

Biological Materials and Recombinant DNA Protocol

Record #: _____ SC#: _____

UNIVERSITY OF WISCONSIN-MADISON

Institutional Biosafety Committee/Office of Biological Safety

Return completed form to OBS as attachment to email (biosafety@fpm.wisc.edu) or via fax **265-8700**

I. CORE REGISTRATION INFORMATION

Name of Principal Investigator (PI): _____ PI status is conferred by virtue of appointment or by being granted explicitly by the Graduate School

Job Title: _____

Office Phone: _____ **Lab Phone:** _____ **Fax:** _____

Department: _____ **Box #:** _____

Campus Address: _____
(Bldg, Rm #, Street)

Email Address: _____

Name of Co-PI(s): _____

Protocol Type	Applicable Registration Number & Date	
New, Amendment, Renewal, Training or Center	Current SC#	Expiration date

General Protocol Title: _____

Title of Grant/Proposal/MTA:	Multiple Donor Acct, Granting Agency or Source:	Grant # (if available):

Signature of Principal Investigator	Date	Chair, Institutional Biosafety Committee	Date
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II. LAY ABSTRACT OF PROPOSED PROJECT(S) (250 word limit) Give a brief explanation of the science for each proposed project.

III. RESEARCH FACILITIES

Location:

Where are experiments performed and animals housed? What is the biosafety level (BSL) of the physical containment? Does the location provide containment equipment such as fume hoods, HEPA-filtered ventilation biological safety cabinets, etc? **NOTE:** The OBS requires prior notification, via written amendment to this protocol, for any change that affects containment (location of lab, animal rooms, and/or BSCs).

Building Name	Room number	Biosafety Level (BSL-1, BSL-2, or BSL-3)	Use of Room (animal housing, lab, surgery, necropsy, growth chamber, etc.)	Protective Equipment* (autoclave, biosafety cabinet, fume hood, other)	Containment Equipment Certification date(s)

* Note that clean air device (CADs) do not provide protection from aerosolized biological, chemical, or radiation hazards and therefore must not be used to handle these materials.

IV. LABORATORY/ADMINISTRATIVE PERSONNEL

List personnel involved with work covered under this research registration; include lab personnel: investigators, students, and research staff. Mark (bold or asterisk) the lab supervisor or administrative coordinator whom you would like OBS to contact for information about this protocol.

Last name, First name	Job Title	Phone number

V. RESEARCH ELEMENTS (Skip sections that do not apply)

- **Complete Section A for recombinant research. The NIH Guidelines defines recombinant DNA molecules as 1) molecules constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or 2) molecules that result from replication of those described in 1) above.**
- **Complete Section B for nonrecombinant research. Nonrecombinant research involves projects that do not fit the NIH definition above such as work with nonrecombinant microbes.**
- **Complete Section C for chemicals used to elicit a biological response**

A. Recombinant DNA subject to the NIH *Guidelines for Research Involving Recombinant DNA Molecules*. (<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>) Avoid or explain acronyms. Human gene therapy studies must also address Appendix M of the *Guidelines*. Reports of any serious adverse events attributed to human gene transfers must be submitted to the Office of Biological Safety (OBS). Additionally, the Institutional Biosafety Committee (IBC) requests a copy of reports that are submitted to the Institutional Review Board (IRB).

A(i) Gene Source(s) List genes of interest and marker traits. All genes conferring antibiotic resistance must be listed; include those constructed in your lab as well as those contained in acquired plasmids or other vectors. All toxins must be listed. Please provide details for EACH higher hazard material (e.g., list high hazard genes such as oncogenes, toxins, antibiotic resistance genes, etc.) Lower hazard genes such as housekeeping genes may be grouped into categories with representative examples given.

Gene Source(s) (Genus, species, strain)	Gene Name Explain acronyms (e.g. GFP - green fluorescent protein)	Nature of Insert or Protein Expressed (Toxin, marker trait, virulence factor, DNA repair gene, oncogene, transcription factor, etc.)	Use of Construct Cloning for sequencing, PCR Expression in a microbe Expression in tissue culture Expression in an Organism

A(ii). Vector Description(s) Provide details for representative examples of each category of vector. It is not necessary to provide details about every construct; categorical descriptions that are useful in assessing risks are acceptable.

