

# **NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules**

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Department of Environment, Health and Safety – 10/2017

This is the print version of the Recombinant or Synthetic Nucleic Acid Molecules Training Module. It does not contain the Knowledge Review, or the test questions. If you have questions, please contact the Department of Environment, Health and Safety at 919-962-5507.

## **Course Overview**

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### **Goals of the Training**

Upon completion of this training, the Principal Investigator/Lab personnel should understand:

- General requirements under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).
- His/her responsibilities under the NIH Guidelines.

### **Principal Investigators and Other Researchers:**

The course provides information in order for the Principal Investigator to make sure that his/her laboratory is in compliance with the National Institute of Health Guidelines for such research.

In addition, the Guidelines identify key ethical principles and key safety reporting requirements, applicable to all researchers who work with recombinant or synthetic nucleic acid molecules.

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### **Training Requirements**

A Knowledge Review will follow each module in order to provide a learning experience for that module. You may skip the learning modules if you choose.

If you do not work with recombinant or synthetic nucleic acid molecules or transgenic animals/plants, the training program will end with a test at the end of Module Two. You may retake the test at any time.

If you work with recombinant or synthetic nucleic acid molecules or transgenic animals/plants, you must also complete modules 3, 4, 5, and 6 to determine what category your research falls under, and if IBC approval is required. The training will end with a test at the end of the last module: Module Six. You may retake the test at any time.

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## Learning Aids

To aid you in your understanding of the NIH Guidelines, you can also find the following documents at any time throughout this module by clicking on the links at the top of each frame.

[NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#)

[NIH Office of Science Policy](#)

[Biosafety Considerations for Research with Lentiviral Vectors](#)

[NIH Report – Viral vectors: from virology to transgene expression](#)

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## Program Modules

There are six training modules:

Module One: Introduction to the NIH Guidelines and Scope

Module Two: Roles and Responsibilities

Module Three: Types of Experiments Covered

Module Four: Safety Considerations or Risk Assessment

Module Five: Submission to the IBC and IBC Review

Module Six: Incident Reporting

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### Module One:

#### Introduction to the NIH Guidelines

NIH Office of Science Policy:

- Oversees recombinant or synthetic nucleic acid research, including human gene transfer.
- Manages the Recombinant DNA Advisory Committee (RAC), a public advisory committee that advises the Department of Health and Human Services and NIH about recombinant nucleic acid research.
- Administers the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

- Partners with Institutional Biosafety Committees in the oversight of recombinant nucleic acid research.

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### **History of the Guidelines Development**

With the emergence of recombinant DNA technology in the mid- 1970s, the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#) grew out of concerns by scientists and the general public for public health and safety, environmental impact, and the ethical and social implications of such research.

The original guidelines were issued in 1976. Multiple revisions have been issued since, with the most recent amendment effective as of April 2016.

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### **Scope of the Guidelines**

The purpose of the NIH Guidelines is to specify practices for constructing and handling:

1. Recombinant nucleic acid molecules
2. Synthetic nucleic acid molecules
3. Cells, organisms and viruses containing recombinant or synthetic nucleic acid molecules
4. Transgenic animals

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### **Definition: Recombinant Nucleic Acid Molecules**

- Molecules that are constructed by joining natural or synthetic nucleic acid molecules to nucleic acid molecules that can replicate in a living cell
- Molecules that result from the replication of the molecules described above
- Recombinant nucleic acid segments likely to yield a potentially harmful polynucleotide or polypeptide (i.e. toxin) are exempt unless they are expressed in vivo as a biologically active product.
- Genomic DNA of plants and bacteria that have acquired a transposable element (even if it was donated from a recombinant vector that is no longer present) are exempt unless the transposon itself contains recombinant nucleic acid molecules.

