Protocols for Wet Lab Anaerobic Work

**Innoculating New Media**

Before Adding the Cells

1. Test for pressure in each tube of media by flame sterilizing all the tubes and then pushing an unattached needle into each one, making sure to hear the hiss of depressurizing.
2. Switch on Nitrogen for sparging, switch it to the gassing canula, and turn on the Bunsen burner
3. Assemble a small syringe (1 mL) for adding Na2S and an additional small syringe for each tube being inoculated
4. Flame sterilize the canula, then sparge the syringe ~10 times. Finish with a full syringe of nitrogen to replace Na2S you’ll be removing
5. Quickly flame sterilize the Na2S, insert the syringe, and push out all the nitrogen.
6. Invert the Na2S bottle and remove 0.1 mL for each tube being inoculated.
7. Pull out the syringe/needle, then flame sterilize each tube of media, insert the syringe, and inject 0.1 mL. Do this until each tube has Na2S

Adding the Cells

1. Flame sterilize the canula, then sparge the syringe ~10 times. Finish with the syringe full of nitrogen (plunger extended) to replace the cells you’ll be taking out
2. Quickly flame sterilize the tube with cells, insert the syringe, and push out all the nitrogen.
3. Invert the tube with the syringe, pull back the syringe to gather cells and push up to clear bubbles. Pull back a second time to take 0.1 mL of cells and hold the syringe plunger in place with the side of the finger. Take out the syringe
4. While holding the syringe, quickly flame sterilize the tube with medium. Insert the syringe, inject the cells, yell “BAMM!”, then remove and dispose of the syringe.

Adding the Nutrients

1. Grab a sterile gassing needle and place it in the test tube holder.
2. Switch from gassing canula to gassing manifold, turn off the Bunsen burner, and turn off the nitrogen. Switch on the H2/CO2 mix so that it’s coming out of the manifold
3. Remove the cap from the gassing needle and insert the manifold needle into the gassing needle top until it’s all the way in.
4. Sterilize the top of the cell + medium tube with ethanol, then quickly blow it off with the gassing needle apparatus and insert the needle into the tube.
5. Hold the gassing needle in the media tube until the pressure equilibrates, then remove the needle apparatus and put it back in its tube.
6. Remove the manifold needle from the gassing needle and place it back in its rubber tube. Place the cap back on the gassing needle and place it in the “Used” tube rack.

**Harvesting Chemostat Cells**

1. In preparation, leave a 1-L collection bottle with a syringe-friendly in the anaerobic hood the day before harvesting