

Nature's dice

3.1 Teacher's and technician's notes

Introduction

About Nature's dice

The *Nature's dice Student's Guide* describes a practical, laboratory-based simulation of genetic screening centred around a fictitious extended family. It aims to enliven the teaching of genetics and to integrate theory, technology and some ethical issues in one activity.

Students are asked to determine the mode of inheritance of a genetic condition by analysing DNA samples that are said to come from individual members of the extended family. Restriction enzymes are used to cut the DNA into fragments and these are separated by gel electrophoresis. The DNA is then stained so that it can be seen and the class results are combined and analysed. This mirrors the way in which genetic analysis has traditionally been carried out.

The science content includes:

- classical Mendelian inheritance (recessive autosomal or sex-linked traits);
- the use of restriction enzymes to cut DNA and help identify genetic variation;
- DNA gel electrophoresis;
- genetic screening.

This activity can also be used to introduce some of the ethical issues that arise from genetic screening and to emphasise the importance of genetic counselling. For example, one of the offspring in the tree has DNA which could not have come from the supposed parents. This can lead to a discussion about the possible reasons for this: a novel mutation; mislabelling of samples; contamination; adoption; re-marriage; infidelity *etc.*

A thought-provoking guide to consent and confidentiality in genetic testing has been produced by the Joint Committee on Medical Genetics in the UK. Entitled *Consent and confidentiality in clinical genetic practice: Guidance on genetic testing and sharing genetic information*, this publication contains numerous clinical scenarios. The issues posed in each scenario could be asked of family members in the *Nature's Dice* pedigree. The document can be downloaded from the

British Society for Human Genetics web site: http://www.bsgm.org.uk/media/678746/consent_and_confidentiality_2011.pdf

Selection of a genetic condition

There are two ways in which the exercise may be used. It can be used to illustrate a true medical condition and its inheritance, such as the single base change (point mutation) that can cause sickle cell anaemia. Alternatively, if teachers do not wish to a 'real' condition and the various concerns it may give rise to, particularly if members of the class may have friends or relatives who are affected, a fictitious trait may be invented.

There are advantages and disadvantages to both approaches. Taking a fictitious condition might be thought of as making light of serious medical issues and, if an inappropriate trait is invented, it could lead students to assume that complex traits are inherited in a Mendelian fashion (*i.e.*, ignoring multifactorial conditions and the impact of environmental influences).

It should be remembered, however, that this activity is intended as part of a teaching programme about genetics and/or DNA technology and it aims merely to introduce issues which can then be explored further.

Additional information that may help in the selection of an appropriate condition may be found on these web sites:

- *The Human Genome project: Exploring our molecular selves:* www.nhgri.nih.gov/educationkit
- *The Human Genome: A guide to online information resources:* www.ncbi.nlm.nih.gov/genome/guide/human
- *Online Mendelian inheritance in man:* omim.org
- *GeneTests — GeneClinics:* www.ncbi.nlm.nih.gov/sites/GeneTests/
- *The Wellcome Trust:* www.wellcome.ac.uk/genome
- *Nuffield Council on Bioethics:* www.nuffieldbioethics.org

Inherited conditions

Single factor (gene)

Clear path of inheritance
High chance of inheritance
Rare in the population
Many known examples

Multifactorial

No clear path of inheritance
Lower chance of transmission
Widespread in the population

Examples

Autosomal dominant

Huntington's disease

Autosomal recessive

Sickle cell disease
Tay-Sachs disease
Thalassaemia
Cystic fibrosis

X-linked recessive

Duchenne muscular dystrophy
Haemophilia A, B

Y-linked

Webbed toes

Procedure

The story (to present to students)

A single gene is being investigated. Blood samples have been taken from each of the 24 members of an extended family and from these DNA has been extracted and amplified using the polymerase chain reaction (PCR)*. For the single locus being investigated there are two different alleles (*i.e.*, types of DNA) possible.

Someone who is homozygous for the dominant allele (genotype DD) will only have DNA of type D. A person who is homozygous for the allele giving rise to the condition (genotype dd) will only have DNA of type d. Heterozygous individuals (genotype Dd) will have DNA of both types. Amplification of the DNA region of interest using PCR gives fragments of the same size for both alleles, in this case 6 500 base pairs (bp).

