
miniPCR™ Crime Lab: Missy Baker Gone Missing

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1. Synopsis

Students will help police investigators solve a crime mystery by analyzing DNA samples using the essential molecular biology techniques of PCR (polymerase chain reaction) and gel electrophoresis. This lab illustrates real-world applications of molecular biology such as DNA analysis in personal identification, forensics, and molecular medicine.

- **Techniques utilized:** PCR, gel electrophoresis, and DNA visualization
- **Time required:** One 90 min. period or two 45 min. periods
- **Reagents needed:** 'Crime Lab' reagents kit (available from miniPCR), gel electrophoresis reagents (See sections 5 and 9)
- **Suggested skill level:** Familiarity with DNA amplification concepts, basic familiarity with micropipetting techniques

2. Learning goals and skills developed

Student Learning Goals:

- 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 polymorphisms (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
- Discuss ethical issues in forensic DNA analysis

Scientific Inquiry Skills:

- Students will create hypotheses and predict results
- Students will compare results to their predictions and to a real-world example
- Students will generate tables to present their results
- Students will use experimental results to make conclusions based on hypotheses
- Students will follow laboratory safety 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

3. Standards alignment

Next Generation Science Standards - Students will be able to...

- 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.
- HS-LS4-1 Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.
- HS-LS4-4 Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

Common Core English Language Arts Standards - Students will be able to...

- RST.11-12.1 Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.
- WHST.9-12.2 Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
- WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.
- SL.11-12.5 Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest.

4. Scenario overview

Missy Baker has gone missing. Two suspects are held by the authorities. Hair samples found in their cars must be analyzed by PCR to evaluate whether they match Missy Baker's DNA.

The DNA Times Free

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.

Boston, October 30th, 2025. Husband Ned reported Missy "Sugar-Cup" 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 (wheat-blond and thin as a breadstick) 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.

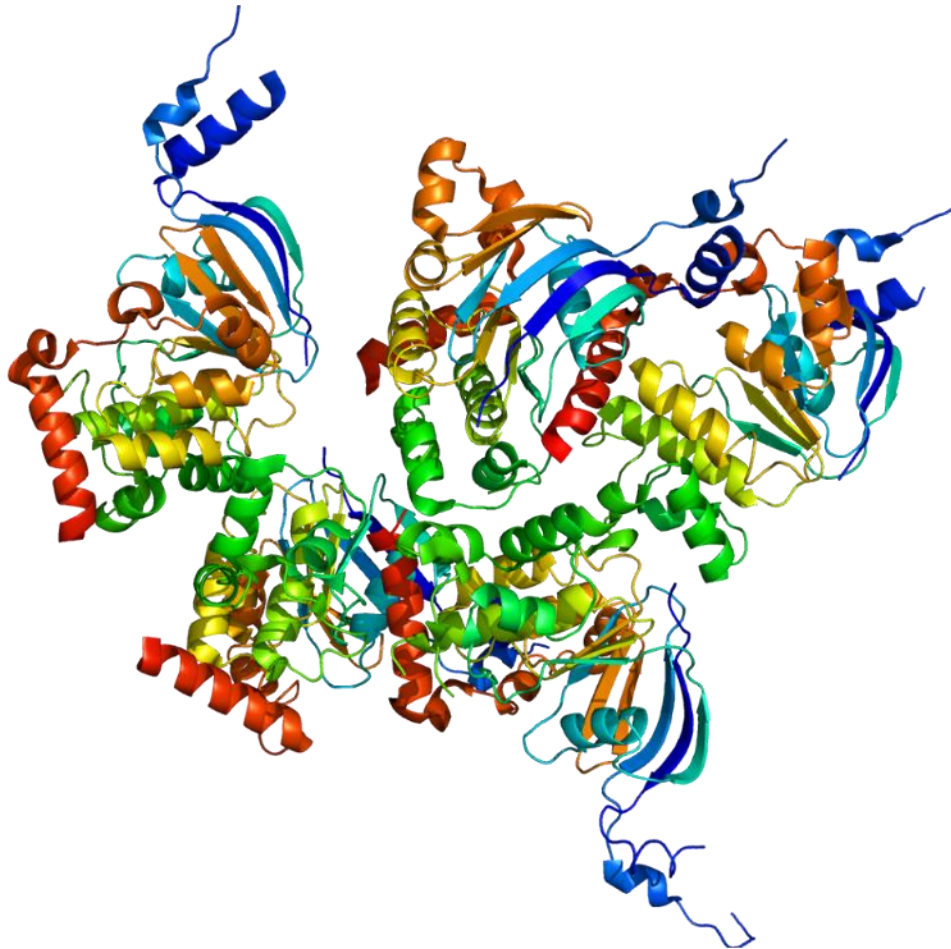
Woolly mammoth finally cloned at
 Brooklyn Zoo p.5



Transgenic kiwi solves
 world hunger p.7



The Cystic Fibrosis Transmembrane Regulator (CFTR) Gene



3-D model of the Cystic Fibrosis Transmembrane Regulator protein, encoded by the CFTR gene. Structure Based on PyMOL rendering of PDB (http://commons.wikimedia.org/wiki/File:Protein_CFTR_PDB_1xmi.png)

Cystic Fibrosis can be caused by mutations in the CFTR gene, which encodes a channel protein involved in the passage of chloride ions through the cell membrane.

A defective CFTR protein interferes with the body's ability to transfer water and salt to and from cells. This causes secretions, which are normally thin and watery in healthy people, to become thick and sticky. Thick secretions clog up organs and prevent them from working properly, often leading to respiratory complications which may be fatal.

Missy Baker carries a deletion mutation in the CFTR gene. We will use this rare mutation to experimentally test whether Missy Baker's genetic material matches DNA samples that were found in the suspects' cars.

