Environmental Health & Safety

The University of Iowa

Biosafety Training

NIH Guidelines for Research Involving Recombinant DNA

Date(s) Revised: 09/2013

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (NIH GUIDELINES)
MARCH 2013

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Visit the OBA Web site at:
http://oba.od.nih.gov/oba/index.html
For current information on Guidelines, protocols, Principal Investigators, meetings, and information about upcoming Gene Therapy Policy Conferences

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
Guidelines for Research Involving Recombinant DNA

About the Course

This training course was designed to inform principal investigators (PIs) about the requirements of the *NIH Guidelines* and to provide information regarding the roles and responsibilities of various personnel involved with recombinant DNA (rDNA) or synthetic nucleic acid research. This course is a general overview of the *NIH Guidelines* and does **not** cover all information applicable to rDNA research. Please refer to the *NIH Guidelines* for all relevant provisions.

The *NIH Guidelines* defines rDNA as either (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.

Who Should Complete this Course?

As a condition for NIH funding, **all rDNA or synthetic nucleic acid research** conducted at or sponsored by the institution, **irrespective of the source of funding**, shall comply with the *NIH Guidelines*.

Non-compliance may result in:

- suspension, limitation or termination of financial assistance for the noncompliant NIH-funded research project **and** of NIH funds for other rDNA research at the institution, or
- a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Do NOT complete this course through the VAMC Staff Training Courses link if you have a Univ. of Iowa HawkID and password. Please access the course through the UI/UIHC ICON Training Courses link on EHS's website and documentation of course completion will be downloaded into your "My Training" record in the Employee Self Service website.

Abbreviations

BSL: Biosafety Level
BSO: Biological Safety Officer
EHS: Environmental Health & Safety Office
FDA: Food and Drug Administration
IBC: Institutional Biosafety Committee
IND: Investigational New Drug Application
IRB: Institutional Review Board
NIH: National Institutes of Health
NSABB: National Science Advisory Board for Biosecurity

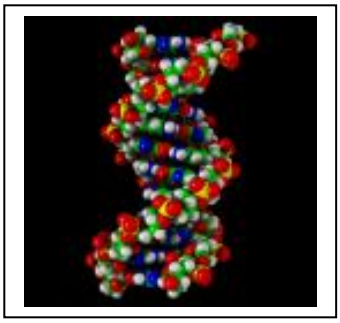
OBA: Office of Biotechnology Activities
PI: Principal Investigator
RAC: Recombinant DNA Advisory Committee, NIH
rDNA: Recombinant DNA
rDNARD: Recombinant DNA Registration Document

How Do I Receive Course Credit?

To receive course credit:

- Read the following material.
- Complete the exam.

What Will This Course Cover?



This course will cover the following topics:

- Responsibilities
- Safety considerations
- Classification of experiments
- Procedures to obtain rDNA approval
- rDNA Research Registration
- Appendices of the *NIH Guidelines*

Introduction

The *NIH Guidelines* outline specific procedures for the construction and handling of rDNA or synthetic nucleic acid molecules and organisms/viruses containing rDNA or synthetic nucleic acid molecules.

The *NIH Guidelines* also outline the responsibilities and relationship between various governmental and University personnel.

The manner in which experiments are classified in the *NIH Guidelines* determines the required review procedures.

General Responsibilities of the Various Entities Involved in rDNA Experiments

Governmental Responsibilities

- It is the responsibility of the NIH Director to establish the *NIH Guidelines* and oversee their implementation and final interpretation.

- The responsibilities of the Office of Biotechnology Activities (OBA) include administrative duties as well as offering scientific and technical advice to the Institutional Biosafety Committee (IBC) or to Principal Investigators (PIs).

- The Recombinant DNA Advisory Committee (RAC) primarily offers scientific, technical and ethical advice to the NIH.

University Responsibilities

- It is the responsibility of the University to:
    - Ensure that all rDNA and synthetic nucleic acid molecule research conducted at or sponsored by the University complies with *NIH Guidelines.*

    - Establish procedures that the Institutional Biosafety Committee (IBC) must follow in the review and approval of applications, proposals and activities.

- File an annual report to NIH that includes a list of the IBC members.

Institutional Biosafety Committee (IBC)

- The IBC will review for compliance with the *NIH Guidelines* all rDNA or synthetic nucleic acid molecule research at the University and approve those projects in conformity with the *NIH Guidelines*.

