might be preferable to other technologies. In light of this public support, RAC is prepared to consider proposals for somatic cell gene transfer.

RAC will not at present entertain proposals for germ line alterations but will consider proposals involving somatic cell gene transfer. The purpose of somatic cell gene transfer is to treat an individual patient, e.g., by inserting a properly functioning gene into the subject's somatic cells. Germ line alteration involves a specific attempt to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring.

The RAC continues to explore the issues raised by the potential of *in utero* gene transfer clinical research. However, the RAC concludes that, at present, it is premature to undertake any *in utero* gene transfer clinical trial. Significant additional preclinical and clinical studies addressing vector transduction efficacy, biodistribution, and toxicity are required before a human *in utero* gene transfer protocol can proceed. In addition, a more thorough understanding of the development of human organ systems, such as the immune and nervous systems, is needed to better define the potential efficacy and risks of human *in utero* gene transfer. Prerequisites for considering any specific human *in utero* gene transfer procedure include an understanding of the pathophysiology of the candidate disease and a demonstrable advantage to the *in utero* approach. Once the above criteria are met, the RAC would be willing to consider well rationalized human *in utero* gene transfer clinical trials.

Research proposals involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer) will be considered through a review process involving both NIH/OBA and RAC. Investigators shall submit their relevant information on the proposed human gene transfer experiments to NIH/OBA. Submission of human gene transfer protocols to NIH will be in the format described in Appendix M-I-A, *Submission Requirements for Protocol Submission*. Submission to NIH shall be for registration purposes and will ensure continued public access to relevant human gene transfer information conducted in compliance with the *NIH Guidelines*. Investigational New Drug (IND) applications should be submitted to FDA in the format described in 21 CFR, Chapter I, Subchapter D, Part 312, Subpart B, Section 23, *IND Content and Format*.

Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

Factors that may contribute to public discussion of a human gene transfer experiment by RAC include: (i) new vectors/new gene delivery systems, (ii) new diseases, (iii) unique applications of gene transfer, and (iv) other issues considered to require further public discussion. Among the experiments that may be considered exempt from RAC discussion are those determined not to represent possible risk to human health or the environment. Full, public RAC review and discussion of a human gene transfer experiment may be (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members, or (b) a Federal agency other than NIH. An individual human gene transfer experiment that is recommended for full RAC review should represent novel characteristics deserving of public discussion. If it is determined that an experiment will undergo full RAC discussion, NIH/OBA will immediately notify the Principal Investigator. RAC members may forward individual requests for additional information relevant to a specific protocol through NIH/OBA to the Principal Investigator. In making a determination whether an experiment is novel, and thus deserving of full RAC discussion, reviewers will examine the scientific rationale, scientific context (relative to other proposals reviewed by RAC), whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. RAC recommendations on a specific human gene transfer experiment shall be forwarded to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate. Relevant documentation will be included in the material for the RAC meeting at which the experiment is scheduled to be discussed. RAC meetings will be open to the public except where trade secrets and proprietary information are reviewed (see Section IV-D-5, *Protection of Proprietary Data – Voluntary Compliance)*. RAC prefers that information provided in response to Appendix M contain no proprietary data or trade secrets, enabling all aspects of the review to be open to the public.

**Note:** Any application submitted to NIH/OBA shall not be designated as 'confidential' in its entirety. In the event that a sponsor determines that specific responses to one or more of the items described in Appendix M should be considered as proprietary or trade secret, each item should be clearly identified as such. The cover letter (attached to the submitted material) shall: (1) clearly indicate that select portions of the application contain information considered as proprietary or trade secret, (2) a brief explanation as to the reason that each of these items is determined proprietary or trade secret.

Public discussion of human gene transfer experiments (and access to relevant information) shall serve to inform the public about the technical aspects of the proposals, meaning and significance of the research, and significant safety, social, and ethical implications of the research. RAC discussion is intended to ensure safe and ethical conduct of gene transfer experiments and facilitate public understanding of this novel area of biomedical research.

In its evaluation of human gene transfer proposals, RAC will consider whether the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of clinical investigation, namely, to protect the health and well being of human subjects being treated while at the same time gathering generalizable knowledge. Two possible undesirable consequences of the transfer of recombinant DNA would be unintentional: (i) vertical transmission of genetic changes from an individual to his/her offspring, or (ii) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, Appendices M-I through M-V request information that will enable RAC and NIH/OBA to assess the possibility that the proposed experiment(s) will inadvertently affect reproductive cells or lead to infection of other people (e.g., medical personnel or relatives).

Appendix M will be considered for revisions as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out periodically as needed.

## Appendix M-I. Requirements for Protocol Submission, Review, and Reporting – Human Gene Transfer Experiments

### Appendix M-I-A. Requirements for Protocol Submission

The following documentation must be submitted (see exemption in Appendix M-VI-A, *Footnotes of Appendix M*) in printed or electronic form to the: Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), E-mail: rosenthg@od.nih.gov. NIH OBA will confirm receipt within three working days after receiving the submission. Investigators should contact OBA if they do not receive this confirmation.

1. A cover letter on institutional letterhead, signed by the Principal Investigator(s), that: (1) acknowledges that the documentation submitted to NIH OBA complies with the requirements set forth in Appendix M-I-A, *Requirements for Protocol Submission*; (2) identifies the Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) at the proposed clinical trial site(s) responsible for local review and approval of the protocol; and (3) acknowledges that no research participant will be enrolled (see definition of enrollment in Section I-E-7) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; IRB approval has been obtained; and all applicable regulatory authorizations have been obtained.

