

# NIH Office of Biotechnology Activities – Recombinant DNA Exemptions (III-F)

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. For example, an experiment involving an otherwise exempt molecule is not exempt from the NIH Guidelines if it is being administered to humans (and thus falls under Section III-C). 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. ***Registration with the UNH Institutional Biosafety Committee (IBC) is required for all recombinant DNA research.***

## Exempt recombinant DNA research includes:

- Recombinant DNA molecules that are not in organisms or viruses.
- Recombinant DNA molecules 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.
- Recombinant DNA molecules 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.
- Recombinant DNA molecules that consist entirely of DNA 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).
- 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. A list of such exchangers has been prepared and will be periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment. See [Appendix A](#) for a list of Natural Exchangers that are exempt from the NIH Guidelines.
- Recombinant DNA molecules that do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See [Appendix B](#) for other classes of experiments which are exempt from the NIH Guidelines.

## Appendix A – Natural Exchangers Exempt from the NIH Guidelines

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. Institutional Biosafety Committee registration is not required for these exempt experiments. For a list of natural exchangers that are exempt from the NIH Guidelines, see the sublists below.

Exempt Experiments are described as 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 shown below.

(See 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.

### 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*

### Sublist B

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

### Sublist C

- *Streptomyces aureofaciens*
- *Streptomyces rimosus*
- *Streptomyces coelicolor*

### Sublist D

- *Streptomyces griseus*
- *Streptomyces cyaneus*
- *Streptomyces venezuelae*

### Sublist E

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

### Sublist F

- *Streptococcus sanguis*
- *Streptococcus pneumoniae*
- *Streptococcus faecalis*
- *Streptococcus pyogenes*
- *Streptococcus mutans*

## Appendix B – NIH Recombinant DNA Exemptions

The following classes of experiments are exempt from the NIH Guidelines.

### 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), that are propagated and maintained in cells in tissue culture are exempt with the following exceptions:

#### Exceptions

The following categories are not exempt:

- (i) Experiments which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation,
- (ii) Experiments which require NIH/OBA and Institutional Biosafety Committee approval before initiation,
- (iii) Experiments involving DNA from Risk Groups 3, 4, or restricted organisms 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, 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.

### Escherichia coli K-12 Host-Vector Systems

Experiments which use *Escherichia coli* K-12 host-vector systems are exempt from the NIH Guidelines provided that:

- A. The *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or
- B. Lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids shall be used as vectors.

However, experiments involving the insertion into *Escherichia coli* K-12 of DNA from prokaryotes that exchange genetic information 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 (BSL) 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.

#### Exceptions

The following categories are not exempt from the NIH Guidelines:

- (i) Experiments which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation,
- (ii) Experiments which require NIH/OBA and Institutional Biosafety Committee approval before initiation,

- (iii) Experiments involving DNA from Risk Groups 3, 4, or restricted organisms or cells known to be infected with these agents may be conducted under containment conditions 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.

## **Saccharomyces Host-Vector Systems**

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems are exempt from the NIH Guidelines. For these exempt experiments, BSL-1 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.

### **Exceptions**

The following categories are not exempt from the NIH Guidelines:

- (i) Experiments which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation,
- (ii) Experiments which require NIH/OBA and Institutional Biosafety Committee approval before initiation,
- (iii) Experiments involving DNA from Risk Groups 3, 4, or restricted organisms or cells known to be infected with these agents may be conducted under containment conditions 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.

## ***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 exceptions those listed below. For these exempt laboratory experiments, BSL-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 it deems necessary.

### **Exceptions**

The following categories are not exempt from the NIH Guidelines:

- (i) Experiments which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation,
- (ii) Experiments which require NIH/OBA and Institutional Biosafety Committee approval before initiation,
- (iii) Experiments involving DNA from Risk Groups 3, 4, or restricted organisms or cells known to be infected with these agents may be conducted under containment conditions 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.

## Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below, propagated and maintained in organisms listed below are exempt from the NIH Guidelines:

- *Bacillus amyloliquefaciens*
- *Bacillus amylosacchariticus*
- *Bacillus anthracis*
- *Bacillus atterimus*
- *Bacillus brevis*
- *Bacillus cereus*
- *Bacillus globigii*
- *Bacillus licheniformis*
- *Bacillus megaterium*
- *Bacillus natto*
- *Bacillus niger*
- *Bacillus pumilus*
- *Bacillus sphaericus*
- *Bacillus stearothermophilus*
- *Bacillus subtilis*
- *Bacillus thuringiensis*
- *Clostridium acetobutylicum*
- *Lactobacillus casei*
- *Listeria grayi*
- *Listeria monocytogenes*
- *Listeria murrayi*
- *Pediococcus acidilactici*
- *Pediococcus dammosus*
- *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*

### Exceptions

The following categories are not exempt:

- (i) Experiments which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation,
- (ii) Experiments which require NIH/OBA and Institutional Biosafety Committee approval before initiation,
- (iii) Experiments involving DNA from Risk Groups 3, 4, or restricted organisms or cells known to be infected with these agents may be conducted under containment conditions 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.

