Guide to the Prather Lab
for New Members

Welcome to the Prather Lab! We hope that your stay here will be an enjoyable one. This brief introduction to our lab has been prepared to make your transition into the group a bit easier. It is NOT intended to be an exhaustive working manual, but rather to provide a few bits of information that should help you become familiar with the lab. There is one main rule that you should follow at ALL times in the lab:

DO NOT BE AFRAID TO ASK QUESTIONS!!!!!

The most important aspect of life in the lab is, first, assuring the safety of yourself and your lab mates. The next is respecting the communal nature of the lab. Although there are not very many chemicals in our lab which pose a severe health hazard, there are many pieces of equipment and chemicals which are shared by and necessary to many of us in the lab. Proper use of both will ensure that our lab functions smoothly. If you are unsure how to operate any piece of equipment or you are uncertain about the use or disposal of chemicals, ask your elder lab mates! We would rather spend 50% of our time assisting you in the lab than lose 95% of our time as the result of an unfortunate accident which could have been prevented. (Remember, too, that we all make mistakes, no matter how long we've been in the lab. Perfection is neither required nor expected!)

Our lab work includes many different aspects of biochemical engineering, biochemistry, and molecular biology, employing a wide variety of experimental techniques. This guide is meant to highlight only those general issues which will affect the vast majority of students in the lab. Happy reading and once again, welcome to the lab.

NOTE: Other resources, including useful protocols, are posted on the lab's OpenWetWare Wiki – http://openwetware.org/wiki/Prather.

Strains and Plasmids

The heart of much of the work in our lab revolves around the use of specific bacterial strains and plasmids. We currently have stocks of over 75 strains. All of the commonly accessible stocks are stored in the -80°C freezer on the top shelf (labeled "Main Storage Box"). Information on each strain is contained in the "Strains Database" file, located on the lab Strainmaster's computer. The database is searchable by any field within a record, so you can easily search for the location and availability of a strain/plasmid of interest.

In order to ensure that any strains or plasmids that you obtain can continue to be useful to future Prather Lab members, you should be sure to store stocks of them in the freezer and register those stocks in the database.

Making Freezer Stocks
Preparing freezer stocks is simple. Strains should be grown in rich liquid medium to mid-exponential phase. Dilute the cultures 1:1 with sterile, cold 30% glycerol (to ~ 15% glycerol) and transfer 1.8 mL of the mixture to 2 mL cryogenic tubes (so put 900µL cells and 900µL of 30% cold, sterile glycerol in each vial). A total of three tubes should be prepared per strain you wish to store. Each tube should be labeled with the strain's name, your initials, the date, and tube number (on the cap as well as the tube face). One tube is placed in the "Main Storage Box" on the top shelf of the -80°C freezer while the other two are placed in the "Back-up Storage Box" located on the bottom shelf of -80°C freezer. You must also register each of your frozen strain stocks with the Strainmaster.

These stocks are for long-term storage and should be accessed infrequently. If you will be using a strain repeatedly over several months (or years!), you should prepare additional cryovials to keep in your personal storage box.

Registering Stocks
There is a folder labeled "Completed Frozen Stock Data Sheets," which is kept on the shelf above the general purpose lab computer. This folder contains information about each strain (box location, strain name, plasmid, characteristics, etc.) in paper form. Similar information is also located in the "Strains Database" group intranet file. There is also a folder in that same location that contains the blank, pre-numbered, data sheets. After strains have been stored properly in the -80°C freezer, a blank form must be filled out and given to the Strainmaster. There are brief instructions on the forms to help you complete them, but be sure to ask for assistance if you are not sure about the proper way to log your strains and plasmids. *Plasmids should always be stored within a strain for our stocks.* You can also keep plasmid DNA frozen in solution in the stocks, but do not store isolated plasmid DNA in place of transformed bacterial strains.

Enzymes

There are a number of enzymes available in the Prather Lab, including a large selection of restriction endonucleases, T4 DNA ligase (for cloning), and Taq polymerase (for

polymerase chain reaction (PCR)). Most of the enzymes we use are stable at -20°C for at least 12 months, but can only be expected to last a few hours at warmer temperatures. *It is crucial that you do not leave enzymes exposed to room temperature or even refrigerated conditions for very long*. Enzymes are stored in coolers in the white (-20°C) freezer. When you need to use an enzyme, remove the entire cooler to your bench, and remove the individual tubes to mix or dispense solution only. Most of the buffer solutions for enzyme reactions are also stored in the 4°C refrigerator in 10X concentrated form. Additional tubes of buffer are kept in the freezer in a box labeled "Extra RE Buffers."

Common Stocks and Solutions

There are some solutions which are needed by many people in the lab which must be prepared prior to use. These include TAE (Tris-Acetate-EDTA) for running agarose gels and the antibiotic ampicillin. Stocks of these solutions are available to anyone who needs them, and we take turns making them for the lab. (If you do not need to use these solutions, you won't be expected to prepare them.). Everyone is assigned refrigerator and bench space for the storage of items that are not being generally made available, i.e. you should ask before removing any items from someone's personal shelf or bench.

