

INSTRUCTIONS - FORM A -
Protocol for Use of
Recombinant DNA in Research

Version 4.0

Office of Animal Care and Institutional Biosafety
(OACIB)

Institutional Biosafety Committee

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I. Instructions

*Important: Form A - Protocol for Use of Recombinant DNA (rDNA) in Research Form A must be completed for ALL work that involves the use of rDNA at UIC with the exception of human gene transfer. **Complete Form D if human gene transfer will be conducted.** NO WORK WITH rDNA IS EXEMPT AT UIC.*

A. General

The NIH Guidelines for Research Involving Recombinant DNA Molecules (*NIH Guidelines*) require the IBC to conduct a comprehensive risk assessment of the project in order to determine the appropriate containment level for the project and the appropriate practices and procedures that should be used in handling the agents. The risk assessment must take into account the following:

- Agent characteristics (e.g., virulence, pathogenicity, environmental stability)
- Types of manipulations planned
- Source(s) of the inserted DNA (e.g., species)
- Nature of the inserted DNA sequences (e.g., structural DNA, oncogenes)
- Host(s) and vector(s) used
- Whether a foreign gene will be expressed and if so, the protein that will be produced
- Containment conditions to be implemented
- Applicable sections of NIH Guidelines

In order to aid the IBC in its assessment and avoid delays in approval, please follow these general rules for each protocol application and the specific instructions for each applicable section.

1. Please read each question and answer all items requested.
2. Be specific and complete in your answers. Vague, incomplete answers may result in additional information being required before protocol can be forwarded onto the committee for review and/or deferral following review.
3. Although all work in a laboratory that has a related purpose can be submitted on one protocol, note that protocols cannot be partially approved and review of higher category research could delay start of the protocol.
4. All Forms must be typed; failure to do so will result in return of the forms
5. All investigators that submit a protocol to the IBC must complete Appendix 1, Personnel and Qualifications.
6. Send the original completed form(s) for review and approval to: Office of Animal Care and Institutional Biosafety (OACIB) at the address listed above.
7. Investigators with questions regarding the completion of forms are encouraged to contact the OACIB.

B. Instructions for Form A

1. **Review Category I-** Experiments involving rDNA that correspond to this category require IBC approval and additional approvals in order to be initiated.
 - a. Check box 1 in this category if research involves both items below.

- i. The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire that trait naturally, **and**
- ii. That acquisition could compromise the use of the drug to control disease agents in humans, animals, or plants. **This category does not apply to the use of standard plasmids for cloning into nonpathogenic prokaryotic sources (e.g., *E. coli* K-12).**

Protocols of this nature must be submitted to the Office of Biotechnology Activities (OBA), have the proposal published in the federal register for a 15-day comment period, be reviewed by the Recombinant Advisory Committee (RAC) and be specifically approved by the NIH Director, prior to receiving final IBC approval and initiation. **Investigators should submit their protocols to the IBC office prior to submission to OBA.** Investigators should note that this category generally refers to the primary, secondary, and potentially tertiary use of the drug to control the disease. In some cases, the drug may not be the standard one used in developed countries, but may be the drug of choice in developing countries. This must be taken into account when determining whether this category applies.

- b. Check box 2 in this category if research involves
 - i. The deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD₅₀ of > 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin).
 - ii. Note- Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules, which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from OBA.

Protocols of this nature must be submitted to the OBA for approval and must obtain final IBC approval prior to initiation. **Investigators should submit their protocols to the IBC office prior to submission to OBA.** A list of common toxic molecules of concern can be found at <http://tigger.uic.edu/depts/ovcr/research/protocolreview/ibc/policies/index.shtml>. Please note that the list is not all-inclusive.

- c. Check box 3 in this category if research involves
 - i. The deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD 50 of greater than 100 nanograms per k/bw, but less than 100 micrograms per kg/bw.
 - ii. Note- Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules, which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from OBA.

Protocols of this nature must be registered with NIH/OBA prior to initiation of the project. Protocol must be approved by IBC prior to registration.

All investigators conducting research that is covered by review category I must sign the assurance statement related to this work.

