

Overview of Experiments Covered by the NIH Guidelines for Research Involving Recombinant DNA Molecules

The NIH rDNA Guidelines describe experiments that must be registered, reviewed, and approved by Yale University's Institutional Biological Safety Committee (the Yale Biological Safety Committee) prior to initiation, as well as those experiments that are exempt from the Guidelines and therefore do not require registration. For institutions that receive funding from the NIH for molecular biology research, the Guidelines become a condition of funding. In order to continue to receive funding from the NIH, all researchers at Yale University must comply with these Guidelines, regardless of funding source. Failure 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 to Yale by the NIH.

CATEGORIES OF WORK THAT REQUIRE REGISTRATION & APPROVAL:

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 is still required even if:

- this drug resistance is acquired naturally;
- 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 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);
- you did not create the antibiotic resistant strain.

Examples:

- Cloning a gene for Erythromycin resistance into *Borrelia burgdorferi*;
- 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:

- Use caution with older plasmids for cloning experiments involving pathogens. Many older plasmids were developed with resistance (marker) 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 OBA FAQ – Major Actions

http://www4.od.nih.gov/oba/IBC/MAJOR_ACTION_FAQS_MARCH_2008.pdf

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 include:

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

Websites: Yale OEHS Table of Toxins:

<http://www.yale.edu/ehs/Documents/Bio/biotoxinexperiments.pdf>

Univ. of Florida – Toxin Lists: <http://www.ehs.ufl.edu/Bio/toxin.htm>

3. Human gene transfer experiments (Section III-C-1)

The deliberate transfer of rDNA, 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.

These experiments require registration with the NIH Office of Biotechnology Activities and also pre-approval from the Yale IRB or Human Investigations Committee and the U.S. Food and Drug Administration.

Websites: Yale HGT experiments:

<http://www.yale.edu/ehs/Documents/Bio/humangenetransfer.pdf>

NIH OBA Frequently Asked Questions on Human Gene Transfer Experiments:

http://www4.od.nih.gov/oba/RAC/RAC_FAQs.htm

4. rDNA experiments involving the use of a human, animal, or plant pathogen (whether the recombinant 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 virus in cell culture or animal 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

A helpful guidance document developed by Stanford University for the classification of experiments involving defective viral vectors can be accessed at the following website:
http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

Note that rDNA experiments involving ≥ 50 % of genetic material from a Risk Group 2 organism 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 one base pair of DNA or RNA from these agents.

Websites: NIH Appendix B (Risk Groups)

http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm#_Appendix_B-I_Risk

American Biological Safety Association Risk Group Classifications of Etiologic Agents:
<http://www.absa.org/riskgroups/index.html>

List of restricted animal pathogens.

<http://www.cdc.gov/od/ohs/biosfty/bmb15/sections/AppendixD.pdf>

Select Agent List:

<http://www.cdc.gov/od/sap/docs/salist.pdf>

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, an other genetically modified materials used in animal, plants or insects.
- Creation of transgenic animals:
 - i. Mice, rats
 - ii. Zebrafish
 - iii. Drosophila, butterflies
 - iv. 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.**

Special Note on 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, review, and approval by the Yale Biological Safety Committee.

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 in excess of 10 liters requires registration with the Yale Biological Safety Committee.

Note: The 10 L limit is the aggregate volume of cell culture at any one time, in either a single vessel or a series of smaller vessels whose aggregate volume exceeds 10 L.