

University of Nebraska Medical Center  
 Biosafety Policies and Procedures

<b>TITLE:</b>	<b>Registration Process for the Use of Exempt Recombinant DNA [UNMC-IBC35]</b>
<b>OVERVIEW:</b>	All recombinant DNA work conducted at or supported by UNMC, UNO, or the Nebraska Medical Center must be registered with the Institutional Biosafety Committee prior to initiation of the project.
<b>APPLIES TO:</b>	All recombinant DNA work performed on the UNMC and UNO campuses.
<b>DEFINITION(S):</b>	Recombinant DNA - are 1] molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or 2] molecules that result from the replication of those described in 1. (based on the <i>NIH Guidelines</i> definition)
<b>PROCEDURES:</b>	<p>1. If your recombinant DNA experiment meets the definition of any of the exempt categories described in <b>Appendix A</b> of this policy, then complete and submit the registration form (see <b>Appendix B</b> or refer to the IBC website at &lt;&lt;<a href="http://www.unmc.edu/ibc/">www.unmc.edu/ibc/</a>&gt;&gt;&gt; for a fillable copy of the registration form).</p> <p>2. The completed form is submitted by e-mail to the IBC at <a href="mailto:ibcora@unmc.edu">ibcora@unmc.edu</a>.</p>
<b>RECORD KEEPING:</b>	All information identifying laboratories conducting exempt recombinant DNA research <sup>0</sup> at UNMC, UNO, and the NMC will be kept on file by the Office of Regulatory Affairs.
<b>OTHER INFORMATION:</b>	<p>The PI is responsible to contact the IBC at <a href="mailto:ibcora@unmc.edu">ibcora@unmc.edu</a> to report any change to the protocol that will make the experiments non-exempt. Under this circumstance, a full IBC protocol will need to be submitted for review by the IBC prior to the initiation of the change. Refer to the IBC website for information on how to submit an IBC protocol.</p> <p>Exempt experiments will be reviewed every 5 years but may be reviewed earlier if the exempt status needs to be re-evaluated.</p>
<b>REFERENCES:</b>	<i>NIH Guidelines</i> , Section III-F, Exempt Experiments
<b>STATUS:</b>	<p>Drafted: August 7, 2007</p> <p>Approved: August 20, 2007</p>

# University of Nebraska Medical Center

## Biosafety Policies and Procedures

### Appendix A: Definitions from the *NIH Guidelines* for the Use of Exempt rDNA Molecules

#### Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the IBC using only the form described in Appendix B is required (a completed IBC protocol is not required to register this type of experimentation):

**Section III-F-1.** Those that are not in organisms or viruses.

##### Interpretation/Examples:

Ligation of recombinant molecules and study of these molecules without transferring to a bacterium, virus, or creating a virus.

Southern blot of plasmid DNA.

Synthetic DNA encapsulated in a synthetic delivery vehicle intended for injection into animals.

The cloning of a DNA segment produced by PCR.

Radiolabeling a probe for *in situ* hybridization.

**Section III-F-2.** Those that consist entirely of DNA segments from a single non-chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

##### Interpretation/Example:

The use of SV40 in tissue culture experiments or lambda bacteriophage DNA in *E. coli* (do not carry a foreign insert but can lead to alterations [mutations] in the sequence.

The cloning of the 5' ends of cDNA (from mRNA) to determine transcriptional start site.

**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.

##### Interpretation/Example:

Cloning *Escherichia coli* DNA using vector (plasmid) derived from *E. coli* or other *Enterobacteriaceae* (i.e. pBR322, pUC19, etc.) and using *E. coli* as a transforming host. The statement "or when transferred to another host by well established physiological means" is not interpreted to mean that "host" is another species and "host" may refer to another *E. coli* isolate/strain.

