EMORY UNIVERSITY | Environmental Health and Safety Office

# HOW CAN WE FACILITATE UNDERSTANDING OF THE r-DNA GUIDELINES TO THE SCIENTIFIC COMMUNITY?

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# Topics

1. *Objective of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Research (The NIH Guidelines)*
2. *Key Players involved in the application of the NIH Guidelines*
3. *Emory EHSO experience with reference documents*
4. *Why do we need a reference sheet for r-DNA Classification?*
5. *Who will benefit?*
6. *What are the components of the document?*
7. *Plans for roll-out*
8. *Ongoing process…*

# 1. The NIH Guidelines

- Provides recommendations on how to conduct research with r-DNA safely
- Revision in 2013
- Section I: Oversight
- Section II: Risk Groups
- Section III: Classification of Experiments
- Section IV: Roles and responsibilities

http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

# 2. Key Players involved in the application of the NIH Guidelines

| NIH | Funded Institution | Investigator |
| --- | --- | --- |
| Office of Biotechnology Activities | Research Administration<br>Biosafety Office<br>& Institutional Biosafety Committee | All Research Community from an institution which receives funding from NIH |
| - Provides Guidance<br>"r-DNA Guidelines"<br>- Oversight (Reporting agency) | - Provides guidance<br>-Reviews research<br>-Ensures compliance | -Informs the type of research to be conducted |

# What is r-DNA?

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

- (i) molecules that a) are constructed by joining nucleic acid molecules **AND** b) that can replicate in a living cell, i.e., recombinant nucleic acids; or

- (ii) 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, or

- (iii) molecules that result from the replication of those described in (i) or (ii) above.

# What type of review is required?

## *In the context of the NIH Guidelines:*

(i) Section III-A: Institutional Biosafety Committee (IBC) approval + RAC + NIH Director approval before initiation

(ii) Section III-B: NIH/OBA + IBC approval before initiation

(iii) Section III-C: IBC + IRB + RAC review before research participant enrollment

(iv) Section III-D: IBC approval before initiation

(v) Section III-E: IBC notification simultaneous with initiation

(vi) Section III-F: exempt from the NIH Guidelines

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# rDNA Experiments Covered by the NIH Guidelines

## Purpose

The table below summarizes the types of experiments involving recombinant or synthetic nucleic acid molecules (rDNA) that are covered in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*). The table also shows the level(s) of review/registration/approval required for each type of experiment.

- **NOTE:** rDNA experiments that are exempt from the *NIH Guidelines* are found in Section III-F and Appendix C of the *NIH Guidelines*.

| *rDNA Experiments Covered by the NIH Guidelines* | *RAC[i] Review* | *NIH Director Approval* | *NIH/OBA[ii] Approval* | *NIH/ORDA[iii] Registration* | *IBC[iv] Approval before Initiation* | *IBC Notice upon Initiation* | *IAUCUC[v] Approval* | *IRB[vi] Approval* |
|---|---|---|---|---|---|---|---|---|
| Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (if such acquisition could compromise ability to control disease agents in humans, veterinary medicine, or agriculture) | ✓ | ✓ | | | ✓ | | | |
| Cloning of toxin molecules with an LD50 of less than 100 ng/kg body weight | | | ✓ | | ✓ | | | |
| Human gene transfer | | | | ✓ | ✓ | | | ✓ |
| Using risk group 2, 3, 4 or restricted agents as host-vector systems | | | | | ✓ | | | |
| Exposing any animal to rDNA modified microbes | | | | | ✓ | | ✓ | |
| DNA from Risk Group 2, 3, 4 or restricted agents is cloned into a nonpathogenic prokaryotic or lower eukaryotic host vector system | | | | | ✓ | | | |
| The use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture | | | | | ✓ | | | |

