13 Research Involving Recombinant and Synthetic Nucleic Acid Molecules

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines) are a set of research requirements for any institutions receiving funding from the NIH. As Yale receives NIH funding, the NIH Guidelines must be followed by every PI at the University, regardless of their source of funding, which is a central condition of the NIH funding agreement.

13.1 Principal Investigator Responsibilities Under the NIH Guidelines

Principal Investigators are responsible for full compliance with the NIH Guidelines. Core responsibilities include:

- Identifying if their research is subject to the NIH Guidelines
- Determining which section of the Guidelines are applicable to their work
- Proposing an appropriate biocontainment level (BSL) to ensure that the research is performed safely
- Obtaining authorization from the Yale Biological Safety Committee prior to starting any research that is subject to the NIH Guidelines

Non-exempt recombinant DNA experiments, which must be registered and approved prior to initiation, are defined in the left hand column of the Registration and Approval of rDNA Experiments document found in appendix E of the Biosafety Manual. These include experiments that involve:

- The deliberately transfer a drug resistance trait to a microorganism
- Human gene transfer
- cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of <100 ug/kg body weight
- human or animal pathogens as host-vector systems
- Cloning of DNA or RNA from certain pathogens
- Recombinant DNA work in whole animals or plants
- Large-scale DNA work with more than 10 liters of culture

More specific information on the classification of non-exempt recombinant DNA experiments is provided below. Please review the examples of non-exempt experiments and ensure that your laboratory is registered for proposed rDNA experiments prior to initiation where applicable. The examples provided include work that is commonly miss-classified by investigators.

You can also review the Yale recombinant DNA registration form which is located in Appendix E of the Manual. The end of this Section of the Biosafety Manual includes relevant fact sheets from the NIH Office of Biotechnology Activities that are useful resources for Principal Investigators. The following table from the NIH provides an overview of rDNA experiments and the level of review required for each category.

13.1.1 Level of Review for rDNA Experiments

Level of Review	Example of rDNA Experiment	NIH Guideline Section
IBC, RAC review and NIH Director Approval	Experiments that compromise the control of disease agents in medicine through deliberate transfer or a drug resistance trait	III-A
IBC Approval and NIH review	Deliberate formation of rDNA containing genes coding for a toxin with an LD50 < 100 ng/kg	III-B
IBC and IRB Approval and NIH review	Introduction of rDNA into human subjects (Human gene transfer)	III-C
IBC Approval before initiation	Wide range (rDNA exp's involving pathogens, defective vectors, animals, plants, large scale)	III-D
IBC Notice at initiation	Creating transgenic rodents, low risk rDNA Plant experiments	III-E
Exempt – registration not required	Those do not represent a significant risk to health or the environment	III-F

Principal Investigators are responsible for ensuring that their researchers are trained in safe work practices and that everyone working in the laboratory is aware of the emergency response procedures that must be followed after an incident. Staff must also be alerted of the rationale for any special medical surveillance restrictions or immunizations. Once work has been initiated, Principal Investigators must also supervise and monitor staff for their adherence to safety protocols.

If the scope of research changes significantly, the Principal Investigator must update their rDNA protocol with the Yale Biological Safety Committee and await confirmation in writing that the protocol change has been approved.

It is also important that Principal Investigators report any significant problem, such as a violation of the NIH Guidelines or any significant research related accidents, exposures or illnesses to the Yale Biological Safety Committee and the Environmental Health and Safety Office (both can be reached at 203-785-3550) and the NIH Office of Biotechnology Activities (301-496-9838).

13.2 Overview of Recombinant DNA Experiments Covered by the NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules (NIH Guidelines), March 2013

The NIH Guidelines describe experiments that must be registered and approved by Yale University's Institutional Biological Safety Committee (the Yale Biological Safety Committee) and those experiments that are exempt from the NIH Guidelines and do not require registration. For institutions that receive funding from the NIH for molecular biology research, the NIH Guidelines become a condition of the receipt of funding. In order to continue to receive funding from the NIH, ALL researchers at Yale University MUST COMPLY with the NIH Guidelines, regardless of funding source. Failure to comply with the NIH Guidelines can lead to suspension of research privileges. Systemic failure to comply with the NIH Guidelines may result in the freezing of funds directed to Yale by the NIH.

The phrase rDNA is used throughout this document. rDNA refers to recombinant and synthetic nucleic acid molecules to reflect changes in the March 2013 version of the NIH Guidelines.

13.2.1 Categories of rDNA Work That Require Registration

1. Cloning a therapeutic antibiotic resistance gene into a human, animal or plant pathogen, if the transfer could compromise the ability to treat or control the disease. (Section III-A-1)

Note: Registration with the Yale Biological Safety Committee is still required even if:

- this drug resistance is acquired naturally;
- the transferred resistance gene is related to a drug that is an end of the line alternative treatment (2nd, 3rd, 4th, or 5th line drug);
- the drug was used years ago, but is not the preferred treatment today (it may be the only treatment in developing countries);
- the drug is only used to treatment a very small portion of the population (i.e. those with specific contraindications to front line drugs)
- Working with antibiotic resistance strains of pathogens also require registration (even if you did not create them)

Examples:

- Cloning a gene for Erythromycin resistance into Borrelia burgdorferi
- Cloning a gene for Chloramphenicol resistance into Rickettsia typhi
- Cloning a gene for Pyrimethamine resistance into Toxoplasma gondii
- Cloning a gene for Rifampin resistance into Mycobacterium tuberculosis

Caution:

- Be careful when using old plasmids for cloning experiments involving pathogens. Many of the old plasmids carry genes for antibiotics that have been used therapeutically or are related to front line drugs.
  - Avoid using these plasmids when working with related pathogens;
  - Verify that the antibiotic resistance gene is not in a location on the plasmid that can be transferred to the pathogen via a double cross over event.

Websites: NIH OBA FAQ – Major Actions
http://oba.od.nih.gov/oba/IBC/MAJOR_ACTION_FAQS_MARCH_2008.pdf
http://oba.od.nih.gov/oba/faqs/Major_Actions_FAQ-Sept-2012.pdf

2. **Cloning DNA encoding for a low LD50 toxin or work with vectors that express toxins with a low LD50 (< 100 ug/kg body weight). (Section III-B-1)**

   Examples of toxins with low LD50's are:

   - Botulinum toxin
   - Staphylococcal enterotoxin B
   - Tetrodotoxin
   - Clostridium tetanus toxin

   Websites: Yale EHS Table of Toxins:
   http://ehs.yale.edu/recombinant-dna-toxin-experiments

   Univ. of Florida – Toxin Lists: http://www.ehs.ufl.edu/Bio/toxin.htm

3. **Human Gene Transfer Experiments (Section III-C-1)**

   The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the NIH Guidelines.

   This includes the transfer of DNA with defective viral vectors, such as retroviral, adenoviral and lentiviral vectors, along with the use of liposomes and other methods of delivery.

   Human gene transfer experiments with synthetic nucleic acid molecules also require registration if any of the following criteria are met: The synthetic nucleic acid molecules:

   - Contain more than 100 nucleotides; or
   - Possess biological properties that enable integration into the genome (e.g. cis elements involved integration); or
   - Have the potential to replicate in a cell; or
   - Can be translated or transcribed

   These experiments require registration with the NIH Office of Biotechnology Activities and also approval from the Yale IRB or Human Investigations Committee and the U.S. Food and Drug Administration.

   Website: Yale HGT experiments: http://ehs.yale.edu/human-gene-transfer-clinical-trials

   NIH OBA Frequently Asked Questions on Human Gene Transfer Experiments:
   http://oba.od.nih.gov/rdna/rdna_faq.html

4. **rDNA Experiments involving the use of a human, animal or plant pathogen (whether the recombinant or synthetic nucleic acid molecules originated from your lab or another). (Section III-D-1, III-D-2, III-D-3)**

Examples:

- Cloning a gene into a pathogen (i.e. expressing a gene into VSV, Vaccinia Virus, Tobacco Mosaic Virus, Mouse Cytomegalovirus)
- Cloning a pathogen into a lower eukaryotic or prokaryotic cell;
- Using a defective pathogen vector with or without helper virus in cell culture or animal experiments, examples include:
  - Poxviruses (Vaccinia)
  - Herpesvirus vectors (HSV)
  - Lentivirus vectors (HIV, FIV based)
  - Retroviruses (murine retroviruses)
  - Adenoviruses
  - Adeno-Associated Virus vectors
  - Vesicular Stomatitis Virus vectors
  - Sindbis Virus vectors

A helpful guidance document developed by Stanford University for the classification of experiments involving defective viral vectors can be accessed at the following website:
http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.

5. **Cloning DNA or RNA from Risk Group 3 or Risk Group 4 human pathogens, restricted animal or plant pathogens, or Select Agents. (Section III-D-2)**

   Any rDNA experiments with these materials must be registered with and approved by the Yale IBC, even if you are working with only one base pair of DNA or RNA from these agents.