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### **Definition: Synthetic Nucleic Acids**

- 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 acids.
- Molecules that result from the replication of the molecules described above

- Synthetic techniques may enable the synthesis of more complex chimeras containing sequences from a number of different sources.
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#### **Definition: SiRNA**

- Small interfering RNA are a class of 20-25 nucleotide-long double-stranded RNA molecules involved in the RNA interference (RNAi) pathway and interferes with the expression of a specific gene.
  - Recombinant DNA experiments include inserting DNA encoding siRNA targeting the gene of interest into an expression vector.
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#### **Definition: Transgenic Animals**

- Animals that have been modified by stable introduction of recombinant or synthetic nucleic acid molecules, or DNA derived therefrom, into the germ-line.
  - Foreign nucleic acid molecules are introduced into the animal using recombinant nucleic acid technology, and then must be transmitted through the germ line so that every cell, including germ cells of the animal, contain the same modified genetic material.
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#### **Example: Cre/loxP System**

- Mice can be made transgenic for the gene encoding Cre attached to a promoter activated only when bound by the appropriate transcription factors:
    - A "target" gene is flanked by loxP sequences.
    - Transcription of the Cre gene. Its protein will then remove the "target" gene under study.
    - The result: a mouse with a particular gene knocked out in only certain cells.
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#### **Module Two:**

##### **The NIH Guidelines are applicable to:**

- Research conducted at or sponsored by an institution that receives any support for recombinant or synthetic nucleic acid research from the NIH;
- Research that involves testing in humans of materials containing recombinant or synthetic nucleic acid molecules developed with NIH funds.

#### **Attention!**

**UNC-CH receives NIH support. Therefore, all recombinant or synthetic nucleic acid molecule work conducted at UNC must be in compliance with the NIH Guidelines.**

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## **Non-Compliance Consequences**

- Non-compliance with the NIH Guidelines can result in:
    - Suspension, limitation or termination of NIH funding for UNC-Chapel Hill.
    - A requirement of prior NIH approval of all recombinant or synthetic nucleic acid projects at UNC.
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## **Roles and Responsibilities**

The NIH Guidelines will never be complete or final since all conceivable experiments involving recombinant or synthetic nucleic acid molecules cannot be foreseen.

Therefore, each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all research with recombinant or synthetic nucleic acid molecules conducted at or sponsored by that institution is conducted in compliance with the NIH Guidelines.

-Section IV-A. Policy, Roles and Responsibilities, NIH Guidelines

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## **Roles and Responsibilities of:**

- Institution (UNC)
  - Institutional Biosafety Committee (IBC)
  - Environment, Health and Safety (EHS)
  - Principal Investigator (PI)
  - NIH
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## **Institutional (UNC's) Responsibilities**

UNC-CH is responsible for:

- Establishing and implementing policies for the safe conduct of recombinant or synthetic nucleic acid research;
  - Establishing an Institutional Biosafety Committee;
  - Assisting and ensuring compliance with the NIH Guidelines by investigators;
  - Ensuring appropriate training for IBC members and staff, PIs and laboratory staff;
  - Reporting any significant problems, violations of the NIH Guidelines or any significant research-related accidents or illnesses to NIH Office of Science Policy.
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## **Institutional Biosafety Committee (IBC) Responsibilities**

The IBC is responsible for:

- Reviewing, approving and overseeing recombinant or synthetic nucleic acid research conducted at UNC to ensure compliance with the Guidelines;
  - Periodically reviewing recombinant or synthetic nucleic acid research;
  - Setting containment levels;
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- Reporting significant problems, violations of the NIH Guidelines or any significant research-related accidents or illnesses to the NIH Office of Science Policy.

Click here for the [UNC IBC website](#)

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### **Environment, Health and Safety Responsibilities**

The Dept. of Environment, Health and Safety is responsible for:

- Conducting periodic inspections of laboratories working with recombinant or synthetic nucleic acid.
- Reporting any significant problems, violations of the NIH Guidelines or any significant research-related accidents or illnesses to the IBC.
- Developing emergency plans for spills and personnel contamination.
- Investigating laboratory accidents.
- Providing advice on lab security.
- Providing technical advice to PIs and IBC on research safety procedures

Click here for the [UNC EHS website](#)

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### **Principal Investigator Responsibilities**

The Principal Investigator (PI) is responsible for:

- Full compliance with the NIH Guidelines
- Determining which category their experiments fall under.
- Determining if experiments require IBC approval.
  - Experiments that require prior IBC approval cannot be conducted until IBC approval is obtained.
  - Experiments that require registration (Section III-E) must not begin until the appropriate paperwork has been submitted to the IBC.
- Making the initial determination of containment levels required for experiments.
- Notifying the IBC of any changes to recombinant or synthetic nucleic acid experiments previously approved by the IBC.
- Immediately reporting any significant problems, violations of the NIH Guidelines or any significant research-related accidents and illnesses to Environment, Health and Safety (919-962-5507) and the IBC.
- Having an updated [Laboratory Safety Plan](#) on file at EHS and in their Laboratory Safety Notebook.
- Describing practices required to ensure safety in the Schedule F and training laboratory staff.
- Describing appropriate medical surveillance required for work with recombinant or synthetic nucleic acid in the Schedule F.

