INSTITUTIONAL BIOSAFETY COMMITTEE
SAN JOSÉ STATE UNIVERSITY
BIOLOGICAL USE AUTHORIZATION APPLICATION

Attachment A.
Recombinant Nucleic Acids, Synthetic Nucleic Acids, and Viral Vectors

Check all of the following that you propose to do in your research:

- ☒ Insertion of foreign nucleic acids into a vector or organism for the purpose of cloning. The nucleic acid to be cloned will:
  - ☐ be from a Risk Group 3 or 4 agent
  - ☐ represents more than two-thirds of the genome of a Risk Group 1 or 2 agent
  - ☐ encode a known oncogene
  - ☐ encode molecules known to be toxic
  - ☒ encode components that impart pharmaceutical resistance
  - ☐ integrate into DNA
- ☐ Use of more than two-thirds of a viral genome
- ☒ Construction and/or use of synthetic DNA/RNA (e.g., probes, DNA or RNA oligonucleotides, base-pair analogs)
- ☐ Creation of cDNA/genomic libraries
- ☐ Recombinants or synthetics are expected to alter virulence or infectivity, change resistance or susceptibility to drug therapy, or change host range
- ☐ Recombinants or synthetics are deliberately created for the biosynthesis of toxin molecules with an LD_{50} of less than 100 ng/kg body weight
- ☒ The host into which foreign DNA is introduced is a cell or organism other than *E. coli* K12 or its derivatives, *Saccharomyces cerevisiae*, *S. uvarum*, *Bacillus subtilis* or *B. licheniformis*.
- ☐ Use of recombinant or synthetic nucleic acids in animals (somatic cells or germ-line transgenics) including insects, nematodes, and mammals.
  - ☐ Creation of a transgenic organism via introduction of recombinant or synthetic nucleic acid molecules or breeding.
- ☐ Use of recombinant or synthetic DNA/RNA in plants (somatic cells or germ-line transgenics)
- ☐ Potential for infectious agents to be produced/released from recombinant cells, animals, or plants.
- ☐ Environmental release or field testing of genetically engineered organisms.

A Standard Operating Procedure (SOP) must be attached that describes your methods for transforming the vector and host. A detailed step-by-step protocol is not necessary, but provide sufficient information on your procedures so that the committee can identify the steps that involve the greatest likelihood of worker or environmental exposure to biohazardous materials. Include the steps that will be conducted in a biological safety cabinet (including reagent and construct preparation). Consult the SOP template and the sample recombinant DNA SOP for other required components.

☐ N/A

1. Vectors

Vector Name	Vector Type	Risk Group	Replication Incompetent (Y/N)	Amphotropic (Y/N)	Used in Animals (Y/N)	Used in Plants? (Y/N)
e.g.: pBAD	*Plasmid*	*1*	*N*	*N*	*N*	*N*
e.g.: Lentivirus	*Viral*	*2*	*Y*	*Y*	*N*	*N*
psiCHECK-2	plasmid	1	N	N	N	N

Complete a below table with additional details for each **viral** vector listed above. Copy and paste additional tables as needed. A blank table is provided after the example.

Vector name:	*e.g.: Lentivirus*
Causes human disease: (Y/N)	*Y*
Used in animals or plants: (Y/N)	*N*
If you derive a new vector from wild-type virus, or similarly use an early generation vector (for example, first generation), please describe the method by which you will verify replication incompetence. Specify the frequency of testing.	
n/a	
Provide an explanation of the hazards to humans and describe the possible modes of transmission:	
The major safety concerns regarding lentivirus vector are listed below: *• Potential generation of replication competent lentivirus (RCL) during the production process* *• In vivo recombination with endogenous lentiviral sequences* *• Insertional mutagenesis of proviral DNA in, or close to, active genes which may trigger tumour initiation or promotion*	
Provide a detailed description of this vector and safety features of its design (such as which generation of viral vector, gene deletions, etc.):	

We use the third generation of lentivirus provided by Invitrogen (ViraPowerTM Lentiviral Expression System is a third-generation system based on lentiviral vectors developed by Dull et al., 1998).

