Escuela Profesional de
Ciencia de la Computación



# Bioinformatics

## DNA sequencing

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# Objectives

- Understand what is DNA sequencing.

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# Objectives

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

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# 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

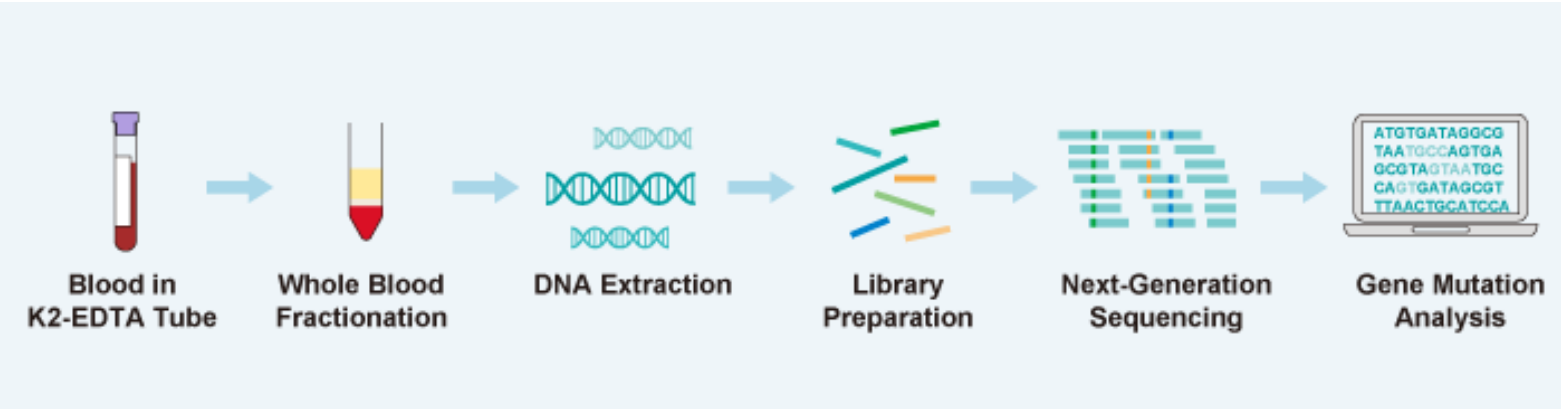


Figure: DNA sequencing in the analysis of mutations

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# 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**.

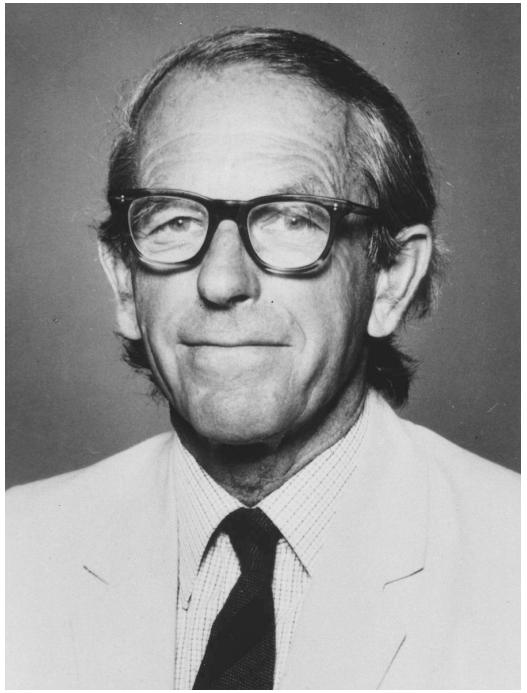

Figure: Frederick Sanger in 1977.

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# Sanger method

## Deoxynucleotide

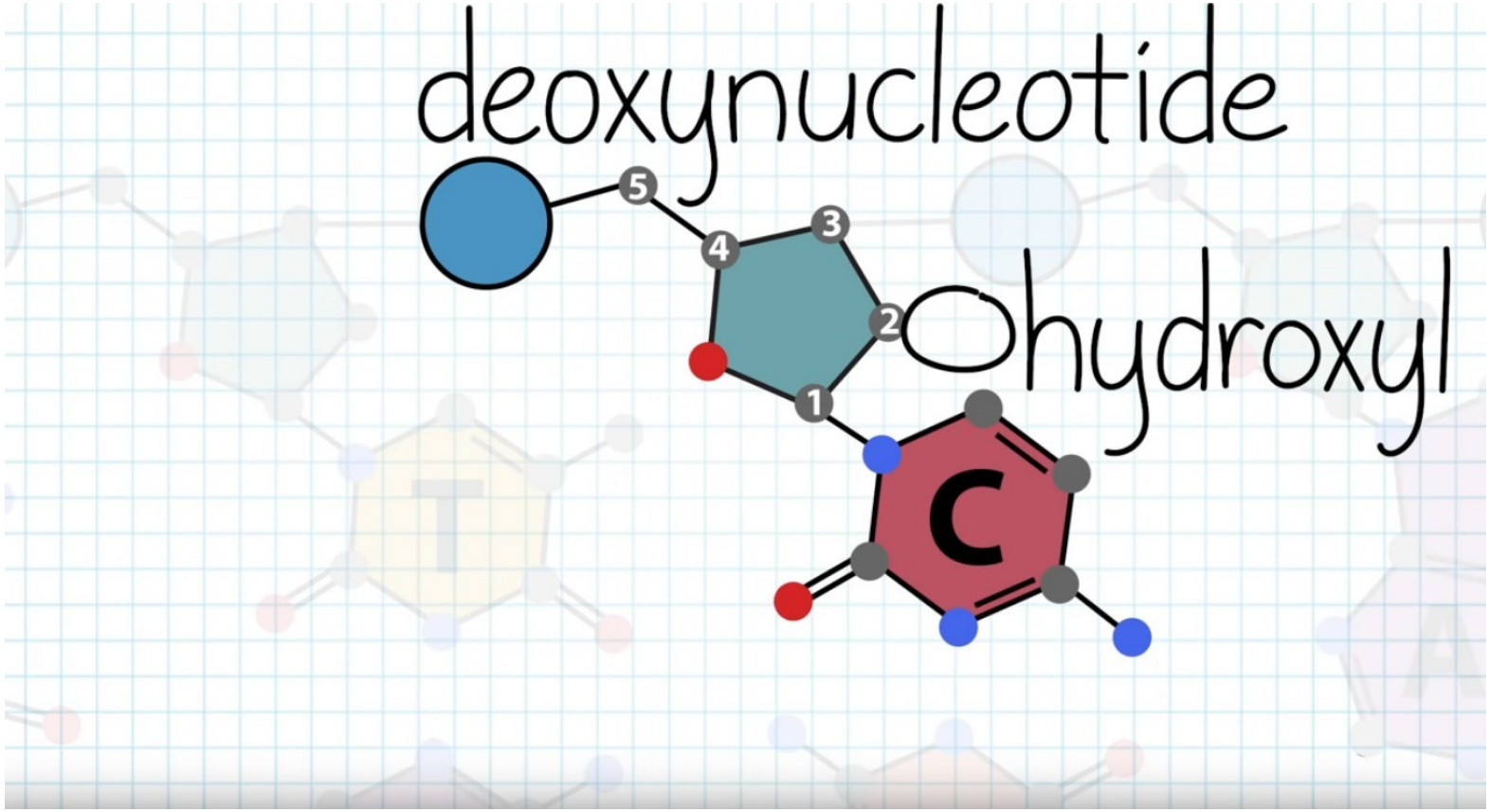


Figure: The nucleotides are also called deoxynucleotides.

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# Sanger method

## dNTP

### dNTP

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

# Sanger method

## Deoxynucleotide

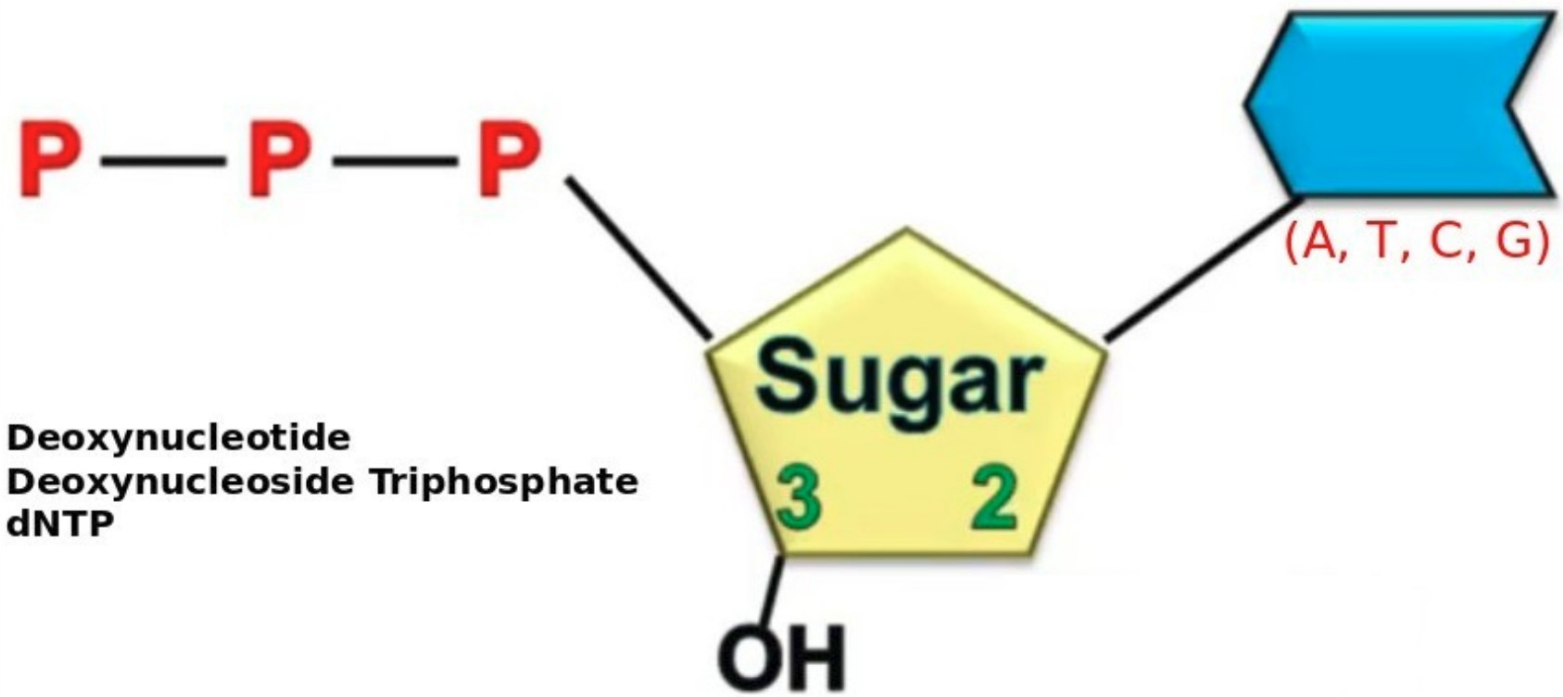


Figure: Deoxynucleotides doesn't have the hydroxyl group at the 2' carbon of sugar.

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# Sanger method

## Nucleotides and nucleosides

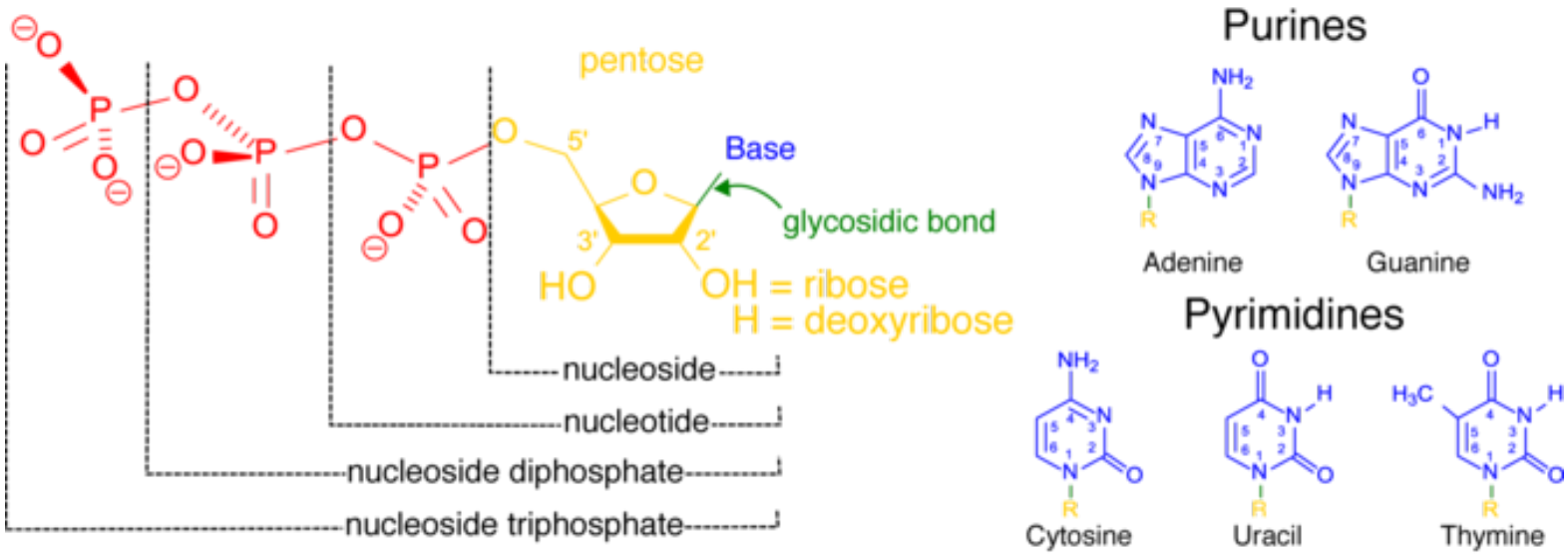


Figure: The difference of nucleotides and nucleosides.

