GTAC Lab Safety, Etiquette and Techniques

IN CASE OF EMERGENCY

Please note the location of fire extinguishers, eye wash, shower and first aid kits in the lab, so that you will know where they are located in case of emergency. The location of this important safety equipment and of hazardous waste accumulation areas is also covered in your GTAC orientation tour.

Who to Call:

Campus Police (be clear you are at **SF State** if calling 911!)	911 or (33)8-2222
Environmental Health and Occupational Safety Dept.	(33)8-1449
COSE Health & Safety Specialist Linda Vadura	(33)8-6892

Evacuation Procedures

When the evacuation alarm sounds…
Stay calm and leave the building through the nearest safe exit and wait there for instructions.
Take the stairs, not the elevators.
Assist persons with injuries or disabilities.
Instruct wheelchair users on upper floors to wait by the stairwell.

- Have them wait until people return or rescue personnel evacuate them.
- Inform people monitoring doors of the existence and location of the wheelchair user so that they can notify campus police.

Fires

In case of fire, immediately notify everyone in the affected area, and GTAC staff is present. If the fire is small and in a contained space you can try to put it out using one of the Fire Extinguishers (located near the entrance doors to HH444 and HH445). If the fire is too big or out of control, pull the lever on a hallway fire pull-station, and evacuate the building. Call Campus Police at 338-2222 from outside of the affected area to advise them of the situation.

Hazardous Materials Spills

Alert lab staff immediately in case of spills. **If the spill presents a hazardous situation** for you o the environment, immediately **notify everyone in the area, the University Police Dept. at 338-2222,** and EHOS at 338-1449.

If no one is around, attempt to clean the spill only if it is safe for you to do so and you feel comfortable and competent to do so. Spill absorbent and dry neutralizers for acids and bases are stored on the shelf above the dish sink in HH445 and on the central bench in HH444. Follow the directions on the jar. Dispose of all hazardous materials in the appropriate containers.

Night/Weekend and Medical Emergencies

Emergencies can include lengthy power outages, flooding, security problems and injuries.
Call Campus Police by dialing 911 or (33)8-2222 from any campus phone.
Plant Operations has personnel on-call to deal with operational emergencies. The campus police dispatcher will contact them.
Campus police have emergency medical technicians (EMTs) on staff.
The campus police dispatcher will contact paramedics and the fire department.

HAZARDOUS MATERIALS IN USE

Several toxic substances are commonly used in the GTAC. Please take the time to become familiar with the toxics you will be using, the appropriate location for their use, their hazards and safe handling and disposal procedures. Listed below are the most commonly used toxic substances:

Phenol, chloroform, and β-mercaptoethanol (BME)

These are used in many DNA/RNA extraction protocols. All three chemicals are dangerous if inhaled or absorbed through the skin, and are NEVER to be handled (opened, dispensed into eppendorf tubes or disposed of) outside of the fume hood. Be sure to wear gloves whenever handling these chemicals. In the base of the hood, you will find the chemicals stored in a small refrigerator, and pipettors and tips available within the hood. Do not bring pipettors from other areas into the fume hood.

Dispose of all tips and tubes which have come into contact with phenol or chloroform in the specially marked containers labeled something like “Tips, tubes, etc. contaminated with Phenol/Chloroform”. Dispose of liquid wastes in the appropriately labeled waste containers. Do you best to minimize liquid in the solid waste disposal.

Ethidium Bromide

Ethidium bromide (EtBr) is used to stain DNA in electrophoretic gels. This chemical is a mutagen and should be handled with extreme care. Always wear gloves when handling any equipment or surface that may have come into contact with a stained gel or the EtBr solution This includes lab benches, gel trays and boxes, and the gel documentation system. Help us contain the spread of the chemical by working only in areas designated for EtBr use.

Dispose of used EtBr solution in the EtBr waste jug, and used gels wrapped in Saran Wrap in the black garbage can next to the gel documentation system. Always throw out your gloves in the hazardous waste garbage can when you are done handling a gel.

Acrylamide/Formamide

Acrylamide and formamide are used in preparing certain gels and fo DNA sequencing. Unpolymerized acrylamide is a neurotoxin, and formamide is a skin irritant and a suspected teratogen. Both are dangerous if inhaled or absorbed through the skin. Always wear gloves when handling acrylamide, formamide or the sequencing polymer. Polymerized acrylamide gels should be disposed of in the labeled hazardous waste bin in HH443. Tips and tubes that have come into contact with TEMED or ammonium persulfate should also be disposed of in the hazardous waste bin labeled for that purpose.

Ultraviolet Light

Ultraviolet (UV) light is used to visualize bands on electrophoretic gels. Even short-term exposure to UV light can cause eye damage and skin burns. Always place the protective plastic cover over the transilluminator before turning on the UV light, or wear one of the clear plastic masks in addition to your eye protection. If you are taking plugs from your gel, wear a mask and a lab coat to protect your arms and face from UV exposure.

