

03 | Pipetting for electrophoresis



Equipment list

Laboratory work station

- P20 micropipette
- Box of micropipette tips
- Waste container
- Microfuge tube of **FD** (food dye)
- Laminated micropipetting target practice sheet
- Non-permanent marker

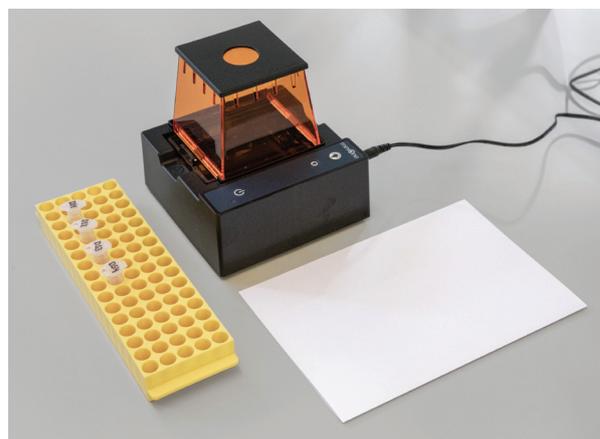
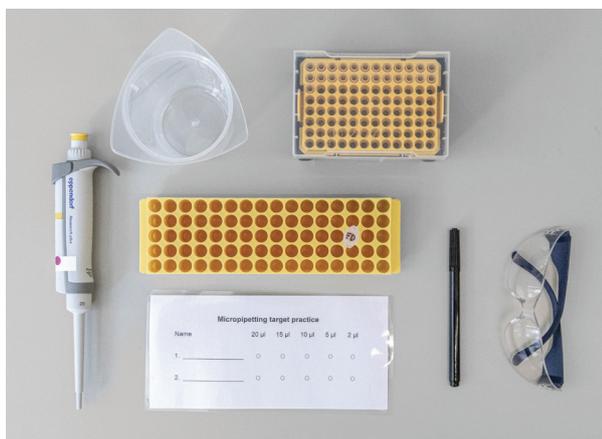
Electrophoresis station

The 4 electrophoresis stations will each have:

- MiniOne electrophoresis gel tank with a 9-well, 1% agarose gel in 1 x TAE buffer
- Dye solutions:
 - Microfuge tube of **DS1** (dye solution 1)
 - Microfuge tube of **DS2** (dye solution 2)
 - Microfuge tube of **DS3** (dye solution 3)
 - Microfuge tube of **DS4** (dye solution 4)
- A white piece of paper

Class equipment

- Gloves
- Safety glasses



Health and safety

All students working in a science lab are expected to follow good laboratory practice, including: not eating or drinking in the lab, tying back long hair, keeping lab benches clear of clutter, clearing up spills immediately, handling materials and equipment with care, and washing hands with soap after completing lab work.

Equipment

Care should be taken when using a micropipette to eject tips downwards into a waste disposal container.

There will be an electrical potential of 42 V across the MiniOne gel and through the buffer during electrophoresis. To prevent access to liquids carrying a 42 V current, the orange top covers the buffer chamber. The electrical supply will not flow if:

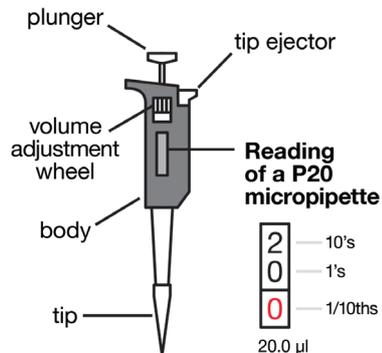
- The photo hood is removed
- The tank is not properly placed inside the base unit, and electrodes are not making contact
- There is no, or insufficient, buffer in the buffer chamber
- The buffer is too dilute

Reagents

Substance	Hazard	Precautions
Food dye (FD)	Food safety standard, but can stain skin.	Use good laboratory practice to avoid contact with skin.
Dye solutions (DS 1, 2, 3, 4) All contain 0.1M Tris pH 8.0 and 10% glycerol. You will determine whether they contain 0.1% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue, and/or 0.1% (w/v) Orange G.	Tris buffer (pH8.0), glycerol, bromophenol blue and Orange G are classed as non-hazardous. Xylene cyanol can cause serious eye irritation in powder form, but at this concentration in solution, it is classed as non-hazardous.	As a precaution students should wear eye protection (safety glasses are sufficient) and gloves when handling.
TAE buffer (Tris, EDTA, Acetic acid)	A 1 x concentration of TAE gel electrophoresis buffer contains no substances at a concentration that is considered to be hazardous to health.	As a precaution students should wear eye protection (safety glasses are sufficient) and gloves when handling.
Agarose gel	Agarose and the 1 x TAE buffer it is melted in are not hazardous substances.	As a precaution students should wear eye protection (safety glasses are sufficient) and gloves when handling.

