



**Recombinant DNA  
and  
Research with Animals**

# **Recombinant DNA and Research with Animals**

**Jan V. Parker-Thornburg, Ph.D.**  
**Director, Transgenic Animal Core Facility**  
**MD Anderson Cancer Center**

**Stephen H. Hughes, Ph.D.**  
**Director, HIV Drug Resistance Program**  
**National Cancer Institute**

**Brenda Wong**  
**Biological Safety Officer**  
**University of California, San Diego**



# Recombinant DNA and Research with Animals

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## NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)

April 2002

Visit the OBA Web site at:  
<http://www4.od.nih.gov/oba>

For current information on Guidelines, Protocols, Principal Investigators, Meetings,  
and information about upcoming Gene Therapy Policy Conferences

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
National Institutes of Health  
Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

These NIH Guidelines supersede all earlier versions and shall be effective thereafter unless  
indicated otherwise.

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<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>



# Content of the *NIH Guidelines*

- **Section I – Scope**
- **Section II – Safety Considerations**
- **Section III – Types of Experiments Covered**
- **Section IV – Roles and Responsibilities**
- **Appendices**



# Section III - Levels of Review

**IBC, RAC, NIH Director**

**IBC, OBA (in consult with experts)**

**IBC, IRB, RAC**

**IBC**

**IBC (notification)**

**Exempt**



# NIH Guidelines - Section III

## Levels of Review

Level of review	Example of recombinant DNA research	Relevant section(s) of the <i>NIH Guidelines</i>
IBC, RAC review, and NIH Director review and approval	Experiments that compromise the control of disease agents in medicine through deliberate transfer of a drug resistance trait	<b>III-A</b>
IBC approval and NIH review for containment determinations	Experiment involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram of body weight	<b>III-B</b>
IBC and IRB approval and NIH review before research participant enrollment	Experiments involving the deliberate transfer of recombinant DNA into a human research participant	<b>III-C</b>
IBC approval before initiation	Creating stable germline alterations of an animal's genome, or testing viable recombinant DNA modified microorganisms on whole animals, where BL-2 containment or greater is necessary	<b>III-D</b>
IBC notice at initiation	Creating stable germline alterations of rodents using recombinant DNA when these experiments require only BL-1 containment	<b>III-E</b>
Exempt from the <i>NIH Guidelines</i> . IBC registration not required if experiment not covered by Sections III-A, III-B, or III-C	Purchase or transfer of transgenic rodents	<b>III-F</b>



## Section III-A

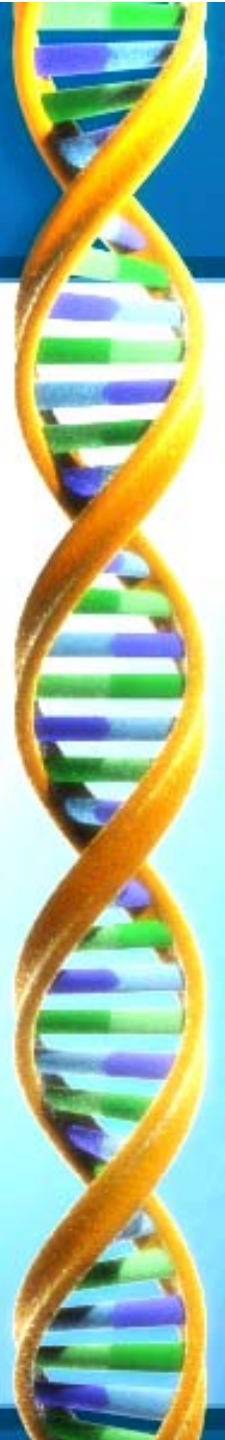
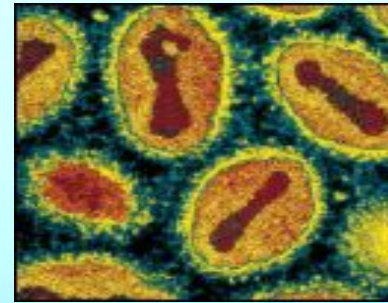
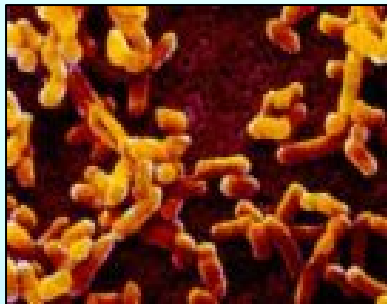
- **Experiments Require IBC Approval, RAC Review and NIH Director Approval Before Initiation**
  - **“Major Action”**
    - **The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, **veterinary medicine**, or agriculture**



