# Cystic Fibrosis Lab

# Missy Baker Missing™

Cystic Fibrosis Lab:
Missy Baker Missing™
KT-1000-03

Version: 2.2
Release: April 2025

# Contents

# At a glance

## Lab overview

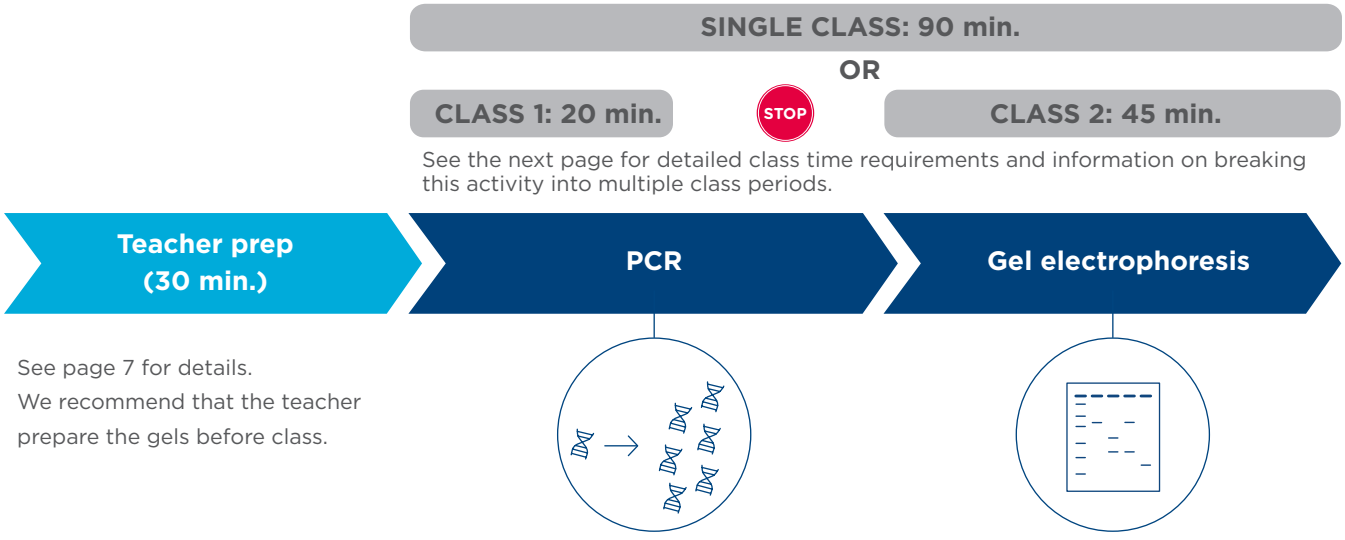
Missy Baker is missing, and police have two suspects. Knowing that Missy Baker has cystic fibrosis, students will test DNA evidence from both suspects' cars to see if it could belong to Missy. Use cystic fibrosis to explore the connection between genes and disease, and the use of biotechnology for genetic analysis.

| TECHNIQUES | TOPICS | LEVEL |
|---|---|---|
| Micropipetting | Genotype to phenotype | General high school |
| PCR | Genetic disease | Advanced high school |
| Gel electrophoresis | Biotechnology | College |

## Required lab skills

- Students must be proficient in accurately pipetting liquids in the 2-20 µl range.
- Instructional videos, worksheets, and free activities to help students build micropipetting skills can be found at https://www.minipcr.com/micropipetting/.

## Planning your time




See page 7 for details.
We recommend that the teacher prepare the gels before class.

## Technical support

If you have any questions about implementing this activity, contact support@minipcr.com.

# Class time requirements

This protocol offers some flexibility to help you manage the class time needed.

| Steps | | Time required |
|---|---|---|
| 1 | **PCR** | |
| | A. Set up PCR samples | 20 minutes |
| | B. Run PCR | 35 minutes<br><br>The PCR program can be started during class and left to run without being monitored. |
| STOP | Optional stopping point: The PCR product is stable at room temperature for several days and can be left in the machine. Transfer tubes to the freezer for long-term storage. | |
| 2 | **Gel electrophoresis** | |
| | Prep: Make gels | We recommend the teacher prepare the gels outside of class (see page 7). Allot 30 minutes of class time if you opt to have students prepare the gels. |
| | A. Load gel | 10 minutes |
| | B. Run gel | 20-25 minutes<br><br>The gel does not need to be actively monitored during this time. |
| | C. Interpret results | 5 minutes |

# Materials needed

## Supplied in kit (KT-1000-03)

- Kit contains DNA samples and PCR reagents for eight lab groups.
- If kept in the freezer, reagents can be stored for 12 months after receipt. If kept in the refrigerator, reagents can be stored for 1 month after receipt.
- Reagents for preparing gels, plastic tubes for distributing reagents to individual groups, plastic tubes for PCR, and pipette tips are sold separately. Refer to the section below for details.

| Contents | Provided | Required per group | Storage |
|---|---|---|---|
| Simulated DNA samples<br>• Suspect A DNA<br>• Suspect B DNA<br>• Control H DNA<br>• Control D DNA | 100 µl each | 10 µl each | Freezer |
| 2X EZ PCR Master Mix Load Ready™ | 700 µl | 75 µl | Freezer |
| 3X Primer Mix | 500 µl | 50 µl | Freezer |
| Fast DNA Ladder 2 | 150 µl | 15 µl | Freezer |

## Electrophoresis reagents and plastics sold separately

- This activity requires:
  - 2% agarose gels with a fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
  - Plastic tubes for distributing reagents to individual groups and 0.2 ml PCR tubes for running PCR.
- The Learning Lab Companion Kit (KT-1510-01) provides sufficient reagents to prepare and run eight gels when using the blueGel or Bandit electrophoresis systems, as well as plastic tubes for distributing reagents to individual groups and plastic tubes for PCR.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

## Required equipment

- This lab is compatible with any thermal cycler.
- This lab is compatible with any horizontal gel electrophoresis system in combination with:
  - A fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
  - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines equipment from miniPCR bio that meets these requirements:

AVAILABLE AT MINIPCR.COM

| Item | Recommended quantity |
|---|---|
| **miniPCR thermal cycler** | Each group will have 4 PCR samples<br>Groups can share machines |
| **Gel electrophoresis and visualization system**<br>Option 1: blueGel™ OR GELATO™ electrophoresis systems with integrated blue light transilluminator | 1 blueGel can be shared by two groups<br>1 GELATO can be shared by four groups |
| Option 2: Bandit™ STEM electrophoresis kit paired with the Viewit™ Illumination Kit | 1 Bandit + 1 Viewit per group |
| Option 3: Bandit™ STEM electrophoresis kit paired with a blueBox™ blue light transilluminator | 1 Bandit per group + 1 blueBox for the class to share |
| **Micropipettes and tips**<br>2-20 µl adjustable micropipette<br>20-200 µl adjustable micropipette | 1 pipette per group<br>1 pipette for teacher prep |

## Other materials supplied by user

- Distilled water
- Microwave or hot plate
- Heat-resistant flask or beaker
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

# Teacher prep

Protective gloves and eyewear should be worn for the entirety of this experiment.

