TEACHING OUTREACH RESOURCE

Genetics on the GO Kit Instructions

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University of Otago
Te Whare Wānanga o Otāgo
NEW ZEALAND

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GO BEYOND WITH GENETICS

Explore *the origins and mechanics of life.* ***Discover*** *causes of disease and their cures.* ***Solve*** *the problems facing our agriculture and natural heritage.* ***Understand*** *the past.* ***Create*** *a better future.* ***Master*** *the world of genetics...*

WELCOME...

…to this Genetics teaching resource, created by Genetics Otago and the Genetics Teaching Programme at the University of Otago.

Our aim is to engage young minds with Genetics and to do this we have developed a range of resources that include information, worksheets and activities or experiments that will help you to plan exciting Genetics classes for your students.

Where possible we have endeavoured to align and link the content of the resources to the New Zealand Curriculum.

If you have any questions relating to the content of the resources or would like to organise an onsite teaching session on one of our topics please contact us at go@otago.ac.nz.

Contents

Overview

Gel Electrophoresis is a very common tool used in all molecular biology laboratories. It is used to separate molecules, such as DNA, by size. The negatively charged DNA is subjected to an electric field causing it to move towards a positive electrode. The size separation is possible due to the matrix like make-up of the agarose gel meaning that smaller molecules are able to move more freely and thus faster than larger ones. This resource provides instructions on how to conduct electrophoresis, please contact us at go@otago.ac.nz if you require the use of one of our electrophoresis kits and to enquire about the experiments we have available.

OBJECTIVES

- Learn the principles of gel electrophoresis
- Use a universal molecular biology technique to assess a scenario
- Observe the migration of dye or DNA through an electrophoresis gel and draw conclusions about the size and charge of the molecules.

CIRRICULUM LINKS

These kits are designed to be adaptable to several different modules of work, allowing them to be used with a wide range of year levels but they broadly feed into the following curriculum areas:

- **Nature of science**
  - Understanding about science – working in groups, with current scientific methods and theories to collect and interpret results..
  - Investigating in science – carrying out more complex investigations, working scientifically with multiple variables and evaluating the suitability of investigative methods.
  - Communicating in science – Using accepted scientific knowledge and vocabulary
  - Participating and contributing – develop an understanding of socio-scientific issues and draw evidence-based conclusions.

- **Living World**
  - Life processes, ecology and evolution –Explore the importance and implications of genetic variation and inheritance and how they relate to diversity, evolution and disease.

Background

Gel electrophoresis for DNA is a technique used routinely in molecular biology laboratories. The gel is made from agarose, a purified form of agar, which is a substance found in red algae. When heated in a solution it sets to a firm jelly–like substance. Gel electrophoresis is used to separate DNA on the basis of size.

When a current is applied across the agarose gel the negatively charged DNA molecules are attracted to the positive (red) electrode. The gel matrix slows the progress of the larger molecules and they move slower through the gel. Smaller molecules are able to move more rapidly.

A dye is loaded with the DNA, the purpose of this is two-fold. Firstly it increases the density of the DNA so that it is heavier than the buffer in the gel tank, this allows the DNA to sink down into the wells of the gel. Secondly, the dye moves very rapidly through the agarose matrix, so it acts as a visual aid of progress of the DNA (the dye front will be ahead of where the DNA actually is).

The DNA is visualised using a specific dye that binds to the DNA molecules and fluoresces under UV light. Different sized bands appear where the each of the samples have migrated too. Samples towards the bottom of the gel are smaller than those towards the top.

