Overview of Recombinant DNA Experiments Covered by
The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)
April 2024

The NIH Guidelines describe experiments that must be registered and approved by Institution's Biological Safety Committee and those experiments that are exempt from the Guidelines and do not require registration. For institutions that receive funding from the NIH for molecular biology research, the Guidelines become a condition of the receipt of funding. For Yale to continue to receive funding from the NIH, ALL researchers MUST COMPLY with the NIH Guidelines, regardless of funding source for molecular biology research. Persistent failure of an institution to comply with the NIH Guidelines can lead to suspension of research privileges. Systemic failure to comply with the Guidelines may result in the freezing of funds directed at an institution by the NIH.

The phrase rDNA is used throughout this document. rDNA refers to recombinant or synthetic nucleic acid molecules to reflect earlier changes in the March 2013 and November 2013 versions of the NIH Guidelines.

Link to current NIH Guidelines: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

CATEGORIES OF RDNA WORK THAT REQUIRE REGISTRATION:

1. ***Cloning a therapeutic antibiotic resistance gene into a human, animal or plant pathogen, if the transfer could compromise the ability to treat or control the disease. (Section III-A-1)***

Note: Registration with the institution's Biological Safety Committee is still required even if:

- this drug resistance is acquired naturally (the Committee will determine if it is a Major Action or not and communicate with the NIH Office of Science Policy (OSP) as needed);
- the transferred resistance gene is related to a drug that is an end of the line alternative treatment (2nd, 3rd, 4th, or 5th line drug) - the Committee will determine if the proposed research qualifies as a Major Action or not;
- the drug was used years ago, but is not the preferred treatment today (it may be the only treatment in developing countries);
- the drug is only used to treatment a very small portion of the population (i.e. those with specific contraindications to front line drugs)
- Working with antibiotic resistance strains of pathogens also require registration (even if you did not create them) – please submit a registration form to the Biological Safety Committee

Examples:

- Cloning a gene for Erythromycin resistance into Borrelia burgdorferi (may not qualify as a Major Action, but the Institutional Biosafety Committee must review prior to initiation)
- Cloning a gene for Chloramphenicol resistance into *Rickettsia typhi*
- Cloning a gene for Pyrimethamine resistance into *Toxoplasma gondii*
- Cloning a gene for Rifampin resistance into *Mycobacterium tuberculosis*

Caution:

- Be careful when using old plasmids for cloning experiments involving pathogens. Many of the old plasmids carry genes for antibiotics that have been used therapeutically or are related to front line drugs.
    - Avoid using these plasmids when working with related pathogens;
    - Verify that the antibiotic resistance gene is not in a location on the plasmid that can be transferred to the pathogen via a double cross over event.

Websites: NIH OSP FAQ – Major Actions
https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy/
https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy/major-actions-under-section-iii-a-of-the-nih-guidelines-for-research-involving-recombinant-or-synthetic-nucleic-acid-molecules-may-2019/

2. Cloning DNA encoding for a low LD50 toxin or work with vectors that express toxins with a low LD50 (< 100 ug/kg body weight). (Section III-B-1)

Examples of toxins with low LD50's are:

- Botulinum toxin
- *Staphylococcal enterotoxin* B
- Tetrodotoxin
- *Clostridium tetanus* toxin

Univ. of Oklahoma and Univ. of Tennessee Knoxville– Toxin LD50 Lists:
https://apps.ouhsc.edu/IBCinventory/toxinLD50.pdf
https://biosafety.utk.edu/biosafety-program/resources/biological-toxins-guidance/

3. Human Gene Transfer Experiments (Section III-C-1)

The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the NIH Guidelines. This includes the transfer of DNA with defective viral vectors, such as retroviral, adenoviral and lentiviral vectors, along with the use of liposomes and other methods of delivery.

Human gene transfer experiments with synthetic nucleic acid molecules also require registration if any of the following criteria are met: The synthetic nucleic acid molecules:

- Contain more than 100 nucleotides; or

- Possess biological properties that enable integration into the genome (e.g. cis elements involved integration); or
- Have the potential to replicate in a cell; or
- Can be translated or transcribed

These experiments also require approval from the institutional review board at the institution (Yale Human Investigation Committee) and the U.S. Food and Drug Administration.