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# Sanger method

## Deoxynucleotide

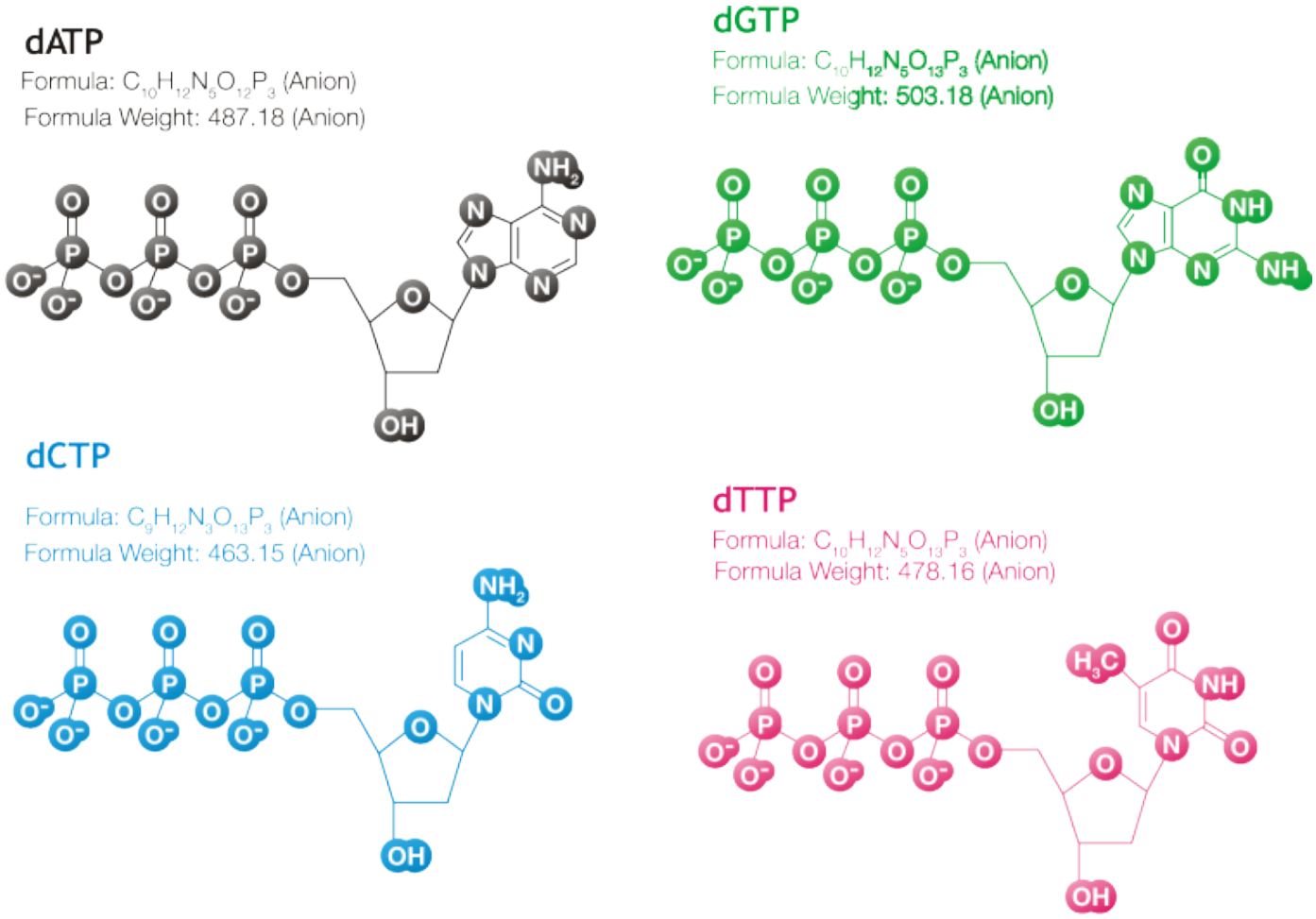


Figure: Deoxynucleotides: dATP, dTTP, dCTP and dGTP.

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# Sanger method

## DNA replication - Di Deoxynucletide principle

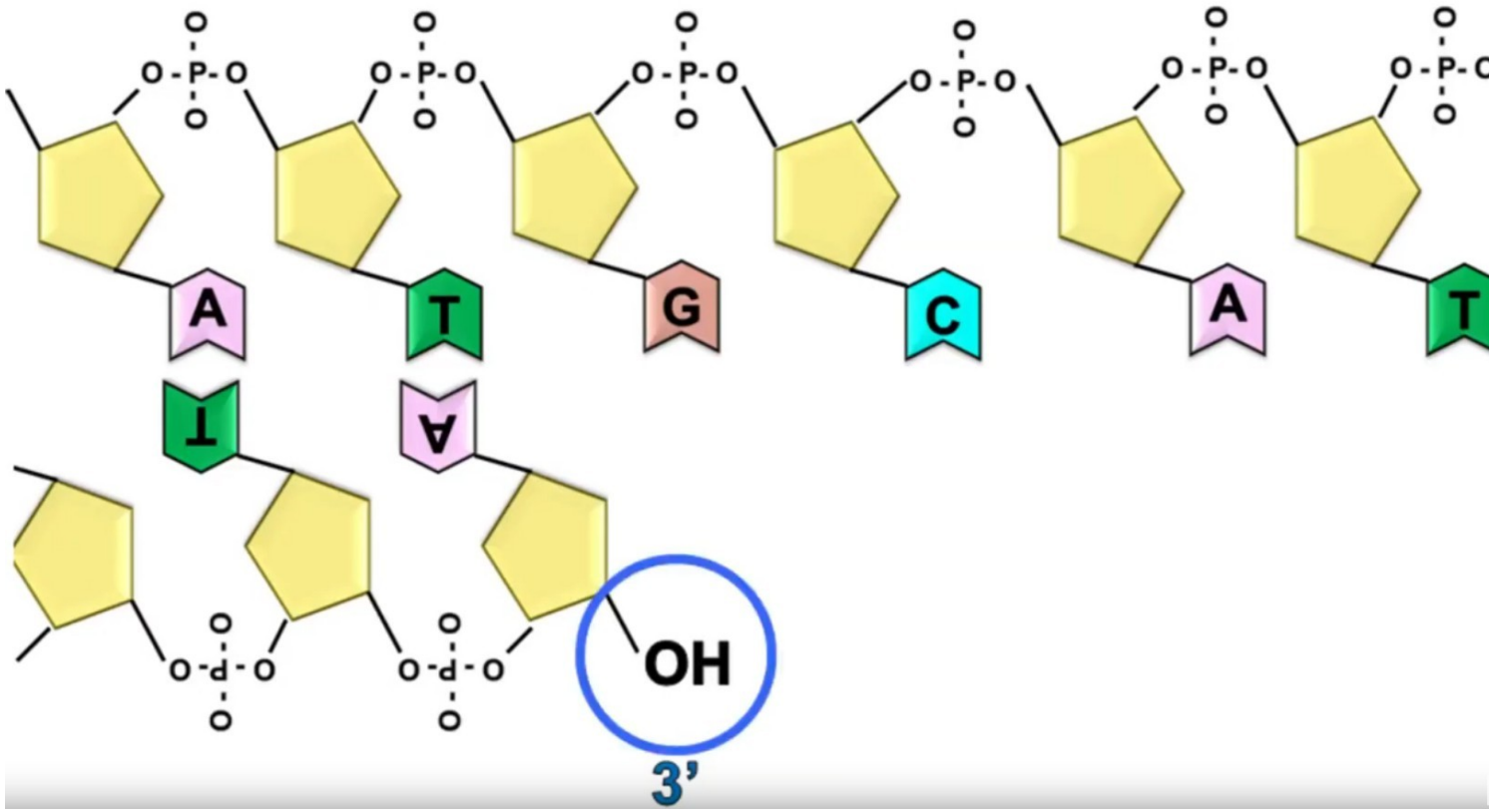


Figure: dNTPs are attached to the 3' carbon of sugar.

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# Sanger method

## DNA replication - Di Deoxynucletide principle

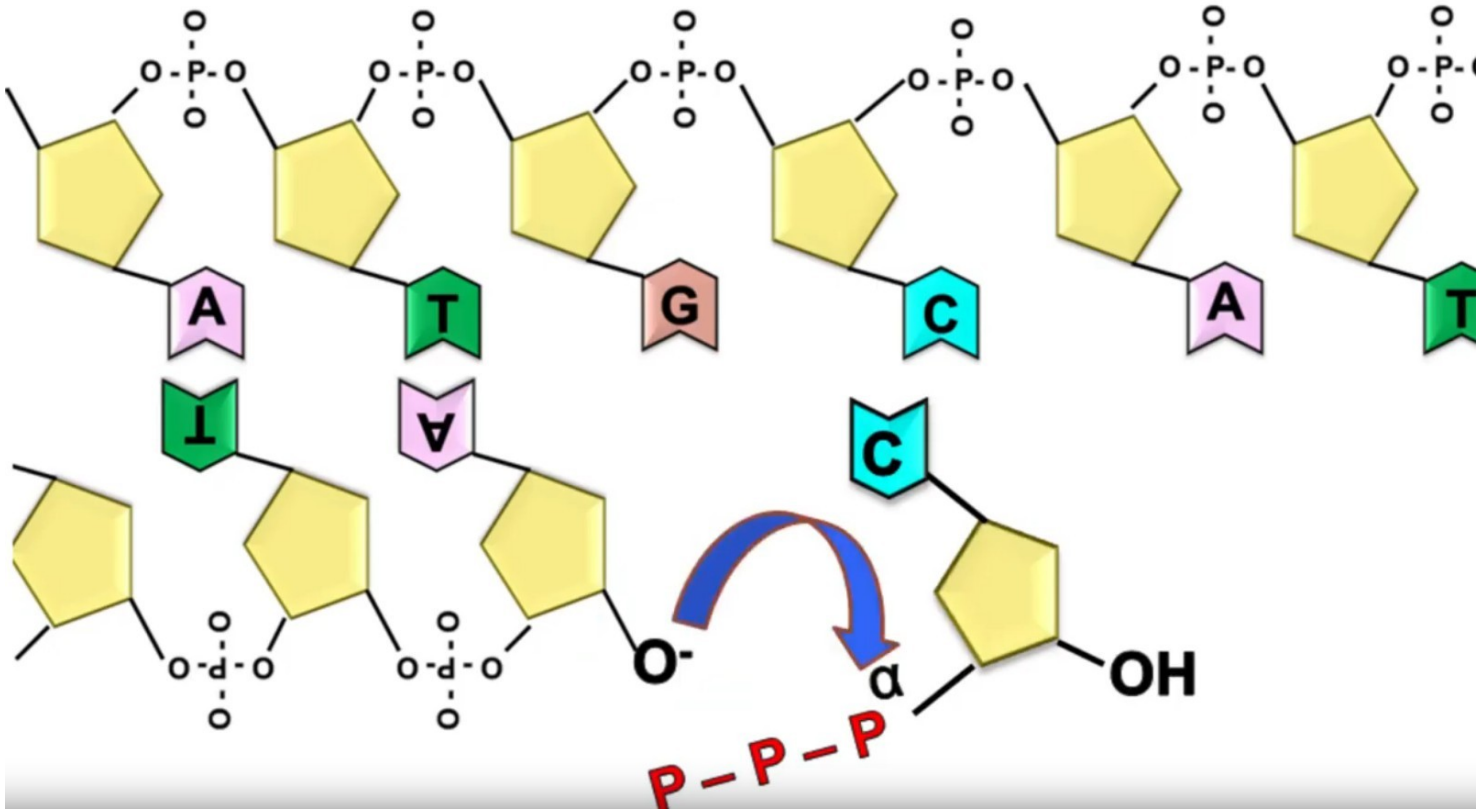


Figure: dNTPs are attached to the 3' carbon of sugar.

