

NIH Guidelines Section III

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 , <i>Footnotes and References of Sections I-IV</i>), if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine, or agriculture
III-B-1	Experiments Involving the Cloning of Toxin Molecules with LD ₅₀ of Less than 100 Nanograms per Kilogram Body Weight
III-B-2	Experiments that have been Approved (under Section III-A-1-a) as Major Actions under the <i>NIH Guidelines</i>
III-C-1	Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants
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)
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
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
III-D-4	Experiments involving whole animals 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 animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals
III-D-5	Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecules, may be conducted under the containment conditions described in Sections III-D-5-a through III-D-5-e .
III-D-6	Experiments Involving More than 10 Liters of Culture
III-D-7	Experiments with influenza viruses generated by recombinant or synthetic methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations)
III-E-1	Experiments Involving the Formation of Recombinant or Synthetic Nucleic Acid Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus
III-E-2	Experiments involving nucleic acid molecule-modified whole plants, and/or experiments involving recombinant or synthetic nucleic acid molecule-modified organisms associated with whole plants, except those that fall under Section III-A , III-B , III-D , or III-F
III-E-3	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) requiring BL1 containment
III-F Experiments Exempt from NIH Guidelines	
III-F-1	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 LD ₅₀ of less than 100 ng 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
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
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
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.
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)
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. See Appendices A-I through A-VI, <i>Exemptions under Section III-F-6--Sublists of Natural Exchangers</i> , for a list of natural exchangers that are exempt from the <i>NIH Guidelines</i> .
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
III-F-8	Those that do not present a significant risk to health or the environment (see Section IV-C-1-b(1)-(c) , <i>Major Actions</i>), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C , <i>Exemptions under Section III-F-8</i> for other classes of experiments which are exempt from the <i>NIH Guidelines</i> .

SECTION III. EXPERIMENTS COVERED BY THE *NIH GUIDELINES*

This section describes six categories of experiments involving recombinant or synthetic nucleic acid molecules: (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 ability to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC.

Consideration should be given as to whether the drug resistance trait to be used in the experiment would render that microorganism resistant to the primary drug available to and/or indicated for certain populations, for example children or pregnant women.

At the request of an Institutional Biosafety Committee, NIH/OBA will make a determination regarding whether a specific experiment involving the deliberate transfer of a drug resistance trait falls under Section III-A-1-a and therefore requires RAC review and NIH Director approval. An Institutional Biosafety Committee may also consult with NIH/OBA regarding experiments that do not meet the requirements of Section III-A-1-a but nonetheless raise important public health issues. NIH/OBA will consult, as needed, with one or more experts, which may include the 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 LD50 of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 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-B-2. Experiments that have been Approved (under Section III-A-1-a) as Major Actions under the NIH Guidelines

Upon receipt and review of an application from the investigator, NIH/OBA may determine that a proposed experiment is equivalent to an experiment that has previously been approved by the NIH Director as a Major Action, including experiments approved prior to implementation of these changes. An experiment will only be considered equivalent if, as determined by NIH/OBA, there are no substantive differences and pertinent information has not emerged since submission of the initial III-A-1-a experiment that would change the biosafety and public health considerations for the proposed experiments. If such a determination is made by NIH/OBA, these experiments will not require review and approval under Section III-A.

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 or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
 - a. Contain more than 100 nucleotides; or
 - b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
 - c. Have the potential to replicate in a cell; or
 - d. Can be translated or transcribed.

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-IB-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 or synthetic nucleic acid molecules 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 or synthetic nucleic acid

molecules 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 or synthetic nucleic acid molecules 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 or synthetic nucleic acid molecules into restricted agents shall be set on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture - [Animal and Plant Health Inspection Service](#) (USDA/APHIS) 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 or synthetic nucleic acid molecules 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 LD50 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 or synthetic nucleic acid molecules or nucleic acid 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 or Synthetic 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 or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecules, 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 or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by [Section 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 [Section III-D-4-a](#), 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecules, 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of <100 nanograms per kilogram body weight fall under [Section III-B-1](#), *Experiments Involving the Cloning of Toxin Molecules with LD50 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 or synthetic nucleic acid molecule-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 or Synthetic Recombinant or synthetic nucleic acid Molecules*, shall be used. [Appendix K](#) describes containment conditions Good Large Scale Practice through BL3-Large Scale.

Section III-D-7. Experiments Involving Influenza Viruses

Experiments with influenza viruses generated by recombinant or synthetic methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the Risk Group of the virus that was the source of the majority of segments in the recombinant or synthetic virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3). Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses) unless indicated below.

Section III-D-7-a. Human H2N2 (1957-1968). Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BL3 enhanced (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant or synthetic virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

Section III-D-7-b. Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1). Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced containment, (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments involving influenza viruses containing a minority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at biosafety level 2 and after they have been excluded pursuant to [9 CFR 121.3\(e\)](#). OBA is available to IBCs to provide consultation with the RAC and influenza virus experts when risk assessments are being made to determine the appropriate biocontainment for experiments with influenza viruses containing a minority of gene/segments from HPAI H5N1. Such experiments may be performed at BL3 enhanced containment or containment may be lowered to biosafety level 2, the level of containment for most research with other influenza viruses. ([USDA/APHIS](#) regulations and decisions on lowering containment also apply.) In deciding to lower containment, the IBC should consider whether, in at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates), there is evidence that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. This should be determined by measuring biological indices appropriate for the specific animal model (e.g., severe weight loss, elevated temperature, mortality or neurological symptoms).

Section III-D-7-c. 1918 H1N1. Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses).

Section III-D-7-d. Antiviral Susceptibility and Containment. The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968). If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc.

Experiments with 1918 H1N1, human H2N2 (1957-1968) or HPAI H5N1 that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational

antiviral agents being developed for influenza) would be subject to [Section III-A-1](#) (Major Actions) and require RAC review and NIH Director approval. As per [Section I-A-1](#) of the *NIH Guidelines*, if the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments.

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 nonpathogenic 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 or Synthetic Nucleic Acid Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

Recombinant or synthetic nucleic acid 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 nucleic acid molecule-modified whole plants, and/or experiments involving recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecule-modified plants.

Section III-E-2-a. BL1-P is recommended for all experiments with recombinant or synthetic recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

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

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

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

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

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*.