## Overview

The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

| Prep | Time required | Timeline |
|---|---|---|
| Dispense reagents | 10 minutes | Can be completed up to one week before before use |
| Prepare electrophoresis buffer and agarose gels | 20 minutes | Varies - If using gel reagents from miniPCR, gels can be prepared and stored for up to five days before use. |

# Dispense reagents

- Reagents can be dispensed up to one week in advance and stored in the refrigerator until use.
- This kit provides sufficient reagents for eight lab groups.

**Materials needed**

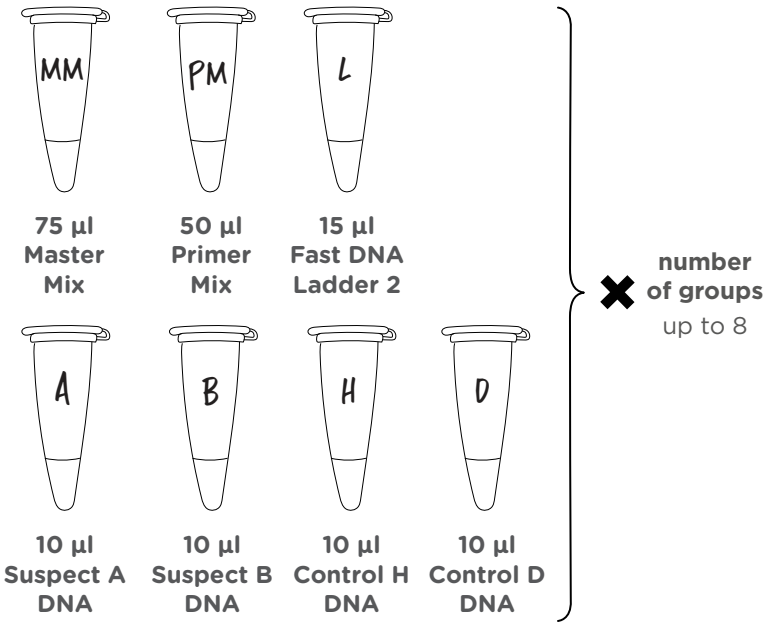
From the lab kit (stored in the freezer):

- 2X EZ PCR Master Mix
- 3X Primer Mix
- Fast DNA Ladder 2
- Suspect A DNA
- Suspect B DNA
- Control H DNA
- Control D DNA

Supplied by user:

- Plastic tubes for dispensing reagents (1.5 ml or 0.2 ml tubes can be used)
- 2-20 µl and 20-200 µl micropipettes and tips
- Fine-tipped permanent marker

1. Thaw reagents by placing tubes at room temperature.
2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.
3. When you open each tube, check for liquid stuck inside the cap. If necessary, put the cap back on and repeat step 2.
4. For each lab group, dispense the following reagents into labeled plastic tubes. 1.5 ml or 0.2 ml plastic tubes can be used.
   - 2X EZ PCR Master Mix (label tube as "MM") 75 µl
   - 3X Primer Mix (tube PM) 50 µl
   - DNA Ladder (tube L) 15 µl
   - Suspect A DNA (tube A) 10 µl
   - Suspect B DNA (tube B) 10 µl
   - Control H DNA (tube H) 10 µl
   - Control D DNA (tube D) 10 µl




Note: Ladder is not needed until the second day of the lab, but you can aliquot it now and store in the refrigerator until needed.

5. If you are dispensing the reagents more than 24 hours before class, store the tubes in the refrigerator until use. Dispensed reagents can be stored in the refrigerator for up to one week.

## Prepare gel electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - Follow the manufacturer's instructions to prepare buffer solution.
   - The volume of buffer needed varies based on the gel electrophoresis system you are using.
     - For the blueGel and Bandit electrophoresis systems, 600 ml of TBE buffer is sufficient for at least eight gel runs.
     - For other systems, refer to the manufacturer's instructions for:
       (1) The buffer volume needed to prepare agarose gels.
       (2) The buffer volume needed for use as running buffer.

2. Prepare 2% agarose gels with fluorescent DNA stain.
   - You will need four lanes plus one lane for DNA ladder per group. If groups are sharing gels, a single lane for ladder per gel is sufficient.
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using. Refer to the manufacturer's instructions.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

**Detailed instructions for preparing buffer and gels for miniPCR electrophoresis systems**

**blueGel**
https://links.minipcr.com/gelpouring

**Bandit**
https://links.minipcr.com/BanditDNAgel

# Student workstation setup

## Part 1: PCR

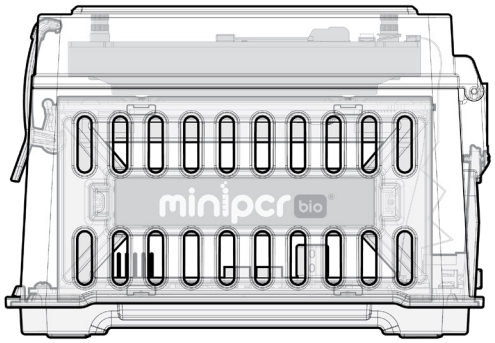
| Every lab group should have: | |
|---|---|
| 2X EZ PCR Master Mix, Load Ready™ (tube MM) | 75 µl |
| 3X Primer Mix (tube PM) | 50 µl |
| DNA samples:<br>• Suspect A DNA (tube A)<br>• Suspect B DNA (tube B)<br>• Control H DNA (tube H)<br>• Control D DNA (tube D) | 10 µl each |
| PCR tubes (0.2 ml) | 4 |
| 2-20 µl micropipette and tips | |
| Space in a thermal cycler for 4 samples | |

If using miniPCR thermal cyclers:

- Groups will need a miniPCR thermal cycler and power supply.
- Machines can be programmed ahead of time by the teacher or during class by the students.
- Once the program has started, the miniPCR will complete the program even if disconnected from the device running the app.
- If you want to monitor the reaction in real-time during the run, groups will need their miniPCR thermal cycler to remain connected to a computer or a compatible phone or tablet.

