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LukemanLab Manual

Acknowledgements

A large portion of this lab manual was inspired and reproduced from the Crews Lab manual found here. <http://www.utexas.edu/research/crewslab/manual.htm>

A lot of the positive wisdom has been provided by teachers of mine including Prof. Elliott at Colorado State, Prof Sanders at Cambridge, Prof Seeman at NYU and their research groups. Their websites are here.

<http://franklin.chm.colostate.edu/cme/>

<http://seemanlab4.chem.nyu.edu/>

<http://www-sanders.ch.cam.ac.uk/>

A lot of the negative wisdom has been provided by my own mistakes.

Emergency Contact Details

Prof Lukeman's Cellphone: six-four-six 322 1751

Prof Lukeman's office: 909 869 3657

Chemistry department office: 909 869 3653

Campus Police/Fire Dept 909 869 3070

*Happy are they who get to know the reasons for things.
Virgil (70-19 BCE) Roman poet.*

Goals

We are predominantly interested in using organic covalent chemistry to control the assembly, stability, and operation of molecular objects and machines constructed from nucleic acids.

While doing so, we intend not to lose sight of the Big Picture. We hope to train inquisitive, responsible citizen-scientists to take an evidence-based approach to all aspects of their lives during and after their time at Cal Poly.

Principles and Hierarchy

Cal Poly's motto is "Learn by Doing". I would add to this that we are here to ask questions: to discover things and learn to be better scientists. I strongly encourage questions about **any and all** aspects of the labs function, both practical and theoretical. It is better to ask questions and learn than blindly follow a wrong path.

The expression "6 months in the lab saved a day in the library" can be reformulated to "ask and think **before** learning by doing".

The structure of the Lukeman Lab is merit based, not hierarchical. It is based on accomplishment. Therefore, an experienced undergraduate can rank an inexperienced graduate student in a particular task. I recognize that every one that comes into the laboratory has little or no prior experience with the work; they have come to the lab for training.

The lab is an egalitarian place: where in the few places it appears dictatorial, it is a benevolent dictatorship. Despite the stern tone of the manual the follows, we are here to have fun and discover things. Relax and read on.

Protocols and re-inventing the wheel

Despite the encouragement of questions, another principle of the laboratory is "if it ain't broke, don't fix it." We have protocols for nearly everything: if try these before making a modification. We have enough work to do getting new science to work, without re-hashing old science.

Tinkering with established procedures should be kept at a minimum. Only if a problem develops should a change be contemplated, and then no action taken until the history of the problem, and the reason for the current procedures are understood and appreciated. It has been said that "there is the way everyone does it, and there is a better way." But "different" does not necessarily mean "better." In other words, changes must be justified by their outcomes, not because of personal preference.

A course of action to take if a problem presents itself is to research the problem and determine why the present policy is in place. Ask yourself: Is it inertia? Does it still get the job done? Will the contemplated change create more problems than it will fix? Most importantly, will it save more precious undergrad time than developing and validating the fix use? An example: the amount of time needed to run gels for purification. The PI spent 6 months *fulltime* trying to develop validate certain purification protocols, none of which proved as good as gel electrophoresis: do you have 6 months (full time) to make things better?

As you progress you'll realize that some protocols can be varied slightly: e.g gels can set for 45 minutes or 1 hour: annealing can take 30 or 40 hours with little change. However, **when you are beginning**, please follow instructions to the letter: once you've gained experience with your system, you can develop the skills it takes to 'improvise'.

Ethics and data ownership

Scientific ethics constitute the norms that help distinguish between acceptable and unacceptable practices in scientific research. General principles of scientific ethics can be found here : <http://www.webguru.neu.edu/ethics/> and should be followed closely.

Data generated by funded research is owned by three entities; the sponsoring organization (e.g. a university), the funding agency, the PI. However, the right to use and publish the data resides in the hands of the PI. Copies of lab notebooks can be stored outside of the lab for safety or reference, but originals do not leave the lab.

Errors and responsibility

Intentional errors (falsifying data, theft or damage of others materials) are at the center of scientific misconduct and cannot be tolerated. Any individual engaging in such actions must and will be dismissed from scientific research.