5. Laboratory set-up manual

| Reagent | Volume needed per lab group | Storage | Teacher's checklist |
|--|------------------------------|---------------|---------------------|
| 2X EZ PCR Master Mix, Load Ready™ <ul style="list-style-type: none"> • 2x Mix with <i>Taq</i> DNA polymerase • dNTPs (included) • PCR buffer with Mg²⁺ (included) • Gel Loading Dye (included) | 60 µL | -20°C freezer | } Supplied in Kit |
| 3X Crime Lab Primer Mix <ul style="list-style-type: none"> • Pair of 'Crime Lab' primers, 3X mix | 50 µL | -20°C freezer | |
| Template DNA <ul style="list-style-type: none"> • <i>Suspect A</i> DNA sample • <i>Suspect B</i> DNA sample • <i>Control H</i> DNA sample ('healthy') • <i>Control D</i> DNA sample ('deletion') | 10 µL each | -20°C freezer | |
| 100 bp DNA Ladder, Load Ready™ <ul style="list-style-type: none"> • DNA molecular weight marker | 15 µL | -20°C freezer | |
| DNA staining agent <ul style="list-style-type: none"> • e.g., GreenView Plus DNA Stain (for Blue light transilluminators) | Follow supplier instructions | -20°C, dark | |
| 1.6% agarose gels <ul style="list-style-type: none"> • Electrophoresis grade agarose | 5 lanes per lab group | Room temp. | |
| Electrophoresis buffer <ul style="list-style-type: none"> • e.g., 1X TBE | Depending on gel apparatus | Room temp. | |


| Equipment and Supplies | Teacher's checklist |
|---|---------------------|
| PCR Thermal cycler: e.g. miniPCR™ machine | |
| Micropipettes <ul style="list-style-type: none"> • 2-20 µL: one per lab group • 20-200 µL: one for the teacher (to dispense reagents) | |
| Disposable micropipette tips | |
| PCR tubes: 4 x 200 µL microtubes per lab group | |
| Plastic tubes: 7 x 1.5 or 1.7 mL tubes (to aliquot reagents for each lab group) | |
| Horizontal gel electrophoresis apparatus: e.g., blueGel™ | |
| DC power supply for electrophoresis apparatus (included with blueGel™) | |
| Transilluminator: UV or Blue light (included with blueGel™) | |
| Scale for weighing agarose | |
| 250ml flasks or beakers to dissolve agarose | |
| Microwave or hot plate to dissolve agarose | |
| Microcentrifuge (optional; only needed to collect liquid at tube bottom) | |
| Gel documentation system (optional, or use cell phone camera instead) | |
| Other supplies: <ul style="list-style-type: none"> • UV safety goggles (if using UV transilluminator) • Disposable laboratory gloves • Permanent marker | |

Planning your time

This experiment has 4 stages:

- A. PCR reaction set up
- B. PCR programming and monitoring
- C. Separation of PCR products by DNA electrophoresis
- D. Size determination of PCR products and interpretation

An overview of the 90-minute experimental plan is represented below:

| Preparatory activity | Experimental stage |
|---|--|
| <p><i>Dispense reagents and prepare equipment</i></p> <ul style="list-style-type: none"> • 20 min | <p>A PCR set up</p> <ul style="list-style-type: none"> • 15 min |
| | <p>B PCR programming & monitoring</p> <ul style="list-style-type: none"> • PCR programming • 40 min PCR monitoring and discussion |
| | <p><i>Possible stopping point</i> Store PCR product in fridge (up to 1 week) or freezer (longer term)</p> |
| <p></p> <p><i>Pour agarose gels (e.g. during stage B)</i></p> <ul style="list-style-type: none"> • 20 min | <p>C Gel electrophoresis</p> <ul style="list-style-type: none"> • 20 min |
| | <p>D Size determination & interpretation</p> <ul style="list-style-type: none"> • 5 min visualization • 5 min discussion |

Quick guide: Preparatory activities

A. PCR set up

- Thaw tubes containing the primers and DNA samples by placing them on a rack or water bath at room temperature
- For each lab group conducting 4 PCR reactions label and dispense six tubes:
 - 2X EZ PCR Master Mix 75 μ L
 - 3X Crime Lab Primer Mix 50 μ L
 - Suspect A DNA 10 μ L
 - Suspect B DNA 10 μ L
 - Control H DNA 10 μ L
 - Control D DNA 10 μ L
- Each lab group will additionally need (at a minimum):
 - Micropipettes (*we recommend 2-20 μ L range*)
 - Disposable micropipette tips and a small beaker or cup to dispose them
 - 4 PCR tubes (200 μ L)
 - A permanent marker (fine-tipped)

B. PCR programming and monitoring

- Ensure each lab group's bench is set up with a miniPCR and power supply
- Ensure the miniPCR machines that are going to be monitored through the PCR reaction are connected to a computer or compatible tablet

C. Gel electrophoresis

- Gels can be poured in advance of the class (as described below)
- Pre-poured gels can be stored in the fridge, in a sealed container or wrapped in plastic wrap, and protected from light
- If doing the gel run on a different day than the PCR run, completed PCR reaction tubes can be stored in the fridge for up to one week until they are used, or in the freezer for longer-term storage

D. Size determination and interpretation

- Have the banding pattern of the 100bp DNA Ladder handy to help interpret the electrophoresis results

6. Instructor laboratory guide

A PCR set up

- Label 4 PCR tubes** (200 μ L tubes) per lab group
 - 1 tube labeled "A": 'Hair DNA' extracted from Suspect A's car
 - 1 tube labeled "B": 'Hair DNA' extracted from Suspect B's car
 - 1 tube labeled "H": 'Control DNA' from a healthy individual
 - 1 tube labeled "D": 'Control DNA' from a CFTR deletion mutant



