For Teagan and Laura on W 1/24:

Today, you need to do three things: (1) take GC samples from Teagan’s trimethyllysine (TML) enrichments (2) feed the yellow-labeled cultures with and (3) flush the headspace of the enrichments with new 100% N2.

**SAFETY stuff – obviously please wear gloves (in theory we could be enriching/growing pathogens in soil enrichments so we do want to treat these carefully!). Also, please prop the door of W204 open the entire time that the gas station is on.**

**Step 1 – turning on the gassing station** - *you will only need 100% N2 today!*

1. Replace the glass syringe with glass wool on the right side of the gassing station with a clean one (we have a white autoclave-safe box of sterile autoclaved syringes to the left of the gassing station).
   1. Please wrap the used syringe you take off with a capped needle on it! - in new foil, place autoclave tape on it, and place it in the white plastic cup on top of the box of sterile syringes.
   2. Take the needle off of the new syringe you just connected to the gassing station (**don’t get rid of it! You’ll need it at the end!).**
2. Open lines/black knobs on the gassing station. At first, you’ll want to only open the line to the syringe with glass wool to help you pull GC samples and feed cultures (*later, you’ll want more lines open to sparge/flush the headspace in cultures*).
3. Next, turn the grey knob connected to the nitrogen tank towards “decrease” until it feels a bit loose.
4. Once that is done, you can open the knob on the N2 tank itself
5. Then, work your way forward from the tank down the “B” line/path, opening the black knob/handle and both green lines/knobs. Once the entire system is opened, you can start to *slowly* turn the grey knob towards increase. You should hear it hissing before you can feel It coming out of the gassing station. Increase the pressure coming out of the gassing station up to 5 psi.
6. Before you start working, wipe the benchtop/counter off with ethanol!

*At this point, the gassing station is on and ready*

**Get cultures and gas sampling supplies**

1. The GC vials are on the side shelves in cardboard boxes, Laura knows where these are. The vacuum pump might not be working in time for this day, so we might have to continue trusting that they come properly evacuated.
2. Twist to tighten the caps of these vials
3. Label tubes as samples (ie OWC E08, STM E07)
4. Take the enrichments out of the drawer they are in; they are currently in the drawer labeled “laura mason” underneath centrifuge 1.
   1. Note, I’ve put two empty bags in the drawer for you to place collected gas samples into (tyou should only need one of these bags, make sure to label it with the date/what sampling day it is)
5. Vortex the tubes (we have a vortex on the bench next to the gas station) to release trapped gas from the soil slurry
6. Get a burner (might be in the flammable cabinet. If it’s one of the metal ones, fill it with ethanol before lighting, but the glass one with the wick might be less annoying/safer to use).
7. Get the glass tight syringe from the side bench (to the right of the analytical balances, under the window. We have two, they’re 10 mL, one has a pink piece of tape with the letter A on it). **Also get the H style needle next to the syringe, it’s the reusable dull metal needle in the plastic cylindrical tube with the red rubber caps.**
8. Make sure the black needle box on the bench top is full; if you run out, we have more stocks on the metal shelves to the left of the flammable cabinet.

**Sampling headspace/taking gas samples**

1. At this point, you should have all your supplies, the nitrogen coming out of the new sterile syringe at 5 PSI, and a burner going. **You should also fill the small glass petri dish at the gassing station with some ethanol (we have a carboy of 70% next to the sink, you can use this). There should also be a swab in this dish.**
2. Flame sterilize the cap of your tube by using the swab to wipe it with ethanol, and then pass the tube through the flame to burn off excess ethanol. Do this for each tube as you go to sample the gas headspace.
3. Prepare the gas tight syringe by placing one of the black disposal needles onto it.
4. Make the gas tight syringe anaerobic by filling it with ~10 mL N2 (using the syringe with gas flowing out of it) and expelling that into the air two times. **For the gas tight syringe to be unlocked/open, the black knob should be pushed *away* from the syringe body.**
5. Then, you can sample your tube by piercing the septum of a culture with the needle on the empty, anoxic syringe, and drawing up 10 mL of sample gas. Lock the syringe (push the black knob in against the glass) and then withdraw the needle from the tube.
6. While keeping the syringe locked, remove the black needle from the syringe, and replace with the reusable H style needle.
7. Then you can carefully inject this needle into the correct labeled GC sample vial, unlock the syringe (black away from body/glass), and inject the sample. Pull out the needle immediately.
8. Remove the H style needle from the syringe, place the no filled sample vial into the labeled sample bag
9. Flame sterilize the septum of the sampled culture tube
10. *Repeat for each culture tube.*

