



University of Tennessee User's Guide for Recombinant DNA Molecules

A reference for researchers of the UT-Knoxville and UT Institute of Agriculture campuses and the Graduate School of Medicine for understanding the NIH "Guidelines for Research Involving Recombinant DNA Molecules" and the Institutional Biosafety Committee registration process

Introduction

This document has been developed to assist researchers in preparing recombinant DNA molecule registration documents for review by the Institutional Biosafety Committee (IBC). To assist researchers with meeting NIH Guideline requirements "in practice", specific portions of the *NIH Guidelines* will be summarized as well.

Topics include:

- Applicability of the *NIH Guidelines*
- Principal Investigator's Responsibilities
- Biosafety Level 1- Standard Microbiological Practices
- Exempt Experiments
- Review Category Summaries
- IBC Registration Process
- Form Preparation Tips
- Resources
- Sample of completed form

Applicability of the *NIH Guidelines*

Applicability of the *NIH Guidelines* does not extend to field releases of transgenic plants that are released in compliance with a USDA Animal Plant Health Inspection Service (APHIS) notification or permit.

Virtually all other research involving recombinant DNA molecules at the University of Tennessee is subject to the National Institutes of Health "Guidelines for Research Involving Recombinant DNA Molecules" (*NIH Guidelines*). These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA molecules and organisms containing them. While the name *NIH Guidelines* may suggest that compliance is optional, compliance with these guidelines is a condition of the contractual agreement that the NIH has with any institution that receives NIH funding. Failure to follow the *NIH Guidelines* can jeopardize certain NIH funding for the entire institution.

NIH's Definition of Recombinant DNA Molecules

Recombinant DNA molecules are defined as either molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from the replication of those previously described.

UT Knoxville, Institute of Agriculture, and Graduate School of Medicine researchers who are using recombinant DNA molecules as part of their research must file a registration document with the Institutional Biosafety Committee (IBC), regardless of the funding source if their work does not meet the exempt criteria outlined in the *NIH Guidelines* (**please contact Brian Ranger, Biosafety Officer for assistance in determining exemption eligibility as interpreted by the IBC**). The IBC will review all registrations and provide recommendations and approvals as appropriate. The UTK/UTIA/GSM Biosafety Officer supports the review process by assisting researchers with registration preparation or revision, training and lab inspections as needed.

Principal Investigator's Responsibilities

Under the *NIH Guidelines*, both the institution and the researchers are charged with specific responsibilities for compliance that involve elements that go beyond general laboratory safety requirements.

NIH Guidelines for Research Involving the Use of Recombinant DNA Molecules (Section IV-B-7)

“On behalf of the institution, the Principal Investigator is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research.”

As part of this general responsibility, the Principal Investigator shall:

- Not initiate or modify any recombinant DNA research which requires Institutional Biosafety Committee (IBC) approval prior to initiation until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;
- Determine whether experiments are covered by [Section III-E](#), *Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation*, and ensure that the appropriate procedures are followed;
- Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), IBC, NIH/OBA, and other appropriate authorities (if applicable) within 30 days;
- Report any new information bearing on the *NIH Guidelines* to the IBC and to NIH/OBA;
- Be adequately trained in good microbiological techniques (including standard microbiological practices);
- Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination; and
- Comply with applicable shipping requirements for recombinant DNA molecules.

To register recombinant DNA molecule use with the IBC, the Principal Investigator shall:

- Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;
- Select appropriate microbiological practices and laboratory techniques to be used for the research;
- Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), or [III-E](#) (*Experiments Covered by the NIH Guidelines*), to the IBC for review and approval or disapproval; and
- Remain in communication with the IBC throughout the conduct of the project.

Before initiating research, the Principal Investigator shall:

- Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
- Instruct and train laboratory staff in the practices and techniques required to ensure safety (including standard microbiological practices), and the procedures for dealing with accidents; and
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

While research is under way, the Principal Investigator shall:

- Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
- Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), IBC, NIH/OBA, and other appropriate authorities (if applicable);
- Correct work errors and conditions that may result in the release of recombinant DNA materials; and
- Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

Biosafety Level 1

Biosafety Level 1 is suitable for work involving agents not reasonably expected to be a hazard, or those agents of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

- ★ **Note:** This level of containment is the minimum standard for all recombinant DNA work that requires IBC registration (Categories III-A through III-E). BL1 containment is also specifically recommended for most of the exempt (Category III-F) subcategories.