Also label each tube with the group's name on the side wall

- Add PCR reagents** to each 200 μ L PCR tube

| | Tube A | Tube B | Tube H | Tube D |
|------------------------------------|--|--|---|--|
| Template DNA | DNA found in Suspect A's car 5 μ L | DNA found in Suspect B's car 5 μ L | Control H 'Healthy' DNA 5 μ L | Control D 'Deletion' DNA 5 μ L |
| 3X Crime Lab Primer Mix | 10 μ L | 10 μ L | 10 μ L | 10 μ L |
| 2X EZ PCR Master Mix | 15 μ L | 15 μ L | 15 μ L | 15 μ L |
| TOTAL VOLUME | 30 μL | 30 μL | 30 μL | 30 μL |



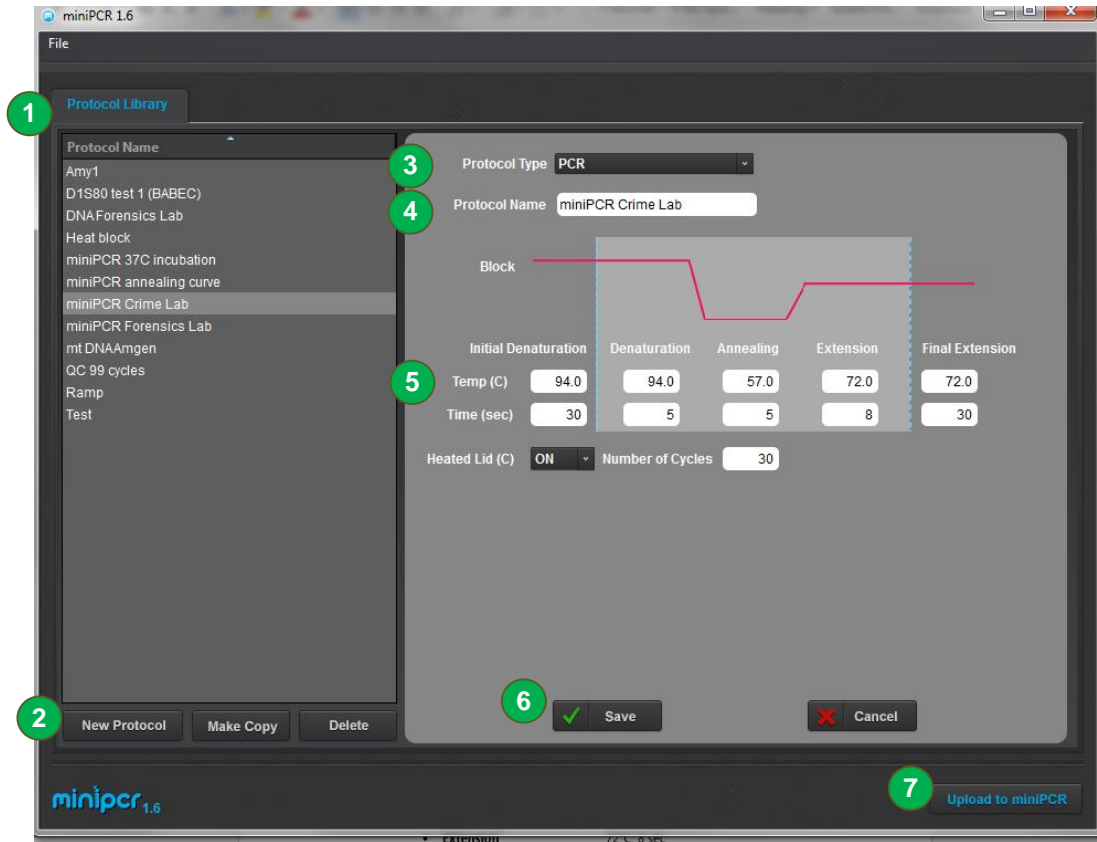
Use a micropipette to add each of the reagents.

Remember to change tips at each step!

- Gently mix the reagents by pipetting up and down 3-4 times, cap the tubes**
 - Make sure all the liquid volume collects at the bottom of the tube (if necessary, spin the tubes briefly using a microcentrifuge)
- Place the tubes inside the PCR machine**
 - Press firmly on the tube caps to ensure a tight fit
 - Close the PCR machine lid and tighten it gently