2. The scientific abstract.

3. The non-technical abstract.

4. The proposed clinical protocol, including tables, figures, and relevant manuscripts.

5. Responses to Appendices M-II through M-V, *Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues*. Responses to Appendices M-II through M-V may be provided either as an appendix to the clinical protocol or incorporated in the clinical protocol. If responses to Appendices M-II through M-V are incorporated in the clinical protocol, each response must refer to the appropriate Appendix M-II through M-V.

6. The proposed informed consent document (see Appendix M-III, *Informed Consent*).

7. Curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format).

**Note:** A human gene transfer experiment submitted to NIH OBA should not contain confidential commercial information or trade secrets, enabling all aspects of the review to be open to the public.

### Appendix M-I-B. RAC Review Requirements

#### Appendix M-I-B-1. Initial RAC Review

The initial RAC review process shall include a determination as to whether the human gene transfer experiment presents characteristics that warrant public RAC review and discussion. During the RAC's initial review, individual committee members may request additional information relevant to the protocol. NIH OBA will immediately notify the Principal Investigator(s) of RAC requests for additional information. In making a determination whether an experiment presents characteristics warranting public RAC review and discussion, reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. Other factors that may warrant public review and discussion of a human gene transfer experiment by the RAC include: (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion.

Initial RAC review shall be completed within 15 working days of receipt of a complete submission (see Appendix M-I-A, *Requirements for Protocol Submission*). At the end of the 15-day review period, NIH OBA will notify the Principal Investigator(s) in writing about the results of the RAC's initial review. Two outcomes are possible: (1) the experiment does not present characteristics that warrant further review and discussion and is therefore exempt from public RAC review and discussion; or (2) the experiment presents characteristics that warrant public RAC review and discussion. Completion of the RAC review process is defined as: (1) receipt by the Principal Investigator(s) of a letter from NIH OBA indicating that the submission does not present characteristics that warrant public RAC review and discussion; or (2) receipt by the Principal Investigator(s) of a letter from NIH OBA after public RAC review that summarizes the committee's key comments and recommendations (if any).

If a human gene transfer protocol is submitted less than eight weeks before a scheduled RAC meeting and is subsequently recommended for public RAC review and discussion, the review of the protocol by the RAC will be deferred until the next scheduled RAC meeting. This eight-week period is needed to ensure adequate time for public notice and comment and thorough review by the committee members.

No research participant shall be enrolled (see definition of enrollment in Section I-E-7) in the human gene transfer experiment until: (1) the RAC review process has been completed; (2) Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained; (3) Institutional Review Board (IRB) approval has been obtained; and (4) all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant numbers(s) if applicable.

#### Appendix M-I-B-2. Public RAC Review and Discussion

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. In making a determination whether an experiment presents characteristics warranting public RAC review and discussion, reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been

resolved. Other factors that may warrant public review and discussion of a human gene transfer experiment by the RAC include: (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion.

After a human gene transfer experiment is reviewed by the full RAC at a regularly scheduled meeting, NIH OBA will send a letter summarizing the RAC key comments and recommendations (if any) regarding the protocol to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate. Completion of RAC review is defined as receipt by the Principal Investigator(s) of a letter from NIH OBA summarizing the committee's findings. Unless NIH OBA determines that there are exceptional circumstances, the RAC summary letter will be sent to the Principal Investigator(s) within 10 working days after the completion of the RAC meeting at which the experiment was reviewed.

RAC meetings will be open to the public except where trade secrets or confidential commercial information are reviewed. To enable all aspects of the protocol review process to be open to the public, information provided in response to Appendix M should not contain trade secrets or confidential commercial information. No application submitted to NIH OBA shall be designated as 'confidential' in its entirety. In the event that an investigator determines that specific responses to one or more of the items described in Appendix M should be considered as confidential commercial information or a trade secret, each item must be clearly identified as such. The cover letter (attached to the submitted material) shall: (1) clearly designate the information that is considered as confidential commercial information or a trade secret; and (2) explain and justify each designation.

### Appendix M-I-C. Reporting Requirements

### Appendix M-I-C-1. Initiation of the Clinical Investigation

No later than 20 working days after enrollment (see definition of enrollment in Section I-E-7) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the FDA Investigational New Drug Application (IND) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

### Appendix M-I-C-2. Additional Clinical Trial Sites

No research participant shall be enrolled (see definition of enrollment in Section I-E-7) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

### Appendix M-I-C-3. Annual Reports

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.

(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number

whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

**Appendix M-I-C-4. Safety Reporting**

Principal Investigators must submit, in accordance with this section, Appendix M-I-C-4-a and Appendix M-I-C-4-b, a written report on: (1) any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events); and (2) any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in Appendix M-I-C-4-b.

Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Principal Investigators may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone and fax numbers of the contact(s). The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

The three alternative mechanisms for reporting serious adverse events to the NIH OBA are: by e-mail to oba@od.nih.gov; by fax to 301-496-9839; or by mail to the Office of Biotechnology Activities, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985.

