**Incubation protocol**

1. **Calibrate EC probe** – use the 12.89 dS/m standard to calibrate at ambient temperature. Press cal until 19.99 appears. Use the up and down arrow keys and press YES to move to next digit until done. Use the temp vs EC table to input expected EC values (reiterate as necessary).
2. **BaCl2** – add 5 mL of BaCl2 to each of the 24 50mL falcon tubes (with white precipitate at the bottom).
3. **Data entry** – computer password is 1504. Sign into my OneDrive (password is oneplus1is@. Go to /projects/SI/Data/Litter incubation/Full experiment/Full incubation experiment.xlsx. Go to the 2nd sheet ‘full incubation measurements’.
4. **Measurement** – Enter the date and time in the first row of the new column by pressing ctrl+; space ctrl+shift+;

Open each jar individually, clean and dry EC probe, dip into KOH solution and wait for stabile reading (~10 sec without change). Enter data in spreadsheet.

1. Transfer the KOH measured in step 4 to the appropriate vial with BaCl2 and put the cap back on the jar. Discard the KOH in the vials that we do not collect (repetitions 4-6 of each treatment).
2. Fill a new glass vial with 15 mL of KOH solution and close the jar shut.
3. Repeat steps 4-6 for jars 49-102.
4. Measure the EC of the KOH solution in the pyrex bottle.
5. **Centrifugation** – insert 4 vials in each of the 4 buckets in pairs (each on both side of the screw…). Centrifuge at 2500 rpm for 5 minutes. Carefully discard supernatant. You need 2 batches to centrifuge all the vials. The last 2 vials are for balancing – they are labeled. Add 20 mL of DDW to each centrifuged vial, vortex and shake until the white solids detaches from vial. Centrifuge again for 5 min at 2500 rpm. Discard supernatant. Cap the vials and return to room 902.
6. **KOH solution**  - in case you need to prepare more KOH solution. Add 20.198 g of KOH to 2L of CO2-free water in volumetric flask. Stir with magnetic bar. It dissolves very quickly.
7. **CO2-free water** – fill the electric water boiler with DI water and boil. Boil 2 batches of 1.5L each - about 3L in total. IT DOES NOT TURN OFF BY ITSELF!! Add boiling water to the big Erlenmeyer flask with the white boiling stones, put on hot plate and turn on hot plate to 300-350C and boil for a couple of minutes. Turn off and unplug both appliances. Put an aluminum foil lid on the boiled CO2-free water. Use this to make KOH solutions.
8. **BaCl2**– Make 1 L of 0.3 M BaCl2 solution in a clean 1L volumetric flask. Wash out bottle that contained BaCl2 and transfer fresh solution to the bottle. Mark date on the bottle.

Thank you so much!!

Itamar