- The IBC is comprised of at least 5 members with a collective expertise in rDNA technology, biological safety and physical containment.
    - At least 2 members are not affiliated with the University.
    - At least 1 member has expertise in animal or plant containment principles when research involves animals or plants, respectively.
    - The Biological Safety Officer (BSO) will be a member when research involves BSL3 containment or large scale production.

IBC Responsibilities

- Responsibilities of the IBC include:
    - Reporting within 30 days to the Institutional Official in the Office of the Vice President for Research and to the NIH all significant **problems** with and **violations** of the *NIH Guidelines* and all significant research-related accidents and illnesses.
    - Prohibiting the initiation of experiments not explicitly covered by the *NIH Guidelines* until NIH establishes the containment required.
    - Adopting emergency plans covering spills and personnel contamination resulting from rDNA or synthetic nucleic acid molecule research.

General Responsibilities of the PI

- In order to insure safety in research activities, ultimate responsibility rests with the PI, which includes:
    - Initiating or modifying rDNA or synthetic nucleic acid molecule research subject to the *NIH Guidelines* **only after** that research or the modification has been approved by the IBC.
    - Following appropriate procedures regarding the classification of the experiment.
    - Reporting within 30 days to the BSO, IBC and NIH/OBA all significant problems with and violations of the *NIH Guidelines* and all significant research-related accidents and illnesses.
    - Being adequately trained in good microbiological techniques.

Responsibilities of the PI to NIH

- Submit information to NIH/OBA for certification of new host-vector systems.

- Petition NIH/OBA and the IBC, for proposed exemptions to the *NIH Guidelines* and for approval to conduct experiments specified in Sections III-A-I and III-B-I.

- Petition NIH/OBA for determination of containment for experiments requiring a case-by-case review and for experiments not covered by the *NIH Guidelines*.

- Reporting new information bearing on the *NIH Guidelines* to the NIH/OBA and IBC.

Responsibilities of the PI to the IBC

- Register all rDNA and synthetic nucleic acid molecule research with the IBC.

- Submit any subsequent changes to the research protocol to the IBC for review and approval or disapproval.

- Remain in communication with the IBC throughout the conduct of the project.

Responsibilities of the PI to their staff

- Make protocols available that describe the potential biohazards and precautions to be taken.

- Inform their staff of the reasons and provisions for any precautionary medical practices.

- Supervise the safety performance of their staff and correct work errors and conditions that may result in the release of rDNA materials.

- Instruct and train laboratory staff in:
    - Practices and techniques required to ensure safety.
    - Protocols for dealing with accidents.
        - Note: Lab staff needs to complete the following online training courses: Lab Chemical Safety, Basic Biosafety, rDNA Research/NIH Guidelines, and PPE Awareness for Labs. Information about these training courses are available on EHS's web site. Bloodborne Pathogens training is required for work with human cells or cell lines.

Safety Considerations: Risk Assessment and Containment

Safety Considerations

During rDNA registration with the IBC, the PI will be asked to determine the containment level appropriate for the proposal.

Note:
In the following information, "Section" and "Appendix" refers to the *NIH Guidelines*. A brief explanation of the appendices follows later in this course.

Risk Assessment

- It is the responsibility of the PI to determine a comprehensive risk assessment of the agent(s) being manipulated (Section II-A).
    - Factors to consider include:
        - Risk Group (see Appendix B)
        - Virulence
        - Pathogenicity
        - Communicability
        - Environmental stability
        - Quantity
        - Toxicity
        - Type of manipulations proposed (e.g., animal inoculation or transmission experiments)
- The development of an organism containing sequences from multiple sources may occur such that the parent organism is not obvious.
    - Factors to consider include:
        - Risk Group of the source(s) of the sequences
        - Assessment of the function s encoded by these sequences (virulence or transmissibility)

Containment

- The appropriate containment levels for experimentation are based on the final risk assessment (Section II-B).
    - Appendices to refer to include:
        - - G, Physical containment
        - - I, Biological containment
        - - P, rDNA research involving plants
        - - Q, rDNA research involving large animals

- The IBC will review the containment levels set by the PI for rDNA or synthetic nucleic acid molecule experiments during review of the rDNA Registration Document and change, if necessary.

