Form I. IU INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)
Protocol Registration Form

Fill out this form, sign it, and submit the original along with the appropriate accompanying forms to IUBIBC@indiana.edu by email (preferred) or to the IBC c/o Office of Research Compliance, 509 E Third St, Bloomington, IN 47401. Keep a copy for your records.

Please note that all research involving recombinant or synthetic nucleic acid molecules MUST be registered with the IUB IBC, even if it is “Exempt” under the NIH Guidelines. Refer to the NIH Guidelines to determine if the work is exempt, or falls under one or more of the Risk Group classifications discussed in this protocol registration form. These guidelines are available for download at: https://osp.od.nih.gov/biotechnology/nih-guidelines/

Part A. Contact and Laboratory Personnel Information

<table>
<thead>
<tr>
<th>Contact Information:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Investigator (PI) and Title:</td>
</tr>
<tr>
<td>School &amp; Department:</td>
</tr>
<tr>
<td>Office Phone &amp; Location:</td>
</tr>
<tr>
<td>Email:</td>
</tr>
<tr>
<td>Lab manager or primary contact for lab, if not PI:</td>
</tr>
<tr>
<td>Lab Phone &amp; Location:</td>
</tr>
<tr>
<td>Location of Animal Facilities (if applicable):</td>
</tr>
</tbody>
</table>

Protocol is: | NEW | RENEWAL |
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>TEACHING</td>
<td>Course #:</td>
<td></td>
</tr>
<tr>
<td>RESEARCH</td>
<td>Project Title:</td>
<td></td>
</tr>
</tbody>
</table>

Source of Funding (agency funding research relevant to this protocol): 

Title and Agency Number of Grant(s):
NIH EXEMPT Transgenic Vertebrate Animal Experiments

If all lab research falls within one of the categories below, check the appropriate box(es), go directly to Part D and sign the form, and submit it to IUBIBC@indiana.edu. NO OTHER INFORMATION OR FORMS ARE NEEDED.

If research also includes recombinant or synthetic nucleic acid molecules or biohazards, fill out the rest of Form I and any other applicable forms, and submit them.

All exempt research below must be conducted in Biosafety Level 1 or Animal Biosafety Level 1 (see part B, below, for definitions of biosafety levels). BSL-2/ABSL-2 research is never exempt.

<table>
<thead>
<tr>
<th>NIH EXEMPT Vertebrate Animal Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>The IUB BIACUC must still grant approval before the research may begin</td>
</tr>
</tbody>
</table>

- **Section III-F-8 Those that do not present a significant risk to health or the environment, as determined by the NIH Director (Appendix C, NIH Guidelines).**
  - Experiments utilizing transgenic rodents under ABSL-1 containment where recombinant or synthetic nucleic acid molecule manipulations are NOT planned

- **Appendix C-VII Purchase or Transfer of Transgenic Rodents**
  - Purchase or transfer of transgenic rodents that utilize ABSL-1 containment (receiving animals including transfers between PI’s)

- **Appendix C-VIII Generation of BL1 Transgenic Rodents via Breeding**
  - Breeding knockout animals from one strain (colony maintenance) at ABSL-1.
  - All breeding and crossbreeding of transgenic rodents housed under ABSL-1 containment that do not meet the following criteria:
    - **NOT EXEMPT:** Breeding/Crossbreeding experiments in which one or both parental rodents, or the offspring, contain more than 50% of the genome of an exogenous eukaryotic virus from a single family (all viruses in the family are considered equal).
    - **NOT EXEMPT:** Breeding experiments in which one or both parental rodents contain a transgene that is under the control of a gammaretroviral long terminal repeat (LTR). A list of gammaretroviruses is provided at the bottom of Form IV.
    - **NOT EXEMPT:** Crossbreeding in which one or both parental rodents is housed under ABSL-2 or ABSL-3 containment

Part B. Resources, Forms and Research Description

**Definitions**

*Recombinant DNA molecules:* 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) above.
**Synthetic DNA segments** that are expressed in vivo are considered to be equivalent to their natural DNA counterparts.

Genomic DNA of plants and bacteria that have acquired a **transposable element**, even if the latter was donated from a recombinant vector no longer present, are not subject to the **NIH Guidelines** unless the transposon itself contains recombinant or synthetic nucleic acid molecules.

**Resources to determine the containment level and applicable section(s) of the NIH Guidelines that apply to the proposed research:**

- **NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules**
- **Biosafety in Microbiological and Biomedical Laboratories** – the “BMBL”, 5th ed
  https://www.cdc.gov/labs/BMBL.html; unsure about Publications link
- Additional resources can be found under “Publications” at
  http://www.researchadmin.iu.edu/Biosafety/UB/bio_home.html

**Containment Levels**

- **Biosafety Level 1 / Animal Biosafety Level 1**: Level of containment for agents not associated with infectious disease in healthy adults or animals. Work is performed on the open benchtop, minimal personal protective equipment.
- **Biosafety Level 2 / Animal Biosafety Level 2**: Appropriate containment for Biohazards including toxins, venoms, and agents associated with infectious disease in healthy adults or animals for which disease is rarely serious. Also human tissues, fluids, and cell lines, and animal tissues known or suspected to be contaminated with disease-causing agents. Additional personal protective equipment, increased technical proficiency, biosafety cabinet and other safety equipment.
- **Biosafety Level 3 / Animal Biosafety Level 3**: Agents associated with serious or lethal infectious disease. Personal protective equipment includes dedicated laboratory clothing, double gloves, respirators, no work is conducted on the open benchtop, directional airflow and restricted access.

**Training**

- Training is required for research with viral vectors, biohazards, or BSL-2, ABSL-2, BSL-3 and ABSL-3 containment prior to initiating work and annually thereafter. Contact the **Biosafety Office** to schedule training and lab inspections.

  **Have all of the personnel listed attended required biosafety training?**
  [ ] Yes  [ ] No  [ ] NA

- A lab inspection is required for work with human tissues, fluids, and cell lines, contaminated animal tissues, BSL-2, ABSL-2, use of viral vectors, BSL-3 and ABSL-3 prior to initiating work and annually thereafter.

  **Date of last biosafety laboratory inspection** ________________
If the laboratory has not been inspected within the last 12 months, an inspection must be completed within 30 days of the IBC meeting in which the protocol is reviewed.

- The Hepatitis B vaccine is recommended for all persons working with human tissues, fluids and cell lines.
- Bloodborne Pathogens training is required prior to initiating work and annually thereafter for all persons working with human tissues, fluids, and cell lines. Contact Environmental Health and Safety to schedule Bloodborne Pathogens training and Hepatitis B vaccines.

**Have all of the personnel listed attended Bloodborne Pathogens training?**
[ ] Yes   [ ] No   [ ] NA

**Have all of the personnel listed been offered the Hepatitis B vaccine?**
[ ] Yes   [ ] No   [ ] NA

- Lab Safety/Chemical Safety is required for all personnel working in research laboratories. Contact Environmental Health and Safety to schedule Lab Safety/Chemical Safety training.

**Have all of the personnel listed attended Lab Safety/Chemical Safety training?**
[ ] Yes   [ ] No

<table>
<thead>
<tr>
<th>List names all IUB researchers who will be involved with this project. PI is responsible for all IUB researchers and liabilities related to use of his/her materials. For courses, list only Instructor and Assistant Instructors.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Researchers working in BSL-1 only:</td>
</tr>
<tr>
<td>Researchers working in BSL-2:</td>
</tr>
</tbody>
</table>

| List names of all IUB centers that will handle or analyze Risk Group 2/BSL-2 samples described in this protocol, e.g microscopy facilities, cell sorting facilities, sample analysis facilities, etc. PI is responsible for all IUB researchers and liabilities related to use of his/her materials. Biosafety will contact these Centers to schedule training, when appropriate. |

**Fill out and submit ALL applicable forms**
- The Biosafety Office provides assistance with filling out these protocol submission forms. Contact Biosafe@indiana.edu.

Please check the boxes for the forms you will need to complete, if your work includes:
- [ ] Form II Human or Nonhuman Primate Tissues, Fluids, or Cell Lines, Biological and Chemical
Hazards (includes culturing pathogenic microorganisms, with or without recombinant or synthetic nucleic acid molecules. Also, animal tissues known, or suspected to be contaminated with infectious viral agents) **Does not include biological toxins (Form IIB) or viral vectors (Form IIIA)**

- **Form IIA** Biological Toxins or Venoms

- **Form III** Recombinant or Synthetic Nucleic Acid Molecules utilizing Microorganisms (Bacteria, viruses, cell culture, fungi, etc) *(Does not include viral vectors; use form IIIA)*
  
  IU does not have the facilities to conduct experiments requiring BSL-4 containment.

- **Form IIIA** Recombinant or Synthetic Nucleic Acid Molecules utilizing Viral Vectors *(including viral sequences present in plasmids)*

- **Form IIIB** EXEMPT Recombinant or Synthetic Nucleic Acid Molecule Research Utilizing Microorganisms *(Exempt recombinant DNA research still requires registration with the IBC)*

- **Form IV** Recombinant or Synthetic Nucleic Acid Molecules Utilizing Vertebrate Animals *(genetically modified animal, or wild type animal with associated genetically modified microorganism)* *The NIH defines “Animal” as everything in the Kingdom Animalia*
  
  IU does not have the facilities to conduct experiments requiring Animal BSL-4 containment.

- **Form IVA** Recombinant or Synthetic Nucleic Acid Molecules Utilizing Invertebrate Animals Including Arthropods *(including Drosophila)* *The NIH defines “Animal” as everything in the Kingdom Animalia*

- **Form V** Recombinant or Synthetic Nucleic Acid Molecules Utilizing Plants *(genetically modified plant or whole plant having an associated animal, arthropod, or microorganism)*
  
  IU does not have the facilities to conduct experiments requiring Plant BSL3 or BSL4 containment.

