Guide to the Prather Lab
for New Members

Updated January 23, 2009

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! (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.

Web and computer resources

The Prather Lab webmaster maintains two websites: the official lab site and the wiki.

The official lab website
http://web.mit.edu/prathergroup/index.html

When you join the lab, please provide the webmaster with some biographical information such as your educational background, CV, list of publications, photo, and a brief research summary so s/he can add you to the "Group Members" section of the site.

There is a secure portion of the website that is available only to lab members and can be accessed using your MIT personal web certificate. This part of the website contains group meeting presentations and a link to the strain database entry form. To access the secure portion of the site, click on the "Research" tab and follow the "Lab Members Only" link on the left side of the page.

The wiki
http://openwetware.org/wiki/Prather

The wiki is hosted by OpenWetWare and contains general information about the lab and its members, protocols, and also an electronic calendar to schedule time on the HPLC. Feel free to edit pages or add things, including a personal page in the "Lab Members" section. All you need to do is sign up for an account by filling out this form:

http://openwetware.org/wiki/OpenWetWare:How_to_join

Once you have an account, you have full editing privileges. The only important guideline is that if you are going to add a page that links to our wiki, be sure to name it in the following form:

Prather:Title

Where "Title" is whatever you want to call your page, and the "Prather" part ensures that the page is associated with our lab, and tells other users that the information on the page is specific to the Prather Lab. You can see examples of this by clicking on the various links on the main Prather Lab page. Other than that, feel free to go nuts! Editing and adding pages isn't too hard… the "Help" sections on OpenWetWare are very useful. You can also click the "edit" tab on the pages we've already made to see existing code.

Data backup
To encourage group members to regularly back up their data, Kris provides a subscription to Tivoli Storage Manager backup service. To enable this service on your computer, go to the following website:

http://web.mit.edu/ist/topics/backup/

Click on the "Register for TSM" link and follow the on-screen instructions. Use account number 2736375. It is recommended that you configure the service to automatically back up your computer at least once per week.

Prather lab shared folder

The lab has a shared folder stored on a network hosted by the computer connected to the RT-PCR machine. This folder can be accessed on any computer connected to the chemical engineering department web server (like your personal computer in the office). You can add a sub-folder labeled with you name to the shared folder and use it to store files. This folder is especially useful for transferring data (e.g. gel images, LC/MS data, RT-PCR data, etc.) from computers in the lab to your personal computer and for storing protocols and solution recipes that you use frequently in the lab.

To access the shared folder from your computer, follow these steps:

1. Open the "My Computer" window
2. From the Tools menu, select "Map Network Drive"
3. Specify the letter you'd like to use for the drive. It doesn't matter what letter you choose, as long as your computer doesn't already have a drive associated with that letter.
4. Click "Browse" to find the shared folder. Open the Microsoft Windows Network group, then click on Prather Lab → Alberta7300 → Prather Lab Shared Folder
5. Click OK
6. Click Finish

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 hundreds of strains stored in the -80°C freezer. Information about each strain is stored in the lab's Strain Database, which is currently stored as a Google Documents spreadsheet. You should be sure to store stocks of any strain or plasmid you obtain or create in the freezer and register those stocks in the database to ensure their usefulness for future Prather Lab members. *Plasmids should always be stored within a strain for our stocks.* You can store plasmid DNA frozen in solution for personal use, but do not store isolated plasmid DNA in place of transformed bacterial strains.

Making freezer stocks

- Grow the strain you'd like to store in rich, liquid medium to mid-exponential phase
- Dilute the cultures 1:1 with sterile, cold 30% glycerol in a 2-mL cryogenic vial by adding 900 μL cells and 900 μL of 30% glycerol to the vial. Invert the vial several times to mix its contents. A total of three vials should be prepared per strain you wish to store.
- Label each vial with the strain's name, your initials, the date, and strain number (on the cap as well as the tube face). Obtain a strain number from the sign-out sheet on top of the filing cabinet in the office.
- Store one vial in the "Main Storage Box" on the top shelf of the -80°C freezer and store two in the "Back-up Storage Box" located on the bottom shelf.
- Add each of your frozen strain stocks to the Strain Database (see below).

These stocks are for long-term storage and should be accessed infrequently. If you will be using a strain repeatedly, you should prepare additional cryovials to keep in your personal storage box.

Registering Stocks

- Access the secure portion of the lab website by clicking on the "Research" tab and then clicking the "Lab Members Only" link
- Under the "Strain Database" heading, click on "Strain entry form"
- Fill out and submit the form
- Your strain has been registered in the Strain Database, and will appear on the Google spreadsheet

Enzymes

There are a number of enzymes available in the lab, including a large selection of restriction endonucleases, T4 DNA ligase, and PCR polymerases. 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 more than a few minutes

Enzymes are stored in the insulated boxes in the -20°C freezer. When you need to use an enzyme, bring the entire box to your bench and remove the individual tubes to mix or dispense solution only. The buffers for restriction enzyme reactions are stored in the 4°C refrigerator. Additional tubes of buffer are kept in the freezer in a box labeled "Extra RE Buffers."

