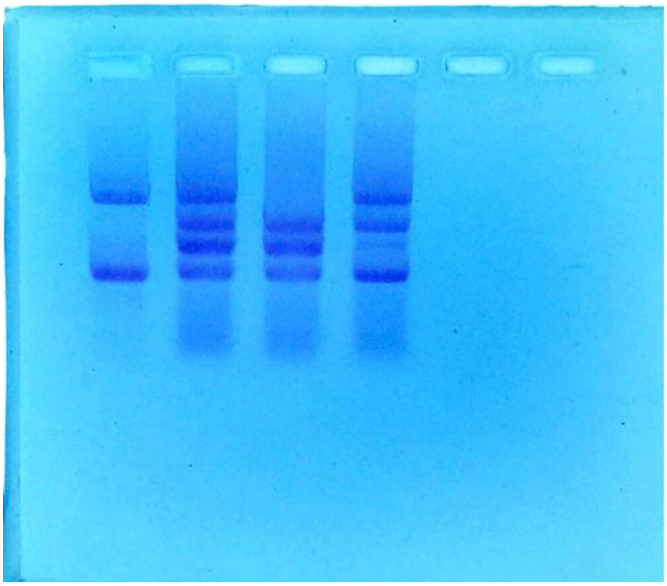


Paternity Testing DNA Kit

BB 103

www.cleaverscientific.com

Table of Contents

1. Introduction

Paternity testing using DNA is achieved by preparing a DNA fingerprint of a child, his mother and potential fathers. DNA electrophoresis is the separation of fragments of DNA through an agarose gel using electricity. DNA is negatively charged, so it migrates toward the positive electrode in an electrophoresis tank. Smaller pieces of DNA migrate through more quickly than big pieces, resulting in the "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 banding pattern resulting banding pattern is unique to each individual.

In this experiment, DNA from a mother, two possible fathers and one child will be tested. Obviously, no real human DNA is used in this experiment. Each individual has a unique profile of bands. A child will get each of their bands from one or as they inherit half their chromosomes from each parent. By comparing the banding profiles, it will be possible to determine who is the biological father of the child.

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 TAE 50x Electrophoresis Buffer
32 1.5 ml Tubes
4X DNA stain concentrate

Samples

335 µl each
Mother's DNA (M)
Child's DNA (C)
Potential Father 1 DNA (F1)
Potential Father 2 DNA (F2)

Equipment required (not supplied)

Electrophoresis tank Power supply
40µl Micropipettes and tips
Gel staining tray

Time

Preparation

Gel pouring, dispensing samples and diluting stain, 30 minutes.

Experiment

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

3. Preparation Guide

Each lab group requires

1 gel
1 set of DNA samples M, C, F1, and F2
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% Gels - bulk preparation for 6 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 (4 each) M, C, F1 and F2.
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 M into well 1.
2) Continue loading consecutive samples into consecutive wells as follows: C, F1 and F2.
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. Run gel until samples are clearly separated, about 30 minutes at 150V.

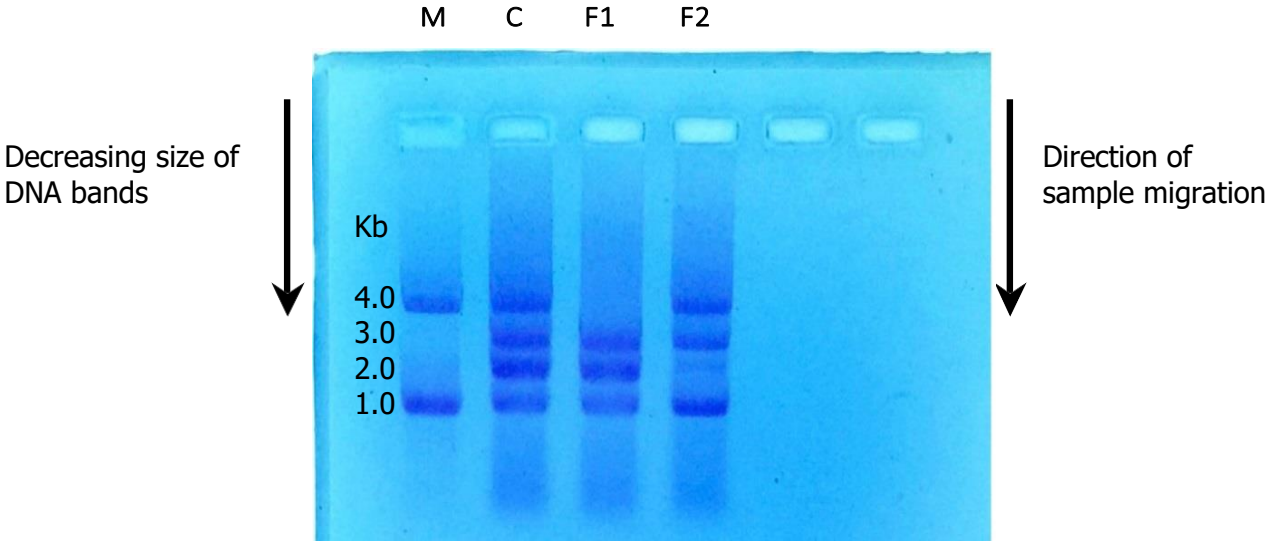
CAUTION: Be careful when using high voltage power supplies! Switch off power supply before removing gels.

4.2 Staining the gel

Gloves are recommended.

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 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 this experiment is to determine who is the father of the child is by comparing DNA banding patterns.

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 (heterozyote), which produces two bands, one or the other (homozygote), which appears as a single band on the gel.

5.3 Result

Who is the father?

The child has bands corresponding to 4.0 3.0, 2.0 and 1.0 kilobases (kb). The mother could only provide bands 4.0 and 1.0 kb so the father had to provide the 3.0 and 2.0 kb band. Father 1 has bands 3.0, 2.0 and 1.0 kb, while father 2 has bands 4.0, 3.0 and 1.0, so father 1 must be the child's biological father.