- **Form VI** Select Agent Identification *(diagnostic or environmental samples)*

**Abstract** (*250 words or less)*, purpose or goal of proposed research experiments in layman’s terms.

**Description of planned experiments**, briefly outline the details of experiments in layman’s terms. This cannot be a grant proposal, may be included as an attachment.
Part C. Containment and Waste Disposal

**Containment Levels**, answer as applicable. Refer to the *NIH Guidelines* for assistance.

<table>
<thead>
<tr>
<th>Biohazards:</th>
<th>☐ BSL-1</th>
<th>☐ BSL-2</th>
<th>☐ BSL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant DNA research:</td>
<td>☐ BSL-1</td>
<td>☐ BSL-2</td>
<td>☐ BSL-3</td>
</tr>
<tr>
<td>Animal Research:</td>
<td>☐ Animal BSL1</td>
<td>☐ Animal BSL2</td>
<td>☐ Animal BSL3</td>
</tr>
<tr>
<td>(vertebrate and invertebrate animals)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Research:</td>
<td>☐ BSL1-Plant</td>
<td>☐ BSL2-Plant</td>
<td>☐ BSL2+-Plant</td>
</tr>
</tbody>
</table>

**Waste Disposal Procedures** check all that apply.

*All genetically modified organisms and biohazards including plants, animals, and arthropods, must be disposed of properly so as to prevent their release into the environment or consumption as food for humans or animals.*

<table>
<thead>
<tr>
<th>Lab or Surface Disinfectant:</th>
<th>☐ 10% commercial bleach (equivalent to .5% sodium hypochlorite), with 10 minutes contact time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐ 70% Ethanol with 10 minutes contact time</td>
</tr>
<tr>
<td></td>
<td>☐ Other: __________________ Contact time_________ Concentration_________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solid waste:</th>
<th>☐ Materials will be autoclaved for a minimum of 15 minutes, at 121°C, under 14 psi (pounds per square inch)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐ Chemical inactivation: Chemical________________ Contact time __________</td>
</tr>
<tr>
<td></td>
<td><em>Final concentration of bleach after addition of biological research materials should be at least 10%</em></td>
</tr>
<tr>
<td></td>
<td>☐ Animal carcasses are frozen, EHS is contacted to pick them up and dispose of them. (Animal carcasses and BSL-3 waste are removed from campus and incinerated)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liquid waste:</th>
<th>☐ 20% commercial bleach (equivalent to 1% sodium hypochlorite), with 10 minutes contact time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Final concentration of bleach after addition of biological research materials should be at least 20%</em></td>
</tr>
<tr>
<td></td>
<td>☐ Materials will autoclaved for a minimum of 15 minutes, at 121°C, under 14 psi (pounds per square inch)</td>
</tr>
</tbody>
</table>

| Infectious sharps: | ☐ Clear puncture resistant container with a biohazard symbol. |
|                   | Clear containers: autoclave, deface the biohazard symbol, disposed of in regular trash |
or left for housekeeping pick-up.
Red or orange containers: autoclave, then contact EHS for pickup.

Non-
infectious
sharps:

☐ Puncture resistant container WITHOUT a biohazard symbol.

Acceptable containers: cardboard or solid walled box, non-hazardous sharps container, white bucket. Disposed of in regular trash or left for housekeeping pick-up.

Equipment decontamination procedures:

Part D. Signatures

Name of individual filling out form (if not PI):

COMPLETION AND SIGNING OF THIS FORM IS THE RESPONSIBILITY OF THE PRINCIPAL INVESTIGATOR

In signing this form, I agree to abide by all university and federal guidelines and regulations regarding recombinant or synthetic nucleic acid molecules, infectious agent and/or human tissues and fluids work.

IU Bloomington researchers must immediately report to the Director of Biological Safety at IU Bloomington any one or more of the following events:

- Any overt or potential personnel exposure to recombinant or synthetic nucleic acid molecule-containing material at any biosafety level, whether or not that exposure leads to illness,
- Any significant spill of recombinant or synthetic nucleic acid molecule-containing material outside of a biological safety cabinet.
  - A spill of recombinant risk group 1 agent-containing material which requires emergency spill response or other environmental remediation, or
  - Any spill of recombinant risk group 2 or 3 agent-containing material.
- Any incident which results in the release of recombinant or synthetic nucleic acid molecules to the environment (including escape of a transgenic animal),
- Any breach of or failure to adhere to BL2 or BL3 containment levels, or
- Any other significant problems with or violations of the NIH Guidelines.

☐ I understand that I am responsible for ensuring compliance with all applicable regulations and the terms of this protocol.

_________________________________________  _____________________________
Principal Investigator Signature         Date
☐ Type name above and check for electronic signature

_________________________________________  _____________________________
Co-Principal Investigator Signature      Date
☐ Type name above and check for electronic signature
Form II. IU Human or Nonhuman Primate Tissues, Fluids, or Cell Lines, Biological and Chemical Hazards

Fill out and submit this form, along with Form I for Biohazards requiring BSL-2 or BSL-3 containment. Use Form IIA Biological Toxins for toxin or venom biohazards.

Definition of Biohazards: Infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. Includes:

- Microorganisms: Parasites, viruses, bacteria, fungi, prions, etc.
- ONLY agents in that cause infectious disease in humans or vertebrate animals
- Human or animal tissues, fluids and cell lines known to be infected with human or animal pathogens or zoonotic infectious agents, or collected from animals known to be sick or carrying infectious disease.
- Toxins, either cloned or in the purified state. Venom, or work with insects or animals that produce toxins or venom. (Use Form IIA)

Bloodborne Pathogens: pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus.
(HBV) and human immunodeficiency virus (HIV).

**Biological materials covered by the OSHA Bloodborne Pathogen Standard:**
- Blood or blood products;
- Human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures;
- Any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;
- Any unfixed tissue or organ (other than intact skin) from a human (living or dead);
- HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

**Not included in Bloodborne Pathogens:** Saliva, urine, sweat, vomit, or feces, when blood is not observed in the sample.

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**Part A. Bloodborne Pathogens and nonhuman primate tissues, fluids or cell lines, and contaminated human or animal tissues** (fill out as appropriate) Be sure to fill out Part C as well.

1. **Bloodborne Pathogens.** Add rows as needed:

<table>
<thead>
<tr>
<th>Human derived material (as defined above)</th>
<th>Source or manufacturer</th>
<th>*Excluding Bloodborne Pathogens, list the Genus species of bacterial or viral infectious agent</th>
<th>If Oncogenic, “X” the box</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

*based on source, or manufacturer product information

2. **Animal tissues, fluids or cell lines, if material is known to be infected with animal pathogens or zoonotic disease-causing agents or collected from animals known to be sick or carrying infectious disease.** Add rows as needed:

<table>
<thead>
<tr>
<th>Animal tissue, fluid, or cell line</th>
<th>Source or manufacturer</th>
<th>* Genus species of bacterial or viral infectious agent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>
3. For materials collected from Old World Macaques, attach documentation that the material has been tested and is negative for Cercopithecine herpesvirus 1 (Herpes B virus), or provide heat inactivation or sterile-filter procedures below:

*PI should maintain documentation of Herpes B virus testing for each vendor materials are received from, or documentation for each lot number if vendor does not routinely test materials. Documentation must be available to the IBC, if requested.

4. If wild type cell lines will be transplanted into wild type animals, list the cell lines and the animals they will be transplanted into, add rows as needed (if the cell line or animal has been genetically modified, skip this question and go to Form IV):

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Part B. Disease-causing microorganisms (fill out as appropriate) Be sure to fill out Part C as well.

1. List all disease-causing organisms that will be cultured.
   - *Genus species*, strain of the wild type or virulent genetically modified organism. Attenuated strains of Risk Group 2 or higher strains must be listed, and attenuation or avirulence EXPLICITLY stated.
   - Do NOT include viral vectors, or infectious non-replicative particles (go to Form IIIA for these).

2. For all biohazards, provide the natural route of transmission/exposure:

3. For all biohazards, provide the laboratory route of transmission or exposure:

4. Host range:

5. Zoonotic: YES NO

Check the box or highlight the procedures that have the potential to generate aerosols, and provide a brief description of procedure and/or method to minimize or contain aerosols.
### Procedure

<table>
<thead>
<tr>
<th>Description of aerosol containment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipetting using serological pipettes</td>
</tr>
<tr>
<td>Grinding or homogenizing</td>
</tr>
<tr>
<td>Pouring or decanting</td>
</tr>
<tr>
<td>Vortexing</td>
</tr>
<tr>
<td>Sonicating</td>
</tr>
<tr>
<td>Shaking or blending</td>
</tr>
<tr>
<td>Streaking culture onto rough agar</td>
</tr>
<tr>
<td>Resuspending packed cells</td>
</tr>
<tr>
<td>Intranasal inoculation</td>
</tr>
<tr>
<td>Other:</td>
</tr>
</tbody>
</table>

### Part C. Risk Mitigation (required)

1. Check or highlight if a biosafety cabinet is in use.
2. Check or highlight if other primary containment device is in use. List device:
3. List Personal Protective Equipment (PPE) in use:
4. List vaccine, if one is available:
Part D. Chemicals (fill out as appropriate)

Include information ONLY for those chemicals used in conjunction with research described in this protocol.


<table>
<thead>
<tr>
<th>Chemical</th>
<th>PPE</th>
<th>Use and disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled substances:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinogens:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive Toxins (mutagens &amp; teratogens):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Chemical Hazards (corrosives, flammables, reactivies, oxidizers, etc.):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical Toxins:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reproductive Toxins include both mutagens (that affect the genetic material of exposed person) and teratogens (that affect the developing fetus).  
Chemical Toxins, refers to those that are not of biological origin.  Fill out Form IIA for biological toxins.

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Part E. Radioactive Materials (fill out as appropriate)

Include information ONLY for those radioactive materials used in conjunction with research described in this protocol.  Add rows as needed.

