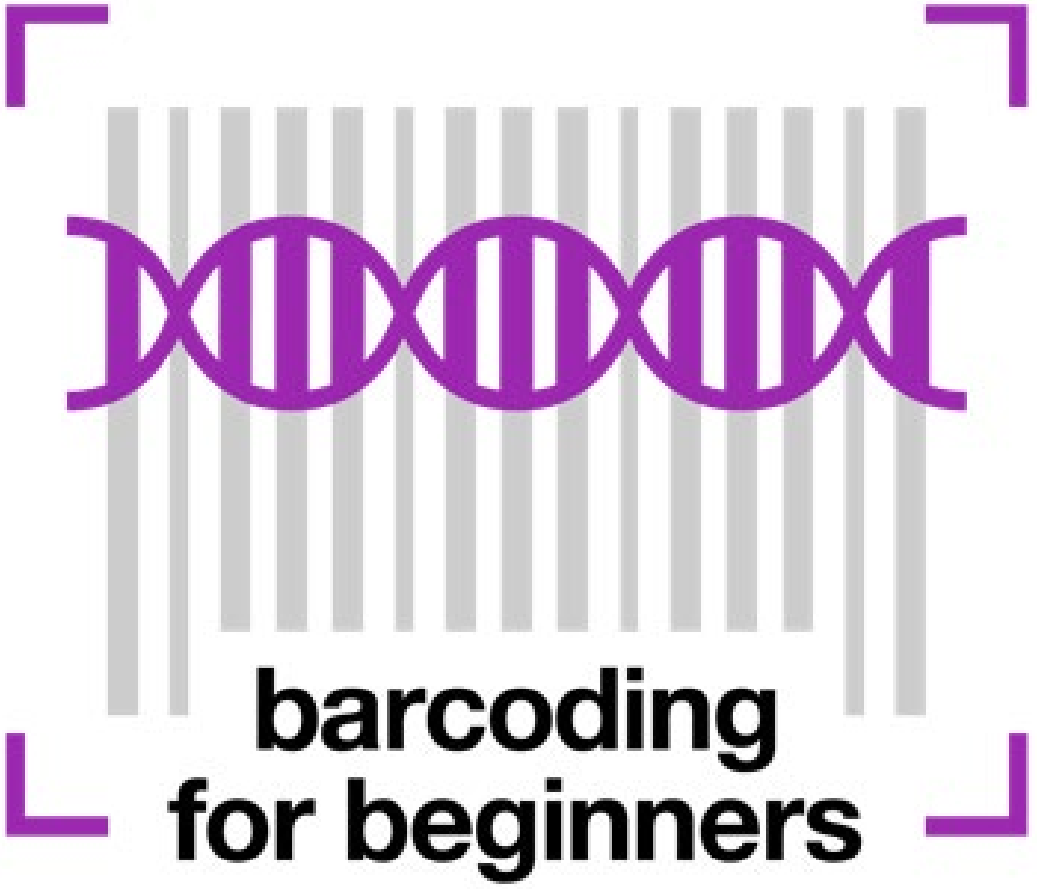
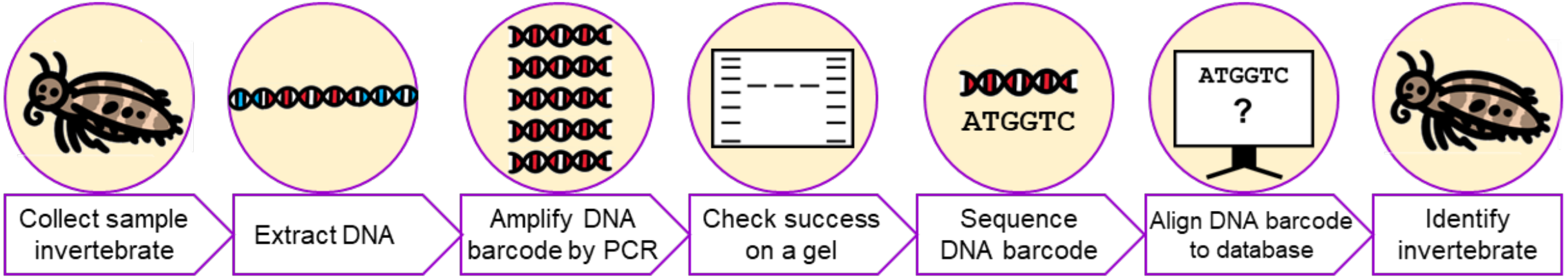
Laboratory safety

Overview of practical activities



Overview of practical activities

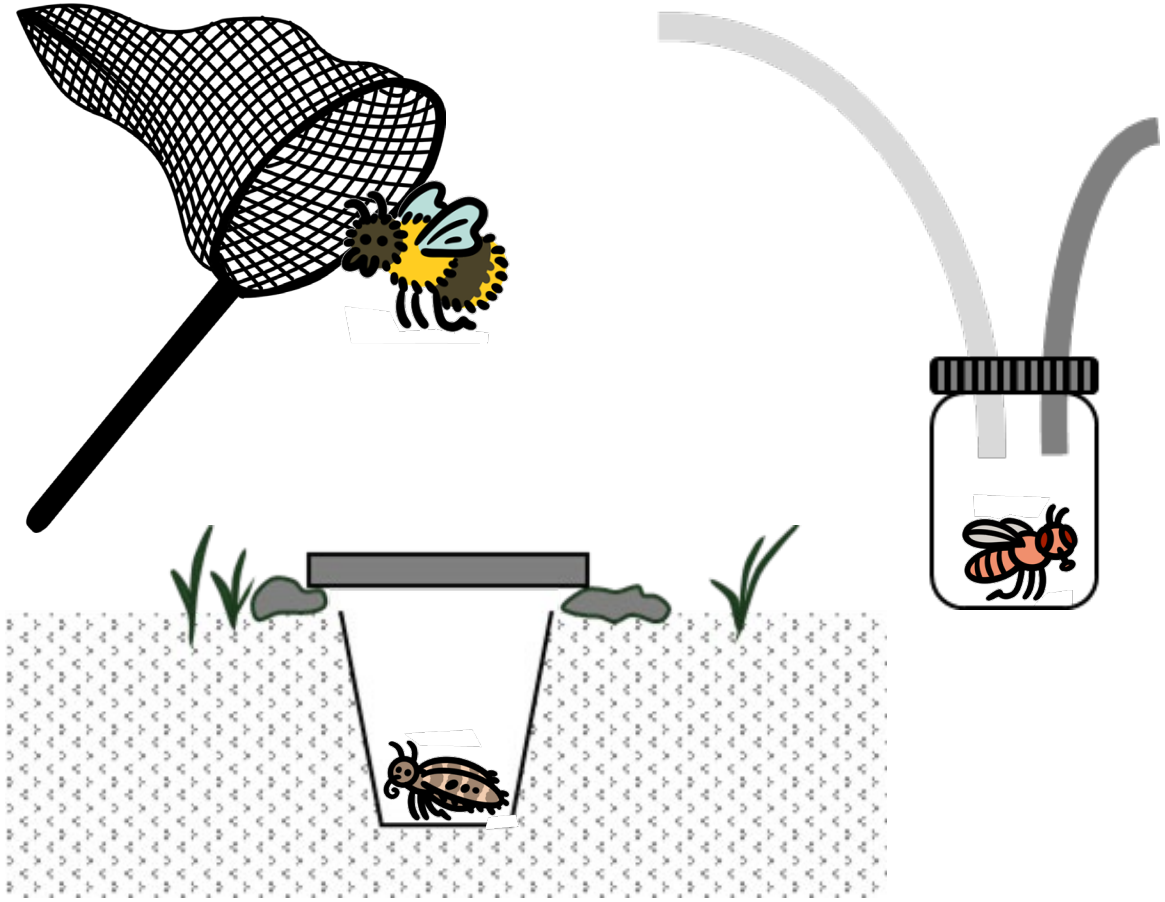
The process for DNA barcoding involves collecting a sample, extracting DNA, amplifying (making more of) the DNA barcode using PCR, checking success using gel electrophoresis, sequencing the DNA barcode then identifying the organism it came from by comparison to an on-line database.



