National Centre for Biotechnology Education

University of Reading

CRIME SCENE DO NOT CROSS CRIME SCENE DO NOT CROSS CRIME SCENE DO NOT CROSS

DNA Detective

Crime Scene Investigation

Teacher's Guide

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Equipment and Materials

Supplied in the kit

8 Student guides

1 teacher/technician guide

5 plasmid tubes with coloured lids: (yellow, orange, blue, green, purple)

1.5 kb ladder: clear lid

8 tubes of loading dye

50 ml bottle of concentrated toluidine blue O

50 ml bottle of concentrated TBE buffer

2 g bottle of agarose

Bag of white tips (100)

8 sheets of carbon fibre

Coloured 1.5 ml tubes (16 yellow, 16 orange, 16 blue, 16 green, 16 purple, 16 clear)

Not supplied in the kit

NCBE electrophoresis base unit (can be purchased separately)

Power pack or batteries to run the gel

Microsyringes (can be found in NCBE electrophoresis base unit)

Scissors for cutting carbon fibre electrodes

A permanent marker pen for labelling tubes and the gel tanks

Black card or paper to go under the gel tank so the wells can be seen for loading

Distilled or deionised water to dilute the TBE buffer and toluidine blue O.

The Story

A body has been found and the students need to investigate who the murderer is using DNA analysis. Forensic scientists have collected blood samples from the crime scene. These include a sample from the victim; so it can be isolated and ruled out of the DNA analysis and a blood sample found on a blunt object near the body, which does not belong to the victim. Additionally, police have collected blood from 3 potential suspects that were all in the vicinity when the crime was committed.

The forensic scientists have already extracted DNA from these 5 blood samples and amplified a specific region using the polymerase chain reaction (PCR). This DNA has been digested using restriction enzymes, so the DNA is linear and not coiled, making it much easier to see on a gel. Each DNA sample should show 2 bands. Only the DNA from one suspect will match up with the unknown DNA found at the crime scene.

The kit provides enough material for 16 groups of students, split into 2 classes of 8 groups (unless you have two base units and so can run 16 gels at once).

Each group of students will get 5 DNA samples (victim, crime scene, suspects 1-3) plus a DNA ladder (consisting of fragments of known sizes), which will be run alongside the other DNA samples allowing the students to assess the size of the DNA bands they can see.

Preparation and timing

Before the practical session

The DNA samples

In the kit there is a master DNA tube for each of the DNA samples. These need to be aliquoted and can be stored at -20 °C until needed for the lesson. Before aliquoting, each master DNA tube should be thawed thoroughly and ideally spun in a centrifuge to make sure all the DNA is at the bottom of the tube and not on the lid. See table below to see which coloured lid corresponds with which DNA sample.

Colour of lid	**DNA sample**
Yellow	Victim
Orange	Crime scene
Green	Suspect 1
Blue	Suspect 2
Purple	Suspect 3

Supplied in the kit are coloured 1.5 ml tubes for the DNA to be aliquoted into; the colours match the master tube lids. For example, DNA from the master tube with the yellow lid should be aliquoted into the yellow 1.5 ml tubes. 20 µl of each plasmid should be aliquoted into the corresponding 1.5 ml tubes. There is enough DNA in the master tube for sixteen 1.5 ml coloured tubes. This needs to be done for each DNA sample, so that you have:

16 yellow tubes

16 orange

16 green

16 blue

16 purple. All with 20 µl of DNA in each.

Additionally, the DNA ladder is supplied in a tube with a clear lid and this should be aliquoted into the 16 clear 1.5 ml tubes. However, only **10** µl of this should be transferred into each tube.

Preparing the agarose

The agarose can be prepared before the lesson either in one bottle, which will be shared and passed around the lesson, or it can be aliquoted into smaller bottles so that each group has its own bottle.

