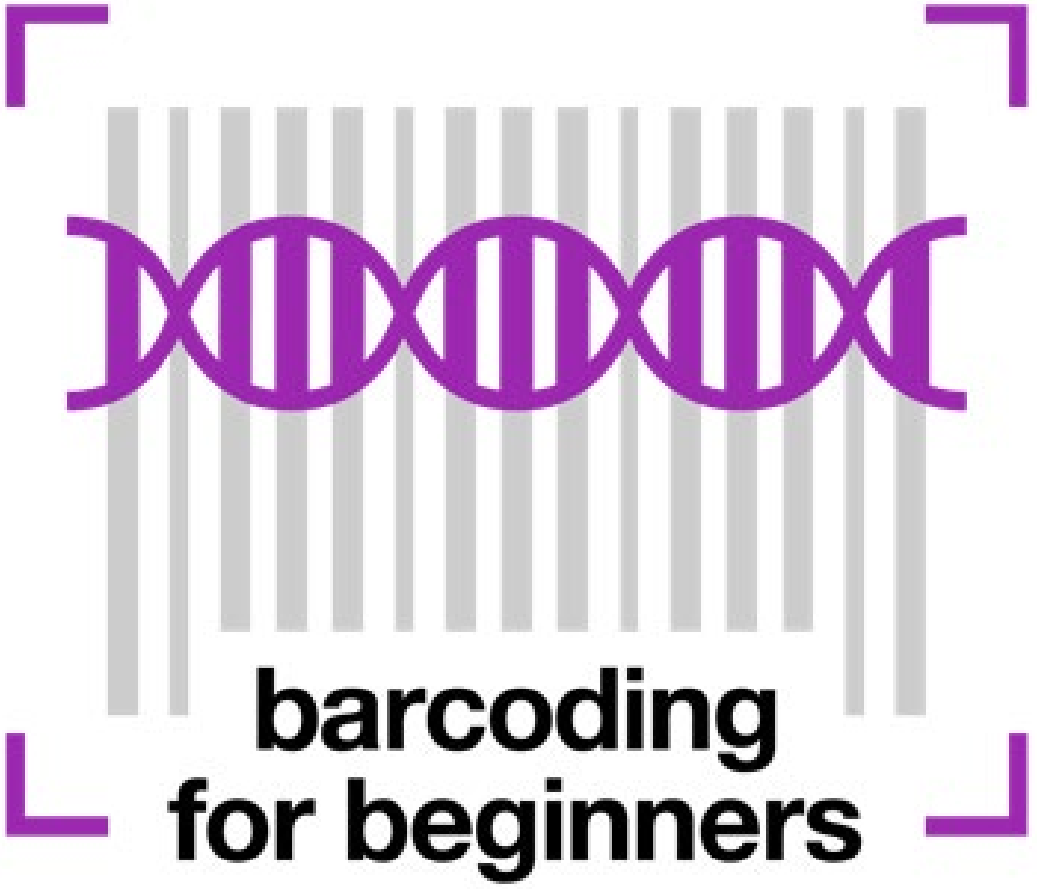
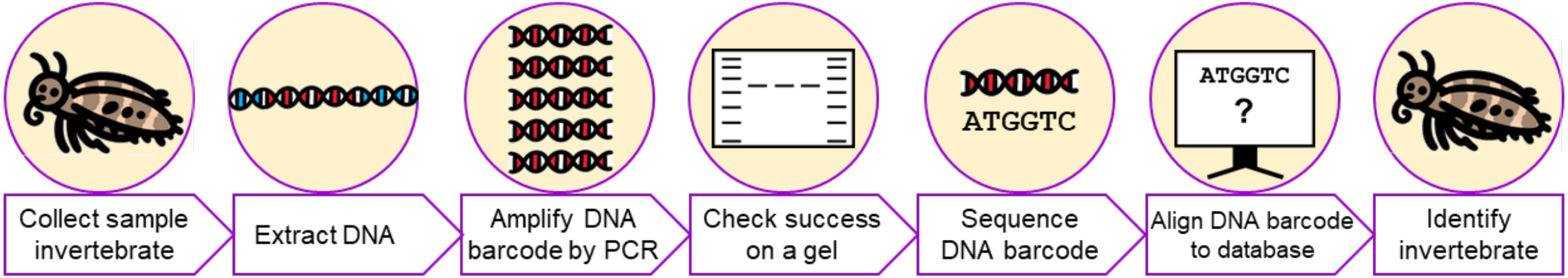
Overview of practical activities

