

Experiments Covered by the NIH Guidelines

III-A Experiments Requiring IBC Approval, RAC Review and NIH Director Approval Before Initiation	Notes & Specific Considerations
<p>Deliberate transfer of drug resistance to microorganisms (not known to acquire the trait naturally) that can compromise the use of the drug to control the microorganism and its disease in humans, veterinary medicine or agriculture.</p>	
III-B Experiments Requiring NIH/OBA and IBC Approval Before Initiation	Notes & Specific Considerations
<p>Cloning of toxin molecules with LD₅₀ <100 nanograms/kg body weight (e.g., botulinum, tetanus, diphtheria toxins).</p>	<p>Specific approvals given for cloning in Escherichia coli K-12 for toxins with LD₅₀ between 100 nanograms/kg and 100 micrograms/kg body weight.</p>
III-C Experiments Requiring IBC and IRB Approval and RAC Review Before Participant Enrollment	Notes & Specific Considerations
<p>Deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human subjects.</p>	<p>Before enrollment, the following must be submitted to NIH/OBA for <u>each</u> clinical trial site:</p> <ol style="list-style-type: none"> 1. IBC Approval (from clinical trial site) 2. IRB Approval 3. IRB-approved informed consent document 4. CV of PIs 5. NIH Grant Numbers (if applicable) <p>**The use of retroviral vectors for gene delivery is further addressed in Appendix B-V-1.</p>

III-D Experiments Requiring IBC Approval Before Initiation	Notes & Specific Considerations
Experiments using Risk Group 2, Risk Group 3, Risk Group 4 or restricted agents as host-vector systems.	This refers to the introduction of recombinant DNA <u>into</u> the pathogenic agent. Containment levels will generally be based on the risk of the unmodified agent.
Experiments in which DNA <u>from</u> Risk Group 2, Risk Group 3, Risk Group 4 or restricted agents is cloned into non-pathogenic prokaryotic or lower eukaryotic host-vector systems.	When cloning is from Risk Group 2 or 3 pathogens, BL2 containment is generally prescribed, but specific lowering to BL1 may be approved by IBC. Some of these applications may be exempt (refer to III-F).
Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of a helper virus in tissue culture systems.	<p>Experiments involving Risk Group 2 viruses (infectious or defective) in the presence of helper virus are generally conducted at BL2.</p> <p>Experiments involving Risk Group 3 viruses (infectious or defective) in the presence of helper virus are generally conducted at BL3.</p> <p>Recombinant DNA or RNA molecules containing < 2/3 of the genome of any eukaryotic virus are considered defective. These viruses, in the absence of a helper virus, are categorized under III-E.</p>
Experiments involving whole animals.	This category applies to whole animals in which the genome has been altered by stable introduction into the germline (transgenic animals). This category also applies to experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. Viral vectors used on animals that do not lead to transmissible infection, either directly or indirectly a result of recombination in animals, may generally be propagated at BL1. Recombinant DNA, or DNA or RNA derived from recombinant DNA, from any source except for >2/3 of eukaryotic viral genome may generally be transferred to animals and propagated at BL1. The purchase of transgenic rodents is exempt under Section III-F.
Experiments involving whole plants.	This category applies to experiments that produce genetically engineered plants by way of recombinant DNA methods. This category also applies to experiments using plants together with microorganisms or insects containing recombinant DNA. This category is <u>restricted</u> to experiments involving exotic infectious agents with recognized potential for serious, detrimental impact on ecosystems and experiments involving sequence encoding potent vertebrate toxins introduced into plants or associated organisms. All other plant experiments are likely to fall under Section III-E.
Experiments involving >10 liters of culture.	Appendix K outlines large-scale containment practices. The IBC will decide upon appropriate containment.

III-E Experiments Requiring IBC Notice Simultaneous with Initiation	Notes & Specific Considerations
The formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus.	Propagation and maintenance in tissue cell culture can be done at BL1, provided that cells lack helper virus of concern.
Experiments involving whole plants.	<p>This category covers all whole plant experiments except those that involve exotic infectious agents with recognized potential for serious detrimental impact on ecosystems (i.e., those covered under Section III-D).</p> <p>Covered: Plant-related experiments involving recombinant DNA-modified organisms that have no recognized potential for rapid and widespread dissemination or detrimental impact on ecosystems (e.g., Agrobacterium, Rhizobium)</p> <p>BL1 may be used where modified plants are not noxious weeds and cannot interbreed with noxious weeds of geographic interest.</p>
Experiments involving transgenic rodents.	This category applies to experiments involving generation of rodents in which the genome has been altered by the stable introduction of recombinant DNA. The purchase of commercially-available transgenic rodents is exempt.
III-F Exempt Experiments	Notes & Specific Considerations
Those not in organisms or viruses.	
Those consisting entirely of DNA segments from a single non-chromosomal or viral DNA source	
Those consisting entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated in that host (or a closely related strain of the same species).	
Those consisting of DNA from eukaryotic hosts, including chloroplasts, mitochondria or plasmids (excluding viruses), when propagated only in that host (or a closely related host)	
Natural exchangers (see Appendices AI-AVI)	

Those that do not pose a significant risk to health or the environment

Appendix C--Exemptions under Section III-F-6:

C-1. Recombinant DNA in tissue culture. Recombinant DNA molecules containing $< \frac{1}{2}$ 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.

C-2. E. coli K-12 host-vector systems (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 (unless the DNA inserted into E. coli K-12 is from a prokaryote that naturally exchanges genetic information with E. coli, in which case any E. coli K-12 vector may be used).

Exceptions include (but may not be limited to):

- Experiments involving Risk Group 3, 4 or restricted organisms
- Large-scale experiments (i.e., >10 liters)
- Cloning of toxin molecule genes coding for biosynthesis of molecules toxic for vertebrates.

C-3. Saccharomyces host-vector systems (BL1 practices generally recommended, IBC can specify higher containment if it deems necessary). Exceptions: See C-2.

C-4. Bacillus subtilis or Bacillus licheniformis host-vector systems (BL1 practices generally recommended, IBC can specify higher containment if it deems necessary).

Any asporogenic strain which does not revert to a spore-former with a frequency $>10^{-7}$ used for cloning DNA is considered exempt. Exceptions: See C-2.

C-5. Extrachromosomal elements of Gram positive microorganisms (refer to list in NIH Guidelines). Exceptions: See C-2.

C-6. The purchase or transfer of transgenic rodents (applicable to experiments requiring only BL1 containment).