**Appendix M-I-C-4-a. Safety Reporting: Content and Format**

The serious adverse event report must include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type , e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo, ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at: http://www4.od.nih.gov/oba/rac/documents1.htm, the FDA MedWatch forms, or other means provided that all of the above elements are specifically included.

Reports from laboratory animal studies as delineated in Appendix M-I-C-4 must be submitted in a narrative format.

### Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Changes in this schedule are permitted only where, under the FDA IND regulations [21 CFR 312(c)(3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity must be reported as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

### Appendix M-I-C-5. Confidentiality

Data submitted in accordance with Appendix M-I-C that are claimed to be confidential commercial or trade secret information must be clearly labeled as such. Prior to making its determination about the confidentiality of data labeled confidential commercial or trade secret, the NIH will contact the Principal Investigator or delegate to ascertain the basis for the claim and subsequently will notify the Principal Investigator or delegate of its final determination regarding the claim.

If NIH determines that the data so labeled are confidential commercial or trade secret and that their public disclosure would promote an understanding of key scientific or safety issues, the NIH will seek agreement from the appropriate party to release such data. Public discussion of scientific and safety issues raised by data submitted in accordance with Appendix M-I-C is vital to informing both investigators and human subjects about the safety of gene transfer research.

To protect the privacy of participants in gene transfer research, any serious adverse event or annual reports submitted to NIH OBA must not contain any information that would identify the human research participants.

### Appendix M-I-D. Safety Assessment in Human Gene Transfer Research

A working group of the RAC, the NIH Gene Transfer Safety Assessment Board, with staff support from the NIH OBA, will: 1) review in closed session as appropriate safety information from gene transfer trials for the purpose of assessing toxicity and safety data across gene transfer trials; 2) identify significant trends or significant single events; and 3) report significant findings and aggregated trend data to the RAC. It is expected that this process will enhance review of new protocols, improve the development, design, and conduct of human gene transfer trials, promote public understanding and awareness of the safety of human gene transfer research studies, and inform the decision-making of potential trial participants.

## Appendix M-II. Description of the Proposal

Responses to this appendix should be provided in the form of either written answers or references to specific sections of the protocol or its appendices. Investigators should indicate the points that are not applicable with a brief explanation. Investigators submitting proposals that employ the same vector systems may refer to preceding documents relating to the vector sequence without having to rewrite such material.

### Appendix M-II-A. Objectives and Rationale of the Proposed Research

State concisely the overall objectives and rationale of the proposed study. Provide information on the specific points that relate to whichever type of research is being proposed.

#### Appendix M-II-A-1. Use of Recombinant DNA for Therapeutic Purposes

For research in which recombinant DNA is transferred in order to treat a disease or disorder (e.g., genetic diseases, cancer, and metabolic diseases), the following questions should be addressed:

**Appendix M-II-A-1-a.** Why is the disease selected for experimental treatment by means of gene transfer a good candidate for such treatment?

**Appendix M-II-A-1-b.** Describe the natural history and range of expression of the disease selected for experimental treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene transfer?

**Appendix M-II-A-1-c.** Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

**Appendix M-II-A-1-d.** What alternative therapies exist? In what groups of subjects are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene transfer?

#### Appendix M-II-A-2. Transfer of DNA for Other Purposes

**Appendix M-II-A-2-a.** Into what cells will the recombinant DNA be transferred? Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using recombinant DNA?

**Appendix M-II-A-2-b.** What alternative methodologies exist? What are their relative advantages and disadvantages as compared to the use of recombinant DNA?

### Appendix M-II-B. Research Design, Anticipated Risks and Benefits

#### Appendix M-II-B-1. Structure and Characteristics of the Biological System

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

**Appendix M-II-B-1-a.** What is the structure of the cloned DNA that will be used?

**Appendix M-II-B-1-a-(1).** Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

**Appendix M-II-B-1-a-(2).** What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)? From what source are these elements derived? Summarize what is currently known about the regulatory character of each element.

**Appendix M-II-B-1-a-(3).** Describe the steps used to derive the DNA construct.

**Appendix M-II-B-1-b.** What is the structure of the material that will be administered to the research participant?

**Appendix M-II-B-1-b-(1).** Describe the preparation, structure, and composition of the materials that will be given to the human research subject or used to treat the subject's cells: (i) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests? (ii) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or contaminating viruses (both replication-competent or replication-defective) or other organisms in the cells or serum used for preparation of the virus stock including any contaminants that may have biological effects? (iii) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being conducted to assess the material to be returned to the subject for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells? (iv) If methods other than those covered by Appendices M-II-B-1 through M-II-B-3, *Research Design, Anticipated Risks and Benefits*, are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are possible sources of contamination? What is the sensitivity of tests used to monitor contamination?

**Appendix M-II-B-1-b-(2).** Describe any other material to be used in preparation of the material to be administered to the human research subject. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

### Appendix M-II-B-2. Preclinical Studies, Including Risk-Assessment Studies

Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.

### Appendix M-II-B-2-a. Delivery System

**Appendix M-II-B-2-a-(1).** What cells are the intended target cells of recombinant DNA? What target cells are to be treated *ex vivo* and returned to the human subject, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?

**Appendix M-II-B-2-a-(2).** Is the delivery system efficient? What percentage of the target cells contain the added DNA?