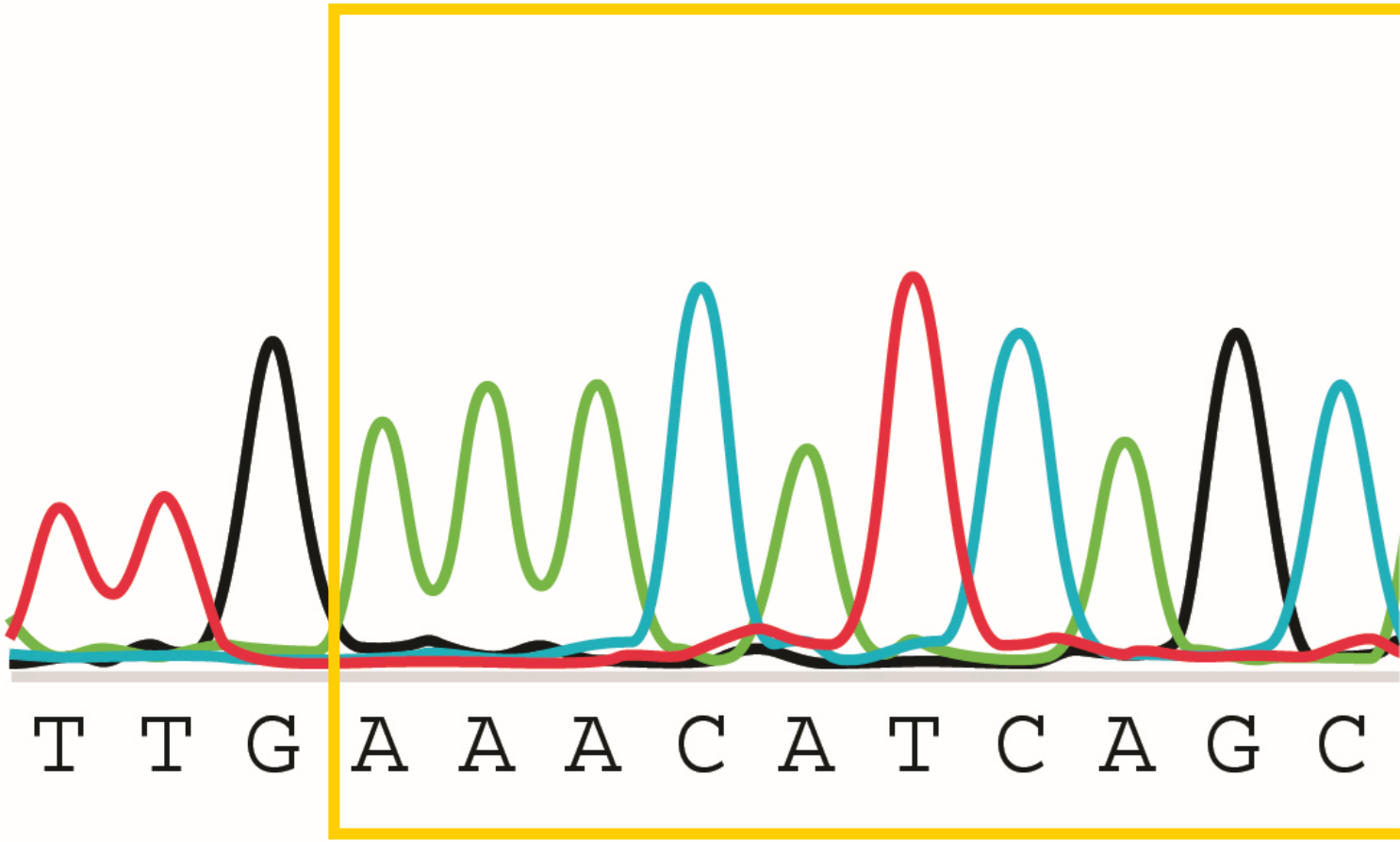


Next practical activities

Use some pre-sequenced invertebrate DNA to develop skills in bioinformatics as you interpret chromatogram quality and identify unknown invertebrates from their DNA barcodes.



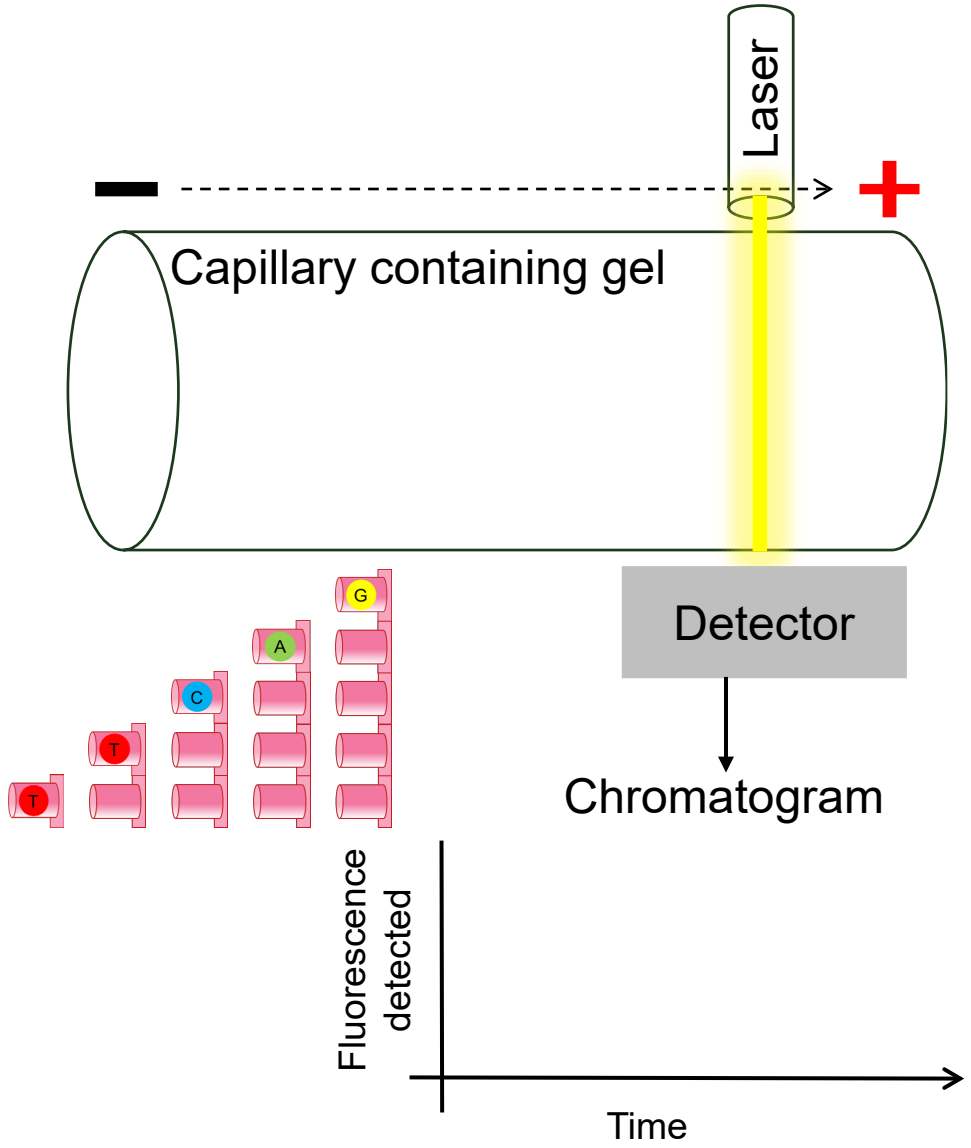
Interpreting chromatograms



Interpreting chromatograms

Sanger sequencing using capillary gel electrophoresis produces a chromatogram:

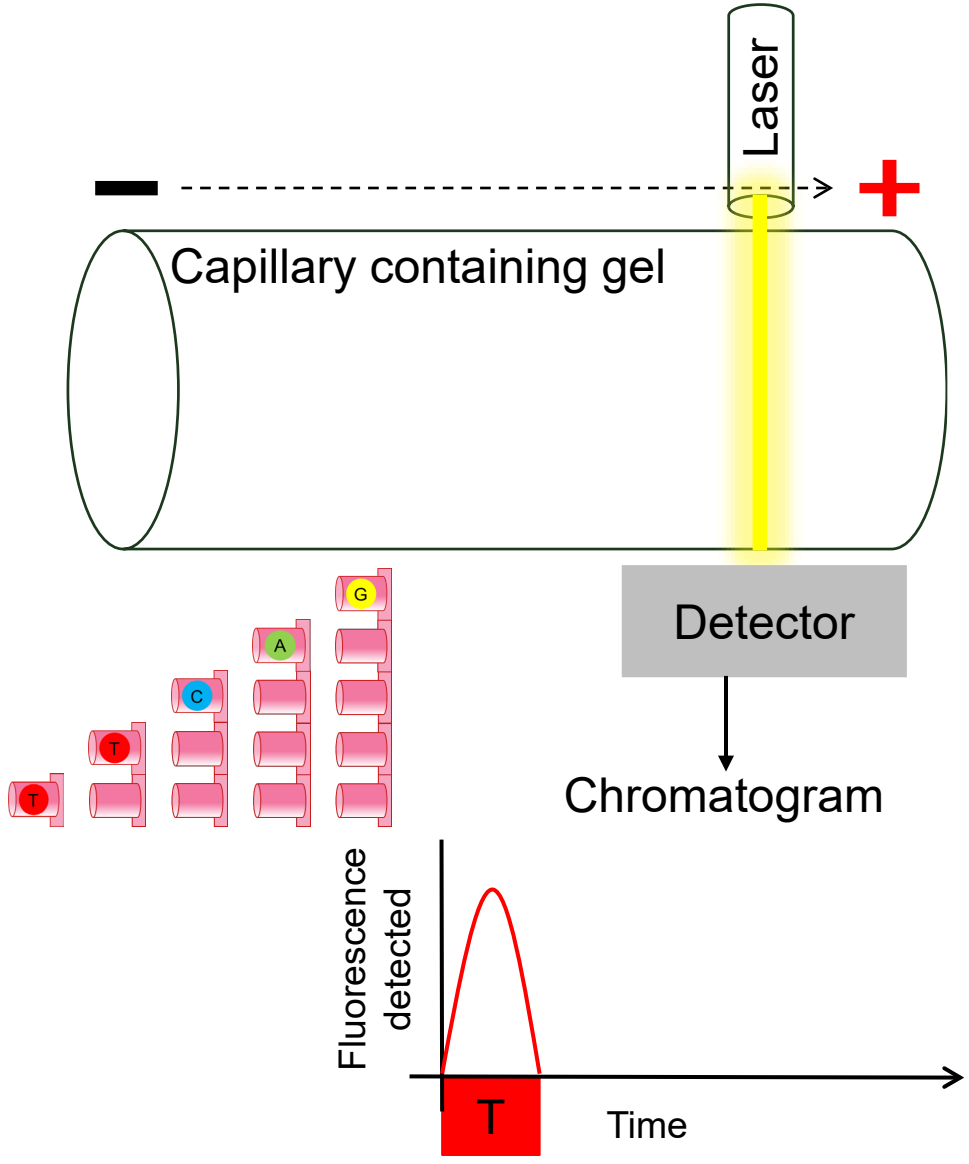
- DNA is denatured and a primer anneals to the DNA (just like in PCR)
- DNA polymerase extends the region of double-stranded DNA (just like in PCR) until a fluorescent terminator nucleotide is added
- This produces fragments of a range of sizes, which can be separated through gel in a narrow capillary by electrophoresis
- As fragments migrate a laser excites the fluorescent terminator base and a detector records the colour on a chromatogram.



Interpreting chromatograms

Sanger sequencing using capillary gel electrophoresis produces a chromatogram:

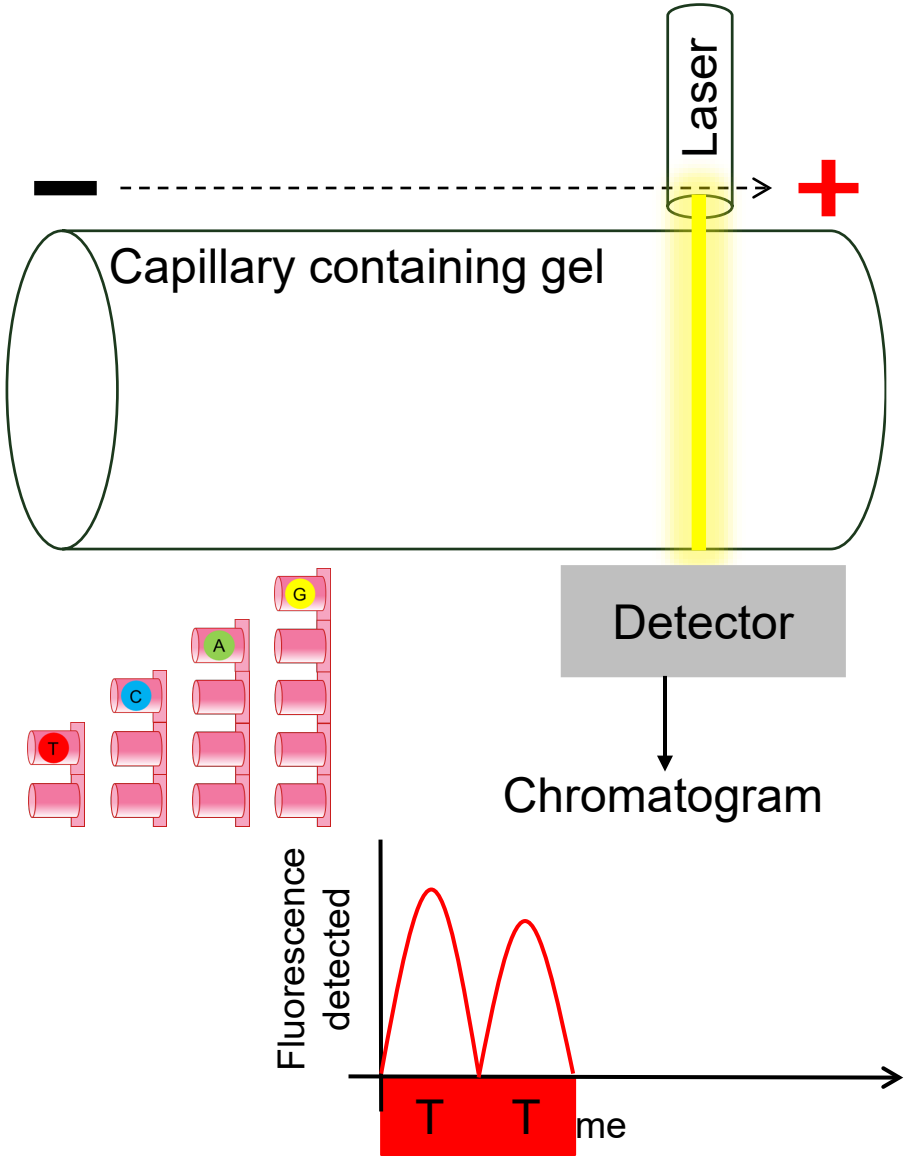
- DNA is denatured and a primer anneals to the DNA (just like in PCR)
- DNA polymerase extends the region of double-stranded DNA (just like in PCR) until a fluorescent terminator nucleotide is added
- This produces fragments of a range of sizes, which can be separated through gel in a narrow capillary by electrophoresis
- As fragments migrate a laser excites the fluorescent terminator base and a detector records the colour on a chromatogram.



Interpreting chromatograms

Sanger sequencing using capillary gel electrophoresis produces a chromatogram:

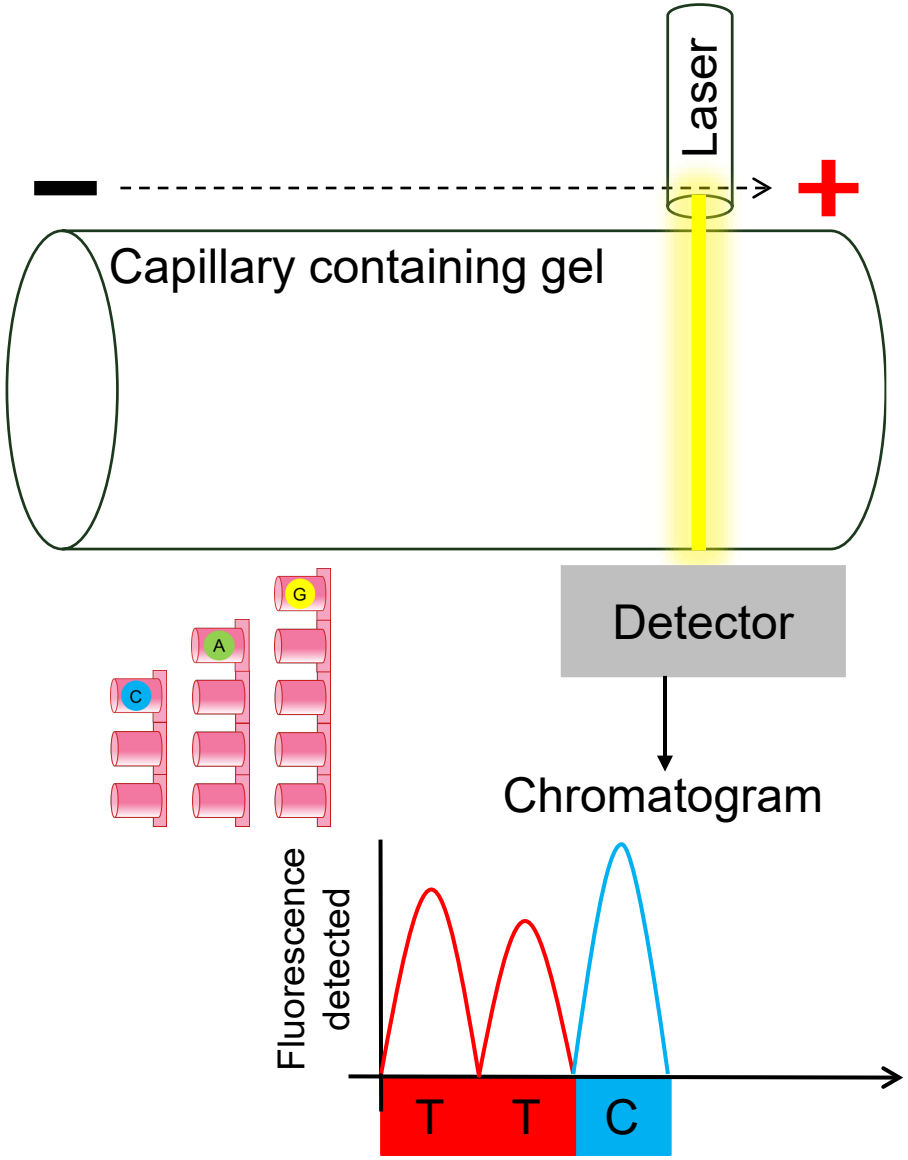
- DNA is denatured and a primer anneals to the DNA (just like in PCR)
- DNA polymerase extends the region of double-stranded DNA (just like in PCR) until a fluorescent terminator nucleotide is added
- This produces fragments of a range of sizes, which can be separated through gel in a narrow capillary by electrophoresis
- As fragments migrate a laser excites the fluorescent terminator base and a detector records the colour on a chromatogram.