Gene Transfer Method (Conjugation; transformation, transduction, liposome; electroporation, viral infection, CaPO ₄ , polyplexes, etc.)	Vector Backbone (Bacterial plasmid, cosmid, phage, virus, synthetic, YAC, BAC, transposon, etc.) Include genus and species of source if applicable	Vector Technical Name Include commercial vendor if applicable (e.g., pLXSN - Clontech)	Risk Attenuation (Replication defective vector? Ecotropic vector? Nonconjugative plasmid? Nontransducing phage? Helper-dependent conjugative plasmid? Suicide vector? Temperature sensitive growth?)

A(iii). Microbes List all microbes (bacteria, virus, fungi, prion, parasite) including those used to propagate recombinant plasmids and vectors or produce foreign proteins (e.g., non-pathogenic *E. coli* K-12 or yeast), as described above. Mark (Y/N) in appropriate categories and specify organisms/cells receiving microbe. Provide strain names or numbers of pathogens when available; Give example representative strain or two of commonly-used nonpathogenic microorganisms such as *E. coli* K12. Provide additional information on toxin production such as LD₅₀ of toxin in Section VIII.A.

Microbe (Genus, species, and strain)	Biosafety Level or Risk Group	Human Pathogen?	Animal Pathogen?	Plant Pathogen?	Toxin Production? (Specify toxin)	Large Scale Production? >10 liters	Recipient of rDNA Construct or Construction of Recombinant Microbes?	Administered to: (e.g., mice, alfalfa, HeLa cells, etc.)

Exposure Prophylaxis and Response For each microbe, describe the prophylactic and response procedures. Consider the consequences of an accidental exposure, i.e., mucosal splash, inhalation, ingestion, or inoculation, which might occur during experimental handling. Organisms normally not pathogenic for healthy humans may become so when the natural barriers to infection are circumvented. Exposure response could be a simple matter of washing the wound with soap and water (if materials not pathogenic to humans), or it could involve medical evaluation (if infectious or potentially infectious materials). Is a particular antibiotic preferred and readily available (a serious concern if the microbe harbors introduced antibiotic resistance)? Is serum tested prior to and/or after exposure? Are personnel immunized? Notify the PI/supervisor of potential exposures to infectious materials and decide whether medical follow up is needed. An information sheet (e.g., a material safety data sheet) should accompany the individual and be provided to medical personnel. The Occupational Health Program (265-5000) and Office of Biological Safety (263-9013) must be informed and an accident report is filed.

Microbe	Exposure Prophylaxis and Response Procedures

A(iv). NIH Guidelines Assessment

Assess the appropriate physical and biological containment for recombinant DNA activities. PIs must thoroughly review Section III of the NIH Guidelines which explains what is and is not subject to the Guidelines. Other sections of the Guidelines that must be reviewed are the roles and responsibilities of PIs (Section IV-B-7), Appendix G for precautions to be used for various biosafety levels, and other sections that pertain to the PI's specific research. The NIH Guidelines training is mandatory for all PIs doing recombinant research and for all research staff doing recombinant research in laboratories with biosafety protocols reviewed by the IBC. The NIH Guidelines training is available from the OBS website (www.fpm.wisc.edu/biosafety). State the appropriate biological safety level(s) for activities with these rDNA elements. **Support your assessment by citing the relevant subsection(s) of the current NIH Guidelines** (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). Contact OBS for assistance in this determination.

Recombinant material and activity	Biosafety Level	Guidelines citation

A(v). Organ, Tissue or Cell Cultures (OTCC) Identify the organism that is the source of the OTCC, the nature of the cell lines, and whether the OTCC is modified by rDNA. Note that established human cell lines are potentially infectious, as are cells from Old World Monkeys and primary human cells and tissues, and biosafety level 2 containment is appropriate for handling these materials.

