Good aseptic technique and general lab safety

Inoculating or transferring cultures

- Swab bench before starting.
- Work close to the Bunsen burner. Use the 'Hot flame' (bright blue cone inside lighter blue cone) to get a strong updraft, to flame the necks of bottles, and to flame your loop. The 'Safety Flame' (yellow) is only good for lighting the ethanol on the glass spreader when you are doing spread plates, it has no other real use.
- Turn off the Bunsen burner whenever you aren't immediately at your bench using it.
- Keep the work area clear of clutter and mess
- Don't do culture work directly on top of your lab book or other paper notes – if there is a spill, you don't want to have to autoclave your lab book !
- If working with slow-growing organisms, it can be helpful to swab hands or gloves with 80% ethanol. If you do this, don't get the ethanol near the flame, and wait for it to evaporate off hands before lighting Bunsen!
- Flame the neck of bottles and tubes when opening and closing them (pass thru the Bunsen flame briefly e.g. back and forth 3 times over 3 seconds).
- Be mindful of where you are putting things down – e.g. don't put down your inoculating loop on the bench with culture in it, flame it first, then put back upright in correct holder
- Secure your lab coat or lab gown sleeves so that they don't flap around – these are a common source of contamination. Sticky tape them or elastic band them to fit snugly, or roll them up a little bit (don't do the later if you are working with corrosives or toxics or UV radiation ! in those cases, its important to have the sleeves all the way down)

Sterile bottles, solutions, and gear

- Make sure everything is LABELLED ! With the chemical, the date, and your name.
- Make sure any hazardous reagents are clearly labelled with the chemical name, the concentration, and the nature of the hazard (e.g. flammable/corrosive/toxic)
- When you take lids off, don't put them down on the bench unless it's absolutely necessary; and if you have to do this, put them upside down, so the screw thread is facing upwards. SURFACES are by far the biggest problem for microbial contamination
- Don't leave the lids off bottles for any longer than is necessary.
- Getting sterile eppi tubes out of a sterile beaker:
    - acceptable way #1. take off foil top, carefully pluck out tube by its base or edge, place carefully in tube rack, repeat if necc., then replace foil
    - acceptable way #2. take off foil top, shake out some tubes into foil, carefully place them in rack, careful not to touch any part of the inside of the tube, replace foil.
    - UNACCEPTABLE: plunge your hand into the beaker and rummage around vigorously, then emerge triumphantly with a tube, leave the foil lid off until tomorrow.

Pipetting with Gilsons

- Swab down your pipettes with 80% ethanol at least once each day, before starting work. Also do this if you change from doing microbiology to DNA work, or when changing from working with one type of microbe to another, or whenever you think it has become contaminated.

- If you have persistent issues with contamination, a more serious cleaning of pipettes is required. Dissemble the pipette and soak in bleach (0.5% hypochlorite = a 1/10 dilution of household bleach, freshly prepared), so that all parts of the pipette are covered. Let them soak for 30 min, then rinse extensively with water, then with 80% ethanol, then allow to dry. Don't leave pipettes in bleach longer than 30 min, or you will rust the metal parts.

- Remember that ONLY THE TIP IS STERILE ! Even if you have swabbed it with ethanol, the main body of the pipette is still contaminated with microbes and enzymes and DNA etc. The body of the pipette should not come into contact with anything that needs to stay sterile (e.g. it the inner wall of bottles or Falcon tubes when you are pipetting liquids out of these)

- Hold the pipette VERTICAL! ….or as close to vertical as possible. If you hold it sideways, the liquids may run up into the barrel of the pipette, which could be catastrophic for your PCR or your culture, when they run back down into the tip, carrying junk with them.

- Plugged pipette tips are not needed for most kinds of work, if you are careful with your pipetting. The only situations where these are really essential are for RNA work, and for 16S rDNA PCRs – the latter is exceedingly sensitive to all kinds of DNA contamination.

- Be careful when getting the tips out of the box. Open it all the way, put on the tip firmly, move the pipette away, then close the box. Don't hit the tip on the edge of the lid, and don't leave the tip box open after you have got the tip out.

Setting up PCRs and other enzyme reactions

- Swab down bench and pipettes before starting. Label all tubes before starting. Read through the procedure carefully before starting.

- Keep all enzymes in the freezer or on ice at all times. This means restriction enzymes, ligase, polymerases, reverse transcriptase, phosphatase, kinase, Gibson assembly mix, etc. The only exceptions here are thermostable polymerases (Taq, Pfu, Phusion); these can be kept on the bench for a little while during setup of a reaction (they are thermostable!).

- Keep all DNAs (plasmids, PCR products, genomic DNAs) either in the fridge or the freezer whenever they are not actively being used. DNA can sit on the bench for a little while (a few minutes up to an hour or so), but don't leave it at room temp longer than this.

- Double check all buffer and incubation requirements (time? temperature?)

- Make up the buffer first (to 1x conc), *then* add the enzymes and/or DNA.

- Handle tubes carefully: don't touch any part of the inside of the eppi tubes or PCR tubes, this includes the inside part of the lids! This takes some care and some practice. Especially be careful when opening Eppi tubes, its easy to push your thumb up onto the inside of the lid; use your thumbnail (not your whole thumb) to carefully flick open the lid from the outside catch

- Be extra careful during any procedures where you are putting tubes on ice. The ice (or melted ice) must not get into your tubes ! So don't push the tubes too far down into the ice, and handle them carefully.