**Appendix M-II-B-2-a-(3).** How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

**Appendix M-II-B-2-a-(4).** How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

### Appendix M-II-B-2-b. Gene Transfer and Expression

**Appendix M-II-B-2-b-(1).** What animal and cultured cell models were used in laboratory studies to assess the *in vivo* and *in vitro* efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

**Appendix M-II-B-2-b-(2).** What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

**Appendix M-II-B-2-b-(3).** Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression.

**Appendix M-II-B-2-b-(4).** To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

**Appendix M-II-B-2-b-(5).** In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

**Appendix M-II-B-2-b-(6).** Is the gene expressed in cells other than the target cells? If so, to what extent?

**Appendix M-II-B-2-c. Retrovirus Delivery Systems**

**Appendix M-II-B-2-c-(1).** What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

**Appendix M-II-B-2-c-(2).** How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the human subject's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

**Appendix M-II-B-2-c-(3).** What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

**Appendix M-II-B-2-c-(4).** Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of these analyses?

**Appendix M-II-B-2-c-(5).** Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

**Appendix M-II-B-2-d. Non-Retrovirus Delivery/Expression Systems**

If a non-retroviral delivery system is used, what animal studies have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells)? How long have the animals been studied after treatment? What safety studies have been conducted? (Include data about the level of sensitivity of such assays.)

**Appendix M-II-B-3. Clinical Procedures, Including Research Participant Monitoring**

Describe the experimental treatment that will be administered to the human subjects and the diagnostic methods that will be used to monitor the success or failure of the experimental treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:

**Appendix M-II-B-3-a.** Will cells (e.g., bone marrow cells) be removed from human subjects and treated *ex vivo*? If so, describe the type, number, and intervals at which these cells will be removed.

**Appendix M-II-B-3-b.** Will human subjects be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy)?

**Appendix M-II-B-3-c.** What treated cells (or vector/DNA combination) will be given to human subjects? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple experimental treatments? If so, over what period of time?

**Appendix M-II-B-3-d.** How will it be determined that new gene sequences have been inserted into the subject's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses?

**Appendix M-II-B-3-e.** What studies will be conducted to assess the presence and effects of the contaminants?

**Appendix M-II-B-3-f.** What are the clinical endpoints of the study? Are there objectives and quantitative measurements to assess the natural history of the disease? Will such measurements be used in human subject follow-up? How will subjects be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will follow-up continue?

**Appendix M-II-B-3-g.** What are the major beneficial and adverse effects of the experimental treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used.

**Appendix M-II-B-3-h.** If a treated human subject dies, what special post-mortem studies will be performed?

### Appendix M-II-B-4. Public Health Considerations

Describe any potential benefits and hazards of the proposed gene transfer to persons other than the human subjects receiving the experimental treatment. Specifically:

**Appendix M-II-B-4-a.** On what basis are potential public health benefits or hazards postulated?

**Appendix M-II-B-4-b.** Is there a significant possibility that the added DNA will spread from the human subject to other persons or to the environment?

**Appendix M-II-B-4-c.** What precautions will be taken against such spread (e.g., subjects sharing a room, health-care workers, or family members)?

**Appendix M-II-B-4-d.** What measures will be undertaken to mitigate the risks, if any, to public health?

**Appendix M-II-B-4-e.** In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to subjects? Are such concerns applicable to health care personnel?

### Appendix M-II-B-5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of recombinant DNA. Describe the laboratory and clinical facilities where the proposed study will be performed. Specifically:

**Appendix M-II-B-5-a.** What professional personnel (medical and nonmedical) will be involved in the proposed study and what is their relevant expertise? Provide a two-page curriculum vitae for each key professional person in biographical sketch format (see Appendix M-I-A, *Requirements for Protocol Submission*).

**Appendix M-II-B-5-b.** At what hospital or clinic will the experimental treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will subjects occupy regular hospital beds or clinical research center beds? Where will subjects reside during the follow-up period? What special arrangements will be made for the comfort and consideration of the research participants. Will the research institution designate an ombudsman, patient care representative, or other individual to help protect the rights and welfare of the research participant?

### Appendix M-II-C. Selection of the Human Subjects

Estimate the number of human subjects to be involved in the proposed study. Describe recruitment procedures and eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable. Specifically:

**Appendix M-II-C-1.** How many subjects do you plan to involve in the proposed study?

**Appendix M-II-C-2.** How many eligible subjects do you anticipate being able to identify each year?

**Appendix M-II-C-3.** What recruitment procedures do you plan to use?

**Appendix M-II-C-4.** What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

**Appendix M-II-C-5.** How will subjects be selected if it is not possible to include all who desire to participate?

### Appendix M-III. Informed Consent

In accordance with the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should indicate how the Informed Consent document makes clear the special requirements of gene transfer research. If a proposal involves children, special attention should be paid to the Protection of Human Subjects (45 CFR Part 46), Subpart D, Additional Protections for Children Involved as Subjects in Research.

### Appendix M-III-A. Communication About the Study to Potential Participants

**Appendix M-III-A-1.** Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?

**Appendix M-III-A-2.** How will the major points covered in Appendix M-II, *Description of Proposal*, be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?

**Appendix M-III-A-3.** What is the length of time that potential participants will have to make a decision about their participation in the study?

