miniPCR® Crime Lab

Missy Baker Missing™

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

Version: 2.1
Release: February 2022
© 2022 by miniPCR bio™

Instructor's Guide Contents

Getting started

At a glance	P.03
Materials needed	P.04
Lab setup	P.06

Student's guide

Background information	P.09
Today's lab	P.12
Laboratory guide	P.13
Study questions	P.22
Extension: CFTR and cystic fibrosis	P.26

Instructor's guide

Expected results	P.35
Notes on lab design	P.36
Differentiation	P.37
Additional student supports	P.37
Extension activities	P.38
Placement in unit	P.39
Learning goals and skills developed	P.40
Standards alignment	P.41
Ordering information	P.42
About miniPCR bio Learning Labs™	P.43

At a glance

Missy Baker has gone missing! Use modern genetic techniques to zero in on a suspect.

This introductory lab will help students gain confidence with important lab techniques such as micropipetting, PCR, and gel electrophoresis. In the process, they will explore cystic fibrosis and the connections between genes and disease, personal identification, and the use of biotechnology for genetic analysis.

TECHNIQUES

Micropipetting
PCR
Gel electrophoresis

TOPICS

Forensics
Genes and disease
Genotype to phenotype
Cystic fibrosis
Biotechnology

LEVEL

General high school through Introductory college

WHAT YOU NEED

Micropipettes
Thermal cycler
Gel electrophoresis and visualization system

AP CONNECTION

AP Biology Units
2.4-2.9, 5.3, 6.7-6.8

Skills and Practices
1.A-1.C, 2.A-2.D, 3.C-3.D, 6.A-6.E

Planning your time

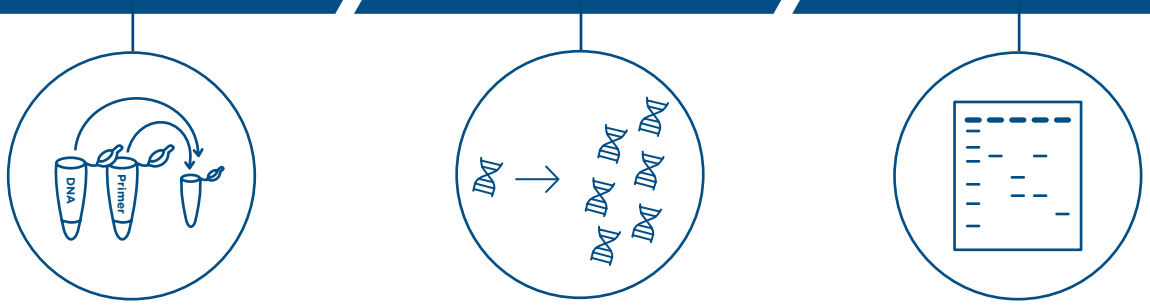
SINGLE CLASS PERIOD - 90 MINUTES

OR

PERIOD 1 - 30 MINUTES → Possible stopping point → **PERIOD 2 - 30 MINUTES**

Once PCR has started, samples may be left unattended.
PCR products are stable at room temperature for at least one week.

Prepare PCR samples → **Run PCR** → **Gel electrophoresis**



Additional Supports

Help your students build proficiency in pipetting, PCR, and gel electrophoresis with additional instructional videos, worksheets, and activities available at: https://www.minipcr.com/tutorials/

Taking it further - extension activities, page 26
Explore the molecular basis of cystic fibrosis and the CFTR mutation.

For answers to the lab study questions and extensions, email answers@minipcr.com. Please include the name of the lab, as well as your name, school, and title in the body of the email.

Materials needed

Supplied in Kit (KT-1510-01)

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
2X EZ PCR Master Mix, Load Ready™	**700 µl**	**75 µl**	-20°C freezer	
Crime Lab 3X Primer Mix	**500 µl**	**50 µl**	-20°C freezer	
Template DNA • Suspect A DNA • Suspect B DNA • Control H DNA • Control D DNA	 **100 µl** **100 µl** **100 µl** **100 µl**	**10 µl each**	-20°C freezer	
Fast DNA Ladder 2	**150 µl**	**15 µl**	-20°C freezer	

Sold Separately in Learning Lab Companion Kit (KT-1510-01)

This lab requires reagents for running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®). The Learning Lab Companion Kit provides enough electrophoresis reagents for 8 groups when using the blueGel™ electrophoresis system. Gels can also be prepared with agarose tabs or agarose powder. Refer to www.minipcr.com/agarose-gel/ for detailed instructions.

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
All-in-one agarose tabs	**8**	**One tab per agarose gel (2% agarose gel)**	Room temp.	
TBE electrophoresis buffer	**Supplied as liquid concentrate or powder** Sufficient for 600 ml of 1X working solution	**30 ml per blueGel™ system**	Room temp.	
PCR tubes (0.2 ml)	**100**	**4**		
Plastic tubes: 1.5 or 1.7 ml tubes to aliquot reagents	**50 microtubes (1.5 ml)**	**6**		

Materials needed (cont.)

