## miniPCR® Crime Lab

# Missy Baker Missing™

**miniPCR® Crime Lab**
Missy Baker Missing™
Instructor's and Student's Guide

Version: 2.1
Release: February 2022

# Student's Guide Contents

# 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.

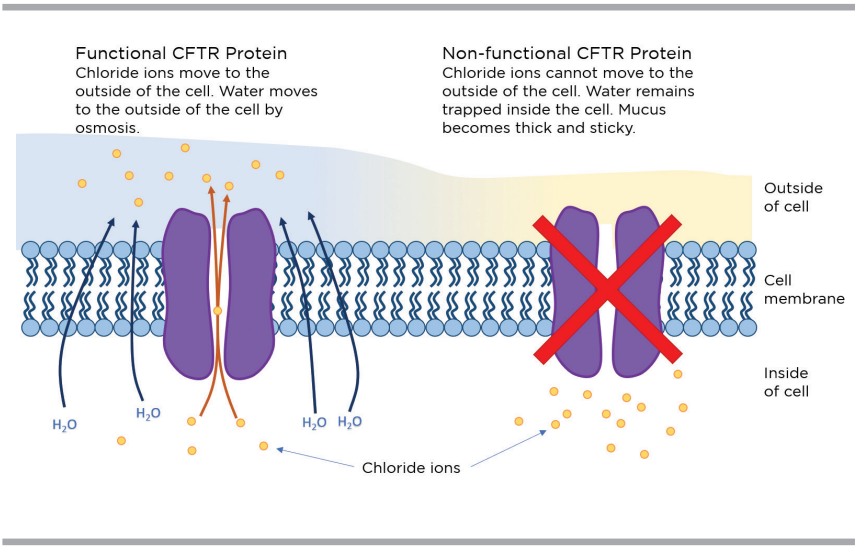
In the case of Missy Baker, there are two suspects, and you will be testing DNA from hair follicles found in each suspect's car to see if either one matches Missy Baker's DNA. We will analyze these hair samples searching for a match to something that makes Missy Baker's DNA unique.

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.



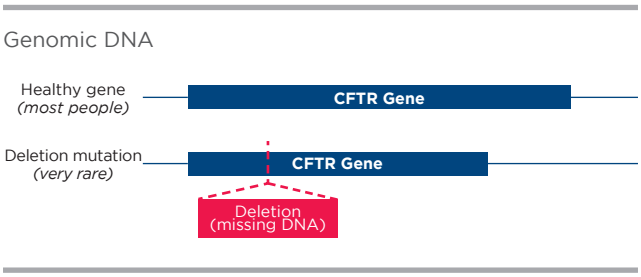

All of the problems a person with cystic fibrosis faces can be tied to mutations in a single gene that is involved in moving molecules in and out of cells. The gene is called CFTR (cystic fibrosis transmembrane regulator), and it codes for a protein that is inserted in cell membranes. In all the cells 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.

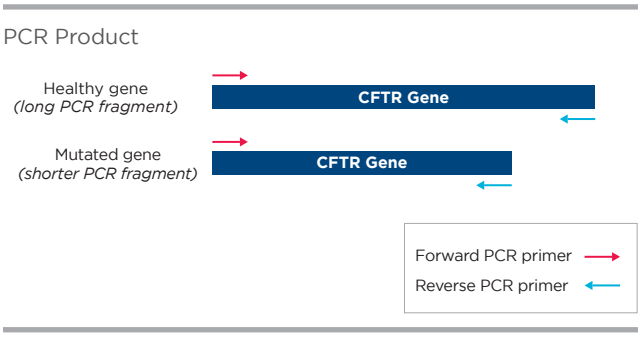
There are over 1,500 different known mutations that can lead to a non-functional CFTR protein and cystic fibrosis, but the most common cystic fibrosis mutation is a deletion. A deletion occurs when part of the normal sequence of DNA is missing. Depending on what is missing from the DNA sequence, deletions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

# Laboratory guide

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

## A. PCR set up

**1. Label 4 PCR tubes** (200 µl tubes)

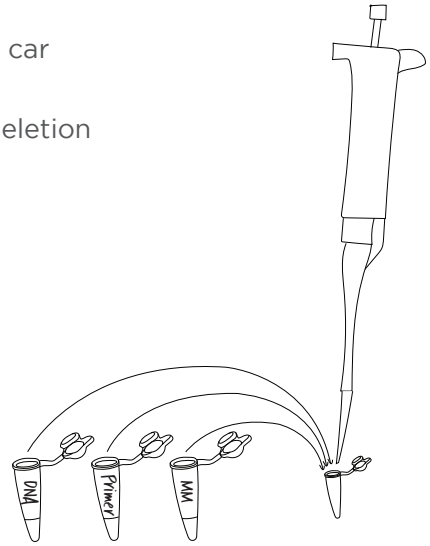
Note: label tubes on the upper side wall, as writing on the cap or lower side wall may rub off during PCR.

