7.02 Fall 2001 Recitation #4

Outline

1. zebrafish observation
2. prehybridization
3. hybridization

I. Zebra fish observations

- T/R: you'll be looking at embryos fertilized this morning. You'll be treating them with retinoic acid at different times during the class.
- W/R: you'll be looking at embryos that were fertilized yesterday morning and treated with retinoic acid yesterday afternoon by your T/R colleagues.

II. Prehybridization

- Pretreats the membrane to reduce background as a result of non-specific binding of the probe

  Hybridization: Single stranded DNA probe recognizes and base-pairs to its complementary RNA sequence.
- *Prehyb/Hyb sol'n reagents:*

  1. 50mM Sodium phosphate (pH 7): buffer

  for *blocking*:

  2. 7% SDS: blocks non-specific binding by neutralizing positive charges on the nylon membrane. **Note:** SDS will precipitate out of solution if left at room temp too long.
  3. 2% blocking reagent (casein): protein, blocks non-specific binding to the membrane.

  for *hybridization*:

  4. 50% Formamide: RNA-DNA hybrids are relatively strong, and imperfect duplexes will remain stable even at high temperatures. Formamide lowers the melting temperature of nucleic acid duplexes allowing lower temperatures to be used to obtain specificity of probe hybridization. Using lower temperatures prevents the degradation of RNA on the blot that could occur at higher temperatures.
  5. 5X SSC: minimizes repulsion between target and probe by neutralizing the negative charges on the backbones.
- by adjusting the conditions that probe can anneal to a target, we are adjusting its **stringency**. Stringency is the relative ability for the probe to bind to imperfect targets. (perfect targets have 100% complementarity. imperfect targets have less than 100%)
  - higher stringency makes probe hybridization less permissive.
    - lower salt, more repulsion → imperfect hybridization more difficult
    - higher temp, more denaturing → imperfect hybridization more difficult
  - Hox gene example (see lecture notes for 12/4)
    - what if you wanted to detected hox gene expression in mice, but you only had sequence for the human hox gene of interest. the human and mice genes are less than 100% identical
      - you could make your probe from the human sequence and then hybridize it to the mice RNA, but then you'd adjust your stringency accordingly
      - you could also use the human probe to look at drosophila hox genes. or any combination of human, mouse, drosophila. all would require different stringencies.

III. Hybridization

- Procedure**:**
  - remember to denature probe! (why?)
  - discard prehyb into appropriate waste
  - add 12 ml of prehyb/hyb solution to the bag
  - squirt denatured probe directly into hyb solution (not onto membrane!)
  - remove most of air bubbles and seal the bag, leaving little room (no more than one inch on each side) around the membrane. **label your bag with permanent marker!**
  - probe will hybridize overnight at 50°C