



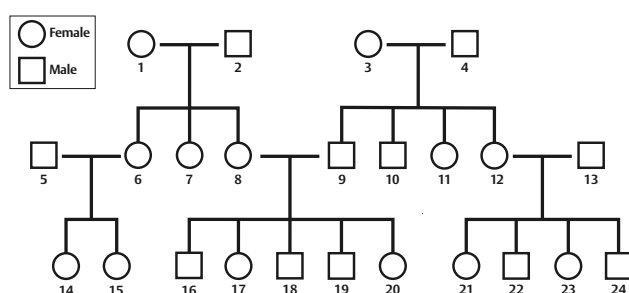
Nature's dice

Student's guide

Family trees and DNA

This investigation involves a genetic condition that is passed from one generation to the next. Twenty-four members of a large family, including people affected by the condition, have donated samples of their DNA to be tested. This DNA will enable you to discover how the condition is passed on and to predict whether individuals in the family will be directly affected by the condition.

The relationships between the relatives are shown in the family tree below. To respect people's confidentiality, the names of individuals have been replaced by numbers in the diagram. By convention, males are represented by squares; females are shown by circles.

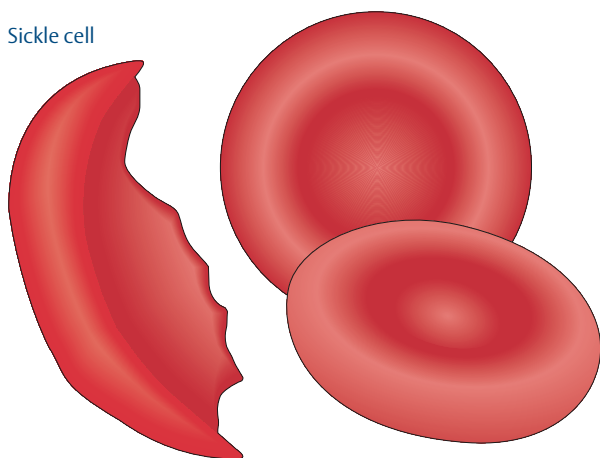


The family tree of the people in this genetic screening exercise.

How genes are passed on

A child inherits half of its DNA from each parent. The DNA includes genes, which are instructions for making proteins. Usually, one copy of each gene comes from the mother and one copy is inherited from the father. Genes come in different forms or *alleles*. Sometimes a person will have two identical alleles, that is, they are *homozygous* for that allele. Alternatively, they may have inherited two different forms of the gene and be *heterozygous*. The different alleles can be *dominant* or *recessive*.

Sickle cell



Normal red blood cells

Sickle cell anaemia is one of the commonest genetic conditions that is inherited in an autosomal recessive manner. A single base-pair change in the DNA encoding haemoglobin causes red blood cells to adopt a sickle shape, reducing their oxygen-carrying capacity and making it difficult for them to pass through small blood vessels.

Autosomal recessive conditions

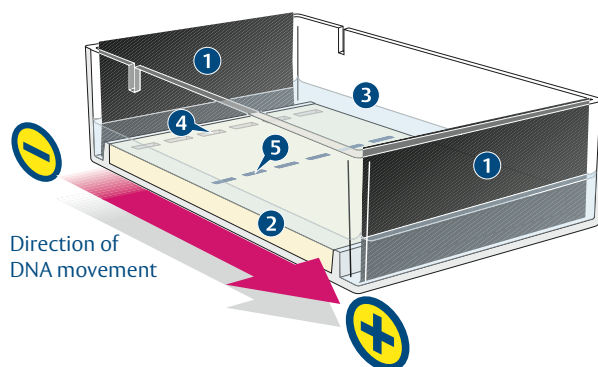
Some of the commonest genetic conditions *e.g.*, cystic fibrosis and sickle cell anaemia, are inherited in an *autosomal recessive* way. This means that the allele responsible is found on one of the 22 autosomes (the chromosomes other than the two sex chromosomes) and that the allele is recessive.

People who are homozygous for a dominant allele will only have DNA of type **D** (genotype **DD**). They will not be affected by the condition. Someone who is homozygous for the allele associated with the condition, however, (genotype **dd**) will only have DNA of type **d**. They will therefore have the disease. Heterozygous individuals (genotype **Dd**) carry DNA of both types, but the recessive allele (**d**) will be masked by the dominant one (**D**) and the disease will not appear. They will, however, be carriers who could pass the condition to their children if their partner was also a carrier.

DNA gel electrophoresis

Gel electrophoresis can be used to separate DNA fragments of different sizes. First, a gel is cast from agarose — a very pure form of agar, which is obtained from seaweed. At one end of the slab of gel are several small wells, made by the teeth of a 'comb' that was placed in the gel before it set. A buffer solution is poured over the gel, so that it fills the wells and makes contact with electrodes at each end of the gel. Ions in the buffer solution conduct electricity. The buffer also stops the gel from drying out.