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# LABORATORY SELF-INSPECTION FORM – CHEAT SHEET

**Instructions:**
This document does not need to be read in its entirety. This document serves as a reference tool for Laboratory Self-Inspections. For each inspection item, EHSO has provided the safety reason, how the lab can comply, and the source of the item. Links are provided to specific forms, pages, manuals, etc. Please contact EHSO (404-727-5922) if you have further questions or notice any broken links.

| # | Item | What's the Safety Reason? | How can I comply? | Source |
|---|---|---|---|---|
| 1.0 | **GENERAL SAFETY** | | | |
| | **Administrative Controls** | | | |
| 1.1 | The external lab doors are posted with EHSO provided signage that reflects the hazards present in the lab and displays current emergency contact information. | This information is very important, not only for the researchers that work in the laboratory, but also for people who work outside of the lab. For example, emergency responders such as firemen need to know what hazards they may encounter when responding to a fire alarm or other emergency. In case of power failure or freezer failure, precious samples may be ruined if lab emergency contacts are not up-to-date and no one can be contacted. | To request a new sign or update an existing sign, complete the Lab Signage Requirements Form and email it to labsign@emory.edu. | OSHA Blood Borne Pathogens Standard 29CFR 1910.1030(e)(2)(C-D)<br><br>Emory's Bloodborne Pathogen Exposure Control Plan<br><br>Emory University's Chemical Hygiene Plan<br><br>Emory University Biosafety Manual |
| 1.2 | All lab personnel are able to verify current training for applicable EHSO training courses. | While lab personnel are performing research they will likely use instrumentation, materials and reagents that have the potential to harm themselves, their co-workers and/or the environment. It is important to spend time outside off the research project learning the safety standards of the discipline and workplace to insure everyone's good health and safety. | Click here to visit the EHSO Training site to see which courses are applicable to your work. You can also use our Training Tracking Sheet to enter your trainings to keep you up to date. | OSHA BBP Standard 29CFR 1910.1030(g)(2)(i-iv)<br><br>NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules<br><br>CDC/NIH: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition<br><br>OSHA Lab Standard<br><br>Emory's Bloodborne Pathogen |

# 4. Why do we need a reference sheet for r-DNA?

- NIH Guidelines are comprehensive and not always easy to understand.
- Investigators must disclose not only the agents but details about the procedures.
- Providing examples for the guidelines facilitates applicability

# 5. Who will benefit from the use of a reference document for r-DNA?

- The investigator/ lab contact
- EHSO-Research Safety
- Institutional Biosafety Committee

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# BIOSAFETY NOTICE OF INTENT FORM

**Notes:**

- Protocol approval is valid for three years but must be updated annually using the Biosafety Protocol – Annual Update, Amendment & Termination Form.
- At the end of three years, a new Notice of Intent must be submitted for extension of an approval.
- Whenever you request an amendment to IACUC, IRB, or chemical safety (to add/delete agents or personnel or to change a procedure), you must apply for a Biosafety Protocol Amendment using the Biosafety Protocol – Annual Update, Amendment & Termination Form.
- To view submission deadlines and meeting dates for the Institutional Biosafety Committee (IBC) and Research Health & Safety Committee (RHSC) refer to the IBC and RHSC Meeting Schedule.
- For your reference, a Sample of a Completed Notice of Intent is available on the EHSO website.

**Instructions:**

- In the first table, mark all sections that will be applicable to your protocol. Go to those applicable sections and answer all questions.
- When answering these questions, think about how your project could affect those not directly involved in the research procedures (i.e., custodial staff, animal care staff, technicians, etc.) and the measures that should be put into place to protect such individuals.
- Complete this form electronically and save as *Bio NOI_PI name*. (Example: Bio NOI_JDoe)
- Access and complete referenced forms as applicable.
- Submit the electronic documents to Biosafety via biosafe@emory.edu. To authenticate, the PI must send from his/her Emory mail account.