The tubes you will be provided with contain the amplified DNA. The task is to detect which forms of DNA are present in each sample by treating the DNA with a restriction enzyme (*Bam*HI) and examining the resulting DNA fragments using gel electrophoresis.

The difference in the DNA sequence of the alleles D and d is such that, in the allele d there is a base sequence that can be 'recognised' and cut by the restriction enzyme *Bam*HI. The allele D has no restriction site for *Bam*HI and thus will not be cut by the enzyme. Thus, after treatment of the DNA with restriction enzyme and electrophoresis, the following results may be seen:

Genotype	No. of bands
DD or AA	1
dd or aa	2
Dd or Aa	3

A DNA 'ruler' (consisting of fragments of known sizes) is run alongside the samples to confirm that the two small fragments would combine to form the large piece (4 000 + 2 500 = 6 500 bp).

A *PowerPoint* or *Keynote* presentation showing the method, *etc* can be downloaded from the NCBE web site: <http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/DNA/dicemodule.html>

**in fact, the samples are plasmid DNA and are not of human origin.*

Preparation and timing

Before the practical session

Decide upon the mode of inheritance for the condition being investigated (autosomal recessive or sex-linked) and dispense the samples as shown on page 6. Store the samples in a freezer at -20 °C until they are required.

Prepare the agarose gel by melting the agarose in TBE buffer. Before the lesson, melt the agarose and keep it molten at 50–65 °C (*e.g.*, in a water bath) until it is required.

You can use either the 4- or 6-toothed comb for this exercise. Using the 4-toothed comb, eight tanks will provide sufficient wells for the 24 samples plus a DNA 'ruler' in each tank. With the 6-toothed comb, fewer tanks are required, or the extra wells can be used for a second run of each sample.

Digestion of the DNA samples and casting of agarose gels

Students place the DNA samples in the enzyme tubes, mix, then immediately incubate them for 30–45 minutes. During incubation, the agarose gels can be cast.

The digested DNA can be frozen at this stage, for loading and running in a later lesson. Prepared gels can be stored for several days, provided they are not allowed to dry out. If you wish to keep gels before use, place a little TBE buffer in the tanks over the gel, place them in a plastic bag and store in a refrigerator.

Loading and running the gels

Loading the gels takes about 10 minutes. At 36 volts it takes about 90 minutes to run the gels. After running, the gels should be stained immediately, because the DNA fragments will diffuse in the gel if they are left.

Staining the gel

Staining takes no more than 10 minutes, with bands normally becoming visible after 20 minutes. Students will require a photocopy of the family tree on which to record the class's results, and a copy of the DNA 'ruler' (page 7).

Equipment and materials

Required by each student or working group

Use straight from the kit

- A copy of the *Student's guide*
- Enzyme *Bam*HI (blue tube) — one per DNA sample
- Carbon fibre tissue from which two electrodes per gel tank, each approximately 42 mm x 22 mm, can be cut
- White, graduated pipette tips — two per DNA sample
- Bromophenol blue loading dye — 2 μ L per DNA sample
- DNA 'ruler' — 2 μ g per well (for the 6-toothed comb)
- Foam floater for holding the tubes in a water bath (shared between groups)
- A photocopy of page 7 of this guide

Supplied in the kit, but prepared in advance

- One or more DNA samples, dispensed by you in advance using the 25 μ L minipipet provided in the kit (see page 6)
- TBE buffer (prepared by diluting one volume of the 10x concentrate from the kit with nine volumes of distilled or deionised water). You will need ~22 mL per tank.
- Tolidine blue O (prepared by diluting the 2x concentrate from the kit with an equal volume of distilled water).
- 0.8% (w/v) DNA electrophoresis-grade agarose, made up in 1x TBE buffer (that is, the 10x TBE concentrate diluted as stated above). You will need 10–12 mL per tank.
IMPORTANT: Do not use water to make up the agarose gel. The ions in the buffer solution are required to conduct the electrical current.