The invisible DNA fragments are mixed with a small volume of loading dye. This dye is dissolved in a dense sugar solution, so that when it is added to the wells, it sinks, taking the DNA with it.



Gel electrophoresis equipment: 1 = electrodes; 2 = agarose gel; 3 = buffer solution; 4 = wells; 5 = loading dye.

A current is applied to the electrodes, setting up an electrical field across the gel. Phosphate groups give DNA fragments a negative electrical charge, so that the DNA migrates through the gel towards the positive electrode. Small fragments move quickly through the porous gel — larger fragments travel more slowly. In this way the pieces of DNA are separated by size. The loading dye also moves through the gel, slightly ahead of the DNA, so that the progress of the electrophoresis can be seen.

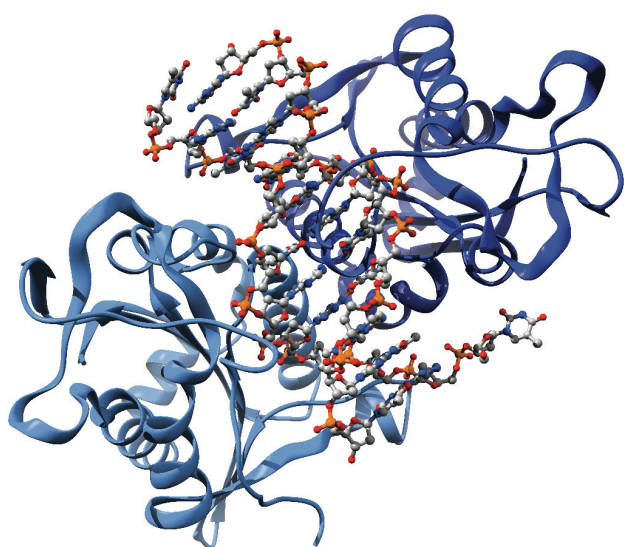
After electrophoresis, the gel is stained to reveal the DNA. Each individual band on the gel is made up of millions of DNA fragments of the same size.

Cutting DNA

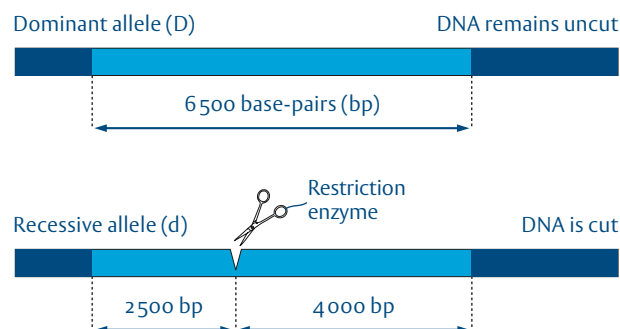
Enzymes that cut specific sequences of DNA are called *restriction enzymes*. They are so called because they are made by bacteria to *restrict* the proliferation of viruses that attack them (the enzymes do this by cutting up the viral DNA).

Restriction enzymes take their names from the bacterial species that produce them. For example, *Bam*HI is obtained from *Bacillus amyloliquefaciens*, strain H, with the Roman numeral I showing that this was the first restriction enzyme found in this bacterium.

Different restriction enzymes 'recognise' and cut DNA at different sequences of bases. Consequently they can be used to help detect the presence of different DNA sequences and hence different forms of genes (that is, different alleles).



A computer-generated model of the restriction enzyme *Bam*HI attached to some DNA. *Bam*HI cuts at the sequence G↓GATCC. The enzyme is shown as two blue ribbons, representing the two chains that comprise the protein. The DNA is shown in the middle of the enzyme as a ball-and-stick model.



How DNA from the two different alleles will be affected by the restriction enzyme *Bam*HI.

Genetic analysis

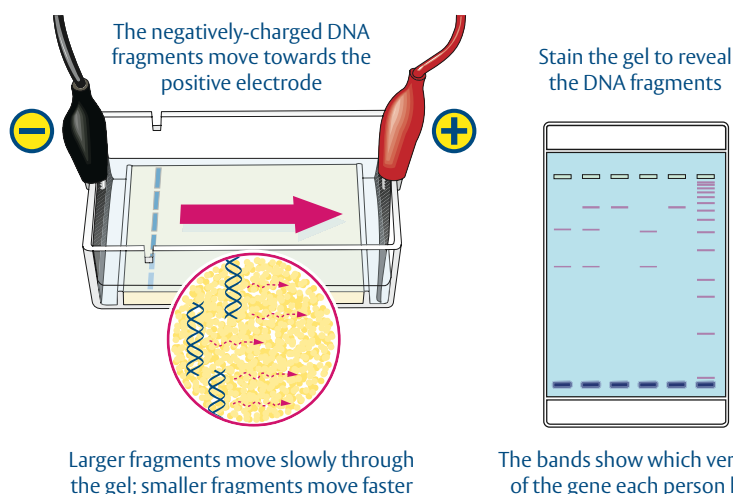
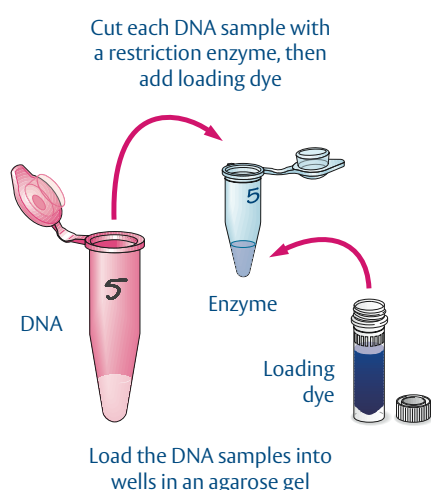
How does gel electrophoresis help with genetic analysis? In the samples provided, there is a difference between the DNA sequences of the alleles **D** and **d**. In **d**, there is a base-pair sequence that can be 'recognised' and cut by the restriction enzyme *Bam*HI. In contrast, **D** has no restriction site and therefore it cannot be cut by the enzyme.

