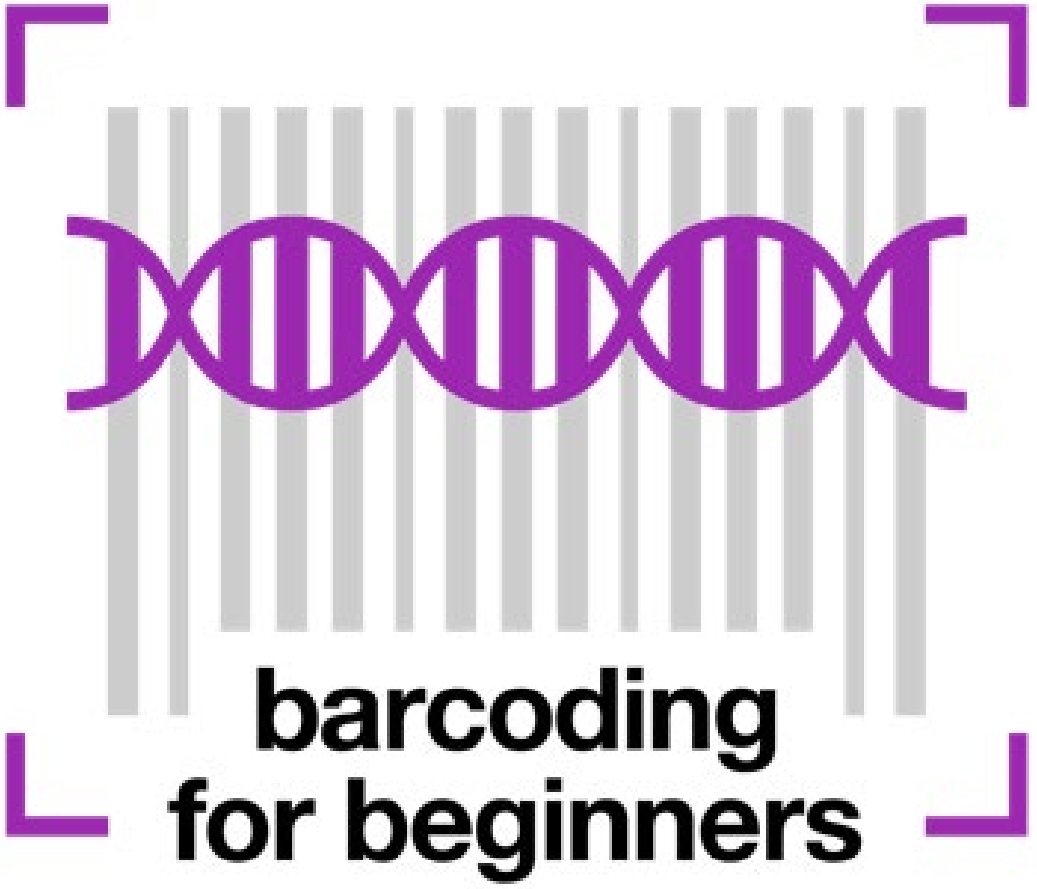
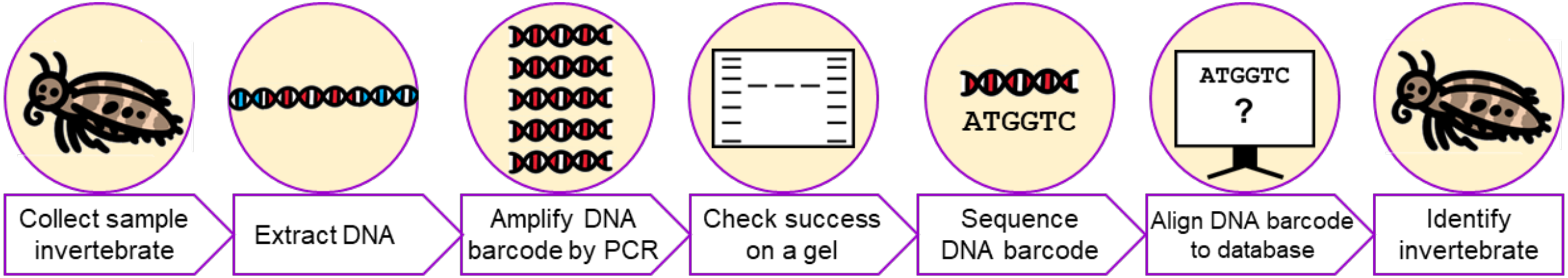
Overview of practical activities



Next practical activities

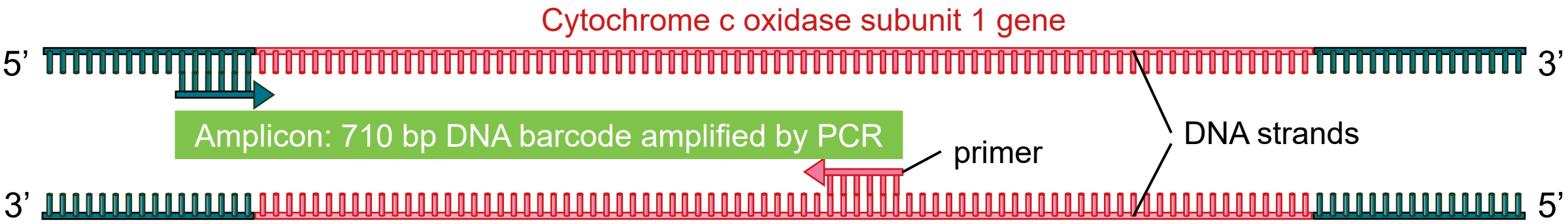
Today you will check your success using gel electrophoresis and use some pre-sequenced invertebrate DNA to develop skills in bioinformatics.



Gel electrophoresis for DNA discovery

Explaining gel electrophoresis

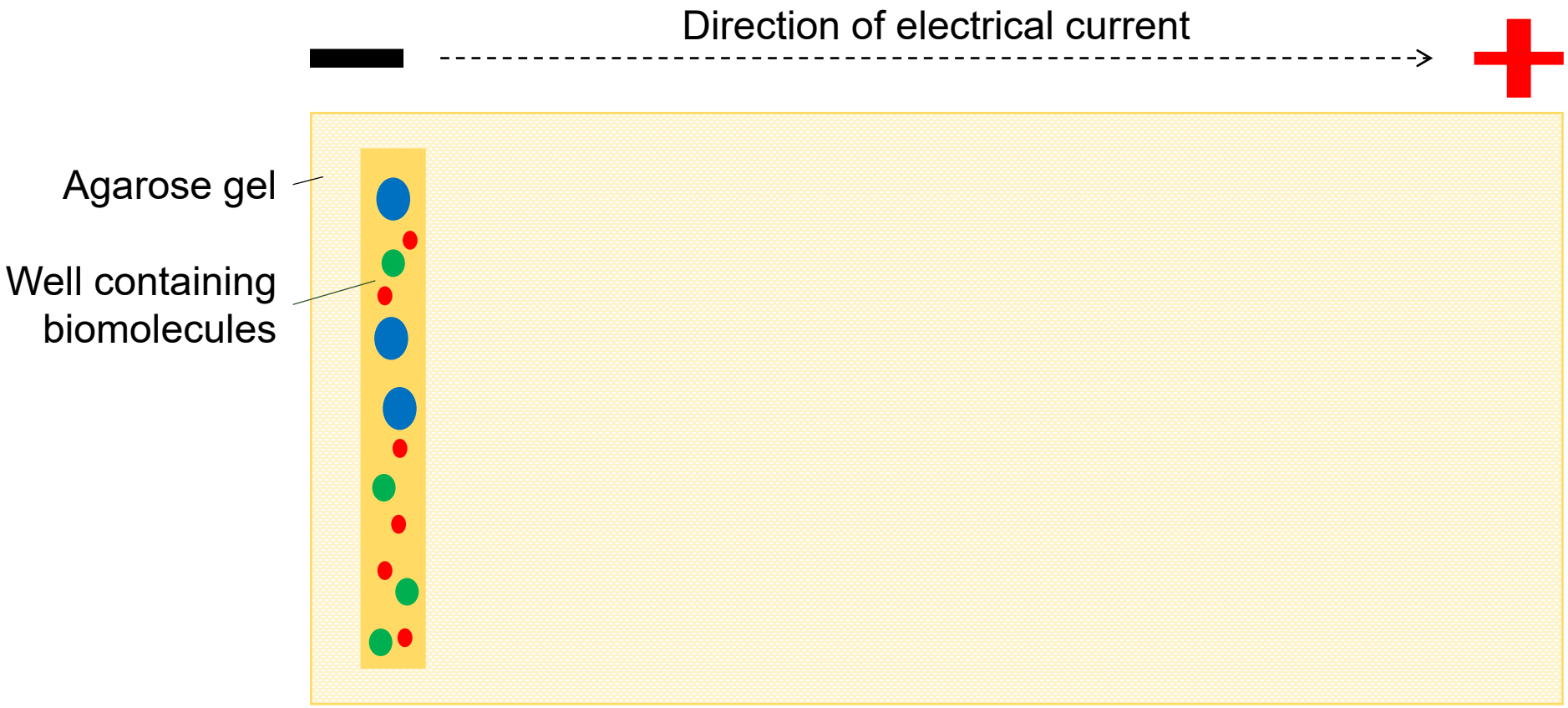
If the DNA extraction and PCR have been successful, the size of the amplicon (amplified DNA barcode using PCR) should be about 710 bp (base pairs).



NOTE: This image is not to scale!

Explaining gel electrophoresis

The primary factor affecting movement through an agarose gel by electrophoresis is size.

