DNA extraction from *N. furzeri* tail tissues

Materials

- 5PRIME Phase Lock Gel Heavy (PLG-Heavy) (VWR, 10847-802)
- Proteinase K (Thermo Fisher Scientific, EO0491)

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. (Switch razor between fish)
2. (Optional) For extraction with higher purity and yield, use the 5PRIME Phase Lock Gel Heavy (PLG-heavy) tube instead of normal Eppendorf. **Centrifuge the tube at max speed for 1 min before use.**
3. 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)
4. Vortex and place on 56'C heat block for 12 hr. Do not vortex if use PLG-Heavy tube.
5. Add 600 ul phenol/chloroform isoamyl alcohol.
6. Vortex for 10 sec until no layers are observed. Do not vortex if use PLG-heavy tube, shake vigorously to mix.
7. Spin at 12,000 rpm for 10 min.
8. Repeat steps 5-7.
9. (Optional) Some black pigments may be present at the interface of the gel and aqueous phase, as well as on top of the aqueous phase. If that is the case and high purity is required, transfer the aqueous phase (try not to pipette the black pigment) to a new PLG-heavy tube and repeat step 8.
10. Carefully remove supernatant and place into a fresh Eppendorf tube containing 50 ul ammonium acetate (10M) and 1,100 ul 100% EtOH.
11. Invert tubes 15x.
12. Place tubes in -80'C for 1 hr.
13. Spin tubes at 4'C 15000 rpm for 15 min.
14. Pour out the supernatant. Don't disturb the pellet.
15. Add 500 ul 70% EtOH (chilled at -20'C).
16. Spin at 12,000 rpm at RT for 8 min.
17. Carefully remove supernatant.
18. Let dry on the bench for 1 hr.
19. Add 40 ul MiliQ water.
20. Resuspend at room temp overnight.
21. 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)

v1.1: Hoang Nguyen, 2022/11/06

Update:
V1.1: Adding optional step of using Phase Lock Gel Heavy tubes.