Manipulating genomes

- DNA profiling
- DNA sequencing and analysis
- Using DNA sequencing
- Genetic engineering
- Gene technology and ethics

DNA Sequencing and Analysis 07 January 2021

Specification points: 6.1.3 Manipulating genomes

(a) the principles of DNA sequencing and the development of new DNA sequencing techniques

(b) (i) how gene sequencing has allowed for genome-wide comparisons between individuals and between species

(ii) how gene sequencing has allowed for the sequences of amino acids in polypeptides to be predicted

(iii) how gene sequencing has allowed for the development of synthetic biology.

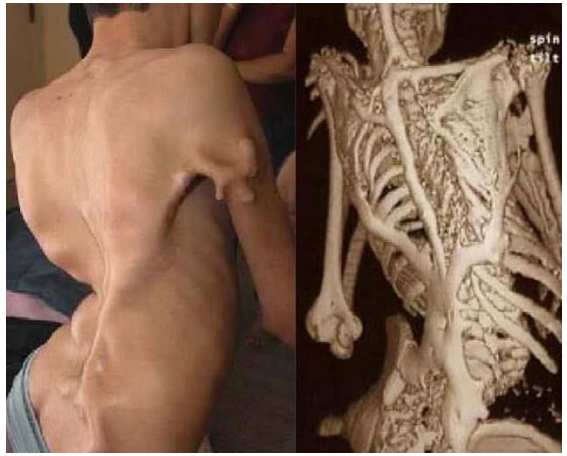
Uses of DNA sequencing

The methods we have looked at so far can compare 2 sets of DNA to see whether they are likely to come from the same source.

However, we may want to know the exact sequence of bases – why / when might this be useful? Discuss....

Uses of DNA sequencing

Clinical applications e.g. Identifying gene mutations involved in hereditary disorders.



Improved drug trialling and pharmacogenomics – personalised drugs.

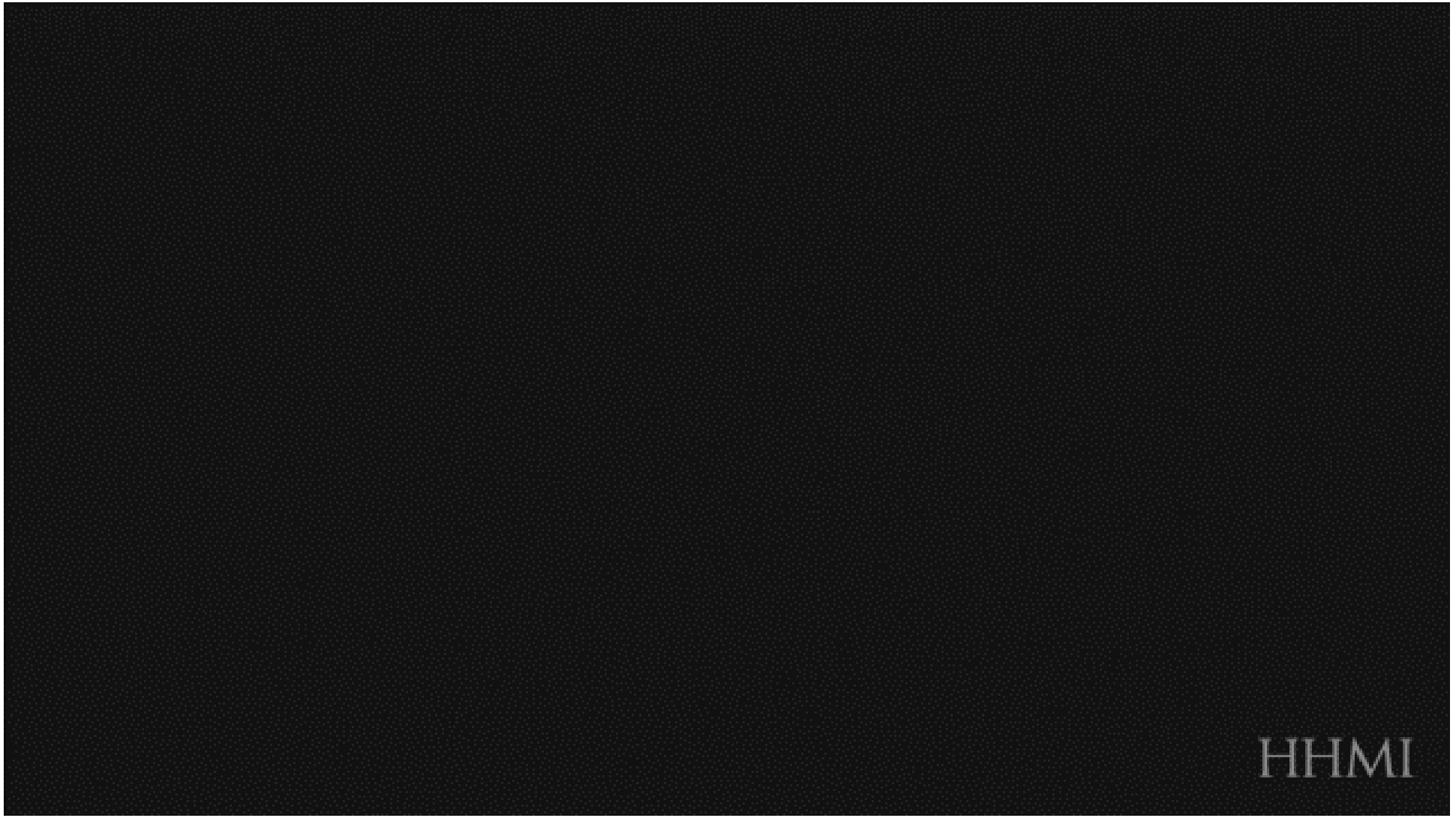
Epidemiology – tracking the spread of viruses during an outbreak.

Wildlife conservation – tracking poaching, reproductive health, biodiversity.

Evolutionary relationships – convergent and divergent evolution.

Biotechnology applications – genes of useful bacterial for fuel production.

