A Summarized Resource of *NIH Guidelines for Research Involving Recombinant DNA Molecules*

Compliance with the *NIH Guidelines for Research Involving Recombinant DNA* (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines) is mandatory for every institution that receives NIH funding for research involving recombinant DNA (rDNA) or synthetic nucleic acids. It is the responsibility of each investigator to make sure that his/her laboratory is in compliance. If your experiments require registration, go to our online protocol submission site: BIOSAFETYNET to register. This outline is intended only to serve as a summarized resource to the *NIH Guidelines*. If you are unsure in which category your experiments fall, please contact us at biosafety@usf.edu or via telephone at 974-0954

CATEGORIES OF RDNA WORK THAT REQUIRE REGISTRATION

Section III-A & B & C – Experiments that require registration and NIHOBA and USF IBC approval Prior to initiation:

1. **III-A-1-** Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease. (Section III-A-1-a).
   Examples:
   - Cloning a gene for Erythromycin resistance into Borrelia burgdorferi (may not qualify as a Major Action, but the Institutional Biosafety Committee must review prior to initiation)
   - 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
2. **III-B-1** Cloning of toxin molecules with LD_{50} of less than 100 ng / kilogram body weight.
   Examples of toxins with low LD50's are:
   - Botulinum toxin
   - Staphylococcal enterotoxin B
   - Tetrodotoxin
   - Clostridium tetanus toxin
3. **III-C-1** Experiments that involve the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participant.
   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.

   Human gene transfer experiments with synthetic nucleic acid molecules also require registration if any of the following criteria are met: The synthetic nucleic acid molecules:
   - Contain more than 100 nucleotides; or
   - Possess biological properties that enable integration into the genome (e.g. cis elements involved integration); or
   - Have the potential to replicate in a cell; or
   - Can be translated or transcribed

   Note: These experiments also require approval from the institutional review board (IRB) and the U.S. Food and Drug Administration.

Section III-D - Experiments that require registration & USF IBC approval Prior to initiation:

1. **III-D-1** Experiments using Risk Group 2, 3, or 4 agents as host-vector systems.
   Examples:
   Using Adenovirus, Adenovirus-luciferase or adeno-associated virus to transfect cells; Typically involves 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, Shigella, Salmonella, Yersinia, and E. histolytica.
2. **III-D-2** Experiments in which DNA from Risk Group 2, 3, or 4 agents or DNA from restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. (Section III-D-2-a)
   Example: *Yersinia pseudotuberculosis* genes encoding outer membrane adhesins are cloned into plasmid vectors for re-introduction into mutant strains of the same bacteria or *E. coli.*

3. **III-D-3** Experiments involving the use of infectious DNA or RNA viruses from Risk Group 2, 3 or 4 in tissue culture systems; or defective recombinant viruses in the presence of helper virus or packaging cells in tissue culture systems (this includes all eukaryotic viruses). (Section III-D-3)
   Example: Insertion of KSHV or RRV genes into defective lentiviral vectors.
4. **III-D-4** Experiments involving whole animals
   a. **III-D-4-a** Experiments involving viable rDNA-modified microorganisms tested on whole animals.
   b. **III-D-4-b** Experiments that generate transgenic animals, including insects.
      Example: Creation of transgenic animals (mice, rats, zebra fish, drosophila,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.
5. **III-D-5** Experiments involving whole plant.
6. **III-D-6** Experiments involving more than 10 liters of culture.
7. **III-D-7** Experiments involving human influenza strains H2N2, 1918 H1N1, and/or highly pathogenic H5N1.

Section III-E - Experiments that require registration simultaneous with initiation requires USF IBC registration:

1. **III-E-1** Introduction into cultured cells of any rDNA containing greater than half but less than 2/3 of a eukaryotic viral genome (with the exception of Risk Group 3 or 4 agents).
2. **III-E-2** Experiments involving whole plants that require BSL1 or BSL2 containment.
3. **III-E** Cloning in non-pathogenic prokaryotes and non-pathogenic lower eukaryotes.

Section III-F - Experiments that are exempt and requires USF IBC registration:

1. **III-F-1** Use of synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g.,oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 ng per kg body weight.
2. **III-F-2** Use of rDNA that is not in organisms or viruses.
3. Introduction into cultured cells of any recombinant DNA containing less than half of a eukaryotic viral genome (with the exception of Risk Group 3 or 4 pathogens). (Appendix C-I)