

SEQUENCING AND "FINGERPRINTING" OF DNA

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

DIDEOXY SEQUENCING: (chain termination) (p 732, 734)

Sanger developed, :

- 1) ss DNA template in each of four tubes with DNA pol I, ³²P labeled primer, four dNTP, and spiked with a small amt of single ddNTP in 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

NOT PRESENTED IN 2009:

Sequencing:

by Maxam and Gilbert::

- 1 **Label** isolated fragment at 5' end with ³²P
- 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.