General Lab Guidelines

1. Lab Hygiene

Lab cleanliness effects lab safety, and the results of both your experiments and others in the lab. Ensuring that you start and end with a clean workspace helps to prevent accidents, and makes the cleanup of any spills much safer. Additionally, there are a lot of us sharing a small space- making sure that shared items are back where they belong and the benches clean ensures that the next person working in lab doesn't have to spend time cleaning and tracking things down. In pursuit of this goal, please adhere to the following guidelines:

1. Clean up after yourself.
2. Put chemicals away properly- ensure spatulas are cleaned and weighing paper disposed of after weighing chemicals.
3. Your bench/hood area should be as clean as possible after you are done working. Ensure all solutions and samples are properly labeled and stored, and all chemicals are put away.
4. Used glassware should be cleaned and dried immediately to prevent adsorption of chemicals onto the glass surface, making cleaning more difficult in the future.
    a. It's fine to clean glassware and leave it on the drying rack, but you should make sure you come back later to move it back to the appropriate location once it has dried.
5. Keep the desk area clean so the next person can use it.
6. Ensure that if you use/remove shared supplies (e.g., pipettors and cuvettes) from their normal place that you return them clean and in good condition as soon as you are finished using them. This saves the next person who needs them from having to track them down, and ensures they are in good, useable condition at all times.

2. Lab Safety

2.1 General Lab Safety

The most important part of lab work is safety. As biological chemists, we frequently work with compounds that are both chemical hazards and biological hazards. Improper use puts not only our personal health at risk, but also the safety of those around us. Accordingly, proper safety training is necessary before you begin work in the lab, as well as yearly thereafter.

Before beginning any experiment, you should make sure you fully understand the hazards of each chemical or biological agent you will be using. The Safety Data Sheets (SDS) are provided at the time of shipping from any company, and outline the potential risks associated with that material- these should be reviewed when you receive a chemical, and stored somewhere you can easily access them in the future. SDS sheets are also available online from the manufacturer, but this should be secondary to having a hardcopy in the lab when you need it. If you are using anything that is especially dangerous- toxic, biohazardous, environmentally dangerous- please come and talk to me about your experiment and containment protocols before performing the experiment. I am always available to review your safety protocols, and you should never hesitate to talk to me if you have any questions at all- it's always preferable to head off problems before they happen.

Additionally, please inform others working in the lab if you are working with particularly dangerous substances or procedures, especially when working in close proximity. It will allow them to help you should a dangerous situation arise, and it will also ensure that they are not working with substances that are incompatible with your own.

Personal protective equipment must be used in lab. This includes clean gloves, changed as needed and appropriate safety glasses for the task at hand. Additionally, a lab coat should be used when working with dangerous or biological samples, as needed.

Never touch "common equipment" while gloved. Gloves are designed to protect us from hazards we handle. If we handle something hazardous and then touch a communal item (door knob, telephone, computer mouse or keyboard, calculator) that either ourselves or someone else will eventually touch without gloves, we are spreading the hazard rather than protecting ourselves from it. Do not touch anything while wearing gloves that you or someone else will touch in the future without gloves.

Finally, lab clothing must be appropriate- this includes **always wearing closed-toe shoes while working in lab**.

2.2 Personal Protective Equipment (PPE)

2.2.1 Safety Goggles

When working with chemical hazards, or in labs where other people will be working with chemicals, **safety goggles or glasses must be worn at all times**. If you have any question about whether safety glasses need to be worn- **wear them**. It's always better to have them on and not need them, rather than need them and not have them on.

For chemical hazards, it is important that safety goggles, not safety glasses, be used. Safety goggles protect from chemical fumes and splashes, in addition to the impact protection offered by safety glasses. When hazardous splashes and fumes are minimal (most biochemical and some biological work), safety glasses can be worn instead.

Special note if you wear contact lenses: Many studies within the past few years have highlighted the hazards of contact lenses in laboratories. If a corrosive liquid gets behind a lens, the process of washing out the eye is not effective unless the lens is removed. However, the natural reflex to close the affected eye makes removal of the lens very difficult. In addition, the newer "soft" lenses can actually absorb and concentrate vapors from the lab atmosphere, leading to eye irritation and, in some case, to damage the lenses themselves. Therefore, the wearing of contact lenses in lab is strongly discouraged.