| SECTION (CTRL + Click to Hyperlink to Applicable Section) | APPLICABLE | NOT APPLICABLE |
|---|---|---|
| Section 1: Administrative | ☐ | ☒ |
| Section 2: Project Description | ☐ | ☒ |
| Section 3: Human & Non-Human Primate Material | ☐ | ☐ |
| Section 4: Microorganisms / Infectious Material | ☐ | ☐ |
| Section 5: Animals | ☐ | ☐ |
| Section 6: Arthropods | ☐ | ☐ |
| Section 7: Plants | ☐ | ☐ |
| Section 8: Biological Toxins | ☐ | ☐ |
| Section 9: Nanoparticles | ☐ | ☐ |
| Section 10: Recombinant & Synthetic Nucleic Acid Molecules | ☐ | ☐ |
| Section 11: Dual-Use Screening | ☐ | ☒ |
| Section 12: Progress Report | ☐ | ☒ |
| Section 13: Investigator's Assurance | ☐ | ☒ |

# 6. Components of the r-DNA Cheat Sheet

- Cites the Section from the NIH Guidelines
- What type of review is required
- Citation in the Notice of Intent (includes verbiage from the NIH Guidelines)
- Examples were benchmarked from multiple academic sources, the NIH Guidelines document, and the OBA website

# Section III A of the NIH Guideline

| Section | Review Required | Question on NOI Form / Mirrors NIH Guidelines | Explanation and Examples |
|---|---|---|---|
| III-A | IBC Approval, RAC Review, NIH Director Approval | (10.2) Do any rDNA experiments involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (if such acquisition could compromise ability to control disease agents in humans, veterinary medicine, or agriculture)? | These experiments are considered “Major Actions”.<br>Includes:<br>Modifying *Mycobacterium tuberculosis* so that it can no longer be treated with the Rifampin.<br>Cloning a gene for Erythromycin resistance into *Borrelia burgdofieri*;<br>Cloning a gene for Chloramphenicol resistance into *Rickettsia typhi*;<br>Cloning a gene for Pyrimethamine resistance into *Toxoplasma gondi*;<br><br>Does not include: Adding an ampicillin resistance gene into a plasmid for selection purposes. |

# Section III B of the NIH Guideline

| Section | Review Required | Question on NOI Form | Explanation and Examples |
|---|---|---|---|
| III-B-1 | OBA and IBC Approval | (10.3) Do any rDNA experiments involve the cloning of toxin molecules with an LD50 of less than 100 ng/kg body weight? | Includes: Cloning experiments for the biosynthesis of toxin molecules lethal for vertebrates.<br>Examples:<br>-*Botulinum toxin* D (LD50=0.4 ng/kg),<br>-*Staphylococcal enterotoxin* B<br>-Ricin<br>-Tetrodotoxin<br>-Tetanus toxin<br>-Diphtheria toxin<br>-*Shigella dysenteriae* neurotoxin<br>-Pertussis toxin<br>Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. |

# Section III C of the NIH Guideline

| Section | Review Required | Question on NOI Form | Explanation and Examples |
|---|---|---|---|
| III-C-1 | IBC & IRB Approval, NIH/ORDA Registration | (10.5) Do any rDNA experiments involve the deliberate transfer of rDNA, into one or more human research participants? | -Exposing humans to recombinant vaccines, lentiviral vectors (HIV vaccines), adeno-associated viral vectors, other retroviral vectors<br>-Deliberate transfer of cell lines to humans<br>-Introduction of Markers in a cell to compensate for defective genes, to produce a potentially therapeutic substance, trigger the immune system to fight disease<br>-Liposome use and other methods of delivery<br>-Synthetic nucleic acid molecules that:<br>1. Contain more than 100 nucleotides, or<br>2. Possess biological properties that enable integration into the genome, e.g. cis elements involved in integration, or<br>3. Have potential to replicate in a cell, or<br>4. Can be translated or transcribed<br><br>-Delivery of shRNA in a plasmid |