## The Purchase or Transfer of Transgenic Rodents

The purchase or transfers of transgenic rodents for experiments that require BSL-1 containment are exempt from the NIH Guidelines.

## DEFINITIONS

### Adenovirus

Adenovirus can be defined as a group of DNA containing viruses, which most commonly cause respiratory disease (ranging from one form of the common cold to pneumonia, croup, and bronchitis), and can also cause gastrointestinal illness, eye infections, cystitis, and rash in humans. Adenoviruses can also be genetically modified and used in gene therapy to treat cystic fibrosis, cancer, and potentially other diseases

### Bacterial Artificial Chromosomes (BAC)

BACs are based on bacterial mini-F plasmids, which are small pieces of episomal bacterial DNA that give the bacteria the ability to initiate conjugation with adjacent bacteria. They have a cloning limit of 75-300 kb.

### Cloning Vectors

A cloning vector is a DNA molecule that carries foreign DNA into a host cell (usually bacterial or yeast), where it replicates, producing many copies of itself along with the foreign DNA. There are three features required for all cloning vectors:

1. Sequences that will permit the propagation of itself in host cell.
2. A cloning site to insert the foreign DNA; the most versatile vectors contain a site that can be cut by many restriction enzymes.
3. A method of selecting a host cell containing a vector with foreign DNA; this is usually accomplished by selectable markers for drug resistance

### Cosmids

Cosmids are extrachromosomal circular DNA molecules that combine features of plasmids and phages. They also have a high transformation efficiency, and their cloning limit of 35-50 kb is larger than that of plasmids or phages.

### Herpesviruses

Herpesviruses include infectious human viruses including herpes simplex virus type-1 (HSV-1), which is common in the general population, but in rare cases can cause encephalitis. It is one of the most commonly used vector systems because it has a broad host cell range, the ability to transduce neurons, and a capacity to receive large inserts.

### Parvovirus

Parvoviruses are small DNA viruses that cause several diseases in mammals, such as canine parvovirus in dogs. Parvovirus B-19, which causes Fifth disease (erythema infectiosum) in humans, is the only form that is pathogenic to humans. In fact, many parvoviruses exhibit oncosuppressive properties (suppression of cancer-causing genes). Parvovirus-based vectors can be used to target the expression of therapeutic genes in tumors.

### Phages

Phages are derivatives of bacteriophage lambda ( $\lambda$  phage), a virus which infects E. coli. They are linear DNA molecules, whose region can be replaced with foreign DNA without disrupting its life cycle.

### Plasmids

Plasmids are small, circular, extrachromosomal DNA molecules found in bacteria, which can replicate on their own, outside of a host cell. They have a cloning limit of 100 to 10,000 base pairs or 0.1-10 kilobases (kb).

## Plasmid Vector

A plasmid vector is made from natural plasmids by removing unnecessary segments and adding essential sequences.

## Poxviruses

Poxviruses are the largest and most complex viruses known. There are at least nine different poxviruses that are pathogenic to humans, the most common being vaccinia and variola virus (smallpox), which has since been eradicated. Poxviral vectors are successful in immunogenetic therapy protocols, due to their strong immunogenicity (ability to induce a high immune response). They cause the activation of immune responses against tumor antigens transported in dendritic cells.

### Recombinant DNA:

Recombinant DNA (rDNA) has various definitions, ranging from very simple to strangely complex. The following are three examples of how recombinant DNA is defined:

1. A DNA molecule containing DNA originating from two or more sources.
2. DNA that has been artificially created. It is DNA from two or more sources that is incorporated into a single recombinant molecule.
3. According to the NIH guidelines, recombinant DNA are molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication.

## Retrovirus

Retroviruses are viruses belonging to the family *Retroviridae*. They are composed of a single RNA strand and use the enzyme reverse transcriptase to copy their genome into the DNA of the host cell's chromosomes. They are relatively genetically simple, and have the ability to infect a wide variety of cell types with high efficiency.

### Risk Group 1 (RG1) Agents:

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include: asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (exceptions are noted in this document) adeno-associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (exceptions are noted in this document) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen); and (2) does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

## Vector

A living organism or molecule, including a recombinant molecule, or a bio-engineered biological product, capable of transporting a biological agent or toxin to a host.

### Viral Vectors

A viral vector is a virus that carries a modified or foreign gene. They are commonly used in gene therapy where the viral vector delivers the desired gene to a target cell.

## Yeast

Yeasts, eukaryotic unicellular fungi, contribute a great deal to the study of molecular genetics. They are popular organisms to clone and express DNA in because they are eukaryotes, and can therefore splice out introns, the non-coding sequences in the middle of many eukaryotic genes.

For the past two decades *Saccharomyces cerevisiae*, a species of yeast, has been an important model system for biological research because its entire genome has been base sequenced, and is used as a reference to human and other higher eukaryotic genes. This is because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved between yeast and larger eukaryotes, including mammals.

### Yeast Artificial Chromosomes (YAC)

YACs are artificial chromosomes that replicate in yeast cells. They consist of:

- Telomeres, which are ends of chromosomes involved in the replication and stability of linear DNA.
- Origin of replication sequences necessary for the replication in yeast cells.