Recombinant DNA FAQs

Introduction: Regardless of funding source, all recombinant DNA (rDNA) research at George Washington University must be reviewed and approved by the Institutional Biosafety Committee (IBC) and comply with the NIH Guidelines for Recombinant DNA Research (available at <http://oba.od.nih.gov/rdna/nih_guidelines_oba.html>. Each investigator working with rDNA is responsible for understanding and following the NIH guidelines. Please contact the Office of Laboratory Safety (OLS) at 202-994-8258 for questions related to rDNA safety and compliance.

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, or other methods. The NIH guidelines cover both rDNA and RNA that is derived from rDNA. The GWU 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 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 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 bacteria used to make recombinant proteins, transgenic mice, and knockout mice.

What is exempt rDNA research?

The NIH Guidelines describe several categories of research as "exempt" from the NIH Guidelines. Examples of exempt activities are: The use of only commercially available deregulated transgenic crops, activities that involve only the in vitro use of nucleic acids (i.e., PCR, synthetic double stranded RNA) and does not involve the cloning and propagation of rDNA in cells. If your work is exempt from the NIH guidelines but does not fall under the previous examples categories it is still subject to IBC review. This includes exempt r/sNA activities under Section III-F of the NIH guidelines which must be reviewed and approved by the IBC.
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 OLS (202-994-8258 or labsafety@gwu.edu) 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* host strains used 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. Research in any E. coli isolate not derived form K12 is NOT exempt.

What is a viral vector?

The GWU 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 is a toxin?

The NIH Guidelines define toxins based on a median lethal dose (LD50) of <100 micrograms per kilogram body weight. This research always requires IBC approval before initiation. It may also require review by the NIH or other regulations. Consult the NIH Guidelines for more details.

What is a Risk Group 3 or 4 pathogen?

Pathogens are characterized into risk groups based on their potential to cause disease in humans and on their routes of transmission. Examples of Risk Group 3 pathogens include *Mycobacterium tuberculosis, Bacillus anthracis, Yersinia pestis*, and monkeypox. Examples of Risk Group 4 pathogens include Ebola virus and smallpox. Some Risk Group 3 and 4 microbes are also to Select Agent registration and other regulations.

The NIH Guidelines assign risk groups to some pathogens. Pathogens not listed in the Guidelines must undergo a formal assessment to determine their risk group. Contact OLS to assist with risk assessment and regulatory compliance.

REGISTRATION PROCESS

How do I register with the IBC?

All IBC registrations must be made electronically via GW iRIS. Forms and instructions are available on the web at labsafety.gwu.edu

How much can be covered on a single rDNA registration?

Each IBC registration requires a description of the work and should cover a single area of research. Researchers who conduct research in multiple areas should have multiple registrations.

Each IBC 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.

Every IBC 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. OLS will confirm that each location is appropriate for the rDNA research described, and will perform a lab audit 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 just 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 work 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 the Standard Operating Procedures (SOP). (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.