### Detailed instructions for using a miniPCR thermal cycler

https://links.minipcr.com/minipcrRUN



## Part 2: Gel electrophoresis

| Every lab group should have: | |
|---|---|
| PCR samples from previous class | 30 µl each |
| Fast DNA Lader 2 (tube L) | 15 µl |
| Electrophoresis buffer<br>*Volume depends on your electrophoresis system | 30 ml TBE if using a blueGel or Bandit |
| 2-20 µl micropipette and tips | |
| 5 wells in a 2% agarose gel with fluorescent DNA stain | |

# Student guide

# Background information

Missy Baker is missing! Two rival bakers, Alan Torte and Brenda Biscotti, are suspected in the disappearance. Hair samples collected from each suspect's car are potential matches for Missy. You must use DNA analysis to help find her!

The DNA Times

**Missy Baker missing, police at a loss**

**The disappearance of a bakery shop owner raises serious concerns. The enigma befuddles police investigators. With two abduction suspects held in custody, local students volunteer to try to find the missing baker.**

**Husband Ned reported Missy Baker missing, fretful after not finding her at the bakery shop following his daily nap. The couple resides in the apartment above the pastry shop at 2 Middleborough Rd. a popular fixture in this usually quiet neighborhood. Anxiety takes hold in the community.**

Quickly following the report of the missing baker (described as having wheat-blond hair) investigators identified two suspects, but both have remained silent after extensive interrogation. Following forensic police searches, investigators found blond hair strands in each of the suspects' cars.

DNA from these hair samples is now in the hands of science students, who will volunteer with DNA analysis equipment to help identify the alleged abductor.

Wooly mammoth finally cloned at Brooklyn Zoo P. 5

Transgenic kiwi solves world hunger P. 7

## DNA as a forensic tool

In virtually every cell of your body, you have a copy of your DNA. This DNA is unique to you. Wherever you go, you leave some of this DNA behind. Hairs that fall out have some cells attached to the root; when you touch things, you leave some skin cells behind. When we need to establish if a person was in a particular place, for example a crime scene, we can look for the DNA they left behind.

In the case of Missy Baker, there are two suspects, and you will be testing DNA from hair follicles found in each suspect's car to see if either one matches Missy Baker's DNA. We will analyze these hair samples searching for a match to something that makes Missy Baker's DNA unique.

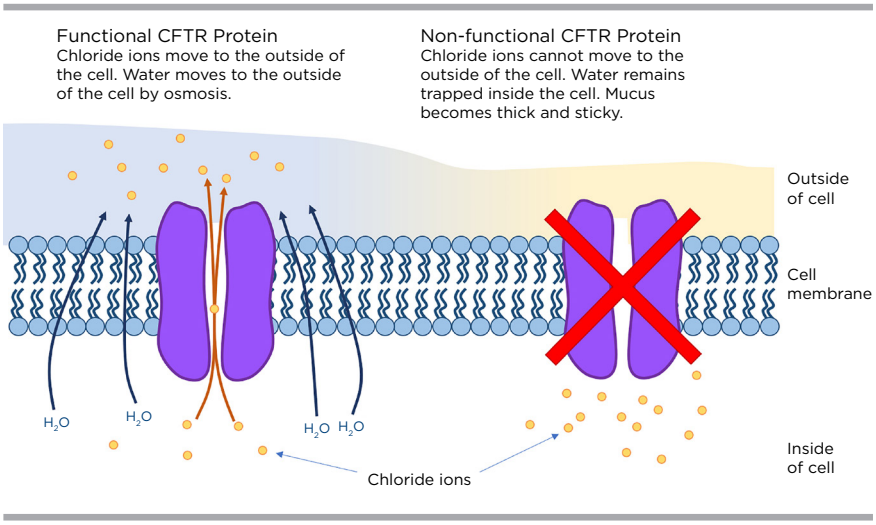
Missy Baker has cystic fibrosis caused by a deletion mutation in her CFTR gene. Using DNA analysis to detect this rare mutation, we will experimentally test whether the hair samples found in either suspect's car might belong to the missing baker.

Note that when police use DNA to identify a suspect, they need to use a technique that can identify any individual, regardless of whether they know anything about them. For that reason, law enforcement uses a broader form of genetic analysis often called "DNA fingerprinting". Rather than focusing on rare genetic mutations, DNA fingerprinting looks at many places in our DNA that are known to vary across most individuals, looking for a match. To learn more about DNA fingerprinting techniques, see the article *DNA Fingerprinting* from DNAdots (https://dnadots.minipcr.com/dnadots/dna-fingerprinting).

# The cystic fibrosis transmembrane regulator (CFTR) gene

Cystic fibrosis is a disease that affects tens of thousands of people in the United States and can lead to many different problems in the body. Most significantly, people with cystic fibrosis suffer from recurrent lung infections. Due to these complications, people with cystic fibrosis have shortened lifespans, usually living only into their 30s or 40s.

All of the problems a person with cystic fibrosis faces can be tied to mutations in a single gene that is involved in moving molecules in and out of cells. The gene is called CFTR (cystic fibrosis transmembrane regulator), and it codes for a protein that is inserted in cell membranes. In all the cells of your body, you have proteins similar to this one that help move important molecules across the cell membrane. Some of these proteins work like pumps, actively moving molecules; others work like channels, just giving specific molecules a passageway through the membrane. Depending on the protein, they may move food molecules into the cell, send waste out, transmit electrical signals, or participate in a host of other processes. The CFTR protein allows chloride ions inside the cell to move outside the cell membrane.




Normally, the CFTR protein is produced in specialized cells of the airway and other places of the body that produce mucus. We often think of mucus as something that we get when we are sick, but really, mucus is produced all the time to keep surfaces throughout the inside of the body moist. When the CFTR protein functions normally, chloride ions move out of the cell through the CFTR protein. Because of the increased number of ions on the outside of the cell, water flows across the cell membrane and out of the cell by osmosis. This leads to the formation of wet and well-hydrated mucus outside the cell. If the CFTR protein isn't working, chloride ions will be trapped inside the cell and water won't move out of the cell and into the mucus.

