Registration of Research with RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

https://osp.od.nih.gov/biotechnology/nih-guidelines/

Rev 04/19

RETURN ORIGINAL FORM TO: Johns Hopkins Biosafety Office
2024 E. Monument Street, Room B-200
Baltimore, MD 21287
410-955-5918 (Fax) 410-955-5929
ibc@jhu.edu

JHU IBC#_____
DATE _____
BIOSAFETY LEVEL _____
ACTION_____
HSE Use Only. Do not write in this space.

Principal Investigator (see * below):	***JH-Badge/ID Number**:***
Academic Title:	***Email Address:***
Department:	***Division:***
Office Address:	***Lab Address:***
Office Phone:	***Office Fax:***
Types of Materials Being Used: ☐ ***Human-derived Material*** ☐ ***Infectious Agent/Pathogen*** ☐ ***Biological Toxin***	
Name of Material:	
Source of Material:	***Repository:*** ☐ ***Yes*** ☐ ***No***
Strain, Genotype, or Vendor Catalog Number if Applicable:	***Freezer Serial No:*** _____
☐ ***Check if non-Baltimore Site and Indicate Location:***	***Location:***

****Work involving the use or possession of INFECTIOUS AGENTS, PATHOGENS, VIRAL VECTORS, BIOLOGICAL TOXINS, or HUMAN TISSUES requires a current Registration Number for the material to be used or you must complete registration form(s) for these materials for review with this form.*

1. Specify gene or name of the recombinant or synthetic nucleic acid(s) _____
2. Identify vector(s), specific phage, plasmid or virus. For novel and viral constructs attach vector map, do not attach gene sequence.
3. Host or Environment: _____ (see #11 below)
4. Is Volume Large Scale, > 10 Liters Culture? ☐ Yes ☐ No
5. If virus source, is it more than 2/3 of the viral genome? ☐ Yes ☐ No
6. Is a helper virus, packaging system, complementary cell used? ☐ Yes ☐ No
7. Are intact animals exposed to the nucleic acid molecules? ☐ Yes ☐ No
8. Are mammalian cells exposed to the nucleic acid molecules? ☐ Yes ☐ No
9. Are Human Subjects exposed to the nucleic acid molecules? ☐ Yes ☐ No

   For submissions involving Human Gene Therapy, please contact ibc@jhu.edu *for additional information.*
10. Will this project involve the use of CRISPR/Cas9 or a similar system? ☐ Yes ☐ No
11. Please check the relevant situation(s) that apply to your project. For "Yes", indicate the Biosafety Level and specify in section 14a.

Host / Environment				**Biosafety Level**	
a. E. coli. K12	☐ Yes	☐ No		☐ BSL-1	☐ BSL-2
b. Other Bacteria	☐ Yes	☐ No		☐ BSL-1	☐ BSL-2
c. Non-pathogen	☐ Yes	☐ No		☐ BSL-1	
d. Pathogen	☐ Yes	☐ No		☐ BSL-2	☐ BSL-3
e. Toxin gene	☐ Yes	☐ No		☐ BSL-2	☐ BSL-3
f. Drug resistance Gene	☐ Yes	☐ No		☐ BSL-2	☐ BSL-3
g. Yeast / YAC	☐ Yes	☐ No		☐ BSL-1	☐ BSL-2
Tissue Culture Cells ☐ Yes ☐ No					
a. R-DNA /plasmids /synth nucleic acids	☐ Yes	☐ No		☐ BSL-2	
b. Segment of virus	☐ Yes	☐ No		☐ BSL-2	☐ BSL-3
c. Virus vector	☐ Yes	☐ No		☐ BSL-2	☐ BSL-3
d. If virus vector:	☐ Adeno	☐ Retro	☐ Vaccinia	☐ Sindbis	☐ Other Virus _____
e. Defective viral vector	☐ Yes	☐ No			
f. Replication competent viral vector	☐ Yes	☐ No			

***Post-doctoral fellows, research associates, & instructors require Department Chair and Laboratory Sponsor (If applicable) co-signature on page 2.**
****JH-Badge/ID number is the number on your ID card. Contact the Biosafety Office if you are unsure of your ID number.**

Intact Lab Animal Recipient ☐ Yes ☐ No If yes, species: _____

a. University Animal Use & Care Committee Protocol Number: _____ Approval Date:_____
b. Animal Housing (building & room no.) _____
c. R-DNA /Plasmid /Synth Nucleic Acid ☐ Yes ☐ No ☐ ABSL-1 ☐ ABSL-2
d. Transgenic ☐ Yes ☐ No ☐ ABSL-1 ☐ ABSL-2
e. Virus Vector ☐ Yes ☐ No ☐ ABSL-1 ☐ ABSL-2 ☐ ABSL-3
f. SCID / Nude ☐ Yes ☐ No ☐ ABSL-1 ☐ ABSL-2 ☐ ABSL-3

