Host-Systems Exempt from the NIH Guidelines for Recombinant or Synthetic Nucleic Acids

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All research with recombinant DNA performed at the University of Kentucky requires at a minimum protocol registration and approval by the Biological Safety Officer (BSO Approval). This type of approval includes experiments which the NIH has deemed "exempt" from the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids. Exempt experiments have been determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee, and following appropriate notice and opportunity for public comment to not present a significant risk to health or the environment. They do not require review by the Institutional Biosafety Committee (IBC). Most experiments involving the use of the following four host-vector systems fall under this classification and may be performed at BSL-1 containment.

- **Escherichia coli K-12 Host-Vector Systems***
- **Saccharomyces Host-Vector Systems**
- **Kluyveromyces Host-Vector Systems**
- **Bacillus subtilis or Bacillus licheniformis Host-Vector Systems**

Experiments involving the host-vector systems described above are not exempt from the NIH Guidelines if they fall into one of the following categories:

- Experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation
- Experiments involving DNA from Risk Groups 3, 4, or restricted organisms or cells known to be infected with these agents
- Large-scale experiments (e.g., more than 10 liters of culture)
- Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates

There are also additional requirements specifically concerning each of these host-vector systems which should be considered when determining if the work involved with the host vector system is exempt from IBC review.

- Additional requirements for Escherichia coli K-12 Host-Vector Systems*
  - The Escherichia coli host does not contain conjugation proficient plasmids or generalized transducing phages
  - Lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids are not used as vectors
- Additional requirements for Kluyveromyces Host-Vector Systems
  - Only laboratory-adapted strains are used
- Additional requirements for Bacillus subtilis or Bacillus licheniformis Host-Vector Systems
  - Any asporogenic Bacillus subtilis or asporogenic Bacillus licheniformis strain which does not revert to a spore-former with a frequency greater than 10^{-7} may be used for cloning DNA

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If any of the above conditions are not met, the experiment requires full IBC review.

*Note concerning the use of Escherichia coli K-12 Host-Vector Systems:

Only experiments that utilize E. coli K-12 strains and strains derived from these ancestral strains as a host-vector system may qualify for exempt status from the NIH Guidelines. While experiments involving other attenuated or laboratory strains of E. coli as host-vector systems may be considered to pose minimal risk to health or the environment, **these experiments do not qualify for exempt status and require full IBC review.**

Ancestral E. coli K-12 Strains	
Strain Designation	**Origin or Collection**
WG1	Wisconsin strain
58	Stanford strain
679	Stanford strain
Examples of E. coli Laboratory Strains Derived from K-12	
Strain Designation	**Origin or Collection**
DH5α	Laboratory strain
DH1	Laboratory strain
XL1-Blue	Stratagene
HMS 174	Novagen
Examples of E. coli Laboratory Strains NOT Derived from K-12	
Strain Designation	**Origin or Collection**
B	Laboratory strain
B-3	Laboratory strain
B/R	Laboratory strain
C	Laboratory strain
TOPP	Stratagene
BL21	Novagen

Kuhnert, P., J. Nicolet, J. Frey. 1995. Rapid and accurate identification of Escherichia coli K-12 Strains. Applied and Environmental Microbiology. 61:4135-4139.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC167724/pdf/614135.pdf

Applicable NIH Guidelines include:

Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required:

Section III-F-8. Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C,

Exemptions under Section III-F-8 for other classes of experiments which are exempt from the *NIH Guidelines*.

Appendix C-II. Escherichia coli K-12 Host-Vector Systems

Experiments which use Escherichia coli K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the NIH Guidelines provided that: (i) the Escherichia coli host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-IX. Footnotes and References of Appendix C, Footnotes and References of Appendix C) shall be used as vectors. However, experiments involving the insertion into Escherichia coli K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-IX. Footnotes and References of Appendix C, Footnotes and References of Appendix C) with Escherichia coli may be performed with any Escherichia coli K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the Escherichia coli K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-III. Saccharomyces Host-Vector Systems

Experiments involving Saccharomyces cerevisiae and Saccharomyces uvarum host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the NIH Guidelines. For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-IV.Kluyveromyces Host-Vector Systems

Experiments involving Kluyveromyces lactis, host-vector systems, with the exception of experiments listed in Appendix C-IV-A, are exempt from the NIH Guidelines provided laboratory-adapted strains are used (i.e. strains that have been adapted to growth under optimal or defined laboratory conditions). For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee may specify higher containment if deemed necessary.

Appendix C-V. Bacillus subtilis or Bacillus licheniformis Host-Vector Systems

Any asporogenic Bacillus subtilis or asporogenic Bacillus licheniformis strain which does not revert to a spore-former with a frequency greater than 10-7 may be used for cloning DNA with the exception of those experiments listed in Appendix C-V-A, Exceptions. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-II to C-V Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates).

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html

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