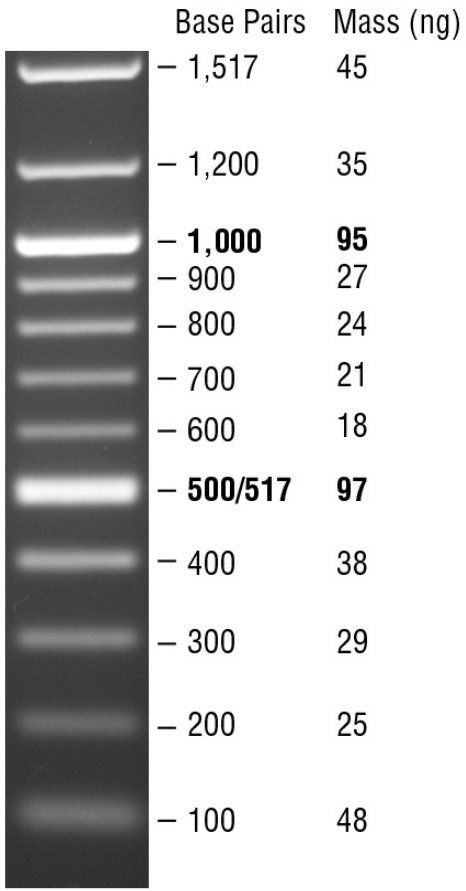


Explaining gel electrophoresis

Gel electrophoresis is used to compare DNA from the PCR with bands of known size, to see whether a band of the expected size (710 bp) has been produced.

The bands of known size are also called a DNA ladder, as they look like a ladder on a gel after electrophoresis We use a DNA ladder that has band sizes of: 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 1200 bp, 1517 bp.

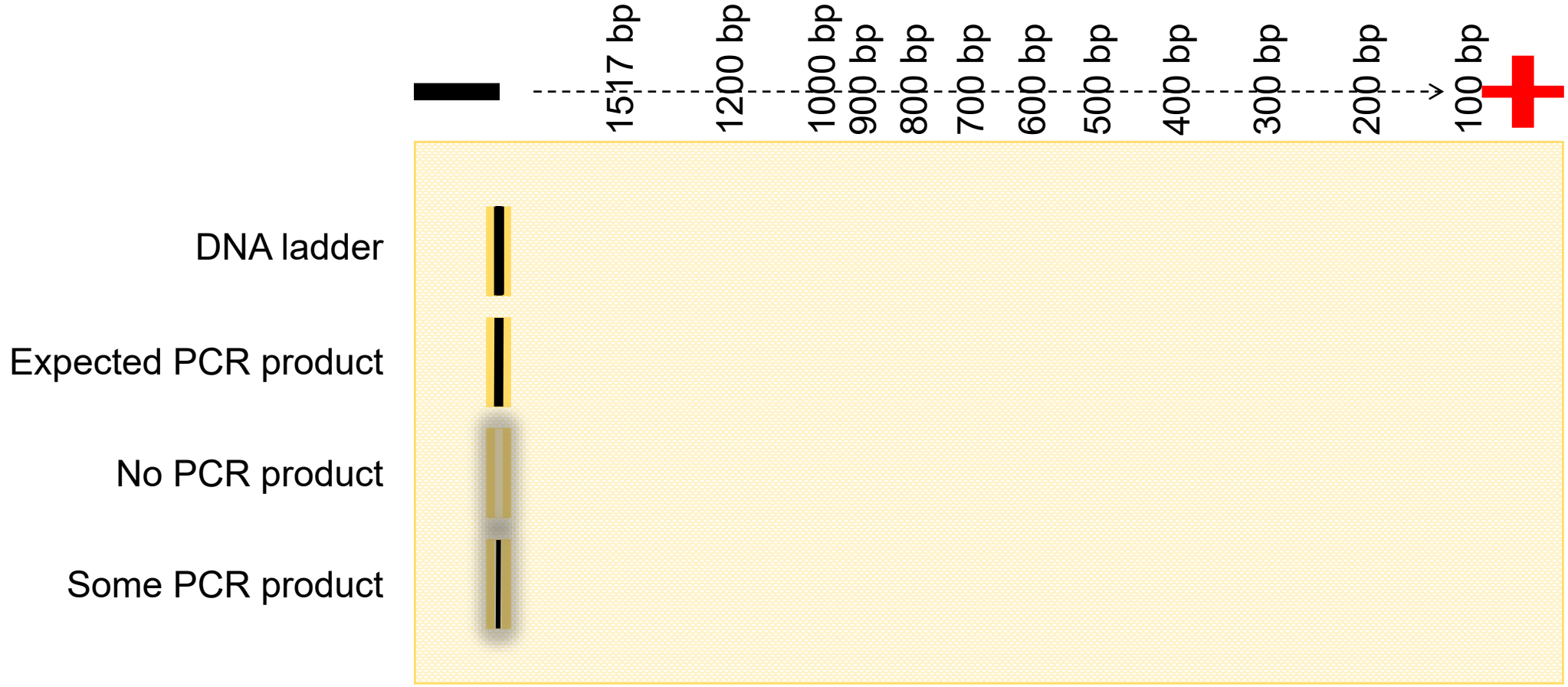
100 bp ladder



Credit: New England Biolabs

Explaining gel electrophoresis

Gels can be used to compare size of the PCR product to a DNA ladder, with bands of known sizes.

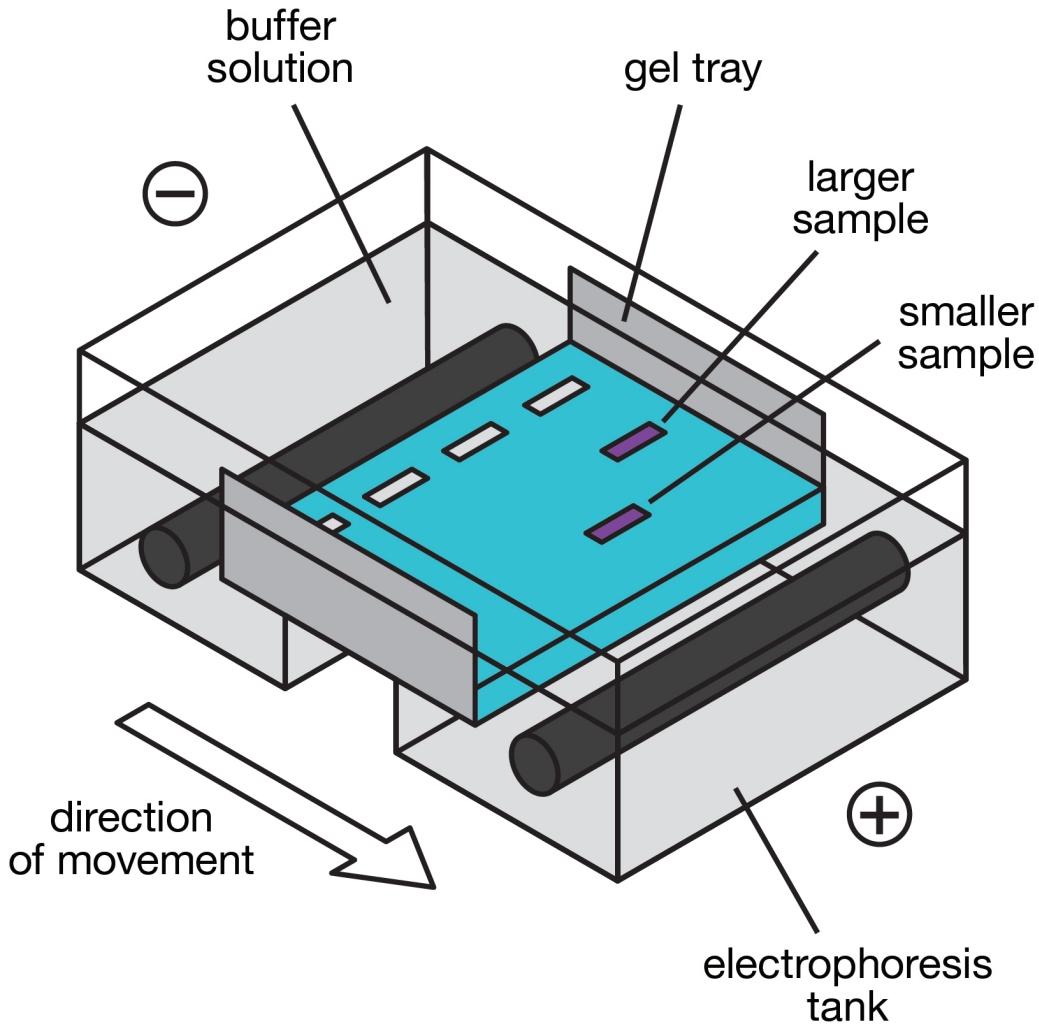


Performing gel electrophoresis

Before performing electrophoresis, you should put on gloves and safety glasses.

Prepare the PCR product for loading onto the agarose gel following the experimental procedure.