Note: Consult the Radiation Safety Manual for disposal of radioactive waste, radioactive/chemical mixed waste, and radioactive/chemical/biological mixed waste.

<table>
<thead>
<tr>
<th>Radioactive Material</th>
<th>PPE</th>
<th>Use and disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
INDIANA UNIVERSITY
Bloomington and Regional Campuses

Form IIA. Biological Toxins or Venoms

Fill out the relevant sections, A, B, or C, and submit this form along with Form I for experiments utilizing biological toxins or venom.

Section A. Toxin Information

Toxin/venom biohazard information (with or without recombinant or synthetic nucleic acid molecule manipulations):

1. Name of toxin/venom:
2. Function of toxin/venom:
3. List at-risk population (humans, animals, plants, etc):
4. List primary routes of transmission/exposure:
5. Symptoms of exposure to toxin:
6. LD$_{50}$ (A list of toxin molecules classified as to LD$_{50}$ are available upon request from the Biosafety Office or the NIH Office of Biotechnology Activities)
7. Toxin inactivation procedures:
8. List all safety equipment used in manipulations:
9. List Personal Protective Equipment (PPE):

Section B. Cloning Toxin Genes

Please select the section of the NIH Guidelines that applies to this work by checking the appropriate box(es) below.

Note: Use Form IIIB if an exempt host vector system will be used, or Form III for cloning in microorganisms.

Note: Toxins that are not lethal to vertebrates are not covered by the NIH Guidelines

<table>
<thead>
<tr>
<th>NIH EXEMPT Toxin Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>These experiments must be registered with the IBC</td>
</tr>
</tbody>
</table>

- **Appendix F-I** Cloning of genes for the biosynthesis of molecules toxic for vertebrates with an LD$_{50}$ equal to or greater than 100ug/kg of body weight

Examples:
- Tetrodotoxin
- T-2 toxin
The experiments listed below require IBC approval, Recombinant DNA Advisory Committee review, and NIH Director approval prior to initiation

- **Section III-A-1-a**  Transferring antibiotic resistance to a microorganism when the antibiotic is currently used to treat the disease caused by the organism in humans or animals. Such transfer compromises the use of the antibiotic to control the disease in humans, veterinary medicine, or agriculture.

- Antibiotic resistance is being used as selectable marker in a disease-causing organism or an attenuated strain of a disease-causing organism.
  If yes, fill out and submit Form II, and list antibiotic resistance selectable markers:

Please select the section of the *NIH Guidelines* that this work will fall under by checking the appropriate box(es) below. Note: Toxins that are not lethal to vertebrates are not covered by the *NIH Guidelines*

<table>
<thead>
<tr>
<th>The experiments listed below require IBC and NIH Director approval prior to initiation</th>
<th>Examples:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloning of genes for the biosynthesis of toxin molecules lethal to vertebrates with:</td>
<td>Tetanus toxin</td>
</tr>
<tr>
<td>LD$_{50}$ less than 100ng/kg of body weight in <em>E. coli</em> K12</td>
<td>Diphtheria toxin</td>
</tr>
<tr>
<td>Section III-B-1, Appendix F-I</td>
<td>Botulinum neurotoxins</td>
</tr>
<tr>
<td>LD$_{50}$ less than 100ng/kg of body weight in Host Vector systems other than <em>E. coli</em> K-12</td>
<td><em>S. dysenteriae</em> neurotoxin</td>
</tr>
<tr>
<td>Section III-B-1, Appendix F-III</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The experiments listed below require IBC approval and registration with the NIH Office of Biotechnology Activities prior to initiation</th>
<th>Examples:</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-II-A  Cloning of genes for the biosynthesis of molecules toxic to vertebrates with an LD$_{50}$ greater than 100ng/kg and less than 1µg/kg of body weight in <em>E. coli</em> K12.</td>
<td></td>
</tr>
</tbody>
</table>
BSL-2 containment when using an EK2 exempt host vector system  
BSL-3 containment when using an EK1 exempt host vector system

<table>
<thead>
<tr>
<th>Cloning of genes for the biosynthesis of molecules toxic to vertebrates with an LD_{50} greater than 1µg/kg and less than 100µg/kg of body weight.</th>
</tr>
</thead>
</table>
| Abrin  
C. perfringens epsilon toxin |

<table>
<thead>
<tr>
<th>F-II-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSL-1 containment when using an EK1 exempt host vector system</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examples:</th>
</tr>
</thead>
</table>
| Conotoxin  
Saxitoxin  
Shigatoxin  
Ricin  
S. aureus alpha toxin  
S. aureus beta toxin  
B. anthracis lethal factor  
P. aeruginosa exotoxin A  
B. pertussis toxin  
Pasteurella pestis/Yersinia pestis murine toxins  
Oxygen-labile hemolysins such as streptolysin O  
Certain neurotoxins present in snake venoms and other venoms |

<table>
<thead>
<tr>
<th>F-II-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSL-1 containment when using an EK1 exempt host vector system</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examples:</th>
</tr>
</thead>
</table>
| Cholera toxin  
Heat stable E. coli toxins  
Heat labile E. coli toxins  
Heat stable Y. enterocolitica toxins  
Klebsiella & related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin |

**Section C. Select Agent Toxins**

**Select Agent toxin:**

1. Amount to be acquired:
2. Existing stock in PI's lab:
3. Largest amount to be used in single experiment:
### HHS Select Agent Toxins [§73.3(d)(3)]

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Exempt from select agent regulation if the total amount in PI’s possession <strong>does not exceed</strong> the amount listed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botulinum neurotoxins</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Staphylococcal enterotoxins</td>
<td>5 mg</td>
</tr>
<tr>
<td>Abrin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Clostridium perfringens epsilon toxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Conotoxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Ricin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Shiga-like ribosome inactivating proteins</td>
<td>100 mg</td>
</tr>
<tr>
<td>Shigatoxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Diacetoxydicirpenol (DAS)</td>
<td>1000 mg</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>1000 mg</td>
</tr>
</tbody>
</table>

---

**INDIANA UNIVERSITY**

Bloomington and Regional Campuses

**Form III. Recombinant or Synthetic Nucleic Acid Molecules Utilizing Microorganisms**

(Bacteria, Whole Viruses, Fungi, Cell Culture, etc.)

Fill out and submit this form along with **Form I** for research utilizing genetically modified microbes.

**Note:** *Research involving viral vectors in a plasmid is described on Form IIIA.* (Use Form IIIA for research using viral sequences in plasmids when packaging elements are present and being used to transfect eukaryotic cells)

- **Section III-D-2-a** DNA from a Risk Group 2 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes.

  Many experiments in **Section III-D-2-a are exempt.** Review Form IIIB to determine if an exemption may be applied. Review the rest of this form to determine if any other sections apply.
  
  - If no other sections apply, go directly to Form IIIB and do not fill out this form.
  - If any other section on this form does apply to the research, fill out and submit this form.

---

**Part A. Cloning Information**
**Required information for all experiments using recombinant or synthetic nucleic acid molecules:**

**Note:** If attenuated/avirulent strains of disease-causing organisms are in use or will be genetically modified, list them on Form II. Attenuation/avirulence must be EXPLICITLY stated on Form II.

1. Source of inserted DNA (including the natural basis if synthetic sequences are used):
2. Name and function of inserted DNA:
3. Name of vector (only those most commonly used, no more than 2 sentences):
4. Name of the host(s) the vector will be transferred into :
5. Foreign genes to be expressed; name and function of protein(s):
6. Anticipated outcome of genetic modification:

**Answer only the applicable questions:**

1. Brief description, if pathogenicity will be enhanced:
2. List of new hosts, if host range will be extended:

Please select the section of the NIH Guidelines that this work fall under by checking the appropriate box(es) below.

**Part B. NIH Guidelines**

The experiments listed below require IBC approval, RAC review, and NIH Director approval prior to initiation

- **Section III-A-1-a.** Transferring antibiotic resistance to a microorganism when the antibiotic is currently used to treat the disease caused by the organism in humans or animals. Such transfer compromises the use of the antibiotic to control the disease in humans, veterinary medicine, or agriculture.

- Antibiotic resistance is being used as selectable marker in a disease-causing organism or an attenuated strain of a disease-causing organism.

  If yes, fill out and submit Form II, and list antibiotic resistance selectable markers:

- Does this research involve culturing pathogens? If yes, please fill out and submit Form II.

The experiments listed below require IBC and NIH Director approval prior to initiation

- Recombinant or synthetic nucleic acid molecules involving DNA from a Restricted Agent: Alastrim, smallpox (variola) and
The experiments listed below require IBC approval prior to initiation

**Note:** Microorganisms that cause disease in healthy adults are Risk Group 2 or 3. Attenuated/avirulent strains must be EXPLICITLY stated on Form II.

- **Section III-D-1-a** Introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2 agents.
- **Section III-D-1-b** Introduction of recombinant or synthetic nucleic acid molecules into Risk Group 3 agents.
- **Section III-D-2-a** DNA from a Risk Group 3 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes.

**Viruses**

**Note:** For defective viruses not in the presence of helper virus, go to Section III-D-1 above, or Section III-E-1 below. For viral vectors, do not fill out this form, go to Form IIIA.

- **Section III-D-3-e** Infectious viruses, or defective viruses in the presence of helper virus or under helper conditions which are not covered in **Section III-D-3-a** or **III-D-3-b**. BSL1.
- **Section III-D-3-a** Infectious Risk Group 2 viruses, or defective Risk Group 2 viruses in the presence of helper virus or under helper conditions. BSL2.
- **Section III-D-3-b** Infectious Risk Group 3 viruses, or defective Risk Group 3 viruses in the presence of helper virus or under helper conditions. BSL3.