- Label each tube with the name of the sample and with the group's name.
  - 1 tube labeled "A": Hair DNA extracted from Alan Torte's car
  - 1 tube labeled "B": Hair DNA extracted from Brenda Biscotti's car
  - 1 tube labeled "H": Control DNA from a healthy individual
  - 1 tube labeled "D": Control DNA from a person with a CFTR deletion

**2. Add PCR reagents to each PCR tube**

| | Tube A | Tube B | Tube H | Tube D |
|---|---|---|---|---|
| **DNA Sample** | DNA A<br>5 µl | DNA B<br>5 µl | Control H<br>5 µl | Control D<br>5 µl |
| **Crime Lab 3X Primer Mix** | 10 µl | 10 µl | 10 µl | 10 µl |
| **2X EZ PCR Master Mix** | 15 µl | 15 µl | 15 µl | 15 µl |
| **TOTAL VOLUME** | 30 µl | 30 µl | 30 µl | 30 µl |

Note: EZ PCR Master Mix™ contains:
• Taq DNA polymerase • dNTPs
• PCR buffer with $Mg^{2+}$ • Gel loading dye

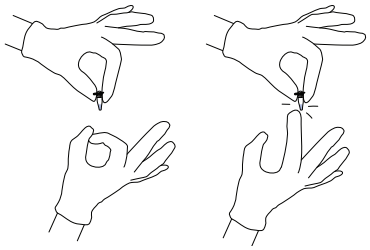



Use a micropipette to add each of the reagents. Remember to change tips at each step!

**3. Cap the tubes and ensure the reagents mix well**

- You may flick each tube with your fingers to ensure proper mixing.
- Gently tap tubes on your bench to collect liquid at the bottom.

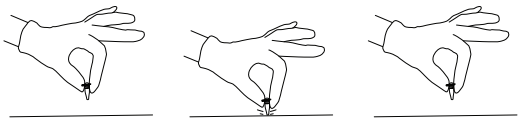
**Flick to mix**



**4. Place the tubes inside the miniPCR® machine**

- Press firmly on the tube caps to ensure a tight fit.
- Close the PCR machine lid and tighten it gently.

**Tap to collect liquid at bottom**



# B. PCR programming and monitoring

These instructions are illustrated using miniPCR® software on a Windows PC. Software interfaces vary slightly by operating system. See the miniPCR® User's Guide for more details. If using a different thermal cycler, PCR protocol parameters should remain the same (step 6).

**1. Open the miniPCR® app and remain on the "Library" window**

**2. Connect your miniPCR® thermal cycler to your device using the supplied USB cable or via Bluetooth®**

- Note: Bluetooth is only available on certain models. To connect via Bluetooth®, select the icon, located by "Devices" at the left of the desktop app or at the top of the mobile app.

**3. Make sure your miniPCR® thermal cycler is plugged in and that the power switch is turned on**

- Note: If your machine begins running a previously loaded protocol, you may stop it by clicking or tapping the "X" symbol in the top left box of the "Monitor" window.

**4. While in the "Library" window, click the ⊕ on to create a new protocol**

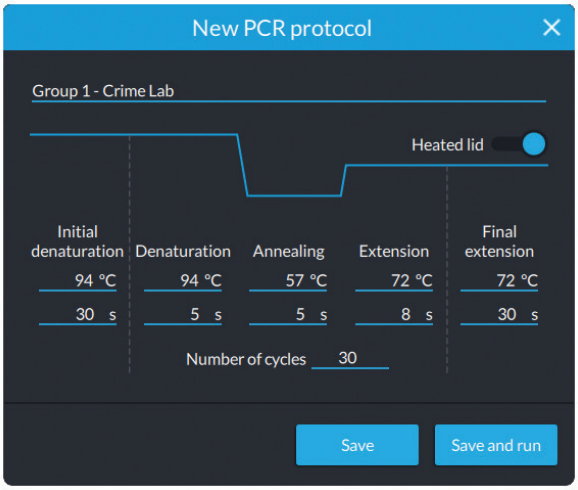
- Button is located in the upper right hand corner of the window.