In our outreach kits we do not use real DNA however, we instead used coloured dyes to represent the DNA. These dyes migrate through the electrophoresis gel in the same way as DNA and provide a suitable alternative to the real thing in a classroom situation. This decision was reached based on a number of factors:

1. Time - without specialist equipment (UV transluminator), the use of real DNA introduces the need for overnight staining steps that extend the length of time it takes to complete the experiments. We are aware that teaching time is precious and in short supply so we have tried to eliminate any unnecessary steps in our protocols.
2. Reliability - Testing of staining in classroom situations produced varied results and in some cases students saw no results at the end of the experiment.
3. Cost - The use of real DNA has costs associated with PCR amplification and technician time to prepare. In order to keep our resources free to schools we must keep our costs at a managable level.

4. Ehtics - most importantly, DNA is a taonga, whether it is from humans, animal or plants. In a research setting this is satisfied by the results and knowledge that is gained by using the DNA. In a classroom setting where the goal is to teach the concepts, we feel that the use of real DNA is not justified because the same outcomes can be achieved using dye samples.

Additional to this, the use of dye allows the students to watch the migration of samples in real time and capture an image of the results with a standard camera or phone.

Electrophoresis kits are available on request from Genetics Otago. These kits contain the hardware required to cast and run a gel as well as the materials and samples required for several different experiments. We are also able to supply sample only, or sample and reagent only kits if you have your own equipment.

Please contact us at go@otago.ac.nz for bookings. Details of our current experiments are available on our website (https://blogs.otago.ac.nz/go/genetics-on-the-go/).

Materials

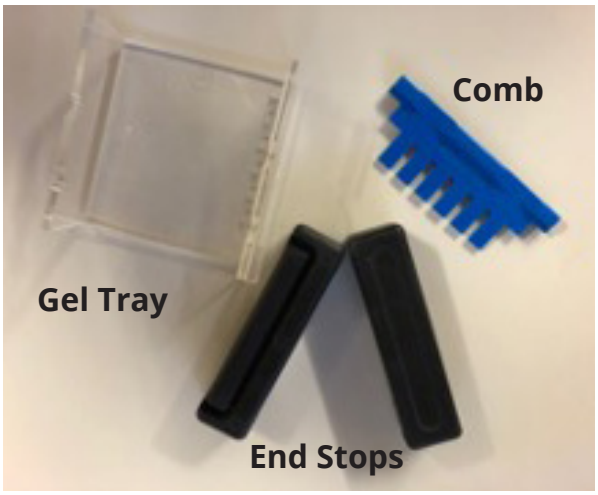
The below are the materials required to complete a gel electrophoresis experiment. The majority of these are provided in our kits, items marked * are not part of the kit but should be easily accessible.

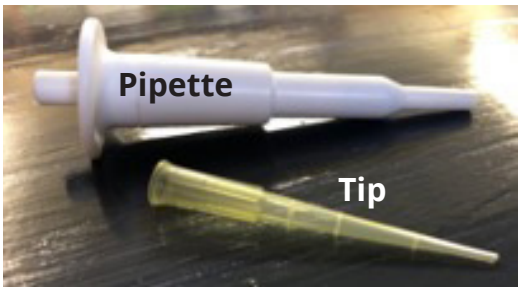
- Gel Cast and comb
- Electrophoresis tank
- Power pack
- Agarose powder
- Buffer
- Water (distilled is recommended if available)*
- 1L bottle with lid*
- Glass flask or similar (must be microwave proof)*
- Heat proof glove*
- Microwave*
- Pipettes and tips
- Samples
- Disposable gloves (the reagents used are quite safe, but we advise against putting hands directly into the buffer)*