### **Principal Investigator's Responsibilities for Human Use Protocols**

- Principal investigators must report any serious adverse events associated with the use of recombinant or synthetic nucleic acid in humans to the IBC.
  - A serious adverse event is an event occurring at any dose that results in any of the following outcomes: death, life threatening event, in-patient hospitalization, disability/incapacity, or congenital anomaly/birth defect.
  - An adverse event is associated with the use of a gene transfer product when there is a reasonable possibility that the event may have been caused by the use of that product.
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### **NIH Responsibilities**

- Managing the RAC.
  - Conducting and supporting training of IBCs.
  - Review of human gene transfer protocols.
  - Review of certain basic recombinant or synthetic nucleic acid experiments
    - 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 LD50<100 ng/Kg bodyweight
    - Nucleic acid from restricted agents transferred to nonpathogenic prokaryotes or lower eukaryotes
    - Nucleic acid from nonpathogenic prokaryotes or lower eukaryotes transferred to restricted agents
    - Use of infectious or defective restricted poxviruses in presence of helper virus
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### **Please review the following instructions before proceeding to the next Module.**

If you do not work with recombinant or synthetic nucleic acid or transgenic animals/plants, you have completed the appropriate modules. You may advance to the next slide, complete the module two knowledge review, and take your final test to complete your training program.

If, however, you work with recombinant or synthetic nucleic acid or transgenic animals or plants, you must complete Modules 3, 4, 5 and 6 to determine what category your research falls under, and if IBC approval is required.

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**Module Three:**  
**Experiments Covered**

Experiment Types	Review Required	Section
Transfer of drug resistance traits that would affect control of disease.	IBC, RAC, NIH/OSP prior to initiation of experiments	III-A
Recombinant or synthetic nucleic acids containing genes for synthesis of toxins with LD50 < 100 ng/kg	IBC, NIH/OSP prior to initiation of experiments	III-B
Transfer of recombinant or synthetic nucleic acids into human subjects	IBC, RAC, IRB prior to initiation of experiments	III-C
Recombinant or synthetic nucleic acids from Risk Group 2, 3, 4 or restricted agents or use as host vector systems. Some experiments involving whole animals or plants. Large scale experiments.	IBC prior to initiation of experiments	III-D (Examples: adenoviral vectors, lentiviral vectors, retroviral vectors, VRP vectors)
Formation of recombinant or synthetic nucleic acids comprised of < 2/3 eukaryotic virus genome (proposed to change to ½ of the genome). Some experiments with whole plants. Creation of transgenic rodents that require BSL1 containment.	IBC notice simultaneous with initiation of experiments.	III-E
Recombinant or synthetic nucleic acids not in organisms or viruses. Purchase or transfer of transgenic rodents that require BSL1 containment.	Exempt from NIH Guidelines. IBC approval required to assess biosafety levels and containment.	III-F Exempt from NIH Guidelines but UNC IBC approval is required to assess biosafety containment of the animals.

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**Next Step for the PI**

- Once the PI has determined which category of the NIH Guidelines their research falls under, they must then conduct a risk assessment to determine the practices and containment that must be used when conducting their experiments.
  - Risk assessment will be covered in the next module.
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## Module Four

### Principal Investigator's Risk Assessment

The Principal Investigator must conduct a comprehensive risk assessment to:

- Determine risk group of the agent - Appendix B of the NIH Guidelines
- Evaluate agent factors
  - Virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, quantity, availability of vaccine or treatment
    - Strain that is known to be more hazardous than wild type should be considered for handling at a higher containment level.
    - Attenuated strains may be able to be handled at lower containment.
- Evaluate gene product effects:
  - toxicity, physiological activity and allergenicity
- Evaluate how the agent will be used:
  - Animal experiments may require higher containment, or large quantity production (>10 L) may require higher containment.
- Determine appropriate laboratory containment and practices required based on the above information.
  - Containment levels are described in Appendix G of the NIH Guidelines

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### Risk Groups

There are four risk groups:

Risk Group 1	Risk Group 2	Risk Group 3	Risk Group 4
Agents are not associated with disease in healthy adult humans.	Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.	Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.	Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are usually not available.

