

Bioinformatics

DNA sequencing

MSc. Vicente Machaca Arceda

Universidad Nacional de San Agustín de Arequipa

May 4, 2020

Table of Contents

1 Introduction

- Objectives
- DNA sequencing

2 Sanger DNA sequencing

- Frederick Sanger
- Di deoxynucleotide
- Sanger Method - Gel Electrophoresis
- Sanger Method - Capilar Electrophoresis

Table of Contents

- 1 Introduction
 - Objectives
 - DNA sequencing
- 2 Sanger DNA sequencing
 - Frederick Sanger
 - Di deoxynucleotide
 - Sanger Method - Gel Electrophoresis
 - Sanger Method - Capilar Electrophoresis

Objectives

- Understand what is DNA sequencing.

Objectives

- Understand what is DNA sequencing.
- Learn the Sanger method for DNA sequencing.

Table of Contents

- 1 Introduction
 - Objectives
 - **DNA sequencing**
- 2 Sanger DNA sequencing
 - Frederick Sanger
 - Di deoxynucleotide
 - Sanger Method - Gel Electrophoresis
 - Sanger Method - Capilar Electrophoresis

DNA sequencing

Definition

DNA sequencing is the process of determining the order of nucleotide bases (As, Ts, Cs, and Gs) in a piece of DNA.

Sanger DNA sequencing

Example

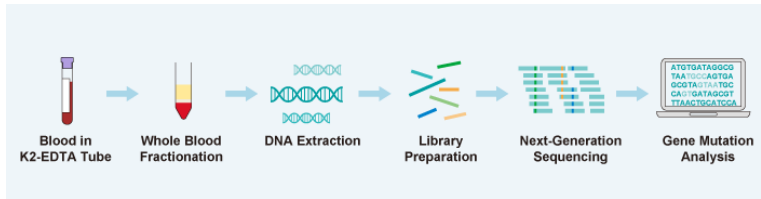


Figure: DNA sequencing in the analysis of mutations

Table of Contents

- 1 Introduction
 - Objectives
 - DNA sequencing
- 2 Sanger DNA sequencing
 - **Frederick Sanger**
 - Di deoxynucleotide
 - Sanger Method - Gel Electrophoresis
 - Sanger Method - Capilar Electrophoresis

Sanger DNA sequencing

Frederick Sanger

Sanger (1975) proposed a method for determining sequences in DNA by primed synthesis with DNA polymerase [1]. It is based on the principle of **Di deoxynucleotide**.

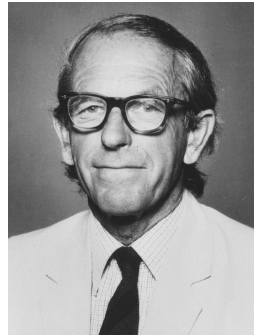


Figure: Frederick Sanger in 1977.

Table of Contents

- 1 Introduction
 - Objectives
 - DNA sequencing
- 2 Sanger DNA sequencing
 - Frederick Sanger
 - **Di deoxynucleotide**
 - Sanger Method - Gel Electrophoresis
 - Sanger Method - Capilar Electrophoresis

Sanger method

Deoxynucleotide

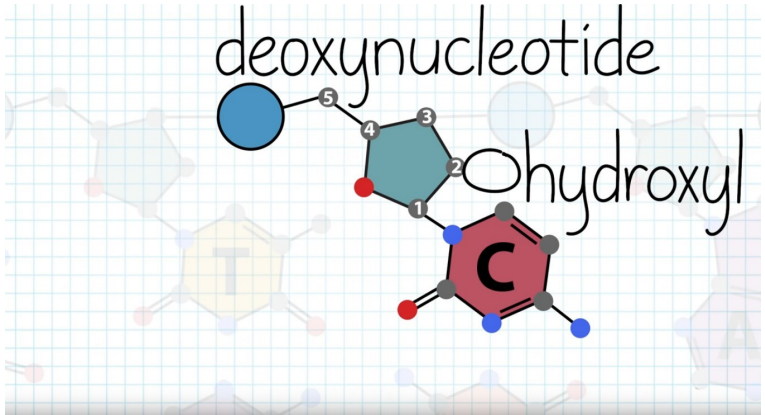


Figure: The nucleotides are also called deoxynucleotides.

