UCMOLAB SURVIVAL MANUAL

(Also Applies to Your Own Lab)

Introduction

Welcome to the Molecular Oncology Laboratory. Now that you've made it here, read the following very carefully and adjust your personal egos/habits accordingly to avoid being "unfairly" accused. Remember that **the Rule No 1 is that you will be blamed for ANYTHING and EVERYTHING** that goes wrong in the lab during your first months here unless there is someone else newer.

Goals for a Molecular Oncology Fellowship

The goals and expectations of clinical training programs and graduate schools are often rather explicit. The similar background and identical career positions of trainees in these programs allow ready comparison among peers. A formal date for the completion of training is set. Virtually none of these structures hold for postgraduate research training. Some guidelines are offered here which serve as a framework for how former trainees view the roles of their laboratory experience in fostering training and career development. These represent minimal expectations for training of a typical high-quality fellow at The University of Chicago, and of course are altered as appropriate for individual differences:

PERSEVERANCE: Good projects may take a year or more to set up. Don't expect publications or preliminary data suitable for grant submissions while setting up a really novel approach. Return is sometimes proportional to risk. Don't count on being done too soon.

QUALITY: The most important question is whether you are meticulous. Care about each individual step in a protocol. Determine how something should be done before doing it. Worry about leaving messes behind for others to clean up. Follow standard lab rules. Become known for identifying problems early.

UNDERSTANDING: Know the reason for every step in every protocol you use. Estimate the relative benefits of competing strategies before launching new efforts.

HELPFULNESS: Problems are infrequent here since everyone is interdependent, and everyone notices. A bigger problem is not asking to be helped (not taking advantage of available resources).

LAB CULTURE: **Successful labs have a successful culture**. Employers that recruit from successful labs hope that they are purchasing the culture along with the person. Whether you are representative of the culture, and whether you can reliably propagate a similar culture, are important.

DEDICATION: The most successful scientists openly enjoy science to a ridiculous degree, and are well rounded enough to briefly excel at a wide variety of other activities outside the lab. Thus, **please do not be surprised if you see somebody works 14-16 hours per day and 7 days per week in the Lab.**

General Principles

1. When you first come to the lab, ask TC for the lab keys., notebooks, and stationary needs.

2. When you are in the lab, wear a lab coat as much as possible. Underneath the coat, wear long pants or long skirt. Shorts and mini-skirts are not allowed unless you are only in the lab for brief period. When handling anything that is radioactive, wear a pair of glasses and/or use the shield. When handling any organic chemicals (e.g., phenol), wear glasses.

3. You can do anything you want with your own bench but please **ALWAYS CLEAN AFTER YOURSELF when you work in common areas**. Clean the gel rigs and put them back where they come from when you are done. Be considerate of others, make up a buffer or put it on the board (where applicable) when it is running low. When you open up a new box or a new vial of anything, make certain that it is not the last one in the lab and if so put the item on the board so it can be ordered before they all run out.

4. When using any centrifuge, stay next to it until it has reached top speed and make sure there are no funny noises when you leave. The bench side microfuges are meant for short spins only. Use the ones in the equipment room if you need to spin for 5 minutes or more. Additionally, it is advised that you ask someone the first time you use the Ultracentrifuge or Sorvall centrifuge.

Hope you can handle this much so far. The following pages were written by members of our group to help make life easier for everyone. Remember to always ask if you have any questions about anything. In case you have not heard: TC loves to show you how to precipitate DNA and run the minigels and stuff like that, so that everyone does it to perfection.

Have a fun and successful adventure.

Specifics

1. All unresolved **problems** get brought to attention when first noticed, generally by writing them on the board. About fixing equipment - don't, unless you have permission. Write the problem on the board.
2. **All data must be dated** on the day obtained. This means that film must be dated on the day it is developed, and include the conditions and duration of exposure. Lab books must have enough detail so that someone else from our lab can reconstruct what was done on a daily basis. Your copies of our lab protocols, as well as data that are voluminous get put onto a ring, into a ring-binder, or into computer files rather than the lab notebook, but all protocols and all data must be referred to in the lab book at the time of protocol use or data acquisition.
3. All tubes and membranes to be stored must include the date of preparation and a unique identification. Permanent markers are used for all permanent labeling of tubes for storage. Sarstedt alcohol-soluble markers are used for all labeling of radiographic film. Photographs of gels (except copies) are not marked in or near the lanes.
4. **All junks** get tossed unless initials and date are marked are on it. Boxes in use for gel staining must have initials and date, too.
5. **About dishwashing**: Rinse out labware and remove all labels before putting on cart. Put glass graduated cylinders on the bottom shelf of the cart. Always consider the washed glasses suspect. They may have soap or whatever still inside. Rinse all nondisposables with deionized water before using for solutions.
6. **Borrowing**: any item borrowed from the lab:

   Must be borrowed from a specific person, who remains responsible

   Must be written on the board, e.g. "Obama borrowed 20ul EcoRI from Jenny on 4-12-10"

   The message stays on the board until returned and erased by the one who lent it
7. **Gifts**: Borrowing of small amounts of a reagent is generally a gift. The exact quantity needed is aliquotted by our lab's person into another container and that container handed over. People from other labs do not handle our stocks.
8. **Inventory control**: If you open the last vial, box, or bottle of something, it must be written on the board. The first use or opening of any bottle requires that the date and person's initials be written on the label.
9. **PCR rules:** always wear gloves. Use filter tips and PCR-clean technique for all genomic samples which may be amplified later. Keep amplified products (phage, plasmids, PCR products, etc.) away from genomic samples and the PCR room.
10. The spectrophotometer must be turned off if there are no initials on it. The quartz cuvettes cannot be borrowed by members of other labs. Don't use the quartz cuvette to measure solutions containing anything other than purified nucleic acids - use plastic cuvettes instead.
11. All waterbaths that are set at >37°C must be turned off if no name is attached to the "on" switch. Except for some boiling experiments, keep the tops of all tubes safely out of the

water - don't trust the seal. Don't leave boiling baths on and unattended for long times as they may run low on water. Keep waterbaths at least 2/3 full. Check before using. Use deionized water to fill; never use tap water.

12. Balance tubes must have a big mark on the cap to prevent confusion with samples.
13. **Waste:** All pipettes (except polypropylene ones) are discarded in separate boxes marked "glass." Toxic chemicals are poured into water-tight bottles. Most things we work with go down the sink and are not hazards. Anything which would look bad if washed up on a beach goes in the biohazard bags.
14. Use gloves to handle specimens, reagents, containers, and lab equipment since DNases and RNases are on your hands, and since you never know when you may touch or splash some phenol or other caustic agent.
15. **Security:** Try not to leave a lab door open if no one is in the lab. **The set of common lab keys don't go in pockets**. Last one out of the lab at the end of the day must check waterbaths and Bunsen burners, lock doors, and turn off and conceal the stereo, etc. Check the list on your way out.
16. Don't leave a centrifuge until it reaches the set speed. Sometimes an imbalance isn't recognized until harmonic vibration sets in at a stable speed.
17. Wash/wipe the tissue culture hood with Windex, followed by 70% alcohol.

Procedures for Reagents

1. Know where **the eyewash/showers** are, and know how to use them.
2. Keep a lid on all supplies in use. This includes the **tips** and **tubes** at your bench, which can accumulate dust; keep them in a drawer or box, and replace the lid on tips between uses.
3. Pour out all chemicals. If you pour too much, discard the excess or you'll contaminate the stocks with DNA or nucleases. **Never use a spatula**.
4. Agarose - dissolve in Erlenmeyer flasks, not in Schott bottles. Agarose can become superheated, and suddenly boil explosively into your eyes.
5. Pay attention to the composition of containers. Glass and polypropylene are chemically resistant to virtually all compounds we use. If you use some other type of container, make sure that it is appropriate for your intended use. If you are using something you are unfamiliar with, ask and learn about it before use. Examples: **chloroform dissolves polystyrene tubes**; **phenol/chloroform (a.k.a., PC-8) dissolves plastic serological pipettes**; polypropylene melts on a hotplate - use glass instead; polyethylene melts when autoclaved; **polystyrene tubes are not as resilient as polypropylene and can crack under moderate forces** (3000 x *g*).
6. **Pipetting by mouth is forbidden**. Pipettemen seals develop leaks with time; check the calibration regularly and get seal replaced when leakage noted (esp. with expulsion of viscous solutions).
7. **Avoid stirbars whenever possible** since they may be contaminated with DNA, etc.