Websites: Yale HGT experiments:
https://ehs.yale.edu/human-gene-transfer

4. ***rDNA Experiments involving the use of a human, animal or plant pathogen (whether the recombinant or synthetic nucleic acid molecules originated from your lab or another). (Section III-D-1, III-D-2, III-D-3)***

Examples:

- Cloning a gene into a pathogen (i.e. expressing a gene into VSV, Vaccinia Virus, Tobacco Mosaic Virus, Mouse Cytomegalovirus)
- Cloning a pathogen into a lower eukaryotic or prokaryotic cell;
- Using a defective pathogen vector with or without helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) in cell culture oranimal experiments, examples include:
    - Poxviruses (Vaccinia)
    - Herpesvirus vectors (HSV)
    - Lentivirus vectors (HIV, FIV based)
    - Retroviruses (murine retroviruses)
    - Adenoviruses
    - Adeno-Associated Virus vectors
    - Vesicular Stomatitis Virus vectors
    - Sindbis Virus vectors
    -

Tools and resources can also be found on Yale EHS's website for rDNA research:
https://ehs.yale.edu/recombinant-dna
A helpful guidance document developed by Stanford University for the classification of experiments involving defective viral vectors can be accessed at the following website:
https://ehs.stanford.edu/topic/biosafety-biosecurity/viral-vectors

Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.

5. ***Cloning DNA or RNA from Risk Group 3 or Risk Group 4 human pathogens, restricted animal or plant pathogens, or Select Agents. (Section III-D-2)***

Any rDNA experiments with these materials must be registered with and approved by the Yale IBC, even if you are working with only short segments of nucleic acid molecules

from these agents.

Websites: Please refer to Appendix B: Classification of Etiologic Agents on the Basis of Hazard for the Risk Groups assigned to the pathogen you intend to work with.

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf
http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

American Biological Safety Association Risk Group Classifications of Etiologic Agents:
https://absa.org/
https://my.absa.org/tiki-index.php?page=Riskgroups

Pathogen Safety Data Sheets from Health Canada are also available for some human pathogens and can be accessed here:
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

List of "Veterinary Pathogens of Significance" (can be found in Appendix D of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, 2009)

Biosafety in Microbiological and Biomedical Laboratories—6th Edition (cdc.gov)

Select Agent List:

Select Agents and Toxins List | Federal Select Agent Program

6. rDNA Experiments involving whole animals, plants, and arthropods (and insects) Section III-D-4, III-D-5, III-E-3

Experiments in this category include:

- Experiments involving toxins, pathogens, defective vectors, and other genetically modified materials used in animal, plants or insects.
- Creation of transgenic animals
    i. Mice, rats
    ii. Zebrafish
    iii. Drosophila, butterflies
    iv. Caenorhabditis elegans
    v. Other

Note: For rodents only, the purchase or transfer of transgenic rodents is exempt from the NIH rDNA Guidelines and does not require registration (if the transgene used does not code for a toxic, virulent or oncogenic sequence).

Purchase is defined as buying a transgenic rodent that has been created by another entity outside of your laboratory.

The transfer of a transgenic rodent to your laboratory is also exempt (provided the

transgene doesn't code for toxic, oncogenic or potentially harmful gene).

Transfer is defined as the acquisition into your research lab of a transgenic animal created (made) by another entity.

Note: In each case above, you may have designed or created the gene that has been inserted into the developing embryo of the transgenic rodent, but if you are not the group that has performed the actual procedure (i.e. the lab that inserted the gene into the embryo), you are exempt from the rDNA Guidelines. **If your lab will insert the gene into the embryo, you must register this work.**

Knock-out Animals
Knock-out (gene silencing, gene ablation, etc.) rodents are exempt from the NIH Guidelines as long as the method to generate the knock-out animal does not leave any "new" genetic material behind in the genome after the procedure. If DNA from the molecule used to create the knock-out is permanently inserted into the genome, the experiment will require registration with the Yale Biological Safety Committee.

Exemption for Breeding Transgenic Rodents
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.

- Both parental rodents can be housed under BSL1 containment; AND
- 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
- 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.

This exemption DOES NOT pertain to other transgenic animals such as zebrafish, drosophila, rabbits, pigs, etc. It also DOES NOT pertain to transgenic experiments involving plants.

7. Large Scale rDNA Experiments (Section III-D-6)

Any rDNA experiments at any level or Risk Group, including exempt and non-exempt experiments that generate a volume of culture that is in excess of 10 liters requires registration with the Yale Biological Safety Committee.