# Section III-D-1

## ■ Experiments IBC Approval Before Initiation

- Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems





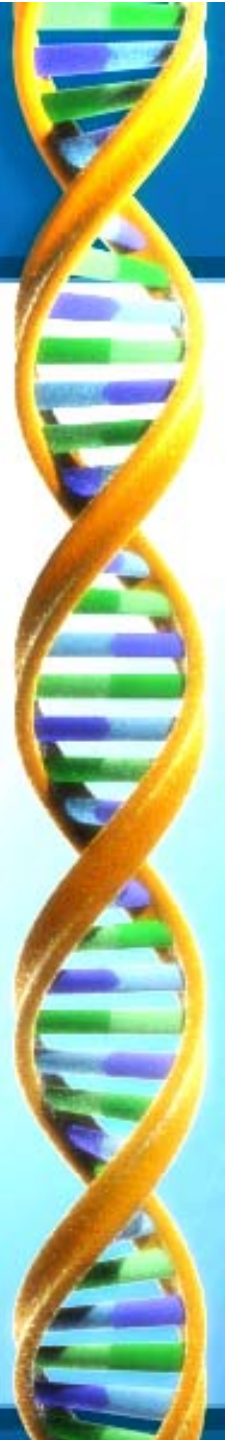
# Section III-D-4 – Experiments Involving Whole Animals

- **Includes experiments in which:**
  - **The animal's genome has been altered by stable introduction of recombinant DNA into germline (transgenic animals)**
  - **Viable recombinant DNA-modified microorganisms are tested on whole animals**
  - **BL2 or BL2-N or higher is required**



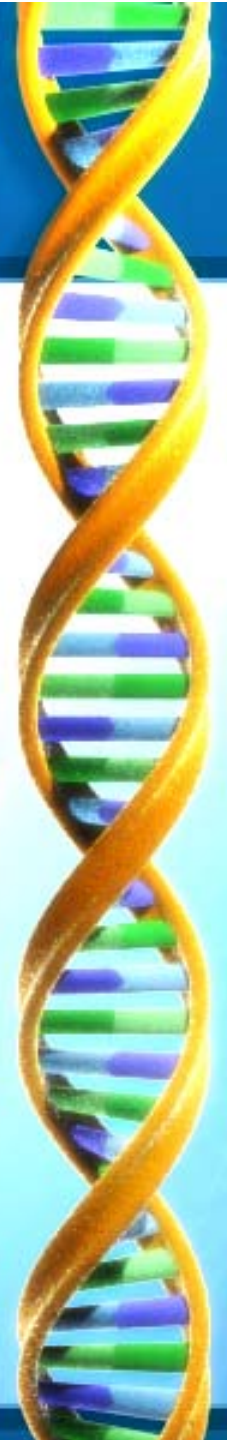
# Section III-D-5 – Experiments Involving Whole Plants

- Includes experiments in which:
  - Plants are genetically engineered by recombinant DNA methods
  - **Plants are used with recombinant DNA-modified insects**
  - Generally BL2-P through BL4-P, depending on risk