   Websites: NIH Appendix B (Risk Groups)
   http://oba.od.nih.gov/rdna/rdna.html
   http://oba.od.nih.gov/oba/rac/Guidelines/APPENDIX_B.htm

   American Biological Safety Association Risk Group Classifications of Etiologic Agents:
   http://www.absa.org/riskgroups/index.html

   List of restricted animal pathogens.
   http://www.cdc.gov/od/ohs/biosfty/bmbl5/sections/AppendixD.pdf

   Select Agent List:
   http://www.cdc.gov/od/sap/docs/salist.pdf
   http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html

6. **rDNA Experiments involving whole animals, plants, and arthropods (and insects)… (Section III-D-4, III-D-5, III-E-3)**

   Experiments in this category include:

   - Experiments involving toxins, pathogens, defective vectors, and other genetically modified materials used in animal, plants or insects.
   - Creation of transgenic animals
     - Mice, rats
     - Zebrafish
     - Drosophila, butterflies
     - Other

   Note: For rodents only, the purchase or transfer of transgenic rodents is exempt from the NIH rDNA Guidelines and does not require registration (if the transgene used does not code for a toxic, virulent or oncogenic sequence).

Purchase is defined as buying a transgenic rodent that has been created by another entity outside of your laboratory.

The transfer of a transgenic rodent to your laboratory is also exempt (provided the transgene doesn't code for toxic, oncogenic or potentially harmful gene).

Transfer is defined as the acquisition into your research lab of a transgenic animal created (made) by another entity.

Note: In each case above, you may have designed or created the gene that has been inserted into the developing embryo of the transgenic rodent, but if you are not the group that has performed the actual procedure (i.e. the lab that inserted the gene into the embryo), you are exempt from the rDNA Guidelines. **If your lab will insert the gene into the embryo, you must register this work.**

Knock-out Animals

Knock-out (gene silencing, gene ablation, etc.) rodents are exempt from the NIH Guidelines as long as the method to generate the knock-out animal does not leave any "new" genetic material behind in the genome after the procedure. If DNA from the molecule used to create the knock-out is permanently inserted into the genome, the experiment will require registration with the Yale Biological Safety Committee.

Exemption for Breeding Transgenic Rodents

Note: Generation of transgenic **rodents** by breeding to create a new strain shall be EXEMPT from the NIH Guidelines if the following criteria are met.

- Both parental rodents can be housed under BSL1 containment; AND
- Neither parental transgenic rodent contains the following genetic modifications:
  - Incorporation of more than 50% of the genome of an exogenous eukaryotic virus from a single family of viruses; OR
  - Incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); AND
- The transgenic rodent that results from this breeding is not expected to contain more than 50% of an exogenous viral genome from a single family of viruses.

This exemption DOES NOT pertain to other transgenic animals such as zebrafish, drosophila, rabbits, pigs, etc. It also DOES NOT pertain to transgenic experiments involving plants.

7. Large Scale rDNA Experiments (Section III-D-6)

Any rDNA experiments at any level or Risk Group, including exempt and non-exempt experiments that generate a volume of culture that is in excess of 10 liters require registration with the Yale Biological Safety Committee.

Note: Work with 10 L may be in a single fermentation vessel (10 L or larger) or a series of flasks whose aggregate volume would exceed 10 L.

Examples include: Growing up five 2 L flasks of E. coli K-12 cultures expressing your gene of interest. Growing 10 L of Sacchryomyces cerevisiae in a fermentation apparatus to get a sufficient yield of the desired protein.

13.3 Synthetic Nucleic Acid Experiments:

The NIH has changed the title of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) to incorporate research with synthetic nucleic acids. The new title of the NIH Guidelines effective March 5, 2013, will be The *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acids*. The change in title was made to reflect that NIH funded locations will have to now review rDNA experiments that involve synthetic nucleic acids. A fact sheet

from the NIH on research with synthetic nucleic acids can be accessed by opening the PDF located at http://oba.od.nih.gov/oba/faqs/Synthetic_FAQs-Sept-2012.pdf.

13.3.1 Synthetic Nucleic Acid Experiments that are covered by the NIH Guidelines:

Research that presents biosafety risks equivalent to rDNA research that is subject to the NIH Guidelines such as research with a genetically modified virus or a vector derived solely by synthetic techniques.

- The molecules can replicate
- They can generate nucleic acids that can replicate in a living cell
- They can integrate into a host cell's DNA
- They produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms/kilogram body weight
- They synthesize an organism that doesn't occur naturally outside of a laboratory setting (i.e. 1918 H1N1 Influenza)

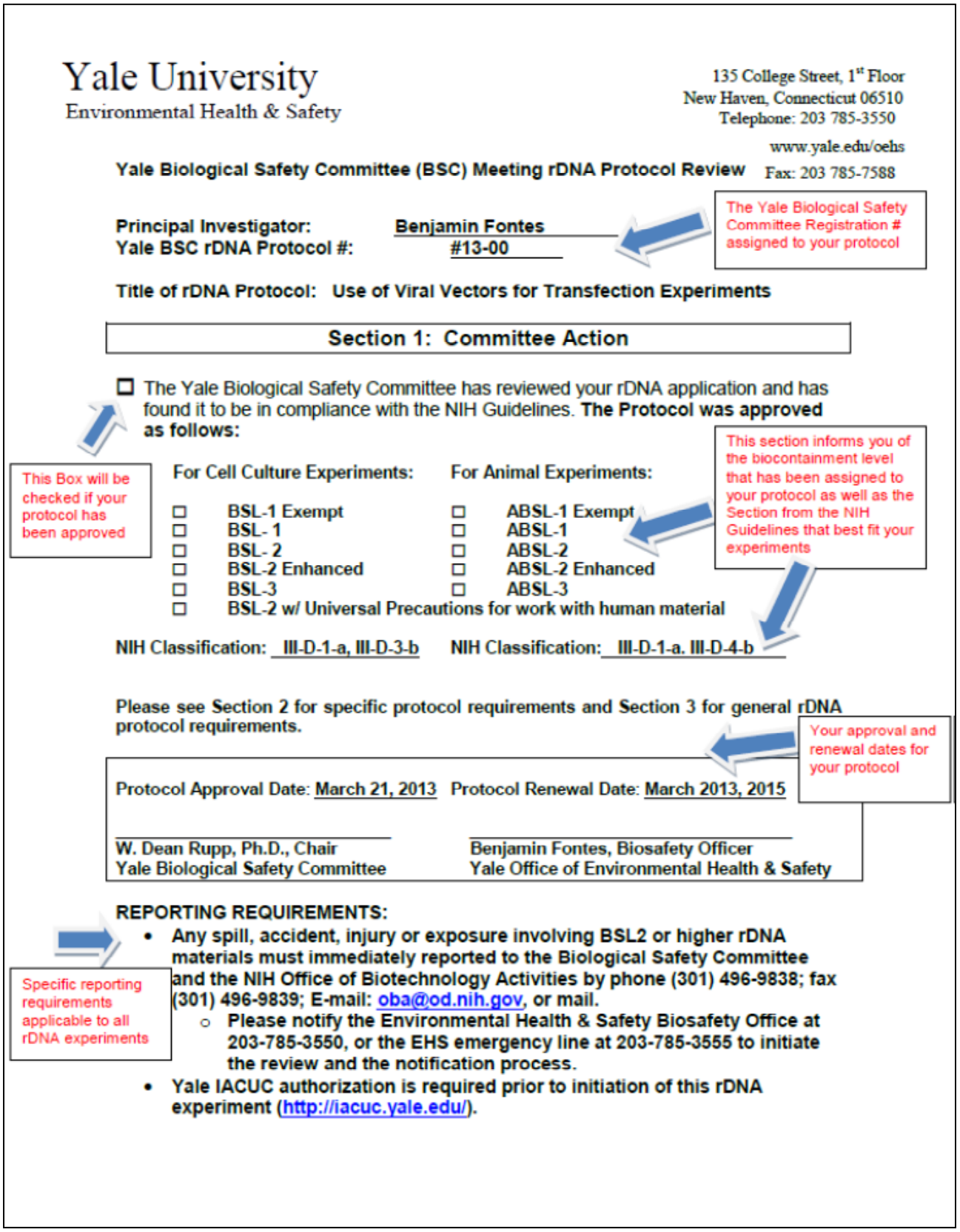
13.3.2 Synthetic Nucleic Acid Experiments that are EXEMPT from the NIH Guidelines:

- Introduction of certain synthetic nucleic acids into a biological system that is not expected to present a biosafety risk that requires review by the IBC
- Introduction of synthetic nucleic acid molecules into biological systems akin to processes of nucleic acid transfer that already occur in nature.
- Experiments with synthetic nucleic acid molecules that are not contained in cells, organisms or viruses
- Those synthetic nucleic acid molecules that meet the following criteria shall be exempt:
    - Those that can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g. oligonucleotides or other synthetic that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and
    - Those that are not designed to integrate into DNA, and
    - Those that do not produce a toxin that is lethal for vertebrates at and LD50 of less than 100 nanograms per kilogram body weight.