**5. Select "PCR" from the drop-down menu**

**6. Enter a name for the protocol; for example: "Crime Lab"**

**7. Enter the PCR protocol parameters:**

| | |
|---|---|
| • Initial denaturation | 94°C, 30 sec |
| • Denaturation | 94°C, 5 sec |
| • Annealing | 57°C, 5 sec |
| • Extension | 72°C, 8 sec |
| • Number of cycles | 30 |
| • Final extension | 72°C, 30 sec |

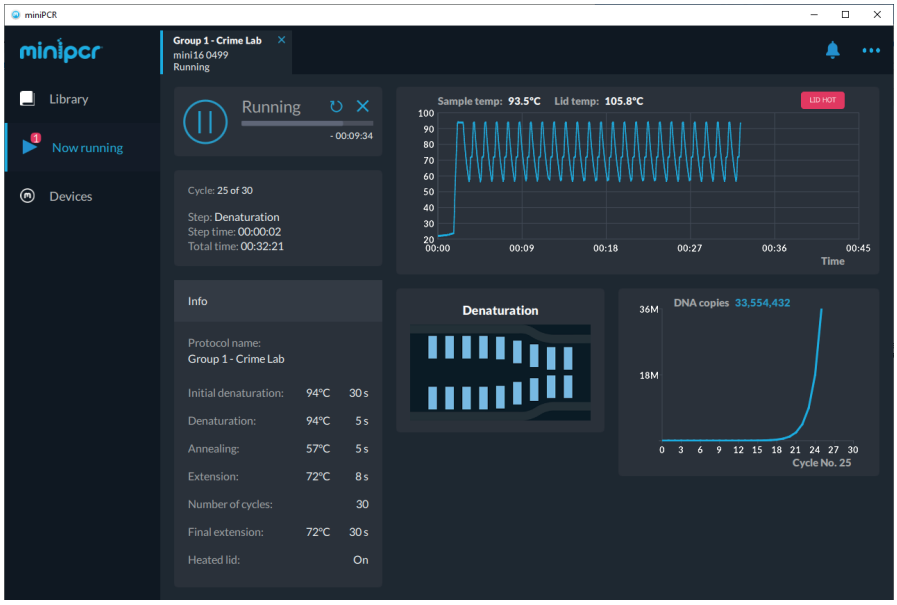



**8. Click "Save and run" to start the protocol**

- If connected to more than one machine, choose from the list the serial number of the miniPCR® thermal cycler you are using. If asked "Do you want to stop the current protocol...?", click "Yes".
- The lights on the front of the miniPCR® thermal cycler will blink 3 times to indicate that the program has been loaded.
- Note: If needed, you may unplug the USB cable or disconnect Bluetooth® once the protocol has been loaded. Even if disconnected from your device, the protocol will continue to completion as normal.

**9. Choose "Monitor" window**

- The "Monitor" window can be selected on the left column in the desktop app and at the top in mobile app.
- If more than one miniPCR® thermal cycler is connected to the same device, choose which machine you would like to monitor using the tabs at the top of the window (desktop app) or bottom of the Library (mobile app).




*The miniPCR® software allows each lab group to monitor the reaction parameters in real time.*

**10. When the PCR run has completed (approximately 35 min), app status will show "Finished" and the red, yellow, and green LEDs on your miniPCR® thermal cycler will light up and stay on**

Be careful not to touch the metal lid which may still be hot.

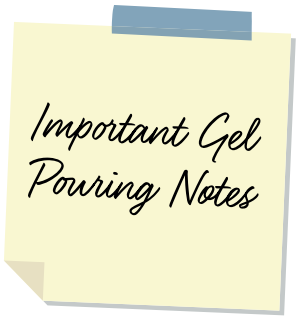
**11. PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer**

- Tubes may remain inside the miniPCR® thermal cycler for several days following protocol completion.

# Laboratory guide

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

## Gel electrophoresis — Pouring gels (before or during class period)



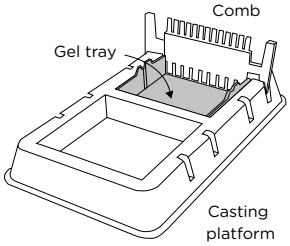

Gels can be prepared up to three days ahead of time and should be stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need four lanes plus one lane for ladder per group. If groups are sharing gels, a single lane for ladder is sufficient.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

**1. Prepare TBE buffer (to be completed by teacher in advance)**

- TBE buffer is often provided as liquid concentrate or powder.
- Follow manufacturer's instructions to prepare 1X TBE buffer solution.