OTCC Source (Genus, species, strain)	Technical Name of OTCC (e.g. 3T3NIH, HepG2)	Passage (e.g., primary, established)	Description (oncogenic, helper/packaging, immortalized, etc.)	Recipient of rDNA? (transient/stable)	Intended Use (admin. to animals, cell culture, etc.)	Potentially Infectious?

A(vi). Vertebrates, Invertebrates, or Plants Identify organisms and mark (y/n) in appropriate categories. If a microbe is administered to the organism, identify it (genus, species). Indicate if animals are immunocompromised.

Organism (Genus, species, strain)	Transgenic? Source?	Immune Status Immuno-competent or -compromised	Recipient of Microbe? (Genus, species)	Recipient of rDNA construct?	Recipient of OTCC? (Specify OTCC)	Animal Care Protocol #, 6-digits (e.g. G0#####, V0#####)

B. Nonrecombinant Research subject to CDC's *Biosafety in Microbiological and Biomedical Laboratories - BMBL*. (<http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm>) IF NONRECOMBINANT INFORMATION IS THE SAME AS RECOMBINANT INFORMATION IN SECTION V.A, PLEASE CUT AND PASTE FROM ABOVE RELEVANT SECTION.

B(i). Microbes List all microbes (bacteria, virus, fungi, prion, parasite). Mark (Y/N) in appropriate categories and specify organisms/cells receiving microbe. Provide strain designations when possible. Provide additional information on toxin production such as LD₅₀ of toxin in Section VIII.A.

Microbe (Genus, species, and strain)	Biosafety Level or Risk Group	Human Pathogen?	Animal Pathogen?	Plant Pathogen?	Toxin Production? (Specify toxin)	Large Scale Production? >10 liters	Administered to: (e.g., mice, alfalfa, HeLa cells, etc.)

Exposure Prophylaxis and Response For each microbe, describe the prophylactic and response procedures. Consider the consequences of an accidental exposure, i.e., mucosal splash, inhalation, or inoculation, which might occur during experimental handling. Organisms normally not pathogenic for healthy humans may become so when the natural barriers to infection are circumvented. Exposure response could be a simple matter of washing the wound with soap and water (if materials not pathogenic to humans), or it could involve medical evaluation (if infectious or potentially infectious materials). Is a particular antibiotic preferred and readily available (a serious concern if the microbe harbors antibiotic resistance)? Is serum tested? Are personnel immunized? Notify the PI/supervisor of potential exposures to infectious materials and decide whether medical follow up is needed. An information sheet (e.g., a material safety data sheet) should accompany the individual and be provided to medical personnel. The Occupational Health Program (265-5000) and Office of Biological Safety (263-9013) must be informed and an accident report is filed.

Microbe	Exposure Prophylaxis and Response Procedures

B(ii). Organ, Tissue or Cell Cultures (OTCC) Identify the organism that is the source of the OTCC and the nature of the cell lines,. Note that established human cell lines are potentially infectious, as are cells from Old World Monkeys and primary human cells and tissues, and biosafety level 2 containment is appropriate for handling these materials.

OTCC Source (Genus, species, strain)	Technical Name of OTCC (e.g. 3T3NIH, HepG2)	Passage (e.g., primary, established)	Description (oncogenic, helper/packaging, immortalized, etc.)	Intended Use (admin. to animals, cell culture, etc.)	Potentially Infectious?

B(iii). Vertebrates, Invertebrates, or Plants Identify organisms and mark (y/n) in appropriate categories. If a microbe is administered to the organism, identify it (genus, species). Indicate if animals are immunocompromised.

Organism (Genus, species, strain)	Transgenic? Source?	Immune Status Immuno-competent or -compromised	Recipient of Microbe? (Genus, species)	Recipient of OTCC? (Specify OTCC)	Animal Care Protocol #, 6-digits (e.g. G0#####, V0####)

B(iv). BMBL Biosafety Assessment

Assess the appropriate physical and biological containment for nonrecombinant activities. Support your assessment by citing the relevant subsection(s) of the current BMBL. Contact OBS for assistance in this determination.

