

To be used if only for exemption or recombinant and synthetic nucleic acid activity. Import of microbes and other use of biological activities use IBC Registration Form.

FAQs about experiments that are exempt from the NIH Guidelines (May 2019)

Are there experiments that are exempt from the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules?

Per Section III-F of the NIH Guidelines, experiments are exempt when they involve recombinant DNA that is:

- For synthetic nucleic acids, those that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), 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. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section. (See Section III-F-1)
- 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. (See Section III-F-2)
- Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature. (See Section III-F-3)
- Those that consist entirely of nucleic acids from a 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 host by well-established physiological means. (See Section III-F-4)
- 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). (See Section III-F-5)
- 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. (See Section III-F-6)
- Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA. (See Section III-F-7)
- Those that do not present a significant risk to health or the environment as determined by the NIH Director (See Section III-F-8). Appendix C of

the *NIH Guidelines* details the specific classes of experiments that may be exempt under Section III-F-8.

2. Are the experiments described in Section III-F and Appendix C of the *NIH Guidelines* always exempt, or are there circumstances in which the research might require IBC review and approval under *NIH Guidelines*?

If an experiment falls under Sections III-A, III-B, or III-C and also III-F, then the research is not exempt (see the note Under Section III of the *NIH Guidelines*). The three types of experiments that would not be exempt under this provision are:

- 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 use of the drug to control disease agents in humans, veterinary medicine or agriculture [Section III-A];
- Deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). [Section III-B]; or
- The deliberate transfer into human research participants of either:
 - Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
 - Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
 - Contain more than 100 nucleotides; or
 - Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
 - Have the potential to replicate in a cell; or
 - Can be translated or transcribed. [Section III-C].

In addition, there are certain exceptions to the exemptions described in Appendix C of the *NIH Guidelines*. [See Appendix C-I-A, through C-VI-A]. In addition to the three types of experiments listed above, these exceptions include experiments involving:

- DNA from Risk Group 3, 4, or restricted organisms or cells known to be infected with these agents

- Whole plants regenerated from plant cells and tissues cultures that do not remain axenic cultures
- Large scale experiments (more than 10 liters of volume in a single culture vessel)
- Deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates with an LD50 greater than 100 nanograms/kg but less than or equal to 100 micrograms/kg (see Appendix F)

The *NIH Guidelines* exempt certain experiments that do not pose a threat to health or the environment. Can an Institutional Biosafety Committee (IBC) or Principal Investigator (PI) determine if an experiment does not pose such a threat and is therefore exempt?

Section III-F-8 of the *NIH Guidelines* refers to categories of experiments that the NIH Director has determined do not present a significant risk to health or the environment and are therefore exempt. **PIs and IBCs cannot make the determination that a class of experiments other than the ones listed below poses no significant risk.**

The following classes of experiments are exempt under Section III-F-8:

- Certain recombinant or synthetic nucleic acid molecules that contain less than one-half of any eukaryotic viral genome when propagated and maintained in cells in tissue culture [Appendix C-I – see below for more information on the limits of this exemption]
- *Escherichia coli* K-12 host-vector systems [Appendix C-II]
- *Saccharomyces cerevisiae* or *Saccharomyces uvarum* host-vector systems [Appendix C-III]
- *Kluyveromyces lactis* host-vector Systems [Appendix C-IV]
- *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems [Appendix C-V]
- Extrachromosomal elements of gram positive organisms [see specific list of organisms in Appendix C-VI]
- The purchase or transfer of transgenic rodents [Appendix C-VII]
- Generation of certain BL1 Transgenic Rodents via Breeding [Appendix C-VIII]

A full description of the exemptions with exceptions can be found in Appendix C of the *NIH Guidelines*.

How do I know if I am working with host-vector system that is exempt from the *NIH Guidelines*?

Only certain experiments that use *E. coli* K-12, *Saccharomyces cerevisiae* or *Saccharomyces uvarum*, *Kluyveromyces lactis*, *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems are exempt from the *NIH Guidelines* (see Appendix C). If you are obtaining an *E. coli* host-vector system from a commercial supplier, genotype information may be available to permit determination of the strain from which the host is derived.

Nucleic acid molecules resulting from the replication of recombinant or synthetic nucleic acids are subject to the *NIH Guidelines*. Are any other materials derived from or produced by genetically engineered organisms subject to the requirements of the *NIH Guidelines*?

No. For example, proteins produced by genetically engineered organisms are not subject to the *NIH Guidelines*.

Are any types of human gene transfer trials exempt from the requirements of the *NIH Guidelines*?

If the investigational product meets the criteria described in Section III-C of the *NIH Guidelines* and involves 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, then the research will be subject to the *NIH Guidelines*.

Synthetic nucleic acid molecules that contain less than 100 nucleotides, or do not possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or do not have the potential to replicate in a cell; or do not have the potential to be translated or transcribed are not covered under Section III-C.