Not in the kit: supplied by you

- An NCBE electrophoresis tank¹
- A six- or four-toothed comb for the electrophoresis tank¹
- A microsyringe¹
- Two electrical leads with crocodile clips¹
- A 36 V mains transformer² or new dry cell batteries with a combined voltage of 36 V e.g., 4 x type 6LR61 (9 volt)
- Scissors for cutting the electrode tissue
- A permanent marker pen for marking the tubes and the lanes on the gel tank
- Access to an incubator or water bath set at 37 °C
- Black card or paper (at least 50 mm wide) to go under the tank so that the wells can be seen
- Distilled or deionised water, for rehydrating the dried lambda DNA
- Protective gloves and eye protection (to protect skin and eyes from the DNA stain)

Notes

1. These non-consumable items can be purchased from the NCBE as a *Base Unit*, which contains eight gel tanks, eight of each type of comb, eight pairs of electrical leads and eight microsyringes. The protocol may be performed with other electrophoresis apparatus, but the volumes of agarose, TBE buffer and other reagents needed will vary. The results will differ too, as the intensity of the DNA bands will be affected by the exact sizes of the wells.
2. A suitable 36 V mains transformer can be purchased from the NCBE. A single transformer of this type can run at least four NCBE gel tanks.

Use of the reagents in the kit

Tris-Borate-EDTA (TBE) buffer

The 10x TBE buffer concentrate should be stored at room temperature. One volume of the concentrate should be diluted with nine volumes of distilled or deionised water before use. To reduce dilution errors it is a good idea to dilute all of the buffer supplied in the kit at once rather than trying to prepare small volumes.

Diluted buffer solution can be stored indefinitely in a refrigerator at about 4 °C in a sealed container. During storage, crystals of EDTA may come out of solution in the concentrate. The loss of a small amount of EDTA should not affect the electrophoresis — simply filter the crystals out of the solution. If the buffer concentrate crystallises significantly, however, you should obtain a new supply.

The TBE buffer can be re-used three or four times if desired. Eventually it becomes 'tired' due to electrolysis and evaporation and should be replaced with a fresh solution. Spent buffer should be washed down the drain with water.

Agarose gel

To prepare the agarose gel, the agarose powder must be dissolved in diluted (1x) TBE buffer. The concentration of agarose required for analysis of lambda DNA fragments is 0.8% (w/v). The liquid must be boiled so that all of the powder dissolves, and that no flecks or lumps can be seen when the solution is held up to the light. The best way of preparing the gel is to use a microwave oven.

IMPORTANT: Do not heat sealed containers of agarose gel in a microwave oven — they are likely to explode! Always loosen the tops on bottles, or heat the agarose solution in a beaker or flask covered with plastic film that has been punctured several times to let any steam escape.

Also note that hot, molten agarose can scald, and it must be handled with care, especially just after it has been heated in a microwave oven, when it can froth up without warning.

Heat-proof gloves should be worn when handling containers of the hot, molten gel.

Once it has been boiled, the agarose solution can be kept molten in a water bath at 55–60 °C until you are ready to cast the electrophoresis gels. Alternatively, the gel can be poured into a container such as a screw-capped bottle, sealed, allowed to set and kept until required. Wide-mouthed glass bottles with plastic tops are ideal, such as *Duran*[®]-style bottles.

Unlike nutrient agar, agarose gel will not support the growth of microorganisms, so it can be stored in sealed containers, set, for many months at room temperature. To re-melt the gel, use a 60 °C water bath or a microwave oven.

It is a good idea to make up all the agarose gel from the kit in one go, rather than trying to prepare small volumes. If you do the latter, the chances are that the gel will be of the wrong concentration, which can significantly affect the time taken to run the gel and the quality of the results obtained.

Agarose is expensive. Damaged, unused, gel *e.g.*, from a poorly-cast gel, may be re-melted and cast again. Care should be taken not to allow excessive evaporation from the gel as it is re-heated, however, as this will alter the concentration of the agarose, which will slow down the movement of the DNA fragments. Do not attempt to re-use agarose that has already been used for electrophoresis.

Used agarose gel is not hazardous (provided it has been stained only with Toluidine blue O stain) and may be disposed of in the normal waste.

Toluidine blue O stain (Replaces Azure A stain)

The *concentrated* DNA stain solution is 0.08% Toluidine blue O dissolved in water. This solution replaces Azure A from previous versions of the kit and is safer to use as it is not flammable. The stain also produces clearer bands on the gel than Azure A.

The concentrate should be diluted with an equal volume of water before use. Both the concentrate and the diluted stain should be stored at room temperature.