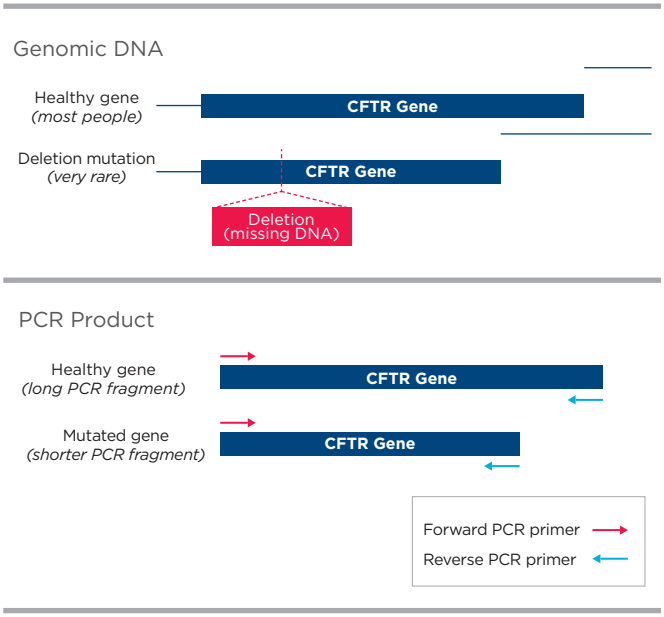
With water not flowing to the extracellular space, mucus, which is normally thin and watery in healthy people, becomes thick and sticky. The thick secretions can be difficult for the body to clear and can clog up organs, preventing them from working properly. In the lungs and airways, this thick sticky mucus can clog passageways and can lead to regular and persistent infections. Respiratory complications arising from cystic fibrosis can be fatal.

There are over 1,500 different known mutations that can lead to a non-functional CFTR protein and cystic fibrosis, but the most common cystic fibrosis mutation is a deletion. A deletion occurs when part of the normal sequence of DNA is missing. Depending on what is missing from the DNA sequence, deletions can result in improperly produced proteins or may prevent the protein from being produced altogether.

Cystic fibrosis is considered a recessive disorder. This means that a person must have two copies of a non-functional CFTR gene in order to have cystic fibrosis. A person with only one non-functional CFTR gene is generally healthy. This is because having just one copy of a functional CFTR gene will make enough normal CFTR protein for cells to function properly.

## PCR analysis of the CFTR gene

Deletions can be detected using PCR (polymerase chain reaction). In PCR, we make billions of copies of very specific pieces of DNA, like a section of the CFTR gene. PCR primers that span the region that may or may not contain the deletion will result in PCR products that vary in length. In this case, a PCR product from a healthy, full length gene will be longer than a PCR product from a gene that has a deletion.



# Today's lab

In this lab, you will be provided with samples of DNA collected from the two potential crime scenes. Sample A is a DNA sample isolated from a hair follicle collected in Alan Torte's car. Sample B is a DNA sample isolated from a hair follicle found in Brenda Biscotti's car. You will test each DNA sample to determine whether it came from a person with a deletion in the cystic fibrosis gene like Missy Baker. You will also be provided with a sample of DNA from a healthy individual and a sample of DNA from an individual with the CFTR deletion mutation to use as controls.

Your job is to perform PCR on each sample and run your results on an agarose electrophoresis gel to visualize your results. By interpreting the size of the DNA products from each sample, you will be able to determine which sample, if any, could have come from Missy Baker!

# Student lab protocol

## Set up PCR samples

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Label four PCR tubes 1-4. Write on the upper sidewall of the tube.

2. Add PCR reagents to the labeled tubes according to the table below. To prevent contamination, use a new tip for each addition.

| | Tube 1 | Tube 2 | Tube 3 | Tube 4 |
|---|---|---|---|---|
| **Master Mix (tube MM)** | 15 µl | 15 µl | 15 µl | 15 µl |
| **Primer Mix (tube PM)** | 10 µl | 10 µl | 10 µl | 10 µl |
| **DNA sample** | Suspect A DNA (tube A) 5 µl | Suspect B DNA (tube B) 5 µl | Control H DNA (tube H) 5 µl | Control D DNA (tube D) 5 µl |

3. Close the caps on the tubes. When they are closed correctly, you should feel the caps “click” into place.

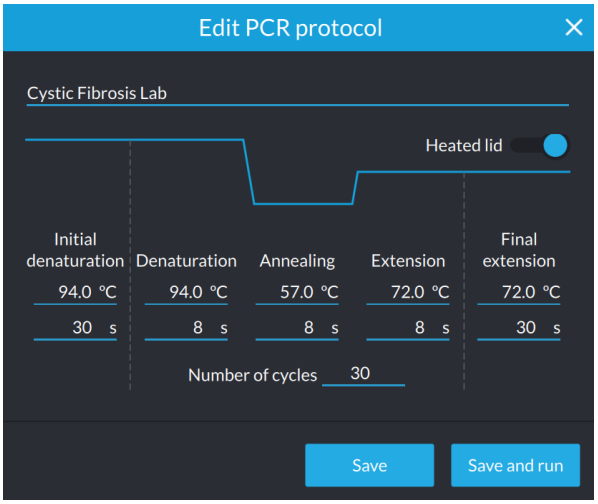
4. Flick each tube to mix the contents. If available, a vortex mixer can be used.

5. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tubes, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.

## Run PCR

- Program your thermal cycler with the following parameters:

| | |
|---|---|
| Initial denaturation | 94°C, 30 sec |
| Denaturation | 94°C, 8 sec |
| Annealing | 57°C, 8 sec |
| Extension | 72°C, 8 sec |
| Number of cycles | 30 |
| Final extension | 72°C, 30 sec |




- The PCR takes approximately 35 min when using a miniPCR® thermal cycler.
- Optional stopping point: The PCR product is stable at room temperature for several days and can be left in the machine. Transfer tubes to the freezer for long-term storage.

# Gel electrophoresis

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample.
   - Well 1: 10 µl Fast DNA Ladder 2
   - Well 2: 10 µl Tube 1 PCR product
   - Well 3: 10 µl Tube 2 PCR product
   - Well 4: 10 µl Tube 3 PCR product
   - Well 5: 10 µl Tube 4 PCR product

4. Run the gel for 20-25 minutes.

5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the bands in the 300-766 bp range to interpret the results.

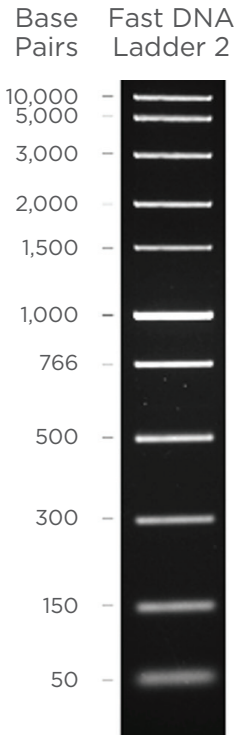
7. If desired, take a photo to document the results.