## Section III-E

- **Experiments Require IBC Notice Simultaneous with Initiation**
  - **E-1** Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus
  - **E-2** Experiments Involving Whole Plants
  - **E-3** Experiments Involving Transgenic rodents
  - **Experiments Not Included in III-A through III-D or III-F**



## Section III-E-3

### Experiments Involving the Generation of Transgenic Rodents

- Experiments in which:
  - Rodent's genome has been altered by stable introduction of recombinant DNA into germline
  - BL1 containment is appropriate



## Section III-F (Animal Example)

- The purchase or transfer of rodents for experiments that require BL-1 containment

*Further manipulations of these animals are not necessarily exempt from the NIH Guidelines*



# ***NIH Guidelines - Appendices***

- **Appendix A – Exemptions: Natural Exchangers**
- **Appendix B – Classification of Etiologic Agents**
- **Appendix C – Exemptions under III-F**
- **Appendix D – Major Actions**
- **Appendix E – Certified Host-Vector Systems**
- **Appendix F – Biosynthesis of Toxic Molecules**
- **Appendix G – Physical Containment**
- ~~Appendix H – Shipment~~ \*
- **Appendix I – Biological Containment**

**\* Use current Dot/IATA regulations**



# Organization of the *NIH Guidelines*

- **Appendix J – Biotechnology Research Subcommittee**
- **Appendix K – Large Scale Physical Containment**
- **Appendix L – Gene Therapy Policy Conferences**
- **Appendix M – Points to Consider in Human Gene Transfer Research**
- **Appendix P – Physical and Biological Containment: Plants**
- **Appendix Q – Physical and Biological Containment: Animals**



# Key Portions of the *NIH Guidelines* for Animal Research

## Appendix G

- Specifies details of containment and confinement for standard laboratory practices
- Defines Biosafety Level 1 through Biosafety Level 4
- Appropriate for animals that are worked with in a laboratory setting





# Key Portions of the *NIH Guidelines for Animal Research*

## Appendix Q

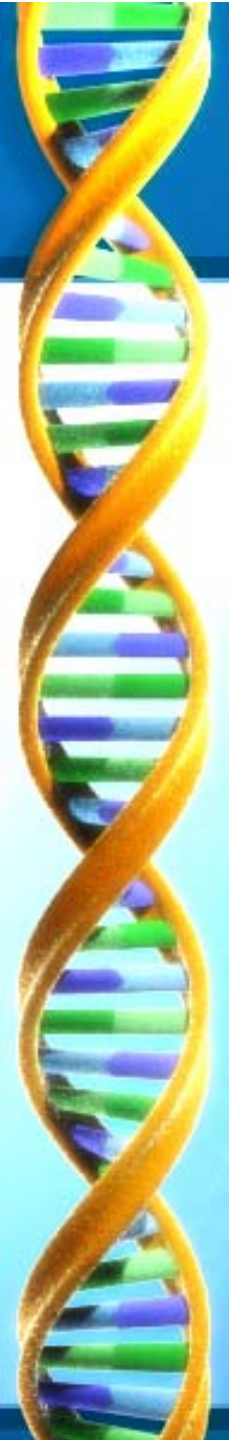
- Applies when research animals are of a size or have growth requirements that preclude laboratory containment
  - For example, cattle, swine, sheep, goats, horses, poultry, etc.
- Addresses containment and confinement practices in animal facilities (BL1-N to BL4-N)



# Key Portions of the *NIH Guidelines* for Animal Research

## Primates - Appendix G or Q?

- Depends on the conditions under which the primates are being housed and used in experimentation
- Primates used in high-level, laboratory containment conditions; Appendix G applies
- In other settings, primates may be worked with in settings akin to those described in Appendix Q
- Professional judgment is key - OBA can help!



# Key Portions of the *NIH Guidelines for Animal Research*

## Appendix M

- Applies to human gene transfer experiments
- Includes many considerations related to **preclinical studies with animals**
- Expedited safety reporting requirements amended in 2002 to include specifically the **reporting of animal data** “that suggest a significant risk for human research participants.”



