MOSS DNA EXTRACTION

Small scale extraction for PCR analysis

1. Harvest 8mm^2 – 1/4 plate moss tissue (< 60mg) and blot the tissue on Whatman paper. Squash and dry as much as possible.
2. Put the tissue in a 1.5ml Eppendorf tube and snap-freeze in liquid N_2.
3. Cool a pestle, made with a blue tip sealed at the narrow end, in liquid N_2 and grind the tissue to powder.
4. Add 500µl prewarmed extraction buffer and grind further.
5. Add a further 200µl extraction buffer and 7µl 10mg/ml RNase A. Mix.
6. Incubate at 65°C for 10 min.
7. Add 600 µl chloroform-isoamyl alcohol (24:1) and shake well.
8. Spin at 13k for 10 min.
9. Transfer the upper aqueous phase to a new tube and add 0.7x volume isopropanol. Mix by shaking.
10. Immediately spin at 13k for 10 min.
11. Wash the pellet with 70% ethanol and air-dry.
12. Resuspend the pellet in 15-30µl TE.
13. Use 1µl for each PCR.

Large scale extraction

1. Harvest 250-350 mg moss tissue and blot on Whatman paper. Squash to dry as much as possible.
2. Snap-freeze the tissue in liquid N_2.
3. Grind tissue to a fine powder in a cooled pestle and mortar.
4. Add the tissue to 3ml prewarmed extraction buffer in a 30 ml tube.

5. Add 30μl 10 mg/ml RNase A.
6. Incubate at 65°C for 15 min.
7. Add 3 ml chloroform-isoamyl alcohol (24:1) and shake well.
8. Spin at 9.5k in SA600 for 10 min.
9. Transfer the upper aqueous phase to a new tube and add 0.7x volume isopropanol. Mix by shaking.
10. Immediately spin at 9.5k in SA600 for 10 min.
11. Remove the supernatant and add 700 μl 70% ethanol. Swirl to lift the pellet up and transfer to a 1.5ml Eppendorf tube with ethanol.
12. Spin at 13k for 5 min in bench-top centrifuge.
13. Air dry the pellet and then resuspend in 70-90 μl TE.
14. Use 20-30 μl prep for genomic digest for southern analysis.

Extraction Buffer

100 mM Tric-HCl pH8.0	25 ml	2 M Tris-HCl pH8.0
1.42 M NaCl	142 ml	5 M NaCl
2% CTAB	10 g	CTAB
20mM EDTA	20 ml	0.5M EDTA
2% PVP-40	10g	PVP-40
dH_2O	to 500 ml	

Autoclave and store at RT
Immediately before use, add 7μl beta-mercaptoethanol and 10 mg ascorbic acid to 10 ml buffer. Prewarm to 65°C.

Note: young tissue gives best yield.