  An example of an exempt synthetic nucleic acid molecule is a synthetic short- interfering RNA (siRNA) that targets an HIV viral protein required for transcription activation, even if this siRNA is injected into animals or used in cell culture.

- Also exempt are those synthetic nucleic acid molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists outside of a laboratory setting. (Research with nucleic acid sequences for organisms that do not currently exist in nature outside of the laboratory setting would not be exempt (e.g. an identical copy of the 1918 H1N1 influenza virus)
- The chemical synthesis of nucleic acids (the NIH Guidelines only apply once synthetic nucleic acids are placed in a biological system).

Yale Biological Safety Committee Meeting rDNA Protocol Review



Yale University
Environmental Health & Safety

135 College Street, 1st Floor
New Haven, Connecticut 06510
Telephone: 203 785-3550
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Fax: 203 785-7588

Yale Biological Safety Committee (BSC) Meeting rDNA Protocol Review

The Yale Biological Safety Committee Registration # assigned to your protocol

Principal Investigator: Benjamin Fontes
Yale BSC rDNA Protocol #: #13-00

Title of rDNA Protocol: Use of Viral Vectors for Transfection Experiments

Section 1: Committee Action

☐ The Yale Biological Safety Committee has reviewed your rDNA application and has found it to be in compliance with the NIH Guidelines. **The Protocol was approved as follows:**

This Box will be checked if your protocol has been approved

This section informs you of the biocontainment level that has been assigned to your protocol as well as the Section from the NIH Guidelines that best fit your experiments

For Cell Culture Experiments:	For Animal Experiments:
☐ BSL-1 Exempt	☐ ABSL-1 Exempt
☐ BSL- 1	☐ ABSL-1
☐ BSL- 2	☐ ABSL-2
☐ BSL-2 Enhanced	☐ ABSL-2 Enhanced
☐ BSL-3	☐ ABSL-3
☐ BSL-2 w/ Universal Precautions for work with human material	

NIH Classification: III-D-1-a, III-D-3-b **NIH Classification:** III-D-1-a. III-D-4-b

Please see Section 2 for specific protocol requirements and Section 3 for general rDNA protocol requirements.

Your approval and renewal dates for your protocol

Protocol Approval Date: March 21, 2013 **Protocol Renewal Date:** March 2013, 2015

W. Dean Rupp, Ph.D., Chair
Yale Biological Safety Committee

Benjamin Fontes, Biosafety Officer
Yale Office of Environmental Health & Safety

REPORTING REQUIREMENTS:

Specific reporting requirements applicable to all rDNA experiments

- **Any spill, accident, injury or exposure involving BSL2 or higher rDNA materials must immediately reported to the Biological Safety Committee and the NIH Office of Biotechnology Activities by phone (301) 496-9838; fax (301) 496-9839; E-mail: oba@od.nih.gov, or mail.**
  - **Please notify the Environmental Health & Safety Biosafety Office at 203-785-3550, or the EHS emergency line at 203-785-3555 to initiate the review and the notification process.**
- **Yale IACUC authorization is required prior to initiation of this rDNA experiment (http://iacuc.yale.edu/).**

☐ The Yale Biological Safety Committee did not approve your protocol at the meeting due to the following:

This box checked if your protocol has not been approved. The specific reason will also be indicted.

- ☐ Incomplete or illegible rDNA Protocol Application
- ☐ Additional information requested, please send the following information:
- ☐ Insufficient training, please complete the following:
- ☐ Lab facility and work practice inspection required, please contact the Biosafety Officer at 785-3550 to schedule an inspection.
- ☐ Other: **State of CT Human Pathogen Authorization Required**

Enclosed is an approved registration document for your files. The general requirements for all approved rDNA protocols are included at the end of the approval letter. Your specific approval requirements are checked off in the following Section.

Additional containment requirements for your protocol will be indicated in the following

Section 2: Protocol Specific Requirements

☐ There are no additional protocol specific requirements for this project. Please refer to Section 3 for the general rDNA protocol requirements for this experiment.

☐ **Additional BSL2 Cell Culture Precautions:**

- The facility should be self-contained, that is, all equipment needed for the experiment must be located in your laboratory;
- All work must be done in a biosafety cabinet;
- Full face protection is required for any procedure that may involve the manipulation of biohazards that could involve splash or splatter to the face;
- Vacuum lines must be protected by filters;
- Eliminate sharps, such as needles or glass Pasteur pipettes, from your experiments;
- Use containment accessories (sealed tubes and safety canisters or rotors with sealed O-rings) for centrifugation;
- A lab coat or back-fastening gown and gloves must be worn (face protection is required for procedures that may involve splash or splatter); and
- Cultures should be monitored for replication competent viruses.

Required criteria for animal rDNA experiments start in this section

☐ Work in animals is approved at Animal Biosafety Level 2 with the requirement that researchers schedule a meeting with Biosafety and representatives from the Yale Animal Resources Center prior to initiating this work in animals. Please contact Ms. Heidi Voegeli at 785-3641 to schedule a meeting.

☐ An approved Institutional Animal Care and Use Committee (IACUC) Protocol is required for this experiment.

☐ A United States Department of Agriculture (USDA) permit is required to obtain this agent from a group outside of your laboratory (including other Yale Investigators).

☐ Researchers handling this agent must avoid contact with livestock (hooved animals) for at least 5 days after last handling the agent in cell culture or 5 days after last handling animals that have been inoculated with the agent.

☐ A successful lab inspection is required prior to the initiation of this experiment. Please contact the Biosafety Office at 785-3550 to schedule the inspection.

☐ New BSL2+ and BSL3 researchers also require approval by the BL3 Subcommittee before initiating independent BSL2+ or BL3 work.

☐ Personnel enrolled in this protocol must complete a work practice observation prior to the initiation of independent research on the project. Please contact the Biosafety Office at 785-3550 to schedule a work practice observation.

☐ This protocol has the following Medical Surveillance Program requirements:

Any special medical requirements will be noted here

☐ As this protocol involves the use of a biohazard, immunosuppressed or immunocompromised individuals may not be assigned to this project without consultation with the Employee Health Physician. Any individual with an immunosuppressive condition may notify the Yale Employee Health Office (432-7978) for a confidential evaluation to request consideration for clearance to work on this project. If the health status of any individual assigned to this project changes after the initiation of the project, the individual must notify the Yale Employee Health Office for a medical clearance review.

☐ Researchers involved in this protocol must be cleared by Yale Employee Health.

☐ This project requires enrollment in the campus serum storage program.

☐ There is an immunization that may be required for enrollment in this protocol and a Medical Consultation with Yale Employee Health is required.

☐ Research involves a reproductive pathogen; men and women of childbearing Age should contact Yale Employee Health at 432-7978 to discuss any concerns they may have regarding work with this agent

☐ This protocol involves the use of a defective Lentivirus vector. As there is a theoretical risk of a false positive result on an HIV antibody test, please immediately document all exposures with this vector with Employee Health.

Section 3: General rDNA Protocol Requirements

The following is required for all rDNA protocols approved by the Yale Biological Safety Committee.

General requirements for all rDNA Protocols are included in this final part of your approval letter

- Please ensure that all new personnel on the protocol fulfill all applicable OEHS training requirements (i.e. Biosafety, Chemical Safety, initial and annual Bloodborne Pathogens training) before initiating work on the protocol.
- It is the responsibility of the Principal Investigator to train new personnel before they begin work
- All new personnel must also demonstrate proficiency with the required work safety practices, engineering controls, and the laboratory techniques utilized in the experiment. The Principal Investigator must ensure that all researchers on this protocol have sufficient experience prior to working independently on this project.
- Should you wish to add personnel to your project, change the scope or location of your work, you must notify the Biosafety Office at 785-3550.
- A Biosafety representative will visit your laboratory periodically to monitor your facility and procedures, and to answer any questions you may have regarding safety.

NIH OBA Frequently Asked Questions

National Institutes of Health • Office of Biotechnology Activities

FREQUENTLY ASKED QUESTIONS

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

1. **Why has the NIH modified the *NIH Guidelines* to include synthetic nucleic acid molecules?**

   The impetus for amending the *NIH Guidelines* was two-fold:

   (1) recognition that appropriate biosafety containment of an agent is critical regardless of the technology used to generate that agent (i.e., recombinant or synthetic techniques), and
   (2) a recommendation by the National Science Advisory Board for Biosecurity (NSABB) that the United States Government work with the scientific community to ensure that current biosafety guidelines are appropriate, adequate, and easily understood with respect to working with synthetic nucleic acids.

2. **What has changed with respect to the scope of the *NIH Guidelines*?**

   The scope of the *NIH Guidelines* has been modified to cover explicitly certain types of basic and clinical research with nucleic acid molecules created solely by synthetic means. Certain classes of basic and clinical research with synthetic nucleic acids will be exempt.

   The new language in Section I-A of the *NIH Guidelines* states "The purpose of the *NIH Guidelines* is to specify the practices for constructing and handling:

   (i) recombinant nucleic acid molecules,
   (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and
   (iii) cells, organisms, and viruses containing such molecules."