# IBCs and IACUCs Animal Research

- **Joint purview, and ideally collaborative review, over certain types of research**
  - **Transgenic or cloned animals**
  - **Use of recombinant DNA molecules in animals**
  - **Pre-clinical studies and data assessment for human gene transfer protocols**



# IBC and IACUC Review of Animal Research Utilizing Recombinant DNA



IBC Review	IACUC Review
<ul style="list-style-type: none"><li>▪ <b>Risks to human health</b><ul style="list-style-type: none"><li>□ <b>Transfer of genetically altered material, viral vectors etc.</b></li></ul></li><li>▪ <b>Risks to the environment</b><ul style="list-style-type: none"><li>□ <b>Escape and establishment in the wild</b></li><li>□ <b>Interbreeding with wild stock</b></li><li>□ <b>Consumption by other animals</b></li></ul></li></ul>	<ul style="list-style-type: none"><li>▪ <b>Animal welfare</b><ul style="list-style-type: none"><li>□ <b>Pain and distress from adverse phenotypes (behavioral, anatomical and physiological abnormalities)</b></li><li>□ <b>Risks to other animals in the facility from the inadvertent spread of vectors</b></li></ul></li></ul>

# Transgenic Animal FAQs



National Institutes of Health  
**Office of  
Biotechnology  
Activities**

## Transgenic Animals and the Use of Recombinant DNA in Animals FAQs for Research Subject to the *NIH Guidelines*



### 1. Under which section of the *NIH Guidelines* does the generation of transgenic rodents fall?

The creation of transgenic rodents falls under one of two portions of the *NIH Guidelines* depending on the containment level required to house the rodents. Experiments involving the creation of transgenic rodents that can be housed under Biosafety Level 1 conditions are covered under **Section III-E-3**. Experiments involving the generation of transgenic rodents requiring BL2, BL3 and BL4 containment are covered under **Section III-D-4**.

### 2. Under which section of the *NIH Guidelines* does the generation of transgenic animals other than rodents fall?

The creation of all transgenic animals (other than rodents that can be housed under BL1 containment conditions) is covered under **Section III-D-4** of the *NIH Guidelines*.

### 3. Would the breeding of two different strains of knock-out mice require IBC approval under the *NIH Guidelines*?

The techniques used initially to create knock-out animals involve the stable introduction of recombinant DNA into the animal's genome, and thus these animals are considered transgenic. As the breeding of two different strains of knock-out mice will potentially generate a novel strain of transgenic animal, the work is covered under the *NIH Guidelines* and as such requires IBC review and approval. Sections in the *NIH Guidelines* that cover work with rodents include **III-E-3** for work that requires Biosafety Level (BL) 1 containment and **III-D-4** for work that requires BL2, BL3 and BL4 containment.

### 4. Is IBC registration and approval needed for the maintenance of a transgenic animal colony?

The maintenance of a transgenic rodent colony (i.e. breeding within a particular transgenic strain) at BL1 is an activity that is exempt from the *NIH Guidelines* and, as such, does not require IBC registration and approval. The maintenance of a transgenic rodent colony at BL2 or higher falls under **Section III-D-4-b** and requires IBC approval. The breeding of all other transgenic animals is subject to the *NIH Guidelines* under Section **III-D-4-a** or **III-D-4-b** depending on the containment level required.

### 5. Is the purchase and transfer of transgenic rodents exempt from the *NIH Guidelines*?

Under **Appendix C-VI** of the *NIH Guidelines*, the purchase or transfer of transgenic rodents may be maintained at BL1 containment are exempt from the *NIH Guidelines*. The purchase or transfer of transgenic rodents that require BL2 or higher containment is not exempt from the *NIH Guidelines*. These animals are covered under **Section III-D-4**, and purchase and transfer of such animals requires IBC registration and approval.