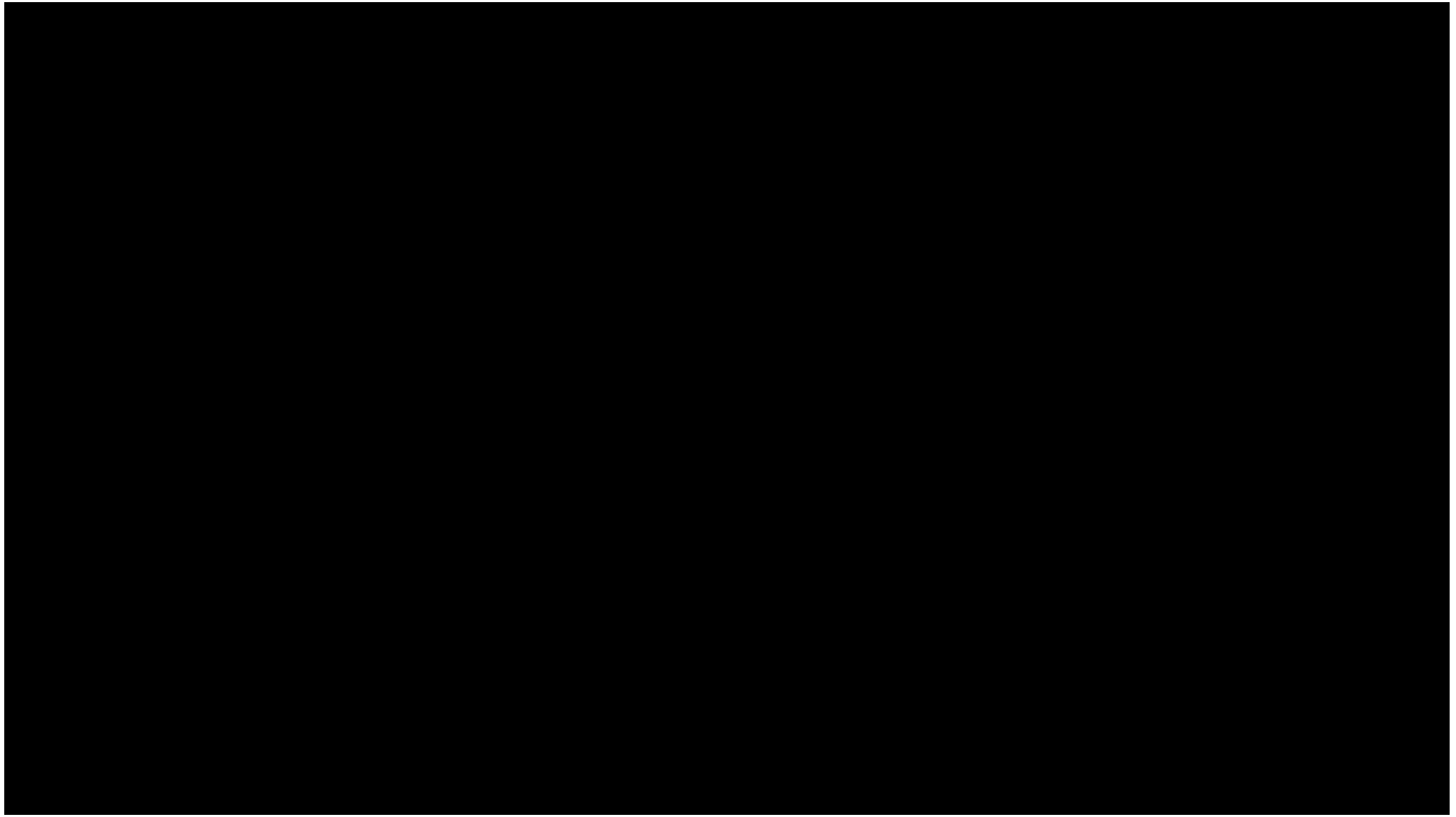
Human Genome Project



https://www.biointeractive.org/classroom-resources/human-genome-sequencing

Into

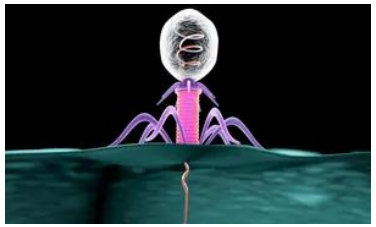
Lessons from the Human Genome Project



https://www.youtube.com/watch?v=qOW5e4BgEa4&t=227s

Brief history of DNA sequencing

- Sanger (1977) developed "DNA sequencing with chain-terminating inhibitors" (first generation sequencing).
- 1st full genome of a bacteriophage 1977.
- Epstein-Barr Virus 172,282 nucleotide sequence published 1984.
- First fully automated sequencer 1987/1988.
- 1990 – Human genome project established.
- Use of florescent labelling of chain-terminating nucleotides 2000 (replaced radioactive labels).
- 2000 shotgun sequencing and advances in computer power leads to a draft human genome (completed 2003).



DNA sequencing – quick check p557

- DNA profiling looks at non-coding sections of DNA and compares the length of repeated sections. But DNA sequencing is different – it is the process of determining the order of _____ within a DNA molecule.
- Frederick Sanger invented a method for DNA sequencing - _____ sequencing in the 1970s.
- Early work sequenced the genomes of _____, bacteria and nematode worms.
- The _____ _____ project sequenced the human genome in 2003.

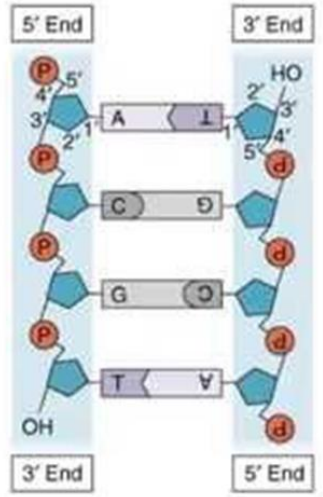
DNA sequencing – quick check p557

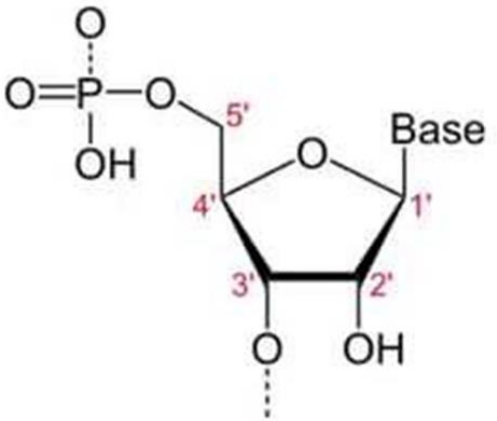
- DNA profiling looks at non-coding sections of DNA and compares the length of repeated sections. But DNA sequencing is different – it is the process of determining the order of **bases** within a DNA molecule.
- Frederick Sanger invented a method for DNA sequencing - **Sanger** sequencing in the 1970s.
- Early work sequenced the genomes of **viruses**, bacteria and nematode worms.
- The **Human Genome** project sequenced the human genome in 2003.

How does sequencing work?

Sequencing relies on some of the unique features of DNA:

- antiparallel strands
- strands have 3′ end and 5′ end (the carbon numbers in the backbone) – C5 has the phosphate, C3 has a hydroxyl group
- only growing from 3′ end
- complementary base pairing





5′ AND 3′ ENDS OF NUCLEIC ACIDS

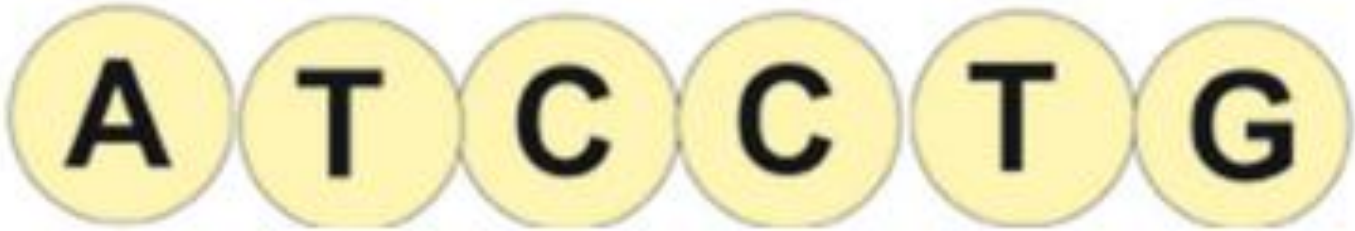
How does Sanger sequencing work?

Sanger sequencing (the earliest form of DNA sequencing) involved combining the 3 techniques which were already used:

- gel electrophoresis to separate out fragments of DNA of different lengths
- fluorescent tags / labels which could highlight a particular base
- PCR to make multiple identical copies of a DNA strand

How does Sanger sequencing work?

Sanger sequencing reads the code **one base at a time**, much as you can read the letters of a sentence – quickly and in the right order.



