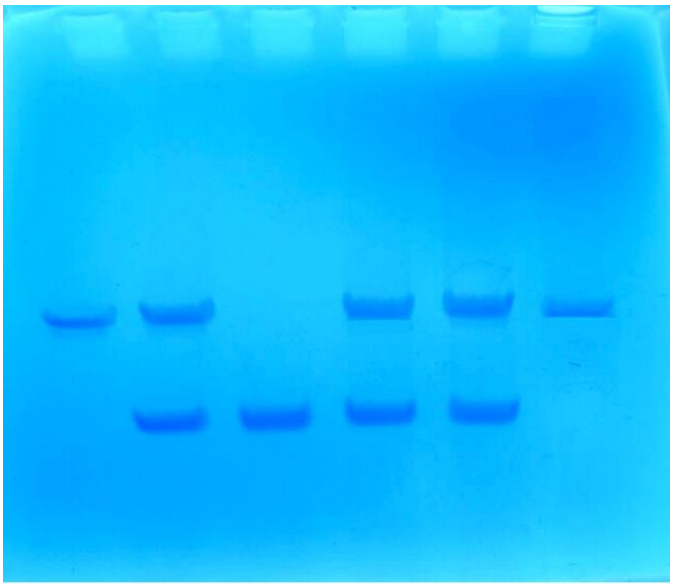

Cystic Fibrosis Testing DNA Kit

BB 102

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1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by one of several possible mutations to the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7. CF is one of the most common single gene genetic conditions. About 1 in 25 people are carriers and 1 in 2500 babies are born in UK with CF. About 9000 people in the UK have CF.

1.1 Symptoms

A mutation in the CFTR gene causes the CFTR protein chloride channel to malfunction which results in sticky mucus being produced in the lungs and digestive system. This causes repeated infections (with some normally harmless bacteria), long-term damage to organs that in severe cases can require a double lung transplant.

1.2 Mutation

The CF phenotype varies depending on the position and nature of the mutation and can give very mild symptoms to very severe. There are at least 1500 possible mutations to the CFTR gene. Delta-F508 (DF508) is most common mutation in Caucasians. It is caused by a 3 base pairs deletion that causes a critical phenylalanine to be lost at position 508. We shall look at this mutation in this activity.

Cystic fibrosis Testing is done using the Polymerase Chain Reaction (PCR). PCR makes millions of copies of DNA from as little as one cell's worth of DNA. After PCR, the DNA can be analysed by electrophoresis.

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 fragments of DNA migrate through more quickly than big fragments, resulting in the “barcode” pattern of bands that are visible on a gel.

For DF508, the PCR test looks for a 3 base pair deletion at the F508 loci that can be detected by gel electrophoresis after the PCR reaction has been carried out. A band shift is seen as the shorter CF PCR product runs more quickly than the normal CFTR PCR product. A non-carrier has one band, a CF patient has one band that is 3 base pairs smaller, and a carrier has two bands one at the smaller position and one at the normal position.

In this experiment, your students will test a family to see if a mother, father and their child are carriers of the DF508 mutation that causes cystic fibrosis. You can use this experiment in the context of genetic testing or embryo pre-implantation genetic diagnosis.

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
4 x DNA stain concentrate

Samples

335 µl each
Normal CFTR homozygote (+/+)
Normal CFTR/DF508 heterozygote (+/-)
DF08 homozygote (-/-)
Mother (M)
Father (F)
Child (C)

Equipment required (not supplied)

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

Time

Preparation

Gel pouring and 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 dye samples +/+, +/-, -/-, M, F, C
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 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) +/+, +/-, -/-, M, F, C. 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 +/+ into well 1.
2) Continue loading consecutive samples into consecutive wells as follows:
   +/-, -/-, M, F, C.
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.

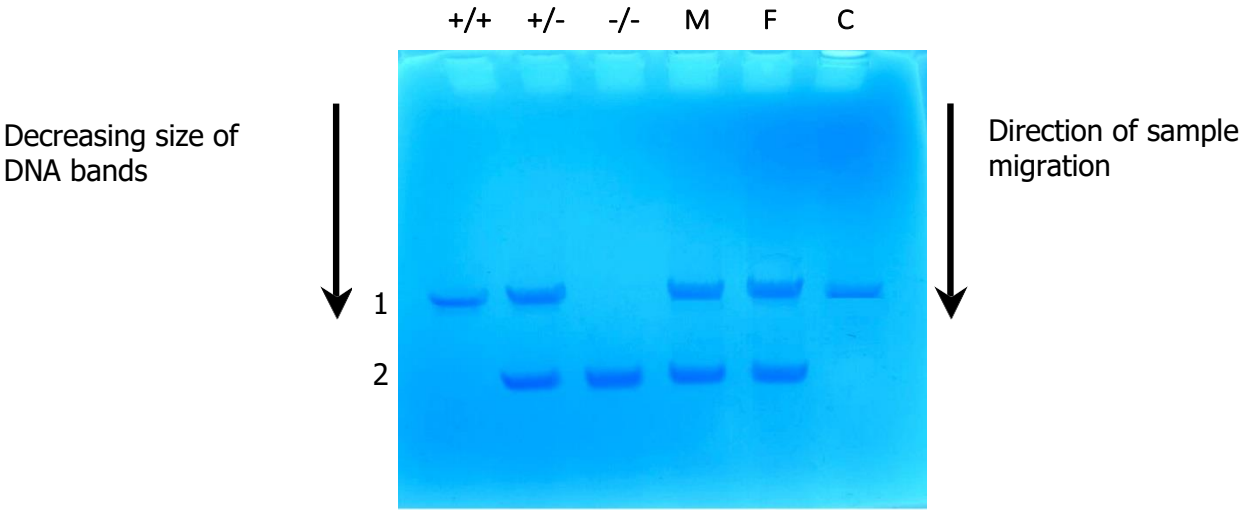
2) Cover the gel with stain and leave for 10 minutes.

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 10-15minutes.

5. Results



5.1 Experimental aim

The aim of the experiment is to determine if members of a family carry the DF508 mutation that causes cystic fibrosis.

5.2 Interpretation

This result simulates a PCR test for the DF508 mutation of the CFTR gene. The normal CFTR gene produces larger piece of DNA than the DF508 mutation (which has a three base pair deletion) and runs as a smaller piece of DNA. Different size pieces of DNA will run at different rates through a gel. Smaller pieces travel faster than big pieces of DNA. An individual may have one of each sized allele (heterozyote), which produces two bands, one or the other (a homozygote), which appears as a single band on the gel. If someone has two copies of the same allele, you will only see one band – but remember there are still two copies present.

The band in position 1 is the normal CFTR gene and the band in position 2 is the DF508 mutation.

5.3 Result

Lane1 – Normal control

Lane 2 – Carrier control

Lane 3 – Cystic fibrosis control

Lane 4 - The mother has bands in both positions indicating that she has one copy of the normal CFTR gene and one copy of the DF508 mutation. She is a carrier.

Lane 5 - The father has bands in both positions indicating that he has one copy of the normal CFTR gene and one copy of the DF508 mutation. He is a carrier.

Lane 6 - The child has one band in the normal CFTR gene position, indicating that they have inherited two copies normal CFTR gene.

They will NOT develop cystic fibrosis