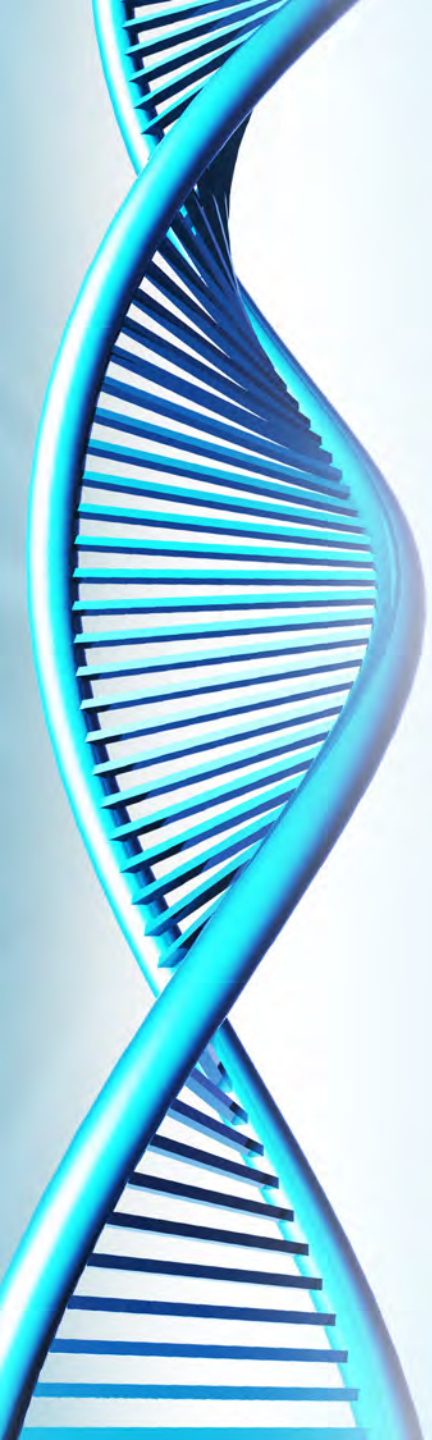


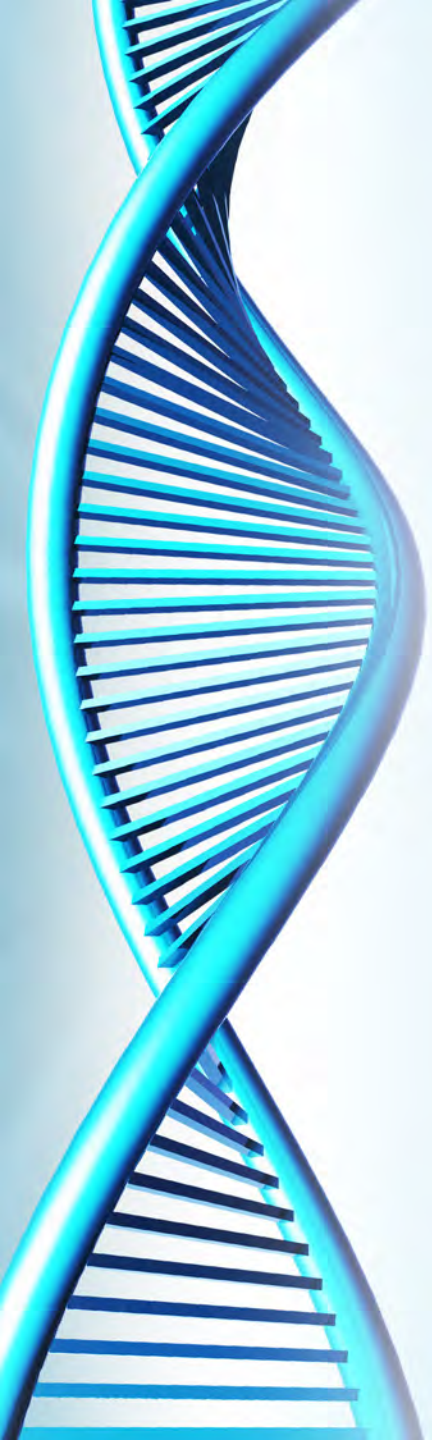
Overview of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*

National Institutes of Health
Office of Biotechnology Activities

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

- **A scientifically-responsive document that will continue to evolve**
  - **Has undergone multiple revisions since 1976**
  - **Latest version – November 2013**

http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines



Content of the *NIH Guidelines*

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

NIH Guidelines – Section I

- **Scope and Applicability**
  - **Specifies practices for constructing and handling**
    - **(i) recombinant nucleic acid molecules,**
    - **(ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and**
    - **(iii) cells, organisms and viruses containing such molecules.**

NIH Guidelines – Section I

- **In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:**
  - **(i) molecules that a) are constructed by joining nucleic acid molecules and b) can replicate in a living cell, i.e. recombinant nucleic acids;**
  - **(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e. synthetic nucleic acids; or**
  - **(iii) molecules that result from the replication of those described in (i) or (ii) above.**

The *NIH Guidelines* Apply to…

- **Research with recombinant or synthetic (or both) nucleic acid molecules that is**
  - **Performed at or sponsored by an institution that receives any NIH funding for such research**
- **Rationale: For biosafety to be meaningful, it has to be observed by all investigators at an institution**