2. **Review Category II-** Experiments involving rDNA that correspond to this category require IBC approval **PRIOR** to be initiated. **Failure to do so is considered serious noncompliance and reportable to OBA.** Check all boxes in this category that apply to the following types of rDNA research if conducted.
- a. All investigators must make an initial determination if the research they are conducting involves the use of vector(s), host(s), and/or cloned DNA from agents that are listed in risk groups (RG) 2, 3 or 4 of Appendix B of the NIH *Guidelines* available at the following URL (http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm). Check box 1 in this category if the vector or host fall into these risk groups and box 2 in this category if the cloned DNA is from agents that fall into this category.
 - i. RG classifications are assigned on the basis of the wild-type organism. They are assigned based on the association of the agent with disease in normal healthy adults, the basis of availability of preventative or therapeutic interventions, and the likelihood of community risk. If any of the above (vector, host or DNA) fall into RG 2-4, then the appropriate box or boxes in review category II must be checked regardless of attenuation.
 - ii. RG 2, 3, and 4 agents will usually be conducted at Biosafety Level (BSL) 2, 3, or 4 containment, respectively. Experiments with such agents will usually be conducted with whole animals at BSL2 or BSL2-N, BSL3 or BSL3-N, and BSL4 or BSL4-N (Animals) containment, respectively. **NO work requiring BSL4 containment may be conducted at UIC.**
 - iii. Transfer of RG 2 or RG 3 into nonpathogenic prokaryotes or lower eukaryotes may be performed under BSL2 containment. Experiments in which DNA from RG 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BSL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BSL4 containment must be used and cannot be conducted at UIC.
 - iv. The IBC may approve the specific lowering of containment for particular experiments to BSL1.
 - b. All investigators must also determine if the research they are conducting involves the use of infectious or defective DNA or RNA viruses in the presence of helper virus in tissue culture. Helper viruses are infectious viruses that allow defective viruses to replicate. If helper viruses will be used in tissue culture, check box 3 in this category.
 - c. If research involves the transfer of rDNA or DNA derived from rDNA into any whole animal then the research falls into this category and box 4 in this category must be checked. The definition of animal for these purposes applies to all animals including vertebrates and nonvertebrates such as insects. Research that involves transfer of rDNA into vertebrate animals also requires the approval of the Animal Care Committee. Check box 4 in this category if this applies. **Transgenic rodents do not fall into this category unless the nature of the DNA being used requires BSL 2 or higher. All creation of transgenic rodents at BSL1 is covered under review category III. Transfer of a biological made from rDNA (e.g., recombinant protein) does not require checking this box.**
 - d. If research involves the transfer of rDNA or DNA derived from rDNA into whole plants and that DNA is from an exotic infectious agent with recognized potential for serious detrimental effects on managed or natural ecosystems then the research falls into this category and box 5 must be checked. Depending on the nature of the research, work must be conducted at BSL2-P+ or above.
 - e. If research involves cloning of rDNA in cultures that are 10 liters or greater then research

falls into this category and box 6 must be checked. The Institutional Biosafety Committee will decide the appropriate containment. When appropriate, Appendix K will be used for determination.

3. **Review Category III-** If research involves work conducted under category III, the work may begin *simultaneously* with submission of IBC protocol. Work conducted in this category cannot also qualify for review category I or II. **The higher review category takes precedence.** Check all boxes in this category that apply to the following types of rDNA research.
- a. Check box 1 in this category if work involves rDNA molecules containing no more than two-thirds of the genome of any eukaryotic virus, with all viruses from a single Family being considered identical. rDNA may be propagated and maintained in cells in tissue culture using BSL1 containment. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of the genome.
 - b. Check box 2 in this category if work involves rDNA molecules containing no more than one-half of the genome of any eukaryotic virus, with all viruses from a single Family being considered identical. rDNA may be propagated and maintained in cells in tissue culture using BSL1 containment. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of the genome.
 - c. Check box 3 if work involves the use of rDNA in plants and the types of experiments do not qualify for BSL2-P+ or above then work is covered by this section. The work listed below must be conducted at BSL2-P or BSL1-P+. All other work with nonpathogenic organisms in plants can proceed at BSL1-P.
 - i. For transgenic plants containing rDNA from noxious weeds or the transgenic plants that could interbreed with noxious weeds in the immediate geographical area.
 - ii. Transgenic plants in which rDNA equals complete genome of a non-exotic infectious agent.
 - iii. Plants associated with rDNA-modified non-exotic microorganism that have no recognized potential for serious detrimental impact on managed or natural ecosystems.
 - iv. Plants associated with rDNA-modified exotic microorganism that have no recognized potential for serious detrimental impact on managed or natural ecosystems.
 - v. Experiments in which arthropods or small animals associated with plants are genetically modified with rDNA or the microorganisms associated with them are genetically modified with rDNA if there is no recognized potential for serious detrimental impact on managed or natural ecosystems.
 - d. Check box 4 if work will involve the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (e.g., transgenic rodents) and experiments can be performed under BSL1 containment, these experiments are covered under this section. If experiments require BSL2 or higher containment, then it is covered under section D4 and experiments cannot be initiated until approved by the IBC.
 - e. Check box 5 if work involves the use of rDNA outside of organisms or viruses (e.g., transfection of cells in culture), then work is covered by this section. If the rDNA is transfected into cells that could be infected or are known to be infected with pathogens work must be conducted at a minimum of BSL2 (e.g., primary culture of cells obtained from human or non-human primate tissue and all cell lines of human origin).

