DNA sequencing with chain-terminating inhibitors

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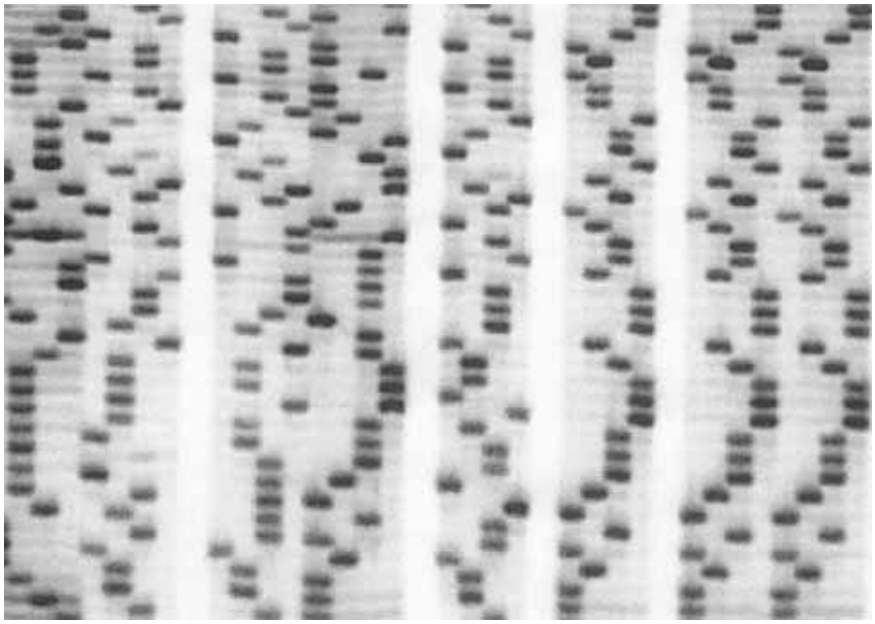

Presented by Kim Butt, Yumiko Komatsu, and Amelia Parrott

Nobel Prize in Chemistry (1980)

"for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA"
"for their contributions concerning the determination of base sequences in nucleic acids"

Paul Berg

Walter Gilbert
Fred Sanger

DNA Sequencing Timeline

1953 – Structure of DNA double helix deduced by Watson and Crick

1972 – Development of recombinate DNA technology by Berg

1975 – Plus and minus method of DNA sequencing developed by Sanger

1977 – DNA sequencing dideoxy method developed by Sanger

1986 – PCR developed by Mullis

1986 – First semi-automated DNA sequencing machine announced

2000 – *Drosophila* genome is completed

2003 – Human genome sequence is released

The Dideoxy Method

PRINCIPLE BEHIND THE METHOD

Sanger method - DNA sequencing using chain-terminating inhibitors to terminate DNA synthesis at a specific site

- also known as the dideoxy method

How did Sanger come up with this method?

Method

1. Preparation of ddNTPs

Complex chemistry

Now: A lot of materials are commercially available

2. Sequencing procedure:

Chain termination method:

- Primer annealed to tDNA in H buffer
- Template DNA from Phage Phi X174

Make 5 separate mixtures and incubate as follows:

dATP chase—put additional **dATP** into each of the tube & incubate.

- A critical step to avoid random termination at A residues

Figure 1: Small primer, no further splitting required

Figure 2: Longer primer, further splitting was necessary to separate the primer from synthesized DNA.

- Used restriction enzyme, Hae III.

Electrophoresis on 12% acrylamide gel to separate fragments of different sizes

Autoradiograph was used to visualize bands

Results

Figure 1 Small primers do not need to be removed before sequencing.

Figure 2 Long primers must be removed before sequencing.

Figure 3 Fragments with multiple restriction sites close together are problematic.

- Problems can be avoided with single site ribosubstitution.

Discussion

The dideoxy method is the **simplest**, **fastest**, and **most effecient** method of sequencing DNA to date.

However

This paper was written in **1977**.

DNA sequencing has significantly advanced since this paper was written.

Modern techniques are based on the **same principles** as the dideoxy method.

See cycle sequencing.

The **Phi X174** genome has been synthesized as of 2003.
