DNA Fingerprinting
(BE-104)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for six groups of five students.

Checklist

- ❑ 1 vial DNA: Victim DNA
- ❑ 1 vial DNA: Suspect 1 DNA
- ❑ 1 vial DNA: Suspect 2 DNA
- ❑ 1 vial DNA: Crime Scene DNA 1
- ❑ 1 vial DNA: Crime Scene DNA 2
- ❑ 2 vials Res. Enz.: HindIII *Longlife* Enzyme
- ❑ 2 vials Res. Enz.: EcoRI *Longlife* Enzyme
- ❑ 2 vials Res. Enz.: EcoRV *Longlife* Enzyme
- ❑ 1 vial Res. Enz.: HindIII Reconstitution Buffer
- ❑ 1 vial Res. Enz.: EcoRI Reconstitution Buffer
- ❑ 1 vial Res. Enz.: EcoRV Reconstitution Buffer
- ❑ 1 vial Res Enz.: R.E. Buffer 2 (4X)
- ❑ 1 tube Sterile Water
- ❑ 1 vial DNA Loading Buffer (6X)
- ❑ 1 pack Agarose
- ❑ 1 bottle TAE Buffer (50X)
- ❑ 1 vial DNA Stain (500X)

SPECIAL HANDLING INSTRUCTIONS

- Store DNA, Restriction Enzymes and Restriction Enzyme Buffers frozen until required.

The majority of reagents and components supplied in the *BioScience Excellence*™ kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
For further details on reagents please review the Material Safety Data Sheets (MSDS).
The following items need to be used with particular caution.

Part #	Name	Hazard
D161	DNA Stain (500X)	Flammable

ADDITIONAL EQUIPMENT REQUIRED

- Agarose electrophoresis equipment

TIME REQUIRED

- **Day 1:** 2-3 hours (includes long incubation times)
- **Day 2**: 1 hour

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OBJECTIVES

- Introduce the technique of DNA fingerprinting & identification.
- Understand DNA digestion, using restriction (cleaving) enzymes.
- Learn agarose gel electrophoresis.

BACKGROUND

The development and application of DNA fingerprinting has had beneficial and far-reaching effects in forensic science, as well as for paternity and maternity cases and the identification of disaster victims. In fact it is hard to listen to the news or read a paper without finding a mention of DNA fingerprinting.

Alec Jeffreys, a British scientist, first coined the phrase DNA fingerprinting in 1984. DNA fingerprinting is a genetic identification of a person, where as regular inkpad fingerprinting is identification by a particular phenotype, how fingertips actually appear.

The genome of two different people is vastly similar and highly conserved, however there are specific genomic regions of highly variable repeats, known as microsatellites. The number of variable repeats at a defined position on a genome varies between two different people. These are the areas analyzed during DNA fingerprinting.

DNA fingerprinting involves the purification of a person's genome from a multitude of biological samples, including skin, hair and blood. The genome is then digested into small fragments, with restriction (cleaving) enzymes, in a process known as restriction fragment length polymorphism (RFLP). The fragments are separated by agarose electrophoresis, which separates the fragments based on their size. In the normal process, the separated fragments are transferred to a DNA binding membrane, which is probed with a specific label that allows forensic scientist to visualize the DNA fingerprint.

DNA fingerprinting in forensic laboratories now utilizes a scientific technique known as the polymerase chain reaction, which allows scientists to amplify small amounts of DNA and then identify the variable regions. This process is highly automated and requires tiny amounts of DNA, such as a single hair follicle.

Below are some examples of how DNA fingerprinting has been used. In 1988, a British baker, Colin Pitchfork, became the first suspect to be convicted using DNA evidence. In the same case, a local boy was the prime suspect in the case and with the help of Alec Jeffreys was cleared and of whom Jeffreys said "*I have no doubt whatsoever that he would have been found guilty had it not been for DNA evidence. That was a remarkable occurrence*".

In 1992, DNA fingerprinting was used to confirm that the Nazi doctor Josef Megele was buried in Brazil under the name of Wolfgang Gerhard.

In addition to convicting criminals, freeing the accused and wrongly impriosoned and identifying human remains DNA fingerprinting has also been used for the following. Paternity testing, to identify birth parents, food identification, to test for purity ground beef, evolutionary studies, and to compare similarities of remains to modern day Homo sapiens, for example the 5000 year old "Iceman".

This kit allows students to analyze DNA samples taken from a fictitious crime scene and attempt to identify the criminal or criminals involved. Students will perform a simplified DNA fingerprinting experiment; they will digest the DNA samples and separate the fragments with agarose electrophoresis, but will visualize the DNA fingerprint directly in the gel.

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PRE EXPERIMENT SET UP

1. Add 130µl Sterile Water to each vial of DNA; incubate at room temperature for 5 minutes. Rehydrate the tube by gently flicking or vortexing the tube.

2. Label 6 sets of 5 tubes with "Crime Scene 1", "Crime Scene 2", "Victim", "Suspect 1" and "Suspect 2". Transfer 20µl of each DNA sample into the appropriately labeled tube and supply each group with one of each sample tube.

3. Transfer 45µl HindIII Reconstitution Buffer to the two Res. Enz.: HindIII *Longlife* Enzyme vials. Wait 5 minutes and then resuspend by gently pipetting up and down.

4. Transfer 45µl EcoRI Reconstitution Buffer to the two Res. Enz.: EcoRI *Longlife* Enzyme vials. Wait 5 minutes and then resuspend by gently pipetting up and down.