** Pipette the reaction up and down 3 or 4 times to mix, so the GelGreen® DNA stain does not settle.*



Performing gel electrophoresis

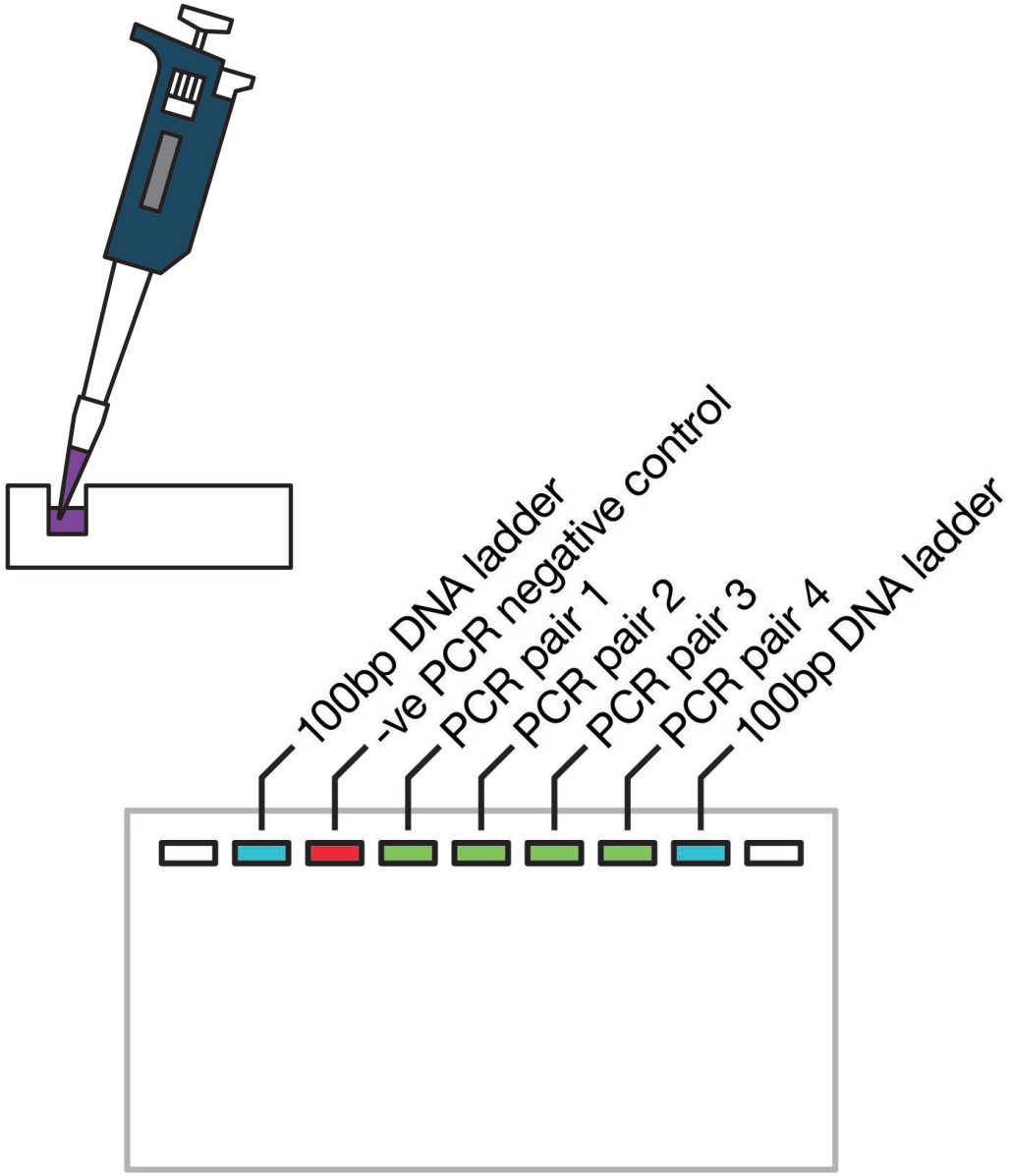
Before performing electrophoresis, you should put on gloves and safety glasses.

- Load 10 µl of prepared mixtures into a well, following the instructions

** Don't put the tip into the well, the samples have greater density than the buffer, so will sink into the well*

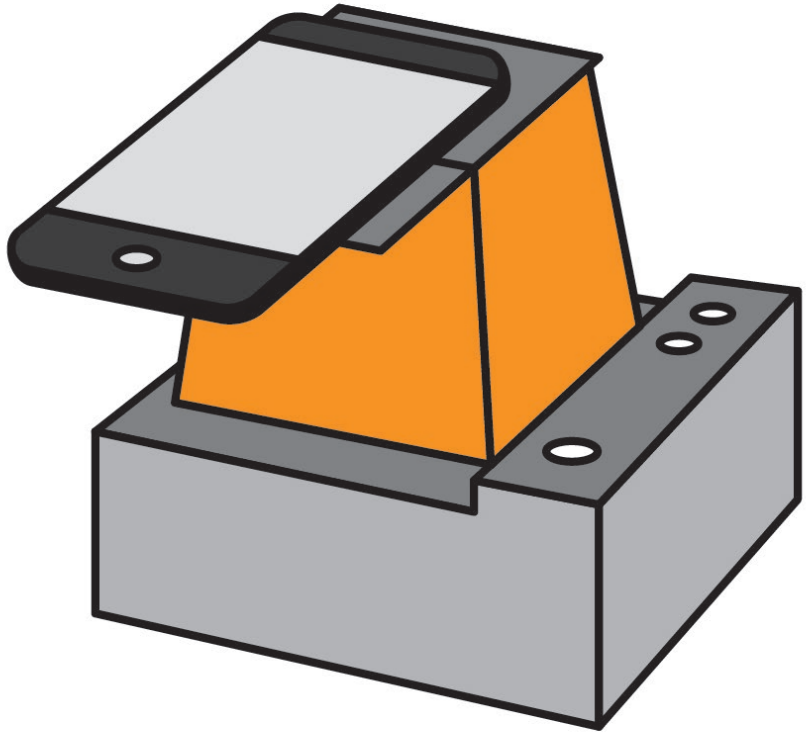
- When the gel is loaded, place the orange lid on and press the power button to start electrophoresis

- Start a timer for 20 minutes



Performing gel electrophoresis

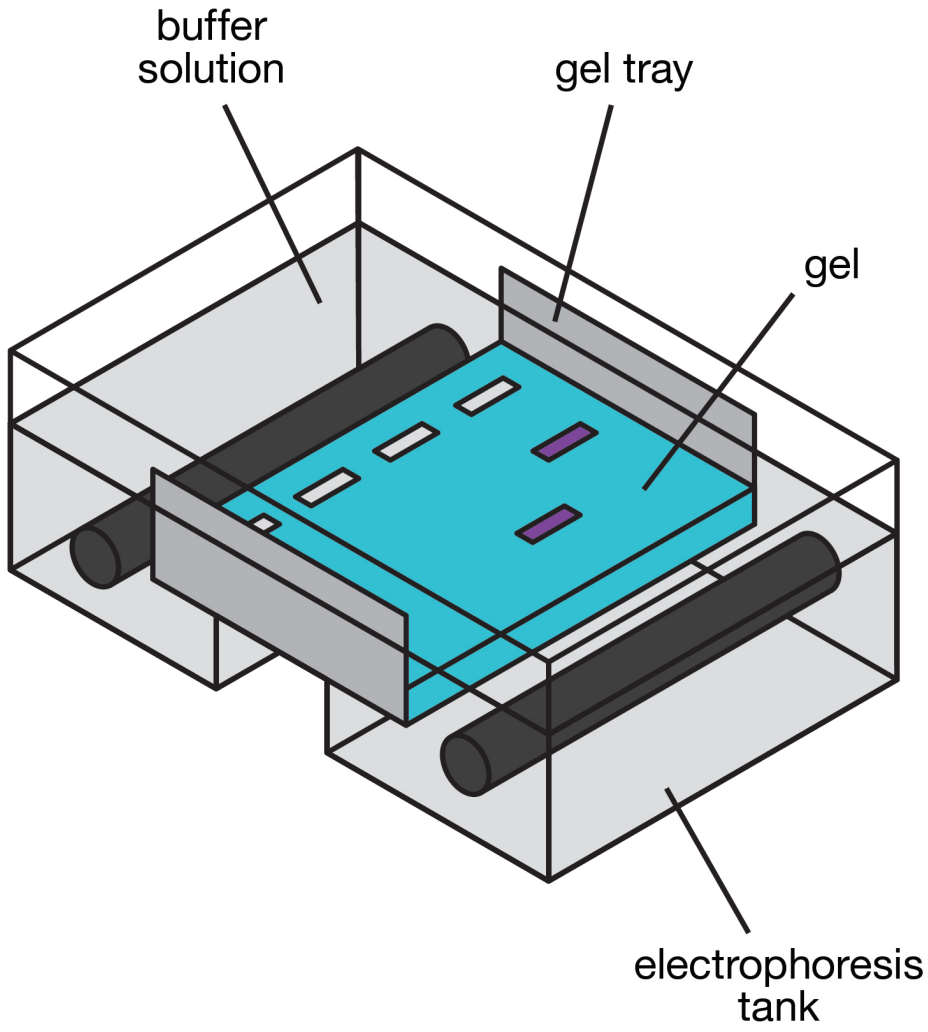
- When 20 minutes have passed, unplug the electrophoresis tank at the socket
- Turn on the blue light and observe the gel through the window in the orange filter
- Place a smart device camera or digital camera lens, directly on the photo hood top
- Focus on the DNA bands in the gel, then take a photo



Performing gel electrophoresis

One person for each tank should put on gloves and safety glasses again.

- This person should carefully lift the gel out of the buffer and place it into the autoclave bag
- The person in gloves and safety glasses should then empty the buffer from the gel carriage into the sink, rinsing it with plenty of water.

