Recombinant and Synthetic Nucleic Acid Molecule Training

Environment Health and Safety
Albert Einstein College of Medicine

Environmental Safety and Health
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The Purpose of the *NIH Guidelines*

- The purpose of the *NIH Guidelines* is to specify the 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
  - (iii) cells, organisms, and viruses containing such molecules.

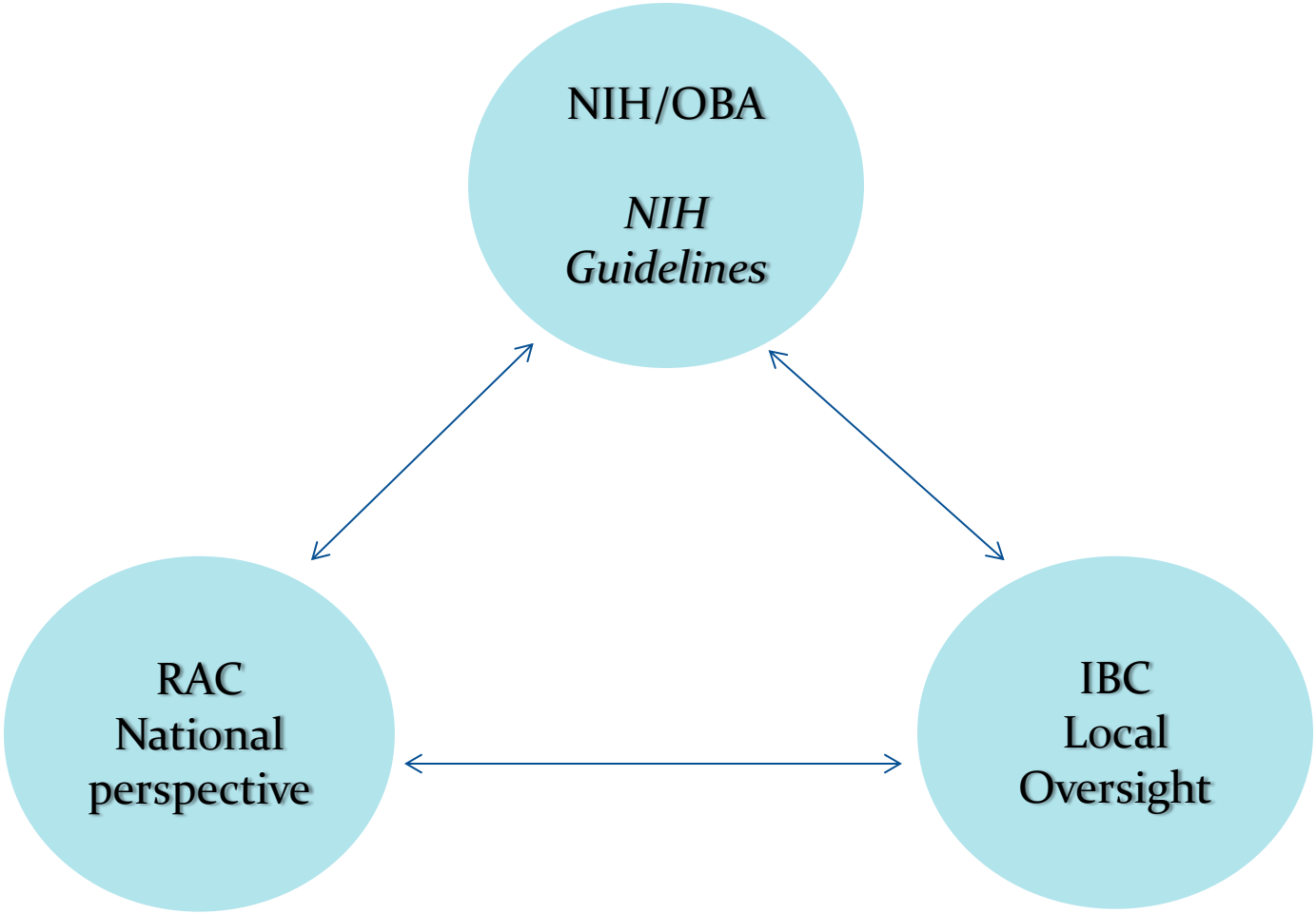
Definition of Recombinant and Synthetic nucleic acids

- Molecules that are constructed outside living cells by joining natural or synthetic DNA to DNA molecules that can replicate in a living cell.