## Risk Group 1

Agents are not associated with disease in healthy adult humans.

### Examples

1. E. coli K-12  
That does not possess a complete lipopolysaccharide  
That does not carry any active virulence or colonization factors or genes encoding these factors
2. Bacillus subtilis host vector systems
3. Adeno-associated virus (AAV) types 1-4
4. Recombinant AAV constructs produced in the absence of helper virus  
transgene cannot encode for tumorigenic gene product or toxin molecule

## Animal Viral Etiologic Agents in Common Use (Risk Group 1)

Animal Viral Etiologic Agents in Common Use

Not associated with disease in healthy human adults

### Examples

1. Murine Cytomegalovirus
2. Bovine Papilloma virus
3. Feline leukemia virus
4. Murine leukemia virus (exception: amphotropic and xenotropic strains are infectious to humans and are considered Risk Group 2).

## Risk Group 2

Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.

### Examples

1. *Staphylococcus aureus*
2. *Streptococcus*
3. *Cryptococcus neoformans*
4. *Giardia* sp.
5. Human adenoviruses
6. Herpesviruses (except Herpes B)
7. Influenza viruses

### **Risk Group 3**

Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.

#### Examples

1. *Mycobacterium tuberculosis*
2. Human immunodeficiency virus (HIV) types 1 and 2
3. Venezuelan equine encephalomyelitis (VEE) virus
4. West Nile virus (WNV)

### **Risk Group 4**

Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are usually not available.

#### Examples

1. Ebola virus
2. Herpes B virus

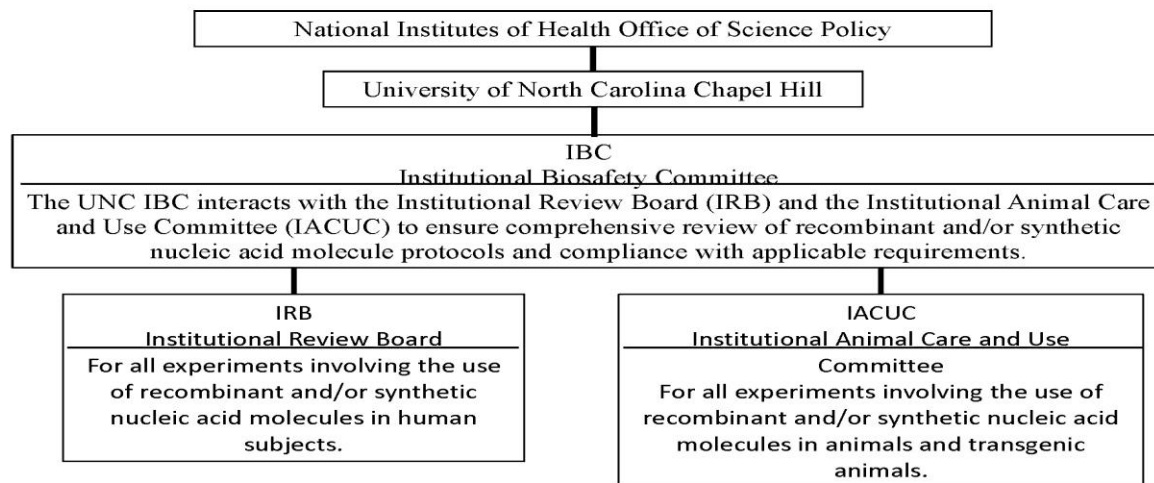
## Module Five

### Submission of rDNA Registrations to IBC

NIH Sections and/or Experiments	Required Schedule or Appendix
III-A, III-B, III-D, III-E	Schedule G
Purchase or transfer of transgenic	Schedule H
III-C (Human Use)	<ol style="list-style-type: none"> <li>1. Appendix 10A, plus,</li> <li>2. Scientific abstract,</li> <li>3. Non-technical abstract,</li> <li>4. Responses to Appendix M-II through M-V,</li> <li>5. Clinical protocol,</li> <li>6. Informed consent document,</li> <li>7. Curriculum vitae--no more than two pages for each key professional person in biographical sketch format.</li> </ol>

### Oversight

The UNC IBC interacts with other committees to ensure comprehensive review of recombinant or synthetic nucleic acid protocols and compliance with applicable requirements.