2.2.2 Gloves

Gloves will be worn for handling of all chemicals that may cause irritation, allergic sensitization or skin absorption of toxic chemicals. You should always check the MSDS for the chemicals you're using to ensure that the type of glove material you have is appropriate for the substance being used.

In addition to wearing gloves to protect yourself in a chemical lab, gloves should be worn at all times when working with biological samples or preparing samples for cell culture work. When working with cell culture, gloves act to protect your samples from as well as yourself from the sample. Human contact has significant effects in the contamination of samples- dead skin cells, hair, and even the RNAses found in the oils on our skin can significantly alter the results of experiments. Protective methods (including gloves) that minimize our contact with biological samples also act to minimize the biological samples contact with us, serving the dual purpose of protection and limiting contamination.

2.2.3 Lab Coats

In addition to safety goggles and gloves, lab coats should be worn as an extra layer of protection in the lab. Lab coats are made of material that has reduced flammability, and increased chemical resistance. Moreover, they have long hems and long sleeves that impart additional protection from accidental splashes. When working with cell culture and biological samples, goggles also help minimize contamination of samples.

2.3 Lab Safety Implements

Listed below are several safety and emergency items generally found in each lab. Familiarize yourself with their location before beginning any lab work.

2.3.1 Fire Extinguisher

Fire is an ever-present hazard. Before lighting a Bunsen burner, make sure that no one is using a flammable solvent nearby. If you are uncertain, ask everyone around you. Also check that all flammable materials such as books, notebooks, paper towels and so forth are moved a safe distance away. Fire extinguishers are located in each lab and the hallways. Before beginning work be sure to note the location of the nearest fire extinguisher. To operate a fire extinguisher remove the safety pin, aim the nozzle at the base of the fire, and squeeze the handle to discharge the chemical flame retardant.

2.3.2 Eye Wash

These fountains are very useful for extended irrigation of eyes that have received a chemical burn. Eye washes are available to the side of the lab sink. If you get a chemical in your eyes, it is imperative that you wash your eyes immediately.

2.3.3 Safety Shower

In the event of a serious chemical spill, where much of the body and/or face is involved safety showers are available. To operate the shower, pull the lever and water will flow from the showerhead until the lever is returned to the off position. To ensure that all traces of the chemical have been removed it will be necessary to remove all contaminated clothing.

2.3.4 First-Aid Kit

A first-aid kit is located in our lab. Even minor injuries should be reported to me, and you should get someone else to help you apply first aid if it is needed.

2.3.5 Spill Kit(s)

A large spill kit is shared between the Battle, Kirk and Duncan labs. This kit contains the elements necessary to safely contain and clean up a chemical spill up to 5 gallons. Any spill, small or large, should be reported to me. If you do not feel comfortable cleaning up a spill on your own, find myself, or another faculty member for assistance.

In biological labs, most spills are contained with bleach (more details below) or ethanol.

3. Biosafety

A comprehensive resource for biosafety is the BMBL5, or "Biosafety in Microbiological and Biomedical Laboratories, 5th Edition" which is published by the National Institutes of Health in conjunction with the Centers for Disease Control. Both synthetic nucleic acids (used in the first part of the lab) and human tissue culture (used later in the lab) are biohazards, and have special precautions that should be followed. Neither is known to pose a serious risk to humans, but just because something is not currently known to pose a specific risk does not mean new research down the rode will not illuminate some previously unknown harm. Accordingly, we will use PPE and protocols designed to minimize contact with these agents. Additionally, human contact has significant effects in the contamination of samples- dead skin cells, hair, and even the RNAses found in the oils on our skin can significantly alter the results of experiments. Protective methods that minimize our contact with biological samples also act to minimize the biological samples contact with us, serving the dual purpose of protection and limiting contamination.

3.1 Synthetic Nucleic Acids:

In our biochemistry work, we will use short (20-23 bp) synthetic DNA fragments as sensors and inhibitors of intracellular micro-RNA. These fragments are not capable of being replicated, and function solely as transient, post-transcriptional modulators of protein expression. The risks in handling synthetic DNA of this type are very low, however, the use of proper PPE in conjunction with good technique should be maintained to minimize exposure.