Human Subject Recipient ☐ Yes ☐ No

a. I.R.B. or RPN Protocol Number:_____ Approval Date:_____
b. R-DNA /Plasmid /Synth Nucleic Acid ☐ Yes ☐No ☐BSL-2
c. Pathogen ☐ Yes ☐ No ☐ BSL-2
d. Virus Vector ☐ Yes ☐ No ☐ BSL-2

Plants ☐ Yes ☐ No **Insects** ☐ Yes ☐ No **Field Release** ☐ Yes ☐ No ☐ BSL-2-P ☐ BSL-3-P

12. Reference your experiment from the NIH Recombinant DNA Guidelines (see attachment). _____ (Required)

** Recombinant DNA inserts in plasmid and phage in E. coli K12, DH5 alpha or in transgenic knockout mice, not involving a viral gene, toxin, or pathogen source, or in large-scale culture (>10L), are EXEMPT from full IBC review and can be approved administratively by the Biosafety Office. rDNA in tissue culture is not exempt. The use of knockouts (by creating or purchase) must be registered prior to use in research.

13. List all staff involved in the project who may come into contact with recombinant or synthetic nucleic acid molecules. List any core labs or facilities that will be used, but do not list the staff members of these groups unless you are filing this form to register such an entity:

Name	***Email or other Contact Address***	***JH-Badge/ID Number****

14. Please attach a summary of the proposed research containing sufficient information to ensure adequate review of the protocol to determine compliance with the JH Biosafety Program, local, state, and federal regulations. Required information to include:

a) The nature and purpose of the research and any key features of the material to be used in the project.
b) Viral vectors; name, source and key features including replication deficient or replication competent, identify marker genes and foreign insert genes. If using CRISPR/Cas9 or a similar system please specify the details including any potential gene drives.
c) An outline of the procedures and techniques to be employed (e.g., cloning, DNA or RNA synthesis, expression, cell culture, etc.
d) Identify known and potential hazards associated with the use of this material including exposure to oncogenic or transforming genes, fragments, or sequences, the use of sharps, hazardous materials, bloodborne pathogens or other potential diseases.
e) Specifically describe safe practices, equipment, facilities, and training that will be used to protect staff from hazards in "d" above.
f) Specifically describe methods of inactivation & disposal of the material and any associated contaminated materials generated.

This registration form (summary and any attachments) must provide sufficient detail for the Institutional Biosafety Committee to understand and evaluate rDNA or other nucleic acid components in order to review the registration. For any attached references, please highlight pertinent paragraphs or sentences. Submissions that lack detail or are illegible will be deferred from action and returned for revision and resubmission. The project registration must be updated annually, and must include a summary of results and changes to the project. Major changes to the project require submission of a new registration form.
Incomplete registration forms will be returned for revision.

As Principal Investigator, I accept responsibility for the safe conduct of work with this material. I will ensure that all personnel receive training on proper safety practices and personal protective equipment that are needed for this work.

Signature (Principal Investigator): _____ Date:_____

*Co-Signature (Dept. Chair): _____ Date:_____

***Post-doctoral fellows, research associates, & instructors require co-signature of Department Chair and Laboratory Sponsor (if applicable).**

Summary of NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules April 2019

(Reference for item #12) https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

The summarized categories below are extracted from the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, the full text of which is available at the hyperlink listed above. After identifying the section below that pertains to the research you propose, access the *NIH Guidelines* at the link above and read the entire section to fully understand your responsibilities. Risk Group Listings are found in Appendix B of the document.

The *NIH Guidelines* are intended to prevent an accidental release of these materials into the environment and ensure safe work practices in the conduct of research. The JH IBC has attempted to summarize the *NIH Guidelines* into a convenient format without altering the original intent. It is possible for a given project be simultaneously classified under more than one of the sections below. Consider that the *NIH Guidelines* have evolved in a somewhat piecemeal fashion since first released in 1976 and are based on the evaluation of technology described in protocols submitted to and reviewed by the Recombinant Advisory Committee (RAC) of the NIH Office of Biotechnology Activities (OBA) (now titled the Office of Science Policy or OSP). As such, the resulting full text of the *NIH Guidelines* is lengthy and sometimes a challenge to interpret. Contact the JHU Biosafety Office or the NIH OSP if you have any questions.

SECTION III-A: Experiments Requiring JHU-IBC Registration and Approval plus NIH Director Approval Before Initiation (see Section IV-C-1-b-(1) of the document linked above.)

