2.2.3. DNA Transfer

1. Nylon membrane (e.g., Zeta Probe GT, Bio-Rad).
2. 10X SSC: 1.5*M* NaCl, 0.15*M* trisodium citrate, pH 7.0.
3. 3MM paper (Whatman, Maidstone, UK).
4. Paper towels.
5. Sponges.
6. Plastic tray.
7. Clingfilm/Sealing film (Whatman).
8. 80°C oven.

2.3. Radiolabeling a DNA Fragment by the Random Primer Method

1. GeneClean II™ DNA purification kit (Bio 101, supplied by Anachem, Luton, UK).
2. Solution O: 1.25*M* Tris-HCl, 0.125*M* $MgCl_2$, pH 8.0 (stored at 4°C).
3. Solution A: 1 mL solution O + 18 μL β-mercaptoethanol + 5 μL of each dATP, dTTP, and dGTP (each triphosphate previously dissolved in TE, pH 7.0, at a concentration of 0.1 *M*; stored at –20°C).
4. Solution B: 2*M* HEPES, titrated to pH 6.6 with 4*M* NaOH (stored at 4°C).
5. Solution C: Hexadeoxyribonucleotides (Pharmacia, St. Albans, UK) dissolved in TE at a concentration of 90 OD_{260} U/mL (stored at –20°C).
6. Oligolabeling buffer (OLB): Prepared by mixing together solutions A, B, and C in a ratio of 100:250:150, respectively. Store in aliquots at –20°C. This protocol assumes that the radiolabel is $\alpha[^{32}P]$dCTP.
7. Sterile dH_2O.
8. BSA (10 mg/mL).
9. $\alpha[^{32}P]$dCTP (10–50 μCi).
10. Klenow fragment of *Escherichia coli* DNA polymerase I.
11. TE: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 7.2.
12. Sephadex G-50 column, equilibrated in TE, pH 7.2.
13. Liquid scintillant: 5% (w/v) POP in toluene.
14. Scintillation counter.

2.4. Hybridization of a DNA Probe to a Southern Blot

1. Church buffer: 0.25*M* Na_2HPO_4, pH 7.2, 7% SDS.
2. Radioactive probe.
3. Wash I: 20 m*M* Na_2HPO_4, pH 7.2, 5% SDS.
4. Wash II: 20 m*M* Na_2HPO_4, pH 7.2, 1% SDS.
5. Saran Wrap (Dow, supplied by SLS, Wilford, UK).
6. X-ray film (e.g., Amersham, Little Chalfont, UK; Hyperfilm-MP).
7. Hybaid (Teddington, UK) oven and glass bottles.
8. Geiger counter.
9. Autoradiography cassette containing signal intensifying screens (e.g., from GRI, Dunmow, UK).