Registration and Approval of rDNA Experiments
RECOMBINANT and SYNTHETIC NUCLEIC ACIDS

YALE BIOLOGICAL SAFETY COMMITTEE **April 2019 (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: 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 virus), such as Adenovirus, Adeno-Associated virus, Baculovirus, Herpes virus, 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.

NIH Guidelines Definitions and Information on Recombinant or Synthetic Nucleic Acids

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.