Instructions | Mastering micropipetting

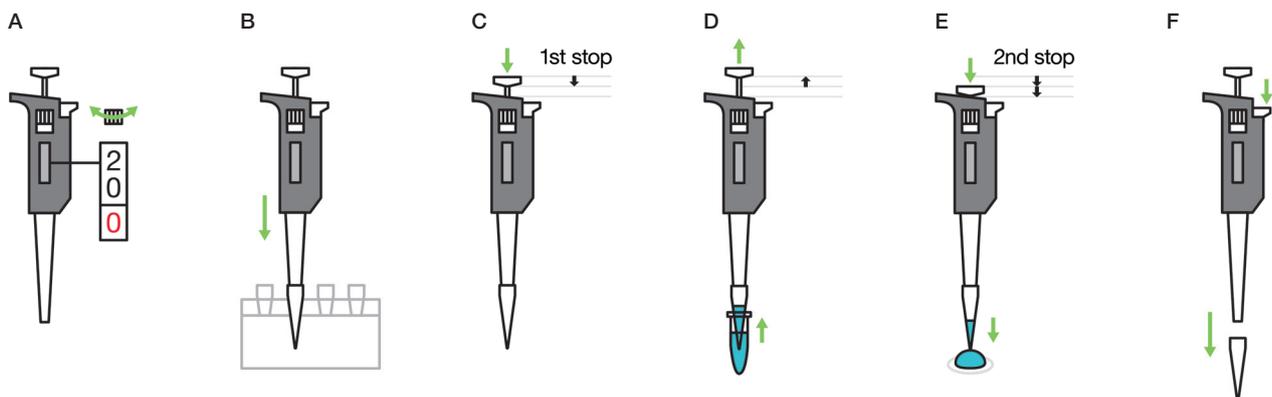
Parts of a micropipette



The volumes that a micropipette can accurately dispense are written on it. In this session you will be using a P20 micropipette, which can accurately dispense between 2 and 20 µl of liquid. Setting the volume outside of this range can damage the accurately calibrated mechanisms inside the micropipette.

The display window shows how many microlitres the micropipette is set to dispense. On a micropipette that dispenses up to 20 microlitres, the largest number will be tens of microlitres, the third digit will show tenths of a microlitre.

Micropipetting method



- To use a micropipette turn the volume adjustment wheel to set the correct volume in the display window.
- Once the volume is set, hold the body comfortably in your hand with your thumb on the plunger. Place a tip on the micropipette, by bringing it down gently into a tip in the tip box.
- Next, push the plunger down gently until you meet resistance. This is known as the first stop. A volume of air equal to the volume of liquid to be dispensed will be pushed out of the tip.
- Place the tip in the liquid to be aspirated (sucked up). Move your thumb slowly upwards to aspirate the correct volume of liquid. Moving your thumb upwards too fast can result in liquid being distributed up the inside of the tip.
- Now place your tip where the sample is to be dispensed. This time push the plunger gently, but firmly beyond the first stop until it can't go any further. This is the second stop and will dispense all of the liquid from the tip. Take care to remove the tip from the liquid before releasing the pressure from your thumb, or the liquid will be aspirated again.
- Position the micropipette over the waste disposal container and press the tip ejector button to remove the tip. When dispensing different quantities of food dye the same tip can be used multiple times. When dispensing DNA samples, it is important to change tips between samples to avoid contamination.

- 1. Pick up the micropipette and set the volume to dispense **20 µl** by twisting the volume adjustment wheel round. When correctly adjusted the display window should read 2-0-0.

- 2. Put a tip onto the end of the micropipette and check it isn't too loose using your fingers.

- 3. Holding the body of the pipette, with your thumb on the plunger, push the plunger down until you first encounter resistance. This is known as the first stop.

- 4. Insert the tip into the blue liquid labelled **FD**, keeping your thumb pressed down to the first stop on the plunger.

- 5. Slowly release the plunger by moving your thumb upwards and the blue food dye should be drawn into the tip.

- 6. Place the tip over the spot for 20 µl on the micropipetting target sheet.

- 7. Press the plunger all the way down to the second stop. This will cause all of the liquid to leave the tip.

- 8. Remove the tip from the liquid before slowly releasing your thumb from the plunger.

- 9. Point the micropipette downwards into a waste disposal container and press the tip ejector button to release the tip.

- 10. Reset the micropipette to dispense **15 µl**. This will read 1-5-0 in the display window. Using the same technique as before, aspirate 15 µl of the blue liquid labelled FD and dispense it onto the micropipetting target sheet.

- 11. Repeat to dispense **10 µl**, **5 µl** and **2 µl**.

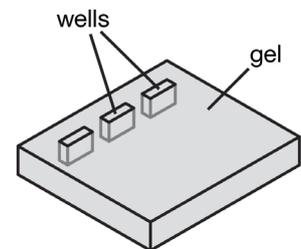
- 12. Observe the difference in volume between the droplets of liquid dispensed.