Standard Microbiological Practices (BL1)

1. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
2. Work surfaces are decontaminated once a day and after any spill of viable material.
Biosafety Note: Cross-contamination can result if work surfaces are not regularly decontaminated. Effective decontamination of work surfaces is achieved through the proper use of disinfectants that are effective for destruction of the microorganisms of concern.
3. All contaminated liquid or solid wastes are decontaminated before disposal.
Biosafety Note: Liquid wastes may be pretreated with chemical disinfectants before disposal via a lab sink that is connected to the sanitary sewer. Alternatively, liquid wastes (without chemical disinfectants) may be autoclaved prior to disposal via a lab sink that is connected to the sanitary sewer. Solid wastes contaminated with infectious agents or recombinant DNA must be managed as solid biohazardous waste for treatment and disposal purposes. Surface disinfection of such wastes is not an appropriate method of treatment for these wastes. Refer to the "Biohazardous Waste Basics" document for further information.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may only be stored in cabinets or refrigerators designated and used for this purpose.
6. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.
7. All procedures are performed carefully to minimize the creation of aerosols.
8. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Special Practices (BL1)

1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
2. An insect and rodent control program is in effect.
Biosafety Note: An insect and rodent control program can not be effective unless lab personnel take action when evidence of pests is discovered. If insects or rodents are detected in the lab area, report this to your building maintenance provider as soon as possible so that corrective actions can be taken.

Containment Equipment (BL1)

Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Laboratory Facilities (BL1)

1. The laboratory is designed so that it can be easily cleaned.
Biosafety Note: The ability to effectively clean a lab space is essential for work with viable biological materials. Open storage of supplies, personal items and nonessential equipment increases the amount of surface area that is susceptible to contamination in the event of a spill or lab practices that create uncontained aerosols. These items should be placed in closed storage whenever possible.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
Biosafety Note: If bench paper is used on bench tops where infectious agents or viable materials containing recombinant DNA molecules are manipulated, this bench paper must be discarded at the conclusion of the procedures and the bench top disinfected at least once a day as noted previously under the "Standard Microbiological Practices" section.
3. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for hand washing.
5. If the laboratory has windows that open, they are fitted with fly screens.

Exempt Experiments (Category III-F)

In general, exempt experiments include those in which the recombinant DNA inserts are in plasmid and phage in *E. coli* K12, DH5 alpha or in transgenic knockout mice; the inserts do not involve a viral gene, toxin, or pathogen source, and are not grown in a large-scale culture capacity (> 10 liters in one vessel).

If you believe your recombinant DNA molecule use is exempt, you need to consult [Section III-F](#) of the *NIH Guidelines* and read carefully to assure that your applications truly meet the criteria for exemption. There are several "exceptions" to the exemptions, and they are not clearly indicated in many cases.

The IBC **strongly** recommends that you register your use of recombinant DNA molecules, even if you think your techniques are exempt. This registration will allow the IBC to verify exemption status and to generate documentation that may be required by funding agencies relative to NIH compliance. **If you do not register your recombinant DNA molecule use because you believe it is exempt, and your funding agency requires you to provide documentation of IBC approval, you will not receive any documentation from the IBC without a completed review of a registration document.** The IBC will not hold a special meeting for review of your registration under these circumstances.

Review Category Summaries (III-A through III-C)

The first 3 categories of studies are those which require a review by the NIH in addition to the local IBC before the work can be initiated. These categories will not be applicable to most recombinant DNA molecule research at UT so they are just briefly captured below. If you feel that your work may fit into one of the categories, please contact the IBC Chair or the UTK/UTIA/GSM Biosafety Officer for assistance.

Category III-A Experiments Requiring IBC Approval, RAC Review and NIH Director Approval Before Initiation
--

This category is limited to studies that involve the deliberate transfer of drug resistance to microorganisms (not known to acquire the trait naturally) that can compromise the use of the drug to control the microorganism and its disease in humans, veterinary medicine or agriculture.

Note: Antibiotic resistance of low risk hosts for genetic screening purposes is not included in this category.

**Category III-B
Experiments Requiring NIH/OBA and IBC Approval Before Initiation**

This category is limited to cloning of genes that encode for toxin molecules with LD₅₀ <100 nanograms/kg body weight (e.g., botulinum, tetanus, diphtheria toxins).

Note: Specific approvals have been granted by NIH for cloning in *Escherichia coli* K-12 for toxins with LD₅₀ between 100 nanograms/kg and 100 micrograms/kg body weight. Refer to the *NIH Guidelines* for further information.

**Category III-C
Experiments Requiring IBC and IRB Approval and RAC Review Before Participant Enrollment**

This category is limited to deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human subjects.

Before enrollment, the following must be submitted to NIH/OBA for each clinical trial site:

1. IBC Approval (from clinical trial site)
2. IRB Approval
3. IRB-approved informed consent document
4. CV of PIs
5. NIH Grant Numbers (if applicable)

Review Category Summaries (III-D through III-E)

The next 2 categories include the recombinant DNA molecule use that is likely to be most applicable to researchers at UT-Knoxville, the Institute of Agriculture and the Graduate School of Medicine. The information regarding the various applications in these 2 categories is presented in table format for quick reference.