**Appendix M-III-A-4.** If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?

### Appendix M-III-B. Informed Consent Document

Submission of a human gene transfer experiment to NIH OBA must include a copy of the proposed informed consent document. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a "marker" or to enhance the power of immunotherapy for cancer.

Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent document should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45 CFR 46). Indicate if each of the specified items appears in the Informed Consent document or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are omitted from the consent process or the Informed Consent document.

### Appendix M-III-B-1. General Requirements of Human Subjects Research

#### Appendix M-III-B-1-a. Description/Purpose of the Study

The subjects should be provided with a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component.

### Appendix M-III-B-1-b. Alternatives

The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches.

### Appendix M-III-B-1-c. Voluntary Participation

The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled.

### Appendix M-III-B-1-d. Benefits

The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others.

### Appendix M-III-B-1-e. Possible Risks, Discomforts, and Side Effects

There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization.

If verbal descriptors (e.g., "rare," "uncommon," or "frequent") are used to express quantitative information regarding risk, these terms should be explained.

The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe.

The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started.

### Appendix M-III-B-1-f. Costs

The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved.

Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury.

## Appendix M-III-B-2. Specific Requirements of Gene Transfer Research

### Appendix M-III-B-2-a. Reproductive Considerations

To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified.

The inclusion of pregnant or lactating women should be addressed.

### Appendix M-III-B-2-b. Long-Term Follow-Up

To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The investigator should request that subjects continue to provide a current address and telephone number.

The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed.

### Appendix M-III-B-2-c. Request for Autopsy

To obtain vital information about the safety and efficacy of gene transfer, subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance.

### Appendix M-III-B-2-d. Interest of the Media and Others in the Research

To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (i) that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (ii) that representatives of applicable Federal agencies (e.g., the National Institutes of Health and the Food and Drug Administration), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records.

### Appendix M-IV. Privacy

Indicate what measures will be taken to protect the privacy of subjects and their families as well as maintain the confidentiality of research data. These measures should help protect the confidentiality of information that could directly or indirectly identify study participants.

**Appendix M-IV-A.** What provisions will be made to honor the wishes of individual human subjects (and the parents or guardians of pediatric or mentally handicapped subjects) as to whether, when, or how the identity of a subject is publicly disclosed.

**Appendix M-IV-B.** What provisions will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual subjects?

### Appendix M-V. Special Issues

Although the following issues are beyond the normal purview of local Institutional Review Boards, investigators should respond to the following questions:

**Appendix M-V-A.** What steps will be taken, consistent with Appendix M-IV, *Privacy*, to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

**Appendix M-V-B.** Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

### Appendix M-VI. Footnotes of Appendix M

**Appendix M-VI-A.** Human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are exempt from Appendix M-I, *Requirements for Protocol Submission*, *Review and Reporting – Human Gene Transfer Experiments*.

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## APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the *NIH Guidelines* apply to plant research activities with the following modifications:

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

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

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

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

### Appendix P-I. General Plant Biosafety Levels

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

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

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

**Appendix P-I-D.** BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in Appendix P-III, *Biological Containment Practices*, provide flexible approaches to ensure the safe conduct of research.

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

**Appendix P-II. Physical Containment Levels**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Appendix P-II-B-1-b-(3).** The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/OBA and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

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

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

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

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

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

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

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

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

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

**Appendix P-II-B-1-f-(1).** A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

**Appendix P-II-B-1-f-(2).** If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

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

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

**Appendix P-II-B-1-g-(1).** Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P)**

**Appendix P-II-C-1. Standard Practices (BL3-P)**

**Appendix P-II-C-1-a. Greenhouse Access (BL3-P)**

**Appendix P-II-C-1-a-(1).** Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

**Appendix P-II-C-1-a-(2).** Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

**Appendix P-II-C-1-b. Records (BL3-P)**

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

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

**Appendix P-II-C-1-b-(3).** The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

**Appendix P-II-C-1-c. Decontamination and Inactivation (BL3-P)**

**Appendix P-II-C-1-c-(1).** All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

**Appendix P-II-C-1-d. Control of Undesired Species and Motile Macroorganisms (BL3-P)**

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

**Appendix P-II-C-1-d-(2).** Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

**Appendix P-II-C-1-e. Concurrent Experiments Conducted in the Greenhouse (BL3-P)**

**Appendix P-II-C-1-e-(1).** Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.

**Appendix P-II-C-1-f. Signs (BL3-P)**

**Appendix P-II-C-1-f-(1).** A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

**Appendix P-II-C-1-f-(2).** If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

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

**Appendix P-II-C-1-g. Transfer of Materials (BL3-P)**

**Appendix P-II-C-1-g-(1).** Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

**Appendix P-II-C-1-h. Greenhouse Practices Manual (BL3-P)**

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

**Appendix P-II-C-1-i. Protective Clothing (BL3-P)**

**Appendix P-II-C-1-i-(1).** Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

**Appendix P-II-C-1-i-(2).** Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

**Appendix P-II-C-1-j. Other (BL3-P)**

**Appendix P-II-C-1-j-(1).** Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

**Appendix P-II-C-1-j-(2).** All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

## Appendix P-II-C-2. Facilities (BL3-P)

### Appendix P-II-C-2-a. Definitions (BL3-P)

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

**Appendix P-II-C-2-a-(2).** The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

### Appendix P-II-C-2-b. Greenhouse Design (BL3-P)

**Appendix P-II-C-2-b-(1).** The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

**Appendix P-II-C-2-b-(2).** Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

**Appendix P-II-C-2-b-(3).** The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

**Appendix P-II-C-2-b-(4).** The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

**Appendix P-II-C-2-b-(5).** Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix P-II-C-2-b-(6).** Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix P-II-C-2-b-(7).** The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