Supplied by teacher

Available at miniPCR.com

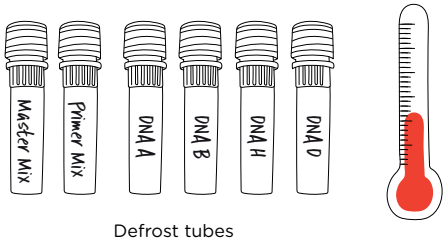
Reagents and supplies	Amount needed per lab group	Teacher's checklist
PCR thermal cycler: e.g. miniPCR® machine	**1 four reactions per group (machines can be shared between groups)**	
Horizontal gel electrophoresis apparatus: e.g. blueGel™ electrophoresis system	**1 If sharing gels, reserve 1 lane for ladder and 4 lanes for each group.**	
Blue light transillumnator *Note: A blue light transilluminator is integrated in the blueGel electrophoresis system	**1**	
Micropipettes • 2-20 µl: one per lab group • 20-200 µl: one for the teacher to dispense reagents	**1**	
Disposable micropipette tips	**At least 17 per group (12 for PCR setup 5 for electrophoresis)**	
Microcentrifuge (optional; only needed to collect liquid at tube bottom)		
Distilled water for making agarose gels and diluting TBE buffer	**50 ml per gel**	
Flask or beaker to dissolve agarose		
Microwave or hot plate to dissolve agarose		
Other supplies: • Disposable laboratory gloves • Protective eyewear • Permanent marker • Cup to dispose of tips		

Lab setup

The following activities can be carried out by the instructor ahead of class. Reagents are sufficient to be used with 8 student groups (4 students per group). Reagents are stable at room temperature for 24 hours, but should remain cold for short term storage and frozen for long term storage.

Gloves and protective eyewear should be worn for the entirety of this lab.

A. Preparation for PCR

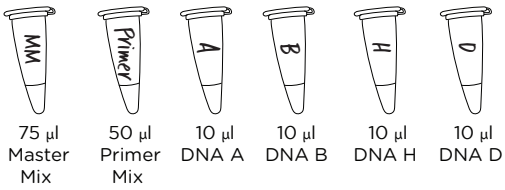


Defrost tubes

- Thaw tubes containing the Crime Lab 3X Primer Mix, DNA samples, and 2X EZ PCR Master Mix by placing them on a rack or benchtop at room temperature.

- For each lab group, label and dispense the following reagents into six labeled 1.7 ml tubes.

- 2X EZ PCR Master Mix	75 µl
- Crime Lab 3X Primer Mix	50 µl
- Suspect A DNA	10 µl
- Suspect B DNA	10 µl
- Control H DNA	10 µl
- Control D DNA	10 µl



75 µl Master Mix | 50 µl Primer Mix | 10 µl DNA A | 10 µl DNA B | 10 µl DNA H | 10 µl DNA D

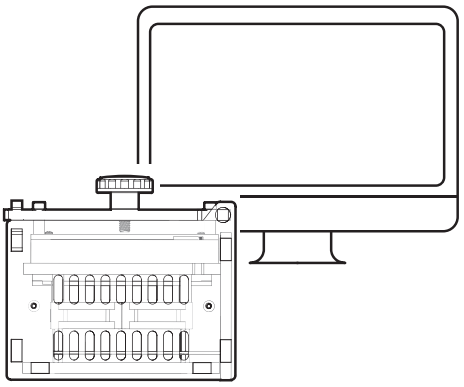
× **number of groups** up to 8

- Distribute supplies and reagents to lab groups.

Check	At the start of this experiment, every lab group should have:	Amount
	2X EZ PCR Master Mix	75 µl
	Crime Lab 3X Primer Mix	50 µl
	Suspect A DNA	10 µl
	Suspect B DNA	10 µl
	Control H DNA	10 µl
	Control D DNA	10 µl
	PCR tubes (200 µl)	4
	2-20 µl micropipette	1
	Micropipette tips	At least 12
	Fine tipped permanent marker	1

Continued on next page.

Preparation for PCR (continued)



- Ensure each lab group's bench is set up with a miniPCR® thermal cycler and power supply.
- Ensure the miniPCR® thermal cyclers that are going to be monitored throughout the PCR reaction are connected to a computer or compatible phone/tablet.
- Machines can be programmed ahead of time by the teacher or during class by the students.

B. Preparation for gel electrophoresis

- Distribute 15 µl Fast DNA Ladder 2 to each group.
- Have the banding pattern of the Fast DNA Ladder 2 handy to help interpret the electrophoresis results (page 19).

15 µl
DNA Ladder

Preparation for gel electrophoresis - preparing gels

- Prepare 1X TBE buffer.
  - TBE buffer is often provided as liquid concentrate or powder.
  - Follow manufacturer's instructions to prepare 1X TBE buffer solution.
  - Volume to prepare depends on method used to prepare gels; see "Important Note" below.
- Gels can be poured in advance of the class.
  - This lab requires running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®).
  - Pre-poured gels can be stored at ambient temperature, in a sealed container or wrapped in plastic wrap, and protected from light for up to three days.

