11.0 RECOMBINANT DNA/RNA

11.1 Definition

Recombinant DNA molecules are either:

- molecules constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or
- molecules that result from the replication described above

11.2 Research Approval Categories

rDNA research may be approved in one of two categories at UTMB:

- experiments which require specific prior approval by the National Institutes of Health (NIH) and the UTMB Institutional BioSafety Committee
- experiments which require prior approval of only the UTMB Institutional BioSafety Committee

See the Biological Safety Policy in this chapter for notification and approval procedures.
Note: The list of recombinant DNA molecules along with the reporting and containment requirements is revised periodically by the Director of the National Institutes of Health. Contact Environmental Health and Safety, Biological and Chemical Safety for current guidelines for research involving recombinant DNA molecules at extension 21781.

11.3 NIH Recombinant Guidelines

- Institutions receiving NIH funding must follow the *NIH guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).*


11.4 Recombinant DNA

- Recombinant DNA can be:
  - DNA constructed *in-vitro* from separate DNA segments that can replicate and/or express a biologically active polynucleotide or polynucleotide *in vivo*
  - Synthetic DNA that has the potential of generating a hazardous product *in vivo*

11.5 What are OBA and RAC?

- NIH OBA (Office of Biotechnology Activities) is an administrative arm responsible for carrying out the orders of the NIH Director with regard with recombinant DNA, genetic testing and xenotransplantation.
- An advisory committee is involved in establishing policies for each of these fields. An called the Recombinant DNA Advisory Committee or fields. For recombinant DNA the committee is called the Recombinant DNA Advisory Committee or “RAC”.

11.6 Roles and Responsibilities

**Principal Investigator (PI)**

- The PI is responsible for registering rDNA work with the Institutional Biosafety Committee (IBC)
- The PI is responsible for ensuring that all their staff are trained and follow all National, Federal, and Local regulations, including but not limited to the NIH Guidelines and the CDC-Select Agent Program.
- The PI is responsible for ensuring proper training of their staff in proper laboratory techniques and procedures related to the work perform.
- The PI is responsible for immediately reporting all incidents and accidents to an Environmental Health and Safety, Biological and Chemical Safety Program, Biosafety Officer

**Laboratory Personnel (LP)**

- The LP is responsible for following all National, Federal, and Local regulations, including but not limited to the NIH Guidelines and the Select Agent Program.
- The LP is responsible for following proper laboratory techniques and procedures related to the work perform.
• The LP is responsible for immediately reporting all incidents and accidents to their PI and/or an Environment Health and Safety, Biological and Chemical Safety Program, Biosafety Officer

Institutional Biosafety Committee (IBC)
• The IBC is responsible for reviewing all Notification of Use for Biological Agent and regulated Recombinant DNA/RNA work performed on campus.
• The IBC is responsible for ensuring that incidents and accidents that need reporting to the appropriate agency (including but not limited to the NIH-OBA and CDC-Select Agent Program) has occurred within the required time frame.

Environmental Health and Safety (EHS)
• EHS Biological and Chemical Safety Program (EHS-B&C) is responsible for auditing all research and clinical laboratories on campus to ensure they meet all the requirements set forth by the Federal, State, and Local agencies.
• EHS-B&C is responsible for assisting the IBC in reviewing all Notification of Use for Biological Agent and regulated Recombinant DNA/RNA work performed on campus.
• EHS-B&C is responsible for ensuring that incidents and accidents needing reporting to the appropriate agency (including but not limited to the NIH-OBA and CDC-Select Agent Program) occurs within the required time frame.
• EHS-B&C is responsible for informing the IBC of reports made to regulatory agencies.

11.7 The 5 NIH Categories
NIH has classified recombinant work into 5 categories, 4 of which require IBC approval or review at a minimum.

• Cat. A Needing NIH, IBC and RAC approval (major actions)
• Cat. B Needing NIH/OBA and IBC approval
• Cat. C Needing IBC-RAC-IRB approval
• Cat. D Needing IBC approval
• Cat. E Needs IBC notification before starting
• Cat. F Exemption- no IBC review needed

Cat. A Needs NIH, IBC and RAC approval (major actions)
• Drug resistance transfer into organisms not known to acquire it naturally

Cat. B Needs NIH/OBA and IBC approval
• Cloning of toxin molecules with LD50 < 100 ng/kg body weight

Cat. C Needs IBC-RAC-IRB approval
• Transfer of rDNA-DNA-RNA derived from rDNA into human

Cat. D Needs IBC approval

Category D is further subclassed into 4 categories
• Category D1
  o Experiment using Risk group 2-3-4 or Restricted Agents as host-vector systems
    a) introduction of rDNA into BSL2 vectors => worked at BSL2/ABSL2
b) introduction of rDNA into BSL3 vectors => worked at BSL3/ABSL3

c) introduction of rDNA into BSL4 vectors => worked at BSL4/ABSL4

d) introduction of rDNA into Restricted Agents is reviewed on a case by case by NIH/OBA. Agriculture permit needed for work with plant or animal pathogen.