Individuals who are homozygous for the dominant allele (**DD**) only have DNA of type **D**, which is not cut by the restriction enzyme. Gel electrophoresis separates DNA fragments by size, so in samples with just the **D** allele, all of the uncut fragments of DNA will move together to form a *single band* on the gel.

Individuals who are homozygous for the recessive allele (**dd**) only have DNA of type **d**, so all of their DNA will be cut by the enzyme. This will create fragments of two different sizes which will produce *two bands* on the gel after electrophoresis.

Finally, heterozygous individuals (**Dd**) will have DNA of *both* types. Half of their DNA fragments (type **D**) will remain uncut and half of their fragments will be cut in two. Thus, after incubation with the restriction enzyme, DNA samples from someone who is heterozygous will contain fragments of three different sizes and so there will be *three bands* on the electrophoresis gel.

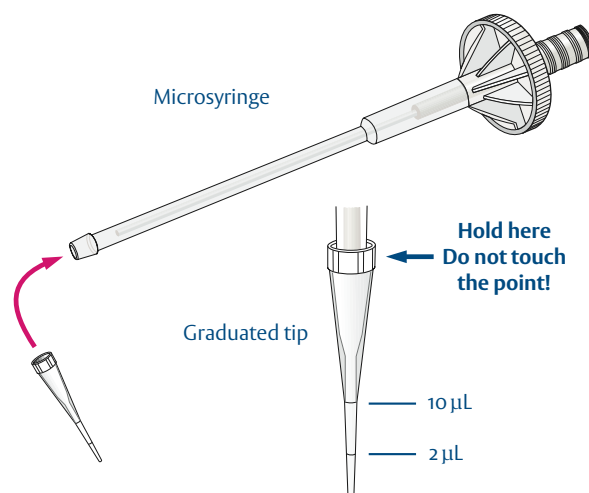
Summary of the protocol



1 Using the microsyringes

The microsyringes are designed to be fitted with disposable tips. Each tip should be used once only, then thrown away. The tips are marked at 2 and 10 microlitres (μL), allowing small volumes of liquid to be dispensed with precision. The best results will be obtained if you observe the following precautions:

- Never pull the plunger right out of the microsyringe — when the plunger is re-inserted the seal may become damaged;
- Before you load the microsyringe, pull the plunger out a little (1–2 mm). This will give you some extra air with which to expel the last drop of liquid from the tip;
- When you dispense liquids, hold the microsyringe as near to vertical as possible, and at eye level so that you can see what you are doing;
- Remove the liquid from the point of the microsyringe tip by holding it against the inner wall of the tube into which you are transferring the liquid;
- Do not touch the point of the microsyringe tip with your fingers. There are proteases and DNases in your sweat which may contaminate and degrade the reactants.



Volume

A microlitre is one millionth of a litre
 1000 microlitres (μL) = 1 millilitre (mL)
 1000 millilitres = 1 litre (L)