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/

Student's Guide Contents

Background information	P.09
Today's lab	P.12
Laboratory guide	P.13
Study questions	P.22
Extension: CFTR and cystic fibrosis	P.26

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.

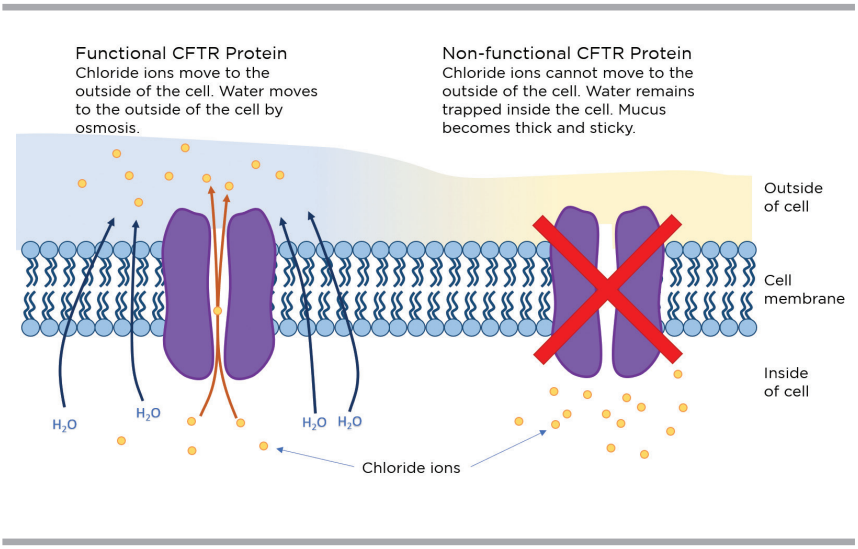
In the case of Missy Baker, there are two suspects, and you will be testing DNA from hair follicles found in each suspect's car to see if either one matches Missy Baker's DNA. We will analyze these hair samples searching for a match to something that makes Missy Baker's DNA unique.

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.



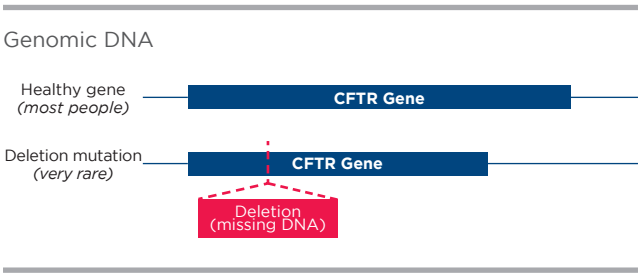
All of the problems a person with cystic fibrosis faces can be tied to mutations in a single gene that is involved in moving molecules in and out of cells. The gene is called CFTR (cystic fibrosis transmembrane regulator), and it codes for a protein that is inserted in cell membranes. In all the cells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

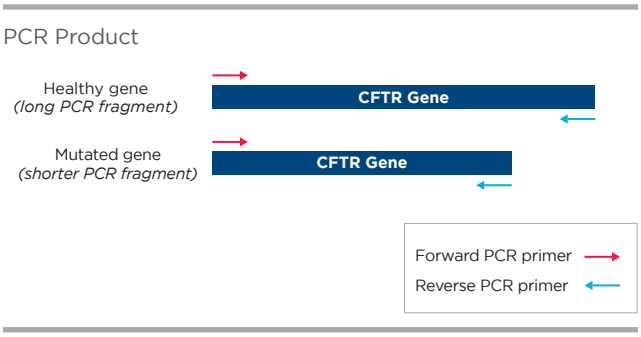
There are over 1,500 different known mutations that can lead to a non-functional CFTR protein and cystic fibrosis, but the most common cystic fibrosis mutation is a deletion. A deletion occurs when part of the normal sequence of DNA is missing. Depending on what is missing from the DNA sequence, deletions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

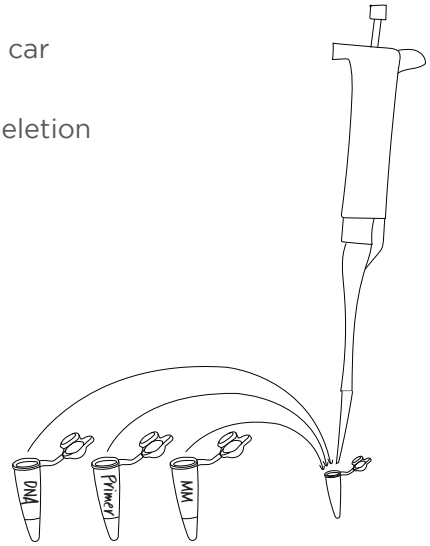
Note: label tubes on the upper side wall, as writing on the cap or lower side wall may rub off during PCR.

