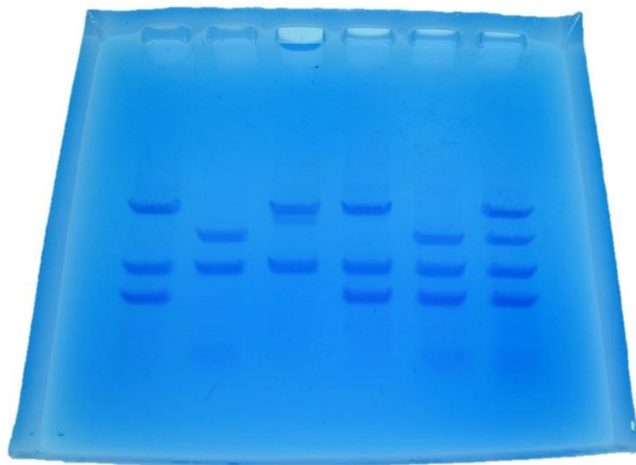


DNA Fingerprinting DNA kit

BB 101

www.cleaverscientific.com

Table of Contents

1. **Introduction** **3**
2. **Experiments components** **3**
3. **Preparation guide** **4**
   3.1 Preparing electrophoresis buffer 4
   3.2 Pouring 0.8% agarose gel 4
   3.3 Sample preparation 4
   3.4 DNA stain preparation 4
4. **Running the experiment** **5**
   4.1 Running the gel 5
   4.2 Staining the gel 5
5. **Result interpretation** **6**
   5.1 Experimental aim 6
   5.2 Interpretation 6
   5.3 Result 6

1. Introduction

DNA fingerprinting is a laboratory technique used to establish a link between biological evidence and a suspect in a criminal investigation. A DNA sample taken from a crime scene is compared with a DNA sample from a suspect. If the two DNA profiles are a match, then the evidence came from that suspect. Conversely, if the two DNA profiles do not match, then the evidence cannot have come from the suspect.

DNA fingerprints are generated using a technique called Polymerase Chain Reaction (PCR). PCR is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions copies of that fragment of DNA. Following PCR the DNA can then be analysed by electrophoresis.

DNA electrophoresis is the separation of DNA through an agarose gel matrix by electricity. The sample containing the DNA fragments is added into a well within a gel and electrical current is passed through the gel. The sugar-phosphate backbone of DNA has a strong negative charge; therefore, the current drives the DNA through the gel towards the positive electrode and the migration distance is dependent on the size of the DNA fragment and percentage of the gel. Smaller fragments of DNA migrate through the gel quicker than bigger fragments, resulting in a "barcode" pattern of bands that are visible on a gel.

The regions of DNA that are analysed in DNA fingerprints are called Short Tandem Repeats (STRs). STRs are not genes, but short repeated sequences that are scattered throughout the genome. PCR uses short lengths of DNA called primers to identify the beginning and the end of the region of DNA to be copied. Many pairs of primers can be used for different regions of DNA at the same time and this is called a multiplex PCR. When the products of a multiplex PCR are run on an agarose gel, the resulting banding pattern is unique to each individual. Thus, PCR can be used to compare DNA found at a crime scene with suspect DNA. If the banding pattern matches, then the suspect was at the scene of the crime.

In this experiment, your students will solve a crime by comparing DNA from a crime scene with 5 suspects using electrophoresis. Capture your students' imagination by using any scenario you like!

2. Experiment Components

This experiment is designed for 8 student groups.

The kit should be stored at room temperature. The DNA samples should be stored in the fridge until use.

Components
5 g Agarose
50ml 50x TAE Electrophoresis Buffer
48 1.5 ml Tubes
4X DNA stain concentrate

Samples
335 µl each
Crime Scene CS)
Suspect 1(S1)
Suspect 2 (S2)
Suspect 3 (S3)
Suspect 4 (S4)
Suspect 5 (S5)

Equipment required (not supplied)
Electrophoresis tank
Power supply
40µl Micropipettes and tips
Gel staining tray

Time:

Preparation
Gel pouring, dispensing samples, 30 minutes.

Experiment
Gels take 5 minutes to load and 30 minutes to run at 150V.
Staining takes 15-20 minutes.

3. Preparation Guide

Each lab group requires

1 gel
1 set of DNA samples (CS, S1, S2, S3, S4 and S5) 1 micropipette and tips
60ml 1x DNA stain
1 gel staining tray

Please refer to your gel electrophoresis tank's manufacturer's guidelines. The following guidelines are for pouring eight 7 x 7 cm gels that each hold 30ml of molten agarose.

CAUTION - Molten agarose can scald!

3.1 Preparing electrophoresis buffer

30ml of 50x stock of TAE electrophoresis buffer will make 1.5L of 1x TAE working buffer solution. To prepare 1x buffer, add 30ml of buffer concentrate and make up to 1.5L with tap water. Electrophoresis buffer can be reused 2-3 times. You will need 1 litre for big tanks (that hold 6-8 gels) and less for smaller tanks. If you require more you can dilute the rest of the concentrated buffer with tap water. You do not need to make of all of the buffer in one go, so you can make up what you need and keep the remainder of the 50X stock solution for a later lesson.