- In order to ensure proper containment of rDNA or synthetic nucleic acid molecules, all wastes involved with recombinant experiments are considered biohazardous and should be properly discarded in biohazardous waste tubs. Additionally, any animals used in rDNA or synthetic nucleic acid molecule research projects are also considered biohazardous wastes and should be discarded according to Office of Animal Resources guidelines.

Classification of Experiments Involving rDNA or Synthetic Nucleic Acids

Classification

During rDNA registration with the IBC, the PI will also be asked to classify the research experiments. The *NIH Guidelines* list 6 Classifications (A through F). Following is a description of each Class, including the specific committees required to approve such work.

IMPORTANT:

A PI does not need to be directly involved in the creation of a recombinant organism to be covered by the *NIH Guidelines*. For example, a PI receiving recombinant bacteria from another investigator must submit an rDNA Registration Document (rDNARD) to the IBC in order to work with the bacteria.

Class III-A

- Experiments that involve the deliberate transfer of a drug resistance trait to microorganisms not known to acquire the trait naturally.
    - Such a trait would compromise the use of drugs to control disease in humans, animals or plants.

- Requires IBC, RAC review and NIH Director approval prior to initiation.

Class III-B

- Experiments that involve the deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight.
- Experiments that have been approved (under Section III-A) as Major Actions. If NIH/OBA determines that there are no substantive differences and pertinent information has not emerged since submission of the initial III-A-1-a experiment, these experiments will not require review and approval under III-A.

- Requires IBC and NIH/OBA approval prior to initiation.

Class III-C

- Experiments that involve the deliberate transfer of recombinant or synthetic nucleic acids, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into human research subjects.
    - Human gene transfer is 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:
            - Contain more than 100 nucleotides; or
            - Possess biological properties that enable integration into the genome; or
            - Have the potential to replicate in a cell; or

        - Can be translated or transcribed.
- IBC approval must be obtained from each institution at which recombinant or synthetic nucleic acids will be administered to human subjects.
- Requires IBC and IRB approvals, RAC review and any other applicable regulatory authorization(s) prior to research participant enrollment.

Class III-D

- Experiments that involve:
    - Human or animal pathogens (Risk group 2 or greater; could include microorganisms classified as select agents and toxins by the CDC and USDA*) as host-vector systems (see Appendix B).
    - DNA from human or animal pathogens (Risk group 2 or greater; including select agents and toxins) is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
    - Using infectious viruses (including replication defective) or helper virus systems.
    - Whole animals.
    - Whole plants.
    - Large scale culture preparations (> 10 liters) (see Appendix K).
    - Influenza viruses.

- Requires IBC approval prior to initiation.

* See CDC's Select agent web page at: http://www.selectagents.gov/ or contact Haley Sinn or Rachel White at 5-8501.

Class III-E

- Experiments include those not listed under Class A-D or F. For example:
    - All rDNA components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes.
    - rDNA molecules that contain no more than two-thirds of the genome of any eukaryotic virus (without helper virus).
    - Creation of transgenic/knockout animals (requiring ABSL1 containment only).
        - On January 19, 2011 the *NIH Guidelines* were revised to exempt the **breeding** of transgenic/knockout rodents with the exception of:
            - Breeding experiments involving transgenic rodents that contain more than 50 percent of the genome of an exogenous eukaryotic virus from a single family, in order to prevent inadvertent reconstitution of an exogenous virus in the resultant transgenic rodent; and
            - Breeding experiments in which the transgenic rodent's transgene is under the control of a gammaretroviral long terminal repeat (LTR), in order to address the small risk of recombination with endogenous retroviruses which could potentially result in mobilization of the transgene via a replication-competent mouse retrovirus.
        - The above two types of experiments must still be registered with, and eventually approved by, the IBC. Please be aware that this exemption only applies to breeding; **the initial production of transgenic/knockout rodents requires registration with and approval from the IBC**.
    - rDNA modified whole plants and associated modified organisms.
    - Experiments using Baculovirus as the vector.

- Requires IBC notification simultaneously with initiation.

Class III-F

- Recombinant or synthetic nucleic acid molecules are exempt from the *NIH Guidelines* if they*:*

- Cannot replicate nor generate nucleic acids that can replicate in living cells, and are not designed to integrate into DNA, and do not produce a toxin lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight.
- Are not in organisms, cells or viruses and are not capable of penetrating cellular membranes.
- Have acquired a transposable element, provided that transposable element does not contain recombinant or synthetic DNA.
- Do not present a significant risk to health or to the environment, as specifically determined by the NIH Director (see Appendix C).
- Consist entirely of a DNA segment from:
  - A single source that exists contemporaneously in nature.
  - A prokaryotic host when propagated only in that host or when transferred to another host by well-established physiological means.
  - A eukaryotic host when propagated only in that host or a closely related strain of the same species.
  - Different species that exchange DNA naturally (see Appendix A).

