Prep

Make Stock Leachate

First make 400mL of each stock solution from the 1000 mg/L prepared leachate

- [ ] Make 10 mg/L stock:

  1000 mg/L * V1 = 10 mg/L * 400mL

  V1 = 4mL

  400mL of 10mg/L stock = 4mL of 1000mg/L stock + 396mL of FSW

- [ ] Make 1mg/L stock:

  10mg/L * V1 = 1mg/L * 400mL

  V1 = 40mL

  400mL of 1mg/L stock = 40mL of 10mg/L stock + 360mL of FSW

- [ ] Make 0.1 mg/L stock:

  1mg/L * V1 = 0.1mg/L * 400mL

  V1 = 40mL

  400mL of 0.1mg/L stock = 40mL of 1mg/L stock + 360mL of FSW

2. Then dilute the stock to each treatment vial/jar

For a 20mL scintillation vial for coral embryo experiments with a final target volume of 15mL:

1mg/L HIGH treatment

10mg/L * V1 = 1mg/L * 19mL

V1 = 1.9mL

19mL of 1mg/L leachate = 17.1mL of FSW + 1.9mL of 10mg/L stock

0.1 mg/L MID treatment

1mg/L * V1 = 0.1mg/L * 19mL

V1 = 1.9mL

19mL of 0.1mg/L leachate = 17.1mL of FSW + 1.9mL of 1mg/L stock

0.01 mg/L LOW treatment

0.1mg/L * V1 = 0.01mg/L * 19mL

V1 = 1.9mL

19mL of 0.01mg/L leachate = 17.1mL of FSW + 1.9mL of 0.1mg/L stock

ZFIX

Going from 18.5% to 4% in a ~200mL final volume!

We want a total of 200 mL volume

0.185 * V1 = 0.04 * 200mL

V1 = 43.24mL

To get 200mL of 4% ZFIX: Add 43.24 mL of 18.5% ZFIX to 156.76mL of millipore water

Freeze (& shield)

To preserve embryos for RNA extraction and sequencing we will first submerge them in Zymo DNA/RNA Shield, and then pronptly transfer them to a -80C freezer. Here, we won't be dunking them in LN2. The combined DNA/RNA Shield and the quick freeze (due to small size) they will experience in the -80 are together sufficient for preserving RNA.

We will first very gently use a tip-clipped transfer pipette to transfer embryos to our 0.5mL screw-cap cryo-tubes. Wewill then carefully decant any excess FSW out of the tube, and then add 500uL of Zymo RNA/DNA Shield. Gently invert cryo-tubes a few times to ensure embryos are fully submerged. Once cryo-tubes are filled with their samples, arrange them in wax paper boxes and promptly shift them to -80°C freezer for storage until samples are ready to be processed.

Spawn Night

19:00 - 19:30

- [ ] Move each colony to a numbered/named cambro chamber bin or 5 gallon bucket
- [ ] Ensure each colony has enough headspace so that bundles can rise to the surface
- [ ] Reduce blue holding tank water level to below the lip of the chambers
- [ ] Decant water in chambers such that bouyant bundles don't spill out

19:30 - 20:00

- [ ] Organize the following collection materials at the spawning tubs:
  - [ ] 50mL falcon tubes (1 for ea. colony) filled with 30mL of 0.22micron FSW
  - [ ] Tip-clipped transfer pipettes
  - [ ] Red Headlamps (new batteries)
  - [ ] Printed waterproof spawn collection data sheet
- [ ] Organize the following collection materials at the dry lab bench:
  - [ ] 20mL scintillation vials prepped with treatments
  - [ ] 50mL falcon tube rack (for working with active bundle-bundle crosses)
  - [ ] Tip-clipped transfer pipettes (for moving bundles to scintillation vials)
  - [ ] Printed waterproof bundle-bundle cross metadata sheet (for recording parent colonies of crosses)

20:50 - 21:20

- [ ] Observe for setting (polyp expansion and bundle visibility) via red headlamp
- [ ] Find a spawner!
- [ ] Collect 24 bundles from each spawning colony and transfer them to a pre-labeled 50mL falcon tube with 30mL of FSW
- [ ] Once we have at least 2 colonies worth of bundles, 1 person will move with any filled falcon tubes to the dry lab bench and begin bundle-bundle crosses

- [ ] 1 person will remain and continue to collect bundles from colonies, periodically transferring them to the bundle-bundle crossing station at the dry lab bench