Instructions | Exploring electrophoresis

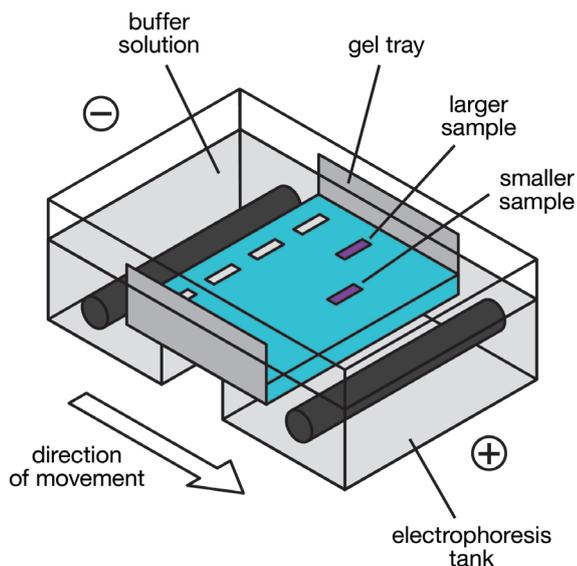
Preparing the agarose gel

An agarose gel is a solid, jelly-like slab, with wells (pocket-like holes that go partway through the gel), which samples are loaded into. It is used in electrophoresis, a procedure using an electrical current to move biomolecules through the agarose gel and acts like a molecular sieve, separating biomolecules by size.

An agarose gel is prepared by heating agarose in a buffer that maintains the pH. This solution is poured into a gel tray, with a comb resting in it. When the gel solidifies, the comb is removed creating the wells for samples to be loaded into.



▲ *Wear nitrile gloves and safety glasses when working with the electrophoresis buffer.*



Performing agarose gel electrophoresis

Agarose gel electrophoresis allows a mixture of DNA molecules to be separated by size. Separation is also affected by charge and shape.

To perform electrophoresis the gel is placed into a gel tank with electrophoresis buffer. Then DNA molecules are loaded into the wells and an electric current applied across the gel. DNA is negatively charged, so when an electrical current flows through the gel, the DNA moves toward the positive electrode.

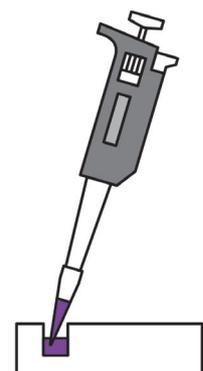
The agarose gel has a molecular structure like a honeycomb, through which smaller samples will move faster and larger samples will move more slowly. This separates the samples by size.

Loading samples for gel electrophoresis

To load samples into the wells of your gel you will need to use a micropipette, positioning the tip **just above** the well. For this exploration of electrophoresis you will load different samples of negatively-charged dye into the wells.

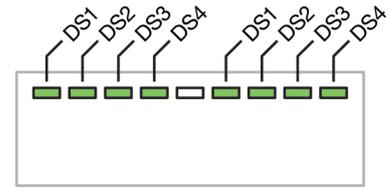
There are 4 dye solutions, labelled **DS1**, **DS2**, **DS3** and **DS4**. Each dye solution is more dense than the electrophoresis buffer, so the solutions will fall into the wells.

▲ *It is important not to put the tip too far into the well, as it can tear the agarose between two wells, or between one well and the bottom of the gel. If this happens your sample will either spread into more than one well or be lost out of the well altogether.*



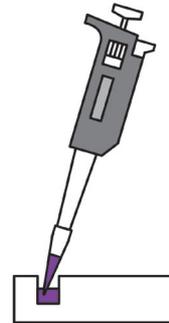
1. Reset the micropipette to dispense 10 μ l.

Your teacher will tell you which dye solution you will load into which well. Each gel has been made with 9 wells, so 2 sets of dye solutions can be loaded onto each gel.

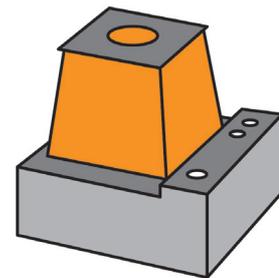


2. Using the same technique as in the previous micropipetting task, aspirate 10 μ l of the dye solution and dispense it carefully into the correct well.

◆ **Top tip:** *If the sample gets splattered all up the sides of the tube, spin for 10 seconds in the microcentrifuge to collect in the bottom of the tube.*



3. When all wells are loaded, put the orange lid on securely and press the power button to start electrophoresis. This will give a 42 V potential difference across the gel and through the electrophoresis buffer. Small bubbles will form near the electrodes.



4. Allow to electrophorese for 20 minutes.

5. Turn off the current, unplug the electrophoresis tank at the socket and remove the lid from the electrophoresis tank.

6. One person, wearing gloves, should carefully lift the agarose gel out of the buffer and place it onto a white piece of paper.

7. People without gloves on should take a photo of the gel. Use this image of the gel to answer the questions.

8. The agarose gel should be placed into an autoclave bag, for autoclaving before disposal. The gel electrophoresis buffer can be disposed of down the sink with copious amounts of water. Both gel tray and buffer tank should be rinsed with water, gently dried and stored until next needed.

Questions

- A. Which dye solution contains only one dye?
- B. Which dye solutions contain 2 dyes?
- C. Which dye solution contains 3 dyes?
- D. What electrical charge do the dyes have? How do you know?
- E. From your gel electrophoresis results, put the dyes into size order from largest to smallest. Explain your reasoning.
- The purple dye is bromophenol blue.
 - The light blue dye is xylene cyanole.
 - The orange dye is Orange G.