K. SCOTT (1999) PROTOCOL FOR BRYOPHYTE DNA EXTRACTION

1. In 6 ml of extraction buffer (see recipes section), grind approximately 0.5 g of clean, live plant material with a cold (4˚ C), sterilized mortar and pestle.

2. Pipette the extract into a 25 ml centrifugge tube. Add 600 µl of 5 M potassium acetate and 50 µl of Triton X100, mix and incubate in a 65˚ C hot water bath for 15 minutes.

3. Centrifuge for 45 minutes at 10,000 rpm.

4. Transfer the supernatant to a new tube, discarding the precipitate.

5. Add 5 ml of Phenol/Chloroform to the supernatant.

6. Centrifuge for 45 minutes at 10,000 rpm.

7. Recover the supernatant into a tube with 5 ml of cold (4˚ C) isopropanol. At this stage the mixture may be stored overnight.at 4° C.

8. Centrifuge for 45 minutes at 10,000 rpm.

9. Pipette off the supernatant and add 1 ml of cold (4˚ C) 75% Ethanol.

10. Centrifuge for 45 minutes at 10,000 rpm.

11. Discard the supernatant, allow the pellet to dry and then resuspend it in 50 µl of autoclaved nanopure water, and the transfer the solution to a 1 ml test tube.

12. Add 2.5 µl of 1µl/ml RNase solution for each 50 µl of DNA stock solution and incubate at 37˚ C for 30 minutes. (We use Sigma Ribonuclease A from bovine pancreas diluted to 1µl/ml with autoclaved nanopure water.)

13. Load 5 µl of DNA with 5 µl of glycerol loading buffer into a 1.4% agarose gel to determine DNA and RNA presence (see electrophoresis protocol). A single high molecular weight band (greater than 12.2 Kb) is indicative of DNA and lower molecular weight bands (between 2 and 3 Kb) are indicative of residual RNA. If RNA is still present, repeat step 12.

14. Determine the concentration of the DNA extract, using Genequant as indicated below:

    a) Dilute 5 µl of the DNA extract to a 100 µl volume; this is a 1:20 dilution of the extract.

    b) Place in Genequant to determine the concentration of DNA in this dilution. This DNA dilution can be recovered and further diluted to use as needed (see c below).

    c) Dilute the remainder of the original DNA extract to a final concentration of 3 ng/µl, based on the information obtained from Genequant. For example, if the diluted DNA sample contains 6 ng/µl (at a 1:20 dilution), then the rest of the extract should be diluted 1:40 in order to achieve a 3 ng/µl concentration. The already diluted sample used in b, should be further diluted 1:2 to achieve a 3 ng/µl concentration.

d) Check the final concentration of the dilutions with Genequant to be sure that you have a 3 ng/µl concentration. Note that a 70 µl sample is needed for quantification.

15. Subdivide the extract into several small aliquotes to avoid repeated freezing and thawing and store appropriately.

DNA STORAGE

1. Undiluted, stock DNA should be stored at -80˚ C and can be maintained indefinitely.

2. Quantified, diluted DNA (about 3 µg/ml) should be stored as follows:
   Stock diluted DNA stored at -80˚ C indefinitely.
   Diluted DNA stored at -20˚ C for use within a few weeks.
   Diluted DNA stored at 4˚ C for immediate use.

RECIPES SECTION FOR DNA EXTRACTION PROTOCOL

A. DNA Extraction Buffer:

Tris HCl 100 mM.....	10 ml
NaEDTA 50 mM.....	20 ml
NaCl 500 mM.....	50 ml
distilled water.....	19 ml

autoclave, then add:

Mercaptoethanol	
10 mM Soln.....	1 ml

B. Phenol/Chloroform Solution:

25 parts Phenol	300ml
24 parts chloroform ...	288 ml
1 part isoamyl alcohol .	1 ml

Store with an upper layer of .1 M Tris HCL (pH 8.0) in a light free container at 4˚ C for up to 1 month.