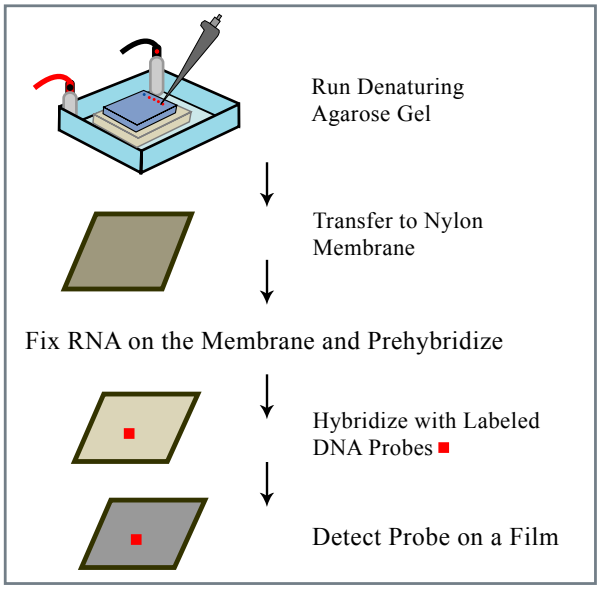
Module overview

Goal	Technique	
• Zebrafish development observation	• Phase contrast microscopy • Teratogenesis	} TODAY
• Gene expression analysis	• RNA isolation • Northern blot	} TODAY

Northern blot: steps



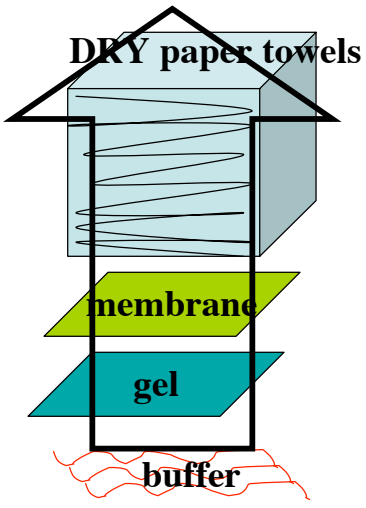
Figures by MIT OCW.

Today's topics

- **Transfer**
- **RNA fixation**
- **Probe labeling**

How is RNA/DNA transferred?

- Through **capillary action**.
- This is possible because **RNA and DNA are soluble at PH 7**.

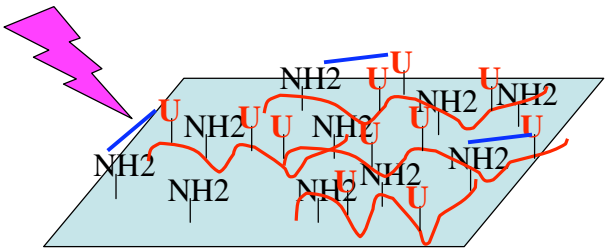


Transfer apparatus

- Whatman paper wick (ends immersed in buffer)
- Gel (inverted; **EVEN** side in touch with membrane)
  - Nylon membrane
  - 1 wet Whatman paper
  - 2 dry Whatman paper
- A stack of DRY paper towels

UV crosslinking

- Purpose: fix RNA/DNA on the membrane.
- Reason: RNA and DNA are soluble.



- Don't want to overcrosslink ➡decreased hybridization efficiency.

Probe labeling

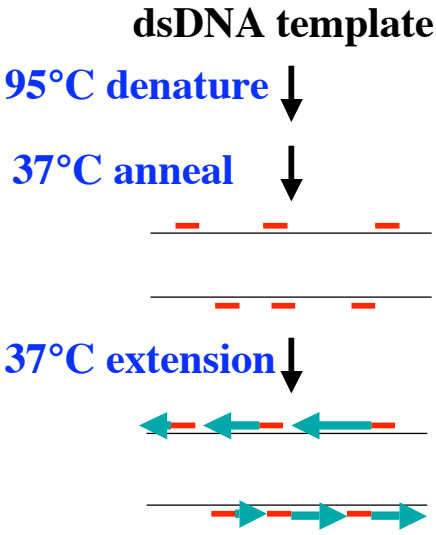
- **Probe: *z-cyt1*□DNA (complimentary to mRNA)**
- **Label: digoxingenin on dUTP**
- **Labeling method: digoxingenin-dUTP is incorporated into DNA probes by random priming.**
- **Random priming: using random hexanucleotide primers that are not gene specific.**

Random priming

Reaction mix:

DNA template,
random hexanucleotides,
dNTP (100μM dATP, dCTP and dGTP; 65 μM dTTP),
DIG-dUTP (35 μM),
Klenow DNA polymerase,
and buffer

Random priming



Probe labeling vs PCR

template	Z-cyt1 cDNA	Genomic DNA
Template amount	1μg	20ng
Denature T (°C)	95	95
#Primers	4^6(in theory)	2
Primer size	6	20
Annealing T (°C)	37	55
Extension T (°C)	37	72
enzyme	Klenow	Taq
dNTPs	DIG-dUTP	dNTP
#cycles	1	30