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### **Animal Use Experiments**

- Experiments involving the use of animals also require UNC IACUC approval before they can be initiated. IACUC applications can be initiated through ACAP.

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### **IBC and IACUC Review of Animal Research Utilizing Recombinant DNA**

#### **IBC Review**

- Risks to human health while working with recombinant or synthetic nucleic acid vectors.
- Risks to the environment including escape of the vector or animal and establishment in the wild and altered animals interbreeding with wild stock.

IBC approval is required for any animal experiments that involve recombinant or synthetic nucleic acid or any transgenic animals before the IACUC protocol is approved.

#### **IACUC Review**

Animal welfare concerns including:

- Pain and distress from adverse phenotypes (behavioral, anatomical and physiological abnormalities)
- Risks to other animals in the facility from the inadvertent spread of vectors

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### **Human Use Experiments**

Human use experiments also require UNC IRB approval before they can be initiated. IRB submission information can be found at:

Research at Carolina  
Office of Human Research Ethics - [Guide to the IRB Process](#)

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### **IBC and IRB Review of Research with Human Subjects**

#### **IRB Review**

- Conducts risk/benefit assessment relative to individual research participants (physical, psychological, social harms)
- Selection of subjects and the informed consent process
- Data monitoring provisions to ensure the safety of subjects

- Provisions to protect subject privacy and confidentiality of data
- Injuries or any other unanticipated problems
- Compliance with regulations

### **IBC Review**

- Recombinant or synthetic nucleic acid research for conformity with the NIH Guidelines
- Potential risk to environment and public health, community, as well as to individual research participants
- Containment levels per NIH Guidelines
- Adequacy of facilities, SOPs, PI and other personnel training
- Institutional and investigator compliance (e.g., adverse event reports)
- Reviews trial design, biosafety and containment, and compliance with NIH Guidelines
- Determines if RAC review is necessary

**IBC approval is required for any human use experiments that involve recombinant or synthetic nucleic acids before the IRB protocol is approved.**

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### **Submission of recombinant or synthetic nucleic acid registrations to IBC**

- Forms must be filled out completely and approved online by the Principal Investigator.
- The Principal Investigator signing the form must have a current [Laboratory Safety Plan](#) on file with EHS.
- All Schedule G or Schedule H forms will be submitted online via the online lab safety plan.
- IBC meets the first Wednesday of each month. Forms should be submitted by the 15th of the month prior to being reviewed at the meeting.
- If registration is approved, the form is signed by the IBC chair and a copy is provided to the Principal Investigator via email.
- A copy of the approval must be maintained in the Principal Investigator's Laboratory Safety Notebook for documentation that experiments are approved.
- Approval is valid for five years or the length of the grant funding period if less than five years.

**Not sure if your plan is current? Please call EHS at 919-962-5507**

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## Module Six

### Requirements for Reporting

- NIH Guidelines require reporting of the following to the Office of Science Policy:
  - Significant problems
  - Violations of the NIH Guidelines
  - Research-related accidents and illnesses

UNC requires reporting of ALL lab related incidents to the Department of Environment, Health and Safety (919-962-5507) on the same business day if the incident occurs during normal work hours, and on the next business day if the incident occurs after normal work hours.