Sanger method

dNTP

dNTP

Deoxynucleoside triphosphate **dNTPs** are standard natural substrates of all DNA polymerases.

Sanger method

Deoxynucleotide

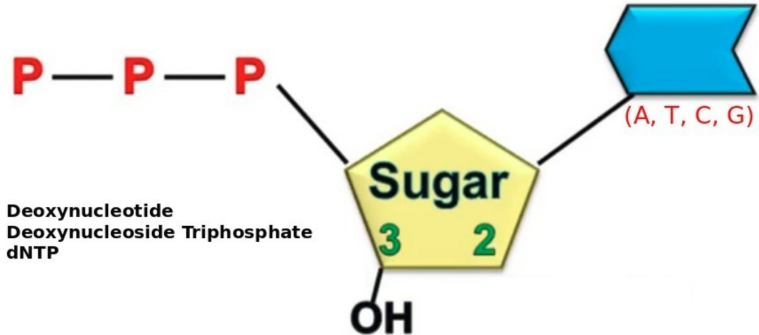


Figure: Deoxynucleotides doesn't have the hydroxyl group at the 2' carbon of sugar.

Sanger method

Nucleotides and nucleosides

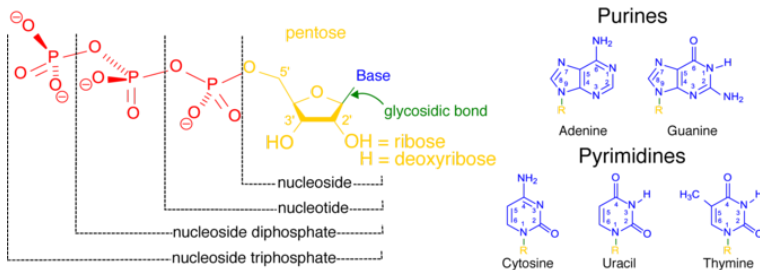


Figure: The difference of nucleotides and nucleosides.

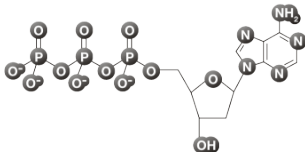
Sanger method

Deoxynucleotide

dATP

Formula: $C_{10}H_{12}N_5O_{13}P_3$ (Anion)

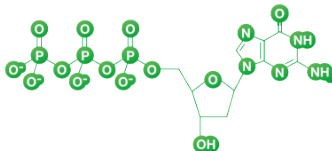
Formula Weight: 487.18 (Anion)



dGTP

Formula: $C_{10}H_{12}N_5O_{13}P_3$ (Anion)

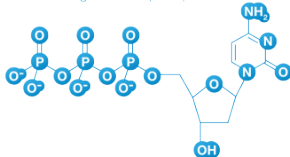
Formula Weight: 503.18 (Anion)



dCTP

Formula: $C_9H_{12}N_3O_{13}P_3$ (Anion)

Formula Weight: 463.15 (Anion)



dTTP

Formula: $C_{10}H_{12}N_5O_{13}P_3$ (Anion)

Formula Weight: 478.16 (Anion)



Figure: Deoxynucleotides: dATP, dTTP, dCTP and dGTP.

Sanger method

DNA replication - Di Deoxynucleotide principle

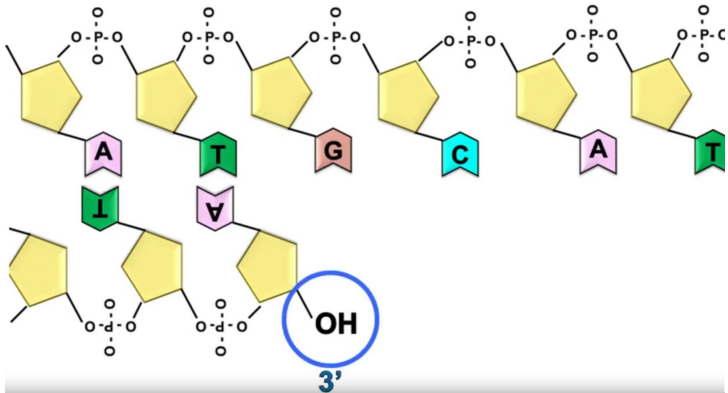


Figure: dNTPs are attached to the 3' carbon of sugar.

