



Cystic Fibrosis Lab

Missy Baker Missing™



Student guide



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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!

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.

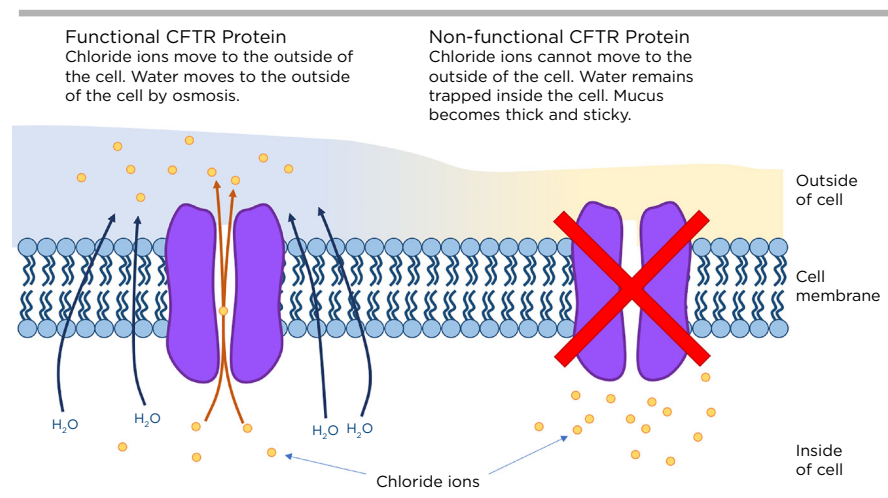
All of the problems a person with cystic fibrosis faces can be tied to mutations in a single gene that is involved in moving molecules in and out of cells. The gene is called *CFTR* (cystic fibrosis transmembrane regulator), and it codes for a protein that is inserted in cell membranes. In all the cells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



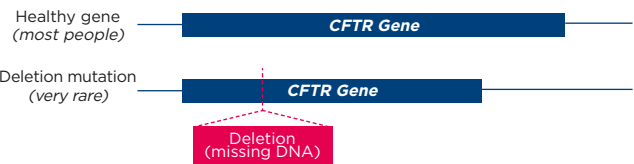
There are over 1,500 different known mutations that can lead to a non-functional CFTR protein and cystic fibrosis, but the most common cystic fibrosis mutation is a deletion. A deletion occurs when part of the normal sequence of DNA is missing. Depending on what is missing from the DNA sequence, deletions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

Genomic DNA



PCR Product



Forward PCR primer →
Reverse PCR primer ←



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 μ l	15 μ l	15 μ l	15 μ l
Primer Mix (tube PM)	10 μ l	10 μ l	10 μ l	10 μ l
DNA sample	Suspect A DNA (tube A) 5 μ l	Suspect B DNA (tube B) 5 μ l	Control H DNA (tube H) 5 μ l	Control D DNA (tube D) 5 μ l

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.

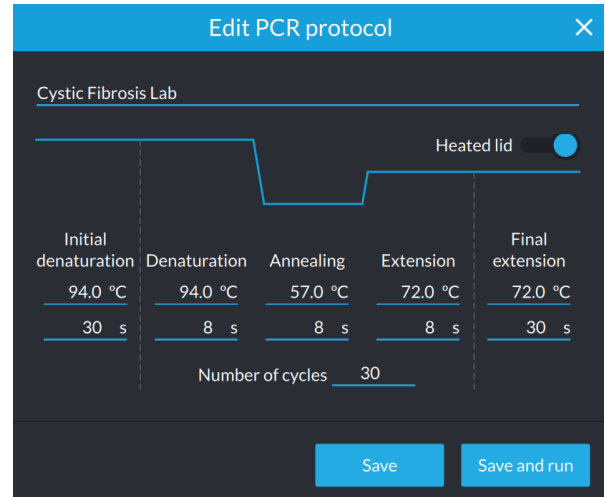


Run PCR

- 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.
 - The blueGel electrophoresis system has an integrated blue light 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