The two main safety concerns surrounding the use of lentiviral vectors noted by the NIH include:
1) The potential for generation of replication-competent lentivirus
2) The potential for oncogenesis

These concerns are primarily addressed by the design of the vectors used and by safe laboratory practice. In terms of vector design, the third generation lentiviral systems provided by Invitrogen separate transfer, envelope, and packaging components of the virus onto different vectors. The transfer vector encodes the gene of interest and contains the sequences that will incorporate into the host cell genome, but cannot produce functional viral particles without the genes encoded in the envelope and packaging vectors. Unless recombination occurs between the packaging, envelope, and transfer vectors, and the resulting construct is packaged into a viral particle, it is not possible for viruses normally produced from these systems to replicate and produce more virus after the initial infection. In this regard, third generation systems are considered safer than second generation systems because the packaging vector has been divided into two separate plasmids (resulting in a four plasmid system in total). In addition, third generation systems do not use the HIV protein tat in order to produce full length virus from the transfer vector during the viral production stage.

Many of the lentiviral transfer vectors are self-inactivating (SIN) vectors. These vectors have a deletion in the 3'LTR of the viral genome that is transferred into the 5'LTR after one round of reverse transcription. This deletion abolishes transcription of the full-length virus after it has incorporated into a host cell.

The potential for oncogenesis is largely based on the specific insert contained within the lentiviral transfer vector (dependent upon whether or not it is an oncogene) and should be considered on a case by case basis.

Vector name:	
Causes human disease: (Y/N)	
Used in animals or plants: (Y/N)	
If you derive a new vector from wild-type virus, or similarly use an early generation vector (for example, first generation), please describe the method by which you will verify replication incompetence. Specify the frequency of testing.	
Provide an explanation of the hazards to humans and describe the possible modes of transmission:	

Provide a detailed description of this vector and safety features of its design (such as which generation of viral vector, gene deletions, etc.):

☐ N/A

2. Genes

Gene (Family) Name	DNA Source(s)	Immunogenic (Y/N)	(Proto)oncogenic (Y/N)	Toxigenic (Y/N)	Pathogenic (Y/N)	Teratogenic (Y/N)
e.g.: fluorescent proteins	*Aequorea victoria, Discosoma coral*	*N*	*N*	*N*	*N*	*N*
Oncogenic kinases Ras, Raf	*Drosophila cDNA*	*N*	*Y*	*N*	*N*	*N*
Fluorescent proteins controlled by short DNA sequence	Renilla reniformis, Photinus pyralis	N	N	N	N	N

Complete a below table with additional details for each gene/gene family listed above that are **immunogenic, (proto)oncogenic, toxigenic** and/or **pathogenic**. Fill out one table per gene/gene family. Copy and paste additional tables as needed. A blank table is provided after the example.

Gene or gene family name:	*oncogenes (Ras, Raf)*
Gene DNA sources:	*Drosophila meloangaster cDNA*
Highest risk group of sources:	*1*
Expressed as RNA/protein: (Y/N); If yes, indicate RNA or protein	*Yes; protein*
Immunogenic: (Y/N)	*No*
(Proto)oncogenic: (Y/N)	*Yes*
Toxigenic: (Y/N)	*No*
Enhances pathogenicity: (Y/N)	*No*
Pathogenic to humans: (Y/N)	*No*
Teratogenic: (Y/N)	*No*
Gene function is uncharacterized: (Y/N)	*No*
Associated vectors:	*pUAST/Plasmid*
Describe the nature of cellular activity that will result if this gene is expressed or knocked down.	
Alterations in cell migration, increased proliferation.	

Provide an assessment of the hazardous potential of cloning, expressing or inhibiting the target of these DNA/RNA segments that encode substances that are immunogenic, (proto)oncogenic, teratogenic, toxigenic or enhance pathogenicity:
These genes are of Drosophila origin and should not be able cause disease in humans to our best knowledge.
Will these genes be expressed in a host organism? If yes, please provide a brief description and any relevant hazards.
These genes will be expressed in transgenic Drosophila. Transgenic animals will be handled according to established protocols.