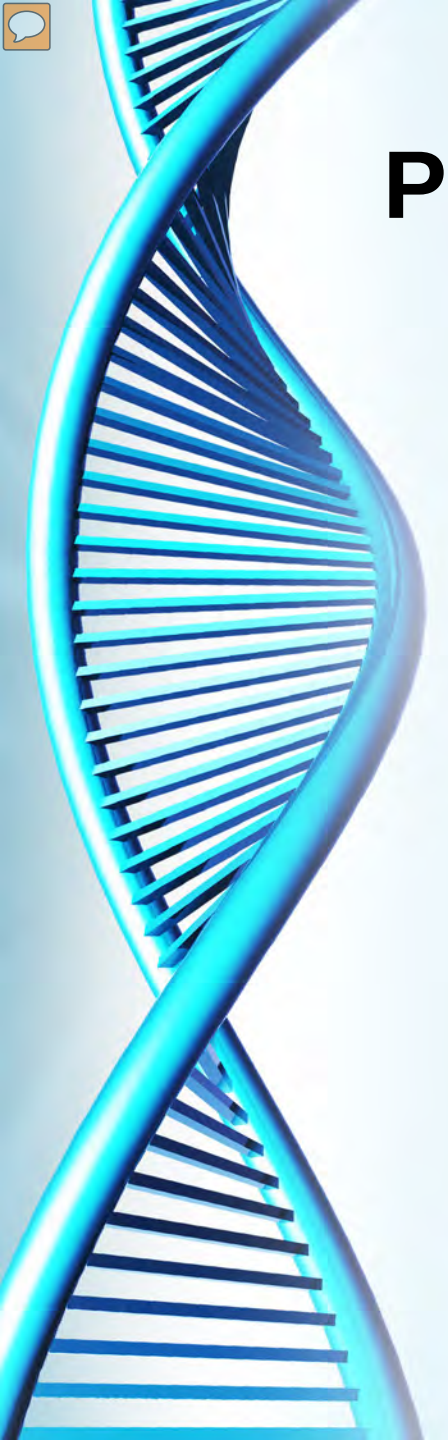
Applicability broader than many NIH grants and contracts requirements

Are the *NIH Guidelines* Optional?

- **“Guidelines” does not mean “optional”**
- **They are a term and condition of NIH funding for research with recombinant or synthetic nucleic acid molecules**

Are the *NIH Guidelines* Optional?

- **What are potential consequences of noncompliance with the *NIH Guidelines*?**
  - **Suspension, limitation, or termination of NIH funds for research subject to the *NIH Guidelines* at the institution, or**
  - **A requirement for prior NIH approval of any or all research subject to the *NIH Guidelines* at the institution.**



Prescription versus Flexibility

- Some matters are left to institutional discretion
- Flexibility is a two-sided coin
  - Accommodates institutional diversity and heterogeneity
  - Can create uncertainty about expectations

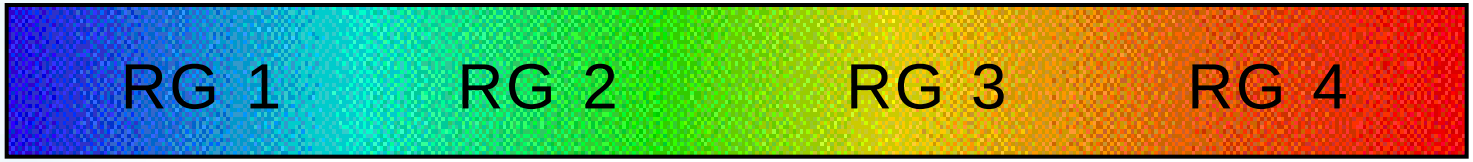
Specifics vs. Intent

- **"The *NIH Guidelines* will never be complete or final since all conceivable experiments involving recombinant or synthetic nucleic acid molecules cannot be foreseen. Therefore, it is the responsibility of the institution and those associated with it to adhere to the intent of the *NIH Guidelines* as well as to the specifics."**
  - **Good judgment is key**
  - **OBA can help**

Section II - Safety Considerations

NIH Guidelines – Section II

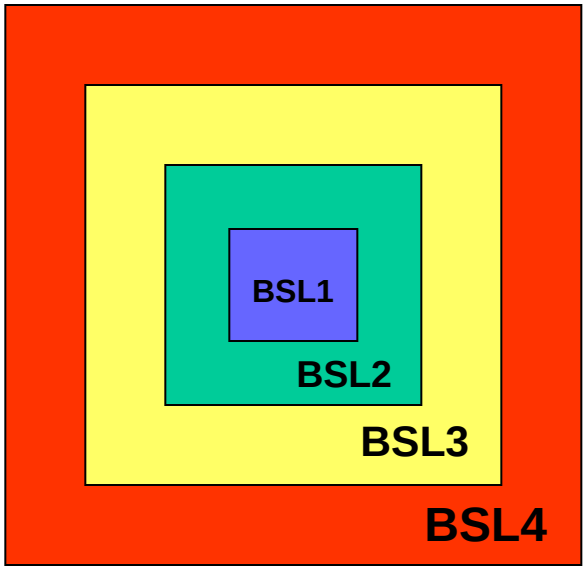
- **Safety Considerations**
  - **Risk assessments: (Appendix B)**



