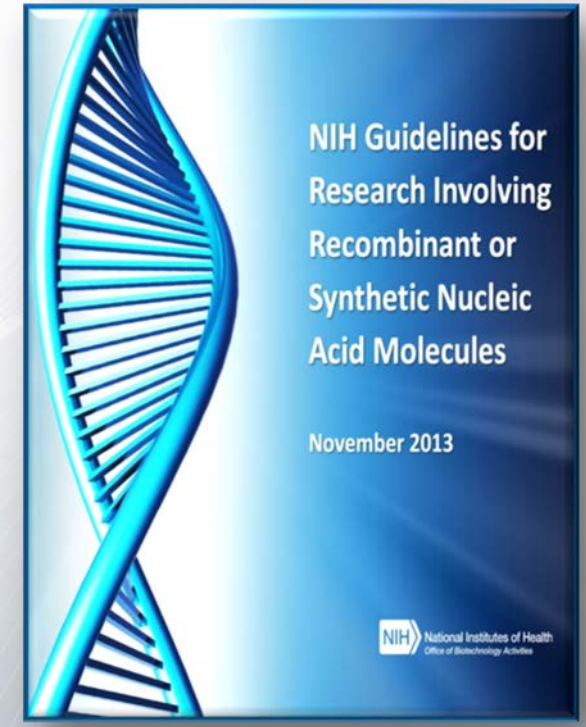
Overview of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* for Principal Investigator Training

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

The NIH Guidelines is a scientifically-responsive document which has undergone multiple revisions since 1976, with the latest version published in November 2013. To view the full version, go to http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.



NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

There are four main sections in the NIH Guidelines. These sections cover:

Section I	The scope of the Guidelines
Section II	Safety Considerations for work with recombinant or synthetic nucleic acid molecules (Risk Groups for infectious agents and containment requirements for experiments)
Section III	Types of experiments covered by the Guidelines (including work with animals, plants) and levels of review
Section IV	Roles and Responsibilities

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section I The scope of the Guidelines

The purpose of the NIH Guidelines is to specify the practices for constructing and handling:

- recombinant nucleic acid molecules
- synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules
- cells, organisms, and viruses containing such molecules

In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:

- molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell (i.e., recombinant nucleic acids)
- nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e., synthetic nucleic acids)
- molecules that result from the replication of those described above

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section I The scope of the Guidelines

"Guidelines" does not mean "optional"

The NIH Guidelines are a term and condition of NIH funding for research with recombinant or synthetic nucleic acid molecules.

As a condition for NIH funding of recombinant DNA projects, institutions must ensure that **ALL** recombinant research, conducted at or sponsored by the institution, complies with the NIH Guidelines, **IRRESPECTIVE OF FUNDING SOURCE**.

Consequences of non-compliance:

(i) suspension, limitation, or termination of financial assistance for the noncompliant NIH-funded research project and/or of NIH funds for other recombinant research at the institution, or

(ii) a requirement for prior NIH approval of any or all recombinant or synthetic nucleic acid molecule projects at the institution.

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Section II Safety Considerations for work with recombinant or synthetic nucleic acid molecules (Risk Groups for infectious agents and containment for experiments)

Appendix B of the NIH Guidelines lists biological agents known to infect humans as well as selected animal agents that have the potential to infect humans. These agents are assigned to one of four risk group based on the potential effect of the agent on a healthy human adult.

RG 1	RG 2	RG 3	RG 4
Agents that are not associated with disease in healthy adult humans	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

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Section II Safety Considerations for work with recombinant or synthetic nucleic acid molecules (Risk Groups for infectious agents and containment for experiments)

In proposing projects, the PI must make an initial determination of the required levels of physical containment as described in **Appendix G** (practices, equipment, facilities) and biological containment as described in **Appendix I (**survivability and transmissibility of the agent).

Four biosafety containment levels are described in **Appendix G**. Each level consists of a combination of lab practices and techniques, safety equipment, and lab facilities appropriate for the operations being performed.

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Section II Safety Considerations for work with recombinant or synthetic nucleic acid molecules (Risk Groups for infectious agents and containment for experiments)

The PI must also propose appropriate microbiological practices and laboratory techniques to be used for the research.

- **Appendix P** specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals.
- **Appendix Q** specifies containment and confinement practices for research involving whole animals, both transgenic animals and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals.
- **Appendix Q** supersedes **Appendix G** when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. The animals covered in Appendix Q include but are not limited to cattle, swine, sheep, goats, horses, and poultry.

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Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Section III describes the categories of review at both the federal and institutional levels necessary for the approval of certain types of recombinant DNA research.

There are six categories of experiments under the NIH Guidelines. These categories reflect the risk of the research, with more stringent review required for the higher risk experiments.

Experiments that are not considered to pose a risk to human health or the environment are exempt from the NIH Guidelines and do not require review, although many institutions may require review by policy.

