EHS Use Only
Protocol#:

Send original to: Yale Environmental Health and Safety
Occupational Health and Safety Section 135
College Street, Suite 100
New Haven, CT 06510
Phone 785-3550 fax: 785-7588

Yale University Biological Safety Committee

Registration of Experiments Involving Recombinant or Synthetic Nucleic Acid Molecules

Principal Investigator: _____

Faculty Rank or Research Appointment: _____

(Faculty Rank must equal Research Scientist or Higher)

Department of Academic Appointment: _____

Institute/Department Section: _____ Telephone #: _____ Fax #: _____

Lab Manager/Supervisor: _____ Telephone #: _____

Locations (Buildings, Room #s): _____

Provide ALL locations where research with recombinant or synthetic nucleic acids will occur, include animal facilities and core research facilities (e.g. Cell Sorting, Electron Microscope, Confocal Microscope, or Genomics Core Laboratories):

Anticipated Starting Date: _____

(The earliest start date for non-exempt research = next Committee Meeting date or 3rd Thursday of current month)

Short Title of Proposal: _____

(Please note that the title of your protocol and brief description may be subject to public review. Thus, it is advisable that the title and description use terminology understandable to the general public and avoid language or descriptions that could be misinterpreted by the general public)

Brief summary stated in non-technical language: (You may attach a summary of your proposed research from a grant application or other document)

I attest that the information in the attached Registration is accurate and complete. I am familiar with and agree to comply with the current edition of the NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules (NIH Guidelines) and accept the Principal Investigator responsibilities listed in the NIH Guidelines Section IV-B-7, as well as any modifications of these guidelines subsequently issued by the Federal Government or Yale University.

As the Principal Investigator, I agree to accept responsibility for training all laboratory workers involved in the project. All research personnel will be familiar with and understand the potential biohazards and relevant biosafety practices, techniques and emergency procedures.

Written reports will be submitted to the Biological Safety Officer, the Biological Safety Committee, and the Office of Biotechnology Activities at NIH (if applicable) concerning: any research related accident, exposure incident or release of recombinant or synthetic nucleic acid molecules or materials containing them to the environment; problems pertaining to the implementation of biological and physical containment procedures; or violations of the NIH Guidelines.

I will not conduct the work described in the attached registration until it has been filed with, and if necessary, approved by the Biological Safety Committee.

Principal Investigator _____ Date _____
Signature

Additional Investigator _____ Date _____
Signature

Reviewed and Accepted _____ Date _____
Institutional Biological Safety Committee

Principal Investigator Protocol Safety Assurance Statements:

☐Yes ☐No	My laboratory has all relevant and required safety equipment for the safe conduct of the research described in this application
☐Yes ☐No	Personal protective equipment has been provided free of charge to those working on this project in the appropriate sizes
☐Yes ☐No	I will supervise lab staff to ensure that the required biocontainment work practices and techniques are employed. I will correct any work practices and conditions that will result in the release of recombinant DNA materials.
☐Yes ☐No	All personnel assigned to this project have completed all applicable EHS laboratory safety training classes (i.e. Biosafety Part 1 and Part 2, Bloodborne Pathogens for lab personnel, Lab Chemical Safety)
☐Yes ☐No	Emergency exposure and spill response procedures have been outlined and reviewed with the laboratory staff
☐Yes ☐No	As the Principal Investigator, I have completed the Yale EHS online class entitled "PI Orientation to the Yale Biological Safety Manual" (and any other applicable training classes if I am participating in the conduct of laboratory research)
☐Yes ☐No	My laboratory will follow the emergency response protocols as detailed in the Yale University Biosafety Manual
☐Yes ☐No	All incidents and exposures involving recombinant or synthetic nucleic acid molecules will be promptly reported to Yale EHS for evaluation
☐Yes ☐No	All non-exempt experiments involving the use of recombinant or synthetic nucleic acid molecules will not be initiated until approved by the Yale University Biological Safety Committee
☐Yes ☐No ☐N/A	Any specific medical surveillance requirements for this protocol (i.e. immunizations, serum storage) have been communicated to all personnel involved in this project.

High Risk Recombinant or Synthetic Nucleic Acid Molecule Research Experiments

☐Yes ☐No	Does this rDNA protocol involve the transfer of recombinant or synthetic nucleic acid molecules to human subjects?
☐Yes ☐No	Does the rDNA protocol involve the cloning of molecules toxic to vertebrates with an LD50 of < 100 ng/kg body weight?
☐Yes ☐No	Does the rDNA protocol involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the ability of the drug to control disease? ***(Example: Placing a gene for Ampicillin resistance in E. coli K12 would not meet this criteria, but placing an gene for Ampicillin resistance in Listeria monoctyogenes would).***
☐Yes ☐No	Does this experiment involve the use of a Select Agent?
☐Yes ☐No	Does this experiment involve the use of greater than 10 Liters of culture?