8. Compare the bands from the DNA samples to the ladder to obtain size estimates.

## Detailed operating instructions for miniPCR electrophoresis systems

**blueGel**
https://links.minipcr.com/blueGelRun

**Bandit**
https://links.minipcr.com/BanditViewit



# Pre-lab questions

## Review

1. What are at least two reasons why DNA analysis can be a good way to identify a person?

2. In your own words, explain what a deletion mutation is.

3. How can PCR help you detect a deletion mutation?

4. What is the role of the CFTR protein in a healthy individual?

5. How does having a non-functional CFTR channel affect mucus production?

## Critical thinking

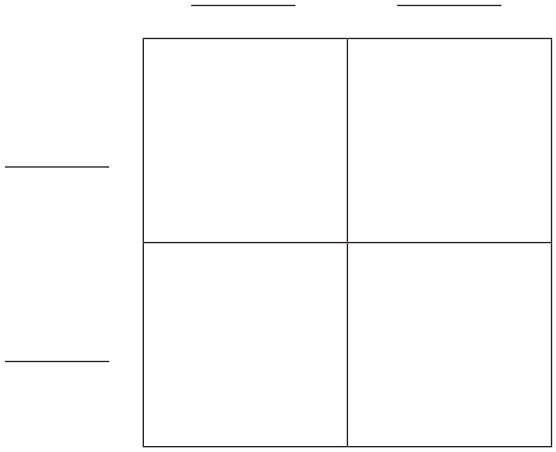
6. Do you think testing for only the CFTR gene would normally be a good way to identify most people?

   If you answered yes to number five, explain why you think it is a good method to identify people. If you answered no, explain what makes it possible for us to use it in the case of Missy Baker.

7. Missy Baker has cystic fibrosis, but both her parents and her two siblings are all healthy. Knowing that cystic fibrosis is a recessive disorder, can you fill in a Punnett square showing how Missy Baker inherited her disease?

   Use F to represent the dominant cystic fibrosis allele.
   Use f to represent the recessive cystic fibrosis allele.

   Write the genotypes of Missy Baker's biological parents here.
   Parent 1: _____
   Parent 2: _____



Fill in the Punnett square. Put a star on the box that represents Missy Baker.

# Post-lab questions


## Interpreting results

1. Use the schematic gel on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel.

2. Next to each band, write approximately how long (in base pairs) the DNA in that band is. Use the image of the ladder from page 19 to help you.

3. Use checkmarks to record the gel electrophoresis results in the first two rows of the table.

| | Tube 1: Evidence from suspect A's car | Tube 2: Evidence from suspect B's car | Tube 3: Healthy CFTR gene control | Tube 4: Deletion mutation control |
|---|---|---|---|---|
| **Normal *CFTR* (700 bp)** | | | | |
| **Mutated *CFTR* (400 bp)** | | | | |

## Critical thinking

4. Does the evidence point to suspect A or B? Justify your answer.

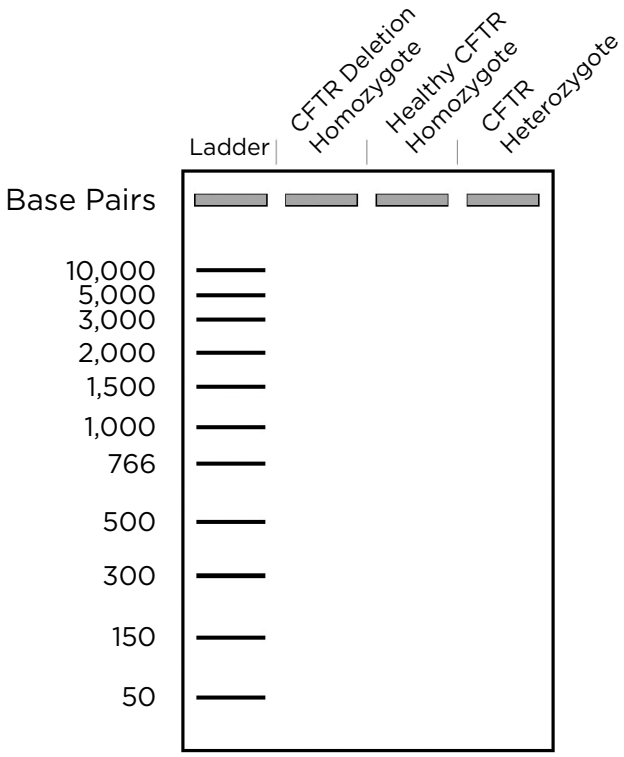
5. Two of the samples you used in this activity were labeled as controls. Explain what specifically each sample tells you and why it was important to include in the experiment:

   Tube H:

   Tube D:

6. Can you say whether Missy Baker was a homozygote or heterozygote for the CFTR mutation? Explain why you think this.

7. On the images of a gel on the right, illustrate what you think the results of this experiment would be for both a homozygote and heterozygote.




8. The mutation investigated today was a deletion mutation. Another class of mutations in the CFTR gene that can cause cystic fibrosis are known as substitution mutations. In a substitution mutation, one or more nucleotides in the DNA sequence are changed, but the overall length of the gene does not change. Would you be able to detect a substitution mutation using PCR and gel electrophoresis like we did in this activity? Explain why you think this.

# CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page.

## Question:

*Which suspect does the evidence point to in the case of Missy Baker?*

### Claim

Make a clear statement that answers the above question.

### Evidence

Provide data from the lab that supports your claim.

### Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

| Score | 4 | 3 | 2 | 1 |
|---|---|---|---|---|
| **CLAIM**<br>A statement that answers the original question/ problem. | Makes a clear, accurate, and complete claim. | Makes an accurate and complete claim. | Makes an accurate but incomplete or vague claim. | Makes a claim that is inaccurate. |
| **EVIDENCE**<br>Data from the experiment that supports the claim.<br>Data must be relevant and sufficient to support the claim. | All of the evidence presented is relevant and sufficient to support the claim. | Provides evidence that is relevant and sufficient to support the claim. May include some non-relevant evidence. | Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence. | Only provides evidence that does not support claim. |
| **REASONING**<br>Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence. | Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning. | Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed. | Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly. | Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly. |

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

| Rubric score | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|
| Equivalent Grade | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 |

