cDNA

Complementary DNA

Problem:
Develop a way of getting the DNA of genes that are actively transcribing.

Enzyme:
RNA Dependent DNA Polymerase (Reverse Transcriptase).

Organism:
Retroviruses, e.g., HIV, SIV, Feline Leukemia Virus. Used to create DNA copy of viral material to be inserted into the nuclear DNA of the host.

Look at Figure 15.19 for details of function.
Key Points:

• mRNA has poly-A tail, so primer is 5’TT...TT3’
• Digest RNA to leave single stranded DNA (DNA has 3’ primer due to unique function of reverse transcriptase -- it “turns the corner when it finishes with RNA template).
• Use DNA Polymerase I to complete the complementary strand.
• Use $S_1$ nuclease to cleave the turn at the end of the DNA.
cDNA Features

• **Resulting DNA contains:**
  ✷ Exons (including start & stop codons)
  ✷ Upstream & Downstream Noncoding Regions
  ✷ Poly-A tail.

• **DNA does not contain:**
  ✷ Introns
  ✷ Upstream regulatory regions
  ✷ Termination Hairpin & Downstream regulatory regions
cDNA Libraries

- Libraries show **active** genes.
- Sensitive to amounts.
- Difficult to make complete.
Probe

• DNA or RNA probe is the **complement** of the sequence of interest.
• Target sequence must be single stranded.
• DNA can be *in vitro* or *in vivo*.
• Increasing the length of the probe increases specificity, but decreases efficiency.
• Usually marked to visualization:
  - Radioactive Phosphorus/Carbon
  - Biotinylated -- Flourescent Dyes
FISH
Flourescent In-Situ Hybridizaton

Metaphase or Interphase Cell

Biotinylated Probe

Treat to Denature DNA & Probe Cells

Visualize Results using Microscope
**RFLP**

**Restriction Fragment Length Polymorphism**

**Linkage Studies with Human Disease**

- Restriction Enzymes recognize sites.
- Presence/Absence of specific sites are neutral & give different lengths of fragments.
- There is a large variability in the population under study in the presence/absence of sites.
- Correlate Disease with pattern of RFLPs.
VNTR

Variable Number of Tandem Repeats

Loci with unknown genetic function with a large polymorphism in the population based on the number of tandem repeats at that locus.

Use Southern Blots to determine number of repeats.

Pattern helps to determine relatedness of samples.
VNTR -- An example

Children (Lanes 5 & 6) are orphans
We suspect they may be related to grandparents through (now deceased) parents.

Remember where each chromosome comes from for each child *if these are the true grandparents*. 