

Cell Lab Protocols:

General

- ANYONE who wants to work in the cell lab, MUST be trained by ME!!!
- if you use something up or it is getting low, MARK it on the shopping list! The shopping list is taped to the fridge. If there are only 2-5 left that is considered low; it only takes 2 people to work in the lab to use this up. If it is something I need to make, find me or send me an email.
- extra stocks of flasks and plates will be kept in the lower cabinets. If you have to open the backup stock, put the item on the shopping list.
- extra containers of autoclaved eppys are kept on my shelf. If you take a container, let me know.
- common lab stocks are kept in door of the fridge. Anything in the door of the fridge is considered common unless specifically marked otherwise.
- label everything with your name and contents. It is in your best interest to label it with the date also. Your materials should be kept in one part of the fridge only (not the door). Falcon tubes should be stored in a proper container and not left loosely on the shelves.
- if you start a new stock of cells, mark it on the cell inventory list.
- goggles/glasses, gloves, and lab coat must be worn while working. The gloves should be put on such that they cover the cuffs of your lab coat.
- your personal protective equipment must stay in the BL2 lab: do not wear your lab coat out of the room. Do not wear gloves out of the room. If you need to leave the room during an experiment you must remove your gloves and lab coat and wash your hands. People touch the doors with their bare hands, so you need to have clean (ungloved) hands before you touch the handle.
- the 309-side sink is for washing hands only. Do not touch the sink, handles, paper towels with gloves. Liquid waste and bottles go on the 307-side sink.
- the paper towels for washing your hands are kept above the sink. DO NOT use these paper towels for wiping down your media, they are only to be touched with clean, bare hands.
- if you need to put live cells in the analyst, place the biohazard magnet on the analyst while it is in use. When you are finished, wipe down the analyst and remove the magnet.

Reserving Instruments and Hoods

- You must reserve an instrument 24hrs in advance. If you forget and want to reserve time that day, you must ask everyone in the lab first and send out an email stating the times of reservation.
- People who have reserved the hood/instrument have priority on the instrument and you must check with them if you want to use it during their time
- Being late for reservation times
 - if you cannot be found in the lab, after 15min a person can use the hood for **short** experiments and you must wait until they are finished. When they are done, the reserved time reverts back to you
 - if you are in the lab you still keep the rights to your reservation and people need to find you before working in the hood, but you should write on the sash of the hood what time you will actually be starting
- A 2nd aspirator and pipettor will be put in the large hood for passaging/feeding cells. The person currently in the large hood has priority and if you wish to use the 2nd setup, you must ask the person currently in the hood
- there are 4 sign up sheets in the lab: current use in the hood, reserving a hood, reserving the centrifuge, and reserving the microscope
- current use in the hood
 - The current use of the hood will be taped to the top of each hood. When you start working in the hood, sign your name and time. When you finish working in the hood, sign out. If someone is using the hood after you, you must still clean the hood and sign out.
- reserving the hood.
 - The reservation list will be taped to the glass cabinet across from the hood. If you are going to need to use the hood exclusively for more than 1hr or at a specific time, you need to reserve it. For times longer than 2 hours, you must reserve the hood a day in advance.
 - If you wish to reserve a hood and the other hood is already reserved, you must send an email to the lab stating the time of overlap when both hoods are in use
 - If you are not willing to share space in the large hood, you should note this on the reservation sheet
 - If you forget to reserve the hood 24hrs in advance and the other hood is not reserved, you can reserve that hood that day without emailing the lab
 - If during your reserved time you have significant breaks, mark on the reservation sheet when those free times are (if you know ahead of time) and write on the sash of the hood until what time the hood is free (during the actual free time)

-reserving the centrifuge

The reservation list is taped to the glass cabinet above the centrifuge. If you need to use the centrifuge at a specific time, reserve it.

-reserving the fluorescent microscope

The reservation sheet is taped to the wall next to the microscope. If you need to reserve the microscope at a specific time, reserve it.

Microscopes

-you must sign in when you turn on the mercury lamps.

-you must sign out when you are finished using the lamp.

-turn off the lights when you are finished with the microscope. The white light should be turned off whenever you are not actively using the microscope. The shutter to the mercury lamp should be closed in between times you are using it, if you leave the actual lamp on for later use that day.

-the camera stays with the microscope. Each room has its own memory card for the camera. When transferring pictures, use the memory card, not the camera. A card reader is located in the computer room in a red folder in the black filing cabinet.

-clean gloves can be worn when handling the microscope and computer. Dirty gloves should not be worn.

Using the Hood

-spray the hood thoroughly with 70% EtOH and let it evaporate

-anything that goes into the hood needs to be sprayed with 70% EtOH. This includes your gloves before you go in the hood. Place it in the hood and let the EtOH evaporate before using it.

-anything that leaves the hood needs to be sprayed with 70% EtOH. If you need to rewarm media in the water bath, it needs to be sprayed with EtOH before being placed in the water bath. The one exception is things going directly into the incubator should not have wet EtOH on them. If you spray them, let the EtOH evaporate before putting it in the incubator.

-try to only put in the hood what you are going to use. If there is anything extra you brought into the hood, but do not use, just leave it in the hood for the next user. Bring the whole package of flasks into the hood, and reseal the package in the hood, leaving any extra flasks for the next users.

-limit the number of times you must enter and exit the hood; put everything you need in the hood before you start working.

-solvents that are placed in the water bath need to be wiped down with a paper towel before they are sprayed with EtOH.

-do not work on or near the vent at the front of the hood, you should work in the middle of the hood to keep the air flowing properly.

-leave the air on in the hood when you are done. If for some reason the air flow is off when you enter the lab, come find me before beginning to work.

Cleaning Up

-if the tip waste in the hood is full, empty it into the waste basket

-if any of the pipette tip boxes are low/empty, replace them with new ones.

-clean the vacuum line by sucking 70% EtOH from the bottle in the hood through the line. After turning off the vacuum, lift the line up to make sure all the EtOH has emptied out of the line. If you empty the EtOH bottle, refill it and put it back in the hood.

-throw any trash from the hood into the biohazard waste, this includes any media bottles that were in the hood and the gloves you were wearing.

-liquid waste must either be sucked through the vacuum line or poured into the liquid waste located next to the sink. Liquids should NOT be placed in the biohazard box waste.

-take the pipettes and put them in the appropriate container. 50mL pipettes go in one container and all other pipettes go in the other container.

-make sure the vacuum line and pipette man thingee are turned off.

-wipe down the hood with 70% EtOH and a paper towel. Then spray down the hood thoroughly with 70% EtOH and let it evaporate.

-check the solvent traps. If they are full, empty them into the waste containers. Add bleach to the bottom of the now empty traps. The final solution should be 10% bleach, so add 100uL bleach to the large flasks and 50uL bleach to the secondary trap.

-if the waste containers are filled with media to the fill line, add bleach to the curve of the container, cover and let sit overnight. The media should turn purple and then yellow after the bleach is added.

-glass bottles that you used need to have ALL tape removed before placing them in the dirty bottle container. The tape goes in the biohazard waste.

-trash that did not enter the hood or touch media or cells can be thrown in the regular trash.

-wash your hands and forearms with soap and water before leaving the lab.