Agents that are not associated with disease in healthy adult humans	**Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available**	**Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)**	**Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)**

NIH Guidelines – Section II

- **Safety Considerations**
  - **Containment**
    - **Physical (Appendix G)**
      - **Practices**
      - **Equipment**
      - **Facilities**
    - **Biological (Appendix I)**
      - **Survival**
      - **Transmission**



Section III - Levels of Review

IBC, RAC, NIH Director

IBC, OBA (in consult with experts)

IBC, IRB, RAC

IBC

IBC (notification)

Exempt

RISK

NIH Guidelines - Section III Levels of Review

Level of review	Example of types of research covered	Relevant section(s) of the *NIH Guidelines*
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	III-A
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	III-B
IBC and IRB approval and NIH review before research participant enrollment	Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules into a human research participant	III-C
IBC approval before initiation	Creating stable germline alterations of an animal's genome, or testing viable recombinant or synthetically modified microorganisms on whole animals, where BL-2 containment or greater is necessary	III-D
IBC notice at initiation	Creating stable germline alterations of rodents by introduction of recombinant or synthetic nucleic acid molecules when these experiments require only BL-1 containment	III-E
Exempt from the *NIH Guidelines*. IBC registration not required if experiment not covered by Sections III-A, III-B, or III-C	Purchase or transfer of transgenic rodents	III-F

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-A

National Institutes of Health • Office of Biotechnology Activities

Major Actions under Section III-A of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)

1. **What experiments are considered "Major Actions" under the *NIH Guidelines*?**

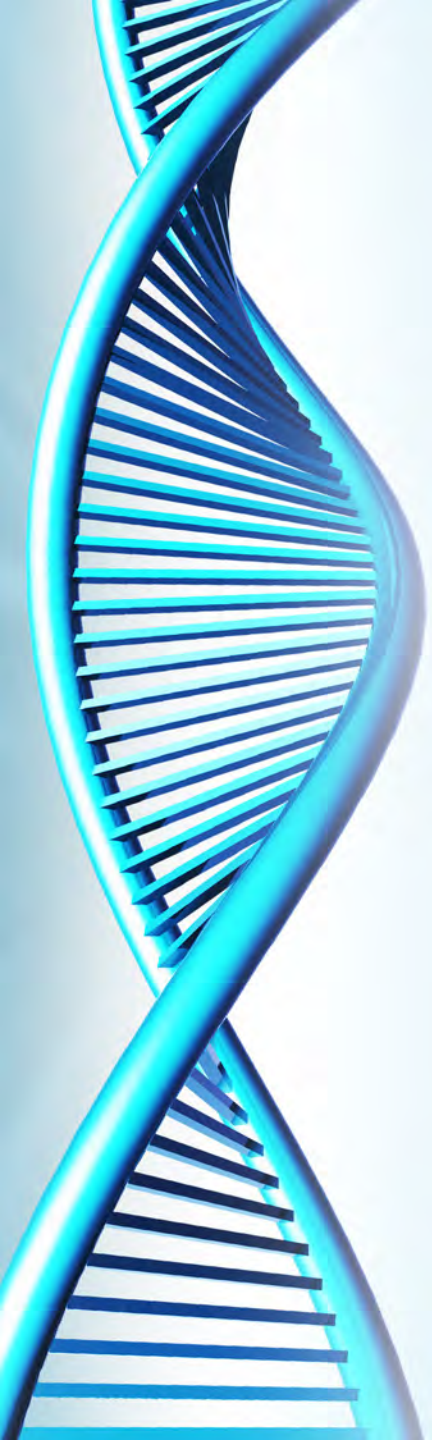
   Under the *NIH Guidelines*, the term "Major Action" means that NIH Director approval is required. Only one type of experiment requires NIH Director approval – the deliberate transfer of a drug resistance trait to a microorganism when such resistance could compromise the ability to control the disease agent in humans, veterinary medicine, or agriculture (see Section III-A-1-a of the *NIH Guidelines*).

2. **What criteria should be used to determine if the transfer of a particular drug resistance trait is considered a Major Action under Section III-A-1-a of the *NIH Guidelines*?**

   An experiment may be considered a Major Action if: 1) it involves the use of recombinant or synthetic nucleic acids to introduce drug resistance into a microorganism, and 2) the drug in question is used to treat disease caused by the organism in humans, veterinary medicine, or agriculture. The experiment would not be considered a Major Action if there is sufficient documentation that resistance to a therapeutically useful concentration of that drug exists in the agent outside of a laboratory setting. Such evidence should be in the form of articles published in the scientific literature.

Section III-B

- **Experiments Require NIH/OBA and IBC Approval Before Initiation**
  - **III-B-1: Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight**
  - **III-B-2: Experiments that have been approved (under Section III-A-1-a) as Major Actions under the *NIH Guidelines***



Section III-C

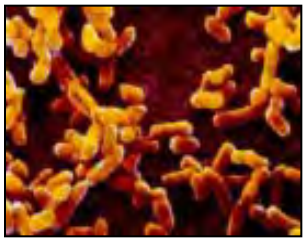
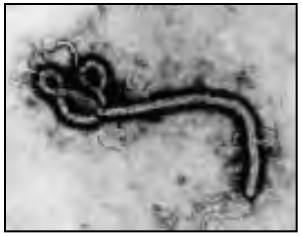
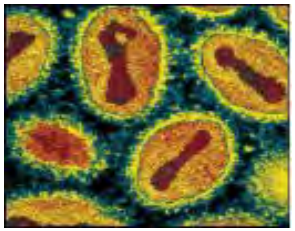
- **Experiments Require RAC Review, IBC Approval and IRB Approval <u>Before</u> Initiation**
- **Human gene transfer - deliberate transfer into human research participants of either:**
  - **Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or**
  - **Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:**

Section III-C

- 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.