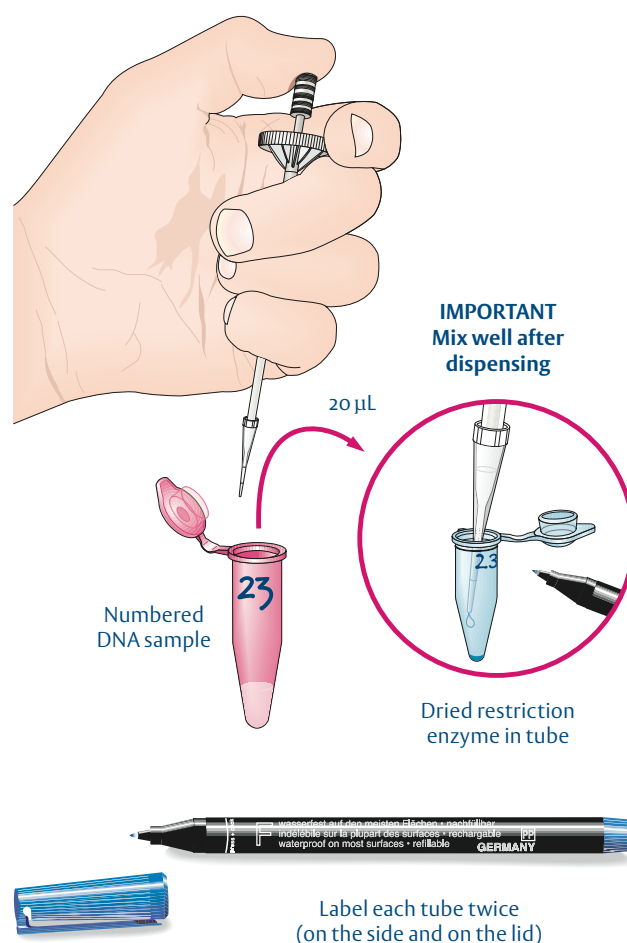
2 Dispensing the DNA samples

- Place a clean tip on the microsyringe. Transfer 20 μL of the DNA solution you have been given into a tube containing dried restriction enzyme.
- Mix the liquid and the dried enzyme carefully by drawing the liquid up and down in the tip a few times. *The liquid in the enzyme tube should have a distinct blue hue, but there should be no concentration of dye at the bottom of the tube.*
- Close the tube tightly.
- Write the number of the sample on the tube with a waterproof pen. *It is a good idea to write the number both on the lid of the tube and the side. On the side, write near the rim where the number is less likely to rub off.*
- Put your tube in a floating rack along with the other DNA samples (perhaps from other students in your class).

Notes

The dried enzyme has been preserved with an appropriate buffer and a blue dye. It is *essential* to add the correct volume of liquid to the tube, and to ensure that its contents are mixed thoroughly. This is because restriction enzymes can be less specific if used in excess or with the wrong concentration of buffer.

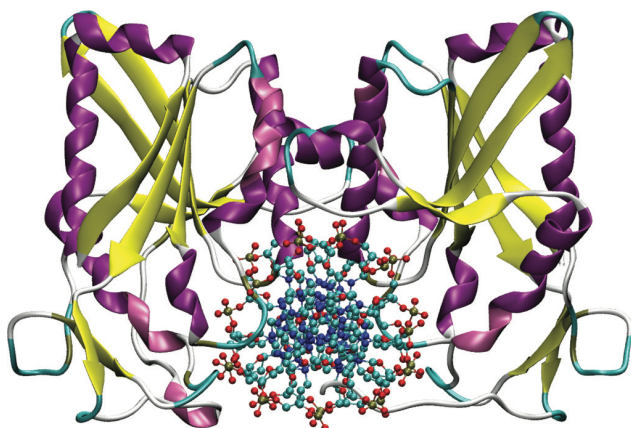
The restriction enzyme provided in this kit works best at 37°C. Restriction enzymes are denatured easily and therefore once the enzyme has been rehydrated it should be used immediately.



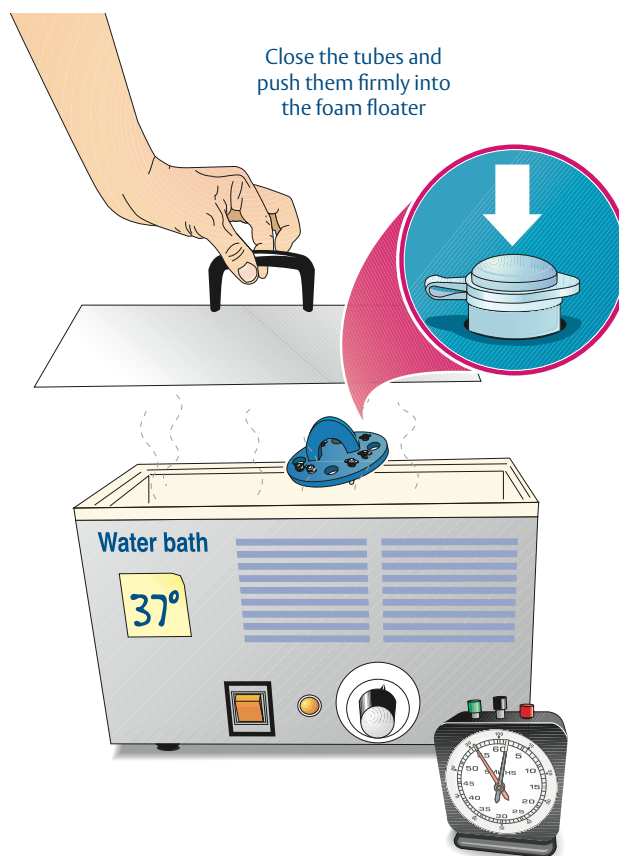
3 Incubation

- a. Check that the tubes containing the DNA are firmly capped, then incubate them in a water bath at 37 °C, for 30 to 45 minutes. *During incubation, the restriction enzyme will cut the DNA molecules at a specific site, if that site is present. This will produce, from the original 6 500 base-pair fragments you were provided with, fragments of 2 500 and 4 000 base-pairs. DNA with no restriction site will remain uncut.*