- 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).

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

NIH Guidelines for Research Involving Recombinant DNA



NIH Office of Biotechnology Activities (OBA)

- “The NIH Office of Biotechnology Activities (OBA) promotes science, safety, and ethics in biotechnology through advancement of knowledge, enhancement of public understanding, and development of sound public policies. OBA accomplishes its mission through analysis, deliberation, and communication of scientific, medical, ethical, legal, and social issues”.

Recombinant DNA Advisory Committee (RAC)

- RAC issues recommendations to the NIH Director that are conveyed through the NIH Office of Biotechnology Activities (OBA), which is responsible for the NIH system of oversight of recombinant DNA research.

- RAC recommends changes to the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*), which outline responsible research practices in basic and clinical recombinant DNA research.

Institutional Biosafety Committee (IBC)

- Originally established under the *NIH Guidelines* specifically to provide oversight of all recombinant and synthetic nucleic acid research.
- The IBC is responsible for the safety and protection of personnel, the general public, and the environment.
- The IBC reviews and approves policies, procedures, training, programs and containment to ensure the safe use of biological agents, other biological materials, and toxins.

Responsibilities of the IBC

- Review and support the activities of the Department of Environmental Health and Safety (EH&S) in providing guidance on the safe use, procurement, storage, and disposal of biohazards.
  - Act as interface between the Research Faculty and EH&S
- Serve as a forum to review, make recommendations, and raise awareness related to biosafety concerns, institutional needs, emerging biosafety issues, and new biosafety requirements.
- Review new safety and health regulations and provide guidance on their application to the Albert Einstein College of Medicine of Yeshiva University.
- Review research activities which raise safety and/or health issues.
- Review those engineering facilities designed to protect the worker from biohazards.
- Review the activities of the Biohazard Facilities (BSL3).

Responsibilities of the IBC (cont'd)

- Review recombinant DNA research to ensure compliance with the *NIH Guidelines*.
  - Adopt emergency plans covering accidental spills and personnel contamination resulting from rDNA research.
  - Notify the Principal Investigator (PI) of the results of the IBC's review and approval.
- Promote a greater awareness and understanding by Faculty and Staff for the need to:
  - Conduct all laboratory procedures and activities with attention to personnel and environmental health and safety.
  - Comply with government health and safety regulations and laws.
  - Lower or increase containment levels for certain experiments as specified in section III-D-2-a of the *NIH Guidelines*.
- Ensure that administrative controls on the use of biohazards, e.g., written guidelines, monitoring personal protection practices, etc. are available and followed.

Responsibilities of the IBC (cont'd)

- Report any significant problems with or violations of the National Institutes of Health, *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH Office of Biotechnology Activities (OBA) within 30 days, unless the IBC determines that a report has already been filed by the Principal Investigator.
- Submit an annual report to NIH/OBA which includes a roster of IBC members and member roles. Inform NIH/OBA of members leaving the Committee or appointed to the Committee.
- Recommend to the Dean (and Executive Dean) measures to decrease the exposure of the Einstein Community to biohazards.
- Support information flow among the IBC, the Internal Review Board (IRB), and the Institutional Animal Care and Use Committee (IACUC).
- Obtain competency training as stipulated by the *NIH Guidelines*.
- Review emergent issues in biosafety.

Who must Comply with the *NIH Guidelines*

- An institution must follow the *NIH Guidelines* if it receives any funding from the NIH.
- Even if only one project of recombinant DNA research benefits from NIH support, all such projects conducted at or sponsored by that institution must comply with the *NIH Guidelines*.
- All Einstein investigators must comply with the *NIH Guidelines*.

