Working in the Transfer Hood

- The hood should remain on continuously. If for some reason it has been turned off, turn it on and let it run for at least 15 minutes before using.
- Make sure that everything needed for the work is in the hood and all unnecessary things are removed. As few things as possible should be stored in the hood.
- Check the bottom of the hood to make sure there is no paper or other debris blocking air intake.
- Remove watches, etc., roll up long sleeves, and wash hands thoroughly with soap (preferably bactericidal) and water.
- Spray or wipe the inside of the transfer hood (bottom and sides, not directly on the filters) with 70% EtOH. Others use disinfectants such as Lysol®. Wipe the work area and let the spray dry.
- Wipe hands and lower arms with 70% EtOH. It is not necessary to flame them (This is a joke.).
- Spray everything going into the sterile area with 70% ethanol. For example, spray bags of petri dishes with 70% alcohol before you open them and place the desired number of unopened dishes in the sterile area.
- Work well back in the transfer hood (behind the line). Especially keep all flasks as far back to the back of the hood as possible. Movements in the hood should be contained to small areas. A line drawn across the distance behind which one should work is a useful reminder.
- Make sure that materials in use are to the side of your work area, so that airflow from the hood is not blocked.
- Don’t touch any surface that is supposed to remain sterile with your hands. Use forceps, etc.
- Instruments (scalpels, forceps) can be sterilized by flaming - dipping them in 95% EtOH and then immediately placing them in the flame of an alcohol lamp or gas burner. This can be dangerous if the vessel holding the alcohol tips over and an alcohol fire results. A fairly deep container, like a coplin-staining jar, should be used to hold the ethanol. Use enough ethanol to submerge the business ends of the instruments but not so much that you burn your hands. Some people wear gloves in the hood for certain procedures. If you do this, be very careful not to get them near the flame. Other methods of sterilization that do not require alcohol are with a bacticinerator or glass bead sterilizer. There is not as much risk from fire with these, but the instruments can still get extremely hot, causing burns.
- Arrange tools and other items in the hood so that your hands do not have to cross over each other while working. For a right-handed person, it is best that the flame, alcohol for flaming, and tools be placed on the right. The plant material should be placed to the left. All other items in the hood should be arranged so that your work area is directly in front of you, and between 8 and 10 inches in from the front edge. No materials should be placed between the actual work area and the filter. Keep as little in the hood as possible.
• Plant material should be placed on a sterile surface when manipulating it in the hood. Sterile petri dishes (expensive), sterile paper towels, or sterile paper plates work fine. Pre-sterilized plastic dishes have two sterile surfaces—the inside top and inside bottom.

• Sterilize your instruments often, especially in between individual petri plates, flasks, etc. The tools should be placed on a holder in the hood to cool or should be cooled by dipping in sterile water or medium before handling plant tissues.

• Wipe up any spills quickly; use 70% EtOH for cleaning. Clean hood surface periodically while working.

• Use of glass or plastic pipettes: Glass pipettes are put into containers or wrapped and then autoclaved. Plastic pipettes are purchased presterilized in individual wrappers. To use a pipette, remove it from its wrapper or container by the end opposite the tip. Do not touch the lower two-thirds of the pipette. Do not allow the pipette to touch any laboratory surface. Insert only the untouched lower portion of the pipette into a sterile container.

• Sterilize culture tubes with lids or caps on. When you open a sterile tube, touch only the outside of the cap, and do not set the cap on any laboratory surface. Instead, hold the cap with one or two fingers while you complete the operation, and then replace it on the tube. This technique usually requires some practice, especially if you are simultaneously opening tubes and operating a sterile pipette. After you remove the cap from the test tube, pass the mouth of the tube through a flame. If possible, hold the open tube at an angle. Put only sterile objects into the tube. Complete the operation as quickly as you reasonably can, and then flame the mouth of the tube again. Replace the lid.

• Inoculating loops and needles are the primary tools for transferring microbial cultures. We use plastic ones that come sterile. If you are moving organisms from an agar plate, touch an isolated colony with the transfer loop. Replace the plate lid. Open and flame the culture tube, and inoculate the medium in it by stirring the end of the transfer tool in the medium. If you are removing cells from a liquid culture, insert the loop into the culture. Even if you cannot see any liquid in the loop, there will be enough cells there to inoculate a plate or a new liquid culture.

• If you don't have to be careful about the volume you transfer, a pure culture or sterile solution can be transferred to a sterile container or new sterile medium by pouring. For example, we do not measure a specific volume of medium when we pour culture plates, although after you have done it for a while, you become pretty consistent. Remove the cap or lid from the solution to be transferred. Thoroughly flame the mouth of the container, holding it at an angle as you do so. Remove the lid from the target container. Hold the container at an angle. Quickly and neatly pour the contents from the first container into the second. Replace the lid.

• If you must transfer an exact volume of liquid, use a sterile pipette or a sterile graduated cylinder. When using a sterile graduated cylinder, complete the transfer as quickly as you reasonably can to minimize the time the sterile liquid is exposed to the air.

• Remove items from the hood as soon as they are no longer needed. All cultures must be sealed before leaving the hood.
• When transferring plant cultures, do contaminated cultures last. Situate the cultures so that the contaminated part is closest to the front of the hood.

• Place waste in the proper containers: Empty (e.g. after transfer) or old petri plates used in transformation experiments go in the big bag to be autoclaved, as do other disposable that were in contact with recombinant bacterial or plant material. All needles go in the sharps box, needles used with bacteria get autoclaved. Small bags used in the hood for waste go in the big bag to be autoclaved; do not overfill the small bags or leave full bags in or on the hood for someone else to dispose of. Glassware that comes in contact with bacteria is placed in a separate pan to be autoclaved.

• When finished in the hood, clean up after yourself. Remove all unnecessary materials and wipe the hood down with 70% EtOH.

• **Be sure when you are finished that you turn off the gas to the burner!**

• It is pointless to practice good sterile technique in a dirty lab. Special problems are contaminated cultures, dirty dishes and solutions where microorganisms can grow.

• Store cultures in a sequestered area. We will discuss this area later. Check cultures every 3-5 days for contamination.