* Monday before sampling:
* -Label **strain** (7803, 8101, and 8018), **replicate** (1, 2, 3), **site** (NB, SB, CC), **dilution** (0, -1, -2, -3) and **date** on 48 well plates
* -Prepare sampling materials

|  |  |
| --- | --- |
|  | * Sample Material |
|  | * 1. Cooler (using ice packs from -20°C) |
|  | * 2. pH meter (calibrate before sampling) |
|  | * 3. Refractometer |
|  | * 4. Autoclaved 500 mL plastic bottles- 3 bottles/site (9 total)- label site and replicate |
|  | * 5. Clip-board with data sheet |
|  | * 6. Pen/pencil |
|  | * 7. 0.2µm filters/syringe -1 /site (3 filters, 3 syringes)- I bring 1 extra of each just in case |
|  | * 8. 2 large jugs to collect water from CC (DNA and for filtering) |
|  | * 9. 2 large jugs to collect water from NP and SB (DNA) |
|  | * 10. HCl washed scintillation vials- 2/site (6 total)- label site, rep and date |
|  | * 11. Urine Cups- 1 cup/ site (3 total)- label site |
|  | * 12. permit |

* Tuesday before sampling:
* -Label **site**, **replicate** and **dilution** on 50mL tubes (on the caps as well)
* -Prepare 2 bottles of SN
* -Fill labeled 50mL tubes with 27mL of SN media and store in 4˚C fridge
* Wednesday (sampling day)
* -Arrive at 7am at the lab
* -Put ice packs in cooler
* -take out 50mL tubes filled with SN from the 4˚C fridge to thaw
* Directions:
* **CRYSTAL COVE**
* -Get on Bison and make a LEFT on East Peltason.
* -Continue on East Peltason and make a RIGHT on Anteater
* -make a RIGHT on Bonita Canyon Dr.
* -make a LEFT on Newport Coast Dr. and go all the way down to the PCH
* -make a LEFT on Coast Highway
* -Make a RIGHT on first light (one side of the street name is Los Trancos)
* **NEWPORT PIER**
* -Make a LEFT on Pacific Coast Hwy
* -Follow signs to Newport Blvd
* -Make right to get on the 1-freeway/Newport Blvd (second entrance-it’s a ramp that turns into the 1-fwy)
* -continue on Newport Blvd and make a RIGHT on 21st street
* -park at one of the metered parking spaces
* **SEAL BEACH**
* -Make a LEFT on Newport Blvd; stay on the RIGHT lane (so you don’t end up on Balboa Blvd)
* -Make a RIGHT on the 1-fwy/PCH North bound (2nd entrance)
* -Continue on until you hit Seal Beach Blvd
* -Make a LEFT on Seal Beach Blvd and drive until you just pass the pier.
* -Park on the street and walk across the street, down the parking lot and straight to the beach.
* -Take Seal Beach Blvd back towards the PCH. Keep going straight until you hit the 405-S
* -Take the 405-S to the 73-S. Exit Bison
* -Make a left on Bison and continue to campus (to drop off the samples)
* Sampling directions:
* -Collect seawater in three 500mL bottles. Be sure to rinse 3 times before collecting the water (get some water, swirl it around in the bottle and dump it out)
* -Collect 1 large jug of seawater for DNA (2 jugs for CC). Also needs to be rinsed 3 times before collecting.
* -Fill a urine cup with seawater (rinse 3 times) take a sterile syringe and fill with a little of the water. Pull the plunger back and swirl the water around. Get rid of the water and draw ~60mL of seawater. Put one of the luer-lock filter (0.2µm) filters on the end of the syringe, twist it tight to close it, and filter the water into 2 acid washed scintillation vials.
* -Use the remaining water in the urine cup to measure the pH and salinity.
  + pH- turn pH meter on and stick the probe into the water to measure pH and water temperature
  + salinity- take a drop of water and place it on the surface of the refractometer and close the lid. Hold it to the light and read the number the line hits
* **MPNs**
* -Whoever is in charge of preparing for MPNs should show up no later than 9:30am to start pipetting the cells.
* -Pipet 100µl of cells (7803, 8101, 8018) to all the wells in the 48-well plate respectively
* -When samplers come back with the seawater, take each 500mL bottle, swirl it around and pour it in the empty (0) rep 50mL tube. Fill to 50mL
  + each site has 3 reps, with a total of 9.
* -Centrifuge the 9 tubes at 4500rpm for 10 minutes. Make sure to check whether the machine is balanced (one of the inserts is 5g heavier than the rest of them)
* -Prepare serial dilutions
  + Pipet 3mL of seawater from the top of the (0) dilution and transfer it to the (-1) dilution. Vortex. From the (-1) dilution, you take 3mL of seawater and transfer to the (-2) dilution, and so on. Be sure to vortex the -1, -2, -3 dilutions well before transferring and change pipet tips between each dilution.
  + DO NOT VORTEX THE (0) DILUTION!!
* -Organize the 48-well plates and 50mL centrifuge tubes according to the replicates
  + i.e. CC rep2 (0, -1, -2, -3), NB rep2 (0, -1, -2, -3), and SB rep2 (0, -1, -2, -3) all go together with the plates that have rep 2 on it
* -Pipet 100µl of seawater in all wells EXCEPT the control row with a stepper-pipet (setting 1 with the 5mL tip)
  + Pipet from highest dilution (-3) to lowest dilution (0) with one tip. Change tips between sites (so you should only have 3 tips on your bench)
* -Let the plates sit for ~15 minutes after adding the seawater
* -Add 500µl fresh SN media to the wells with a 25mL tip at setting 1 on the stepper-pipet

-Parafilm CAREFULLY to avoid cross contamination (splashing), and place in random order in the light incubator.