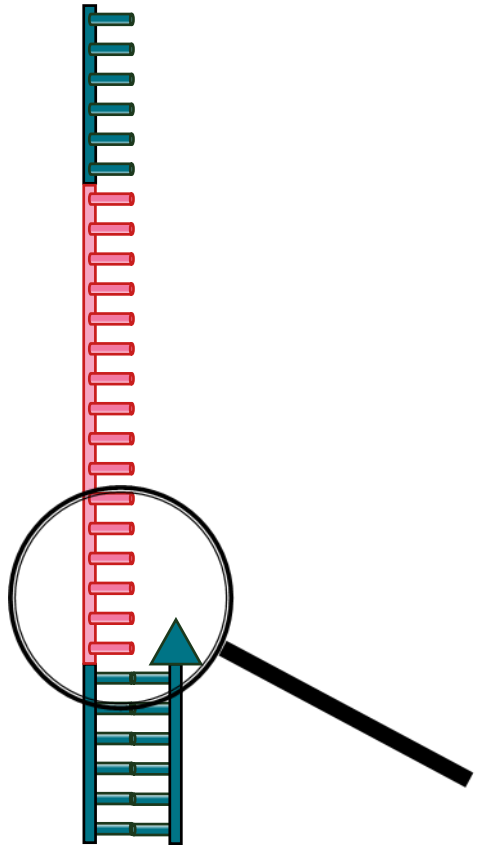


DNA – Sanger sequencing using capillary gel electrophoresis

Preparing for DNA sequencing

An overview of the stages of Sanger sequencing using capillary gel electrophoresis:

- DNA is denatured and a primer anneals to the DNA (just like in PCR)



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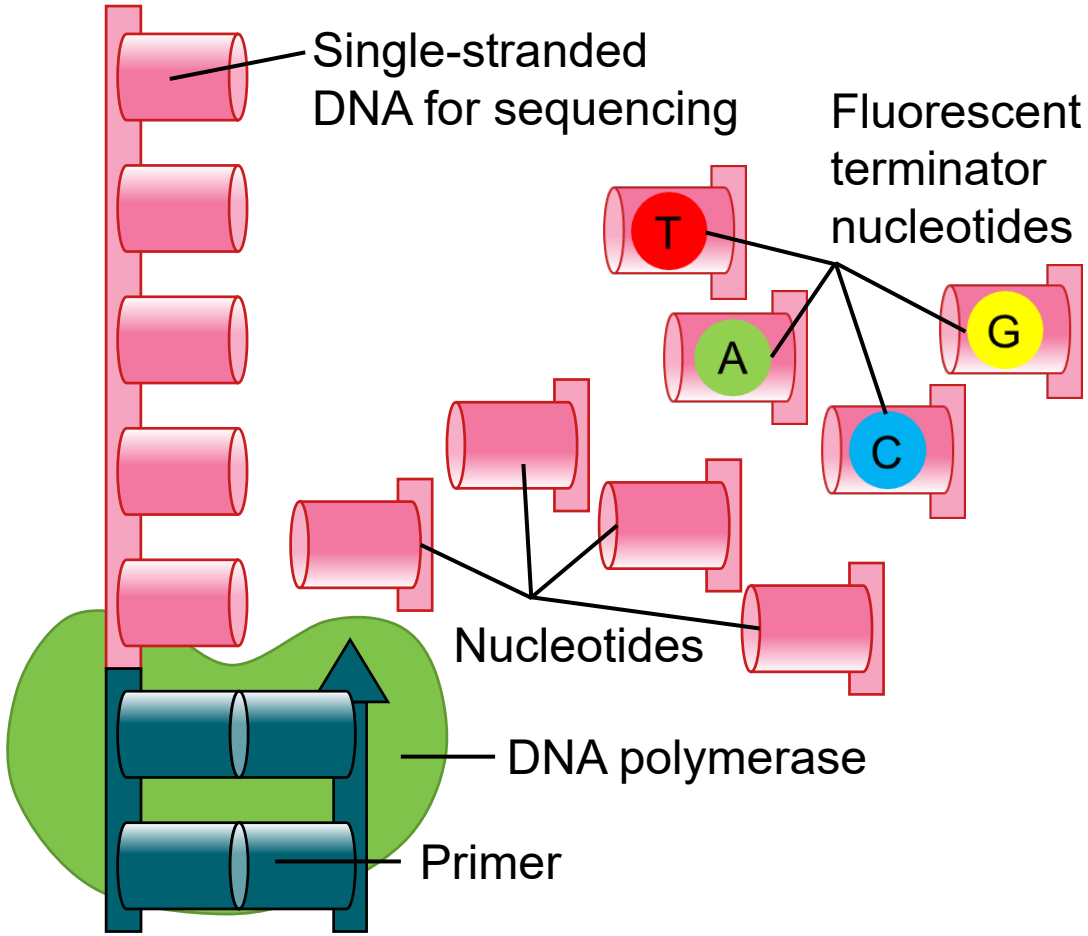

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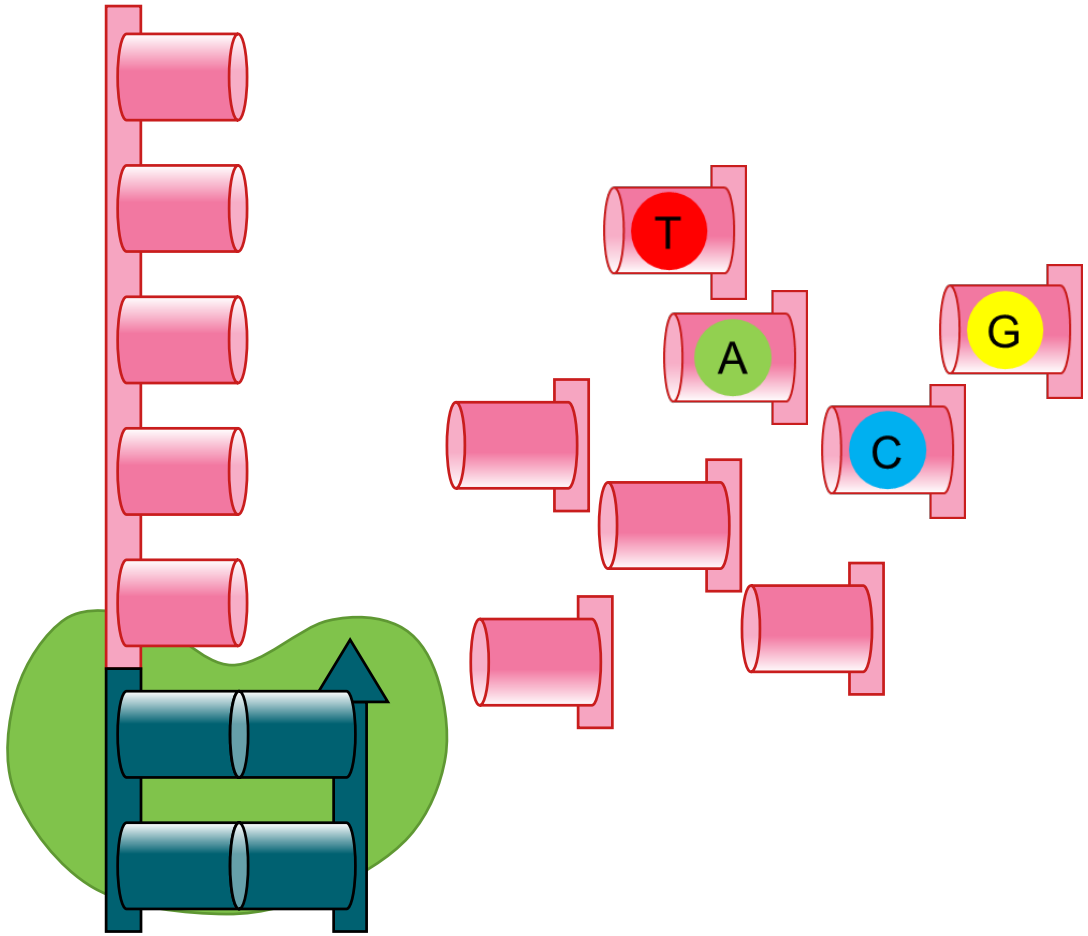

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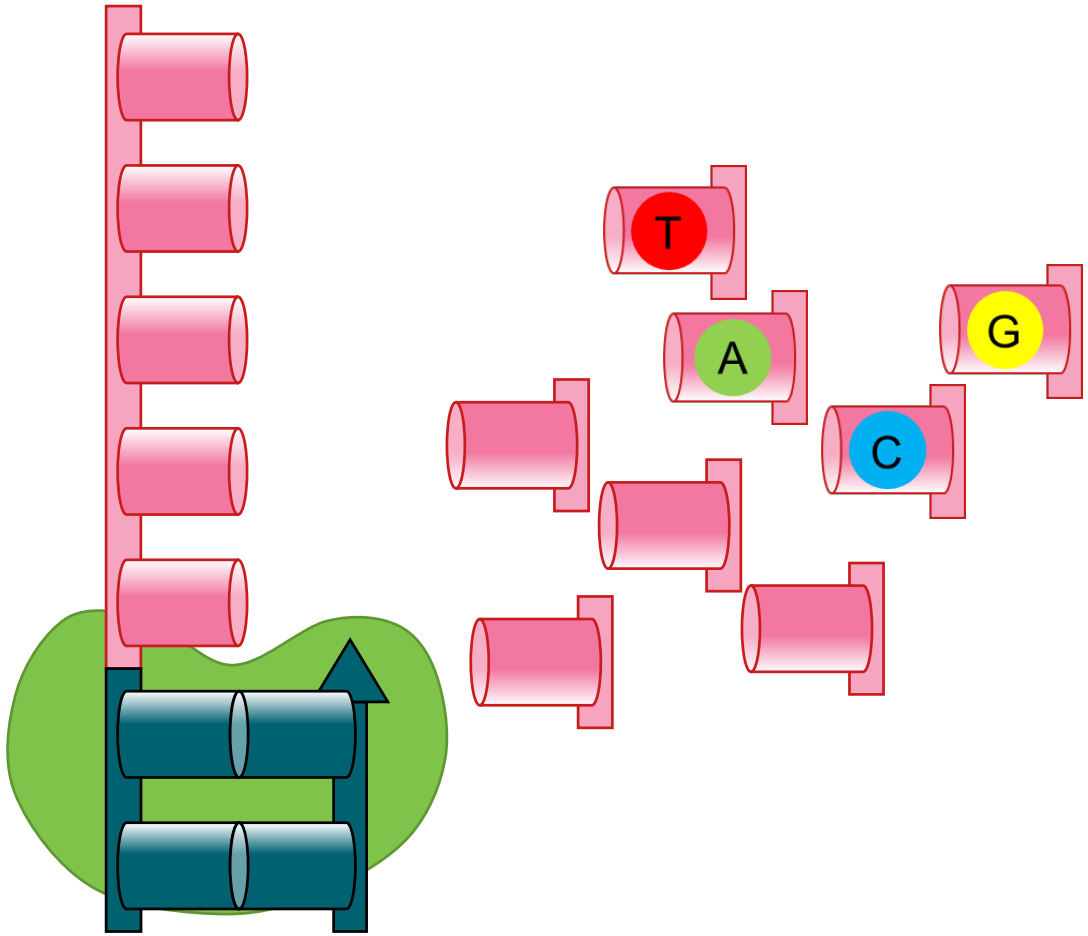