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# Sanger method

## DNA replication - Di Deoxynucletide principle

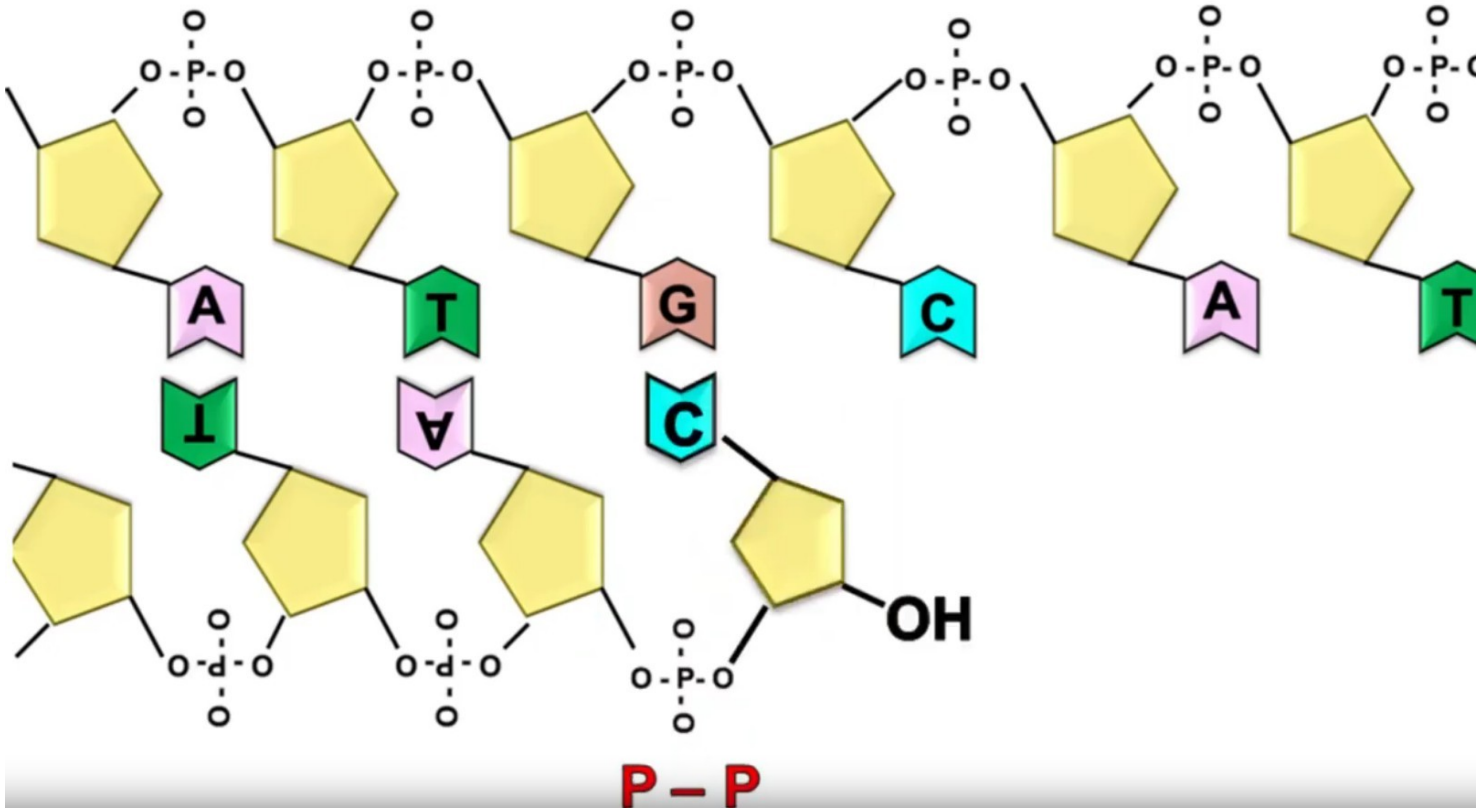


Figure: dNTPs are attached to the 3' carbon of sugar.

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# Sanger method

## DNA replication - Di Deoxynucletide principle

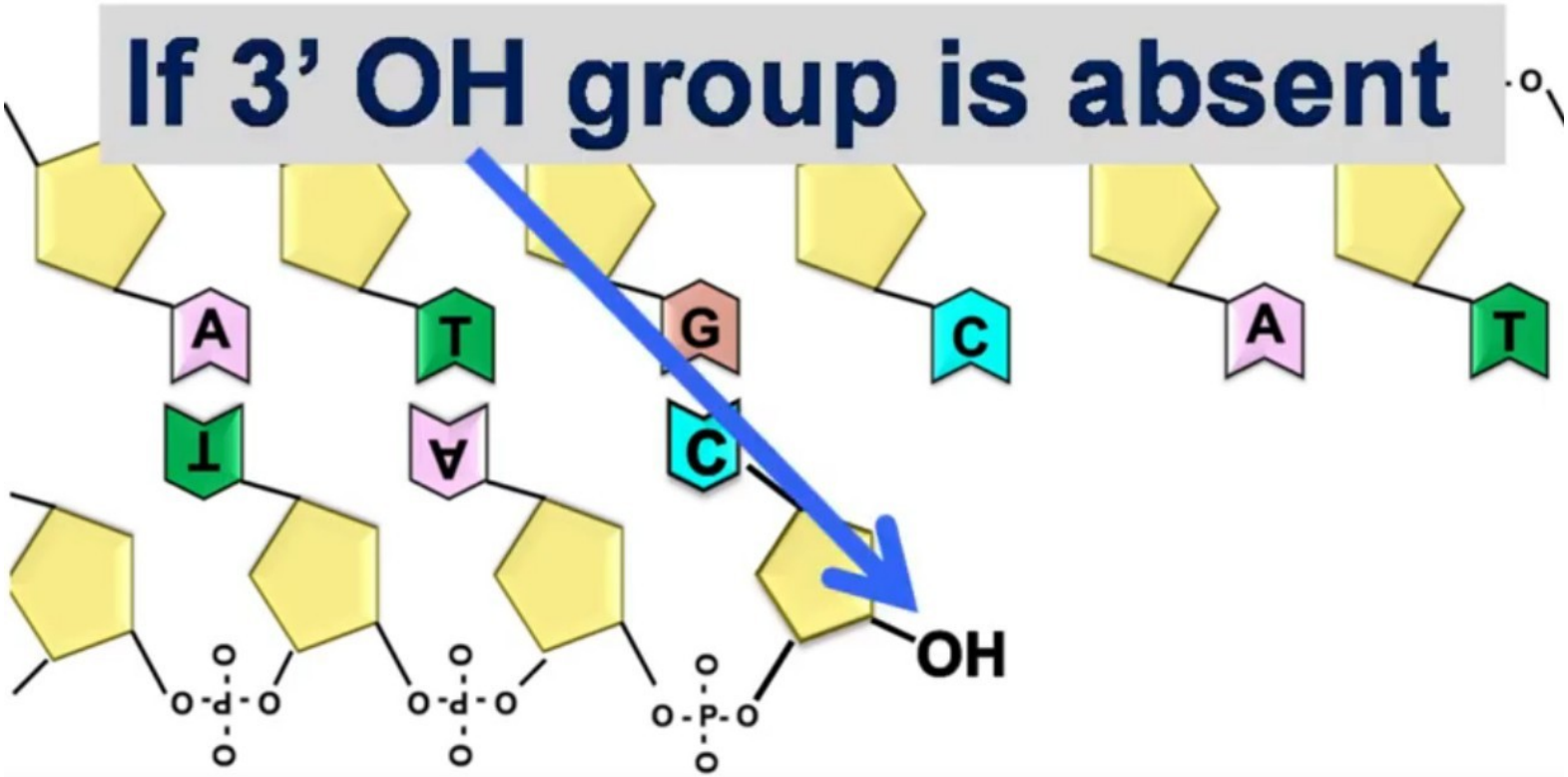


Figure: If 3' OH group is absent, the reaction stop.

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# Sanger method

## DNA replication - Di Deoxynucletide principle

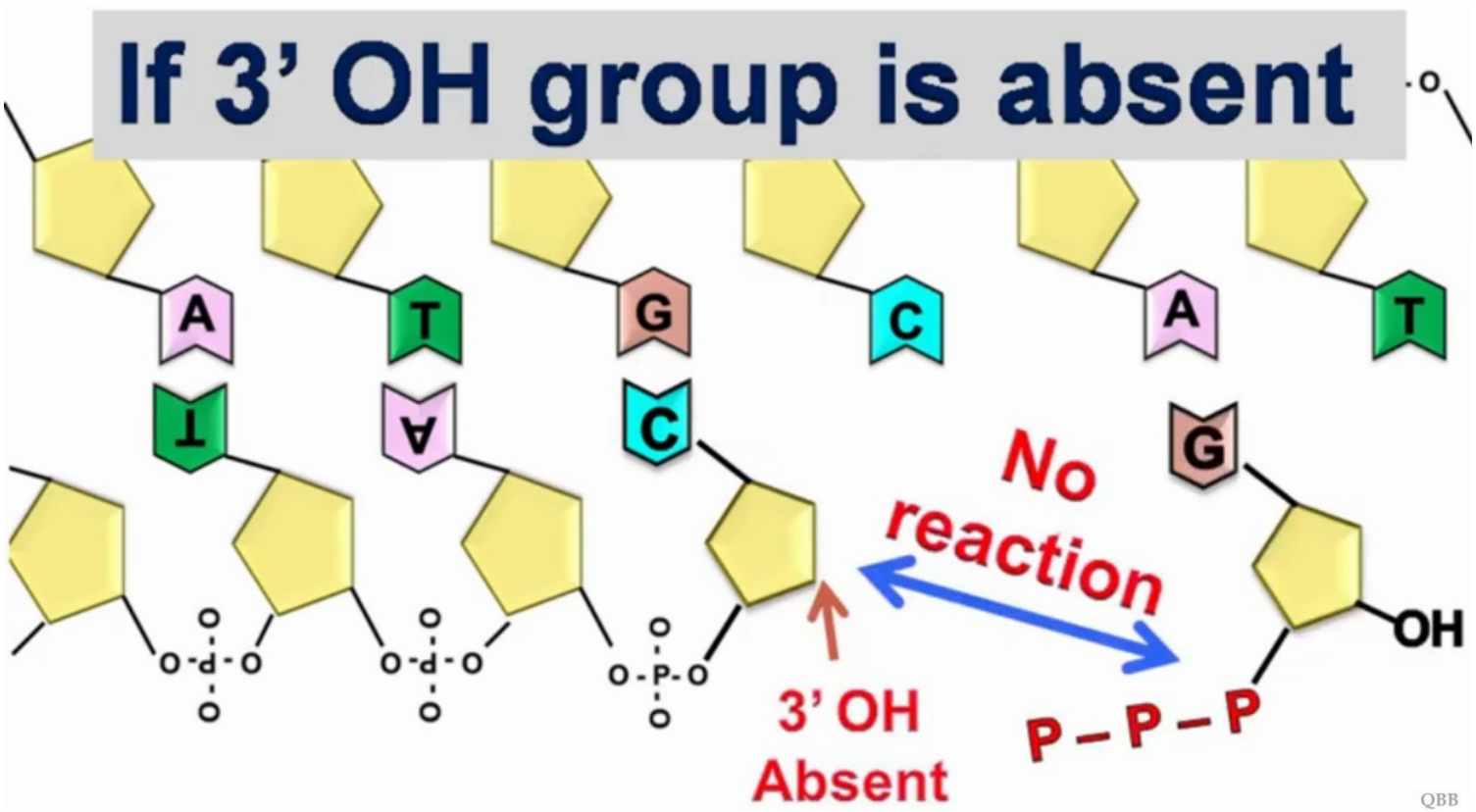


Figure: If 3' OH group is absent, the reaction stop.

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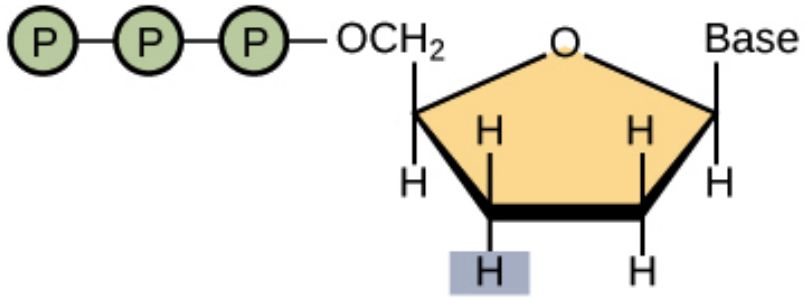
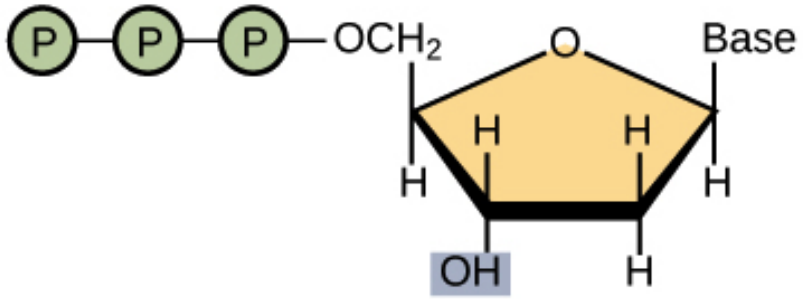
# Sanger method

## Deoxynucleotide




**Dideoxynucleotide (ddNTP)**




**Deoxynucleotide (dNTP)**

Figure: Deoxynucleotide and Di Deoxynucletide.

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# Sanger method

## Double helix DNA


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

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# Sanger method

## Single strand DNA

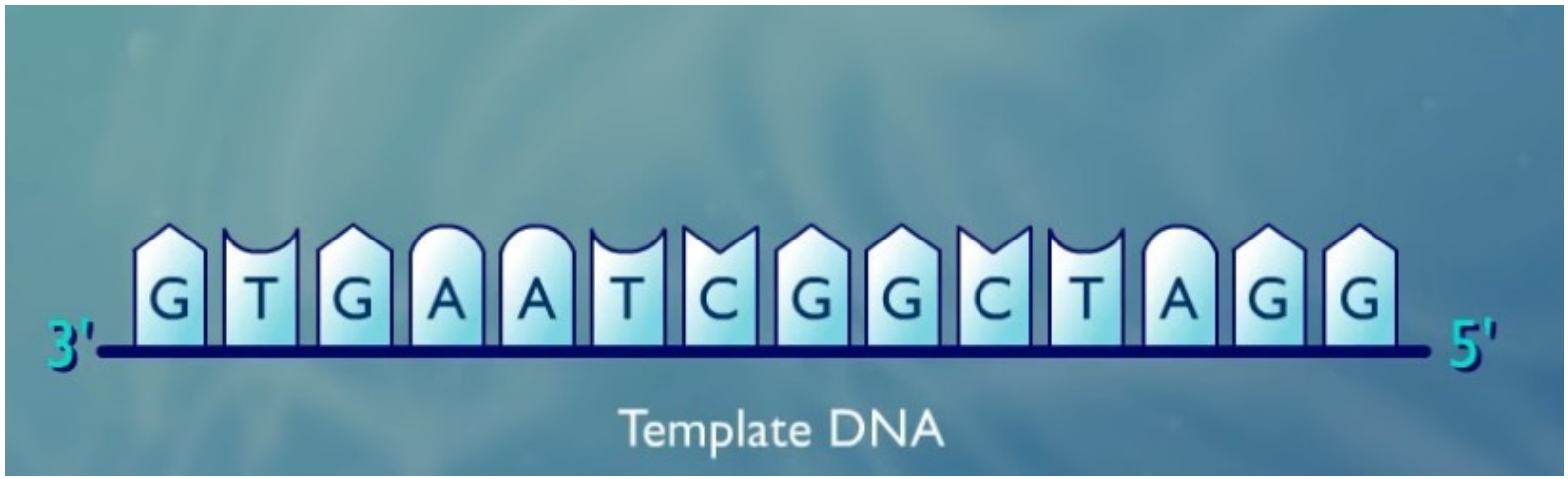


Figure: The double helix DNA is separated with high temperatures.