Consequences for Noncompliance with the *NIH Guidelines*

- Suspension, limitation, or termination of financial assistance for:
  - non-compliant NIH projects;
  - NIH funding for other recombinant DNA research at the institution;
- Having to obtain prior NIH approval for any recombinant DNA projects.

Classification of Biohazardous Agent by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans.
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are *often* available.
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk by low community risk).
Risk Group 4 (RG4)	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).

Risk Assessment and Risk Groups

- The PI is required by the NIH Guidelines to conduct an initial risk assessment. The PI should:
  - Determine the risk group of the agent or material being researched (See Appendix B)of the Guidelines,
  - Evaluate the virulence, pathogenicity, infectious dose, environmental stability, exposure route, communicability, concentration, availability of prophylactic, and
  - Evaluate gene product.
  - IBC makes the final risk group determination.

Experiments Covered by the *NIH Guidelines*

Experiment	Section of Guidelines
Require IBC approval, RAC Review, and NIH Director Approval before initiation	Section III-A
Require NIH/OBA and IBC approval before initiation	Section III-B
Require IBC and IRB approvals and RAC review before research participant enrollment	Section III-C
Require IBC approval before initiation	Section III-D
Require IBC approval simultaneous with initiation	Section III-E
Exempt Experiments	Section III-F

Section III-A

- These experiments require IBC approval, RAC Review and NIH Director approval before initiation and is considered a Major action.

- Deliberate transfer of a drug resistance trait to a microorganism that is not known to acquire that trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture.

- Consideration should be given as to whether the drug resistance trait to be used in the experiment would render that microorganism resistant to the primary drug available to and/or indicated for certain populations, for example children or pregnant women.

Section III-B

- Require NIH/OBA and IBC approval before initiation.

- Deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin).

Section III-C

- Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment.

- Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into one or more human research participants.

Section III-D

- Require Institutional Biosafety Committee approval **before** initiation.
  - Experiments using Risk Group 2, Risk Group 3, Risk Group 4, or restricted agents as host-vector systems.
  - 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.
  - 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.
  - Experiments involving whole animals.
  - Experiments involving whole plants.
  - Experiments involving more than 10 liters of culture.
  - Experiments involving influenza viruses.

Section III-E

- Experiments that require IBC notice simultaneous with initiation.
  - Experiments involving the formation of recombinant or synthetic Nucleic Acid Molecules containing no more than two-thirds of the Genome of any Eukaryotic Virus.
  - Experiments involving whole plants (non-applicable).
  - Experiments involving transgenic rodents.
    - This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents).
    - Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4, *Experiments involving whole animals*.

Section III-F

- Experiments in this category are exempt from NIH Guidelines. However:
  - Still require PIs to submit their application to the IBC for review and approval.
  - Require IBC approval simultaneous with initiation of experiments.

- Those 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 (cont'd)

- If a synthetic nucleic acid is deliberately transferred into one or more human research participant and meets the criteria of Section III-C, it is not exempt under this Section.
  - 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.
  - Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.
  - 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.
  - Those that consist entirely of nucleic acids from a 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).
  - 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.
  - Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.
  - 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.

Principal Investigator Responsibilities

- The PI must :
  - Be proficient in good microbiological techniques.
  - Supervise staff to ensure safety practices are followed.
  - Instruct laboratory staff on:
    - Practices and techniques required to ensure safety.
    - Emergency procedures for spills and exposures.
    - Inform laboratory staff of the reasons for any occupational health/medical surveillance.
    - Correct conditions that may result in release of recombinant or synthetic nucleic acid molecule materials.
    - Ensure the integrity of physical containment and biosafety cabinets.
    - Comply with permit and shipping requirements for recombinant or synthetic nucleic acid molecules materials.