Many chemicals that bind to DNA are mutagens, because they intercalate within the double helix like an extra pair of bases, causing frame-shift mutations. In contrast, Toluidine blue O and similar stains (such as Azure A, Azure B and Methylene blue) are not thought to be mutagens.

The concentrate should be diluted before use with an equal volume of distilled or deionised water. At this concentration it presents no serious safety hazard, although care should be taken to prevent splashes onto the skin and eyes *e.g.*, wear protective gloves and eye protection (see the accompanying Safety Data Sheet).

Exposure of the solution to strong sunlight may weaken the stain. The diluted Toluidine blue O solution may be re-used several times. After many uses its effectiveness will be reduced as it is gradually diluted with buffer solution washed from the gel. With elderly solution it may be necessary to stain the gel for longer than the four minutes that is suggested in the *Nature's dice Student's guide*.

Used or unwanted Toluidine blue O solution may be washed down the drain with plenty of water.

DNA and restriction enzymes, tubes and pipette tips

The DNA and restriction enzymes provided in this kit are safe for use in schools. No living organisms are used, so there is no need to follow strict aseptic techniques. Cleanliness is important, however, to prevent cross-contamination and ensure success.

The dried DNA and restriction enzymes **MUST** be stored at room temperature, with silica gel desiccant in the foil pouch provided. The pouch should be tightly-sealed to prevent the dried enzymes and DNA from absorbing moisture from the air. In dry conditions, the DNA and enzymes will remain stable for many years.

Under no circumstances should the pouch be stored in a fridge or freezer as moisture will condense inside, damaging the dried DNA and enzymes.

Used plastic (polypropylene) tubes and microsyringe tips together with any remaining DNA or enzyme solutions can be disposed of in the normal waste.

Bromophenol blue loading dye

When used as directed, this loading dye presents no hazard. It should be stored at room temperature. Used loading dye can be washed down the drain.

Carbon fibre electrode tissue

The electrode tissue is essentially carbonised cotton. The fibres comprising the tissue are bound together with PVAc (polyvinyl acetate), which is also known as 'wood glue', 'carpenter's glue' or 'school glue'.

The tissue may release small fibres, which can cause skin irritation if you handle it 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.

Used electrode tissue should be disposed of in the normal waste.

Electrical safety

The NCBE 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.