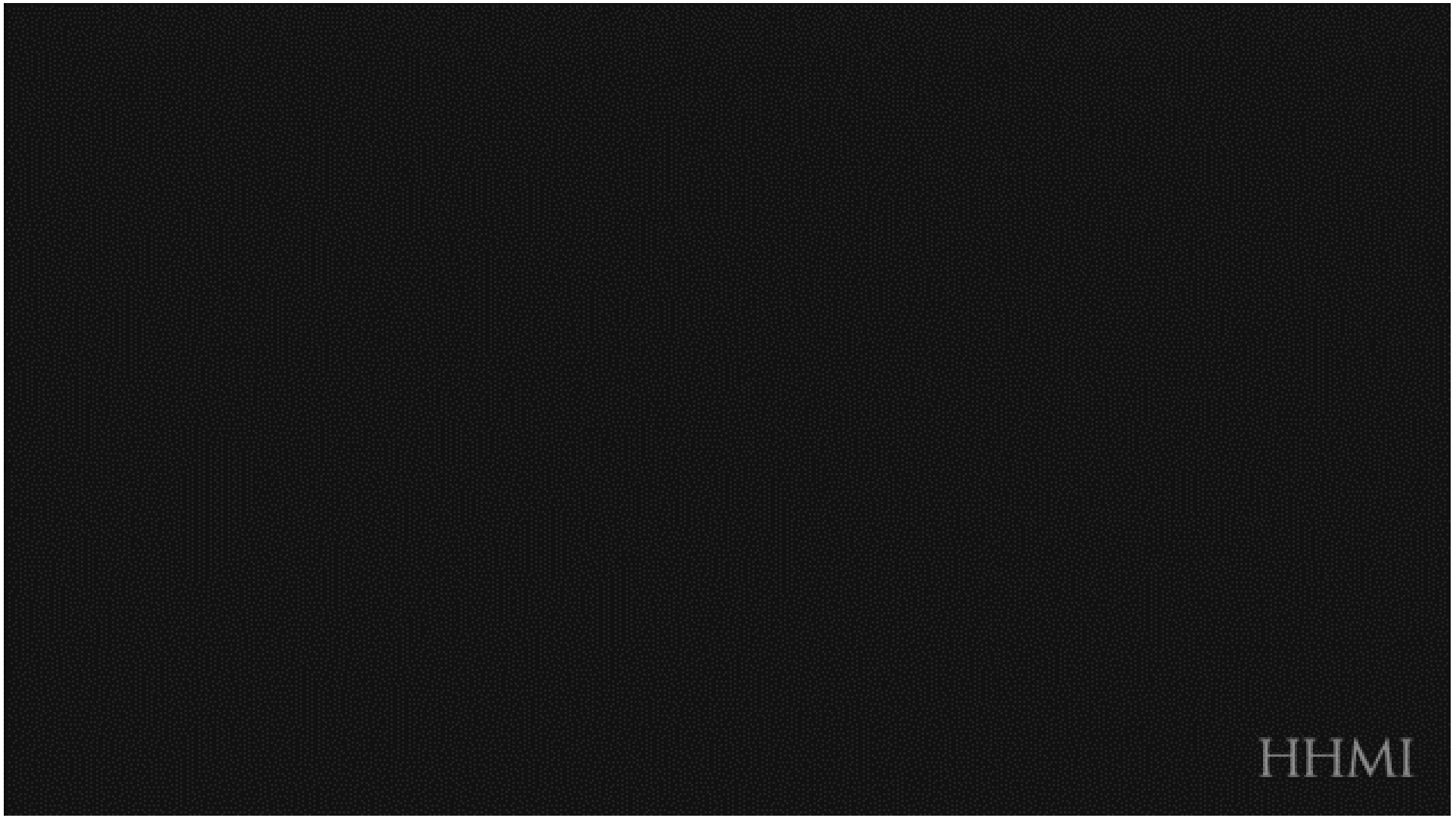
So how do we pick out base so we know whether they are A, T, C or G? at a time so that we can "read" it?

We make them fluoresce different colours for different bases.

Won't that be a mess of colour if they are all glowing?

Sanger sequencing solved this by just making one base glow at a time and in order.

How does Sanger sequencing work?



https://www.youtube.com/watch?v=H0z8CSokkjU&feature=emb_logo

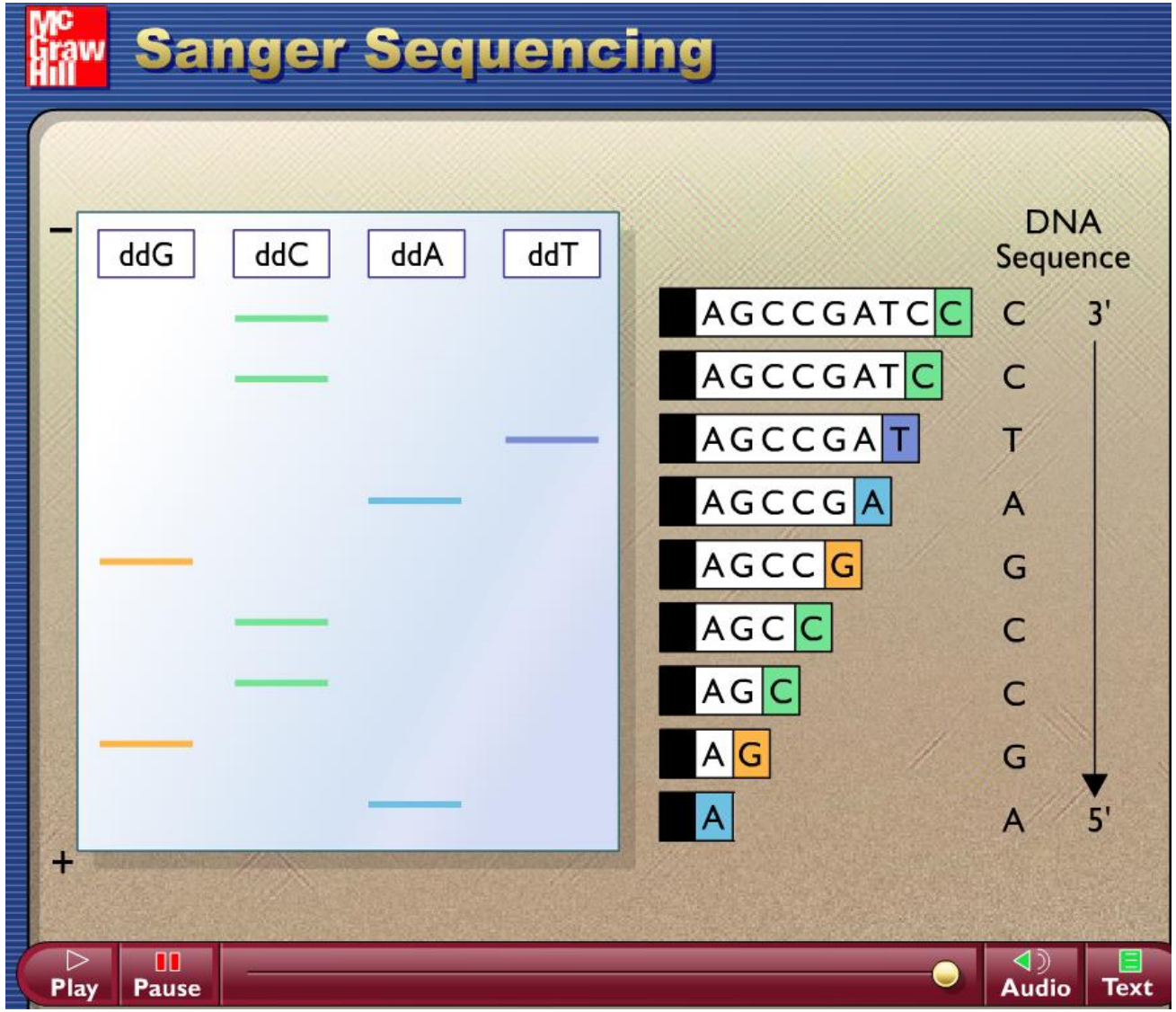
How does Sanger sequencing work?

Two types of nucleotides are involved;

- **Normal nucleotides dNTPs** – in the A, C, T and G versions which allow new DNA strands to be built.

- **Modified nucleotides - ddNTPs** - dideoxynucleotides (ddGTP, ddATP, ddTTP and ddCTP). These have **terminator bases** which stop the strand being built at specific bases – either at A, C, G or T.