Vaccine trials, like other human gene transfer trials subject to the *NIH Guidelines* and must be reviewed and approved by an IBC before research participants can be enrolled.

The deliberate transfer of recombinant or synthetic nucleic acids into one human research participant, conducted under an FDA regulated individual patient expanded access IND or protocol, including for emergency use, is not research subject to the *NIH Guidelines* and thus does not need to be submitted to an IBC for review and approval.

There is a note at the beginning of Section III of the *NIH Guidelines* that states “If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.” What is meant by this note?

If an experiment falls into Section III-D or III-E of the *NIH Guidelines* and also falls into section III-F, it is exempt. An example of such an experiment is the following:

Staphylococcus aureus (a Risk Group 2 bacterium) contains a recombinant plasmid. The plasmid is indigenous to *S. aureus*, was created in vitro, and contains only DNA from *S. aureus* (i.e., the DNA inserted into the plasmid was *S. aureus* DNA).

Rationale: The introduction of recombinant DNA into Risk Group 2 agents is usually covered under Section III-D-1-a. However, because the experiments are only performed in *S. aureus*, this work would fall under III-F-4 (experiments that consist entirely of DNA from a prokaryotic host including its indigenous plasmids when propagated only in that host or a closely related strain of the same species). Thus, this experiment falls into both Sections III-D and III-F and is exempt, due to the above note, from the requirements of the *NIH Guidelines* for IBC review and approval.

It should be noted that only experiments covered by both III-D or III-E and III-F are considered to be exempt. If an experiment falls into Section III-A, III-B or III-C and any one of the other sections, then the requirements pertaining to Sections III-A, III-B or III-C must be followed.

Appendix C-I of the *NIH Guidelines* exempts experiments involving recombinant or synthetic nucleic acid molecules in tissue culture. I have a cell line that was created by the introduction of recombinant DNA. Are all experiments that I conduct with this cell line exempt from the requirements of the *NIH Guidelines*?

No. Although Appendix C-I does exempt the use of recombinant or synthetic nucleic acid molecules in tissue culture, there are exceptions to this exemption. Existing tissue culture cell lines created by the introduction of recombinant or synthetic nucleic acid molecules are exempt from the *NIH Guidelines* unless, the cell line:

- was modified using DNA from Risk Group 3 or 4 agents, or from restricted agents [Section III-D];
- contains a toxin with an LD50 of less than 100 nanograms per kilogram/kg body weight [Section III-B-1];
- contains viral DNA in a quantity exceeding 50% of any viral genome [Appendix C-I];
- is used in conjunction with defective viruses in the presence of helper virus [Section III-D-3];
- is used in an experiment involving the deliberate transfer of the cell line into humans [Section III-C-1]; or
- is grown in a volume exceeding 10 liters of culture [Section III-D-6].
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- **Where can I get more information about the *NIH Guidelines*?**
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- **Questions about the *NIH Guidelines* may be directed to NIH OSP staff at NIHguidelines@od.nih.gov. Staff may also be reached at (301) 496-9838.**

University of Hawaii
Institutional Biosafety Committee

PROJECT EXEMPTION REQUEST FORM

Section I – Administrative Data

PI/Faculty Name: _____ Signature: _____

Email Address: _____

Office Room No.: _____ Department: _____

Telephone No.: _____ [] Office [] Lab [] Cellular

PROJECT TITLE:

Laboratory Location

Most Recent Biosafety Inspection date:

Section II – NIH Exemptions (Check Box which your research is most applicable)

<input type="checkbox"/>	Section III-F-1. Those that are not in organisms or viruses.
<input type="checkbox"/>	Section III-F-2. Those that consist entirely of DNA segments from a single non-chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
<input type="checkbox"/>	Section III-F-3. Those that consist entirely of DNA from a 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 host by well-established physiological means.
<input type="checkbox"/>	Section III-F-4. Those that consist entirely of DNA 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).
<input type="checkbox"/>	Section III-F-5. 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. Appendix A of the guidelines shows a current list of natural exchangers that are exempt from the NIH Guidelines- see below
<input type="checkbox"/>	<p>Section III-F-6. Those that do not present a significant risk to health or the environment, as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. Appendix C of the guidelines shows a current list of classes of experiments which are exempt from the NIH Guidelines – see below</p> <p><input type="checkbox"/> Recombinant DNA in tissue culture (Appendix C-I and C-IA) <i>Recombinant DNA molecules containing < ½ of any eukaryotic viral genome that are propagated and maintained in cells are exempt, with the following exceptions-Risk Group 3, 4 or restricted organisms</i></p> <p><input type="checkbox"/> <i>Escherichia coli</i> K-12 host-vector systems (Appendix C-II and C-II-A) <i>BL1 practices generally recommended, IBC can specify higher containment if it deems necessary). These experiments are exempt provided that: The E. coli host does not contain conjugation proficient plasmids or generalized transducing phages (unless a non-conjugative vector is used). Lambda or lambdoid bacteriophages or Ff non-conjugative plasmids are used as vectors</i></p>