Plates

We have allocated a common space in the refrigerator for the storage of plates; however, you must make your own plates and you should ask before borrowing plates that someone else has made. Plates should be stored upside down in the refrigerator to prevent condensation from collecting on the surface of the agar.

Making Plates

Preparing plates is fairly straightforward. Autoclave agar (Luria Broth (LB) for rich medium or Select Agar for minimal medium) in water to sterilize and mix the solutions, let the agar cool until you can hold the flask in your hand, then pour warm agar into plastic Petri dishes. You should not autoclave antibiotics or any other heat labile additives for your medium. These should be filter sterilized and added to the agar medium prior to pouring the plates. You can also spread solutions on the plates after they have been poured.

Disposal of Solid and Liquid Waste

There are 8 places for waste disposal:

Garbage Cans

- Non-chemically contaminated solid waste
- Gloves which have not been used for handling hazardous materials

- Paper towels used to clean non-chemical spills.
- Packaging materials

Blue Recycling Bins
- Plastic (Pipet Tip Boxes) – lab recycle bin
- Paper – office recycle bin

Biosharps Bin
- Any form of item that is considered "sharp", i.e. pipet tips, glass pipets, etc.
- Other items should not be placed in this bin, as we are charged for removal.

White Bio Waste containers
- Any dry item that has come in contact with a bio-hazardous material, i.e. tubes, gloves, etc.
- Any sharp items, including pipets and pipet tips should go into the biosharps bin.

Black Chemical Waste Container
- Located near the digital imager/gel staining and imaging station.
- For disposal of gels only (wrapped in plastic).

White Chemical Waste Containers
- Located in the chemical hood.
- Two bottles for Aqueous Waste and Organic Waste.
- After discarding waste, be sure to update tag with the name(s) of the chemicals being discarded.

Sink
- LB/Bio liquid waste which has been treated with by 10% by volume (final concentration) bleach and allowed to sit for a period of time (more than 30 minutes).
- Salts which have been pH-adjusted, to between 7 and 9

Clear Benchtop Biohazardous Waste Bags
- Biohazardous Pipette Tips.
- Biohazardous Cuvettes (no liquid left inside).
- Other small biohazardous objects.
- Once full, these should be discarded in the white Biohazard bins.

Clear or Red Benchtop Plastic Waste Containers
- All pipette tips.
- All syringes.
- Any other sharp items that may puncture plastic bags.
- Once full, these should be discarded in the Biosharps bins.

If you have any questions about waste disposal or management, please feel free to ask the lab's Student Safety Coordinator.

Ethidium Bromide and UV Light Table (for viewing gels)

Resolving DNA on agarose gels is a staple technique for much of the Prather Lab. While the process of running gels is very straightforward, the method we use for viewing gels does involve two of the more serious aspects of the lab with regard to safety. The DNA is stained with ethidium bromide (EtBr) and then illuminated with ultraviolet light using the UV light table. EtBr is a powerful mutagen (this should come as no surprise since it binds tightly to DNA in low concentrations). You should always wear gloves and safety glasses when you work in the EtBr area. You should also consider everything stored in that area to be contaminated. If you are splashed with EtBr wash any exposed areas immediately and thoroughly. The good news about EtBr is that it is light sensitive, so lightly contaminated surfaces will not remain contaminated forever. Always cover the UV light table with plastic wrap before viewing gels to prevent EtBr contamination of the surface.

Remember that UV light also causes damage to DNA. You should protect your skin and eyes from exposure to the light. The yellow table cover (hinged to the viewing table) does protect against exposure, so in general you do not need UV-protectant face shield when viewing gels. The digital imager keeps the UV light completely encased, so there is no danger here. (A safety switch turns the light off if the cabinet is opened and the switch has not been deactivated.) However, if you need to work behind the cover (e.g. to cut bands from a gel), you should wear UV-protectant face mask when you do so.

General Supplies

General supplies such as pipettes, gloves, microfuge tubes, and pH paper are stored throughout the lab in labeled drawers. The Quartermaster is responsible for ordering general supplies. When you notice that we are low on or out of some stock item, make a note of the item on the lab chalkboard under "Requests" so that the Quartermaster can have it reordered. *Please try to make an order request before we are completely out of the most commonly used items.* Items that you need only for yourself, including specialty chemicals and enzymes, should be ordered by you.

As of June 2007, we have adopted the following policies for ordering general supplies:

a. Perishable items should be ordered by the individual who requests them. These tend to be needed quickly and are also able to be delivered quickly. Included are enzymes (restriction, ligase), PCR master mix, PAGE gels, etc. Essentially, anything that is frozen or refrigerated and has an expiration date.

b. Perishable items that are ordered should still be listed on the board under "Already Ordered" with a line drawn through it so that multiple orders are not mistakenly placed.

c. The Quartermaster will continue to order non-perishable items. A weekly walk-through will be conducted on Fridays to check the status of commonly used items. *This does not mean you are not still responsible for requesting items that are running low.*

This new policy should help to prevent running out of critical supplies while still keeping the Quartermaster's job reasonable. S/he still needs to do research!