Sanger sequencing with gel elctrophoresis

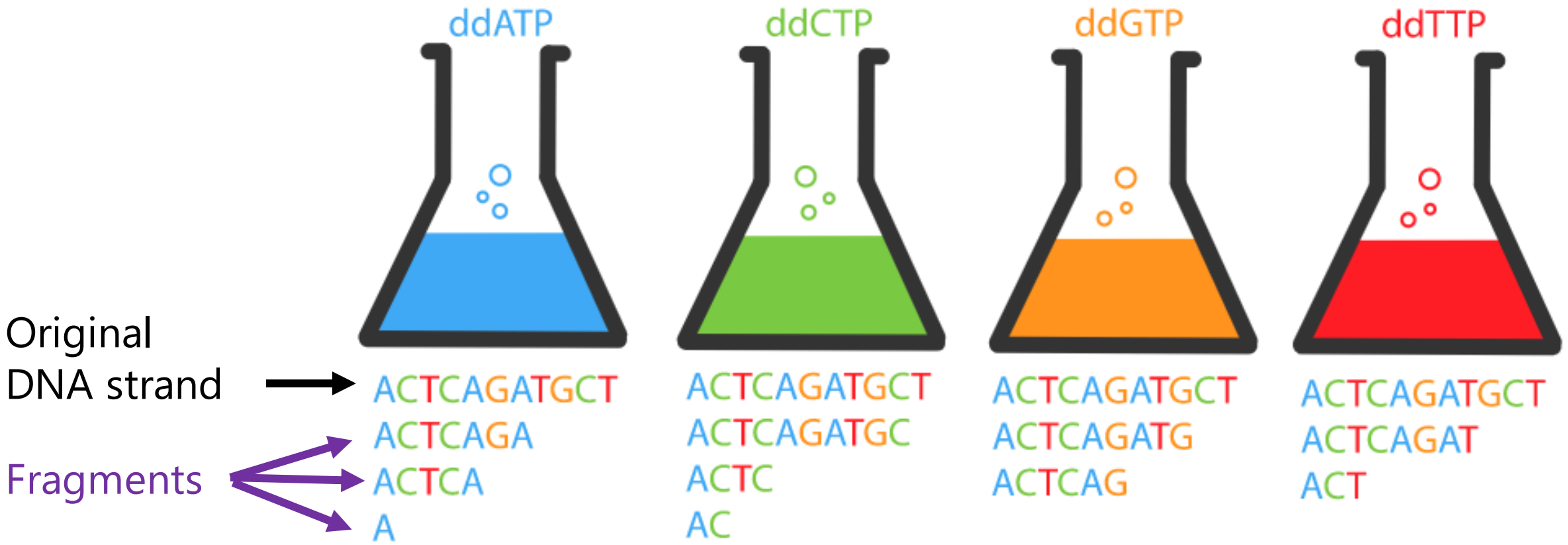


This animation shows how the DNA can be cut into fragments of different lengths using the terminator bases and then separated using gel electrophoresis.

Watch out for how the terminator bases stop the rest of the strand being copied.

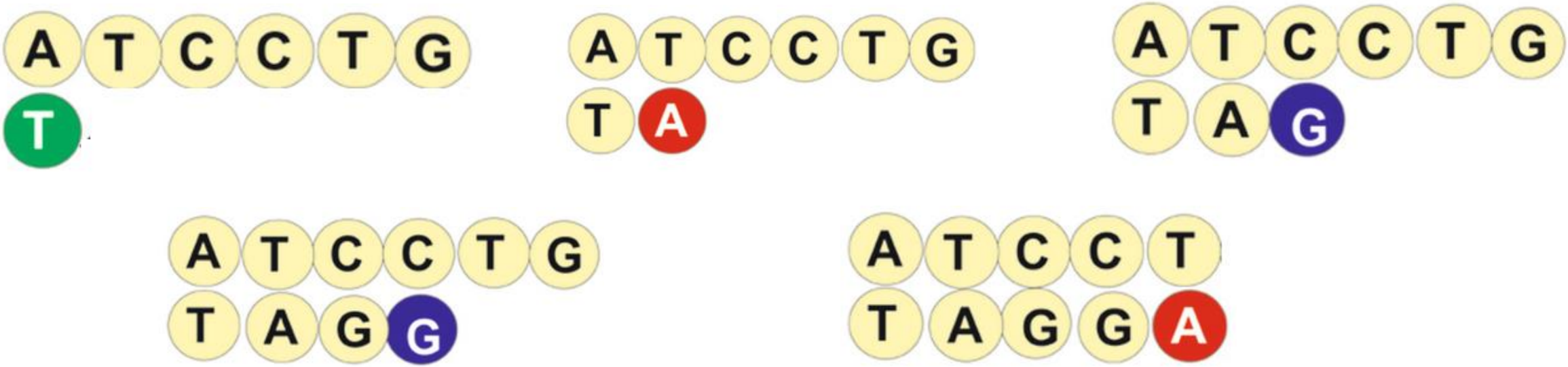
https://highered.mheducation.com/sites/9834092339/student_view0/chapter18/sanger_sequencing.html

How does Sanger sequencing work?



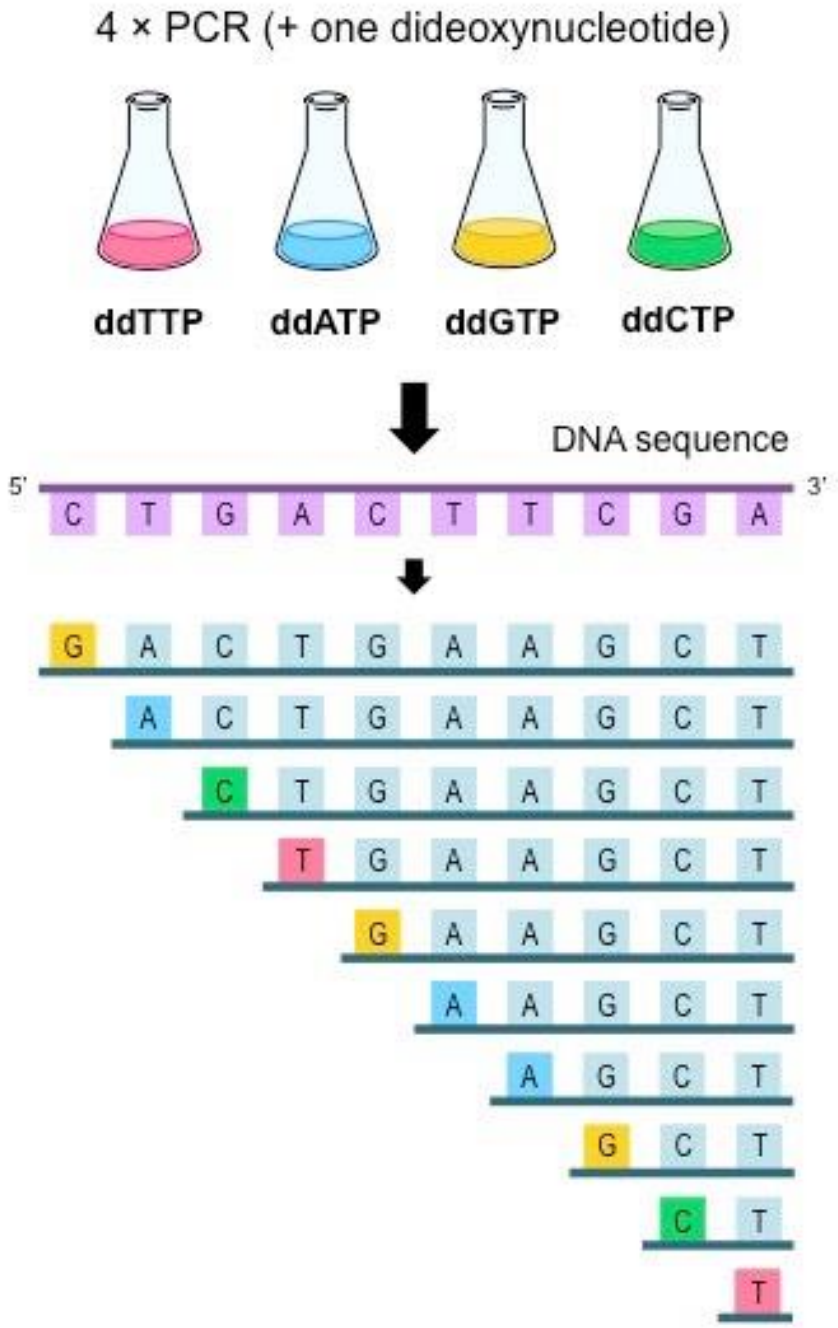
This diagram shows all the fragments you would get for the 4 terminator base mixtures. You repeat the copying multiple times to get all the possible lengths from one base to thousands (and all the lengths in between).

