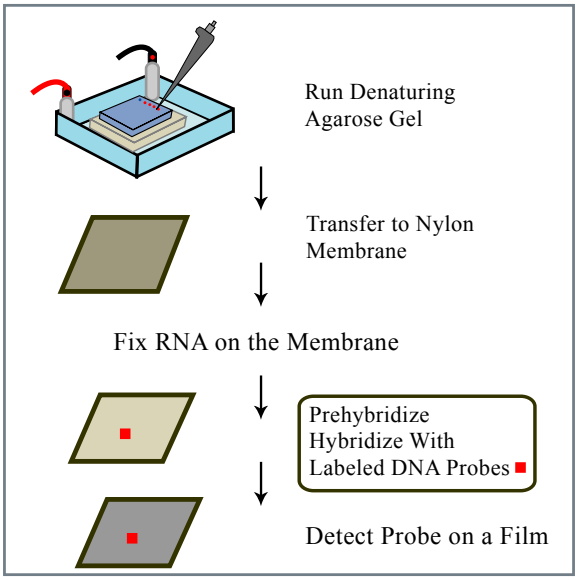
Northern blot: steps



Figures by MIT OCW.

Purpose of pre-hyb

- Prevent **non-specific** binding by using molecules that **fill in the spots on the membrane where the nucleic acid has not bound**

Pre-hyb/hyb buffer

- **2% casein** Block non-specific binding
- **7% SDS** Neutralize ⊕ charged membrane
- **5X SSC** Provide proper ionic strength for hyb
- **50% formamide** Allow lower hyb temperature
- **50mM Na phosphate** Buffer

Hybridization temperature

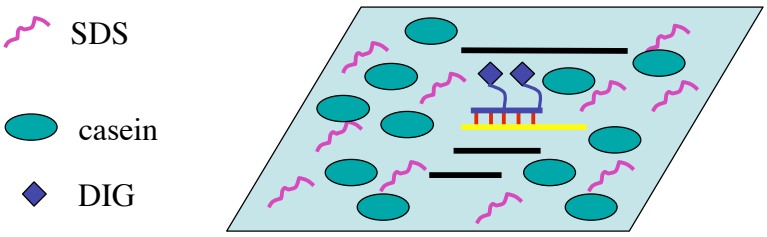
- Too high: **RNA hydrolyzes**
- Too low: **non-specific binding**
- We use: **50°C**

Tm = 81.5 + 16.6(log [Na+]) + 0.41(%G+C) - 0.72(% formamide)

Experimental hyb T is typically (Tm - 20 °C) because

Tm is the temperature at which 50% of the nucleic acids are in duplexes.

During pre-hyb and hyb….



What can Northern blot tell you?

- Size of RNA of interest
- Amount of RNA transcribed
- Stages of transcription

Northern vs Western

Gel	**Denaturing agarose gel**	**SDS-PAGE**
Transfer	**Capillary action**	**Electroblot**
Pre-hyb	**Casein + SDS**	**blotto**
Probe	**DIG labeled DNA**	**Specific antibody**
Hyb T (°C)	**50**	**4**

Zebrafish development observation

- Your TA has:
- 2 plates, Mon AM
- 2 plates, Sun AM

- 1 plate, WT Tue 8-9AM
- 1 plate, LiCl@ 1:30PM

} **5h post fertilization**