Interpreting chromatograms

Sanger sequencing using capillary gel electrophoresis produces a chromatogram:

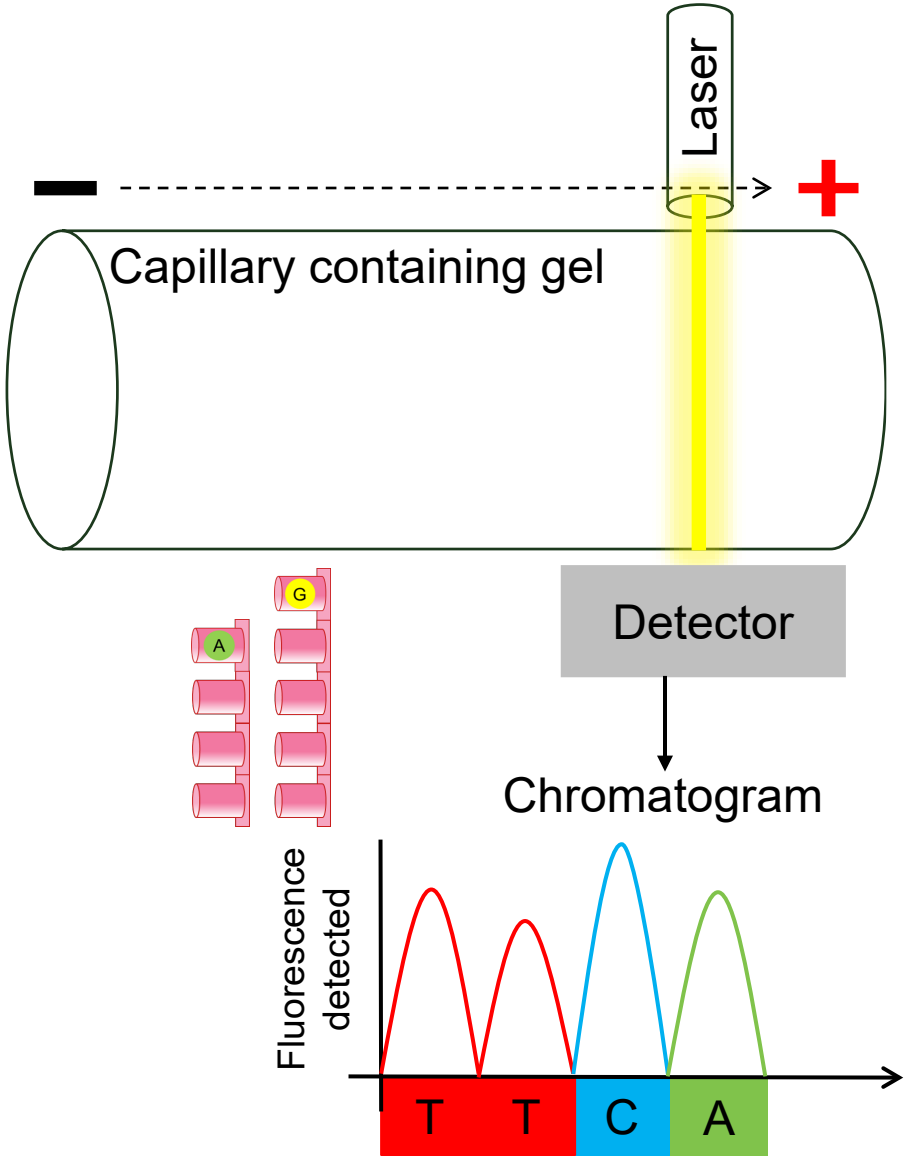
- DNA is denatured and a primer anneals to the DNA (just like in PCR)
- DNA polymerase extends the region of double-stranded DNA (just like in PCR) until a fluorescent terminator nucleotide is added
- This produces fragments of a range of sizes, which can be separated through gel in a narrow capillary by electrophoresis
- As fragments migrate a laser excites the fluorescent terminator base and a detector records the colour on a chromatogram.



Interpreting chromatograms

Sanger sequencing using capillary gel electrophoresis produces a chromatogram:

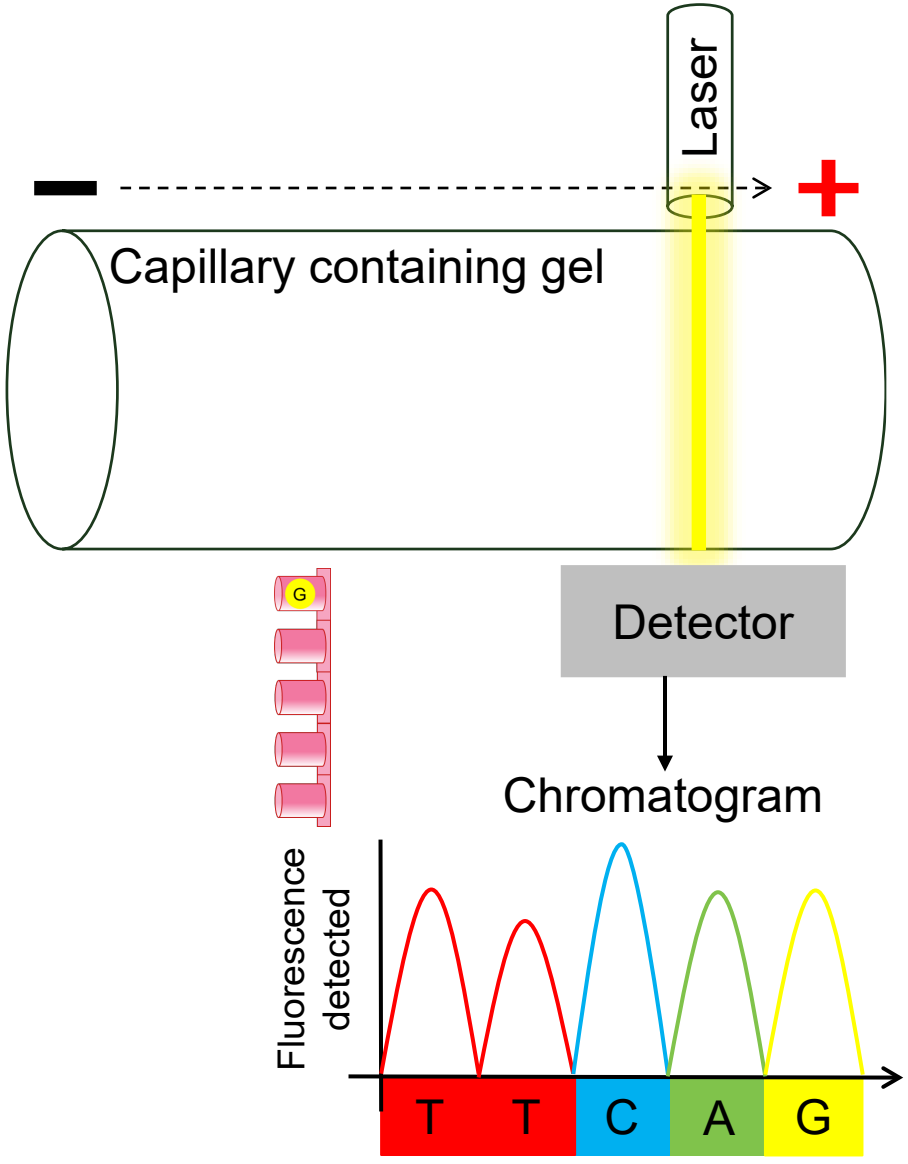
- DNA is denatured and a primer anneals to the DNA (just like in PCR)
- DNA polymerase extends the region of double-stranded DNA (just like in PCR) until a fluorescent terminator nucleotide is added
- This produces fragments of a range of sizes, which can be separated through gel in a narrow capillary by electrophoresis
- As fragments migrate a laser excites the fluorescent terminator base and a detector records the colour on a chromatogram.



Interpreting chromatograms

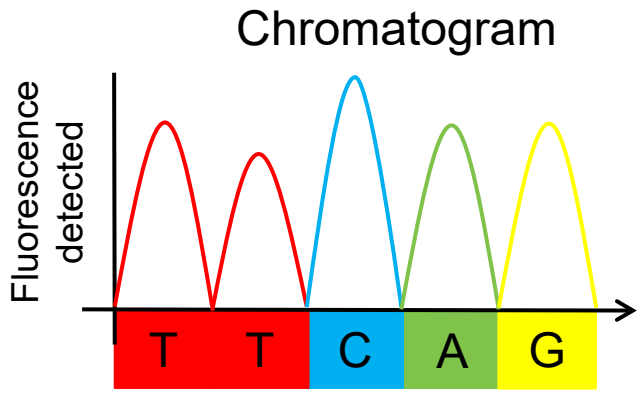
Sanger sequencing using capillary gel electrophoresis produces a chromatogram:

- DNA is denatured and a primer anneals to the DNA (just like in PCR)
- DNA polymerase extends the region of double-stranded DNA (just like in PCR) until a fluorescent terminator nucleotide is added
- This produces fragments of a range of sizes, which can be separated through gel in a narrow capillary by electrophoresis
- As fragments migrate a laser excites the fluorescent terminator base and a detector records the colour on a chromatogram.



Interpreting chromatograms

Sanger sequencing using capillary gel electrophoresis produces a chromatogram. The results of the chromatogram are used to produce a FASTA (Fast-All) file too.



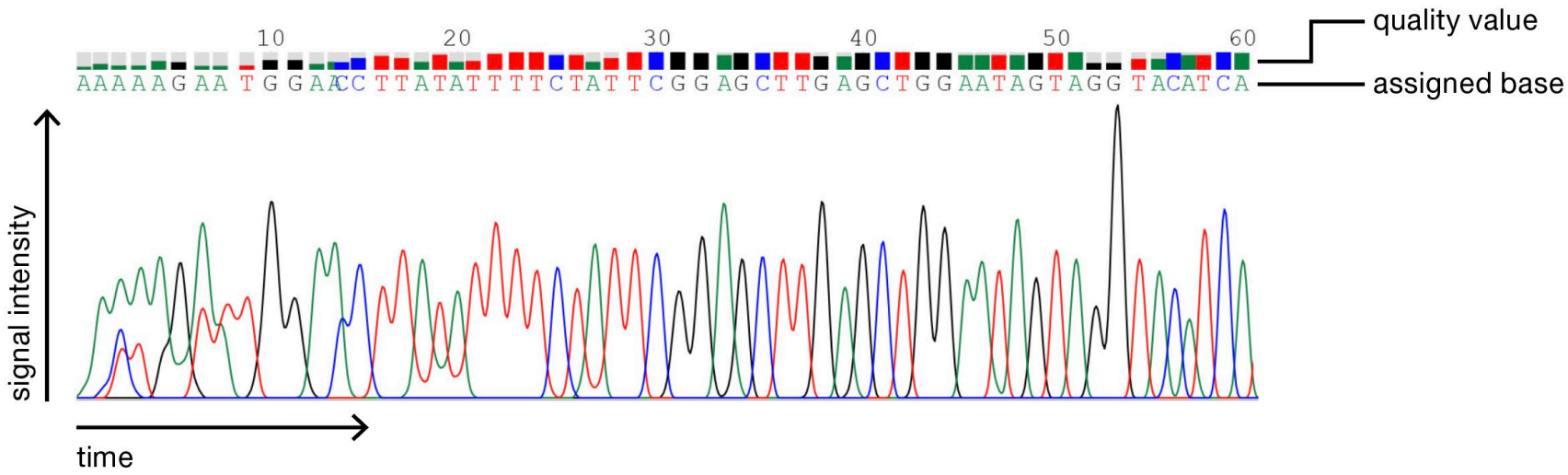

```
>Sequence name
TTCAGGTACAGGATACAATAG
ACTTAGACATAGGCATAGCAC
CCAGATAGGCGGCTGCAATGC
ATACAGCCCCGAGGGGTTACA
```

