DNA extraction with dispersion buffer

We have found that this protocol works to extract large quantities of high-quality, high molecular weight DNA from benthic marine invertebrate (coral and sponge) tissue preserved in 100% molecular grade ethanol. A brief soak of the sample in TRIzol reagent appears to get rid of inhibitors and greatly improves DNA quality and downstream enzymatic reactions. This extraction protocol is relatively time consuming but may work well for people who are having trouble with their extractions (especially with pigmented pellets, poor downstream digestions or amplification).

Pre-Steps

- Set a refrigerated centrifuge to 4 °C
- Set heat block to 55 °C

Required Supplies

For each sample:

- FastPrep tube with glass beads
- 4 sets of 2 mL tubes (3 if samples were preserved in TRIzol)
- 1 set of Zymo DCC-5 preps
- 1 set of 1.5 mL tubes

1. Prepare dispersion buffer (can be kept in the 4 °C refrigerator in an opaque tube protected from light for several days to a week).
2. Scrape tissue from coral fragment (tissue from 1–2 polyps is plenty) and place into a 2 mL tube. Add 300–800 μL of T RIzol reagent to the tube, let sit for at least 5–10 min but can refrigerate or freeze the tissue in TRIzol if doing the extractions later.
3. Make an extraction buffer master mix.

Reagent	**Volume (μL)**	**Master Mix for 24 samples (+10% error)**
Dispersion Buffer	1000	26.4 mL
Proteinase K	10	264 μL
RNAse A	1	26.4 μL
Total	**1011**	**26.69 mL**

4. Add 1000 μL of extraction buffer and 0.2 mL (~.075 g) of 0.5 mm glass beads to a FastPrep bead tube.
5. Carefully transfer tissue from TRIzol into FastPrep tube with beads and buffer.

6. Bead beat for 2–3 mins (6 M/s, three 45 sec intervals w/ 2 min cool down between).
7. Incubate at 55°C for 90 min while mixing.
8. Centrifuge at 20,000 x *g* for 3 min at 4°C to pellet beads and debris.
9. Transfer 800 µL of supernatant to a clean 2 mL tube, being careful to avoid debris at the bottom and fat/mucus on top.
10. Add 800 µL phenol:chloroform:isoamyl alcohol (25:24:1). Do this in the hood and remember to pipette from the bottom layer, invert to mix, place samples on ice.
11. Vortex samples for a few seconds and leave on ice for 1 min. Vortex samples again for 1–2 s. You want to make sure the two phases are homogenized, it may be necessary to shake the tubes before vortexing.
12. Centrifuge at 20,000 x *g* for 15 min at 4°C.
13. Transfer aqueous phase to new tube (~ 600 µL), taking care not to disturb interphase layer. **Only get as much as you can w/o disturbing the interphase layer.**
14. Add 600 µL chloroform:isoamyl alcohol (24:1), place samples on ice. Repeat step 11.
15. Mix and centrifuge at 20,000 x *g* for 15 min at 4 °C.
16. Transfer aqueous phase to new tube (~ 500 µL), taking care not to disturb interphase layer which should be non-existent or much thinner. **Only get as much as you can w/o disturbing the interphase layer.**
17. Add 800 µL 100% isopropanol, invert samples to mix 25–30 times. Incubate for 10 min @ RT.
18. Centrifuge at 20,000 x *g* for 30 min at 4 °C to pellet the DNA.
19. Remove supernatant (carefully pour off, quick spin the samples, and pipette off the remaining isopropanol avoiding pellet).
20. Add 1000 µL of 70% EtOH @ RT. Gently wash EtOH around tube and invert to mix.
21. Centrifuge at 20,000 x *g* for 10 mins at 4°C.
22. Remove supernatant (carefully pour off, quick spin the samples, and pipette off the remaining ethanol avoiding pellet).
23. Dry for 15–20 min upside down on a KimWipe @ RT.
24. Elute in 50–100 µL of NFW.
25. Incubate for 10 min @ 55°C.
26. Purify DNA extractions using the Zymo Clean and Concentrate Kit according to manufacturer's protocol.

Cleaning genomic DNA with Zymo DNA Clean & Concentrator-5 Kit

After extracting genomic DNA, Zymo DNA Clean & Concentrator-5 (D4014) is used to clean DNA and remove inhibitors prior to running PCR, this protocol has a few slight modifications from the manufacturer's protocol.

1. Set NFW for elution step in heat block at 65° C
2. Add to your eluted DNA a 2:1 volume of Binding Buffer:DNA (e.g. 200 μL BB:100 μL DNA) and vortex thoroughly, spin down.
3. Transfer the entire mixture (~300 μL) to a provided Zymo-Spin Column in a collection tube.
4. Centrifuge 16,000 x *g* for 2 minutes at room temperature. Discard flow through.Check to make sure all of the solution has passed through the filter, if not then spin the filter column again. Issues with getting binding buffer to pass through the filter suggests that there may be too much DNA for the filter and it is getting clogged. If this is happening for a lot of your samples, consider scraping less tissue in the beginning of the protocol, or consider switching to a larger filter column set-up, which Zymo has available.
5. Add 200 µL DNA Wash Buffer to the column. Centrifuge at 16,000 x *g* for 1 min at room temperature. **Repeat.**
6. Transfer the column to a new labeled 1.5 mL tube. Elute by adding 15–20 μL of heated NFW directly to the column matrix and incubate at room temperature for 3–5 min.
7. Centrifuge for 2 min to elute DNA. Ensure the DNA has completely eluted before discarding the column, if there is too much DNA you may have to spin the column twice to ensure you have all of the sample.
8. Nanodrop cleaned DNA, if 260/280 values are < 1.8 and 260/230 values are below 2.0 then re-clean and elute in a smaller volume (8 μL).
9. Quantify via picogreen or Qubit.

Dispersion buffer recipe

Handle all reagents under fume hood

- Materials: Guanadine thiocyanate (light sensitive), sodium citrate dihydrate, beta-mercaptoethanol (stored in flammable cabinet), milliQ, NFW, or DEPC water.
- Equipment: Plate with stirring rod, beaker for mixing, amber glass bottle for storage.

Reagent	**Target Concentration**	**Molecular Mass**	**For 50 mL Buffer**
Guanadine thiocyanate	4 M	118.16	23.632 g
Sodium Citrate dihydrate	30 mM	294.10	0.441 g
β-mercaptoethanol	30 mM	Stock concentration = 14.3 M	105 μL

1. Set up stirring plate under hood.
2. Set 100 mL beaker with 50 mL of milli Q water stirring.
3. Add reagents slowly to stirring liquid.
4. Transfer dispersion buffer to labeled storage buffer tube.
5. Store at 4 °C protected from light.