OPTIONAL STOPPING POINT



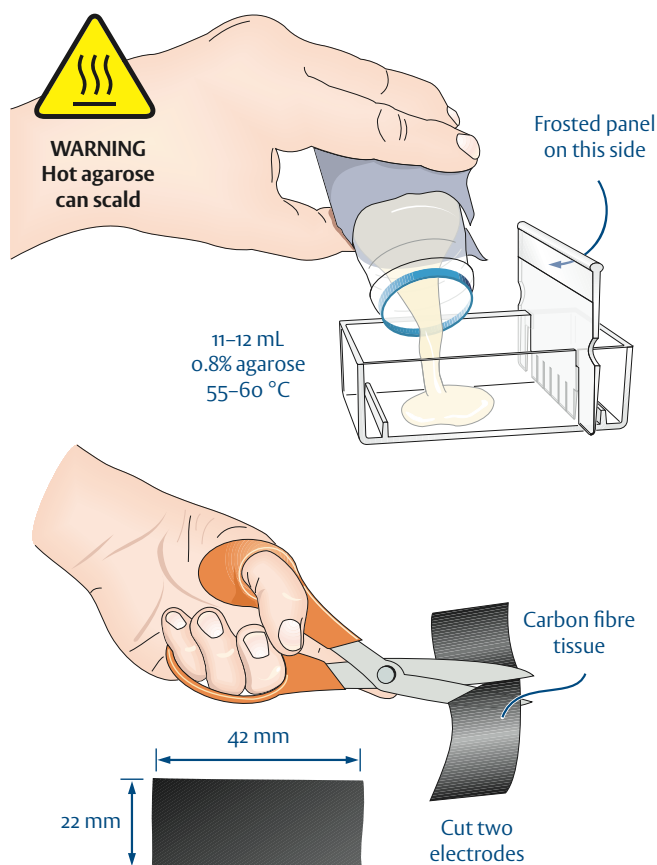
A computer-generated model of the enzyme BamHI attached to a short length of DNA. The enzyme is shown as a ribbon structure on either side, with one chain facing forwards, the other back. In the protein structure, β -pleated sheets are shown in yellow; α -helices in magenta. The DNA being cut is in the centre, depicted as ball-and-stick model, looking down the axis of the DNA molecule.