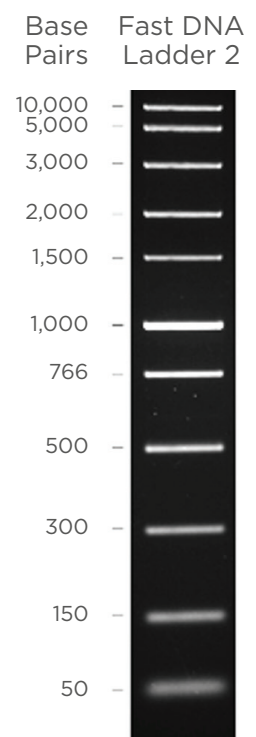
blueGel

<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: _____

Parent 2

Parent 1

	_____	_____

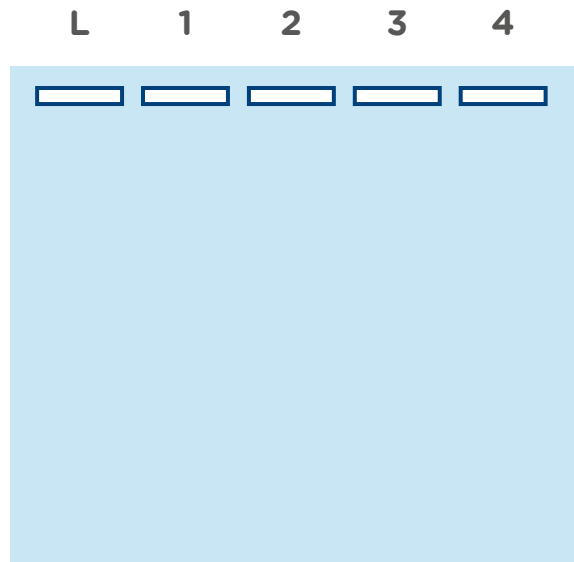
Fill in the Punnett square. Put a star on the box that represents Missy Baker.



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: Functional <i>CFTR</i> gene control	Tube 4: Deletion mutation control
Functional <i>CFTR</i> (700 bp)				
Mutated <i>CFTR</i> (400 bp)				

Critical thinking

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



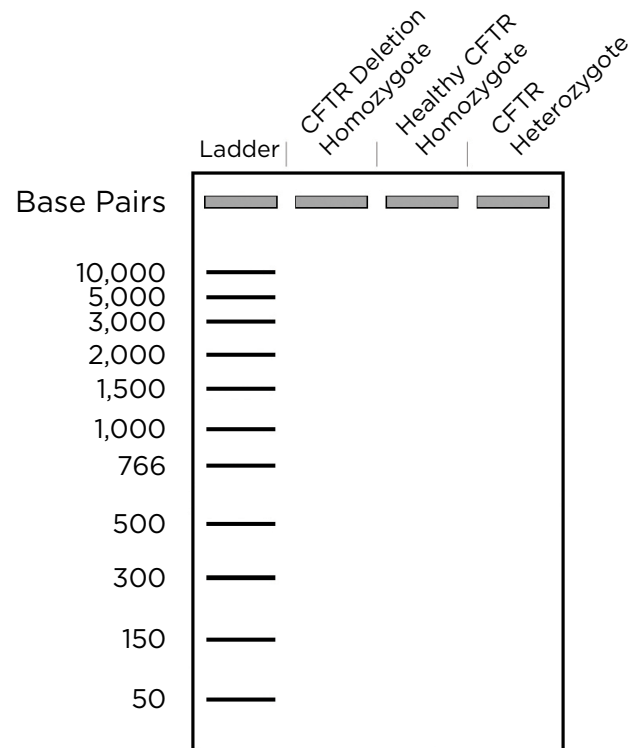
5. Two of the samples you used in this activity were labeled as controls. Explain what specifically each sample tells you and why it was important to include in the experiment:

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 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.

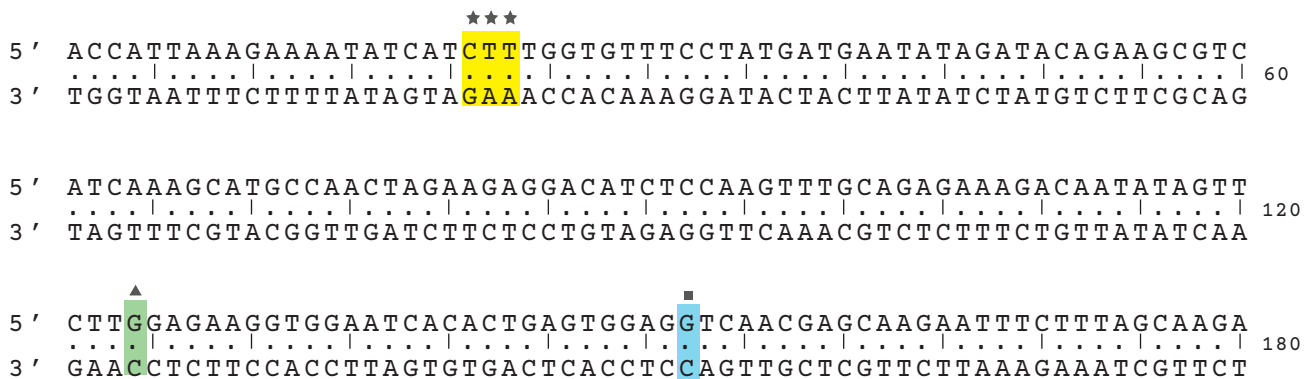
Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100



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.