Common stocks and solutions

There are a few reagents in the lab that we take turns preparing and store as common stocks because they are used frequently by a majority of the lab members. These reagents include antibiotics and IPTG (-20°C), X-gal (4°C), and electrocompetent DH10B cells (-80°C). Instructions for preparation and maintenance of these stocks are discussed below.

Antibiotics

Table 1: Information about common antibiotic stock solutions

Name	Powder storage	Stock concentration	Diluent
Ampicillin	4°C	100 mg/mL	Water
Kanamycin	RT	10 mg/mL or 100 mg/mL	Water
Chloramphenicol	-20°C	34 mg/mL	Ethanol

Antibiotic stock solutions made in water should be filtered through a 0.2 μm filter and then aliquoted into labeled, sterile 1.7-mL tubes. Ethanol-based solutions do not need to be sterile filtered.

IPTG (Isopropyl β-D-1-thiogalactopyranoside)
Stock concentrations typically used in the lab are 0.1M or 1M. Dissolve the appropriate amount of IPTG powder (stored at -20°C) in water and sterile filter before aliquoting into sterile, 1.7-mL tubes. Store IPTG stock solutions at -20°C.

X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)
The stock concentration is usually 20 mg/mL. Dissolve the appropriate mass of X-gal powder (stored at -20°C) in N,N-dimethylformamide (DMF). Sterile filter the solution through a 0.2 μm

polypropylene membrane.

You can add IPTG/X-gal to agar plates if you are plating a strain that has blue-white selection. First make a puddle of 40 µL of stock (0.1 M) ITPG on the plate. On top of the ITPG puddle, add 50 µL of 20 mg/mL X-gal stock for a total of 1 mg X-gal. Use sterile glass beads to spread the IPTG and X-gal evenly over the plate. Allow the plates to dry uncapped in the biosafety hood for about 20 minutes.

Electrocompetent DH10B

The following rules should be followed when working with competent cells to ensure that these cells are always available in the lab.

How they arrive from the supplier:

Electrocompetent DH10B Cells (Electromax from Invitrogen) are packaged in 100 µl aliquots, 5 tubes per box.

How they are prepared for use:

Perform these steps on ice: thaw a tube of electrocompetent cells, dilute with equal volume (100 µl) cold, sterile 10% (v/v) glycerol, and distribute 20 µl aliquots in sterile, chilled 0.6-ml tubes. One tube is used per transformation.

How to ensure the stocks don't run out

If you plan to use electrocompetent cells, you should follow these rules:

1. Go to the white box labeled "Electrocompetent Cells" in the -80°C freezer. If there are at least N+1 aliquotted tubes, where N is the number you need for a transformation, go for it.

2. If there are N tubes or less, then you have the pleasure of preparing 20 µl aliquots. (That is, don't leave the box empty.) You should remove one (or more) yellow-capped tube(s) from the white box and dilute and distribute as described above.

3. If you remove the last yellow-capped tube from the white box, you should open a new Invitrogen box (red) of cells and transfer all 5 of those tubes to the white box. If there is only one new box or less remaining, order another box. This should ensure that we always have sufficient back-up.

Plates

Plates should be stored in the cold room (56-459) or on your shelf in the 4°C refrigerator. Everyone in the lab should make their own plates, so please ask permission 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.

Waste disposal

The lab has several different receptacles for waste disposal depending on the type of waste. If you have a question regarding how to dispose of a particular type of waste, consult the lab student safety representative.

Garbage cans

- Solid waste that is not contaminated with chemicals or biologicals
- Gloves which have not been used for handling hazardous materials
- Paper towels used to clean non-chemical and non-biological spills.
- Packaging materials that can't be recycled (Styrofoam, plastic bags)

Blue recycling bin

- Plastic (e.g. pipet tip boxes)
- Paper
- Cardboard (large boxes should be flattened and placed behind the bin)
- Note that MIT does **not** recycle Styrofoam or plastic bags

Biosharps bins

- Any sharp item (e.g. pipet tips, pipets, LC vials, broken glass) that is not chemically-contaminated
- Other items should not be placed in this bin, as we are charged for removal
- Small sharps can be discarded in the red, benchtop biosharps containers
- Full benchtop biosharps containers should be discarded in the large biosharps bins

White biohazardous waste containers

- Any item (e.g. tubes, gloves, plates, etc.) that has come in contact with a bio-hazardous material
- Please do not dispose of tubes or cuvettes containing liquid in these bins, as they are for dry waste. Biohazardous liquid waste should be disinfected with bleach and poured down the drain.
- Small biohazardous objects can be disposed of in the clear plastic benchtop bags
- Full benchtop biohazard bags should be discarded in the white biohazard bins

Chemical waste bucket

- Located near the digital imager/gel staining station
- For disposal of gels (wrapped in Saran Wrap) and other solid waste contaminated with ethidium bromide or other hazardous chemicals

Chemical waste containers in the fume hood

- There are often bottles in the fume hood that are used to collect the different classes of incompatible chemical waste: aqueous acidic, aqueous basic, organic, and oxidizer. If your waste falls into one of these categories and is compatible with the current contents of the bottle you can add your waste to the bottle and update the red tag with the name(s) of the chemicals being discarded