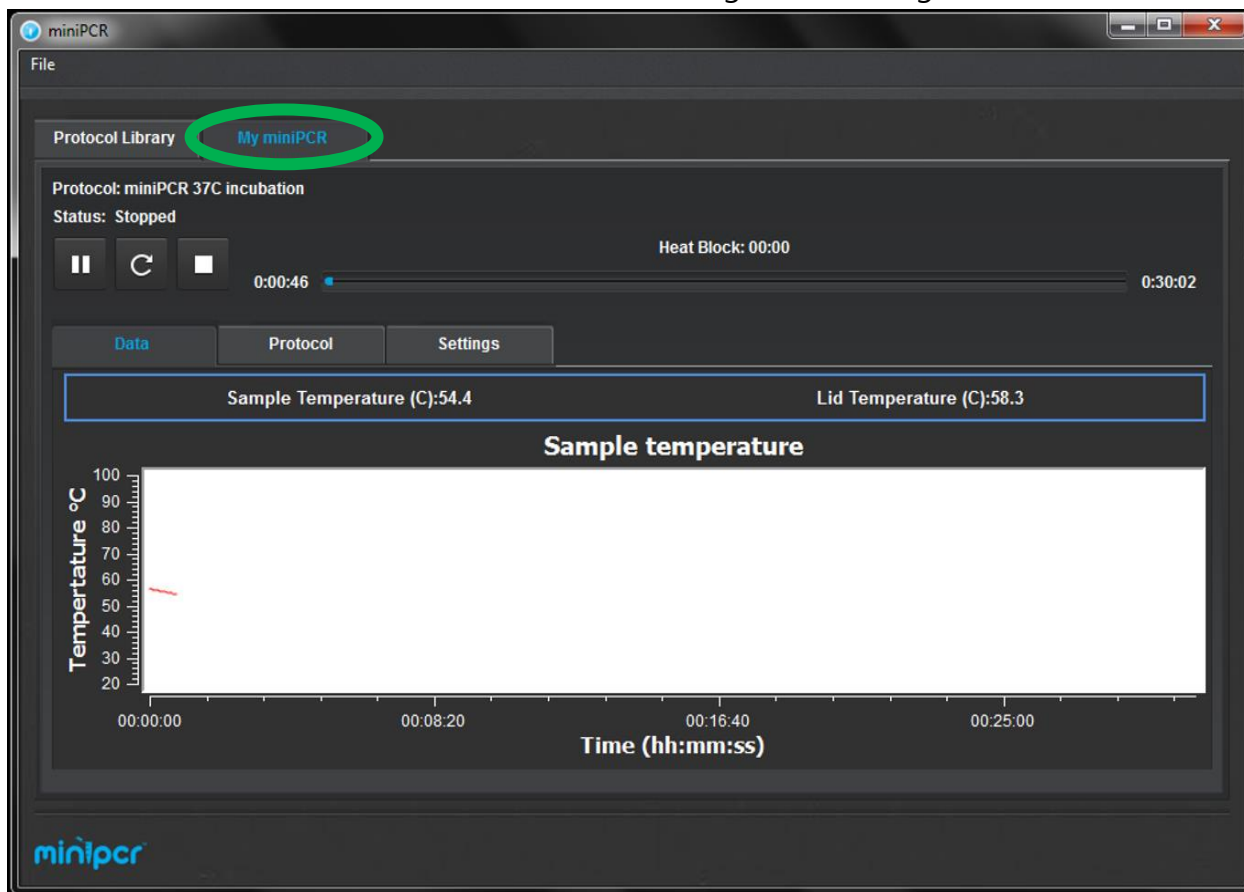
B PCR programming and monitoring (illustrated using miniPCR™ software)

1. Open the miniPCR software app and remain on the "Protocol Library" tab



2. Click the "New Protocol" button on the lower left corner
3. Select the **PCR "Protocol Type"** from the top drop-down menu
4. Enter a name for the Protocol; for example "**Group 1 - Crime Lab**"
5. 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
 - **Heated Lid** ON

6. Click "**Save**" to store the protocol
7. Click "**Upload to miniPCR**" (and select the name of your miniPCR machine in the dialogue window) to finish programming the thermal cycler. Make sure that the power switch is in the ON position
8. Click on "**miniPCR [machine name]**" tab to begin monitoring the PCR reaction



The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, and to export the reaction data for analysis as a spreadsheet.

Once the PCR run is completed (approximately 30-40 min), the screen will show "**Status: Completed**" and all LEDs on your miniPCR machine will light up.

You can now open the miniPCR lid and remove your PCR tubes.



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



The PCR product can now be stored for up to 1 week in the fridge or 1 year in a freezer.

C Gel electrophoresis – Pouring agarose gels (*Preparatory activity*)




TIME MANAGEMENT TIP

If the lab is going to be completed in a single time block, agarose gels should be prepared during the PCR run to allow the gels to settle.


If the lab is going to be performed over two periods, gels can be prepared up to one day ahead of the second period and stored in a refrigerator, covered in plastic wrap and protected from light.

1. Prepare a clean and dry agarose gel casting tray
 - Seal off the ends of the tray as indicated for your apparatus
 - Place a well-forming comb at the top of the gel (5 lanes per group)

2. For each lab group, prepare a 1.6% agarose gel using 1X TBE buffer
 - Adjust volumes and weights according to the size of your gel tray
 - e.g., add 0.32 g of agarose to 20 ml of 1X TBE for blueGel™ system
 - Mix reagents in glass flask or beaker and swirl to mix

3. Heat the mixture using a microwave or hot plate
 - Until agarose powder is dissolved and the solution becomes clear
 -  Use caution, as the mix tends to bubble over the top and is very hot

4. Let the agarose solution cool for about 2-3 min at room temperature.
 - Swirl the flask intermittently

5. Add gel staining dye (e.g. GreenView Plus)
 - Follow dye manufacturer instructions
 - Typically, 1.0 µL of staining dye per 10 mL of agarose solution
 -  Note: Follow manufacturer's recommendations and state guidelines if handling and disposing of ethidium bromide

6. Pour the cooled agarose solution into the gel-casting tray with comb

7. Allow gel to completely solidify (until firm to the touch) and remove the comb
 - Typically, 15-20 minutes

8. Place the gel into the electrophoresis chamber and cover it with 1X TBE buffer

Gel electrophoresis – Running the gel

1. Make sure the gel is completely submerged in 1X TBE electrophoresis buffer
 - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged)
 - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to cover the gel and wells

2. Load PCR samples onto the gel in the following sequence
 - **Lane 1:** 10µL DNA ladder
 - **Lane 2:** 15µL **Suspect A** PCR
 - **Lane 3:** 15µL **Suspect B** PCR
 - **Lane 4:** 15µL **Control H** PCR
 - **Lane 5:** 15µL **Control D** PCR



Note: there is no need to add gel loading dye to your samples.
miniPCR EZ PCR Master Mix and *100 bp DNA Ladder* are Load-Ready™!