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.



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 $\Delta F508$ is a deletion mutation. In $\Delta F508$, the CTT in positions 21-23 of the DNA sequence (highlighted in yellow and marked with \star) 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 C[■]CA GTT 5'
 RNA : _____
 AA Seq : _____

7. A second mutation known as G542X changes the cytosine (C) (highlighted in green and marked with a \blacktriangle) 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.

R N A : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
 A A S e q : _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

- 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:

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

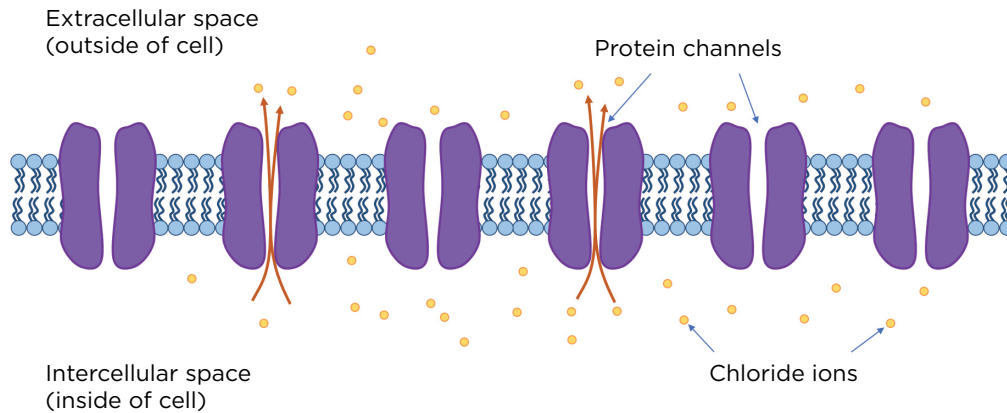
G542X:

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.

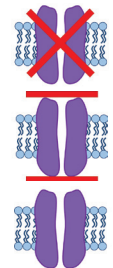


Every person has two copies of the *CFTR* gene, one on each copy of chromosome 7. This means that typically half of the CFTR proteins in a cell are encoded by one copy of the gene, while half of the proteins are encoded by the other copy of the gene. Cystic fibrosis is considered a recessive disorder. This means that 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.

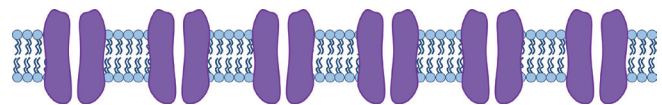
Below are membranes with CFTR proteins present. Each membrane represents the cell membrane from a different person. The individual's genotype is described to the left of the membrane.

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.

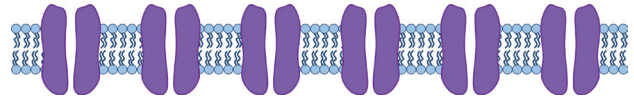


10. A person with a F508 mutations on both chromosomes. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?

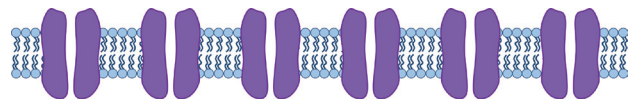




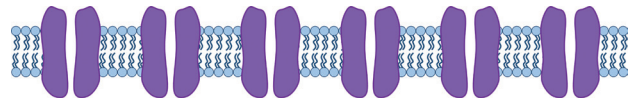
11. A person with a F508 mutation on one chromosome and a G551D mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



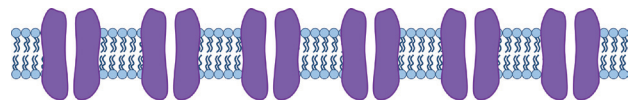
12. A person with a G551D mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



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?

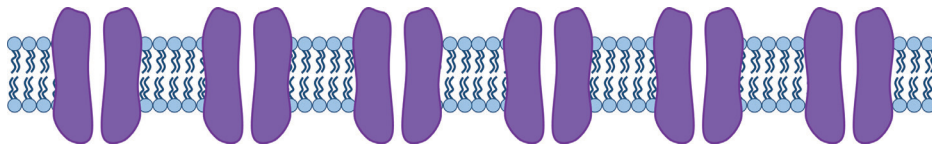




15. Cystic fibrosis is a recessive disorder. Is it correct to describe everyone with cystic fibrosis as a homozygote? Explain your answer.

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

		Second Position Nucleotide									
		U		C		A		G			
First 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	