- Research involving rDNA or synthetic nucleic acid molecules which is exempt according to the *NIH Guidelines* must still be registered with the IBC.

Procedures to Obtain Approval for rDNA and Synthetic Nucleic Acid Research

Initial Procedures for Protocols Not Involving Human Subjects

- A completed rDNA Registration Document must be submitted to the Environmental Health & Safety's Biosafety Office for all rDNA or synthetic nucleic acid molecule experiments..
  Note: Throughout the rest of this document, "Biosafety Office" refers to the Environmental Health & Safety's Biosafety Office.

- The online rDNA Registration Document is available through UIRIS (https://login.uiowa.edu/uip/login.page?service=https://uiris.research.uiowa.edu/index.php). Instructions for completing the form are located within the online process towards the top right of the EHS rDNA Research Registration webpage.

- **IMPORTANT:**
  Experiments in Classes III-A through III-D may **not** proceed until the rDNA Registration Document is **approved** by the IBC.
  - The primary contact person for rDNA registration is Debbie Kratz, 3-5678. You may also direct questions to: ehs-rdna@uiowa.edu.

Procedures for Annual Review

- Near the end of the first and second year of registration, an Annual Review request will be emailed to the PI from EHS to determine if:
  - The protocol is active.
  - Any changes have been made to the protocol (includes changes in the host, insert or vector).
  - The project no longer involves rDNA or synthetic nucleic acid molecules.
  - The protocol is inactive and no longer valid.

- The PI should mark the appropriate response and note any changes before responding via email to EHS.

Procedures for Document Renewal

- The IBC approves recombinant or synthetic nucleic acid protocols for 3 years.

- Near the end of the third year of registration, EHS will email a request to the PI to inquire if a new document will be submitted, as the current rDNA Registration Document will soon expire.

- The PI should indicate the appropriate response, email the reply to EHS and submit a new rDNA Registration Document, if appropriate.

Procedures for Amendment Requests

- Minor changes to rDNA protocols can be approved as amendments by the IBC Chair without full committee review.
    - Changes that would increase the biosafety level of what is already approved, adding animals to a protocol not approved for animal work, or adding a viral vector to a protocol only approved for bacterial plasmids would be considered a major change and would require submission of a new protocol.

- In order to initiate the process of amending an approved protocol, the **PI** must send an email to the Biosafety Officer at ehs-rdna@uiowa.edu indicating the rDNA protocol that is to be amended and detailing the proposed change(s). To facilitate review and record keeping, it is requested that, if appropriate, the PI insert the changes in highlighted form within a copy of the protocol or within a copy of the table associated with the protocol.

- The Biosafety Officer will review the request and forward the email to the IBC Chair. If the IBC chair agrees that the changes are appropriate for an amendment and approves them as such, he/she will communicate this by return email. The Biosafety Officer then will forward the email indicating approval to the PI.

Initial Procedures for Protocols Involving Human Subjects

- For protocols involving human research participants, please review the supplemental information entitled “Human Gene Transfer Protocols,” by clicking the back button twice in the upper left hand corner of the screen.

rDNA Review - IBC

- Criteria used to review submitted rDNA Registration Documents include assessment of:
    - Compliance with *NIH Guidelines*
    - Containment levels: physical and biological
    - Facilities
    - Procedures
    - Practices, training and expertise of personnel

- PIs will be notified by an email generated through UIRIS when their research protocol has been reviewed and approved by the committee.

- The IBC meets bi-monthly to review and approve submitted rDNA Registration Documents.

- Correspondence regarding the document will take place through the Biosafety Office at EHS.

rDNA Registration Document

Completing the Registration Document

- General information that is required on the rDNA Registration Document includes:
    - **Project Title:** This is the title of your research project.
    - **Funding Agency:** List the agencies that will fund this research.
    - **Name of PI**: In order to be considered a PI, a person must be a Professor (including Assistant, Associate, Emeritus, or Full). Research Scientists and Assistant Research Scientists may be listed as the PI if they have their own funding.

