4.3. Radiolabeling a DNA Fragment by the Random Primer Method

17. During the time that the oligolabeling reaction is proceding, set up prehybridization of the blot (*see* **Subheading 3.4.**).
18. This type of labeling reaction is usually very efficient, so the unincorporated [^{32}P]dCTP does not have to be separated from the probe DNA before adding to the hybridization solution. However, genomic Southerns often require a long exposure time, so a clean background is necessary. In this case, probe fractionation can be beneficial.

    When fractionating a probe for the first time, it is helpful to add blue dextran (10 mg/mL) to the reaction mixture just prior to fractionation. The dye co-migrates with the labeled DNA fragment, thereby facilitating identification of the desired fractions (the blue dextran does not interfere with the subsequent hybridization). It is now possible to buy ready made columns for DNA fractionation (e.g., Stratagene NucTrap purification columns).
19. It is important to boil the DNA probe thoroughly, because a double-stranded probe will not bind to sequences on the Southern blot.

4.4. Hybridization of a DNA Probe to a Southern Blot

20. If a Hybaid oven is not available, the hybridization can be carried out in a plastic sandwich box with a tight-fitting lid, such as a Boehringer (Lewes, UK) box (cat. no. 800058). To prevent the membrane drying out during the hybridization period, a piece of Saran Wrap just bigger than the box should be floated on top of the hybridization solution. Alternatively, a heat-sealed plastic bag can be used.
21. It is often useful to be able to alter the stringency (amount of nucleotide mismatch allowed) of the hybridization reaction and the washes. With the Church buffer system, this can be acheived by altering the temperature of the hybridization and washes. Alternatively, the following buffer system can be used, in which the stringency can be altered by changing the salt concentration. Hybridization solution: 6X SSC, 10X Denhardts (50X Denhardts: 1% Ficoll [type 400], 1% PVP, 1% BSA), 0.5% SDS, 6% PEG 6000, 0.5 mg/mL sheared and denatured herring sperm DNA. Wash solution I: 3X SSC, 0.5% SDS. Wash solution II: 0.5X SSC, 0.5% SDS.
22. The probe solution can be stored at –20°C and reused after boiling for 5 min.

4.5. Probe Stripping and Rehybridization

23. The Zeta-Probe membrane should be stripped as soon as possible after autoradiography. If this is not possible, ensure that the blot is well-wrapped with Saran Wrap and store in the freezer.
24. A 1-L Duran bottle is useful for stripping the probe from Southern blots.