Phenol Chloroform Extraction

1. Turn on heat block to 55°C.
2. Add 500 µl of STE buffer – the same tip can be used for all tubes.
3. Pick out one piece of muscle (~3x2 mm), liver or a fin clip. Use an open flame to clean the cutting equipment after each sample is taken.
4. Add 15 µl of 1% proteinase K to each tube. Proteinase K is heat stable, and therefore it is not necessary to keep on ice. Use a new tip for each tube.
5. Add 75 µl of 10% SDS to each tube.
6. Thoroughly mix contents of the tubes by inversion.
7. Place samples in the heat block (55°C) till the tissue is completely dissolved (up to overnight or add more Prot. K). Shake samples to dislodge any solid pieces.
8. Add 600 µl of equilibrated phenol.
9. Mix by inversion for 2 minutes.
10. Centrifuge samples at full speed (14K) for 10 minutes.
11. Label new tubes.
12. Collect aqueous phase into the newly labeled tubes – be conservative by leaving what can not be cleanly collected.
13. Add 600 µl of phenol:chloroform:isoamyl alcohol (24:24:1) mix.
14. Mix by inversion for 2 minutes.
15. Centrifuge samples at full speed (14K) for 10 minutes.
16. Label new tubes.
17. Collect aqueous phase into the newly labeled tubes – be conservative by leaving what can not be cleanly collected.
18. Add 600 µl of chloroform.
19. Mix by inversion for 2 minutes.
20. Centrifuge samples at full speed (14K) for 10 minutes.
21. Label new tubes.
22. Collect aqueous phase into the newly labeled tubes – be conservative by leaving what can not be cleanly collected.
23. After this step, absolutely no chloroform should remain the tubes.
24. Add 55 µl of 5M NaCl to each tube (10% total volume).
25. Add 1000 µl of absolute alcohol (or 95%) to the aqueous phase.
26. Thoroughly mix by inversion.
27. Precipitate the samples overnight in the -20°C freezer.
28. Centrifuge for 10 minutes at full speed (14K).
29. Pour off alcohol, leaving the pellet of DNA behind.
30. Wash the pellet by adding ~500 µl of 70% alcohol. The same tip can be used if the alcohol is dribbled in from above the tube.
31. If the DNA pellet becomes loose, spin for 2 minutes at 14K.
32. Remove all alcohol, leaving the pellet of DNA behind.
33. Let the pellets either air dry or dry in the speedwac.
34. When the tubes are dry resuspend the pellet in 50 µl of water and let dissolve.
35. Store suspended DNA at -20°C freezer.