I. Review Category

Select the review category of your protocol based on the following information. If your project does not fall within one of the listed categories or you need clarification, reference the NIH Guidelines Nov 2013 edition (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines) or contact the ISU IBC.

Review Category I - If any box in this review category is checked, your project requires ISU IBC approval, RAC review, and/or NIH director approval prior to the initiation of experiments. Submit this form and a representative from the Office of Research and will contact you concerning NIH review.

☐ The deliberate transfer of a drug resistant trait to microorganisms that are not known to acquire this trait naturally, if such acquisition could compromise the use of the drug to control disease agent in humans, veterinary medicine, or agriculture [Section III-A of NIH Guidelines].

☐ The deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (kg/bw) (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin) [See Section III-B and Appendix F of NIH Guidelines].

☐ Cloning of toxic molecules with LD_{50} of greater than 100 nanograms per kg/bw, but less than 100 micrograms per kg/bw [See Section III-B and Appendix F of NIH Guidelines].

Review Category II - If any box in this review category is checked, your project requires ISU IBC approval prior to the initiation of experiments.

☐ Experiments using risk group 2, 3, 4, or restricted agents as host-vector systems [See Section III-D-1 and Appendix B of NIH Guidelines].

☐ Experiments in which DNA from risk group 2, 3, 4, or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems [See Section III-D-2 and Appendix B of NIH Guidelines].

☐ Experiments involving the use of infectious or defective DNA or RNA viruses in the presence of helper virus in tissue culture [See Section III-D-3 of NIH Guidelines].

☐ Experiments involving whole animals [See Section III-D-4 of NIH Guidelines]:

  ☐ Experiments involving introduction of recombinant or synthetic DNA into whole animals (non-transgenic experiments). ISU IACUC Protocol No. _____.

  ☐ Generation, purchase, transfer, or crossing of rodent transgenic (includes transgenic, knockout or knockin) animals requiring ABSL2 or higher containment. ISU IACUC Protocol No. _____.

  ☐ Generation, purchase, or transfer of nonrodent vertebrate transgenic animals (i.e., fish, pigs). ISU IACUC Protocol No. _____.

  ☐ Generation of nonvertebrate transgenic animals (i.e., nematodes, fruit flies)

☐ Experiments involving whole plants requiring BSL2-P+ or higher containment [See Section III-D-5 of Guidelines]

☐ Experiments involving greater than 10 liters for an individual culture [See Section III-D-6; Appendix K of NIH Guidelines]
Review Category III - If any box in this review category is checked, your project may be initiated simultaneously with the submission of this form to the IBC.

☐ Using recombinant or synthetic nucleic acid molecules containing less than 2/3 of the genome of any eukaryotic virus [See Section III-E-1, See Appendix C-1-A for exceptions in NIH Guidelines].

☐ Using whole plants at BSL1-P or BSL2-P containment [See Section III-E-2 of NIH Guidelines].

☐ Generation of new transgenic rodents at ABSL1 containment (IACUC protocol No. _______). This includes knockin and knockout rodent models [See Section III-E-3 of NIH Guidelines].

Note: Crossing of existing lines in which the following applies are considered review category II: 1) both parental lines can be housed at ABSL1, and 2) neither parental line contains the following: a) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or b) incorporation of transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and 3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses. If line(s) exists and are not being cross-bred to produce new lines, this research does not apply.

Review Category IV - If any box in this review category is checked, registration with the ISU IBC is not required; however, other federal or state standards of biosafety may still apply. Your project may be initiated simultaneously with the submission of this form.

☐ Using recombinant or synthetic nucleic acid molecules that can neither replicate nor generate nucleic acids in any living cell, cannot integrate into DNA, or does not produce a toxin that is lethal for vertebrates at an LD₅₀ of less than 100 ng/kg body weight [See Section III-F-1 of NIH Guidelines].

☐ Those synthetic nucleic acids that are not in organisms, cells, or viruses and that have not been modified or manipulated to render them capable of penetrating cellular membranes [See Section III-F-2 of NIH Guidelines].

☐ Using recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature [See Section III-F-3 of NIH Guidelines].

☐ Using recombinant or synthetic nucleic acid molecules from a prokaryotic source including its indigenous plasmids or viruses and propagating it only in the same host (or a closely related strain of the same species) or when transferred to another host by well established physiological means [See Section III-F-4 of NIH Guidelines].