**Note:** Helper conditions, infectivity or virulence is restored through a plasmid-mediated mechanism or viral sequences in a plasmid

- **Section III-D-2-a** DNA from a Risk Group 4 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes. BSL-2 containment if PI can demonstrate that only a totally and irreversibly defective fraction of the agent’s genome is present in a given recombinant. Failure to meet this requirement moves the research to BSL-4 containment. Describe testing procedure:

**Large Scale**

- **Section III-D-6** Large scale, more than 10 liters of culture (in one container).

**The experiments listed below may be initiated when the paperwork is registered with the IBC. IBC approval is still required.**

These experiments are conducted under BSL-1 containment. If an experiment described in the section requires BSL-2 containment it falls under section III-D and requires IBC approval prior to initiation.
Section III-E  ALL experiments not included in Sections III-A, III-B, III-D, or III-F are non-exempt from the NIH Guidelines and fall under Section III-E.

Section III-E-1  recombinant or synthetic nucleic acid molecules that contains no more than 2/3 of the genome of any eukaryotic virus propagated and maintained in cells in tissue culture. The PI must demonstrate cells lack helper virus or helper conditions (OR demonstrate the absence of a productive infection) for the specific Families of defective viruses used. Failure to meet this requirement moves the research to Section III-D-3 and requires IBC approval prior to initiation. Describe testing procedure:

Note: All viruses in a Family are considered to be identical. Fragments of more than one eukaryotic virus may be present if the total sequence is less than 2/3 of the genome

EXEMPTION to Section III-E-1
Appendix C-I
Recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome propagated and maintained in cells in tissue culture.

Note: Does apply if source of DNA is a Risk Group 2 agent.
Note: Does not apply if source of DNA is a Risk Group 3, Risk Group 4 or Restricted Agent, or if cells are known or reasonably expected to be infected with Risk Group 2, Risk Group 4, or Restricted agents.
Part C. Examples, Risk Group 2 Bacteria and Viruses

(Provided for informational purposes only, Part C does NOT need to be included with the protocol submission) Attenuated strains of Risk Group 2 (and Risk Group 3) organisms MUST be listed on Form II and attenuation EXPLICITLY stated.

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD
- “This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive.” Pg 37
- “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.” Pg. 38

Note: Part III includes examples of Rick Group 2 bacteria and viruses, the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules also include fungal and parasitic agents.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)</td>
<td>Adenoviruses, human - all types</td>
</tr>
<tr>
<td>Actinobacillus</td>
<td><strong>Alphaviruses (Togaviruses) - Group A</strong></td>
</tr>
<tr>
<td>Actinomycyes pyogenes (formerly Corynebacterium pyogenes)</td>
<td>Arboviruses</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>Eastern equineencephalomyelitis virus</td>
</tr>
<tr>
<td>Amycolata autotrophic</td>
<td>Venezuelan equineencephalomyelitis vaccine strain TC-83</td>
</tr>
<tr>
<td>Archanobacterium haemolyticicum (formerly Corynebacterium haemolyticum)</td>
<td>Western equineencephalomyelitis virus</td>
</tr>
<tr>
<td>Arizona hinshawii - all serotypes</td>
<td>Arenaviruses</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>Lymphocytic choriomeningitis virus (non-neurotropic strains)</td>
</tr>
<tr>
<td>Bartonella henselae, B. quintana, B. vinsonii</td>
<td>Tacaribe virus complex</td>
</tr>
<tr>
<td>Bordetella including B. pertussis</td>
<td>Bunyaviruses</td>
</tr>
<tr>
<td>Borrelia recurrentis, B. burgdorferi</td>
<td>Bunyamwera virus</td>
</tr>
<tr>
<td>Burkholderia (formerly Pseudomonas species) except those listed in Appendix B-III-A (RG3)</td>
<td>Rift Valley fever virus vaccine strain MP-12</td>
</tr>
<tr>
<td>Campylobacter coli, C. fetus, C. jejuni</td>
<td>Caliciviruses</td>
</tr>
<tr>
<td>Chlamydia psittaci, C. trachomatis, C. pneumoniae</td>
<td>Coronavirus</td>
</tr>
<tr>
<td>Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani</td>
<td>Flaviviruses (Togaviruses) - Group B</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale</td>
<td>Arboviruses</td>
</tr>
<tr>
<td>Dermatophilus congolensis</td>
<td>Dengue virus serotypes 1, 2, 3, and 4</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>Yellow fever virus vaccine strain 17D</td>
</tr>
<tr>
<td>Erysipelothrix rhusiopathiae</td>
<td>Hepatitis A, B, C, D, and E viruses</td>
</tr>
<tr>
<td>Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli O157:H7</td>
<td>Herpesviruses - except Herpesvirus simiae (Monkey B virus)</td>
</tr>
<tr>
<td>Haemophilus ducreyi, H. influenzae</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Epstein Barr virus</td>
</tr>
<tr>
<td>Klebsiella - all species except K. oxytoca (RG1)</td>
<td><strong>Herpes simplex</strong> types 1 and 2</td>
</tr>
<tr>
<td>Legionella including L. pneumophila</td>
<td><strong>Herpes zoster</strong></td>
</tr>
<tr>
<td></td>
<td>Human herpesvirus types 6 and 7</td>
</tr>
<tr>
<td></td>
<td>Orthomyxoviruses</td>
</tr>
<tr>
<td></td>
<td>Influenza viruses types A, B, and C</td>
</tr>
<tr>
<td></td>
<td>Papovaviruses</td>
</tr>
<tr>
<td></td>
<td>All human papilloma viruses</td>
</tr>
<tr>
<td></td>
<td>Paramyxoviruses</td>
</tr>
<tr>
<td></td>
<td>Newcastle disease virus</td>
</tr>
</tbody>
</table>
- **Leptospira interrogans** - all serotypes
- **Listeria**
- **Moraxella**
- **Mycoplasma** (except those listed in Appendix B-III-A (RG3)) including
  - *M. avium* complex
  - *M. bovis* BCG vaccine strain
  - *M. chelonei*, *M. fortuitum*, *M. kansasii*,
  - *M. leprae*, *M. malmoense*, *M. marinum*,
  - *M. paratuberculosis*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, *M. xenopi*
- **Mycoplasma**, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- **Neisseria gonorrhoeae**, *N. meningitidis*
- **Nocardia asteroides**, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- **Rhodococcus equi**
- **Salmonella** including *S. arizonae*, *S. cholerasuis*,
  - *S. enteritidis*, *S. gallinarum-pullorum*, *S. meleagridis*, *S. paratyphi*, A, B, C,
  - *S. typhi*, *S. typhimurium*
- **Shigella** including *S. boydii*, *S. dysenteriae*, type 1, *S. flexneri*, *S. sonnei*
- **Sphaerophorus necrophorus**
- **Staphylococcus aureus**
- **Streptobacillus moniliformis**
- **Streptococcus** including *S. pneumoniae*, *S. pyogenes*
- **Treponema pallidum**, *T. carateum*
- **Vibrio cholerae**, *V. parahemolyticus*, *V. vulnificus*
- **Yersinia enterocolitica**

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**INDIANA UNIVERSITY**

Bloomington and Regional Campuses

**Form IIIA. Recombinant or Synthetic Nucleic Acid Molecules Utilizing Viral Vectors**

Includes use of viral sequences to create non-infectious, non-replicative virus particles. Fill out and submit this form along with **Form I** for research utilizing viral vectors. Research utilizing whole viruses is described on **Form III**.

---

**SAMPLE**
Part I. Provide as much information as possible about the viral vector system:

1. PI must demonstrate lack of replication competent virus. Failure to meet this requirement may increase biosafety containment by one level. Describe testing procedure:

2. Name of viral vector:

3. Agent characteristics (e.g. virulence, pathogenicity, environmental stability)

4. Manufacturer, product manual title and number:

5. Name and Risk Group of functional, or wild type, virus:

6. What is the envelope protein?

7. Vector and packaging functions segregated onto how many plasmids?

8. How many recombination events are necessary to reassemble a replication competent virus?

9. Vector titer and total amount of vector:

10. Which essential genes have been deleted from the vector/packaging system?

11. HIV-based vectors only: Is TAT encoded on any system component?

12. Animal research, viral vector introduced into an animal:
   a. Biosafety containment level of the host animal immediately after vector delivery:
   b. Biosafety containment level of the host animal after shedding has ended:

Please select the section of the NIH Guidelines that this work falls under by checking the appropriate box(es) below.

Part II. Cloning into a viral vector system

The experiments listed below require IBC approval, RAC review, and NIH Director approval prior to initiation

☐ Section III-A-1-g. Transferring antibiotic resistance to a microorganism when the antibiotic is currently used to treat the disease caused by the organism in humans or animals. Such transfer compromises the use of the antibiotic to control the disease in humans, veterinary medicine, or agriculture.

☐ Antibiotic resistance is being used as selectable marker in a disease-causing organism or an attenuated strain of a disease-causing organism.
If yes, fill out and submit Form II, and list antibiotic resistance selectable markers:
Required information for all experiments using recombinant or synthetic nucleic acid molecules:

7. Source of inserted DNA (including the wild type basis for synthetic sequences):
8. Name and function of inserted DNA:
9. Is the inserted DNA an oncogene?
10. Name of the host(s) or cell line the vector will be transferred into:
11. Foreign genes to be expressed; name and function of protein(s):
12. Anticipated outcome of genetic modification:
13. Cell line [ ] is [ ] is not potentially contaminated with an animal strain of HIV (FIV, SIV, MIV). If contamination is possible, PI must first test the cell line to demonstrate retrovirus is not present (consult manufacturer product information). Describe testing procedure:

The experiments listed below require IBC and NIH Director approval prior to initiation

☐ Recombinant or synthetic nucleic acid molecules involving DNA from a Restricted Agent. Restricted Agents are Variola major (smallpox), variola minor (Alastrim), and whitepox. CDC/APHIS Select Agents are NOT Restricted Agents. Immediately contact the Biosafety Office at Biosafe@indiana.edu or call 812-856-5360

The experiments listed below require IBC approval prior to initiation

☐ 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. Infectious, non-replicative particles, where wildtype virus, or parent strain, is Risk Group 2 or 3. BSL-2

☐ Section III-D-1-a Introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2 agents.