**Category III-D
Experiments Requiring IBC Approval Before Initiation**

NIH Subcategory	Research Application Description	Notes & Specific Considerations
D-1	Experiments using Risk Group 2, Risk Group 3, Risk Group 4 or restricted agents as host-vector systems.	This refers to the introduction of recombinant DNA <u>into</u> the pathogenic agent. Containment levels will generally be based on the risk of the unmodified agent.
D-2	Experiments in which DNA <u>from</u> Risk Group 2, Risk Group 3, Risk Group 4 or restricted agents is cloned into non-pathogenic prokaryotic or lower eukaryotic host-vector systems.	When cloning is from Risk Group 2 or 3 pathogens, BL2 containment is generally prescribed, but specific lowering to BL1 may be approved by IBC. Some of these applications may be exempt (refer to III-F).
D-3	Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of a helper virus in tissue culture systems.	Experiments involving Risk Group 2 viruses (infectious or defective) in the presence of helper virus are generally conducted at BL2. Experiments involving Risk Group 3 viruses (infectious or defective) in the presence of helper virus are generally conducted at BL3. Recombinant DNA or RNA molecules containing < 2/3 of the genome of any eukaryotic virus are considered defective. These viruses, in the absence of a helper virus, are categorized under III-

		E.
D-4	Experiments involving whole animals.	<p>This category applies to whole animals in which the genome has been altered by stable introduction into the germline (transgenic animals). This category also applies to experiments involving viable recombinant DNA-modified microorganisms tested on whole animals.</p> <p>Viral vectors used on animals that do not lead to transmissible infection, either directly or indirectly a result of recombination in animals, may generally be propagated at BL1.</p> <p>Recombinant DNA, or DNA or RNA derived from recombinant DNA, from any source except for >2/3 of eukaryotic viral genome may generally be transferred to animals and propagated at BL1.</p> <p>The purchase of transgenic rodents is exempt under Section III-F.</p>
D-5	Experiments involving whole plants.	<p>This category applies to experiments that produce genetically engineered plants by way of recombinant DNA methods. This category also applies to experiments using plants together with microorganisms or insects containing recombinant DNA.</p> <p>This category is <u>restricted</u> to experiments involving exotic infectious agents with recognized potential for serious, detrimental impact on ecosystems and experiments involving sequence encoding potent vertebrate toxins introduced into plants or associated organisms. All other plant experiments are likely to fall under Section III-E.</p>
D-6	Experiments involving >10 liters of culture.	Appendix K outlines large-scale containment practices. The IBC will decide upon appropriate containment.

**Category III-E
Experiments Requiring IBC Notice Simultaneous with Initiation**

This category includes several different types of applications, which can typically be carried out using biosafety level 1 containment facilities, equipment and practices.

NIH Subcategory	Research Application Description	Notes & Specific Considerations
E	Experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic eukaryotes.	BL1 containment
E-1	The formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus.	Propagation and maintenance in tissue cell culture can be done at BL1, provided that cells lack helper virus of concern.
E-2	Experiments involving whole plants.	<p>This category covers all whole plant experiments except those that involve exotic infectious agents with recognized potential for serious detrimental impact on ecosystems (i.e., those covered under Category III-D).</p> <p>Includes: Plant-related experiments involving recombinant DNA-modified organisms that have no recognized potential for rapid and widespread dissemination or detrimental impact on ecosystems (e.g., <i>Agrobacterium</i>, <i>Rhizobium</i>)</p>

		BL1 may be used where modified plants are not noxious weeds and cannot interbreed with noxious weeds of geographic interest.
E-3	Experiments involving transgenic rodents <u>only</u> .	This category applies to experiments involving generation of rodents in which the genome has been altered by the stable introduction of recombinant DNA. The purchase of commercially-available transgenic rodents is exempt.

IBC Registration Process

To register your recombinant DNA molecule use with the Institutional Biosafety Committee, you must complete a registration document and submit it to the UTK/UTIA/GSM Biosafety Officer, 336 Ellington Plant Sciences Bldg. A copy of the completed form can be submitted via fax to 946-2574 to initiate the review process but final IBC approval of the registration will not be granted unless the original hard copy is received by the Biosafety Office before the scheduled IBC review date.

[Click here to access the "Registration for Use of Recombinant DNA Molecules" form](http://biosafety.tennessee.edu) or visit the UT Biosafety Program website (<http://biosafety.tennessee.edu>) to access this form under the "Forms" link on the main page.