The concentrated TBE buffer provided in the kit is sufficient to make 500 ml of diluted TBE buffer. The 2 g of agarose provided in the kit should be mixed with 250 ml of the diluted TBE buffer to give a 0.8 % agarose gel. To get the agarose and buffer to combine, you need to heat them. This should be done in the microwave. We would recommend that you swirl the bottle regularly to help mix the agarose and bring it to boiling. When using a microwave, be careful not to let the agarose boil over. All the agarose should be in solution, so the solution appears clear; with no lumps of solid agarose. If you are going to use half the agarose for another class this can be put in a separate bottle and allowed to solidify at room temperature, it can be heated back up when needed either in a microwave or in a water bath at 50–65 °C.

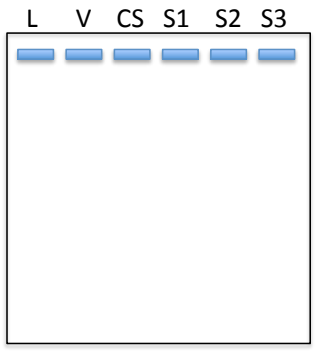
If the agarose is to be shared out into smaller bottles, this should be done when the agarose is molten. Be careful when pouring, since it will be hot. The agarose for can be kept molten at 50–65°C (*e.g.*, in a water bath) until it is required for the lesson. For more information, see agarose gel in the reagents section.

The students will need to use the 6-toothed combs provided in the NCBE electrophoresis base unit. These will need to be inserted into the gel tanks with the rough side facing the top of the gel, before the agarose is poured in. The students will also need to cut 2 carbon fibre electrodes per gel. These should be cut carefully and fit snugly at the ends of the gel tank (see student guide).

Loading and running the gels

Each group of students will get a yellow, orange, green, blue and purple tube plus a clear tube containing the ladder. The teacher should put a key on the board so the students know what is in each tube and they can label them appropriately if the technician has not already done so. Once they have poured their gels and they are waiting for them to set (this should take around 15-20 minutes at room temperature), the students can prepare their DNA samples.

The students need to add 2 ul of loading dye to the ladder and each of their DNA samples and mix them well. Once the gels are set, they should pour on 15-20 ml of diluted TBE buffer and then gently remove the comb. The gels should be labelled with L for ladder, V for victim, CS for crime scene and S1, S2 and S3 for the suspects. They should be loaded left to right as shown below:



The students should load 10 µl of each DNA sample onto their gel. Loading the gels takes 10-15 minutes. Once loaded, the gels can be attached to the power source. The red positive wire should be attached to the carbon fibre electrode at the bottom of the gel tank and the black wire attached to the top of the gel tank. This is because DNA is negatively charged and will run towards the positive electrode, if the red wire is attached to the top of the gel tank the DNA will run off the top of the gel. At 36 volts it takes about 90-120 minutes to run the gels.

Staining the gel

After running the gels, they should be stained immediately because the DNA fragments will diffuse in the gel if they are left. For each gel, the electrodes should be thrown away and the gel should be washed 2-3 times in water. Toluidine blue O stain should be poured onto the gel, so that the gel is covered. This should be left for 4 minutes. The toluidine blue can be poured back into the bottle and reused, be careful not to get it on your hands and clothes since it does stain. The gel should then be rinsed again in water 3-4 times and then left without water for the bands to develop. Bands start to become visible after 20-30 minutes, but they can be seen clearly after 24 hours. Use the gel comb as a lid to the gel tank and place an elastic band round to secure. This can then be kept in a sealed small plastic bag to prevent the gel from drying out.

Analysis of the results

The students should see two bands for each blood sample and be able to match one of the suspects to that of the crime scene DNA (the bands should look the same size in both, showing the DNA to be from the same person). See if the students notice anything else about the two other suspects. Do these bands match each other? Ask the students under what circumstances 2 individuals may share the same DNA? (Identical twins!).

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.

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 the CSI DNA 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 may 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 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 gel may be the wrong concentration, which can significantly affect the time taken to run the gel and the quality of the results obtained. 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

The concentrated DNA stain solution is 0.08 % Toluidine blue O dissolved in water. 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 reused several times. After many uses its effectiveness will be reduced as it is gradually diluted with buffer solution washed from the gel. Both the concentrate and the diluted stain should be stored at room temperature. Used or unwanted toluidine blue O solution may be washed down the drain with plenty of water.

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. 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.