3.2 Human Tissue Culture:

For our biological projects, we will use human breast cancer cells to study the role of micro-RNA in cancer progression as a means of learning techniques relating to the manipulation of nucleic acids. Both cell lines used in the lab (MCF-7 and MDA-MB-231) are well characterized cell lines commonly used for studying cancer. Both are classified as Biosafety Level 1 by the American Type Culture Collection (a central supplier and repository of cell lines). Neither cell line is known to cause human disease. As with synthetic nucleic acids, however, precautions will be taken in both PPE and protocols to minimize exposure. All handling of cells will follow sterile techniques and will use a biosafety cabinet as an additional primary barrier over the normal BSL1 requirements when techniques with the potential for generating aerosol are performed.

3.3 Decontamination & Disposal:

Human tissue culture will generate solid and liquid waste products that are potentially biohazardous. Accordingly, they will be decontaminated and disposed of differently than other waste products from the lab.

All waste generated from contact with cells (e.g., pipettes, culture plates, gloves and tubes) or that contains cells, cell by-products (cell-extracts, cellular RNA) will be considered biohazardous waste.

- Liquid waste will be decontaminated by treatment with a 10% bleach solution for 1 hour.
- Solid waste will be decontaminated by autoclaving.

Any waste generated that contains synthetic nucleic acids with no other biological products or chemical waste will be treated as biohazardous liquid waste. Synthetic nucleic acid waste containing other chemicals (buffers, etc.) will be disposed of as appropriate for the chemical hazard.

3.4 Accidental Spills & Personnel Decontamination:

In accordance with Biosafety Level 1 (BSL-1) practices, the following response should be used for any spill containing biohazardous material:

- Notify others in the area, to prevent contamination of additional people or the environment.
- Report the spill to the PI.
- Remove any contaminated clothing and wash exposed skin with soap and water.
- Wearing gloves and lab coat, cover spill with paper towels, pour 10% bleach solution around the spill allowing it to mix with spilled material. Allow suitable contact time, at least 15 min.
- Pick up any pieces of broken glass with forceps and place in a sharps container.
- Discard all disposable materials used to clean up the spill into a biohazard bag.
- Wash hands with soap and water.

3.5 Sharps:

While needles, broken glass, and any other sharp object poses a risk of harm in any setting, this risk is much higher in the presence of biohazardous materials or chemical hazards, due to the potential for accidental introduction of those hazards into your system through a cut or puncture.

All needles should be disposed of in a puncture-proof container. Needles should **not** be re-capped after use, as capping a contaminated needle carries a significant risk of puncture. Instead, the uncapped needle (and syringe, if connected) should be immediately disposed of in a sharps container.

4. Experimentation & Documentation

It is important to perform safe, well-planned, and well-documented procedures in the lab. Planning carefully ahead of time ensures that your time in the lab is well spent and productive, and minimizes any potential safety hazards. That said, over-planning can prevent getting in the lab and performing experiments. Research is inherently pushing the

boundaries of our knowledge, and as such, planning will only take a project so far- results are frequently not accurately predictable when working with new systems. The results we don't anticipate are frequently those that are the most interesting, and can lead to great discoveries.

Recording your results is one of the most important parts of doing good science- frequently, we have to refer back months (or even years) to verify new data against old. You will be provided with a laboratory notebook, and it should be with you at all times in lab. **Write everything down**- the literature you're basing an experiment off of, the planned procedure, the reagents you're using (chemical name, supplier), the exact amounts you measure out, and any deviations from the planned procedure. You should also record your thoughts and observations. If you aren't sure whether you should record something or not, err on the side of writing it down. You may not need it in the future, but if you do you will be very glad you did.

It is not important that your lab notebook be perfect- mistakes in a lab notebook are common. Simply mark through mistakes with a single line so what was there can still be read, but is noted as incorrect. Lab notebooks are a true record of what happened- and mistakes are a natural part of that. Don't let a fear of making mistakes keep you from using your lab notebook.

All data gathered through experiments should be described in your notebook, and both hardcopies and digital copies saved. If you don't want to tape data sheets into your notebook, it is perfectly appropriate to keep a 3-ring binder full of data, but the data **must** be cross-referenced to the lab notebook so it is easily findable by anyone else looking through your notebook. Digital copies of data should be backed up regularly to prevent any losses.

Computer data files should include a reference to the lab notebook page in the name. Your lab notebook is identified by your initials, and if you have multiple lab notebooks, each will carry a number in order of when you started it. So for example, my first lab notebook was CHB01. For an experiment that appeared on the 35th page of my 1st notebook, the data file would include CHB01-35 somewhere in the file name. The date the data was collected on should also be included.

