General Lab Procedures
(KWK/BV Lab Survival Manual)

Jin Jen & Scott Kern October 7, 1996

Introduction

Welcome to the Molecular Genetics lab. Now that you've made it here, read the following and the pages that follow very carefully and adjust your personal egos/habits accordingly to avoid being "unfairly" accused. You need to 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.**

General Principles

1. When you first come to the lab, ask Tina or Kathy for a set of keys to the main entrance, the lab and postdoc fellow rooms. Also ask her to show you the lab security system and, above all, KNOW THE CODES!

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, Sorvall, or large IEC 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: Bert loves to show you how to precipitate the 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. Labbooks 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 labbook 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. VWR permanent markers are used for all permanent labelling of tubes for storage. Sarstedt alcohol-soluble markers are used for all labelling of radiographic film. Photographs of gels (except copies) are not marked in or near the lanes.

4. **Radiation** rules: Filter tips are used for all pipetting of fresh radioactive label, but are not used generally for routine radioactive solutions. Use of radioactivity is restricted to the following areas: the radiation room, the Speedvac, the PCR machines, and the sequencing rig bench. At all other times, radioactivity should be in an acrylic box, autoradiography cassette, or gel drier. Sign in and out of the radiation room for all uses of radioactivity. Check all areas you have used when you are done and leave nothing behind. Tolerable limits are <5000 in the microfuge, <200 cpm above background anywhere else; higher readings require more cleaning, or label the spot with tape containing your initials, the level of counts detected, and the date; leaving things to soak is not allowed. When aliquotting label, write the assay date and your initials on the tube, not the date you aliquotted it. Don't let label sit at RT once the ice melts; thawing at 4 C is best, and use of a timer is mandatory. The Geiger counter should always be on and nearby whenever radioactivity is used. Use only the equipment and racks marked for radioactive use.

5. All junk gets tossed unless **initials and date** are marked are on it. Boxes in use for gel staining must have initials and date, too..

6. 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.

7. **Borrowing**: any item borrowed from the lab:
   Must be borrowed from a specific person, who remains responsible
   Must be written on the board, example: "JQP borrowed a small cassette from SEK on 1-21-94"
   The message stays on the board until returned and erased by the one who lent it

8. **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.

9. Do not remove journals from the kitchen/waiting room/hallway areas. If you must read an article elsewhere, copy it. Try to read journals in the kitchen/waiting room; that's what these rooms are for.

10. **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.

11. **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.

13. 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.

14. All circulating **waterbaths** must be turned off if no name is attached to the "on" switch. Except for some boiling preps, keep the tops of all tubes safely out of the water - don't trust the seal. Don't leave circulating 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.

15. **Balance tubes** must have a big mark on the cap to prevent confusion with samples.

16. **Waste**: All pipettes (except polypropylene ones) are discarded in separate boxes marked "glass." Toxic chemicals are poured into water-tight bottles. Ethidium bromide (EthBr) solutions are poured into the Schleicher and Schuell thing or into a 4 l bottle containing 1 g Amberlite; when full, the Amberlite is captured by pouring through layered cheesecloth, and the cheesecloth then put in a toxic chemical waste container. Alternatively, use a carboy with Amberlite. 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.

17. 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.

18. **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.

19. 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.

20. Autoradiography **cassettes** should be sealed and labeled with a piece of tape opposite the hinged side, with your initials and the date the film will be developed. Cassettes without tape or past their date will be assumed empty or ready to develop by anyone needing a cassette. Film cassettes can be stored only in the designated cassette drawer. This means they can't be kept at your bench between uses

21. Wash the tissue culture hood with a 1:100 dilution of **Wescodyne**, not with expensive alcohol.

Procedures for Reagents

22. Know where the **eyewash/showers** are, and know how to use them.

23. 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.

24. **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.**

25. **Agarose** - dissolve in screw-capped Erlenmeyer flasks, not in Schott bottles or in open flasks. Agarose can become superheated, and suddenly boil explosively into your eyes.

26. 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 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).

27. **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).

28. Avoid **stirbars** whenever possible since they may be contaminated with DNA, etc.

29. All solutions are made with 18 Mega **water.** Don't consider water pure after a day of storage - get new water.

30. **Labelling** 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 labelled on both the top and the side. All tubes in the box must have at least a brief unique label (such as "L" for L-STET). All agar plates must be labelled with the antibiotic color code and be stored in a bag labelled 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.

31. 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.

32. 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.

33. 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.

34. 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. Radioactive compoundsl always gets mixed by pipetting before use.

35. **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.

36. 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 labelled with the your initials and the date.

37. 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 often 10 l 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.

38. 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.

Hot Room

1. Sign your name, date and radioisotopes on sign up list mounted on the side of hot room refrigerator.

2. Record amount of radioisotope consumed on recording book and hot drum.

3. Always check and clean the hot room hood or area each time before and after you finish your work in hot room.

4. Don't forget to ask someone else to check and sign you out each time.

5. Place your labeled probes, primers into the specially marked plexiglass radioisotope storage racks before you put them in the hot room freezer.
   For stuff that can not fit into storage racks, place them in white storage boxes labeled with your name and date.

6. Hot tubes and plates not stored in the storage boxes or racks can be trashed into hot drum any time without warning.

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. For counting ^{32}P. The way we usually count is called Cerenkov. It's efficiency is approximately 50%. The numbers you get from a scintillation counter are always cpm. They can be converted to dpm by correcting for efficiency (e.g., 500 cpm = 1000 dpm if efficiency is 50%). Note that 1 Ci = 2.2 x 10 E12 dpm.

3. 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).
   Also 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 (Protocol is in book).

Protocol on Protocols

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

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 BV prior to handing out at a lab meeting.

Goals for a Molecular Biology 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 Scott Kern views the role of his laboratory in fostering training and career development. These represent minimal expectations for training of the typical high-quality fellow at Hopkins, 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.

***Reading**:* At least four hours a week of quiet reading in molecular biology is recommended

***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 since 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.