Sanger method

DNA replication - Di Deoxynucleotide principle

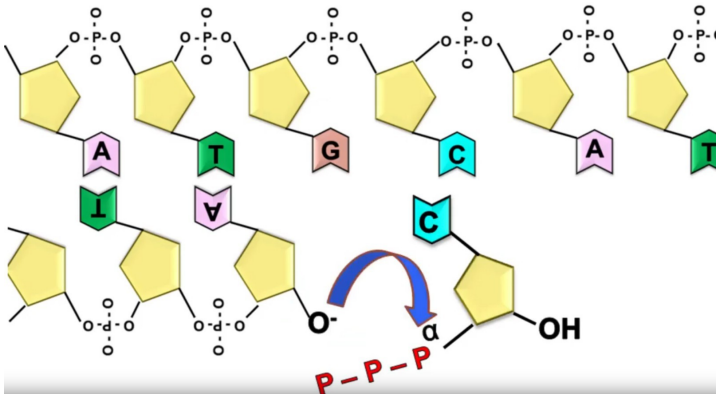


Figure: dNTPs are attached to the 3' carbon of sugar.

Sanger method

DNA replication - Di Deoxynucleotide principle

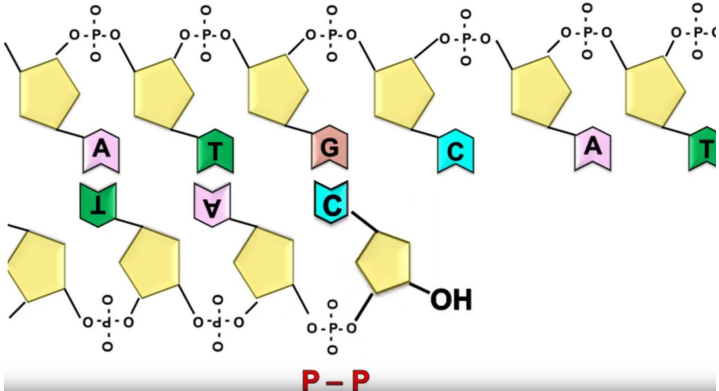


Figure: dNTPs are attached to the 3' carbon of sugar.

Sanger method

DNA replication - Di Deoxynucleotide principle

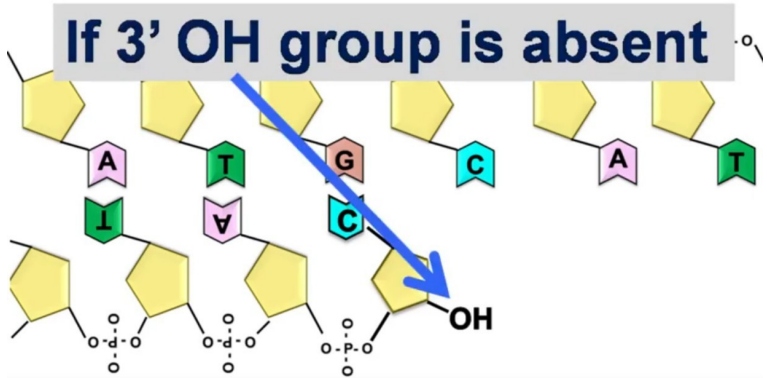


Figure: If 3' OH group is absent, the reaction stop.