3. Place the cover on the gel electrophoresis box
 - Ensure the electrode terminals fit snugly into place

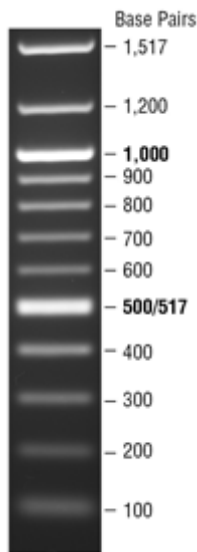
4. Insert the terminal leads into the power supply (not needed if using blueGel™)

5. If using blueGel™, simply press the “Run” button. Otherwise, set the voltage at 100-130V. Conduct electrophoresis for 15-20 minutes, or until the colored dye has progressed to about half the length of the gel
 - Check that small bubbles are forming near the terminals in the box
 - Longer electrophoresis times will result in better size resolution

6. Once electrophoresis is completed, turn the power off and remove the gel from the box (not needed if using blueGel™ which has a built-in illuminator)

D *Size determination and interpretation*

1. Place the gel on the transilluminator (or turn on the blueGel™ illuminator)
 - *Wear UV-protective goggles if using UV light*
2. Verify the presence of PCR product
3. Ensure there is sufficient DNA band resolution in the 400-800 bp range of the 100bp DNA ladder

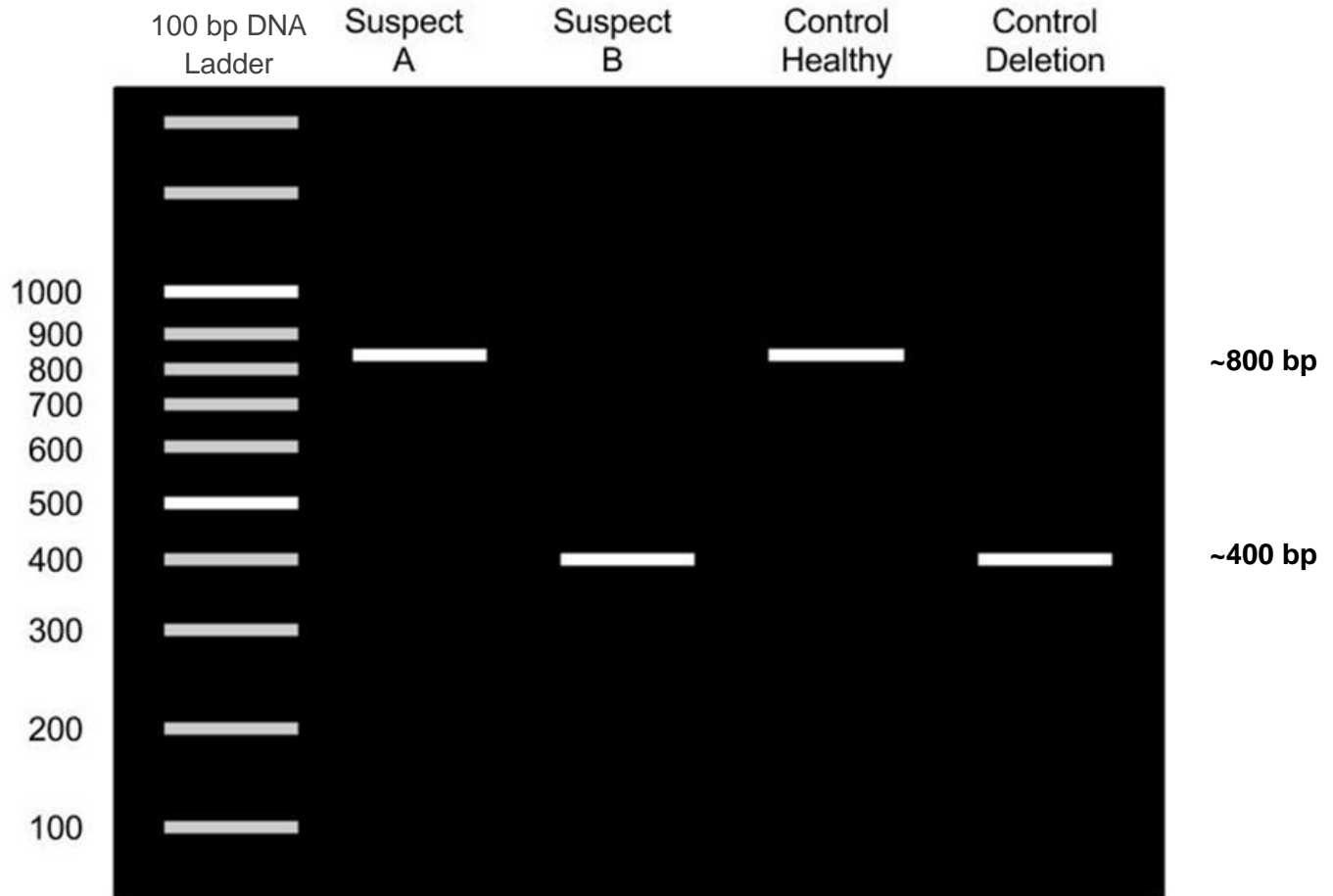


- Run the gel longer if needed to increase resolution
- DNA ladder should look approximately as shown

4. Document the size of the PCR amplified DNA fragments by comparing the PCR products to the molecular weight reference marker (100bp DNA ladder)
 - Capture an image with a smartphone camera
 - If available, use a Gel Documentation system

Expected experiment results

Expected results:



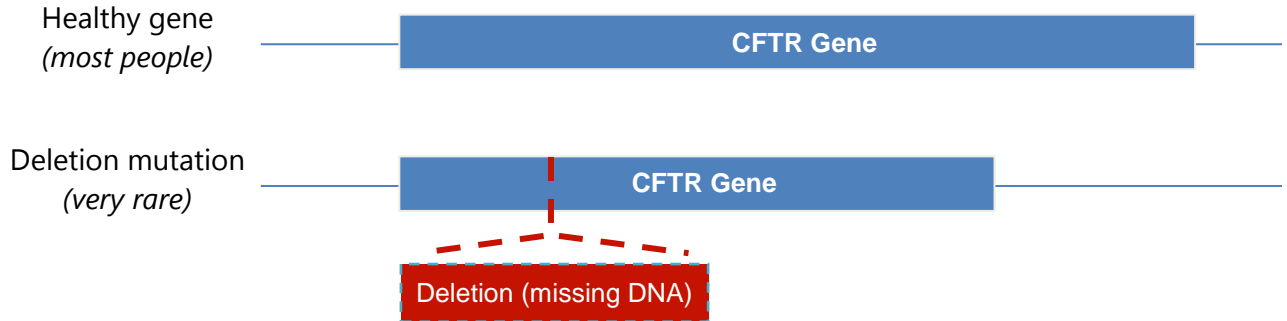
This schematic image shows the idealized experimental results

