# SOUTHERN BLOT PROTOCOL USING DIGOXIGENIN LABELED PROBE, *RHODOCOCCUS* OPTIMIZED

selflessly tested , optimized and illustrated with love by xian o'brien

Recipes for reagents in **bold** included at the end of this protocol, in *italics* are included with the Roche DIG labeling and detection kit

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

- dilute 10ng-3µg probe DNA (genomic, plasmid or gene clean fragment) in ds$H_2O$ 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

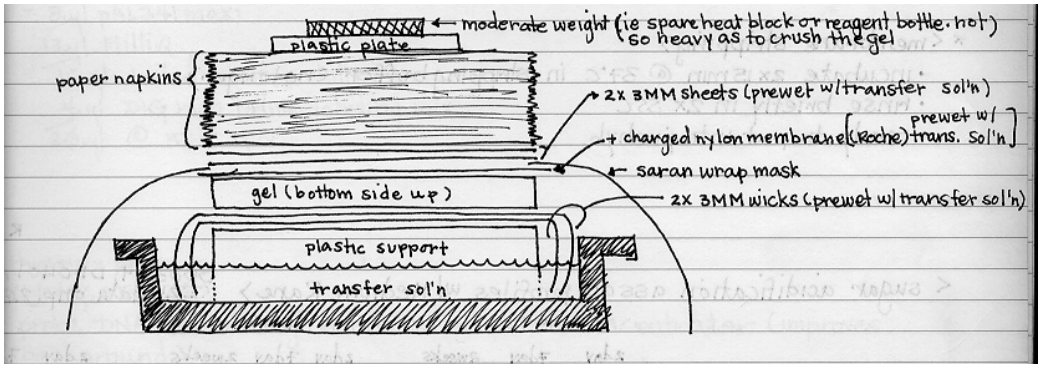
- run gel at appropriate voltage to obtain clean bands; stain and photograph with size reference
- smaller fragments transfer more efficiently. for very large DNAs, a 2min UV nicking step on a short wave transilluminator can be added
- transfer gel to a sealable Tupperware container

*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° 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
- wash 2X 15min at room temperature with 100ml **Washing Buffer** on rocker
- equilibrate 5min at room temperature with **Detection Buffer**
- 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 remove as much as possible to ensure low background on film
- expose to file (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

## SOUTHERN BLOT (CONT.)

### REAGENTS

**0.25N HCl**
25ml conc. HCl
MilliQ to 1L

**Denaturization Solution**
88g NaCl (1.5M)
20g NaOH (0.5N)
MilliQ to 1L

**Neutralization Solution**
176g NaCl (3M)
6.7g Tris base (0.5M)
70.2g Tris·HCl
MilliQ to 1L

**20X SSC**
176g NaCl (3M)
88g $Na_3Citrate$ (0.3M)
MilliQ to 1L
pH to 7.0 w/ 1M HCl

**2X SSC**
50ml 20X SSC
MilliQ to 500ml

**10X Maleic acid Buffer**
116g maleic acid (1X is 0.1M)
88g NaCl (1X is 0.15M)
MilliQ to 1L
PH to 7.5 w/ solid NaOH

**2X SSC; 0.1% SDS**
100ml 20X SSC
10ml 10% SDS
MilliQ to 1L

**0.5X SSC; 0.1% SDS**
25ml 20X SSC
10ml 10% SDS
MilliQ to 1L

**10X Detection Buffer**
1M Tris·HCl
1M NaCl
pH to 9.5

**Washing Buffer**
3ml Tween-20 (0.3%)
100ml 10X maleic acid buffer
MilliQ to 1L

**Blocking Solution**
50ml 10X Blocking Reagent*
50ml 10X maleic acid buffer
MilliQ to 1L

**Hybidization Buffer**
250ml 20X SSC (5X)
100ml 1% lauryl sarcosine (0.1%)
2ml 10% SDS (0.02%)
100ml 10X Blocking Reagent*
MilliQ to 1L

**10N NaOH**
200g NaOH
MilliQ to 500ml

**0.4N NaOH**
40ml 10N NaOH
MilliQ to 1L

**Stripping Buffer**
8g NaOH (0.2N)
10ml 10% SDS (0.1%)
MilliQ to 1L

** included in Roche DIG kit*