University of Nebraska Lincoln

Safe Operating Procedure

(Revised 7/11)

RECOMBINANT DNA – IBC AND OTHER REVIEW REQUIREMENTS

(For assistance, please contact EHS at (402) 472-4925, or visit our web site at http://ehs.unl.edu/)

All recombinant DNA (rDNA) projects at the University of Nebraska-Lincoln (UNL) must adhere to the requirements of *the NIH Guidelines for Research Involving Recombinant DNA Molecules*. UNL has also adopted policies and procedures that describe how the NIH Guidelines are implemented at this institution. These policies and procedures are more stringent than the NIH Guidelines. For example, UNL requires IBC notification and review of rDNA projects that are specifically exempt from the NIH Guidelines, as well as projects involving pathogenic agents and bloodborne pathogens. In no case are UNL's policies and procedures less stringent than the NIH Guidelines. Links to the NIH Guidelines and UNL Biosafety Guidelines are provided on the EHS web page.

Principal Investigators (PIs) submitting a protocol to the IBC must include a reference to the appropriate section of the NIH Guidelines. The table below summarizes experiments covered by the NIH Guidelines, including the relevant reference to the appropriate section of the Guidelines. This table also specifies if more stringent review requirements are mandated by the UNL Biosafety Guidelines.

Type of rDNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
NIH Exempt Experiments			
rDNA molecules that are not in organisms or viruses	Registration with IBC required by UNL policy. BSL-2 or higher requires registration and approval by IBC prior to initiation. BSL-1 requires IBC notice simultaneous with initiation.	Exempt	III-F-1
rDNA molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent			III-F-2
rDNA molecules that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means			III-F-3
rDNA molecules that consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species)			III-F-4
rDNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent (Appendix A)			III-F-5

rDNA molecules that do not present risk to health or environment as determined by the NIH Director (Appendix C). E.g., certain rDNA molecules containing less than one-half of any eukaryotic viral genome propagated and maintained in tissue culture, certain *Escherichia coli* K-12 host-vector systems; certain *Saccharomyces* host-vector systems; certain *Kluyveromyces lactis* host-vector systems; certain *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems; certain rDNA molecules derived entirely from extrachromosomal elements of listed gram positive organisms.	Registration with IBC required by UNL policy. BSL-1 requires IBC notice simultaneous with initiation.	Exempt	III-F-6
Purchase and transfer of transgenic rodents that require BSL-1 containment			III-F-6 (Appx C-VII)
rDNA Experiments Subject to NIH Guidelines			
Experiments that don't fall into any other section of the NIH Guidelines, e.g. experiments involving the introduction of risk group 1 DNA into risk group 1 organisms such as E. coli BL-21, or non-viral risk group 1 or risk group 2 rDNA used in tissue culture systems.	Requires IBC notice simultaneous with initiation	Requires IBC notice simultaneous with initiation (all are typically conducted at BSL-1)	III-E
rDNA molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated in maintained in cells in tissue culture using BSL-1 containment so long as it is demonstrated that the cells lack helper virus for the specific families of defective viruses being used.			III-E-1
Experiments involving whole plants, and/or rDNA-modified organisms associated with whole plants (unless specified in III-A, III-B, III-D, or III-F)			III-E-2
Experiments involving the generation of **rodents** in which the animal's genome has been altered by stable introduction of rDNA, or DNA derived therefrom, into the germ-line so long as the experiment can properly be conducted at BSL-1 containment.			III-E-3
Experiments involving the introduction of rDNA into risk group 2 or higher agents [so long as the containment level specified in this section of the NIH Guidelines is observed]	Requires IBC approval prior to initiation	Requires IBC approval prior to initiation	III-D-1
Experiments in which DNA from risk group 2 or higher agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems [certain conditions exist for DNA from RG 4]			III-D-2
Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus [so long as the containment level specified in this section of the NIH Guidelines is observed]			III-D-3
Experiments involving whole animals in which the animal's genome has been altered by stable introduction of rDNA, or DNA derived therefrom, into the germ-line; **AND** experiments involving viable rDNA-modified microorganisms tested on whole animals.			III-D-4

