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Salting-out Protocol for Extracting HMW Genomic DNA from frozen octocorals

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High molecular weight DNA extraction from all kingdoms Long Read Club

Santiago Herrera

DISCLAIMER

Tom Jenkins' protocol modified by Santiago Herrera, 20191016.

ABSTRACT

Salting-out protocol for extracting high-molecular weight (HMW) genomic DNA from frozen octocorals for genomic methods. Suitable for genome sequencing**, RAD-seq and other methods that** require **HMW DNA. Starting material must be alive when flash-frozen in liquid nitrogen and always stored below -80C.**

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GUIDELINES

Follow steps 1-12

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MATERIALS TEXT

Reagents
Proteinase K(20 mg/ml; Invitrogen; optimal digestion at 65°C)
RNase A (100 mg/ml) optional
Cell lysis buffer (100mM Tris-Cl; 50 mM EDTA; 1 % SDS)
0.5M EDTA
β-Mercaptoethanol (very toxic, handle with extreme care in fume hood only)
7.5Mammonium acetate (cooled at 4°C)
100% isopropanol (cooled at 4°C)
70% ethanol (cooled at 4°C)
TE pH 8.0 or AE (Qiagen) buffer
Liquid N2
Dry Ice
Distilled water
Crushed ice

Supplies
Kim-wipes(small)
Aluminium foil
Whirl-pak bags (4oz)
1.5 ml microcentrifuge tubes (low-binding)
2.0 ml screw capped microcentrifuge tubes
Lighter
2x Microcentrifuge racks

Equipment
Liquid Nitrogen Cooled Mini Mortar & Pestle Set
3L Stainless Steel Dewar Flask
2x Small Styrofoam coolers
IncuShaker
Hot plate
Ultracentrifuge
Vortex
Fume hood
Small forceps
Squirt bottle for distilled water
Alcohol burner
Small beaker

SAFETY WARNINGS

Discard all liquids and solids downstream of addition of **β-Mercaptoethanol** (step 6) in appropriate hazardous waste containers. Wear full PPE when handling this chemical and be careful. Only manipulate in fume hood. Avoid any contact with skin. Re-seal stock bottle with parafilm.

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Preparation

1 Make sure that there are enough Materials and Supplies available and at the right temperature conditions.

2 Make sure that the Ultracentrifuge, Heat Block and IncuShaker are set up in the fume hood.

3 Place tube rack in IncuShaker.

4 Turn on the Heat Block and the IncuShaker and set to 65°C.

5 Pre-label 3 sets of 1.5ml microcentrifuge tubes for each sample (label one of the three tubes [the last one to be used] with a green tough-spot and full SH ID in addition to labelling the side of the tube with the date of the extraction.

6 Prepare the following digestion mix for each tube (mix in a 2.0ml tube, scale accordingly for up to 4 samples) and pre-heat in hot plate: 350µl cell lysis buffer, 42µl 0.5 M EDTA.

7 Fill 3L Stainless Steel Dewar Flask with liquid N2

8 Fill a small Styrofoam cooler with dry ice

9 Fill a small Styrofoam cooler with crushed ice

10 10. Take cryovials containing frozen tissues out of -80C freezer and place in styrofoam cooler with dry ice.

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11 11. Take Proteinase K, 7.5M ammonium acetate, 100% isopropanol, and 70% ethanol stocks out of fridge and place in cooler with crushed ice.

12 12. Pour liquid N2 from the Stainless Steel Dewar Flask into the Liquid Nitrogen Cooled Mini Mortar base up to the fill line and then place the metal microcentrifuge tube mortar holder in the base to chill. Digestion

Digestion

13 13. Sterilize the small forceps and the metal Pestle by exposing to the flame of the alcohol burner for ~10 seconds. Cool each tool by squirting distilled water on the tools (place collection beaker below to catch the water). Dry up the tools by shaking them vigorously.

14 14. Place forceps on top of a clean piece of aluminium foil.

15 15. Place the tip of the metal Pestle in a new Whirl-Pak bag

16 16. Place a 1.5 mL microcentrifuge tube (corresponding to the right sample) in the chilled MiniMortar.

17 17. Break a small piece of coral tissue (~3mm3) with the forceps (it should be very brittle and easy to break) and place in the microcentrifuge tube that is sitting in the MiniMortar.

18 18. Wipe forceps with clean Kim-wipe and place on the Aluminium foil.

19 19. Chill the Pestle by immersing the tip in liquid N2 (~10s in the Dewar Flask) while keeping it in the Whirl-pak bag.

20 20. Grind the sample to a powder with the Pestle until you don't feel any more crunching.

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21 21. Wipe Pestle with clean Kim-wipe and place on Aluminium foil.

22 22. Loosen pulverized tissue by quickly flicking the bottom end of the tube with your finger.

23 23. Add pre-heated 392µl of digestion mix and quickly mix by inverting and flicking. Place tube in heat block.

24 24. Add 10µl of Proteinase K to the mix. Place in tube rack in IncuShaker (minimize door opening).

25 25. Repeat steps 13-24 for each sample.

26 26. Incubate at 65oC for 2.5-3 hours. Mix digestion rack by invesion every 20-30min

27 27. Optional Add 2 µl RNase A. Mix by vortexing. Incubate at 37oC for 30 minutes.
Protein and Cellular Debris Precipitation

Protein and Cellular Debris Precipitation

28 28. Add 140 µl 7.5 M ammonium acetate to each tube. Mix by inversion. Incubate at 4oC (in the fridge) for 10 minutes.

29 29. Centrifuge at 12,000xg for 10 minutes.

30 30. Transfer supernatant (500 µl) to a new 1.5 ml microcentrifuge tube. Be careful not to disturb waste pellet.

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31 31. Repeat steps 28-29.

32 32. Transfer supernatant to a new 1.5 ml microcentrifuge tube (with the green tough spot label). Be careful not to disturb waste pellet.

DNA Precipitation

33 33. Add 680 µl cold isopropanol (volume ratio ~1:1). Mix by inverting gently ~50 times. Centrifuge at 8000 xg for 5 minutes.

34 34. Carefully discard the supernatant, by pouring out. Keep an eye on DNA pellet to avoid avoiding loosing it.

35 35. Pulse centrifuge the tubes and pipette out remaining supernatant while avoiding contact with the DNA pellet. DNA Washing

DNA Washing

36 36. Add 800 µl 70 % ethanol. Invert the tube several times to wash the DNA pellet. Centrifuge at 8000 xg for 1 minute.

37 37. Carefully discard the supernatant, by pouring out. Keep an eye on DNA pellet to avoid avoiding loosing it.

38 38. Repeat steps 36-37.

39 39. Pulse centrifuge the tubes and pipette out remaining ethanol while avoiding contact with the DNA pellet.

40 40. Allow pellet to air dry for 20 minutes. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve. DNA Rehydration

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DNA Rehydration

41 41. Re-suspend dried pellets with 150 µl TE or AE buffer. Invert tube to mix and spin down using a centrifuge.

42 42. Incubate at room temperature for 60 minutes or incubate in the fridge overnight.

43 43. Pulse centrifuge the tubes and store at -20oC.

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