3.2 Pouring 0.8% agarose gels - bulk preparation for 8 gels

1) In a microwave safe bottle mix 2.2g of agarose with 270 ml of diluted TAE electrophoresis buffer
2) Swirl to mix. Mark a line on the bottle at the level of the liquid to indicate 270ml.
3) Heat in a microwave for 1 minute, swirl and repeat until all agarose is dissolved and there are no granules of agarose left.
4) Allow to cool to 50°C (hand hot). Check for evaporation by looking at the liquid level and the line you drew earlier. Top up with water if needed.
5) Pour 30ml into each 7 x 7 cm gel tray.
6) Leave to set for 15 - 20 minutes.

Gels can be poured up to one week in advance and stored wrapped in cling film or foil in the fridge. You can keep any made up agarose in the bottle for use later on.

3.3 Sample preparation

Label 1.5ml tubes (6 tubes for each students' group) CS, S1, S2, S3, S4 and S5. Dispense 40μl of each sample into the appropriate tube.

3.4 DNA stain preparation

Dilute the 4x DNA stain to a 1x working concentration solution by mixing 120ml of the concentrated stain with 360ml of water.

4. Running the Experiment

4.1 Running the gel

1) Load 40µl sample CS into well 1.
2) Continue loading consecutive samples into consecutive wells as follows: S1, S2, S3, S4 and S5.
3) Place gel into electrophoresis tank.
4) Carefully cover gel with diluted electrophoresis buffer.
5) Cover tank with lid and switch power on.

Or, you can load the gels whilst they are in the tank covered with buffer.

6) Run gel until the loading dye is two thirds of the way down the gel, about 30 minutes at 150V.

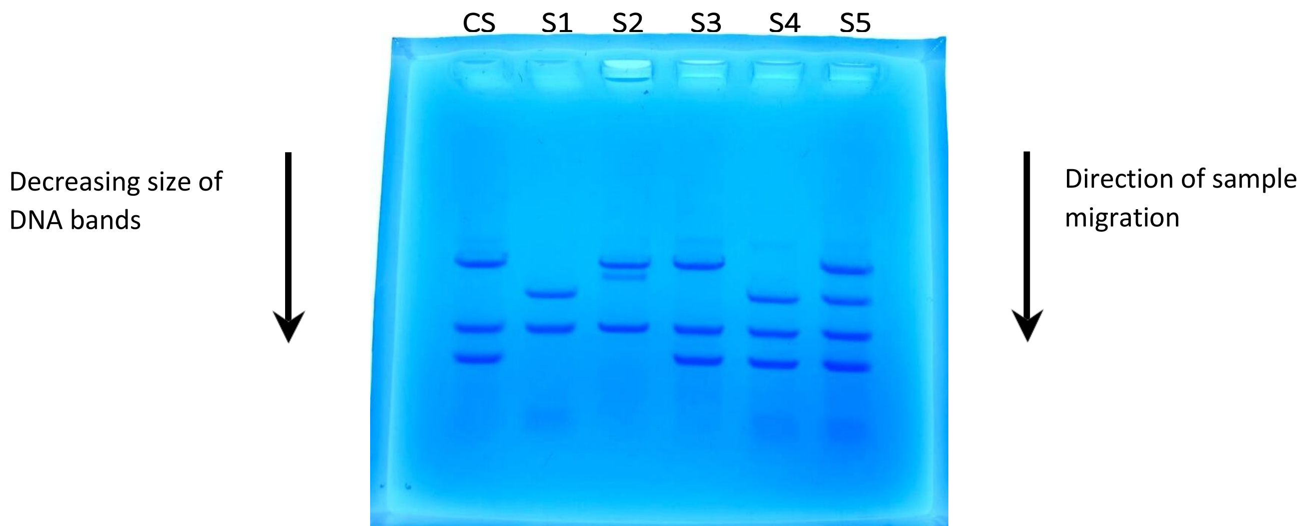
CAUTION: Be careful when using high voltage power supplies! Switch off power supply before removing gels.

4.2 Staining the gel

<u>Gloves are recommended.</u>

1) Remove the gel from the casting tray and place it in the staining tray.
2) Cover the gel with stain and leave for 10 minutes.
3) Remove all of the stain.
4) Dry the surface of the gel with blotting paper by gently positioning it on top of the gel.
5) Remove the blotting paper from the gel and allow gel to develop. Bands will appear in 10-15 minutes.

5. Result interpretation



5.1 Experimental Aim

The aim of the experiment is to match the crime scene DNA banding pattern with one of the suspects.

5.2 Interpretation

Everyone gets two copies of each allele (STR in this scenario) at each region of DNA, one from their mother and one from their father. There are two possible alleles, big or small. If someone has two copies of the same allele, you will only see one band – but remember there are still two copies present.

This result simulates a PCR test for two regions of DNA. For each primer set, there are two possible alleles, that is, different sized fragments of DNA. Different size fragments of DNA will run at different rates through a gel. Smaller fragments travel faster than big fragments of DNA. An individual may have one of each sized allele (heterozygote), which produces two bands; one or the other (homozygote), which appears as a single band on the gel.

Primer set one produces either a 4 kilobase (kb) or 3 kb fragment of DNA. Primer set two produces either a 2 kb or 1 kb fragment of DNA.

5.3 Result

The result shows that suspect 3 was at the scene of the crime because the DNA banding patterns are the same.