Northern Blotting

Transfer the gel O/N

1. Prewet the membrane for 5-10 minutes in SSC 20X.
2. Place the membrane on the Whatman paper.
3. UV Crosslink.
4. Rinse the membrane with distilled water.
5. Dry the membrane.

Pre IMB- HYB (Temp= (49.82 + 0.41) (Divided by 6 + C) – (600/length)) –20 to 25°C)

1. Prehybridize for 30 minutes in pre-heated DIG EASY HIB (10 mL/100 cm^2).
2. Denature the probe by boiling for 5 minutes and cool on ice fast.
3. Dilute in 3.5 mL of DIG EASY HIB.
4. Pour off the pre-HYB and add the HYB soln.
5. Incubate for 4 hours O/N.

Washes

1. Wash twice on 2X SSC + 0.1% SDS for 5 minutes each at 15-25°C.
2. Wash twice on 0.5X SSC to 0.1 SDS for 15 minutes at 65-68°C. (The soln. must be pre-warmed, the temperature high with high 6 c and high probe length).

Detection

1. Rinse membrane in washing buffer.
2. Incubate for 30 minutes in 100 uL of blocking soln.
3. Incubate for 30 minutes in 20 uL of antibody soln.
4. Wash twice in 100 uL of washing buffer for 15 minutes each.
5. Equilibrate 2-5 minutes in 20 uL of detection buffer.

6. Incubate in 10 uL of color substrate soln. for a few hours in the dark. (200 uL of BCIP vial 5 into 40 of detection buffer.)

7. Stop reaction by washing in distilled water for 5 minutes.