☐ Section III-D-2 Viral vector is being used to transfer DNA from Risk Group 2 or 3 agents into lower prokaryote, eukaryote, or cell line.

☐ Section III-D-2 Viral vector is being used to transfer DNA from Risk Group 4 agent into lower prokaryote, eukaryote, or cell line. PI must demonstrate that only a totally and irreversibly defective fraction of the agent’s genome is present in a given recombinant. Failure to meet this requirement moves the research to BSL-4 containment. Describe testing procedure:

☐ Section III-D-6 Large scale, more than 10 liters of culture (in one container).
Form IIIB. EXEMPT Recombinant or Synthetic Nucleic Acid Molecule Research Utilizing Microorganisms

Exempt recombinant or synthetic nucleic acid molecule research MUST still be registered with the IBC. Fill out and submit this form with Form I for exempt research utilizing genetically modified microbes.

<table>
<thead>
<tr>
<th>Part A. Section III-F-1 through Section III-F-5 Exemptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part B. Section III-F-6 Exemptions, Cloning in E. coli K12 and other Exempt Host Vector Systems</td>
</tr>
<tr>
<td>Part C. Experimental Information</td>
</tr>
<tr>
<td>*Part D. List of Exempt Gram Positive Organisms</td>
</tr>
<tr>
<td>*Part E. Sublists of Natural Exchangers</td>
</tr>
<tr>
<td>*Parts D and E provide information to assist with submission of Parts A, B, and C and contain no questions. Do not include these Parts with the protocol submission.</td>
</tr>
</tbody>
</table>

Note: Research experiments described in Section III-D-2-a of the NIH Guidelines that also meet the criteria for Section III-F exemptions, are exempt from the NIH Guidelines.

☐ Section III-D-2-a DNA from a Risk Group 2 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes. A representative list of Risk Group 2 agents is provided at the end of Form III.

Part A. Section III-F-1 through Section III-F-5 Exemptions (fill out as appropriate)

Section III-F-1
Recombinant or synthetic nucleic acid molecules that are not in organisms or viruses. Registration with the IBC is not required for this exemption.

Section III-F-2, III-F-3, III-F-4
Exemptions in this section refer to experiments in which nucleic acids are
- cloned from a host into a plasmid indigenous to that host
- the plasmid carries NO foreign genes
- the plasmid is propagated in the host or a closely related strain of the same species
- the plasmid is re-introduced back into the original host or into a closely related strain of the same species
- exemption includes synthetic nucleic acids matching natural sequences

Note:
- III-F-2, III-F-3, III-F-4 exemptions do NOT apply if E.coli K12 or another exempt host vector system is used
- Plasmids must be able to transfer through natural physiological means: transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. This definition is more stringent than the exemption described under Section III-F-5
- Examples of sequences that DO NOT meet these criteria: antibiotic resistance genes or other selectable markers, promoters, regulatory elements, etc. that do not occur in the wildtype plasmid.
III-F-2

- Recombinant or synthetic nucleic acid 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.

  Non chromosomal or viral DNA source:
  List vector(s):

III-F-3

- Recombinant or synthetic nucleic acid 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 natural physiological means.

  Prokaryote:
  List vector(s):

III-F-4

- Recombinant or synthetic nucleic acid molecules 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).

  Eukaryote:
  List vector(s):

Section III-F-5

Exemptions in this section refer to experiments utilizing organisms listed in Appendix A of the NIH Guidelines, Sublists of Natural Exchangers

- Recombinant or synthetic nucleic acid molecules are 1) composed entirely of DNA segments from one or more of the organisms within a sublist; and 2) propagated in any of the organisms within that sublist

Note: Cloning in virulent or attenuated disease-causing organisms requires submission of Form II

Section III-F-2 through III-F-5 exemptions do NOT apply if the plasmid contains sequences from organisms not listed within the applicable sublist; or if *E. coli* or other intermediate host is used to facilitate cloning

- Examples of sequences that DO NOT meet these criteria: antibiotic resistance genes or other selectable markers, promoters, regulatory elements, etc that do not occur in the wildtype plasmid.

- Recombinant or synthetic nucleic acid 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.

  List natural exchanger(s) in use:
  List vector(s):
Part B. Section III-F-6 Exemptions, Cloning in *E. coli* K12 and other Exempt Host Vector Systems (fill out as appropriate)

### Section III-F-6

Recombinant or synthetic nucleic acid molecules that do not present a significant risk to health or the environment

as determined by the NIH Director, with advice from the NIH Recombinant DNA Advisory Committee.

*NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*

- Consult Appendix C, Appendix E, and Appendix I for details about exempt host vector systems

### Appendix C Exceptions to Exemptions

**Section III-F-6 exemptions do NOT apply if:**
- The experiments fall under Section III-A
- The experiments fall under Section III-B
- The experiments involve DNA from Risk Group 3, Risk Group 4, or Restricted agents
- Large Scale experiments (more than 10L in one container)
- Toxin molecule genes coding for biosynthesis of molecules toxic to vertebrates are being cloned

---

**Section III-F-6** The host and the vector must be listed in the same category (e.g. "EK1 Host Vector Systems" for the exemption to apply.

**Appendix C-I Escherichia coli K-12 Host Vector Systems**

**EK1 Host Vector Systems:** The host is always *Escherichia coli* K-12 or a derivative thereof, and the vectors include non-conjugative plasmids and variants of bacteriophage, such as lambda. The *Escherichia coli* K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

- [ ] Host: *E. coli* K12
- [ ] Host: Derivative of *E. coli* K12
- [ ] Vector: non-conjugative-plasmid *circle or highlight the vector(s) in use* pSC101 Co1E1
- [ ] Vector: derivative of non-conjugative plasmid *list vector(s):*

**Source of inserted DNA (including the wild type basis for synthetic sequences):**

**EK2 Host Vector Systems**

- [ ] Host: *E. coli* K12
- [ ] Host: Derivative of *E. coli* K12
- [ ] Vector: exempt plasmid *circle or highlight the vector(s) in use* pSC101 pMB9 pBR313 pBR322 pDH24 pBR325 pBR327 pGL101 pHB1
- [ ] Vector: derivative of exempt plasmid *list vector(s):*

**Source of inserted DNA (including the wild type basis for synthetic sequences):**

**EK2 Host Vector Systems**

- [ ] Host: *E. coli* K12 or [ ] Host: *Saccharomyces cerevisiae* strain SHY1, SHY2, SHY3, or SHY4
- [ ] Host: Derivative of *E. coli* K12 or [ ] Host: *S. cerevisiae* strain SHY1, SHY2, SHY3, or SHY4
- [ ] Vector: exempt hybrid plasmid *circle or highlight the vector(s) in use* YIp1 YEp2 YEp4 YIp5 YEp6 YRp7 YEp20 YEp21 YEP24
Sn/sm 5/11 rev7 Page 27


Vector: derivative of exempt hybrid plasmid  list vector(s):
Source of inserted DNA (including the wild type basis for synthetic sequences):

Section III-F-6

Appendix C-III  
Saccharomyces Host Vector 2 Systems

Host:  Saccharomyces cerevisiae strain SHY1, SHY2, SHY3, or SHY4

Vector:  exempt plasmid  circle or highlight the vector(s) in use

YIp1 YEp2 YEp4 YIp5 YEp6 YRp7 YEp20 YEp21 YEP24

Vector: derivative of exempt plasmid  list vector(s):
Source of inserted DNA (including the wild type basis for synthetic sequences):

Section III-F-6

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

Host Vector 1 Systems

Host:  B. subtilis strain RUB 331 or  B. subtilis strain BGSC 1553

Host:  Derivative of RUB 331 or  BGSC 1553

Vector:  exempt plasmid  circle or highlight the vector(s) in use

pUB110 pC194 pS194 pSA2100 pE194
pT127 pUB112 pC221 pC223 pAB124

Vector: derivative of exempt plasmid  list vector(s):
Source of inserted DNA (including the wild type basis for synthetic sequences):

Host Vector 2 Systems

Host:  Bacillus subtilis ASB 298 asporogenic mutant

Host:  Derivative of Bacillus subtilis ASB 298 asporogenic mutant

Vector:  exempt plasmid  circle vector(s) in use

pUB110 pC194 pS194 pSA2100 pE194
pT127 pUB112 pC221 pC223 pAB124

Vector: derivative of exempt plasmid  list vector(s):
Source of inserted DNA (including the wild type basis for synthetic sequences):

Section III-F-6

Appendix C-V  
Extrachromosomal Elements of Gram Positive Organisms

Recombinant or synthetic nucleic acid molecules derived entirely from extrachromosomal elements from one of the organisms on the list, propagated and maintained in the same organism or in another organism on the list. Exemption includes all shuttle vectors described in Appendix C (Part B of this form) and derivatives of those shuttle vectors. Consult Part D of this form for a full list of exempt gram positive organisms.

List exempt gram positive organisms in use:
List vector(s):

**Additional exempt Host Vector Systems**
Contact the Biosafety Office for assistance with *Neurospora crassa*, *Streptomyces*, or *Pseudomonas putida* exempt Host Vector Systems, or consult Appendix E of the NIH Guidelines.

