



IBC eProtocol Guidance Document

The Institutional Biosafety Committee (IBC) is responsible for reviewing all research conducted at The Ohio State University involving recombinant or synthetic nucleic acid molecules and biohazards. Biohazards requiring an IBC protocol include all human source material (blood, tissue, cell lines, etc.), human pathogens and, in some cases, animal and exotic plant pathogens. An IBC protocol is submitted by the Principal Investigator (PI) and is a comprehensive risk assessment of work involving biohazards or recombinant or synthetic nucleic acid molecules. IBC protocols are intended to ensure compliance with Federal Regulations outlined in the NIH Guidelines for Recombinant DNA, which ensure that novel, dangerous organisms are not created by genetic engineering.

Additionally, IBC protocols ensure that all research involving biohazards is conducted in a manner that minimizes risk to personnel, the community, and the environment.

The purpose of this Guidance Document is to provide PIs with information that will help ensure that the submitted IBC protocol contains the information that the IBC requires to make a decision, thereby expediting the approval process. The goal of the protocol is not to convince the committee that the work itself is without risk, but to demonstrate that all of the PIs understand the risks involved and are operating at the proper biosafety level to mitigate those risks.

1. **Getting Started:** The “Getting Started” step is a very important one. You will be given a list of YES/NO questions to answer pertaining to your proposed research. How you answer these questions will generate the “SmartForm” that you will complete for review by the IBC. It is extremely important that you read these questions and any sub-questions that appear, very carefully. The form that is generated is specific to the type of research you specify so all questions should be relevant if the Getting Started questions are answered correctly.
 - a. Do not check “YES” to the first question “Will your research involve the use of human source material” unless you are not using any other pathogens (i.e. bacteria, viruses, etc) or rDNA. This question should only be checked “YES” if human blood, tissue or cell lines are the only reason you are submitting the protocol.

2. **Descriptive Summary:** The purpose of the Descriptive Summary is to provide the IBC with adequate but concise information regarding the proposed research. There are four sections in the Descriptive Summary. Listed below each section are important components of each.



IBC eProtocol Guidance Document

- a. Provide a hypothesis / goals for the proposed research.
 - i. Be concise.
 - ii. Use non-technical language because not all of the committee members will be familiar with your research area and its jargon.
 - iii. Do not simply cut and paste sections from your grant proposals.
 - iv. Include how the use of biohazards and/or rDNA is relevant to the outlined research.

- b. Provide an abstract of the proposed research; including descriptions of any rDNA constructs, definitions of all acronyms used in the protocol and a brief description of the laboratory procedures to be conducted.
 - i. Include how the use of recombinant or synthetic nucleic acid constructs is relevant to the proposed work.
 1. Why is it a gene of interest?
 2. Why is a particular vector being used?
 - ii. Do not use abbreviations that have not been defined.
 - iii. A step-by-step detailed SOP for specific techniques is not required but enough detail that the committee will understand the type of experiments being performed should be provided.
 - iv. The Descriptive Summary should accurately reflect the rest of the protocol. For example, if the Animal section indicates that mice are being used, the Descriptive Summary should also include an experimental description of mouse work.

- c. Discuss the risks associated with the biohazards outlined in this protocol and what will be done to mitigate these risks, i.e. engineering controls, work practices, personal protective equipment (PPE), etc.
 - i. Include a risk assessment for each agent/biohazard; include relevant information regarding potential risks of transmission, dose, containment in environment, etc.



IBC eProtocol Guidance Document

surrogate mother, ultimately gives rise to a new strain that is homozygous for the inert gene¹.

The IBC requires this information to ensure compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

The proposed work may be classified as “EXEMPT” but the registration of this work via an IBC protocol is necessary to confirm that IBC approval per the NIH Guidelines is not required. Registration of commercially available genetically modified animals is required by OSU’s IBC to cover the potential that a new strain created as a result of breeding registered animals would require approval under the NIH Guidelines.

- a.** Include a brief description of the gene of interest. If transgenic or knock-out / knock-in animals are being purchased commercially, provide a description of the strain, including the gene of interest / gene that has been knocked out/in.
 - i.** Simply listing the strains of rodents, etc is not acceptable. The committee should be able to ascertain from the information provided in your protocol, what the genetic modification to the animal is and why it is important to your research.

The OSU Institutional Biosafety Committee requires a protocol submission for all research involving the use of recombinant or synthetic nucleic acid molecules. In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:

- a.** Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e. recombinant nucleic acids;
- b.** Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e. synthetic nucleic acids, or
- c.** Molecules that can result from the replication of those described in (a.) or (b.) above.