# Section III D of the NIH Guideline

| Section | Review Required | Question on NOI Form | Explanation and Examples |
|---|---|---|---|
| III-D-1 | IBC Approval | (10.6) Using risk group 2, 3, 4 or restricted agents as host-vector systems?<br><br>(10.7) Exposing any animal to rDNA modified microbes? | The NIH Guideline provides risk group classification.<br><br>Does not include: exposing mice to human cells that have been transfected with a lentiviral vector previously. |

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| | | (10.7) Exposing any animal to rDNA modified microbes? | Does not include: exposing mice to human cells that have been transfected with a lentiviral vector previously. |
| III-D-2 | | (10.8) DNA from Risk Group 2, 3, 4 or restricted agents is cloned into a nonpathogenic prokaryotic or lower eukaryotic host vector system? | Cloning DNA from *Mycobacterium tuberculosis* into a plasmid and expressing the gene in *E. coli* (non-K12 derived strain). |

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| III-D-2 | | (10.8) DNA from Risk Group 2, 3, 4 or restricted agents is cloned into a nonpathogenic prokaryotic or lower eukaryotic host vector system? | Cloning DNA from *Mycobacterium tuberculosis* into a plasmid and expressing the gene in *E. coli* (non-K12 derived strain). |
| III-D-3 | | (10.9) The use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems? | A helper virus is a virus used to aid in the replication of a viral vector. |

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| | | (10.7) Exposing any animal to rDNA modified microbes? | Does not include: exposing mice to human cells that have been transfected with a lentiviral vector previously. |
| III-D-2 | | (10.8) DNA from Risk Group 2, 3, 4 or restricted agents is cloned into a nonpathogenic prokaryotic or lower eukaryotic host vector system? | Cloning DNA from *Mycobacterium tuberculosis* into a plasmid and expressing the gene in *E. coli* (non-K12 derived strain). |
| III-D-3 | | (10.9) The use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems? | A helper virus is a virus used to aid in the replication of a viral vector. |
| III-D-4 | | (10.10) The creation of, (10.11) breeding of, (10.12) experiments with, (10.13) purchase or transfer of transgenic rodents, or other small animals | Includes: Creating transgenic fruit flies, monkeys, zebrafish, pigs, mosquitos |

# Section III D of the NIH Guideline

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| | | (10.7) Exposing any animal to rDNA modified microbes? | Does not include: exposing mice to human cells that have been transfected with a lentiviral vector previously |
| III-D-2 | | (10.8) DNA from Risk Group 2, 3, 4 or restricted agents is cloned into a nonpathogenic prokaryotic or lower eukaryotic host vector system? | Cloning DNA from *Mycobacterium tuberculosis* into a plasmid and expressing the gene in *E. coli* (non-K12 derived strain) |
| III-D-3 | | (10.9) The use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems? | A helper virus is a virus used to aid in the replication of a viral vector |
| III-D-4 | | (10.10) The creation of, (10.11) breeding of, (10.12) experiments with, (10.13) purchase or transfer of transgenic rodents, or other small animals | Includes: Creating transgenic fruit flies, monkeys, zebrafish, pigs, mosquitos |
| III-D-5 | | (10.14) Plant research | Exotic infectious agents, synthetic nucleic acid molecule techniques, cloned genomes of exotic agents, cloned toxins, pathogens of insects |
| III-D-6 | | (10.15) Volume | |
| III-D-7 | | (10.16) The use of influenza viruses? | Any rDNA work with flu |

# Special cases (many of them!)

- **Appendix B-V. Animal Viral Etiologic Agents in Common Use**
  - *Murine leukemia virus*
    - Not associated with disease in healthy adult humans;
    - Commonly used in laboratory experimental work.