Overview of practical activities

We have collected an invertebrate to use in your project.

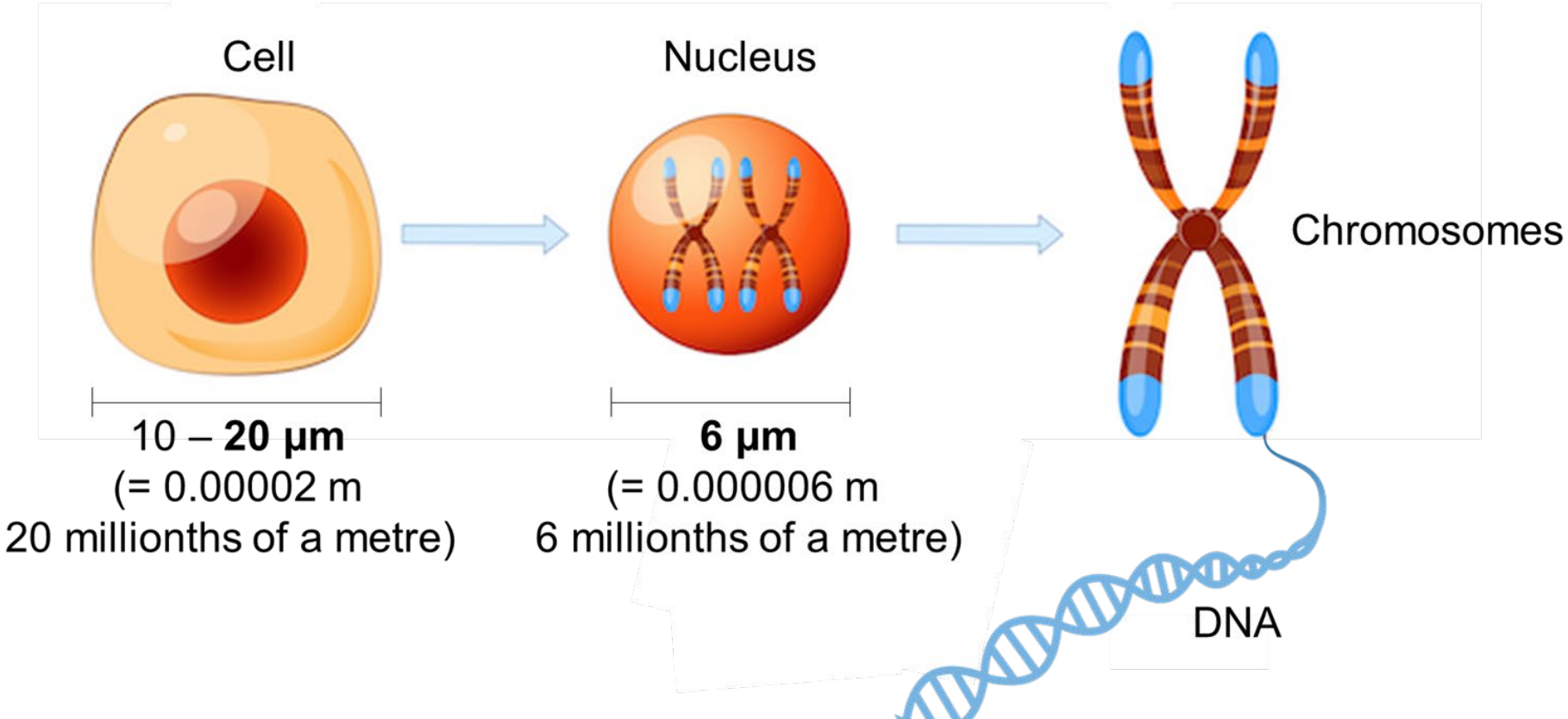
Whilst there are lots of ways to do this, we have found freshly deceased invertebrates on windowsills. These have been stored in a Ziplock bag in the freezer (at -20°C).



Mastering micropipetting

Mastering micropipetting

Molecular biology involves working with very small samples of material.



Mastering micropipetting

DNA is a very important, but very small molecule.

The DNA from **one** human body cell (a human genome) is:

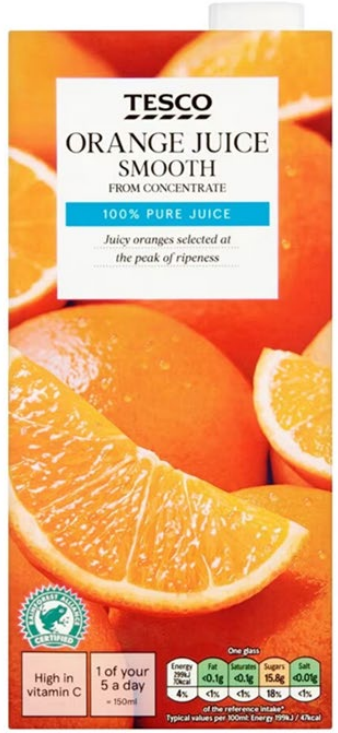
- ~ 2 metres long
- ~ 2.5 nm wide (0.0000000025 metres)
- ~ 6 picograms of DNA (0.000000000006 g)

Mastering micropipetting

Working with very small molecules, like DNA, means small samples, and requires accurate measurement of very small quantities.