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# Sanger method

## Primer - dNTPs - DNA Polymerase

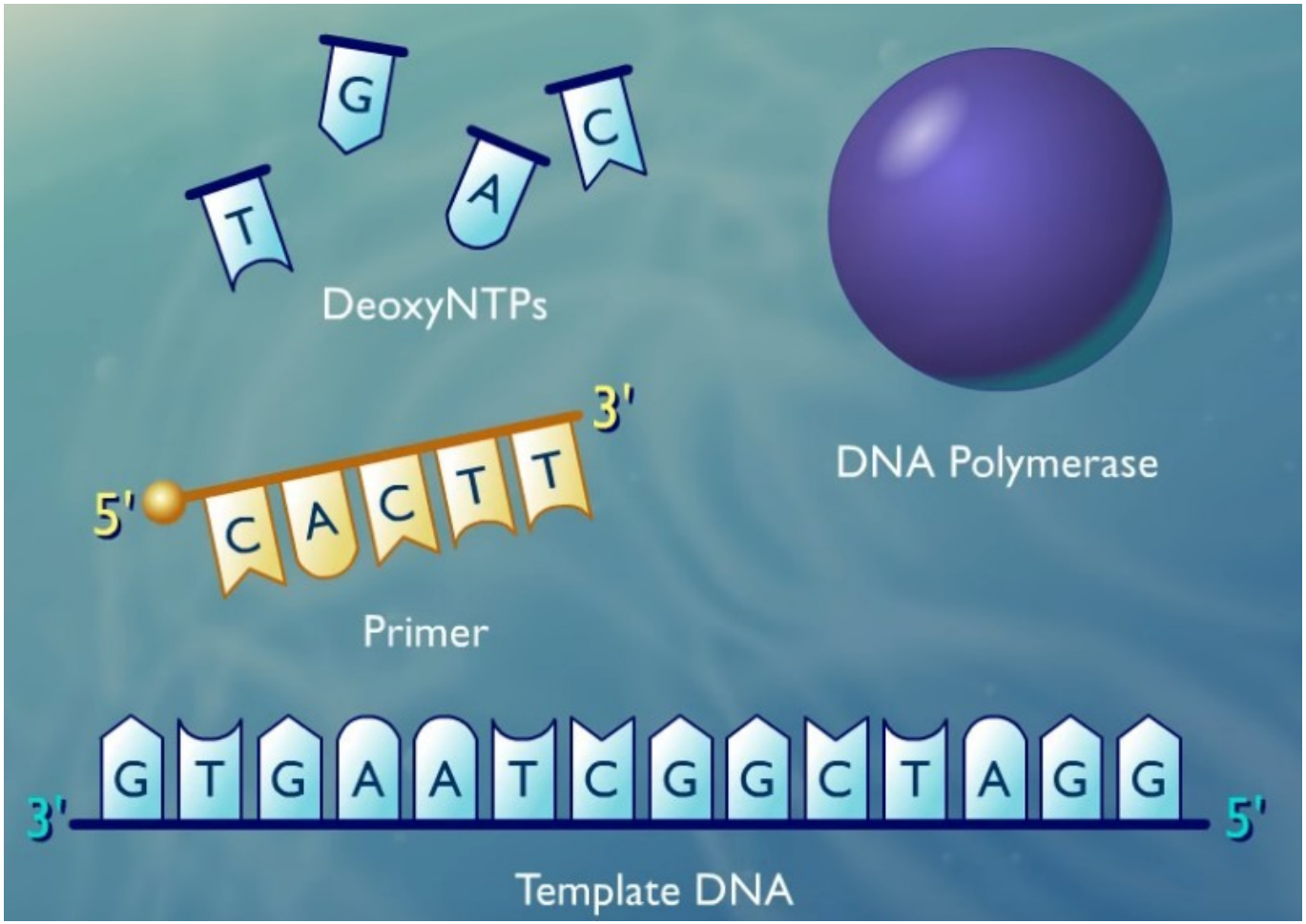


Figure: Three components are prepared in vitro. dNTPs, DNA Polymerase and a Primer.

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# Sanger method

## Primer - dNTPs - DNA Polymerase

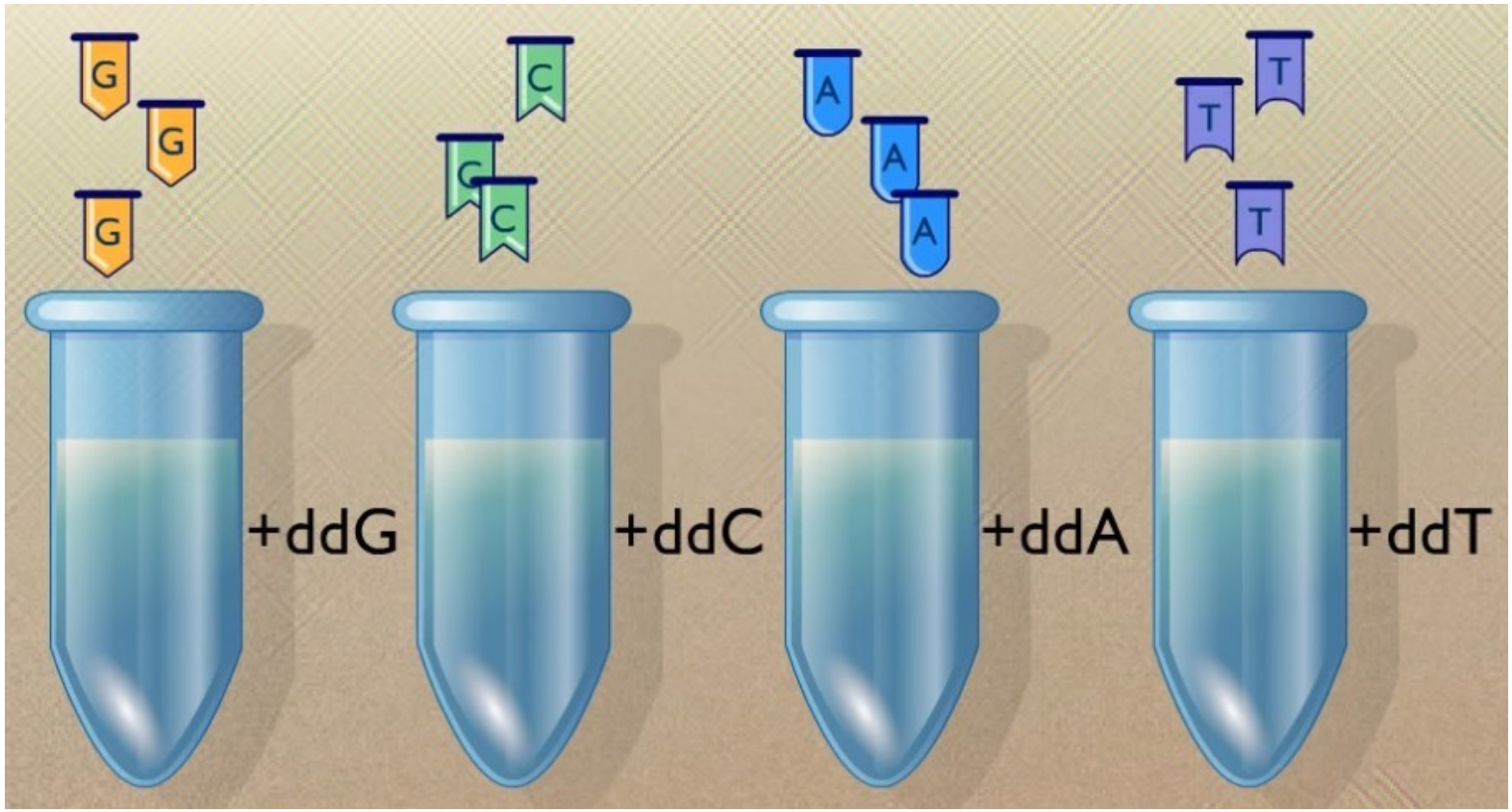


Figure: Four tubes are used. Each tube contains: DNA template, primer, dNTPs, DNA polymerase and one type of ddNTP (ATP or TTP or CTP or GTP).

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# Sanger method

## Radioactive phosphorus labeled

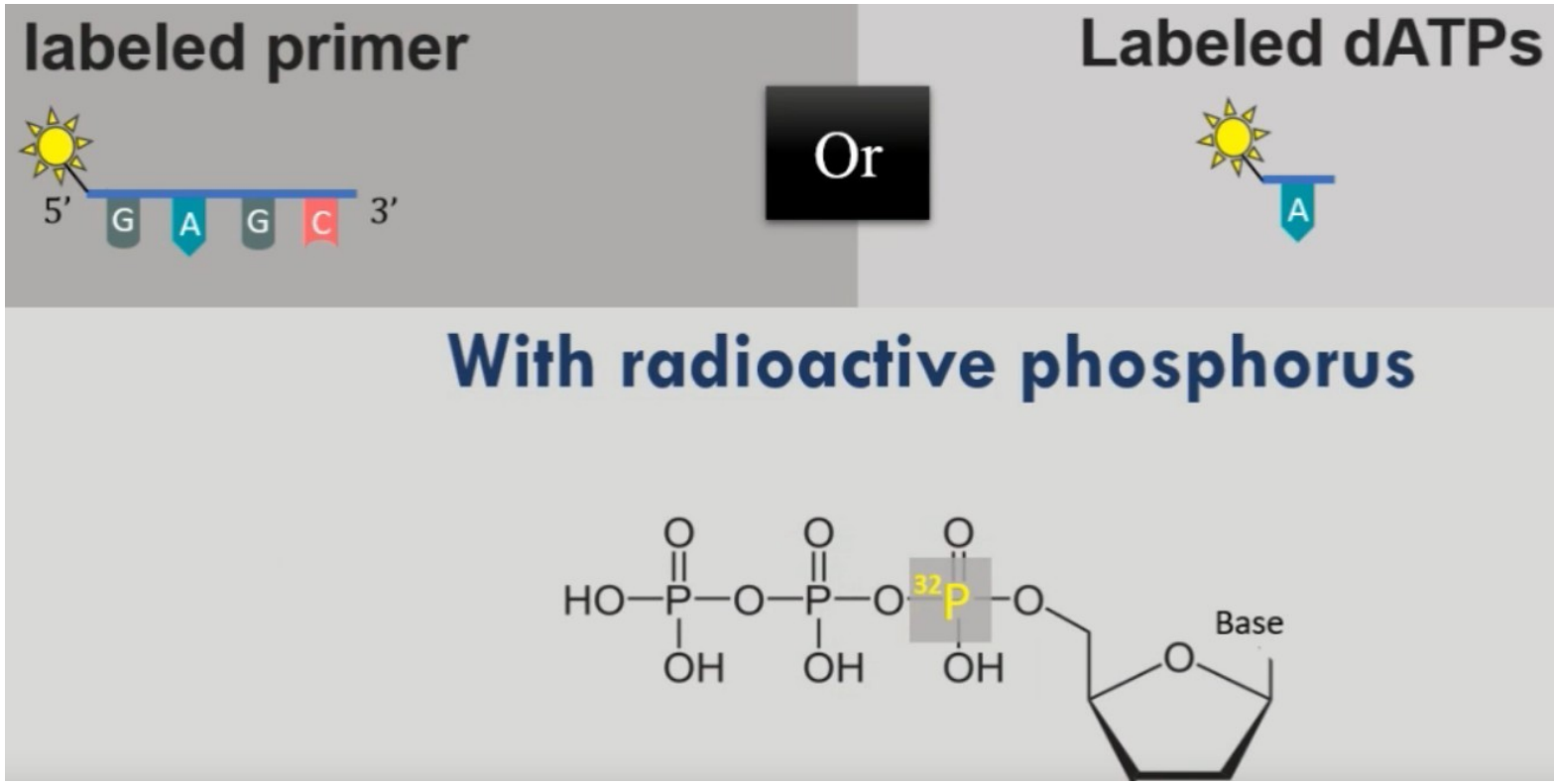


Figure: Before starting the reaction, the primer or one of dNTPs are labeled.