Incubate for 30–45 minutes at 37 °C

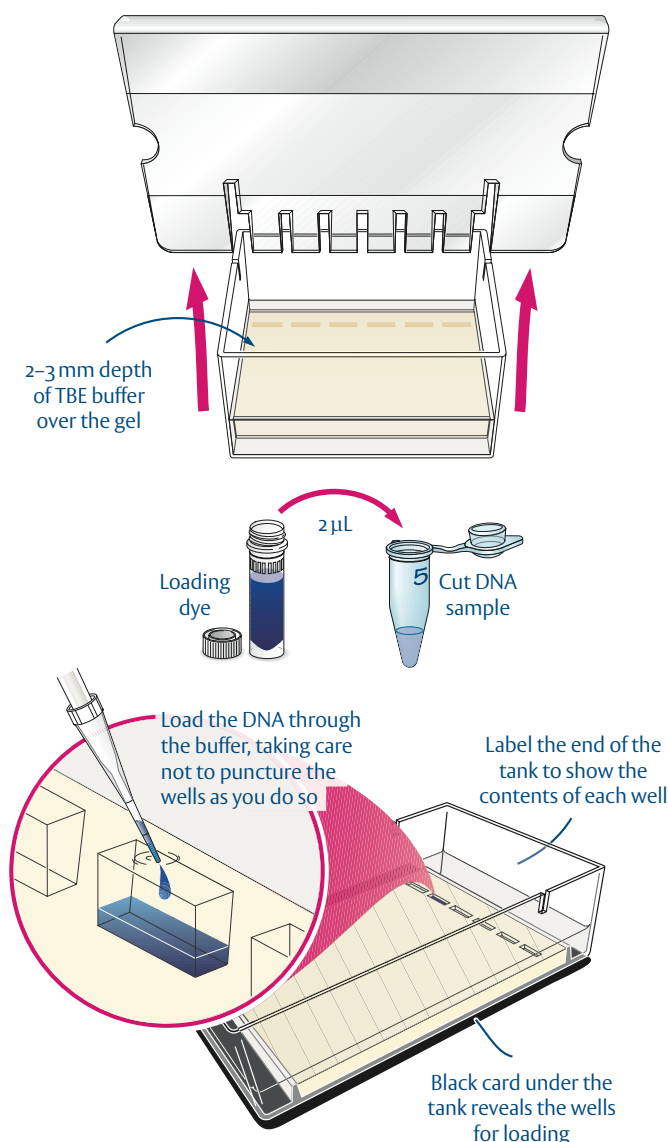
4 Preparing the agarose gel

- a. If it has not already been done, use a microwave oven or a boiling water bath to melt some agarose gel (0.8% w/v in TBE buffer). *Swirl the liquid and hold the bottle up to the light to check that no lumps or fibres of undissolved agarose remain.* Store the molten agarose in a water bath or incubator at 55–60 °C until it is needed.
- b. Cut two pieces of the carbon fibre tissue, each about 42 mm x 22 mm. These will be the electrodes at either end of the gel tank. Test that the electrodes fit neatly and without curving or folding, then take them out and put them to one side.
- c. Place a 6-toothed comb into the slot at one end of the gel tank, then place it on a *level* surface. This is important because if the gel sets at an angle, the DNA fragments will not run evenly through the gel.
- d. Taking care not to scald yourself, swirl the bottle again and pour 11–12 mL of molten agarose into the gel tank so that it fills the central cavity and flows under and between the teeth of the comb. Try not to add too much agarose — you need a flat gel, not one that curves upwards or downwards. If you spill agarose solution into the areas at either end of the tank, just leave it — you can scoop the extra agarose out later, once it has set.
- e. Leave the tank undisturbed for 20–30 minutes, until the gel hardens (the gel will turn opaque as it sets).



5 Loading the gel

- Pour slightly more than 10 mL of TBE buffer solution into the gel tank. The liquid should just cover the surface of the gel and flood into the areas at either end.
- Very gently ease the comb from the gel, allowing the buffer solution to fill the wells left behind. Take care not to tear the wells as you lift the comb out.
- Place the gel tank on a dark surface, such as a piece of black paper. Alternatively, a strip of black tape can be stuck onto the bottom of the tank beneath the wells. Put the gel tank where it will remain undisturbed while the gel is being 'run'.
- Put a clean tip on the microsyringe. Add 2 μL of loading dye to the tube containing the DNA you wish to load. Mix the dye and the DNA sample thoroughly by drawing the mixture up and down in the microsyringe tip.
- Pipette the mixture of loading dye and DNA into one of the wells, holding the tip above the well but under the buffer solution (see illustration). Take great care not to puncture the bottom of the well with the microsyringe tip.
- Make a note of which DNA you have put into the each well (write on the side of the tank with a marker pen).
- Repeat steps d-f with the other digested DNA samples (perhaps from other students in your class). To avoid cross-contamination, it is **essential** to use a new tip for each sample.
- Add 1 μg of DNA 'ruler' or 'ladder' to one of the six wells in each tank.

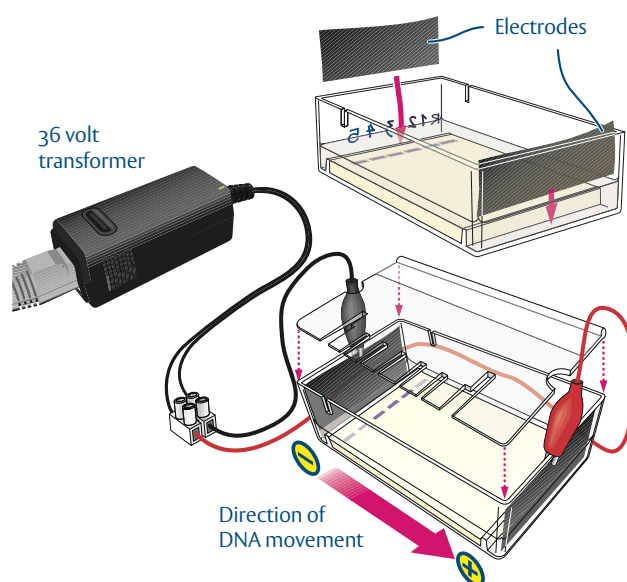


6 'Running' the gel

- Fit one electrode at each end of the tank as shown in the illustration. Attach crocodile clips to the top of the electrodes. A 36 volt transformer or sufficient batteries (in series) should be used, giving a total voltage of no more than 36 volts. Ensure that the positive terminal of the battery is connected to the electrode **furthest** from the wells.
- Check that contact is made between the buffer solution and the electrodes (with care, you can add more buffer if necessary). Place the comb over the tank to reduce evaporation during the electrophoresis. Leave the gel to 'run', undisturbed, for several hours. At 36 volts, it will take about two hours to run a gel.
- Disconnect the power supply once the blue loading dye has reached the end of the gel. If you leave the batteries connected, the DNA will run off the end of the gel!
- Rinse the crocodile clips in tap water and dry them thoroughly after use to prevent corrosion.