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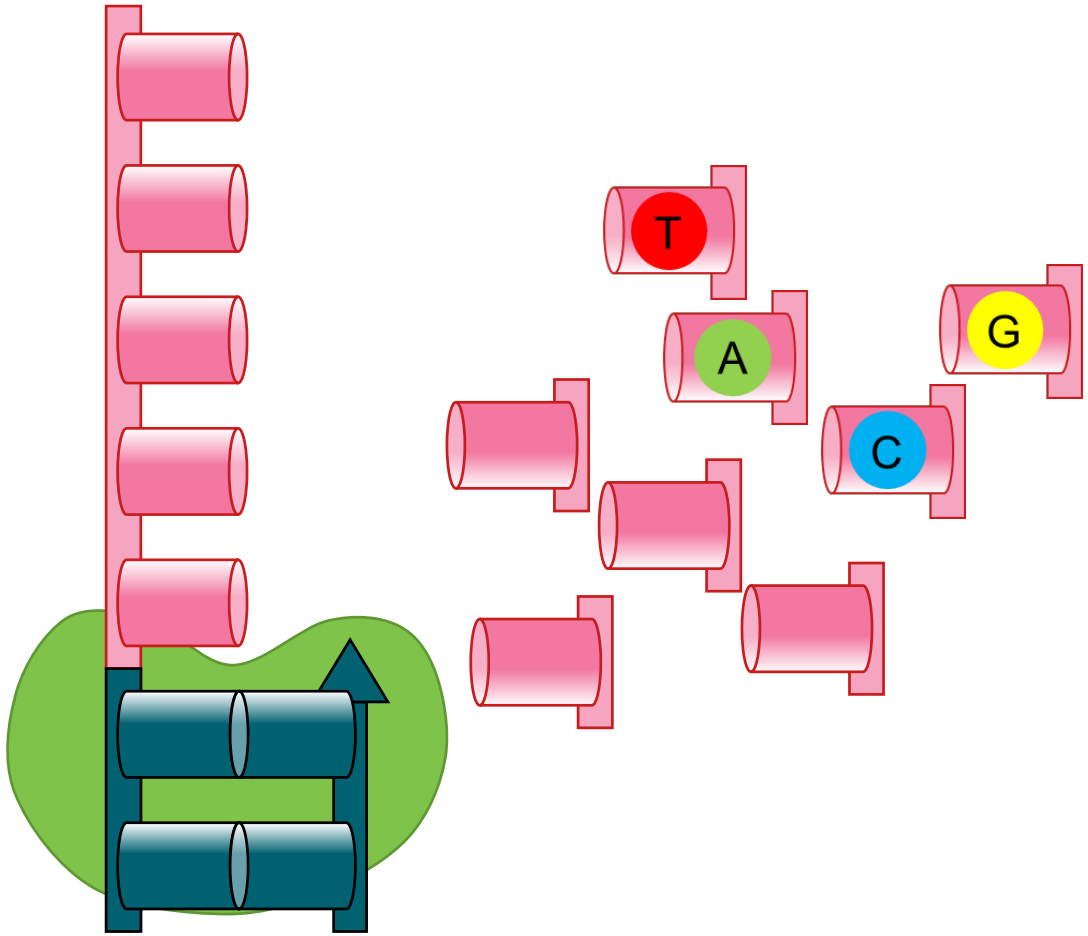

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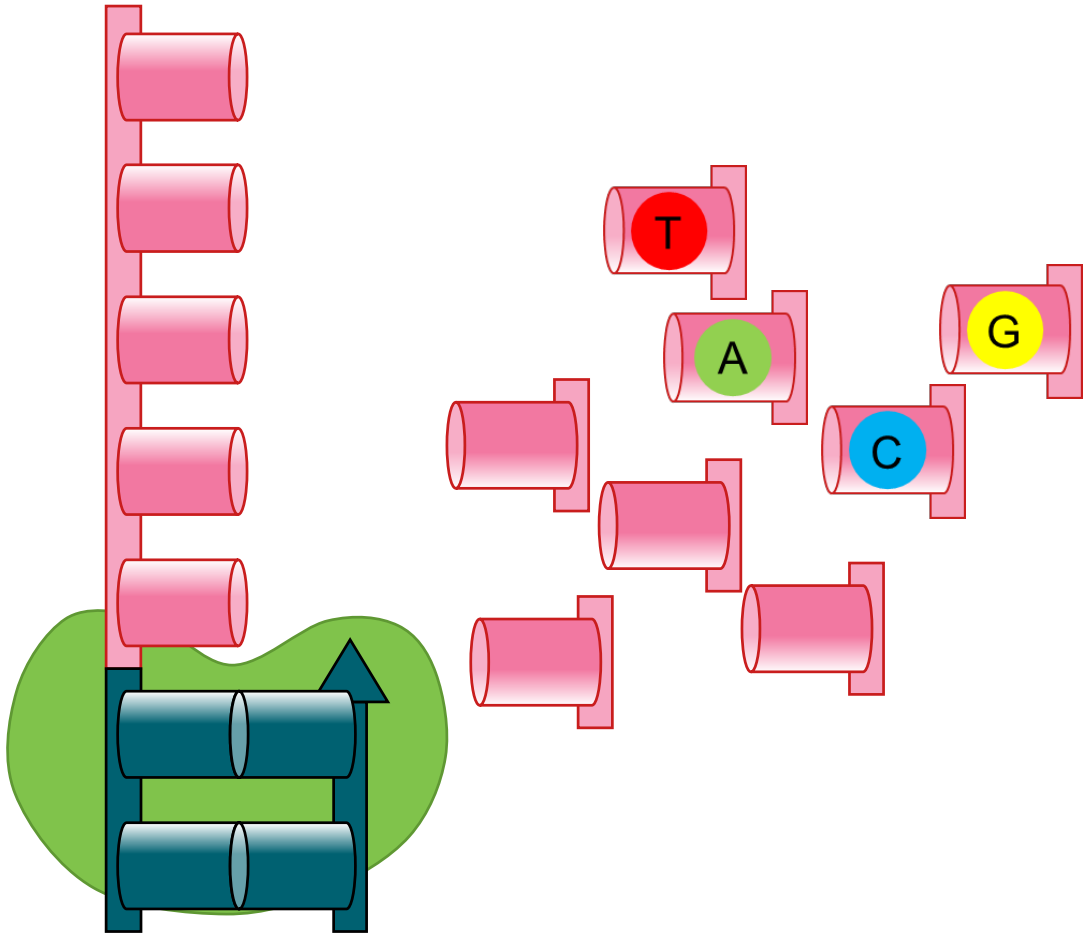

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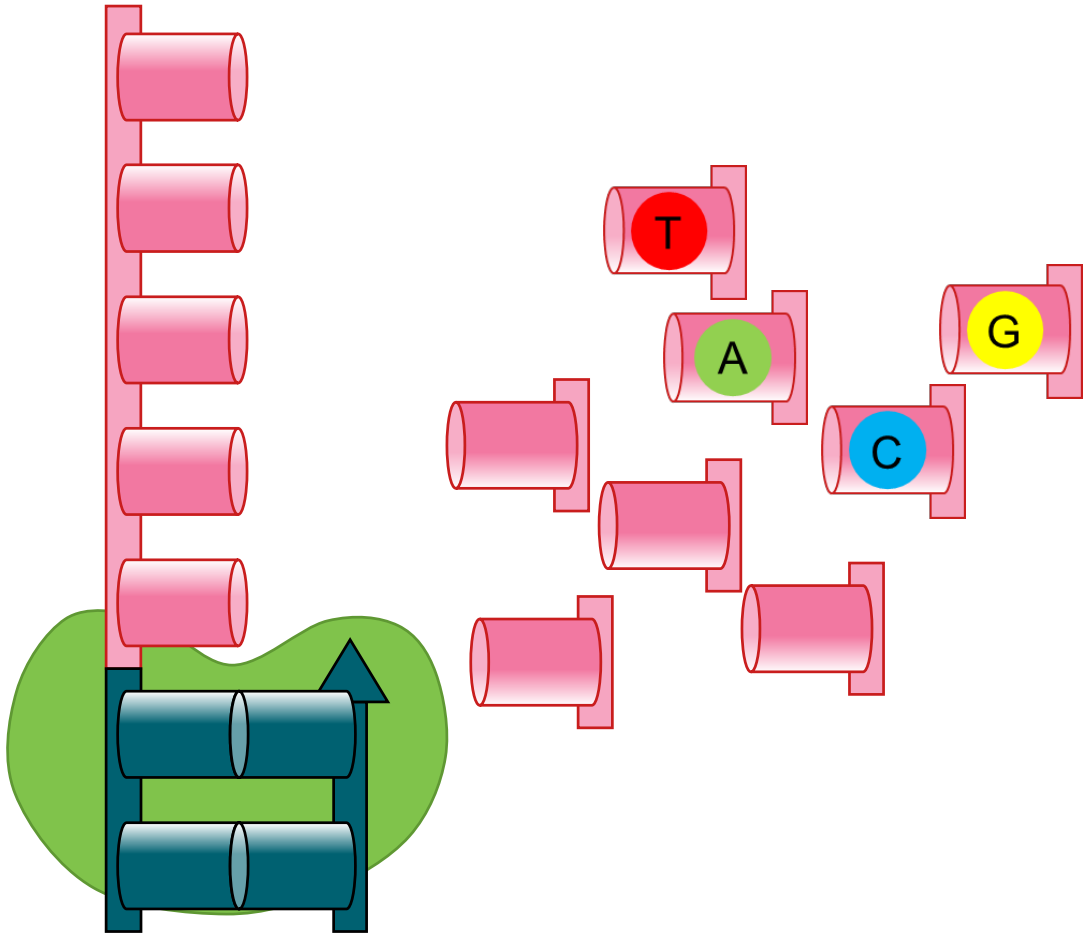