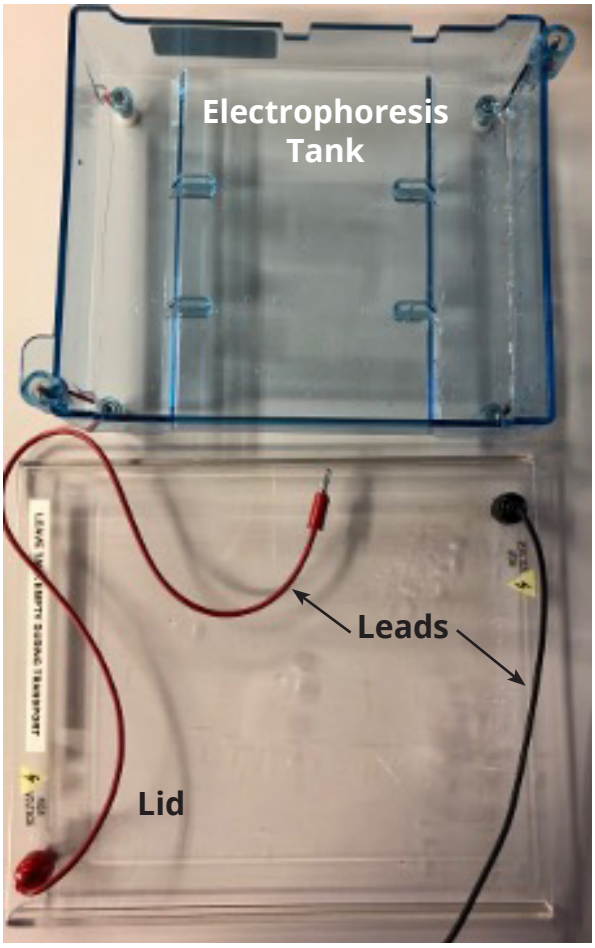
Note

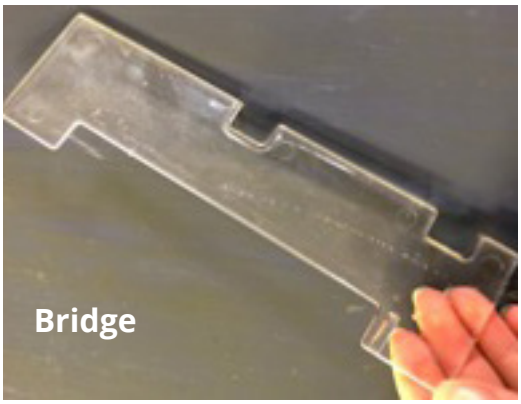
The kit provides enough materials and samples to run six gels at once and enough reagents for your class to work in groups of up to 6. If you have more than 36 students the experiment will need to be run in several sessions

Equipment Names Used in This Document









Buffer

The buffer supplied is Sodium Borate (SB) Buffer. SB buffer has a lower conductivity than other electrophoresis buffers so can be used to make and run gels at a higher voltage, increasing the speed of running.

For your reference the recipe used to make this buffer is as follows:

For 20X concentrate add 45g Boric Acid and 8g NaOH to a bottle, make up to 1L in water and mix to dissolve.

The SB buffer provided is at 20X concentration, this must be diluted to 1X before use.

Protocol

To prepare 1L of 1X SB buffer from the 20X stock solution:

1. Add the pre-measured 50mL of concentrated 20X stock SB buffer into a bottle with a capacity of at least 1L
2. Add 950mL of water (distilled if possible)
3. Mix well before use

You will use this 1X SB buffer to both make the gels and fill the electrophoresis tank. The electrophoresis tank holds 1L of buffer, and this can be used up to three times (you should only need to fill it once, but it may require topping up if you are running several experiments over a few days as some evaporation will occur.

You will also require an additional 300mL of 1X SB buffer to make each set of six gels.

Gel Preparation

The kit contains pre-weighed 0.5 gram aliquots of agarose and one electrophoresis tank with six gel casting trays. The below protocol can be used in the class for the students to make their own gels. The following page details methods for preparing the gels ahead of the class.