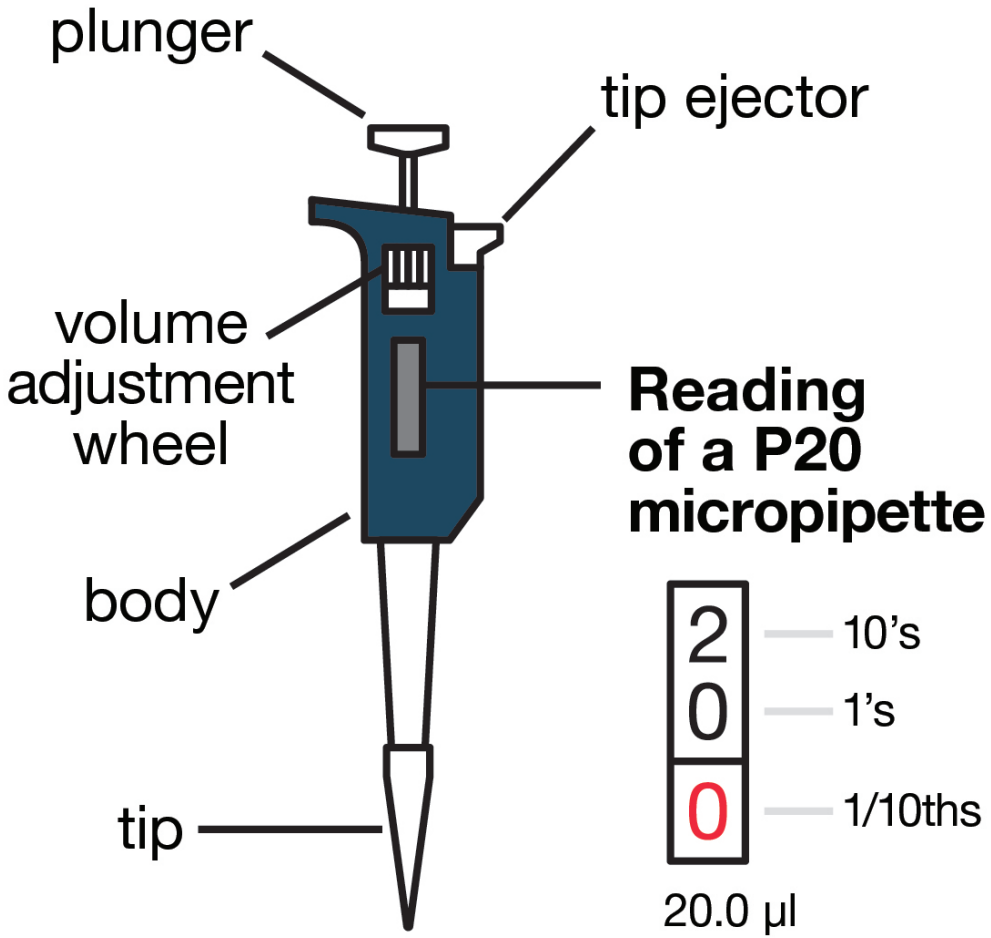
DNA samples are commonly used in microlitre volumes. To give an idea of how small this is, the diagram shows:

- 1 litre of orange juice
- 1 millilitre (ml) in a tube = 1 x 10^{-3} litre
- 1 microlitre (µl) in a dot = 1 x 10^{-6} litre



Mastering micropipetting

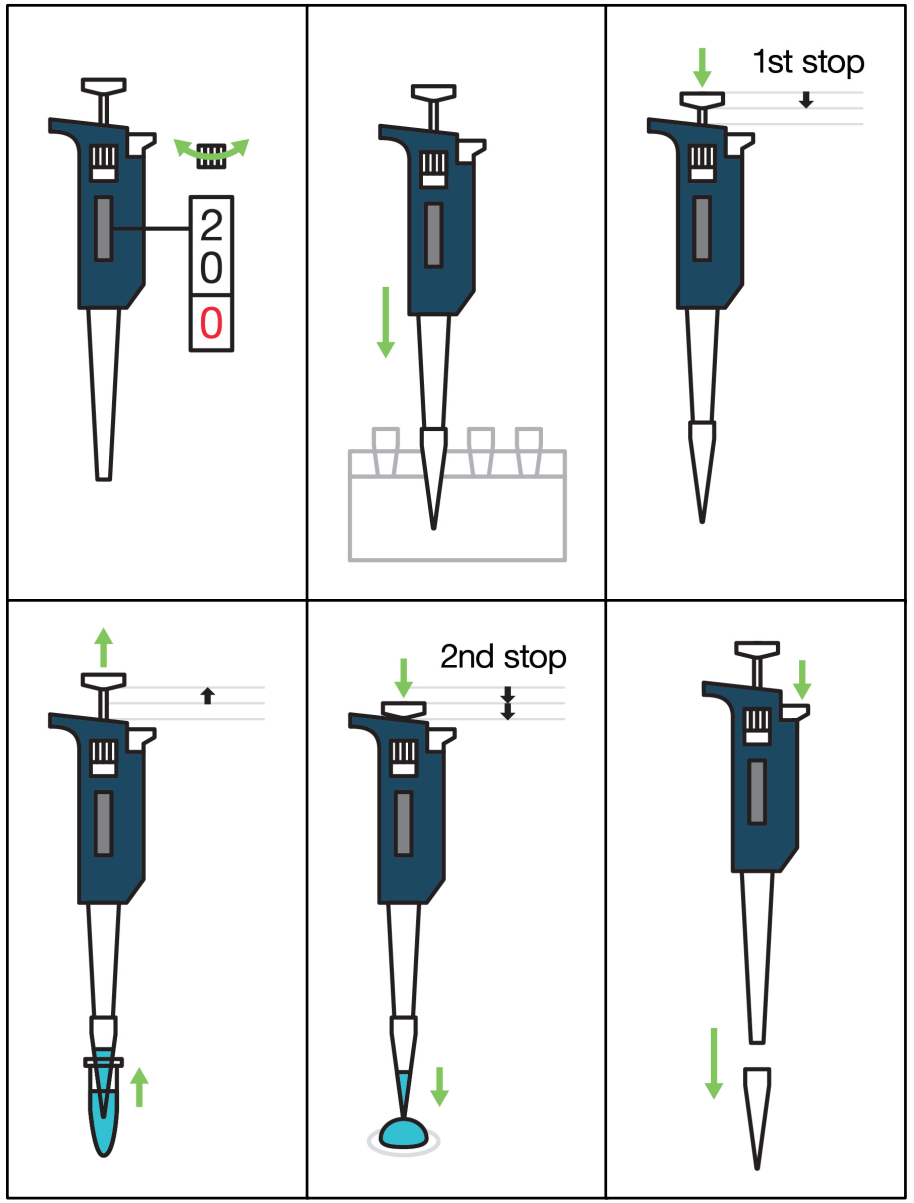
A micropipette is used to dispense small volumes accurately.