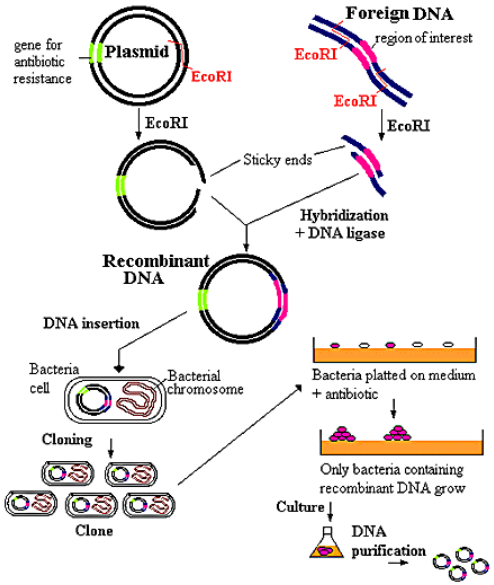
Section III-D-1

- **Experiments IBC Require Approval <u>Before</u> Initiation**
  - **Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems**

Section III-D-2

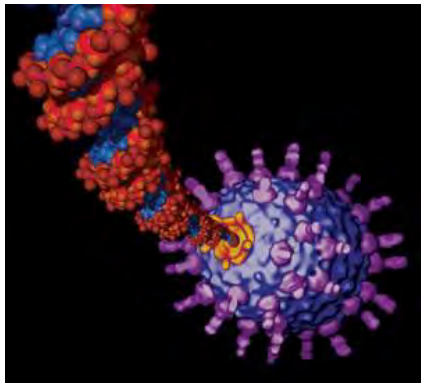
- **Experiments Require IBC Approval <u>Before</u> Initiation**
  - **Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems**



Cloning into a plasmid

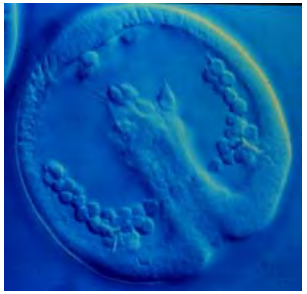
Section III-D-3

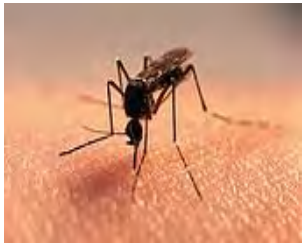
- **Experiments Require IBC Approval <u>Before</u> Initiation**
  - **Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems**



Section III-D-4: Experiments Involving Whole Animals

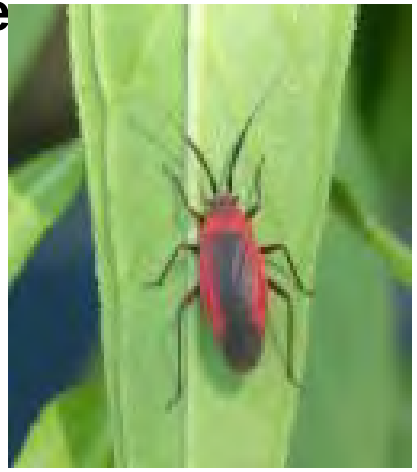
- **Includes experiments in which:**
  - **The animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acids into germline (transgenic animals)**
  - **Viable recombinant or synthetic nucleic acid molecule-modified microorganisms are tested on whole animals**





Section III-D-5: Experiments Involving Whole Plants

- **Includes experiments in which:**
  - **Plants are genetically engineered by recombinant or synthetic nucleic acid molecule methods**
  - **Plants are used with recombinant or synthetic nucleic acid molecule containing insects**
  - **Generally BL2-P through BL4-P, depending on risk**



Section III-D-6: Experiments Involving More Than 10L of Culture

Also See Appendix K

Section III-D-7

- **Experiments Involving Influenza Viruses**
  - **Generated by recombinant or synthetic methods (e.g., reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the risk group of the virus that was the source of the majority of segments in the recombinant virus**
  - **Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at BL3 enhanced containment**

