DIG WASH AND DETECTION

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Wash

1. Pour hybridization solution into 15 ml tube (it can be reused). Label with date and store in freezer.
2. Wash membrane (in hyb tube) with 2xSSC, 0.1% SDS at room temperature 8 minutes. Pour off and repeat. (approx. 25-50 ml of wash each time)
3. Wash membrane 2x with preheated 0.2xSSC, 0.1% SDS at 68°, rotating 20 minutes.

Detection

1. Place membrane in tray (just larger than membrane if possible).
2. Wash with approx. 100 ml wash* solution/ 100 cm^2 membrane for 5 minutes, with shaking.
3. Block membrane in blocking* solution: 100 ml/100 cm^2 of membrane for 30-40 minutes with shaking.
4. Incubate in antibody* solution: 20 ml/100 cm^2 of membrane for 30 minutes, with shaking.
5. Rinse membrane briefly in a small amount of wash solution. Transfer membrane to clean tray. Wash 2x15-20 minutes in approx. 100 ml of wash* solution, with shaking.
6. Incubate in 20 ml detection* buffer/100 cm^2 of membrane for 5 minutes with shaking.
7. Transfer membrane to between two sheets of acetate page protector cut about 1 inch larger than membrane on each side. (Before using the page protector rinse the insides with sterile water and wipe with a Kimwipe, then wipe the outside with a Kimwipe wetted with 95% ethanol) . Apply approx. 1 ml CSPD, ready to use solution/100 cm^2 of membrane. Apply most of the solution at the top and some near the middle. Immediately lower top sheet to spread substrate evenly and without air bubbles. Do not press now.
8. Let incubate 5 min. Press out excess solution using a Kimwipe wetted with 95% ethanol, and seal edges with tape.
9. Incubate at 37° for 15 minutes.
10. Expose to X-ray film at room temp for 15-25 minutes initially, then as needed.

*Solutions

- Wash Solution
  0.3% Tween 20 in Maleic Acid buffer

- Maleic Acid Buffer
  0.1M maleic acid
  0.15M NaCl
  pH 7.5 (autoclaved)

- Blocking Solution
  2% blocking reagent (Boehringer's) in Maleic Acid Buffer
  For 100ml use 20 ml 10% Block , 80 ml maleic acid buffer

- Antibody Solution
  1/10,000 anti-DIG-AP conjugate in new blocking solution
  Before diluting antibody, spin the antibody tube for 1 min in mini-centrifuge.

- Detection Buffer
  0.1M tris

0.1M NaCl
pH 9.5 (autoclaved)