Principal Investigator

Signature: _____ Date _____

Dual Use Research

In reviewing registrations, the Yale IBC considers "dual use" potential, namely the potential for research projects with a beneficial purpose to provide knowledge, products or technologies that could be directly misapplied to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or material. For a full discussion of this topic, consult Dual Use Research and the National Science Advisory Board https://oir.nih.gov/sourcebook/ethical-conduct/special-research-considerations/dual-use-research

Will you be conducting research that directly uses nonattenuated forms of one or more of the following agents? ☐Yes ☐No.

If yes, please check the agent involved:

- ☐Avian influenza virus (highly pathogenic)
- ☐Bacillus anthracis
- ☐Botulinum neurotoxin (in any quantity)
- ☐Burkholderia mallei
- ☐Burkholderia pseudomallei
- ☐Ebola virus
- ☐Foot-and-mouth disease virus
- ☐Francisella tularensis
- ☐Marburg virus
- ☐Reconstructed 1918 influenza virus
- ☐Rinderpest virus
- ☐Toxin-producing strains of Clostridium botulinum
- ☐Variola major virus
- ☐Variola minor virus
- ☐Yersinia pestis

Do any of your experiments fall into any of the followingexperimental categories? ☐Yes ☐No

If yes, please check all that apply:

- ☐ Enhances the harmful consequences of the agent or toxin;
- ☐ Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/ or agricultural justification;
- ☐ Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
- ☐ Increases the stability, transmissibility, or the ability to disseminate the agent or toxin;
- ☐ Alters the host range or tropism of the agent or toxin;
- ☐ Enhances the susceptibility of a host population to the agent or toxin; and
- ☐ Generates or reconstitutes an eradicated or extinct listed agent or toxin.
- ☐ Provide other knowledge, products or technologies that could be directly misapplied to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or material.

Comment on aspects of your research, if any, with potential for dual use:

Registration of Research Experiments with Recombinant or Synthetic Nucleic Acid Molecules

I. General Information

For all Recombinant or Synthetic Nucleic Acid Molecule Experiments Provide:	
Type and name of rDNA Vector (provide name and company or person providing it). Provide reference also if available.	
Vector Map (attach from website) **Also include all packaging vectors** that will be used with your expression vector.	
Percent of the pathogen genome present in the vector (kilobases of the parent pathogen in the vector **plus** packaging plasmids and/or packaging cells combined)	
Please indicate the tropism of your vector (i.e. pantropic, amphotropic, ecotropic, xenotropic)	
Has your vector been pseudotyped with the VSV-G gene or another gene that can extend the tropism of your vector?	
Please indicate the gene that extends tropism?	
Can your vector infect a human cell?	

II. Specific Information:

Complete the applicable parts of Section II based on the answers to the following questions and then proceed to part D of Section II.

Will experiment be carried out in E. coli or other prokaryotic host? ☐Yes ☐No
If Yes, complete part A.

Will experiment be carried out in eukaryotic cells? ☐Yes ☐No
If Yes, complete part B.

Will whole plants or animals (any member of the kingdom Animalia) be used as hosts? ☐Yes ☐No
If Yes, complete part C.

A. E. coli or other prokaryotic host

Specify:

Host strains:

Vectors:

Inserted DNA
(**include names of genes and organisms from which they were cloned**):

Will an attempt be made to express a foreign gene? ☐Yes ☐No

If yes, what protein will be produced?

Will whole virus or provirus be cloned? ☐Yes ☐No

Will the research involve the use of antibiotic selection markers? ☐Yes ☐No
If yes, list the markers and microbial agents used (e.g. neomycin resistance marker in *E. coli*)

NIH Guideline Section: Recommended Biosafety Level:

B. Eukaryotic cells

Type:

Host cells:

Vectors:

All Packaging Plasmids used with your expression vectors:

Inserted DNA:

Will an attempt be made to express a foreign gene? ☐Yes ☐No

If yes, what protein will be produced?

Helper virus or packaging cells if used:

What fraction of a eukaryotic viral genome is contained in the recombinant or synthetic nucleic acid molecules (including vector and/or packaging plasmids or packaging cell lines)?

Check appropriate range: ☐ <1/2 ☐ >1/2 but <2/3 ☐ >2/3

NIH Guideline Section: Recommended Biosafety Level:

Will the research involve the use of antibiotic selection markers? ☐Yes ☐No

If yes, list the markers and microbial agents used (e.g. neomycin resistance marker in *E. coli*)

C. Whole plants or animals (any member of the kingdom Animalia) be used as hosts

Type:

Plant or animal hosts:

Vectors:

Inserted DNA:

What fraction of a eukaryotic viral genome is contained in the recombinant or synthetic nucleic acid molecules (including vector and packaging cell line)?

☐ <2/3 ☐ >2/3

Will transgenic plants or animals be constructed or used? ☐Yes ☐No

Has a Yale IACUC registration been filed for your research experiments with vertebrate animals?

☐Yes ☐No

NIH Guideline Section: Recommended Biosafety Level:

Will you cross-breed two or more genetically modified rodents or plants? ☐Yes ☐No
If yes, please answer the questions below:

Note: Generation of transgenic rodents by breeding to create a new strain shall be EXEMPT from the NIH Guidelines if the following criteria are met.