### Appendix P-II-C-2-c. Autoclaves (BL3-P)

**Appendix P-II-C-2-c-(1).** An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

### Appendix P-II-C-2-d. Supply and Exhaust Air Ventilation Systems (BL3-P)

**Appendix P-II-C-2-d-(1).** An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

**Appendix P-II-C-2-d-(2).** The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

**Appendix P-II-C-2-e. Other (BL3-P)**

**Appendix P-II-C-2-e-(1).** BL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

**Appendix P-II-C-2-e-(2).** Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

**Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P)**

**Appendix P-II-D-1. Standard Practices (BL4-P)**

**Appendix P-II-D-1-a. Greenhouse Access (BL4-P)**

**Appendix P-II-D-1-a-(1).** Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

**Appendix P-II-D-1-a-(2).** Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

**Appendix P-II-D-1-a-(3).** Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

**Appendix P-II-D-1-a-(4).** Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

**Appendix P-II-D-1-a-(5).** Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4-P practices and procedures.

**Appendix P-II-D-1-b. Records (BL4-P)**

**Appendix P-II-D-1-b-(1).** A record shall be kept of all experimental materials brought into or removed from the greenhouse.

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

**Appendix P-II-D-1-b-(3).** A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

**Appendix P-II-D-1-b-(4).** The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

**Appendix P-II-D-1-c. Decontamination and Inactivation (BL4-P)**

**Appendix P-II-D-1-c-(1).** All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

**Appendix P-II-D-1-c-(2).** Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

**Appendix P-II-D-1-c-(3).** Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

**Appendix P-II-D-1-d. Control of Undesired Species and Motile Macroorganisms (BL4-P)**

**Appendix P-II-D-1-d-(1).** A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

**Appendix P-II-D-1-d-(2).** Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4-P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

**Appendix P-II-D-1-e. Concurrent Experiments Conducted in the Greenhouse (BL4-P)**

**Appendix P-II-D-1-e-(1).** Experiments involving organisms that require a containment level lower than BL4-P may be conducted in the greenhouse concurrently with experiments that require BL4-P containment provided that all work is conducted in accordance with BL4-P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4-P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

**Appendix P-II-D-1-f. Signs (BL4-P)**

**Appendix P-II-D-1-f-(1).** A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

**Appendix P-II-D-1-f-(2).** If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

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

**Appendix P-II-D-1-g. Transfer of Materials (BL4-P)**

**Appendix P-II-D-1-g-(1).** Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

**Appendix P-II-D-1-g-(2).** Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

**Appendix P-II-D-1-h. Greenhouse Practices Manual (BL4-P)**

**Appendix P-II-D-1-h-(1).** A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

**Appendix P-II-D-1-i. Protective Clothing (BL4-P)**

**Appendix P-II-D-1-i-(1).** Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

**Appendix P-II-D-1-i-(2).** Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

**Appendix P-II-D-1-i-(3).** All laboratory clothing shall be autoclaved before laundering.

**Appendix P-II-D-2. Facilities (BL4-P)**

**Appendix P-II-D-2-a. Greenhouse Design (BL4-P)**

**Appendix P-II-D-2-a-(1).** The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.

**Appendix P-II-D-2-a-(2).** Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

**Appendix P-II-D-2-a-(3).** Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

**Appendix P-II-D-2-a-(4).** Access doors to the greenhouse shall be self-closing and locking.

**Appendix P-II-D-2-a-(5).** The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

**Appendix P-II-D-2-a-(6).** The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix P-II-D-2-a-(7).** Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix P-II-D-2-a-(8).** A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

**Appendix P-II-D-2-b. Autoclaves (BL4-P)**

**Appendix P-II-D-2-b-(1).** A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

### Appendix P-II-D-2-c. Supply and Exhaust Air Ventilation Systems (BL4-P)

**Appendix P-II-D-2-c-(1).** An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

**Appendix P-II-D-2-c-(2).** Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

### Appendix P-II-D-2-d. Other (BL4-P)

**Appendix P-II-D-2-d-(1).** Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

**Appendix P-II-D-2-d-(2).** A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

**Appendix P-II-D-2-d-(3).** Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

**Appendix P-II-D-2-d-(4).** If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

## Appendix P-III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

### Appendix P-III-A. Biological Containment Practices (Plants)

**Appendix P-III-A-1.** Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

### Appendix P-III-B. Biological Containment Practices (Microorganisms)

**Appendix P-III-B-1.** Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

### Appendix P-III-C. Biological Containment Practices (Macroorganisms)

**Appendix P-III-C-1.** Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

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## APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING ANIMALS

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G (*Physical Containment*) when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q-III-D, *Footnotes and References for Appendix Q*). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

### Appendix Q-I. General Considerations

### Appendix Q-I-A. Containment Levels

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in Section III, *Experiments Covered by the NIH Guidelines*. For the purpose of animal research, four levels of containment are established. These are referred to as BL1-Animals (N), BL2-N, BL3-N, and BL4-N and are described in the following appendices of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

**Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N)**

**Appendix Q-I-B-1.** When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

**Appendix Q-I-B-2.** A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

**Appendix Q-II. Physical and Biological Containment Levels**

**Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N)**

**Appendix Q-II-A-1. Standard Practices (BL1-N)**

**Appendix Q-II-A-1-a. Animal Facility Access (BL1-N)**

**Appendix Q-II-A-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-A-1-a-(2).** Access to the containment area shall be limited or restricted when experimental animals are being held.