1 (unless the DNA inserted into *E. coli* K-12 is from a prokaryote that naturally exchanges

	<p><i>genetic information with E. coli, in which case any E. coli K-12 vector may be used).</i></p> <p>Note: This exemption is for the <u>system</u>; if foreign DNA is introduced into the system, then these experiments do not meet the exemption criteria.</p> <p><input type="checkbox"/> <i>Saccharomyces</i> host-vector systems (Appendix C-III and C-III-A)</p> <p><input type="checkbox"/> <i>Kluyveromyces</i> Host-Vector Systems (Appendix C-IV and C-IV-A)</p> <p><input type="checkbox"/> <i>Bacillus subtilis</i> or <i>Bacillus licheniformis</i> host-vector systems (Appendix C-V and C-V-A)</p> <p><input type="checkbox"/> Extrachromosomal elements of gram positive organisms (Appendix C-VI and C-VI-A)</p>
	<p>Section III-F-6. The purchase or transfer of transgenic rodents for experiments that require Biosafety Level 1 containment (The Purchase or Transfer of Transgenic Rodents - Appendix C-VII)</p>
<p><input type="checkbox"/></p>	<p>Section III-F-6. The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at Biosafety Level 1 containment if:</p> <p>1) Both parental rodents can be housed under BL1 containment; and</p> <p>2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and</p> <p>3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses. (Generation of BL1 Transgenic Rodents via Breeding - Appendix C-VIII).</p>
<p><input type="checkbox"/></p>	<p>Knock-out (gene silencing, gene ablation, etc.) rodents are exempt from the NIH Guidelines as long as the method to generate the knock-out animal does not leave any “new” genetic material behind in the genome after the procedure. If DNA from the molecule used to create the knock-out is permanently inserted into the genome, the experiment will require registration, review, and approval by an IBC.</p>

If this project involves the use of animals or human tissue/human subjects, you must submit an application for review by the IACUC or IRB.

Section III Project Details

1. Does this project involve any of the following activities?

No Yes (this project is not exempt, please contact IBC Coordinator at uhibc@hawaii.edu for further instruction)

- The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (NIH Guideline Section III-A)
- The deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD₅₀ or less than 100 nanograms per kilogram body weight). (NIH Guidelines III-B)
- The deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic molecules, into one (1) or more human research participants (NIH Guidelines Section III-C).

2. Does this project involve any of the following activities? (NIH Guidelines Section III-D)

No Yes (this project is not exempt, please contact IBC Coordinator at uhibc@hawaii.edu for further instruction)

- Using risk group 2, 3, 4 or Restricted agents as host-vector systems.
- DNA from risk group 2, 3, 4 or restricted agents being cloned into non-pathogenic prokaryotic or lower eukaryotic host-vector systems.
- The use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.
- Involve whole animals 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 germline (transgenic animals) and require BSL2 or higher containment.
- Viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals
- Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (e.g., responses to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules.
- Involve research or production of more than 10 liters of cultures of viable organisms containing recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom into germline (transgenic animals) and require no higher than BSL1 containment.
- Involve influenza viruses generated by recombinant or synthetic nucleic acid molecules (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations).

3. Does the project involve any of the following activities? Please complete IBC Protocol Registration Application.

No Yes (this project is not exempt, please contact IBC Coordinator at uhibc@hawaii.edu for further instruction)

The formation of recombinant or synthetic nucleic acid molecules containing not more than 2/3 of the genome of any eukaryotic virus.

- Experiments involving nucleic acid molecules-modified whole plants, and/or experiments involving recombinant or synthetic nucleic acid molecules-modified organisms associated with whole plants.
- Experiments involving generations 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) and require no higher than BSL1 containment.

4. Does this project involve any of the following activities (NIH Guideline Section III-F)

No Yes (this project is not exempt, please contact IBC Coordinator at uhibc@hawaii.edu for further instruction)

- Recombinant or synthetic nucleic acid molecules that 1) can neither replicate or generate nucleic acids in any living cells, (2) are not designated to integrate into DNA and 3) do not produce a toxin that is lethal for vertebrate at an LD₅₀ or less than 100 nanograms per kilogram body weight, and 4) no deliberately transferred into one (1) or more human research participants.
- Those that are not in organisms, cell, 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.
- Consist solely of exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.
- Consists entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in their host (or closely related strain of the same species) or when transferred to another host by well established physiological means.
- Consist entirely of DNA segments from different species that exchange DNA by known physiological process, though one or more of the segments may be a synthetic equivalent.
- Involve genomic DNA molecules that have acquired a transposable element, provided transposable elements does not contain any recombinant and/or synthetic DNA.

5. Please provide a detailed description of your project

IBC Exemption Approval No.: _____

Biosafety Officer (Signature): _____

Biosafety Officer (Print Name) : _____

Date: _____

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IBC Chair (Signature): _____

IBC Chair (Print Name: _____