The "Form Preparation Pointers" section of this document will provide you with guidance for form completion to assure that all necessary information is completed appropriately to minimize the potential for delays in the IBC review process. A sample of a completed form is included for your reference as well. Please note that incomplete forms will not be sent on to the IBC for review, but will be returned to the submitting PI.

The IBC meets on a monthly basis to review and act on registrations submitted during the previous calendar month. Therefore, if you want to have your registration on the next month's agenda, you should take actions to have your completed registration documents on file in the Office of Research by the end of the current month.

If your work will require biosafety level 2 (BSL-2) practices and containment, or your work falls into a Category III-D, III-C, III-B, or III-A, it is strongly recommended that you contact the UTK/UTIA/GSM Biosafety Officer for assistance with form preparation. When BSL-2 work is proposed, you should attend the IBC meeting when your proposed work will be reviewed if at all possible. Your presence at the meeting will greatly facilitate the information exchange process that is likely to accompany the review at these levels. If you do not attend, and questions arise that cannot be addressed, you will most likely receive a request for more information and further review of your proposed protocol will be delayed.

Once approval is granted by the IBC, an approval letter and a copy of the approved version of the registration document will be generated by the Office of Research. Whenever possible, approval letters will be prepared and sent to PI's within the same business month. If you do not receive any communication regarding the outcome of the review process by the end of the month for which your review was scheduled, please contact the Research Compliance Officer.

IBC approval of protocols will be valid for 3 years from the approval date. However, in order to maintain your approval, you must complete and submit an annual assurance form to document any changes in the recombinant DNA materials or techniques listed on the original registration. You will receive a notice from the Office of Research approximately one month before the anniversary date of your approval with instructions for form completion and submission. If you do not receive such a notice before your approval anniversary date, please contact the Research Compliance Officer.

Please note that if your recombinant DNA work changes to the degree where the work falls into a different review category, or presents new considerations for containment requirements, you must notify the IBC Chair before initiating these changes to determine if further review actions are required.

It is your responsibility and in your best interest to take measures to assure that your work is properly documented and currently approved to assure compliance with regulations that can impact funding monies.

Form Preparation Pointers

Click here to access the “Registration for Use of Recombinant DNA Molecules” form or visit the UT Biosafety Program website (<http://biosafety.tennessee.edu>) to access this form under the “Forms” link on the main page.

Section 1: Protocol Profile

Principal Investigator

The *NIH Guidelines* refer to the researcher who is responsible for documentation, training and reporting relative to the recombinant DNA molecule use as the “Principal Investigator”. For registration with the UT IBC, the Principal Investigator is someone who has a faculty appointment with UT and meets funding agency requirements as a lead researcher. If you do not have such an appointment, you must partner with someone who does and work with that individual to have the work appropriately documented through that individual on your behalf.

Research Protocol Title

This title should represent the protocol to be used for generation and use of the recombinant DNA molecules captured in the protocol. It should not be specific for a particular grant proposal if the protocol is intended to cover multiple grant proposals. IBC approval letters will list the general protocol title. If you find that you need an approval letter that specifies a particular grant title, you can request such a letter from the Biosafety Office if this grant title is listed on the approved general protocol. If you submit grant proposals that would be covered by the approved general protocol but are not listed on the original general protocol, you can have the general protocol updated by contacting the Biosafety Office.

Question 1

Please assure that you place an “X” or a check mark in the appropriate box for each item in the table.

- Regarding deliberate transfer of drug-resistance traits to microorganisms, this item refers to microorganisms of clinical, environmental or agricultural significance that could compromise the use of drugs to control disease and does not refer to antibiotic-resistance that may be employed as a screening tool of low-risk organisms.
- Regarding work with a Risk Group 2 or higher pathogens, this category applies to any microorganisms that are infectious to humans as well as some animal pathogens. Work with such infectious materials is likely to require completion of additional information if the work will require biosafety level 2 containment. Please contact the UTK/UTIA/GSM Biosafety Officer at 974-1938 for assistance if you suspect your work may fit into this category.

Question 2

You can determine your review category and subcategory by referring to the category summary tables provided previously in this document. Assure that you place an “X” in the Review Classification column for the category that applies to your work. Additionally, list the subcategory that applies to your work. If you need further assistance in determining your review category, please contact the IBC Chair or the UTK/UTIA/GSM Biosafety Officer.

Question 3

Please provide the biosafety level under the biosafety level category that applies to your work.

- Lab biosafety levels apply to work conducted in the lab such as those activities involving microbiological and cell cultures, and plant work carried out in the lab or in growth chambers. A description of the requirements for these containment levels can be found in Appendix G of the *NIH Guidelines*.
- Large-scale biosafety levels only apply to work that involves 10 liters or more of culture in one vessel. A description of the requirements for these containment levels can be found in Appendix K of the *NIH Guidelines*.

