G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

DNA Fingerprinting

Teacher's Guidebook

(Cat. # BE-104)

MATERIALS INCLUDED

- 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
- 6 vials Cleaving Enzyme Mix
- 1 tube Sterile Water
- 1 vial DNA Loading Buffer (6X)
- 1 pack Agarose
- 1 bottle TAE Buffer (50X)
- 1 vial LabSafe™ Nucleic Acid Stain
- 50 centrifuge tubes (1.5ml)

SPECIAL HANDLING INSTRUCTIONS

- Store DNA, Cleaving Enzyme Mix frozen until required.
- Store LabSafe™ Nucleic Acid Stain at 4°C.

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).

ADDITIONAL EQUIPMENT REQUIRED

- Agarose electrophoresis equipment
- UV Light box or transilluminator

TIME REQUIRED

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

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 imprisoned 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. This kit does not use the carcinogen ethidium bromide for visualization, but a non-toxic fluorescent dye that allows students to visualize the DNA migrating during electrophoresis.

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. 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, add the entire contents of the LabSafe™ Nucleic Acid Stain to the agarose and swirl to mix.

5. Pour the agarose into the gel casting mould as per the manufacturer's instructions. You will need 25 wells that each holds 30µl for each group, use an appropriate size comb.

6. Once the gels have set, remove the comb, transfer to the running apparatus and cover with the running buffer until ready to use.

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
- 1 vial (120µl) Cleaving Enzyme Mix
- 55µl DNA Loading Buffer

PROCEDURE

1. Transfer 20µl Cleaving Enzyme Mix 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.

   The incubations can be left at 37℃ overnight. If longer incubations are required remove from 37℃ and store at 4℃, in a fridge.

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

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

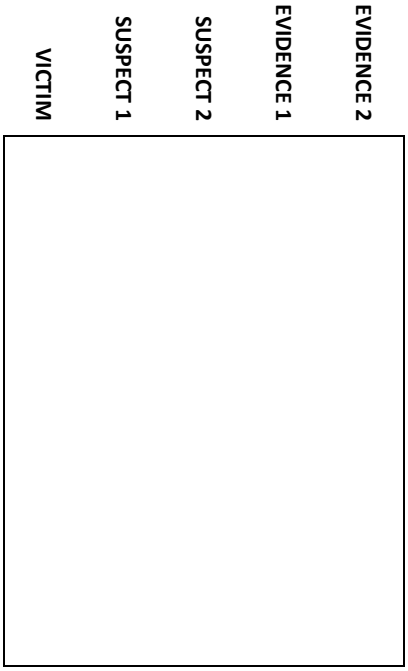
5. Once the samples are all loaded, apply a current at 12-15V/cm. For an 8cm long gel run at 96-120 volts.

6. Once the blue dye front has migrated ¾ the length of the gel, turn off the power and carefully transfer the gel to a UV Light box.

CAUTION: Wear UV Safety Specs when working around the UV Light Box.

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.

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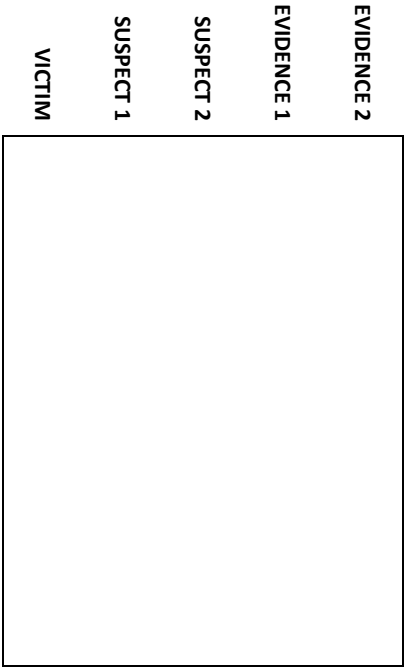

Describe your results:

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