Sanger method

DNA replication - Di Deoxynucleotide principle

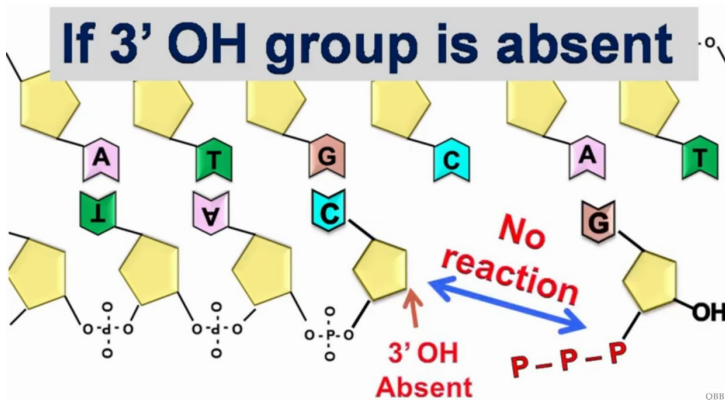
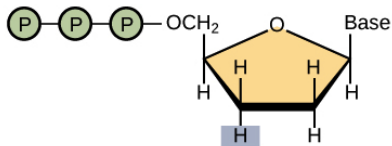


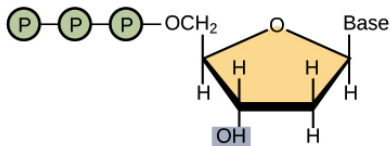
Figure: If 3' OH group is absent, the reaction stop.

Sanger method

Deoxynucleotide



Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

Figure: Deoxynucleotide and Di Deoxynucleotide.

Table of Contents

- 1 Introduction
 - Objectives
 - DNA sequencing
- 2 Sanger DNA sequencing
 - Frederick Sanger
 - Di deoxynucleotide
 - **Sanger Method - Gel Electrophoresis**
 - Sanger Method - Capilar Electrophoresis

Sanger method

Double helix DNA



Figure: The Sanger method start from a double helix DNA.

Sanger method

Single strand DNA

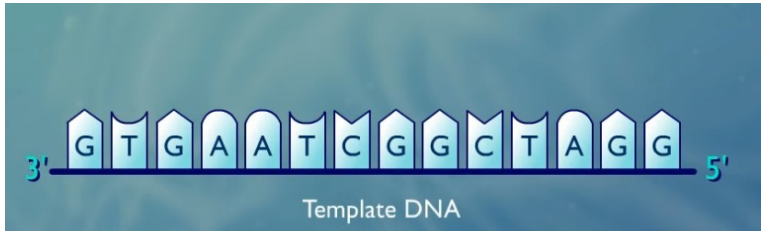


Figure: The double helix DNA is separated with high temperatures.

Sanger method

Primer - dNTPs - DNA Polymerase

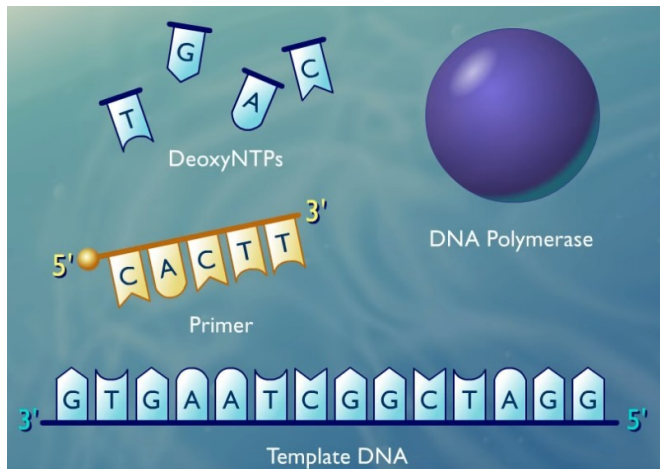


Figure: Three components are prepared in vitro. dNTPs, DNA Polymerase and a Primer.

Sanger method

Primer - dNTPs - DNA Polymerase

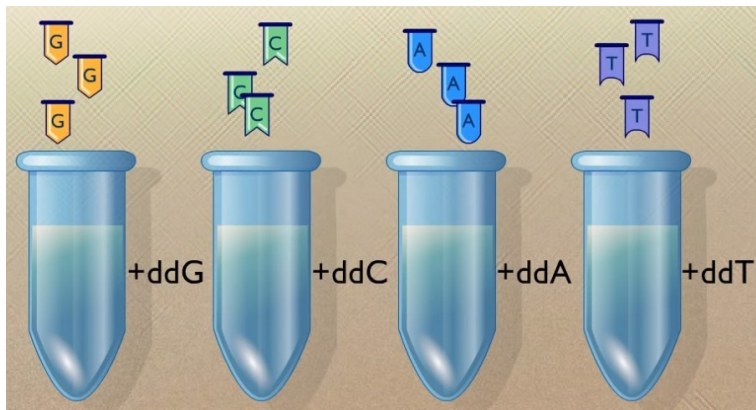


Figure: Four tubes are used. Each tube contains: DNA template, primer, dNTPs, DNA polymerase and one type of ddNTP (ATP or TTP or CTP or GTP).