Mastering micropipetting

To use a micropipette:

- Set the volume
- Put on the tip
- Press plunger to first resistance
- Insert tip into solution
- Slowly release plunger
- Place tip ready to dispense
- Press and hold plunger right down
- Remove tip from liquid
- Release plunger
- Eject tip



Mastering micropipetting

- Use the micropipette to accurately dispense the liquid labelled **FD** onto the laminated micropipetting target practice sheet in the volumes shown.

Micropipetting target practice

Name	20 µl	15 µl	10 µl	5 µl	2 µl
1. _____					
2. _____					

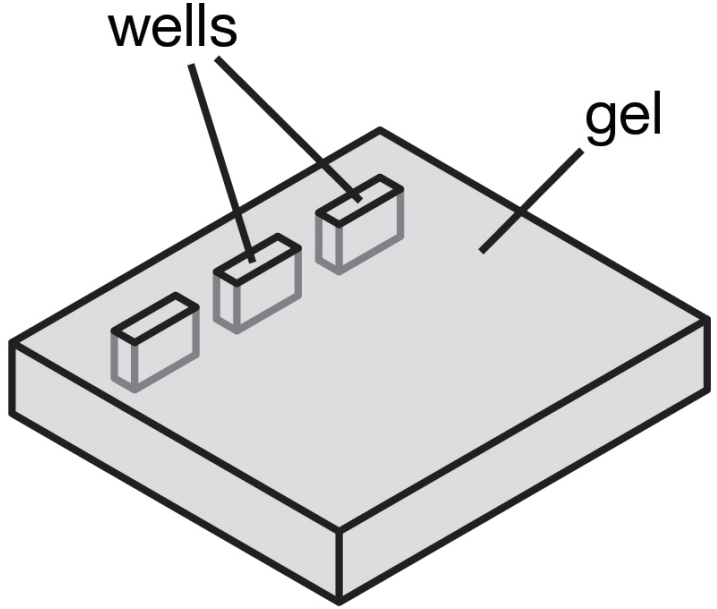
Exploring gel electrophoresis

Exploring gel electrophoresis

Agarose gel electrophoresis allows separation of a mixture of biomolecules (often DNA) by size.

An agarose gel is a slab of jelly, which acts like a molecular sieve, with wells (pocket-like holes that go partway through the gel) that samples are loaded into.

Electrophoresis is the use of an electrical current to move the biomolecules through the agarose gel. DNA is negatively charged, so it moves toward the positive electrode.



Exploring gel electrophoresis

The agarose gel matrix is visible under a scanning electron microscope.

The rate at which molecules can move through the tunnel-like matrix depends on:

- size – smaller molecules move more rapidly
- electrical charge – molecules with greater charge to mass ratio move more rapidly
- shape – compact molecules move more rapidly

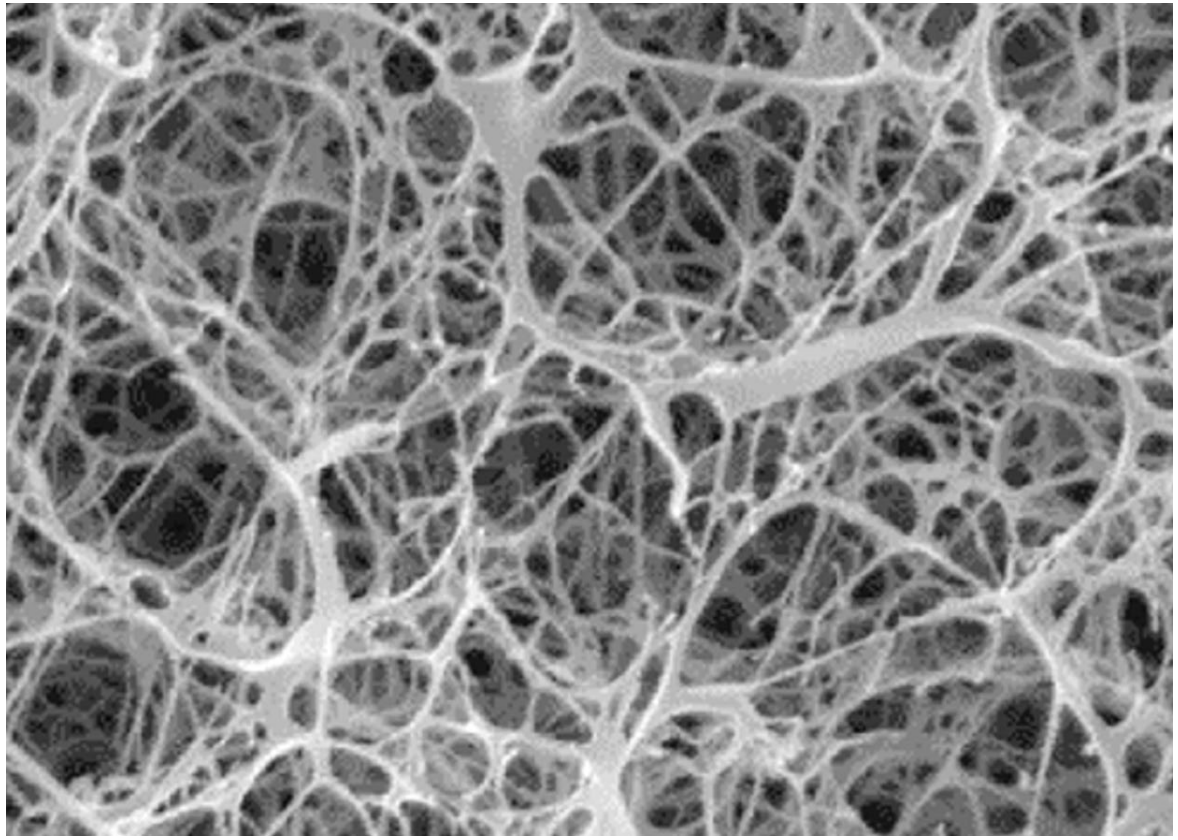
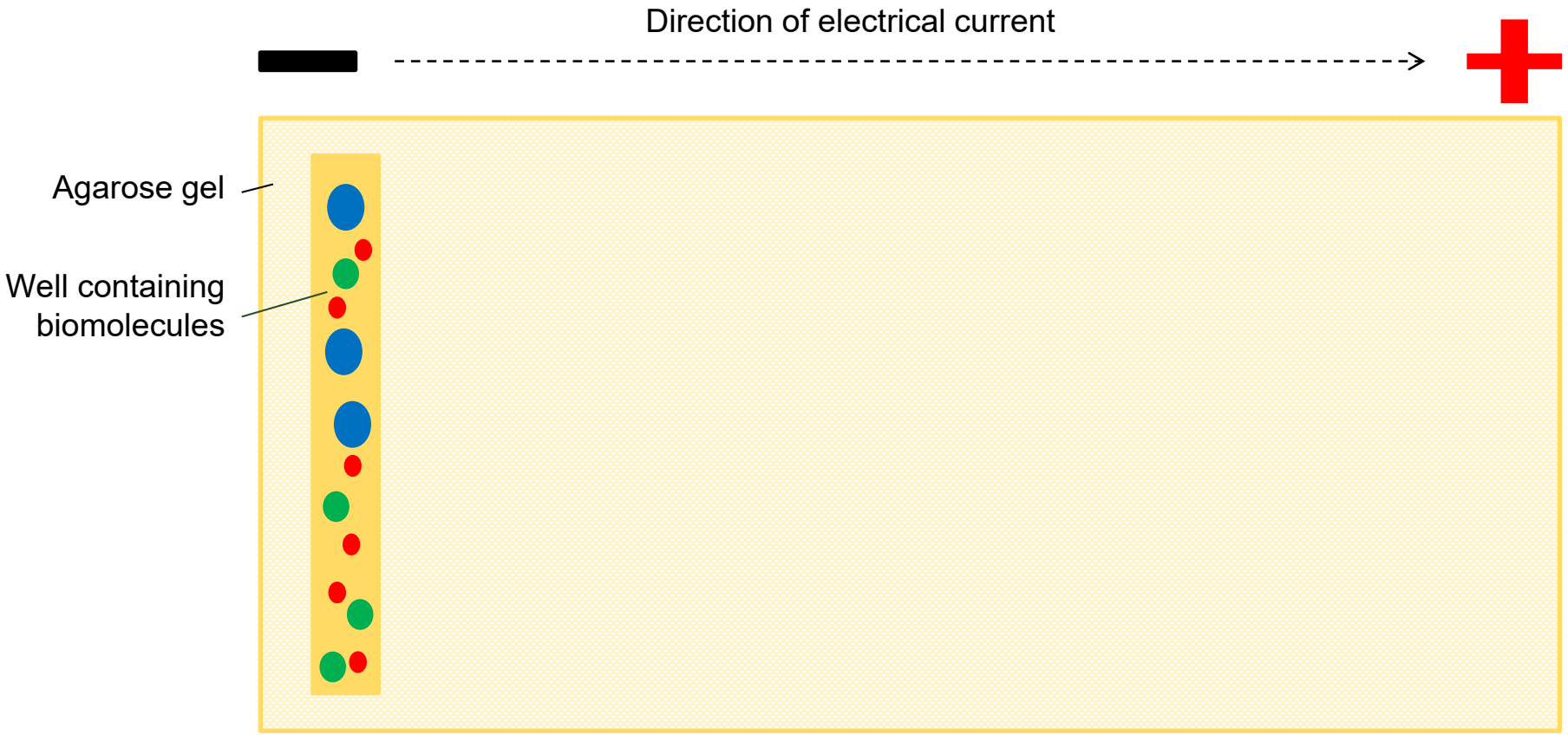


