General Laboratory procedures

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General Laboratory Procedures, Equipment Use, and Safety Considerations

- Wash hands and arms with commercial disinfectant, before starting and at the end of working in hoods, or use latex gloves.
- Swab hood with 70% ethanol/water before and after use. Clean up spills immediately. Always assume a hood is contaminated before you start. Don't trust others to have cleaned the hood before you use it.
- Do not share culture media with others – theirs may be infected even if yours is not.

TISSUE CULTURE METHODS

- Each student should maintain his own cells throughout the course of the experiment. These cells should be monitored daily for morphology and growth characteristics, fed every 2 to 3 days, and subculture when necessary.
- A minimum of two 25 cm2 flasks should be carried for each cell line; these cells should be expanded as necessary for the transfection experiments.

TISSUE CULTURE METHODS

- Each time the cells are subculture, a viable cell count should be done, the subculture dilutions should be noted, and, after several passages, a doubling time determined.
- As soon as you have enough cells, several vials should be frozen away and stored in liquid N_2. One vial from each freeze down should be thawed 1-2 weeks after freezing to check for viability.
- These frozen stocks will prove to be vital if any of your cultures become contaminated.

Media preparation.

- Each student will be responsible for maintaining his own stock of cell culture media; the particular type of media, the sera type and concentration, and other supplements will depend on the cell line.
- Do not share media with you partner (or anyone else) because if a culture or a bottle of media gets contaminated, you have no back-up. Most of the media components will be purchased prepared and sterile.

Media preparation.

- In general, all you need to do is sterile combine several sterile solutions. To test for sterility after adding all components, pipet several mls from each media bottle into a small sterile Petri dish or culture tube and incubate at 37ºC for several days.
- Use only media that has been sterility tested. For this reason, you must anticipate your culture needs in advance so you can prepare the reagents necessary. But, please try not to waste media.

Media preparation.

- Anticipate your needs but don't make more than you need. Tissue culture reagents are very expensive; for example, bovine fetal calf serum cost ~ $200/500 ml.
- Some cell culture additives will be provided in a powdered form. These should be reconstituted to the appropriate concentration with double-distilled water (or medium, as appropriate) and filtered (in a sterile hood) through a 0-22 μm filter.

Media preparation.

- All media preparation and other cell culture work must be performed in a laminar flow hood.
- Before beginning your work, turn on blower for several minutes, wipe down all surfaces with 70% ethanol, and ethanol wash your clean hands. Use only sterile pipettes, disposable test tubes and autoclaved pipette tips for cell culture. All culture vessels, test tubes, pipette tip boxes, stocks of sterile ependorfs, etc. should be opened only in the laminar flow hood.

Media preparation.

- If something is opened elsewhere in the lab by accident, you can probably assume its contaminated. If something does become contaminated, immediately discard the contaminated materials into the biohazard container and notify the instructor.

A. Chemicals

- **A number of chemicals used in any molecular biology laboratory are hazardous.**
- **All manufacturers of hazardous materials are required by law to supply the user with pertinent information on any hazards associated with their chemicals.**
- **This information is supplied in the form of Material Safety Data Sheets or MSDS. This information contains the chemical name, CAS#, health hazard data**

A. Chemicals

- It also include first aid treatment, physical data, fire and explosion hazard data, reactivity data, spill or leak procedures, and any special precautions needed when handling this chemical.
- A file containing MSDS information on the hazardous substances should be kept in the lab. In addition, MSDS information can be accessed on World Wide Web.

A. Chemicals

- You are strongly urged to make use of this information prior to using a new chemical and certainly in the case of any accidental exposure or spill.
- The instructor/lab manager must be notified immediately in the case of an accident involving any potentially hazardous reagents.
- The following chemicals are particularly noteworthy:
  - Phenol - can cause severe burns
  - Acrylamide - potential neurotoxin
  - Ethidium bromide - carcinogen

A. Chemicals

- These chemicals are not harmful if used properly:
  - Always wear gloves when using potentially hazardous chemicals
  - Never mouth-pipette them.
  - If you accidentally splash any of these chemicals on your skin, immediately rinse the area thoroughly with water and inform the instructor.
  - Discard the waste in appropriate containers.

