

03 | Pipetting for electrophoresis



Aim Develop proficiency in dispensing small volumes of liquid accurately using a micropipette. Understand the principles of gel electrophoresis, then run and interpret gels based on understanding of the technique.

Activity outline Learn the parts of the micropipette (plunger, body, display window, barrel, pipette tip and tip ejector). Build confidence in adjusting the volume dispensed and visualise small quantities of food dye using the laminated micropipetting target practice sheet.

After instructions on the technique of gel electrophoresis, practice loading wells with dye solutions, separate the dyes using electrophoresis and interpret the results of the electrophoresis.

Age range Key stage 4 and above (14 years and older)

Timing

- 15 min - micropipetting
- 05 min - instruction on gel electrophoresis
- 10 min - loading the gel
- 20 min - running the gel
- 10 min - interpreting results of the gel electrophoresis using questions

Venue This practical activity needs to be carried out in a science laboratory

Resources

- Micropipetting target practice sheet
- Student protocol: Mastering micropipetting and exploring electrophoresis (including questions on interpretation of electrophoresis)
- **Presentation:** 03_P_Pipetting-for-electrophoresis

Preparation

Before the session

For each pair of students, prior to the session:

- ☐ Prepare a microfuge tube, labelled **FD**, containing 350 µl of the supplied food dye.

In addition, prior to the session for the class:

- ☐ Prepare 4 microfuge tubes, each labelled **DS1** and each containing 30 µl of the supplied dye solution 1.
- ☐ Prepare 4 microfuge tubes, each labelled **DS2** and each containing 30 µl of the supplied dye solution 2.
- ☐ Prepare 4 microfuge tubes, each labelled **DS3** and each containing 30 µl of the supplied dye solution 3.
- ☐ Prepare 4 microfuge tubes, each labelled **DS4** and each containing 30 µl of the supplied dye solution 4.

Summary of aliquotting:

Label	Tube contents	No. of tubes	Aliquot	Used
FD	Food dye	1 per pair	350 µl	105 µl
DS1	Dye solution 1: 0.1 M Tris buffer (pH8.0), 10% glycerol, 0.1% xylene cyanol FF and 0.05% bromophenol blue	4	30 µl	20 µl
DS2	Dye solution 2: 0.1 M Tris buffer (pH8.0), 10% glycerol and 0.05% bromophenol blue	4	30 µl	20 µl
DS3	Dye solution 3: 0.1 M Tris buffer (pH8.0), 10% glycerol, 0.1% xylene cyanol FF, 0.1% Orange G and 0.05% bromophenol blue	4	30 µl	20 µl
DS4	Dye solution 4: 0.1 M Tris buffer (pH8.0), 10% glycerol, 0.1% Orange G and 0.05% bromophenol blue	4	30 µl	20 µl

- ☐ Check that you have 15 laminated copies of the micropipetting target practice sheet.
- ☐ Dilute the 10 x TAE electrophoresis buffer to make up 200 ml of buffer, by adding 20 ml of 10 x TAE buffer to 180 ml of distilled water.

- Pour 4 agarose gels. Each should be a 1% agarose gel and needs to be poured with a 9-well comb. Each MiniOne gel is made with 20 ml of molten agarose.

To pour 4 gels:

- Weigh 0.8 g of agarose into a conical flask.
- Add 80 ml of 1 x TAE buffer.
 - ▲ *Ensure that a conical flask that holds at least a volume of 250 ml is used, so that the agarose does not bubble over during heating.*
- Microwave, or heat over a Bunsen, until the agarose is melted into the buffer (remember to check that there are no clear solid pieces left by holding the flask against the light before pouring).
 - ▲ *Take care as the liquid becomes superheated and can bubble out of the conical flask.*
- Allow to cool before pouring into the gel tanks to prevent crazing of the plastic. The gels take about 30 minutes to set. Once set, remove the combs by pulling gently directly upwards.

If poured in advance gels can be left for up to 12 hours in the electrophoresis tank, covered in 1 x TAE buffer to prevent drying out. For storage up to 5 days, gels can be placed in a watertight ziplock bag with 1 x TAE buffer.

Laboratory set up

Laboratory work stations

At the start of the session set up the laboratory work station for each pair of students, so that it contains:

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

Electrophoresis stations

Set up 4 gel electrophoresis stations to contain:

- An electrophoresis tank containing a 1% agarose gel with 9 wells, submerged in 1 x TAE buffer.
- A microfuge tube rack with:
 - 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

Set up the science laboratory to contain:

- Vortex
- Microcentrifuge
- Gloves
- Safety glasses

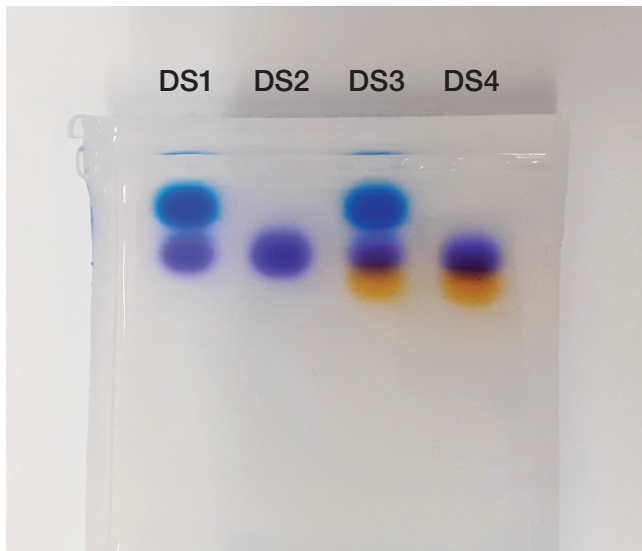
▲ *There is no need for gloves and goggles for the 'mastering micropipetting' exercise, but when working with the loading dye solution and electrophoresis buffer during 'exploring electrophoresis', students should wear nitrile gloves and safety glasses.*

Disposal

Used tips and microfuge tubes should be placed into a sealed plastic jar for autoclaving later. Electrophoresis buffer can be disposed of down the sink with copious amounts of water. Despite not containing biological materials it is good practice to place gels into an autoclave bag for autoclaving and disposal.

Answers to questions

The gel is expected to look like this:



A. Which dye solution contains only one dye?

2

B. Which dye solutions contain 2 dyes?

1 and 4

C. Which dye solution contains 3 dyes?

3

D. What electrical charge do the dyes have? How do you know?

Negative. In an electrical field they migrate / move towards the positive electrode.

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

Light blue, purple, orange.

The orange dye (Orange G) has moved furthest through the agarose matrix, so must be smallest. The light blue dye (xylene cyanol) has moved the least distance through the agarose matrix, so must be largest.

The remaining questions, F and G, are not in the student notes, they are only on the slides.

This is because you may wish to stop at question E for KS4 students, but it gives the option of using questions F and G to extend KS5 students.

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

- The purple dye is bromophenol blue, molecular weight 669.98 au
- The light blue dye is xylene cyanole, molecular weight 538.62 au
- The orange dye is Orange G, molecular weight 452.38 au

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.

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.

The movement of molecules during agarose gel electrophoresis can also be affected by shape.

When separating DNA the charge to mass ratio remains the same, so will not influence the rate of migration through the gel.