**2. Prepare a clean and dry casting platform with a gel tray and comb**

- Place the clear gel tray in the white casting platform.
- Place a well-forming comb at the top of the gel tray.

**3. Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

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**IMPORTANT NOTE: There are several ways to prepare agarose gels.**

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

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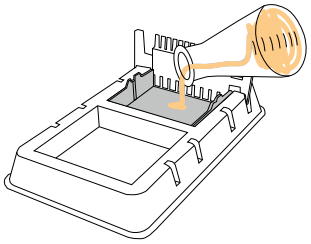
**4. Pour the agarose solution into the prepared casting platform with a gel tray and comb**

- The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).



**5. Allow gel to solidify completely and remove the comb by pulling firmly upwards**

- Gels will typically be ready in about 10 minutes.
- Gel is ready when cool and firm to the touch.

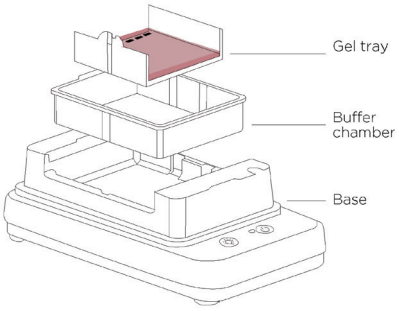
Protective gloves and eyewear should be worn for the entirety of this experiment.

# C. Gel electrophoresis — Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

**1. Place the gel tray containing your gel in the buffer chamber**

- Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
- The wells of the gel should be on the same side as the negative electrode, away from the power button.



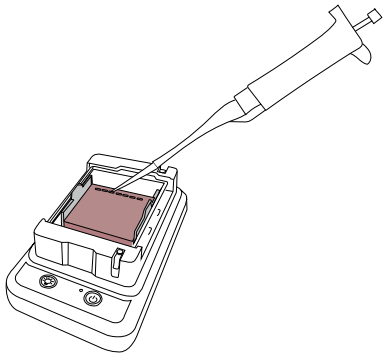

**2. Add 30 ml of 1X TBE electrophoresis buffer**

- The buffer should just cover the gel and fill the wells.
- Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

**3. Load samples onto the gel in the following sequence**

- Lane 1: 10 µl Fast DNA Ladder 2
- Lane 2: 15 µl Suspect A PCR product
- Lane 3: 15 µl Suspect B PCR product
- Lane 4: 15 µl Control H PCR product
- Lane 5: 15 µl Control D PCR product

Note: Samples already contain loading dye.

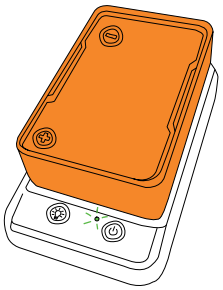


**4. Place the orange cover on the blueGel™ electrophoresis system**

- To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
- Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
- The orange lid should sit flush with the blue base using little force.

**5. Press the "Run" button**

- Check that the green light beside the power button remains illuminated.

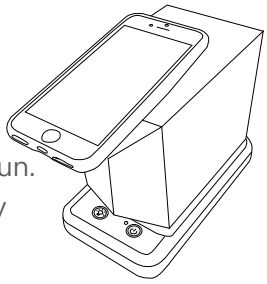


**6. Conduct electrophoresis for 20-25 minutes**

- Note: Check the progress of your samples every 10 minutes to monitor the migration of your DNA samples.
- Longer electrophoresis times will result in better separation of similar sized DNA fragments. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

# C. Gel electrophoresis – Visualizing results

**1. Press the "light bulb" button to turn on the blueGel™ transilluminator**



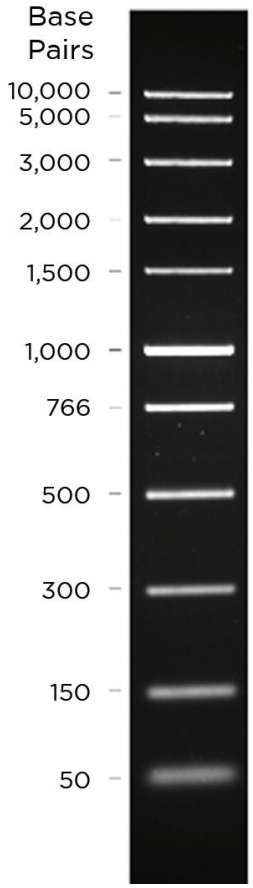
- For best viewing, dim ambient lights or use **Fold-a-View™** photo documentation hood with a smartphone camera.
- Gels may be viewed at the end of the run or periodically throughout the run.
- If image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

**2. Ensure that the bands in your gel have separated enough to clearly interpret your results**

- Run the gel longer if needed to increase resolution.

