ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

# Safe Operating Procedure

(Revised 5/21)

# RECOMBINANT AND/OR SYNTHETIC NUCLEIC ACID MOLECULE EXPERIMENTS REQUIRING IBC REVIEW

## Scope

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 Institutional Biosafety Committee (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.

## References

- **NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules** (*NIH Guidelines*). Dept. of Health and Human Services, Office of Science Policy, National Institute of Health. https://osp.od.nih.gov/biotechnology/nih-guidelines/
- **University of Nebraska - Lincoln Biosafety Guidelines.** UNL Office of Research and Economic Development and UNL Environmental Health and Safety. https://ehs.unl.edu/Biosafety_Guidelines.pdf

Principal Investigators (PIs) submitting a protocol to the IBC must indicate the appropriate section of the NIH Guidelines that apply to the experiments described in the protocol. The information below is adapted from Section III of the most recent revision of the NIH Guidelines. Please review the criteria listed in the full version of the *NIH Guidelines* to ensure that your experiments meet the criteria of the summarized versions listed below.

# EXPERIMENT DESCRIPTIONS

## Section III-A: Experiments That Require IBC Approval AND NIH Director Approval BEFORE INITIATION Of Experiments.

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.

## Section III-B: Experiments That Require IBC AND NIH OSP Approval BEFORE INITIATION.

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.

- Experiments that involve cloning genes coding for toxin molecules toxic for vertebrates that have an LD50 of > 100 nanograms per kilogram bodyweight and < 100 micrograms per kilogram body weight require **IBC approval and registration with NIH OSP prior to initiation** per Appendix F of the *NIH Guidelines*.

## Section III-C: Experiments Involving Human Gene Transfer That Require IBC Approval BEFORE INITIATION.

Experiments involving the deliberate transfer of r/sNA, or DNA or RNA derived from r/sNA, into human research participants.

## Section III-D: Experiments that Require IBC Registration and Approval BEFORE INITIATION

### III-D-1 Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems

III-D-1-a: Introduction of rDNA into Risk Group 2 agents; BSL-2/ABSL-2
*Generally, involves the use of viral vectors where more than 2/3 of the original viral genome is intact in the vector.*

III-D-1-b: Introduction of rDNA into Risk Group 3 agents; BSL-3/ABSL-3

III-D-1-c: Introduction of rDNA into Risk Group 4 agents; BSL-4/ABSL-4
(**NOT ALLOWED AT UNL**)

III-D-1-d: Introduction of rDNA into restricted agents at BSL-4/ABSL-4 not permitted except on a case-by-case basis following NIH/OSP review and USDA permit application.
(**NOT ALLOWED AT UNL**)

### III-D-2 Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

III-D-2-a: Any DNA from Risk Group 2 or Risk Group 3 agents transferred into nonpathogenic prokaryotes or lower eukaryotes or exempt from the *NIH*

*Guidelines* (see Section III-F). May be performed at BSL-1 or BSL-2 depending on the risk assessment by the IBC. Example: Cas-9 gene from *S. pyogenes* (RG-2 organisms) into *Agrobacterium* or non-K-12 strains of *E. coli*.

- Experiments in which DNA from RG-4 organisms transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed at BSL-2 containment **only** after demonstration that the fraction of DNA transferred is a totally and irreversibly defective fraction of the agent's genome.
- Experiments involving the formation of recombinant or synthetic nucleic acid molecules for genes coding for molecules toxic to vertebrates must be approved by NIH OSP or conducted under NIH specified conditions as described in Appendix F of the *NIH Guidelines*.

III-D-2-b: DNA from Risk Group 4 and restricted agents transferred into nonpathogenic prokaryotes or lower eukaryotes not permitted except on a case-by-case basis following NIH OSP review and USDA permit application. (**NOT ALLOWED AT UNL**)

### III-D-3 Experiments Involving the Use Of Infectious DNA Or RNA Viruses Or Defective DNA Or RNA Viruses In The Presence Of Helper Virus In Tissue Culture Systems

III-D-3-a: Use of Infectious or defective (defective eukaryotic viruses contain < 2/3 of the genome) Risk Group 2 viruses in the presence of helper virus in tissue culture may be conducted at BSL-2.

*Examples: Insertion of genes into defective lentiviral, retroviral, or adenoviral vectors (creation of recombinant vectors).*

III-D-3-b: Use of Infectious or defective (defective eukaryotic viruses contain < 2/3 of the genome) Risk Group 3 viruses in the presence of helper virus in tissue culture may be conducted at BSL-3.

