**APPENDIX A – RECOMBINANT/SYNTHETIC NUCLEIC ACIDS**

1. Briefly describe, in lay terms, the work to be performed with recombinant or synthetic nucleic acid molecules.

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1. List the vectors included in this project:

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| --- | --- | --- |
| **Vector Type**(plasmid, viral, phage, etc.) | **Technical Name**(include vendor if applicable) | **Gene Transfer Method** (transformation, transfection, electroporation, etc.) |
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1. List the genes/nucleic acids included in this project:

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| **Gene/Nucleic Acid**(specific name of gene, promoter, regulatory sequence, etc.) | **Source Organism**(genus and species or common name) | **Nature of Insert or Protein Expressed**(reporter gene, virulence factor, DNA repair, etc.) | **Use/Purpose**(cloning for sequencing, PCR, expression, etc.) |
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1. Attach and submit a detailed map of a representative sample of the vectors and inserts to be used. Indicate any regions that increase the safety of this construct. Provide copies of key references that describe the construction of the vector(s) used.
2. List any proteins that are produced:

**Click to enter text**

1. Will the nucleic acids contain genes for the biosynthesis of toxic molecules lethal to vertebrates?

[ ] No [ ] Yes (If yes, list the toxin’s LD50): **Click to enter text**

1. Will the nucleic acids contain genetic material from [select agents or toxins](https://www.selectagents.gov/SelectAgentsandToxinsList.html)?

[ ] No [ ] Yes: **Click to enter text**

1. What is the maximum volume of culture per container used at any one time?

**Click to enter text**

1. Is secondary containment used?

[ ] No [ ] Yes (If yes, describe the containment): **Click to enter text**

1. If using a virus vector:
	1. Describe how nucleic acids will be used to create the virus:

**Click to enter text**

* 1. What will host the virus vector?

**Click to enter text**

* 1. Are nucleic acid molecules containing ≥ 2/3 of the genome of any eukaryotic virus created?

[ ] No [ ] Yes: **Click to enter text**

* 1. Do experiments involve the use of infectious human, animal, or plant viruses?

[ ] No [ ] Yes: **Click to enter text**

* 1. Do experiments involve the use of defective animal or plant viruses in the presence of a helper virus?

[ ] No [ ] Yes (if yes, attach your procedures to determine the relative proportions of helper virus and defective virus): **Click to enter text**

* 1. Are the nucleic acids likely to make the virus more pathogenic?

[ ] No [ ] Yes: **Click to enter text**

1. Are oncogenic genes being used?

[ ] No [ ] Yes (If yes, identify which are oncogenic): **Click to enter text**

1. Does your research involve a gene editing technology?

[ ] No [ ] Yes (If yes, select the technology/technologies being proposed):

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| --- | --- | --- | --- |
| [ ]  CRISPR/Cas9 | [ ]  ZFN | [ ]  TALENS |  [ ]  Meganucleases |
| [ ]  Other: **Click to enter text** |

1. Will the genome editing technology be used in prokaryotes, eukaryotes, or mammalian cells?

[ ] No [ ] Yes (If yes, specify which): **Click to enter text**

1. How is the gene editing technology being delivered (e.g., nanoparticles, plasmid, lentivirus, adeno-associated virus, etc.)?

**Click to enter text**

1. Will the gene editing technology target embryos or germ line cells?

[ ] No [ ] Yes: **Click to enter text**

1. Will the gene editing technology be used for human gene transfer research?

[ ] No [ ] Yes: **Click to enter text**

1. For CRISPR/Cas9 systems, are the guide RNA (gRNA) and nuclease on the same plasmid, vector, or delivery vehicle?

[ ] No [ ] Yes (If yes, explain if this plasmid, vector, or delivery vehicle can transfect or infect a human cell and explain if the gRNA or CRISPR nuclease can be expressed in human cells): **Click to enter text**

1. For CRISPR/Cas9 systems, are you inserting the gene for Cas9 (or a similar nuclease) and a guide RNA into the chromosome of a sexually reproducing organism?

[ ] No [ ] Yes: **Click to enter text**

1. For CRISPR/Cas9 research involving viral vectors, a Genome Target Scan (GT-Scan) for off target effects by your gRNA must be completed. This is necessary to determine if there is homology to human DNA and for assessing the risk of potential exposure in the event of an unanticipated incident (Bae et al., 2014; O’Brien and Bailey, 2014). An off-target database is available [here](http://www.rgenome.net/cas-offinder/).
2. Will the inserted nucleic acid sequences contain a guide RNA that can target the insertional locus of the unmodified chromosome?