- 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 5 µl	DNA B 5 µl	Control H 5 µl	Control D 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

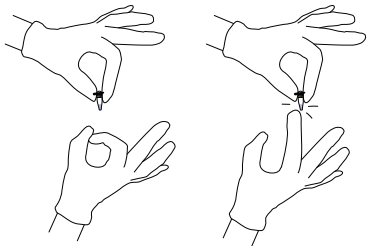


Use a micropipette to add each of the reagents. Remember to change tips at each step!

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.

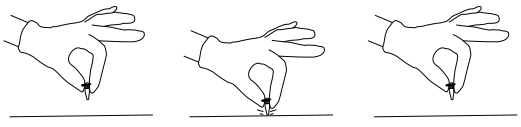
Flick to mix



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

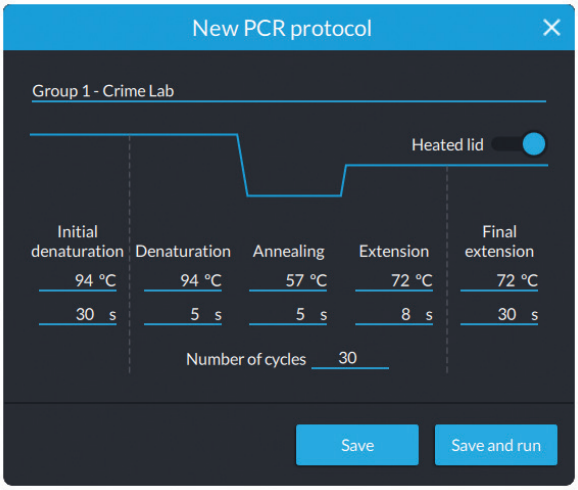
- Button is located in the upper right hand corner of the window.

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

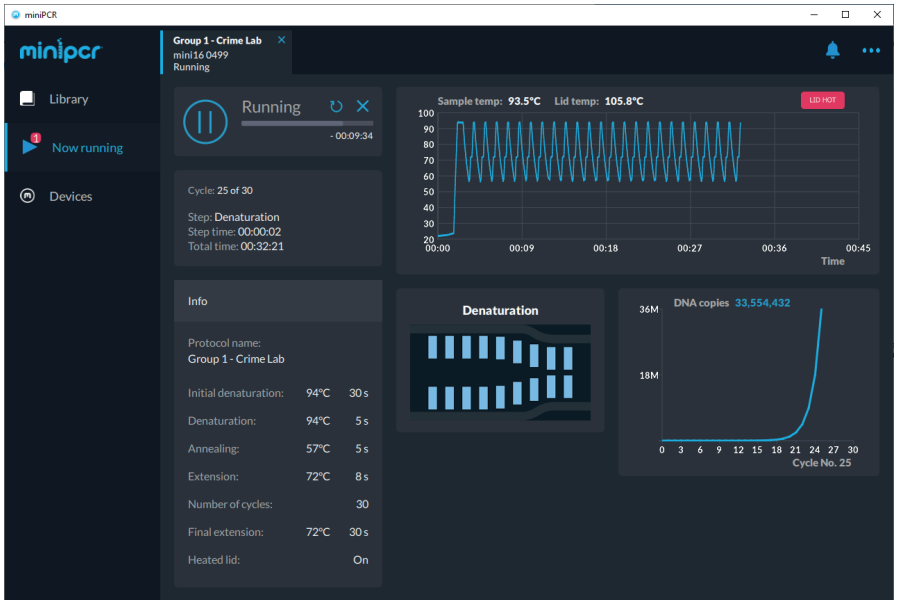


8. Click "Save and run" to start the protocol

- 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.

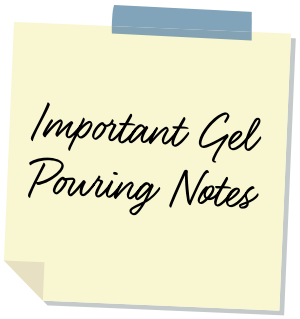
11. PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer

- 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)



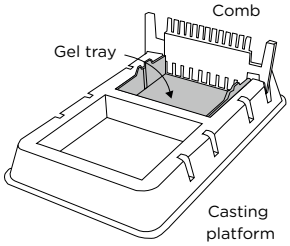
Gels can be prepared up to three days ahead of time and should be stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

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

---

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

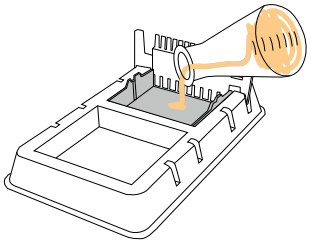
- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

---

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.

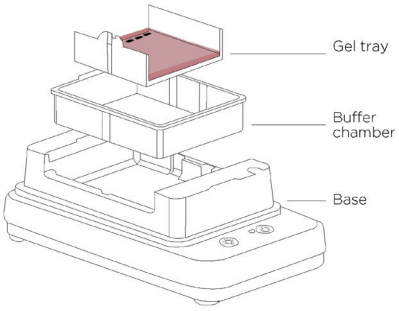
Protective gloves and eyewear should be worn for the entirety of this experiment.

