Investigating how gel electrophoresis is used to separate DNA fragments of different lengths

Transcript

The study of DNA has a number of practical applications.

These range from medical diagnosis and the study of evolutionary relationships, to forensic science.
One technique that allows scientists to study DNA is a process called gel electrophoresis.

This is a technique that separates different DNA molecules on the basis of their size.

The simple principle behind gel electrophoresis is that DNA is negatively charged. Therefore, DNA molecules will move from a negative electrode, the cathode, to a positive electrode, the anode, over a period of time.
The technique of gel electrophoresis involves placing a mixture of DNA molecules into wells cut into a special gel. These may be obtained directly from a living source or organism, but more usually they are the products of the polymerase chain reaction, or PCR.

A power supply is set up across the gel. DNA molecules are negatively charged. Therefore, they will move from the negtive pole, the cathode, to the positive pole, the anode.

The distance travelled by the DNA molecule in a given time is dependent on its length. Longer fragments of DNA will move more slowly and therefore a shorter distance than shorter fragments, which will travel further towards the end of the gel.

This produces a characteristic band pattern, from which scientists can draw conclusions.

The separation of DNA by electrophoresis requires a gel made from agarose, a polysaccharide found in seaweed. The gel is prepared by dissolving it in hot water…and pouring the mixture into a casting tray. A sample comb is placed into the tray before the mixture is poured in, which provides a series of wells to which the DNA samples will be added later. Once it has solidified, the sample comb is removed from the gel, and the gel is placed into the electrophoresis tank.

The end of the gel that has the wells is placed closest to the negative electrode, or cathode, which has a black surface. This is because the DNA molecules will move towards the positive pole, which is red, during electrophoresis.

The electrophoresis tank is then filled with a buffer solution, which contains a high concentration of ions. Enough buffer solution is added so that the gel is completely submerged.

Next, the DNA samples that will be analysed in this investigation are prepared. If there is more than one DNA sample, these should be placed in the order they will be put into the gel.

The DNA is usually mixed with a small volume of tracking dye, usually blue in colour. DNA is colourless. During electrophoresis, the tracking dye indicates the position of the DNA in the gel.

To transfer the DNA and dye from the tube into the wells of the gel, a special micropipette is used. This allows for the precise measurement of volumes of liquid to be transferred. A plastic tip is attached to the end of the micropipette. An airtight seal is ensured by pressing down firmly. The micropipette is held in a vertical position and the plunger is pressed to the first stop. Air equal to the volume of the setting is displaced. The tip is immersed into the sample of DNA. The plunger is released back to the rest position and a moment is allowed

for the liquid to be sucked up into the tip. The volume of liquid in the tip will now equal the volume of the setting of the micropipette. In this case, this volume is 35 microliters.

The micropipette is then held against the hand to guide the tip into the well at an angle. The tip is placed just a few millimetres into the well to avoid puncturing the bottom of the well. The plunger is pressed to the first stop, before waiting a second, and then pressed again to expel the full volume of liquid. Care is taken to avoid pushing the plunger any further, which would bubble air into the well and disturb the sample. It is important that the tips are replaced or washed between loading, so that the DNA samples are not mixed.

Once all samples have been added to the wells in the gel, the lid is paced on the electrophoresis tank.

The power supply is connected to the tank using the leads, again ensuring that the electrodes match the leads: black to black and red to red. The voltage is set to 75 V, and charge begins to flow through the buffer solution. The power supply generates an electric field. Bubbles should start to appear to indicate that charge is flowing.

After a few minutes, the samples are seen to migrate from the wells into the gel as they are attracted to the positive electrode. As time passes, the position of the tracking dye, and the DNA, will move closer to the positive electrode. When the loading dye has nearly reached the end of the gel, switch off the power supply. Around 60 minutes is normally sufficient to allow the fragments of DNA to separate fully from one another.

The gel is removed from the tank… and placed into a small tray.

DNA is colourless, so it is only after staining the DNA that a pattern of bands can be seen. These represent fragments of DNA of different sizes.

In this example, a scientist investigated the inheritance of the recessive genetic disorder sickle cell anaemia.

In this family, both parents (4 and 6) are heterozygous carriers of the mutant recessive allele. Their child (5) is affected by the disease.

This was determined by comparison with three lanes containing reference samples of DNA. The first lane, which has a single band, has a sample of DNA taken from a known sufferer of sickle cell anaemia. They have two copies of the mutant allele. The second lane contains a sample of DNA taken from an individual with no copies of the mutant allele. This contains two bands, because the DNA sample has been hydrolysed into two fragments by a restriction enzyme. The third lane contains a sample of DNA taken from a carrier of sickle cell anaemia, who has one copy of both alleles. They therefore have two bands associated with the normal allele, and the band associated with the mutant allele.