Gene or gene family name:	
Gene DNA sources:	
Highest risk group of sources:	
Expressed as RNA/protein: (Y/N); If yes, indicate RNA or protein	
Immunogenic: (Y/N)	
(Proto)oncogenic: (Y/N)	
Toxigenic: (Y/N)	
Enhances pathogenicity: (Y/N)	
Pathogenic to humans: (Y/N)	
Teratogenic: (Y/N)	
Gene function is uncharacterized: (Y/N)	
Associated vectors:	
Describe the nature of cellular activity that will result if this gene is expressed or knocked down.	
Provide an assessment of the hazardous potential of cloning, expressing or inhibiting the target of these DNA/RNA segments that encode substances that are immunogenic, (proto)oncogenic, teratogenic, toxigenic or enhance pathogenicity:	

☐ N/A	**3. Hosts**

Host Name	Infectious Agent (Y/N)	Bloodborne Pathogen (Y/N)
Drosophila melanogaster	*N*	*N*
Human cancer cell lines	*N*	*Y*
Human cancer cell line	N	Y

For each host listed above, fill out a table with additional details. A blank table is provided after the examples.

Host name:	*Drosophila melanogaster*
Risk group:	*1*
Infectious agent: (Y/N)	*No*
Bloodborne pathogen: (Y/N)	*No*
Host details (if applicable):	*n/a*
Vectors (from section 1 above)	*pUAST*
Genes (from section 2 above)	*Oncogenic kinases Ras, Raf*

Host name:	*Human cancer cell lines*
Risk group:	*2*
Infectious agent: (Y/N)	*No*
Bloodborne pathogen: (Y/N)	*Yes*
Host details (if applicable):	*MCF10A-Ras; MDA-MB-213*
Vectors (from section 1 above)	*peGFP*
Genes (from section 2 above)	*Oncogenic kinases Ras (human)*

Host name:	Human cancer cell lines
Risk group:	2
Infectious agent: (Y/N)	No
Bloodborne pathogen: (Y/N)	Yes
Host details (if applicable):	HeLa
Vectors (from section 1 above)	psiCHECK-2
Genes (from section 2 above)	Luciferases (firefly and Renilla)

4. Potential Environmental Impact

Describe aspects of the protocol which could have potential or deliberate environmental impact. Discuss the possible consequences of a release into local agricultural areas or natural ecosystems. If you plan to conduct a field trial, include the location and size of environmental release

psiCHECK-2 plasmid confers ampicillin resistance to host E. coli (XL-10 Gold), though the likelihood of plasmid transfer to pathogenic E. coli in the environment is extremely low, due to experimental design and work practices. Release of ampicillin-resistant E. coli could increase antibiotic-resistant E. coli populations.

5. Section III of NIH Guidelines

Check all of the registration categories that best describe your experiments involving recombinant/synthetic nucleic acids. You may need to check more than one category. For more detailed information about the types of recombinant/synthetic nucleic acid experiments covered under the Office of Science Policy (OSP) NIH Guidelines, refer to https://osp.od.nih.gov/biotechnology/nih-guidelines/.

Note: While certain recombinant/synthetic nucleic acid experiments are considered by the *NIH Guidelines* to be exempt (Section III-F and Appendix C), research of this nature is not exempt from review and oversight from the SJSU IBC.

III-A. Experiments that require IBC Approval, RAC (Recombinant DNA Advisory Committee) Review, and NIH Director Approval Before Initiation

☐	1. The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine, or agriculture. Note: Consideration should be given as to whether the drug resistance trait to be used in the experiment would render that microorganism resistant to the primary drug available to and/or indicated for certain populations (e.g., children or pregnant women).

III-B. Experiments that require NIH OSP and IBC Approval Before Initiation (Check all that apply)

☐	1. Experiments involving 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 ng/kg body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin).
☐	2. Experiments that have been Approved (under Section III-A-1-a and Appendix D) as Major Actions under the *NIH Guidelines*.

