SOUTHERN BLOTTING

Materials:

Whatman 3 mm Blotting Paper
nitrocellulose (Schleicher & Schuell, Amersham) or nylon membrane filter (Amersham).
Paper towels (preferably C-fold "cheap-o" variety)
Pyrex or Tupperware dish, glass plates and rubber stoppers
A weight (lead pigs work well)

Stock Solutions:

20x SSC (3M NaCl, 0.3M NaCitrate pH7.4)
20% w/v SDS
1 M. Tris pH8.0
100x Denhardt's solution (2% Ficoll, 2% polyvinylpyrroliolone, 2% BSA)
100 µg/ml SS sheared DNA (Sigma Herring Sperm)

Procedure:
Setting up the blot

1. After the gel run and photograph, soak the gel in the appropriate volume (enough to cover the gel well) of the following solutions. The gel can be placed on the shaker and gently agitated during soaking periods.
   The solutions, especially the NaOH/NaCl, should be made fresh.
   Place the gel in:

   1. 0.25 M HCl for 15' depurination step for high MW DNA,

   Important: do not soak times > 15 minutes--times can be shorter.

   2. 0.5M NaOH/1.5M NaCl 3 x 20' (3 solution changes) denaturation step
   3. 0.5M Tris pH8.0/1.5M NaCl 3 x 20' (again 3 changes) neutralization step (this solution can be reused if the pH is closely monitored. Once it drops below neutral make a fresh stock)

2. After the neutralization step, the gel is ready to be blotted. Leave gel in neutralization solution until blot apparatus is assembled.

3. Place a glass plate, supported by rubber stoppers, into the Pyrex or Tupperware dish. Cover the plate with 2 sheets of Whatman 3mm paper which have been cut longer than the plate so they hang over:

4. Fill the dish with 10x SSC so the level of SSC is just below the glass plate.

5. Place the gel onto the filter paper covering glass such that the open wells are face

down and the "back" of the gel is up. Make sure no bubbles are trapped between the gel and filters.
Cut a piece of nitrocellulose to the exact size of the gel, soak the filter in 6X SSC until it is fully wet.
Place nitrocellulose on top of the gel - again take great care to insure that no bubbles are trapped between gel and nitrocellulose.
Cut 4-6 pieces of Whatman 3 mm paper to the same size as gel and nitrocellulose. Soak quickly in 6x SSC. Place these on top of
nitrocellulose, again watching for trapped bubbles.
Cut paper towels to same size as gel and filters - place these on top of the stack. Top with a glass plate and a weight - should look like this:

6. Allow the blot to proceed, changing paper towels when the stack becomes wet. Approximate blotting times:
Minigel w/plasmid DNA - 5-10 hours.
Large gel - Plasmid DNA - 8 hours - overnight
Large DNA (g) - overnight - 14-16 hours.
Genomic DNA - 20-24 hours.

Taking the blot apart

1. Peel off paper towels and Whatman 3 mm filters, trying not to dislodge the nitrocellulose from the gel.
2. Flip the nitrocellulose/gel over and mark the positions of the gel wells onto the nitrocellulose using a ball-point pen along the lower edge of the well. After marking wells, peel the gel off and discard.
3. Rinse the filter in 6xSSC and air dry completely (~60' @ RT)
4. Bake the filter in between two sheets of Whatman 3 mm for 2 hours @ 80°C in a vacuum oven.

After baking, the filter is ready to be prehybridized and hybridized with the desired probe.

References:

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-518.

Maniatis, T., E.F. Fritsch, and J. Sambrook (1982 Molecular Cloning: A Laboratory Manual. pp. 383-386.