   Throughout the *NIH Guidelines*, the term "recombinant DNA molecules" has been replaced as appropriate with "recombinant or synthetic nucleic acid molecules" which encompasses research with both recombinant and/or synthetic nucleic acids. As a result, the amended *NIH Guidelines* apply (unless otherwise exempted by other sections of the *NIH Guidelines*, e.g. III-F) to research with recombinant or synthetically derived nucleic acids, including those that are chemically or otherwise modified analogs of nucleotides (e.g., morpholinos), or both.

Synthetic Amendment FAQ/September 2012

3. **How are recombinant and synthetic nucleic acids defined under the amended *NIH Guidelines*?**

In the amended *NIH Guidelines*, recombinant and synthetic nucleic acid molecules are defined as:

(i) molecules that a) are constructed by joining nucleic acid molecules and b) can replicate in a living cell (i.e. recombinant nucleic acids);
(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.

4. **What institutions are subject to the amended *NIH Guidelines*?**

Institutions that receive NIH funding for any research involving recombinant or synthetic nucleic acids, unless such research is specifically exempted by the *NIH Guidelines*, must comply. Previously, institutions were required to follow the *NIH Guidelines* only if they received NIH funding for recombinant DNA research. If an institution is receiving NIH funding for only research with synthetic nucleic acids, and that research is covered under the amended *NIH Guidelines*, any research with synthetic nucleic acids or recombinant DNA conducted at the institution, regardless of the source of funding, will need to comply with all of the requirements of the *NIH Guidelines*. For further information on institutional requirements under the *NIH Guidelines* see:
http://oba.od.nih.gov/oba/ibc/FAQs/IBC_Frequently_Asked_Questions7.24.09.pdf/.

Other Federal agencies, including the Departments of Energy, Veterans Affairs, and Agriculture, may have policies in place stating that recombinant or synthetic nucleic acid research conducted by or funded by these agencies must comply with the *NIH Guidelines*, and investigators receiving funding from other Government agencies need to check with those agencies regarding the applicability of the amended *NIH Guidelines*. While the *NIH Guidelines* may not govern all Government funded and privately funded research, it may be used as a tool for the entire research community to understand the potential biosafety implications of this type of research.

5. **When do the requirements under the amended *NIH Guidelines* go into effect?**

The changes are effective March 5, 2013, which is six months from the date of publication. All ongoing and proposed experiments with synthetic nucleic acids newly subject to the amended *NIH Guidelines* need to be registered by the Principal Investigator with the Institutional Biosafety Committee (IBC) by the effective date listed above. The six month time frame was deemed sufficient to allow institutions to develop new procedures, as well as

outreach and training for investigators whose research now will be subject to the amended *NIH Guidelines*.

6. **What basic research with synthetic nucleic acids is covered under the amended *NIH Guidelines*?**

The amended *NIH Guidelines* apply to research with synthetic nucleic acids that presents biosafety risks equivalent to recombinant DNA research that is subject to the *NIH Guidelines*. For example, research with a genetically modified virus or a vector derived solely by synthetic techniques is subject to the amended *NIH Guidelines*.

7. **What impact is this change projected to have on institutions?**

Presently, most research with synthetic nucleic acids also involves the use of recombinant techniques and is already covered by the *NIH Guidelines*. Thus, the NIH anticipates that in the near-term there will not be a significant increase in the amount of research subject to the amended *NIH Guidelines* due to these amendments.

8. **What basic research with synthetic nucleic acids is exempt from the amended *NIH Guidelines*?**

In keeping with the exemptions for recombinant DNA molecules described in Section III-F of the previous version of the *NIH Guidelines*, certain synthetic nucleic acid molecules are exempt from the amended *NIH Guidelines* because:

(1) their introduction into a biological system is not expected to present a biosafety risk that requires review by an IBC, or
(2) the introduction of these nucleic acid molecules into biological systems would be akin to processes of nucleic acid transfer that already occur in nature, so that the appropriate biosafety practices would be the same as those used for the natural organism.

Even though these molecules are exempt from the amended *NIH Guidelines*, other federal and state standards of biosafety may still apply to research with such molecules (e.g., the Centers for Disease Control and Prevention/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*). The exemptions that were in the previous version of the *NIH Guidelines* under Section III-F also will apply to research with synthetic nucleic acids. This includes an exemption for any research with synthetic nucleic acids that are not contained in cells, organisms, or viruses.

In addition, in review of the *NIH Guidelines*, it was recognized that most biosafety risks arise from recombinant molecules because they are designed to replicate or are derived from molecules that can replicate. Synthetic nucleic acid molecules may not be designed to replicate and therefore may not pose the same biosafety risks. Also, a biosafety risk may arise due to the ability of nucleic acid molecules to integrate into the genome or produce a toxin. Therefore, an

exemption was made for research with certain low-biosafety-risk synthetic nucleic acid molecules. Specifically, synthetic nucleic acids molecules that meet the following criteria will be exempt:

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.

An example of an exempt molecule would be research with a synthetic short-interfering RNA (siRNA) that targets an HIV viral protein required for transcription activation, even if this siRNA is injected into animals or used in cell culture.

9. Does the synthesis of a naturally occurring organism fall under the amended *NIH Guidelines*?

The *NIH Guidelines* always have exempted research with recombinant molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, although one or more segments may be a synthetic equivalent. This language exempted nucleic acid sequences that are essentially copies of those found in nature. The language has been modified in the amended *NIH Guidelines* by limiting this exemption to those nucleic acid sequences that exist contemporaneously in nature. The new exemption now applies to those molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists outside of a laboratory setting.

Research with nucleic acid sequences for organisms that do not currently exist in nature outside of the laboratory setting would not be exempt (e.g., an identical copy of the 1918 H1N1 influenza virus).

10. Is the chemical synthesis of nucleic acids subject to the *NIH Guidelines*?

No. The amended *NIH Guidelines* do not cover the chemical synthesis of nucleic acids. While the scope of the amended *NIH Guidelines* refers to "constructing" nucleic acids, the amended *NIH Guidelines* exempts research with nucleic acids that are not contained in cells, organisms, or viruses. Therefore, the chemical synthesis of nucleic acids is exempt. The amended *NIH Guidelines* only apply once synthetic nucleic acids are placed in a biological system.

11. Will recombinant DNA protocols still need to be reviewed by an Institutional Biosafety Committee?

Yes. The existing requirements to review recombinant DNA research that were previously covered under the *NIH Guidelines* have not changed.

12. Does research need to encompass both recombinant and synthetic nucleic acids to be subject to the amended *NIH Guidelines*

No. The amended *NIH Guidelines* apply to research involving either recombinant or synthetic nucleic acid molecules (or both in combination) unless the research is specifically exempted under one or more conditions listed in Section III-F.

13. What clinical research with synthetic nucleic acids is covered under the amended *NIH Guidelines*?

Section III-C-1 of the amended *NIH Guidelines* covers human gene transfer experiments, i.e., research that involves the deliberate transfer into human research participants of either:

- Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
- Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:

    - Contains more than 100 nucleotides; or
    - Possesses biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
    - Have the potential to replicate in a cell; or
    - Can be translated or transcribed.

The above criteria are designed to capture synthetic nucleic acid agents that share common characteristics with current gene transfer agents that are delivered by vectors. The key characteristics of gene transfer agents are their ability to be translated or transcribed, potential for replication, even if engineered to be replication incompetent; and their ability to integrate and persist. In contrast, synthetic oligonucleotides, such as short interfering RNAs or micro-RNAs, that are directly administered or delivered in chemical vehicles, such as nanoparticles, are very small molecules and do not share these same characteristics. This definition of human gene transfer is designed to exclude these constructs from the amended *NIH Guidelines* and instead to only capture agents that would be the equivalent of recombinant gene transfer agents, (e.g., short-hairpin RNA that is delivered in a plasmid, bacterial, or other viral vector).

14. How do the changes affect what gene transfer protocols need to be registered?

OBA does not anticipate a significant increase in the number or types of gene transfer protocols that will need to be registered. At this time, recombinant techniques are predominately used to create vectors, such as a plasmid. It is likely that over the next few years or longer recombinant techniques will continue to be used to create large nucleotides that are designed to integrate, replicate, or be translated or transcribed. However, as synthetic techniques evolve, this may change and it may be more efficient to produce synthetic vectors for gene transfer.

15. How does a risk assessment for research with synthetic nucleic acid molecules differ from a risk assessment for research with recombinant nucleic acid molecules?

The risk assessment framework of the *NIH Guidelines* uses the risk group of the parent organism as a starting point for determining the necessary containment level. For example, genetic modifications of a Risk Group 3 organism (defined as agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available) would generally be carried out at Biosafety Level 3 (BL3) containment but the containment level might be raised or lowered depending on the specific construct and the experimental manipulations.