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# Sanger method

## DNA replication in each tube

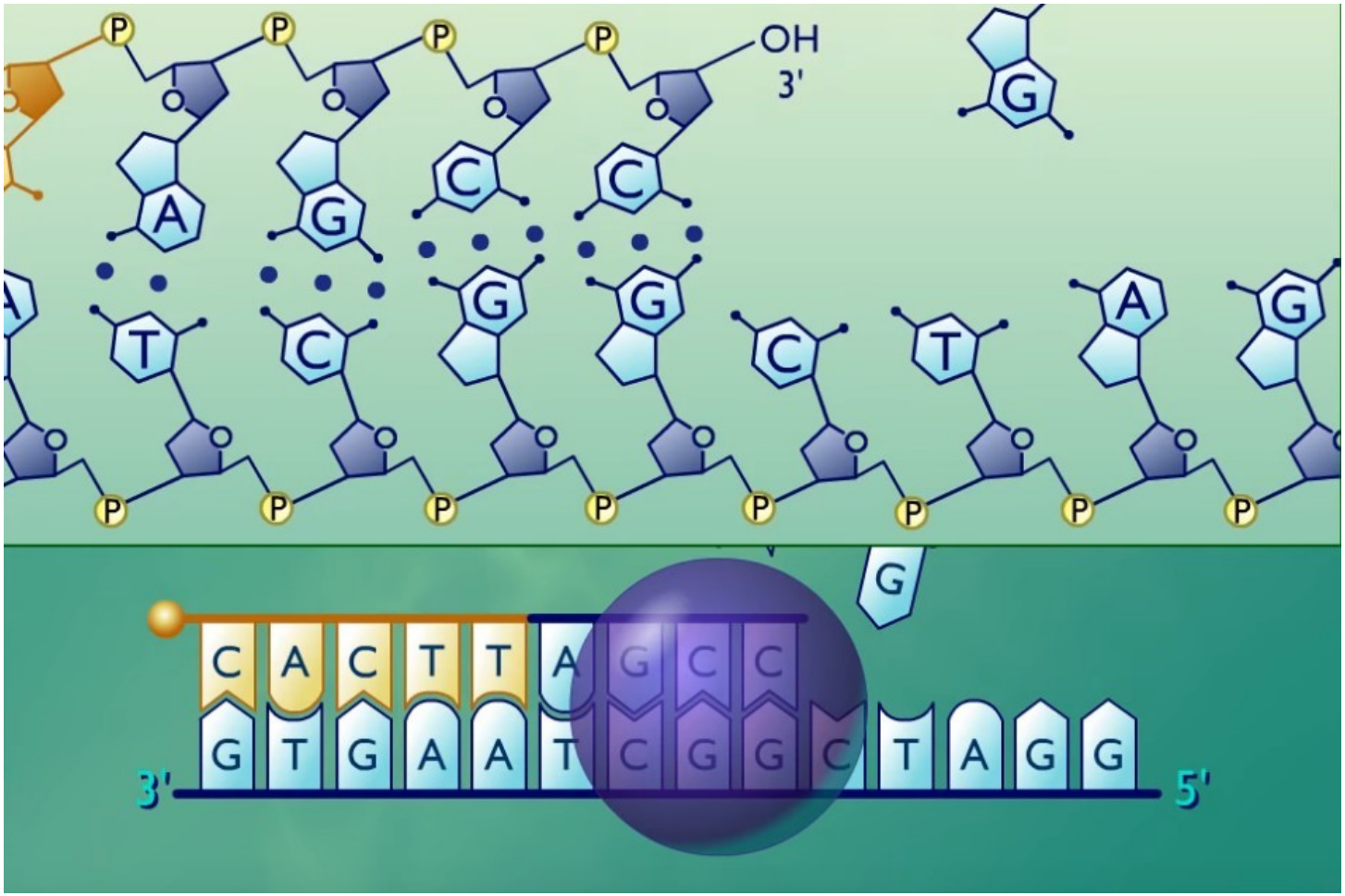


Figure: DNA replication occurs in each tube. The tubes contains the dNTPs.

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# Sanger method

## DNA replication stop when ddNTP arrives

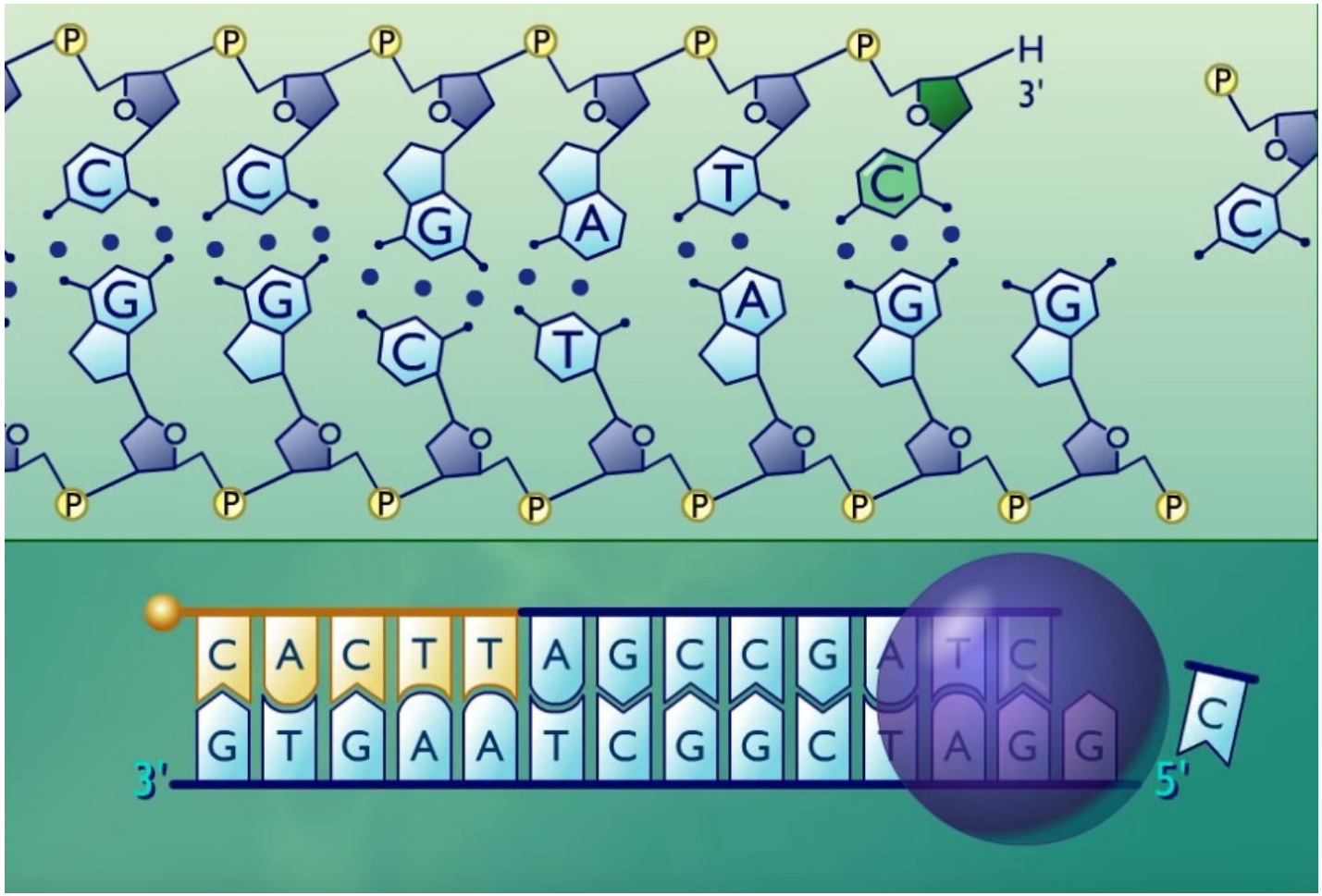


Figure: DNA replication stop when a ddNTP arrives.

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# Sanger method

## DNA replication starts again

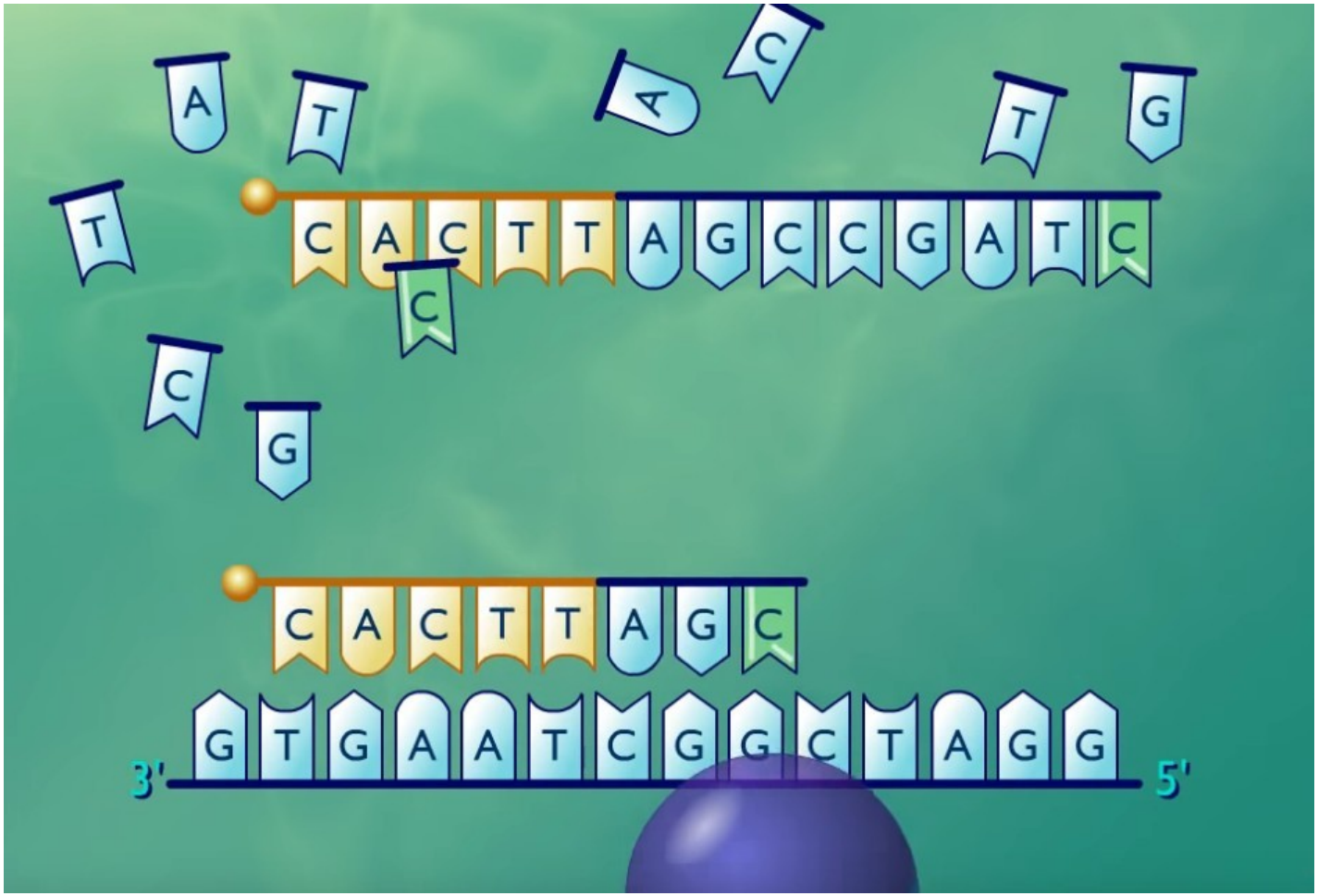


Figure: DNA replication starts again and different fragments length are obtained.

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# Sanger method

## DNA replication starts again

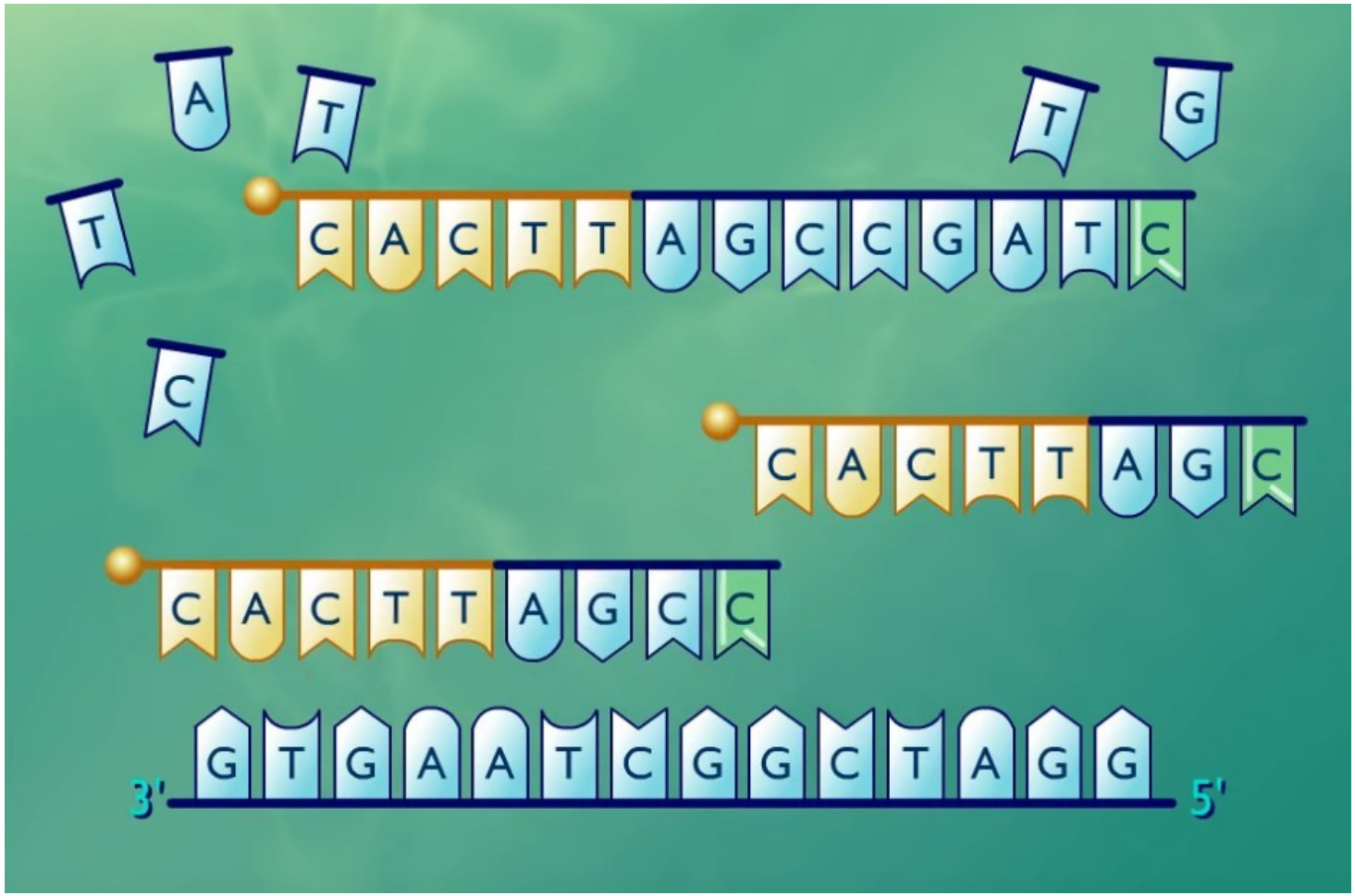


Figure: DNA replication starts again and different fragments are obtained.

