BL1 Laboratory Practices

1. Keep laboratory door closed when experiments are in progress.
2. Use procedures that minimize aerosols.
3. Do not smoke, eat, drink or store food in BL1 areas.
4. Wear laboratory gowns or coats when appropriate.
5. Do not mouth pipette. Always use mechanical pipetting devices.
6. Avoid using hypodermic needles.
7. Wash hands after completing experimental procedures and before leaving laboratory.
8. Disinfect work surfaces daily and immediately after a spill.
9. Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
10. For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
11. Control insect and rodent infestations.
12. Keep areas neat and clean.

BL2 Laboratory Practices

1. Keep laboratory door closed.
2. Post a universal biohazard label on equipment where infectious agents are used/stored.
3. Allow only persons informed of the research to enter BL2 areas.
4. Keep animals not used in BL2 experiment out of the laboratory.
5. Do not smoke, eat, drink, or store food in BL2 areas.
6. When appropriate, wear laboratory gowns or coats.
7. Do not mouth pipette. Always use mechanical pipetting devices.
8. Use procedures that minimize aerosol formation.
9. Avoid using hypodermic needles.
10. Use biological safety cabinets to contain aerosol-producing equipment.
11. Wash hands after completing experimental procedures and before leaving laboratory.
12. Disinfect work surfaces daily and immediately after a spill.
13. Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
14. For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
15. Control insect and rodent infestations.
16. Keep areas neat and clean.

Registration and Approval of rDNA Experiments
RECOMBINANT and SYNTHETIC NUCLEIC ACIDS

YALE BIOLOGICAL SAFETY COMMITTEE **March 2013 (rev.)**

This outline provides an overview of the "Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules" (NIH Guidelines). It is the responsibility of each investigator to make sure that their laboratory is in compliance with these Guidelines. If your experiments require registration, check the NIH Guidelines for the relevant regulatory section and the appropriate biosafety level or contact the Biosafety Office or your Safety Advisor for assistance. For copies of the NIH Guidelines or rDNA registration forms, please call the Office of Environmental Health & Safety (OEHS) at 785-3550.

OEHS contacts: Phone: (203) 785-3550 Fax: 785-7588 Website: www.yale.edu/ehs
Yale rDNA Forms and Information Regarding rDNA: http://www.yale.edu/oehs/bioreqIII.htm
NIH Office of Biotechnology Affairs website: http://oba.od.nih.gov/oba/index.html

Experiments which must be registered and approved prior to initiation:	**Examples:**
1. Deliberate transfer of a drug resistance trait to a microorganism (if it could compromise the use of the drug to control disease agents in human, animals, or agriculture);	1. Transferring a drug resistance trait that is used, had previously been used, may be used (outside the U.S.), or that is related to other drugs that are used to treat or control disease agents. Examples include: Transfer of Erythromycin resistance into Borrelia burgdorferi; Transfer of Pyrimethamine resistance into Toxoplasma gondii; Transfer of Chloramphenicol resistance into Rickettsia conorii; Transfer of Tetracycline resistance into Porphyromonas gingivalis.
2. Human gene transfer experiments;	2. Use of a defective adenoviral vector to deliver the CFTR gene intranasally to patients with Cystic Fibrosis; Introduction of a HSV-TK transduced cell line into patients with epithelial ovarian carcinoma, followed by therapy with Gancyclovir.
3. Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of < 100 ug/kg body weight;	3. Cloning toxins (or using plasmids that express toxins with low LD50's) such as Botulinum, Tetrodotoxin, Ricin, T-2, Saxitoxin, Abrin, Tetanus, Shigella Dysenteriae, Pertussis, Staph Aureus Beta, ShigaToxin, and Conotoxins;
4. Experiments using human or animal pathogens as host-vector systems;	4. Use of pathogens or defective pathogen vectors (with or without helper virus), such as Adenovirus, Adeno-Associated virus, Baculovirus, Herpes virus, Lentivirus, Retrovirus, Vaccinia and Vesicular Stomatitis Virus.
5. Cloning of DNA or RNA from all Risk Group 3, 4, or restricted pathogens (includes HIV and human tumor viruses), as well as Risk Group 2 experiments involving ≥ 50 % of genetic material;	5. rDNA experiments involving any quantity of genetic material from a Risk Group 3 or higher pathogens (e.g., HIV, HTLV-1 & II, Prions, Mycobacterium tuberculosis, West Nile Virus, Lymphocytic Choriomeningitis Virus, and Rickettsia typhi. Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.
6. Recombinant DNA experiments involving whole animals or plants:	6. Creation of transgenic animals or plants (mice, rats, zebra fish, drosophila, etc.), or knockout animals that leave genetic material in the animal as part of the silencing of the gene. Note: the purchase (or transfer to your lab) of previously created transgenic rodents is exempt from the regulations.
7. Large-scale DNA work (i.e., ≥ 10 liters of culture combined).	7. Use of a 10 L fermenter or growing up five 2 L flasks of rDNA culture (i.e. E. coli K-12) qualifies as a large scale experiment at Yale University.