Examples include: Growing 10.1 L of Sacchryomyces cerevisiae in a fermentation apparatus to get a sufficient yield of the desired protein.
Growing up five 2 L flasks of E. coli K-12 cultures expressing your gene of interest would not qualify as a large scale experiment under the NIH Guidelines, but given the

high volume, please register this volume of work with the Institutional Biological Safety Committee in order for an appropriate risk assessment of the proposed work.

8. Experiments Involving Influenza Viruses (III-D-7)

Experiments with influenza viruses generated by recombinant or synthetic methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the Risk Group of the virus that was the source of the majority of segments in the recombinant or synthetic virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3).

Examples:

Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1), including, but not limited to, strains of HPAI H5N1 virus that are transmissible among mammals by respiratory droplets, as demonstrated in an appropriate animal model or clinically in humans (hereinafter referred to as mammalian-transmissible HPAI H5N1 virus), shall be conducted at BL3 enhanced containment (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses) unless indicated below.

Section III-D-7-a. Human H2N2 (1957-1968). Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BL3 enhanced (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant or synthetic virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

Section III-D-7-b. Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1). Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced containment, (see Appendix G-II-C-5, Biosafety

Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses).

Section III-D-7-c. 1918 H1N1. Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BL3 enhanced containment (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses).

9. Experiments Involving Gene Drive Modified Organisms (III-D-8)

Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of Biosafety Level (BL) 2, BL2-N (Animals) or BL2-P (plant) containment.

Examples

Suppression drives force deleterious traits into a population, leading those populations to crash or be much diminished. If the mosquito is eliminated, so too will all the diseases it can transmit.

Population modification or replacement, leaves the insect in place in the environment but blocks disease transmission by modifying the insect vector to prevent pathogen transmission.

April 2024 Changes to the NIH Guidelines
Gene Drive Modified Organisms

NIH has amended the NIH Guidelines to ensure the continued responsible research involving Gene Drive Modified Organisms (GDMOs) in contained research settings. The changes take effect at the end of September 2024.

Specifically, the NIH Guidelines will be amended to:

1. Clarify minimum containment requirements for research involving Gene Drive Modified Organisms (GDMOs);
2. Provide considerations for risk assessment;
3. Define additional institutional responsibilities for IBCs and BSOs.

In addition to the amendments related to contained research involving GDMOs, the NIH Guidelines will also be amended to:

4.Replace the term ‘‘helper viruses’’ with the broader term ‘‘helper systems’’;

‘‘The potential for reversion or generation of replication competent virus should be considered when generating or using defective viruses or vectors in the presence of helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems).’’

And

5.. Reclassify West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) as risk group 2 agents for consistency with containment guidance provided in the BMBL.

Gene Drive Modified Organisms (GDMO’s)

Definition of a Gene Drive:
Section I-E-7. “Gene drive” is defined as a technology whereby a particular heritable element biases inheritance in its favor, resulting in the heritable element becoming more prevalent than predicted by Mendelian laws of inheritance in a population over successive generations.

Field Release or field work with GDMO’s not allowed:
NIH does not currently support field release of GDMOs and the NIH Guidelines pertain to contained research. All research involving GDMOs must be conducted within a research, plant or animal research laboratory after authorization from the Yale Biological Safety Committee.

GDMO Research Must be Registered with and Approved by the Yale Biological Safety Committee

GDMO research is NOT EXEMPTED from the NIH GUIDELINES:
Research subject to the NIH Guidelines, including research with GDMOs, requires review and approval by an IBC that is registered with the NIH Office of Science Policy prior to initiation.

The following categories are not exempt from the NIH Guidelines:

(i) experiments described in Section III-B, which require NIH OSP and Institutional Biosafety Committee approval before initiation;

(ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval;

(iii) large-scale experiments (e.g., more than 10 liters of culture),

(iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates), and

(v) experiments involving gene drive modified organisms (Section III-D-8).

This means that any GDMO research in E. coli K12, saccharomyces cerevisiae, B. subtilis, purchased transgenic rodents, or other exempt research must be registered and approved by the Yale Biological Safety Committee prior to initiation.

GDMO Research Requires a Minimum of BSL-2 Containment:
Research involving GDMOs must be conducted at a minimum containment level of BSL-2, BL2-N (Animal BSL-2), or BL2-P (Plant BSL-2). Based on the risk assessment of a specific research protocol, the IBC may require enhancements or a higher level of containment. Review and approval from NIH OSP are required to lower containment below the minimum specified in the NIH Guidelines.