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Section III Types of experiments covered and levels of review

Level of review	Example of types of research covered	Relevant section(s) of the *NIH Guidelines*
IBC, RAC review, and NIH Director review and approval	Experiments that compromise the control of disease agents in medicine through deliberate transfer of a drug resistance trait	III-A
IBC approval and NIH review for containment determinations	Experiment involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram of body weight	III-B
IBC and IRB approval and NIH review before research participant enrollment	Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules into a human research participant	III-C
IBC approval before initiation	Creating stable germline alterations of an animal's genome, or testing viable recombinant or synthetically modified microorganisms on whole animals, where BL-2 containment or greater is necessary	III-D
IBC notice at initiation	Creating stable germline alterations of rodents by introduction of recombinant or synthetic nucleic acid molecules when these experiments require only BL-1 containment	III-E
Exempt from the *NIH Guidelines*. IBC registration not required if experiment not covered by Sections III-A, III-B, or III-C	Purchase or transfer of transgenic rodents	III-F

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Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

PIs must submit a research proposal for Institutional Biosafety Committee (IBC) review and obtain IBC approval ***prior to the initiation*** of work if the work is subject to Section III-A, III-B, III-C, III-D or III-E of the NIH Guidelines.

The majority of recombinant work performed at UGA falls under Sections III-D, III-E and III-F. IBC approval for research subject to Section III-E may be obtained ***simultaneous with initiation***. Research that falls under Section III-F, Exempt Experiments, must be submitted to the IBC for review.

The following slides provide a brief description of the work covered in Sections III-D, III-E and III-F.

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III — Types of experiments covered (includes work with animals and plants) and levels of review

Recombinant work at UGA falls, primarily into three sections (D, E, and F):

Section III-D All experiments in this section require IBC approval ***prior to initiation*** of work.

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

Section 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

Section 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

Section III-D-4 Experiments Involving Whole Animals (includes experiments in which 1) the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acids into germline (transgenic animals), 2) viable recombinant or synthetic nucleic acid molecule-modified microorganisms are tested on whole animals).

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Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Section III-D-5 Experiments Involving Whole Plants (includes experiments in which 1) plants are genetically engineered by recombinant or synthetic nucleic acid molecule methods, 2) plants are used with recombinant or synthetic nucleic acid molecule containing insects, and 3) are generally BL2-P through BL4-P, depending on risk)

Section III-D-6 Experiments Involving More Than 10L of Culture (also see **Appendix K**)

Section III-D-7 Experiments Involving Influenza Viruses

- Generated by recombinant or synthetic methods (e.g., reverse genetics of chimeric viruses with reassorted 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 virus
- Experiments with influenza viruses containing genes or segments 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) shall be conducted at BL3 enhanced containment

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Section III-E Experiments in this section require IBC notice ***simultaneous with initiation***

Section III-E-1 Experiments involving the formation of recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus

Sections III-E-2 Experiments involving whole plants

Section III-E-3 Experiments involving the generation of transgenic rodents (rodents whose genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules into germline. BL1 containment is appropriate.)

Also includes experiments not included in III-A through III-D or III-F that can be conducted at BSL-1

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Section III-F This section describes experiments that are exempt from the NIH Guidelines. According to the NIH Guidelines, registration with the IBC is not required (unless required by institutional policy). *Submission and approval of protocols for experiments covered in this section is required by the UGA IBC.*

Section III-F-1 Synthetic nucleic acids that 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 are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Section III-F-2 Those 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

Section III-F-3 Those that consist entirely of recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature

Section III-F-4 Those that consist entirely of nucleic acids 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

Section III-F-5 Those that consist entirely of nucleic acids from an 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)

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Section 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. *Meaning recombinant DNA molecules that are composed entirely of DNA segments from one or more of the organisms within a sublist, and to be propagated in any of the organisms within the same sublist*

Section 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

Section III-F-8 Those that do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the RAC (Recombinant DNA Advisory Committee), and following appropriate notice and opportunity for public comment (see **Appendix C**, ***Exemptions under Section III-F-8***)

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Appendix C Exceptions under Section III-F-8

Appendix C-I Recombinant or Synthetic Nucleic Acid Molecules in Tissue Culture

Recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical (see **Appendix C-IX-E**) that are propagated and maintained in cells in tissue culture are exempt (*with the exceptions listed in* **Appendix C-I-A**)

Appendix C-II Escherichia coli K-12 Host-Vector Systems

Experiments which use *Escherichia coli* K-12 host-vector systems are exempt (*with the exception of those experiments listed in* **Appendix C-II-A**)

Appendix C-III Saccharomyces Host-Vector Systems

Experiments involving *S. cerevisiae* and *S. uvarum* host-vector systems (*with the exception of experiments listed in* **Appendix C-III-A**) are exempt

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Appendix C-IV Kluyveromyces Host-Vector Systems

Experiments involving *K. lactis* host-vector systems (*with the exception of experiments listed in* **Appendix C-III-A**) are exempt

Appendix C-V Bacillus subtilis or Bacillus licheniformis Host-Vector Systems

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than 107 may be used for cloning DNA (*with the exception of those experiments listed in* **Appendix C-IV-A**, Exceptions).