III-D-3-c: Use of Infectious or defective (defective eukaryotic viruses contain < 2/3 of the genome) Risk Group 4 viruses in the presence of helper virus in tissue culture may be conducted at BSL-4. (**NOT ALLOWED AT UNL**)

III-D-3-d: Use of Infectious or defective restricted poxviruses (alastrim, smallpox (variola, and whitepox) in the presence of helper virus in tissue culture shall be determined on a case-by-case basis following NIH OSP review and USDA permit application. (**NOT ALLOWED AT UNL**)

III-D-3-e: Use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BSL-1.

### III-D-4 Experiments Involving Whole Animals

III-D-4-a: Recombinant/synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome transferred to any non-human vertebrate or any invertebrate organism (Conducted at BSL-1 or ABSL-1). *Example: making transgenic mice.*

III-D-4-b: For experiments involving recombinant or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Section III-D-1, Experiments Using Human or Animal Pathogens (Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems), or Section III-D-4-a, the appropriate containment shall be

determined by the Institutional Biosafety Committee

### III-D-5 Experiments Involving Whole Plants (not typically done at UNL)

III-D-5-a: Recombinant techniques with exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants. Requires BSL3-P containment.

III-D-5-b: Plants with cloned genomes of readily transmissible exotic infectious agents that may reconstitute by genomic complementation.

III-D-5-c: Readily transmissible exotic infectious agents, such as the soybean rust fungus, maize streak or other viruses in the presence of their specific arthropod vectors. BSL-4P

III-D-5-d: Sequence encoding vertebrate toxins introduced into plants or associated organisms. BSL-3P

III-D-5-e: Microbial pathogens of insects or small animals associated with plants if the rDNA-modified organism has a recognized detrimental impact on ecosystems.

### III-D-6 Experiments involving more than 10 liters of culture.

IBC determines containment level. (See Appendix K of the NIH Guidelines for Good Large Scale through BSL-3 Large Scale containment conditions).

### III-D-7 Experiments Involving Influenza Viruses

Experiments with influenza viruses generated by recombinant or synthetic methods (e.g. generation by revers genetics of chimeric viruses with reasserted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the Risk Group of the virus that was the source of the majority of segments in the recombinant or synthetic virus. Experiments with influenza viruses containing genes from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1), including but not limited to, strains of mammalian-transmissible HPAI H5N1 virus, shall be conducted at BSL-3 enhanced containment unless indicated below. *(Any recombinant or*

*synthetic nucleic acid work with influenza viruses falls under the parent III-D-7 if not explicitly described in subsection below.)*

III-D-7-a: **Human H2N2 (1957-1968).** Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BSL-3 enhanced (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BSL-2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant or synthetic virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BSL-2.

III-D-7-b: **Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).** Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BSL-3 enhanced containment, (see Appendix G-IIC- 5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments involving influenza viruses containing a minority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BSL-3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at Biosafety Level 2 and after they have been excluded pursuant to 9 CFR 121.3(e). Such experiments may be performed at BSL-3 enhanced containment or containment may be lowered to Biosafety Level 2, the level of containment for most research with other influenza viruses. (USDA/APHIS regulations and decisions on lowering containment also apply.)

III-D-7-c: **1918 H1N1.** Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BSL-3/ABSL3.

III-D-7-d: **Antiviral Susceptibility and Containment.** The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968). If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc.

Experiments with 1918 H1N1, human H2N2 (1957-1968) or HPAI H5N1 that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational antiviral agents being developed for influenza) would be subject to Section III-A-1 (Major Actions). As per Section I-A-1 of the NIH Guidelines, if the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments.

## Section III-E: Experiments Requiring IBC form submission <u>SIMULTANEOUS WITH INITIATION</u>

Experiments not included in Sections III-A, III-B, III-C, III-D, or III-F and their subsections are considered in Section III-E. All such experiments are conducted at BSL-1 containment. *Example: experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes may be conducted at BSL-1.) [E. coli BL21 stains and Agrobacterium tumefaciens fall under this parent section]*

**III-E-1 Formation of r/sNA molecules containing no more than 2/3 of the genome of any eukaryotic virus in tissue culture.** Can be conducted at BSL-1 with no helper virus. The IBC classifies retroviral vectors with packaging system capable of infecting human cells as BSL-2.

