

06 | DNA sequencing



Aim To check that the DNA extraction and PCR have been successful, using agarose gel electrophoresis to determine the size of the DNA products and compare them to the expected sizes. Prepare successful PCR products for DNA sequencing.

Activity outline Run PCR samples next to the DNA ladder on agarose gels. Visualise the size of the products from PCR by comparison to bands of known size in the DNA ladder. Check the PCRs give the expected results, both the negative control and reactions with invertebrate DNA as template.

For invertebrate samples where DNA extraction and PCR have been successful, samples need to be labelled and aliquotted ready for DNA sequencing.

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

Timing

- 10 min - explanation of gel electrophoresis to find the size of DNA bands by comparison to a DNA ladder
- 15 min - sample preparation and loading the gel
- 20 min - running the gel
- 15 min - interpreting the results of gel electrophoresis and preparing samples to send off for DNA sequencing

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

Resources

- Student protocol: Electrophoresis
- Instructions for sending samples for DNA sequencing
- **Presentation:** 06_P_DNA-sequencing

Preparation

Before the session

For each pair of students, prior to the session:

- ☐ Prepare a microfuge tube, labelled **GG**, containing 2 µl of loading dye with GelGreen®.
 - ▲ *It is important that the supplied tube of GG is vortexed between each aliquot, as GelGreen® is more dense than the other components of the loading dye and will sink within the mixture.*
- ☐ Prepare a microfuge tube, labelled **H₂O**, containing 10 µl of nuclease-free water.

In addition, prior to the session for the class:

- ☐ Prepare 4 microfuge tubes, each labelled **-ve** and each containing 4 µl of nuclease-free water, 2 µl of loading dye with GelGreen® and 4 µl of the PCR negative control.
- ☐ Prepare 4 microfuge tubes, each labelled **LAD** and each containing 22 µl of the supplied 100 bp ladder combined with loading dye containing GelGreen®.

Summary of aliquotting:

Label	Tube contents	No. of tubes	Aliquot	Used
GG	Loading dye with GelGreen®	1 per pair	2 µl	2 µl
H₂O	Nuclease-free water	1 per pair	10 µl	4 µl
-ve	PCR negative control + loading dye with GelGreen™	4	10 µl	10 µl
LAD	100 bp ladder	4	22 µl	20 µl

- ☐ 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 2% 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 1.6 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 tank 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 labelled GG containing loading dye with GelGreen®
- Microfuge tube labelled H2O containing nuclease-free water
- The students' PCR tube
- Non-permanent marker

Electrophoresis stations

Set up 4 gel electrophoresis stations to contain:

- A microfuge tube rack with:
 - Microfuge tube of **-ve** (PCR negative control pre-mixed with loading dye containing GelGreen®)
 - Microfuge tube of **LAD** (100 bp DNA ladder, premixed with loading dye containing GelGreen®)
- An electrophoresis tank containing a 2% agarose gel with 9 wells, submerged in 1 x TAE buffer.

Class equipment

Set up the science laboratory to contain:

- Gloves
- Safety glasses

⚠ *When working with the loading dye solution and electrophoresis buffer, 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. These agarose gels also contain biological materials (DNA from the PCRs), so need to be placed into an autoclave bag for autoclaving prior to disposal. Electrophoresis buffer can be disposed of down the sink with copious amounts of water.

Instructions | Sending samples for DNA sequencing

- ✓ Any PCR that shows a single band of about 710 bp when visualised by gel electrophoresis, should have sufficient DNA present for sequencing.

For interest, when loading 10 µl of 100 bp ladder, there is 500 ng of DNA loaded onto the gel. The brighter bands at 500 bp and 1000 bp contain about 100 ng of DNA each. DNA sequencing requires a concentration of 10-50 ng/µl of unpurified PCR product. So if 4 µl of PCR product is loaded in a lane, and the band is of equal brightness to the 500 / 1000 bp bands in the 100 bp DNA ladder, then the PCR is approximately 25 ng/µl. This is within the 10-50 ng/µl concentration required.

- ✗ If a PCR product is not visible at 710 bp, then please do not send the sample for sequencing, as it is incredibly unlikely to yield a result.

We will be using a company called **GeneWiz Azenta** to perform rapid sequencing of our DNA samples. A volume of 20 µl of unpurified PCR product is needed for DNA sequencing.

To prepare samples for sequencing:

- ☐ Check that the PCR product forms a band at about 710 bp when observed by gel electrophoresis.
- ☐ Ensure that students transfer 20 µl of PCR product into a clean, labelled microfuge tube.
- ☐ Make sure that none of the 7 letter codes of the samples (from the sample record sheet), used to label the microfuge tubes, are the same.
- ☐ Make sure that the 7 letter codes of the samples are legible on the student tubes.
- ☐ Store the labelled tubes containing the student samples in the Isofreeze box at 4°C (in the fridge), until you are ready to deliver them.

On the day of your visit to the Wellcome Genome Campus, you need to bring:

- ☐ The Isofreeze box containing your DNA samples.
- ☐ The completed sample record sheet, which will link the samples to school, student initials and the information about the invertebrate used in preparation of the DNA sample for barcoding.

You can also bring the equipment and any unused consumables back with you, as the sessions following the campus visit do not require laboratory work.

We will use the 7 letter codes to submit the DNA samples for sequencing. The DNA will be purified, then sequenced using capillary gel electrophoresis.

Your DNA sequence results should be returned by email within 4 working days of your visit to the Wellcome Genome Campus.