Application for Biohazard Use Authorization

ATTACHMENT #2 – RSNA Details

- Any experiment using Recombinant and Synthetic Nucleic Acid molecules involving tissue culture, transgenic plants or animals, including, but not limited to, rodents. zebrafish, *Drosophilia*, and *C. Elegans* must be approved by the Campus Biosafety Committee.
- You do not need to complete Attachment #2 if your request does not include RSNA or other genetically modified materials.
  Useful Sources: ABSA Risk Group Database   NIH Guidelines Involving RSNA, April 2019
- **Instruction:** Fill in the cells in the table with the appropriate information. They will expand as needed.

Section 2.1 NIH Guidelines Section That Applies

Select the section of the *NIH Guidelines* that your project may fall under by selecting the type of recombinant or synthetic nucleic acid experiments that are part of your project.

Section III-**F**

☐	Recombinant or synthetic nucleic acid molecules (RSNA) that **will not** be used inside an organism or virus such as use of probes
☐	Recombinant or synthetic nucleic acid (RSNA) molecules that are propagated or maintained in E. coli K12 host vector systems except for RSNA from RG 3 and 4 viruses or organisms or in systems where conjugation or transduction can take place
☐	RSNA molecules containing less than 1/2 of any eukaryotic virus genome from a single family that are propagated or maintained in tissue culture except for RSNA from RG 3 and 4 viruses or organism
☐	Experiments involving *Saccharomyces cerevisiae* and *S. uvarum* host-vector systems except experiments involving except for RSNA from RG 3 and 4 viruses or organism
☐	Purchase of transgenic animals that can be housed at ABSL-1
☐	Other exempt experiments -- See Appendix C and Section IV-F of the NIH Guidelines for other exempt experiments. Include this information here in the shaded box below.

Section III-**E**

☐	RSNA molecules that are propagated or maintained in E. coli B or W host vector systems except for RSNA from RG 3 and 4 viruses or organism or in systems where conjugation or transduction can take place
☐	Experiments involving generation of transgenic rodents that require ABSL-1 containment
☐	Experiments involving RSNA molecules containing no more than 2/3 of any eukaryotic virus genome and are maintained in tissue culture using BSL-1 containment. Cells must lack helper viruses. (Example: Adeno-associated viral vectors)
☐	Experiments involving plants and RSNA molecules. See the *NIH Guidelines* Section III-E description of these experiments

Section III-**D**

- ☐ Experiments using Risk Group (RG) 2 or 3 agents as host-vector systems
- ☐ Experiments where RSNA molecules are introduced into RG 2 or 3 agents
- ☐ Experiments where RSNA molecules from RG 2 or 3 is transferred into nonpathogenic prokaryotes or lower eukaryotes
- ☐ Use of helper viruses with RG 2 or 3 viruses
- ☐ Experiments where RG 2 or 3 agents containing RSNA molecules are introduced into animal subjects
- ☐ Experiments involving plants. See Section III-D-5 of the NIH Guidelines for a description of these types of experiments

Section III-**C**

- ☐ Transfer of RSNA molecules into one or more human subjects

Section III-**B**

- ☐ Deliberate formation of RNSA molecules containing genes for the biosynthesis of toxic molecules for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight

Section III-**A**

- ☐ Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally and could compromise the ability to control disease agents in humans, veterinary medicine or agriculture

Section 2.2 RSNA Information

***Host**: Caenorhabditis elegans, E-coli*
***Vector**: pUC19*
***Nature of inserted sequences:** marker, gfp cDNA, antibiotic resistance, ampicillin and kanamycin*
***Source of inserted sequences:** bacterial*
***Types of manipulation:** standard tissue culture, growth of worms occur using E-coli agar gel plates*
***Attempt to express foreign gene:** yes, AmpR, KanR, bacterial resistance, gfp*
***Protein produced:** Green Florescent Protein*
Containment: BSL1
***Section of Guidelines:** (Section III-D-4-a): Experiments Involving Whole Animals*

Host	
Vector	
Nature of inserted sequences	
Source of inserted sequences	
Types of manipulation	
Attempt to express foreign gene	
Protein produced	
Containment	
Section of Guidelines that applies	

Host	
Vector	
Nature of inserted sequences	
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Protein produced	
Containment	
Section of Guidelines that applies	

If you have more than 2, please attach the above information for each material to this form.

Section 2.3 Description of Gene(s), include but not limited to: genes overexpressed, expressed in transgenic animals or plants and/or silenced by RNA interference

Gene Sources (organism-genus, species, strain, e.g., E-coli, K12)	**Gene Name and Protein Produced** (acronym & full name, e.g., GFP, green florescent protein)	***Gene category**	**Expression of construct in Host**	
			In vitro cultured Cells - define	**In vivo Animals Define species**

*Gene category examples: structural, enzymatic proteins, metabolic enzymes, cell growth/housekeeping, cell cycle/cell division, DNA replication, membrane proteins, tracking genes (GFP, luciferase), toxins, regulatory genes, oncogenes

Section 2.4 Viral Vectors Used (check all that apply)

☐ Adenovirus, genes deleted:
☐ Adeno-Associated virus (AAV); helper virus used ☐ Yes ☐ No
☐ Epstein-Barr Virus (EBV)
☐ Herpesvirus: ☐ HSV-1 ☐ HSV-2
☐ Baculovirus
☐ Poxvirus -Vaccinia Virus
☐ Sindbis (alpha) virus ☐ helper virus used
☐ Retrovirus: ☐ ecotropic ☐ amphotrophic
☐ MMLV
☐ pseudotype virus, (e.g, VSV Glycoprotein Envelope expressed):
☐ Lentivirus: ☐ HIV ☐ SIV ☐ Other:
☐ helper virus used
☐ genes separated on separate plasmids
☐ Other, please list:

Section 2.5 Vector Description (check all that apply)

Vector backbone (organism-genus, species, strain)	**Vector name** (e.g. PBr322)	**Gene Transfer Method** (e.g. gene gun, transfection)	**Host to be used in** (e.g. E. coli K-12, D.melanogaster)		**Expression**	
			In vitro- define cultured Cells	Animal/Plant species	**Stable**	**Transient**
					☐	☐
					☐	☐
					☐	☐

Attach a construct map and clearly indicate what viral sequences are being deleted from the wild-type vector, and the description and location of inserted viral or cellular sequences.

Section 2.6 Packaging Cell Line(s) and Helper Plasmids (check all that apply)

Name of Cell Line(s) and helper plasmids (co-transfection) (e.g., HEK 293)	**Source(s)** (e.g., viral, human)	***Source of envelope glycoprotein*** If retro-or lentivirus (e.g. vsv-g pseudotype in retroviral system)	**Characterization with respect to host range** (e.g. retro - ecotropic, amphotrophic or lentivirus)	**Host Cells**