However, unintentional errors do not constitute scientific misconduct. Should such errors occur, it is the responsibility of the parties concerned to try and remedy the situation as soon as possible by informing the appropriate person, usually the PI. **I do not punish or harshly judge people for unintentional errors:** I'd much rather hear about an error than have it covered up. I will be forthright when I make a mistake: I expect you to be so too.

Lab management

We do not have a dedicated technician or majordomo, so there is no overall coordinator of laboratory activities except the PI. It is essential that all involved in laboratory activity fulfill their assigned obligations with efficiency and reliability. The duties of students in the lab are assigned by the PI: every member of the lab participates in the general care and maintenance of the laboratory.

My "management style" is not to define goals in general terms and then ignore how they are accomplished. I like to keep in close touch with the progress (and problems) associated with all of the projects. One of my "pet peeves" is not knowing what an individual is doing in the laboratory. I have seen the result of excellent undergraduate students working diligently on a project without providing me with regular progress reports. When we do get together in conference, it is not uncommon to find that there has been a fatal error in the logic, design, conduct, or analysis stages and the effort must be repeated. So, progress depends upon efficient and timely communication: no secrets! I will try to meet with each of you on a fortnightly basis to discuss progress and future directions: these meetings are mandatory.

Conflict and external communications

There inevitably will be instances in which conflicts arise in the laboratory (e.g. authorship, areas of interest, etc.). In such instances, I reserve the right to make all final decisions. It is preferable that areas of conflict be worked out among the individuals concerned, but if this is not possible, I will listen to all evidence and perspectives before making this decision.

On the off-chance that we have to deal with the outside world, communications with authorities and media (i.e. anyone outside Cal Poly) regarding laboratory activities and research must have received my written approval before being sent out. There is a lot of nonsense spoken about nanotechnology and chemistry these days and the web is rife with rumor and foolishness: being in the *unwanted* spotlight of the media/blogosphere is a waste of time and energy and something I'd very much like to avoid.

Lab safety rules

Rule 0 : **The golden rule:** You must perform your duties in such a manner that you and your peers can come back tomorrow and continue your life, research and study ! Personal safety is paramount in running a lab: the only heroism I'm interested in is intellectual heroism, not macho nonsense or make-it-up-as-you-go-along cowboysim.

Most of the materials and equipment we use are not intrinsically dangerous but they are hazardous. Something intrinsically dangerous means you experience risk from being in its vicinity. Something hazardous means that you experience risk under a certain set of circumstances, usually to do with how the material is treated. If rules are followed, risk and hazard can be minimized.

In order to minimize potential hazards and provide for the safety of both regular staff and visitors, the following precautions are to be adhered to at all times:

1. All members of the Lab must complete the requisite courses offered by the Cal Poly safety office.
2. No unaccompanied visitors, friends and relatives of employees and students are allowed to enter the Lab at any time. Guests are the responsibility of the hosting student: they must not perform experiments or use the equipment.
3. No food, drink or smoking is permitted in the working areas of the lab at any time.
4. All disposable items (glass, plastic, etc.) used for hazardous materials are to be placed in the appropriate containers provided in each lab room: i.e NOT in the trash. Syringe needles, razor blades and broken glass must go in the sharps containers. Organic chemical waste must go in an appropriate waste bottle.
5. Unlike most organic chem. labs, most of the chemicals we work with are *relatively* innocuous: providing you don't inhale or ingest them they are extremely benign. There are three exceptions to this:
 - i) Acrylamide. The **poly**acrylamide gels you make are relatively benign. However, the acrylamide monomer solutions (denaturing and native) contain **potent** neurotoxins and are probably carcinogens. This means they must be treated with respect: be aware of when gel solutions are being transferred: watch for drips and cleanup immediately. Gels must **ONLY** be poured in the gel pouring area in 339 and protected by a drip tray.
 - ii) Ethidium Bromide (EB). This UV active gel stain works by intercalating (sliding between) the bases of the DNA you've just separated on a gel. Guess what? If ingested, it also slides between **your** DNA bases acting as a mutagen and carcinogen. Whenever working with EB, always use Nitrile gloves (in solution it slowly penetrates latex gloves). When making a solution to stain gels, use enough so that the solution is super-super-super-super faintly pink or almost clear (this corresponds to about 0.3 mg in 1 L of ddH₂O). If your solution has anything except the slightest pink color to it, it is too concentrated.

