RADIATION

DAY 1

- Make pre-hyb and hyb solutions
- **Prehyb**
- Sign into workstation. Boil 800µl salmon sperm DNA (green lid) for 5 mins at 100˚C, then to pre-hyb solution
- Pour pre-hyb solution into warmed hyb tube and use to ‘stick’ membranes onto sides of hyb tube.
- Incubate at 65˚C for >3 hours (shorter is OK for colony hyb)

DAY 1 CONTINUED

- **Hyb**

- Label all tubes if using more than one probe

  - Add 30µl TE to a 1.5ml eppendorf – keep on ice
    For Colony Hyb – Add 3µl probe DNA and 12µl SDW to a screw cap tube, keep on ice
    For Southern Blot – Add 3µl probe DNA, 3µl diluted 1kb ladder (1ng/µl) and 8µl SDW to a screw cap tube, keep on ice

- Get 450µl salmon sperm DNA (red lid) out of freezer and keep on ice

- Boil probe DNA for 5mins at 100˚C then keep on ice – pulse spin.

- Add 4µl HiPrime and 2µl ^{32}P-dCTP (1µl for colony hyb) to DNA, mix gently and incubate at 37˚C for 15-20mins.

DAY 1 CONTINUED

- **Hyb continued**
  - Get gel column from fridge – 'flick' tube to get packing buffer out of the lid. Spin out packing buffer from column: snap bottom off, take the lid off, put in collection tube and spin for 4 mins at 3600 rpm
  - When incubation is finished, add the 30µl TE to probe before loading onto column (column minimum volume is 50µl).
  - Spin column for 4 mins at 3600 rpm (with lids on) to purify probe away from unincorporated dCTPs – probe is the flow through!
  - Add probe to 450µl salmon sperm DNA and boil for 5 mins at 100˚C, then put on ice
  - Pour pre-hyb solution down the sink (with lots of water) out of small hole in hyb tube
  - Use syringe barrel to add hyb solution to hyb tube. Then use p1000 pipette to add probe vertically into hyb tube using small hole, replace cap
  - Incubate at 65˚C overnight. Put wash solutions at 65˚C overnight. Sign out of workstation and document isotope usage

DAY 2

- **Washes:** Sign into workstation. Pour out hyb solution down sink using small hole in hyb tube (with lots of water). Wash blot sequentially, pouring old wash solutions down sink as above:
  - 50ml Wash 1 for 10 mins
  - 50ml Wash 1 for 30 mins
  - 50ml Wash 2 for 30 mins
    - For mismatched probes, adjust Wash 2 for reduced probe annealing temperature
  - 50ml Wash 2 for 30 mins
    - While last wash is incubating, blank screen(s) with white light
- Blot off excess liquid using Whatman paper – do not allow membranes to dry completely Wrap in cling film and insert into cassette with screen, exposing to white side
- Leave for >24hours (depending on age of radiation) to expose
- Wash up all hyb tubes and leave to drain and sign out of workstation
- **Tidy up:** Dispose of tips, wash thoroughly with water/Decon and put in tub in bin
- Record radiation use/disposal on Isostock

DAY 3

- **Scan image:**
  - Typhoon phosphorimager in D69. Door code C148YZ
  - Stick screen to solid metal tray white side up. Slide tray into phosphorimager (screen on underside).
  - Open Typhoon software. Drag area to scan to correct size (usually J9).
  - Pixel size 100 μM. Sensitivity 1000 for colony hyb and 4000 for Southern.
  - Choose name and location to save and start scan.
  - Fill in Typhoon user book and radiation monitoring book (monitor bench with Geiger counter).
  - Blank screen on light box
  - Open image in Affinity photo to adjust levels

PRE-HYB SOLUTION

Note: make solutions fresh in 50ml falcon

- 25ml dH_2O
- microwave on high 10sec/tube
- 12ml 20X SSPE
- 2ml 20% SDS 
- 2ml 100X Denhardt's Solution

Total = 40ml

Pre-warm at 65°C in hyb oven

20X SSPE

- 175.3g NaCl
- 27.6g $NaH_2PO_4.H_2O$
- 7.4g EDTA (solid) or 40ml 0.5M EDTA (liquid)
- 800ml dH_2O
- Adjust pH to 7.4 with NaOH 
- make up to 1L with dH_2O
- Autoclave

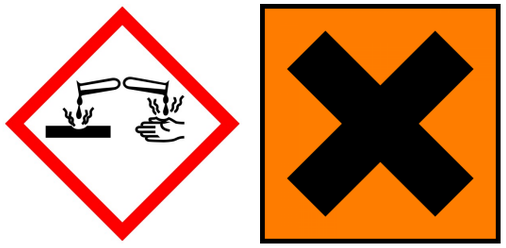
100X DENHARDT'S SOLUTION

- 1g Ficoll 400
- 1g PVP 360
- 1g BSA (fraction V)
- Make up to 50ml with dH_2O
- Leave shaking in water bath until all solids are in solution
- Syringe filter 0.8µm
- Aliquot and store at -20°C

HYB SOLUTION

Note: make solutions fresh in 50ml falcon

- 1.5g Dextran Sulphate (omit for colony hybs)
- ~18ml dH_2O
- microwave on high 10sec/tube
- 9ml 20X SSPE
- 1.5ml 20% SDS

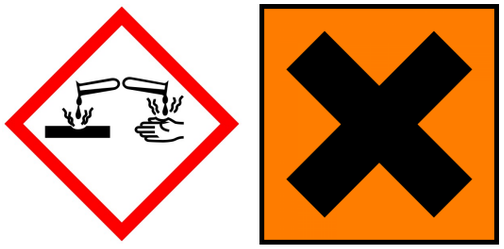


Total = 30ml

Pre-warm at 65°C in hyb oven

WASH 1

- 50ml 20X SSPE
- 5ml 20% SDS
- dH_2O up to 500ml



WASH 2

- 5ml 20X SSPE
- 5ml 20% SDS
- dH_2O up to 500ml

For mismatched probes, adjust Wash 2 for reduced annealing temperature

PROBE ANNEALING TEMPERATURE VS SSPE/SDS CONCENTRATION

Length	% GC	%	[Na+]	%	Tm (°C)
100	65	100	50	0	80.3
25	65	100	50	0	62.3
50	65	100	50	0	74.3
75	65	100	50	0	78.3
100	65	100	50	0	80.3
125	65	100	50	0	81.5
150	65	100	50	0	82.3

X SSPE	% SDS	[Na+]	% GC	Length Tm (100 bp)	100 Tm (°C)
6	0.5	1009	65	98.4	98.4
2	0.5	348	65	93.0	93.0
0.5	0.5	100	65	85.1	85.1
0.5	0.1	86	65	84.0	84.0
0.2	0.5	50	65	80.3	80.3
0.2	0.1	37	65	78.2	78.2
0.2	0	33	65	77.4	77.4
0.1	0.5	34	65	77.6	77.6
0.1	0.1	20	65	73.8	73.8
0.1	0	17	65	72.7	72.7