- Animal biosafety levels apply to work involving animals in a laboratory animal setting. Animal biosafety levels apply to activities such as experimental infection of animals with recombinant microorganisms and recombinant vaccine trials. A description of the requirements for these containment levels can be found in Appendix Q of the *NIH Guidelines*.
- Plant biosafety levels apply to work conducted in greenhouses with plants and their pests. A description of the requirements for these containment levels can be found in Appendix P of the *NIH Guidelines*.

Question 4

Please list all locations where the work described in the general protocol will take place.

Question 5

Please list the names and professional credentials of all personnel that will participate in the recombinant DNA research described in this protocol. Professional credentials include job title, degree, date of “Standard Microbiological Practices” training, and the most recent BSL-2 training date (if the work will require BSL-2 practices).

Section 2: Protocol Description

Question 6

This should be a 1 or 2 sentence description of the purpose of recombinant DNA molecule generation and use.

Question 7

This table is intended to simply serve as a list of the materials included in the recombinant DNA molecule generation and use described in this protocol. You should be as specific as possible with listing the materials that you may use but groups of materials may also be listed if adequately described and examples are provided. It is understood that this list is likely to change through the research process. For this reason, you will be expected to complete an annual assurance document to update your information as described in the “IBC Review Process” section of this guide.

PLEASE NOTE: If your approved recombinant DNA work changes to the degree where the work falls into a different review category, or presents new considerations for containment requirements, you must notify the IBC Chair before initiating these changes to determine if further review actions are required.

Question 8

The instructions for completion of this section are detailed on the form. Please note that proper completion of this section is the key to facilitating completion of the IBC review and approval process. If the proposed work is classified under Category III-F (exempt experiments) you do not need to complete this question.

Question 9

The response to this question will help to clarify the information provided in Question 7 in a manner that can be easily understood by reviewers who are not experts in your field including regulatory auditors.

Section 3: Biological Containment, Health & Safety

NOTE: If your recombinant DNA molecule use is Category III-E or Category III-F, you do NOT need to complete this section!

However, the *NIH Guidelines* require that ALL non-exempt recombinant DNA molecule use be carried out in accordance with Biosafety Level 1 containment requirements as a minimum standard. You MUST assure that

these requirements are adopted in your laboratory's standard operating procedures and that you are able to demonstrate compliance with these requirements in the event of a regulatory audit.

Biosafety Level 1 containment is also recommended for exempt experiments.

Question 10

The *NIH Guidelines* have specific provisions for the IBC to review and approve associated emergency/incident response procedures, including spill response. Contact the UTK/UTIA/GSM Biosafety Officer to assist you with developing your spill response plan and to provide guidance for summarizing the information for your registration document.

Question 11

The information provided here should identify the disinfectant product to be used, and how it will be used. A statement such as "treat with bleach" is not an adequate response to this question. Please remember that your completed registration document can be used as a training and guidance tool in your lab's biosafety manual. Therefore, the response to this question should summarize the procedure to be used by your lab personnel for disinfection.

Here is an example of the appropriate level of information:

"Environmental surfaces where the recombinant organisms will be manipulated will be cleaned and then disinfected with a 1:20 solution of household bleach to water. The solution will be freshly prepared at the time of use. Surfaces will be left wet with this solution for a minimum of 10 minutes to assure adequate disinfection."

Question 12

The response to this question should include information regarding waste segregation, means of treatment, and method of disposal. A statement such as "collected in biohazard bag, autoclaved, and disposed of as trash" is not an adequate response to this question. Please remember that your completed registration document can be used as a training and guidance tool in your lab's biosafety manual. Therefore, the response to this question should summarize the procedure to be used by your lab personnel for disinfection.

Here is an example of the appropriate level of information:

"Solid lab wastes (excluding sharps) contaminated with recombinant DNA molecules or recombinant organisms will be collected in a biohazard-labeled, leak-proof container with a lid. The container will be lined with a biohazard bag. Biohazard bags will be biologically inactivated by sterilization. The autoclave to be used for this process has been recently validated for waste treatment with a minimum cycle time of 40 minutes on the gravity cycle and these parameters will be applied for our waste treatment. Once treated by autoclave, bags of waste will be placed in a non-see-through bag for disposal as regular trash. No biohazardous sharps or liquids will be generated."

Visit the UT Biosafety Program website (<http://biosafety.tennessee.edu>) for more information regarding biohazardous waste disposal procedures.