Image credit: MiniPCR
(https://www.minipcr.com/choosing-the-right-agarose-percentage/)

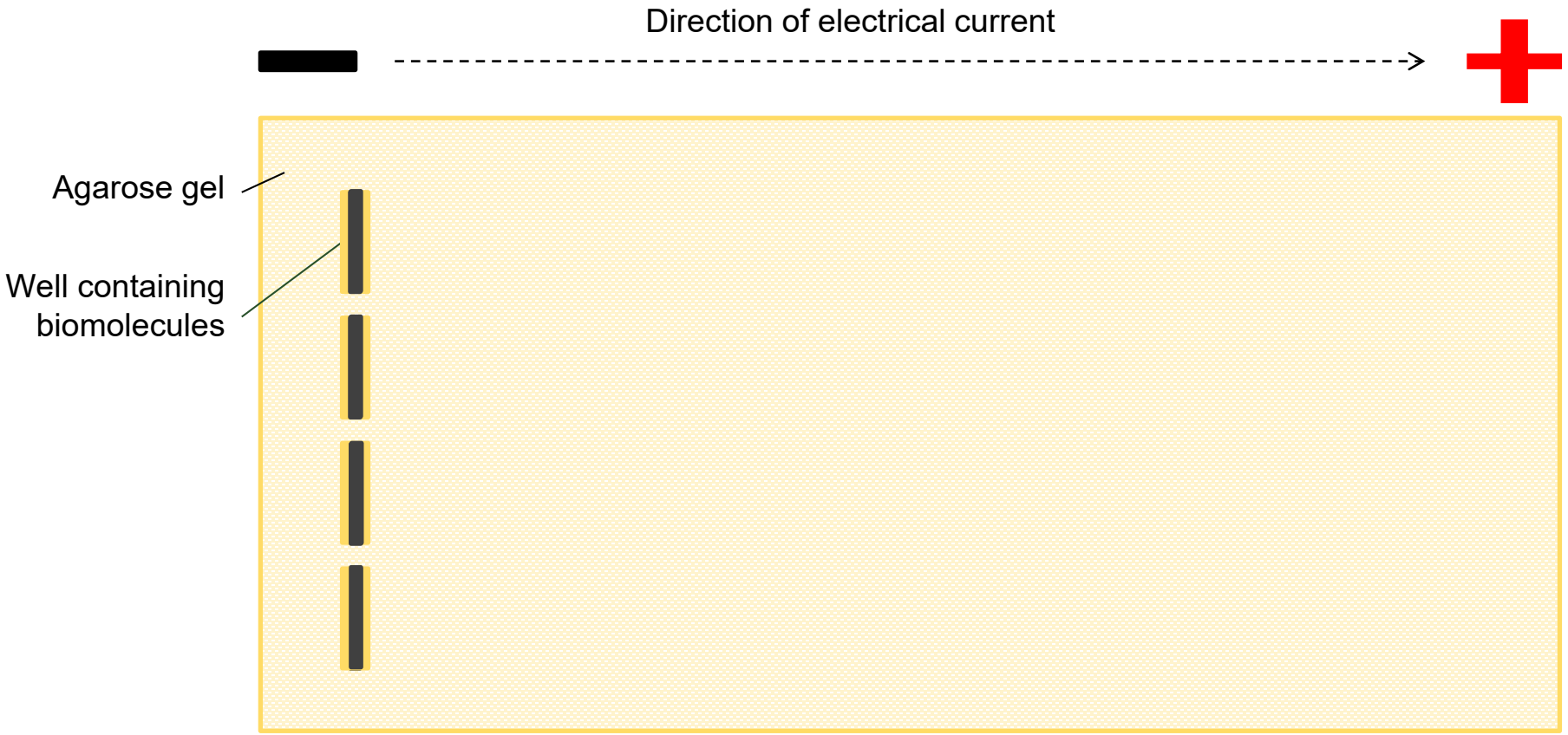
Exploring gel electrophoresis

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



Exploring gel electrophoresis

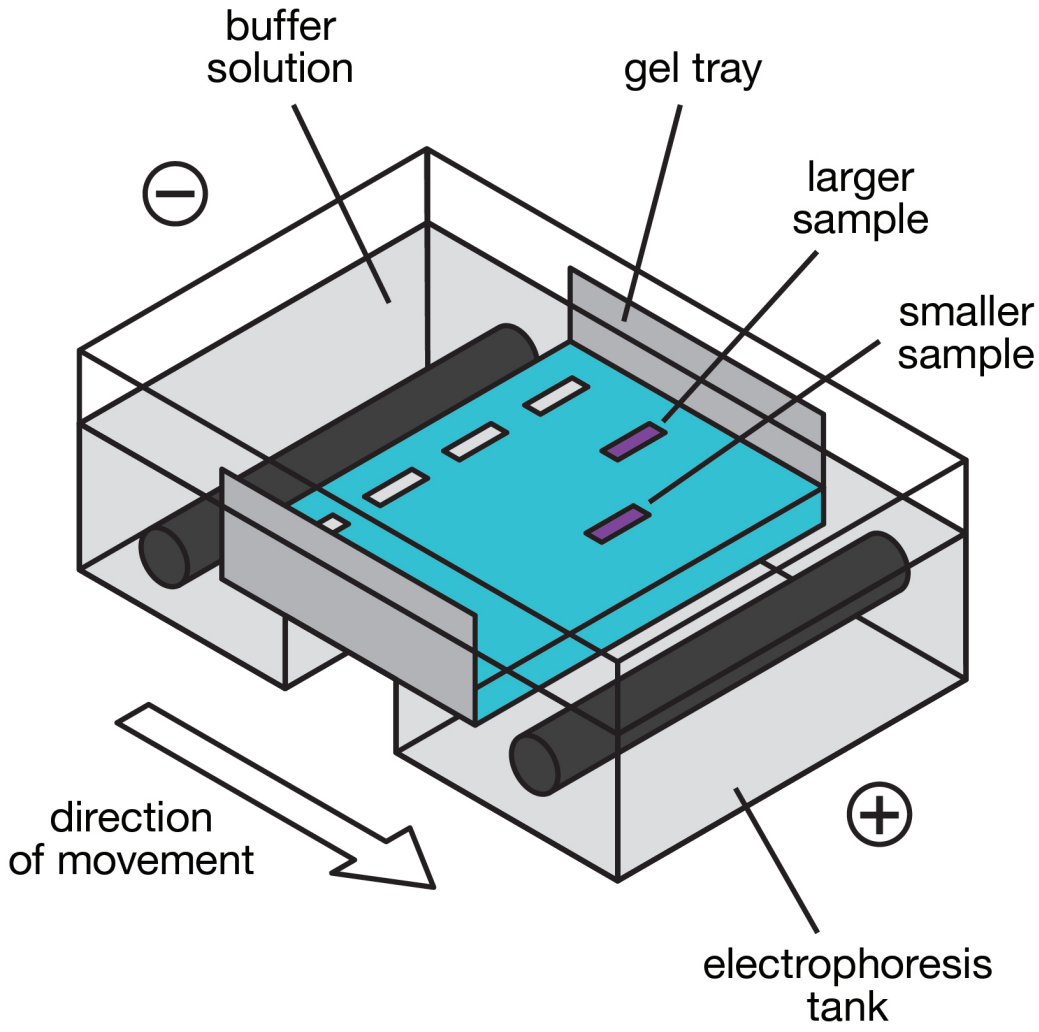
Gels can determine the number and relative size of biomolecules in several samples at once.