# Extension: CFTR and cystic fibrosis

## The CFTR mutation

Cystic fibrosis is a genetic disease caused by mutations in the CFTR gene. CFTR is a protein made of 1,480 amino acids, but a change to just one amino acid can cause the protein to become nonfunctional, or stop it from being produced altogether. Below is a section of DNA sequence from the CFTR gene. The 180 nucleotides listed here come from a middle section of the gene and code for 60 amino acids, specifically, amino acids 501-560. The top strand represents the coding strand and is written in the 5' ⟶ 3' direction. The bottom strand represents the template strand and is written in the 3' ⟶ 5' direction.

```
                                  ★★★
5'  ACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAATATAGATACAGAAGCGTC
    ....|....|....|....|....|....|....|....|....|....|....|....| 60
3'  TGGTAATTTCTTTTATAGTAGAAACCACAAAGGATACTACTTATATCTATGTCTTCGCAG

5'  ATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTT
    ....|....|....|....|....|....|....|....|....|....|....|....| 120
3'  TAGTTTCGTACGGTTGATCTTCTCCTGTAGAGGTTCAAACGTCTCTTTCTGTTATATCAA

        ▲                                 ■
5'  CTTGGAGAAGGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGA
    ...•|....|....|....|....|....|....|.••.|....|....|....|....| 180
3'  GAACCTCTTCCACCTTAGTGTGACTCACCTCCAGTTGCTCGTTCTTAAAGAAATCGTTCT
```

Highlighted above are the locations of three of the over 1,500 different mutations known to cause cystic fibrosis. You will look at each one individually. These three sites happen to be the sites of three of the most common mutations that cause cystic fibrosis.

1. Transcribe the first 30 nucleotides of DNA sequence into mRNA. To help you, the first 30 nucleotides from the template strand have been written for you below. For convenience, they have already been broken up into codons.

```
                                                  ★   ★★
DNA:  3'  TGG  TAA  TTT  CTT  TTA  TAG  TAG  AAA  CCA  CAA  5'
RNA:      ___  ___  ___  ___  ___  ___  ___  ___  ___  ___
```

2. Use your mRNA codon table (page 32) to translate this portion of mRNA into an amino acid sequence. You can use the one-letter abbreviations for amino acids. Remember, this sequence comes from the middle of a gene, so there is no start codon.

```
AA seq:  ____  ____  ____  ____  ____  ____  ____  ____  ____  ____
```

3. A mutation known as ΔF508 is a deletion mutation. In ΔF508, the CTT in positions 21-23 of the DNA sequence (highlighted in yellow and marked with ★) are deleted from the DNA. The new sequence has been written below. Transcribe and translate this new DNA sequence.

```
DNA:  3′  TGG  TAA  TTT  CTT  TTA  TAG  TAA  CCA  CAA  5′
RNA:      ___  ___  ___  ___  ___  ___  ___  ___  ___
AA seq:   ____ ____ ____ ____ ____ ____ ____ ____ ____
```

4. What change occurred in the amino acid sequence?

5. The first amino acid you translated corresponds to amino acid 501 of the CFTR protein. Which number amino acid is affected by the mutation you just made?

6. Now transcribe nucleotides 121-156 into mRNA. Then use your mRNA codon table to translate this portion of mRNA into an amino acid sequence. You can use the one-letter abbreviations for amino acids. Remember, this sequence comes from the middle of a gene, so there is no start codon.

```
DNA:  3′ GAA CCT CTT CCA CCT TAG TGT GAC TCA CCT CCA GTT 5′
RNA:     ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___
AA Seq:  ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____
```

7. A second mutation known as G542X changes the cytosine (C) (highlighted in green and marked with a ▲) to an adenine (A). What effect would this change have on the final protein? Remember, you will first need to transcribe into mRNA and then use your codon table to translate your mRNA sequence into amino acids.

```
RNA:
AA Seq:
```

8. In the third mutation, known as G551D, the cytosine (C) (highlighted in blue and marked with a ▪ ) in position 152 is changed to a thymine (T). What effect would this change have on the final protein? You will need to use your codon table to translate your mRNA sequence into amino acids.

RNA: ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___

AA Seq: ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____

CFTR mutations are divided into six classes. We will look at the first three classes.

- Class I mutations prevent translation from completing properly, and a full amino acid chain is never produced.
- Class II mutations result in a complete, or nearly complete, amino acid chain, but one that cannot fold properly so the protein is never inserted into the cell membrane.
- Class III mutations result in a fully formed protein that is inserted into the cell membrane, but whose ion channel does not open properly, effectively making the protein non-functional.

9. Of the three mutations we discussed, G542X (highlighted in yellow and marked with ★ ) is a class I mutation; ΔF508 (highlighted in green and marked with a ▲ ) is a class II mutation, G551D (highlighted in blue and marked with a ▪ ) is a class III mutation. Based on your analysis and the information provided:

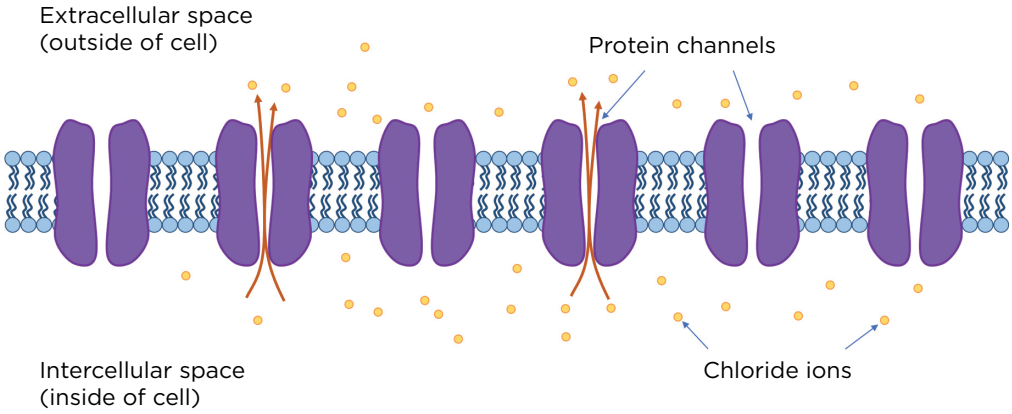
   a. Summarize the effect each mutation has on the amino acid sequence.
   b. Then, relate how this change could cause the protein to be classified as a class I, II, or III mutation.

G542X:

ΔF508:

G551D:

The CFTR protein allows for chloride ions to exit the cell. The diagram below shows an illustration of a cell membrane with six normal CFTR protein channels.

