7. 20X SSC (1 L; can store for months at room temperature): 175 g NaCl, 88.23 g Na citrate, to 1 L with ddH_2O.
8. Southern base (make fresh before use): 850 mL ddH_2O, 20 g NaOH, 87.7 g NaCl (after NaOH has dissolved), adjust volume to 1 L.
9. Southern neutralization solution (1 L; can store for months at room temperature): 800 mL ddH_2O, 60.5 g Trizma base, 175.3 g NaCl, adjust pH to 7.0 with HCl, adjust volume to 1 L.
10. Prehybridization solution (100 mL; can store for months at –20°C): 30 mL 20X SSC, 20 mL 50X Denhardt's solution, 50 mL ddH_2O.
11. Hybridization solution (10 mL; can store for months at –20°C): 3 mL 20X SSC, 1 mL 50X Denhardt's, 200 µL 1*M* Tris-HCl, pH 8.0, 100 µL 10% SDS, 100 µL 0.5*M* EDTA, 250 µL 0.1*M* Na pyrophosphate, 5.35 mL ddH_2O.
12. Salmon sperm DNA (10 mg/mL in water) (*see* **Note 3**).
13. 6X Wash mix (1 L, can store for months at room temperature): 300 mL 20X SSC, 10 mL 20% SDS, 10 mL 0.5*M* EDTA, 680 mL ddH_2O.
14. 0.3X Wash mix (1 L, can store for months at room temperature): 15 mL 20X SSC, 5 mL 20% SDS, 980 mL ddH_2O.
15. Scientific imaging film (e.g., Kodak X-OMAT XAR-5, Eastman Kodak, Rochester, NY).

3. Methods

3.1. Extraction of DNA for PCR Reactions

3.1.1. DNA Extraction from Leaves for PCR

Unless otherwise noted, all steps should be carried out at room temperature.

1. Add liquid nitrogen to a collected leaf sample (e.g., *see* Chapter 40; **Subheading 3.4.**) and grind to a fine powder in a 1.5-mL microcentrifuge tube with a pellet pestle.
2. Add 300 µL of extraction buffer, mix, and incubate extract in a water bath (65°C, 30–60 min).
3. Centrifuge briefly (15,000*g*, 30 s) to pellet cellular debris.
4. Transfer 150 µL of supernatant to another 1.5-mL microcentrifuge tube.
5. Add 150 µL of isopropanol and mix by inversion.
6. Incubate at room temperature for at least 5 min.
7. Centrifuge (15,000*g*, 5 min) to pellet precipitate.
8. Aspirate and discard each supernatant with a fresh pipet tip.
9. Dry pellets briefly under vacuum.
10. Dissolve each pellet in 30–50 µL TE (depending on the size of the DNA pellet).

3.1.2. DNA Extraction from Callus for PCR

This is a modification of the method of Agudo et al. ***(3)***. If scaled up, sufficient quantities of digestable DNA can be isolated from callus for genomic blot analysis.

1. Put 10–100 mg of callus tissue in a 1.5-mL microcentrifuge tube, add 300 µL of extraction buffer, and homogenize, using a pellet pestle.
2. Incubate the extract in a water bath (65°C, 30–60 min).