7. For blot analysis, we normally load 2 μg of DNA per gel lane, although higher amounts can be used. Routinely, DNA aliquots are run undigested to confirm that the introduced sequences comigrate with rice genomic DNA, indicating integration in the genome. Additional aliquots are digested with ~8 U of restriction enzyme for 4 h in a total volume of 20–30 μL. After adding loading buffer, the entire reaction can then be loaded in a single well of a gel. Positive and negative controls should always be included. As a negative control, we use wild-type T309 DNA digested with the same restriction enzyme as the test samples. A one copy/haploid genome reconstruction is included as a positive control. This consists of 2 μg of wild-type rice DNA that has been spiked with plasmid DNA. The amount of plasmid to add can be determined by the following equation:

$$n*D*P*660 / K*6.02 \times 10^{23} = M$$

where: n = the number of gene copies desired in the reconstruction; D = μg genomic DNA loaded per lane; P = plasmid size in bp; $K = 0.6 \times 10^{-12}$ μg, the mass of a haploid copy of the rice genome; and M = μg of plasmid DNA to use.

The reconstruction and putative transgenic rice DNAs should be digested with an enzyme(s) to release a DNA fragment that would indicate whether the introduced gene is intact. When compared to the intensity of the reconstruction, the intensity of this fragment can also be used to estimate the copy number of the introduced gene. Additional copy number information can be obtained by digesting the DNA with an enzyme that cuts once in the center of the introduced gene. If the probe hybridizes only to the sequences on one side of the site, the minimum copy number of the introduced gene will equal the number of hybridizing fragments. The copy number would be half that number if the hybridization probe is complementary to sequences on both sides of the restriction site. After blotting and hybridization with a high specific activity probe (~1×10^9 cpm/μg DNA), single copy/haploid genome fragments can be visualized by overnight exposure (–70°C, one enhancing screen) to Kodak X-OMAT XAR-5 film, or 2 h exposure to a phosphoimager plate.

8. Cover the lower portion of the gel with a folded paper towel to avoid nicking the low-mol-wt DNA (~6 kb and less). Expose the uncovered portion of the gel to a UV light (254 n*M*). The time of exposure and distance from the light source must be determined empirically to optimize DNA transfer, but allow efficient hybridization once blotted. (Presumably, thymidine dimers or other products produced during UV exposure interfere with hybridization.) Begin with a 15-s exposure at ~10–15 cm. For this step, we use a Stratagene UV crosslinker (3000 μW/cm^2) and expose for 0.6 min. The upper 2 cm of the gel should be exposed for another 0.4 min to further nick the uncut genomic DNA.
9. Binding of radiolabeled probe to contaminants (body oils, proteins, and so on) on the membrane can lead to high background. Therefore, care should be taken when handling the membranes. Always use gloves or clean, blunt forceps, and avoid contacting dirty surfaces.