Equipment

Most of the equipment we have in the lab is fairly standard and not difficult to operate, but you should ask someone to show you how to operate a piece of equipment before using it for the first time, *even if you have used similar items in the past*. Larger items like the high speed centrifuge remain on most of the time, but remember to turn off small equipment like heating blocks when you are finished using them.

Note that you are required to reserve the HPLC due to high demand for its use. You may reserve time on the instrument through the lab Wiki, http://openwetware.org/wiki/Prather:HPLC.

General Cleanliness and Orderliness

The rules for general cleanliness and orderliness are quite simple. You may keep your own space as clean or filthy as you please, so long as you are not endangering the safety of your lab mates. For other areas, these rules apply:

1. **If you spill it, clean it up.** This includes even small spills. We don't have the advantage of knowing what you spilled and whether we should be concerned for our safety.
2. **If it is on someone else's bench, shelf, or other work area, ask before you take it.** Exceptions, of course, can be made for items such as graduated cylinders and other common use items which are clearly not in use.
3. **If you use the last of it, you should replace it.** This includes items such as 1X TAE, towels, and other general lab commodities. If there is no more of the item left in the lab, it is your responsibility to ask the lab Quartermaster to purchase more of it.
4. **If you take it to a common area, put it back.** Space is limited and should not be occupied by unnecessary items. This includes flasks and plates in incubators (which should not be left until all the media evaporates) and cuvettes in the spec area. If you leave old items in common areas for longer than two weeks, you risk having them thrown out.

5. **If you store it in a common area, label it.** This is most important for flasks in the shakers and plates in the incubators, and for any beakers or bottles of chemicals left in the hoods. For cultures (flasks and plates), write your initials (so we know who to find to ask about it), the date (so we can decide to throw it away if it's been there for an unreasonably long time if we can't find you), and the contents (so we know how to dispose of it). The strain name and any hazardous media components is sufficient for "contents." Storage in common areas is, of course, short-term since other items should be kept at your bench.
6. **If you break it, replace it.** You can either fix it or order a new one.
7. **If you are the last person to leave at night, lock the doors and close down the biosafety hoods.** Also check the spec and Biosafety Cabinets and turn them off if necessary.

In general, please remember that we share space and equipment. Life in the lab will proceed much more happily and, most importantly, more safely if we respect on another and follow a few simple rules.

Laboratory Jobs

The day-to-day operation of a laboratory is, to say the least, laborious. Experiments generate waste that must be dealt with, strains and plasmids must be cataloged and stored, and supplies must be purchased and distributed. These general tasks and others are delegated to specific students in the lab to help the lab run smoothly and effectively. All Prather Lab graduate students (and post-docs, if necessary) are expected to have at least one job at all times. Jobs are re-distributed in February of each year, when new grad students join the group. Currently the lab jobs are:

Handyman: The handyman assists the lab in repairing simple equipment and in building, moving, and working on pieces of lab equipment.

Quartermaster: Responsible for the purchase of general laboratory supplies. General lab supplies include items like soap, pipette tips, towels, gloves, and other materials that are commonly used by all members of the lab. While specialty items, like reagents or cells needed for a specific project, are not the responsibility of the quartermaster, it is the quartermaster's responsibility to teach new members of the lab how to order these things for themselves.

Strainmaster/Keeper of the -80 °C freezer: Maintains a paper and electronic database of all cells and plasmids in the lab. The strainmaster distributes and processes all forms related to strain storage. In addition, the strainmaster maintains the -80^{o}C freezer.

Enzymemaster/Keeper of the -20 °C freezer: Maintains a paper and electronic database of enzymes in the lab. The enzymemaster regularly defrosts and/or replaces the enzyme boxes and maintains the -20 ^{o}C freezer.

Student Safety Coordinator: Responsible for keeping the lab in line with current safety regulations and practices. The student safety coordinator helps the lab manage its waste, ensures that the lab complies with the department's safety guidelines, and instructs members of the lab how to dispose of their wastes properly.

Webmaster: Responsible for the creation, maintenance, and expansion of the Prather Group website.

Lab Security and Shutting Down the Lab

The outer lab door should always be locked and pulled shut (sometimes it doesn't shut all the way), regardless of whether there are students present in the lab or not. To avoid lockouts, always keep your keys on your person when leaving the lab.

When leaving for the day, your laptop should be locked inside your desk.

The last person to leave the lab for the day should ensure the following:

-Both of the lamps on the spectrophotometer are turned off.
-Both of the biosafety hoods are closed and irradiated with UV light.
-All lights in the lab are turned off.
-No other equipment (hot plates, thermal cyclers, heating blocks, etc.) is left on.
-Both the office and outer lab doors are closed and locked.

Adhering to these practices will both conserve energy and help prevent malicious persons from gaining access to the lab.