B. Ultraviolet Light

- Exposure to ultraviolet light can cause acute eye irritation. Since the retina cannot detect UV light, you can have serious eye damage and not realize it until 30 min to 24 hours after exposure. Therefore, always wear appropriate eye protection when using UV lamps.

C. Electricity

- The voltages used for electrophoresis are sufficient to cause electrocution.
- Cover the buffer reservoirs during electrophoresis. Always turn off the power supply and unplug the leads before removing a gel.

D. General Housekeeping

- All common areas should be kept free of clutter and all dirty dishes, electrophoresis equipment, etc should be dealt with appropriately.
- Since you have only a limited amount of space to call your own, it is to your advantage to keep your own area clean.
- Since you will use common facilities, all solutions and everything stored in an incubator, refrigerator, etc. must be labeled.

D. General Housekeeping

- **In order to limit confusion, each person should use his initials or other unique designation for labeling plates, etc. Unlabeled material found in the refrigerators, incubators, or freezers may be destroyed.**
- **Always mark the backs of the plates with your initials, the date, and relevant experimental data, e.g. strain numbers.**

E. Glassware and Plastic Ware.

- Glass and plastic ware used for molecular biology must be scrupulously clean. Dirty test tubes, bacterial contamination and traces of detergent can inhibit reactions or degrade nucleic acid.
- Glassware should be rinsed with distilled water and autoclaved or baked at 150 ° C for 1 hour.
- For experiments with RNA, glassware and solutions are treated with diethyl-pyrocarbonate to inhibit RNases which can be resistant to autoclaving.

E. Glassware and Plastic Ware

- Plastic ware such as pipettes and culture tubes are often supplied sterile.
- Tubes made of polypropylene are turbid and are resistant to many chemicals, like phenol and chloroform
- polycarbonate or polystyrene tubes are clear and not resistant to many chemicals.
- Make sure that the tubes you are using are resistant to the chemicals used in your experiment. Micro pipette tips and micro-centrifuge tubes should be autoclaved before use.

Disposal of Buffers and Chemicals

- Any uncontaminated, solidified agar or agarose should be discarded in the trash, not in the sink, and the bottles rinsed well.
- Any media that becomes contaminated should be promptly autoclaved before discarding it. Petri dishes and other biological waste should be discarded in Biohazard containers which will be autoclaved prior to disposal.
- Organic reagents, e.g. phenol, should be used in a fume hood and all organic waste should be disposed of in a labeled container, not in the trash or the sink.

Disposal of Buffers and Chemicals

- Ethidium bromide is a mutagenic substance that should be treated before disposal and should be handled only with gloves. Ethidium bromide should be disposed of in a labeled container.
- Dirty glassware should be rinsed, all traces of agar or other substance that will not come clean in a dishwasher should be removed, all labels should be removed (if possible)

Disposal of Buffers and Chemicals

- the glassware should be placed in the dirty dish bin.
- Bottle caps, stir bars and spatulas should not be placed in the bins but should be washed with hot soapy water, rinsed well with hot water, and rinsed three times with distilled water.

Micropipettes

- Most of the experiments you will conduct in this laboratory will depend on your ability to accurately measure volumes of solutions using micropipettors.
- The accuracy of your pipetting can only be as accurate as your pipettor and several steps should be taken to insure that your pipettes are accurate and are maintained in good working order.

Micropipettes

- Each pair of students will be assigned a set of pipettors and upon receipt, they should be labeled with the students' name. They should then be checked for accuracy following the instructions given by the instructor. If they need to be recalibrated, do so.
- Since the pipettors will use different pipette tips, make sure that the pipette tip you are using is designed for your pipettor.
- DO NOT DROP IT ON THE FLOOR.
- If you suspect that something is wrong with your pipettor, first check the calibration to see if your suspicions were correct, then notify the instructor.