Notes

Ions in the TBE buffer used in and above the gel conduct electricity. This buffer is alkaline. Under alkaline conditions the phosphate groups of the DNA are negatively charged and therefore the DNA moves towards the positive electrode (anode) when a current is applied. EDTA in the buffer 'mops up' or chelates divalent cations. This helps to prevent damage to the DNA as such ions are required (as co-factors) by DNA-degrading enzymes. The loading dye does not affect the DNA, but moves through the gel slightly ahead of all but the smallest of DNA fragments. The loading dye moves at about the same rate as DNA fragments of 300 base pairs.



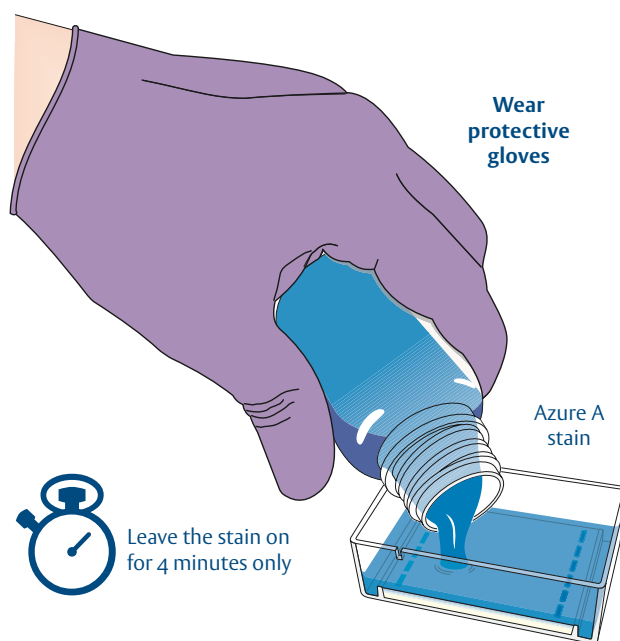
APPLY NO MORE THAN 36 VOLTS!



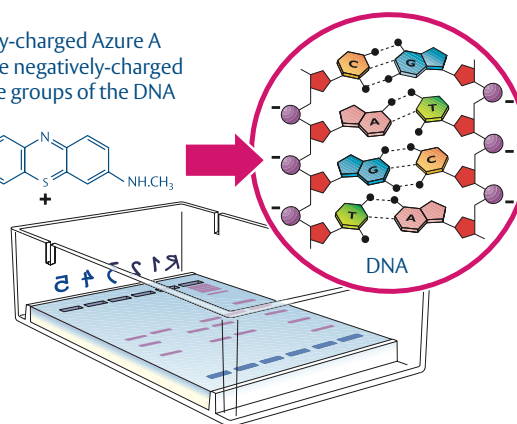
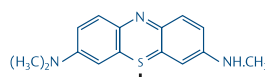
Serious or lethal electrical shock may occur if you connect the equipment directly to a mains electricity supply.

7 Staining the gel

- Remove and dispose of the electrodes. Pour off the buffer solution. The buffer may be retained and re-used several times, but it will not last indefinitely.
- Put on some protective gloves to prevent the stain from touching your skin (the stain is not harmful, but who wants blue fingers?)
- Leave the gel in the tank. Pour about 10 mL of staining solution (0.04% Azure A in 20% ethanol) onto the surface of the gel.
- Leave it for *exactly* 4 minutes, then return the stain to a bottle for re-use. (Like the buffer, the DNA stain can be re-used several times before you need to replace it. You may find, however, that older stain needs to be left on the gel for a little longer than 4 minutes.)
- Wash surplus stain from the gel surface with about 5 mL of 70% ethanol for a few seconds (industrial denatured alcohol, IDA, can be used for this task).
- Pour away the alcohol, then rinse the surface of the gel with water 3 or 4 times, *but do not leave any water on the gel, as this will remove stain from the top of the gel*. The remaining stain will gradually move down through the gel, staining the DNA as it does so. Faint bands should start to appear after 10 minutes. The best results will be seen if the gel is left to 'develop' overnight (put the tank in a plastic bag if you do this, to prevent the gel from drying out). Stained gels may be stored indefinitely in a sealed plastic bag, kept in a dark, cool place such as a refrigerator.



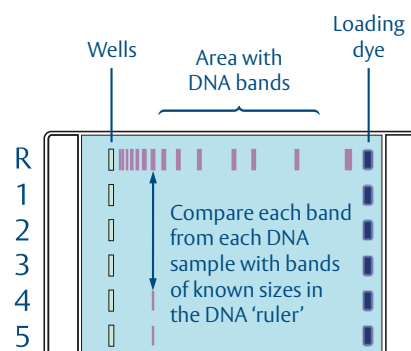
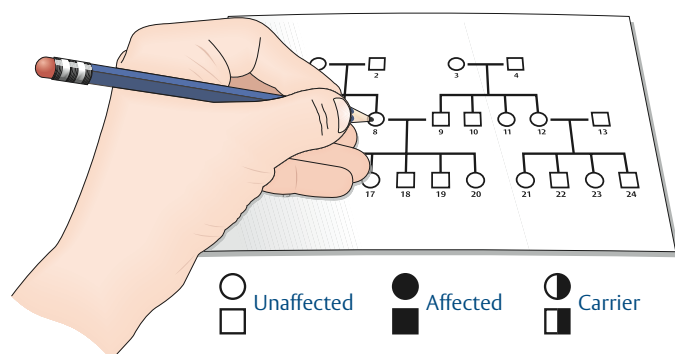
Positively-charged Azure A binds to the negatively-charged phosphate groups of the DNA



