Decoding the Double Helix: Frederick Sanger and Sanger Sequencing

Creighton A. Jackson

Parkland College

Recommended Citation
http://spark.parkland.edu/nsps/103

Open access to this Poster is brought to you by Parkland College's institutional repository, SPARK: Scholarship at Parkland. For more information, please contact spark@parkland.edu.
Decoding the Double Helix: Frederick Sanger and Sanger Sequencing

Creighton Jackson
Che 101-004, Mullen, Parkland College

DNA Sequencing

- The field of DNA sequencing began with Watson & Crick's discovery of the double-helix structure of DNA.
- Sanger’s work with sequencing the amino acid structure of insulin provided direction to DNA researchers, who began considering how DNA directed protein formation (Marrs retrieved 2016).
- Early methods of sequencing DNA focused on methods for labeling specific nucleotides. (Sanger 1977)
- Sanger built on this method in 1977. (Sanger 1977)

- In 1977, the genome of the bacteriophage ΦX174 (172,282 bases) was fully sequenced. (Marrs retrieved 2016)
- The sequencing process was refined and eventually automated.
- As improvements were made to the Sanger method, more and more complex genomes were sequenced. In 1995, the genome of Haemophilus influenzae was sequenced (1,830,137 bases). (Marrs retrieved 2016)
- Further improvements led to the production of the first human genome in 2001 using Sanger Sequencing. (Venter 2001)

- Advances in DNA sequencing have improved techniques and reduced costs to less than a $10,000^9 of what large-scale Sanger Sequencing cost initially.

Modern Sequencing Methods

- Massively Parallel Signature Sequencing
- Polony Sequencing
- 454 Pyrosequencing
- Illumina Sequencing
- SOLiD Sequencing
- Ion Torrent semiconductor sequencing
- and many others

Sanger Sequencing (Sanger 1977)

- Sanger developed the "plus and minus" method, which utilized DNA polymerase to transcribe specific regions of DNA under controlled conditions.
- In 1977, Sanger proposed a new method for sequencing DNA using chain terminating inhibitors to stop the transcription of DNA when a particular base would be implemented.
- By doing this for each of the four bases, then analyzing and organizing the resultant chain fractions, the overall sequence of the DNA can be determined.

Method (Simplified)

- Prepare a bulk DNA sample and four solutions of normal dNTPs and polymerase.
- Add one modified dNTP to each solution.
- Add a sample of the DNA to be sequenced to each solution and incubate, allowing the DNA to replicate.
- Denature the DNA samples using heat.
- Organize the chain fragments by size using gel electrophoresis.
- Visualize DNA bands using autoradiography or UV light.

Chain-Terminating Inhibitors

Chain-terminating inhibitors are analogues of deoxynucleoside triphosphates (dNTPs) that contain no 3'-hydroxyl group. This prevents DNA polymerase from continuing the transcription past the base where the inhibitor is incorporated.

- 2',3'-dideoxythymidine triphosphate (dSTTP) was commercially available.
- 2',3'-dideoxyadenosine triphosphate (dDATP) had been prepared by another research team.
- 2',3'-dideoxyguanosine triphosphate (dGTP) and 2',3'-
dideoxyuridine triphosphate (dUTP) were synthesized based on method for dSTTP

Improvements to Sanger Sequencing

- Dye-terminator Sequencing (Smith 1986)
  - Use of fluorescent dyes to tag chain-terminating dNTPs, allowing sequencing to take place in a single reaction, rather than 4 separate reactions.
  - Improves efficiency of the reaction and enables automatic reading through optical systems.
- Automation
  - Process has been automated with the development of DNA sequencers.
  - Automated interpretation of sequencing output is not as accurate as human judgment when it comes to recognizing suboptimal results. (Smith 1986)
- Microfluidic Sanger Sequencing (Kan 2004)
  - "Lub-on-a-chip"

References