Protocol

1. Assemble the casting equipment
    - Clip a black rubber end stop onto each end of the gel tray
    - Insert a blue comb into each gel tray NB: Combs are normally inserted towards one end of the casting tray to maximise the amount of gel available to run the samples through
2. Set the assembled equipment on a level horizontal surface.
3. Measure 50mL of 1X SB Buffer and pour it into the microwave safe flask
4. Add 0.5g of Agarose powder to the flask
5. Swirl to disperse clumps
6. Heat the solution on high in the microwave for 30 second bursts
    - This will take an estimated 1-2 minutes depending on your microwave
    - Swirl the mixture to mix every 30 seconds

Caution: solution will become very hot, possibly superheated, gloves must be worn

7. Heat the solution until all glass-like particles have dissolved and the solution appears clear when held up to the light.
8. Allow the solution to cool on the bench until it reaches approximately 60°C. An infrared thermometer is provided, simply point it at the side of the bottle and press read.
9. Carefully pour the agarose solution in to the gel cast. Remove any air bubbles using a disposable pipette tip or similar
10. Leave to set for 15-20 minutes (time will vary), you will know it is set once it is uniformly cloudy.
11. Gently remove the black end stops and the comb
12. If you want to store the gel overnight wrap it in plastic wrap and keep refrigerated, or submerged in 1x buffer in a sealed container.

In Advance Gel Preparation Procedures

If you wish to pour all six gels at once the protocol can be adjusted simply by adding 3g of agarose (6 aliquots) to 300mL of 1X SB buffer, rather than the 0.5g in 50mL stated on the previous page. This is the most time efficient way to make the gels if you are planning to prepare them prior to the class. The following times are given as a guide:

- Prepare diluted SB buffer (10 mins)
- Make and pour agarose gels (30 mins)
- Setting time for gels (20 mins)

So the total pre-class preparation time is approximately 1 hour

Alternatively, if you have experience with making and pouring gels you can use the super quick method detailed below:

1. Assemble gel casting equipment as described previously
2. Measure 15mL of 20X SB buffer into microwave safe bottle or flask
3. Make up to 150mL with water
4. Add 3g of agarose (6 aliquots)
5. Heat in microwave until the agarose is dissolved, mixing every 30 secs
6. Once the agarose is completely dissolved immediately add 150mL water to the hot agarose solution
7. Check the temperature of the agarose solution (it should be about 60 °C straight away)
8. Pour the gels as described previously
9. While the gels set dilute the running buffer by adding 50mL of 20X SB stock solution to 950mL water.

Total time for this method is approximately 20-30 minutes.

Electrophoresis

Before you start this protocol make sure you have prepared the required number of agarose gels and diluted sufficient SB buffer to fill the electrophoresis tank (1L).

