

The following protocol describes a user developed lysis procedure followed by DNA purification using the QIAGEN® Genomic-tip.

The protocol has been successfully applied for the isolation of HMW genomic DNA from plants and filamentous fungi, as well as clam symbionts.

User-Developed Protocol:

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

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

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

Procedure

- 1. Estimate the approximate DNA content per unit weight (e.g., mg or g) of tissue. Which particular QIAGEN-tip is used will depend upon the amount of DNA required (Genomic-tip 20/G: up to 20 µg; 100/G: up to 100 µg; 500/G: up to 500 µg). Freeze the appropriate amount of tissue in liquid nitrogen and pulverize.**
- 2. Suspend powder in lysis buffer (Genomic-tip 20/G: 2ml; 100/G: 15 ml; 500/G: 40 ml) containing:**
 - 20 mM EDTA
 - 10 mM Tris-Cl, pH 7.9
 - 0.5 mg/ml of an enzyme for digesting cell wall material
 - 1 % Triton® X-100
 - 500 mM Guanidine-HCl
 - 200 mM NaCl.
- 3. Incubate at 37–45°C for 1–2 h with gentle agitation.**
- 4. Supplement with DNase-free RNase A (20 µg/ml) and incubate for 30 min at 37°C.**
- 5. Add Proteinase K to 0.8 mg/ml and incubate for 2 h at 50°C with gentle agitation.**
- 6. Centrifuge for 20 min at 12–15,000 x g to pellet insoluble debris.**
- 7. Transfer clarified lysate to the appropriate buffer QBT-equilibrated QIAGEN Genomic-tip (Genomic-tip 20/G: 1 ml; 100/G: 3 ml; 500/G 10 ml).**
- 8. Wash with Buffer QC (Genomic-tip 20/G: 4 x 1 ml; Genomic-tip 100/G: 2 x 10 ml; Genomic-tip 500/G: 2 x 30 ml).**
- 9. Elute with Buffer QF (Genomic-tip 20/G: 0.8 ml; Genomic-tip 100/G: 5 ml; Genomic-tip 500/G: 15 ml).**

**User-developed
protocol**

10. **Precipitate DNA by adding 0.7 volumes of room-temperature isopropanol.**
11. **Centrifuge for 20 min at 15,000 x g to pellet DNA, or spool out DNA on a glass rod.**
12. **Wash with ice-cold 70% ethanol.**
13. **Air-dry and resuspend in TE.**

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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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