Exploring gel electrophoresis

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

- 4 dye solutions are provided
- The agarose gel is in the electrophoresis tank ready for use



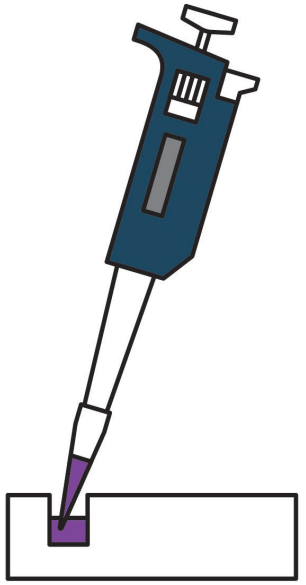
Exploring gel electrophoresis

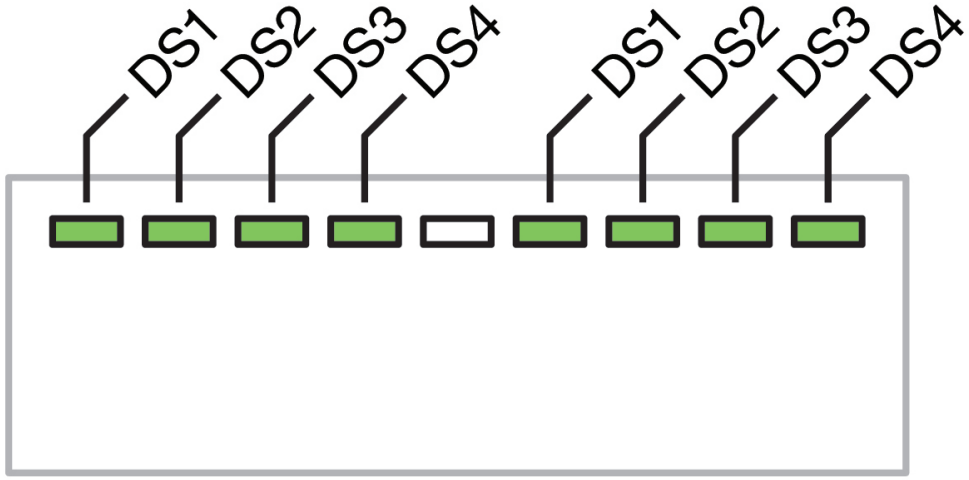
Before performing electrophoresis, you should put on gloves and safety glasses.