Interpreting chromatograms

Chromatograms show the strength of the fluorescent signal from a nucleotide, plotted against time (as an indication of where that nucleotide is in the DNA sequence).

Each base has a differently coloured fluorescent tag:

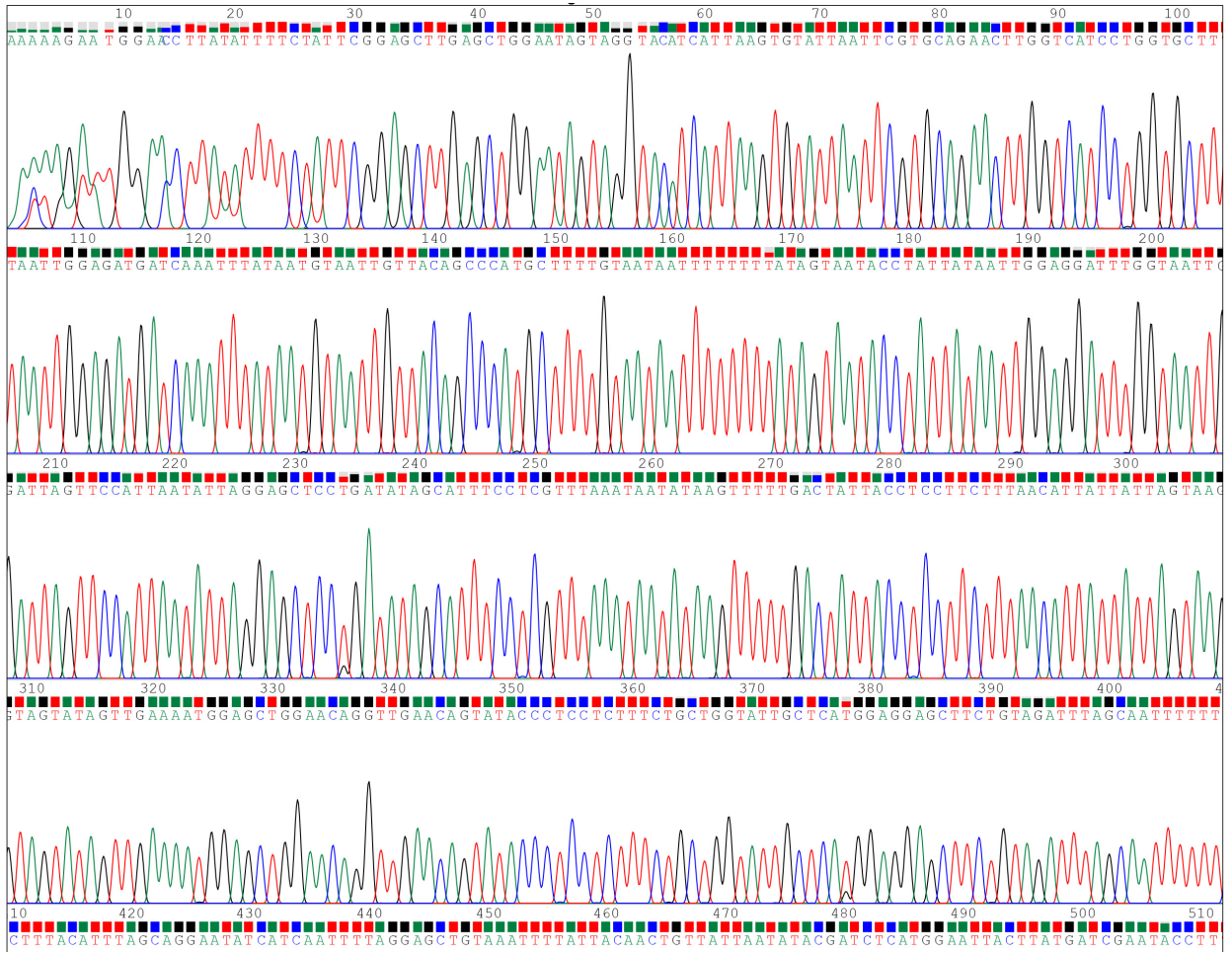
A = green
C = blue
G = yellow
T = red



Interpreting chromatograms

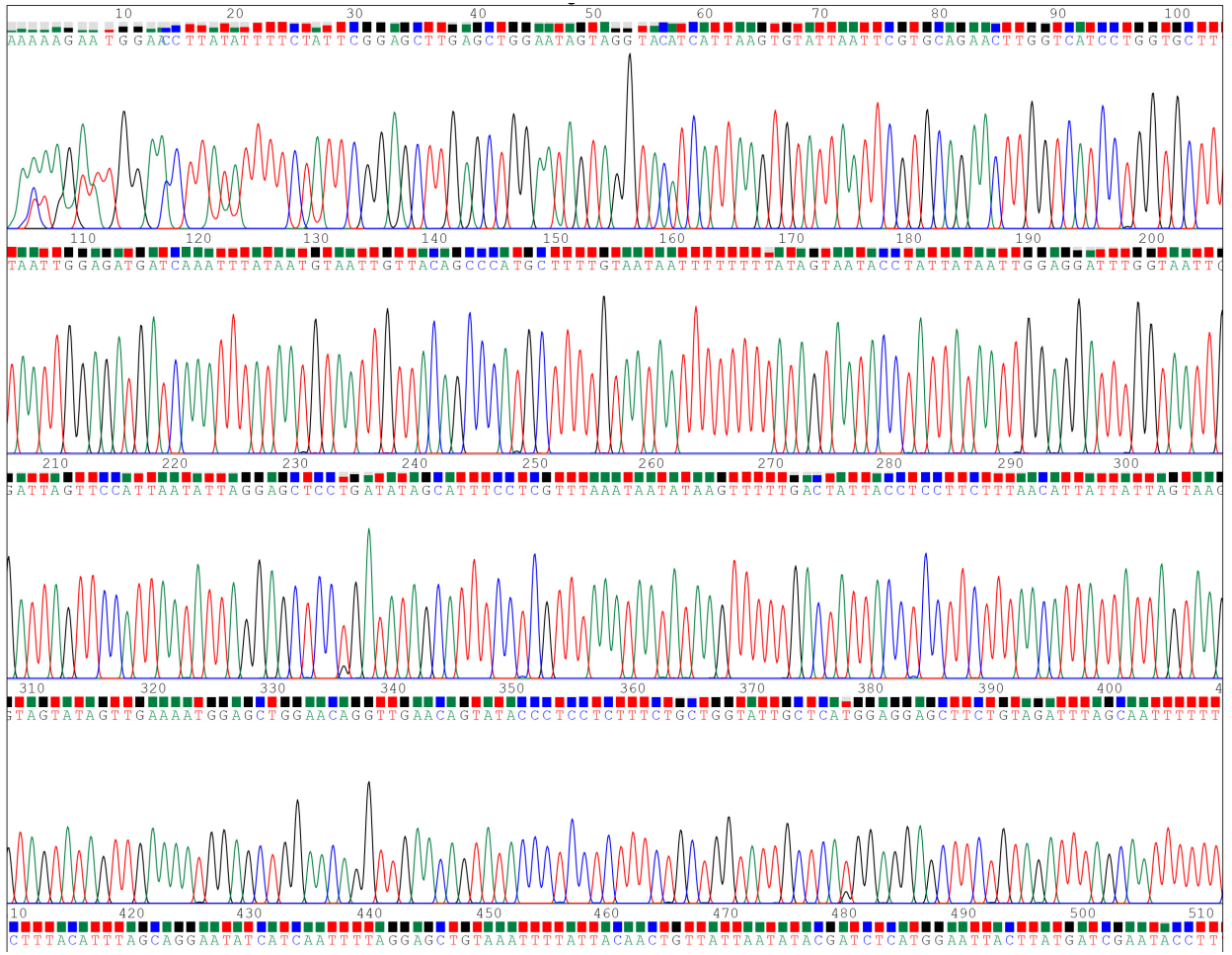
On a chromatogram:

- Good quality, accurate DNA sequence is seen as sharp, evenly-spaced peaks
- The 1st 40 base sequence is often poor
- The best DNA sequence data is usually found between 100 and 500 bases
- The quality of DNA sequence data often deteriorates towards the end of the trace



Interpreting chromatograms

- Find a chromatogram file for the DNA barcode from a fly leg or marmalade hoverfly.
- Follow the printed or digital instructions to view your chromatogram
- Discuss the accuracy of your DNA barcode sequence (using information from the chromatogram)



Identifying invertebrates

Bioinformatics: BLAST

Bioinformatics is the development of software and computing tools to organise and analyse raw biological data.

One way in which bioinformatics is used is for the comparison of DNA sequence.

- Comparison of DNA sequence from known organisms determines how similar DNA sequence is and relatedness.
- Comparison of DNA sequence from an unknown organism to a database of DNA sequences assists identification.

Bioinformatics: BLAST

To compare DNA sequences, a BLAST is used.

