APPENDIX 1

DNA Extraction from Fin Clip

Extraction Buffer	Volume (mL)	Volume (mL)
1M Tris pH 8.0	0.5	5
0.5M EDTA pH 8.0	1	10
10% SDS	1	10
5M NaCl	1	10
Water	46.5	465
Total	**50**	**500**

1. **Cut** 1 cm^2 of fin tissue into small pieces. **Place** in a 1.5mL centrifuge microtube and **add** 500 µL extraction buffer, 5 µL DTT (1M), and 6 µL Pro-K (10mg/mL).
2. **Store** at 56 ˚C for a few hours or 37 ˚C overnight on a *rotary shaker*. If digestions are incomplete, add more Pro-K and **store** at 56 ˚C.
3. **Spin** in *centrifuge* for 2 min at 14,000rpm. **Transfer** supernatant to a new 1.5mL tube.
4. **Add** 200 µL 5M NaCl. **Mix** thoroughly, but do not vortex.
5. **Spin** in *centrifuge* for 10 min at 14,000 rpm.
6. **Transfer** clear supernatant to a new 2mL screw-cap tube containing 1 mL cold 100% EtOH.
7. **Store** on ice for 10 min or in -20 ˚C *freezer*.
8. **Spin** in *centrifuge* for 10 min at 14,000 rpm and 4 ˚C.
9. **Aspirate**, or pour out supernatant.
10. Perform steps A B, and C at least once. Repeat as needed.
    A. **Rinse** the pellet in 1 mL of 70% EtOH.
    B. **Spin** for 5 min. at 14,000 rpm and 4 ˚C.
    C. **Aspirate**, or pour out supernatant.
11. **Dry** in open air for 10-20 min.
12. **Add** 100 µL Low TE (10 mM Tris, pH8.0 and 0.1 mM EDTA). **Store** in 56 ˚C water bath for 1 hour or overnight at 37 ˚C.
13. **Quantify** DNA on *spectrophotometer*.
14. For PCR, **dilute** an aliquot to 12.5 ng/µL.

- *Or maximum rpm*
- *~40% of starting volume*
- *May store overnight in freezer at -20 ˚C*
- *Pellet should adhere to wall of tube.*
- *Dislodge pellet from wall of tube.*
- *Do not overly dry pellet.*
- *A260/A280 ratio should be around 1.75-2.0.*