Nonrecombinant material and activity	Biosafety Level	BMBL citation

C. Chemicals Used to Elicit a Biological Response

Identify chemicals that are potential human health hazards and are used to elicit a biological outcome. Include routine uses (anesthetics, antibiotics, etc.) only if the chemical is hazardous (e.g., halothane, urethane, bromodeoxyuridine, chloramphenicol, tamoxifen, cyclosporine, rapamycin, etc.). In Section VIII below, describe precautions used to prevent inadvertent exposure to staff when handling these chemicals and materials exposed to them. Specify organisms/cells receiving each chemical and where it is prepared and administered (e.g., fume hood, BSC, or lab bench)..

Chemical Name (avoid acronyms)	Nature of Chemical (carcinogen, mutagen, drug, pesticide, teratogen, toxin, etc.)	Where Prepared (e.g., BSC, fume hood, lab bench)	Highest Quantity Handled	Administered to: (e.g., mice, alfalfa, HeLa cells, bacteria)	Where Administered (e.g., BSC, fume hood, lab bench)	Regimen for Dosing Animals: Route of Admin., (IV, IP, etc.), highest dose, # of doses, # of animals

VI. DISPOSAL AND DISINFECTION

Describe the method(s) used to inactivate hazardous materials within your research facility (e.g., autoclaving, disinfection with a chemical). If a disinfectant is used, state its name, the concentration used, and the exposure time. **If different methods/disinfectants are used for different agents, specify method for each.** If disposal involves pickup of the hazardous material for off-site management, who provides this service? Disposal of medical waste, including sharps is managed under a contract with MERI. The Safety Department will pick up animal tissues and carcasses and hazardous chemicals. See the UW Chemical Safety and Disposal Guide for instructions.

Material to be Disinfected/Inactivated	Disposal method/Procedure (e.g., autoclave, disinfectant type, or picked up for off-site disposal by MERI or Safety Dept)	Disinfectant concentration and exposure time, or autoclave time and temp
Surfaces (counters and equipment)		
Cell lines, infected material, rDNA materials		
Plasticware and glass		
Animal bedding/wastes		
Other:		

VII. OTHER REGULATORY REQUIREMENTS

USDA/APHIS Permits The Animal and Plant Health Inspection Service of the US Department of Agriculture regulates plant and animal pests and exotic organisms through a permit process. These permits often have conditions concerning the facility and containment procedures. OBS can assist with these stipulations. Are materials used in this protocol subject to federal permit requirements?

YES: _____ NO: _____ If yes, provide a copy of the current permits.

OSHA Bloodborne Pathogens Standard Research involving the use of human-derived substances (e.g. blood or blood components, tissues, secretions) or human-derived cell lines, may be subject to the OSHA *Bloodborne Pathogens Standard*. Does your laboratory have a registered OSHA Bloodborne Pathogen *Exposure Control Plan*? If **yes**, please indicate the binder number from the UW Occupational Health Program *Bloodborne Pathogens Reference and Training Manual*. If **no**, please contact Occupational Health Program at 265-5000.

YES, Binder #: _____ NO: _____

Respiratory Protection Depending on the situation, use of a respirator could involve fit testing, training, and medical clearance. For more information, contact Occupational Health Program at 265-5000 or Bill Deppen at 262-9179.

Are there conditions for which staff must wear respiratory protection?

YES: _____ NO: _____ If yes, explain: _____

Animal Contact Risk Questionnaire Have members of your research group having contact with lab animals completed and submitted an *Animal Contact Risk Questionnaire*? If not, contact Occupational Health Program at 265-5000.

YES: _____ NO: _____

Chemical Hygiene Plan Does your laboratory have a Chemical Hygiene Plan? If no, please contact Chemical Safety at 265-5000 for information.

YES: _____ NO: _____

DOT HazMat Shipping Certification The U.S. Department of Transportation requires that all persons involved in shipping hazardous materials in commerce be trained and certified in proper handling of these materials. The Safety Department offers two programs to meet this requirement. The Chemical and Radiation Protection Office provides training and certification for shipping and receipt of hazardous chemicals; call 265-5518 for further information. The Office of Biological Safety provides training and certification for shipping Infectious Substance, Patient Specimens, and other biological materials; call OBS (263-2037) for more information.