III-A-1 Major Actions Under the *NIH Guidelines.*

III-A-1-a Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture.

SECTION III-B: Experiments Requiring JHU-IBC Registration plus NIH OSP Approval Before Initiation.

III-B-1 Cloning of Toxin Molecules with LD_{50} < 100ng/kg of body weight.

III-B-2 Experiments that have previously been approved under Section III-A-1-a above as Major Actions. OSP will decide if the proposed experiments are equivalent to the previously approved work.

SECTION III-C: Experiments Requiring JHU-IBC Registration plus IRB and FDA Approval before Research Participant Enrollment

III-C-1 Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into human subjects.

SECTION III-D: Experiments Requiring JHU-IBC Registration and approval Before Initiation

III-D-1 Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems. (See appendix B of the document linked above for full listing)

III-D-1a Introduction of recombinant or synthetic nucleic Acid molecules into Risk Group 2 agents, BSL-2 or BSL2-N (ABSL-2N) Containment.

III-D-1-b Introduction of recombinant or synthetic nucleic Acid molecules into Risk Group 3 agents, BSL-3 or BSL3-N (ABSL-3N) Containment.

III-D-1-c Introduction of recombinant or synthetic nucleic Acid molecules into Risk Group 4 agents, BSL4 or BSL4-N (ABSL4N) Containment.

III-D-1-d Introduction of recombinant or synthetic nucleic Acid molecules into Restricted Agents is permitted on a case-by-case basis approval from NIH OSP and USDA. Permit Required for Plant or Animal Pathogens.

III-D-2 Experiments in which DNA From Risk Group Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host Vector Systems.

III-D-2-a Experiments in which DNA from Risk Group 2 or 3 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes Some experiments in this category may be exempt from the guidelines and fall under Section III-F. Experiments involving DNA from Risk Group 4 agents can only involve a totally and irreversibly defective fraction of the agent's genome. Else, BSL4 containment will be required – not available at JHU.

III-D-2-b Experiments in which DNA from Restricted Agents is transferred into nonpathogenic prokaryotes or lower eukaryotes is permitted on a case-by-case basis approval from NIH OSP and USDA. Permit Required for Plant or Animal Pathogens.

III-D-3 Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

III-D-3-a Experiments involving Infectious or defective [defective eukaryotic viruses contain less than 2/3 of the genome] Risk Group 2 viruses in the presence of helper or packaging system in tissue culture BSL-2 or higher at JHU-IBC discretion.

III-D-3-b Experiments involving Infectious or defective Risk Group 3 viruses and prions in the presence of helper functions in tissue culture. These will be conducted at BSL3

III-D-3-c Experiments involving Infectious or defective Risk Group 4 viruses in the presence of helper functions in tissue culture. These will be conducted at BSL4 – not available at JHU

III-D-3-d Experiments involving Infectious or defective restricted pox viruses in the presence of helper functions in tissue culture is permitted on a case-by-case basis approval from NIH OSP and USDA. Permit Required for Plant or Animal Pathogens..

III-D-3-e Experiments involving Infectious or defective viruses in the presence of helper virus in tissue culture or other host systems not covered in III-D above. The IBC reserves the right to determine Risk Group Classification for novel agents.

III-D-4 Experiments Involving Whole Animals

III-D-4-a Recombinant or synthetic nucleic acid molecules or DNA or RNA molecules derived therefrom (including the creation and use of transgenic animals) except where greater than two-thirds of eukaryotic viral genome transferred to any non-human vertebrate or an invertebrate organism. Animals with sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly. BSL-1 or ABSL-1N. Introduction of other sequences from eukaryotic viral genomes are covered in Section III-D-4-b. Experiments involving Recombinant or synthetic nucleic acid molecule-modified Risk Group 2 and higher agents in animals are covered in Sections V-A, V-G and V-L.

III-D-4-b Recombinant DNA, or DNA or RNA molecules derived from DNA involving whole animals, including transgenic animals, not covered in Section IIID-1, (human or animal pathogen Risk Group 2 and higher or Restricted Agents as host vector systems) or Section III-D-4-a. Containment determined by IBC.

III-D-4-c Exceptions Under Section III-D-4 (Experiments Involving Whole Animals)

III-D-4-c-(1) Generation of transgenic rodents that require BSL1/ABSL1 containment are described in Section III-E-3 (Experiments involving transgenic rodents)

III-D-4-c-(2) The purchase or transfer of existing transgenic rodents is exempt from the NIH Guidelines under Section III-F but still registered with the JHU Biosafety Office and open to review by the JH IBC.