- Intensity of the bands will depend on
 - the efficiency of the PCR reaction
 - the efficiency of gel-loading
 - the quality of the detection reagents and system
- The migration patterns of the PCR product will vary with
 - the length of electrophoresis
 - the electrophoresis voltage

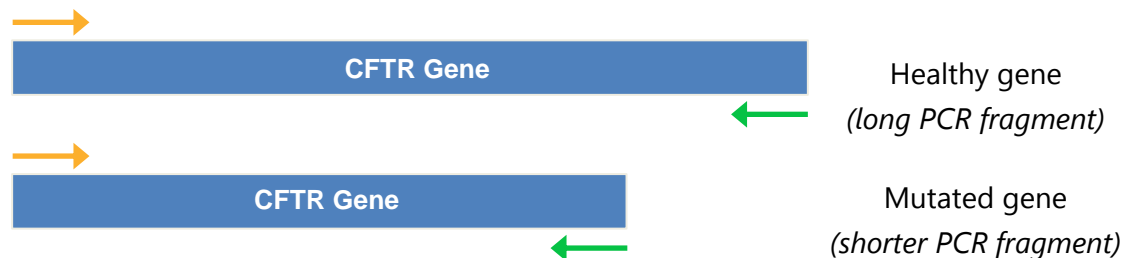
7. Study questions

1. Questions before experimental set-up

- What is a deletion mutation? We take the CFTR gene as a model

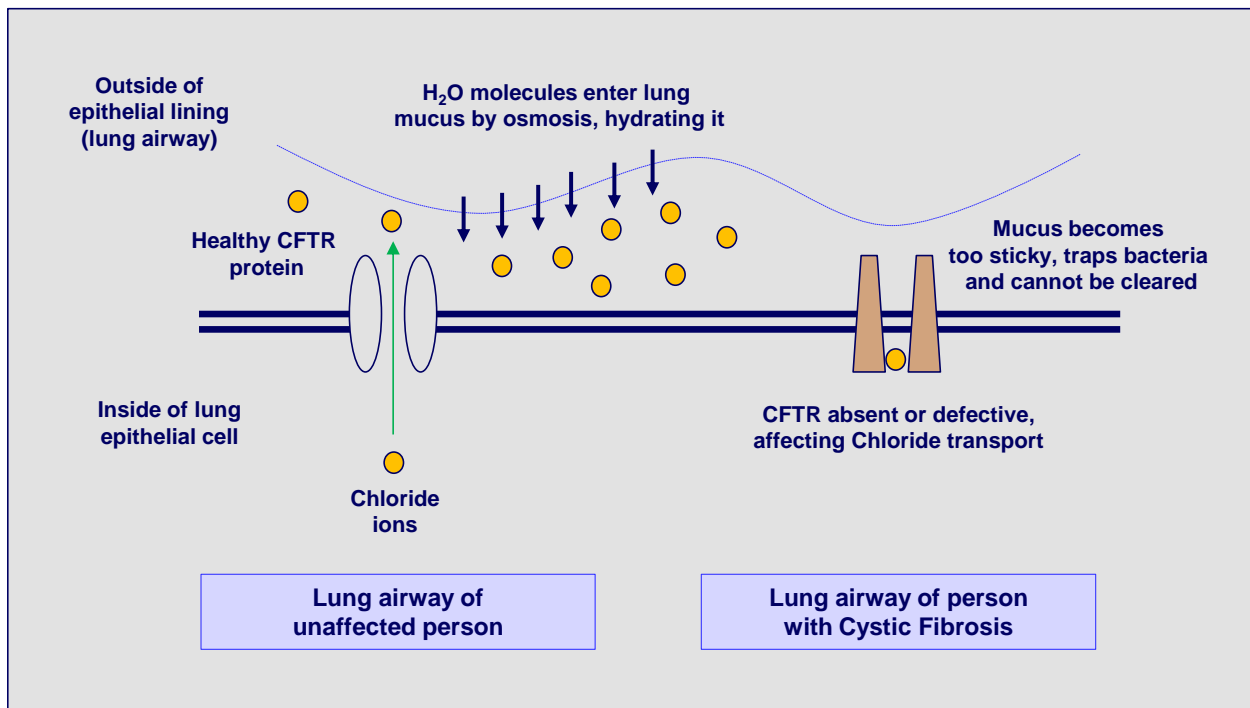


- How can PCR help detect a genetic deletion?
 - Why do we use primers that anneal to regions flanking the deletion?
 - What would happen if the primers targeted the deletion itself?



- How can we identify other types of genetic mutations?
 - Insertion mutations add a piece of DNA
 - Missense mutations change (substitute) a single DNA base pair
 - Nonsense mutations: missense mutations that result in a stop codon
- What is the physiological role of the CFTR protein?
 - The CFTR gene encodes a transporter-class ion channel protein that conducts chloride ions across epithelial cell membranes

- In normal physiology the CFTR protein is essential for increasing electrolyte concentration in the mucus, resulting in the movement of water out of cell by osmosis, and conferring the extracellular mucus its optimal hydration
 - Hence CFTR is necessary for the production of thin, freely flowing mucus. Mucus is a slippery substance that lubricates and protects the lining of the airways, digestive system, reproductive system, and other organs and tissues.
- What are the effects of Cystic Fibrosis (CF) genetic mutations?
 - CFTR mutations interfere with the cell's ability to transport chloride into the extracellular space, and hence lower water and salt accumulation
 - This causes mucus secretions, which are normally thin and watery in healthy people, to become thick and sticky
 - Thick secretions clog up organs and prevent them from working well
 - An increase in the viscosity of cell secretions often causes respiratory complications, and eventually death in many CF patients



