Qiagen DNeasy Kit Extractions
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Qiagen DNeasy kit:

1) This protocol is relatively simple and can yield very clean DNA for PCR analyses.

Freeze Thaw Method for Cultures

a. Put 200ul of culture in a 1.5 ml microfuge tube. Freeze at –80C for 10min.
b. Remove from freezer and place in a 70 °C heat block for 10min.
c. Repeat the freezing and thawing two more times.
d. Proceed to step 2.

SLB proteinase K Method for Filtered Seawater

a. Add 200 ul 10% SDS, 10 ul proteinase K (20 mg/ml) to each tube. Incubate in shaker at 37 °C for 30 min.
b. Heat to 55 °C for 30 min in an incubator.
c. Transfer 200 ul pooled Lysis Buffer/SDS solution into an autoclaved micro centrifuge tube.

2) Proceed with the Qiagen DNeasy Tissue kit protocol starting on page 29, "Protocol for isolation of genomic DNA from crude lysates". The freeze/thaw is the sample-specific lysis so start with step 2 for cultures, adding 20ul proteinase K. Start with step 3 for filtered seawater preparations.

3) Add 200ul Buffer AL and mix immediately by vortexing.

4) Incubate at 70C for 10min.

5) Check the pH with pH paper. The lysate must be acidic. Usually, adding 2ul of 10% HCl is sufficient.

6) Add 200ul 95-100% Ethanol to the sample and mix thoroughly by vortexing.

7) Pipette the mixture into the DNeasy spin column placed in a 2ml collection tube. During the first few steps, the collection tube collects waste products so it is possible to use a previously used, dirty tube. Centrifuge at 8000 rpm for 1min. Discard flow-through.

8) Add 500ul Buffer AW1, and centrifuge for 1min at 8000 rpm. Discard flow-through.

9) Add 500ul Buffer AW2 and centrifuge for 1min at 8000 rpm. Discard flow-through.

10) Place spin column in a dry collection tube (used is OK) and spin at full speed for 1min to dry the DNeasy membrane. Discard flow-through.

11) Place spin column in a new, clean collection tube and pipette 50ul TE directly onto the DNeasy membrane. Incubate at room temperature for at least 1min, then centrifuge at 8000 rpm to elute. Alternatively, DNA can be eluted twice with 30-50ul.

12) Transfer eluate to a microfuge tube and store at –20C.