

SLOT BLOT PROCEDURE

1. For each sample, add equal amounts of DNA to the well of a 96 well plate (If possible, try to keep volume as small as possible (~10 μ l)).
2. Add 100 μ l of 0.1 N NaOH to each well. Incubate plate at 65°C for 30 min.
3. Add 100 μ l of 2 M ammonium acetate to each well.
4. Prepare Nylon membrane(s): Cut to the appropriate size. Label. Place membrane in ddH₂O. Pour out ddH₂O and add 20X SSC. Set up the slot blot apparatus as follows: Wet 2 sheets of blotting paper in 20X SSC and place both sheets on the bottom of the slot blot apparatus. Then place the Nylon membrane on top of the blotting paper and remove the air bubbles. Close slot blot apparatus.
5. Transfer samples to the well of the slot blot apparatus. Suck samples into wells by applying house vacuum to the slot blot apparatus.
6. Remove filter and bake in a vacuum oven for 2 hours.
7. Hybridize with the appropriate probe using the Southern hybridization procedure.