How does Sanger sequencing work?



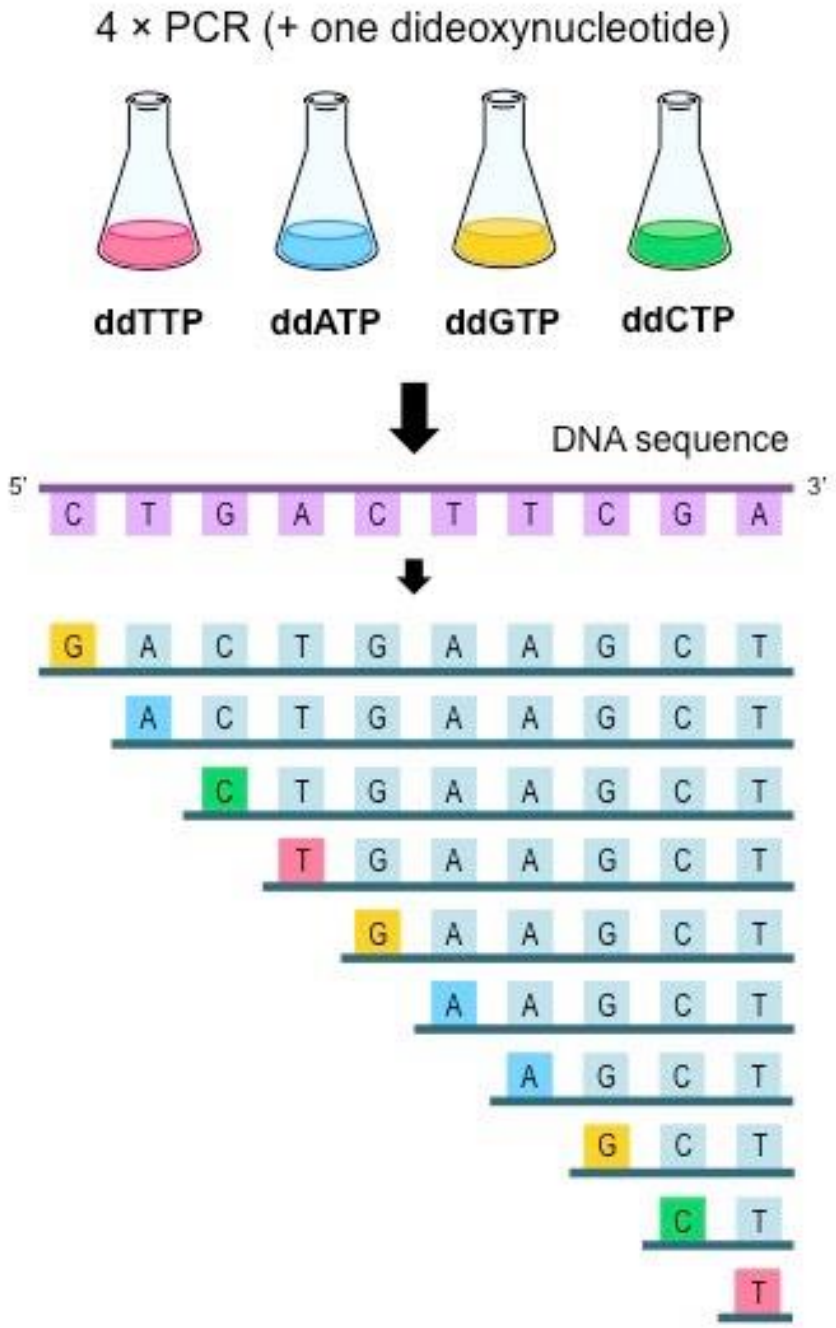
To read the fluorescent tags in order, electrophoresis is used.
The shortest piece of DNA travels to the end of the gel fastest.
A laser reads the colour of the fluorescent base.

