10. It is essential that bubbles are not present to assure uniform flow of buffer, and thereby uniform transfer of the DNA, to the membrane.
11. Parafilm or other hydrophobic material can be butted up to the gel on all sides to prevent overhanging paper from absorbing buffer without it going through the gel.
12. Use $5 \times 10^6$ cpm denatured probe/mL hybridization solution. To denature, dilute the volume of probe needed to at least 50 µL with $H_2O$, heat denature (95–100°C, 5 min), and fast chill for 2–3 min on wet ice prior to adding to the hybridization solution.
13. If the membrane becomes dry, it is virtually impossible to remove the hybridized probe. If kept moist, ~90–100% of the probe can be removed and the blot reused. (We have reused nitrocellulose blots 6–7 times with only limited loss in sensitivity.) Strip the probe by incubating it in 200–300 mL of 30 m*M* NaOH, 1 m*M* EDTA for 15 min at room temperature, with gentle agitation. Decant and neutralize with a similar volume of Southern neutralization solution. Use a radiation monitor before and after stripping to evaluate the effectiveness of probe removal. If some radioactivity remains, expose the blot for a specific time to determine the background level. To re-use the blot, the prehybridization step can be omitted. Add the hybridization solution (without probe) and incubate at 65°C for 1–2 h before adding the probe.

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