

Duke University Recombinant DNA FAQs (Revised January 2021)

Introduction: Regardless of funding source, all research at Duke University using recombinant DNA (rDNA) materials must comply with the NIH Guidelines for rDNA Research (available at <https://osp.od.nih.gov/biotechnology/nih-guidelines-faqs/>). Each investigator working with rDNA is responsible for understanding and following the NIH guidelines. Please contact the Duke Occupational and Environmental Safety Office (OESO) at 919-684-8822 for questions about rDNA compliance and safety.

What are rDNA materials?

Recombinant DNA (rDNA) was originally created by cutting DNA segments with restriction enzymes, then re-combining them in a ligation reaction. Today, rDNA is commonly generated by PCR, *de novo* synthesis, and other methods. The NIH guidelines cover both rDNA and RNA that is derived from rDNA. The Duke IBC includes the following as rDNA materials:

- Plasmids and viral vectors
- Any synthetic DNA or RNA
- Any RNA produced from rDNA, including messenger RNA (mRNA), small interfering RNA (siRNA), micro RNA (miRNA), etc.
- Genetically-modified organisms (animals, plants, bacteria, viruses, fungi, etc.)

All non-exempt research with rDNA materials must be registered with the Institutional Biosafety Committee (IBC) before it is initiated. **This includes work with rDNA materials obtained from other scientists or from commercial sources.**

What is a genetically-modified organism (GMO)?

A genetically-modified organism (GMO) is any organism whose genetic material has been altered using rDNA technology. Note that descendants of GMOs are also considered GMOs. The term GMO includes any form of life (vertebrate or invertebrate animal, plant, bacteria, fungus, virus, etc.). Common examples of GMOs include transgenic mice, knockout mice, and bacteria used to make recombinant proteins.

What is exempt and non-exempt rDNA research?

The NIH Guidelines describe several categories of research as "exempt" from the NIH Guidelines. The annual Faculty rDNA Compliance Screen is intended to help researchers to correctly identify common examples of exempt and non-exempt rDNA research.

Note that BOTH exempt and non-exempt rDNA materials are subject to reporting requirements in the event of loss, theft, release, and human exposure. Please contact Occupational and Environmental Safety Office (OESO, phone 684-8822) to report rDNA events.

What is an *E. coli* K12 derivative?

Early rDNA research was carried out in the K12 strain of *E. coli*. Most popular *E. coli* strains using in rDNA research are derived from this strain. This can be confirmed by

contacting the commercial source. Some rDNA research in *E. coli* K12 strains is exempt. All research using other *E. coli* isolates is non-exempt.

What is a viral vector?

The Duke IBC defines a viral vector as any rDNA molecule or molecules used to deliver nucleic acids to cells using viral proteins or their equivalents for cell entry. Examples of viral vectors include replication-deficient forms of adenoviruses and retroviruses. Typically one or more plasmids are transfected into cells. The plasmids express viral proteins in the cells. These cells produce replication-deficient viral particles containing both nucleic acids and viral proteins. The replication-deficient viral particles are used to efficiently deliver the nucleic acids to other cells either *in vitro* or *in vivo*. Replication-competent viruses are sometimes used as hosts for recombinant DNA research.

What experiments involving toxin molecules are subject to the NIH Guidelines?

The NIH Guidelines apply to certain experiments involving the deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules. All experiments subject to the NIH Guidelines that involve toxin molecules must be reviewed and approved by an Institutional Biosafety Committee (IBC) prior to initiation. In addition, certain experiments involving genes coding for functional recombinant or synthetic nucleic acid toxin molecules must be registered with the NIH Office of Science Policy (OSP), and in some instances approved by NIH prior to initiation. Find more information at <https://osp.od.nih.gov/biotechnology/faqs-on-toxin-experiments/>

What is a Risk Group 3 or 4 microbe?

Microbes are characterized into risk groups based on their routes of transmission, and their potential to cause disease in humans. Examples of Risk Group 3 microbes include *Mycobacterium tuberculosis*, *Bacillus anthracis*, *Yersinia pestis*, and monkeypox. Examples of Risk Group 4 microbes include Ebola virus and smallpox. These microbes may also be subject to Select Agent registration and other regulations. The NIH Guidelines assign risk groups to some microbes. Microbes not listed in the Guidelines must undergo a formal assessment to determine their risk group. Contact OESO (phone 919-684-8822) for help with a risk assessment and regulatory compliance.

REGISTRATION PROCESS

How do I register with the IBC?

Forms and registration instructions are available at <https://www.safety.duke.edu/biological-safety/institutional-biosafety-committee-ibc>, or can be obtained from OESO (phone 919-684-8822).

How much work should be included on a single rDNA registration?

Each rDNA registration requires a descriptive title, and should cover a single area of research. Researchers who conduct rDNA research in multiple areas should have multiple registrations.

Each rDNA registration has a single PI who takes responsibility for assuring compliance for all work on the registration, and for assuring that all workers in all locations are appropriately trained to conduct the research.

Each rDNA registration must list every location (laboratory, animal housing location, core facility, clinical trial site, etc.) where rDNA will be manipulated under the registration. Existing protocols can be amended to add new locations. The procedures for safe work with rDNA, as described in the IBC registration, must be followed by everyone listed on the protocol in all locations. OESO will confirm that each location is appropriate for the rDNA research described, and will perform a lab evaluation if none has been completed in the past year.

I am using rDNA in collaboration with another faculty member. Do I have to register separately, or can I use the other faculty member's approved IBC registration?

A single registration can cover collaborative research in more than one lab if ALL the following conditions are met. (1) The collaborative research is described in the registration. (2) All locations and faculty members are listed on the registration. (3) All work is conducted in compliance with the IBC registration including any Standard Operating Procedures (SOPs). (4) The PI of the registration assumes responsibility for assuring that all work in all labs is compliant with the registration, and for assuring that all workers in all locations are appropriately trained to conduct the research.

Existing protocols can be amended to describe collaborative research and to add additional faculty members.