3. Buffer 2: Blocking stock solution (*see* **item 5**) diluted 1 in 10 in buffer 1. This buffer should be made fresh every time it is used, because it deteriorates quickly.
4. Buffer 3: 100 m*M* Tris-HCl, 100 m*M* sodium chloride, pH 9.5.
5. Blocking stock solution: Make up 10% blocking reagent in buffer 1 and heat until almost boiling, mixing occasionally, then autoclave and store at 4°C.
6. Wash buffer: Buffer 1 + 0.3% Tween-20. Make fresh, as required.
7. RNA extraction buffer: 100 m*M* Tris-HCl, 100 m*M* lithium chloride, 10 m*M* EDTA, 1% SDS, pH 8.0.
8. MOPS buffer (10X): 0.4*M* MOPS, pH 7.0, 100 m*M* sodium acetate, 10 m*M* EDTA.
9. SSC (20X): 0.3*M* sodium citrate, 3*M* sodium chloride, pH 7.0.
10. Tris-SDS: 100 m*M* Tris-HCl, 0.2% SDS, pH 8.0.
11. Agarose gel loading buffer: 50% glycerol, 1 m*M* EDTA, 0.4% bromophenol blue, and 0.4% xylene cyanol. Do not autoclave.

2.3. Apparatus

In addition to general laboratory apparatus and a thermocycler for labeling probes by PCR, the following are required.

1. Several steps in the hybridization procedure require the use of heated solutions and constant movement of the solutions over the membrane. This can be achieved using a dish on an orbital shaker in an incubator, but is much better done in a purpose-made hybridization oven with rotating hybridization tubes. At other stages, when the membrane is incubated in a sealed plastic bag, this should be agitated on an orbital shaker.
2. RNA is bound to nylon membranes by heating at 120°C for 15–30 min or by UV-irradiation (*see* **Note 2**) using a purpose-made UV crosslinker. In the absence of such a machine, an appropriate UV lamp or transilluminator could be used, but the time of exposure should be determined empirically.
3. Glass dishes are required at several stages for incubating membranes. These, and all other glassware, should be sterilized before use by dry heat at 145°C.

3. Method

3.1. Labeling Probes by PCR

The use of a Boehringer PCR-DIG labeling kit is recommended. *See* **Note 3** for discussion of some theoretical and practical aspects.

1. Resuspend up to 1 μg template DNA in 5 μL sterile dH_2O.
2. Mix, on ice, in a PCR reaction tube: 66.5 μL sterile dH_2O, 10 μL PCR buffer, 6 μL $MgCl_2$, 10 μL dNTP mix (containing 2 m*M* dGTP, dATP, dCTP, 1.3 m*M* dTTP, and 0.7 m*M* DIG-11-dUTP), 1 μL each of upstream and downstream primers (1 mg/mL).
3. Add to PCR reaction tube: 5 μL template DNA and 0.5 μL *Taq* polymerase.
4. Mix PCR reaction constituents thoroughly and centrifuge briefly to collect the liquid. Overlay with 100 μL of light liquid paraffin, if required by the