

EHS Use Only
Protocol#:

Send original to: Yale Environmental Health and Safety
135 College Street, Suite 100
New Haven, CT 06510
Phone 203-785-3550 fax: 203-785-7588

Yale University Biological Safety Committee
Registration of Experiments Involving Recombinant or Synthetic Nucleic Acid Molecules

Note: Registrations with hand written responses will not be accepted.

Principal Investigator: _____

Faculty Rank or Research Appointment: _____
(Faculty Rank must equal Research Scientist or Higher)

Academic Department Appointment: _____

Institute/Department Section (if relevant): _____

Telephone #: _____ Fax #: _____

Lab Manager/Supervisor (if relevant): _____ Telephone #: _____

Locations (Buildings, Room #s): _____
Provide ALL locations where research in this registration 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 Representative

I. Principal Investigator Protocol Safety Assurance Statements:

<input type="checkbox"/> Yes <input type="checkbox"/> No	My laboratory has all relevant and required safety equipment for the safe conduct of the research described in this application
<input type="checkbox"/> Yes <input type="checkbox"/> No	Researchers working on this project will be provided personal protective equipment in the appropriate sizes at no cost to them.
<input type="checkbox"/> Yes <input type="checkbox"/> 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.
<input type="checkbox"/> Yes <input type="checkbox"/> 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)
<input type="checkbox"/> Yes <input type="checkbox"/> No	Emergency exposure and spill response procedures have been outlined and reviewed with the laboratory staff
<input type="checkbox"/> Yes <input type="checkbox"/> 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)
<input type="checkbox"/> Yes <input type="checkbox"/> No	My laboratory will follow the emergency response protocols as detailed in the Yale University Biosafety Manual
<input type="checkbox"/> Yes <input type="checkbox"/> No	All incidents and exposures involving recombinant or synthetic nucleic acid molecules will be promptly reported to Yale EHS for evaluation
<input type="checkbox"/> Yes <input type="checkbox"/> 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
<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> 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.

II. High Risk Recombinant or Synthetic Nucleic Acid Molecule Research Experiments

<input type="checkbox"/> Yes <input type="checkbox"/> No	Does this rDNA protocol involve the transfer of recombinant or synthetic nucleic acid molecules to human subjects?
<input type="checkbox"/> Yes <input type="checkbox"/> No	Does the rDNA protocol involve the cloning of molecules toxic to vertebrates with an LD50 of < 100 ng/kg body weight? Examples: Botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin
<input type="checkbox"/> Yes <input type="checkbox"/> 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 criterion but placing a gene for Ampicillin resistance in Listeria monocytogenes would).
<input type="checkbox"/> Yes <input type="checkbox"/> No	Does this experiment involve the use of a Select Agent?
<input type="checkbox"/> Yes <input type="checkbox"/> No	Does this experiment involve the use of greater than 10 Liters of culture?

Principal Investigator's
Signature: _____

Date: _____

III. 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 <http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern>

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:

- | | |
|--|--|
| <input type="checkbox"/> Avian influenza virus (highly pathogenic) | <input type="checkbox"/> Marburg virus |
| <input type="checkbox"/> <i>Bacillus anthracis</i> | <input type="checkbox"/> Reconstructed 1918 influenza virus |
| <input type="checkbox"/> Botulinum neurotoxin (in any quantity) | <input type="checkbox"/> Rinderpest virus |
| <input type="checkbox"/> <i>Burkholderia mallei</i> | <input type="checkbox"/> Toxin-producing strains of <i>Clostridium botulinum</i> |
| <input type="checkbox"/> <i>Burkholderia pseudomallei</i> | <input type="checkbox"/> Variola major virus |
| <input type="checkbox"/> Ebola virus | <input type="checkbox"/> Variola minor virus |
| <input type="checkbox"/> Foot-and-mouth disease virus | <input type="checkbox"/> <i>Yersinia pestis</i> |
| <input type="checkbox"/> <i>Francisella tularensis</i> | |

Do any of your experiments fall into any of the following experimental 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;
- 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:

IV. Registration of Research Experiments with Recombinant or Synthetic Nucleic Acid Molecules

Note: All requested information refers solely to the research described and included in this registration

A. General Information

Please provide all information requested below. If an item is not applicable, indicate that by placing N/A in the space provided.

