**071020 PCJ**

**Hematodinium assay gels**

1. Use 2% agarose in 1X TBE.
2. Set up gel rig. Remember to level gel rig before pouring gel. **DOUBLE comb gel**.
3. Heat agarose in microwave.
4. Cool agarose with cold tap water.
5. Let bubbles pop before pouring.
6. Pour, lift combs, replace combs (breaks bubbles), double check combs for bubbles.
7. Once agarose looks opaque, put gel in fridge – doesn’t have to be perfectly level, but should look flat.
8. Remove gel from fridge at Step 13 (don’t leave gel in fridge overnight)

**Preparing PCR products**

1. Locate 2 paired PCR plates in freezer. Pairs are plates with same plate number, same date, but different loci: “2014-0026 mid 040616” & “2014-0026 long 040616”.
2. Thaw PCRs
3. Label 1 new PCR plate (mark so can’t get plate turned upside down)
4. Vortex loading buffer (LB) & put 3.0 LB in each well of 96-well plate.
	1. LB recipe: 135 ul 10X tartrazine (yellow) + 3.0 ul 6X xc/bb. Gives the yellow tartrazine a greenish color so it’s easier to see what is being loaded.
5. **Vortex PCR plates. Spin down PCR plates in salad spinner.**
6. Carefully peel mat/seal off 1 PCR plate & set aside on paper towel
7. Check labels & orientation of plates. Pipet 8.5 ul of PCR product into corresponding wells of LB plate. Pipet up & down once to mix. Use new tip for each PCR. Will now have 11.5 ul in each well.
8. Seal PCR plate with shipping tape.
9. Remove mat from 2nd PCR plate & set on paper towel.
10. Check labels & orientation of plates. Pipet 8.5 ul of PCR product from 2nd plate into corresponding wells of LB plate. Pipet up & down twice to mix. Use new tip for each PCR. Will now have 20.0 ul in each well.
11. Put one of the PCR mats on LB plate – make sure mat is in ‘proper’ orientation. Or use tape.
12. Spin LB plate in salad spinner.
13. Get gel; carefully remove combs. Inspect for damaged lanes. Remove gel tray from leveling board. Put gel tray into gel box so plugs are on **RIGHT** & wells are across top of gel.
14. Get electronic m-c pipet.
15. Remove mat from LB plate (put on paper towel). To clean mats (when gel is running). Hold under hot running tap water & ‘wash’ both sides with hands. Rinse under running DI. Dry in pile of paper towels by UV hood in prep room. UV to destroy DNA.
16. Load total volume (19.5 ul on pipet to avoid bubbles) into gel wells.
	1. first & last well on each comb is reserved for ladder.
17. Vortex & load 6.0 ul HiLo ladder into the 4 ladder lanes.
18. Carefully carry gel to electrophoresis bench. Position on cafeteria tray so lid can be plugged in & connected to power supply w/o moving gel rig again.
19. Carefully pour 1X TBE running buffer into top & bottom reservoirs until buffer almost runs over gel. Dribble more running buffer into bottom reservoir so it slowly spreads across gel & into wells. Add running buffer until gel is covered. It’s OK to mix buffer from bottles, but add ~equally to upper & lower buffer chambers.
20. Plug in lid. Black electrode is at top of gel & in black socket of power supply.
21. Run at ~115 **volts** for ~3 minutes & check to see if LB is moving in correct direction.
22. Run gel until yellow tartrazine in LB from upper comb is at lower comb (~45 minutes, but quicker if running buffer is fresher).
23. Stop electrophoresis. Transfer gel to EtBr bath.
24. Turn on camera (needs to warm up for 20-30 min).
25. Pour running buffer back into bottles (mix if multiple bottle) for reuse. Rinse gel rigs with tap water.
26. Soak gel in EtBr 20 minutes.
27. Transfer gel to imaging tray.
28. Leave door open on dark room to position gel.
29. Close door & set black level.
30. Turn on UV.
31. Image gel.
	1. Expose until ladder is proper brightness.
		1. Save as “PCR *number* long mid *date* rt.tif” for right side.
	2. Overexpose gel until no more sample bands appear (don’t go crazy & obliterate image)
		1. Save as “PCR *number* long mid *date* OE rt.tif” for right side.
	3. Open door & move gel so can image left side; check gel position. Close door.
	4. Expose until ladder is proper brightness.
		1. Save as “PCR *number* long mid *date* lft.tif” for left side.
	5. Overexpose gel until no more sample bands appear (don’t go crazy & obliterate image)
		1. Save as “PCR *number* long mid *date* OE lft.tif” for left side.
32. Turn off UV. Turn off camera (can leave on until after last gel of the day)
33. Dispose of gel in metal pan under hood. Under hood, rinse imaging tray with tap water & thoroughly (both sides) w DI water.
34. Please back up gels on a flash drive (in case PC dies).