- **Co-Investigator:** Co-investigators should include faculty members or research scientists who will also be creating, manipulating or otherwise utilizing the recombinant or synthetic nucleic acid or the resulting recombinant organism.
- **Overall goals**: Briefly (1-5 sentences) describe the overall goal or purpose of this project.

- Lab location and training information is required, including:
  - **Lab location:** all locations in which the rDNA or synthetic nucleic acid molecule will be utilized, including the PI and Co-PI's laboratories.
  - **Other laboratory involvement**: State whether any other labs/core facilities will be involved with this rDNA or synthetic nucleic acid work (e.g., vector construction, propagation, creation of transgenics, etc.). Check the appropriate box identifying which labs will be involved with the proposal and subsequently describe the work that will be carried out by this lab/facility.
  - If **employee health surveillance** is recommended, a description of the surveillance program is required. A statement regarding laboratory staff being offered any applicable immunizations should be included here. Depending on the research proposed some other examples include:
    - No immunocompromised or pregnant individuals will be allowed to work in the laboratory with vaccinia strains.
    - Baseline serum will be collected from all individuals working in the BSL3 laboratory.
    - Individuals working with TB will undergo PPD testing twice a year.
  - **Training:** For all laboratory staff involved in the project, please identify if they have completed the basic courses listed in the question; this includes the PI and associated staff.
  - **Experience:** During review of the rDNA Registration Document, the IBC is required to take into consideration the relevant experience of all personnel involved in the project. Provide information on relevant experience the PI and applicable personnel have in relation to the proposal. Any listed Co-PIs and associated staff will be asked to identify their laboratory experience in a separate email.
    - If personnel do not have experience, please explain who will train personnel, the associated qualifications of the trainer, and how lab staff will be trained.

- Insert, vector and host information is required, including:
  - **Insert/Vector/Host/BSL Table**:
    - By clicking on the button you will be shown the following 6 fields as described below:
    - **Insert**: List the genes (recombinant or synthetic nucleic acid) used in the proposal.
    - **Source of insert**: List the species from which the rDNA or sequence of the synthetic nucleic acid insert originates.
    - **Function**: List the function of the expressed protein or the gene in which you are targeting.
    - **Vector**: Include a list of all possible vectors that will be used in the experiments; you may use broad categories by specifying a parental vector and its derivatives, e.g., "cloning vectors such as pBluescript and its derivatives" or "Saccharomyces expression vectors such as pESC and its derivatives."
    - **Host**: Include an exhaustive list of all possible hosts that will be used in the experiments; you may use broad species categories, e.g., "human and mouse cell lines" or "InvSc1 and similar strains."
    - **BSL/ABSL**: Assess the biosafety level of the work to be done; add animal (ABSL) if appropriate, see Risk Assessment and Containment.
      - If the facilities are under construction the PI must include an assurance that no rDNA or synthetic nucleic acid work will occur until the Biosafety Office staff has surveyed the completed laboratory facility.
  - **Replication status of the vector:** explain the molecular basis for the designation of each vector as replication defective, e.g., adenovirus: E1, E3 deleted; FIV: gag, pol and env deleted, if appropriate.

- Details of the project are required, including:
  - **Antibiotic resistance**: Include all antibiotic resistance genes that will be expressed in bacteria or cell culture; this includes genes that are already present in a commercially available vector.

If the transfer of these antibiotic genes is clinically relevant, NIH/OBA must approve this research prior to IBC review; please contact the Biosafety Office at EHS (5-8501) for further guidance.

- **Will this research involve**:
  - **Human subjects**: check the appropriate box and list the IRB protocol number (which must be current) if appropriate. Describe the rDNA or synthetic nucleic acids and/or recombinant organisms that will be administered to the subjects and the route of administration;
  - **Animal subjects**: check the appropriate box.
  - **Transgenic/knockout animals**: check the appropriate box.
  - Where appropriate, state the species of the animal involved in the proposal, include the ACURF approval number (must be current), and describe the rDNA, synthetic nucleic acid molecule or recombinant organisms that will be administered to the animals and the route(s) of administration.
  - For transgenics/knockouts, describe the genetic alterations that will be made to the animal, either through the creation of the transgenic/knockout or through cross breeding.

- An assessment of the classification is required:
  - **Classification**: select the appropriate classification for your project from the drop down list provided (see Classification of Experiments).

