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

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

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

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 or synthetic nucleic acid molecules, or nucleic acids 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-E-3-a. Experiments involving the breeding of certain BL1 transgenic rodents are exempt under Section III-F, Exempt Experiments (See Appendix C-VII, Generation of BL1 Transgenic Rodents via Breeding).

Section III-F. Exempt Experiments

The following recombinant or synthetic nucleic acid molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required; however, other federal and state standards of biosafety may still apply to such research (for example, the Centers for Disease Control and Prevention (CDC)/NIH publication Biosafety in Microbiological and Biomedical Laboratories).

Section III-F-1. Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.

Section III-F-2. Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.

Section III-F-3. Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

Section III-F-4. Those that consist entirely of nucleic acids 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-5. Those that consist entirely of nucleic acids from a 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-6. 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-6--Sublists of Natural Exchangers, for a list of
natural exchangers that are exempt from the *NIH Guidelines*.

**Section III-F-7.** Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

**Section III-F-8.** 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-8 for other classes of experiments which are exempt from the *NIH Guidelines*.

**************************************************************************

**SECTION IV. ROLES AND RESPONSIBILITIES**

**Section IV-A. Policy**

The safe conduct of experiments involving recombinant or synthetic nucleic acid molecules 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 or synthetic nucleic acid molecules cannot be foreseen. The utilization of new genetic manipulation techniques may enable work previously conducted using recombinant means to be accomplished faster, more efficiently, or at larger scale. These techniques have not yet yielded organisms that present safety concerns that fall outside the current risk assessment framework used for recombinant nucleic acid research. Nonetheless, an appropriate risk assessment of experiments involving these techniques must be conducted taking into account the way these approaches may alter the risk assessment. As new techniques develop, the *NIH Guidelines* should be periodically reviewed to determine whether and how such research should be explicitly addressed.

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. Therefore, each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all research with recombinant or synthetic nucleic acid molecules conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines*. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant or synthetic nucleic acid 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecule research at Biosafety
APPENDIX A. EXEMPTIONS UNDER SECTION III-F-6--SUBLISTS OF NATURAL EXCHANGERS

Certain specified recombinant or synthetic nucleic acid 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-6, 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-6 Sublists of Natural Exchangers. Section III-F-6, Exempt Experiments, describes recombinant or synthetic nucleic acid 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/synthetic organism (see Biosafety in Microbiological and Biomedical Laboratories, 5th edition, 2007, 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
Streptomycyes rimosus
Streptomycyes coelicolor

Appendix A-IV. Sublist D

Streptomycyes griseus
Streptomycyes cyaneus
Streptomycyes 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)

<table>
<thead>
<tr>
<th>Risk Group 1 (RG1)</th>
<th>Agents that are not associated with disease in healthy adult humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Group 2 (RG2)</td>
<td>Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available</td>
</tr>
<tr>
<td>Risk Group 3 (RG3)</td>
<td>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)</td>
</tr>
<tr>
<td>Risk Group 4 (RG4)</td>
<td>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)</td>
</tr>
</tbody>
</table>

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 – all serotypes); and recombinant or synthetic 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.
Polyoma viruses
--Polyoma 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-8

Section III-F-8 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-8, for other classes of experiments which are exempt from the NIH Guidelines." The following classes of experiments are exempt under Section III-F-8:

Appendix C-I. Recombinant or Synthetic Nucleic Acid Molecules in Tissue Culture

Recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see Appendix C-VIII-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-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) 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, (iii) 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 (iv) 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-VIII-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-VIII-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 or synthetic nucleic acid molecule 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-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) 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, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the 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-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 unmodified host organism; 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-B, which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) 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, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) 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. Kluyveromyces Host-Vector Systems

Experiments involving Kluyveromyces lactis host-vector systems, with the exception of experiments listed in Appendix C-IV-A, are exempt from the NIH Guidelines provided laboratory-adapted strains are used (i.e. strains that have been adapted to growth under optimal or defined laboratory conditions). 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 unmodified host organism; the Institutional Biosafety Committee may specify higher containment if deemed necessary.

Appendix C-IV-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B, which require NIH/OBA and Institutional Biosafety Committee approval before initiation; (ii) 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; (iii) 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.  **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-V-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 unmodified host organism; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-V-A.  **Exceptions**

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) 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, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) 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.  **Extrachromosomal Elements of Gram Positive Organisms**

Recombinant or synthetic nucleic acid 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 amylo liquefaciens*
*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*
*Staphylococcus 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 salivarius
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Appendix C-VI-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) 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, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) 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-VII. 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-VIII. Generation of BL1 Transgenic Rodents via Breeding

The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the NIH Guidelines if:
(1) Both parental rodents can be housed under BL1 containment; and
(2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
(3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

Appendix C-IX. Footnotes and References of Appendix C

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

Appendix C-IX-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
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 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: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E-III. *Escherichia coli*

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


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

The following are certified EK2 systems based on bacteriophage lambda:

<table>
<thead>
<tr>
<th>Vector</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>λgt WESAB*</td>
<td>DP50supF</td>
</tr>
<tr>
<td>λgt WESAB+</td>
<td>DP50supF</td>
</tr>
<tr>
<td>λgt ZJ virλB*</td>
<td><em>Escherichia coli</em> K-12</td>
</tr>
<tr>
<td>λgtALO-λB</td>
<td>DP50supF</td>
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<td>DP50 or DP50supF</td>
</tr>
<tr>
<td>Charon 4A</td>
<td>DP50 or DP50supF</td>
</tr>
<tr>
<td>Charon 16A</td>
<td>DP50 or DP50supF</td>
</tr>
<tr>
<td>Charon 21A</td>
<td>DP50supF</td>
</tr>
<tr>
<td>Charon 23A</td>
<td>DP50 or DP50supF</td>
</tr>
<tr>
<td>Charon 24A</td>
<td>DP50 or DP50supF</td>
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</tbody>
</table>

*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 DP50supF 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, plJ101, 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, diptheria 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$_{50}$ $\geq 100$ 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).