☐ Using recombinant or synthetic nucleic acid molecules entirely from a eukaryotic source including chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in the same host (or a closed related strain of the same species) [See Section III-F-5 of NIH Guidelines].

☐ Using recombinant or synthetic nucleic acid molecules and propagating it in hosts where the source and host exchange DNA by known physiological processes (natural exchangers only) [See Section III-F-6 of NIH Guidelines]. Exchangers must be listed in APPENDIX A of NIH Guidelines.

☐ Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA [See Section III-F-7 of NIH Guidelines].

☐ Those that do not present significant risk to health or the environment as determined by the NIH director [See Section III-F-8 and Appendix C of NIH Guidelines].

II. Background
a. **Purpose of the research project:** Describe the overall purpose of the project in a short paragraph. Use language and define scientific terminology in lay terms.

b. **Scientific background:** Describe the scientific background and expertise of the personnel listed in this protocol as it relates to the work with recombinant or synthetic DNA.

### III. Project Information

a. **Nucleic Acids, Strains, and Virus Information**

**Table 1 – Plasmids, Vectors, and Genes**

- Plasmid/Source Column - List all plasmids used and their source. For those that are used for knockdown of a gene target, identify by adding (KD).
- Antibiotic Resistance Gene Column – List, if any, the antibiotic resistance gene encoded in the plasmid
- Gene/Host/Source Column – List the gene(s) inserted for expression, the species of origin, and the source of the DNA/RNA.
- Name/Function Column - List the name and function of the gene product.
- Host Risk Group Column - Determine the risk group of the parental species from which the gene was cloned (e.g. green fluorescent protein is cloned from *Aequorea Victoria*, which is risk group 1).

**Table 2 – Bacterial Strains**

- Strain/Source Column – List the genus and species of the bacterial strain and where it was obtained.
- Relevant Characteristics, Attenuation Column – List all relevant characteristics.
- Native Antibiotic Resistance Gene Column – List, if any, the antibiotic resistance gene encoded endogenously
- Risk Group Column - Determine the risk group of the bacterial species (e.g. *Salmonella typhi* is risk group 2).
- Plasmids to be Transfected Column – List all plasmids, from table 1, that will be transfected into the strain

**Table 3 – Non-Bacterial Strains and Cell Lines**

- Strain/Source Column – List the genus and species name of the organism and where it was obtained.
- Relevant Characteristics Column – List all relevant characteristics.
- Native Antibiotic Resistance Gene Column – List, if any, the antibiotic resistance gene encoded endogenously
- Risk Group Column - Determine the risk group of the non-bacterial species (e.g. *Saccharomyces cerevisiae* is risk group 1).
- Plasmids to be Transfected Column – List all plasmids, from table 1, that will be transfected into the cell

**Table 4 – Viral Packaging and Attenuation**

- Virus Class and Name Column – List the virus classification and its name
- Source Column – List where the virus (vector) was obtained
- Packaging Cell Line Column – List the cell line, from table 3, that will be used to propagate the virus
- Host Range Post Packaging Column – List the natural host(s) of the virus
- Replication Defection Column – Check the box if the virus cannot replicate
- Relevant Characteristic Column – List all relevant characteristics such as which viral genes have been deleted or exogenous genes inserted.

b. **Project Description**
In this section, briefly describe your protocol so that the ISU IBC and other regulatory agencies understand how you intend to use rDNA in cells or cell lines.

**IV. Animal Work and V. Plant Work**

Describe your protocol so that the ISU IBC and other regulatory agencies understand how you intend to use rDNA in animals or plants.

**V. Additional Information**

Provide information regarding the locations for the storage and use of nucleic acid molecules in the project, and the highest BSL for any work involved.

**VI. Personnel: Provide information for personnel, and training, for this project.**

4) **BST** = Biosafety Training

5) **BBP** = Bloodborne Pathogen Training

6) **ST** = Shipping Training

7) **Procedures/Experience with Procedures** – In this section, briefly discuss which procedures (transfection, transformation, etc) the employee/student will conduct. If the employee/students will be trained to perform such procedures, list the initials of the trainer in box 8.

**Definitions**

- **RG1** - Agents that are not associated with disease in healthy adult humans
- **RG2** - Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
- **RG3** - Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
- **RG4** - Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)