BLAST stands for **B**asic **L**ocal **A**lignment **S**earch **T**ool.

Compare the DNA barcode sequence from your invertebrate sample to a database of DNA sequences to try and identify what it is.

Bioinformatics: BLAST

- Find the DNA barcodes in a file named '02-R-UnknownBarcodes'

- Follow the printed or digital instructions to compare these DNA barcodes to the NCBI (**N**ational **C**entre for **B**iotechnology **I**nformation) database using a BLAST

- Complete the table in the file named '02-R-BLASTResults' identifying the 5 invertebrates

Identifying unknown organisms using DNA barcodes

Barcode number	Gene used	Binomial classification	Common name	E value	% identical
1	Cytochrome oxidase subunit 1				
2	Cytochrome oxidase subunit 1				
3	Cytochrome oxidase subunit 1				
4	Cytochrome oxidase subunit 1				
5	Cytochrome oxidase subunit 1				

Bioinformatics: BLAST

- Check whether you managed to successfully identify the 5 unknown species using DNA barcode data.

Barcode number	Gene used	Binomial classification	Common name	E value	% identical	
1	Cytochrome oxidase subunit 1	*Coccinella septempunctata*	7-spot ladybird	0.00	100	
2	Cytochrome oxidase subunit 1	*Oniscus asellus*	Common woodlouse	0.00	100	
3	Cytochrome oxidase subunit 1	*Agelena labyrinthica*	Labyrinth spider	0.00	100	
4	Cytochrome oxidase subunit 1	*Myzus persicae*	Green peach aphid	0.00	100	
5	Cytochrome oxidase subunit 1	*Gonepteryx rhamni*	Brimstone butterfly	0.00	100