- Load 10 µl of dye solutions 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*

- Each person should load 1 sample
- When the gel is loaded, place the orange lid on and press the power button to start electrophoresis
- Start a timer for 20 minutes



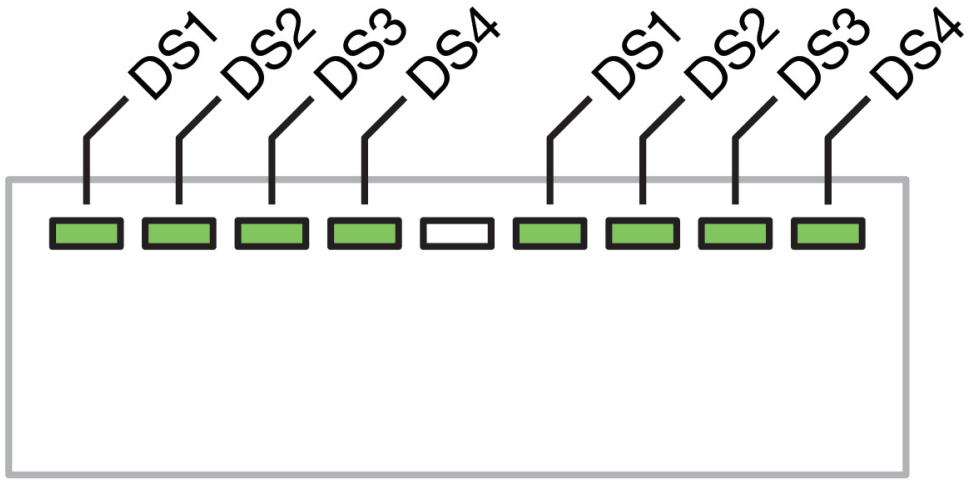


Interpreting gel electrophoresis

- When 20 minutes have passed, unplug the electrophoresis tank at the socket

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

- This person should carefully lift the gel out of the buffer and onto a white piece of paper
- Other people from the group should take a photograph of the results for analysis
- The person in gloves and safety glasses should then place the gel into the autoclave bag and empty the buffer from the gel carriage into the sink, rinsing it with plenty of water



Interpreting gel electrophoresis

Using the photo of the gel answer the questions:

A. Which dye solution contains only one dye?

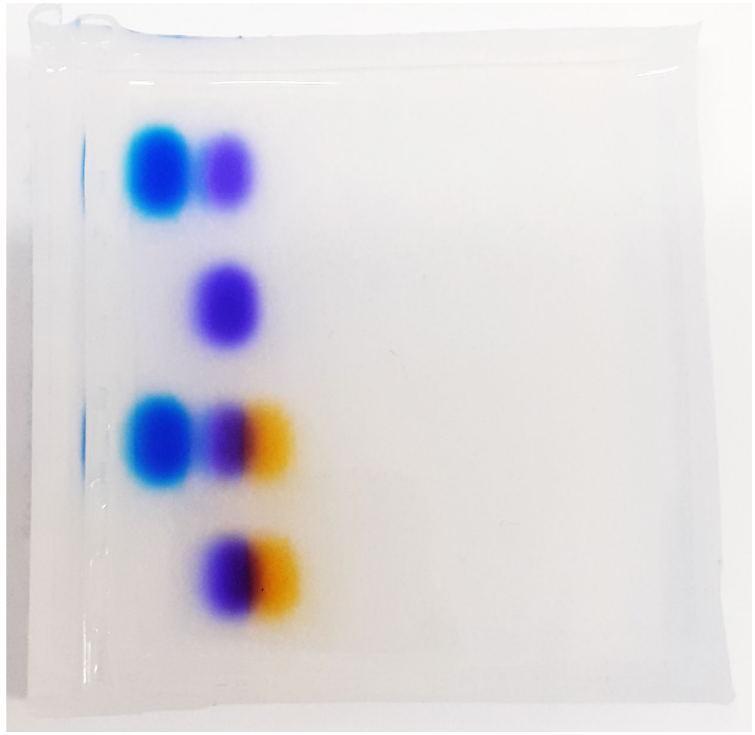
Dye solution 2.

B. Which dye solutions contain 2 dyes?

Dye solutions 1 and 4.

C. Which dye solution contains 3 dyes?

Dye solution 3.



Interpreting gel electrophoresis

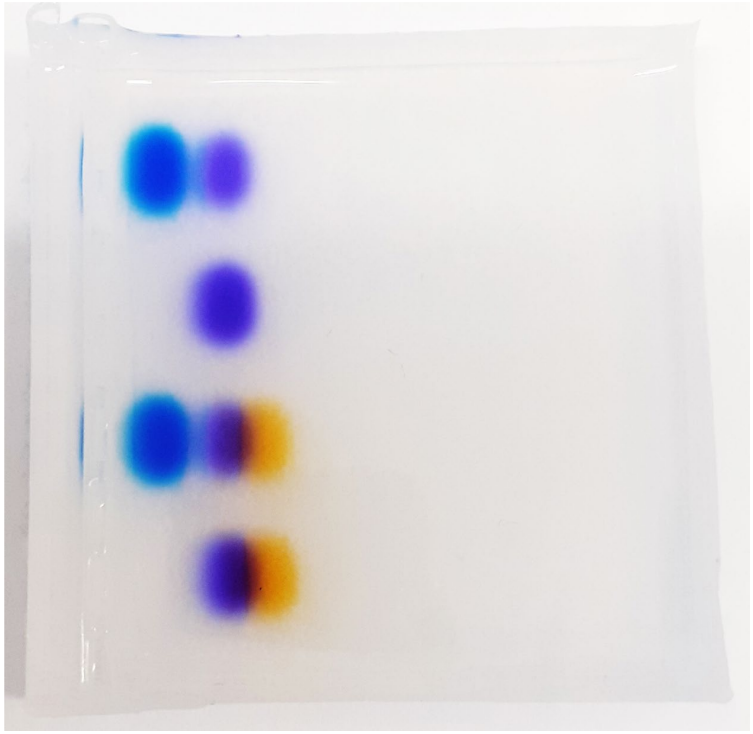
Using the photo of the gel answer the questions:

D. What electrical charge do the dyes have?

Negative.

How do you know?

In an electrical field they move towards the positive electrode.