Question 13

Please answer this question with a NO or a YES. If your response is YES, please provide a brief description of what will be transported where it will be transported to, and how you are proposing to transport it. This information will be used to assess what transportation regulations may apply to your work.

Question 14

If your work does not involve the use of pathogens that are infectious to humans, or the use of research animals, this question will most likely not apply to your work. However, you still need to provide a response to this question to complete your application. You may simply state: "Medical surveillance requirements do not apply to this work because no human pathogens or research animals will be used as part of this work."

If the proposed work involves the use of human-derived materials (including cells), it will most likely be covered by the OSHA Bloodborne Pathogens standard which requires that employers offer hepatitis B vaccination to exposed personnel. Under this circumstance, you may simply state: "All personnel have been offered hepatitis B

vaccination and will follow universal precautions and post-exposure procedures as required for compliance with the OSHA Bloodborne Pathogens standard.”

If the proposed work involves research animals, you and your personnel will be enrolled in the Occupational Health Program associated with the UT Institutional Animal Care and Use Committee (IACUC) registration process. Please note this enrollment in your response if applicable to the proposed recombinant DNA work.

If your work involves the use of pathogens infectious to humans, review the current literature regarding vaccination recommendations and identified risk populations and summarize your findings in your response to this question. There are several resources on the UT Biosafety Program website (<http://biosafety.tennessee.edu>) that can be used for this assessment as well.

Section 4: Principal Investigator/Department Head Acknowledgment

The Principal Investigator and the Department Head must review the applicable statements in this section and provide signatures to acknowledge their responsibilities. These signatures must be provided before the registration document can be distributed to the IBC for review. Once the registration is reviewed and approved, the IBC Chair will sign the form. A copy of the approved registration document with all signatures will be provided to the submitting PI as described previously in this guide.

A sample of a completed form for a Category III-E protocol can be found on the following pages.

UT Resources

John Sanseverino, IBC Chair	jsansev@utk.edu	974-8080
Bonnie Ownley, Former Chair	bownley@utk.edu	974-0219
Brian Ranger, UTK/UTIA/GSM Biosafety Officer	branger@utk.edu	974-1938
Sarah DiFurio, UTK/UTIA/GSM Asst. Biosafety Officer	sbottoml@utk.edu	974-9836

IBC Registration #: _____

Initial receipt date: _____

Final revision date: _____

University of Tennessee-Knoxville
Registration for Use of Recombinant DNA Molecules

Principal Investigator: Please complete all applicable sections of this form. Incomplete forms will not be sent on to the IBC for review. If you choose to use acronyms or other scientific shorthand, please define these terms the first time they are used. For assistance with completion of the form, please refer the "UT User's Guide for Recombinant DNA Molecules" or contact the IBC Chair or the UTK/UTIA/GSM Biosafety Officer at 974-1938 (branger@utk.edu).

SECTION 1: PROTOCOL PROFILE

Principal Investigator: John Jones

Title: Research Assistant Professor

Mailing Address: 123 Science & Arts

Department: The Center for

Phone: 974-00XX

Email Address: jonesy@utk.edu

Research Protocol Title: Bioluminescent Bioreporter for the Specific Detection of Mercury (II)

Applicable Grant Titles (if any)

Development of a Bioluminescent Bioreporter for the Specific Detection of Mercury (II)

Funding Agencies

National Center for Mercury Exposure Prevention

1. Does this project involve any of the following?

(Check the appropriate box for each category)

	YES	NO
Deliberate transfer of drug-resistance traits to microorganisms of clinical, environmental or agricultural significance that could compromise the use of drugs to control disease		√
Clinical trial of human gene therapies		√
Work with human-derived materials (i.e., cells including cell lines , body fluids or tissues)		√
Work with genetic materials from a Risk Group 2 or higher pathogen (i.e., any agent infectious to humans; refer to Appendix B of the NIH Guidelines; if YES, contact the UTK/UTIA/GSM Biosafety Officer for further documentation requirements.)		√
Work with a select agent or high-consequence pathogen (Refer to current select agent list, contact the UTK/UTIA/GSM Biosafety Officer immediately if YES for this category.)		√
Work with live animals (i.e., genetic modification of animals or challenge of animals with recombinant DNA or viable recombinant DNA-modified microorganisms)		√
Work with live plants (i.e., genetic modification of plants or challenge of plants with recombinant DNA-modified organisms to include microbes and arthropods)		√
Work involving cloning of genes that encode for toxins		√
Work that will involve more than 10 liters of culture in one vessel		√
Radioisotope use in conjunction with recombinant DNA use (if YES, provide an explanation of this use in your summary of techniques to be used.)		√

2. Which NIH review classification and category applies to this work?

Note: NIH review classifications and categories are summarized in the “UT User’s Guide for Recombinant DNA Molecules” and can also be found in Section III of the NIH Guidelines.