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.



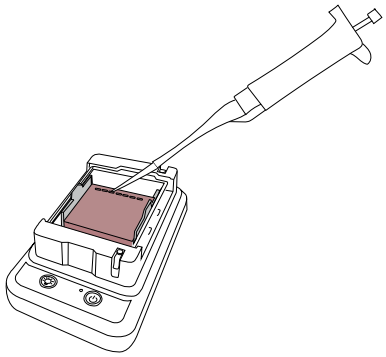
2. Add 30 ml of 1X TBE electrophoresis buffer

- 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.

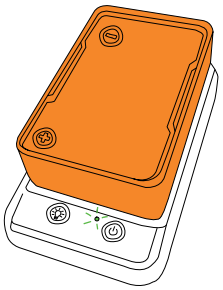


4. Place the orange cover on the blueGel™ electrophoresis system

- 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.

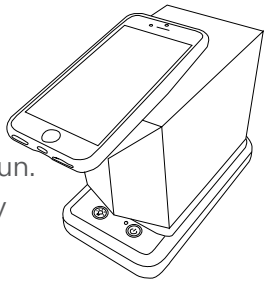


6. Conduct electrophoresis for 20-25 minutes

- 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



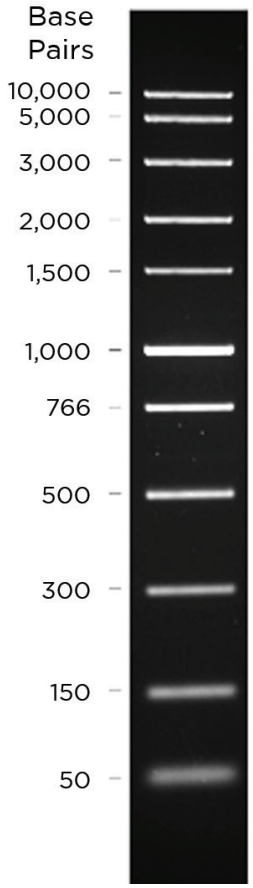
- For best viewing, dim ambient lights or use **Fold-a-View™** photo documentation hood with a smartphone camera.
- Gels may be viewed at the end of the run or periodically throughout the run.
- If image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

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 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

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

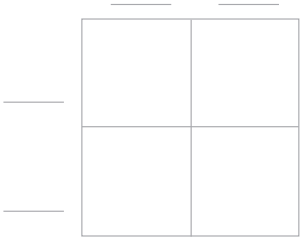
6. Do you think testing for only the CFTR gene would normally be a good way to identify most people?

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: _____

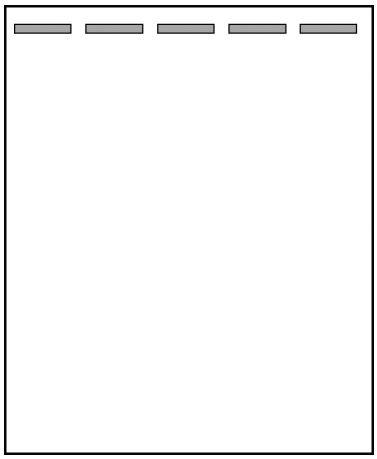


Fill in the Punnett square. Put a star on the box that represents Missy Baker.

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.

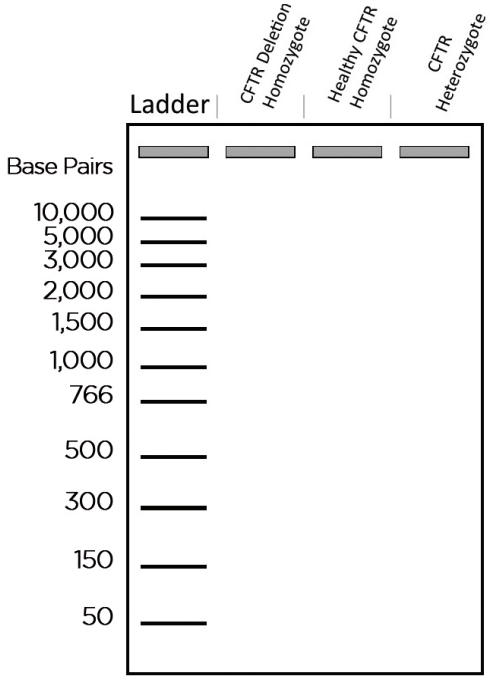
4. Two of the samples you used in this activity were labeled as controls. Explain what specifically each sample tells you and why it was important to include in the experiment:

- 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:

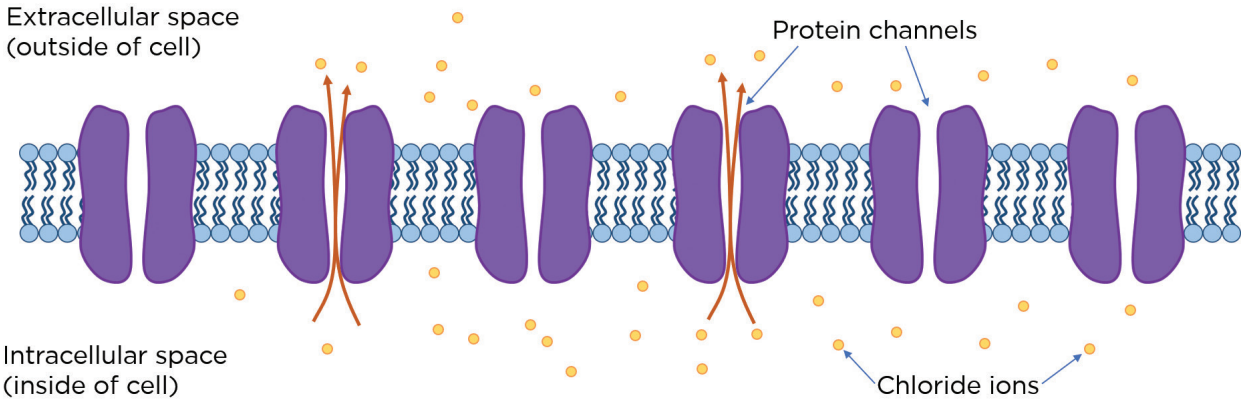
a. Summarize the effect each mutation has on the amino acid sequence.
b. Then, relate how this change could cause the protein to be classified as a class I, II, or III mutation.

G542X:

ΔF508:

G551D:

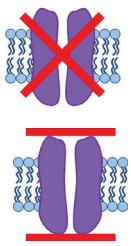
The CFTR protein allows for chloride ions to exit the cell. The diagram below shows an illustration of a cell membrane with six normal CFTR protein channels.



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.

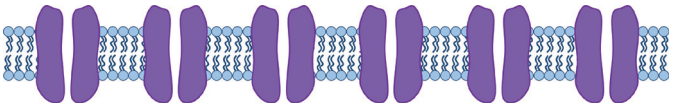
Below are membranes with CFTR proteins present. Each membrane represents the cell membrane from a different person. The individual's genotype is described to the left of the membrane.

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

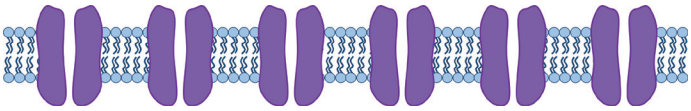


- Mark the protein with an X if it would not be present in the membrane.
- Draw lines blocking the channel if the protein would be present, but not functional.
- Leave the protein alone if it would be present and functional.

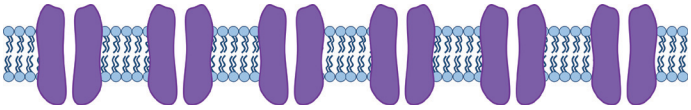
10. A person with a ΔF508 mutations on both chromosomes. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



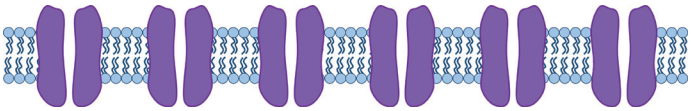
11. A person with a ΔF508 mutation on one chromosome and a G551D mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



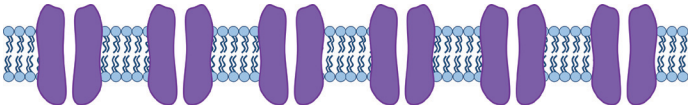
12. A person with a G551D mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



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?



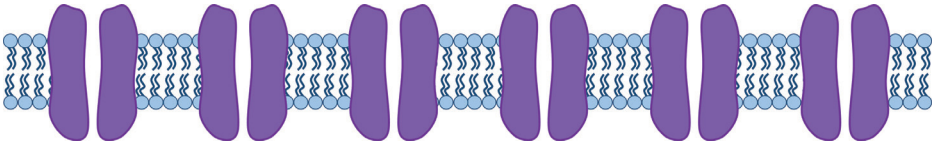
14. A person with a G542X mutation on one chromosome and no mutation on the other. Mark the proteins as described in the directions above. Based on how you marked the proteins, do you think this person will have cystic fibrosis?



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

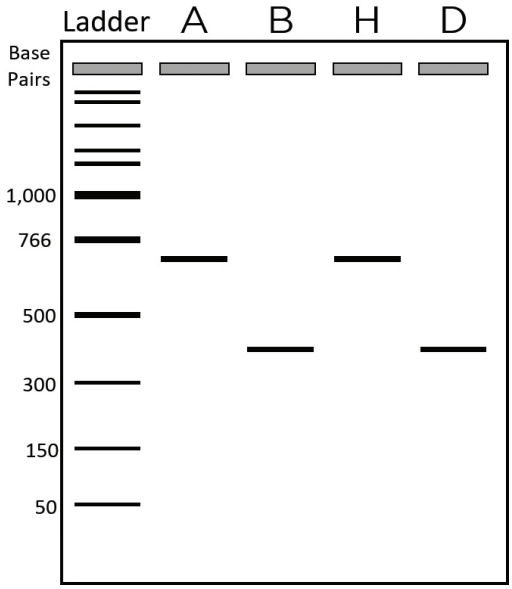
First Position Nucleotide	Second Position Nucleotide: U		Second Position Nucleotide: C		Second Position Nucleotide: A		Second Position Nucleotide: G		Third Position Nucleotide
U	UUU	Phenylalanine (Phe, F)	UCU	Serine (Ser, S)	UAU	Tyrosine (Tyr, Y)	UGU	Cysteine (Cys, C)	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine (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