- Liquid chemical waste for which there is not already a designated bottle can be put in a compatible container, labeled with a red hazardous waste tag, and stored in the satellite accumulation area in the fume hood.
- If you fill a chemical waste container, complete the red tag with the date and request a chemical waste pickup (see below)

Qiagen & Zymo Kit Waste

- There are two containers next to the aspirator, one for kit buffers containing guanidine thiocyanate (QG, ADB) and the another for all other buffers

Sink

- Consult the "Sink Disposal" sign located under the main lab sink
- Biohazardous liquid waste (e.g. cell culture) which has been disinfected by treatment with 10% by volume (final concentration) bleach for at least 20 minutes at room temperature

Chemical sharps

- Small bins located at gel staining station and in fume hood
- Large bin located next to solid chemical waste bucket
- For disposal of sharp objects contaminated with chemicals

HPLC

- Bottles in the process of being filled should be labeled "HPLC effluent"
- Empty LC waste bottles are usually stored on the shelves above the large, benchtop centrifuge
- Full bottles should be labeled with a red, hazardous waste tag and a waste pickup should be requested (see below)

Requesting chemical waste pickup

If you fill a chemical waste container:

1. Complete the red tag with the date the container was filled
2. Go to the Chemical Waste Collection Form on the EHS website: http://web.mit.edu/environment/ehs/chem_collection.html
3. Fill out the required fields
4. Click submit

Call EHS at 2-3477 to request additional red, hazardous waste tags.

Gel staining and visualization: Ethidium bromide and UV light

Agarose gel electrophoresis is a staple technique for many members of the Prather Lab. The method we use to view nucleic acids on gels involves two of the more serious aspects of the lab with regard to safety; DNA is stained with ethidium bromide (EtBr) and then illuminated with ultraviolet light using the UV light table or the AlphaInnotech digital imager.

EtBr is a powerful mutagen because it binds tightly to DNA, even at low concentrations. EHS is strict about keeping particularly hazardous chemicals, like EtBr, and all equipment that comes in contact with them sequestered in one part of the lab. In our lab, the bench near the chalkboard with the gel imager on it is the only part of the lab designated for EtBr use. We don't put EtBr in the gels while they're running because then all gels would have to be run on this bench. **You should always wear nitrile 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.

UV light also causes damage to DNA, so you should protect your skin and eyes from exposure. The light table has a plastic cover (hinged to the viewing table) that protects against exposure, so in general you do not need to wear the UV-protectant face shield when viewing gels. However, if you need to work behind the cover (e.g. to cut bands from a gel), you should wear the face shield. 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.)

General supplies

General supplies such as pipets, pipet tips, gloves, microfuge tubes, and falcon tubes are stored throughout the lab on shelves and in labeled drawers. The Quartermaster is responsible for ordering general supplies and will perform a weekly walk-through to determine what items need to be ordered. However, if you notice that we are running out of an item, make a note of the item on the lab chalkboard under "Requests" so that the Quartermaster can reorder it. *Please try to make an order request before an item is completely gone.* You should order any items that you need only for yourself, including specialty chemicals and enzymes.

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

- 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.
- Perishable items that are ordered should be listed on the board under "Already Ordered" so that multiple orders are not mistakenly placed.
- The Quartermaster will continue to order non-perishable items. A weekly walk-through will be conducted to check the status of commonly used items. Items ordered after each week's walk-through will be posted on the Quartermaster board. *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

Please 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/MS in advance 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

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 or the integrity of your experiments. For common lab spaces, please remember that we all share the same space, resources, and equipment. Life in the lab will proceed much more happily and, most importantly, more safely if we respect one another and follow these few simple rules:

1. **If you spill it, clean it up.**
   If you spill something in the biosafety cabinet please clean it up immediately and sterilize the area of the spill with 70% isopropanol to maintain the sterility of the cabinet.
2. **If it is on someone else's bench, shelf, or other work area, ask before you take it.**
3. **If you use the last of it, you should replace it.**
   This includes items such as antibiotics, paper 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 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. Label these items with your initials, the date, and a description of the contents
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, close down the biosafety hoods, turn off the spec, and turn off the lights.**

Lab jobs

To help keep the lab running smoothly and effectively, all Prather Lab graduate students are assigned 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. The handyman is also responsible for promptly making service calls and requesting pipet calibration.

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 database of all cells and plasmids in the lab. In addition, the strainmaster maintains the -80°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°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 waste properly.

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

Lab chores

A spreadsheet of lab chores is posted near the door. All chores should be performed on an "as-needed" basis by the next person on the list. The only exception is autoclaving biohazardous waste which is performed on a weekly basis. Initial and date the spreadsheet after you have completed a particular chore. Instructions for performing chores are described in the "Prather Lab Chore Protocols" document.

Lab security and shutting down the lab

The outer lab door should always be closed and locked, 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 pieces of equipment (hot plates, thermal cyclers, heating blocks, etc.) are 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.