**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).

##### Interpretation/Example:

Same interpretation as section III-F-3 above, except using a eukaryotic host (i.e. a yeast such as *Saccharomyces cerevisiae*)

**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*.

##### Interpretation/Example:

Certain microorganisms are known to exchange genetic information through a variety of mechanisms including conjugation, transduction, etc. Recombinant DNA experiments are exempt if cloning DNA from, for instance, *Pseudomonas aeruginosa* and transferring that DNA to *E. coli*. These two species are known to exchange DNA naturally. A list of those organisms that are known to exchange DNA are shown below (Identified as Appendix A by the *NIH Guidelines*, Exemptions under section III F-5 sub-lists of natural exchanges).

#### Sublist A

Genus *Escherichia*

Genus *Shigella*

Genus *Salmonella* - including *Arizona*

# University of Nebraska Medical Center

## Biosafety Policies and Procedures

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*

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**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 below, *Exemptions under Section III-F-6* for other classes of experiments which are exempt from the *NIH Guidelines*.

#### Interpretation/Example:

**Recombinant DNA experiments associated with specific host systems are not considered a public health threat and are therefore exempt. These exemptions are listed below (Identified as Appendix C by the *NIH Guidelines*, Exemptions under section III-F-6).**

**Exemption C1:** Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed

# University of Nebraska Medical Center

## Biosafety Policies and Procedures

below

### Exceptions to Exemption C1:

- 1) Cloning a drug resistance marker not naturally known to be found in the organism.
- 2) Cloning of toxins with an LD<sub>50</sub> of less than 100 ng/kg (botulinum toxin, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin)
- 3) Cloning of DNA from any Risk group 3 or 4 pathogen or cloning from cells known to be infections with a risk group 3 or 4 pathogen.
- 4) Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates.
- 5) 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.

**Exemption C2:** Recombinant DNA experiments which use *E. coli* K12 host-vector systems (almost all *E. coli* strains purchased from molecular biology sources are from the K12 lineage) provided that: 1) the *E. coli* strain does not contain conjugative plasmids or prophages that are able to undergo transduction. 2) Lamboid or non-conjugative plasmids are used as cloning vectors (this includes pUC19, pGEM, and most all cloning vectors used in *E. coli* genetic experiments). However, a conjugative plasmid may be used if the DNA that is inserted is from organisms that naturally exchange DNA with *E. coli* (see Appendix A-1 sublist A above).

### Exceptions to Exemption C2:

- 1) Experiments involving DNA from Risk Groups 3 or 4 organisms.
- 2) Large-scale experiments (e.g., more than 10 liters of culture).
- 3) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.

**Exemption C3:** Recombinant DNA experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems.

### Exceptions to exemption C3:

- 1) Experiments involving DNA from Risk Groups 3 or 4 organisms.
- 2) Large-scale experiments (e.g., more than 10 liters of culture).
- 3) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.

**Exemption C4:** Recombinant DNA experiments involving asporogenic *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems. *Bacillus* strains used must not form spores at a frequency greater than 10<sup>-7</sup>.

### Exceptions to exemption C4:

- 1) Experiments involving DNA from Risk Groups 3 or 4 organisms.
- 2) Large-scale experiments (e.g., more than 10 liters of culture).
- 3) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.

**Exemption C5:** Recombinant DNA molecules derived entirely from extrachromosomal elements (i.e. plasmids) of the gram-positive organisms listed below (including shuttle vectors comprised of vectors listed in Exemption C2 above), 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*

# University of Nebraska Medical Center

## Biosafety Policies and Procedures

*Bacillus pumilus*  
*Bacillus sphaericus*  
*Bacillus stearothermophilus*  
*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 ferus*  
*Streptococcus mitior*  
*Streptococcus mutans*  
*Streptococcus pneumoniae*  
*Streptococcus pyogenes*  
*Streptococcus salivarius*  
*Streptococcus sanguis*  
*Streptococcus sobrinus*  
*Streptococcus thermophilus*

### Interpretation/Example:

Recombinant DNA and cloning experiments using shuttle plasmids constructed from *Staphylococcus aureus* (i.e. pE194) and *Escherichia coli* (pUC19) are exempt if the plasmids are maintained in one of the species listed above. These recombinant DNA molecules can also be transferred to *E. coli* K12 lineage strains as discussed in exemption C2 above (through the use of the shuttle plasmids).