Does at least one member of your lab have current certification for shipping hazardous materials?

YES: _____ NO: _____

Who certified? _____ Certification date: _____

Human Embryonic Stem Cells in Animals Animals that receive human embryonic stem cells may not be bred. By checking the box below, investigators who administer human embryonic stem cells to animals confirm that they are aware of this prohibition and will not allow the animals to reproduce. If you do not handle these materials or perform these procedures, mark the box NA.

YES, I confirm that the animals receiving human embryonic stem cells will not be allowed to reproduce. _____

Select Agents Agents that can be considered potential bioterrorism agents are regulated by CDC and USDA [Provide link to SA list]. Do you have SA materials or plan to have SA materials in your facilities?

YES: _____ NO: _____

If so, are you a SA-registered user?

YES: _____ NO: _____

VIII. RESEARCH PROTOCOL DESCRIPTION

A. Design and Objectives

Briefly describe the experimental design and objectives, tying together all the research elements listed in Section V (Recombinant, Nonrecombinant, and Chemicals). Describe in vivo as well as in vitro research. Explain your intended use of these materials. Describe the rDNA constructs listed in Section V.A. in molecular terms (e.g. promoter[s], genes of interest, ORFs, selectable markers). **Attach representative examples of construct maps for each category of vector used. For replication-deficient viral vectors, indicate how the vector is rendered replication-deficient. Include additional information in description of toxin production such as LD₅₀ of the toxin and whether there is deliberate cloning of toxins.**

B. Potential environmental impact

Please describe aspects of the protocol that may have potential environmental impact, or indicate that no impact is anticipated. **At the minimum, indicate if all hazardous materials are appropriately disinfected or inactivated prior to release to the environment.** If you plan to conduct a field trial, include the location and correspondence from the appropriate regulatory agency showing approval for the environmental release.

C. Description of safety precautions.

Describe precautions used for handling materials listed in Section V by providing a detailed response to each of the following points. Each box can be expanded as needed to provide appropriate details. Appropriate precautions for biohazardous materials are described in the institution's biosafety manual, *Biohazard Recognition and Control*, which is based on *Biosafety in Microbiological and Biomedical Laboratories* and *NIH Guidelines for Research Involving Recombinant DNA Molecules*. These guidance documents are available from the OBS website. **Guidance documents giving recommended precautions for handling of viral vectors are also available from the OBS website. Reference materials that describe appropriate precautions for handling hazardous chemicals include toxnet (<http://toxnet.nlm.nih.gov/>) and the MSDS that accompanies the chemical. A detailed manual is required for work under Biosafety Level 3 containment; guidance for points to consider in developing a BSL-3 manual is posted on the OBS website. Standard operating procedures (SOPs) may be provided to supplement the description of precautions.**

C(i). Laboratory Facilities

a.	Describe adequacy of facility design and containment equipment ¹ .
b.	Describe measures that prevent or minimize expression of pathogenic/infectious sequences (risk attenuation).
c.	Describe evaluation of antibiotic resistance traits. <ol style="list-style-type: none">1. What antibiotic resistance trait(s) are introduced to each species of microorganism2. Could this modification compromise treatment of an infection?3. Do nonrecombinant microorganisms harbor antibiotic resistance trait(s) that could compromise treatment of an infection?
d.	For what procedures is containment (e.g., a BSC or fume hood) used? What precautions are used when working outside containment?
e.	Specify safeguards used during procedures that could generate aerosols, such as flow cytometry, centrifugation, electroporation, etc.
f.	Describe precautions used for handling human blood and tissues; for potentially infectious materials such as established human or nonhuman primate cell lines.
g.	Describe the use of PPE-personal protective equipment (e.g., eye protection, type of mask recommended or required, gloves, lab coat, etc). If PPE varies by material handled or procedures done, specify which PPE is used for different procedures.
h.	Is efficacy testing done for each disinfection method (e.g., autoclave monitored with biological indicator such as spore strips)?
i.	Describe training of personnel for handling hazardous and/or recombinant materials. Training on the NIH Guidelines must include a verification that the trainee understands what recombinant research is subject to the Guidelines and what research is not allowed without approval of the IBC and/or NIH. The NIH Guidelines training is mandatory for all PIs doing recombinant research and for all research staff doing recombinant research in laboratories with biosafety protocols reviewed by the IBC. Who provides training and how often is it done? Describe how you document this training.
j.	Describe methods used to prevent escape into the environment of exotic and transgenic organisms.
k.	Describe precautions used during transport of hazardous materials between labs within a building and between buildings
l.	Describe precautions used for procedures that involve large volumes of a microbe (i.e., production quantities or more than 10 liters ²).
m.	Describe hazard communication for biological and chemical hazards in laboratory areas. Include which signage is used for what materials and whether the signage is posted on the door or posted locally (e.g., work area, fume hood, biosafety cabinet, potentially-contaminated equipment).
n.	Describe or attach the laboratory's spill protocol(s) for hazardous materials. (Generic spill protocols are available from the OBS website).