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# Sanger method

## Each tube have fragments of DNA

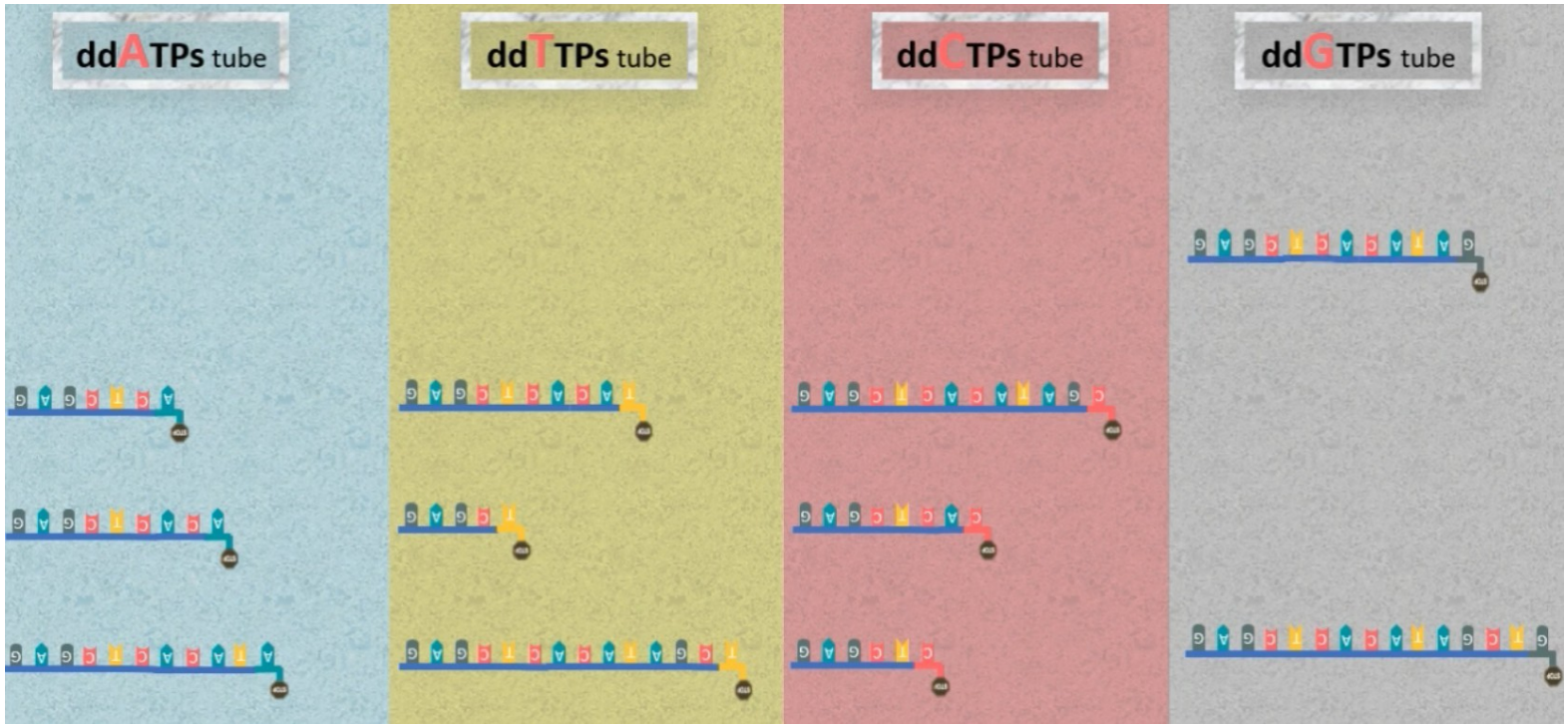


Figure: On each tube, there are DNA fragments, each one with different length.

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# Sanger method

## Gel electrophoresis

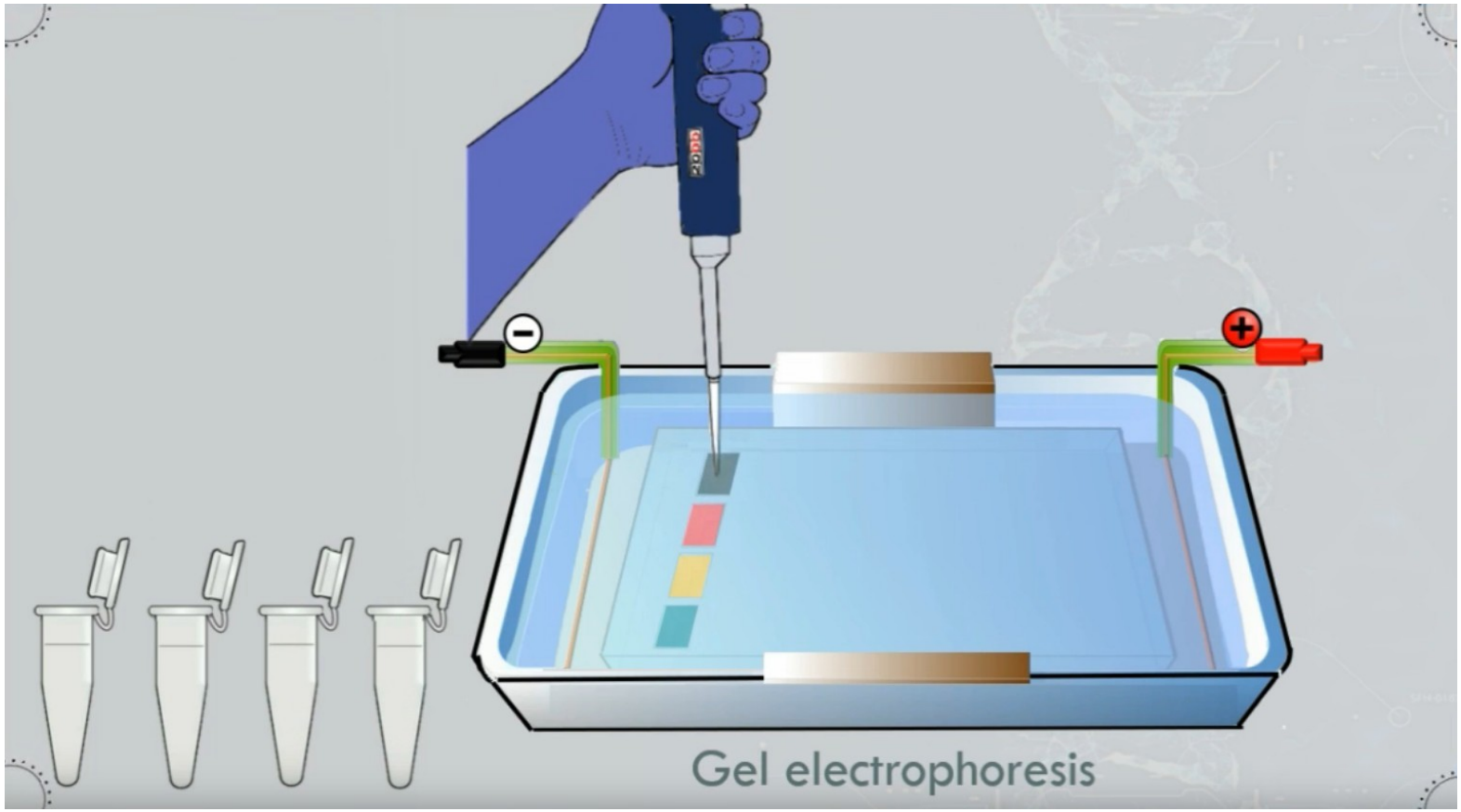


Figure: Gel electrophoresis. The negative charge of its phosphate backbone moves the DNA towards the positively charge anode.

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# Sanger method

## Gel electrophoresis

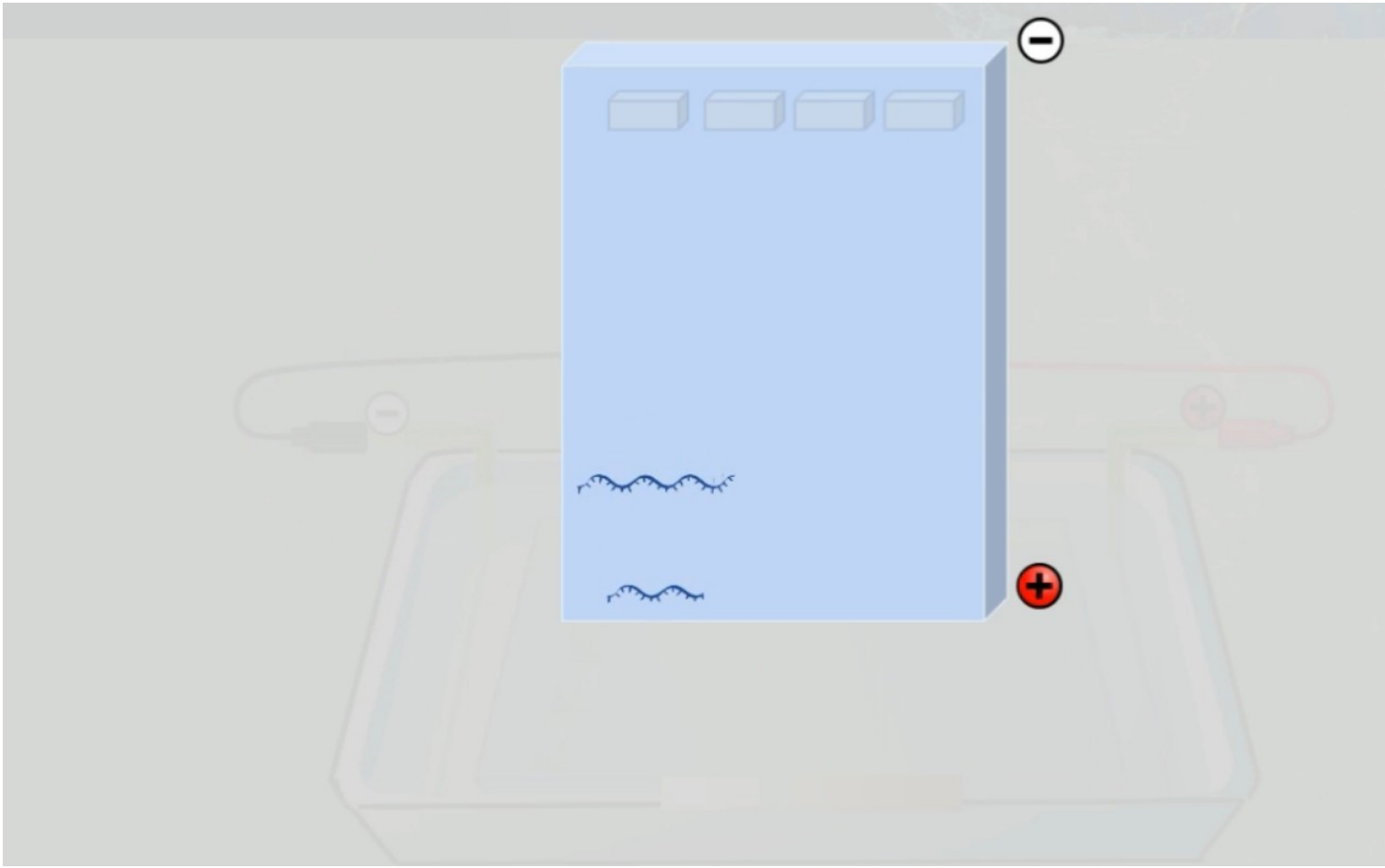

Figure: Shorter DNA molecules can travel farther than longer counterparts.

