65°C for 15–30 min in a Hybaid oven (*see* **Note 20**). Occasionally, it may be necessary to alter the hybridization conditions (*see* **Note 21**).
2. Add the denatured radioactive probe to some fresh Church buffer (10 mL) and mix carefully.
3. Pour away the prehybridization buffer and replace it with the probe solution. The hybridization should be carried out at 65°C, with constant agitation, for at least 12 h.
4. Remove the probe solution (*see* **Note 22**) and replace with Wash I. Incubate at 65°C, with constant agitation, for 30 min. Remove the wash and repeat.
5. Remove Wash I and repeat the procedure with Wash II.
6. If the activity is not localized and the background is still high, repeat **step 5**.
7. Remove excess wash solution, wrap the membrane in Saran Wrap, and expose to X-ray film at –80°C, in a cassette containing signal intensifying screens.
8. If desired, after autoradiography, the blot can be stripped and reprobed by following the protocol in the next section.

3.5. Probe Stripping and Rehybridization

1. If reprobing is desired, do not let the membrane dry out between hybridizations (*see* **Note 23**).
2. Wash the membrane 2 × 20 min each in a large volume of 0.1X SSC/0.5% SDS at 95°C (*see* **Note 24**).
3. Check that all of the probe has been removed from the blot by exposing overnight.

4. Notes

4.1. Genomic DNA Extraction

1. It is important not to crush the plant material or let it wilt, because this may affect the efficiency of the extraction. The protocol can be successfully scaled up or down, depending on the amount of tissue available.
2. After adding the 2X CTAB buffer to the ground plant material, the suspension is sometimes very viscous. This is dependent on the source of the material, and the amount of polysaccharide present in the tissue. If very viscous, it may be advantageous to dilute the extract further with aliquots of CTAB extraction buffer.
3. CTAB will precipitate at temperatures below 15°C, so ensure that the centrifuge and rotor are at room temperature before inserting the tubes.
4. This step precipitates CTAB–nucleic acid complexes by reducing the NaCl concentration; if no precipitate forms, add a little more precipitation buffer. Precipitates may be stringy or fine suspensions. The solution can be left for a longer time than indicated to allow precipitation, even overnight, as long as the temperature is maintained over 20°C.
5. The CTAB–DNA pellet may not resuspend immediately, and it may be neccessary to heat the solution to 50°C. It is often easier to resuspend the CTAB–DNA complexes if they have been pelleted in a low-speed benchtop centrifuge.