o. Provide additional relevant details about how risks are mitigated.

Notes:

¹ See Table 2 of *Biohazard Recognition and Control* for a summary of facility standards.

² See Appendix K of the NIH Guidelines for standards that apply to large scale production of recombinant organisms

C(ii). Animal Facilities

a. Describe adequacy of facility design and containment equipment¹.

b. Describe precautions used such as containment (e.g., a BSC or fume hood) and PPE-personal protective equipment (e.g., eye protection, type of mask recommended or required, gloves, lab coat, etc) for handling animals that have been administered infectious materials. Specify safeguards used during procedures that could generate aerosols such as administration, cage changes, necropsy, etc.

c. Describe precautions used including containment, PPE, and safeguards for aerosol-generating activities for handling animals that have been administered potentially infectious materials such as established human or nonhuman primate cell lines.

d. Describe precautions used including containment, PPE, and safeguards for aerosol-generating activities for handling animals that have potential to carry zoonotic disease (e.g., Old World monkeys such as macaques, pregnant sheep, reptiles, etc.).

e. Describe precautions used including containment, PPE, and safeguards for aerosol-generating activities for handling animals that have been administered potentially-hazardous chemicals.

f. Is efficacy testing for disinfectants done (e.g., autoclave monitored with biological indicator such as spore strips)?

g. Describe training of personnel for handling hazardous and/or recombinant materials. Training on the NIH Guidelines must include a verification that the trainee understands what recombinant research is subject to the Guidelines and what research is not allowed without approval of the IBC and/or NIH. The NIH Guidelines training is mandatory for all PIs doing recombinant research and for all research staff doing recombinant research in laboratories with biosafety protocols reviewed by the IBC. Who provides training and how often is it done? Describe how you document this training.

h. Describe methods used to prevent escape into the environment of hazardous materials and organisms.

i. Describe precautions used during transport of animals between facilities within a building and between buildings.

j. Describe hazard communication for biological and chemical hazards in animal facilities. Include which signage is used for what materials and whether the signage is posted on the door and posted locally (e.g., cage cards, fume hood, biosafety cabinet).

k. Provide additional relevant details about how risks are mitigated.

Notes:¹. See Table 2 of *Biohazard Recognition and Control* for a summary of facility standards

Appendix 1

Inventory of Stored Biological Materials

List pathogens and toxins that are stored and not actively used in your current research projects. Include:

- Human pathogens classified as risk group 2, 3, or 4 (see Appendix B of the NIH Guidelines for risk group listing)
- Animal and plant pathogens, including those regulated by USDA APHIS PPQ and VS
- Select agent pathogens and toxins. A consolidated list is available at the OBS website (→Select Agents)

Human, Animal and Plant Pathogens (Genus, species, strain)	Select Agent Toxin (Toxin name)

Appendix 2

If this is a renewal protocol or amendment to a protocol, provide the Office of Biological Safety's current risk assessment for your laboratory. A summary risk assessment is provided on your current biosafety protocol registration form. Additional risk assessment information for individual projects is communicated on IBC memos sent to PIs after IBC protocol reviews.