For computerized data, you should save two formats: the format of the instrument used to collect it, and one that's accessible off the instrument. For spectroscopic data, the latter format is a .csv file that can be opened in Excel. You should add basic experimental data to your raw data in Excel, so the important parameters (concentrations, sequences, times, temperatures, instrument parameters) are always tied to the data that was collected.

It is important to remember that what you record (both your lab notebook and your data) are not just useful to you, but to myself, your colleagues, and those students who come after you. Make sure you organize them such that they are comprehensible to someone else reading through them.

5. Regular Maintenance

Closely related to lab cleanliness, maintenance of group equipment in your workspace is important- this includes pipettors, cuvettes, vacuum pumps, schlenk lines, rotatory evaporators, and shared glassware. All equipment should be regularly cleaned, maintained, and inspected for signs of wear and proper function.

Expected Maintenance:

- **Belt-driven, oil-cooled vacuum pumps** are intended for long periods of use and drive a high-vacuum system. The oil levels should be checked before every use, and oil added as necessary. Additionally, the oil should be removed, the system flushed, and new oil added **every 3 Months**. All oil pumps must use a cold-trap with a temperature maintained at -78 °C with a dry ice/acetone slurry as long as the pump is on.
- **Membrane pumps**, which are intended for short-term use (several hours at most) and provide moderate vacuum, do not need regular maintenance. They should, however, be regularly checked for signs of wear, as corrosive solvents can erode the PTFE membrane in the pump, resulting in a loss of vacuum efficiency.

- **Schlenk Lines** and the associated glassware and tubing should be regularly maintained, including thorough cleaning, oven drying, and re-greasing of all seals after any material has entered the line. Regular cleaning of the line should be done **yearly** using water followed by acetone and isopropanol. Drying columns between the inert gas tank and the schlenk line should incorporate indicating drierite, and should be changed as soon as the drierite is no longer fully dry. All hoses should be regularly checked for tears or breaks to ensure the system is gas-tight, and the mineral oil in the gas bubbler should be maintained at 1-3 inches.
- **Rotatory Evaporators** should be cleaned at least **monthly**, with the water in the bath replaced **once per month**, with **distilled water**. The bump trap should be cleaned after every use, even if no noticeable product entered the trap. A full cleaning includes cleaning and re-greasing vacuum joints.
- **Cuvettes** should be cleaned after every use, with water followed by ethanol, and allowed to dry before storage. Solutions should **never** be stored in the cuvette, but rather should be removed as soon as the assay is finished. Many compounds can adsorb onto the walls of the cuvette, permanently altering the surface and background absorbance.
- **Pipettors** should be cleaned **every 3 months** by disassembling, cleaning the interior mechanism, and re-greasing and re-assembling the barrel according to the user manual. **Pipettors should be immediately cleaned if any solvent enters the barrel of the pipettor**. To avoid solvent entering the barrel, pipettes should never be laid on their sides with a tip attached, tips should not be left on when the pipettor is not in use, and solvents should be taken up into the pipette tip gently, rather than rapidly to avoid splashing. Highly volatile solvents and any biohazardous reagents should only be pipetted using barrier-tipped pipettes to prevent contamination of the barrel.

6. Computers

Maintaining our shared computer in good order requires good computer hygiene on the part of everyone using them. You should regularly scan USB flash drives with antivirus software before using them in shared computers, and you should not download programs or visit questionable websites on the lab computer.

Additionally, with multiple people sharing computer workstations, it is important to keep data organized and well labeled. You should not leave files on the desktop- if they are no longer needed, they can be deleted when you are done with them. If you wish to keep a copy on the shared computer, you should locate it in a directory under your name, with a descriptive file name detailing what the file contains.

These safety precautions are especially important on computers used to drive instrumentation (spectrophotometers, mass-spectrometers, HPLC, etc.). You should always back up your data as soon as you are finished collecting it, and you should under no circumstances browse the internet or install programs of any sort on an instrument computer. Most have operating systems and operating parameters specially designed to control the instruments they connect to.

Finally, ensure that when using group computers that you **do not change settings unless you have obtained specific permission to do so**. Changing settings in programs that control instruments, or modifying the operating system or hardware configuration of the computer can cause equipment or programs to stop working as intended.

All data should be backed up to the “Battle Lab DropBox” folder in our shared DropBox, into a folder with your name. This is in addition to keeping a hard copy in your notebooks.