**Feeding cultures trimethyllysine (TML)**

1. You need to feed the cultures with yellow tape on them 1 mM TML. This is equal to 0.2 ml (or 200 uL) of your 50 mM stocks. Your stocks of TML are in the same drawer that your cultures were in.
2. You are going to want a few of the 1 mL syringes from the metal shelves to the left of the flammable cabinet.
3. Leave the gas running pure N2 out of the tip of the glass syringe
4. First, put a black disposable needle on a 1 mL syringe
5. Make this needle anaerobic by drawing up N2 and expelling it into the air twice
6. Flame sterilize the septum of the TML stock
7. Flame sterilize the septa of ALL your 8 culture tubes that will be fed TML
8. Then, use the anoxic needle to draw up TML. You are going to feed 8 cultures 0.2 mL each, so I would recommend drawing up 0.8 mL and using one needle to feed four cultures.
9. Once you have TML in your syringe, you can inject 0.2 mL at a time into each culture tube. If you draw up 0.8 mL substrate and feed four cultures with the first needles, you’ll only need to do this twice!
10. Flame sterilize the septa of tubes after feeding!

**Lastly, sparge the headspace of your cultures with fresh N2.**

1. You will need to adjust the gassing station here. You should have. 4 black knobs with two lines each open. You can also close the knob leading to the syringe, and go ahead and replace the capped needle on the syringe (you’re done with it).
2. Onto each black gas line opened (n=8), you should place one of the green PES syringe filters from the box on the metal shelves behind you. Doing this will constrict gas flow **so keep a careful eye on not letting the gassing station get much above 5 psi!!**
3. Flame sterilize the septa of your tubes. You are going to do two rounds of 8 tubes at a time, so only sterilize 8 at a time
4. You want then, one at a time, to
   1. Place a sterilize black needle onto the gas line with a syringe filter on it
   2. Make sure you can gently feel some gas flowing out/that you have the right knobs opened
   3. Place that needle into the septum of one of your sterile tubes.
   4. Place a loose sterile black needle into the same septum as an outlet needle (so you have filtered N2 flowing into the tube, and an outlet for the old headspace gas to be purged out)
   5. Set this up for all 8 of your tubes for round one, and then set a time for 5 minutes
   6. After these sit for 5 minutes, you can take your tubes off of the gas station. Try to pull both needles (inlet and outlet) out of one septum at the same time.
      1. Sometimes, the entire filter with needle will fall off the black line and this depressurizes the system. If it happens, just get it back on asap.
      2. Be careful not to prick yourself, there will be a lot of needles coming off suddenly
      3. **Leave the syringe filters on the gas lines!**
      4. **Flame sterilize the tubes as they come off**
   7. You are going to repeat this exact routine for the other 8 tubes. Once these are done, you can take the syringe filters off the gas lines. It’s time to shut the gas station down
   8. Extinguish your burner and let it sit on the benchtop to cool down.

**Shutting the gas station off**

1. Turn the knob on the tank itself to shut the tank. Do nothing for at least 3-4 minutes while the system bleeds itself dry of gas (you should ultimately be able to watch the pressure gauge above the black knobs drop to 0 **AND** not feel any gas coming out of the station.

Sometimes, I might close like one or two of the four black knows/lines I have open just to make sure I can see the gas pressure up at like 5 psi, and watch it drop (versus having 4 knobs/8 lines open, and its running at 1 psi and barely has a visible drop. Doesn’t matter/up to you).

1. Once you are confident that the gas is fully done running, go turn the grey knob towards decrease until ti feels a bit loose.
2. Then, move the black knob to close it, and close both green lines.
3. Lastly, go close all knobs on the gas station itself
4. Swoop the black lines over the gas station so they aren’t hanging over the counter.
5. Put your enrichments back in the same drawer, with gas samples and substrate
6. Ethanol the counter top

If you have any gas station questions while I’m out, Emily has probably used it the most beyond me!