In most instances, this risk assessment framework can be effectively applied to assess the biosafety risks of experiments with synthetic nucleic acids. However, synthetic biology research has the potential to create complex, novel organisms for which identification of a parent organism may be more difficult or may not be as relevant to the risk assessment as it is with more traditional recombinant organisms. The risk assessment may also be complicated by the limitations in predicting gene function from sequence(s) or the synergistic effects from combining sequences from different sources in a novel context. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest risk group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The risk assessment should also consider that the combination of certain sequences in a new biological context may result in an organism for which the risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through the combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

16. Will the composition and expertise of IBCs need to change to address the changes to the amended *NIH Guidelines*?

No. The required composition of an IBC has not changed under the amended *NIH Guidelines*. In general, when constituting an IBC, institutions should consider their research portfolios and constitute the IBC so that it has the necessary expertise to review research that is subject to the amended *NIH Guidelines*.

17. Where can I get more information about the *NIH Guidelines*?

Further information is available on the NIH OBA website at:

http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

If you have specific questions about the *NIH Guidelines*, please contact the NIH Office of Biotechnology Activities by e-mail at oba@od.nih.gov, by telephone at 301-496-9838, by fax to 301-496-9839, or by mail to:

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750
Bethesda
Maryland 20892-7985 (20817 for non-USPS mail).

NIH OBA Exempt Experiment FAQs

National Institutes of Health ▪ Office of Biotechnology Activities

FAQs about experiments that are exempt from the *NIH Guidelines*

1. Which experiments are exempt from the *NIH Guidelines for Research Involving Recombinant DNA Molecules*?

Per Section III-F of the *NIH Guidelines*, experiments are exempt when they involve recombinant DNA that is:

- Not in organisms and viruses;
- Entirely DNA segments from a single nonchromosomal or viral DNA source;
- Entirely from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host or when transferred to another host by well established physiological means;
- Entirely from a eukaryotic host including its chloroplasts, mitochondria, or plasmids when propagated only in that host or a closely related strain of the same species,
- Entirely segments from different species that exchange DNA by known physiological processes, though one or more may be a synthetic equivalent; see Appendix A of the *NIH Guidelines*; or
- Not a significant risk to health or the environment as determined by the NIH Director, with advice from the RAC and public comment; see Appendix C of the *NIH Guidelines* for a detailed listing;

Unless these experiments also 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 the use of the drug to control disease agents in humans, veterinary medicine or agriculture [Section III-A];
- Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram of body weight [Section III-B]; or
- The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into one or more human research subjects [Section III-C].

Details on certain other experiments that may be exempt, as well as exceptions, may be found in Appendix C of the *NIH Guidelines*.

2. The *NIH Guidelines* exempt certain experiments that do not pose a threat to health or the environment. Can an Institutional Biosafety Committee (IBC) or Principal Investigator (PI) determine if an experiment does not pose such a threat and is therefore exempt?

Section III-F-6 of the *NIH Guidelines* lists categories experiments that do not present a significant risk to health or the environment and are therefore exempt. The determination of the types of experiments that fall into this category is made by the NIH Director with the advice of the RAC, following appropriate notice and opportunity for public comment. **PIs and IBCs can not make the**

6705 Rockledge Drive, Suite 750, Bethesda MD 20892-7985 • Tel (301)496-9838 • Fax (301)496-9839
Email: oba@od.nih.gov • http://www4.od.nih.gov/oba

determination that a class of experiments other than the ones listed below poses no significant risk.

The following classes of experiments are exempt under Section III-F-6:

- Recombinant DNA in tissue culture [Appendix C-I]
- *Escherichia coli* K-12 host-vector systems [Appendix C-II]
- *Saccharomyces* host-vector systems [Appendix C-III]
- *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems [Appendix C-IV]
- Extrachromosomal elements of gram positive organisms [Appendix C-V]
- The purchase or transfer of transgenic rodents [Appendix C-VI]

A full description of the exemptions with exceptions can be found in Appendix C of the *NIH Guidelines*.

3. How do I know if I am working with host-vector system that is exempt from the *NIH Guidelines*?

Only certain experiments that use *E.coli* K-12, *Saccharomyces*, *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems are specifically exempted from the *NIH Guidelines* (see Appendix C-II). If you are obtaining a host-vector system from a vendor, genotype information may be available and permit determination of the strain from which your host is derived.

4. DNA molecules resulting from the replication of recombinant DNA are subject to the *NIH Guidelines*. Are any other materials derived from or produced by genetically engineered organisms subject to the requirements *NIH Guidelines*?

No. For example, proteins produced by genetically engineered organisms are not subject to the *NIH Guidelines*.

5. I have heard that certain kinds of human gene transfer trials are exempted from the requirements of the *NIH Guidelines* – is this true?

No. All trials involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the *NIH Guidelines*. Appendix M-VI-A of the *NIH Guidelines* exempts certain types of vaccine trials from the requirements for **submission of the protocol to NIH OBA, RAC review, and subsequent reporting** (Appendix M-I). Specifically, this exemption applies to "human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected." Trials with these characteristics do not have to be registered with NIH OBA or undergo RAC review, but can be submitted on a voluntary basis, particularly if the investigator believes that a trial presents scientific, safety, or ethical concerns that would benefit from RAC review and public discussion. Investigators that submit trials voluntarily will be expected to comply with all aspects of the protocol review and reporting requirements. OBA encourages investigators and institutional review bodies to contact us (oba@od.nih.gov) for assistance in determining whether this exemption applies to a particular trial.

It is important to note that Appendix M-VI-A does not exempt these vaccine trials from other requirements specified in the NIH Guidelines, including biosafety review. Thus, vaccine trials, like other human gene transfer trials subject to the NIH Guidelines, must be reviewed and approved by an IBC before research participants can be enrolled.

6. **There is a note at the beginning of Section III of the *NIH Guidelines* that states "If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines.*" What is meant by this note?**

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. An example of such an experiment is the following:

Staphylococcus aureus (a Risk Group 2 bacterium) contains a recombinant plasmid. The plasmid is indigenous to S. aureus, was created in vitro, and contains only DNA from S. aureus (i.e., the DNA inserted into the plasmid was S. aureus DNA).

Rationale: The introduction of recombinant DNA into Risk Group 2 agents is usually covered under Section III-D-1-a. However, because the experiments are only performed in the *S. aureus strain*, this work would fall under III-F-3 (experiments that consists entirely of DNA from a prokaryotic host including its indigenous plasmids when propagated only in that host or a loosely related strain of the same species). Thus this experiment falls into both Sections III-D and III-F and is exempt, due to the above note from the requirements of the *NIH Guidelines* for IBC review and approval.

It should be noted that only experiments covered by both III-D or III-E and III-F can be exempted. If an experiment falls into Section III-A, III-B or III-C and any one of the other sections, then the rules pertaining to Sections III-A, III-B or III-C must be followed.

7. **Appendix C-1 of the *NIH Guidelines* exempts experiments involving recombinant DNA in tissue culture. I have a cell line that was created by the introduction of recombinant DNA. Are all experiments I conduct with this cell line exempt from the requirements of the *NIH Guidelines*?**

No. Although Appendix C-1 does exempt the use of recombinant DNA in tissue culture, there are exceptions to this exemption. Existing tissue culture cell lines created by the introduction of recombinant DNA are exempt from the *NIH Guidelines* unless, the cell line:

- was modified using DNA from Risk Group 3 or 4 agents, or from restricted agents. [Section III-D]
- contains a toxin with an LD50 of less than 100 ng/kg body weight. [Section III-B-1]
- contains viral DNA in a quantity exceeding 50% of any viral genome. [Appendix C-I]
- is used in conjunction with defective viruses in the presence of helper virus. [Section III-D-3]
- is used in an experiment involving the deliberate transfer of the cell line into humans. [SectionIII-C-1]
- is grown in a volume exceeding 10 liters of culture. [Section III-D-6]

NIH OBA Major Actions Under Sections III-A of the NIH Guidelines

National Institutes of Health • Office of Biotechnology Activities

Major Actions under Section III-A of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)

1. **What experiments are considered "Major Actions" under the *NIH Guidelines*?**

   Under the *NIH Guidelines*, the term "Major Action" means that NIH Director approval is required. Only one type of experiment requires NIH Director approval – the deliberate transfer of a drug resistance trait to a microorganism when such resistance could compromise the ability to control the disease agent in humans, veterinary medicine, or agriculture (see Section III-A-1-a of the *NIH Guidelines*).

2. **What criteria should be used to determine if the transfer of a particular drug resistance trait is considered a Major Action under Section III-A-1-a of the *NIH Guidelines*?**

   An experiment may be considered a Major Action if: 1) it involves the use of recombinant or synthetic nucleic acids to introduce drug resistance into a microorganism, and 2) the drug in question is used to treat disease caused by the organism in humans, veterinary medicine, or agriculture. The experiment would not be considered a Major Action if there is sufficient documentation that resistance to a therapeutically useful concentration of that drug exists in the agent outside of a laboratory setting. Such evidence should be in the form of articles published in the scientific literature.

