SJSU SAN JOSÉ STATE UNIVERSITY

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 libraries or genomic libraries
- ☒ Transgenes 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 (vertebrates and invertebrates).
  - ☒ 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 (vertebrates and invertebrates), 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 (e.g., pET28b)	Vector Description (e.g., bacterial expression plasmid)	Risk Group	Replication Incompetent (Y/N)	Amphotropic (Y/N)	Used in Animals (vertebrates and invertebrates)? (Y/N)	Used in Plants? (Y/N)
pN15C200	bacterial expression plasmid	1	N	N	N	N
Lentivirus	viral vector	2	Y	Y	N	N
pUAST	fly expression plasmid	1	N	N	Y	N

Complete a below table with additional details for each **viral** vector listed above. Copy and paste additional tables as needed.

Viral vector name:	*Lentivirus*
Causes human disease: (Y/N)	*Y*
Used in animals (vertebrates and invertebrates) or plants: (Y/N)	N
Replication incompetent: (Y/N)	Y
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 tumor 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.

☐ N/A	**2. Genes**

Gene (Family) Name (e.g., fluorescent proteins)	DNA Source(s) (e.g., *Aequorea victoria, Discosoma coral*)	Immunogenic (Y/N)	(Proto)oncogenic (Y/N)	Toxigenic (Y/N)	Pathogenic (Y/N)	Teratogenic (Y/N)
fluorescent proteins (e.g., eGFP)	Aequorea victoria, Discosoma coral	N	N	N	N	N
phenazine biosynthetic enzymes	P. aeruginosa	Y	N	Y	N	N

Due to enhanced potential risk to personnel, additional details for each gene/gene family listed above that are **immunogenic, (proto)oncogenic, toxigenic** and/or **pathogenic** are required. Fill out one table per gene/gene family. Copy and paste additional tables as needed.

Gene or gene family name:	phenazine biosynthetic enzymes (*phzA2, phzB2, phzC2, phzD2, phzE2, phzF2, phzG2*)
NCBI Gene ID:	*Locus tags:* PA14_39970, PA14_39960, PA14_39945, PA14_39925, PA14_39910, PA14_39890, PA14_39880
Gene DNA sources:	*P. aeruginosa UCBPP-PA14*
Highest risk group of sources:	2
Expressed as RNA/protein: (Y/N); If yes, indicate RNA or protein	Y, protein
Immunogenic: (Y/N)	Y (the final product of the biosynthetic pathway encoded by the phzA2-G2 genes is phenazine 1-carboxylic acid (PCA, [2538-68-3]), which elicits an immune response during P. aeruginosa infection. We are not concentrating/isolating PCA from culture, nor are we producing higher levels in the recombinant system than in the wild-type P. aeruginosa strain (<1 mM concentrations).)
(Proto)oncogenic: (Y/N)	N
Toxigenic: (Y/N)	Y (the final product of the biosynthetic pathway encoded by the phzA2-G2 genes is phenazine 1-carboxylic acid [2538-68-3], which is harmful in a concentrated form (acute oral/dermal/inhalation toxicity, irritant), but we are not concentrating/isolating from culture, nor are we producing higher levels in the recombinant system than in the wild-type P. aeruginosa strain (<1 mM concentrations).)

Enhances pathogenicity: (Y/N)	Y (phenazine production in P. aeruginosa contributes to virulence, but we are expressing in non-pathogenic E. coli)
Pathogenic to humans: (Y/N)	N (E. coli host used is not pathogenic, phz gene expression does not make pathogenic)
Teratogenic: (Y/N)	N
Gene function is uncharacterized: (Y/N)	N
Associated vectors:	pN15C200

Describe the nature of cellular activity that will result if this gene is expressed or knocked down.

The *phzA2-G2* operon encodes the biosynthetic enzymes PhzA2-G2, which convert shikimate to phenazine 1-carboxylic acid (PCA). Recombinant expression in E. coli allows the recombinant strain to produce PCA.

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:

PCA is a virulence factor from *P. aeruginosa*, which elicits an immune response during infections and is harmful in a concentrated/purified form (acute oral/dermal/inhalation toxicity; irritant). We are using expression of this operon in the recombinant E. coli to test direct inhibition of PhzA2-G2 with small molecules made in our lab. Recombinant levels of PCA do not exceed levels in wild-type *P. aeruginosa* (< 1 mM). PCA is not isolated or concentrated, the cell-free supernatant is just sampled by HPLC to quantitate PCA levels (pellet 1 mL samples in closed vials in biological safety cabinet, decant supernatant and filter through 0.22 µm syringe-driven filter, and place in HPLC vial). Recombinant strains are handled at BSL-2 level to minimize risk to lab personnel.

Will these genes be expressed in a host organism? If yes, please provide a brief description and any relevant hazards.