Section III-E

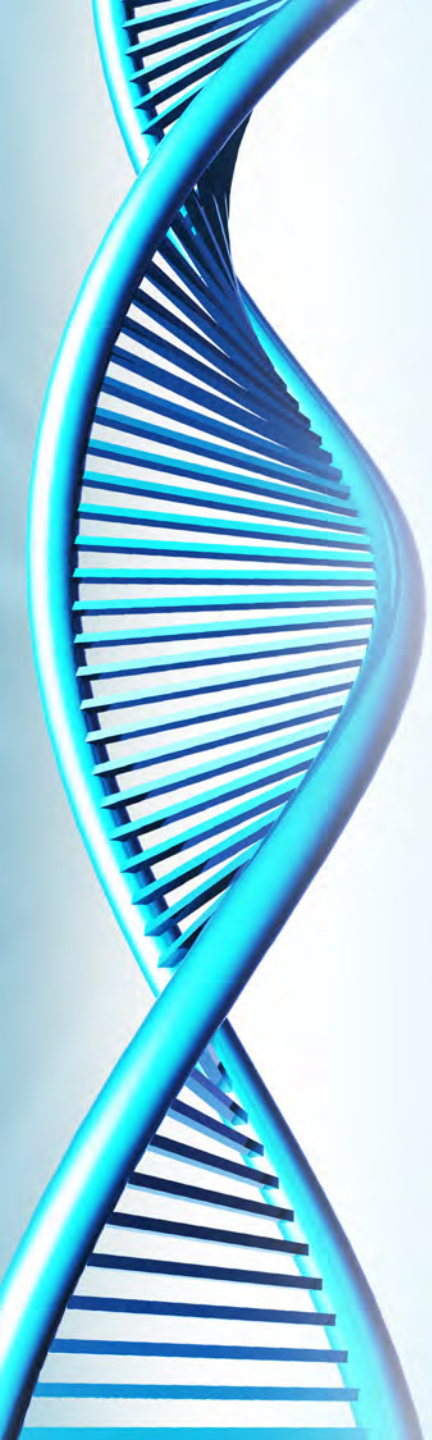
- **Experiments Require IBC Notice Simultaneous with Initiation**
  - **E-1** Experiments Involving the Formation of Recombinant or Synthetic Nucleic Acid Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus
  - **E-2** Experiments Involving Whole Plants
  - **E-3** Experiments Involving Transgenic rodents

Also - Experiments not included in III-A through III-D or III-F that can be conducted at BSL1

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 or synthetic nucleic acid molecules into germline**
  - **BL1 containment is appropriate**



Section III-F: Exempt Experiments

Registration with the Institutional Biosafety Committee is not required (although many institutions may require this by policy)

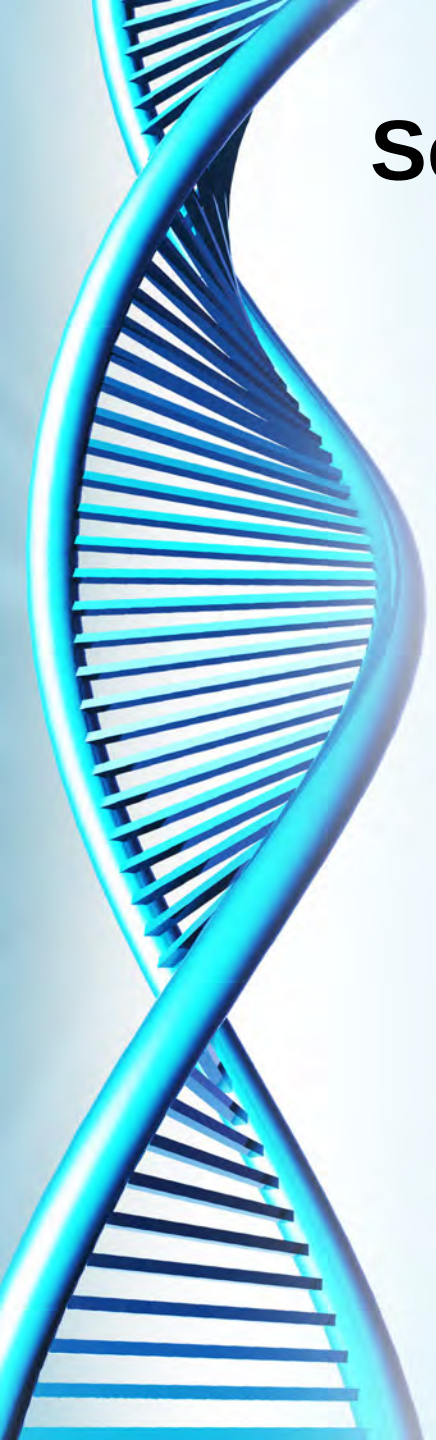
Section III-F-1: Exempt Experiments

- **Synthetic nucleic acids 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.