- [ ] Appendix E-III-B *E. coli* bacteriophage lambda (EK2 host vector system)
- [ ] Appendix E-IV *Neurospora crassa* (Host Vector-1)
- [ ] Appendix E-V *Streptomyces* (Host Vector-1)
- [ ] Appendix E-VI *Pseudomonas putida* (Host Vector-1)

**Part C. Experimental Information** (Required)

Required information for all experiments using recombinant or synthetic nucleic acid molecules:

14. Name and function of inserted DNA:
15. Provide the Genus species of the final host the recombinant or synthetic nucleic acid molecules will be transferred into:
16. Anticipated outcome of genetic modification:

If YES is checked for any question below, fill out and submit Form II along with this form:

- [ ] Yes [] No  Cloned DNA will be transferred from an exempt host vector system into a Risk Group 2 or 3 agent. If yes, fill out and submit Form III
- [ ] Yes [] No  Does this research involve culturing pathogens?
- [ ] Yes [] No  Antibiotic resistance is being used as selectable marker in a disease-causing organism or an attenuated strain of a disease-causing organism. If yes, list antibiotic resistance selectable markers:
### Part D. List of Exempt Gram Positive Organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Organism</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>Clostridium acetobutylicum</td>
<td>Streptococcus equisimilis</td>
</tr>
<tr>
<td>Bacillus amylosacchariticus</td>
<td>Lactobacillus casei</td>
<td>Streptococcus faecalis</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>Listeria grayi</td>
<td>Streptococcus ferus</td>
</tr>
<tr>
<td>Bacillus aterrimus</td>
<td>Listeria monocytogenes</td>
<td>Streptococcus lactis</td>
</tr>
<tr>
<td>Bacillus brevis</td>
<td>Listeria murrayi</td>
<td>Streptococcus ferrns</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Pediococcus acidilactici</td>
<td>Streptococcus mitior</td>
</tr>
<tr>
<td>Bacillus globigii</td>
<td>Pediococcus damnosus</td>
<td>Streptococcus mutans</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>Pediococcus pentosaceus</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>Staphylococcus aureus</td>
<td>Streptococcus pyogenes</td>
</tr>
<tr>
<td>Bacillus natto</td>
<td>Staphylococcus carnosus</td>
<td>Streptococcus salivarivus</td>
</tr>
<tr>
<td>Bacillus niger</td>
<td>Staphylococcus epidermidis</td>
<td>Streptococcus sanguis</td>
</tr>
<tr>
<td>Bacillus pumilus</td>
<td>Streptococcus agalactiae</td>
<td>Streptococcus sobrinus</td>
</tr>
<tr>
<td>Bacillus sphaericus</td>
<td>Streptococcus anginosus</td>
<td>Streptococcus thermophylus</td>
</tr>
<tr>
<td>Bacillus stearothermophilis</td>
<td>Streptococcus cremoris</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Streptococcus dorans</td>
<td></td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Part E. Sublists of Natural Exchangers

#### 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*, *Pseudomonas mendosa*
- *Serratia marcescens*
- *Yersinia enterocolitica*

#### Appendix A-II. Sublist B
- *Bacillus subtilis*
- *Bacillus licheniformis*
- *Bacillus pumilus*
- *Bacillus globigii*
- *Bacillus niger*
- *Bacillus nato*
- *Bacillus amyloliquefaciens*
- *Bacillus aterrimus*

#### 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*
Form IV. Recombinant or Synthetic Nucleic Acid Molecules Utilizing Vertebrate Animals

Fill out and submit this form with Form I for research utilizing genetically modified animals or recombinant or synthetic nucleic acid molecule experiments using wild type or transgenic animals.

Experiments to transplant genetically modified cell lines into a transgenic or wild type animal should be discussed as part of the research description on Form I. Unmodified cell lines that are contaminated, or potentially contaminated with disease causing agents are considered biohazards and should be described on Form II.

DEFINITIONS

Under the NIH Guidelines transgenic vertebrate animals include:
- Knockout animals
- Inbred lines of animals, when one or both of the parent strains was a transgenic animal or a knockout animal
- DNA modifications to the somatic cells of non-transgenic animals
- Gene ablation, when recombinant or synthetic nucleic acid molecules are used to knock out the gene
- Transplanting genetically modified cell lines into a transgenic or wild type animal.

Note: Cloning animals is not covered under the NIH Guidelines

Animal Biosafety Levels (ABSL)

ABSL-1 Most common animal containment used.
- Animals are not experimentally infected with human or animal pathogens.
- Standard cages, personal protective equipment used to prevent contact with animal allergens, recombinant or synthetic nucleic acid molecules, and bites or scratches

ABSL-2
- Animals experimentally infected with Risk Group 1 or 2 organisms, those that are pathogenic to humans or other animals but do not cause serious or lethal disease. Viral vectors introduced directly into an animal.
- Enhanced physical or biological containment practices, or cages have filters or bonnet tops, BSL-2 personal protective equipment and practices.

ABSL-3
- Animals experimentally infected with Risk Group 3 organisms, those that cause serious or lethal disease in humans or other animals. Some Risk Group 2 pathogens require ABSL-3 containment when introduced into animals.
- Cages offer a high level of containment, BSL-3 practices and personal protective equipment including respirators. Facility has directional airflow, an airlock, and restricted access only to those required for program support.

For ABSL-3 Animal Research, Contact the Biosafety Office for assistance
- to purchase or receive wild type or transgenic animals that utilize Animal Biosafety Level 3;
- for assistance with protocol submission, when animals utilize ABSL-3, or when microbes associated with animals utilize BSL-3;
- if proposed research includes genetic sequences from Risk Group 4 viruses.
SECTIONS TO COMPLETE

For all research, Brief description of animal research experiments. Use Form III or IIIB to describe cloning in microorganisms prior to introduction into animals.

1) Planned animal experiments, in layman’s terms, may be included as an attachment. This cannot be a grant proposal. (Skip this question if experiments are described on Form I)

Answer the following questions, as appropriate

1. Recombinant or synthetic nucleic acid molecules used to create new lines of transgenic animals. Provide lists of
   a. Names or classes of vectors:
   b. Species sources of DNA:
   c. Genes or gene classes to be introduced:

1. List, add lines as needed:

<table>
<thead>
<tr>
<th>Name of Transgenic Animal (Genus species, MUST match the names given on the BIACUC protocol form)</th>
<th>Description of modification in each line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If genetically modified cell lines or transgenic animals are in use, answer these questions:

1. List cell lines to be transplanted into an animal, and the animal it will be transplanted into, add lines as needed:

<table>
<thead>
<tr>
<th>*Cell line</th>
<th>Wild type or transgenic animal, list each line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Form II is required for human cell lines, or animal cell lines known or suspected to be contaminated with infectious viral agents.

2. Crossbreeding experiments: List transplanted animals, animals containing sequences from viral vectors, or animals housed in ABSL-2 containment that will be crossbred. Add lines as needed:

<table>
<thead>
<tr>
<th>(Animal X) crossed to (Animal Y)</th>
<th>Description of trait(s) that are being selected following crossbreeding:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Animal containment practices or equipment for animals housed in ABSL-2 (may be included as
Please select the section of the NIH Guidelines that this work falls under by checking the appropriate box(es) below.

Animal Biosafety Level 1 Research

If BIACUC approval has been granted, these experiments may be initiated when the paperwork is registered with the IBC. IBC approval is still required.

If BIACUC approval has NOT been granted these experiments may NOT begin until BIACUC has approved the research, even if the IBC grants approval.

These experiments are conducted under BSL-1 containment. If an experiment described in this section requires BSL-2 containment, the research falls under Section III-D.

III-E-3 ANIMALS other than rodents
☐ Cross breeding knockout animals from 2 different strains (generating a new strain)

III-E-3 RODENTS
☐ Creating knockout rodents
☐ Creating transgenic rodents
☐ Breeding or Crossbreeding experiments in which one or both parental rodents, or the resulting offspring, contain more than 50 percent of the genome of an exogenous eukaryotic virus from a single family (all viruses in the family are considered equal).
☐ Breeding experiments in which one or both parental rodents contain a transgene that is under the control of a gammaretroviral long terminal repeat (LTR). A list of gammaretroviruses are provided at the end of this form.

The experiments listed below require BIACUC approval and IBC approval prior to initiation.
Research using Viruses or Viral Vectors in animals

All animals including rodents

Section III-D
☐ Experiments in any animal, including non-transgenic, with genetically modified viruses that are only transmitted vertically (transmitted from parent to offspring). ABSL-1

Section III-D-4-a
☐ Propagation of animals that contain viral vector sequences that do not lead to transmissible infection directly or indirectly through complementation or recombination. PI must demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. Failure to meet this requirement may increase biosafety containment by one level. Describe testing procedure:

☐ Less than 2/3 of a eukaryotic viral genome is introduced into any non human vertebrate or invertebrate. PI must demonstrate that the virus is defective. Failure to meet this requirement may increase biosafety containment by one level. Describe testing procedure:

---------------------------------------------------------------

Experiments Utilizing Transgenic Animals

ANIMALS other than rodents

III-D-4
☐ Breeding transgenic animals other than rodents
☐ Purchase or transfer of transgenic animals other than rodents (receiving animals including transfers between PI’s)

III-D-4-a
☐ Creating transgenic animals, other than rodents, that utilize ABSL-1
☐ Experiments with transgenic animals other than rodents that utilize ABSL-1

Animal Biosafety Level 2 Research

The experiments listed below require BIACUC approval and IBC approval prior to initiation

☐ Check this box if viral vectors (infectious, non-replicative particles) will be introduced into any animal.

☐ Check this box for all experiments utilizing genetically modified animals that are not exempt and are not described elsewhere on this form.