Sanger method

Radioactive phosphorus labeled

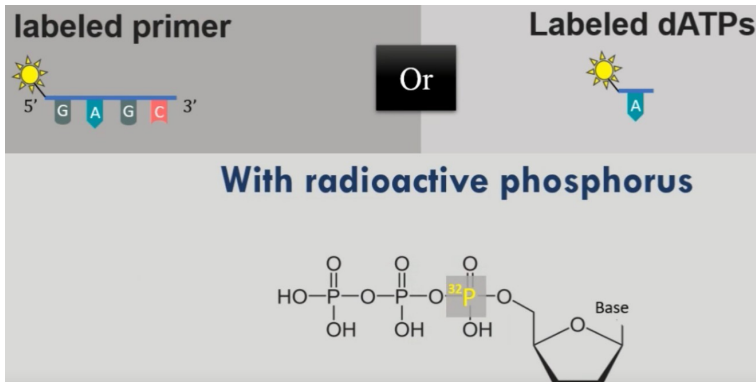


Figure: Before starting the reaction, the primer or one of dNTPs are labeled.

Sanger method

DNA replication in each tube

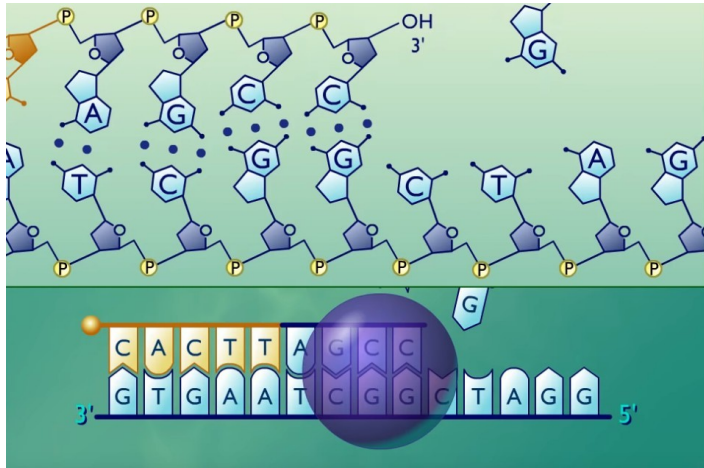


Figure: DNA replication occurs in each tube. The tubes contains the dNTPs.

Sanger method

DNA replication stop when ddNTP arrives

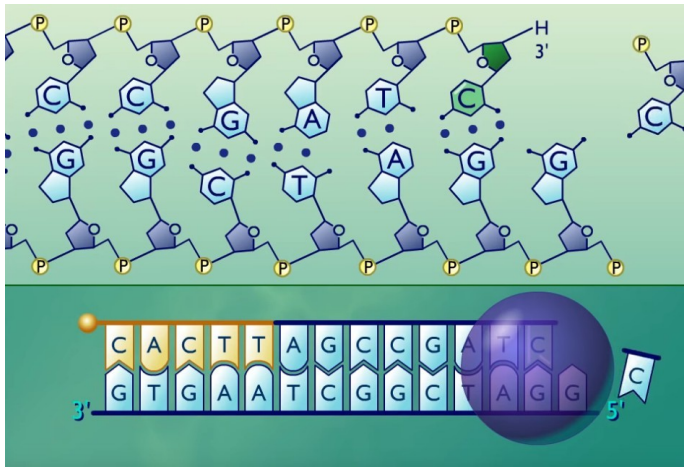


Figure: DNA replication stop when a ddNTP arrives.