3. **What is considered a therapeutically useful drug?**

   A drug is therapeutically useful if it is effective in the treatment of the disease caused by the microorganism. It does not have to be the 'first line' agent, but should be recognized in the scientific literature as a useful drug. *In vitro* sensitivity to the drug is not sufficient; it must be useful *in vivo*. In addition, if a drug is not therapeutically useful but will confer cross resistance to a therapeutically useful drug then this also is considered a III-A-1-a experiment.

   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 pregnant women), or it is primarily used for treatment outside of the United States where alternative drugs are not available (e.g., chloramphenicol is not in common use in the U.S. but is used in many other countries).

4. **Can an Institutional Biosafety Committee (IBC) determine if a particular experiment meets the criteria of Section III-A-1-a or must NIH make the determination?**

   The determination regarding whether a particular experiment constitutes a Major Action initially should be made by the investigator and confirmed by the IBC. IBCs are encouraged to contact the NIH Office of Biotechnology Activities (OBA) if they need assistance in determining whether a specific experiment involving the deliberate transfer of a drug resistance trait falls under Section III-A-1-a and therefore requires NIH Recombinant DNA Advisory Committee (RAC) review and NIH Director approval. IBCs may also consult with OBA regarding experiments that raise important public health issues but do not meet the criteria of Section III-A-1-a (e.g., because there is low frequency of resistance to a drug). OBA will consult, as needed, with experts who may include members of the RAC.

Major Actions FAQ/September 2012

5. **What are the steps in the review process for a Section III-A-1-a experiment?**

The steps in the review process are 1) submission of relevant information on the proposed experiment to OBA, 2) publication of the proposal in the *Federal Register* for 15 days of comment, 3) review by the RAC at a public meeting, and 4) approval by the NIH Director who stipulates the containment conditions and any special requirements for the experiments.

6. **Does the IBC need to review the proposal before it is sent to OBA?**

The IBC may initiate its review of these experiments before or after submission to OBA and review by the RAC. The IBC's final determination should occur after this process, because the IBC must take into account any special conditions that the NIH Director (or OBA) has stipulated as a condition of approval and ensure that they are implemented. Research that meets the criteria for Section III-A-1-a cannot be initiated unless approval is granted by the NIH Director (or OBA in the case of an equivalent experiment). See question 9.

7. **What information needs to be submitted to OBA for review of research that involves the transfer of drug resistance that may ultimately be reviewed and approved by the NIH Director?**

Information about the proposed experiment that OBA requires to determine whether the experiment meets the criteria of Section III-A-1-a of the *NIH Guidelines* includes technical information about the proposed transfer of drug resistance (e.g., the vector(s), gene(s) encoding the resistance, degree(s) of resistance, cross-resistance to other drugs, and other pertinent characteristics of the recombinant construct).

If the experiment is considered to be a III-A-1-a experiment and therefore will be reviewed by the RAC, the following additional information must be submitted to OBA:

- Rationale for why the work should go forward, including an assessment of how the scientific and public health benefits outweigh the potential risks for humans, animals, or agriculture.
- A discussion of whether there are alternative approaches to this research that would not involve conferring resistance to a drug that has utility in the treatment of disease caused by the organism in question. This should include a statement whether the submitting investigator or others have considered any alternatives.
- A description of the proposed risk mitigation strategies that will be implemented to minimize risk to laboratory personnel as well as to the public.
- Minutes, if available, of any IBC discussion of the research in question (IBC review is not required prior to RAC review and NIH Director approval, but a preliminary review is desirable).
- IBC contact information.

In addition, OBA may also request that the following information be submitted:

- Description of the biosafety features of the room(s) in which the research will be conducted.
- Most recent inspection report(s) of the room(s) in which the research will be conducted, including any reports of biosafety equipment failures or biosafety-related problems that have occurred in these rooms in the last two years.
- Biosafety manual for the proposed work.
- Description of any additional biosafety training that laboratory personnel will receive specific to the research question.
- Description of any special occupational health requirements for the laboratory personnel involved in the research (e.g., vaccination, medical surveillance).

Submission of relevant information on a proposed Section III-A-1-a experiment should be made to:

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892-7985 (20817 for non-USPS mail)
Telephone: (301) 496-9838
Fax: (301) 496-9839
Email: oba@od.nih.gov

8. **Who approves Section III-A-1-a experiments?**

   The NIH Director approves these experiments after consideration of the RAC's recommendations and any public comments. These experiments may not proceed unless approved by the NIH Director and the IBC.

9. **Once a Section III-A-I-a experiment has been approved by NIH, do equivalent experiments also need to be reviewed by the RAC and approved by the NIH Director?**

   Once a Section III-A-I-a experiment has been reviewed by the RAC and approved by the NIH Director, equivalent experiments may not need to follow the same approval process to determine the appropriate biosafety containment level for the work. Under Section III-B-2 of the *NIH Guidelines* (Experiments that Require OBA and IBC Approval Before Initiation), OBA may determine that a proposed experiment is equivalent to one that has previously been approved by the NIH Director as a Major Action. An experiment will be considered equivalent only if, as determined by OBA, there are no substantive differences in methodology and other pertinent information has not emerged since submission of the initial III-A-1-a experiment that would change the biosafety and/or public health considerations for the proposed experiment. If OBA makes such a determination, experiments deemed to be equivalent will not require review and approval under Section III-A-1-a. These experiments will have been approved by OBA and may proceed once approved by the appropriate IBC.

10. **My research involves the transfer of a drug resistance trait into an organism on the Department of Health and Human Services (HHS) and/or the United States Department of Agriculture (USDA) Select Agent list (i.e. is a "restricted experiment") and thus requires approval from those agencies. Do I also need to submit a request to OBA for approval?**

    Experiments utilizing recombinant or synthetic nucleic acid molecules that involve the deliberate transfer of a drug resistance trait into a Select Agent (that meet the definition of a restricted experiment) may be subject to the regulatory authority of, and review by, HHS and/or USDA under their respective rules (found in 42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121). Review and approval by the appropriate Federal agency (HHS or USDA) supersedes the requirement for RAC review and NIH Director approval under the *NIH Guidelines*. However, other provisions of the *NIH Guidelines* - for example, IBC review and approval - are still applicable.

11. **I am conducting research that involves the transfer of resistance to certain drugs in a strain of a Select Agent that has been excluded or exempted from the Select Agent regulations. From which agency do I need to get approval?**

    Potential III-A-1-a experiments in strains of Select Agents that have been excluded or exempted from the Select Agent regulations should be submitted to OBA.

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Useful Links and Resources Regarding the Control of Disease Agents

Government Resources

PubMed:
http://www.ncbi.nlm.nih.gov/sites/entrez/

Centers for Disease Control and Prevention:
http://www.cdc.gov/

World Health Organization:
http://www.who.int/en/

Medline:
http://www.nlm.nih.gov/medlineplus/

Private Resources (fees may apply)

Up to Date Online
http://www.uptodateonline.com/utd/index.do

Control of Communicable Diseases Manual
Editor: David L. Heymann
Publisher: American Public Health Association
ISBN-13: 978-0875530345

Sanford Guide to Antimicrobial Therapy 2011 (or updated editions)
Authors: David N. Gilbert, Robert C. Moellering, and Merle A. Sande
Publisher: Antimicrobial Therapy
ISBN-13: 978-1930808300

Principles and Practice of Pediatric Infectious Disease
Authors: Sarah S. Long, Larry K. Pickering, and Charles G. Prober
Publisher: Saunders
ISBN-13: 978-0443066870

Johns Hopkins ABX Guide
http://www.hopkins-abxguide.org/

Red Book: Report of the Committee on Infectious Diseases
Publisher: American Academy of Pediatrics
ISBN-13: 978-1581101942

Principles and Practice of Infectious Diseases
(Available on MDConsult.com)
Authors: Gerald L. Mandell, John E. Bennett, and Raphael Dolin
Publisher: Churchill Livingstone
ISBN-13: 978-0443066436

Merck Manual
http://www.merckmanuals.com/professional/index.html

RAC discussions of Major Actions involving the transfer of drug resistance:

http://oba.od.nih.gov/rdna_rac/rac_meetings.html

June 2007 RAC Meeting: Transfer of tetracycline resistance to *Chlamydia trachomatis*
September 2007 RAC Meeting: Transfer of chloramphenicol resistance to *Rickettsia conorii* and *Rickettsia typi*
December 2007 RAC Meeting: Transfer of chloramphenicol resistance to *Rickettsia conorii* and *Rickettsia typi*
December 2009 RAC Meeting: Transfer of tetracycline resistance to *Chlamydia trachomatis*

Major Actions FAQ/September 2012

NIH OBA Transgenic Animals and rDNA Use in Animals

National Institutes of Health
Office of Biotechnology Activities

Transgenic Animals and the Use of Recombinant DNA in Animals

FAQs for Research Subject to the *NIH Guidelines*

- **Under which section of the *NIH Guidelines* does the generation of transgenic rodents fall?**

  The creation of transgenic rodents falls under one of two portions of the *NIH Guidelines* depending on the containment level required to house the rodents. Experiments involving the creation of transgenic rodents that can be housed under Biosafety Level 1 conditions are covered under **Section III-E-3**. Experiments involving the generation of transgenic rodents requiring BL2, BL3 and BL4 containment are covered under **Section III-D-4**.