Check the appropriate box for your review category and indicate the subcategory that applies to your work.

Review Category	Subcategory
	III-A: Requires IBC, RAC & NIH approval before initiation Subcategory:
	III-B: Requires NIH/OBA and IB approval before initiation Subcategory:
	III-C: Requires IBC and IRB approvals and RAC review before participant enrollment. (This category is for human gene therapy trials only.) Subcategory:
	III-D: Requires IBC approval before initiation Subcategory:
√	III-E: Requires IBC notice simultaneous with initiation Subcategory: E
	III-F: IBC registration is not required but recommended to assure that work is properly classified Subcategory:

3. What Biosafety Level are you proposing to use for this work?

Note: Recommendations for Biosafety Levels are generally outlined under Section III of the NIH Guidelines and in the “[UT Guide for Recombinant DNA Molecule Use](#)”. Full Descriptions of the Biosafety Levels are found in Appendix G for lab applications, Appendix K for large-scale use of recombinant organisms, Appendix P for plant applications, and Appendix Q for animal applications.

Place the number of the proposed biosafety level for the recombinant DNA procedures under the appropriate box or boxes.

Laboratory Biosafety level	Large-Scale Biosafety Level	Animal Biosafety Level	Plant Biosafety level
1			

Note: If Biosafety Level 2 is indicated, contact the UTK/UTIA/GSM Biosafety Officer at 974-1938 (branger@utk.edu) for assistance with supplementary form preparation.

4. Where will this work be conducted? (Rooms & Buildings)

2212 Science Tower

5. List all personnel who will be working with recombinant DNA covered under this registration, their expertise, and training:

Name	Title	Degree	Date of Standard Microbiological Practices Training	Date of Most Recent BSL-2 Training (if applicable)
John Jones	Assistant Professor	PhD	2/5/2005	N/A
Janet Davis	Research Associate	MS	1/31/2006	N/A
William Wright	Graduate Student	BS	1/31/2006	N/A

SECTION 2: PROTOCOL DESCRIPTION

6. Provide a purpose statement for the protocol:

The contract established by USACEHR was to develop a stable, bioluminescent reporter for mercury (Hg²⁺) detection.

7. List all hosts, vectors and inserts used as part of this protocol in the table below. Please be as specific as possible (e.g. *E. coli* DH5- α rather than *E. coli* or IPTG-inducible *lac* promoter rather than inducible promoter).

Hosts (bacteria, yeast, cell lines, etc.; include technical names)	<i>E. coli</i> EC100 competent cells (Epicenter, Madison, WI) <i>E. coli</i> DH5 α
Vectors (describe the class of vector, source organism or derivative, etc.)	pDG106 –contains the mer operon Plasmid pFSP3 – EZ::TN containing the promoterless <i>lux</i> cassette
Promoters (include source and activity)	<i>PmerT</i>
Insert genes (name of gene or name of protein it codes for, specify natural source, activity/ phenotype)	Final chromosomal insert <i>merR PmerT luxCDABE</i> Phenotype is production of bioluminescence in the presence of Hg ²⁺

8. Provide a technical description of this proposed recombinant DNA protocol. Briefly describe the procedures that will result in recombinant DNA molecule generation and how these products will be used. Please include attachments if appropriate. The content of this section (at a minimum) must include information that supports the category that you selected under #2 and reflects the materials identified under #6.

*****Note:** If your proposed recombinant DNA molecule use is described under Category III-F, you do not need to complete this question.

A 505 bp merR fragment was PCR amplified from the mer operon and cloned into the TA Cloning Vector (pCR2.1; Invitrogen, San Diego, CA). Primers (Table 1) for the amplification were synthesized based on the *merRo/p* sequence listed in GenBank (Accession #AF071413; nucleotides 19133-19638).

Table 1. Primers for amplification of *mer* components.

Primer	Sequence*	Notes
19145- <i>NotI</i>	5'GCGGCCGCttgaattggattgtagatgcgtaaccttacttccg	Incorporation of NotI site at 5'-end of merRo
19638- <i>XbaI</i>	5'AGATCTctaagcatagctgacc	Opposite strand; incorporation of XbaI site at 3'-end of merRo

*Capital letters denote the addition of the restriction site.