IBC eProtocol Guidance Document

While some recombinant and synthetic nucleic acid molecule research is exempt under the NIH Guidelines, the OSU IBC requires a protocol for all work of this nature to ensure that the proper reviews are taking place and that the work is truly exempt. Exempt protocols do not require an annual review or 5-year renewal.

4. Recombinant and/or Synthetic Nucleic Acids: Your research is **NOT** exempt from the NIH Guidelines if you work involves the any of the following:

- a.** the deliberate transfer of drug resistance into organisms that do not acquire them naturally
- b.** the deliberate transfer of recombinant or synthetic nucleic acids into humans
 - i.** Human gene transfer is the deliberate transfer into human research participants of either:
 - 1.** Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
 - 2.** Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic molecules, that meet any one of the following criteria:
 - a.** Contain more than 100 nucleotides; or
 - b.** Possess biological properties that enable integration into the genome (e.g., cis elements involved in integration); or
 - c.** Have the potential to replicate in a cell; or
 - d.** Can be translated or transcribed
 - e.** Genes that produce vertebrate toxins with an LD50 of less than 10ng/kg of body weight
 - f.** Human or animal pathogens used as host-vector systems
 - g.** Human or animal pathogen DNA cloned into a non-pathogenic prokaryote or lower eukaryote
 - h.** Infectious human or animal DNA or RNA viruses used in tissue culture or defective viruses used in the presence of a helper virus



IBC eProtocol Guidance Document

- i.* Altering the genome of an animal by recombinant or synthetic nucleic acids
- j.* Viable genetically-modified (with recombinant or synthetic nucleic acids) microorganisms tested in whole animals
- k.* Genetic engineering of plants by rDNA methods or use of plants with microorganisms or insects containing recombinant or synthetic nucleic acids
- l.* Experiments involving more than 10 liters of culture containing recombinant or synthetic nucleic acid molecules
- m.* A deliberate release of genetically-modified (insertion of recombinant or synthetic nucleic acids) plants or animals into the environment

The IBC requires this information to ensure compliance with the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#). There are six categories of experiments involving recombinant or synthetic nucleic acid molecules described by the NIH Guidelines. It is the responsibility of the Principal Investigator to understand under which category, and specifically which section, of the NIH Guidelines his/her research belongs. For more information on these categories please review the NIH Guidelines, Section III (Experiments Covered by the NIH Guidelines) or the Institutional Biosafety Committee NIH Guideline Training available on the [ORRP website](#).

The following information must be provided in the Recombinant DNA section of the protocol for research in all six NIH categories, including exempt research:

- a.* For each experiment, list genes, inserts, gene products and key regulatory elements to be cloned. Provide a brief description of each gene's activity/function and indicate the species of origin for each. If you do not know the function of a gene, then indicate how the library/clone bank has been constructed and the phenotypes you are screening for. Provide an explanation of any acronyms.
- b.* Describe all vectors (plasmids, viruses, RNA/DNA constructs, promoters, tropism) to be used. Provide a written description and include a URL or



IBC eProtocol Guidance Document

- upload a map if available. Are any viral vectors defective or replication incompetent in the absence of a helper virus?
- c. For each experiment identify all applicable host systems to be used, i.e. bacterial strains or eukaryotic cell lines. Be sure to indicate if *E. coli* will be used as a final or intermediary cloning host and whether or not is a derivative of strain K-12. (Many experiments may be exempt from the NIH Guidelines if they are in certain cell lines or K-12.)
 - d. Under the NIH Section indicate if any viral vectors contain less than 2/3 of the wild-type virus genome and choose the appropriate section designation for your research. More than one section may be appropriate.
5. **Safety Equipment:**
- a. Work with any biohazards (excluding human cell lines) that will involve centrifugation will require the use of aerosol-proof safety cups.
6. **Exposure Assessment and PPE:** The main purpose of the Exposure Assessment and PPE section is to detail the consequences of accidental exposure to each agent listed in the protocol. This includes assessing effects on personnel, community, and the environment.
- a. Describe potential exposure routes for personnel.
 - b. If the work described involves animals, please detail the risk to staff handling the animals.
 - i. Is the agent shed or excreted into bedding from feces or urine?
 - ii. Can the agent be transmitted via a bite?
 - iii. Is the agent present in the blood?
 - c. Describe associated symptoms of exposure.
 - d. If an exposure occurs, what procedures will be followed?
 - i. How will the spill be cleaned up?
 - ii. What steps will be taken to ensure the safety of exposed personnel?
 - iii. What treatment options are available?



