First Generation Sequencing

http://www.nature.com/scitable/topicpage/the-order-of-nucleotides-in-a-gene-6525806
Outline

• Introduction
• Maxam-Gilbert Sequencing
  • Principle, Method, Result and Discussion
• Sanger Sequencing
  • Principle, Method, Result and Discussion
• ABI automate sequencing
Francis Crick (left) and James Watson (right) proposed that the DNA molecule has a double-helical structure.
Sequencing in 1977

Chain termination method and Chemical degradation method

Walter Gilbert (1932 - )

http://www.slideshare.net/jordanfuller32/dna-sequencing-10632407
• Chemical Degradation Method

• **Principle-Chemical Degradation of Purines and Pyrimidines**
  - Purines (A, G) damaged by dimethyl sulfate
  - Alkali(formic acid) cleaves G

- Dimethyl sulfate (DMS)

- Hydrazine
  - Hydrazine+NaCl cleaves C

- Pyrimidines (C, T) are damaged by hydrazine
1977 Maxam-Gilbert Sequencing
1977 Maxam-Gilbert Sequencing

5'-labeled ssDNA

Sequencing gel

Deduced DNA sequence

Electrophoresis

http://www.digplanet.com/wiki/Maxam-Gilbert_sequencing
Advantages

• No premature termination due to DNA sequencing. So, no problem with polymerase to synthesize DNA.
• Stretches of DNA can be sequenced which can not be done with enzymatic method.

Disadvantages

• Not widely used.
• Use of radioactivity and toxic chemicals.
Principle

• Partial copies of DNA fragments made with DNA polymerase
• Collection of DNA fragments that terminate with A, C, G or T using ddNTP
• Separate by gel electrophoresis
• Read DNA sequence
The 3′-OH group necessary for formation of the phosphodiester bond is missing in ddNTPs.

Dideoxynucleotide

PPP — O — CH₂

5′

BASE

3′

no hydroxyl group at 3′ end prevents strand extension

Growing strand

Template strand

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www.fadavis.com

http://www.mikeblaber.org/oldwine/BCH4053/Lecture20/Lecture20.htm
• A sequencing reaction mix includes labeled primer and template.

http://www.mikeblaber.org/oldwine/BCH4053/Lecture20/Lecture20.htm
• Dideoxynucleotides are added separately to each of the four tubes.
1977 Sanger Sequencing

Gel electrophoresis

Movement of DNA through acrylamide gel due to applied voltage

Largest
Mixture of DNA fragments separated on polyacrylamide gel electrophoresis
Smallest

ddA mix  ddT mix  ddC mix  ddG mix

Largest

Smallest
• Chain termination sequencing

1. Single-stranded DNA with unknown sequence (blue) serves as a template.
   + DNA polymerase
   + dATP, dCTP, dTTP, and dGTP
   + Radioactively labeled primer

2. Prepare four reaction mixtures:
   + ddATP
   + ddCTP
   + ddTTP
   + ddGTP

3. DNA synthesis followed by gel electrophoresis.

4. Longer fragments and shorter fragments.
   - Reaction products
   - Read sequence of new strand
   - and deduce sequence of template

http://www.bio.utexas.edu/faculty/sjasper/bio212/biotech2.html
Advantages

• Most popular method.
• Simpler and quicker allowing large output. Within an hour the primer-annealing and sequencing reactions can be completed.

Disadvantages

• Yielding of poor results owing to secondary structure in the DNA as sometimes DNA polymerases terminate chain elongation prematurely.
• The sequence is obtained not from the original DNA molecule but from an enzymatic copy. So, there is a chance of incorporation of wrong bases.
ABI PRISM

• The development of automated sequencing

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/DNAsequencing.html
http://www.ebay.com/sch/sis.html?_nkw=ABI%20PRISM%203700%20DNA%20ANYLYZER%20SEQUENCER%20fully%20automated&_itemId=330512964596