DNA sequencing with chain-terminating inhibitors

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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 recombinant 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
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 efficient 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.