Hounslow, UK), and Hybond N (Amersham). For ease of use, high signal-to-noise ratio, and durability, we have found Zeta-Probe GT to be the most suitable. It is important to follow the manufacturer's guidelines for each type of membrane used. However, the methods for use are all broadly similar, and the protocols given below are suitable for all three membranes mentioned above.

1. Cut the nylon membrane to the same size as the gel and presoak the membrane in either distilled water or the transfer buffer (10X SSC), according to the membrane manufacturer's instructions.
2. Cut three pieces of 3MM paper to the same size as the gel, and soak in the transfer buffer.
3. Lay sponges to a thickness of 6–8 cm in a plastic tray and flood with 10X SSC.
4. Lay two pieces of 3MM paper, larger than the gel, on the sponges (*see* **Note 13**).
5. Lay the gel on top of the 3MM paper, checking that there are no air bubbles. Cover the remaining surface of the 3MM and sponges with cling film (*see* **Note 14**).
6. Carefully place the membrane on the gel, ensuring that there are no air bubbles present (*see* **Note 15**).
7. Place the presoaked pieces of 3MM paper on top of the gel, followed by a stack (approx 10 cm thick) of paper towels. Place a glass plate and a suitable weight (e.g., a 500-mL medical flat) on top of the stack and leave standing overnight to allow DNA transfer to take place.
8. If necessary, top up the transfer buffer in the tray before leaving the blot overnight.
9. Remove the membrane, wash it briefly in 10X SSC, and then leave it to air-dry on 3MM paper, DNA-side up.
10. Follow the manufacturer's instructions to irreversibly crosslink the DNA to the membrane (*see* **Note 16**). The membrane is now ready for hybridization.

3.3. Radiolabeling a DNA Fragment by Random Primer Method

A method for radiolabeling DNA fragments in agarose gel slices to high specific activity was devised by Feinberg and Vogelstein ***(4)***. However, the specific activity of the DNA fragments can be significantly increased by purification of the DNA from the agarose gel, by electroelution or by using purification kits (e.g., GeneClean II DNA purification kit available from Bio 101). To estimate the amount of DNA that should be labeled, the hybridization membrane manufacturer's guidelines should be consulted; typically, the probe should be at a concentration of 10–50 ng/20 mL of hybridization solution. It is also important to generate a probe with a good specific activity, to produce a high signal-to-noise ratio during hybridization. A specific activity of 108 cpm/μg probe is generally recommended.