Appendix C-VI Extrachromosomal Elements of Gram Positive Organisms

Recombinant or synthetic nucleic acid molecules derived entirely from extrachromosomal elements of the organisms, when propagated and maintained in those organisms (***see NIH Guidelines for listing***).

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section III **Types of experiments covered (includes work with animals and plants) and levels of review**

Appendix C-VII The Purchase or Transfer of Transgenic Rodents The purchase or transfer of transgenic rodents for experiments that require BL1 containment (*Further manipulations of these animals are not necessarily exempt from the NIH Guidelines)*

Appendix C-VIII Generation of BL1 Transgenic Rodents via Breeding

The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the NIH Guidelines if 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.

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Section IV **Roles and Responsibilities**

This section covers the roles and responsibilities for compliance as specified in the NIH Guidelines:

for the Institution

for the Institutional Biosafety Committee (IBC)

for the Biological Safety Officer (BSO)

for the Principal Investigator (PI)

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section IV **Roles and Responsibilities**

Under the NIH Guidelines, the **Institution** shall:

- Establish and implement policies for the safe conduct of research subject to the *NIH Guidelines*
- Establish an Institutional Biosafety Committee
- Assist and ensure compliance with the *NIH Guidelines* by principal investigators (PIs)
- Ensure appropriate training for IBC members and staff, PIs, and laboratory staff
- Determine necessity for health surveillance of laboratory personnel
- Report any significant accidents, incidents or violations to NIH-OSP (NIH Office of Science Policy) within 30 days
- ***Immediately*** report any spills and accidents occurring at BSL-2 or BSL-3 which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules to the BSO, the IBC, and to NIH-OSP

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Section IV Roles and Responsibilities

Under the NIH Guidelines, on behalf of the Institution, the **Institutional Biosafety Committee** shall:

- Review recombinant or synthetic nucleic acid molecule research conducted at or sponsored by the institution for compliance with the NIH Guidelines
- Approve those research projects that are found to conform with the NIH Guidelines. This review shall include independent assessment of the containment levels required by the NIH Guidelines for the proposed research, assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant or synthetic nucleic acid molecule research
- Notify the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval
- Lowering containment levels for certain experiments, as applicable
- Adopting emergency plans covering accidental spills and personnel contamination

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Section IV Roles and Responsibilities

Under the NIH Guidelines, the **Biological Safety Officer's** duties include, but are not limited to:

- Performing periodic inspections of laboratories performing rDNA work
- Reporting to the IBC and the Institution any significant problems, violations of the NIH Guidelines and any significant research-related accidents or illnesses
- Report any spills and accidents occurring at BSL-2 or BSL-3 which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules are *immediately* reportable to the BSO, the IBC, and to NIH-OSP.

- Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving rDNA research
- Providing advice on laboratory security
- Providing technical advice to PIs and the IBC on general research safety procedures

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Section IV — Roles and Responsibilities for the Institution and the Principal Investigator

Principal Investigator responsibilities as defined in the NIH Guidelines:

- Initiate or modify no research subject to the *NIH Guidelines* which requires IBC approval until approval is granted (at UGA, most of this work is covered under **Section III-D**)
- Determine whether experiments are covered under **Section III-E (IBC notification simultaneous with initiation of project)** and notify the IBC
- Be adequately trained in good microbiological techniques
- Adhere to IBC emergency plans for spills and personnel contamination
- Report any significant problems or violations to the Biosafety Officer (BSO), greenhouse or animal facility director (as applicable), the Institutional Biosafety Committee, NIH-OSP, and other appropriate authorities (if applicable) within 30 days. Incidents involving overt exposure to organisms containing recombinant or synthetic nucleic acid molecules occurring at BSL-2 or BSL-3 are *immediately* reportable to the BSO, IBC and NIH-OSP. Report any new information bearing on the NIH Guidelines to the Institutional Biosafety

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Section IV Roles and Responsibilities for the Institution and the Principal Investigator

Responsibilities of the **Principal Investigator** prior to initiating research and during the conduct of research:

- Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken
- Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection)
- Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed
- Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) correct work errors and conditions that may result in the release of recombinant or synthetic nucleic acid molecule materials
- Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

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Section IV **Roles and Responsibilities for the Institution and the Principal Investigator**

For submissions by the **Principal Investigator** to the Institutional Biosafety Committee (using the IBC protocol form or the IBC renewal/modification form) for projects requiring IBC approval ***prior to initiation*** (Sections III-A, III-B, III-C, III-D, or III-E) :

- Make an initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines
- Select appropriate microbiological practices and laboratory techniques to be used for the research
- Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the Institutional Biosafety Committee for review and approval or disapproval
- Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

This training is an overview of the directives provided by the NIH Guidelines, as they apply to the bulk of recombinant research being performed at the University of Georgia. It is the responsibility of each Principal Investigator conducting this research or conducting teaching laboratories involving recombinant DNA techniques to be familiar with the guidelines that apply to their work and to understand their responsibilities under the guidelines. If you have questions or need assistance in determining which guidelines apply to your work, please contact the Office of Biosafety at 706.542.2697 or 706.542.9347.