MOSS DNA EXTRACTION

Small scale extraction for PCR analysis
1. Harvest 8mm² - 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 N₂.

3. Cool a pestle, made with a blue tip sealed at the narrow end, in liquid N₂ 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 N₂.

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**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mM Tric-HCl pH8.0</td>
<td>25 ml</td>
<td>2 M Tris-HCl pH8.0</td>
</tr>
<tr>
<td>1.42 M NaCl</td>
<td>142 ml</td>
<td>5 M NaCl</td>
</tr>
<tr>
<td>2% CTAB</td>
<td>10 g</td>
<td>CTAB</td>
</tr>
<tr>
<td>20mM EDTA</td>
<td>20 ml</td>
<td>0.5M EDTA</td>
</tr>
<tr>
<td>2% PVP-40</td>
<td>10 g</td>
<td>PVP-40</td>
</tr>
<tr>
<td>dH₂O</td>
<td>to 500 ml</td>
<td></td>
</tr>
</tbody>
</table>

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.