DNA extraction from *N. furzeri* tail tissues

For use on fresh tissues or fish preserved in 100% EtOH

1. Cut out a portion of tail tissue and place in 600 ul tail digestion buffer in 1.5 ul Eppendorf tube. (Clean scissors between fish)
2. After all fish have been extracted, add 10 ul proteinase K to each Eppendorf tube. (Adding up to 16 ul proteinase K when resultant DNA concentrations are not high enough)
3. Vortex and place on 56'C heat block for 12 hr, or 4 hr with vortexing every 30 min until no tissue fragments are observed.
4. Add 600 ul phenol/chloroform isoamylalcohol
5. Vortex for 10 sec until no layers are observed
6. Spin at 12,000 rpm for 10 min
7. Carefully remove supernatant and place into a fresh Eppendorf tube containing 50 ul ammonium acetate (10M) and 1,100 100% EtOH
8. (Optional) Repeat steps 4-6 before putting into ammonium acetate/EtOH.
9. Invert tubes 15x
10. Place tubes in -80'C for 1 hr
11. Spin tubes at 4'C for 15 min
12. Pour out the supernatant. Don't disturb the pellet.
13. Add 500 ul 70% EtOH (chilled at -20'C)
14. Spin at 12,000 rpm at RT for 8 min
15. Carefully remove supernatant
16. Let dry on the bench for 20 min
17. Add 40 ul RNase, DNase free water
18. Resuspended at RT overnight
19. Determine DNA concentration

Tail digestion buffer recipe

- 450 mL dH2O
- 5mL 1M Tris, ph 8 (buffer w/ hcl)
- 10mL 5M NaCl (2.92g NaCl in 8.7 mL dH2O)
- 10mL 0.5M EDTA
- 25mL 10% SDS (2.78g in 22.22mL dH2O)

1. Place all ingredients together, using stir bar if necessary.
2. Filter at 0.45um
3. Autoclave buffer: Transfer buffer to a glass jar, unscrew lid, place jar and long trough of water in a big tray of autoclave. Use 1,1 20 minutes cycle
4. Let cool at RT overnight

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