☐ Check this box for breeding or crossbreeding where one rodent that is housed under ABSL-2 containment will be bred with a rodent housed in ABSL-1 containment.
Experiments Utilizing Transgenic Animals

III-D-4
☐ Purchase or transfer of transgenic rodents requiring ABSL-2 (receiving animals including transfers between PI's)

III-D-4-a
☐ Recombinant or synthetic nucleic acid molecule experiments with transgenic rodents that utilize ABSL-2

III-D-4-b
☐ Creating transgenic animals, other than rodents, that utilize ABSL-2
☐ Experiments with transgenic animals other than rodents under ABSL-2
☐ Creating transgenic rodents that utilize ABSL-2
☐ Breeding rodents from one strain (colony maintenance) under ABSL-2
☐ Crossbreeding rodents from 2 different strains (generating a new strain) under ABSL-2
☐ Experiments with transgenic rodents under ABSL-2

Experimentally Infecting Animals, ABSL-2 or ABSL-3

Section III-D
☐ All experiments involving Risk Group 2 viruses in animals. PI must demonstrate the fraction of the viral genome being utilized does not lead to productive infection. Failure to meet this requirement may increase biosafety containment by one level. Describe testing procedure:

Section III-D-1-a
☐ Experiments in any animal, including non-transgenic, with genetically modified viruses that can be transmitted. ABSL-2
☐ Experiments in any animal, including non-transgenic, with Risk Group 2 genetically modified microbes. ABSL-2

Section III-D-4-a
☐ Propagation of animals that contain viral vector sequences that lead to transmissible infection directly or indirectly through complementation or recombination.

Breeding or Crossbreeding of animals, ABSL-2

Section III-D-4-b
☐ Creating knockout animals that utilize ABSL-2
☐ Breeding knockout animals (colony maintenance) under ABSL-2
☐ Crossbreeding knockout animals from 2 different strains (generating a new strain) under ABSL-2
**Gammaretroviruses**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moloney murine leukemia virus</td>
<td>Xenotropic MuLV-related virus</td>
<td>AVian reticuloendotheliosis virus</td>
</tr>
<tr>
<td>AKV murine leukemia virus (AKR (endogenous) murine leukemia virus)</td>
<td>Abelson murine leukemia virus</td>
<td>Cas-Br-E murine leukemia virus</td>
</tr>
<tr>
<td>Baboon endogenous virus (strain M7)</td>
<td>Friend murine leukemia virus (isolate S7) (FrMLV)</td>
<td>FBJ murine osteosarcoma virus (FBJ-MSV) (Finkel-Biskis-Jenkins murine osteosarcoma virus)</td>
</tr>
<tr>
<td>Feline leukemia virus</td>
<td>Friend murine leukemia virus (isolate FB29) (FrMLV)</td>
<td>Feline endogenous virus ECE1</td>
</tr>
<tr>
<td>Feline sarcoma virus (strain Gardner-Arnstein) (Gardner-Arnstein feline leukemia oncovirus B)</td>
<td>Gibbon ape leukemia virus (GALV)</td>
<td>Feline leukemia virus (isolate CFE-6)</td>
</tr>
<tr>
<td>Friend murine leukemia virus (isolate VP42) (XMRV)</td>
<td>Xenotropic MuLV-related virus (isolate VP35) (XMRV)</td>
<td>Feline leukemia virus (strain A/Glasgow-1)</td>
</tr>
<tr>
<td>Friend spleen focus-forming virus (isolate 502) (FSFFV)</td>
<td>Avian reticuloendotheliosis virus</td>
<td>Feline leukemia virus (isolate B/lambda-B1)</td>
</tr>
<tr>
<td>Hortulanus murine leukemia virus (HoMuLV) (Mus hortulanus virus)</td>
<td>Friend spleen focus-forming virus (isolate 502) (FSFFV)</td>
<td>Feline leukemia virus (strain C/Sarma)</td>
</tr>
<tr>
<td>Moloney murine sarcoma virus (strain ts110) (MoMSV)</td>
<td>Friend spleen focus-forming virus (strain BB6) (FSFFV)</td>
<td>Feline leukemia virus FTT</td>
</tr>
<tr>
<td>Woolly monkey sarcoma virus (WMSV) (Smian sarcoma-associated virus)</td>
<td>Friend spleen focus-forming virus (strain Lilly-Steeves) (FSFFV)</td>
<td>Feline sarcoma virus (strain Hardy-Zuckerman 2)</td>
</tr>
<tr>
<td>AKT8 murine leukemia virus</td>
<td>Harvey murine sarcoma virus</td>
<td>Feline sarcoma virus (strain Hardy-Zuckerman 4)</td>
</tr>
<tr>
<td>Cas-NS-1 murine leukemia virus</td>
<td>Kirsten murine sarcoma virus</td>
<td>Feline sarcoma virus (strain SM) (Sm-FeSV)</td>
</tr>
<tr>
<td>Duplan murine leukemia</td>
<td>Mink cell focus-forming murine leukemia virus</td>
<td>Friend spleen focus-forming virus (strain Lilly-Steeves) (FSFFV)</td>
</tr>
<tr>
<td>FBJ murine osteosarcoma virus (FBJ-MSV) (Finkel-Biskis-Jenkins murine osteosarcoma virus)</td>
<td>Mink cell focus-forming murine leukemia virus (isolate Cl-3)</td>
<td>Feline sarcoma virus (strain Hardy-Zuckerman 4)</td>
</tr>
<tr>
<td>Friend spleen focus-forming virus (strain BB6) (FSFFV)</td>
<td>Moloney murine sarcoma virus (strain HT-1) (MoMSV)</td>
<td>Feline sarcoma virus (strain SM) (Sm-FeSV)</td>
</tr>
<tr>
<td>Moloney murine sarcoma virus (strain m1) (MoMSV)</td>
<td>Moloney murine sarcoma virus (strain m1) (MoMSV)</td>
<td>Friend spleen focus-forming virus (strain Lilly-Steeves) (FSFFV)</td>
</tr>
<tr>
<td>Murine leukemia virus (strain BM5 eco)</td>
<td>Murine leukemia virus (strain DEF27)</td>
<td>Feline sarcoma virus (strain Hardy-Zuckerman 2)</td>
</tr>
<tr>
<td>Murine sarcoma virus 3611</td>
<td>Murine sarcoma virus NS.C58</td>
<td>Feline sarcoma virus (strain Hardy-Zuckerman 4)</td>
</tr>
<tr>
<td>Murine sarcoma virus NS.C58</td>
<td>Myeloproliferative sarcoma virus (isolate ts159)</td>
<td>Feline sarcoma virus (strain SM) (Sm-FeSV)</td>
</tr>
<tr>
<td>Rauscher mink cell focus-inducing virus</td>
<td>Radiation murine leukemia virus (strain Kaplan)</td>
<td>Friend spleen focus-forming virus (strain Lilly-Steeves) (FSFFV)</td>
</tr>
<tr>
<td>Rauscher spleen focus-forming virus (RSFFV)</td>
<td>Rasheed rat sarcoma virus</td>
<td>Friend spleen focus-forming virus (strain Lilly-Steeves) (FSFFV)</td>
</tr>
</tbody>
</table>

**INDIANA UNIVERSITY**

**Bloomington and Regional Campuses**

**Form IVA. Recombinant or Synthetic Nucleic Acid Molecules Utilizing Invertebrate Animals Including Arthropods**

Fill out and submit this form along with Form I for research utilizing genetically modified invertebrate animals including arthropods and/or treating invertebrate animals or arthropods with genetically modified microbes or viruses. “Genetically modified” means modified by recombinant or synthetic nucleic acid molecule techniques.
Experiments to transplant genetically modified cell lines into a transgenic or wild type animal should be discussed as part of the research description on Form I. Unmodified cell lines that are contaminated, or potentially contaminated with disease causing agents are considered biohazards and should be described on Form II.

**Under the NIH Guidelines transgenic invertebrate animals include:**
- Knockout animals
- Inbred lines of animals, when one or both of the parent strains was a transgenic animal or a knockout animal
- DNA modifications to the somatic cells of non-transgenic animals
- Gene ablation, when recombinant or synthetic nucleic acid molecules are used to knock out the gene
- Transplanting genetically modified cell lines into a transgenic or wild type animal.

**Invertebrate animal research that includes any of the following may utilize BSL-2 or higher containment.**
- Wildtype or attenuated strains of microorganisms that can infect and cause disease in humans, animals or plants.
- Research utilizing any viruses that can be transmitted horizontally, including Risk Group 1 viruses.
- Human cell lines, (Bloodborne Pathogens, see Form II).
- Functional toxins/holotoxins or venom (*Does not apply to subunits of toxins*)
  - Includes crossing lines in which progeny have the potential to produce a functional toxin (*transgenic fly lines genetically modified or crossbred to carry genes coding for all of the subunits necessary to produce a functional toxin/holotoxin*).

**For BSL-2 or BSL-3 Research, Contact the Biosafety Office for assistance**
- to purchase or receive transgenic invertebrate animals that utilize Biosafety Level 2 or Biosafety Level 3;
- for assistance with protocol submission, when invertebrate animals, or microbes associated with invertebrate animals, utilize BSL-2 or BSL-3 (see the list above);
- if proposed research includes genetic sequences from Risk Group 4 viruses.

For all Biosafety Level 1 invertebrate animal research including exempt research, review Parts A, B, and C and select the section(s) of the *NIH Guidelines* that this work falls under by checking the appropriate box(es) below.

**Part A. Cloning Information**
Part B. All work described in Part B is conducted under BSL-1 containment. Contact the Biosafety Office if research requires higher containment

The experiments listed below require IBC approval prior to initiation

NOTE: BSL-1 containment is NOT permitted for research involving any viruses that can be transmitted, except those which are transmitted only vertically.

Purchase or transfer of genetically modified invertebrate animals
Section III-D-4
☐ Purchase or transfer of genetically modified invertebrate animals (receiving, including transfers between PI’s) that utilize BSL-1 containment.

Creation of transgenic invertebrate animals.
III-D-4-a
☐ Creation of genetically modified invertebrate animals using recombinant or synthetic nucleic acid molecule techniques that utilize BSL-1.

Experiments with transgenic or non-transgenic invertebrate animals.

III-D-4-a. Check the applicable boxes if the work involves:

☐ Experiments with genetically modified invertebrate animals that utilize BSL-1.