Centrifuge Safety

- Each operator must be trained on the proper operating procedures
- Keep a log book detailing operation records for centrifuges and rotors
- Do not exceed safe rotor speed
- Place a biohazard label on the centrifuge if used for infectious agents
- Always use sealed safety buckets or sealed rotors with O-rings
- Load and unload safety buckets or rotors within the biosafety cabinet
- Check tubes and bottles for cracks and deformities before each use
- Examine O-ring and replace if worn, cracking or missing
- Never overfill primary containers; do not exceed ¾ full
- Wipe exterior of tubes or bottles with disinfectant prior to loading into safety buckets or rotor
- Wipe the exterior of safety buckets or rotors with disinfectant before removing from biosafety cabinet
- Stop the centrifuge immediately if an unusual condition, such as noise or vibration, begins
- Wait five minutes after the run before opening the centrifuge to allow aerosols to settle in the event of a breakdown in containment
- Decontaminate safety buckets or rotors and centrifuge interior after each use
- Wash hands after removing gloves

Centrifuge Spill

If you notice that there has been a leak outside the safety bucket or rotor when opening centrifuge:

First:

- Hold Breath
- Close centrifuge lid
- Notify others to evacuate the lab

Then:

- Immediately leave the lab
- Post biohazard spill sign

Notify PI or Supervisor:

- DO NOT re-enter lab until PI and OEHS have given clearance (at least 30 minutes)
- Follow centrifuge spill instructions in the Biosafety Manual or Spill Response Guide

Decontaminate:

- Remove PPE turning exposed areas inward
- Place disposable PPE in biomedical waste (autoclave reusable PPE)
- Wash any exposed areas with antiseptic soap and water
- Wash hands thoroughly

For Centrifuge Explosion:

Evacuate room immediately; notify PI and OEHS

Autoclave Safety

Steam sterilization has been an indispensable tool in biological research since Pasteur's time. Despite this importance, many people are unaware of some basic autoclave operating procedures that can improve the quality of sterilization as well as reduce the risk of personal injury.

- Never autoclave nitrocellulose tubes – they can explode!
- Carefully prepare items for autoclaving. Loosely cover or cap containers to avoid over-pressurization.
- Keep loads small – overloading hinders steam penetration.
- Bags should be left partially open and should be contained within a tray.
- If time allows let the load cool before removing it from the autoclave. Otherwise, open the door about ½ inch and vent for 5-10 minutes before emptying autoclave.
- Wear shoes/sneakers, pants, lab coat, face shield, and long sleeved insulated gloves when operating an autoclave. A heavy, rubberized insulated apron is further recommended for those who autoclave frequently.
- Periodically verify autoclave effectiveness with biological and chemical indicators that are available from the Biosafety Office.
- If you experience any problems or unusual occurrences please report them to your supervisor or manager, Building Operations Coordinator, or the Office of Environmental Health & Safety (785-3550).

Toxins

Safe Working practices to minimize exposure via ingestion, inhalation, mucous membrane contact, and absorption or penetration through the skin.

BL2 Work Practices

- Label toxin work areas within lab
- Cover work surface with plastic-backed absorbent paper
- Avoid generating aerosols; handle the powdered form carefully
- Use a chemical fume hood or biosafety cabinet when feasible
- Avoid the use of needles or Pasteur pipettes
- Substitute plastic for glass wherever possible
- Decontaminate work surfaces with 5-10% household bleach or 0.1N sodium hydroxide
- Treat liquid waste with 50% household bleach (soak overnight.) For T-2 mycotoxin use a combination of 50% household bleach and 0.25N sodium hydroxide.
- Collect and autoclave waste at the end of the day
- Autoclave or chemically disinfect contaminated protective clothing before reuse

Personal Hygiene

- Keep your hands away from your face
- Do not eat, drink, or smoke in the lab
- Do not mouth pipette
- Always wash hands after removing protective clothing and before leaving the lab

Labels and Transport

- Post BL2 biohazard sign at lab entry
- Restrict access to the lab
- Label equipment used with or storing toxins
- For transport, use sealed, unbreakable, leakproof containers with a biohazard label and full toxin name

Protective Clothing Requirements

- Lab coat buttoned to the top with knit or grip cuffs, or use gloves that are long enough to cover the sleeves; a back-fastening gown is suitable; sleeve covers offer additional protection
- Gloves (consider double gloving)
- Face protection such as a face shield or safety glasses and a mask to cover the eyes, nose and mouth
- Dedicate protective clothing for work with toxins and do not wear outside the lab
- Avoid skin contact when removing gloves

Work with Powdered Form of Toxin

- Carefully weigh and convert to aqueous form as soon as possible
- Store powdered form in an unbreakable secondary container labeled with the complete toxin name to identify the hazard
- Change gloves after handling powdered toxin being sure to avoid skin contact with the toxin while removing gloves; wash hands prior to donning new gloves

Emergency Response

- Flush skin or eyes with running water for 15 minutes, notify PI immediately, seek medical assistance
- Follow BL2 spill procedures: leave lab for 30 minutes, upon return, decontaminate spill with 25% household bleach solution for 30 minutes, collect and autoclave waste