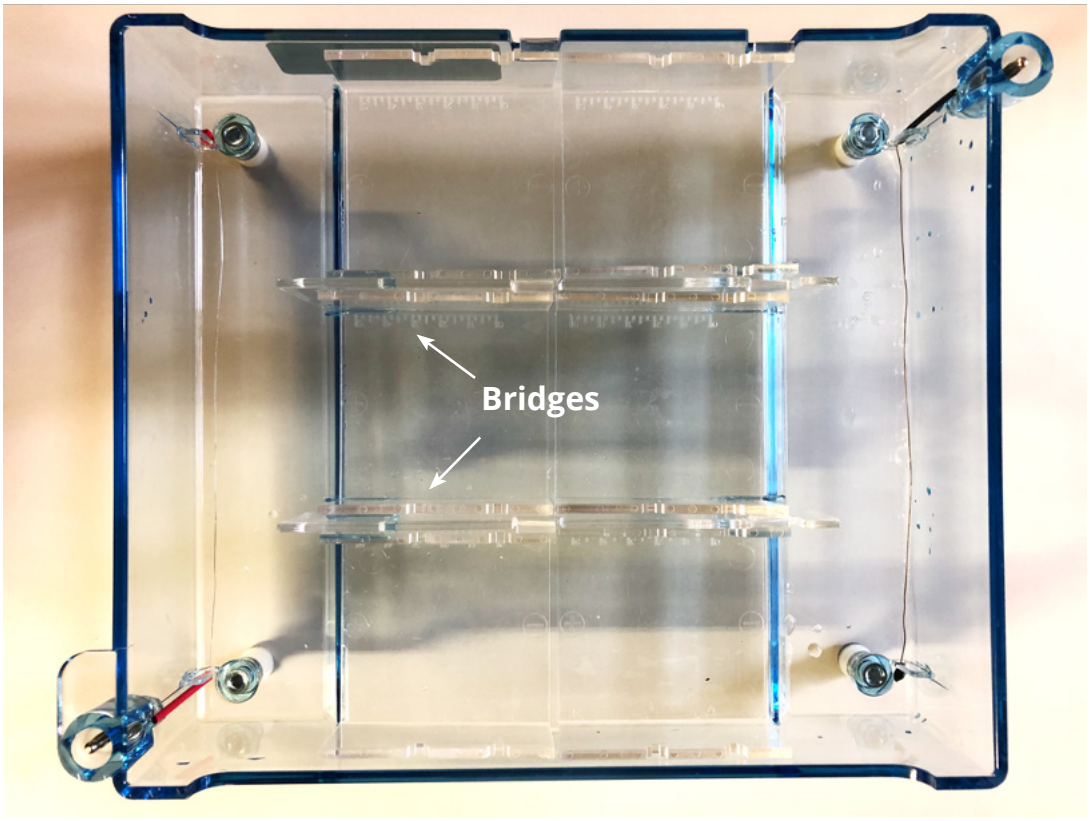
Protocol

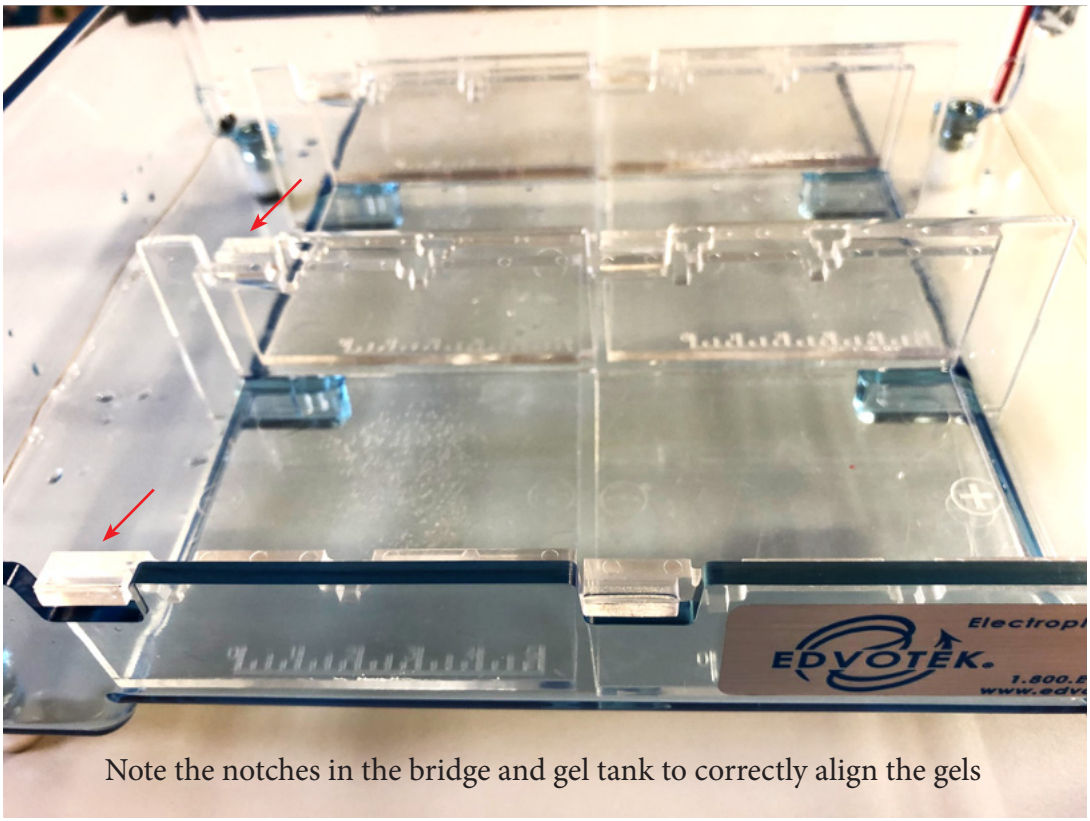
1. Load the samples into the wells of the agarose gel using the provided pipette, remember to change the tip between samples.
    - This can be done by the students at their own benches
    - Use dark paper under the gels to see the wells more easily