For recombinant or synthetic nucleic acid molecule experiments in this registration, please provide:	
Type of vector (e.g. defective lentivirus, defective adenovirus), name of rDNA vector, and the name of the company or person providing it. Provide reference 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, or will, your vector been pseudotyped with the VSV-G gene or another gene that can extend the tropism of your vector? If yes, please provide name of gene(s).	
Can your vector or other delivery vehicle infect or transduce a human cell?	
Do your recombinant or synthetic nucleic acids contain elements known to interact with either DNA or RNA polymerase? If Yes, please describe.	
Does this recombinant DNA protocol involve oligonucleotides or other synthetic nucleic acids that contain elements known to interact with the DNA repair apparatus? If yes, please describe.	
Are the recombinant or synthetic nucleic acids designed to integrate into DNA?	
Do the recombinant or synthetic nucleic acids product a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight? If yes, please list the toxin(s)?	

B. 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 on page 8.

Will experiment be carried out in *E. coli* or another prokaryotic host? Yes No
If Yes, complete part B1 below.

Will experiment be carried out in eukaryotic cells? Yes No
If Yes, complete part B2 on page 7.

Will whole plants or animals (any member of kingdom Animalia) be used as hosts? Yes No
If Yes, complete part B3 on page 8.

Will nanoparticles be used as a delivery mechanism in this research? Yes No
If Yes, please complete part B4 on page 9.

B1. *Escherichia coli* or other prokaryotic host(s) – Please provide the information below:

Host strains: _____

Vectors: _____

Inserted recombinant or synthetic nucleic acids; include names of genes and source organisms:

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(s): _____ Recommended Biosafety Level: _____

The EHS document "[Overview of Recombinant DNA Experiments Covered by The NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules](#)" provides descriptions of the NIH Guidelines sections that may be helpful. The full text of the [NIH Guidelines](#) is available on the NIH Office of Science Policy web site.

End of Part B1.

B2. Eukaryotic cells – Please provide the information below:

Host cells: _____

Vectors: _____

All Packaging Plasmids used with your expression vectors:

Inserted recombinant or synthetic nucleic acids; include names of genes and source organisms:

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

If yes, what protein will be produced? _____

Will helper virus or packaging cells if used? Yes No

If yes, list helper virus or packaging cells: _____

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

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(s): _____ Recommended Biosafety Level: _____

The EHS document "[Overview of Recombinant DNA Experiments Covered by The NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules](#)" provides descriptions of the NIH Guidelines sections that may be helpful. The full text of the [NIH Guidelines](#) is available on the NIH Office of Science Policy web site.

End of part B2.

B3. Whole plants or animals (any member of the kingdom Animalia) used as hosts – Please provide the information below:

Plant or animal hosts: _____

Vectors: _____

Inserted recombinant or synthetic nucleic acids; include names of genes and source organisms:

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: _____

The EHS document "[Overview of Recombinant DNA Experiments Covered by The NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules](#)" provides descriptions of the NIH Guidelines sections that may be helpful. The full text of the [NIH Guidelines](#) is available on the NIH Office of Science Policy web site.

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.

End of part B3.

B4. Nanoparticles – Please provide the information below:

Note: If nanoparticles will be used in animals please also complete section C.

Describe the type of nanoparticle used: _____

Host strains: _____

Vectors: _____

Inserted recombinant or synthetic nucleic acids; include names of genes and source organisms:

Describe what is being delivered by the nanoparticle (e.g. what recombinant or synthetic nucleic acids) and what it's function is.