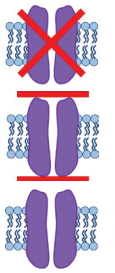



Every person has two copies of the CFTR gene, one on each copy of chromosome 7. This means that typically half of the CFTR proteins in a cell are encoded by one copy of the gene, while half of the proteins are encoded by the other copy of the gene. Cystic fibrosis is considered a recessive disorder. This means that a when a person is heterozygous for the disease, the one functional gene can still produce its share of healthy CFTR protein. Let's investigate what that means at the cellular level.

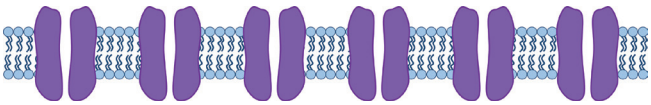
Below are membranes with CFTR proteins present. Each membrane represents the cell membrane from a different person. The individual's genotype is described to the left of the membrane.

For each membrane below, depending on the class of mutation, you are going to:

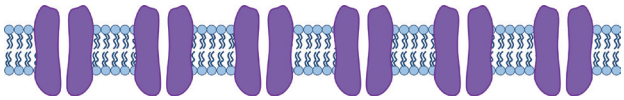
- Mark the protein with an X if it would not be present in the membrane.
- Draw lines blocking the channel if the protein would be present, but not functional.
- Leave the protein alone if it would be present and functional.



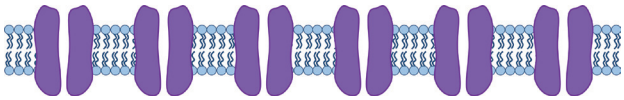
10. A person with a ΔF508 mutations on both chromosomes. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



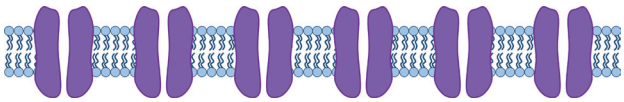
11. A person with a ΔF508 mutation on one chromosome and a G551D mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



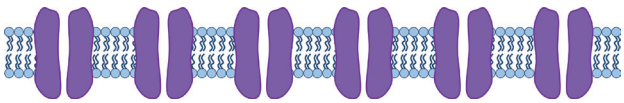
12. A person with a G551D mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



13. A person with a G542X mutation on one chromosome and a G551D mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



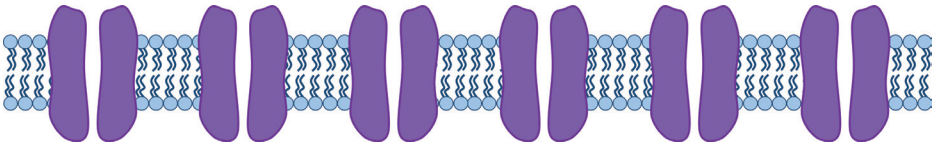
14. A person with a G542X mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



15. Cystic fibrosis is a recessive disorder. Is it correct to describe everyone with cystic fibrosis as a homozygote? Explain your answer.

16. A common and sometimes deadly symptom of cystic fibrosis is persistent lung infections. This is because the mutation leads to the mucus of the lungs becoming sticky and more viscous (less watery). In your own words, explain how a mutation in the CFTR gene will lead to sticky mucus in the lungs.

17. Some individuals who have a normal allele for CFTR and one allele with a mutation show minor symptoms of cystic fibrosis even though they are generally healthy. Use the diagram below to explain why you think that might be. Include the movement of chloride ions and water in your answer. Explain what you drew in the space provided.



18. Which one of the diagrams above (in #s 10-14) is most like what would be found in Missy Baker's cell membranes? Explain why you think this.

# Amino acid codon table

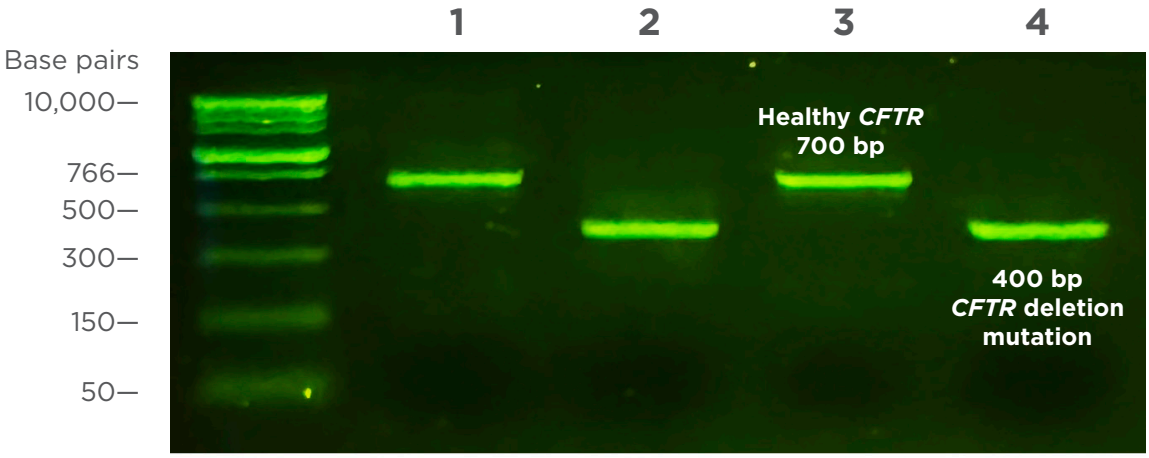
| First Position Nucleotide | Second Position Nucleotide: U | | Second Position Nucleotide: C | | Second Position Nucleotide: A | | Second Position Nucleotide: G | | Third Position Nucleotide |
|---|---|---|---|---|---|---|---|---|---|
| U | UUU | Phenylalanine (Phe, F) | UCU | Serine (Ser, S) | UAU | Tyrosine (Tyr, Y) | UGU | Cysteine (Cys, C) | U |
| | UUC | | UCC | | UAC | | UGC | | C |
| | UUA | Leucine (Leu, L) | UCA | | UAA | STOP | UGA | STOP | A |
| | UUG | | UCG | | UAG | | UGG | Tryptophan (Trp, W) | G |
| C | CUU | Leucine (Leu, L) | CCU | Proline (Pro, P) | CAU | Histidine (His, H) | CGU | Arginine (Arg, R) | U |
| | CUC | | CCC | | CAC | | CGC | | C |
| | CUA | | CCA | | CAA | Glutamine (Gln, Q) | CGA | | A |
| | CUG | | CCG | | CAG | | CGG | | G |
| A | AUU | Isoleucine (Ile, I) | ACU | Threonine (Thr, T) | AAU | Asparagine (Asn, N) | AGU | Serine (Ser, S) | U |
| | AUC | | ACC | | AAC | | AGC | | C |
| | AUA | | ACA | | AAA | Lysine (Lys, K) | AGA | Arginine (Arg, R) | A |
| | AUG | Methionine (Met, M) START | ACG | | AAG | | AGG | | G |
| G | GUU | Valine (Val, V) | GCU | Alanine (Ala, A) | GAU | Aspartic Acid (Asp, D) | GGU | Glycine (Gly, G) | U |
| | GUC | | GCC | | GAC | | GGC | | C |
| | GUA | | GCA | | GAA | Glutamic Acid (Glu, E) | GGA | | A |
| | GUG | | GCG | | GAG | | GGG | | G |

