2. The extracts are clarified by centrifugation in a microcentrifuge at 15,900*g* for 15 min. The supernatant is then ready for application to a nylon membrane. Denaturation of the samples at this step can help to increase sensitivity (*see* **Note 3**).
3. Cut a piece of a nylon membrane to the appropriate size. Before removing the two pieces of paper that protect the membrane, mark it into 1 × 1-cm squares with a pencil. The membrane below will be sufficiently marked by the pencil's pressure for the squares to be visible. Cut a corner of the membrane so that the samples can be located after detection. It is important to handle the membrane with gloved hands or with forceps to avoid undesirable background.
4. A 5-μL sample of the supernatant obtained in **step 2** is then applied to each marked square of the membrane. Nylon membranes does not need any previous treatment. Nitrocellulose membranes must be prepared by presoaking in water and then in 20X SSC. They must also be dried before being loaded with RNA. Larger volumes can be applied under vacuum with the aid of a Bio-dot blotting apparatus (Bio-Rad, Hercules, CA). The sample volume must be then adjusted to avoid saturation of the membrane. The nucleic acids are bound covalently to the membrane by baking at 80°C for 2 h (*see* **Note 5**).
5. Nonspecific binding sites are blocked by incubating the membrane in the prehybridization solution (10 mL/100 cm^2 of membrane) twice for 1 h at 68°C.
6. The prehybridization mixture is then removed and replaced by 10 mL/100 cm^2 of the same solution, plus 0.2–0.5 μg/mL of the DIG-labeled RNA probe, and incubated overnight at 68°C. DNA–DNA hybridizations must be made at 42°C; hybridizations of the RNA probes to DNA blots are usually performed at 50°C. Preferably, the prehybridization and hybridization steps are carried out in a hybridization oven, if available. Alternatively, they can be carried out in heat-sealable plastic bags in a water bath, with gentle shaking, in which case care must be taken that no air bubbles are trapped in the plastic bag. The solution containing the probe can be reused several times without loss of sensitivity. In addition, probes stored at –20°C in the hybridization solution can be used for several months without significant loss of sensitivity. In our case, probes stored up to 2 yr, which have had no more than four or five uses, gave satisfactory results.
7. Finally, the membrane is washed 2 × 5 min at room temperature with washing solution I, followed by 2 × 15 min at 68°C in washing solution II, to eliminate the nonhybridized probe.

3.3. Immunological Detection

All the steps are performed at room temperature with gentle shaking. Put the membrane in a plastic box or in hybridization bags, and make sure that the solution covers the membrane totally.

1. The membrane is washed briefly (approx 2 min) in washing buffer.
2. Blocking reagent (buffer 2) is then added and the membrane is incubated for 30 min at room temperature to block nonspecific detection.