Describe what has been done to increase the specificity to the target cell and minimize off target effects:

End of part B4

C. Additional Information

For recombinant or synthetic nucleic acid molecule experiments in this registration, 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	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Cell cycle/Cell division regulators	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Regulatory genes for transcription and cell activators (cytokines, lymphokines, and tumor suppressors)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Structural Proteins, Membrane Proteins	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Enzymatic Proteins and Metabolic enzymes	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Cell growth	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Tracking genes (i.e. GFP)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Allergenicity or other risk to research personnel	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Oncogenicity (cellular or viral oncogenes) or tumor suppressor genes	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	
Other potential risk to researchers handling the recombinant or synthetic nucleic acid molecules in this application?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	

D. CRISPR or Other Gene Editing Technology applicable to this registration

Does your research in this registration involve CRISPR or other gene editing technology? Yes No

If No, go to section E. If Yes, complete the rest of section D.

Indicate in the space below which gene editing technology will be used (CRISPR/Cas9, Zinc Finger nucleases, TALENS, Meganucleases, other - specify).

For CRISPR systems, are 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? Yes No

If Yes, were there any significant off-target effects noted? Please provide information below; it is helpful in assessing the risk of potential exposure in the event of an incident:

E. Human Gene Transfer Research Using Gene Editing Technologies Related to this Registration

Are you planning human gene transfer research using gene editing technologies related to this registration? 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.

If Yes, please describe the project below or in an attachment:

F. Gene Drive Experiments

1. Does the experiment involve or can it result in transgenic, sexually reproducing organisms?
Yes No
2. Is an entire CRISPR (or other gene editing technology) system encoded in a single DNA construct?
Yes No
3. Could the entire CRISPR (or other gene editing technology) system self-insert into the genome?
Yes No
4. If you answered “yes” to all three above questions, this appears to be a gene drive experiment. Provide the following additional information. (A document with this information may be attached.):
 - A) Explain why you believe that the proposed experiment will not create a gene drive.

B) If you do not intend to create a gene drive, contact EHS to discuss the protocol and submit a revised version of the proposal where the answer to one of the three questions above is ‘no’.

C) If the intent is to create a gene drive, answer the following questions C1-C7 (A document with this information may be attached.):

C1. Explain why a gene drive is required and why an alternative approach that does not involve a gene drive cannot be utilized.

C2. Is there potential that the organism will possess enhanced fitness or a transmission advantage?

C3. Describe methods of containment and confinement to reduce the potential for unintended release or persistence of gene-drive modified organisms.

C4. Describe mitigation strategies to address potential harmful off-target and non-target effects.

C5. In the event of an accidental release to the environment, what mechanism could be used to prevent spread of the organism? (Examples include a split gene drive, the insertion of an artificial sequence in the target gene of the laboratory organism, or a reversible gene drive.)

C6. Describe the potential risks to humans or the environment, or why this does not present a risk to humans or the environment.

C7. If you have additional information with respect to the gene drive, provide it here:

G. Describe the Biohazard Potential of these Experiments:

Provide the information below related to the risks associated with the work in this registration; the transgene and chance of generating replication competent virus must be considered. A document with this information may be attached.

Note: 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?

Please note: Anyone who is currently pregnant or immunosuppressed must contact Employee Health (432-7978) before working with these research materials.

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

H. Description of Experiment:

Please provide a description of the experiment including the points below.

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.

I. Personnel (names, netIDs, title, and telephone number):

Yale *Environmental Health & Safety*

Researcher Experience Form

Please send the completed form to:
Yale Environmental Health & Safety
Attn: Biosafety Office
135 College Street, Suite 100
New Haven, CT. 06510
OR
email-to ehs@yale.edu.

Today's Date:			
Name:			
Job Title:		NetID:	
Principal Investigator:			
Education			
Date	Institution	Major Area	Degree
Laboratory Experience			
<i>Please list your laboratory experience related to your work with microorganisms, cell culture and/or human pathogens. Provide the approximate date of your work, the institution where the work took place and a description of the work including the names of organisms you worked with. Continue on page 2, if necessary.</i>			
Approximate Date:		Institution:	
Description of work:			
Approximate Date:		Institution:	
Description of work:			

Laboratory Experience-Continued

Approximate Date:	Institution:
Description of work:	
Approximate Date:	Institution:
Description of work:	
Approximate Date:	Institution:
Description of work:	
Approximate Date:	Institution:
Description of work:	
Approximate Date:	Institution:
Description of work:	