FAQs about experiments that are exempt from the *NIH Guidelines*

1. **Which experiments are exempt from the *NIH Guidelines for Research Involving Recombinant DNA Molecules*?**

   Per Section III-F of the *NIH Guidelines*, experiments are exempt when they involve recombinant DNA that is:

   - Not in organisms and viruses;
   - Entirely DNA segments from a single nonchromosomal or viral DNA source;
   - Entirely from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host or when transferred to another host by well established physiological means;
   - Entirely from a eukaryotic host including its chloroplasts, mitochondria, or plasmids when propagated only in that host or a closely related strain of the same species,
   - Entirely segments from different species that exchange DNA by known physiological processes, though one or more may be a synthetic equivalent; see Appendix A of the *NIH Guidelines*; or
   - Not a significant risk to health or the environment as determined by the NIH Director, with advice from the RAC and public comment; see Appendix C of the *NIH Guidelines* for a detailed listing;

   Unless these experiments also involve:

   - The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture [Section III-A];
   - Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram of body weight [Section III-B]; or
   - The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into one or more human research subjects [Section III-C].

   Details on certain other experiments that may be exempt, as well as exceptions, may be found in Appendix C of the *NIH Guidelines*.

2. **The *NIH Guidelines* exempt certain experiments that do not pose a threat to health or the environment. Can an Institutional Biosafety Committee (IBC) or Principal Investigator (PI) determine if an experiment does not pose such a threat and is therefore exempt?**

   Section III-F-6 of the *NIH Guidelines* lists categories experiments that do not present a significant risk to health or the environment and are therefore exempt. The determination of the types of experiments that fall into this category is made by the NIH Director with the advice of the RAC, following appropriate notice and opportunity for public comment. **PIs and IBCs can not make the**

determination that a class of experiments other than the ones listed below poses no significant risk.

The following classes of experiments are exempt under Section III-F-6:

- Recombinant DNA in tissue culture [Appendix C-I]
- *Escherichia coli* K-12 host-vector systems [Appendix C-II]
- *Saccharomyces* host-vector systems [Appendix C-III]
- *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems [Appendix C-IV]
- Extrachromosomal elements of gram positive organisms [Appendix C-V]
- The purchase or transfer of transgenic rodents [Appendix C-VI]

A full description of the exemptions with exceptions can be found in Appendix C of the *NIH Guidelines*.

3. How do I know if I am working with host-vector system that is exempt from the *NIH Guidelines*?

Only certain experiments that use *E.coli* K-12, *Saccharomyces*, *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems are specifically exempted from the *NIH Guidelines* (see Appendix C-II). If you are obtaining a host-vector system from a vendor, genotype information may be available and permit determination of the strain from which your host is derived.

4. DNA molecules resulting from the replication of recombinant DNA are subject to the *NIH Guidelines*. Are any other materials derived from or produced by genetically engineered organisms subject to the requirements *NIH Guidelines*?

No. For example, proteins produced by genetically engineered organisms are not subject to the *NIH Guidelines*.

5. I have heard that certain kinds of human gene transfer trials are exempted from the requirements of the *NIH Guidelines* – is this true?

No. All trials involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the *NIH Guidelines*. Appendix M-VI-A of the *NIH Guidelines* exempts certain types of vaccine trials from the requirements for **submission of the protocol to NIH OBA, RAC review, and subsequent reporting** (Appendix M-I). Specifically, this exemption applies to "human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected." Trials with these characteristics do not have to be registered with NIH OBA or undergo RAC review, but can be submitted on a voluntary basis, particularly if the investigator believes that a trial presents scientific, safety, or ethical concerns that would benefit from RAC review and public discussion. Investigators that submit trials voluntarily will be expected to comply with all aspects of the protocol review and reporting requirements. OBA encourages investigators and institutional review bodies to contact us (oba@od.nih.gov) for assistance in determining whether this exemption applies to a particular trial.

It is important to note that Appendix M-VI-A does not exempt these vaccine trials from other requirements specified in the NIH Guidelines, including biosafety review. Thus, vaccine trials, like other human gene transfer trials subject to the NIH Guidelines, must be reviewed and approved by an IBC before research participants can be enrolled.

6. **There is a note at the beginning of Section III of the *NIH Guidelines* that states "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.*" What is meant by this note?**

   If an experiment falls into Section III-D or III-E of the *NIH Guidelines* and also falls into section III-F, it is exempt. An example of such an experiment is the following:

   Staphylococcus aureus (a Risk Group 2 bacterium) contains a recombinant plasmid. The plasmid is indigenous to S. aureus, was created in vitro, and contains only DNA from S. aureus (i.e., the DNA inserted into the plasmid was S. aureus DNA).

   Rationale: The introduction of recombinant DNA into Risk Group 2 agents is usually covered under Section III-D-1-a. However, because the experiments are only performed in the *S. aureus strain*, this work would fall under III-F-3 (experiments that consists entirely of DNA from a prokaryotic host including its indigenous plasmids when propagated only in that host or a loosely related strain of the same species). Thus this experiment falls into both Sections III-D and III-F and is exempt, due to the above note from the requirements of the *NIH Guidelines* for IBC review and approval.

   It should be noted that only experiments covered by both III-D or III-E and III-F can be exempted. If an experiment falls into Section III-A, III-B or III-C and any one of the other sections, then the rules pertaining to Sections III-A, III-B or III-C **must** be followed.

7. **Appendix C-1 of the *NIH Guidelines* exempts experiments involving recombinant DNA in tissue culture. I have a cell line that was created by the introduction of recombinant DNA. Are all experiments I conduct with this cell line exempt from the requirements of the *NIH Guidelines*?**

   No. Although Appendix C-1 does exempt the use of recombinant DNA in tissue culture, there are exceptions to this exemption. Existing tissue culture cell lines created by the introduction of recombinant DNA are exempt from the *NIH Guidelines* unless, the cell line:

   - was modified using DNA from Risk Group 3 or 4 agents, or from restricted agents. [Section III-D]
   - contains a toxin with an LD50 of less than 100 ng/kg body weight. [Section III-B-1]
   - contains viral DNA in a quantity exceeding 50% of any viral genome. [Appendix C-I]
   - is used in conjunction with defective viruses in the presence of helper virus. [Section III-D-3]
   - is used in an experiment involving the deliberate transfer of the cell line into humans. [SectionIII-C-1]
   - is grown in a volume exceeding 10 liters of culture. [Section III-D-6]

TABLE OF CONTENTS

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 amylosaccharíticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylcoccus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarious
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Appendix C-V-A. Exceptions

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

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

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

Appendix C-VII. Footnotes and References of Appendix C

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

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

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

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

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