Type of rDNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
Experiments to genetically engineer whole plants by rDNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing rDNA (unless otherwise specified in III-A, III-B, III-D, or III-F)	Requires IBC approval prior to initiation	Requires IBC approval prior to initiation.	III-D-5
Experiments involving more than 10 L of culture.			III-D-6
Experiments involving influenza viruses (applies to experiments with recombinant influenza viruses containing genes and/or segments from human H2N2 (1957-1968), HPAI H5N1, and 1918 H1N1.) (*Generally conducted at BSL-3 enhanced containment.*)			III-D-7
Experiments involving the deliberate transfer of rDNA, or DNA or RNA derived from rDNA, into human research participants.	Requires review and approval by the IBC, IRB, and federal NIH/OBA-RAC prior to initiation	Requires review and approval by the IBC, IRB, and federal NIH/OBA-RAC prior to initiation.	III-C-1
Experiments involving the deliberate formation of rDNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 ng/Kg body weight.	Requires review and approval by the IBC and federal NIH/OBA-RAC prior to initiation.	Requires review and approval by the IBC and federal NIH/OBA-RAC prior to initiation.	III-B
Major Actions, defined as the 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 agents in humans, veterinary medicine, or agriculture.	Requires review and approval by the IBC, federal NIH/OBA-RAC, and NIH Director prior to initiation.	Requires review and approval by the IBC, federal NIH/OBA-RAC, and NIH Director prior to initiation.	III-A

Interpretative guidance provided by NIH/OBA relevant to the above categories of experiments is summarized below:

Exempt Experiments (Section III-F)

- Materials derived from or produced by genetically engineered organisms (i.e., proteins) are not subject to NIH Guidelines (other than DNA molecules resulting from replication of rDNA).
- If an experiment falls into Section III-D or III-E of the NIH Guidelines and also falls into section III-F, it is exempt.

- Although Appendix C-1 exempts the use of rDNA in tissue culture, there are exceptions to the exemption. Existing tissue culture cell lines created by the introduction of rDNA are exempt from the NIH Guidelines unless, the cell line:
    - Was modified using DNA from RG 3 or 4 agents
    - Contains a toxin with an LD50 or less than 100 ng/kg body weight
    - Contains viral DNA in a quantity exceeding 50% of any viral genome
    - Is used in conjunction with defective viruses in the presence of helper virus
    - Is used in an experiment involving the deliberate transfer of the cell line into humans
    - Is grown in a volume exceeding 10 liters of culture

Major Actions (III-A) [Deliberate Transfer of a Drug-Resistance Trait]

- A drug is considered to be useful for treatment even if its use is limited to the treatment of a specific patient population (for example, children or immunocompromised individuals), or it is primarily used for treatment outside of the US (for example chloramphenicol is not in widespread use in the US but it is a commonly used antibiotic in many other countries).
- Approval from the NIH Director is limited to the investigator that sought the approval.

Animal Experiments

- The purchase and transfer of transgenic rodents that may be maintained at BSL-1 is exempt under the NIH guidelines. However, subsequent use involving rDNA or requiring BSL-2 or higher containment is not exempt.
- The purchase or transfer of animals other than rodents, regardless of containment level, is not exempt.
- With respect to gene ablation studies, when recombinant techniques are used to knock out genes, the experiments are subject to the NIH Guidelines.

Experiments with Animals or Plants

Activity	Minimum BSL	NIH Guidelines Section
Creation of Transgenic Animals		
Rodents	BSL1	III-E-3
Rodents	BSL2 or higher	III-D-4-b
Animals other than rodents	BSL-1	III-D-4-a
Animals other than rodents	BL 2 or higher	III-D-4-b
rDNA modified arthropods	BSL-1	III-D-4-a
rDNA modified arthropods	BSL-2 or higher	III-D-4-b
Knock-out rodents	BSL-1	III-E-3
Knock-out rodents	BSL-2 or higher	III-D-4-b
Breeding of Transgenic Animals		
Rodents from one strain (propagation/colony maintenance)	BSL-1	Exempt (III-F-4)
Rodents from one strain (propagation/colony maintenance)	BSL-2 or higher	III-D-4-b
Rodents from two strains (generating a new strain) if ***neither*** parental rodent contains the following genetic modifications: (1) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (2) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and (3) the rodent that results from the breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.	BSL-1	Exempt (Appendix C-VIII)