III-C. Experiments that require IBC, IRB, and RAC review registration before research participant enrollment

☐	1. Experiments involving the deliberate transfer of recombinant/synthetic nucleic acid molecules, or DNA/RNA derived from recombinant nucleic acid molecules, into human subjects (human gene transfer).
☐	2. Experiments involving the deliberate transfer into human research participants synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria: a. Contain more than 100 nucleotides; or b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or c. Have the potential to replicate in a cell; or d. Can be translated or transcribed.

III-D. Experiments that require IBC approval before initiation (Check all that apply)

☒	1. Experiments using Risk Group 2, Risk Group 3, Risk Group 4, or restricted agents as host-vector systems.
☐	2. Experiments in which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
☐	3. Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.
☐	4. Experiments involving any transgenic animal or the transfer of recombinant/synthetic nucleic acid molecules to any whole animal (vertebrate or invertebrate), at any biosafety level, excluding the generation or purchase of transgenic rodents under BSL-1 containment. Experiments involving viable recombinant/synthetic nucleic acid molecule-modified microorganisms tested on whole animals are also covered in this section. Examples of animal experiments covered under the *NIH Guidelines.*
☐	5. Experiments involving whole plants to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules.
☐	6. Experiments involving more than 10 liters of culture
☐	7. Experiments involving influenza viruses generated by recombinant or synthetic methods.

III-E. Experiments that require IBC notice simultaneous with initiation

For experiments in this category, a BUA shall be dated and signed by the investigator and filed with the IBC at the time (or before) the experiment is initiated. The IBC reviews and approves all such proposals, but IBC review and approval prior to initiation of the experiment is not required. **(Check all that apply)**

☐	1. Experiments not classified in III-A, III-B, III-C, III-D, or III-F and involving the formation of recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus. It must be demonstrated that the cells lack helper virus for the families of defective viruses being used.
☐	2. Experiments not classified in III-A, III-B, III-D, or III-F and involving recombinant/synthetic nucleic acid molecule-modified whole plants, and/or experiments involving recombinant/synthetic nucleic acid molecule-modified organisms associated with whole plants
☐	Experiments involving the generation of rodents in which the animal's genome has been altered by a stable introduction of recombinant/synthetic nucleic acid molecules into the germ-line (transgenic rodents) with BSL-1 containment. For BSL-1 experiments that involve the introduction of recombinant/synthetic nucleic acids to somatic cells of animals, check this selection (please do not select a box in Section III-F). Note: Experiments involving the breeding of certain BSL-1 transgenic rodents are exempt under Section III-F, *Exempt Experiments* (See Appendix C-VIII, *Generation of BL1 Transgenic Rodents via Breeding*).

III-F. Experiments exempt from IBC review (IBC notification required)

Some investigations involving recombinant/synthetic nucleic acid molecules may be exempt, but the IBC must still be notified of the research through submission of the BUA and attachment A. The following eight categories of recombinant/synthetic nucleic acid molecules are exempt from the NIH Guidelines and registration with the IBC. Note: Other federal and state standards of biosafety may still apply to such research (e.g., standards outlined in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*). **(Check all that apply)**

☐	1. Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD_{50} of less than 100 ng/kg body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.
☐	2. Experiments with recombinant or synthetic nucleic acid molecules that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes
☐	3. Experiments with recombinant or synthetic nucleic acid molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature
☐	4. Experiments with recombinant or synthetic nucleic acid molecules that consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means**.**
☐	5. Experiments with recombinant or synthetic nucleic acid molecules that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or closely related strain of the same species)**.**
☐	6. Experiments with 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. See Appendices A-I through A-VI, *Exemptions under Section III-F-6--Sublists of Natural Exchangers*, for a list of natural exchangers that are exempt from the *NIH Guidelines*.
☐	7. Genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA
☐	8. Experiments that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Section III-F-8* for other classes of experiments which are exempt from the *NIH Guidelines*.