Key: Repeated step, **Section header**

When kit arrives:

1. Acquire 100% ethanol, 100% isopropanol, and a 24:1 chloroform: isoamyl alcohol solution.
2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3373-00	6 mL
D3373-01	60 mL
D3373-02	100 mL per bottle

3. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D3373-00	1.6 mL
D3373-01	10 mL
D3373-02	32 mL

Before starting an extraction:

1. Set water baths or heat blocks to 60°C and 70°C.
2. Heat elution buffer to 70°C.

Extraction

1. Take a whole flash-frozen oyster, pulverize to powder in a mortar and pestle
2. Weigh out 30 mg of powder and put into a clean 1.5 mL microcentrifuge tube
3. Add 350 µL ML1 Buffer and 25 µL Proteinase K Solution. Vortex to mix thoroughly.
4. Incubate at 60°C for 1 hour (check in 30 min, add 30 if large chunks)
5. Add 350 µL chloroform:isoamyl alcohol (24:1). Vortex to mix thoroughly.
6. Centrifuge 10,000 x *g* for 2 minutes at room temperature.
7. Transfer the UPPER AQUEOUS LAYER to a clean 1.5 mL microcentrifuge tube. Avoid the milky interface/pellet that contains contaminants and inhibitors.
   Note: If the upper aqueous phase is SMALL after centrifugation, add 200 µL ML1 Buffer and vortex to mix thoroughly. Repeat Step 5 (centrifugation) and Step 6 (transfer the upper aqueous phase).
8. Add equal volume of BL Buffer and 10 µL RNase A. Vortex at maximum speed for 15 seconds.

Note: For example, to 350 µL upper aqueous solution from Step 6, add 350 µL BL Buffer.

9. Incubate at 70°C for 10 minutes.
10. Cool the sample to room temperature.
11. Add that same volume (from steps 8 and 6) of 100% ethanol. Vortex at maximum speed for 15 seconds.
12. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
13. Transfer 750 µL sample from Step 9 (including any precipitate that may have formed) to the HiBind® DNA Mini Column.
14. Centrifuge at 10,000 x *g* for 1 minute.
15. Discard the filtrate and reuse the collection tube.
16. Repeat Steps 13-15 until all of the sample has been applied to the HiBind® DNA Mini Column (2 -3 washes)
17. Discard the filtrate and the Collection Tube.
18. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.
19. Add 500 µL HBC Buffer (make sure it's been diluted, see **When kit arrives**)
20. Centrifuge at 10,000 x *g* for 30 seconds.
21. Discard the filtrate and reuse the Collection Tube.
22. Add 700 µL DNA Wash Buffer. (make sure it's been diluted, see **When kit arrives**)
23. Centrifuge at 10,000 x *g* for 1 minute.
24. Discard the filtrate and reuse the Collection Tube.
25. Repeat Steps 21-23 for a second DNA Wash Buffer wash step.
26. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.
    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.
27. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).
28. Add 50-100 µL Elution Buffer (or sterile deionized water) preheated to 70°C.
    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.
29. Let sit at room temperature for 2 minutes.
30. Centrifuge at 10,000 x *g* for 1 minute.
31. Repeat Steps 28-30 for a second elution step.
    Note: Any combination of the following steps can be used to help increase DNA yield.
    a. After adding the Elution Buffer, incubate the column for 5 minutes.
    b. Increase the elution volume.
    c. Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).

d. Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

32. Store DNA at -20°C.