8. All solutions are made with dd-H2O. Don't consider water pure after a day of storage - get new water.

9. Labeling and storage: All bottles of solutions or boxes of aliquotted solutions must have 1) **solution name** or a list of all components and pH if no common name is available, 2) **initials of maker**, and 3) **date of making**. Concentrated solutions should have the "x" value on the box or bottle label. Example: "10% SDS/Proteinase K 5 mg/ml 10X 1/92 AS". Freezer boxes must be labeled on both the top and the side. All tubes in the box must have at least a brief unique label. All agar plates must be labeled with the **antibiotic color code** and be stored in a bag labeled as above. All freezer boxes must have a rubber band. They will on occasion be accidentally dropped by someone else, and it is unfair for you to turn their mistake into a catastrophe just because you omitted the rubber band.

10. About condensation: After incubations, centrifuge tubes briefly to bring down condensate from the lid before opening the tube. Warm up all dry chemicals to room temperature before opening to prevent condensation from forming in the reagent. Condensation on dry ice converts the 95% ethanol in the dry ice traps to a suspension of ice crystals, which impairs trap efficiency.

11. Don't let liquids touch lids of bottles, as the rim and lid are best considered contaminated. Prevent liquid touching rim or lid of test tubes also, if possible.

12. Common sterile stock solutions must be poured out or pipetted with a sterile pipette in the laminar flow hood. Other solutions should be poured out or pipetted with a new disposable pipette. **Never put a Pipetteman into a bottle**. Pipettemen are not pipettes and can contaminate solutions with plasmids or PCR products. Gibco-style bottles don't stand heat well, and break, so they generally can't be heat-sterilized.

13. About frozen aqueous solutions: they expand, fractionate, and sublimate. Don't aliquot more than 1 ml in a 1.5 ml tube. Tightly cap all tubes, and use a screw-cap tube for long-term storage. Use a small tube for small volumes. Don't fill glass bottles more than half full, or they will crack from the expansion. No frozen solution gets used until all of it thaws and is mixed.

14. pH adjustments must be done with the solution at room temp unless specified otherwise. Note: solutions are often made by heating to improve dissolution and heat is often given off upon dissolving or pH titration, and a cooling period is necessary before pH is adjusted further. Don't use pH meter on protein solution or detergents. They can coat the electrode.

15. **Put a mark on top of any tube** that is opened and placed back in a stock box. This marks the tubes currently in use and those having possible contamination. **When a reagent bottle is opened for the first time, it gets labeled with your initials and the date**.

16. **Microfuge all enzyme tubes** to get the residual enzyme off the sides when the tube has not been stored upright, when the volume is low, or before tossing out as "empty" as there are of 10 ul or more left. All enzymes go from freezer directly to one of the coolers that are kept in the freezer, and are put back immediately after use.

17. Use 10 units of restriction enzyme for small digests of $\leq$ 1 ug DNA. This is the maximum allowed. DNA concentration must be < 100 ug/ml. Enzyme must be < 10% of total volume. In general, use 10 units of cheaper enzymes per ug of DNA for 30 min., use 4 units of more

expensive enzymes per ug DNA for 2-4 hours. Most digests don't exceed 3 ug DNA per 100 ul, exceptions include screening of plasmids with minute (~10 ul) digests. **Most failed digests are due to an inhibitor in the DNA sample** (such as salt) which is not diluted out adequately by small reaction volumes; repeat using a larger volume digest.

Common Mistakes & Problems

1. In PCR, the optimum annealing temperature for reactions run in plates is ~ 5 degrees less than that in tubes. So if you work out optimum temperature in tubes, don't assume it will work the same in plates. So always do PCR in plates, never use tubes.

2. When reading O.D. on spec, it is imperative to measure a spectrum, not just the 260/280 ratio. The cuvettes change their baseline rather dramatically in comparison to the O.D. value when the O.D. values are low. Therefore you should subtract the 300 nm reading from the other readings to get a much more accurate value (e.g., if A260 is 0.11 and A300 is 0.03, the real A260 is 0.08).

Note that both RNA and DNA absorb approximately equally at 260 and 280 nm. So the readings you get likely reflect RNA, DNA, and any nucleic acid in the preps. If you want to find out how much DNA you actually have, use the Cytofluor or similar protocols.

Protocol on Protocols

1. Find out if the lab has a protocol for what you will be doing in MOLab website.

2. Follow the protocol directions.

3. If the protocol doesn't work, re-read the protocol to see what you did wrong.

4. Modifications to a protocol are best considered as experiments, to be done **side-by-side** with the original procedure. If there is no protocol, or if you find an improvement for the protocol, you should prepare a new protocol Review it with TC prior to handing out at a lab meeting.