

3. Centrifuge briefly (15,000g, 30 s) to pellet cellular debris.
4. Transfer 300 μL of supernatant to another 1.5-mL microcentrifuge tube.
5. Add 75 μL 5M NaCl and 120 μL 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 μL 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 μL 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 μL 10X PCR buffer, 2 μL sense primer @ $\text{OD}_{260} = 2$, 2 μL antisense primer @ $\text{OD}_{260} = 2$, 0.05 μL *Taq* DNA polymerase (0.25 U), 40 μL H_2O , yielding a total volume of 49 μL per sample.
2. Aliquot 49 μL of mix into a 0.5-mL microcentrifuge tube for each sample, and overlay with 25 μL of mineral oil. (Put all PCR stock solutions back into the -20°C freezer before handling any DNA samples.)
3. Add 1 μL 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 μL 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 μL of the PCR reaction to another tube containing 3 μL 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 μg 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