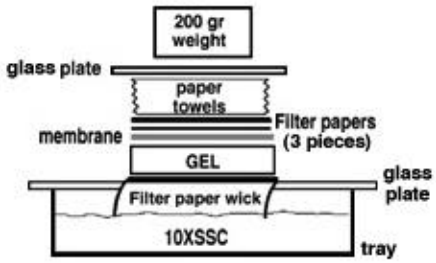
Southern-blot Analysis

Protocol

I. Salt transfer

- Cut membrane (GeneScreen Plus, NEN Research Products, Cat# NEF-976) to size of gel.
- Wet membrane by placing in a tray containing distilled water.
- Transfer to a tray containing 10X SSC and allow to equilibrate for at least 15 minutes.
- Agitate gel in a tray containing a fair amount of 0.25N HCl (10 minutes).
- Pour off 0.25N HCl solution and rinse briefly with distilled water.
- Agitate gel in a tray containing 0.4N NaOH/0.6M NaCl (30 minutes).
- Pour off 0.4N NaOH/0.6M NaCl solution and rinse gel with distilled water.
- Agitate gel in a tray containing 1.5M NaCl/0.5M Tris pH 7.5 for 30 minutes.
- Set up a capillary transfer using 10xSSC as transfer solution -see figure- (Be sure to remove all bubbles between filter paper wick, gel and membrane, by rolling with a 10ml pipette).



Capillary transfer set-up

II. DNA fixation

- After transfer is complete (over night), remove membrane and incubate for 1 minute in 0.4N NaOH to denature membrane-bound DNA (only single stranded DNA binds membrane so this denaturation step enhances avidity of weakly bound DNA molecules).
- Neutralize membrane by incubating for 1 minute in 0.2M Tris-HCl pH 7.5/1xSSC.
- Rinse membrane with 2xSSC.
- Fix DNA to membrane by UV-crosslinking:
  - Place wet membrane on a piece of wet filter paper [GeneScreen membranes do not require drying prior to or after crosslinking].
  - Put membrane into UV-crosslinker (Stratalinker), DNA facing-up.
  - Select AUTO and press START.
  - Remove membrane from chamber and place it in 2xSSC.

III.Hybridization

- Incubate membrane in pre-warmed “Prehybridization solution” (see below for recipe) for a couple hours at 65^{o}C.
- Boil radiactive-labeled probe for 5 minutes [note: we typically label our probes using a T7 QuickPrime Kit from Pharmacia (cat# 27-952-01) as per vendor’s instructions].
- Place immediately on ice for 5 minutes.
- Add probe to pre-warmed “Hybridization solution” (see below for recipe) (we typically use probe at between 0.5-2x10^{6} cpm/ml).

- Remove prehybridization solution and add radiactive (i.e. probe containing) hybridization solution.
- Incubate over night at 65°C.
- Remove hybridization solution and transfer immediately to a tray containing "Washing solution" [0.2xSSC/0.1%SDS]. Incubate for 10 minutes at 65°C.
- Wash membrane three times with "Washing solution" for 20 minutes each at 65°C.
- Remove membrane and cover it with plastic-seal wrap.
- Place it in a cassette and expose film [Biomax-MR Kodak cat#8701302] over night at –70°C.

Solutions

Stock solutions

1M Tris pH 7.4 (see Trizma table)

0.5M Sodium phosphate buffer pH 7.6 (see Sodium Phosphate Buffers table)

10% SDS		500ml
Sodium Dodecyl Sulfate		50 g
ddH_2O	Up to	500 ml

20xSSC	1 liter
3M NaCl	175.3 g
0.3M Sodium Citrate-$2H_2O$	88.2 g

I. Salt transfer

0.25N HCl	500ml
12.1N HCl	10.3 ml
ddH_2O	489.7 ml

0.4N NaOH/0.6M NaCl	500ml
0.4N NaOH	8 g
0.6M NaCl	17.5 g
ddH_2O	500 ml

1.5M NaCl/0.5M Tris pH 7.4	500ml
1.5M NaCl	43.8 g
0.5M Tris pH 7.4	250 ml 1M Tris pH 7.4
ddH_2O	250 ml

10xSSC	100ml
20xSSC	50 ml
ddH_2O	50 ml

II. DNA fixation

0.4N NaOH	500ml
NaOH	8 g
ddH_2O	500 ml

0.2M Tris pH 7.4/1xSSC	500ml
0.2M Tris pH 7.4	100 ml 1M Tris pH 7.4
1xSSC	25 ml 20xSSC
ddH_2O	375 ml

2xSSC	100ml
20xSSC	10 ml
ddH_2O	90 ml

III.Hybridization

Prehybridization solution		100ml
0.72M NaCl		4.2 g
0.04M Phosphate buffer pH 7.6		8.0 ml 0.5M Phosphate buffer pH7.6
4 mM EDTA pH 8.0		0.8 ml 0.5M EDTA pH 8.0
0.2% Polyvinylpyrrolidone		0.2 g
0.2% Ficoll		0.2 g (Pharmacia cat#17-0400-01)
0.1% SDS		1.0 ml 10% SDS
0.2 mg/ml Salmon Sperm DNA		2.0 ml 10mg/ml Salmon Sperm DNA
ddH_2O	Up to	100 ml

Hybridization solution	50ml
Prehybridization solution	50 ml
90 mg/ml Dextran Sulphate	4.5 g (Pharmacia cat#17-0340-01)

Washing solution	500ml
0.2xSSC	5 ml 20xSSC
0.1% SDS	5 ml 10% SDS
ddH_2O	490 ml