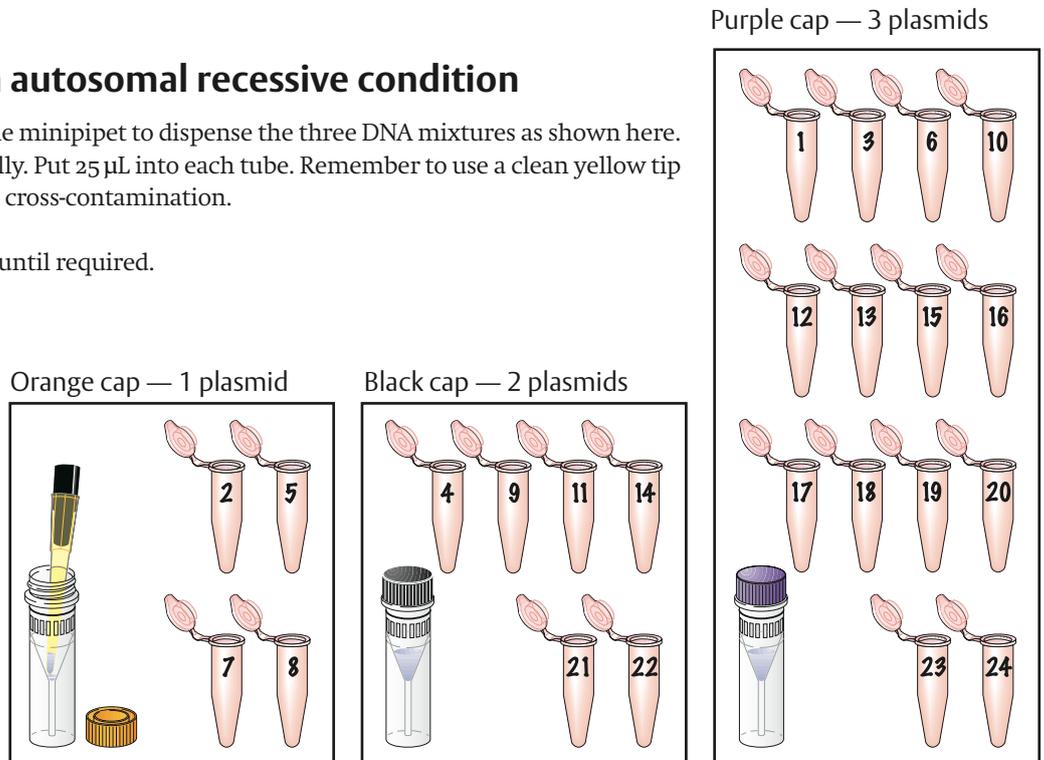
Preparing the DNA samples

This should be done in advance by the teacher or technician

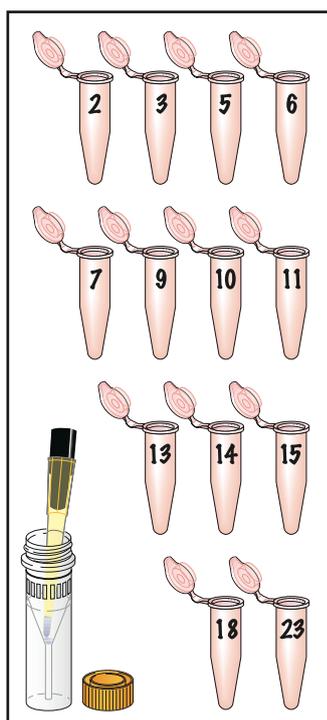
Simulation of an autosomal recessive condition

Use the 25 μ L fixed volume minipipet to dispense the three DNA mixtures as shown here. Number the tubes carefully. Put 25 μ L into each tube. Remember to use a clean yellow tip for each mixture to avoid cross-contamination.

The tubes may be frozen until required.



Orange cap — 1 plasmid

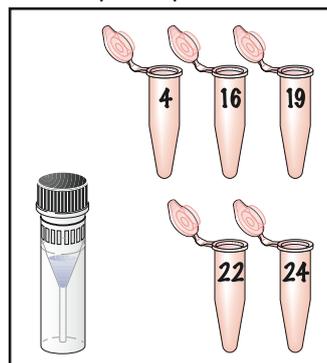


Simulation of a sex-linked recessive condition

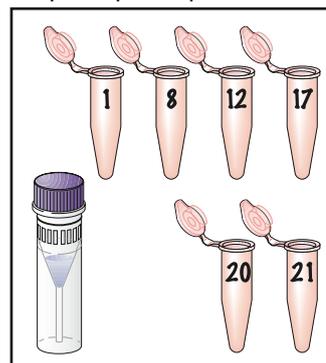
Use the 25 μ L fixed volume minipipet to dispense the three DNA mixtures as shown here. Number the tubes carefully. Put 25 μ L into each tube. Remember to use a clean yellow tip for each mixture to avoid cross-contamination.

The tubes may be frozen until required.