- When you have completed all requested fields and submit the document to EHS, instructions will direct you to the attestation statement. Carefully read the attestation statement that appears; by clicking on the "**Submit Application**" button you indicate your acceptance of all statements and your electronic signature will be applied to the document. Biosafety staff at EHS will be alerted to the submission of your document.

Major Changes

- The PI is responsible for notifying the IBC of any changes to the rDNA Registration Document, including changes in the:
  - Host or vector.
  - Donor species or nature of the DNA insert.
  - Physical location of the experiments.
  - Responsible investigator.

Appendices of the *NIH Guidelines*

Appendix A

- Appendix A of the *NIH Guidelines:*
  - Includes exemptions under Section III-F-6: Natural Exchangers.
  - Exempt experiments include rDNA molecules that are: (i) composed entirely of DNA segments from one or more of the organisms within a sublist, and (ii) to be propagated in any of the organisms within a sublist.
  - Sublists of natural exchangers are listed in this appendix.

Appendix B

- Appendix B of the *NIH Guidelines:*
  - Describes the classification of biological agents based on their potential effect on a healthy human adult.
    - **Risk Group 1**: **not** associated with disease (e.g. all serotypes of adenovirus-associated viruses, Baculovirus).
    - **Risk Group 2**: associated with disease that is **rarely** serious and interventions are **often** available (Adenovirus, *Listeria*).

        - **Risk Group 3**: associated with disease that is **serious or lethal** and interventions **may be** available (*Brucella*).
        - **Risk Group 4**: associated with disease that is **serious or lethal** and interventions are **not usually** available (Ebola virus).
    - Commonly encountered agents are listed according to their associated risk group.
    - For questions regarding the rDNA Registration Document and biosafety level assessment please refer to Appendix B or contact EHS Biosafety personnel via email: ehs-rdna@uiowa.edu, or call 5-8501.

Appendix C

- Appendix C of the *NIH Guidelines:*
    - Includes those exemptions under Section III-F-8: rDNA molecules that "do not present a significant risk to health or the environment as determined by the NIH Director."
    - The following experiments are exempt:
        - Recombinant or synthetic DNA containing < one-half of any eukaryotic viral genome propagated and maintained in cells in tissue culture.
        - *E. coli K-12, Saccharomyces, Kluyveromyces, B. subtilis* or *B. licheniformis* host-vector systems.
        - Extrachromosomal elements of gram positive organisms.
        - Purchase or transfer of transgenic rodents.
        - Generation of ABSL1 transgenic rodents via breeding.
    - **Exemptions for each group** are also listed in this appendix**.**

Appendix D

- Appendix D of the *NIH Guidelines:*
    - Includes a listing of the major actions taken under the *NIH Guidelines* after the issues have been considered by the RAC.

Appendix E

- Appendix E of the *NIH Guidelines:*
    - Includes a listing of the certified Host-Vector systems. These are exempt from the *NIH Guidelines*.
    - These host-vector systems were previously classified as Host-Vector systems 1 or 2.
        - Specific listed systems include:
            - *Bacillus subtilis*
            - *Saccharomyces cerevisiae*
            - *Escherichia coli*
            - *Bacteriophage systems*
            - *Neurospora crassa*
            - *Streptomyces*
            - *Pseudomonas putida*

Appendix F

- Appendix F of the *NIH Guidelines:*
    - Specifies the containment conditions for the cloning of genes coding for the biosynthesis of molecules toxic for vertebrates.

Appendix G

- Appendix G of the *NIH Guidelines:*
    - Specifies the physical containment for standard lab experiments and defines Biosafety levels (BSL) 1 - 4.
    - Standard Practices and Training includes:
        - Training personnel in microbiological techniques.
        - Adopting emergency plans for work with biohazards.

        - Making vaccines available, when appropriate.
    - Physical Containment includes:
        - Primary containment: lab practices and containment equipment.
        - Secondary containment: special lab design.