# Special cases (many of them!)

- **Section III D-4**
  - *Caenorhabditis elegans*
    - Free-living, non-parasitic soil nematode
    - Commonly used in laboratory experimental work to generate mutants
  - Lamprey

National Institutes of Health
Office of Biotechnology Activities

# Animal experiments covered under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*

| ACTIVITY | MINIMUM BSL | SECTION |
|---|---|---|
| **CREATION OF TRANSGENIC ANIMALS** | | |
| Creation of transgenic rodents | BL1 | III-E-3 |
| Creation of transgenic rodents | BL2 or higher | III-D-4-b |
| Creation of transgenic animals other than rodents | BL1/BL1-N | III-D-4-a |
| Creation of transgenic animals other than rodents | BL2/BL2-N or higher | III-D-4-b |
| Creation of recombinant DNA modified arthropods | BL1 | III-D-4-a |
| Creation of recombinant DNA modified arthropods | BL2 or higher | III-D-4-b |
| Creation of knock-out rodents | BL1 | III-E-3 |
| Creation of knock-out rodents | BL2 or higher | III-D-4-b |
| **BREEDING OF TRANSGENIC ANIMALS** | | |
| Breeding rodents from one strain (propagation/colony maintenance) | BL1 | Exempt |
| Breeding rodents from one strain (propagation/colony maintenance) | BL2 or higher | III-D-4-b |
| Breeding rodents from two strains (generating a new strain) providing neither parental 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 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. | BL1 | Exempt (Appendix C-VII) |
| Breeding rodents from two strains (generating new strain) if the parental 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); 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. | BL1 | III-E-3 |
| Breeding rodents from two strains (generating new strain) | BL2 or higher | III-D-4 |
| Breeding of transgenic animals other than rodents | BL1 | III-D-4 |
| Breeding of transgenic animals other than rodents | BL2 or higher | III-D-4 |
| Breeding of recombinant DNA modified arthropods | BL1 | III-D-4 |
| Breeding of recombinant DNA modified arthropods | BL2 or higher | III-D-4 |
| Breeding of knockout rodents from one strain (propagation/ colony maintenance) | BL1 | Exempt |
| Breeding of knockout rodents from two strains (propagation/colony maintenance) | BL2 or higher | III-D-4 |
| Breeding of knockouts from two strains (generating new strain) providing neither parental 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 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. | BL1 | Exempt (Appendix C-VII) |

http://osp.od.nih.gov/sites/default/files/TableAnimalResearchCoveredUnderNIHGuidelines-Aug2011.pdf

# Section III E of the NIH Guideline

| Section | Review Required | Question on NOI Form | Explanation and Examples |
|---|---|---|---|
| III-E | IBC Notice | 10.33 – 10.37:<br>(10.33) Not fall under Sections III-A, B, C, D, F<br><br>(10.34) Involve the formation of rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus<br><br>(10.35) Involve whole plants<br><br>(10.36) Involve the creation of transgenic or knock out rodents in which the animal's genome has been altered by stable introduction of rDNA or derived from, into the germ-line<br><br>(10.37) Involve breeding of rodents from 2 strains to generate a new strain or knock-out that can be housed at ABSL1 and does not fall under the exempt Appendix C-VIII | All other research…<br>Experiments in which the components are nonpathogenic prokaryotes and non-pathogenic lower eukaryotes |

# 7 Plans for roll-out

- Tested with investigators
- Obtain feedback from Investigators and IBC
- Revise accordingly

# 8 Ongoing Process...

- Feedback from investigators
- New technology
- New Questions from Investigators
- Revision on NIH Guidelines

# Other resources available to investigators when classifying their research under the NIH Guidelines

- Emory University
  - Institutional Review Guidelines
    IACUC, IRB, Chemical Safety, Radiation Safety
  - Use of viral Vectors in vivo
  - Biosafety Officer
- NIH
  - OBA website
  - FAQs
  - NIH Guidelines and the use of animals (Very Useful!)

# Questions

Contact Information

Esmeralda Meyer & Kalpana Rengarajan
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Emory University