**3. Document your results**

- Compare the bands from samples A, B, H and D to your DNA ladder to obtain size estimates.
- Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.



## CER Table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

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

| | |
|---|---|
| **Claim**<br>Make a clear statement that answers the above question | |
| **Evidence**<br>Provide data from the lab that supports your claim | |
| **Reasoning**<br>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** 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** Data from the experiment that supports the claim. 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** 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 |

# Study questions - pre-lab

## 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

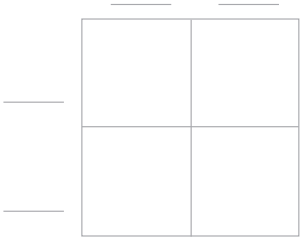
**6. Do you think testing for only the CFTR gene would normally be a good way to identify most people?**

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: _____

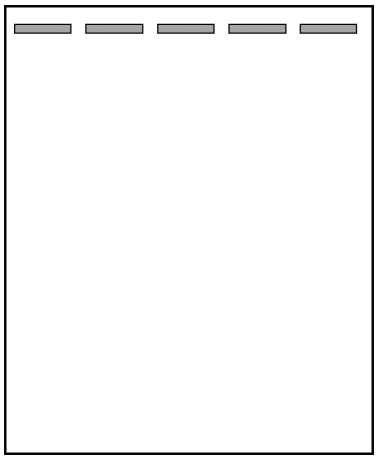


Fill in the Punnett square. Put a star on the box that represents Missy Baker.

# Study questions - post-lab

## Interpreting results

**1. Use the gel to the right to illustrate the results you saw on your gel. There are five lanes on the gel: one for your ladder, and one for each PCR reaction that you performed.**



**2. Next to each band, write how many base pairs you think the DNA in that band is. Use the image of the ladder from page 19 to help you.**

Write "F" next to bands from individuals who you think produce a functional CFTR protein.

Write "f" next to bands from individuals who you think produce a non-functional CFTR protein.

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

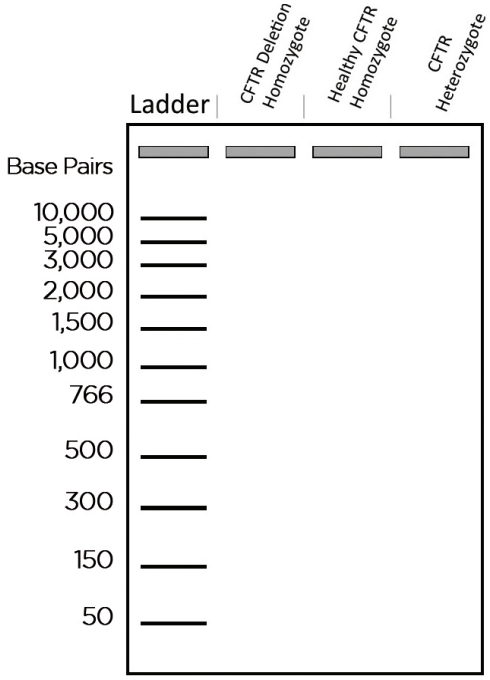
**4. Two of the samples you used in this activity were labeled as controls. Explain what specifically each sample tells you and why it was important to include in the experiment:**

- Tube H:
- Tube D:

# Critical thinking

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

**6. On the images of a gel below, illustrate what you think the results of this experiment would be for both a homozygote and heterozygote.**




