

Guidelines for Protocols Involving Recombinant DNA and/or Pathogenic Organisms:

Criteria for Review Status

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*.

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

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.

Section III-E. Expedited Review – 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 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. 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) 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-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-D. Full Review – 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.

Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems

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. 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 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 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

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.

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.

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. 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.

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](#).

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.