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### Incident Definition

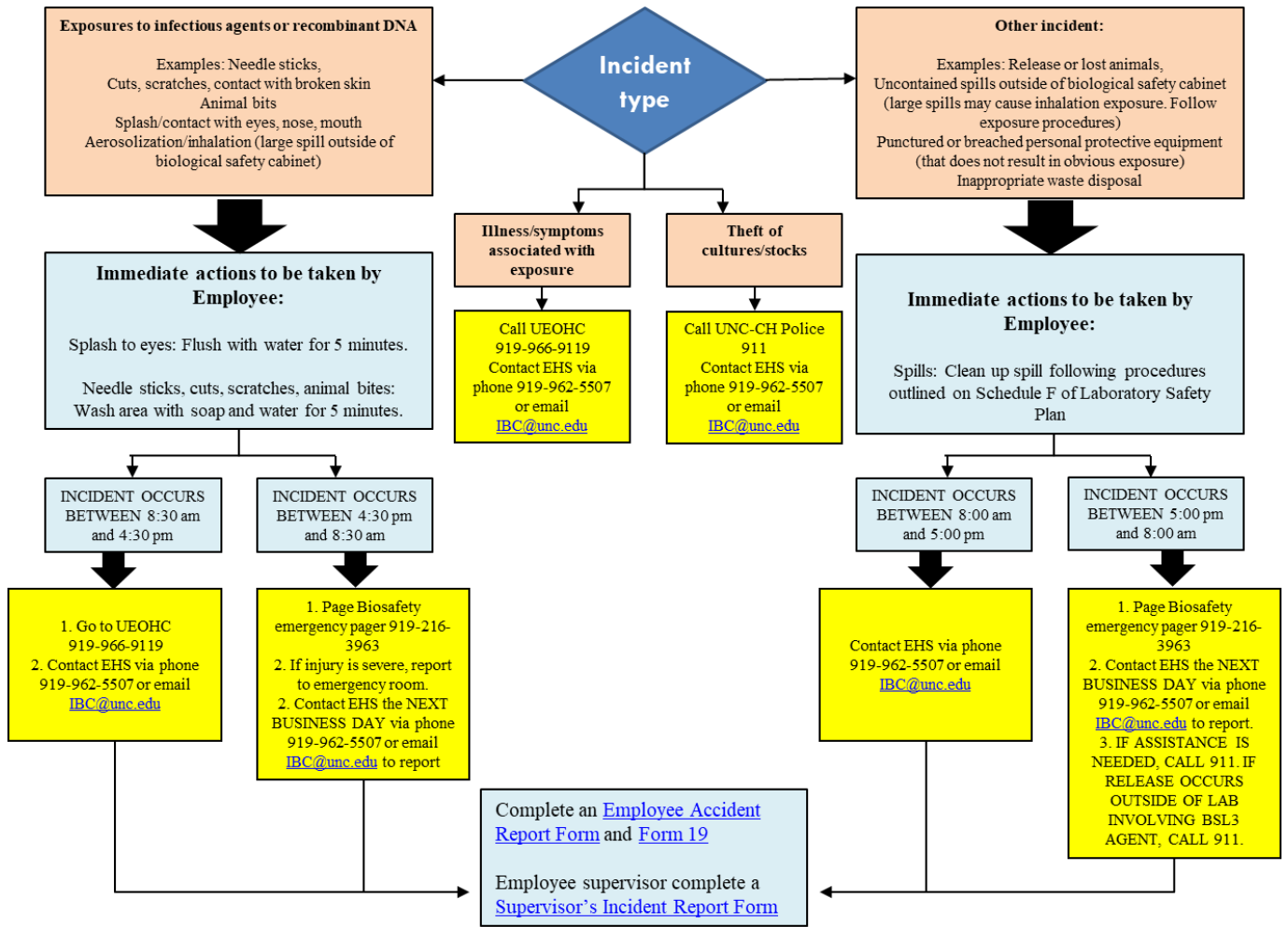
Any event that leads to unintended exposure to humans or the environment:

- Needlesticks
- Animal bites
- Released or lost transgenic or infected animals
- Uncontained spills
- Punctured or breached PPE
- Splashes to eyes/mucous membranes
- Accidental aerosolization/inhalation
- Inappropriate waste disposal
- Illness/symptoms related to agents in lab
- Any other incident in which researcher is unsure about exposure potential.

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### Incident Procedures

- PIs are required to describe procedures for spills and exposures on Schedule F of their Laboratory Safety Plan.
- These procedures must include what steps to take after-hours (i.e. go to Emergency Room or report next business day)





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## How to Report Incidents

Following the Initial Report of the Incident

- Employees will be required to fill out an [Employee Accident Report Form](#).
- Supervisors are required to fill out a [Supervisors Incident Report Form](#).
- The Employee will also have to complete a [NCIC Form 19](#).

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## Additional Information in the 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](#)

[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](#)

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## Recombinant or Synthetic Nucleic Acid Test

- In order to fulfill NIH Guidelines for recombinant or synthetic nucleic acid research, and to obtain credit for this training program, you are expected to complete the following test.
- If you do not wish to complete the test at this time, you may return to this site at a later time. You can again proceed through the modules, or click [Test](#) upon entering the Training program and go directly to the test.
- If you have questions about recombinant or synthetic nucleic acid experiment, the NIH Guidelines, or UNC requirements for submission, please contact the Department of Environment, Health and Safety at 919-962-5507, or by email at: [ibc@unc.edu](mailto:ibc@unc.edu)

Thank You!

