

3. Centrifuge briefly (15,000g, 30 s) to pellet cellular debris.
4. Transfer 300 μ L of supernatant to another 1.5-mL microcentrifuge tube.
5. Add 75 μ L 5M NaCl and 120 μ L 5M KOAc and incubate on ice 5–10 min.
6. Centrifuge (15,000g, 5 min) to pellet precipitate.
7. Transfer supernatant to another 1.5-mL microcentrifuge tube.
8. Add 180 μ L 30% PEG to the supernatant fraction, mix and incubate on ice for at least 20 min.
9. Centrifuge (15,000g, 5 min) to pellet nucleic acids.
10. Aspirate and discard supernatant with a fresh pipet tip.
11. Dry pellets briefly under vacuum.
12. Dissolve each pellet in 30–50 μ L TE (depending on the size of the DNA pellet).

3.2. Running the PCR Reactions

Prior to analyzing putative transgenic rice plants, primers should be designed and synthesized (*see Note 4*). Also, a PCR amplification program should be optimized (*see Note 5*). The following steps should be done sequentially in order to limit possibilities of contamination.

1. Set up a single homogenous reaction mix that contains the following components (per sample). Always include negative and positive control reactions (*see Note 6*). Reaction mix required per sample: 5 μ L 10X PCR buffer, 2 μ L sense primer @ OD₂₆₀ = 2, 2 μ L antisense primer @ OD₂₆₀ = 2, 0.05 μ L *Taq* DNA polymerase (0.25 U), 40 μ L H₂O, yielding a total volume of 49 μ L per sample.
2. Aliquot 49 μ L of mix into a 0.5-mL microcentrifuge tube for each sample, and overlay with 25 μ L of mineral oil. (Put all PCR stock solutions back into the –20°C freezer before handling any DNA samples.)
3. Add 1 μ L of rice DNA for each reaction. (Put away all DNAs from test samples before handling any positive control DNA.)
4. For the positive-control reaction, add 10 pg (1 μ L of a 10 ng/mL) plasmid DNA to ~100 ng of wild-type rice DNA.
5. Run the appropriate PCR program for fragment amplification.
6. Transfer 15 μ L of the PCR reaction to another tube containing 3 μ L of gel loading dye.
7. Size-fractionate PCR products by agarose gel electrophoresis (1–2% gel, depending on the size of the amplification product expected).
8. Results of typical reactions are shown in **Fig. 1** and discussed in **Section 3.5.1**.

3.3. Extraction of Genomic DNA for Blotting

The volumes in this protocol are adjusted for 0.5–1.5 g samples and will yield 50–100 μ g DNA. For larger samples, use appropriately scaled-up volumes. Unless otherwise noted, all steps should be carried out at room temperature.

1. Weigh out approx 1 g of rice leaves (rapidly growing tissues such as young leaves are preferable) and slice into 0.5-cm sections. Place leaf cuttings into a cold mortar, add enough liquid nitrogen to cover the leaves and grind them to a