**7. 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.**

# Extension: CFTR and cystic fibrosis

# 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 non-functional, 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'  CTTGGAGAAAGGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGA
    ...•|....|....|....|....|....|....|....|....|....|....|....| 180
3'  GAACCTCTTTCCACCTTAGTGTGACTCACCTCCAGTTGCTCGTTCTTAAAGAAATCGTTCT
```

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 33) 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 | ▲C | CT | CTT | CCA | CCT | TAG | TGT | GAC | TCA | CCT | C■A | 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:**

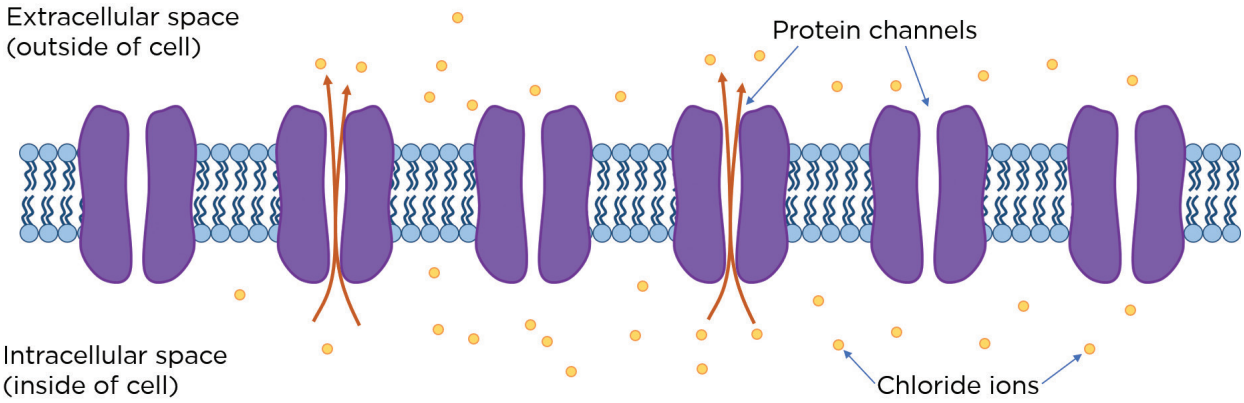
a. Summarize the effect each mutation has on the amino acid sequence.
b. Then, relate how this change could cause the protein to be classified as a class I, II, or III mutation.

G542X:

ΔF508:

G551D:

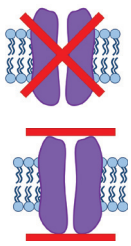
The CFTR protein allows for chloride ions to exit the cell. The diagram below shows an illustration of a cell membrane with six normal CFTR protein channels.




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.

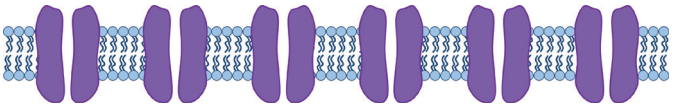
Below are membranes with CFTR proteins present. Each membrane represents the cell membrane from a different person. The individual's genotype is described to the left of the membrane.

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

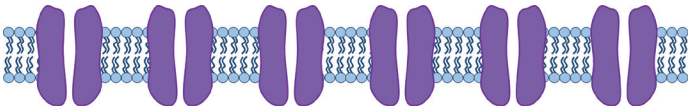


- Mark the protein with an X if it would not be present in the membrane.
- Draw lines blocking the channel if the protein would be present, but not functional.
- Leave the protein alone if it would be present and functional.

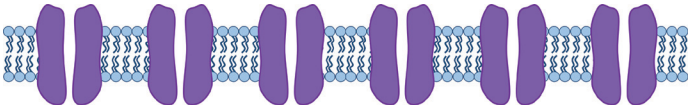
**10. A person with a ΔF508 mutations on both chromosomes. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?**



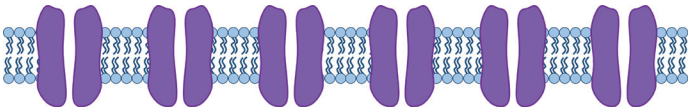
**11. A person with a ΔF508 mutation on one chromosome and a G551D mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?**



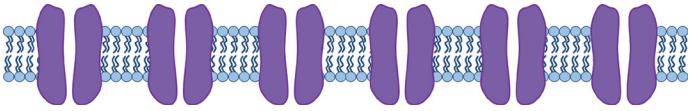
**12. A person with a G551D mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?**



**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?**



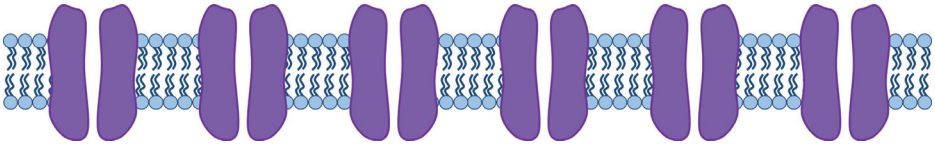
**14. A person with a G542X mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?**



**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 11-15) 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 |