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# Sanger method

## Gel electrophoresis

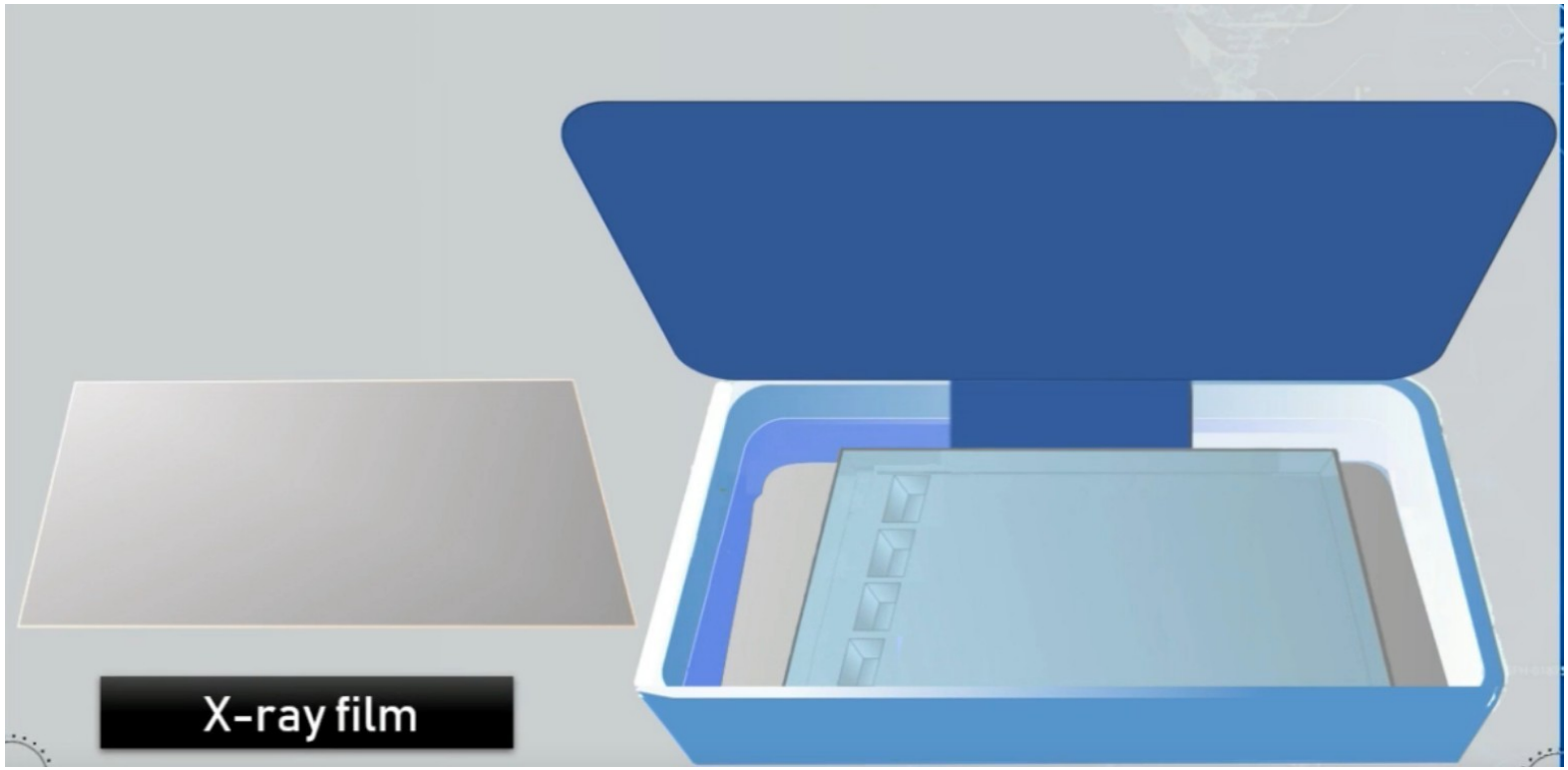


Figure: Tha DNA bands are visualized by autoradiography using the X-ray film.

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# Sanger method

## Gel electrophoresis

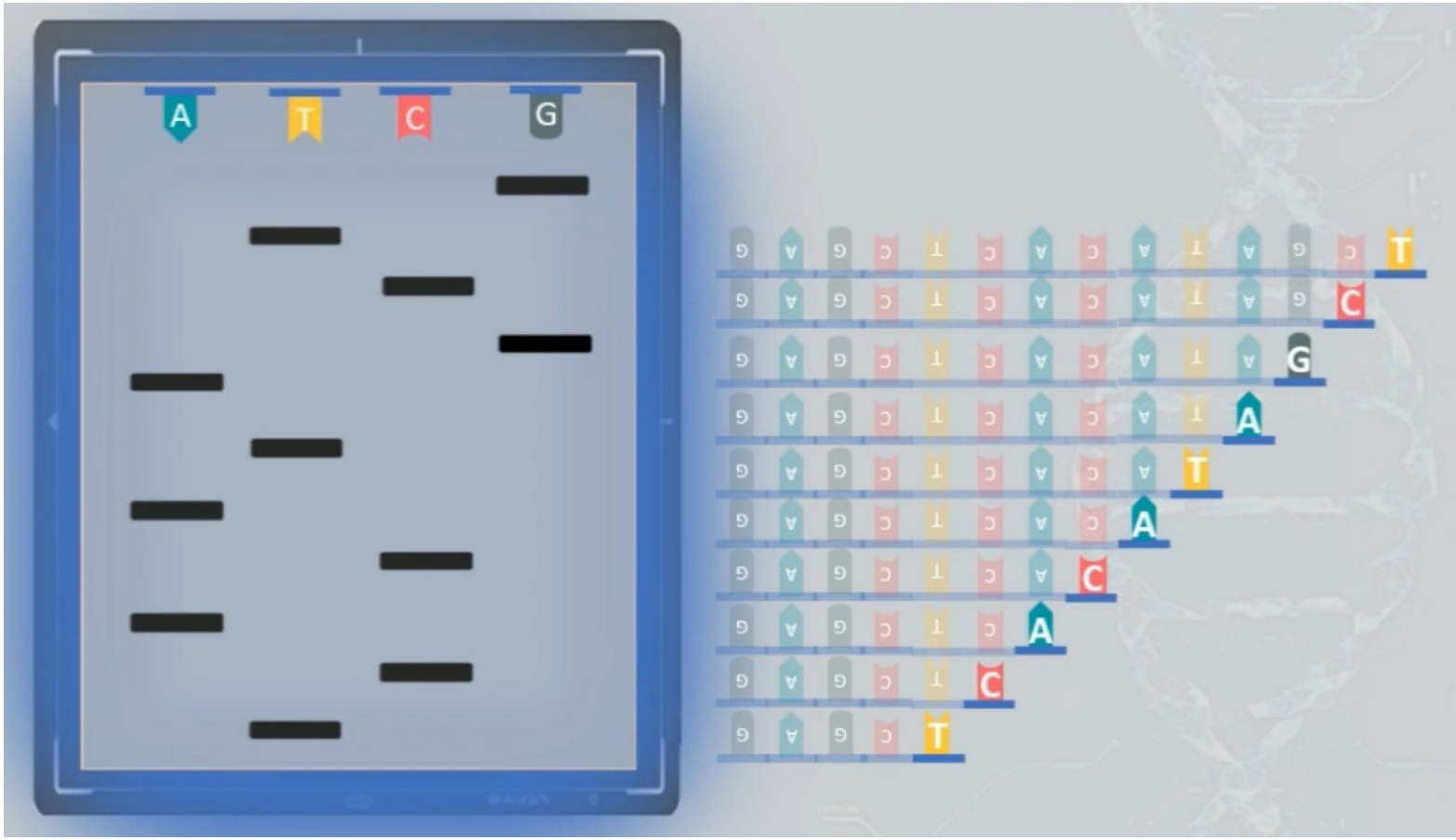


Figure: Tha DNA bands are visualized by autoradiography using the X-ray film.

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# Sanger method

## Gel Electrophoresis Animation

Gel Electrophoresis Animation.

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# Sanger method

## Capilar Electrophoresis

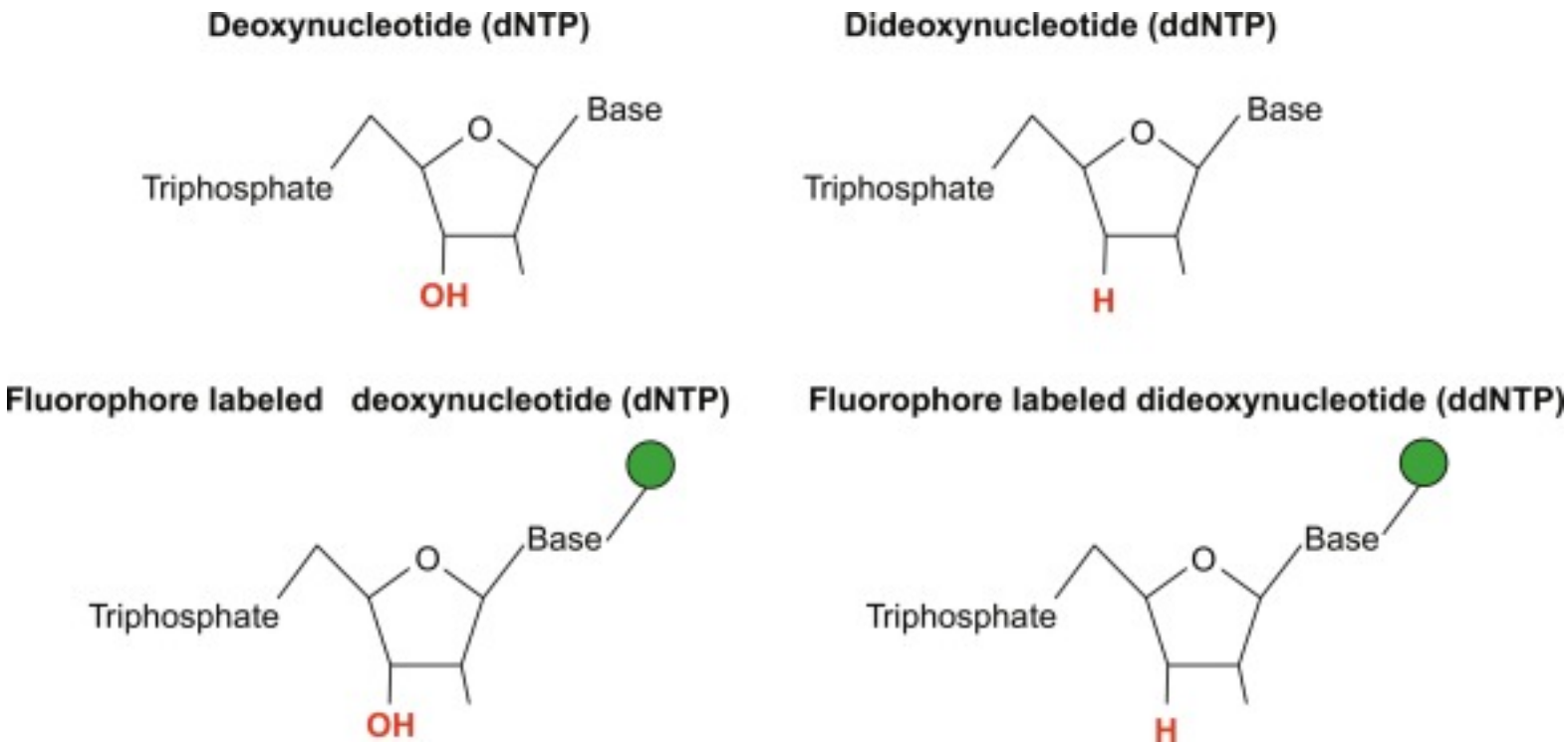


Figure: The ddNTPs are fluorophore labeled.

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# Sanger method

## Capilar Electrophoresis

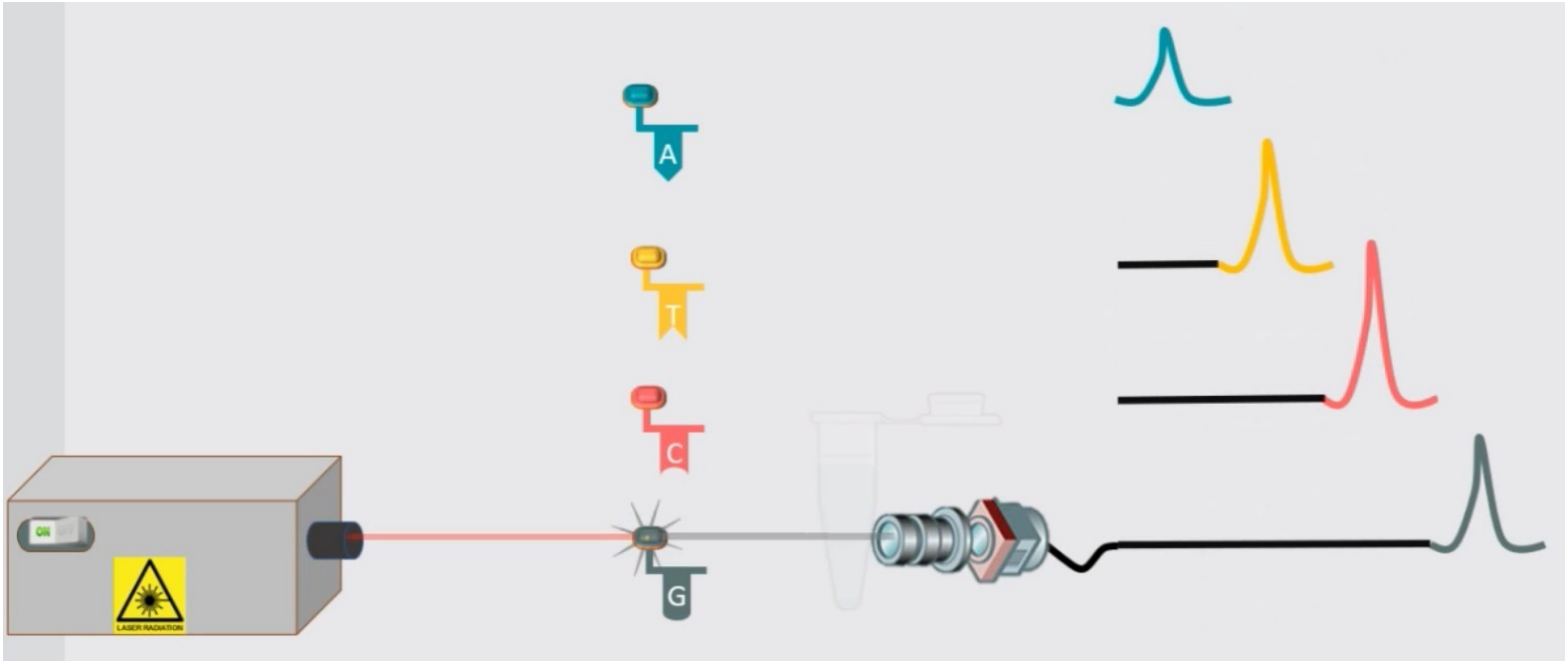


Figure: Each of the four ddNTPs chain terminator are labelled with fluorescent dyes.

