Registration and Approval of rDNA Experiments
RECOMBINANT and SYNTHETIC NUCLEIC ACIDS

YALE BIOLOGICAL SAFETY COMMITTEE **April 2024 (rev.)**

This outline provides an overview of the "Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules" (NIH Guidelines). It is the responsibility of each investigator to make sure that their laboratory is in compliance with these Guidelines. If your experiments require registration, check the NIH Guidelines for the relevant regulatory section and the appropriate biosafety level or contact the Biosafety Office or your Safety Advisor for assistance. For copies of the NIH Guidelines or rDNA registration forms, please call Environmental Health & Safety (EHS) at 785-3550.

OEHS contacts: Phone: (203) 785-3550 Fax: (203) 785-7588 Website: https://ehs.yale.edu/Yale rDNA Forms and Information Regarding rDNA: https://ehs.yale.edu/recombinant-dna

NIH Office of Science Policy website: https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/

Experiments which must be registered and approved prior to initiation:	**Examples:**
1. Deliberate transfer of a drug resistance trait to a microorganism (if it could compromise the use of the drug to control disease agents in human, animals, or agriculture);	1. Transferring a drug resistance trait that is used, had previously been used, may be used (outside the U.S.), or that is related to other drugs that are used to treat or control disease agents. Examples include: Transfer of Erythromycin resistance into Borrelia burgdorferi; Transfer of Pyrimethamine resistance into Toxoplasma gondii; Transfer of Chloramphenicol resistance into Rickettsia conorii; Transfer of Tetracycline resistance into Porphyromonas gingivalis.
2. Human gene transfer experiments;	2. Use of a defective adenoviral vector to deliver the CFTR gene intranasally to patients with Cystic Fibrosis; Introduction of a HSV-TK transduced cell line into patients with epithelial ovarian carcinoma, followed by therapy with Gancyclovir.
3. Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of < 100 ug/kg body weight;	3. Cloning toxins (or using plasmids that express toxins with low LD50's) such as Botulinum, Tetrodotoxin, Ricin, T-2, Saxitoxin, Abrin, Tetanus, Shigella Dysenteriae, Pertussis, Staph Aureus Beta, Shiga Toxin, and Conotoxins;
4. Experiments using human or animal pathogens as host-vector systems;	4. Use of pathogens or defective pathogen vectors (with or without helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems), such as Adenovirus, Adeno-Associated virus, Baculovirus, Herpesvirus, Lentivirus, Retrovirus, Vaccinia and Vesicular Stomatitis Virus.
5. Cloning of DNA or RNA from all Risk Group 3, 4, or restricted pathogens (includes HIV and human tumor viruses), as well as Risk Group 2 experiments involving ≥ 50 % of genetic material;	5. rDNA experiments involving any quantity of genetic material from a Risk Group 3 or higher pathogens (e.g., HIV, HTLV-1 & II, Prions, Mycobacterium tuberculosis, West Nile Virus, Lymphocytic Choriomeningitis Virus, and Rickettsia typhi. Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.
6. Recombinant DNA experiments involving whole animals or plants:	6. Creation of transgenic animals or plants (mice, rats, zebra fish, drosophila, C. elegans etc.), or knockout animals that leave genetic material in the animal as part of the silencing of the gene. Note: the purchase (or transfer to your lab) of previously created transgenic rodents is exempt from the regulations.
7. Large-scale DNA work (i.e. ≥ 10 liters of culture combined).	7. Use of a 10 L fermenter or growing up five 2 L flasks of rDNA culture (i.e. E. coli K-12) qualifies as a large-scale experiment at Yale University.
8. Gene Drive Modified Organisms research	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.

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.Rreplace 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.

NIH Guidelines Definitions and Information on Recombinant or Synthetic Nucleic Acids

April 2019 Update

Section I-B. Definition of Recombinant and Synthetic Nucleic Acid Molecules
In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:
(i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;
(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
(iii) molecules that result from the replication of those described in (i) or (ii) above.

Section III-C-1. Human gene transfer is the deliberate transfer into human research participants of either:
1.Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acidmolecules, or
2.Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acidmolecules, 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.

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. Research involving synthetic nucleic acid molecules will require registration if:
    - 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)
- Human gene transfer experiments or clinical protocols with synthetic nucleic acid molecules if any of the following criteria are met - the synthetic nucleic acid molecules:
    - Contains more than 100 nucleotides; or
    - Possess biological properties that enable integration into the genome (e.g. cis elements involved in integration); or
    - Have the potential to replicate in a cell; or
    - Can be translated or transcribed.

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.