DNA extraction
(using the Omega Biotek E.Z.N.A. Mollusc DNA Kit)

What's needed:

- Tissue for extraction, scalpel, wax paper or plastic weighing dish
- Heating block set to 60°C
- 2.0 ml (boil-proof) centrifuge tubes (1.8 ml tubes can be used too though they may leak during lysis)
- **Buffer ML1** (from kit)
- **Proteinase K** (from kit – prepare by adding 1.5 ml of **Proteinase storage buffer** to 30 mg of **Proteinase K** (already pre-weighed in kit vials) (store according to instructions)
- **Chloroform** (kept in fume hood, main stock in flammable cabinet below hood; you can use chloroform:isoamyl alcohol (24:1), but it doesn't make a huge difference if you only have straight chloroform)
- 1.8 ml centrifuge tubes
- **Buffer MBL** (from kit)
- **Absolute ethanol** (95-100%, 95% works fine) and **100% isopropanol**
- **HiBind DNA column** (from kit)
- **2 ml collection tube** (from kit)
- **HBC Buffer** (make sure the isopropanol has been added – should be checked on lid of supply vial)
- **DNA Wash Buffer** (make sure the EtOH has been added – should be checked on lid of supply vial)
- **Elution Buffer** (from kit)

Useful tips:

- This is a several hour to two-day procedure depending on length of incubation needed to completely break down tissue.
- Multiple samples can be done at once; label tubes accordingly (*e.g.*, keep same numbers for different sets of tubes.
- Label all tubes prior to use.

PROCEDURE (*modified from Omega Biotek's protocol*)

[Steps 1-6 can be done in < about 1 hr depending on the number of samples being extracted.]

1. Prepare 2.0 ml centrifuge tubes for subsequent steps by numbering them and placing them in a rack (1 per sample!).
2. Dispense 350 ml of **Buffer ML1** into each numbered tube.
3. Remove approximately 10-25 mg of tissue from specimen (*no more than about 5 mm^3 of tissue*). Mince tissue by making cross-cuts if practical (if not, don't worry the extraction not working—a piece of tissue or whole specimen if you're working with a small individual should completely dissolve). Do dissections and cross cuts on wax paper or in a plastic weighing dish (or under more sterile conditions if desired). [*Although the kit's protocol calls for pulverizing the tissue in liquid nitrogen, this seems like overkill.*]
4. Place tissue in centrifuge tube that already contains the 350 ul of **Buffer ML1**.
5. Add 25 ul **Proteinase K** (20 mg/ml) to tube.
6. Incubate tube at 60°C for at least 30 minutes or until majority or all of tissue is dissolved. [*Overnight incubations can be done 60°C or 37°C.*]

[This is a good stopping point; incubation can be run from 30 minutes to several hours or even overnight if needed.]

[The remaining steps shouldn't take more than about 1 hr.]

7. Add 350 ul **chloroform** to each tube (in fume hood!). Gently mix solution in tube by inverting tube (*up and down*) 5-10 times.
8. Centrifuge tube at 10,000 g for 2 minutes.
9. Prepare 1.8 ml centrifuge tubes (1 per sample!) for next step by numbering them and placing them in a rack.
10. Prepare **Elution Buffer** (for final steps) by placing ~55 ul of the buffer (per each sample to be extracted) in a 1.8 ml centrifuge tube and incubating the solution at 60°C in the heating block.
11. Carefully remove 250 ul of the upper aqueous layer from tube and place in a centrifuge tube. **Avoid taking any of the whitish material at the bottom of the aqueous layer even if this means not being able to remove all of the upper phase.** [*If there is not much of an aqueous layer or the aqueous and organic phases do not separate well, add about 200 ul of Buffer ML1 to tube, mix and centrifuge again.*] [*Discard organic phase in appropriate container and discard all tubes and tips that came into contact with the chloroform in appropriate contaminated plastics bin.*]
12. Add 250 ul of **MBL** to aqueous phase in each centrifuge tube.
13. Add 250 ul of **absolute ethanol** to each centrifuge tube (that contains aqueous phase + MBL).
14. Close tube and gently mix the solution by inverting the tube (*up and down*) 10 or so times. Look for spooled DNA that will look like a small white, cloudlike substance—having spooled DNA is a good sign of having high quality DNA, but not having it is ok too!
15. Prepare **HiBind DNA columns** by placing each column in an empty **collection tube** and numbering each column. [*or place columns in vacuum manifold if available*]
16. Add solution from step 14 (ethanol+MBL+aqueous layer) to each **HiBind DNA column**.
17. Centrifuge **HiBind DNA columns** in **collection tubes** for 1 min at 10,000 x g. [*not needed if using vacuum manifold but make sure all of solution is removed from the columns via the vacuum*]
18. Discard liquid from **collection tubes** and place **HiBind DNA columns** back in tubes. [*not needed if using vacuum manifold*]
19. Add 500 ul of **HBC buffer** to each **HiBind DNA column**.
20. Centrifuge **HiBind DNA columns** in **collection tubes** for 30 s at 10,000 x g. [*not needed if using vacuum manifold but make sure all of solution is removed from the columns via the vacuum*]
21. Discard liquid from **collection tubes** and place **HiBind DNA columns** back in tubes. [*not needed if using vacuum manifold*]

22. Do first wash: Add 700 ul **DNA Wash Buffer** (*make sure ethanol was added*) to **HiBind DNA columns**.

23. Centrifuge **HiBind DNA columns** in **collection tubes** at 10,000 g for 1 min. [*not needed if using vacuum manifold but make sure all of solution is removed from the columns via the vacuum*]

24. Discard liquid from **collection tubes** and place **HiBind DNA columns** back in tubes. [*not needed if using vacuum manifold*]

25. Do second wash: Add 700 ul **DNA Wash Buffer** (*make sure ethanol was added*) to **HiBind DNA columns**. [*use vacuum manifold for this step to remove as much of the wash solution as possible from the columns; proceed with the following centrifugation step even if using the vacuum manifold]*

26. Centrifuge **HiBind DNA columns** in **collection tubes** at 15,000 g for 2 min (*note longer spin time than above!*). [*this step is needed if using vacuum manifold to remove all the wash buffer from columns*]

27. Label new 1.8 ml centrifuge tubes with sample names, date of extraction and extraction numbers.

28. Place **HiBind DNA columns** into the new, labeled 1.8 ml centrifuge tubes.

29. Add 50 ul of preheated (to 60-70°C) **Elution Buffer** (or 10 mM Tris buffer, pH 9.0) to each **HiBind DNA column**.

30. Let solution soak for approximately 2 min at room temperature.

31. Centrifuge **HiBind DNA columns** in labeled centrifuge tubes at 10,000 g for 1 minute.

32. Store extracted DNA at -20°C (*or use now as template for pcr!*).

The final tube has your final genomic DNA (gDNA) extract. To check quality/quantity of gDNA you can run 2 ul of solution (with 2 ul of loading dye) on a 2% agarose/0.5X TBE gel (*or do an amplification and check to see if it can be pcr'd from–gDNA can also be run on the gel used to assay the pcr*). You should be able to use 1 ul of solution as the template for pcr; if the DNA is very concentrated (or if inhibitors are present), you will need to dilute the gDNA about 10-100 fold.