6. The CTAB is soluble in ethanol, but the nucleic acids are not. The ethanol washes remove any remaining CTAB from the nucleic acids, which may otherwise inhibit restriction-enzyme digestion.
7. If highly pure DNA is required, the DNA can be purified on a CsCl/EtBr gradient at this stage.
8. Instead of removing the RNA at this stage, RNase can be added to the DNA during restriction enzyme digestion. In this case, 2 μL of 0.5 mg/mL RNase A (diluted from the 10 mg/mL stock, **Subheading 2.1.**) can be added to each reaction.

4.2. Southern Blotting

4.2.1. Restriction Enzyme Digestion of Genomic DNA and Agarose Gel Electrophoresis

9. The amount of DNA digested varies with the source of the DNA. Typically, 1–10 μg are digested to detect a single copy T-DNA, depending on the species. For *Arabidopsis*, 500 ng–1 μg is sufficient, but for tobacco, 10 μg is usually required. It is best to digest genomic DNA overnight.
10. When photographing the gel, it is useful to put a ruler alongside to provide a reference for sizing the hybridizing bands later.

4.2.2. Preparation of the Gel for DNA Transfer

11. The incubation period of 30 min is the minimum time required for denaturation, and can be increased to 2 h without any detrimental effects, providing high quality agarose has been used to make the gel.
12. The incubation period of 30 min is the minimum time required for neutralization. It is better to increase this incubation time, especially if nitrocellulose is being used, since this type of membrane becomes fragile if put in contact with a gel that is not completely neutralized.

4.2.3. DNA Transfer

13. Ensure that the 3MM paper is saturated with buffer and that there are no air bubbles present, which would prevent local transfer of the DNA.
14. Push the clingfilm 2 mm under the gel; this will prevent the buffer short circuiting the gel, which would result in poor transfer.
15. Cut a corner off the membrane to allow orientation later, and mark the positions of the wells with a soft pencil.
16. The DNA can be bound to the membrane by baking the membrane at 80°C for 30 min in a vacuum oven. Alternatively, the DNA can be bound to the membrane by UV crosslinking. Before using this method, the UV source has to be calibrated for the particular membrane ***(6)***. Automatically regulated UV crosslinking machines are now commercially available from several manufacturers (e.g., Stratagene, Cambridge, UK).