

## RECOMBINANT OR SYNTHETIC NUCLEIC ACIDS – 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 or synthetic nucleic acid molecule (r/sNA) projects at the University of Nebraska-Lincoln (UNL) must adhere to the requirements of *the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid 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 r/sNA 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 r/sNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
<b>NIH Exempt Experiments</b>			
Those 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 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.	Registration with IBC required by UNL policy.	Exempt from the NIH Guidelines	III-F-1
r/sNA molecules that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.	BSL-2 or higher requires registration and approval by IBC prior to initiation.		III-F-2
r/sNA 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	BSL-1 requires IBC notice simultaneous with initiation.		III-F-3

Type of r/sNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
r/sNA 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	<p>Registration with IBC required by UNL policy.</p> <p>BSL-2 or higher requires registration and approval by IBC prior to initiation.</p> <p>BSL-1 requires IBC notice at the same time as initiation.</p>	Exempt from the NIH Guidelines	III-F-4
r/sNA 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-5
r/sNA 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-6
Genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.			III-F-7
r/sNA molecules that do not present risk to health or environment as determined by the NIH Director (Appendix C). E.g., certain r/sNA molecules containing less than one-half of any eukaryotic viral genome propagated and maintained in tissue culture, certain <i>Escherichia coli</i> K-12 host-vector systems; certain <i>Saccharomyces</i> host-vector systems; certain <i>Kluyveromyces lactis</i> host-vector systems; certain <i>Bacillus subtilis</i> or <i>Bacillus licheniformis</i> host-vector systems; certain r/sNA molecules derived entirely from extrachromosomal elements of listed gram positive organisms.			III-F-8
Purchase and transfer of transgenic rodents that require BSL-1 containment			III-F-8 Appx C-VII
Generation of BL1 Transgenic Rodents via Breeding			III-F-8 Appx. C-VIII

Type of r/sNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
<b>Experiments Subject to NIH Guidelines</b>			
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 <i>E. coli</i> BL-21, or non-viral risk group 1 or risk group 2 r/sNA used in tissue culture systems.	Requires IBC notice at the same time as initiation	Requires IBC notice at the same time as initiation (all are typically conducted at BSL-1)	III-E

(Created 1/09; Revised 10/09, 3/11, 7/11)

Type of r/sNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
r/sNA molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated and 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.	Requires IBC notice at the same time as initiation	Requires IBC notice at the same time as initiation (all are typically conducted at BSL-1)	III-E-1
Experiments involving whole plants, and/or r/sNA-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 <b>rodents</b> in which the animal's genome has been altered by stable introduction of r/sNA, 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 r/sNA 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 r/sNA, or DNA derived therefrom, into the germ-line; <b>AND</b> experiments involving viable r/sNA-modified microorganisms tested on whole animals.			III-D-4
Experiments to genetically engineer whole plants by r/sNA 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 r/sNA (unless otherwise specified in III-A, III-B, III-D, or III-F)			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.) ( <i>Generally conducted at BSL-3 enhanced containment.</i> )			III-D-7
Experiments involving the deliberate transfer of r/sNA, or DNA or RNA derived from r/sNA, 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

(Created 1/09; Revised 10/09, 3/11, 7/11)

Type of r/sNA Experiment	UNL Biosafety Guidelines Requirement	Status Under NIH Guidelines	Relevant Section(s) of NIH Guidelines
Experiments involving the deliberate formation of r/sNA 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 r/sNA).
- Although Appendix C-1 exempts the use of r/sNA in tissue culture, there are exceptions to the exemption. Existing tissue culture cell lines created by the introduction of r/sNA 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 r/sNA 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	Recommended BSL	NIH Guidelines Section
<b>Creation of Transgenic Animals</b>		
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
r/sNA modified arthropods	BSL-1	III-D-4-a
r/sNA 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
<b>Breeding of Transgenic Animals</b>		
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 <i>neither</i> 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 III-F-8 (Appendix C-VIII)
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
r/sNA modified arthropods	BSL-1	Exempt (III-F-4)
r/sNA 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 <i>neither</i> parental rodent contains the following genetic modifications: (1) incorporation of more than one-half of the genome of an exogenous	BSL-1	Exempt III-F-8 (Appendix C-VIII)

(Created 1/09; Revised 10/09, 3/11, 7/11)

Activity	Recommended BSL	NIH Guidelines Section
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.		
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
<b>Experiments with Transgenic Animals</b>		
Transgenic rodents (use after purchase or transfer that <i>does not</i> involve the use of r/sNA)	BSL-1	Exempt (III-F-4)
Transgenic rodents (use of r/sNA 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
r/sNA modified arthropods associated with plants	BSL-1	III-E-2-b-5
r/sNA modified arthropods associated with plants	BSL-2 or higher	III-E-2
r/sNA modified arthropods not associated with plants	BSL-1	III-D-4-a
r/sNA modified arthropods not associated with plants	BSL-2 or higher	III-D-4-b
<b>Experiments with r/sNA in an Animal (transgenic or otherwise)</b>		
r/sNA modified microbes in any animal	BSL-1	BSL-1 restricted to viruses transmitted vertically
RG2 r/sNA modified microbes in any animal	BSL-2	III-D-1-a
RG3 r/sNA modified microbes in any animal	BSL-3	III-D-1-b
RG4 r/sNA modified microbes in any animal	BSL-4	III-D-1-c
r/sNA modified restricted agent (e.g., pox) in an animal	BSL-4	III-D-1-d
r/sNA 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
r/sNA involving whole animals not covered by sections III-D-1 or III-D-4-a	Set by IBC	III-D-4-b
<b>Cloning animals</b>		
Cloning animals	BSL-1 or higher	Not covered
<b>Purchase or Transfer of Transgenic Animals</b>		
Rodents	BSL-1	Exempt III-F-8 (Appendix C-VII)
Rodents	BSL-2 or higher	III-D-4
Animals other than rodents	BSL-1	III-D-4

(Created 1/09; Revised 10/09, 3/11, 7/11)

Activity	Recommended BSL	NIH Guidelines Section
Animals other than rodents	BSL-2 or higher	III-D-4
r/sNA modified arthropods	BSL-1	III-D-4
r/sNA modified arthropods	BSL-2 or higher	III-D-4
<b>Plant Experiments with Animals or Arthropods</b>		
Experiments with microorganisms or insects containing r/sNA 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 r/sNA modified plants	BSL-1	III-E-2
Experiments with r/sNA-modified arthropods or small animals associated with plants	BSL-1	III-E-2-b-(5)