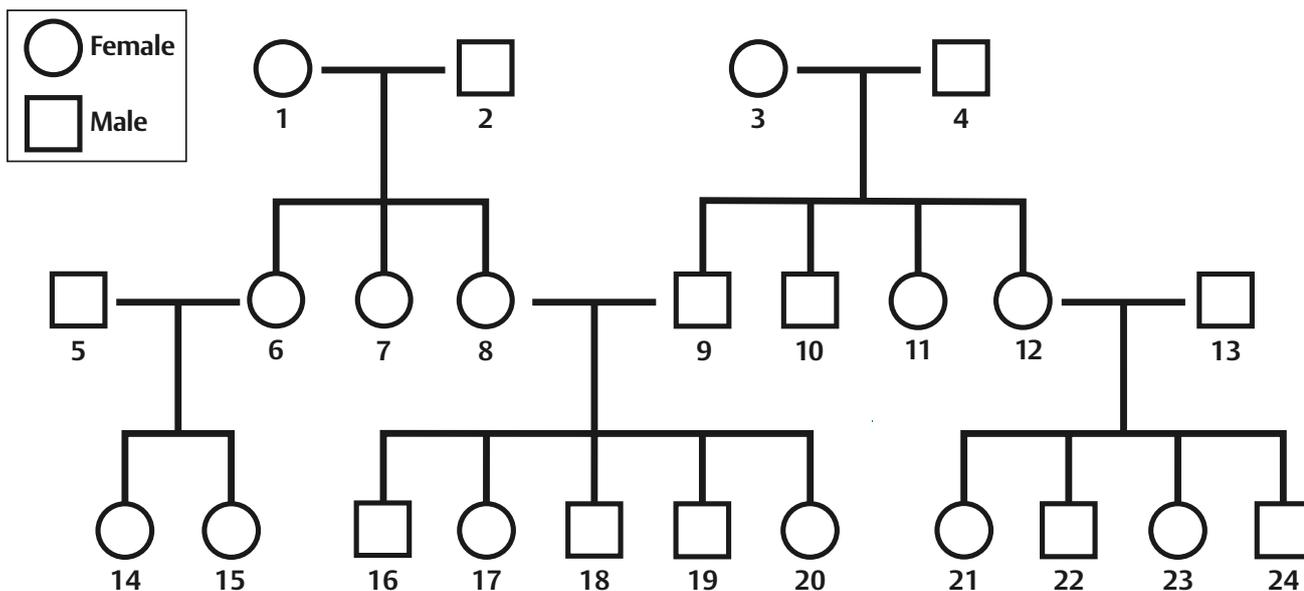
Black cap — 2 plasmids



Purple cap — 3 plasmids

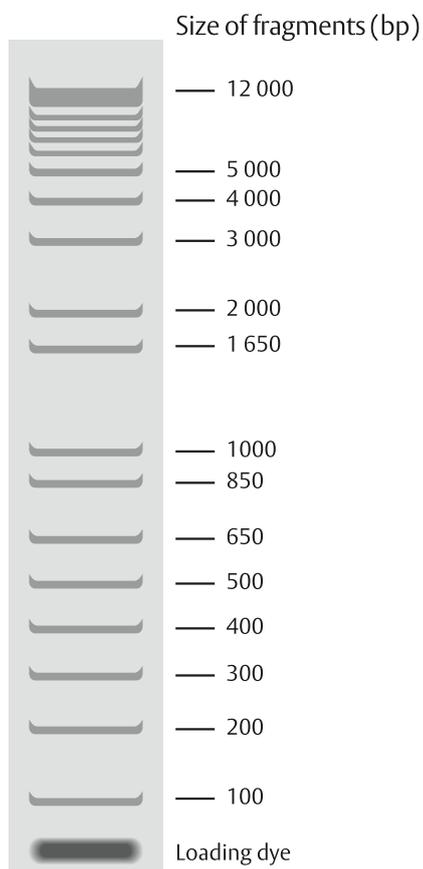


The family tree



Fill in the results (that is, the number of bands on the gel) on this diagram.

The DNA ruler



A DNA 'ladder' or 'ruler' consisting of fragments up to 1.2 kb is included so that the DNA under investigation can be compared with fragments of known sizes.

The fragment sizes are given in base pairs (bp).

The bromophenol blue loading dye moves through the gel as if it were made of 500 bp DNA fragments.

With a six-tooth comb, you should use 2 µg of 'ruler' solution plus 2 µg of loading dye per well. With the 4-tooth comb you will need about 3 µg of 'ruler' plus 2 µg of loading dye per well.

Note: This DNA ruler is NOT the same as the one shown in the student's guide. It is an improved version with more regularly-distributed DNA fragment sizes.

Hints and tips

Using microsyringes

Students' ability to dispense precise volumes using the microsyringes will improve with experience. It is therefore a good idea to practise transferring liquids (*e.g.* food colouring) before starting work with expensive DNA, enzymes and other reagents. Some extra microsyringe tips are provided in the kit for this purpose.

Loading the gel

A steady hand is required to load gels. Practice gels may be cast from cheaper agar (use a 1% solution) rather than agarose. When practising, use water rather than buffer over the gel. Sufficient loading dye has been included in the kit for such attempts. Practice gels can be washed out and re-used several times. *Note: agarose, dissolved in diluted TBE buffer, not water, must be used for the 'real' gels with DNA.*

Melting agarose

Ensure that the container used to prepare the agarose gel is clean. Tiny flecks of dust will not affect the way the gel runs, but they can prove a nuisance when you are trying to see faint bands in the gel.