Sanger method

DNA replication starts again

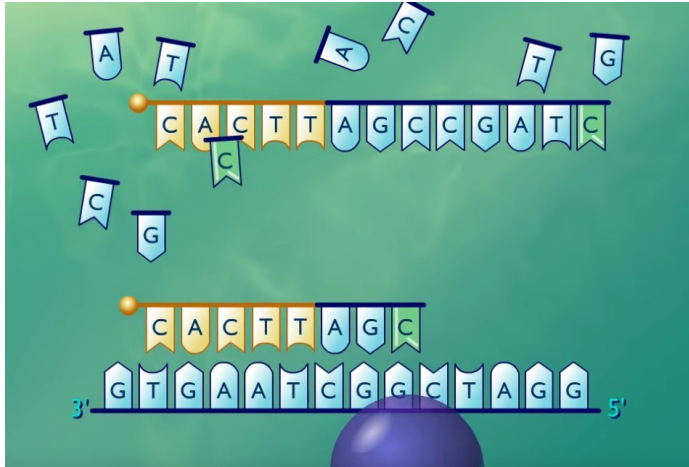


Figure: DNA replication starts again and different fragments length are obtained.

Sanger method

DNA replication starts again



Figure: DNA replication starts again and different fragments are obtained.

Sanger method

Each tube have fragments of DNA

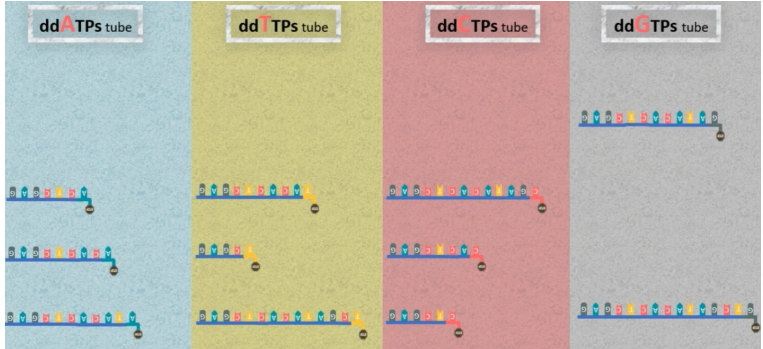


Figure: On each tube, there are DNA fragments, each one with different length.

Sanger method

Gel electrophoresis

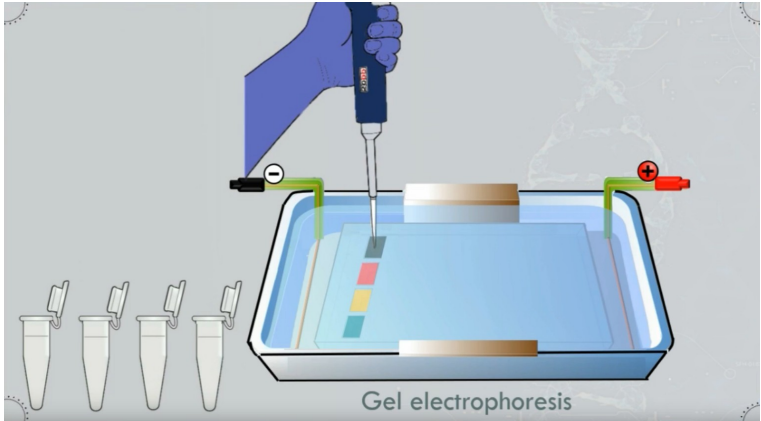


Figure: Gel electrophoresis. The negative charge of its phosphate backbone moves the DNA towards the positively charge anode.

Sanger method

Gel electrophoresis

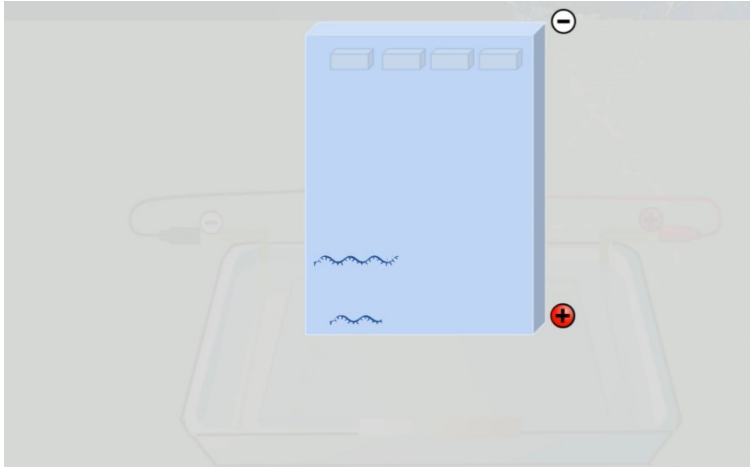


Figure: Shorter DNA molecules can travel farther than longer counterparts.

