7.02 Fall 2001 DEV Recitation #3

Outline:

1. Zebrafish observation
2. Northern blot gel and transfer
3. probe labeling

Zebrafish observation:
-observe and draw RA-treated and control embryos. fertilized yesterday at 9am, treated with retinoic acid at 2:15pm or 3:15pm.

Northern blot transfer:

Materials:
Positively charged nylon membrane--RNA is negatively charged, attracted to the membrane. Nylon membranes are very durable, can be reprobed several times

1. Take a quick picture of your gel before you set up the blot
   - leave the gel in the tray when taking a picture
2. rinse the gel with DEPC'd water (formaldehyde will hinder transfer)
3. set up blot as described in your lab manual, make sure to:
   - invert the gel, because RNA is closer to the bottom of the gel
   - cut off a small piece from the lower left-hand corner of gel and membrane for orientation
   - never handle the membrane with your fingers, because oils on your fingers will prevent transfer
   - remove all the air bubbles between gel and wick and between gel and nylon by rolling a glass pipette over each layer. Air bubbles will prevent transfer of fluid by capillary action thus causing RNA transfer problems.
4. surround the gel closely with Saran wrap, covering the buffer chambers and the wick, to prevent SSC from reaching the paper towel stack directly, without going through the gel.
5. secure the paper towels and the heating block by using tape

Tomorrow, we will dismantle the set up for you.
- locations of wells and ladder bands will be marked with pencil, relative amounts of RNA in each lane will be noted
- blot will be air-dried and RNA cross-linked to the membrane (UV cross-linking covalently links some RNA bases to the positively charged amine groups on the surface of the membrane)

Probe labeling:

<u>Theory: Random priming</u>
1. A complex mixture of random hexamers is added to the denatured DNA template
2. Primers anneal throughout the length of the template, to both the sense and the anti-sense strands.

- Which strand of the labeled probe will bind to the *zcyt-1* mRNA?

3. Klenow polymerase extends by adding dNTPs to the primer.
   - Klenow is a fragment of the DNA polymerase from E. coli
   - Does it make a full-length product? No. Klenow lacks 5'-3' exonuclease activity. It will fall off the template when it reaches the next annealed primer.

PCR		Probe
95°C	denature	95°C
50-60°C	anneal	37°C
72°C	extend	37°C

- why do we extend at 72°C during PCR and 37°C during probe labeling?
- is the probe template amplified exponentially or linearly? what about pcr?

Reagents:

1. hexanucleotide mixture:
   - hexamers- primers
   - Tris-HCl- buffer
   - MgCl2- Mg2+ is a cofactor for Klenow enzyme
   - DTT- reducing agent
   - BSA- bovine serum albumin, creates a good environment for the enzyme
   - dNTPs: (dATP, dGTP, dCTP, and dTTP/dUTP-DIG)
     (DIG=digoxigenin-11-dUTP, see structure in your manual)
     This structure resembles uracil, with a large moiety added to the C-11 carbon
     Why not add all dUTP-DIG? It's too large, will cause problems due to steric hinderance. also, the klenow enzyme uses dTTP more efficiently and we don't want it to stall and fall off the template.
   - template- 600ng of 1kb z-cyt 1 cDNA
   - Klenow DNA polymerase

   What are the advantages of random priming?
   1) don't have to know the sequence of template
   2) make labeled probe corresponding to the whole length of the template