*Examples: Inserting DNA sequences that encode reporters that are measured (lacZ, luciferase, eGFP, dsRed2, etc), or that encode enzymes that are potentially therapeutic (nitric oxide synthases, superoxide dismutase, siRNA) against mRNAs that promote disease, etc into viral vectors that retain no more than 2/3 of the original viral genomic sequence. The cDNAs will be driven by the following promoters: CMV IE, RSV LTR, and cardiac Troponin T (cTnT).*

**III-E-2 Experiments involving Whole Plants (Experiments involving recombinant or synthetic nucleic acid molecules-containing whole plants and/or organisms associated with whole plants not covered by Sections III-A, III-B, III-D, or III-F)**

**III-E-2-a BL1-P is recommended for all experiments with recombinant or synthetic recombinant or synthetic nucleic acid molecule-containing plants and plant-associated microorganisms not covered in Section III-E-2-b or other sections of the NIH Guidelines**

**III-E-2-b BL2-P or BL1-P + biological containment is recommended for the following experiments:**

**III-E-2-b-(1) Plants modified by r/sNA molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.**

**III-E-2-b-(2) Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent.**

**III-E-2-b-(3) Plants associated with r/sNA molecule-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems.**

**III-E-2-b-(4) Plants associated with r/sNA molecule-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems.**

**III-E-2-b-(5) Experiments with r/sNA acid molecule-modified arthropods or small animals associated with plants, or with arthropods or small animals with r/sNA molecule-modified microorganisms associated with them if**

**the r/sNA molecule-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems**

**III-E-3** **Formation of rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus in tissue culture.** Can be conducted at BSL-1 if no helper virus is present. The IBC classifies retroviral vectors with packaging system capable of infecting human cells as BSL-2.

## Section III-F: Exempt Experiments (requires IBC form submission SIMULTANEOUS WITH INITIATION)

**III-F-1** **Synthetic nucleic acids that can a) neither replicate nor generate nucleic acids that can replicate in a living cell, and b) are not designed to integrate into DNA, and c) do not produce a toxin with an LD50 of less than 100ng/kg body weight in vertebrate animals.**

Synthetic nucleic acids deliberately transferred to one or more human research participants and meets the criteria of Section III-C of the NIH Guidelines is not exempt under this section.

**III-F-2** **Not in cells, organisms or viruses and have not been modified or manipulated to make them able to penetrate cellular membranes.** *Example: encapsulated into synthetic or natural vehicles.*

**III-F-3** **Consist solely of the exact r/sNA sequence from a single source that exists in nature.**

**III-F-4** **Consist entirely of nucleic acids from a prokaryotic host when propagated only in that host or when transferred to another host by well-established physiological means.** Includes indigenous plasmids or viruses.

**III-F-5** **Consist entirely of nucleic acids from a eukaryotic host when propagated only in that host (or a closely related strain of the same species).** Includes chloroplasts, mitochondria or plasmids (but excluding viruses).

**III-F-6** **Those 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.**

A list of such exchangers will be prepared and periodically revised by the NIH Director after appropriate notice and opportunity for public comment. Appendices A-I through A-VI of the ***NIH Guidelines***, for a list of natural exchangers that are exempt from the ***NIH Guidelines***.

**III-F-7** **Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA**

**III-F-8** **Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), Major Actions), as determined by the NIH Director following appropriate notice and opportunity for public comment.**

**Appendix C: Exemptions under III-F-8**

- **C-I:** r/sNA in tissue culture (must contain less than 50% of any eukaryotic viral genome). Exceptions apply (See C-I-A).
- **C-II:** *E. coli* K-12 host-vector systems. Exceptions apply (See C-II-A)
- **C-III:** *Saccharomyces cerevisiae* and *uvarum* host-vector systems. Exceptions apply (See C-III-A).
- **C-IV:** *Kluyveromyces lactis* host-vector systems. Exceptions apply (See C-IV-A).
- **C-V:** Asporogenic *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems that do not revert to spore-formers. Exceptions apply (See C-V-A).
- **C-VI:** Extrachromosomal elements of Gram positive organisms. Must be derived entirely from a listed organism and be propagated and maintained in a listed organism (see ***NIH Guidelines*** for list of organisms). (Exceptions apply (See C-VI-A).
- **C-VII:** Purchase or transfer of transgenic rodents.
- **C-VIII:** Generation of BL1 transgenic rodents via breeding. The following conditions must be met to qualify:
    1) Both parental rodents can be housed under BL1 containment; and
    2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
    3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

Interpretative guidance provided by NIH/OSP 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 U.S. (for example chloramphenicol is not in widespread use in the U.S. 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.