Sanger method

Gel electrophoresis

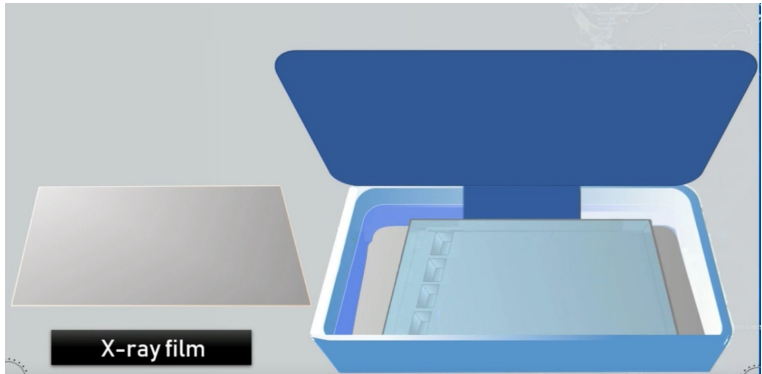


Figure: The DNA bands are visualized by autoradiography using the X-ray film.

Sanger method

Gel electrophoresis

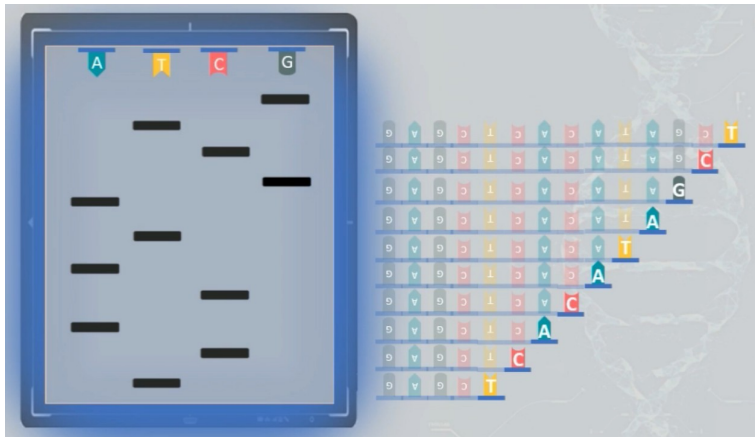


Figure: The DNA bands are visualized by autoradiography using the X-ray film.

Sanger method

Gel Electrophoresis Animation

Gel Electrophoresis Animation.

Table of Contents

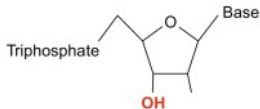
- 1 Introduction
 - Objectives
 - DNA sequencing

- 2 Sanger DNA sequencing
 - Frederick Sanger
 - Di deoxynucleotide
 - Sanger Method - Gel Electrophoresis
 - **Sanger Method - Capilar Electrophoresis**

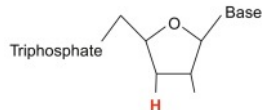
Sanger method

Capilar Electrophoresis

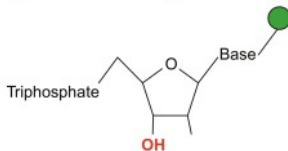
Deoxynucleotide (dNTP)



Dideoxynucleotide (ddNTP)



Fluorophore labeled deoxynucleotide (dNTP)



Fluorophore labeled dideoxynucleotide (ddNTP)

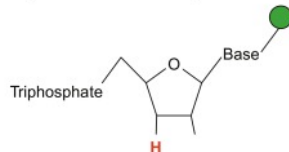


Figure: The ddNTPs are fluorophore labeled.

