The equipment available for carrying out hybridizations has improved over recent years, allowing the reactions to be carried more safely and with greater ease. The rotisserie-type hybridization oven (e.g., Hybaid) can be very useful. Multiple hybridization reactions can be carried out simultaneously (provided the same temperature is required), and the glass bottles used in the oven mimimize the risks associated with the handling of radioactively labeled solutions and membranes.

The protocol presented here is designed to allow the detection of a specific DNA fragment using a homologous, radioactively labeled DNA sequence as probe. Both the hybridization reaction and washes are carried out under stringent conditions, at 65°C, to minimize nonspecific hybridization. There are various hybridization buffers that can be used, but Church buffer ***(5)*** seems to produce the best results. The SDS within the buffer acts as a blocking agent, which, in conjunction with the reaction temperature of 65°C, eliminates problems of background hybridization. This buffer can be used successfully with any of the membranes mentioned above. It is convenient to make up 2–3 L of both the hybridization buffer and the wash solutions and store them at 65°C.

1. Purify the DNA fragment by electroelution or using the Gene Clean kit (Bio 101), and resuspend in distilled water at a concentration of 10–100 ng/μL. This stock can be stored for several months at –20°C.
2. To a sterile Eppendorf tube, add 0.6 μL of BSA, 3 μL of OLB, 10–50 μCi [^{32}P]dCTP, and 0.6 μL (0.5 U) of the large fragment of DNA polymerase I (Klenow fragment). Store on ice.
3. The final volume of the oligolabeling reaction will be 15 μL. Therefore, add an appropriate volume of distilled water to 10–50 ng of the DNA probe stock solution and boil for 5 min to denature the DNA. Centrifuge briefly to collect the contents to the bottom of the tube, and cool on ice before adding to the reaction mix.
4. Incubate the reaction at 37°C for approx 1 h (*see* **Note 17**).
5. Add 200 μL TE, pH 7.2, to stop the reaction.
6. Fractionate the probe to remove the unincorporated [^{32}P]dCTP by running the reaction mixture through a Sephadex G-50 column, equilibrated in TE, pH 7.2 (*see* **Note 18**).
7. Pool the labeled fractions.
8. Denature the probe by boiling for 5 min (*see* **Note 19**) immediately prior to adding to the hybridization solution (*see* **Subheading 3.4.**).

3.4. Hybridization of DNA Probe to Southern Blot

1. Place the membrane in a Hybaid glass bottle and add enough Church buffer to cover the membrane (typically, ca. 10–25 mL). Prehybridize the membrane at