Tips for Gel Loading

1. Hold the pipette in your dominant hand
2. Pipette the solution up and down a couple of times to mix the sample
3. Draw the sample up into the pipette
4. Rest the elbow of your dominant arm on the bench to stabilise your hand.
5. Using your other hand gently guide the yellow tip so that it is positioned above the well.
6. Move the tip down 1-2mm inside the well (be careful not to put it in too far or you will stab the agarose and block the tip)
7. Slowly press down on the pipette button to drop the sample into the well (gravity is on your side, the sample will drop down into the well so remember not to put the tip in too far).
8. If there is more sample remaining in the tube, repeat the process to get as much of it in the well as possible
9. Repeat the process for each sample working from left to right
10. Remember to change your tip between samples to avoid contamination
11. Be sure to record the order that you have loaded the samples so that you know which is which when you come to analyse them.

2. Carefully place the loaded gels, still in their casting trays into the middle section of the electrophoresis tank (see images on the following page)
    - Ensure that the two clear plastic bridges are in place along the middle section of the tank before placing the gels
    - The gels should be placed in the tank so that the wells are closest to the black electrode (negatively charged DNA will move through the gel towards the positive, red, electrode).
3. Pour the 1X SB buffer into the black end of the tank and then the red, when filling the red end allow the buffer to spill over the top of the gel (filling in this way will minimise the chance of the samples being disrupted in the wells.
4. Place the lid on the tank, matching the electrodes on the lid to those on the tank.
5. Double check that the gels are completely covered and that the electrodes are in the correct orientation.
6. Plug the leads into the power pack, again ensure that you match the colours.
7. Turn on the powerpack and set the Voltage to 150V.
    - Once the power is started small bubbles should form on the metal wire at the red end of the electrophoresis tank
    - It is normal for condensation to form on the lid
8. Monitor the gel and run it until the fastest moving sample is about 1cm from the end of the gel
9. Turn off the power supply before removing the lid from the tank.





Note the notches in the bridge and gel tank to correctly align the gels

After the gels have been run:

1. Remove the gels, still on their casting trays for analysis
2. Running buffer can be left in the tank with the lid on or it can be poured into a bottle or container for storage (it can be used 3 times). If you have completed all of the experiments, you can discard the buffer down a drain
3. Analyse gels by looking at the bands and taking photos as you wish
4. Once analysed gels can be disposed of in the rubbish bin
5. Wash the tank, bridges, casting trays, combs and end stops in hot water and rinse with distilled water where available. Wipe the tank lid with cloth or paper towels.
    - Please make sure that the tank lid is not submerged and that the leads don't get wet

Koha

While these kits will remain free for schools in need, we ask that you please consider a koha of whatever your school can afford to help in the continued development and upkeep of these ever-popular kits. Each kit costs us $50 - $120, depending on class size and location. Our resources have been used by more than 1500 students, from Invercargill to Whangarei, annually over the past few years.

You can donate by simply visiting:
https://alumni.otago.ac.nz/donate/genetics-otago-on-the-go

Thank you!

Feedback

We hope you have enjoyed this resource.

Feedback is very welcome to:
go@otago.ac.nz