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University of Nebraska Medical Center  
Biosafety Policies and Procedures

**Appendix B - Registration form for the Use of Exempt Recombinant DNA**

**Institutional Biosafety Committee (IBC)  
EXEMPT Recombinant DNA**

*\*\*All recombinant DNA work conducted at or supported by the University of Nebraska Medical Center/University of Nebraska at Omaha (UNO) must be registered with and approved by the Institutional Biosafety Committee (IBC).*

**Section I**

**IBC #** \_\_\_\_\_ (to be completed in IBC Office)

**1. APPLICATION DATA**

TITLE OF PROTOCOL:

PRINCIPAL INVESTIGATOR:

DEPARTMENT:

TELEPHONE #:

E-MAIL ADDRESS:

CAMPUS ZIP:

*The following information must be provided to the IBC. See specific instructions in each subpart. Email this form to [IBCORA@UNMC.EDU](mailto:IBCORA@UNMC.EDU) to have it reviewed and approved by the IBC Chair.*

**CERTIFICATION OF PRINCIPAL INVESTIGATOR**

I recognize that as the PI it is my responsibility to ensure that this research and the actions of all project personnel involved in conducting the study will conform with the IBC approved protocol and the provisions of the *NIH Guidelines for Research Involving Recombinant DNA*, the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* manual, and the *Select Agent Rule* (<http://www.cdc.gov/od/sap/docs/42cfr73.pdf>) where appropriate.

- I will inform the IBC of any unanticipated biosafety related problems encountered while doing the research.
- I will notify the IBC of any change in the protocol that may change the status of the study.
- I will maintain all required research records on file, and I recognize that representatives of the IBC are authorized to inspect these records.
- I accept responsibility for the safe conduct of the experiments to be conducted and will see that all associated personnel are trained in the safe laboratory practices required for this work.
- I will oversee the development and implementation of standard biosafety operating procedures for the laboratory.
- I understand that IBC approval is valid for 5 years for exempt research.

\_\_\_\_\_  
Signature of Investigator

\_\_\_\_\_  
Date

# University of Nebraska Medical Center

## Biosafety Policies and Procedures

### Section II

1. Does the construct contain viral DNA that represents more than 2/3 of any eukaryotic viral genome?
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)
  
2. Is the viral construct from DNA of Risk Group 2, 3, 4, or restricted agents?
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)
  
3. Does the Study involve the deliberate transfer of Recombinant DNA into one or more Human Subjects?
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)
  
4. Does the Study involve generation of Transgenic Animals or Plants
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)
  
5. Does the Study involve the generation of Toxin Molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight?
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)
  
6. Does the Study involve the generation of more than 10 Liters of Culture?
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)
  
7. Does the Study involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally and if so, could this acquisition compromise the use of the drug to control disease agents in human, animals, and/or plants?
  - a.  No.
  - b.  Yes. This registration is not exempt. Please complete the IBC Protocol for Research Involving Biohazardous Materials located on the IBC website – [www.unmc.edu/ibc](http://www.unmc.edu/ibc)

# University of Nebraska Medical Center

## Biosafety Policies and Procedures

8. Please provide the following information:

Nature of Inserted DNA Sequence/Expressed gene	Host(s)	Vector(s)	Intended Use of rDNA

9. Briefly describe your research project (less than 50 words) and include the biological function of the gene product (protein) that you wish to express.

10. Indicate the Biosafety Containment Level at which the project will be conducted (defined in Appendix G of the NIH Guidelines)

\*\*\* Email this form to [IBCORA@UNMC.EDU](mailto:IBCORA@UNMC.EDU) in order to have it reviewed and approved by the IBC Chair. \*\*\*