LABELING DNA PROBE USING DIG HIGH PRIME LABELING MIX (ROCHE)

- dilute 10ng-3µg probe DNA (genomic, plasmid or gene clean fragment) in dsH₂O to a final volume of 16µl
- denature DNA by boiling 10min; quickly chill on ice to prevent reannealing of strands
- add 4µl DIG high prime labeling mix; mix briefly and tap spin
- incubate overnight at 37°C.
- stop reaction by adding 2µl 0.2M EDTA (pH 8) and heat inactivate at 65°C 10min
- boil probe 10-20min before using
- used probe can be stored at -20°C in Hybridization Buffer and used repetedly

TRANSFER OF DNA FROM GEL TO MEMBRANE

- run gel at appropriate voltage to obtain clean bands; stain and photograph with size reference
- smaller fragments transfer more efficiently. for very large DNAs, a 2min UV nicking step on a short wave transilluminator can be added
- transfer gel to a seable Tupperware container

traditional method of transfer

- incubate 40min at room temperature in 0.25 HCl (sufficient to cover gel) to depurinate
- rinse 2X in MilliQ
- incubate 2X 20min in Denaturation Solution to cleave depurination sites
- incubate 30min in Neutralization Solution
- set up capillary transfer as shown in the schematic below using 20X SSC as the Transfer Solution
- transfer overnight (48hr for pulsed field gels)

alternative quick transfer method (Phil’s favorite)

- incubate at room temperature in 0.25M HCl until dye bands turn yellow (ca. 20min)
- rinse 2X in MilliQ
- set up capillary transfer as shown in the schematic below using 0.4N NaOH as the Transfer Solution
- transfer overnight (48hr for pulsed field gels)
SOUTHERN BLOT (cont.)

Fix DNA to membrane
- cut off left top corner of gel and membrane for orienting blot
- restain and photograph gel to assess transfer efficiency
- rinse membrane briefly in 2X SSC and transfer to sealable Tupperware container
  *optional*: prestrip membrane using BLOT STRIPPING PROTOCOL. Often yields cleaner blots

Probe membrane
- prehyb membrane on rocker for a minimum of 3hr at 68°C in Hybridization Buffer
  - boil probe in 40ml Hybridization Buffer at least 10min to denature
- hybridize membrane DNA side down overnight at 68°C (42°C for lower homology) in boiled Hybridization Buffer/Probe mix, rocking optional
  - used Hybridization Buffer/Probe mix can be stored at -20°C and used repeatedly

Membrane detection
- wash 2X 15min at room temperature with 2X SSC, 0.1% SDS on rocker
- wash 2X 15min at 42°C with 0.5X SSC, 0.1% SDS on rocker (washes can be modified to control stringency – this is fairly stringent)
- rinse in Washing Buffer
- incubate 30min at room temperature in Blocking Solution, rocking optional
- incubate 30min at room temperature with 30ml Blocking Solution + 2µl anti-DIG-AP Conjugate (premix before adding to blot), rocking optional
- lay membrane on saran wrap; add 20 drops Ready-To-Use CSPD Reagent
- cover with a second piece of saran wrap
- let stand 3min; squeeze out excess CSPD Reagent from between sheets of plastic wrap
- expose to film (enzymatic reaction is accelerated at 37°C and slowed at 4°C)
- after developing, membranes can be stored as is between sheets of plastic wrap at room temperature indefinitely

Membrane stripping
- remove membrane from plastic wrap and place in a sealable Tupperware container
- wash 2X 15min at 37°C in Stripping Buffer (no longer!)
- rinse briefly in 2X SSC
- membrane is now ready to prehyb
### REAGENTS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>0.25N HCl</strong></td>
<td>25ml conc. HCl, MilliQ to 1L</td>
</tr>
<tr>
<td><strong>Denaturization Solution</strong></td>
<td>88g NaCl (1.5M), 20g NaOH (0.5N), MilliQ to 1L</td>
</tr>
<tr>
<td><strong>Neutralization Solution</strong></td>
<td>176g NaCl (3M), 6.7g Tris base (0.5M), 70.2g Tris-HCl, MilliQ to 1L</td>
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<tr>
<td><strong>20X SSC</strong></td>
<td>176g NaCl (3M), 88g Na3Citrate (0.3M), MilliQ to 1L, pH to 7.0 w/ 1M HCl</td>
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<tr>
<td><strong>2X SSC</strong></td>
<td>50ml 20X SSC, MilliQ to 500ml</td>
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<tr>
<td><strong>10X Maleic acid Buffer</strong></td>
<td>116g maleic acid (1X is 0.1M), 88g NaCl (1X is 0.15M), MilliQ to 1L, PH to 7.5 w/ solid NaOH</td>
</tr>
<tr>
<td><strong>2X SSC; 0.1% SDS</strong></td>
<td>100ml 20X SSC, 10ml 10% SDS, MilliQ to 1L</td>
</tr>
<tr>
<td><strong>0.5X SSC; 0.1% SDS</strong></td>
<td>25ml 20X SSC, 10ml 10% SDS, MilliQ to 1L</td>
</tr>
<tr>
<td><strong>2X SSC; 0.1% SDS</strong></td>
<td>50ml 10X Blocking Reagent*, MilliQ to 1L</td>
</tr>
<tr>
<td><strong>Blocking Solution</strong></td>
<td>50ml 10X Maleic acid buffer, MilliQ to 1L</td>
</tr>
<tr>
<td><strong>Hybridization Buffer</strong></td>
<td>250ml 20X SSC (5X), 100ml 1% lauryl sarcosine (0.1%), 2ml 10% SDS (0.02%), 100ml 10X Blocking Reagent*, MilliQ to 1L</td>
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<tr>
<td><strong>Washing Buffer</strong></td>
<td>3ml Tween-20 (0.3%), 100ml 10X maleic acid buffer, MilliQ to 1L</td>
</tr>
<tr>
<td><strong>Stripping Buffer</strong></td>
<td>8g NaOH (0.2N), 10ml 10% SDS (0.1%), MilliQ to 1L</td>
</tr>
<tr>
<td><strong>10N NaOH</strong></td>
<td>200g NaOH, MilliQ to 500ml</td>
</tr>
<tr>
<td><strong>10X Detection Buffer</strong></td>
<td>1M Tris-HCl, 1M NaCl, pH to 9.5</td>
</tr>
<tr>
<td><strong>0.4N NaOH</strong></td>
<td>40ml 10N NaOH, MilliQ to 1L</td>
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* included in Roche DIG kit