Extraction of DNA and RNA from filters using CTAB protocol

1. Filter water first through 0.8 μm polycarbonate filters if necessary to minimize eukaryotic nucleic acids. We usually use 47 mm filters (< 0.8 um fraction). Triplicate samples of at least 500 ml each.
2. Filter water through 0.2 μm Durapore (25 mm diameter). Do in batches of ~100-250 ml until clogs. Goal: 1-3 filters in a single 2 ml screw cap tube.
3. Add 1 ml of CTAB (without beta-mercaptoethanol) to each tube.
4. Freeze overnight at -20^{o}C (or longer at -80^{o}C).
5. Thaw room temperature ~5 min.
6. Add 4 μl (0.4% (v/v)) beta-mercaptoethanol per tube.
7. Vortex briefly. Incubate 65^{o}C for 15 minutes, with occasional inversion of the tube.
8. Cool to room temperature. Add equal volume of chloroform/isoamyl alcohol (24:1).
9. Put on rotating platform or mixer for 20 minutes at room temperature.
10. Spin 15 minutes 12,500 rpm.
11. Put aqueous layer (top) in new 2 ml tube, avoid interface.
12. Extract one more time with chloroform/isoamyl alcohol, spin 5 minutes at 12,500 rpm.
13. Put aqueous layer (top) in 2 new 1.5 ml tubes (split sample), avoid interface.
14. Add ½ volume (~250 μL) 5M NaCl to each tube, mix.
15. Add 1 volume (~500 μl) isopropanol to each tube. Invert to mix.
16. Incubate at -80^{o}C for 1-2 hours. Leave one of the tubes at -80^{o}C, for long term storage/stability of RNA.
17. Spin other tube at 12,500 rpm for 30 minutes.
18. Wash pellet (nucleic acids) with 500 ul of 70% ETOH. Spin 5 minutes, 12,500 rpm. Remove ETOH carefully from the pellet with a pipet.
19. Air dry. Do not vacuum dry.
20. Resuspend in 50 μl RNAase free H_2O.
21. Incubate RT with gentle vortexing (or ice 1-2 hours) to ensure dissolution.
22. Determine quality and quantity of DNA/RNA by spectrophotometer. Dilute 5 μL with 45 μL Milli-Q H_2O. Measure and record 230, 260, 280, 320, and ratios. Run ~250 ng on 1% agarose gel (1x TAE, DEPC water, use RNAase free gel setup).

Wear gloves at all times!
Use tips with filters, especially after step 14.

CTAB Buffer

100 mM TrisHCl pH 8.0
1.4 M NaCl
2% (w/v) CTAB
0.4% (v/v) beta-mercaptoethanol (but see step 3)
1.0% PVP (polyvinyl pyrrolidone)
20 mM EDTA

RNAase free water

1. Add diethylpyrocarbonate (DEPC) (0.1%) to Milli-Q water
2. Stir overnight
3. Autoclave