Lab safety rules contd.

5. contd. **Small** spills (< 5 ml) of both of these materials must be cleaned up by absorbing the majority of the sample on absorbent material and then wiping the area down repeatedly with paper towels soaked in water. The towels can then be rinsed in the sink and thrown away. For large spills, the waste must be separated and go in the solid waste box.

EB and Acrylamide are not acute toxins: rather they cause cumulative damage. Exposure to these molecules is like smoking a few cigarettes per day : it will likely shorten your lifespan and potentially increase your risk of nasty diseases. Care is required not only for you, but your fellow lab users and workers.

iii) The chemicals used on the DNA synthesizer. As the PI will be operating this, you just need to know to stay clear of this area. Note also that the chemicals used there are moisture-sensitive: **no** aqueous solutions should be brought into the DNA synthesis area.

7. When working with the UV light box, you must use face/eye protection in the form of a UV protecting faceshield. When the lightbox is on, you must turn the sign on the door to 331A to “DO NOT ENTER”. When done with the UV lightbox, you must turn the sign back to “ENTRY OK”.

8. All large containers (>200ml) of flammable liquids should be stored in safety-cabinets in 331A. Concentrated acid and base solutions should be kept separate in spill contained trays.

9. Eye protection and the use of disposable gloves is mandatory in the labs. Labcoats are strongly recommended.

10. Familiarize yourself with the Fire Evacuation Plan and the policies of the university

These regulations are for the safety of everyone working in the lab. They are also necessary to protect the lab from liability and legal jeopardy and, as such, are not voluntary. Failure to comply with these and the general regulations on chemicals, biohazardous or carcinogenic compounds will be brought to the attention of the Environmental Safety Office and, after one warning, will result in dismissal from the lab.

Lab space policies and protocols

We have two lab spaces: the left side of 339 and the whole of 331A.

Please enter 331A through the research lab on the left (lab 341) and **not** through the teaching lab:

- a) it avoids disruption in the teaching lab and
- b) it allows people using the UV light box to use 331A as a darkroom and signal whether it is safe to go into 331A using the sign on the 331A door.

With the exception of the Genesys UV spectrometer, the material and instrumentation in 341 is off-limits to us.

The computer in 331A is for lab work: scanning gels and preparing reports. If no-one is using it for these purposes, it can be used for personal use (websurfing, email etc.): you must give way to someone who needs to use it for work under these circumstances.. However, NO programs are to be installed w/o permission from the PI. Any found will be deleted. You will have personal folders on the machine: anything kept outside those folders is considered temporary and can also be deleted. Please backup your data (scanned gels, reports etc.): USB thumb drives are cheap and save you a lot of hassle.

In Lab 339, our work area is in the leftmost aisle in the lab. This includes the front half of the leftmost bench against the wall and the left half of the first bench. Other areas are off-limits.

Reagents, Chemicals and Equipment

Please familiarize yourself with the safety data sheet for all new chemicals you encounter.

If a reagent is ABOUT to run out (i.e. you see that there are approx 2 uses more left in the contained), email the PI: he'll order some more. Please don't wait until a reagent is empty to request an order: this causes delays.

Communal reagents should be replaced in the designated area: they shouldn't live on your bench or in your cupboard. Personal reagents should be clearly labeled. If you run out, you should ask before using someone else's stock: never finish someone else's reagents without replacing them and notifying them of the replacement.

Equipment should not be used until you have been trained on it. If problems occur, contact the PI or another user. Do not try and repair the equipment yourself without being familiar with its repair. In case of malfunction you **MUST** note the time and problem and email PI.

Other important resources

The website <http://www.webguru.neu.edu/> has lots of advice on how to conduct scientific research as an undergraduate. Worth 30 minutes of your time.

Campus safety and security

<http://dsa.csupomona.edu/orientation/newgrad/safety.asp>

Cal Poly Policy Guidelines

<http://www.csupomona.edu/~guidebook/policy/index.htm>

This lab manual is a work in progress: the latest version will be posted on www.csupomona.edu/~psl frequently.

*The universe is full of magical things patiently waiting
for our wits to grow sharper.
Eden Phillpotts, A Shadow Passes*