

Student Guide – Cystic Fibrosis Testing DNA Kit

The aim of the experiment is test a family to see if a mother, father and their child are carriers of the DF508 mutation that causes cystic fibrosis using electrophoresis.

Each lab group requires

- 1 gel
- 1 set of dye samples +/+, +/-, -/-, M, F, C
- 1 micropipette and tips
- 60ml 1X DNA stain

Running the Experiment

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.

Alternatively, you can place the gel into the tank, cover it with buffer and then load the samples through the buffer.

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

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

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 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.

Result 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 (heterozygote), 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.

On your gel:

Lane 1 +/+ Normal control, lane 2 +/- carrier control, lane 3 -/- cystic fibrosis control.

What are the genotypes of the mother, father and child?