**Appendix Q-II-A-1-a-(3).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-A-1-b. Other (BL1-N)**

**Appendix Q-II-A-1-b-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-A-1-b-(2)** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-A-1-b-(3).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-A-2. Animal Facilities (BL1-N)**

**Appendix Q-II-A-2-a.** Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

**Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N)** (See Appendix Q-III-A, *Footnotes and References for Appendix Q*)

**Appendix Q-II-B-1. Standard Practices (BL2-N)**

**Appendix Q-II-B-1-a. Animal Facility Access (BL2-N)**

**Appendix Q-II-B-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-B-1-a-(2).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-B-1-a-(3).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-B-1-a-(4).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

**Appendix Q-II-B-1-a-(5).** Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

**Appendix Q-II-B-1-b. Decontamination and Inactivation (BL2-N)**

**Appendix Q-II-B-1-b-(1).** Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

**Appendix Q-II-B-1-b-(2).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-B-1-c. Signs (BL2-N)**

**Appendix Q-II-B-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-B-1-d. Protective Clothing (BL2-N)**

**Appendix Q-II-B-1-d-(1).** Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

**Appendix Q-II-B-1-d-(2).** Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

**Appendix Q-II-B-1-e. Records (BL2-N)**

**Appendix Q-II-B-1-e-(1).** Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-B-1-e-(2).** When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

**Appendix Q-II-B-1-f. Transfer of Materials (BL2-N)**

**Appendix Q-II-B-1-f-(1).** Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

**Appendix Q-II-B-1-g. Other (BL2-N)**

**Appendix Q-II-B-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-B-1-g-(2).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-B-1-g-(3).** Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

**Appendix Q-II-B-1-g-(4).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-B-1-g-(5).** Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-B-1-g-(6).** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-B-1-g-(7).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-B-1-g-(8).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-B-2. Animal Facilities (BL2-N)**

**Appendix Q-II-B-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

**Appendix Q-II-B-2-b.** Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix Q-II-B-2-c.** The animal containment area shall be designed so that it can be easily cleaned.

**Appendix Q-II-B-2-d.** Windows that open shall be fitted with fly screens.

**Appendix Q-II-B-2-e.** An autoclave shall be available for decontamination of laboratory wastes.

**Appendix Q-II-B-2-f.** If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

**Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N)** (See Appendix Q-III-B, *Footnotes and References for Appendix Q*)

**Appendix Q-II-C-1. Standard Practices (BL3-N)**

**Appendix Q-II-C-1-a. Animal Facility Access (BL3-N)**

**Appendix Q-II-C-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-C-1-a-(2).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-C-1-a-(3).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-C-1-a-(4).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

**Appendix Q-II-C-1-a-(5).** Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

**Appendix Q-II-C-1-b. Decontamination and Inactivation (BL3-N)**

**Appendix Q-II-C-1-b-(1).** The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

**Appendix Q-II-C-1-b-(2).** All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

**Appendix Q-II-C-1-b-(3).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-C-1-b-(4).** Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

**Appendix Q-II-C-1-b-(5).** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

**Appendix Q-II-C-1-c. Signs (BL3-N)**

**Appendix Q-II-C-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-C-1-d. Protective Clothing (BL3-N)**

**Appendix Q-II-C-1-d-(1).** Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3-N area and wearing of personal clothing.

**Appendix Q-II-C-1-d-(2).** Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

**Appendix Q-II-C-1-d-(3).** Appropriate respiratory protection shall be worn in rooms containing experimental animals.

**Appendix Q-II-C-1-e. Records (BL3-N)**

**Appendix Q-II-C-1-e-(1).** Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

**Appendix Q-II-C-1-e-(2).** Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-C-1-e-(3).** When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

**Appendix Q-II-C-1-f. Transfer of Materials (BL3-N)**

**Appendix Q-II-C-1-f-(1).** Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

**Appendix Q-II-C-1-f-(2).** Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

**Appendix Q-II-C-1-g. Other (BL3-N)**

**Appendix Q-II-C-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-C-1-g-(2).** Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

**Appendix Q-II-C-1-g-(3).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-C-1-g-(4).** Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-C-1-g-(5).** Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3-N practices.

**Appendix Q-II-C-1-g-(6).** Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

**Appendix Q-II-C-1-g-(7).** All procedures shall be performed carefully to minimize the creation of aerosols.

**Appendix Q-II-C-1-g-(8).** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-C-1-g-(9).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-C-1-g-(10).** All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

**Appendix Q-II-C-1-g-(11).** Personnel shall be required to shower before exiting the BL3-N area and wearing personal clothing.