Instructor's Guide

Expected results	P.35
Notes on lab design	P.36
Differentiation	P.37
Additional student supports	P.37
Extension activities	P.38
Placement in unit	P.39
Learning goals and skills developed	P.40
Standards alignment	P.41
Ordering information	P.42
About miniPCR bio Learning Labs™	P.43

Expected results

Gel electrophoresis results are expected to resemble the gel image to the right:



- Sample A, hair sample from Alan Torte's car, should produce a 700 bp band.
- Sample B, hair sample from Brenda Biscottis's car, should produce a 400 bp band.
- Sample H, control from a healthy individual, should produce a 700 bp band.
- Sample D, control from a person with a CFTR deletion, should produce a 400 bp band.

Interpretation:

- Sample H is a control from a healthy individual and produced a 700 bp band.
- Sample D is a control from an individual with a CFTR deletion and produced a 400 bp band.
- As we know that Missy Baker has a deletion in her CFTR gene, we expect MIssy Baker's DNA to produce a 400 bp band.
- Sample B is a match for Missy Baker, where Sample A is not a match.
- This suggests that the evidence points to Suspect B, Brenda Biscotti.

Notes on lab design

This lab serves as an introduction to how genetic technologies can be used broadly to investigate many types of questions including personal identification, forensics, genetic disease and the effect of mutations. Specifically, this lab introduces how genetic technologies can be used for DNA analysis 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:

- We use plasmid DNA as the PCR template in order to increase the protocol's robustness rather than procuring "real" forensic human genomic DNA. The primers used are therefore designed to amplify plasmid DNA and not the human CFTR gene.

- Real-world forensic biotechnology often uses DNA profiling of tandem repeats (variable number tandem repeats, VNTRs, or short tandem repeats, STRs). Instead, we chose to use the example of a rare mutation in a single human gene (CFTR). Our goal is to engage students with a type of mutation with which they will be more familiar and that has broader implications beyond forensics, providing an opportunity to discuss the molecular pathophysiology of cystic fibrosis.

- 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.

We suggest discussing these differences with students after finishing this lab to give a deeper understanding of how such technologies are used outside of the classroom.

Differentiation

This lab serves as an introductory look at how DNA and biotechnology can be used across several related fields. With simple modifications, this activity can be used effectively in classes ranging from middle school through introductory college.

Introductory classes: Choose to focus on DNA as a unique identifier, as an underlying cause of our traits and even some diseases, or both. Avoid discussion of how specific sequence changes affect specific phenotypes. The standard introduction and review questions for this lab take this approach.

Advanced classes: Use the extension CFTR and Cystic Fibrosis to explore the links between DNA sequence changes and changes in cell physiology and disease. First, students analyze DNA sequences to link mutations to changes in the CFTR protein. Next, students use visual representations to model how such protein changes affect cell physiology and cause disease.

Additional student supports

At miniPCR bio™, we are committed to preparing students to be successful in the laboratory through high quality curriculum and training. We have created an extensive set of resources to help your students succeed in molecular biology techniques, all of which are available for free download at the miniPCR bio™ tutorials page of our website.
-
https://www.minipcr.com/tutorials/

Those activities most relevant to this lab are listed below.

Micropipetting: Video and 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.

Extension activities

The following optional extension activities are 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.

Forensics and DNA Fingerprinting: miniPCR® Crime Lab allows students to investigate a fictional crime using DNA analysis. Have your students explore in more detail how criminal investigators typically investigate crimes using DNA fingerprinting and the FBI CODIS system.
Link includes article as well as classroom questions.

DNAdots™
Simple explanations of modern genetic technologies

https://dnadots.minipcr.com/dnadots/dna-fingerprinting

miniPCR® in Forensics: See how miniPCR® machines are used in actual forensics investigations. Instead of bringing evidence to the lab, some investigators are bringing miniPCR® machines to the evidence, reducing the chance of contamination or mishandling.

https://www.minipcr.com/case-studies/forensic-dna-analysis-evidence/

Placement in unit

Biotechnology
This lab can be used as a culminating activity to demonstrate mastery of micropipetting, PCR and gel electrophoresis. For this approach, we recommend spending more time on some of the activities discussed in the Additional Student Supports (p. 37) section of this lab before beginning the experiment.

