autoradiogram indicates the number of copies present. Tandemly linked T-DNAs can also be diagnosed by cutting with restriction enzymes that cut once within the construct. In this case, a single intense band is generated from internal fragments within the tandem repeat. Border fragments of different sizes are also generated. This experiment is important in order to differentiate between single and multiple insertion events when segregation data have indicated a single locus insertion. In all cases it is important to run a negative control using nontransformed genomic DNA, to check that, under the experimental conditions used, there is no background hybridization that could confuse the analysis.

1. Digest genomic DNA with appropriate restriction enzymes (*see* **Note 9**).
2. Prepare a 0.8% agarose gel in 1X TAE buffer. Before pouring into casting tray, add ethidium bromide solution (to a final concentration 0.05 mg/mL).
3. Mix digested DNA with a 0.1 vol gel loading buffer.
4. Place the gel in electrophoresis apparatus, and cover with 1X TAE buffer.
5. Add ethidium bromide solution to the 1X TAE buffer (to a final concentration of 0.05 mg/mL).
6. Load the DNA samples and 1 μg 1-kb ladder on the gel.
7. Run the gel at 60–100 V until the bromophenol blue visual dye marker has migrated approx three-fourths of the way down the gel.
8. Visualize the DNA on a UV transilluminator and photograph, using Polaroid Land Camera or video camera (Flowgen) with an orange G filter to maximize the contrast (*see* **Note 10**).

3.2.2. Preparation of Gel for DNA Transfer

1. After photographing, incubate the agarose gel in depurinating solution for 15 min at room temperature, with gentle agitation. This acts to depurinate or acid-nick the DNA, and increases the efficiency of DNA transfer to the membrane.
2. Remove the depurinating solution and rinse the gel in distilled water.
3. Incubate the gel in denaturing solution for 30 min at room temperature, with gentle agitation (*see* **Note 11**).
4. Rinse the gel in distilled water.
5. Incubate the gel in neutralizing solution for 30 min at room temperature, with gentle agitation (*see* **Note 12**).
6. The gel is now ready for DNA transfer.

3.2.3. DNA Transfer

There are many different membranes commercially available for nucleic acid blotting. The nylon-based ones are the most practical, because they are very strong and can withstand repeated use (which is important if the membranes are to be probed sequentially, such as with more than one DNA sequence), with minimal loss of sensitivity. The products that we have used in our laboratory are Zeta-Probe GT (Bio-Rad), Gene Screen Plus (Dupont,