21:20 - 22:00

- [ ] Cross fertilize by taking one bundle each from two distinct colonies and transferring them to the same scintillation vial
- [ ] Record the parent colonies of each cross on the embryonic development metadata sheet
- [ ] Only transfer intact bundles! Once eggs break up and begin to hydrate, the spawn party is over

22:00 - 22:30

- [ ] Observe scintillation vials for bundle breakup and egg hydration
- [ ] Record the times when the first and last vials experience hydration
    - [ ] First Egg Hydration Time_____
    - [ ] Last Egg Hydration Time_____
- [ ] Average the times above , and add 4 hours, 9 hours, and 14 hours
    - [ ] Average Egg Hydration Time_____
    - [ ] 4 hours post-fertilization[1] _____
    - [ ] 9 hours post-fertilization_____
    - [ ] 14 hours post fertilization_____
- Set alarms for ~40 minutes prior to the times above

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-   [ ] (& on July 6th make another batch of leachate!)
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22:30 - 23:00

- [ ] Return each colony to blue tank and remove chambers
- [ ] Return water height to normal
- [ ] Clean up anything left outside

23:00 PM - 01:45

- [ ] 2.5 hr nap time!

02:15 - 03:30

- [ ] ZFIX 4hpf samples (4Z)
    - [ ] Use a transfer pipette to gently move embryos from scintillation vials to microcentrifuge tubes
    - [ ] Decant any excess FSW with transfer pipette
    - [ ] Add ~1mL of 4% ZFIX to each microcentrifuge tube in the fume hood!
    - [ ] Gently invert each capped scintillation vial to mix the ZFIX into the filtered seawater and sample
    - [ ] Record time of fixin' : _____

- [ ] Add 8-12 hours to fixin' time to transfer from ZFIX to 70% ethanol: _____
- [ ] Set fixed vials in 4C fridge

- [ ] Freeze 4hpf samples (4F)
  - [ ] Gently transfer live embryos to cryo-vials
  - [ ] Decant excess FSW using a transfer pipette
  - [ ] Add 500uL of Zymo DNA/RNA Shield
  - [ ] Promptly transfer to -80C freezer
- [ ] Make PVC Leachate (on July 6th!)

03:30 - 06:45

- [ ] 3.25 hr nap time!

07:15 - 08:30

- [ ] ZFIX 9hpf samples (9Z)
  - [ ] Use a transfer pipette to gently move embryos from scintillation vials to microcentrifuge tubes
  - [ ] Decant any excess FSW with transfer pipette
  - [ ] Add ~1mL of 4% ZFIX to each microcentrifuge tube in the fume hood!
  - [ ] Gently invert each capped scintillation vial to mix the ZFIX into the filtered seawater and sample
  - [ ] Record time of fixin' : _____
  - [ ] Add 8-12 hours to fixin' time to transfer from ZFIX to 70% ethanol: _____
  - [ ] Set fixed vials in 4C fridge
- [ ] Freeze 9hpf samples (9F)
  - [ ] Gently transfer live embryos to cryo-vials
  - [ ] Decant excess FSW using a transfer pipette
  - [ ] Add 500uL of Zymo DNA/RNA Shield
  - [ ] Promptly transfer to -80C freezer

08:30 - 11:45

- [ ] 3.25 hr nap time!

12:15 - 13:30

- [ ] ZFIX 14hpf samples (14Z)
  - [ ] Use a transfer pipette to gently move embryos from scintillation vials to microcentrifuge tubes
  - [ ] Decant any excess FSW with transfer pipette
  - [ ] Add ~1mL of 4% ZFIX to each microcentrifuge tube in the fume hood!
  - [ ] Gently invert each capped scintillation vial to mix the ZFIX into the filtered seawater and sample

- [ ] Record time of fixin' : _____
- [ ] Add 8-12 hours to fixin' time to transfer from ZFIX to 70% ethanol: _____
- [ ] Set fixed vials in 4C fridge

- [ ] Freeze 14hpf samples (14F)
  - [ ] Gently transfer live embryos to cryo-vials
  - [ ] Decant excess FSW using a transfer pipette
  - [ ] Add 500uL of Zymo DNA/RNA Shield
  - [ ] Promptly transfer to -80C freezer

Ethanol Transfer Times

4hpf: _____

9hpf:_____

14hpf:_____

::: callout-tip
You can do this!
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