The source of the *mer* DNA was pDG106 (Gambill and Summers, 1985). The merR was excised from pCR2.1-*merR* with *EcoRV* and *BamHI*. Plasmid pFSP3 (a gift from B.M. Applegate, Purdue University) was prepared by digesting with *SmaI* and *BamHI*, dephosphorylation by shrimp Page 4 of 6 alkaline phosphatase (USB, Cleveland, Ohio) and purification by GeneClean (Bio 101, Carlsbad, California). The *merR* fragment was ligated into pFSP3 overnight at 16°C followed by chemical transformation into chemically competent *E. coli* DH5 α cells. Transformants were subjected to miniprep plasmid isolation and further screened by restriction digestion with *BamHI* and *KpnI*. A positive clone designated #7 contained the *merR* gene in the proper orientation to induce

bioluminescence in the presence of Hg²⁺ ions. Following large-scale preparation, the transposon vector was digested with *PshAI* overnight at 25°C. The 8.5 kb fragment containing the *mer-lux* reporter transposon was gel purified with Gene Clean. The transposome was formed by incubating the 8.5 kb fragment (*mer lux* EZ::TN) with transposase according to manufacturer's directions. The resultant transposome was then electroporated into *E. coli* EC100 competent cells (Epicenter, Madison, WI). Electroporants were plated on LB agar plates with Km (50 mg/L). Three colonies were recovered which produced bioluminescence in the presence of Hg²⁺ ions. These strains were designated *E. coli* ARL1, *E. coli* ARL2, and *E. coli* ARL3.

- 9. Provide a non-technical summary of the recombinant DNA use for this protocol.** This should be written so that a non-scientist or someone who is not an expert in your particular field can understand your recombinant DNA use.

The objective is to make a bioluminescent sensor responsive to mercury (Hg²⁺). This is accomplished through standard genetic engineering procedures. A genetic element that responds to mercury (*merR PmerT*) is fused with the genes that mediate bioluminescence (*luxCDABE*). This construct is inserted into the chromosome of a host microorganism, in this case an innocuous strain of *E. coli* (EC100).

SECTION 3: BIOLOGICAL CONTAINMENT, HEALTH & SAFETY

*If your recombinant DNA molecule use is described under Category III-E or Category III-F, you do **NOT** need to complete this section. However, please note that the NIH Guidelines require that ALL non-exempt recombinant DNA molecule use be carried out in accordance with Biosafety Level 1 containment requirements as a minimum standard. You **MUST** assure that these requirements are adopted in your laboratory's standard operating procedures and that you are able to demonstrate compliance with these requirements in the event of a regulatory audit.*

*Biosafety Level 1 containment is also recommended for exempt experiments. **Please proceed to Section 4.***

- 10. Summarize your written biological spill response procedure for the materials covered by this protocol which has been approved by the UT Biosafety Officer. (Contact the UTK/UTIA/GSM Biosafety Officer for assistance.)**
- 11. Summarize your surface disinfection procedures. Include information relative to dilution, contact time, shelf life and personal protective equipment requirements. (Contact the UTK/UTIA/GSM Biosafety Officer for assistance if needed.)**
- 12. Summarize how wastes contaminated with recombinant DNA products or other biological materials will be segregated, treated and disposed of.**

- 13. Will any biological materials be transported outside of the lab area (i.e., out of the building where the lab is located), or shipped off-site? If YES, please describe.**
- 14. Describe any health surveillance measures that are required for personnel working on this protocol (i.e., vaccination offers, health conditions that could increase exposure or disease risk, etc.)**

SECTION 4: PRINCIPAL INVESTIGATOR/DEPARTMENT HEAD ACKNOWLEDGMENT

Principal Investigator

As the Principal Investigator for this protocol, I understand and acknowledge my responsibilities under the NIH Guidelines to:

- Be adequately trained in good microbiological techniques (including standard microbiological practices) and assure that all personnel working on this protocol are also trained in these techniques;
- Instruct and train laboratory staff in the practices and techniques required to ensure safety, and the procedures for dealing with accidents;
- Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
- Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).
- Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), IBC, NIH/OBA, and other appropriate authorities (if applicable);
- Correct work errors and conditions that may result in the release of recombinant DNA materials;
- Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination;
- Comply with applicable shipping requirements for recombinant DNA molecules;
- Maintain communication with the IBC including reporting of any releases, accidents or procedural modifications relative to this recombinant DNA use.

Signature- Principal Investigator

Date

Department Head

I have reviewed this document and will assure that this work is carried out in accordance with applicable health & safety regulations and guidelines.

Signature- Department Head

Date

Submit the final version of this form with above signatures to Brian Ranger, Biosafety Officer at 336 Ellington Plant Sciences for review and approval by the UT IBC.

IBC Chair's Acknowledgment

The Institutional Biosafety Committee has reviewed the proposed project involving recombinant DNA molecules and has approved the use of these research materials using the containment facilities practices outlined based on the NIH "Guidelines for Research Involving Recombinant DNA Molecules".

Signature- IBC Chair

Date