APS Conch Shell DNA Extraction Protocol

Equipment/Consumables List

- 220 grit sandpaper/similarly abrasive disposable material
- Something to mechanically process the shells into powder – we use a custom-made stainless-steel smasher
- Bleach and ethanol for cleaning
- Forceps, weigh boats, scale
- 15- and 50-mL nuclease-free falcon tubes
- 1.5-mL nuclease-free microcentrifuge tubes
- Centrifuge(s) that can accommodate all previously listed tubes + vacuum manifold (optional, see Step 22)
- Shaking heat block
- Serological and micropipettes, and associated tips
- Extraction buffer reagents[1–5] (see Step 3)
- DNA binding apparatus:
    - Detach column from Zymo-Spin V with Reservoir.
    - Attach QIAquick® Spin Column to Zymo Reservoir and secure with parafilm.
    - Place entire binding apparatus into nuclease-free 50-mL falcon tube; lid should be able to enclose apparatus.
- QIAquick® PCR Purification Kit [50][1,2]:

Consumable	28104	Storage	Consumable	28104	Storage
QIAquick® Spin Columns	50	Room temp	Sodium acetate (3 M, pH 5.5)*	+ 100 mL	Room temp
2 mL Collection Tubes	50	Room temp	*Pellet Paint*®**	125 µL	Freezer [-20°C]
Buffer PB*	+ 500 mL	Room temp	Buffer PE***	mL	Room temp
pH Indicator I	mL	Room temp	Buffer EB	mL	Room temp

*Volume used in this protocol requires additional Buffer PB and sodium acetate to be purchased separately.
**Pellet Paint® NF Co-Precipitant increases likelihood that smaller DNA fragments will bind to column, but is optional.
***Record date alcohol added in notebook.

DNA Extraction and Purification from Marine Snail Shell

This method is suitable for the isolation of DNA from up to 1 g shell powder. Yields vary depending on source. The protocol can be scaled up to accommodate larger samples, but additional QIAquick® PB Buffer (with pH indicator), sodium acetate (pH 5.2), and Pellet Paint will need to be added in Steps 15 and 17.

☐ 1. Prepare subsampled shell material.
   - Remove exterior surface of shell with 220 grit sandpaper. Rinse throughout with DI water.
   - Clean stainless-steel smasher and forceps with detergent and DI water.
   - Rinse smasher with 10% bleach, then freshly prepared 70% ethanol. Dry with lab wipes.
   - Smash shell into a powder (anything easily picked up by forceps is likely too large).
   - Store powder in a *labeled* nuclease-free 15-mL falcon tube.

☐ 2. Weigh out up to 1000 mg (1 g) shell powder and transfer to a *labeled* nuclease-free 50-mL falcon tube.

☐ 3. Prepare extraction buffer[1–5] in *labeled* nuclease-free falcon tube.

Reagent	Volume (µL) per Sample	Final Concentration	Vol. (µL) per ___.5 Samples
EDTA 0.5 M, pH 8	3600	~0.5 M	
10% SDS solution	200	0.5%	
Proteinase K (20 mg/mL)	50	0.25 mg/mL	

☐ 4. Vortex extraction buffer, and warm at 37°C until all precipitate has dissolved.

☐ 5. Add 4 mL fresh extraction buffer to each sample.

☐ 6. Vortex to mix thoroughly, then briefly spin down.

☐ 7. Incubate samples at 55°C in shaking heat block (≥ 750 rpm) for at least 24 hours.

☐ 8. Centrifuge at 1500 x *g* for 2 minutes, and set heat block to 37°C.

☐ 9. Transfer ~4 mL supernatant to a *labeled* nuclease-free 15-mL falcon tube. **Do not** disturb or transfer any of the insoluble pellet or remaining shell.

☐ 10. Repeat Steps 3-6, then incubate samples at 37°C for at least 24 hours.
   - Prepare binding apparatus (Zymo extender + QIAquick column) as described above during the 24-hour wait.

☐ 11. Centrifuge at 2000 x *g* for 5 minutes, and set heat block to 55°C.

☐ 12. Transfer ~4 mL supernatant to the same *labeled* nuclease-free 15-mL falcon tube as Step 9. **Do not** disturb or transfer any of the insoluble pellet or remaining shell – store at -20°C.
☐ 13. Centrifuge at maximum speed (2520 x *g*) for 5 minutes. Meanwhile, attach binding apparatus to your vacuum manifold (if using, see Step 22).
☐ 14. Transfer ~7 mL supernatant to a *labeled* nuclease-free 50-mL falcon tube. **Do not** transfer any of the pellet.
☐ 15. Add 5 volumes * ~7 mL = 35 mL QIAquick® PB Buffer (with pH indicator) to each sample.
☐ 16. Vortex to mix thoroughly, then briefly spin down.
☐ 17. Add 700 µL sodium acetate (3 M, pH 5.5) to each sample, along with 15 µL Pellet Paint®.
☐ 18. Vortex to mix thoroughly, then briefly spin down.
☐ 19. Aliquot 50 µL* of qH_2O **per sample** into a nuclease-free 1.5-mL microcentrifuge tube [ex: 4 samples = heat 200 µL of qH_2O; *see Step 28].
  – Could also use QIAquick® Buffer EB for elution, but beware of salts when concentrating DNA extractions for later protocols.
☐ 20. Heat qH_2O aliquot(s) to 55°C on block.
☐ 21. Transfer 12 mL of extraction solution to QIAquick® Spin Column in DNA binding apparatus.
☐ 22. Choose the option best suited to you/your lab:
  – Centrifuge binding apparatus in falcon tube at 1500 x *g* for 30 seconds – 1 minute. Discard filtrate and **reuse** binding apparatus falcon tube until all of the extraction solution has passed through the column.
  – Turn on suction in vacuum manifold, and top off until all of the extraction solution has passed through the column.
☐ 23. Transfer the QIAquick® Spin Column to the reserved 2 mL Collection Tube.
☐ 24. Wash DNA on column with 750 µL QIAquick® PE buffer.
☐ 25. Centrifuge at 12,800 x *g* for 1 minute.
☐ 26. Discard filtrate and **reuse** collection tube. Centrifuge the empty QIAquick® Spin Column at 12,800 x *g* for 1 minute to dry the column.
  – This step is critical for removal of trace ethanol that may interfere with downstream applications.
☐ 27. Transfer the QIAquick® Spin Column into a *labeled* nuclease-free 1.5-mL microcentrifuge tube.
☐ 28. Add 25 µL qH_2O heated to 55°C directly to filter of column.
☐ 29. Let sit at room temperature for 2 minutes, then centrifuge at 12,800 x *g* for 1 minute.
☐ 30. Repeat Steps 28-29 for a second elution step.
☐ 31. Check concentration of samples via Qubit, then store samples accordingly.
  – When necessary also check protein content via Nanodrop, and distribution of fragments on electrophoresis gel.
  – Store eluted DNA at 4°C if entering library preparation within 1-2 days, -20°C for long-term storage.

References

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3. Gamba, C. *et al.* Genome flux and stasis in a five millennium transect of European prehistory. *Nat. Commun.* **5,** 5257 (2014).
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5. Dabney, J. *et al.* Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci.* **110,** 15758–63 (2013).