OPTIONAL STOPPING POINT

8 Recording the results

- Make a careful note of your results. For each DNA sample, you should see one, two or three bands on the gel. Record the number of bands you see alongside the number of the DNA sample that you have written on the side of the gel tank.
- Obtain the results for all of the other DNA samples from other people in your class.
- Write the number of bands obtained for each person by their square or circle in the family tree, then mark the squares or circles to show whether they are unaffected, affected or heterozygous gene carriers.
- Compare the sizes of the fragments obtained from the samples with the sizes of the bands in the DNA 'ruler'.



DNA RULER or LADDER

These are the sizes of the fragments in the DNA 'ruler' or 'ladder' in base-pairs (bp) and their relative positions on the electrophoresis gel.



11 198	12 216
10 180	9 162
8 144	7 126
6 108	5 090
	4 072
	3 054
	2 036
	1 636
	1 018
	506 + 517
	Loading dye

Safety guidelines

DNA and restriction enzyme

The DNA and restriction enzyme provided with this kit are safe for use in schools. No living organisms are used, so there is no need to follow strict aseptic techniques. However, cleanliness is important to prevent cross-contamination and ensure success. Used plastic (polypropylene) tubes and microsyringe tips can be disposed of in the normal waste.

Agarose gel

If a microwave oven is used to melt the agarose gel, ensure that the gel is placed in an *unsealed* container. A boiling water bath or hotplate may be used instead, but the gel must be swirled as it melts to prevent charring. The use of a Bunsen burner to melt agarose is *not* recommended.



Molten agarose can scald and it must be handled with care, especially just after it has been heated in a microwave oven.

TBE buffer (Tris-Borate-EDTA)

When used as directed, this buffer presents no serious safety hazards. Spent buffer can be washed down the drain.

Loading dye (Bromophenol blue)

When used as directed, this loading dye presents no hazard. Used loading dye can be washed down the drain.

Electrode tissue

The carbon fibre electrode tissue may release small fibres, which can cause skin irritation if you handle the tissue a lot. Wear protective gloves if you find the tissue unpleasant to handle. The fibres are too large to enter the lungs however, so it is not necessary to wear a face mask. The fibres are soluble in body fluids and are completely biodegradable.

Electrical supply

The gel electrophoresis equipment was designed to be used with direct current at low voltages (≤ 36 volts) from batteries or a transformer such as that supplied by the NCBE. ***Under no circumstances should this voltage be exceeded, as the live electrical components are not isolated from the user.***



Serious or lethal electrical shock may occur if you connect the equipment directly to a mains electricity supply.

DNA stain (Azure A)

The concentrated DNA stain solution is flammable and it must be kept away from naked flames. The stain is Azure A, which when diluted as directed, forms a 0.04% solution in 20% ethanol. At this concentration it presents no serious safety hazard, although care should be taken to prevent splashes on the skin or eyes *e.g.*, wear protective gloves and eye protection. Used solution can be diluted with water and washed down the drain.

Further information

Printed publications

When a gene makes you smell like a fish by Lisa Seachrist Chiu (2007) Oxford University Press. ISBN: 978 0195327069.
Is it in your genes? by Philip R. Reilly (2004) Second edition. Cold Spring Harbor Laboratory Press. ISBN: 978 0879697211.
An ABC of clinical genetics by Helen M. King (2002) Third edition. British Medical Journal Publishing Group. ISBN: 978 0727916273.

Web sites

- Genetic Alliance UK
www.geneticalliance.org.uk
- Nuffield Council on Bioethics
www.nuffieldbioethics.org
- Online Mendelian Inheritance in Man
www.ncbi.nlm.nih.gov/sites/entrez?db=omim

Molecular structure data

The images of molecules on pages three and five of this booklet show the enzyme *Bam*HI bound to DNA. They were created using data from: Newman, M., *et al* (1995) Structure of *Bam*HI endonuclease bound to DNA: partial folding and unfolding on DNA binding. *Science* **269**, 656–663. This data can be downloaded from the Protein Data Bank [PDB ID: 1BHM]: www.rcsb.org/pdb

The software used to produce these pictures was UCSF Chimera and VMD (Visual Molecular Dynamics), both of which can be downloaded free-of-charge from: www.cgl.ucsf.edu/chimera/ and: www.ks.uiuc.edu/Research/vmd/ respectively.

For more information about restriction enzymes, an excellent source is David Goodsell's 'Molecule of the Month' series at the Protein Data Bank web site: www.rcsb.org/pdb/101/motm.do?momID=8