For convenience, dissolve and melt the agarose using a microwave oven. Less than a minute at full power in a 940 watt oven is sufficient for 100 mL of gel. The container (flask or beaker) used to hold the molten agarose must not be sealed, but covered lightly with plastic film that has been punctured with one of two small holes. Swirl the gel half way through the heating cycle to ensure that it is mixed thoroughly.

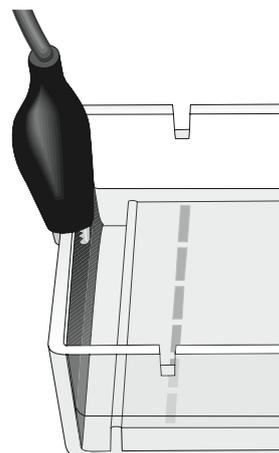
The DNA bands are very faint

If you are certain that the DNA has been dispensed and mixed correctly, another cause of missing or faint bands is incorrect use of the Toluidine blue O stain. Once you have applied the Toluidine blue O solution to the gel, poured off the stain, then rinsed off the excess, take care not to leave any water on the gel. If necessary, wipe over the gel gently with a finger tip to disperse beads of water. The blue stain that starts in the upper layer of the gel can re-dissolve even in small drops of water left on the surface. 'Soaking' away the stain into water drops on the surface prevents it from passing down through the gel to stain the nucleic acid.

Under no circumstances should you attempt to de-stain the gel. Although this is necessary with methylene blue (to remove 'background' stain), the method using Toluidine blue O has been specifically devised to avoid the need for de-staining. The bands on gels stained with Toluidine blue O should not fade for several months.

Corrosion of crocodile clips

The crocodile clip used at the anode will slowly corrode due to electrolysis. This can discolour the buffer solution and gel, but will not interfere with the electrophoresis. The chances of corrosion can be reduced by ensuring that only the first two teeth of the clip are in contact with the electrode tissue, as shown here:



Eventually, you will need to replace corroded clips. Gold-plated crocodile clips are not subject to such corrosion. These are available at relatively low cost from high street electronic component retailers as well as the usual school suppliers.

Viewing the stained gel

The bands are seen most easily by holding the gel at an angle against a well-lit surface. A light box is ideal, but if one is not available, you can put a sheet of white paper on an overhead projector, and put the stained tanks on that for direct observation. Several authors suggest that a yellow or orange filter improves the contrast between stained bands and the background. While this may be true if you are taking photographs, filters can make it more difficult to spot very faint bands.

A magnifying lens can be useful; flat fresnel lenses the size of a credit card (sold for use with atlases) are ideal.

If the contrast between the background and the DNA bands is strong, an image of the gel may be projected onto a screen using an overhead projector.

There's no DNA on the gel

A 'blank' lane on the gel or very faint bands usually indicate a pipetting error. It is essential that the DNA is correctly dispensed for the students to use and that it is thoroughly mixed with the restriction enzyme and the loading dye. To ensure that this is done, always draw the DNA solution up and down in the microsyringe tip a few times.

The bands on the gel are very close together

There are several possible causes of this. Provided the buffer has not evaporated excessively while the gel was running (slowing the electrophoresis down), it may be that the concentration of agarose you used was wrong. Always make up more gel than you need to ensure that its concentration is accurate.

There is a very significant difference between say, a 1% and a 0.8% agarose gel — only a slight error in weighing a small amount of agarose powder can produce inaccuracies of this scale. The concentration of agarose you should use with this practical activity is 0.8% (mass to volume).

The gel takes too long to run

At 36 V, the loading dye should move about 5 mm per hour. At a lower voltage the loading dye will move more slowly. If, after the first 10 minutes, the loading dye does not seem to have moved and bubbles are not visible at the cathode, check the electrical contacts between the batteries and the electrodes. Ensure that there is enough buffer above the gel to cover the plastic ridges at each end of the tank, but not so much that most of the current passes through the buffer solution rather than the gel. In a warm environment liquid may evaporate from the buffer. The tank should be covered with the comb to reduce such evaporation.