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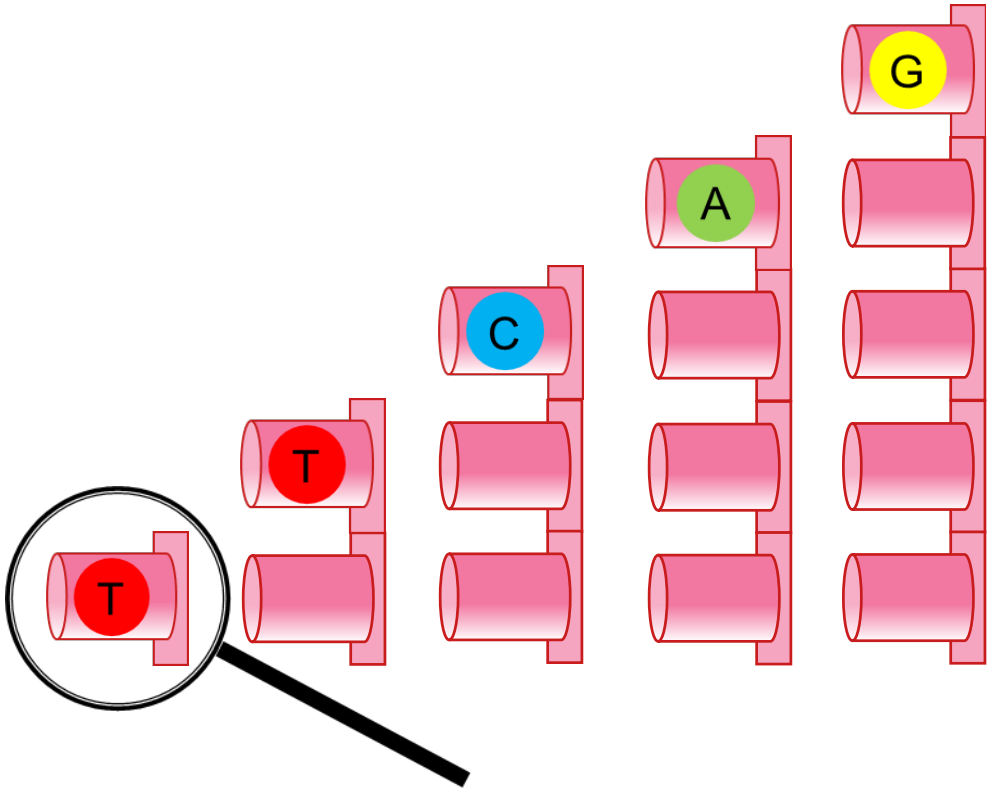

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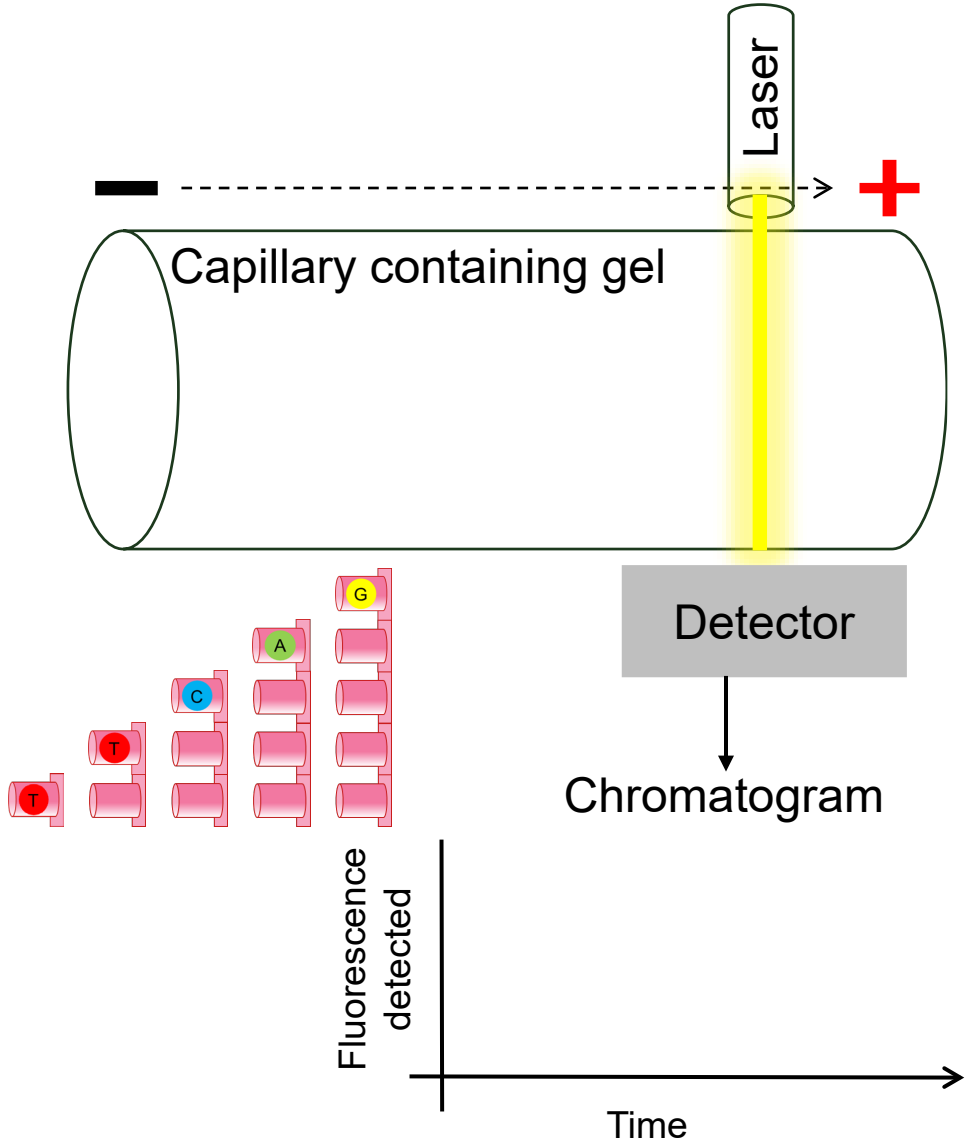

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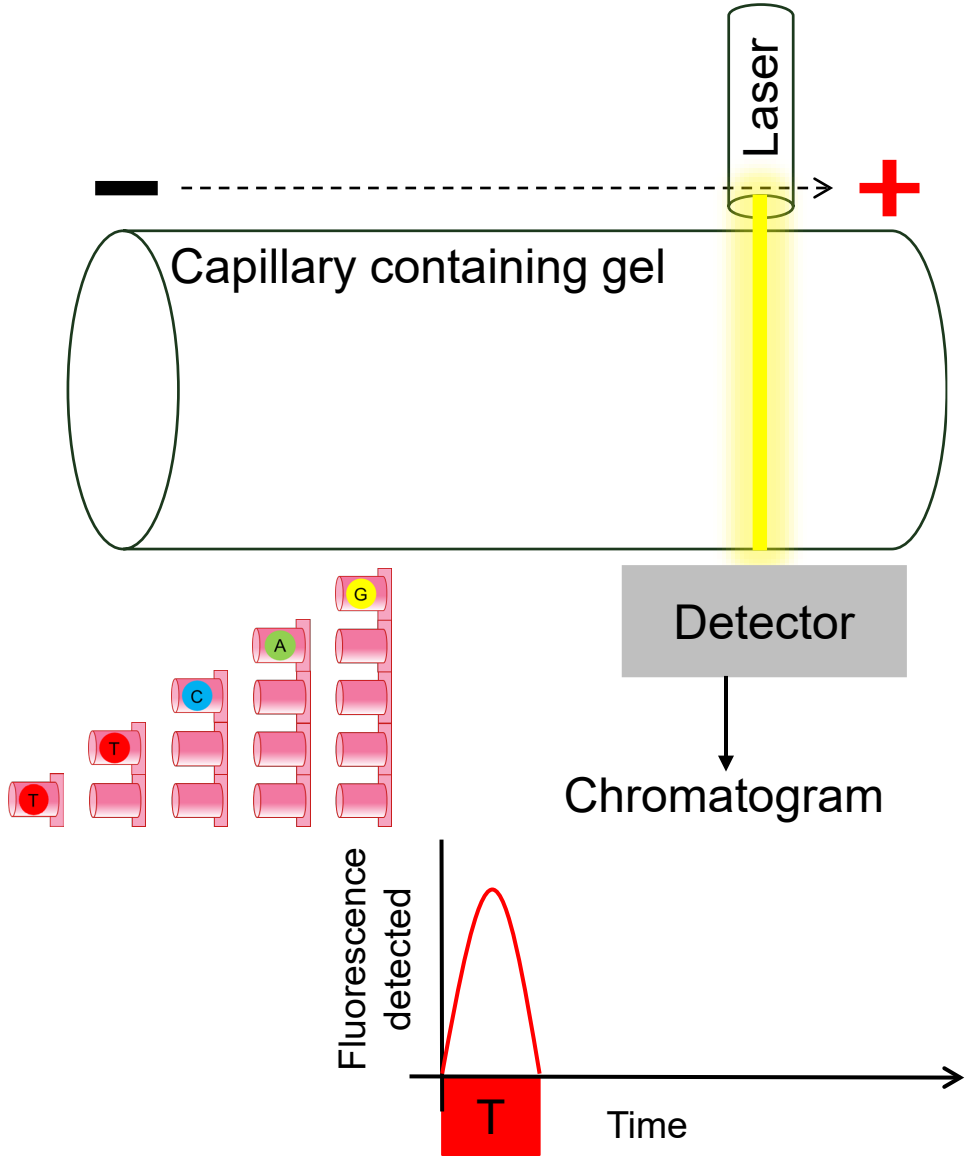