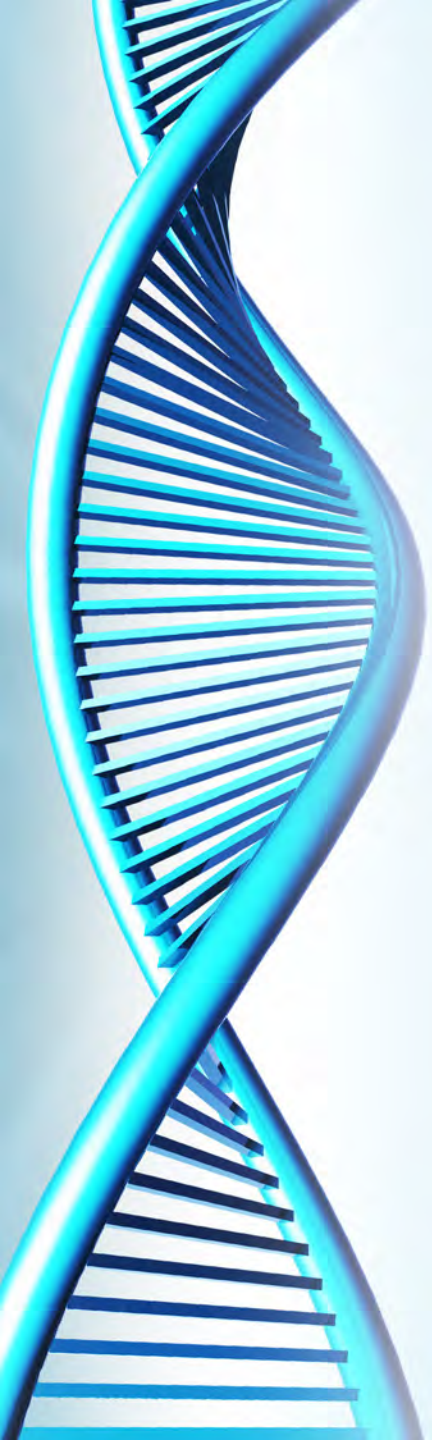


Section III-F-1: Exempt Experiments

Note: **If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the amended criteria of Section III-C, it is not exempt under the *NIH Guidelines*.**

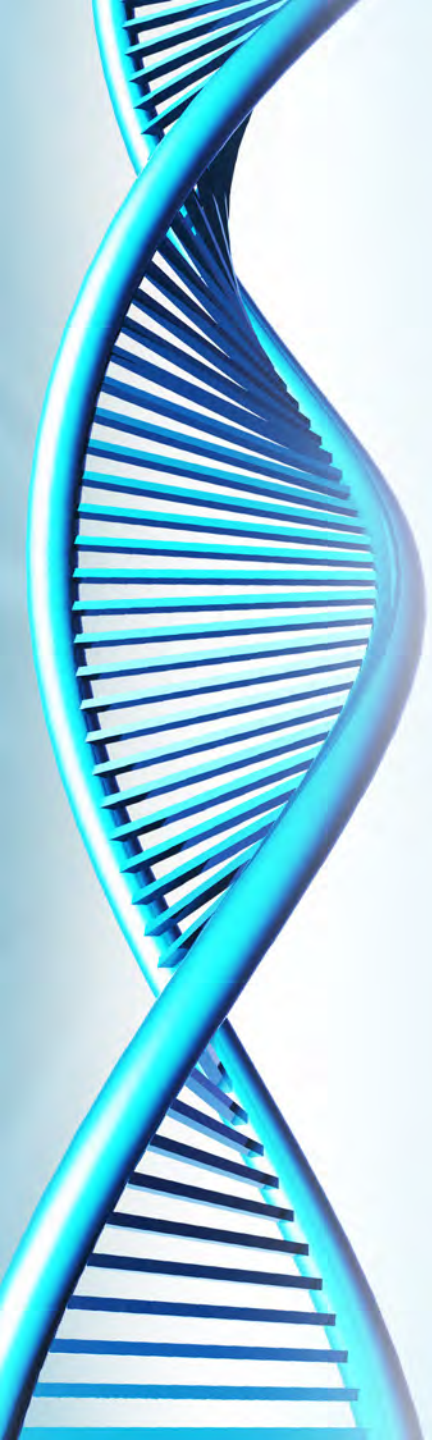
Section III-F-2

- **Exempts the following experiments:**
  - **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.**



Section III-F-3

- **Those that consist entirely of recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature**



Section III-F-4

- **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.**

Section III-F-5

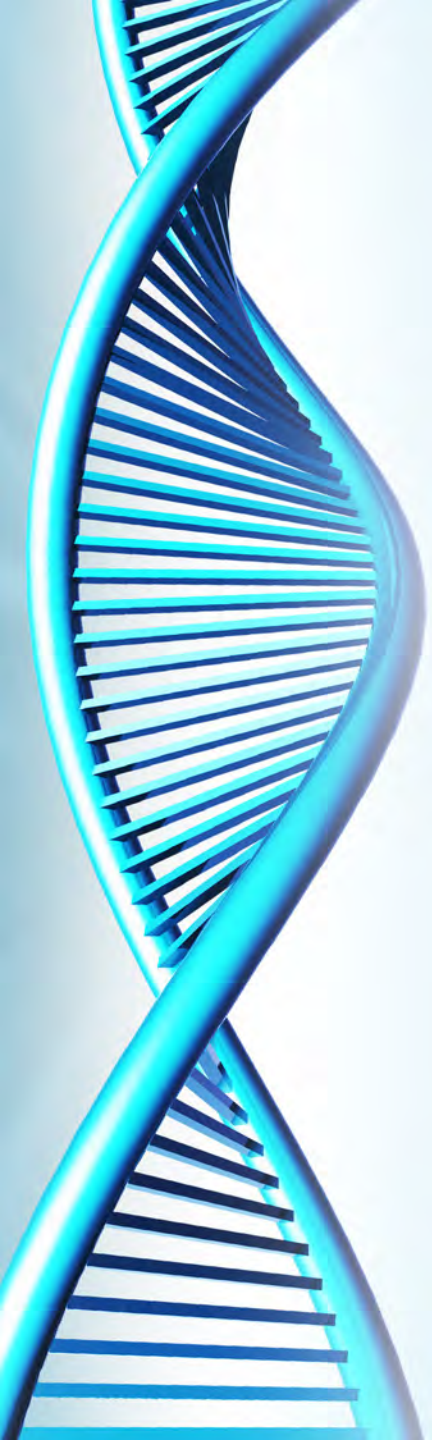
- **Those that consist entirely of nucleic acids from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).**