IBC eProtocol Guidance Document

- e. *If the agent is a plant pathogen that is not found in Ohio, what are the potential impacts of its accidental release on the plants in the surrounding area? Is a vector involved? If the agent is exotic to Ohio or the USA, have necessary permits and inspections been obtain from USDA APHIS for its importation and use?*
 - f. *If the agent is strictly an animal pathogen (not zoonotic), what biosecurity procedures are in place to prevent exposure to other animals? For animal pathogens transmitted by waste or bedding, describe how the risks are mitigated to prevent environmental contamination. Include carcass disposal procedures.*
 - g. *The PPE section should be a list of PPE that will be used for these studies.*
7. **Occupational Exposure Assessment and Risk Groups:** When completing this section, be sure to check the highest risk group of the agents that you will be using in your research. Only one risk group should be selected. Based on the highest risk group selected, consider and select the appropriate biocontainment level for the proposed experiments. In some cases, you may select multiple biocontainment levels if work with agents of differing risk groups will be used in separate lab facilities. Otherwise, always select the highest biocontainment level appropriate.
8. **Animals:** When completing the animal section, please consider both the innate hazard of working with animals as well as the agent being delivered to the animals. What is the source of the hazard to animal handlers and animal care staff?

Note: Projects involving the collection and analysis of animal feces may require an IBC protocol. A risk assessment should be done in conjunction with the EHS Biosafety staff to determine if zoonotic pathogens may be present in the feces and potential occupational exposures could occur.



IBC eProtocol Guidance Document

Appendix A

Appendix A covers specific information that should be included in an IBC protocol if LENTIVIRAL VECTORS (LVV) are being used.

1. **Getting Started:** The answers supplied in this section are important to ensure that the correct subsequent sections are populated in eIBC. The use of LVV requires:
 - a. check no to the question “Will your research involve the use of human source material as the ONLY biohazard”
 - b. check yes to the question “Will you be using biohazards in your research”
 - c. check yes to the question “Will you be using recombinant or synthetic nucleic acids in your research”
 - i. There are 11 additional sub questions under this heading. Answers will depend on the specific experiments being conducted but at a minimum, check yes to the question “Will human or animal pathogen DNA be cloned into a non-pathogenic prokaryote or lower eukaryote”.

2. **Descriptive Summary:** This section should include the specific risks of working with LVV (ie. insertional mutagenesis, the oncogenic potential of the transgene, and/or recombination resulting in replication-competent virus) and the safety measures that will be implemented to mitigate these risks. Specific information that should be included in the protocol’s risk assessment are as follows:
 - a. How will lentiviral vectors be used to accomplish the goals of the protocol?
 - b. Describe the transgene(s):
 - i. Function?
 - ii. Is the transgene an oncogene?
 - iii. Does the transgene silence a tumor-suppressor?
 - c. Describe the LVV design:
 - i. What generation of LVV is being used?
 - ii. How many plasmids are required to generate virions?
 - iii. Describe the promoters.



IBC eProtocol Guidance Document

- iv. Describe the host range (does the LVV target human cells?)
- v. What percentage of the viral genome is present in the LVV?
- d. Describe the production:
 - i. What concentration will be used?
 - ii. What is the maximum volume that will be used?
- e. Are LVV or LVV modified cells being used in animals?
- f. What are the likely routes of exposure?
- g. Determination of the vector as being a “high-risk” LVV or “low-risk” LVV based on the table below:

Information about LVV	Low-Risk Examples	High-Risk Examples
Transgene Function	Protein-based fluorescence (eg, GFP)	Silence a tumor-suppressor or express an oncogene (i.e., <i>Ras</i> , <i>Myc</i> , etc.)
Number of plasmids used to generate virions	3-4 plasmids	2 or less plasmids
Mutations within LVVs	Use self-inactivating long terminal repeats (LTRs) and other deleterious mutations	Wild-type LTRs
Expression control elements	Weak promoters	Strong promoters present (CMV, SV40)
Host range	Nonhuman tropism	Extended host range (i.e., VSV-g)
Concentration	<1 x 10 ⁹ infectious units/mL	>1 x 10 ⁹ infectious units/mL
Production volume	<100 mL	>100 mL
Percentage of genome deleted or substituted	>2/3	<2/3



IBC eProtocol Guidance Document

- h. Describe the risk mitigation strategies:*
 - i.* What type of lab specific training is provided for staff?
 - ii.* What type of engineering controls are required?
 - iii.* What type of PPE is required?
- 3. Recombinant and/or Synthetic Nucleic Acids:** Include a statement regarding the generation of the LVV system being used and tropism of the LVV.



IBC eProtocol Guidance Document

References

1. Manis, J. (n.d.). Knock Out, Knock In, Knock Down -- Genetically Manipulated Mice And The Nobel Prize. *New England Journal of Medicine*, 2426-2429.
2. Vyas, J. Risks Associated With Lentiviral Vector Exposures and Prevention Strategies. *JOEM*, 58:12 (1159-1166). 2016.