2. Bind the RNA to the membrane by exposure to UV or by heating (*see* **Note 2**). The membrane should be irradiated on both sides before hybridization with DIG probes, as described in **Subheading 3.7**. Following nucleic acid detection, quantitative estimates of the amount of RNA can be made (*see* **Note 9**).

3.7. Hybridization of DIG-Labeled DNA Probes to RNA on Nylon Membranes

See **Note 10** for discussion of hybridization buffers.

1. Prewarm to 37°C the roller containing 20 mL DIG Easy Hyb solution and incubate the membrane for 30 min to prehybridize (*see* **Note 11**).
2. Denature the DIG-labeled DNA probe by boiling for 10 min and then rapidly chilling on an ice–water mix. Add probe to fresh prewarmed DIG Easy Hyb solution (2.5 mL is sufficient for a 100-cm^2 membrane; if using a roller, the minimum volume required is 8 mL) and mix gently. Do not allow foaming to occur, because bubbles cause increased background.
3. Pour off and discard the prehybidization solution from the membrane and place it in a plastic bag; immediately add the probe in DIG Easy Hyb. Do not allow the membrane to dry out.
4. Incubate overnight at 37°C.
5. Pour off the hybridization probe solution and retain. Probes can be re-used three to four times after storage at –20°C. When reusing a probe diluted in DIG Easy Hyb, it must be denatured at 68°C for 10 min and fast chilled before use (*see* **Note 12**). Hybridized nucleic acid should be detected as described in **Subheading 3.8.**

3.8. Detection of Hybridized Nucleic Acid

1. After hybridization, wash the membrane for 2 × 5 min at room temperature with at least 50 mL 2X SSC containing 0.1% SDS to remove unbound probe. It is preferable to do this in a dish on an orbital shaker, rather than using a hybridization oven. Ensure there is enough buffer for the membrane to move freely.
2. Wash 2 × 15 min at 68°C with at least 50 mL of 0.1X SSC containing 0.1% SDS. The buffer should always be prewarmed, and, if using a hybridization oven, both the hybridization tube and buffer should be preheated (*see* **Note 13**).
3. Now proceed from **steps 4–11** of **Subheading 3.3.** Use 1 mL of diluted CSPD substrate for a 100-cm^2 blot.
4. Expose to standard X-ray film for 30 min–24 h, as necessary. Exposures can be obtained for up to 2 d after the addition of the chemiluminescent substrate.

4. Notes

1. Filtration of many solutions is recommended by Boehringer, but we commonly autoclave without apparent problems.
2. RNA and DNA can be bound to the membrane by heating at 120°C for 30 min or by exposure to 120,000 μJ/cm^2 UV. Always UV crosslink both sides of the membrane, because this will reduce background.