SEQEUNCING AND "FINGERPRINTING" OF DNA

28 Feb 1994, 1 March 1996, 3 Mar 97, 5 Mar 03, 29Feb08, 4Mar09, 25Feb11, 5Mar12
gmslg: p. 427, 6th, , 9th: 732-

DIDEOXY SEQUENCING: (chain termination) (p 732, 734)
Sanger developed:
1) In four different reaction tubes:
   a) ss DNA template in each of four tubes with DNA pol I,
   b) $^{32}$P labeled target primer to initiate polymerization
   c) four dNTP
   d) each tube is spiked with a small amt of one of the four ddNTPs which halts polymerization at
      the site of its incorporation.
2) As synthesis proceeds, varying lengths of new strand will be generated for each site of given base.
3) Run the four samples in four lane gel, Southern blot, autoradiography.
4) Read sequence directly as on p 734.

DNA Sequencing machine employs fluorescent labeling with four different colors. This can be run in the
same test tube, greatly decreasing time and effort. Read automatically by a scanner...

Chromosome walking: (p 730)
1) Probe eukaryotic gene bank with probe
2) select two different clones, each carry probed sequence
3) prepare new probe from end of selected dyad (contains a pair), reprobe library
4) select new clones

ALREADY COVERED:
Variable number tandem repeats: short sequence (15-100 bp long) repeated in variable numbers to yield
1 kb to 5 kb sequences at different loci, often found as introns.

RFLP: restriction length polymorphism (p 148, 728)
forensic applications to identify individuals from DNA from blood, semen, other tissue:
1) Isolate and purify DNA (if inadequate amts, polymerase chain reaction used). Check for integrity by
   preliminary electrophoresis, should form major single 15 Kb band, not smear indicating degradation
2) Digest with restriction enzyme which does not cut within the tandem repeat sequence (Commonly
   Pst I, Hae III or Hin fl used). Run on gel to ensure digestion complete.
3) Transfer by Southern Blot to nylon membrane, fix. (728)
4) Hybridize with labeled probe for hypervariable regions of VNTR (variable number tandem repeats) often
   have minor differences in sequence, but consensus sequence is conserved. with 7 or 8 such loci, nearly
   impossible for two people to randomly have identical pattern.

Polymerase Chain Reaction:
Uses DNA polymerase from Thermus aquaticus, temp resistant polymerase = Taq polymerase
1) select 2x DNA to be amplified
2) select or create set of flanking primers to bind at each end of target sequence (ad in excess)
3) heat 2x DNA, allow primer to anneal (at selected annealing temperature).
4) Use Taq to synthesize complimentary strands
5) heat denature again to produce ssDNA (Taq not affected)
6) synthesis cycle again, this time, newly synthesized strand = length between primers
7) heat and synthesize repeatedly to geometrically increase amt of DNA
NOT PRESENTED IN 2009:

**Sequencing:**

by Maxam and Gilbert:

1. **Label** isolated fragment at 5' end with $^{32}$P
2. **Separate into single strand**, labeled components
3. **Split into four aliquots**
4. **Treat different aliquot with reagents which destroy about 1/50th of bases, cleaves chain at that spot:**
   a. guanine
   b. adenine and guanine
   c. thymine and cytosine
   d. cytosine
5. **Generates strand one base shorter** than where destroyed base was located.
6. **Electrophorese all four samples** on same gel to separate varying lengths of bands
7. Identify bands with autoradiography

Appearance of band in given row means base specific to that treatment occurred there.

Note that two bands will appear for guanine and cytosine, single bands for adenine and thymine.