Seawater nucleic acid extraction protocol vs. 1.4

1. Add 1.6 ml of SET lysis buffer directly into top of Sterivex using a 2.5-ml syringe with a 25G 5/8" needle.

2. Add 180 µl of fresh lysozyme.

3. Seal large end with a cut 1-ml syringe tip (containing plunger fragment).

4. Cover narrow end with blue tac.

5. Incubate at 37°C for 30 min with rotation in a hybaid oven.

6. Add 200 µl of SDS.

7. Add 55 µl 20 mg/ml fresh proteinase K.

8. Incubate at 55°C for 2 h with rotation in a hybaid oven.

9. Withdraw lysate into a 5 ml syringe.

10. Add 1 ml SET buffer to Sterivex and rotate to rinse.

11. Withdraw rinse buffer into same 5 ml syringe.

12. Add lysate to 15-ml Phase-Lock tube containing 2 ml of phenol:chloroform:isoamyl alcohol (25:24:1), pH 8 (in Maxtract gel lock system, QIAGEN). Shake gently until mixed.

13. Centrifuge @ 1,500 x g for 5 min.

14. Add an additional 2 ml of phenol:chloroform:isoamyl alcohol (25:24:1). Shake gently until mixed.

15. Centrifuge @ 1,500 x g for 5 min.

16. Add 2 ml of chloroform:isoamyl alcohol (24:1). Shake gently until mixed.

17. Centrifuge @ 1,500 x g for 5 min.

18. Decant aqueous phase to a sterile and DEPC-treated (if RNA needed) JA-20 centrifuge tube.

18b. Add 5 ul of glycogen (Roche).

19. Add 0.5V of 7.5 M NH4acetate. Mix briefly.

20. Add 2.5V of pure ethanol.

21. Mix and leave @ -20°C for > 1 h (overnight is fine).

22. Centrifuge in a JA-20 rotor @ 20,000 rpm for 30 minutes @ 4°C.

23. Decant ethanol.

24. Add 2 ml 80% ethanol and rinse tube.

25. Centrifuge in a JA-20 rotor @ 20,000 rpm for 20 minutes @ 4°C.

26. Decant ethanol.

27. Add 2 ml 80% ethanol and rinse tube.

28. Centrifuge in a JA-20 rotor @ 20,000 rpm for 20 minutes @ 4°C.

29. Decant ethanol and leave inverted.

30. Dry inverted tube for 15 minutes in fume hood (provides air flow).

31. Suspend invisible pellet in 200 µl DEPC-treated sterile water. Leave on ice for approximately 1 h with frequent finger-tapping to rinse tube walls.

33. Store @ -20°C (DNA) or -80°C (RNA).

SET buffer

40 mM EDTA

50 mM Tris-HCl, pH 9

0.75 M sucrose

autoclave and store @ RT

Lysozyme

990 µl sterile water

9 mg lysozyme

9 µl 1M Tris-HCl, pH 8

store on ice

SDS

10% SDS

Proteinase K

950 µl sterile water

50 µl 1M Tris-HCl, pH 8

20 mg proteinaseK

7.5 M NH4acetate

According to molecular cloning manual