Activity	Minimum BSL	NIH Guidelines Section
Rodents from two strains (generating new strain) if the parental rodent contains the following genetic modifications: (1) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (2) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); or (3) the rodent that results from the breeding contains more than one-half of an exogenous viral genome from a single family of viruses.	BSL-1	III-E-3
Rodents from two strains	BSL-2 or higher	III-D-4-b
Transgenic animals other than rodents	BSL-1	III-D-4
Transgenic animals other than rodents	BSL-2 or higher	III-D-4
rDNA modified arthropods	BSL-1	Exempt (III-F-4)
rDNA modified arthropods	BSL-2 or higher	III-D-4-b
Rodent knockouts (propagation)	BSL-1	Exempt (III-F-4)
Rodent knockouts (propagation)	BSL-2 or higher	III-D-4-b
Rodent knockouts from two strains (generating a new strain) if ***neither*** parental rodent contains the following genetic modifications: (1) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (2) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and (3) the rodent that results from the breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.	BSL-1	Exempt (Appendix C-VIII)
Rodent knockouts from two strains (generating new strain) if the parental rodent contains the following genetic modifications: (1) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (2) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); or (3) the rodent that results from the breeding contains more than one-half of an exogenous viral genome from a single family of viruses.	BSL-1	III-E-3
Rodent knockouts from two strains	BSL-2 or higher	III-D-4-b
Experiments with Transgenic Animals		
Transgenic rodents (use after purchase or transfer that ***does not*** involve the use of rDNA)	BSL-1	Exempt (III-F-4)
Transgenic rodents (use of rDNA subsequent to purchase)	BSL-1	III-D-4-a
Rodents	BSL-2 or higher	III-D-4-b
Animals other than rodents	BSL-1	III-D-4-a
Animals other than rodents	BSL-2 or higher	III-D-4-b
rDNA modified arthropods associated with plants	BSL-1	III-E-2-b-5
rDNA modified arthropods associated with plants	BSL-2 or higher	III-E-2
rDNA modified arthropods not associated with plants	BSL-1	III-D-4-a
rDNA modified arthropods not associated with plants	BSL-2 or higher	III-D-4-b
Experiments with rDNA in an Animal (transgenic or otherwise)		
rDNA modified microbes in any animal	BSL-1	BSL-1 restricted to viruses transmitted vertically
RG2 rDNA modified microbes in any animal	BSL-2	III-D-1-a
RG3 rDNA modified microbes in any animal	BSL-3	III-D-1-b

Activity	Minimum BSL	NIH Guidelines Section
RG4 rDNA modified microbes in any animal	BSL-4	III-D-1-c
rDNA modified restricted agent (e.g., pox) in an animal	BSL-4	III-D-1-d
rDNA modified animal pathogens in an animal	BSL-4	III-D-1-d
Introduction of less than 2/3 of eukaryotic viral genome into a non-human vertebrate or invertebrate	BSL-1	III-D-4-a
Propagation of animals containing viral vector sequences not leading to transmissible infection	BSL-1	III-D-4-a
rDNA involving whole animals not covered by sections III-D-1 or III-D-4-a	Set by IBC	III-D-4-b
Cloning animals		
Cloning animals	BSL-1 or higher	Not covered
Purchase or Transfer of Transgenic Animals		
Rodents	BSL-1	Exempt (Appendix C-VII)
Rodents	BSL-2 or higher	III-D-4
Animals other than rodents	BSL-1	III-D-4
Animals other than rodents	BSL-2 or higher	III-D-4
rDNA modified arthropods	BSL-1	III-D-4
rDNA modified arthropods	BSL-2 or higher	III-D-4
Plant Experiments with Animals or Arthropods		
Experiments with microorganisms or insects containing rDNA with potential for detrimental impact to ecosystems	BL3-P or BL2-P+	III-D-5-a or III-D-5–b
Experiments with exotic infectious agents in the presence of arthropod vectors	BL4-P	III-D-5-c
Experiments with microbial pathogens of insects or small animals associated with plants with the potential for detrimental impact to ecosystems	BL3-P or BL2-P+	III-D-5-e
Small animals associated with rDNA modified plants	BSL-1	III-E-2
Experiments with rDNA-modified arthropods or small animals associated with plants	BSL-1	III-E-2-b-(5)