- **Under which section of the *NIH Guidelines* does the generation of transgenic animals other than rodents fall?**

  The creation of all transgenic animals (other than rodents that can be housed under BL1 containment conditions) is covered under **Section III-D-4** of the *NIH Guidelines*.

- **Would the breeding of two different strains of knock-out mice require IBC approval under the *NIH Guidelines*?**

  The techniques used initially to create knock-out animals involve the stable introduction of recombinant DNA into the animal's genome, and thus these animals are considered transgenic. As the breeding of two different strains of knock-out mice will potentially generate a novel strain of transgenic animal, the work may be covered under the *NIH Guidelines* and require IBC review and approval. Sections in the *NIH Guidelines* that cover work with rodents include **III-E-3** for work that requires Biosafety Level (BL) 1 containment and **III-D-4** for work that requires BL2, BL3 and BL4 containment. Certain breeding experiments are exempt under Appendix C-VII the NIH Guidelines. This exemption covers 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 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.

- **Is IBC registration and approval needed for the maintenance of a transgenic animal colony?**

  The maintenance of a transgenic rodent colony (i.e. breeding within a particular transgenic strain) at BL1 is an activity that is exempt from to the *NIH Guidelines* and, as such, does not require IBC registration and approval. The maintenance of a transgenic rodent colony at BL2 or higher falls under **Section III-D-4-b** and requires IBC approval. The breeding of all other transgenic animals is subject to the *NIH Guidelines* under Section **III-D-4-a** or **III-D-4-b** depending on the containment level required.

- **Is the purchase and transfer of transgenic rodents exempt from the *NIH Guidelines*?**

  Under **Appendix C-VI** of the *NIH Guidelines*, the purchase or transfer of transgenic rodents may be maintained at BL1 containment are exempt from the *NIH Guidelines*. The purchase or transfer of transgenic rodents that require BL2 or higher containment is not exempt from the *NIH Guidelines*. These animals are covered under **Section III-D-4**, and purchase and transfer of such animals requires IBC registration and approval.

It should be noted that the subsequent use of transgenic rodents may not be exempt from the *NIH Guidelines*. Experiments using transgenic rodents at BL1 are exempt from the *NIH Guidelines* if the experiment does not involve the use of recombinant DNA. If the protocol does involve the use of recombinant DNA or is conducted at BL2 or higher then the work falls under **III-D-4** of the *NIH Guidelines* and as such requires IBC review and approval prior to initiation.

- **Is the purchase and transfer of transgenic animals other than rodents exempt from the *NIH Guidelines*?**

  No, only the purchase or transfer of transgenic rodents that may be maintained at BL1 containment is exempt from the *NIH Guidelines*. The purchase or transfer of any other animal for research purposes at any biosafety level (including BL1) is not exempt, nor is the purchase and transfer of transgenic rodents that require BL2 or higher containment.

- **Are gene ablation studies covered by the *NIH Guidelines*?**

  The answer to this question depends on the technique employed in the study. If recombinant techniques are used to knock out the gene, then work would be covered under the *NIH Guidelines*.

- **Who has the responsibility to review the generation of transgenic animals if an institution is generating animals for investigators who are not affiliated with that institution?**

  The generation (creation) of transgenic animals is an activity covered under the *NIH Guidelines*. The IBC at the institution where that activity is occurring has the responsibility to review and approve that activity (if the institution is subject to the requirements of the *NIH Guidelines*). The subsequent use of the animals by investigators not at that institution would need to be reviewed and approved by the IBC at the investigator's institution if that institution conducts or supports recombinant DNA research that receives NIH support and the activity covered under the *NIH Guidelines*.

- **When a core facility generates transgenic mice as a "fee for service" for Principle Investigators (PIs), is it the responsibility of the PI or the core facility to register the generation of the mice with the IBC?**

  Section IV-B-7-a-(1) of the *NIH Guidelines* articulates one of the responsibilities of the PI as 'initiating no recombinant DNA research which requires IBC approval prior to initiation until that research has been approved by the IBC and has met all other requirements of the *NIH Guidelines*.' It would be acceptable for either the PI of the core facility or the PI purchasing the transgenic animals to fulfill the responsibility to register the generation of the animals. In many cases, the animals being generated will be subsequently used in experiments that are subject to the *NIH Guidelines*, and the registration of the research with the IBC may encompass both the generation and subsequent experimentation with the animals.

- **When existing transgenic animals at an institution are purchased or transferred to an investigator outside the institution, who should review and approve the use of these animals?**

  An institution's IBC does not need to review and approve the use of transgenic animals at another institution. If the receiving institution is subject to the *NIH Guidelines* (i.e. conducts or supports recombinant DNA research that receives NIH support), then the purchase and transfer of animals (other than rodents that can be housed under BL1 containment), along with any experiments subject to the *NIH Guideline*, would require review and approval by the IBC at that institution.

- **What are the *NIH Guidelines* requirements for research with large transgenic animals (sheep, pigs, etc.), or research with recombinant DNA microorganisms in such animals?**

  When conducting recombinant DNA work with large animals, the work is covered under Appendix Q of the *NIH Guidelines*. **Appendix Q** specifies containment and confinement practices when animals are of a size or have growth requirements that preclude the use of laboratory containment of animals. The *NIH Guidelines* include provisions for tracking and inventorying these animals (**Appendix Q-1-B-2** states that a permanent record must

be maintained of the experimental use and disposal of each animal). Animal carcasses must be disposed of as to avoid their use as food for human beings or animals unless food use is specifically authorized by an appropriate federal agency (**Appendix Q-1-B-1**). An acceptable method, for example, would be incineration.

- **Are recombinant DNA modifications to the somatic cells of non-transgenic animals subject to the *NIH Guidelines*?**

  Yes, these experiments are subject to the *NIH Guidelines*.

  - **Sections III-D-1-a** through **III-D-1-d** cover experiments using Risk Group 2, 3, 4, or restricted agents in whole animals. See the *NIH Guidelines* for the appropriate containment for such experiments
  - **Section III-D-4-a** covers experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. DNA from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any animal and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study.
  - **Section III-D-4-b** covers recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals that are not covered by **Sections III-D-1** or **III-D-4-a**. The appropriate containment for these experiments is determined by the IBC.
  - Experiments not included in **Sections III-A, III-B, III-C, III-D, III-F**, fall into **Section III-E**. Experiments covered by **Section III-E** may be conducted at BL1 containment.

The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) are available at http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

For further information about the requirements of the *NIH Guidelines*, please visit the NIH Office of Biotechnology Activities web page at http://oba.od.nih.gov or write to us at oba@od.nih.gov

NIH OBA Animal Experiments Covered Under the NIH Guidelines

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)

Breeding of knockouts 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 of knockouts from two strains (generating new strain)	BL2 or higher	III-D-4-b
EXPERIMENTS WITH TRANSGENIC ANIMALS		
Experiments with transgenic rodents	BL1	III-D-4-a* (see note)
Experiments with transgenic rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with transgenic animals other than rodents	BL1	III-D-4-a
Experiments with transgenic animals other than rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with recombinant DNA modified arthropods associated with plants	BL1	III-E-2-b-(5).
Experiments with recombinant DNA modified arthropods associated with plants	BL2 or higher	III-E-2
Experiments with recombinant DNA modified arthropods not associated with plants	BL1	III-D-4-a
Experiments with recombinant DNA modified arthropods not associated with plants	BL2 or higher	III-D-4-b
*The purchase or transfer of transgenic rodents requiring BL1 containment is exempt under Appendix C-VI. Subsequent use of these animals is also exempt providing the experimental protocol does not involve the use of recombinant DNA. If the protocol does involve the use of recombinant DNA then the research is covered under III-D-4-a. All experiments involving the use of other transgenic animals at any Biosafety Level and the use of transgenic rodents requiring BL2 or higher containment are subject to the *NIH Guidelines*. See above for applicable sections.		
EXPERIMENTS WITH R-DNA IN AN ANIMAL (TRANSGENIC OR OTHERWISE)		
Experiments with r-DNA modified microbes in any animal (transgenic or otherwise)	BL1/BL1-N	Not permitted at BL1*
Experiments with RG2 r-DNA modified microbes in any animal (transgenic or otherwise)	BL2/ BL2-N	III-D-1-a
Experiments with RG3 r-DNA modified microbes in any animal (transgenic or otherwise)	BL3/ BL3-N	III-D-1-b
Experiments with RG4 r-DNA modified microbes in any animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-c
Experiments with r-DNA modified restricted agent in an animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Experiments with r-DNA modified animal pathogens in an animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Introduction of less than 2/3 of eukaryotic viral genome into a non-human vertebrate or invertebrate	BL1/BL1-N	III-D-4-a
Propagation of animals containing viral vector sequences not leading to transmissible infection	BL1/BL1-N	III-D-4-a
Experiments with R-DNA involving whole animals not covered by Sections III-D-1or III-D-4-a	Set by IBC	III-D-4-b
* Other than viruses which are only transmitted vertically, the experiments may not be conducted at BL1. A minimum of BL2 or BL2-N is required.		
CLONING ANIMALS		
Cloning animals	BL1 or higher	Not covered
PURCHASE OR TRANSFER OF TRANSGENIC ANIMALS		
Purchase or transfer of transgenic rodents	BL1	Exempt (Appendix C-VI)
Purchase or transfer of transgenic rodents	BL2 or higher	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL1	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL2 or higher	III-D-4
Purchase or transfer of recombinant DNA modified arthropods	BL1	III-D-4
Purchase or transfer of recombinant DNA modified arthropods	BL2 or higher	III-D-4

