DNA Extraction

Center for Coastal Margin Observation & Prediction (www.stccmop.org)

Solutions:

DNA extraction buffer: Contains 0.1 M EDTA @ pH 8, 1% SDS and 200 µg/mL proteinase K. Make a stock of 50 mL 0.1 M EDTA-1% SDS by combining 10 mL EDTA pH8, 5 mL 10% SDS and 35 mL MilliQ water for a total volume of 50 mL. Mix well by vortexing. Before adding DNA extraction buffer to field sample make a DNA EXTRACTION BUFFER WORKING SOLUTION. Add 10 µl of 20 mg/mL Proteinase K to 1 mL of 0.1M EDTA-1% SDS, mix by gently shaking tube up and down and quickly centrifuge to bring down the liquid.

Notes: 1) DNA extraction buffer contains SDS, if the room temperature is lower than 25ºC, SDS will usually precipitate. If precipitate forms, microwave the buffer for 10 seconds and mix to dissolve. Repeat one more time if needed.

2) Proteinase K can be purchased from Fisher Scientific as solution: cat. no. NC9499957, 20 mg/mL Proteinase K (easiest to use)

CTAB (N-cetyl-N,N,N,-trimethyl ammonium bromide): CTAB is a light powder, somewhat similar to SDS, used eliminate contaminants in field samples. Make a 10% CTAB working solution (50 mL): Combine 50 mL of 0.7 M NaCl and 2.5 g of CTAB into a 50 mL polypropylene tube (Fisher Scientific cat no. 06-443-18). Rotate slowly at 60˚C for several hours to dissolve powder completely. Store at Room Temperature for up to 6 months. Can also make by dissolving 4.1 g NaCl in 80 mL MilliQ water. While stirring, add 10 g CTAB. To dissolve, heat the solution at 65ºC. Adjust the volume to 100 mL with MilliQ water. Store at RT for no longer than 6 months.

Notes: 1) 10% CTAB is very sticky at RT and cannot accurately be pipetted. Therefore it is convenient to make 1 mL aliquots. When you need to use 10% CTAB, pre-heat a 1 mL aliquot at 55-60ºC. When adding to DNA solution pipet immediately, trying to keep the aliquot heated. Pipet up and down for several times to make sure all the CTAB in the tip is washed out. You can also microwave the 10% CTAB solution for several seconds, but be careful not to overheat the solution so that the liquid will boil out.

2) CTAB can be purchased from Fishier Scientific: cat. no. AC22716-1000, 100 g CTAB

DNA cleaning kit: Zymo DNA Clean & Concentrator kit- 25 columns (Cat. No. D4006, Zymo Research, Orange, CA)

Field sample DNA isolation:

1. Filter water sample through a 0.22 µm Sterivex filter
2. When ready to process sample, break Sterivex following DNA extraction protocol in blue notebook.
3. Add the cut up filter membrane into a 2 mL mircocentrifuge tube (with orange cap), trying to get the filter as close to the bottom as possible
4. Add 0.5 mL **DNA extraction buffer**, making sure the filters are completely covered by the buffer
5. Incubate at 55ºC for 16 hours to 3 days
6. Add 165 µl 5M NaCl and 165 µl **pre-warmed 10% CTAB**, mix well by vortexing
7. Centrifuge briefly and incubate at 55ºC for 10 minutes
8. Pipette out the DNA solution, making sure to avoid taking any of the filter, into a 1.5 mL epindorf tube (~650 µl)
9. Add 600 µl chloroform, close the cap and vortex for 1 minute
10. Centrifuge at the highest setting (>13000 rpm) for 10 minutes
11. Transfer the supernatant (~600 µl) into a 2 mL collection tube, being careful to not suck up the interface
12. This is the crude DNA solution, purify DNA using **DNA cleaning kit**
13. To purify crude DNA, add 2 volumes of DNA binding buffer (1.2 mL) to DNA solution, mix well by vortexing
14. Transfer 700 µl of DNA/buffer mixture into a filter column and centrifuge at top speed (>13000 rpm) for 15 seconds
15. Discard the flow through and return the column back to the collection tube
16. Repeat steps 14-15 until all the DNA/buffer mixture has been transferred to the filter cartridge
17. Add 300 µl DNA wash buffer to the column (make sure you have added 100% ethanol into the concentrated washing buffer), centrifuge at top speed (>13000 rpm) for 15 sec
18. Discard flow through and add another 200 µl DNA wash buffer, centrifuge at top speed for 1 min
19. Transfer the column into a clean 1.5 mL epindorf tube
20. Add 15-50 µl elution buffer (10mM Tris-Cl pH 8) to the white base of column and incubate at RT for 1 minute
21. Centrifuge at 10,000 rpm for 15 seconds
22. Add another 15-50 µl elution buffer to the base of the column, incubate at RT for 1 minute, centrifuge at 10000 rpm for 15 seconds
23. Discard column and vortex elution briefly and store DNA at -20ºC