Chlamydomonas DNA Preparation

Solutions:

1 X TE, 10 mM Tris-CL pH 8.0, 1mM EDTA
7.5 M Ammonium Acetate pH 7.5
Chloroform
TE saturated 50% Phenol/50% Chloroform
100% Ethanol
80% Ethanol
Death Buffer:
    20 mM Tris pH 7.5
    20 mM EDTA
    5% SDS
    1 mg/ml Protease K

Note:
The theoretical yield is 1 microgram of DNA from 1.3 x 10^7 cells.

Protocol:

1. Use P1000 pipette and special rack dedicated to Protease K work when aliquoting Death Buffer.

2. Spin down about 100 ml of culture and transfer to a microfuge tube. Pellet again and discard the supernatant. There should be about 0.2-0.4 ml of packed cells. Spin down cultures first in 50 ml conical and then transfer to microfuge tubes to get packed cell pellet.

3. Resuspend cell pellet in 0.5 ml of Death Buffer. Resuspend using a clean toothpick. You may need to adjust the volume of Death Buffer if the cell pellet is larger or smaller than 0.4 ml.

4. Incubate at 50 degrees C overnight (12-16 hrs).

5. Next day, add 100 µl of 7.5 M ammonium acetate pH 7.5 and 500 µl of phenol/chloroform and mix by inversion.

6. Spin at 10K RPM for 5 minutes in centrifuge dedicated to Protease K work. We use a Fisher Micro-Centrifuge Model 235A.

7. Transfer supernatant to a new 2.0 ml tube.

8. I have added an additional protocol for the removal of polysaccharides. See http://www.chlamy.org/methods/digest.html. After the phenol/chloroform extraction, add to the supernatant:
    a. 1/7 volumes of 5 M NaCl (100 µl) and mix VERY WELL.
    b. Add 0.1 volumes (70 µl) of CTAB solution (10% in 0.7 M NaCl). Mix again well by inversion. CTAB Cetyltrimethylammonium bromide
    c. Extract with an equal volume of 24:1 chloroform: isoamyl alcohol (700 µl). Spin at 10K RPM for 2 minutes.
    d. This is in place of the chloroform extraction step.

9. If you choose not to do the polysaccharide elimination step, add 500 µl of chloroform and mix by inversion. Spin at 10K RPM for 2 minutes.

10. Transfer supernatant to a new tube, add 1 ml 100% ethanol and mix by inversion.

11. Spin at 10K RPM for 5 minutes and aspirate off supernatant.

12. Wash pellet with 80% ethanol, spin at 10K RPM for 5 minutes, and remove remaining ethanol by aspiration.

13. Air dry pellet until odor of ethanol is dissipated.

14. Resuspend the pellet in 100 µl of 1X TE. Other choices of buffers would be sterile ddH_2O or 10 mM Tris-Cl, pH 8.5 depending on the final use of the DNA. It is not recommended to use TE as a buffer if you plan to use this DNA for sequencing.