Principal Investigator Responsibilities
(cont'd)

- Adhere to IBC approved emergency plans for handling accidental spills and personnel contamination.
- Complete administrative procedures (e.g. Evaluation Form and Document of Registration forms - DOR).
- Determine whether their research is subject to Section III-A, B, C, D, E, F of the NIH Guidelines.
- Conduct risk assessments and propose containment levels in accordance with NIH Guidelines when registering research with the IBC.
- Submit research registration (DOR) to IBC for review (III-A through F); obtain approval before initiating research if section III-A through D applies.
- Seek NIH approval for research falling under sections III-A, III-B, III-C.

Principal Investigator Responsibilities (cont'd)

- Determine the need for IBC review before modifying registration that was already approved.
- Submit any changes to the IBC for review and approval.
- Report any significant problems to the Biosafety Officer concerning:
  - Containment or operational procedures.
  - Accidents and illnesses.
  - Violation of the NIH Guidelines.

NIH Guideline - Appendices

Appendix A	Exemptions: Natural Exchangers
Appendix B	Classification of Etiologic Agents
Appendix C	Exemptions under IIIF
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 an d Biological Containment: Animals

Appendix B of the *NIH Guidelines*

- Includes biological agents known to infect humans and selected animal agents that may pose theoretical risks if inoculated into humans.
- Includes lists of representative agents known to be pathogenic
- Mutated, recombined and non-pathogenic species and strains are not considered.
- Non-infectious life cycle stages of parasites are excluded.

Appendix G of the *NIH Guidelines*

- Specifies physical containment for standard laboratory practices.
- Defines Biosafety Level 1 through Biosafety Level 4.
- Appropriate for research involving animals within a laboratory setting.

Appendix M of the *NIH Guidelines*

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

Registering with Einstein's IBC

- All recombinant DNA work, work involving the use of microorganisms pathogenic to humans or animals, and any work with Select Agents and Toxins at Einstein will be initially registered through EH&S. See DOR Form.
  - A Document of Registration (DOR) will be completed by the Principal Investigator for their research and provided to the BSO for review.
  - The BSO will check for completeness, provide the project with a pending protocol number, provide a biosafety level and enter the registration information into a computer database.
  - If the project involves Risk Group (RG2) agents or above, a letter or notation on the DOR will accompany the DOR which is sent back to the PI informing them that the project is pending IBC approval.
  - A DOR spreadsheet is provided to the IBC members before each meeting. With a quorum present, the DORs are discussed, approved, delegated, or rejected.

IBC Approval/Notification to PI

- DORs are valid for a period of three years unless there has been a change in the research. If there has been a change in the research, the PI must complete a new DOR. DORs expire on December 31st of the third year.
- At the beginning of each calendar year, the PI is asked to renew the signature on the DOR to verify that no changes have occurred throughout the year.
- Once the IBC has reviewed a project, a letter or e-mail will be sent to the PI stating that the project has been reviewed by the IBC and the project was either approved, delegated, tabled or rejected.
- No work will commence prior to the approval.

Incident Reporting to NIH

- Incidents are reported only once to NIH/OBA and coordinated through BSO.
- The report must include the response made to mitigate the problem and preclude its reoccurrence.
- Report of incidents to NIH/OBA must occur within 30 days of the incident and include:
  - Any significant problems or violations of the NIH Guidelines.
  - Any significant research related accidents and illnesses.
- Minor spills of low-risk agents, contained and properly disinfected, generally do not need to be reported to NIH but should be report to the BSO.

Immediate Incident Reporting to NIH

- Spills or accidents involving rDNA requiring BSL 2 containment resulting in an overt exposure.
  - Needlestick, splash to the eyes, nose, mouth or accidental aerosolization/inhalation
- Spills or accidents involving rDNA requiring BSL 3 containment resulting in overt exposure or potential exposure.
  - Spills of high risk recombinant materials occurring outside of a biosafety cabinet.

Good Judgment

- "The *NIH Guidelines* will never be complete or final since all conceivable experiments involving recombinant DNA 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."

- If unsure, consult with the BSO.

Questions

- Biosafety Officer (BSO) x3560
- Environmental Health and Safety x4150

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