- How many different CFTR mutations have been identified?
 - Nearly two thousand cystic fibrosis-causing mutations have been described
 - The most common mutation, $\Delta F508$ results from a deletion of three nucleotides which cause a loss of the amino acid phenylalanine (F) at the 508th position on the protein
 - As a result the protein does not fold normally and is more quickly degraded
 - Read more about CFTR mutations: <http://ghr.nlm.nih.gov/gene/CFTR>

- Can Cystic Fibrosis be treated?
 - There still is no cure for cystic fibrosis (CF), but treatments have improved greatly in recent years
 - The goals of CF treatment are to prevent and control infections in the lungs, loosening and removing the thick, sticky mucus
 - Can the type of CFTR mutation of a C
 - New classes of more personalized drugs are emerging to treat CF differentially depending on the underlying CF mutation in each patient

2. Questions during PCR run

- Do you expect deletion mutations in CFTR to be dominant? Recessive?
- What is happening to DNA molecules at each step?
 - Denaturation
 - Annealing
 - Extension
- Why do we need to add an enzyme (*Taq* DNA polymerase) to the PCR mix?
- What temperature do you think is optimal for most enzymes?
- What makes *Taq* DNA polymerase unique?
- How many more molecules of DNA will we have at the end of each cycle?
 - And at the end of the entire PCR reaction?
- How will we know whether the PCR reaction has worked?
 - And how can we recover the product?

3. **Questions after gel electrophoresis and visualization**

- How do you think the investigation turned out?
- Does the evidence point to suspect A or B being guilty?
- Why? What is the role of the experimental controls?
- Do you think the evidence is incriminating?
- What caveats should the investigators consider?
- What DNA migration pattern would we have seen for a heterozygotic person?

8. Student-centered investigations and extension activities

Student Centered Investigations

Option 1: Divide class into 2 sets of lab groups with 2 tubes each

“Detectives” groups (2 PCR tubes each)

- 1 tube labeled “A”: ‘Hair DNA’ extracted from Suspect A’s car
- 1 tube labeled “B”: ‘Hair DNA’ extracted from Suspect B’s car

“Reference Labs” groups (2 PCR tubes)

- 1 tube labeled “H”: ‘Control DNA’ from a healthy individual
- 1 tube labeled “D”: ‘Control DNA’ from a CFTR deletion mutant

Note: Use half the volumes specified in section 6 for each group

Option 2: Ask students to propose and experimentally test new PCR conditions

- Increase extension times
- Lower or raise annealing temperature
- Increase denaturation times

Post-Lab Extension Activities

Lab Report – report on the findings of the written lab or further investigations

- Title
- Introduction
- Materials
- Procedure
- Results
- Discussion

Student writing exercise 1 – write a persuasive article about the use of forensic DNA evidence in our criminal justice system, and its use to overturn wrongful convictions.

- Address arguments for and against re-opening cases
- State an opinion
- Defend that opinion with facts and cited resources

Student writing exercise 2 – write a persuasive report about the role of the CFTR gene in the pathophysiology of Cystic Fibrosis, and therapeutic advances in the field.

- Discuss the CFTR gene, protein, and its role in normal physiology
- Present the different types of known CFTR mutations, and their role in disease
- Share recent therapeutic advances to combat cystic fibrosis linked to specific CFTR mutations
- Develop an opinion on the advent of personalized or precision medicine (based on an individual's own genetic makeup)
- Defend that opinion with facts and cited resources

Additional teacher resources:

- Understanding CFTR mutations
<http://ghr.nlm.nih.gov/gene/CFTR>
<http://www.cftrscience.com/>
<http://www.cftr.info/about-cf/role-of-ctfr-in-cf/>
- The Innocence Project
Dedicated to exonerating wrongfully convicted individuals through DNA testing
<http://www.innocenceproject.org/>
- History of the use of DNA in crime solving
<http://www.forensicmag.com/articles/2005/01/evolution-dna-evidence-crime-solving-judicial-and-legislative-history>
- Deutsche Welle: 30 years of DNA Fingerprinting
<http://www.dw.de/from-paternity-to-criminal-cases-dna-fingerprinting-has-been-30-years-of-eureka/a-17911987>

9. 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-1000-01) contains the following reagents:

- 2X EZ PCR Master Mix, Load-Ready™
 - including *Taq* DNA polymerase, dNTPs, PCR buffer, and gel-loading dye
- 3X Crime Lab Primer Mix
- Suspect A DNA sample
- Suspect B DNA sample
- Control H DNA sample
- Control D DNA sample
- 100bp DNA ladder (50µg/ml)

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 (not included in the kit):

- Agarose (electrophoresis grade)
- DNA intercalating agent (e.g., GreenView Plus)
- Gel electrophoresis buffer (e.g., 1X TBE)
- Distilled or deionized H₂O (to dilute 20X TBE buffer concentrate)

10. About miniPCR Learning Labs™

This Learning Lab was developed by miniPCR™ 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 1-to-2-hour PCR-based experiment that recapitulates a real-life biotechnology application (in this case, a simulated criminal investigation), provides the right balance between intellectual engagement, inquiry, and discussion. The design of this lab has simplified certain elements to achieve these goals. For example, we use plasmid DNA instead of human genomic DNA as the PCR template in order to increase the protocol's robustness, rather than implausibly procuring "real" forensic genomic DNA. Real-world forensic biotechnology often uses DNA profiling of tandem repeats (variable number, VNTRs, or short tandem repeats, STRs). Instead, we chose to use the example of a rare mutation in a single human gene (CFTR) to also offer the opportunity to discuss the molecular pathophysiology of cystic fibrosis.