- **BSL-1:** Appendix G-II-A:
    - **Standard Microbiological Practices**
        - Access is limited or restricted at the discretion of the PI when experiments are in progress.
        - Work surfaces are decontaminated daily and after spills.
        - Wastes are decontaminated before disposal.
        - Eating and drinking are not permitted in the lab.
        - Personnel wash their hands after handling recombinant or synthetic molecules/organisms and before leaving the lab.
        - All procedures are performed carefully to minimize the creation of aerosols.
        - Hand washing sink, shower, changing room and protective clothing are provided if appropriate.
    - **Special Practices**
        - Contaminated material that is to be decontaminated at a site away from the lab is transported in a closed, durable leak-proof container.
        - Insect and rodent control program is in effect.
    - **Containment Equipment**
        - NONE
    - **Laboratory Facilities**
        - The laboratory is easily cleaned.
        - Bench tops are impervious to water and resistant to acids, alkalis, organic solvents and moderate heat.
        - Each lab contains a sink for hand-washing.
        - Fly screens are present on all windows that open.
        - Furniture is sturdy and spaces between benches, equipment and cabinets are accessible for cleaning.

- **BSL2:** Appendix G-II-B:
  These practices are in addition to those described for BSL1 containment.
    - **Standard Microbiological Practices**
        - Access is limited or restricted at the discretion of the PI when work with organisms containing recombinant or synthetic nucleic acid molecules is in progress.
        - All procedures are performed carefully to minimize the creation of aerosols.
    - **Special Practices**
        - Hazard warning signs are posted identifying the agents.
        - Protective laboratory clothing is worn in the lab and removed before exiting the lab to non-lab areas.
        - Gloves are worn when handling animals or agents.
        - Spills and accidents resulting in exposure are immediately reported to the IBC and NIH/OBA.
        - Needles and syringes are placed in puncture resistant containers.
        - Baseline serum samples for lab personnel are obtained when appropriate.
    - **Containment Equipment**
        - Biological safety cabinets are used when:
            - procedures with a high potential for generating aerosols are performed or
            - high concentrations or large volumes of organisms containing recombinant/synthetic nucleic acids are used.
    - **Laboratory Facilities**
        - An autoclave is available for decontaminating wastes.

- **BSL3:** Appendix G-II-C:
  These practices are in addition to those described for BSL1 and BSL2 containment.

- **Standard Microbiological Practices**
  - Persons under 16 are not allowed in the laboratory.
  - If other experiments are being conducted in the lab at the same time as those requiring BSL3 containment, they will be conducted in accordance with BSL3 level practices.
- **Special Practices**
  - The PI restricts access to the lab to those personnel required for support or program purposes.
  - All activities are conducted in a biological safety cabinet.
  - All work surfaces are decontaminated when work with rDNA or synthetic nucleic acid containing organisms is finished.
  - Laboratory clothing is decontaminated prior to laundering.
  - Surgical masks or respirators are worn when handling animals.
  - A biosafety manual is prepared.
- **Containment Equipment**
  - Biological safety cabinets are used for all activities.
- **Laboratory Facilities**
  - A ducted exhaust air ventilation system provides directional airflow that draws air into the laboratory.
  - High efficiency particulate air (HEPA) filtered exhaust from biological safety cabinets is discharged through the building exhaust system.
- **Enhancements for Research Involving Risk Group 3 Influenza Viruses**
  - Additional personal protective equipment and procedural requirements.
  - Animal containment enhancements.
  - Occupational health plan requirements.

Appendix H through J

- Appendix H of the *NIH Guidelines:*
  - Is applicable to the shipping of rDNA modified organisms or viruses.
    - Online training for shipping infectious substances/diagnostic specimens and shipping with dry ice can be found at: http://ehs.research.uiowa.edu/icon-training-information

- Appendix I of the *NIH Guidelines:*
  - Describes levels of biological containment with regard to the certified host-vector systems.

- Appendix J of the *NIH Guidelines*
  - Describes the responsibilities of the Biotechnology Research Subcommittee.

Appendix K

- Appendix K of the *NIH Guidelines:*
  - Describes the physical containment for research or production of viable organisms containing recombinant or synthetic nucleic acids at a large scale (>10 Liters).

Appendix M

- Appendix M of the *NIH Guidelines* applies to research involving human subjects.

- If your research involves a Human Gene Transfer Protocol please review the supplemental information for requirements regarding Appendix M.
  - To view this information, click on the “Back” button to exit this course.

Appendix P and Q

- Appendix P of the *NIH Guidelines:*
  - Describes the physical and biological containment for rDNA involving plants (BSL-P).

- Appendix Q of the *NIH Guidelines:*
  - Describes containment and confinement practices for research involving large animals (BSL-N).

Course Exam

Please complete the course exam; to receive credit for this online training course, you must receive a score of 80% or greater. This exam will serve as a record that you have fulfilled *NIH Guidelines* training.

- Click **HERE** to go to the exam.