**Appendix Q-II-C-1-g-(12).** Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

**Appendix Q-II-C-1-g-(13).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-C-1-g-(14).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-C-2. Animal Facilities (BL3-N)**

**Appendix Q-II-C-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

**Appendix Q-II-C-2-b.** The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix Q-II-C-2-c.** Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

**Appendix Q-II-C-2-d.** An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-C-2-e.** If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

**Appendix Q-II-C-2-f.** Access doors to the containment area shall be self-closing.

**Appendix Q-II-C-2-g.** The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

**Appendix Q-II-C-2-h.** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

**Appendix Q-II-C-2-i.** An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

**Appendix Q-II-C-2-j.** If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

**Appendix Q-II-C-2-k.** Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix Q-II-C-2-l.** In lieu of open housing in the special animal room, animals held in a BL3-N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

**Appendix Q-II-C-2-m.** Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

**Appendix Q-II-C-2-n.** Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

**Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N)** (See Appendix Q-III-C, *Footnotes and References for Appendix Q*)

**Appendix Q-II-D-1. Standard Practices (BL4-N)**

**Appendix Q-II-D-1-a. Animal Facility Access (BL4-N)**

**Appendix Q-II-D-1-a-(1).** Individuals under 16 years of age shall not be permitted to enter the animal area.

**Appendix Q-II-D-1-a-(2).** The containment area shall be locked.

**Appendix Q-II-D-1-a-(3).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-D-1-a-(4).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-D-1-a-(5).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

**Appendix Q-II-D-1-a-(6).** Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

**Appendix Q-II-D-1-a-(7).** Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

**Appendix Q-II-D-1-a-(8).** Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

**Appendix Q-II-D-1-b. Decontamination and Inactivation (BL4-N)**

**Appendix Q-II-D-1-b-(1).** All contaminated liquid or solid wastes shall be decontaminated before disposal.

**Appendix Q-II-D-1-b-(2).** The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

**Appendix Q-II-D-1-b-(3).** All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

**Appendix Q-II-D-1-b-(4).** No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix Q-II-D-1-b-(5).** When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

**Appendix Q-II-D-1-b-(6).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-D-1-b-(7).** Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

**Appendix Q-II-D-1-b-(8).** An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-D-1-b-(9).** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

**Appendix Q-II-D-1-c. Signs (BL4-N)**

**Appendix Q-II-D-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-D-1-d. Protective Clothing (BL4-N)**

**Appendix Q-II-D-1-d-(1).** Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4-N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

**Appendix Q-II-D-1-d-(2).** A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

**Appendix Q-II-D-1-d-(3).** Appropriate respiratory protection shall be worn in rooms containing experimental animals.

**Appendix Q-II-D-1-e. Records (BL4-N)**

**Appendix Q-II-D-1-e-(1).** Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

**Appendix Q-II-D-1-e-(2).** A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-D-1-e-(3).** When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

**Appendix Q-II-D-1-e-(4).** A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

**Appendix Q-II-D-1-f. Transfer of Materials (BL4-N)**

**Appendix Q-II-D-1-f-(1).** No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix Q-II-D-1-f-(2).** Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4-N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4-N animal facility to one with a lower containment classification.

**Appendix Q-II-D-1-f-(3).** Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

**Appendix Q-II-D-1-g. Other (BL4-N)**

**Appendix Q-II-D-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-D-1-g-(2).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-D-1-g-(3).** Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-D-1-g-(4).** Experiments involving other organisms that require containment levels lower than BL4-N may be conducted in the same area concurrently with experiments requiring BL4-N containment provided that they are conducted in accordance with BL4-N practices.

**Appendix Q-II-D-1-g-(5).** Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

**Appendix Q-II-D-1-g-(6).** All procedures shall be performed carefully to minimize the creation of aerosols.

**Appendix Q-II-D-1-g-(7).** A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

**Appendix Q-II-D-1-g-(8).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-D-1-g-(9).** The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

**Appendix Q-II-D-1-g-(10).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall

not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-D-1-g-(11).** An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

**Appendix Q-II-D-1-g-(12).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-D-1-g-(13).** Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix Q-II-D-2. Animal Facilities (BL4-N)**

**Appendix Q-II-D-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

**Appendix Q-II-D-2-b.** The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix Q-II-D-2-c.** Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

**Appendix Q-II-D-2-d.** An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-D-2-e.** Access doors to the containment area shall be self-closing.

**Appendix Q-II-D-2-f.** All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

**Appendix Q-II-D-2-g.** The BL4-N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

**Appendix Q-II-D-2-h.** A necropsy room shall be provided within the BL4-N containment area.

**Appendix Q-II-D-2-i.** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

**Appendix Q-II-D-2-j.** A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

**Appendix Q-II-D-2-k.** Exhaust air from BL4-N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4-N containment area.

**Appendix Q-II-D-2-l.** All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least $1x10^{-3}$ micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80-120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

**Appendix Q-II-D-2-m.** If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally $1x10^{8}$ organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilis*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

**Appendix Q-II-D-2-n.** All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

**Appendix Q-II-D-2-o.** Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

**Appendix Q-II-D-2-p.** Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

**Appendix Q-II-D-2-q.** The supply water distribution system shall be fitted with a back-flow preventer or break tank.

**Appendix Q-II-D-2-r.** All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

**Appendix Q-II-D-2-s.** Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

### Appendix Q-III. Footnotes and References for Appendix Q

**Appendix Q-III-A.** If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

**Appendix Q-III-B.** Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3-N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

**Appendix Q-III-C.** Risk Group 4 and restricted microorganisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import animal or plant pathogens, special approval must be obtained from U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, MD 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

**Appendix Q-III-D.** Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix Q, may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in Appendices G and P.

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