Forensics
Use this lab as an introduction to how genetic technologies allow forensic scientists to make personal identifications. Continue with the extension: DNA Fingerprinting (https://dnadots.minipcr.com/dnadots/dna-fingerprinting), to introduce the tools forensic scientists use more regularly. Once students have mastered their molecular biology skills with Missy Baker, continue with the miniPCR Forensics Lab (https://www.minipcr.com/product/minipcr-forensics-lab-d1s80-vntr/), which allows students to sample their own DNA to solve a forensic mystery.

Genes and disease
Missy Baker is described as having cystic fibrosis. Use this lab as an engaging scenario to talk about the relationships between genes and human health. Continue with the extension CFTR and cystic fibrosis (page 26) to explore this connection in more depth.

Mutations
Cystic fibrosis is caused by mutations in the CFTR gene. Using the extension CFTR and cystic fibrosis (page 26), explore different types of mutations and how they can affect gene function. Discuss what different effects deletions, insertions, and substitutions can have on a protein's function.

Learning goals and skills developed

Student Learning Goals – students will:

- Understand the basic structure of DNA and its role in genetic inheritance
- Comprehend how traits are passed from parent to offspring
- Understand that PCR is a technique for amplifying specific parts of the genome
- Learn about the existence of genetic mutations (through the cystic fibrosis transmembrane regulator CFTR gene) and their biomedical significance
- Explore the role of the CFTR gene in cystic fibrosis
- Correlate genotype to phenotype
- Learn about the use of DNA analysis in personal identification and forensics

Scientific Inquiry Skills – students will:

- Create hypotheses and predict results
- Compare results to their predictions and to a real-world example
- Generate tables to present their results
- Use experimental results to make conclusions based on hypotheses
- Follow laboratory protocols

Molecular Biology Skills:

- Micropipetting skills
- Principles and practice of PCR
- Preparation of agarose gels
- Agarose gel DNA electrophoresis
- Staining, visualization, and molecular weight analysis of DNA fragments

Standards alignment

Next Generation Science Standards

Students who demonstrate understanding can:

HS-LS1-1.	Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
HS-LS3-1.	Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
HS-LS3-2.	Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

Science and Engineering Practice	Disciplinary Core Ideas	Crosscutting Concepts
• Asking Questions and Defining Problems • Developing and Using Models • Planning and Carrying Out Investigations • Analyzing and Interpreting Data • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	**LS1.A: From Molecules to Organisms: Structures and Processes** **LS3.A: Inheritance of Traits** **LS3.B: Variation of Traits**	• Patterns • Cause and Effect • Systems and System Models • Structure and Function • Stability and Change • Interdependence of Science, Engineering, and Technology • Influence of Engineering, Technology, and Science on Society and the Natural World

Common Core ELA/Literacy Standards

RST.9-10.1	Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
RST.9-10.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.
RST.9-10.4	Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics
RST.9-10.5	Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
RST.9-10.9	Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts.
WHST.9-10.1	Write arguments focused on discipline-specific content.
WHST.9-10.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
WHST.9-10.9	Draw evidence from informational texts to support analysis, reflection, and research.

*This activity has been aligned to high school NGSS and grades 9-10 Common Core standards. For information aligning this activity to middle school or other grade levels, please contact curriculum@minipcr.com.

Ordering information

To order miniPCR® Crime Lab reagent kits, you can:

Call (781)-990-8PCR

email us at orders@minipcr.com

visit www.minipcr.com

miniPCR® Crime Lab kit (catalog no. KT-1000-03) contains the following reagents:

- 2X EZ PCR Master Mix, Load-Ready™
  - including Taq DNA polymerase, dNTPs, PCR buffer, and gel-loading dye
- Crime Lab 3X Primer Mix
- Suspect A DNA sample
- Suspect B DNA sample
- Control H DNA sample
- Control D DNA sample
- Fast DNA Ladder 2

Materials are sufficient for 8 lab groups, or 32 students
All components should be kept frozen at -20°C for long-term storage
Reagents must be used within 12 months of shipment

Other reagents needed

Additional reagents can be sourced independently or are available as part of our Learning Lab Companion Kit (catalog no. KT-1510-01) at miniPCR.com.

- Agarose (electrophoresis grade)
- Fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®)
- Gel electrophoresis buffer (e.g., 1X TBE)
- Distilled or deionized H_2O (to dilute TBE buffer concentrate – not included in Lab Companion Kit)

About miniPCR bio Learning Labs™

This Learning Lab was developed by the miniPCR bio™ curriculum team in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop Learning Labs™ to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The guiding premise for this lab is that a 90 minute PCR-based experiment can recapitulate a real-life biotechnology application and provide the right balance between intellectual engagement, inquiry, and discussion.

Starting on a modest scale working with Massachusetts public schools, miniPCR bio Learning Labs™ have been well received, and their use is growing rapidly through academic and outreach collaborations across the world.

Acknowledgement - This lab was originally inspired by the following article:
Bouakaze C, et al. "OpenLAB": A 2-hour PCR-based practical for high school students. Biochem Mol Biol Educ. 2010 Sep;38(5):296-302. doi: 10.1002/bmb.20408. PubMed PMID: 21567848