c. Use PCR-designated reagents, preferably stored in small aliquots. For those not aliquotted in advance (e.g., organic solvents), aliquot into a new disposable chemical resistant container (e.g., Falcon 2059 tubes) just prior to use and discard any excess.
d. Use separate designated areas for sample workup (e.g., lab bench), PCR set up (e.g., laminar flow hood), and PCR product analysis (e.g., electrophoresis bench).
e. Use disposable pestles for grinding tissue. Periodically decontaminate pipeters, microcentrifuge racks, and so on, by irradiation with a germicidal lamp.

2. To assemble a blotting table, place a 20 × 30-cm glass plate on four size 11½ rubber stoppers in a 26.5 × 37.5 × 5.5-cm glass baking dish. Cut chromatography paper 26 × 36 cm, and notch the corners so the edges can hang over the sides of the glass plate and touch the bottom of the baking dish. Saturate the paper with 20X SSC. Cut another paper 20 × 30 cm and place over first on top of the glass. Saturate with 20X SSC and roll any bubbles out with a pipet. The table can be stored for weeks by sealing the top with plastic film. Replace the upper layer of paper prior to reuse.
3. The salmon sperm DNA solution should be denatured and sheared by heating to 100°C for 10 min with intermittent vigorous vortexing. It can be stored for months at –20°C. Once denatured, avoid conditions that allow renaturation and occasionally repeat heat denaturation.
4. Most primers we use have 20–24 nt and are ~50–60% G + C. Two of the last 3 nt at the 3' end of the primers, especially the ultimate nt, should be G or C if possible. We generally amplify fragments that range from ~400–2000 bp.
5. Optimization of a PCR program can be done by using 10 pg (1µL of a 10 ng/mL solution) purified plasmid DNA as substrate. A good starting point for determining the annealing temperature (T_a) of a typical (i.e., ~20 nt) PCR primer in standard PCR buffer is to use the equation T_a = (4 × G + C residues) + (2 × A + T residues). Twenty cycles are usually adequate to amplify a fragment from purified plasmids. Once conditions are defined for amplification of the target sequences from plasmid DNA, add 10 pg of plasmid to 50–100 ng of wild-type rice DNA (isolated using the above protocol) and run the reaction for 30–40 cycles. (This number of amplification cycles is usually required because of the relatively low purity of DNA prepared using the above method.) Further adjustments to the PCR program may be necessary.
6. For every set of PCR reactions, include two negative controls (no DNA and wild-type rice DNA) and a positive control. Preferably, the positive control DNA should be from a transgenic plant known to contain the target sequences. Alternatively, plasmid added to wild-type rice DNA (~10 pg into 100 ng) can be used. The DNAs used for controls should be isolated in parallel with the samples to be tested. At least until the DNA isolation technique becomes routine, those extracts yielding negative results should be tested to confirm that the DNA can serve as a suitable PCR substrate. This is easily done by amplifying a fragment from an endogenous single-copy gene (e.g., cytosolic triose phosphate isomerase) (*see* **Fig. 1**).