- f. Check box 6, if the experiments consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent. Work must be conducted at the appropriate biosafety level. If work must be conducted at BSL2, it must be approved prior to initiation.
 - g. Check box 7 if experiments consist of cloning of DNA from a **eukaryotic or prokaryotic source** not containing a pathogenic agent and propagating it using plasmids in **nonpathogenic prokaryotic source**
 - h. Check box 8 if experiments consist of cloning DNA from a **prokaryotic source including its indigenous plasmids or viruses** and propagating it **only** in the **same host (or a closely related strain of the same species)** or when transferred to another host by well established physiological means.
 - i. Check box 9 if experiments cloning DNA **entirely** from a **eukaryotic source** including chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated **only** in the **same host (or a closed related strain of the same species)**.
 - j. Check box 10 if DNA will be cloned from (source) and propagated in (host) a natural exchanger (source and host exchange DNA by known physiological processes in nature) then work is covered by this section. Sublists of natural exchangers can be found in Appendix A of the *NIH Guidelines* available at the following URL (http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_A.htm). The work may be conducted at BSL1 if both the source and host are nonpathogenic. All other experiments must be performed at the appropriate biosafety level for the host or recombinant organism.
 - k. Check box 11 if research does not fit into review category I, II or III and provide an explanation of the research.
4. **Section IV- Purpose and Background-** This section must be completed for all review categories. In section a, investigators should describe the purpose of their work in lay language. This is important, as the IBC is required to have two non-affiliated members on the Committee who are often non-scientists. In section b, the investigators must briefly indicate the scientific background for the work
5. **Section V- Project Description-** All applicable sections must be completed to describe the agent characteristics (e.g., virulence, pathogenicity, environmental stability), types of manipulations planned, source(s) of the inserted DNA (e.g., species), nature of the inserted DNA sequences (e.g., structural DNA, oncogenes), host(s) and vector(s) used, and whether a foreign gene will be expressed and if so, the protein that will be produced.
- a. Section Va- List all laboratory E. coli hosts, the vectors used in those hosts, the inserted genes, species of origin of the genes, and the functions of the genes. In addition, the sources of the E. coli hosts, vectors, and genes should be listed. Only BSL1 E. coli strains should be listed here.
 - b. Section Vb- List all other organisms and their strains, as well as, any cells and species of origin of the cells that are used as hosts. List the risk group of any host that is a microorganism, and all plasmids or vectors used in these hosts, and gene inserted and the function of the gene. Sources for all hosts, vectors, and genes should be listed. For each organism listed above describe the pathogenicity or attenuation of the organisms. For all pathogenic strains, list the antibiotic selection markers that will be used in those strains.
 - c. Section Vc- List all viral vector class and name, source, packaging cell line, plasmids required for replication, and host cell range, replication competence and attenuation if applicable.

- d. Section Vd- Provide a description of the in vitro work that will be conducted with the reagents listed above.
- e. Section Ve- Describe all transgenic/knockin/knockouts to be developed or the crossing of existing lines. If rDNA will be administered to animals provide a description of the construct to be administered, amount administered, the route of administration, where the work will be conducted, where the animals will be housed post-administration, the duration of experiment, and the biosafety level for this portion of the project.
- f. Section Vf- Describe all transgenics to be developed or the crossing of existing lines. If rDNA will be administered to plant provide a description of the construct to be administered, amount administered, the route of administration, where the work will be conducted, where the plants will be housed post-administration, the duration of experiment, and the biosafety/containment level for this portion of the project.

6. Section VI- Locations, BSL, Storage, and Shipping

- a. Section VIa- List all locations in which rDNA and/or infectious agents work will occur.
- b. Section VIb- List type of housing for animal work if it is applicable.
- c. Section VIc- List all locations in which rDNA and/or infectious agents will be stored.
- d. Section VI d- List highest BSL at which work will be conducted. Note that * refers to conducting project in facilities at the BSL indicated, but incorporating some or all procedures and practices from the next highest BSL.
- e. Section VIe- Indicate if any potentially infectious material will be shipped off campus, indicate so on the form, indicate where it will be shipped, and who is responsible for shipping.

A LABORATORY-SPECIFIC BIOSAFETY MANUAL MUST BE COMPLETED AND SUBMITTED WITH ALL PROTOCOLS. For work conducted at BSL1 or BSL2, complete the appropriate biosafety manual template provided on IBC website. For BSL3, contact IBC office to discuss biosafety manual requirements. **FAILURE TO SUBMIT A MANUAL WITH THE PROTOCOL WILL RESULT IN PROTOCOL BEING REVIEWED.**

7. **Section VII- Funding-** List all funding support for this protocol.
8. **Section VIII- Conflict of Interest-** List all potential financial conflicts of interest. *“Investigators” include the principal investigator, co-investigators, and any other person who is responsible for the design, conduct, or reporting of research. **For conflict of interest guidance and information, please email COI@uic.edu or call 312/996-4070.***
9. **Section IX- Assurances-** All investigators must sign all assurances