PLANT EXPERIMENTS WITH ANIMALS OR ARTHROPODS		
Experiments with microorganisms or insects containing recombinant DNA with the potential for detrimental impact to ecosystems.	BL3-P or BL2-P plus biological containment	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 plus biological containment	III-D-5-e
Small animals associated with recombinant DNA-modified plants.	BL1	III-E-2
Experiments with rDNA-modified arthropods or small animals associated with plants	BL1	III-E-2-b-(5).
OTHER		
Transfer of a drug resistance to microorganisms compromising the use in veterinary medicine	Set by NIH (case by case)	III-A-1-a

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NIH OBA Reporting Incidents Involving rDNA to the NIH OBA

National Institutes of Health ▪ Office of Biotechnology Activities

Information for Labs Conducting Recombinant DNA Research

Reporting of Incidents Involving Recombinant DNA to the NIH Office of Biotechnology Activities (OBA)

- **What kinds of incidents involving recombinant DNA must be reported to the NIH OBA?**

  The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) states that "...any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" must be reported to NIH OBA within 30 days. Certain types of accidents must be reported on a more expedited basis. Spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported to NIH OBA. Spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately reported to NIH OBA.

- **How serious must a problem be to warrant reporting to OBA?**

  Any spill or accident involving recombinant DNA research of the nature described above or that otherwise leads to personal injury or illness or to a breach of containment must be reported to OBA. These kinds of events might include skin punctures with needles containing recombinant DNA, the escape or improper disposition of a transgenic animal, or spills of high-risk recombinant materials occurring outside of a biosafety cabinet. Failure to adhere to the containment and biosafety practices articulated in the *NIH Guidelines* must also be reported to OBA.

  Minor spills of low-risk agents not involving a breach of containment that were properly cleaned and decontaminated generally do not need to be reported. OBA should be consulted if the Institutional Biosafety Committee (IBC), investigator, or other institutional staff are uncertain whether the nature or severity of the incident warrants reporting; OBA can assist in making this determination.

- **Who is responsible for reporting incidents involving recombinant DNA to NIH OBA?**

  Under the *NIH Guidelines* incident reporting is articulated as a responsibility of the Institution, IBC, Biological Safety Officer, and Principal Investigator. Institutions have the discretion to determine which party should make these reports, and one report for each incident or set of information is generally sufficient.

6705 Rockledge Drive, Suite 750, Bethesda MD 20892-7985 • Tel (301)496-9838 • Fax (301)496-9839
Email: oba@od.nih.gov • http://www4.od.nih.gov/oba

- **What information should incident reports include?**

  Incident reports should include sufficient information to allow for an understanding of the nature and consequences of the incident, as well as its cause. A detailed report should also include the measures that the institution took in response to mitigate the problem and to preclude its reoccurrence.

- **What other information needs to be provided?**

  Depending on the severity of the incident, OBA staff may request the IBC meeting minutes documenting approval conditions for the research, minutes of IBC meetings where the incident was reviewed, policies in place at the time the incident occurred, or any revised policies prepared in response to the incident. Training records for the personnel involved in the incident may also be requested.

- **What does OBA do with this information?**

  OBA staff review incident reports to assess whether the institutional response was sufficient. Depending on the adequacy of the institutional response, OBA may ask the institution to take additional measures as appropriate to promote safety and compliance with the *NIH Guidelines*.

- **Do adverse events experienced by participants in human gene transfer trials fall under this incident reporting requirement?**

  No, adverse events in human gene transfer trials are subject to a separate set of reporting requirements. These are found in Appendices M-1-C-3 and M-1-C-4 of the *NIH Guidelines*. Serious adverse events that are unexpected and possibly associated with the gene transfer product should be reported to OBA within 15 calendar days of sponsor notification, unless they are fatal or life threatening, in which case they should be reported within 7 calendar days. Other serious adverse events should be reported to OBA as part of the Principal Investigator's annual report to OBA.

- To report an incident involving an exposure, loss of containment, a violation of the *NIH Guidelines* or other compliance issue to OBA contact:

  Kathryn Harris, Ph.D., RBP
  Senior Outreach and Education Specialist
  6705 Rockledge Drive, Suite 750
  Bethesda, MD 20892
  Phone: 301-496-9838
  Fax: 301-496-9839
  Email: harriskath@od.nih.gov

NIH OBA Investigator Responsibilities Brochure

PIs conducting human gene transfer research must:

- Ensure that all aspects of Appendix M have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA for review by the NIH Recombinant DNA Advisory Committee (RAC).
- Provide a letter signed by the PI(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OBA complies with the requirements set forth in Appendix M.
- Not enroll research participants in a human gene transfer experiment until the RAC review process has been completed; IBC approval (from the clinical trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained.
- Comply with reporting requirements for human gene transfer experiments (see Appendix M-I-C of the *NIH Guidelines).*

For More Information

To receive updates on current initiatives, policies, and news from OBA, subscribe to our listserv, "OBA_NEWS," by sending a message to: listserv@list.nih.gov with the message: subscribe OBA_NEWS

Visit the following websites for additional information:

NIH Office of Biotechnology Activities
http://oba.od.nih.gov

NIH Guidelines for Research Involving Recombinant DNA Molecules
http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

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Fax: 301-496-9839

National Institutes of Health
Office of Biotechnology Activities

Investigator Responsibilities

under the

NIH Guidelines for Research Involving Recombinant DNA Molecules

What are the *NIH Guidelines for Research Involving Recombinant DNA Molecules*?

The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) detail procedures and practices for the containment and safe conduct of various forms of recombinant DNA research, including research involving genetically modified plants and animals, and human gene transfer.

Who must comply with the *NIH Guidelines*?

All institutions that receive NIH funding for recombinant DNA research must comply with the *NIH Guidelines*. Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if their individual projects are not funded by NIH.

What is an Institutional Biosafety Committee?

Institutional Biosafety Committees (IBCs) provide local review and oversight of nearly all forms of research utilizing recombinant DNA. They ensure that recombinant DNA research conducted at or sponsored by the institution is in compliance with the *NIH Guidelines*. A requirement of the *NIH Guidelines* is that an IBC must review and approve all research subject to the *NIH Guidelines*.

What is the NIH Office of Biotechnology Activities?

The NIH Office of Biotechnology Activities (OBA) promotes science, safety, and ethics in biotechnology through the advancement of knowledge, enhancement of public understanding, and development of sound public policies. A core responsibility of OBA is to foster awareness of, and adherence to, the standards and practices set forth in the *NIH Guidelines*.

Safety and science go hand in hand

Principal Investigator Responsibilities

Principal Investigators (PIs) are responsible for full compliance with the *NIH Guidelines* during the conduct of recombinant DNA research. As part of this general responsibility, the PI should:

- Be adequately trained in good microbiological techniques.
- Provide laboratory research staff with protocols describing potential biohazards and necessary precautions.
- 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 laboratory staff to ensure that the required safety practices and techniques are employed.
- Correct work errors and conditions that may result in the release of recombinant DNA materials.
- Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment (e.g., purity and genotypic and phenotypic characteristics).
- Comply with permit and shipping requirements for recombinant DNA molecules.
- Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination.

Before initiating research subject to the *NIH Guidelines*, the PI must:

- Determine whether the research is subject to Section III-A, III-B, III-C, III-D, or III-E of the *NIH Guidelines*.
- Propose physical and biological containment levels in accordance with the *NIH Guidelines* when registering research with the IBC.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.
- Submit a research protocol to the IBC for review and approval.
- Seek OBA's determination of containment for experiments that require case-by-case review.
- Petition OBA, with notice to the IBC, for proposed exemptions from the *NIH Guidelines*.
- Obtain IBC approval before initiating research subject to the *NIH Guidelines*.
- Seek NIH approval, in addition to IBC approval, to conduct experiments specified in Sections III-A and III-B of the *NIH Guidelines*.

While conducting research subject to the *NIH Guidelines*, the PI must:

- Determine the need for IBC review before modifying recombinant DNA research already approved by the IBC.
- Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval.
- Remain in communication with the IBC throughout the duration of the project.
- Report any significant problems pertaining to the operation and implementation of containment practices and procedures, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the IBC, OBA, and, as applicable, the Biological Safety Officer, Greenhouse or Animal Facility Director, and other appropriate authorities.