Reduced Formaldehyde RNA Gels for Northern Transfer

Rgts

① 10X MOPS (make fresh everytime)

8 g MOPS sodium salt
3.2 ml 3M NaOAc pH 5.2
4.0 ml 0.5 M EDTA
↑ volume to 200 ml
add 0.5 ml glacial acetic acid to bring pH to 7.0 - 7.5 *check ↑ pH paper

② Agarose - Formaldehyde Gel

1 g agarose } bring to boil + cool to 65°
88 ml ddH_2O }

→ add 10 ml 10X MOPS buffer
1.9 ml formaldehyde (no cootes)
5 µl EtBr of a 10 mg / ml stock
→ Pour in a fume hood until solidified
* use 1mm thick comb wells for best resolution

③ 1.33 X Sample buffer (make fresh)

500 µl formamide
169 µl formaldehyde
100 µl 10X MOPS buffer

6X RNA loading Buffer

30% Glycerol
pinch BPB

Procedure

- 15 µl sample buffer
  + 5 µl RNA sample (to give 10-20 µg total RNA or 0.5 - 1.0 µg polyA+)
  - Combine + heat to 65° 5' + immediately place tube on ice to "quench"
- Add 4 µl 6X loading buffer, mix + load into gel
- Run gel overnight 25 V ± buffer recirculation for best resolution
  or 3 hr at 84 V (in cold room)
- When complete, leave gel in casting tray + gently soak/agitate in 3 changes of ddH_2O = to 30'
- Photograph gel over saran wrap
- Agitate gel in 10X SSC 15-30'

Transfer

- Place blotting sponge in dish of 10X SSC
- cover ± whatman 3M cut to size
- invert gel on top of 3M (get all air out by rolling wet pipet over it)
- have a pre wetted gene screen cut to size soaking in 10X SSC → overlay gel + remove air
- overlay ± 2 sheets wet 3M

- cover c blotting paper/papertowels
- cover c a glass plate + weight c 250 ml H_2O bottle
- cover whole thing in saran → o/n +
- invert gel + membrane + mark wells c pencil
- remove membrane → 10X SSC + rub off agarose c clean gloved hands

→ dry + bake 80° 2hr

Prehyb + Hyb

5X SSPE
1% SDS
10X Denhardt's
50% Formamide
Sheared salmon sperm DNA at 100 µg/ml heat denatured
i.e. boil it! + quench

- put dry filter in bag
- add prehyb → chase out bubbles + seal

→ 42° 4hr

- remove ½ prehyb → 15-50 ml tube c probe waiting
want $10^6 - 2.5 \times 10^6$ cpm/ml of heat denatured

probe → mix + readd to bag, heat seal + mix up

→ 42° o/n +

Washes

2X SSC
0.1% SDS preheat 1 Hr. to 42°

0.2X SSC
0.1% SDS preheat 1 Hr. to 60°

- remove filter + place in ~ 300 ml 2X SSC 0.1% SDS + wash 10-15'
  repeat 2X

⇒ ~300 ml 0.2X SSC 0.1% SDS → 60° ~15'

→ blot dry + expose to film

Northern Blot using Formamide

20 mls prehybridization Buffer

Formamide	-	10 mls	=> 50%
20x SSC	-	6 mls	=> 6x
50x Denharts	-	2 mls	=> 5x
20% SDS	-	1 mls	=> 1%
SSS 10mg/ml	-	0.5 mls	=> 250mg/ml
ddH2O	-	0 mls	
Tris 2M pH 7-8	-	0.5 mls	=> 50 mM
		20 mls	

- leave out formamide and do hybridization at 68°C
- Wash 65°C, 1% SDS
  2x 30 mins

Low Stringency Wash:

0.1% SDS, 47°C

2x 1 hour then
2x 1 hour at R. temp
in 0.1x SSC

Stripping

0.1x SSC 2% SDS. (20g/liter)
Boil 30 minutes
prehybridize
(keep wet).

Northern Blots

Water	191 cc	222 cc	318.3 cc
Mops 5X	60 cc	70 cc	100 cc
Agarose	3 gms	3.5 gms	5.0 gms

Melt agarose in water and MOPS, then carefully add formaldehyde and cool in 55C water bath.

Formaldehyde	49 cc	57.2 cc	81.7 cc
Total Volume	300 cc	350 cc	500 cc

37% Formaldehyde is about 12.3 M. Use at 2.2 M so dilute by 5.6 fold

water	62.2 cc
5X MOPS	20 cc
agarose	1.3 gms
Formaldehyde	17.8 cc

NEN loading buffer

Formamide	720 ul
5X MOPS	320 ul
37% Formaldehyde	260 ul
dH2O	100 ul
80% Glycerol	100 ul

Dry RNA down and resuspend in 20 ul loading buffer. Heat to 95C for 2 minutes.

5X MOPS

0.2 M MOPS	88.7 g
50 mM sodium acetate*3H2O	13.6 g
5 mM EDTA	7.45 g

to 4 liters with water. pH to 7.0 with NaOH

Loading per sample

Formamide	7.5 ul
Formaldehyde	2.5 ul
MOPS 5X	3.0 ul
Water	2.0 ul

Hybridization buffer for 50C.

Formamide	5 cc	50%	
SSC 20 x	2.5 cc	5X	
SDS 20%	0.5 cc	1%	
PVP/Ficoll	1.0 cc	0.2%/0.02% final concentration	
Pipes 0.5M	0.8 cc	40 mM	
SS DNA 10 mg/ml	0.1 cc	100 ug/ml	