



Guidelines for Research Involving Recombinant DNA Molecules *Extract from Department of Health and Human Services*

The newest version of the “Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)” is dated September 2009. In time, these Guidelines will be updated and the newest published version will dictate our College’s Guidelines for r-DNA research applications for approval where an IBC decision or input is mandated. The PI leading the research is responsible for reading the most recent Guidelines and implementing the required activities. When the Guidelines indicate that a r-DNA protocol must be registered and/or approved by the IBC, the PI must recognize this requirement, and file the r-DNA registration application materials. Note that Appendix B now terms Etiologic Agents by RISK GROUPS [RGs 1-4]. New emerging pathogens may be added in the future and these can be identified by going to http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

The following is an excerpt Appendix A and C are provided for quick reference to “exempt hosts”.

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

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

Appendix A-I. Sublist A

Genus *Escherichia*
Genus *Shigella*
Genus *Salmonella* - including *Arizona*
Genus *Enterobacter*
Genus *Citrobacter* - including *Levinea*
Genus *Klebsiella* - including *oxytoca*
Genus *Erwinia*

Pseudomonas aeruginosa, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Pseudomonas mendocina*
Serratia marcescens
Yersinia enterocolitica

Appendix A-II. Sublist B

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus atterimus

Appendix A-III. Sublist C

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor
Appendix A-IV. Sublist D
Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

Appendix A-V. Sublist E

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

Appendix A-VI. Sublist F

Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

APPENDIX C. EXEMPTIONS UNDER SECTION III-F-6

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

Appendix C-I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see [Appendix C-VII-E](#), *Footnotes and References of Appendix C*), that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed in Appendix C-I-A.

Appendix C-I-A. Exceptions

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

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

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

Appendix C-II-A. Exceptions

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

Appendix C-III. Saccharomyces Host-Vector Systems

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

Appendix C-III-A. Exceptions

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

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

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

Appendix C-IV-A. Exceptions

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

Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms

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

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus atterrimus
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 thermophilus

Appendix C-V-A. Exceptions

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

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

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

Appendix C-VII. Footnotes and References of Appendix C

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

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

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

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

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