5. Transfer 45µl EcoRV Reconstitution Buffer to the two Res. Enz.: EcoRV *Longlife* Enzyme vials. Wait 5 minutes and then resuspend by gently pipetting up and down.

6. Label six tubes with "Cleaving Enzyme". Aliquot 55µl R.E. Buffer 2 (4X) into each tube. Next add 15µl HindIII (Step 3), 15µl EcoRI (Step 4) and 15µl EcoRV (Step 5) into each tube. Gently flick or shake the tube to mix. Supply each group with one tube.

7. Label 6 tubes with "Loading Buffer". Aliquot 55µl DNA Loading Buffer into each tube and supply each group with a single tube.

Preparation of agarose gel

Make 1-2 hours before the experiment.

Wear heat protective gloves throughout the agarose melting and pouring procedure.
Each group requires 5 wells on a 1% agarose gel. The well must accommodate 30µl sample.

1. Prepare running buffer: In a clean two liter container, add the entire contents of the TAE Buffer (50X) and make up to two liters with ultra pure water to make a 1X TAE Buffer solution. Stir until thoroughly mixed.

2. Prepare 1% agarose: In a clean, glass 500ml container add 1gm Agarose for every 100ml of the TAE Buffer from step 1. The pack has 5gm Agarose.

3. Heat the solution in a microwave on full power, using 10-second bursts. Check to see if all the agarose has dissolved. Repeat until agarose has dissolved.

DO NOT BOIL. The agarose gets very hot, very quickly and can cause severe burns. Wear heat protective gloves throughout the melting and pouring procedure.

4. Once the agarose has cooled to the point it can be held comfortably in your hand, pour the agarose into the gel casting mould as per the manufacturer's instructions. You will need 5 wells that each holds 30µl for each group.

5. Once the gels have set, transfer to the running apparatus and cover with the TAE Buffer.

Preparation of staining buffer

1. In a clean 500ml glass container, add 500ml of ultra pure water and the entire contents of the DNA Stain (500X) vial. Stir until thoroughly mixed.

2. For a destaining solution, prepare a 10-fold dilution of TAE Buffer in a one-liter container; add 100ml TAE Buffer and 900ml of ultra pure water.

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MATERIALS FOR EACH GROUP

20µl Victim DNA
20µl Suspect 1 DNA
20µl Suspect 2 DNA
20µl Crime Scene 1 DNA
20µl Crime Scene 2 DNA
105µl Cleaving Enzyme
55µl DNA Loading Buffer
500ml Staining Buffer (Shared with class)

PROCEDURE

1. Transfer 20µl Cleaving Enzyme into the five tubes of DNA. Use a clean tip for each DNA sample.

2. Place each tube in a waterbath or incubator at 37°C for one hour.
3. *The incubations can be left at 37℃ overnight. If longer incubations are required remove from 37℃ and store at 4℃, in a fridge.*

4. Following incubation, add 10µl DNA Loading Buffer to each tube.

5. The agarose gels should have been prepared by your teacher/supervisor. Each student takes turns in loading 30µl sample into a well.

6. Once the samples are all loaded, apply a current at 12-15V/cm. For an 8cm long gel run at 96-120 volts.

7. Once the blue dye front has migrated ⅔-¾ the length of the gel, turn off the power. Carefully transfer the gel to a staining tray. CAUTION: Agarose gels are very fragile, handle with extreme care.

8. Add sufficient Staining Buffer to cover the gels and place on a slow (less than 60rpm) shaker for 1-4 hours at room temperature or overnight at 4°C.

If shaker is too fast the gels will break. As an alternative, leave the gel at room temperature for 2-4 hours or overnight at 4℃ without shaking.

9. If DNA bands are hard to see after staining, due to a high background, then destain with 0.1X TAE for 30-60 minutes. To help visualize bands, place gel on a sheet of white paper.

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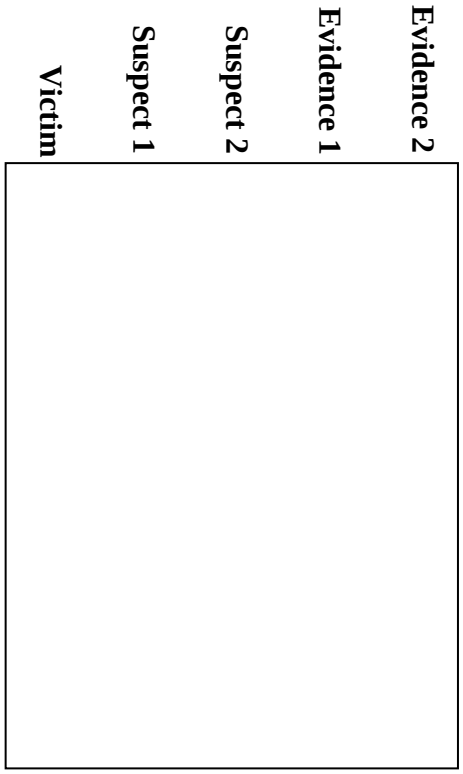
RESULTS, ANALYSIS & ASSESSMENT

Draw a representation of the bands visualized on the gel below:



Describe your results:
Evidence 2 collected at the crime scene identifies Suspect 2 as being at the crime scene; Evidence 1 belonged to the victim. The results show that Suspect 2 was present at the crime scene, where as no DNA for suspect 1 was found at the crime scene, implying that the suspect was not there.