**SOUTHERN BLOTTING ONTO A POSITIVELY CHARGED NYLON MEMBRANE WITH AN ALKALINE BUFFER**

With a positively charged nylon membrane, the transferred DNA becomes covalently linked to the membrane if an alkaline transfer buffer is used. This transfer technique is not suitable for nitrocellulose membranes, as these do not retain DNA at pH >9.0 and fall apart after long exposure to alkali. The resistance of the nylon membrane to alkali should be checked before use, as some types of nylon membrane are less resistant than others.

***Materials***

0.25 M HCl
0.4 M NaOH

***Prepare the gel***

*CAUTION:* Wear gloves to protect your hands from the acid and alkali solutions and to protect the membrane from contamination.

1. Digest the DNA samples with appropriate restriction enzyme(s), run in an agarose gel with appropriate DNA size markers, stain with ethidium bromide, and photograph with a ruler laid alongside the gel so that band positions can later be identified on the membrane.

*The gel should contain the minimum agarose concentration needed to resolve bands in the area of interest and should be ≤7 mm thick, preferably less. The amount of DNA that must be loaded depends on the relative abundance of the target sequence that will subsequently be sought by hybridization probing.*

2. Rinse the gel in distilled water and place in a clean glass dish containing ~10 gel volumes of 0.25 M HCl. Shake slowly on a platform shaker for 30 min at room temperature.

*This step results in partial depurination of the DNA fragments, which in turn leads to strand cleavage. The length reduction improves the transfer of longer molecules. The step is not necessary with PCR products <4 kb in length, or if efficient transfer of molecules >5 kb is not required. The time needed for the acid treatment depends on the concentration and thickness of the gel. To check that the treatment has been sufficient, watch the xylene cyanol and bromophenol blue dyes. When these change color to green and yellow respectively, the gel has equilibrated with the acid. Adequate depurination takes a further 10 min.*

3. Rinse the gel with distilled water. Pour 10 gel volumes of 0.4 M NaOH into the dish and shake slowly on a platform shaker for 20 min.

4. Set up transfer. 0.4 M NaOH is used to do the transfer. Use 2 pieces of thin filter paper to make a wick. Place wick over plastic plate on support platform. Remove air

bubbles at all steps in the set up procedure. Put gel face down on wick. Put nylon down correctly the first time. Use 4 peices of Whatman 3 MM paper and place on top. Also add a large stack of paper towels to the top. Use the 50-100g glass plate weights on the top. Place plastic wrap around gel to prevent evaporation.

*Alkaline transfer is quicker than high-salt transfer, so the blot can be taken apart any time after 2 hr.*

*A positively charged nylon membrane does not have to be prewetted, but can be placed directly onto the gel. Check that the paper towels are resistant to the alkali solution; some types go brown and should not be used.*

5. Remove the paper towels and filter paper and recover the membrane. Rinse the membrane in 2x SSC, place on a sheet of Whatman 3MM filter paper, and allow to air dry.

*The rinse has two purposes: to remove agarose fragments that may adhere to the membrane and to neutralize the membrane. Baking or UV cross-linking is not needed with a positively charge membrane; in fact, UV cross-linking is detrimental.*

17. Store membranes dry between sheets of Whatman 3MM paper for several months at room temperature. For long-term storage, place membranes in a desiccator at room temperature or 4°C.