> **Section III-D-8.** Experiments Involving Gene Drive Modified Organisms
> Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of Biosafety Level (BL) 2, BL2-N (Animals) or BL2-P (plant) containment.

When the institution conducts research involving GDMOs, the institution must ensure that the IBC has adequate expertise (e.g., specific species containment, ecological or environmental risk assessment) using ad hoc consultants if necessary. (The Yale Biological Safety Committee has added consultants to our Committee to assist with the ecological risk assessment of GDMO in the event of an accidental release from the laboratory).

In addition, when such research is being conducted, a Biological Safety Officer (BSO) shall be appointed to the IBC. Yale University's Biosafety Officer is a representative of the Yale Biological Safety Committee.

Research involving gene drive modified organisms may require risk assessments that incorporate a broader scope of considerations because of greater uncertainty of the technology and potential uncertainty of the impact of the newly modified organism.
Specific attention must be paid to risks of an unintended release from the laboratory and the potential impact on humans, other populations of organisms, and the environment.

Considerations for conducting risk assessments for research involving gene drive modified organisms might include:

1) The specific types of manipulations based on:

   a) Function or intended function of the genetic/gene drive construct (i.e., a designed or engineered assembly of sequences);

   b) Source of the genetic material (e.g., sequences of transgenes) in the construct;

   c) The modifications to the construct;

   d) Whether it is possible to predict the consequences of a construct, including the recognition of an unintended gene drive (i.e., construct not specifically designed as a gene drive but nonetheless having properties of a gene drive) and the possible consequences of escape into the environment;

   e) The potential ability of the gene drive to spread or persist in local populations;

2) Options for approaches to risk mitigation for specific types of risks in experiments or when dealing with a high degree of uncertainty about risks;

3) Considerations for implementing more stringent containment measures until biosafety data are accrued to support lowering containment.

The conduct of risk assessments for research involving GDMOs presents challenges in addition to those associated with other genetically modified organisms (GMOs) or vectors because the preferentially inherited traits of GDMOs spread and persist in the environment, are intended to modify natural populations, and may have associated impacts on the environment and society.

The potentially broad and long-lasting impacts of the use of this technology on humans, other populations of organisms, and the environment are not seen with research involving clinical research participant cohorts or even with other GMOs

not designed to survive outside of laboratory containment. As such, research involving GDMOs requires risk assessments that incorporate a broader scope of issues because of the greater uncertainty in terms of risks in the event of an unintended release from the laboratory.

April 2019 Changes to the NIH Guidelines

Synthetic Nucleic Acid Experiments that are covered by the Guidelines:

- Research that presents biosafety risks equivalent to rDNA research that is subject to the NIH Guidelines such as research with a genetically modified virus or a vector derived solely by synthetic techniques.
    - The molecules can replicate
    - They can generate nucleic acids that can replicate in a living cell
    - They can integrate into a host cell's DNA
    - They produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms/kilogram body weight
    - They synthesize an organism that doesn't occur naturally outside of a laboratory setting (i.e. 1918 H1N1 Influenza)

Synthetic Nucleic Acid Experiments that are EXEMPT from the Guidelines:

- Introduction of certain synthetic nucleic acids into a biological system that is not expected to present a biosafety risk that requires review by the IBC
- Introduction of synthetic nucleic acid molecules into biological systems akin to processes of nucleic acid transfer that already occur in nature.
- Experiments with synthetic nucleic acid molecules that are not contained in cells, organisms or viruses
- Those synthetic nucleic acid molecules that meet the following criteria shall be exempt:
    1) Those that can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g. oligonucleotides or other synthetic that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and
    2) Those that are not designed to integrate into DNA, and
    3) Those that do not produce a toxin that is lethal for vertebrates at and LD50 of less than 100 nanograms per kilogram body weight.

  An example of an exempt synthetic nucleic acid molecule is a synthetic short-interfering RNA (siRNA) that targets an HIV viral protein required for transcription activation, even if this siRNA is injected into animals or used in cell culture.

- Also exempt are those synthetic nucleic acid molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists outside of a laboratory setting. (Research with nucleic acid sequences for organisms that do not currently exist in nature outside of the laboratory setting would not be exempt (e.g. an identical copy of the 1918 H1N1 influenza virus)
- The chemical synthesis of nucleic acids (the NIH Guidelines only apply once synthetic nucleic acids are placed in a biological system).