III-D-5 Experiments Involving Whole Plants

III-D-5-a Recombinant techniques with exotic infectious agents with recognized potential for serious detrimental impact on ecosystems using whole plants. BSL-3P or BSL2-P+

III-D-5-b Plants with cloned genomes of readily transmissible exotic infectious agents that may

reconstitute by genomic complementation. BSL3-P or BSL2-P+

III-D-5-c Readily transmissible exotic infectious agents such as the soybean rust fungus, maize streak or other viruses in the presence of specific arthropod vectors. BSL-4P

III-D-5-d Sequences coding vertebrate toxins introduced into plants or associated organisms, BSL-3P

III-D-5-e Microbial pathogens of insects or small animals associated with plants if the recombinant or synthetic nucleic acid molecule-modified microorganism has a recognized detrimental impact on managed or natural ecosystems. BSL-3-P or BSL2-P+

III-D-6 Experiments involving more than 10 liters of culture. IBC determines containment level. (See Appendix K)

III-D-7 Experiments Involving Influenza Viruses

III-D-7-a Human H2N2 (1957-1968) See *NIH Guidelines* for specifics

III-D-7-b Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1). See *NIH Guidelines* for specifics

III-D-7-c 1918 H1N1. See *NIH Guidelines* for specifics

III-D-7-d Antiviral Susceptibility and Containment. Virus that may be resistant to antiviral drugs may require higher containment levels. Experiments that may create resistance to antiviral drugs in any of the influenzas listed above in III-D-7-a-c are considered a Major Action and are subject to Section III-A-1 and any applicable select agent regulations.

SECTION III-E: Experiments Requiring JHU-IBC Registration Before Initiation

III-E-1 Formation of recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus in tissue culture, BSL-1 with no helper virus. JHU-IBC classifies Retroviral vectors with packaging system capable of infecting human cells as BSL-2.

III-E-2 Experiments Involving Whole Plants

III-E-2-a Recombinant or synthetic nucleic acid molecules-modified whole plants or experiments involving recombinant or synthetic nucleic acid molecule-modified organisms not covered in Section III-E-2-b. BSL-1-P

III-E-2-b BSL2-P or BSL1-P+ for the following experiments involving plants

III-E-2-b-(1) Plants modified by recombinant or synthetic nucleic acid molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

III-E-2-b-(2) Plants in which the introduced DNA is the complete genome of a non-exotic infectious agent.

III-E-2-b-(3) Plants associated with recombinant or synthetic nucleic acid molecule-modified non-exotic microorganisms with a recognized potential for serious detrimental impact on managed or natural ecosystems.

III-E-2-b-(4) Plants associated with recombinant or synthetic nucleic acid molecule-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems

III-E-2-b-(5) Experiments with recombinant or synthetic nucleic acid molecule-modified arthropods or small animals associated with plants, or with arthropods or small animals associated with recombinant or synthetic nucleic acid molecule-modified microorganisms that are associated with them, if the modified microorganisms have no serious impact on managed

or natural ecosystems.

III-E-3 Experiments Involving Transgenic Rodents. Generation of rodents with stable introduction of DNA into the animal's genome if BSL-1, otherwise Section III-D-4

III-E-3-a Breeding of certain BSL-1 transgenic rodents are exempt under Section III-F.

SECTION III-F: Exempt experiments Note: The JHU-IBC Requires Registration before Initiation (BSL-1)

III-F-1 Synthetic nucleic acids that:

1. can neither replicate nor generate nucleic acids that can replicate in any living cell
2. are not designed to integrate into DNA
3. do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100ng/kg body weight

Synthetic nucleic acids introduced into human subjects and meet Section III-C are not exempt

III-F-2 Those that are not in organisms or viruses and have not been modified or manipulated to render them capable of penetrating cellular membranes.

III-F-3 Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

III-F-4 Those that consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host or when transferred to another host by well-established physiological means.

III-F-5 Those that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

III-F-6 Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes though one or more of the segments may be a synthetic equivalent..

III-F-7 Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

III-F-8 Those that do not present a significant risk to health or the environment.

Appendices in the *NIH Guidelines* you may need to refer to:

Appendix A: Exemptions Under Section III-F-6, Sublists of Natural Exchangers
Appendix B: Classification of Human Etiologic Agents on the Basis of Hazard
Appendix C: Exemptions Under Section III-F-8
Appendix D: Major Actions Taken Under the NIH Guidelines
Appendix E: Certified Host-Vector Systems
Appendix F: Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates
Appendix G: Physical Containment
Appendix H: Shipment
Appendix I: Biological Containment
Appendix J: Biotechnology Research Subcommittee
Appendix K: Physical Containment for Large Scale Uses of Organisms Containing Recombinant or Synthetic Nucleic Acid Molecules
Appendix L: Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants
Appendix M: Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Animals