Yes, they will be expressed in E. coli. See the assessment above.

☐ N/A

3. Hosts

Host Name (e.g., *E. coli*)	Infectious Agent (Y/N)	Bloodborne Pathogen (Y/N)
BSL-1 *E. coli* strains	N	N
Drosophila melanogaster	N	N
Human cancer cell lines	N	Y

For **each host** listed above, fill out a table with additional details.

Host name:	E. coli

Risk group:	1
Infectious agent: (Y/N)	N
Bloodborne pathogen: (Y/N)	N
Host details (if applicable):	NEB Turbo, BL21(DE3), Top10, Rosetta, s17 λpir, DH5α (cloning and protein expression strains of E. coli)
Vectors (from section 1 above):	pN15C200
Genes (from section 2 above):	phenazine biosynthetic enzyme genes, fluorescent protein genes

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)	fluorescent proteins

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)	Lentivirus
Genes (from section 2 above)	fluorescent protein genes

4. Potential Environmental Impact

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

For all of the recombinant bacterial strains, they are likely to lose the plasmid without the selective pressure of antibiotic treatment. Assuming the plasmid remained in the cell, only the recombinant strains with the full phenazine 1-carboxylic acid (PCA) biosynthetic pathway could have an impact in their production of (PCA), however, the levels produced do not exceed wild-type *P. aeruginosa* strains (which are ubiquitous in the soil). Human cancer cell lines are unable to survive outside the lab environment. Regardless, rigorous BSL-2 (for recombinant strains producing PCA, recombinant cancer cell lines) and BSL-1 (all other recombinant strains) protocols will minimize the risk of release.

For recombinant Drosophila melanogaster, accidental release could result in introduction of transgenes into wild populations. However, this is unlikely due to the reduced fitness of inbred lab strains. In addition, rigorous protocols for fly use with minimize the risk of accidental release.

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.
☐	**III-B. Experiments that require NIH OSP and IBC Approval Before Initiation** Experiments involving the cloning of toxin molecules with LD_{50} of less than 100 ng per kg body weight (e.g., **microbial toxins** such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin).
☐	**III-C. Experiments that require IBC, IRB, and RAC review registration before research participant enrollment** Experiments involving the transfer of recombinant/synthetic nucleic acids into human subjects (**human gene transfer**)
☒	**III-D. Experiments that require IBC approval before initiation** • Experiments using Risk Group 2, Risk Group 3, Risk Group 4 or restricted agents as host-vector systems, including **adenoviral and lentiviral vectors** • Experiments in which DNA from Risk Group 2, 3, 4, or restricted agents is cloned into nonpathogenic bacteria or lower eukaryotic host-vector systems • Experiments involving the use of DNA or RNA viruses (infectious or defective) **in the presence of a helper virus** in tissue culture systems (This includes **AAV production** with adenovirus or HSV) • Experiments involving **any transgenic animal** (vertebrate or invertebrate) or the transfer of rDNA to any whole animal (vertebrate or invertebrate), at any biosafety level, **excluding** the generation or purchase of transgenic **rodents** under BSL-1/ABSL-1 containment. **Click here** for examples of animal experiments covered under the *NIH Guidelines* • Experiments 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. • Experiments involving more than **10 liters of culture** • Experiments involving influenza viruses generated by recombinant or synthetic methods.
☒	**III-E. Experiments that require IBC approval before initiation** • 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. This includes non-exempt (section III-F) host-vector systems (e.g., *E. coli* B strains like BL21; click here for resource on *E. coli* strain derivatives). This also includes experiments containing no more than 2/3 of the genome of a eukaryotic virus (in tissue culture only, no helper virus). • Experiments not classified in III-A, III-B, III-D, or III-F and involving recombinant/synthetic nucleic acid molecule 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/ABSL-1** containment **excluding** breeding
☒	**III-F. Experiments that require IBC approval before initiation** • Synthetic nucleic acids that (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (no origin of replication), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin • Experiments 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 • Experiments that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature

	• Experiments 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
	• Experiments 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)
	• Experiments 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*).
	• Genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA
	• Experiments that do not present a significant risk to health or the environment not described above and as listed in Appendix C of the *NIH Guidelines*
	  • Certain recombinant or synthetic nucleic acid molecules that contain less than one half of any eukaryotic viral genome when propagated and maintained in cells in tissue culture
	  • Experiments using the following host-vector systems: *E. coli K-12, Saccharomyces cerevisiae, Saccharomyces uvarium, Kluyveromyces lactis, Bacillus subtilis, Bacillus licheniformis*, or extrachromosomal elements of gram positive organisms. Click here for resource on *E. coli* strain derivatives.
	  • Purchase, transfer, and breeding (with exceptions) of transgenic rodents under BSL-1/ABSL-1 containment