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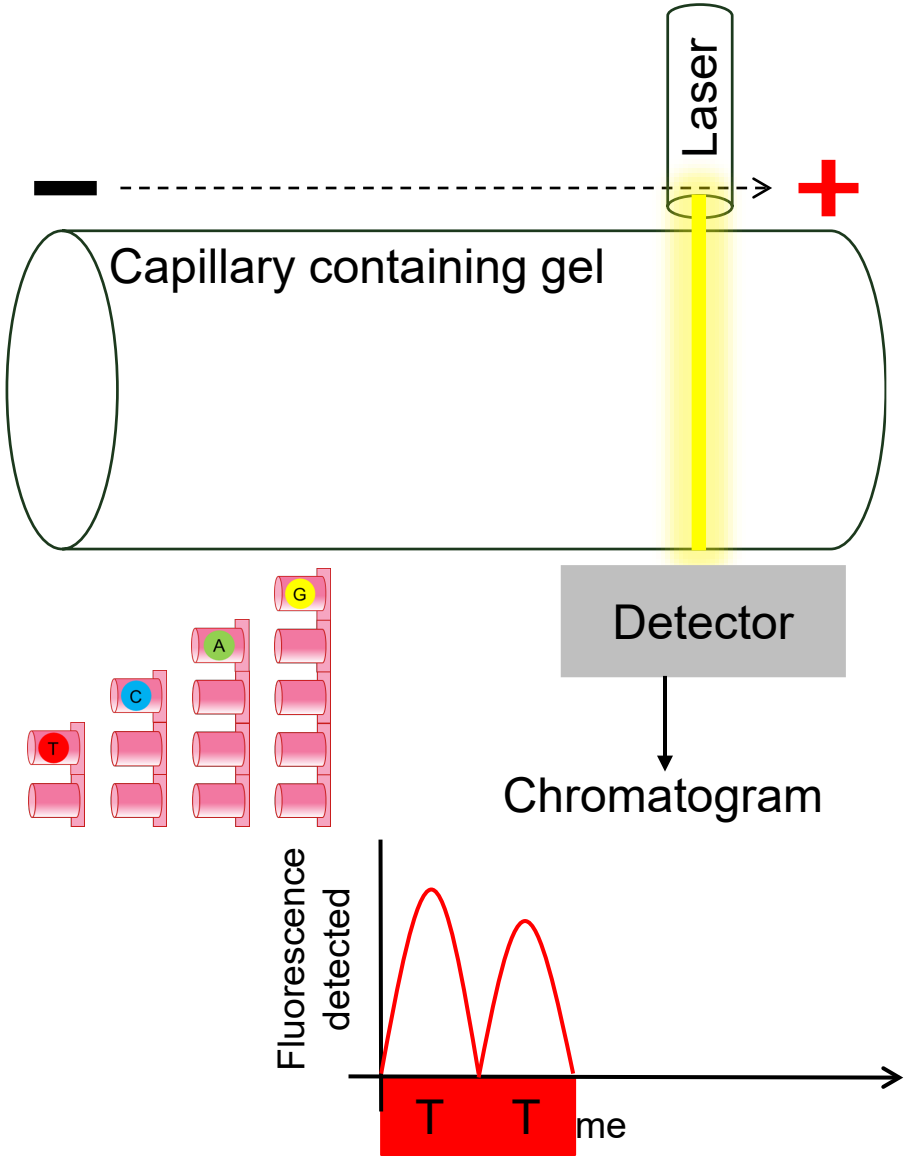

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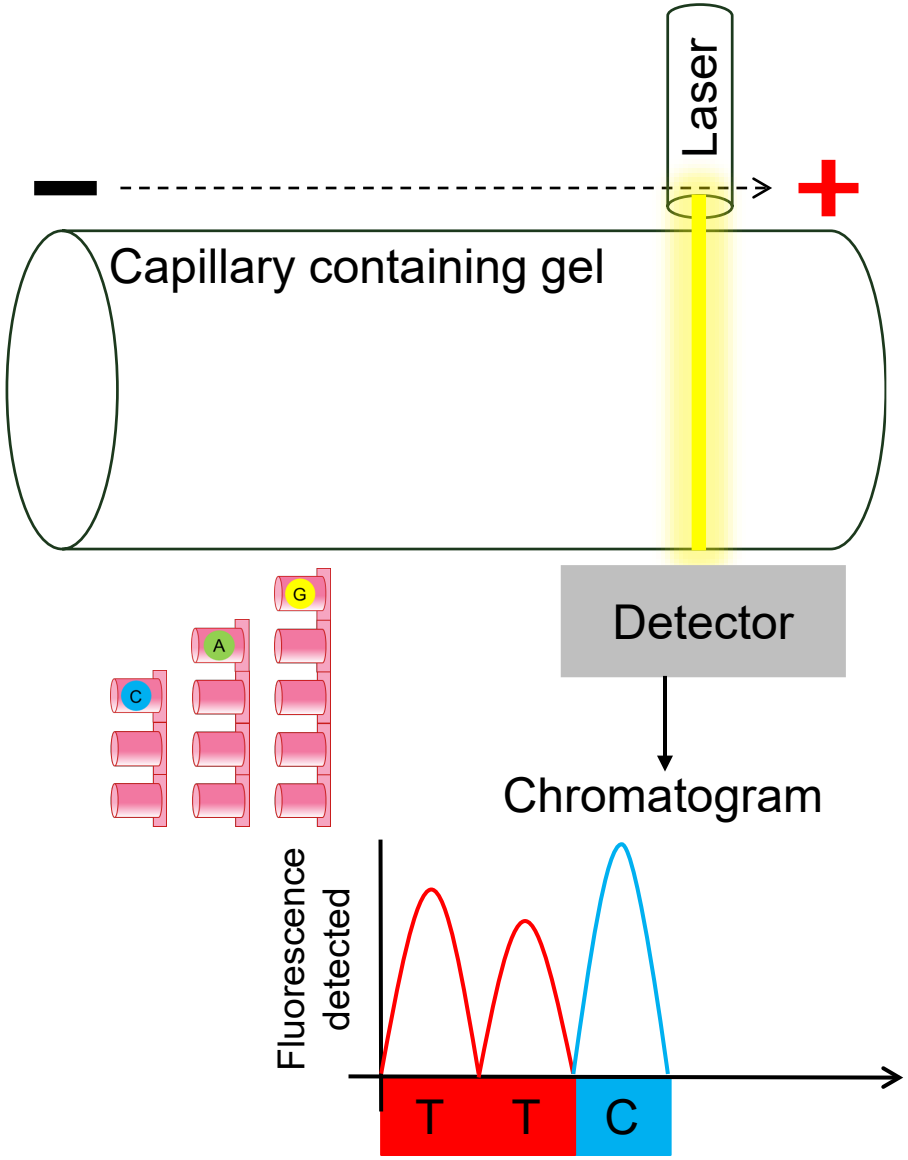

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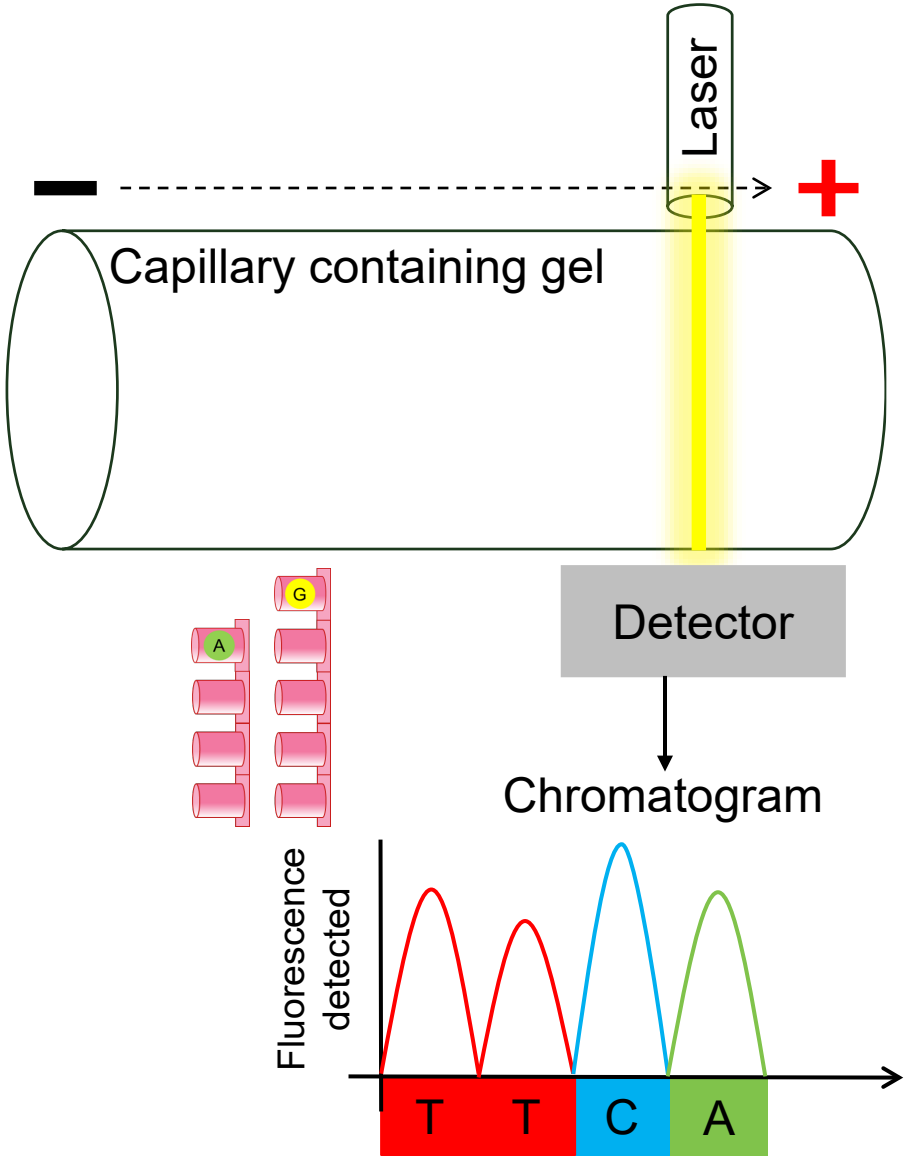