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# Sanger method

## Capilar Electrophoresis

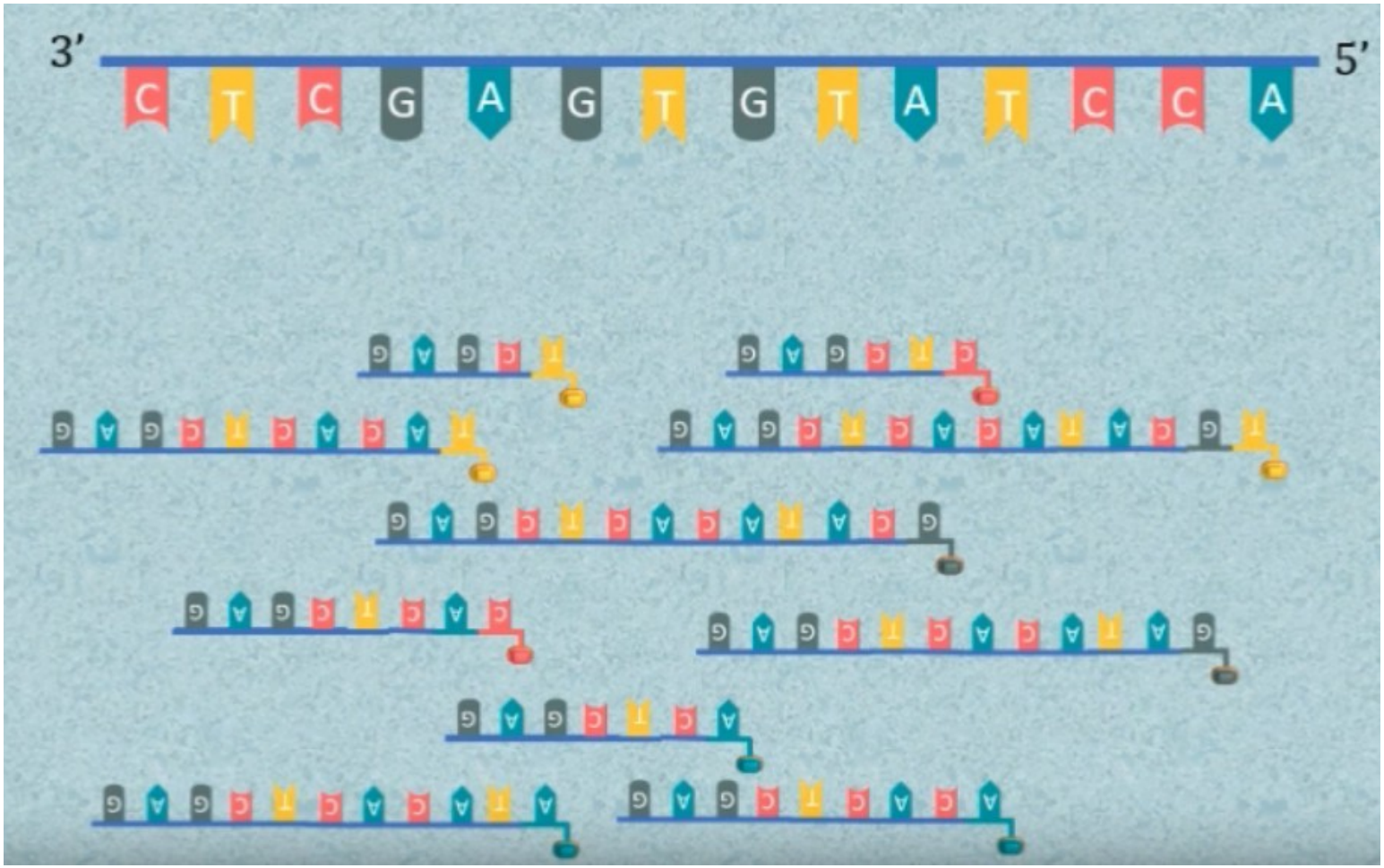


Figure: DNA replication occurs in one tube (Gel electrophoresis uses four).

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# Sanger method

## Capilar Electrophoresis

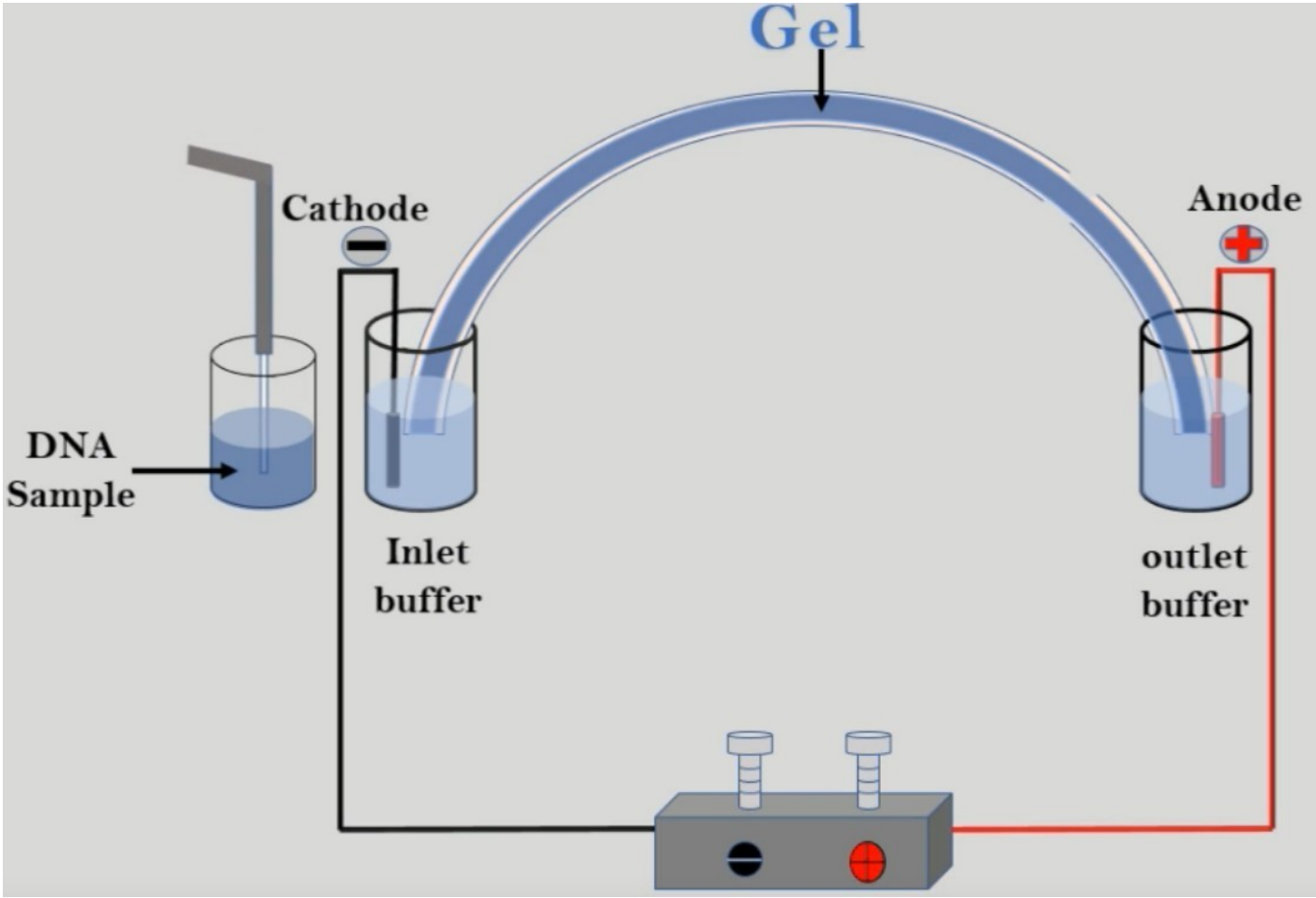


Figure: DNA fragments are placed in order move through a gel conductor.

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# Sanger method

## Capilar Electrophoresis

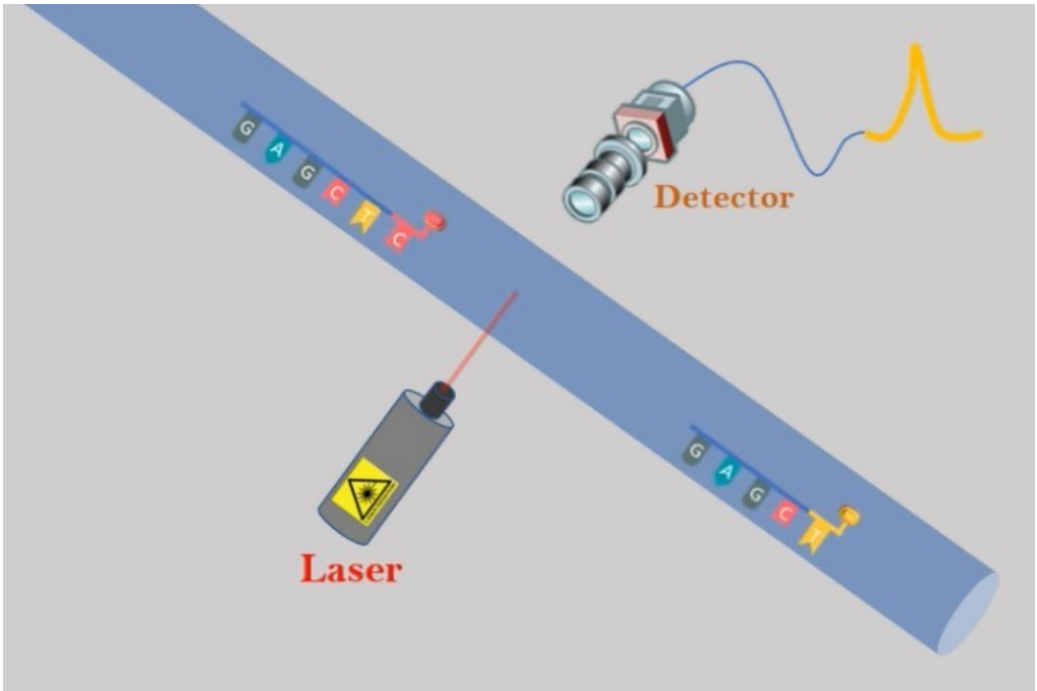


Figure: The fragment are charged and they move over a conductor. Smaller moves rapidly and they are read by a laser.

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# Sanger method

## Many samples read

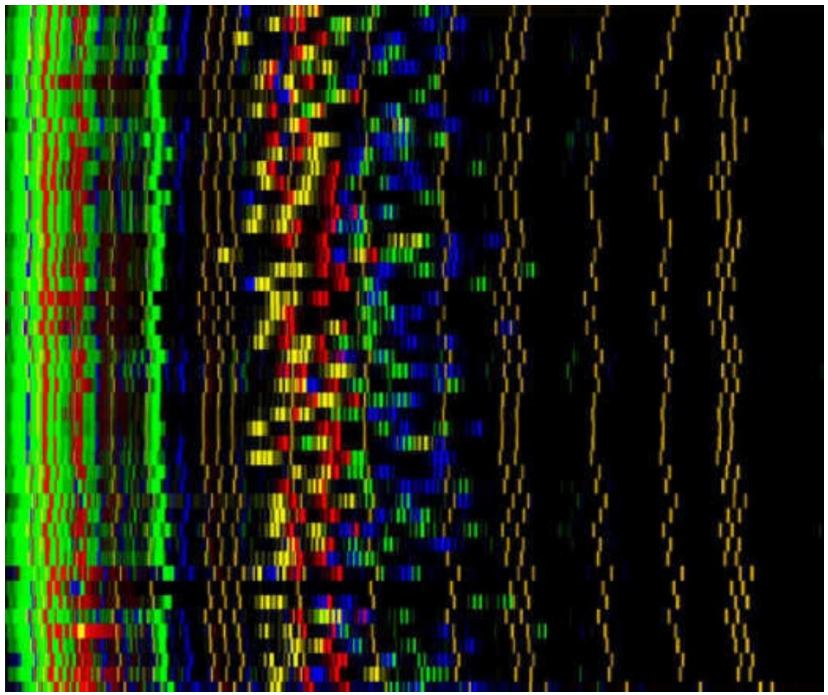

Figure: The process is repeated several times and many samples are obtained.

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# 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.

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