It should be noted that the subsequent use of transgenic rodents may not be exempt from the *NIH Guidelines*. Experiments using transgenic rodents at BL1 are exempt from the *NIH Guidelines* if the experiment does not involve the use of recombinant DNA. If the protocol does involve the use of recombinant DNA or is conducted at BL2 or higher then the work falls under **III-D-4** of the *NIH Guidelines* and as such requires IBC review and approval prior to initiation.

### 6. Is the purchase and transfer of transgenic animals other than rodents exempt from the *NIH Guidelines*?

No, only the purchase or transfer of transgenic rodents that may be maintained at BL1 containment is exempt from the *NIH Guidelines*. The purchase or transfer of any other animal for research purposes at any biosafety level (including BL1) is not exempt, nor is the purchase and transfer of transgenic rodents that require BL2 or higher containment.



# Animal Experiments Covered Under the *NIH Guidelines*



## Animal experiments covered under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*



ACTIVITY	MINIMUM BSL	SECTION
<b>CREATION OF TRANSGENIC ANIMALS</b>		
Creation of transgenic rodents	BL1	III-E-3
Creation of transgenic rodents	BL2 or higher	III-D-4-b
Creation of transgenic animals other than rodents	BL1/BL1-N	III-D-4-a
Creation of transgenic animals other than rodents	BL2/BL2-N or higher	III-D-4-b
Creation of recombinant DNA modified arthropods	BL1	III-D-4-a
Creation of recombinant DNA modified arthropods	BL2 or higher	III-D-4-b
Creation of knock-out rodents	BL1	III-E-3
Creation of knock-out rodents	BL2 or higher	III-D-4-b
<b>BREEDING OF TRANSGENIC ANIMALS</b>		
Breeding rodents from one strain (propagation/colony maintenance)	BL1	Exempt (III-F-4)
Breeding rodents from one strain (propagation/colony maintenance)	BL2 or higher	III-D-4-b
Breeding rodents from two strains (generating new strain)	BL1	III-E-3
Breeding rodents from two strains (generating new strain)	BL2 or higher	III-D-4-b
Breeding of transgenic animals other than rodents	BL1	III-D-4
Breeding of transgenic animals other than rodents	BL2 or higher	III-D-4
Breeding of recombinant DNA modified arthropods	BL1	Exempt (III-F-4)
Breeding of recombinant DNA modified arthropods	BL2 or higher	III-D-4-b
Breeding of knockouts (propagation)	BL1	Exempt (III-F-4)
Breeding of knockouts (propagation)	BL2 or higher	III-D-4-b
Breeding of knockouts from two strains (generating new strain)	BL1	III-E-3
Breeding of knockouts from two strains (generating new strain)	BL2 or higher	III-D-4-b
<b>EXPERIMENTS WITH TRANSGENIC ANIMALS</b>		
Experiments with transgenic rodents	BL1	III-D-4-a* (see note)
Experiments with transgenic rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with transgenic animals other than rodents	BL1	III-D-4-a
Experiments with transgenic animals other than rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with recombinant DNA modified arthropods associated with plants	BL1	III-E-2-b-(5).
Experiments with recombinant DNA modified arthropods associated with plants	BL2 or higher	III-E-2
Experiments with recombinant DNA modified arthropods not associated with plants	BL1	III-D-4-a
Experiments with recombinant DNA modified arthropods not associated with plants	BL2 or higher	III-D-4-b

\*The purchase or transfer of transgenic rodents requiring BL1 containment is exempt under Appendix C-6. Subsequent use of these animals is also exempt providing the experimental protocol does not involve the use of recombinant DNA. If the protocol does involve the use of recombinant DNA then the research is covered under III-D-4-a. All experiments involving the use of other transgenic animals at any Biosafety Level and the use of transgenic rodents requiring BL2 or higher containment are subject to the *NIH Guidelines*. See above for applicable sections.