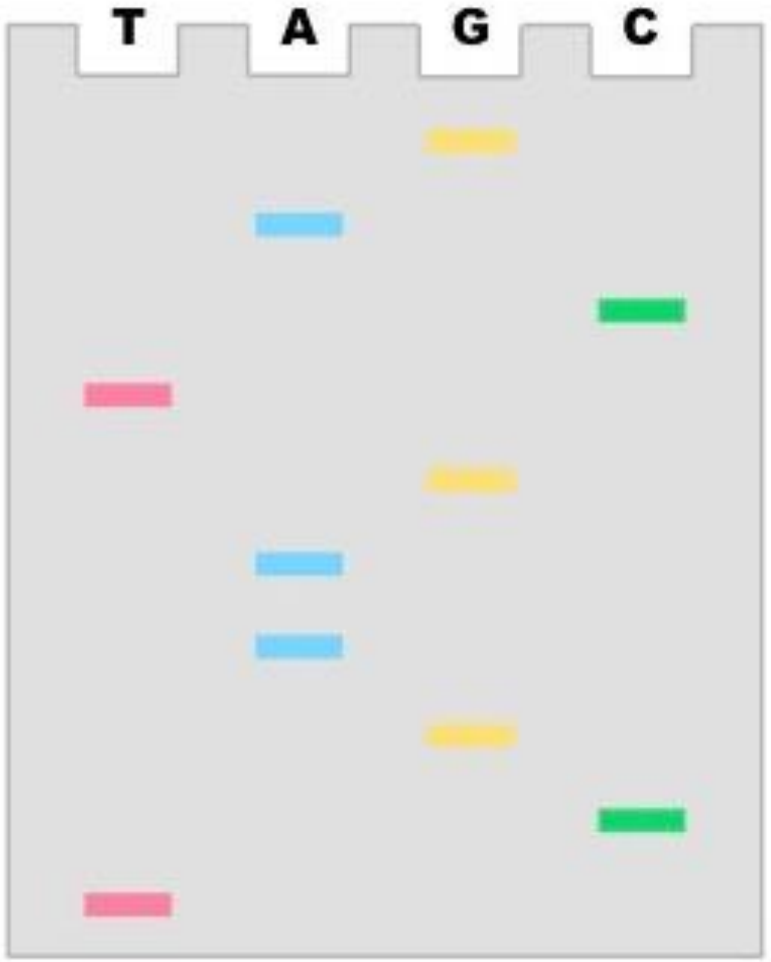
Gel plate method

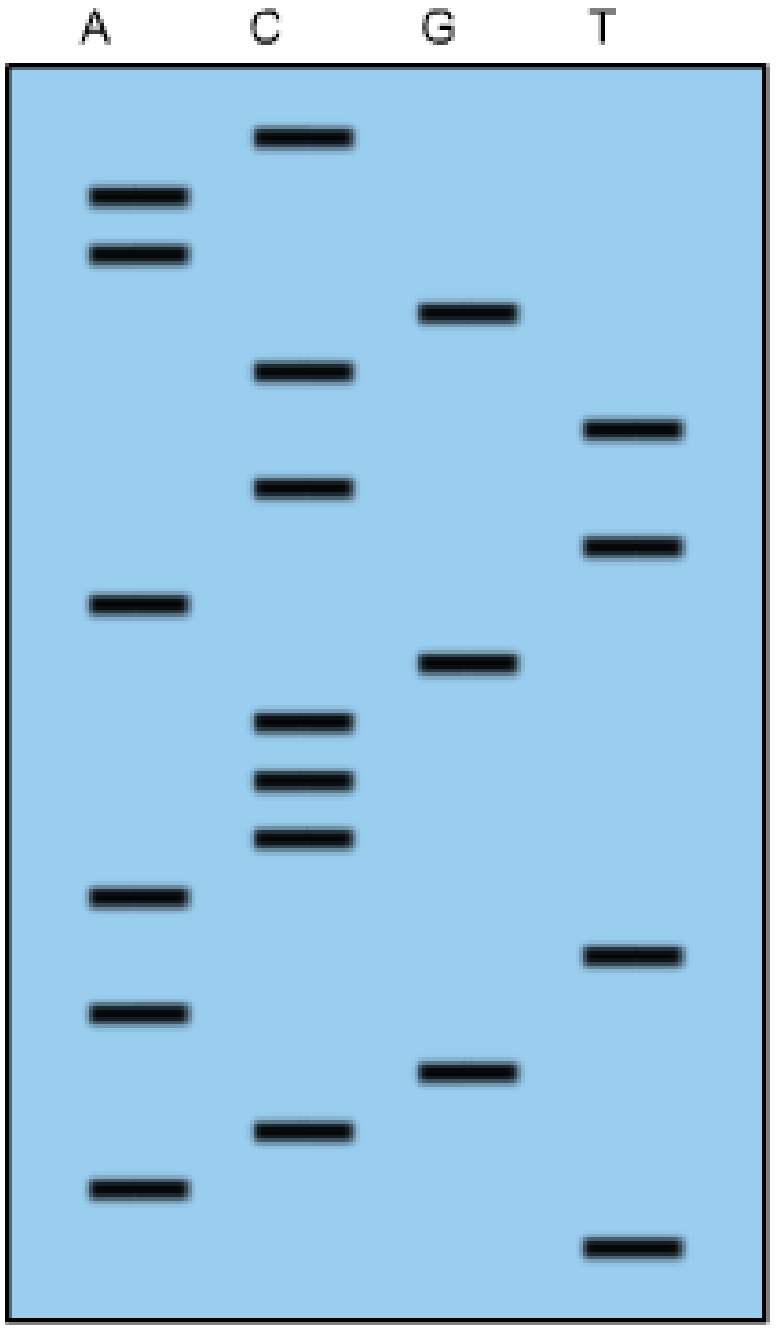


What would the gel plate look like for this sample?

Gel plate method







Gel plate method

Deduce the base sequence order for this DNA sequencing gel.

T A C G A T A C C C G A T C T C G A A C

Sanger Sequencing – copy and complete

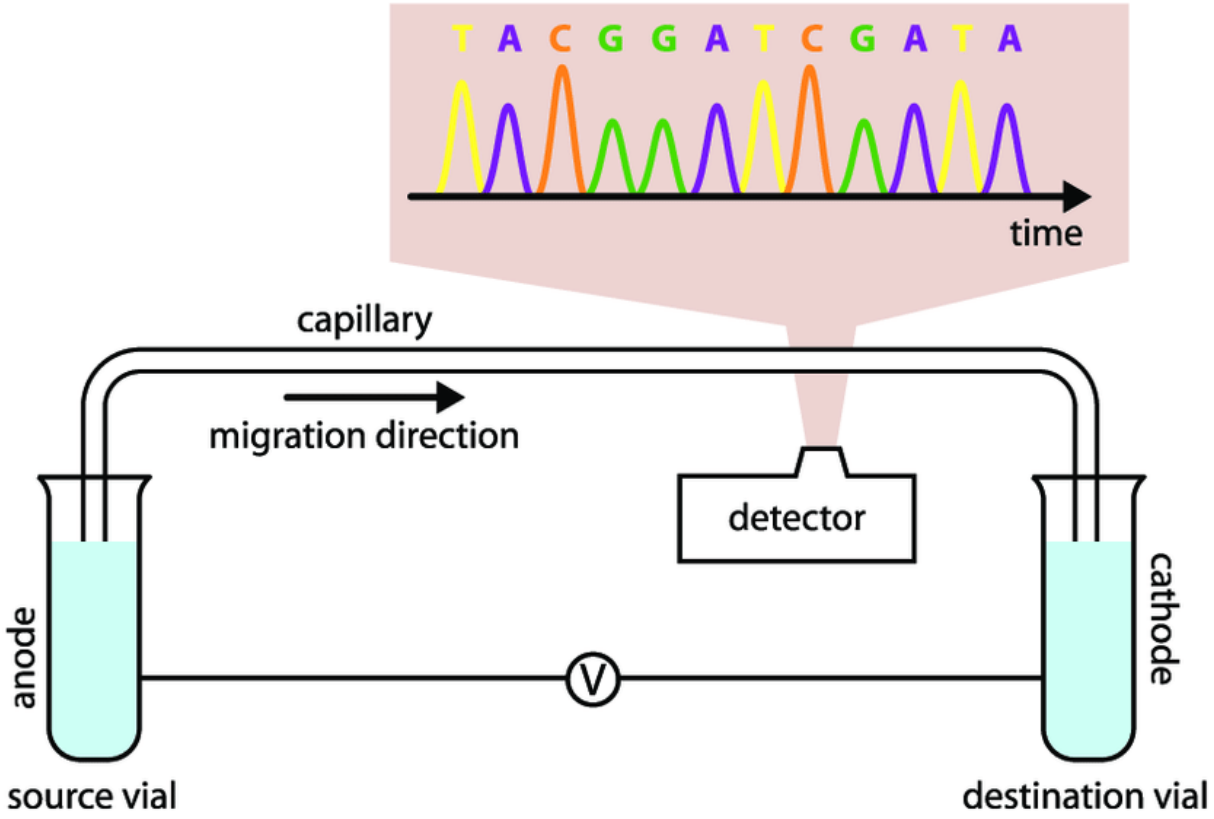
- DNA is mixed with; _____, DNA polymerase, normal nucleotides, fluorescent terminator bases / terminator / chain terminating nucleotides.
- DNA strand heated to _____ , unwinds.
- Cool to 50°C, primers attach.
- 60°C _____ builds new strand.
- When a terminator base is reached, no further _____ can be added due to an ___ rather than OH on the 3rd carbon.
- Different length _____ are produced.
- As PCR can produce 1 billion molecules we should get all the possible fragments for a DNA sample.

Sanger Sequencing

- DNA is mixed with; **primer**, DNA polymerase, normal nucleotides, fluorescent terminator bases / terminator / chain terminating nucleotides.
- DNA strand heated to **95°C**, unwinds.
- Cool to 50°C, primers attach.
- 60°C **DNA polymerase** builds new strand.
- When a terminator base is reached, no further **bases** can be added due to an **H** rather than OH on the 3rd carbon. .
- Different length **fragments / oligonucleotides** are produced.
- As PCR can produce 1 billion molecules we should get all the possible fragments for a DNA sample.