[ ] No [ ] Yes [ ] I don’t know

1. Can the inserted nucleic acids act as a gene drive (i.e., can the inserted sequences in any way alter Mendelian inheritance at this locus)?

[ ] No [ ] Yes [ ] I don’t know

**NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES**

The *NIH Guidelines* can be found at: <http://osp.od.nih.gov/office-biotechnology-activities/biosafety>

On the following pages, select all *NIH Guidelines* categories that apply to your research:

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| **SECTION III-F includes recombinant or synthetic nucleic acid molecules that are exempt from the *NIH Guidelines*, but still require registration with the Institutional Biosafety Committee:** |
| [ ]  | **III-F-1:** | Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell, and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. |
| [ ]  | **III-F-2:** | Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) 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 non-pathogenic prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another non-pathogenic 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 as determined by the NIH Director. Check the appropriate appendices below. |
| **SECTION III-F-8 Appendices** |
| [ ]  | **C-I:** | Recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome that are propagated and maintained in cells in tissue culture. |
| [ ]  | **C-II:** | Experiments that use *Escherichia coli* K-12 host-vector systems. |
| [ ]  | **C-III:** | Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems. |
| [ ]  | **C-IV:** | Experiments involving *Kluyveromyces lactis* host-vector systems. |
| [ ]  | **C-V:** | Experiments involving *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems. |
| [ ]  | **C-VI:** | Extrachromosomal Elements of Gram Positive Organisms. |
| [ ]  | **C-VII:** | The purchase or transfer of transgenic rodents, BSL1 only. |
| [ ]  | **C-VIII:** | Generation of BSL1 transgenic rodents via breeding. |

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| **SECTION III-E covers experiments not found in sections A, B, C, D, and F, and are not exempt from the *NIH Guidelines*, but still require Institutional Biosafety Committee notice simultaneous with initiation of work:** |
| [ ]  | **III-E:** | Experiments not included in Sections III-A, III-B, III-C, III-D, III-F; and experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes and may be conducted at BSL1. |
| [ ]  | **III-E-1:** | Recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated and maintained in cells in tissue culture (BSL1). For such experiments, it must be demonstrated that the cells lack helper virus for the specific families of defective viruses being used. |
| [ ]  | **III-E-2:** | Experiments involving nucleic acid molecule-modified whole plants, and/or experiments involving recombinant or synthetic nucleic acid molecule-modified organisms associated with whole plants. |
| [ ]  | **III-E-3:** | Experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). BSL1 containment only. Experiments BSL2 or higher are covered under Section III-D-4. |

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| **SECTION III-D covers experiments that require Institutional Biosafety Committee notice prior to initiation of work:** |
| [ ]  | **III-D-1:** | Experiments using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as host-vector systems. |
| **Select Risk Group:** [ ]  **RG2** [ ]  **RG3** [ ]  **RG4** |
| [ ]  | **III-D-2:** | Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. |
| **Select Risk Group:** [ ]  **RG2** [ ]  **RG3** [ ]  **RG4** |
| [ ]  | **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. |
| **Select Risk Group:** [ ]  **RG2** [ ]  **RG3** [ ]  **RG4** |
| [ ]  | **III-D-4:** | Recombinant or synthetic nucleic acid experiments involving whole animals (e.g., non-human vertebrate or invertebrate organism, including arthropods). |
| **Select Risk Group:** [ ]  **RG1** [ ]  **RG2 or RG3** |
| [ ]  | **III-D-5:** | Experiments involving whole plants or insects. Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules (BSL2 or higher). |
| [ ]  | **III-D-6:** | Experiments involving more than 10 liters of culture in a single culture vessel. |
| [ ]  | **III-D-7:** | Experiments involving influenza viruses. |
| **Risk Group Definitions** |
| **Risk Group 1 (RG1):** Agents that are not associated with disease in healthy adult humans.**Risk Group 2 (RG2):** Agents are associated with human disease which is rarely serous and for which preventative or therapeutic interventions are often available.**Risk Group 3 (RG3):** Agents that are associated with serious or lethal human disease for which preventative or therapeutic interventions may not be available.**Risk Group 4 (RG4):** Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available. |

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| **Experiments that fall under sections A, B, and C require NIH pre-approval. Contact the IBC Office for assistance.** |
| [ ]  | **III-A-1:** | The 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, animals, or plants. |
| [ ]  | **III-B-1:** | Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight. |
| [ ]  | **III-C-1:** | Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participants. |