Sanger method

Capilar Electrophoresis

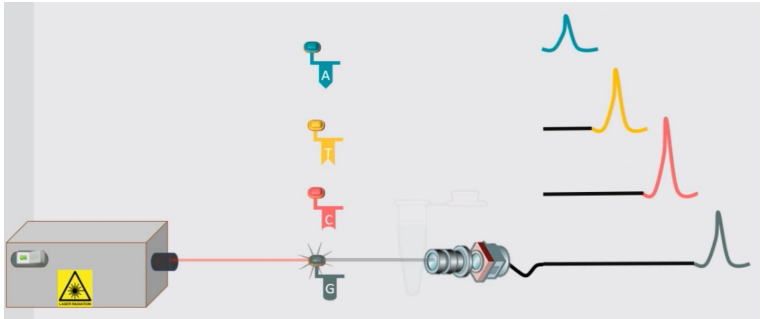


Figure: Each of the four ddNTPs chain terminator are labelled with fluorescent dyes.

Sanger method

Capilar Electrophoresis



Figure: DNA replication occurs in one tube (Gel electrophoresis uses four).

Sanger method

Capilar Electrophoresis

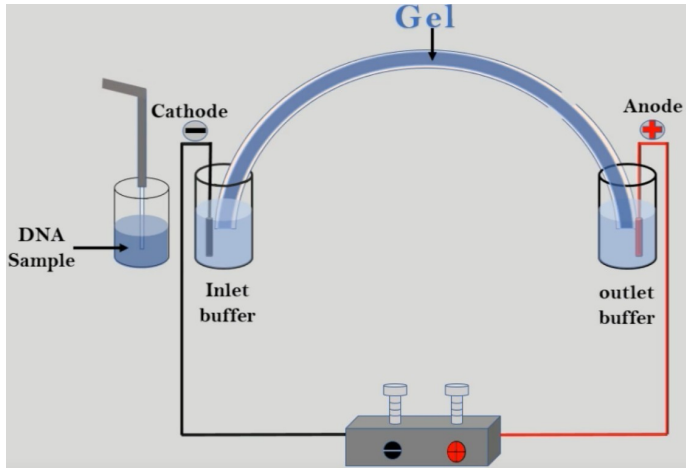


Figure: DNA fragments are placed in order move through a gel conductor.

Sanger method

Capilar Electrophoresis

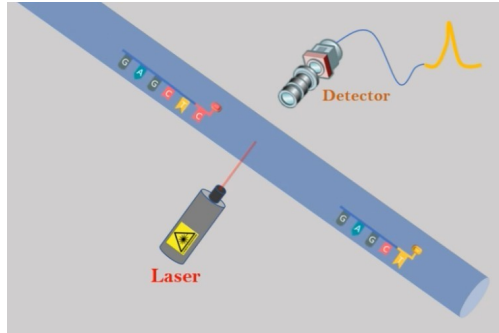


Figure: The fragment are charged and they move over a conductor. Smaller moves rapidly and they are read by a laser.

Sanger method

Many samples read

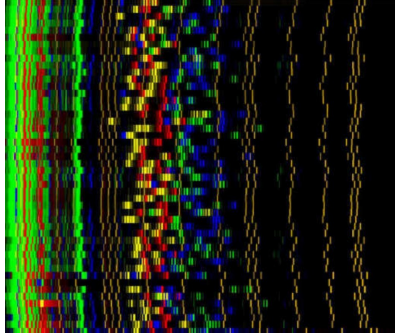


Figure: The process is repeated several times and many samples are obtained.

Sanger method

Capillary Electrophoresis Animation

Capillary Electrophoresis Animation.

Sanger method

Gel Electrophoresis vs Capillary Electrophoresis

- Capillary Electrophoresis is faster than Gel Electrophoresis.
- In Gel Electrophoresis the X-ray film is read by eyes meanwhile, in Capillary Electrophoresis a machine records the DNA sequence.
- Gel Electrophoresis reads DNA fragments of 150-200 bp meanwhile, Capillary Electrophoresis reads DNA fragments of 800-1000 bp.

References I



F. Sanger, S. Nicklen, and A. R. Coulson, "Dna sequencing with chain-terminating inhibitors," *Proceedings of the national academy of sciences*, vol. 74, no. 12, pp. 5463–5467, 1977.