Sanger Sequencing – capillary method

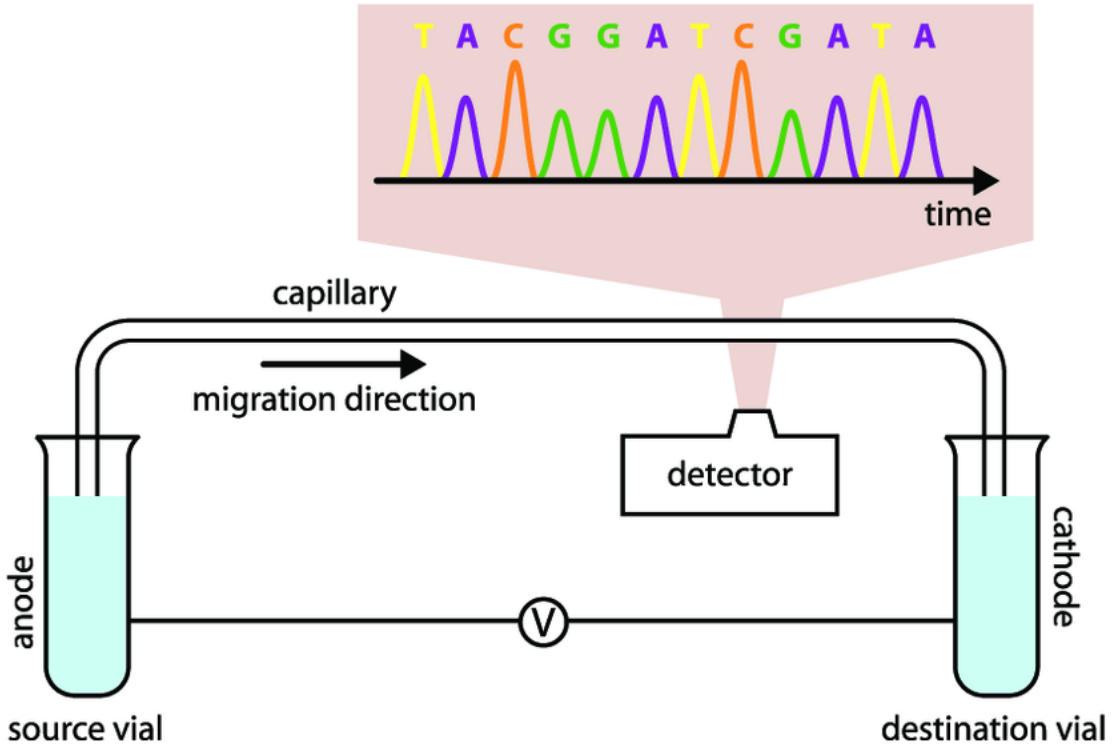
In 1981 a different method was proposed to separate the fragments more rapidly than the slab gel plate method.



The fluorescent fragments are placed in a source via. Gel runs from the source vial to a destination vial via a narrow capillary tube. A potential difference is applied between the two vials.

Sanger Sequencing – capillary method

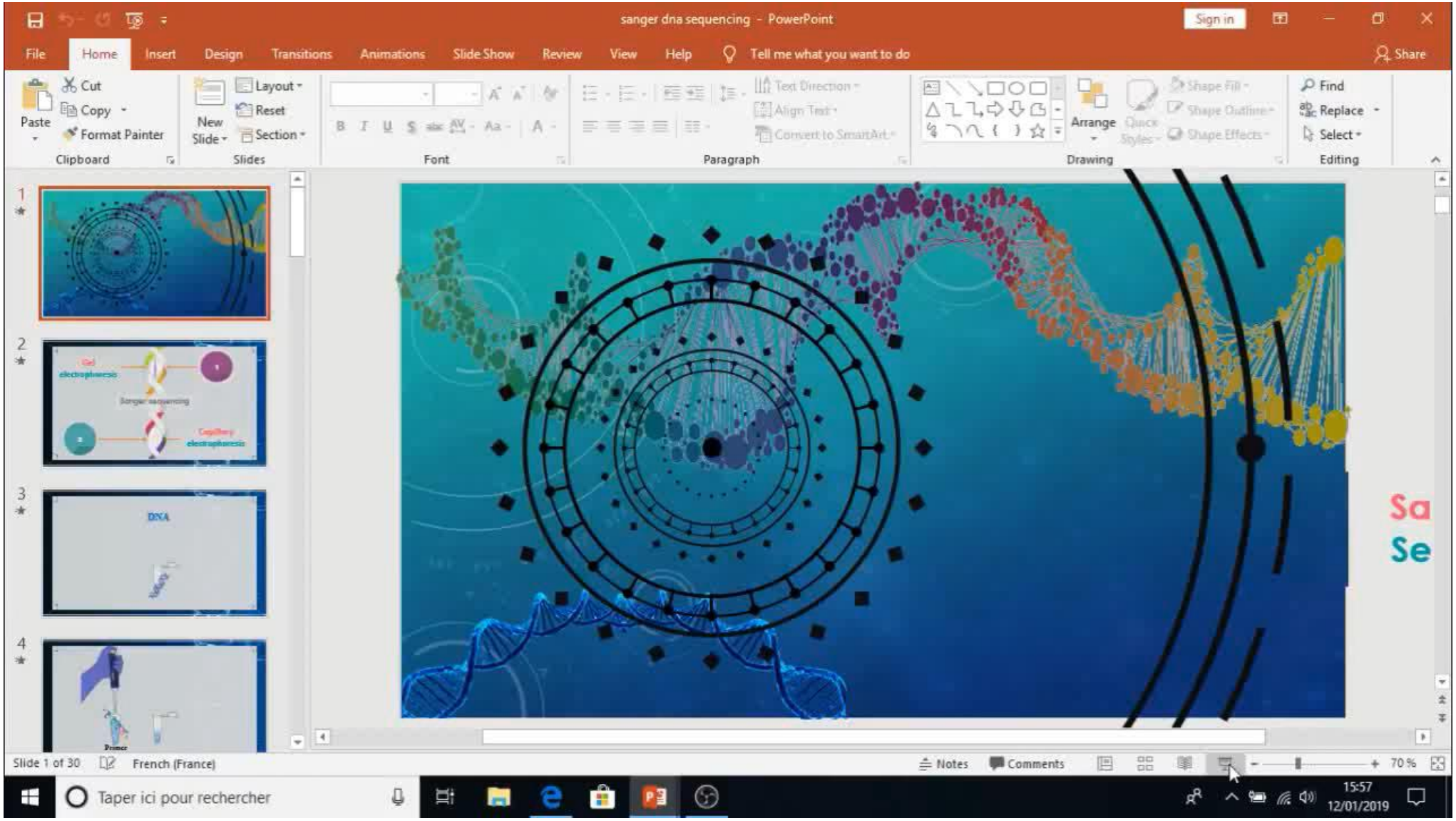
The fragments move from the source to the destination vial through the capillary tube continuously. As before, shorter fragments move fastest and reach the end of the capillary tube quickest.



