User-Developed Protocol:

Isolation of genomic DNA from plants using the QIAGEN® Genomic-tip

This procedure has been adapted by customers from the QIAGEN® Genomic-tip Protocols, and is for use with QIAGEN Genomic-tips**. It has not been thoroughly tested or optimized by QIAGEN.**

The protocol has been successfully used by customers for genomic DNA isolation from tobacco, *Arabidopsis*, maize, cotton, tomato, pine, rhododendron, oak, fir, elm, and poplar. Genomic DNA prepared by this method is generally suitable for restriction enzyme digestions, Southern blotting, and PCR.

The protocol below is designed for isolation of genomic DNA from 1–2 g of starting material with a QIAGEN Genomic-tip 500/G. However, it can be scaled down for use with QIAGEN Genomic-tip 100/G.

Please be sure to read the *QIAGEN Genomic DNA Handbook* and the detailed Protocol for Isolation of Genomic DNA from Blood, Cultured Cells, Tissue, Yeast, or Bacteria.

Procedure

1. **Grind up to 1–2 g of leaf material in liquid nitrogen with a mortar and pestle.**
2. **Transfer tissue powder into a 50 ml screw-cap tube.**
3. **Add 20 ml of Carlson lysis buffer pre-warmed to 74°C.**

   Carlson lysis buffer:

100 mM	Tris ·Cl, pH 9.5
2 %	CTAB
1.4 M	NaCl
1 %	PEG 6000 or 8000
20 mM	EDTA

4. **Add 50 µl ß-mercaptoethanol and 200 µl of RNase A (20 mg/ml). Vortex at full speed for 5–10 s**

   Note: ß-mercaptoethanol is toxic; dispense in a fume hood and wear appropriate protective clothing.

5. **Incubate at 74°C for 20 min in a shaking water bath.**

   Note: If a shaking water bath is not available, gently shake the samples every 5 min during incubation.

6. **Cool the samples to room temperature, add 1 volume of chloroform/isoamylalcohol (24:1), and vortex at full speed for 5–10 sec.**

7. **Centrifuge at 5000 x *g* for 10 min at 4°C.**
8. **Transfer aqueous upper phase to a fresh 50 ml screw-cap tube.**
9. **Add 1 volume of distilled water and adjust the pH to 7.0 using HCl.**
   Note: Generally 100–200 µl of 25% HCl is required.
10. **Follow the QIAGEN Genomic-tip 500/G procedure in the *QIAGEN Genomic DNA Handbook*.**

Reference

*Carlson et al. (1991) Theor. Appl. Genet. **83**, 194–200.*

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.

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