Section 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.**

Meaning recombinant DNA molecules that are:

1) **composed entirely of DNA segments from one or more of the organisms <u>within a sublist</u>, and**
2) **to be propagated in any of the organisms <u>within the same sublist</u>**



Section 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**

Section III-F-8

- **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.**

See Appendix C, *Exemptions under Section III-F-8*

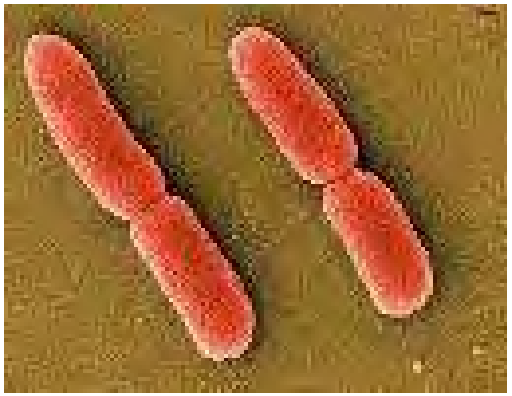
Appendix C-I

- **Recombinant or Synthetic Nucleic Acid Molecules in Tissue Culture**
  - **Recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical that are propagated and maintained in cells in tissue culture are (with exempt (with the exceptions listed in Appendix C-I-A)**

Appendix C-II

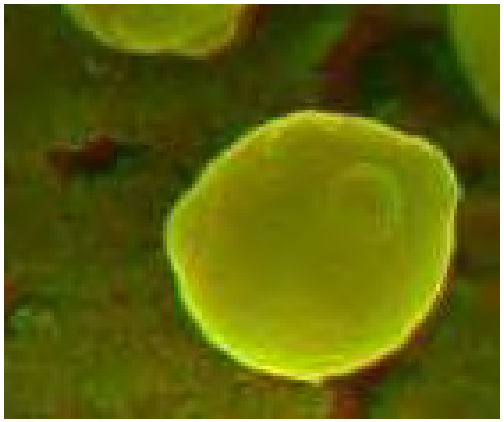
- ***Escherichia coli K-12*** **Host-Vector Systems**

  - **Experiments which use *Escherichia coli* K-12 host-vector systems (with the exception of those experiments listed in Appendix C-II-A) exempt.**



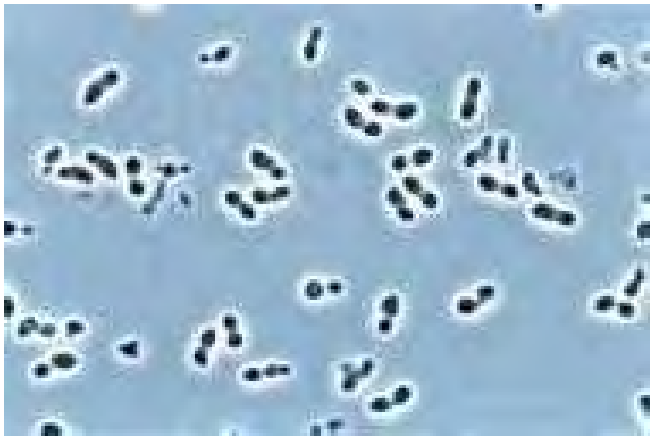
Appendix C-III

- ***Saccharomyces* Host-Vector Systems**
  - **Experiments involving *S. cerevisiae* and *S. uvarum* host-vector systems (with the exception of experiments listed in Appendix C-III-A) are exempt**



Appendix C-IV

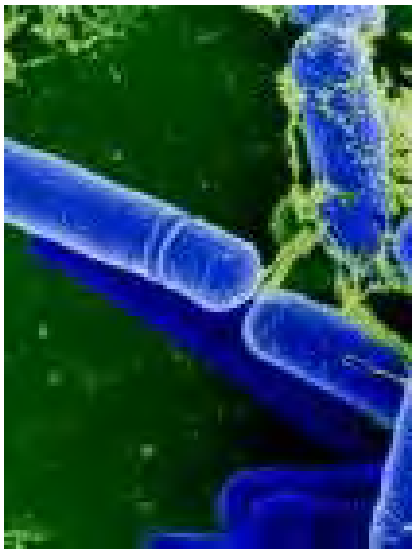
- ***Kluyveromyces* Host-Vector Systems**
  - **Experiments involving K. lactis host-vector systems (with the exception of experiments listed in Appendix C-III-A) are exempt**



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-IV-A, *Exceptions*)**



Appendix C-VI

- **Extrachromosomal Elements of Gram Positive Organisms**
  - **Recombinant or synthetic nucleic acid molecules derived entirely from extrachromosomal elements of the organisms listed below, propagated and maintained in organisms listed below are exempt.**
    - ***Bacillus amyloliquefaciens***
    - ***Bacillus amylosacchariticus***
    - ***Bacillus anthracis***
    - ***Bacillus aterrimus***
    - ***Bacillus brevis***
    - ***Bacillus cereus***
    - ***Bacillus globigii***
    - ***Bacillus licheniformis***
    - ***Bacillus megaterium…….*** **(see *NIH Guidelines* for complete list)**

Appendix C-VII

- **The Purchase or Transfer of Transgenic Rodents**
  - **The purchase or transfer of transgenic rodents <u>for experiments that require BL1 containment</u>**

Further manipulations of these animals are <u>not necessarily</u> exempt from the NIH Guidelines

Appendix C-VIII

- **Generation of BL1 Transgenic Rodents via Breeding**

  - **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 BL1 containment will be exempt from the *NIH Guidelines* if:**

    (1) Both parental rodents can be housed under BL1 containment; and

    (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

    (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.

