

Table 1
10X PCR Buffer (for 10 mL)

For a final (10X) concentration of:	use:	of a:
100 mM Tris-HCl (pH 8.0)	1 mL	1M Tris (pH 8.0)
500 mM KCl	2 mL	2.5M KCl
15 mM MgCl ₂	0.75 mL	0.2M MgCl ₂
0.1% Gelatin	10 mg	Powdered gelatin
2 mM dATP	0.2 mL	0.1M dATP
2 mM dGTP	0.2 mL	0.1M dGTP
2 mM dTTP	0.2 mL	0.1M dTTP
2 mM dCTP	0.2 mL	0.1M dCTP
1% Triton X-100	0.1 mL	100% Triton
H ₂ O	5.35 mL	55.55M Stock
Total	10.0 mL	

brane. Radiolabeled single-stranded DNA probe is hybridized to complementary sequences present in the filter bound DNA. Nonspecifically bound probe is washed off under conditions that allow specifically bound probe to remain hybridized. The presence of complementary sequences is then visualized by autoradiography.

2. Materials for Molecular Analyses

2.1. Isolation of DNA for PCR Analysis

1. Disposable pellet pestles (e.g., no. 749520, Kontes Glass, Vineland, NJ).
2. Extraction buffer: 200 mM Tris-HCl, pH 8.0, 250 mM NaCl, 25 mM Ethylenedinitrilotetraacetic acid (EDTA), 0.5% sodium dodecyl sulfate (SDS).
3. Isopropanol (IpOH).
4. TE: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

These additional reagents are needed if DNA is being isolated from callus tissue:

5. 5M NaCl.
6. 5M KOAc.
7. 30% polyethylene glycol 8000 (no. P 5413, Sigma, St. Louis, MO) in TE.

2.2. Supplies for PCR Reactions

1. PCR buffer (*see Table 1*).
2. Sense and antisense primers.
3. Thermostable polymerase (e.g., AmpliTaq, Perkin-Elmer, Norwalk, CT).
4. Mineral oil.
5. Thermocycler (e.g., PTC-100, MJ Research, Watertown, MA).