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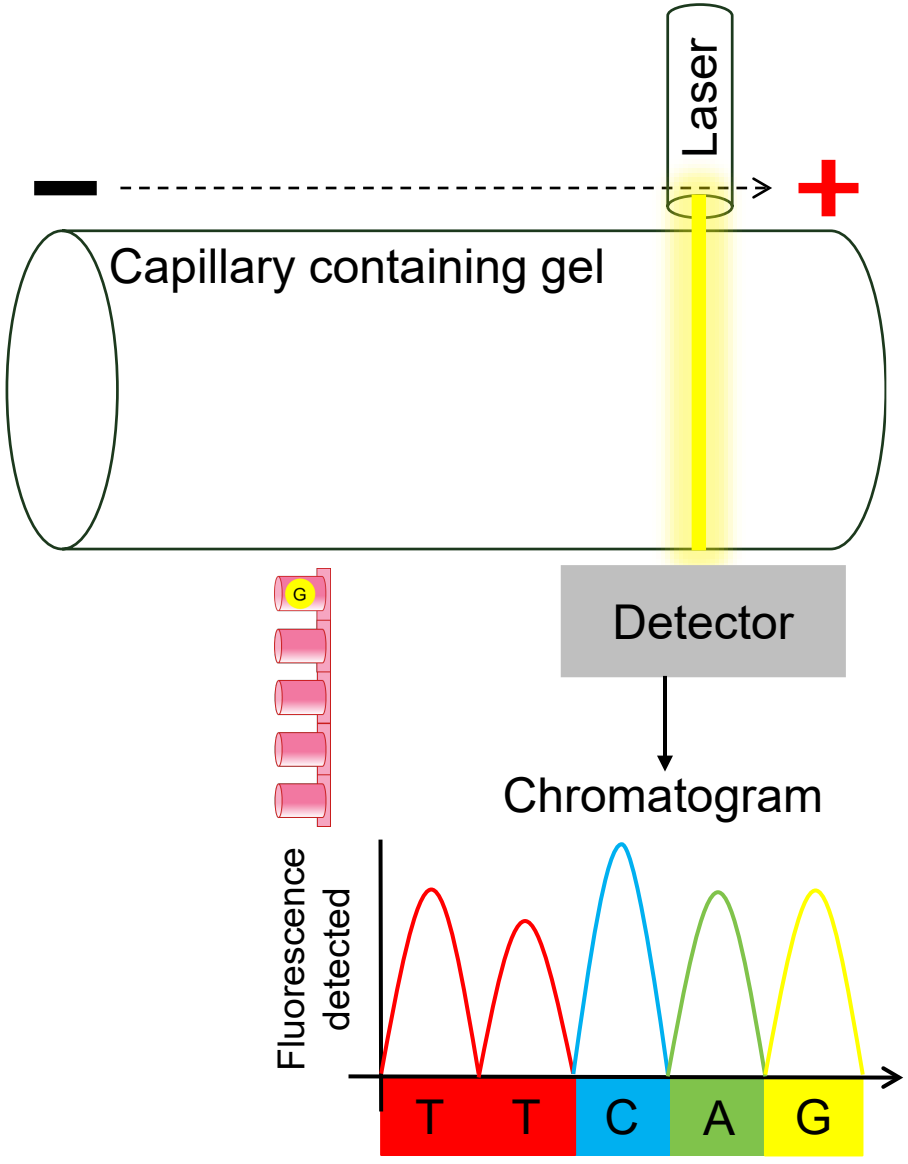
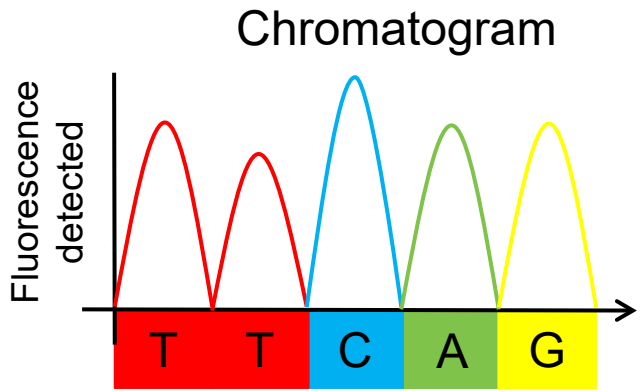


Preparing for DNA sequencing

After Sanger sequencing using capillary gel electrophoresis a chromatogram and a FASTA file will be produced.

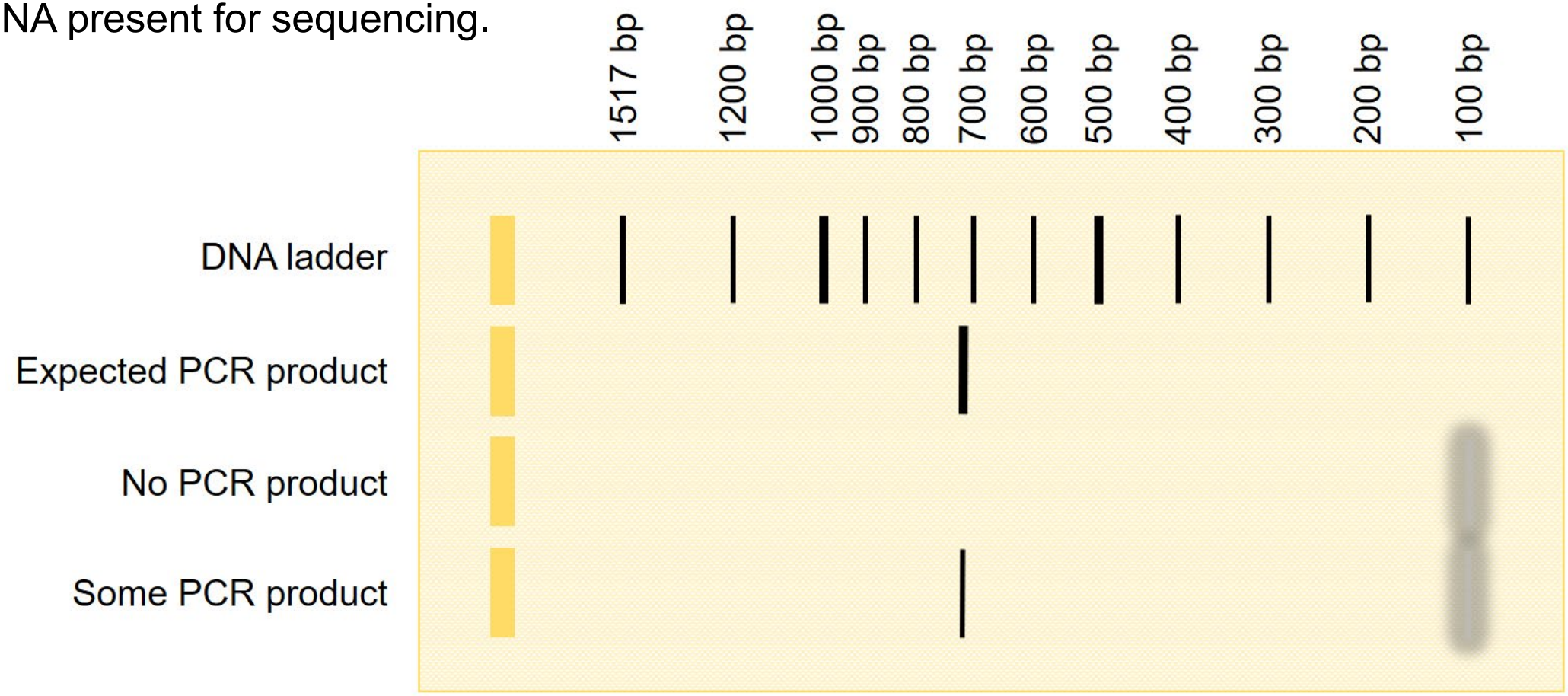



```
>Sequence name
TTCAGGTACAGGATACAATAG
ACTTAGACATAGGCATAGCAC
CCAGATAGGCGGCTGCAATGC
ATACAGCCCCGAGGGGTTACA
```

Gel electrophoresis for DNA discovery

Interpreting gel electrophoresis

If you have a band visible on your gel at about 710 bp after gel electrophoresis, you have sufficient DNA present for sequencing.



Preparing for DNA sequencing

One person for each sample should put on gloves and safety glasses again.

- This person should label a clean microfuge tube with the 7 letter code from the sample record sheet then transfer 20 µl of PCR product into this tube.
- Store the labelled tubes containing the samples in the Isofreeze box at 4°C (in the fridge), until you are ready to send them for DNA sequencing.