We follow a proven model of experimental design¹ which has been incredibly effective for educational lab courses, and owe them for the inspiration.

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

Authors: Ezequiel Alvarez Saavedra, Ph.D., and Sebastian Kraves, Ph.D.

¹ See, for example: 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.

11. Case Study

"It makes DNA analysis an even more powerful tool for personal ID. The impact [of miniPCR] can be felt from forensics to public safety, to countless other applications."



- Mary Heaton, Signature Science LLC

miniPCR™ : Bringing forensic DNA analysis closer to the evidence

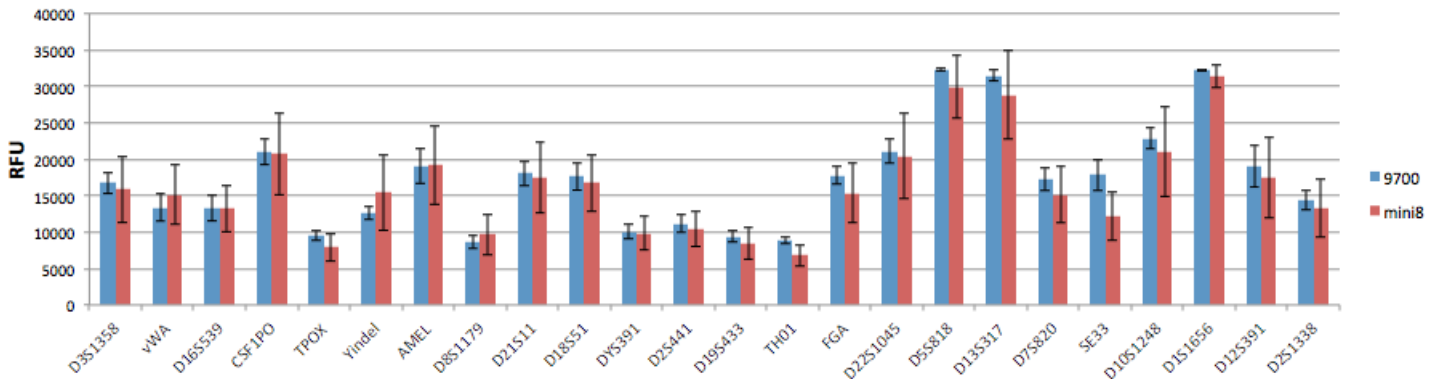
Through her tenure at Signature Science LLC, Mary Heaton had seen the company's portfolio of DNA applications grow hand-in-hand with the power of genetic technologies. Founded in 2001, Signature Science is engaged in the collection, analysis, and interpretation of DNA data for use in personal identification, including applications in homeland security, public safety, criminal justice and other elements of law enforcement. As genetic analysis has rapidly become the most reliable tool in personal identification, Signature Science's Forensic DNA Laboratory has grown in tune.

But Heaton also grew aware of practical limitations to the use of DNA evidence. In the path from collection in the field to processing in the lab, samples could get degraded, contaminated, or simply mixed up. These risks grew greater with increasing distance to the lab, as more sample handling and transit time were needed. Case in point, millions of Americans witnessed the 1995 acquittal of football player OJ Simpson in a televised murder investigation. Blood found on Simpson's sock matched the victim's DNA with a certainty of 1 in 21 billion, and a bloodied glove found next to the victim matched Simpson's blood. However, Simpson was acquitted because the defense argued that forensic evidence had been mishandled. It had not been correctly logged, entered into the chain of custody, bagged appropriately, or analyzed in a timely manner.

Heaton knew that shortening the distance and time to sample processing were key to opening the field to broader uses of DNA samples. When Heaton learned about miniPCR, a portable thermal cycler manufactured by Cambridge-based Amplyus, she thought she might have found the ideal solution. "I figured, instead of bringing samples into the lab, why can't one bring the lab closer to the samples", says Heaton. The diminutive footprint and weight of miniPCR made it the ideal candidate to test this premise. But there was one catch: Heaton and her team needed to prove that DNA samples amplified with miniPCR could meet the rigorous quality standards of forensic science. Could tiny miniPCR deliver the same performance as 20-times larger rivals? Heaton decided to find out the only way a forensic scientist would: experimentally.

Forensic DNA samples are analyzed by examining genetic markers known as short tandem repeats (STRs). STRs are short DNA regions that vary uniquely across individuals. They are the gold standard in personal identification because multiple STRs can be analyzed simultaneously, reducing the probability of a random match to virtually null. The CODIS database managed by the FBI uses 13 different STRs, reducing the chances of spuriously matching two individuals to less than one in one trillion. Genetic databases now offer access to 25 and more loci used in personal ID.

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To ensure that the fieldable miniPCR machine could study STRs as accurately as benchmark technologies, Signature Science compared results obtained using the miniPCR and the large benchtop AB 9700 thermal cycler. PCR amplicons were separated by capillary electrophoresis and detected in a 3500 xL Genetic Analyzer, and the resulting fluorescence readings were analyzed using GeneMapper ID-X software. Electropherogram plots quantifying the intensity of fluorescence signals (relative fluorescent units, RFUs), a function of the number of copies amplified for each STR by PCR, were compared statistically between miniPCR and the 9700. The results were clear: DNA amplification produced by miniPCR and benchtop PCR machines were essentially identical. These results held true after analyzing 40 loci from multiple DNA donors using three industry-standard personal ID kits (Global Filer, Global Filer Express, and YFiler, Applied Biosystems). In other words, miniPCR can perform forensic analysis in the field with the same accuracy and precision as much larger PCR equipment can in the lab.

Heaton was elated. The Signature Science team ordered several miniPCR machines that can be brought closer to the source of genetic material, minimizing sample degradation and contamination risks. "It makes DNA analysis an even more powerful tool for personal ID", says Heaton. "The impact [of miniPCR] can be felt from forensics to public safety, to countless other applications."

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