☐Yes ☐No Both parental rodents can be housed under BSL1 containment; AND

☐Yes ☐No Neither parental transgenic rodent contains the following genetic modifications:

- Incorporation of more than 50% of the genome of an exogenous eukaryotic virus from a single family of viruses; OR
- Incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); AND

☐Yes ☐No The transgenic rodent that results from this breeding is not expected to contain more than 50% of an exogenous viral genome from a single family of viruses.

D Additional Information

For recombinant or synthetic nucleic acid molecule experiments, do the genes involved or expressed involve potential:		**Provide full name of genes in each category** **(**provided the full scientific names for all the abbreviations on the form)
Toxicity	☐Yes ☐No ☐N/A	
Cell cycle/Cell division regulators	☐Yes ☐No ☐N/A	
Regulatory genes for transcription and cell activators (cytokines, lymphokines, and tumor suppressors)	☐Yes ☐No ☐N/A	
Structural Proteins, Membrane Proteins	☐Yes ☐No ☐N/A	
Enzymatic Proteins and Metabolic enzymes	☐Yes ☐No ☐N/A	
Cell growth	☐Yes ☐No ☐N/A	
Tracking genes (i.e. GFP)	☐Yes ☐No ☐N/A	
Allergenicity or other risk to research personnel	☐Yes ☐No ☐N/A	
Oncogenicity (cellular or viral oncogenes) or tumor suppressor genes	☐Yes ☐No ☐N/A	
Other potential risk to researchers handling the recombinant or synthetic nucleic acid molecules in this application?	☐Yes ☐No ☐N/A	

CRISPR or Other Gene Editing Technology

☐Yes ☐No If yes, answer questions **below**.	Does your research involve CRISPR or other gene editing technology?
	Please indicate which technology (CRISPR/Cas9, Zinc Finger nucleases, TALENS, Meganucleases, other?)
☐Yes ☐No	For CRISPR systems, is the gRNA and nuclease on the same plasmid, vector or delivery vehicle?
☐Yes ☐No	Can this plasmid, vector or delivery vehicle transfect or infect a human cell?
☐Yes ☐No	For CRISPR research involving viral vectors, has a GT Scan for off target effects by your gRNA been completed? This is helpful in assessing the risk of potential exposure in the event of an incident.

Gene Transfer Research Using Gene Editing Technologies

Are you planning human gene transfer research using gene editing technologies? ☐Yes ☐No

Note: No gene editing of the germ line human embryos or germ cells for clinical application is allowed. Gene editing of human embryos and germ cells for scientific purpose may be allowed, but must be evaluated on a case by case basis by the appropriate federal and local scientific review committees.

Gene Drive Experiments

☐Yes ☐No	Will the experiment make transgenic, sexually reproducing organisms?
☐Yes ☐No	Is an entire CRISPR system encoded in a single DNA construct?
☐Yes ☐No	Could it self-insert into the genome?
	If you answered yes to all three questions above, answer yes to the following question.
☐Yes ☐No	Are you performing a gene drive experiment?

III. Describe the Biohazard Potential of these Experiments:

The risks associated with the transgene and chance of generating replication competent virus must be considered.

If using defective lentiviral or retroviral vectors risks also include insertional mutagenesis, which can lead to activation of oncogenes, inactivation of tumor suppressor genes, and gene disruption.

A. Provide the risks associated with the vector(s) being used including ability of the vector to infect researchers.

B. Address the risks associated with the inserted nucleic acid. (If using defective lentiviral or retroviral vectors risks also include insertional mutagenesis, which can lead to activation of oncogenes, inactivation of tumor suppressor genes, and gene disruption).

C. What are the consequences of exposure if a researcher is exposed to the recombinant molecules described on this protocol?

D. What are the treatment options in the event of an occupational exposure?

E. What are the suggested medical follow-up steps required after an exposure?

F. Are there any medical surveillance requirements for the research materials described in this protocol?

G. What are the chances of the formation of a replication competent vector (if your vector is replication defective)?

Please note: Anyone who is currently pregnant or immunosuppressed must contact Employee Health (432-7978) before working with the agent in question.

IV. Description of Experiment:

1) Describe the goals of the research and potential benefits in sufficient detail to clarify the scientific basis of the work:

2) Indicate the desired start date for each phase of the work:

3) Provide key and relevant references to assist the Committee in their review of your request to perform the described experiment:

V. **PERSONNEL** (Names, Status and Telephone):

RESEARCHER EXPERIENCE FORM
(Required for the PI and each researcher listed in Section V - Personnel)

Name:_____ Job Title: _____

Principal Investigator:_____ Date: _____

Education (Degrees and programs completed)**:**

Date	**Institution**	**Major Area**	**Degree**

Laboratory experience related to work with microorganisms or cell culture:

Dates	**Institution**	**Description of work and name of microorganisms (include the genus and species)**