CONTAMINATION CONTROL

Possible sources of contamination of PCR reactions in clued PC products and DNA fro other sources, such as your other samples, airborne fungal spores, bacteria, human skin, etc. The lab is organized to prevent contamination in a number of ways.

1. Fume hoods are available for performing DNA/RNA extractions and for PCR reaction setup. These hoods actively draw air in through the front openings, and are thus not ideal for setting up PCR, as contaminates may be actively drawn into the hood. Working with the hood **off** will at least provide a relatively clean environment.

2. A PCR reaction produces millions of copies of DNA. Keep the preparations areas separate from where PCR products are handled to minimize the possibility of contamination. **No PCR products** (or items which may have come into contact with PCR products) **can enter the HH444 pre-PCR "clean" room**.

3. It is very important that you maintain the cleanliness of this troom by being conscious that movement between them and rest of the lab is unidirectional. **Absolutely no laboratory materials from elsewhere can be brought into the pre-PCR room.** We keep the clean room stocked with all the equipment and reagents you will need for DNA extraction and PCR prepping. Plastic micro-tube trays, sample tubes and other stocks can be taken out of the clean room as needed, but cannot be brought back in.

4. Other DNA contaminants can easily get into a PCR reaction tube, by pipette transfer or by airborne agents. Standard sterile techniques prevent most contamination from these sources.

Sterile technique should be used when handling any shared stock (Taq Polymerase in particular), anything in the hoods, and tip and micro-tube containers in HH444.

Pipettor Use

Because pipettors can come into direct contact with DNA, we use filtered tips for all extractions and PCR setup in HH444. These tips are costly, so please minimize waste. Rapid pumping up and down of the pipettes will increase the chance of contaminants getting in to the pipette shaft, and also is less accurate. Be slow and gentle!

As an extra precaution, the pre-PCR room has separate sets of pipettors for handling DNA containing extracts, those which are only used with reagents, those used only with enzymes, and those used for setting up cloning reactions:

1) Red-labeled "Pre-PCR" pipettes should only be used for measuring reagents that do not contain DNA.
2) Yellow-labeled "DNA Extraction" pipets should be used for handling DNA/RNA – containing materials during Extraction.
3) A special yellow-labeled "Template Only" pipet is provided in each PCR setup hood for loading DNA-containing genomic extracts into the PCR tubes just prior to cycling.
4) White-labeled "Enzyme Only" pipets are provided in PCR setup hoods for dispensing Taq. Only open tubes of Taq polymerases inside the hoods.
5) Use "Cloning Only" pipets for setup of cloning reactions and for use with reagents, competent cells, and media used for cloning.

Work done in HH445 is done with pipettors that regularly see lots of amplified PCR product. The pipettors on the mini-gel loading bench are continually used to pipet concentrated DNA, and so should be used for nothing else. **No other pipets from the lab should be moved to the mini-gel bench.**

Sterile Techniques

1. Hood use

The hoods in HH444 are not ideal for sterile technique, as they are actually fume hoods. When they are turned on, air is drawn in through the front opening(s). This may draw in any contaminants on your skin or from the outside environment. Therefore, for creating a mostly sterile working environment:

a) **keep the hoods off** if not working with toxic chemicals
b) **clean the bench surface** with bleach and ethanol

2. Pipette tips
Be conscious of what your pipette tip has touched. If you transfer a reagent into anything but an empty eppindorf tube, change tips before reusing the pipettor.

3. Micro-tube containers
Do not pick micro-tubes directly out of the containers. Pour tubes out onto the container lid. Pick each tube out one at a time and avoid touching the other tubes.

4. Gloves
If you have been working in HH445, remove your gloves before entering the pre-PCR room.

Stock Usage
DNA extraction stocks are stored in shelves in HH444. Take your own personal stock tube or dispense and aliquot from tubes found in the trays labeled "Stocks in Current Use."

PCR stocks are aliquotted into micro-tubes and kept in racks in the "PCR" freezer in HH444. Take tubes as you need them, and store them in a tray labeled on the front with your name, in the racks provided inside the freezer.

It is very important to the smooth functioning of the lab that you help us keep track of inventory. If you only see a few (five or less) stock tubes left, let us know by writing the name of the stock, the amount left, and your initials on the white bulletin board in HH445.

Broken Equipment
Please notify GTAC staff if you know or suspect that a pipettor or other piece of equipment is not working properly. Place a note on large pieces of equipment or tape a note onto pipets or other small pieces of equipment and move them to:

The chemical bench in HH444 (items from pre-PCR room)
The work bench under the backup tip shelf in HH445 (items from post-PCR room)

Sample Storage
All your sample tubes should be labeled clearly and stored in a tray or closed box **with your name and lab on it.** Please **store different types of materials in the appropriate fridge or freezer** (DNA extracts, PCR products, cleaned products ready for cycle-sequencing, sequence reactions ready for loading – all these storage areas are clearly labeled with the type of materials that can be stored inside.

Every single tray or box that is kept in the GTC storage areas **MUST be labeled with your name** (preferably on both ends of the tray or on top and side of boxes). DNA extract MUSt be stored in closed boxes, not open racks.