Section III-F (and Appendix C)

National Institutes of Health • Office of Biotechnology Activities

Experiments that are Exempt from the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*
Frequently Asked Questions

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

   Section III-F of the *NIH Guidelines* details experiments that are exempt from the requirements of the *NIH Guidelines*. The following molecules are exempt:

   - 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)

NIH Guidelines – Section IV

- **Roles and Responsibilities**
  - **Institution**
  - **Institutional Biosafety Committee (IBC)**
  - **Biological Safety Officer (BSO)**
  - **Principal Investigator (PI)**
  - **NIH**

Institutional Responsibilities under the *NIH Guidelines*

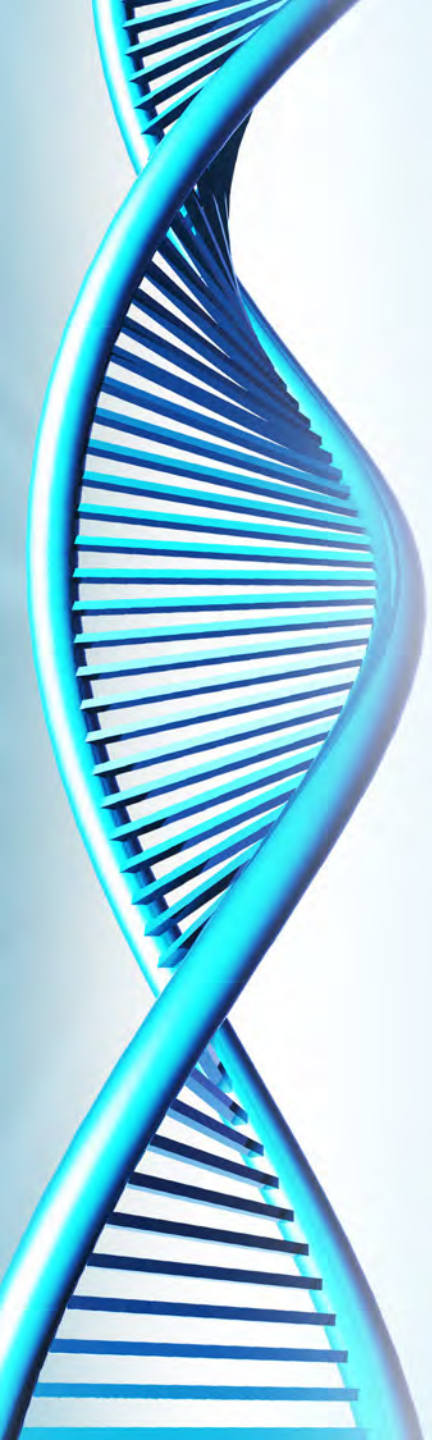
- **The Institution shall:**
  - **Establish and implement policies for the safe conduct of research subject to the *NIH Guidelines***
  - **Establish an Institutional Biosafety Committee**
  - **Assist and ensure compliance with the *NIH Guidelines* by investigators**
  - **Ensure appropriate training for IBC members and staff, PIs, laboratory staff**
  - **Determine necessity for health surveillance of personnel**
  - **Report any significant accidents, incidents or violations to OBA within 30 days (or immediately as required)**

PI Responsibilities under the *NIH Guidelines*

- **The Principal Investigator shall (among other things):**
  - **Initiate or modify no research subject to the *NIH Guidelines* which requires IBC approval until approval is granted**
  - **Determine whether experiments are covered under III-E and notify the IBC as appropriate**
  - **Be adequately trained in good microbiological techniques**
  - **Adhere to IBC emergency plans for spills and personnel contamination**
  - **Report any significant problems or violations to OBA within 30 days (or immediately as required)**

NIH Responsibilities under the *NIH Guidelines*

- **NIH OBA (*on behalf of the NIH Director*)**
  - **Managing the RAC**
  - **Conducting and supporting training of IBCs, BSOs, investigators, laboratory staff**
  - **Convening Scientific Symposia and Gene Therapy Policy Conferences**
  - **Review of:**
    - **Human gene transfer protocols**
    - **Certain basic recombinant or synthetic nucleic acid molecule experiments**
  - **"Minor actions"**
    - **Changes not requiring approval by the NIH Director**



NIH OBA Responsibilities under the *NIH Guidelines*

- **Basic experiments reviewed by NIH OBA**
  - **Deliberate transfer of drug resistance trait to microorganisms not known to acquire the trait naturally, if it could compromise disease control**
  - **Cloning of toxin molecules with LD_{50} <100 ng/Kg bodyweight**
  - **Recombinant or synthetic nucleic acid molecules from restricted agents transferred to nonpathogenic prokaryotes or lower eukaryotes**
  - **Recombinant or synthetic nucleic acid molecules from nonpathogenic prokaryotes or lower eukaryotes transferred to restricted agents**
  - **Use of infectious or defective restricted poxviruses in presence of helper virus**

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**

Questions?