# Instructor guide

# Expected results

Gel electrophoresis results are expected to resemble the photo below:




This photo represents a 20-minute run using a blueGel. Note that it is likely that the top of the Fast DNA Ladder 2 will not fully separate with a short gel run, but this is ok! You only need the ladder to separate in the 300-766 bp range to be able to interpret the experimental results.

| | Tube 1: Evidence from suspect A's car | Tube 2: Evidence from suspect B's car | Tube 3: Healthy CFTR gene control | Tube 4: Deletion mutation control |
|---|---|---|---|---|
| **Normal *CFTR* (700 bp)** | ✓ | | ✓ | |
| **Mutated *CFTR* (400 bp)** | | ✓ | | ✓ |

- We know Missy Baker is homozygous for this specific CFTR mutation, so a DNA sample from Missy Baker would show only a 400 bp band.
- The sample from Suspect B's car could belong to Missy Baker.

For technical support, contact **support@minipcr.com**

For answers to the student questions, email **answers@minipcr.com**
Please include in the body of the email:

- The name of the lab
- Your name, school, and job title

# Unexpected results and troubleshooting

**If fluorescent DNA bands are faint or entirely absent from one or more student sample lanes,** the following may have occurred:

- Suboptimal PCR amplification: Pipetting errors during PCR setup can lead to suboptimal amplification for individual student samples.

- Failure to load the DNA samples on the gel: Loading DNA samples for gel electrophoresis takes a little practice. The bands will appear faint if students do not successfully deposit the full sample volume into the well. Refer to https://www.minipcr.com/how-to-load-a-gel-electrophoresis/ for gel loading tips.

**If fluorescent DNA bands are not visible on the gel, even for the DNA ladder,** the following may have occurred:.

- Failure to use a fluorescent DNA stain: This lab requires agarose gels made with a fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®). DNA stains that reveal DNA with a visible blue compound are less sensitive and are not compatible with this lab kit.

- Incorrect visualization conditions: Fluorescent DNA stains (*e.g.*, SeeGreen™ or GelGreen®) must be viewed using a blue light or UV transilluminator. The blueGel system has an integrated blue light transilluminator. For DNA visualization, ensure that you have turned on the blueGel's blue light by pressing the light bulb button.

- Samples were run off the gel: If you run the gel too long, DNA samples may migrate off the gel. Monitor progress by occasionally checking the DNA samples under a transilluminator or tracking the loading dye, which is visible to the eye. Stop the run before the colored loading dye reaches the end of the gel.

- Reagents were stored improperly and/or are expired: DNA samples can be stored in the freezer for up to twelve months after receipt or in a refrigerator for six months. Storage under different conditions or in excess of this guidance may impair performance.

**For tips on picture-perfect gels,** see https://www.minipcr.com/gel-electrophoresis-troubleshooting/.

**For additional technical support,** contact support@minipcr.com.

# Notes on lab design

This lab serves as an introduction to DNA testing, and genetic disease using the CFTR gene and cystic fibrosis as a model. We believe this approach provides the right balance between intellectual engagement, inquiry, and accessibility. The design of this lab has simplified certain elements to achieve these goals. Some of these elements include:

- This lab uses prepared template DNA to simulate human genomic DNA.

- The most common cystic fibrosis allele involves a deletion of three base pairs. While such a deletion would be detectable using PCR, it would require using more sensitive electrophoresis techniques than are available in a typical classroom. We have used a length difference of approximately 300 base pairs between PCR fragments to create a more robust and easily observable result.

- While this lab uses a forensic investigation storyline to make it engaging, forensic DNA analysis relies on analyzing areas of the genome that contain polymorphic short tandem repeats. For more information, visit https://dnadots.minipcr.com/dnadots/dna-fingerprinting/.

# Additional student supports

**E-worksheets:** The student questions accompanying this lab are available for download here as editable text documents you can customize and upload to your LMS. E-worksheets can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/minipcr-crime-lab-missy-baker/.

**miniPCR tutorials:** Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources most relevant to this lab are listed below.

- **Micropipetting:** Video, worksheet, and hands-on activity resources to train students in the basic use of a micropipette.
- **PCR:** Video and worksheet activity instructing students on the fundamentals and practice of PCR.
- **Gel electrophoresis:** Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.

**miniPCR Digital:** Interactive tools for experiment-based learning with or without hands-on lab kits. Visit https://digital.minipcr.com/.

# Extension activities

The following optional extension activity is provided for students to explore topics more deeply.

**CFTR and Cystic Fibrosis** (page 26): Missy Baker has the genetic disease cystic fibrosis. Explore the link between genetic mutation and disease more deeply in this activity. Students will need to know how to transcribe mRNA sequences from DNA and how to translate mRNA sequences using an mRNA codon table. An mRNA codon table is provided.

# Learning goals and skills developed

**Student learning goals**

- Correlate genotype and phenotype
- Identify how amino acid composition affects protein function
- Relate changes in DNA and amino acid sequence to human disease
- Solve real-world problems using genetic analysis
- Understand the principles and practice of PCR

**Scientific inquiry skills**

- Identify or pose a testable question
- Formulate hypotheses
- Identify dependent and independent variables and appropriate experimental controls
- Follow detailed experimental protocols
- Create tables or graphs to present their results
- Interpret data presented in a chart or table
- Use data to evaluate a hypothesis
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

**Molecular biology skills**

- Micropipetting
- PCR
- Agarose gel electrophoresis

# Standards alignment

The standards alignment document for this activity is available for download here. This document can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/minipcr-crime-lab-missy-baker/.

This activity is aligned to the following standards:

- Next Generation Science Standards: High School Life Science
- Advanced Placement Biology
- Texas Essential Knowledge and Skills: Biology
- Texas Essential Knowledge and Skills: Biotechnology
- Texas Essential Knowledge and Skills: Forensic science
- Biotechnician Assistant Credentialing Exam
- Common Core ELA/Literacy Standards (9-10)

For additional information on alignment to state standards, please contact support@minipcr.com.