☐ Experiments with genetically modified Risk Group 1 microbes in a transgenic or nontransgenic invertebrate animal (excludes Risk Group 1 viruses that are transmitted horizontally).

☐ Experiments in any invertebrate animal with genetically modified viruses that are transmitted only vertically following initial infection (transmitted from parent to offspring). (Excludes all viruses that are transmitted horizontally after infection).

☐ Recombinant or synthetic nucleic acid molecules/RNA that contains no more than 2/3 of the genome of any eukaryotic virus transferred to an invertebrate animal host. Failure to meet this requirement may increase biosafety containment.
  • Vertical transmission (parent to offspring) is permitted in Biosafety Level 1 (Section III-D-4-a, above).
  • PI must demonstrate the fraction of the viral genome being used does not lead to productive infection.

Creation of new transgenic strains. For strains requiring only BSL-1 containment, provide lists of

d. Species to be modified:

e. Names or classes of vectors (phage-, transposon- or virus-based):

f. Species sources of DNA:

g. Genes or gene classes to be introduced. If human, animal, or insect cell lines will be introduced, list them as well:

Required information for all experiments involving recombinant or synthetic nucleic acid molecules and invertebrate animals is described on this form. Use Form III or IIIB to describe cloning in microorganisms prior to introduction into invertebrate animals. Include Form V to describe experiments involving transgenic plants.
Describe testing procedures:

- Propagation of invertebrate animals that contain **viral vector sequences** that do not lead to a horizontally transmissible infection directly or indirectly through complementation or recombination. Failure to demonstrate that a viral construct is not transmitted horizontally may increase biosafety containment.
  - Infectious non-replicative viral vector particles may be tested prior to introduction into the invertebrate animal (PCR, ELISA, plaque assay, etc). **Describe testing procedures:**

---

**INDIANA UNIVERSITY**

*Bloomington and Regional Campuses*

**Form V. Recombinant or Synthetic Nucleic Acid Molecules Utilizing Plants**

Fill out and submit this form with Form I for experiments with genetically modified plants, or whole plants with associated genetically modified animals, arthropods, or microorganisms.

**DEFINITIONS**

**Exotic plant pathogen:** a plant pathogen not known to occur within the U.S.

**Noxious weed:** a plant species that has been designated by state or national agricultural authorities as a plant that is injurious to agricultural and/or horticultural crops and/or humans and livestock.
  - Often an invasive species, can be a native species.
  - Grow aggressively, multiply quickly, and adversely affect desirable plants or are somehow injurious to livestock or humans either by contact or when ingested.

**Required information for all plant experiments involving recombinant or synthetic nucleic acid molecules described on this form.** Use Form III or IIIB to describe cloning in microorganisms prior to introduction into plants:

- h. Name and function of DNA to be inserted into plant:
  - i. As plant research often involves large numbers of transgenic plants, provide a list of the *Genera* and a summary, or summaries, of the main types of genetic modifications:

- New lines of transgenic plants will be created throughout the duration of the approved protocol, and will fall into one of the categories already described. (An amendment **must** be approved prior to initiating research with recombinant or synthetic nucleic acid molecules and plants if the research is not already described on this form)

**Answer only the applicable questions.**

[ ] Yes  [ ] No  Does transformation utilize a non exotic plant pathogen?  If Yes, list:
Fill in the table for experiments in which arthropods or small animals are associated with plants:

<table>
<thead>
<tr>
<th>Plant</th>
<th>Arthropod (include appropriate forms)</th>
<th>Animal (include appropriate forms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

List Genus species strain Add rows for each experiment

Please select the section of the NIH Guidelines that this work falls under by checking the appropriate box(es) below.

**NIH EXEMPT Plant Experiments**

- [ ] Appendix C-I-A-(v)
  Whole plants regenerated from plant cells and tissue cultures are exempt provided they remain pure cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

The experiments listed below require IBC approval prior to initiation

For the following questions containment levels may lowered through the use of biological containment practices (contact Biosafe@indiana.edu for assistance).

**GENERAL QUESTIONS**

*Section III-D-5*

- [ ] Creation of genetically modified plants
- [ ] Propagation of genetically modified plants
- [ ] Experiments utilizing genetically modified plants

**ASSOCIATED MICROORGANISMS, INSECTS, OR SMALL ANIMALS**

*Section III-D-5*

- [ ] Experiments utilizing genetically modified animals, arthropods, or microorganisms with
The experiments listed below may be initiated when the paperwork is registered with the IBC. However IBC approval is still required. For the following questions containment levels may be raised if genetic modification increases pathogenicity or converts a nonpathogen into a pathogen, depending on the organism, mode of dissemination, and target organisms (contact biosafe@indiana.edu for details).

MICROORGANISMS ASSOCIATED WITH PLANTS

- **III-E-2-b-(2)** Genetically modified plants in which introduced DNA represents the complete genome of a non-exotic plant pathogen. BSL1-P+ or BSL2-P
- **Section III-E-2-a** Plants with associated genetically modified non-exotic microorganisms that have no potential for rapid and widespread dissemination or serious detrimental impact on managed or natural ecosystems (Examples: *Rhizobium* spp, *Agrobacterium* spp). BSL1-P
- **III-E-2-b-(4)** Plants with associated genetically modified exotic microorganism that have no recognized potential for serious detrimental impact on managed or natural ecosystems. BSL1-P+ or BSL2-P
- **III-E-2-b-(3)** Plants with associated genetically modified non-exotic microorganism that has a recognized potential for serious detrimental impact on managed or natural ecosystems. BSL1-P+ or BSL2-P. Describe potential impact on natural or managed ecosystems:

INSECTS OR SMALL ANIMALS ASSOCIATED WITH PLANTS

**Section III-E-2-b-(5)** Check the applicable boxes if the work involves:
- Plant experiments with genetically modified arthropods that use BSL1-P
- Plant experiments with genetically modified small animals that use BSL1-P
- Plant experiments with genetically modified microorganisms associated with arthropods or small animals where the genetically modified microorganism has no potential for serious detrimental impact on managed or natural ecosystems. BSL1-P+ or BSL2-P

**Section III-E-2** Check the applicable boxes if the work involves:
- Experiments with small animals in genetically modified plants in BSL1-P
- Experiments with genetically modified arthropods associated with plants that use BSL2-P

WEEDS

**Section III-E-2-a** BSL1-P Check the applicable boxes if the work involves:

unmodified plants.
Genetically modified plants are **not** noxious weeds  
Genetically modified plants **cannot interbreed** with noxious weeds in geographic area

*Section III-E-2-b-(1) BSL1-P+ or BSL2-P*  
Check the applicable boxes if the work involves:  
- Genetically modified plants are noxious weeds  
- Genetically modified plants **can interbreed** with noxious weeds in the geographic area.  
  List the local plants noxious weed can interbreed with:

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The experiments listed below require BSL2-P+, BSL3-P or BSL-4 P containment, and IBC approval PRIOR to initiating the research

Indiana University does not have the facilities to support BSL3-P or BSL4-P research.

The experiments listed below require BSL2-P+ or BSL3-P. Contact the Biosafety Office at mailto:biosafe@indiana.edu for assistance in determining if BSL2-P+ is appropriate for your research

- **Section III-D-5-a**  
  Experiments involving an exotic plant pathogen with recognized potential for detrimental impact on managed or natural ecosystems when recombinant or synthetic nucleic acid molecule techniques are associated with whole plants

- **Section III-D-5-b**  
  Plant experiments with genetically modified microorganisms or insects that have potential for detrimental impact on managed or natural ecosystems

- **Section III-D-5-c**  
  Experiments involving plants containing cloned genomes of readily transmissible exotic plant pathogens with recognized potential for detrimental impact on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*

- **Section III-D-5-e**  
  Plant experiments with microbial pathogens of insects or small animals if the genetically modified organism has recognized potential for detrimental impact on managed or natural ecosystems

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**Prohibited**  
BSL4-P  
*Section III-D-5-c*  
Experiments with a small number of transmissible exotic plant pathogens that have the potential of being serious pathogens of major U.S crops (soybean rust fungus-*Phakospora pachyrhizi*, maize streak, other viruses) in the presence of their arthropod vectors
Prohibited  BSL3-P  Section III-D-5-d  Experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms.

INDIANA UNIVERSITY
Bloomington and Regional Campuses

Form VI. IU Select Agent Identification

Fill out and submit this form with Form I for the identification of CDC/USDA Select Agents in Diagnostic Samples. A security risk assessment and registration are not required for research described on this form. 42 CR 73 Possession, Use, and Transfer of Select Agents and Toxins; Final Rule can be viewed at https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=8a4be60456973b5ec6bef5dfeaffd49a&r=PART&m=42y1.0.1.6.61

1. List the select agents for which there exists the potential for identification:

2. Brief description of sample source(s) and manipulation procedures:

3. Brief description of identification procedures:

Which method will be used to facilitate the research:

Option 1:

☐ Samples will be verified to contain no viable organism prior to performing analysis that might lead to the identification of select agent organisms.

☐ A copy of the procedure and result of samples positively identified to contain select agent organism(s) will be provided to the Responsible Official within 14 calendar days where it will be maintained on file for 3 years.

Option 2:

☐ Samples will be destroyed after positive identification of select agent(s).

☐ Samples will be immediately secured against theft, loss, or release until destruction.

☐ Destruction will occur as soon as reasonably possible, but within 4 calendar days of identification.

☐ Destruction must be witnessed by the Responsible Official.

Contact Biosafe@indiana.edu for detailed destruction procedures.

☐ Heat Sterilization (autoclave, 121°C 1 hour).

OR

☐ Chemical inactivation.

☐ A copy of the destruction procedure and results of samples positively identified to contain select agent(s) will be provided to the Responsible Official where it will be maintained on file for 3 years.
☐ CDC Form 4 filled out, signed by Responsible Official, submitted to the CDC within 7 calendar days of identification:
https://www.selectagents.gov/form4.html