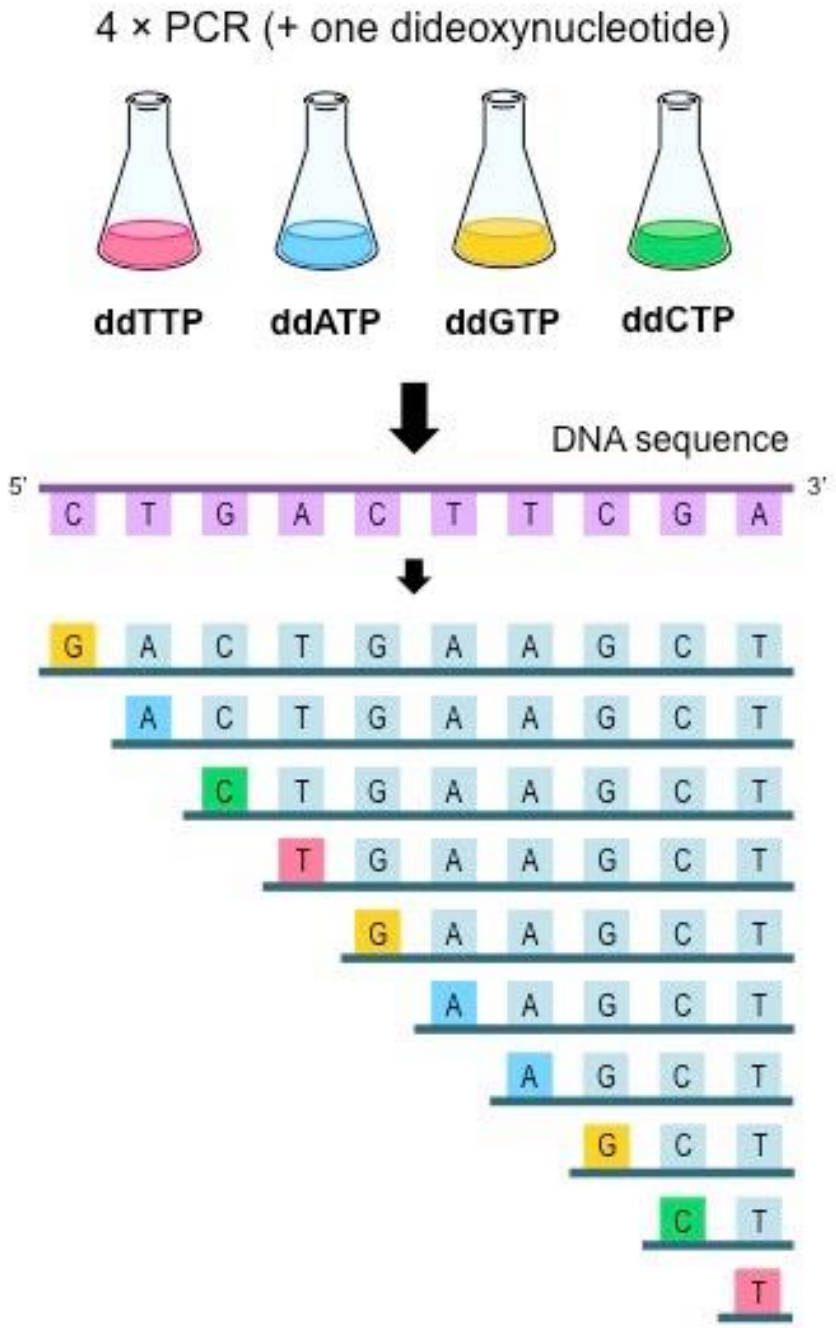
As they pass through the tube, a laser detector identifies the terminator base.

The detector picks up the order of the bases coming through from smallest to largest fragment and records it.

Sanger sequencing – capillary electrophoresis

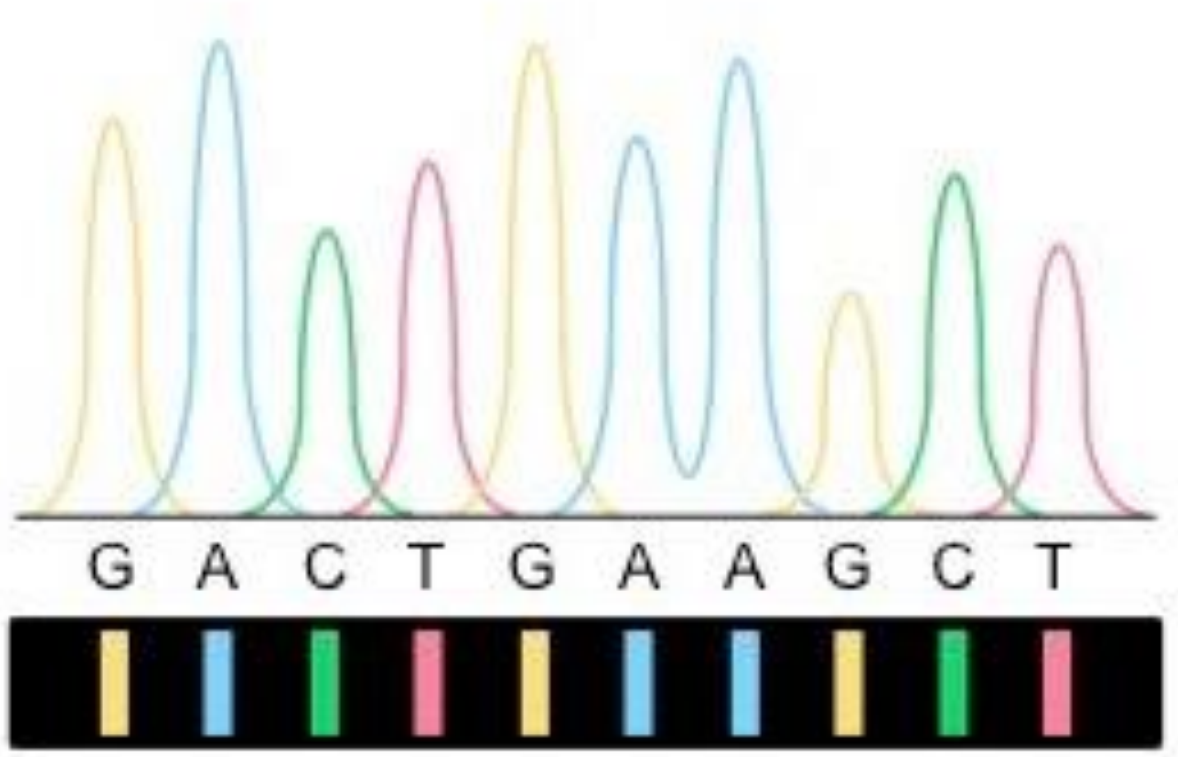


https://www.youtube.com/watch?v=wdS3j0TgbjM

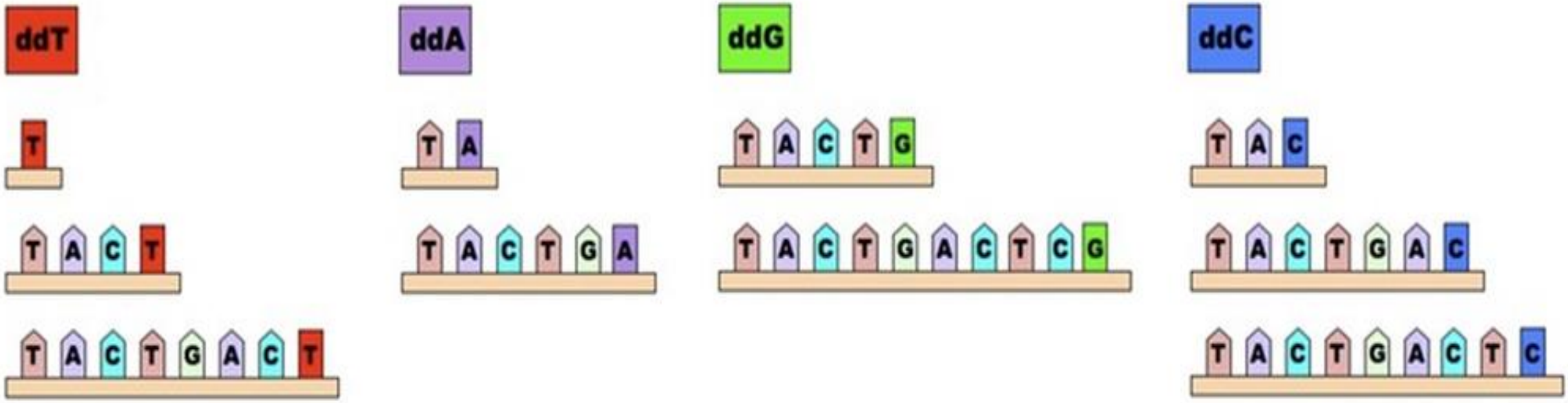


Capillary method

What would the output trace look like for this sample with the capillary method?



Sanger Sequencing – gel plate or capillary method



What is the DNA sequence for this sample?

T A C T G A C T C G

Tasks for you to complete

Complete - the notes from slides 5 and 21 and any other notes and diagrams to help you understand and remember this process.

Check that you have completed:

MG1 – The human genome

MG2 – Producing a DNA profile

MG3 – Restriction endonucleases

MG4 – Gel electrophoresis

Complete:

MG5 – PCR

MG6 – Uses and reliability of DNA evidence

MG7 – DNA profiling exam questions