empirically and depends on whether the target is DNA or RNA, the GC content, and homology of probe to target, further details can be found in **ref. *1***. Although the use of DIG Easy Hyb buffer is probably the easiest option, if buffer containing formamide is required, the following can be used: 5X SSC, 0.1% lauryl-sarcosine, 0.02% SDS, 1% blocking reagent, and 50% deionized formamide.

11. Prehybridization can be longer than 30 min, but do not exceed 2 h.
12. Probe denaturation is more efficient if done in a water bath rather than a dry block.
13. Unbound probe is removed in washing **steps 1** and **2**. If using probes >100 bp, the washes in **step 2** must be at 68°C; for shorter probes, the washing temperature must be determined empirically. Membranes are most easily washed by shaking in a dish in an incubator, rather than in a hybridization oven. An exception to this are the washes in **step 2** at 68°C, which is difficult to achieve in an incubator. It is important to preheat the roller and buffer before use in this step. Never allow the membrane to dry out at any time, because this will increase background.

References

1. DIG System User's Guide for Filter Hybridization. Boehringer Mannheim, Mannheim, Germany.
2. Verwoerd, T. C., Dekker, D. M. M., and Hoekema, A. (1989) A small-scale procedure for the rapid isolation of plant RNAs. *Nucleic Acids Res.* **17,** 2362.