- Category D2
  - DNA from risk group 2-3-4 or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host-vector system
    a) DNA from risk group 2-3 => worked at BSL2
       DNA from risk group 4 => worked at BSL2 after proof of defective irreversible fragment present.
       If not => worked at BSL4
       IBC may lower to BSL1 work. Many are NIH exempt
    b) DNA from SA transferred => NIH/OBA case by case review Agricultural permit needed

- Category D3
  - Use of infectious DNA-RNA virus or defective DNA/RNA virus in presence of helper virus in tissue culture
    - Be aware that this may enhance path, or change host range
      Risk group 2 agents => BSL2
      Risk group 3 agents => BSL3
      Risk group 4 agents => BSL4
      if not Risk group 2-3-4 agents => BSL1

- Category D4
  - Experiment involving whole animals
    - transgenic animal and animal testing => BSL2

Cat. E  Needs IBC notification before starting
  - Experiments with <2/3 viral genome and host cells are lacking the helper virus from defective virus
    - Creation of transgenic animal

Cat. F  Exemption- no IBC review needed
  - not in organism or viruses
  - DNA sequence from a single non-chromosomal or viral DNA slice
  - Eukaryotic host expressed in same host
  - Prokaryotic host - expressed in that host
  - natural recombination possible
  - no risk to environment

11.8 IBC Notification
- Before any work can be started, the PI needs to ensure that the proposed work meets NIH Guidelines.
• PI must then fill out the Notification of Use for Biological Agents and rDNA (NOW) describing the type of work proposed and providing a comprehensive risk assessment and specific information about the work conducted.
• The NOU must be forwarded to EHS, Route 1111, to be placed on the IBC agenda.

11.9 Information Needed
• Information needing to be submitted
  o source of DNA
  o nature of inserted DNA sequence
  o host and vector
  o protein products
  o Biosafety level
• NIH category in which research will fall
• Risk assessment on the work proposed
• Addressing the “Fink tank” report and Dual Use

11.10 Fink Committee Report
• The following questions must be answered
  – Would this research demonstrate how to render a vaccine (if applicable) ineffective?
  – Would this research confer resistance to therapeutically useful antibiotics and antiviral agents?
  – Would this research increase transmissibility of this pathogen?
  – Would this research alter the host range of this pathogen?
  – Would this research enable the evasion of diagnostic/detection modalities of this agent?
  – Would this research enable the weaponization of a biological agent or toxin?

11.11 Risk Assessment
  – Describe pathogenicity, including disease incidence and severity.
  – Describe route of transmission.
  – Describe agent stability.
  – What is the infectious dose?
  – What is the concentration (number of infectious organism per unit volume) and the volume of the concentrated material being handled?
  – What is the origin of the infectious material (may refer to geographic location, host or nature of source).
  – What is the availability of data from animal studies (pathogenicity, infectivity and route of transmission in animal)?
  – Is there an effective prophylaxis or therapeutic intervention available? Specify prophylaxis or therapeutic intervention.

11.12 Additional Information
  – Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
  – Does the inserted gene encode a known oncogene?
  – Does the viral DNA integrate into the host genome?
– Does the modification have the potential to increase the replication capacity of virus?
– Does the modification increase the pathogenicity of the agent?
– Does the inserted gene have the potential for altering the cell cycle?
– Does the modification change the host range of the agent?
– Use of infectious DNA / RNA?
– Use of defective DNA / RNA with Helper virus?
– Is there a probability of generating replication-competent viruses?
– Will the infectious DNA / RNA be used in tissue culture?
– Will the infectious DNA/RNA be use in whole animals?
– Will the infectious DNA / RNA be used in whole plants?

11.13 Dual Use

– Dual use applies to any development or use of research material: biological, chemical, equipment or intellectual that could be used for both the advancement of science and knowledge and the harm of others.