Specimen results

Sex-linked inheritance

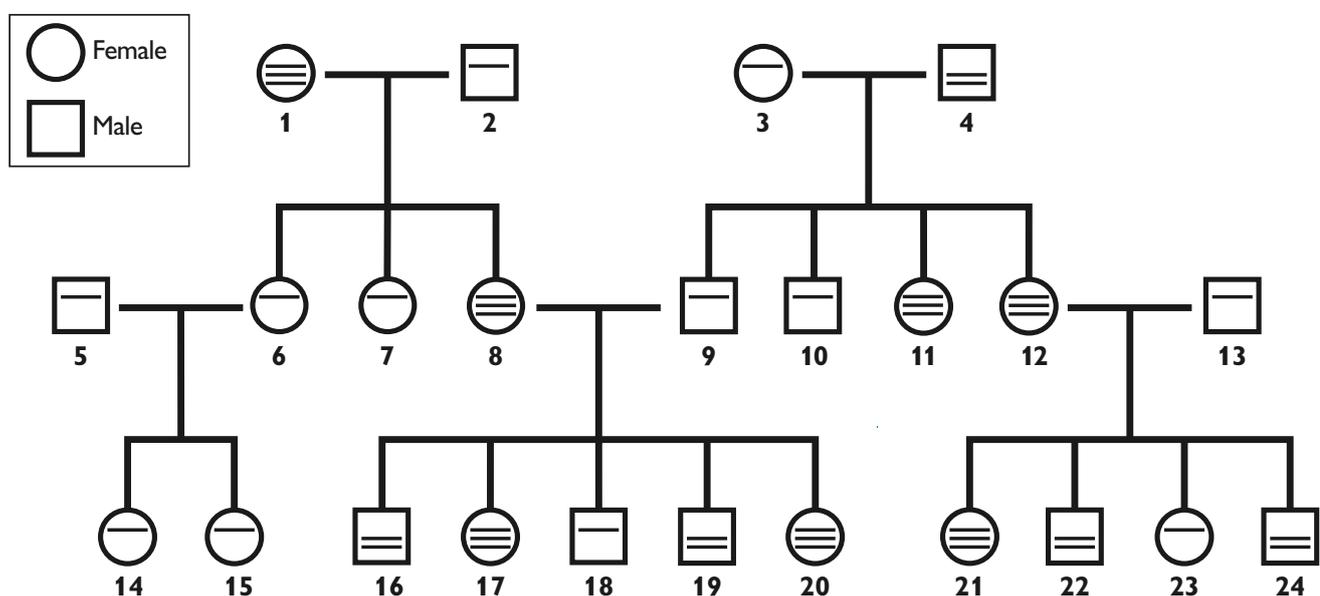
The family tree below shows the inheritance of a sex-linked recessive condition.

It has been designed to pose the question 'Is the inheritance of the condition a normal recessive inheritance

pattern or is it sex-linked?' Students have to examine the gel electrophoresis results and the family tree and make deductions about the mode of inheritance. This task is more complex than analysing an autosomal recessive condition.

Results for a sex-linked condition

Showing the number of DNA bands to be expected on the electrophoresis gels



Specimen results

Autosomal recessive inheritance

The family tree below shows the results for the inheritance of an autosomal recessive condition. Several points can be made while discussing these results with students:

The mother (8) is homozygous for the dominant allele while the father (9) is homozygous for the recessive allele. One would therefore predict that all their children will be heterozygous.

12 and 13, being heterozygous, will produce gametes carrying either dominant or recessive alleles (in a 50 : 50 ratio). The children from 12 and 13 show only two of the three possible genotypes for this locus. This should lead to a discussion about probability and reality (avoiding possible misconceptions that every fourth child must be of a particular genotype if the previous three were of another type).

There is a deliberate problem posed by the analysis of the gels. Individuals 5 and 6 have two children, one of whom turns out not to follow the expected pedigree.

As the mother (6) is heterozygous and the father (5) is homozygous for the dominant allele, one child (15) presents no problems; however, child 14 is unexpectedly found to be homozygous for the recessive allele. Students usually suggest several possible explanations:

- the child may be adopted and therefore the parents are not her biological parents;
- the child could be the offspring of a previous relationship;
- there was a mix-up in the samples and the test should be repeated;
- there was an unexpected mutation (after some thought the students may realise that this must have occurred during the formation of the father's sperm);
- finally some students will, no doubt, suggest that the mother has had an affair with an unknown man.

Results for an autosomal recessive condition

Showing the number of DNA bands to be expected on the electrophoresis gels