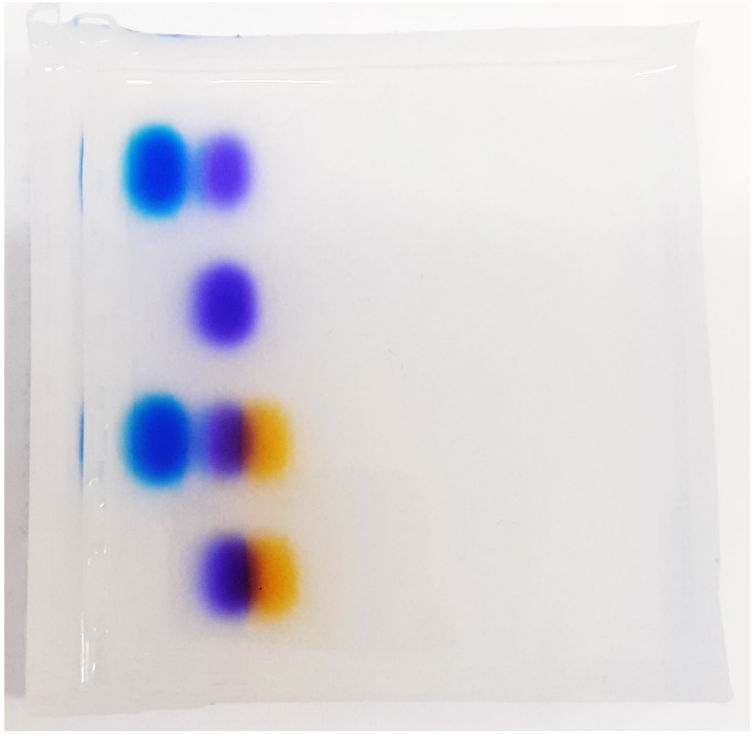


Interpreting gel electrophoresis

- The purple dye is bromophenol blue.
- The light blue dye is xylene cyanol.
- The orange dye is Orange G.

Use the photo of the gel and information above to answer the question:

E. From your gel electrophoresis results, put the dyes into size order from largest to smallest. Explain your reasoning.

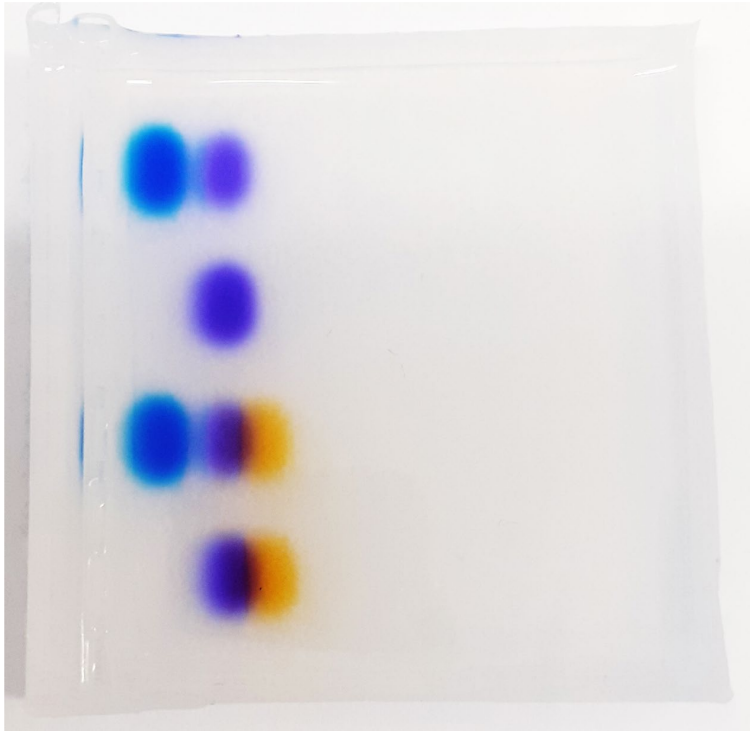


Light blue (xylene cyanol), purple (bromophenol blue), orange (Orange G)
Xylene cyanol has moved least distance, so is the largest, whilst Orange G has moved the furthest, so is the smallest.

Interpreting gel electrophoresis

- The purple bromophenol blue has molecular weight 669,98 au.
- The light blue xylene cyanol has molecular weight 538.62 au.
- The orange, Orange G has molecular weight 452.38 au.

F. How do these weights compare with your original conclusions about the sizes of the dye molecules?



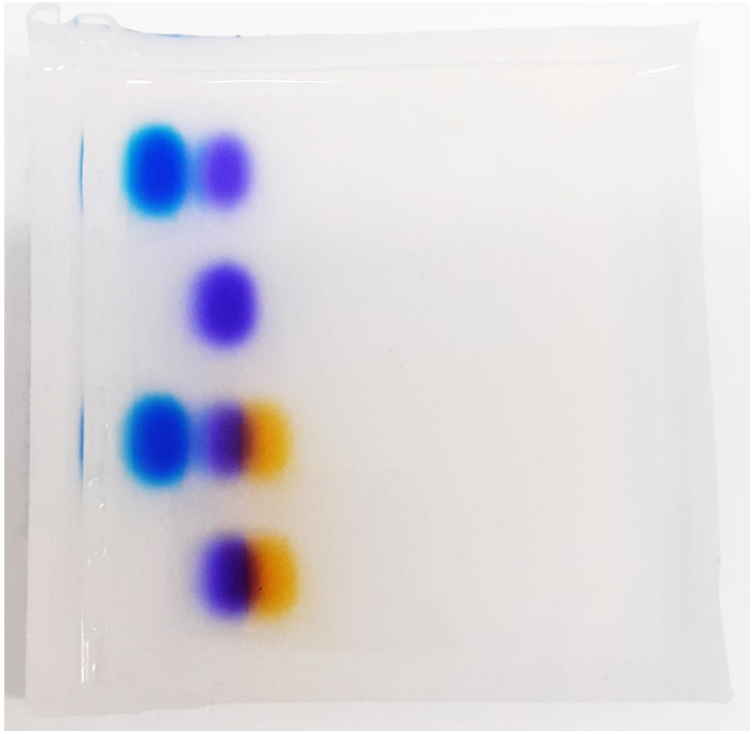
The light blue dye (xylene cyanol) and purple dye (bromophenol blue) are the opposite way around compared to expectations. They have not separated based on size alone.

Interpreting gel electrophoresis

G. What factors other than molecular weight may have played a role in the separation of these dyes by electrophoresis?

Charge and shape.

Whilst larger molecules will move less distance than smaller molecules if both have the same charge, molecules with more negative charge will move faster than molecules with less negative charge if both have the same weight.



Despite, xylene cyanole (538.62 au) having a smaller size than bromophenol blue (669.98 au) it migrates more slowly than bromophenol blue because xylene cyanole has a smaller charge to mass ratio.

Next practical activities

After lunch you will be applying your skills in micropipetting and gel electrophoresis to extract DNA from invertebrates and amplify the DNA barcode by PCR.