8. Place gel back in the baking dish and add Southern base solution (use 1 L of solution for an 11 × 14-cm gel) and incubate with gentle agitation on an orbital shaker for 1 h (4-mm-thick gels) to 1.5 h (>4-mm-thick gels) to denature the DNA.
9. Briefly rinse excess base from the gel a few times with ddH_2O.
10. Add Southern neutralization solution (~500 mL). Gently agitate for 45 min, pour off, and replace with fresh neutralization solution. Gently agitate for another 1 h (1.5 h for thicker gels).

3.4.2. Blotting Gel

1. Cut two pieces of chromatography paper and one piece of nitrocellulose or nylon membrane to the exact size of the gel to be blotted. Cut off the top right corner of the membrane and label the blot with a soft lead pencil on the bottom center (*see* **Note 9**).
2. Wet membrane in hot (60–90°C) ddH_2O. When hydrated, decant water and saturate membrane and chromatography paper with 20X SSC.
3. Assemble a blotting table (*see* **Note 2**).
4. Put a puddle (~20–30 mL) of 20X SSC on the blotting table and lay the gel upside down in the puddle, being careful to avoid trapping bubbles. Gently rub the gel, or roll with a pipet, to remove any bubbles trapped between the gel and the chromatography paper (*see* **Note 10**).
5. Put a puddle of 20X SSC on the gel. Carefully lay the membrane (labeled side down) on the gel and align the cut corner for orientation and the top of the membrane precisely with the wells to allow accurate measurements. Avoid trapping bubbles, as above.
6. Put a puddle of 20X SSC on the membrane. Carefully lay one piece of chromatography paper on the membrane, avoiding bubbles. Lay the second piece on the first and then stack paper towels (that are cut to the same size as the gel) on top until the towels are 15–20 cm high (*see* **Note 11**).
7. Fill dish with 20X SSC, until the level is just to the bottom of the glass plate.
8. Cover the dish and paper towel stack with plastic wrap to prevent evaporation. If desired, a weight can be placed on the paper towels. A 15 × 20-cm glass plate with a small water bottle balanced in the center is adequate (total weight ~400 g).
9. Blot for 24–48 h. Replace wet paper towels (do not disturb the bottom 1–2 cm of the stack) and replenish the 20X SSC in the dish as needed.
10. When blotting is complete, remove the paper towels. Leave the two sheets of chromatography paper with the nitrocellulose. Lift the gel off the blotting table and turn it over. Mark or cut the membrane as needed, and remove and discard the gel. Note that the gel should be quite flattened. Evaluate transfer efficiency by staining the gel with EtBr, if desired.
11. Dry the filter between sheets of chromatography paper and further dry for at least 2 h at 80°C, under vacuum. Be careful: Nitrocellulose will be very brittle at this stage. (Alternatively, while the membrane is still damp, but not shiny wet, and on the chromatography paper, place it with the DNA side up inside UV crosslinker and fix the DNA to the membrane.)