

## miniPCR™ Forensics Lab: Analysis of the D1S80 VNTR

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## 1. Synopsis

In this miniPCR Learning Lab™, students will amplify their own DNA and compare it to a sample obtained from a hypothetical crime scene in order to try to rule themselves out as a suspect. Students will use essential molecular biology techniques of PCR (polymerase chain reaction), gel electrophoresis, and VNTR (variable number tandem repeat) analysis. This lab illustrates real-world applications of molecular biology in personal identification and forensics and in the study of inheritance, human genetics, DNA polymorphisms, and genetic diversity.

- **Techniques utilized:** PCR, gel electrophoresis, DNA visualization, VNTR analysis
- **Time required:** Single 150 min. block or two 45-min. periods
- **Suggested skill level:** Familiarity with DNA amplification and gel electrophoresis concepts; basic familiarity with micropipetting techniques

### Teacher's note:

The crime in this lab is deliberately fairly innocuous. This is because the “crime scene” DNA may match DNA amplified from your students. For some forensics classes, you may wish to present the lab as a murder or other more serious crime. In this case, we recommend randomizing student DNA samples, labeling them as “Individual 1”, “Individual 2”, etc. and have each student analyze the DNA of another random, anonymous classmate.



# Materials needed

## Supplied in Kit (KT-1009-01)

Reagents and supplies	Amount provided in kit	Amount needed per student	Storage	Teacher's checklist
2X EZ PCR Master Mix, Load Ready™	600 µl	12 µl	Freezer	
D1S80 Lab Primer Mix	600 µl	12 µl	Freezer	
X-Tract™ DNA Extraction Buffer	1200 µl	50 µl	Freezer	
Crime Scene DNA Control Sample	200 µl	15 µl <u>per gel</u>	Freezer	
100 bp DNA Ladder, Load Ready™	100 µl	12 µl <u>per gel</u>	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 <https://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., protected from light	
TBE electrophoresis buffer • 1X working solution	Supplied as liquid concentrate or powder Sufficient to prepare 600 ml of 1X working solution	30 ml of 1X solution per blueGel™ system	Room temp.	
PCR tubes (0.2 ml)	100	2 <u>per student</u>		
1.7 ml plastic tubes	50	4 <u>per group</u>		



# Materials needed (cont.)

## Supplied by teacher

Available at miniPCR.com

Reagents and supplies	Amount needed per lab group	Teacher's checklist
<b>PCR thermal cycler:</b> e.g. miniPCR <sup>®</sup> machine	<b>1</b> One reaction <u>per student</u> Can be shared between groups	
<b>Horizontal gel electrophoresis apparatus:</b> e.g., blueGel™ electrophoresis system	<b>1</b> Can be shared between groups	
<b>Blue light transilluminator</b> *Note: A blue light transilluminator is integrated in the blueGel™ electrophoresis system.		
<b>Micropipettes</b> • 2-20 µl adjustable volume	<b>1</b>	
<b>Disposable micropipette tips</b>	<b>At least 3 <u>per student</u></b>	
<b>Flathead toothpicks</b> for cheek swabs	<b>1 toothpick <u>per student</u></b>	
<b>Distilled water</b> for making agarose gels and diluting TBE buffer	<b>60 ml per gel</b>	
<b>Flask or beaker</b> to dissolve agarose		
<b>Microwave or hot plate</b> to dissolve agarose		
<b>Other supplies:</b> • Disposable laboratory gloves • Protective eyewear • Permanent marker • Cup to dispose of tips		

## 3. Lab setup


### Planning your time

This experiment has 5 stages:

- A. Cheek Cell Collection and DNA Extraction
- B. PCR Set up
- C. PCR Programming and monitoring
- D. Separation of PCR products by DNA electrophoresis
- E. Size determination of PCR products and interpretation

This lab is designed to be completed in two 45-min periods.

An overview of the experimental plan is represented below:

Preparatory activity	Experimental stage
<p><i>Dispense reagents and prepare equipment</i></p> <ul style="list-style-type: none"> <li>• 15 min</li> </ul>	<p><b>A Cheek Cell Collection and DNA Extraction</b></p> <ul style="list-style-type: none"> <li>• 15 min</li> </ul> <p><b>B PCR set up</b></p> <ul style="list-style-type: none"> <li>• 10 min</li> </ul> <p><b>C PCR programming &amp; monitoring</b></p> <ul style="list-style-type: none"> <li>• 5 min PCR programming</li> <li>• 15 min PCR monitoring, discussion</li> </ul>
<p> <i>Possible stopping point. miniPCR will run for approximately 1h. PCR product is stable after amplification at room temperature. For long term storage, move tubes to a freezer.</i></p>	
<p><i>Pour agarose gels</i></p> <ul style="list-style-type: none"> <li>• 20 min</li> </ul>	<p><b>D Gel electrophoresis</b></p> <ul style="list-style-type: none"> <li>• 5 min load samples</li> <li>• 30 min run samples</li> </ul> <p><b>E Size determination &amp; interpretation</b></p> <ul style="list-style-type: none"> <li>• 5 min visualization</li> <li>• 5 min discussion</li> </ul>

## Quick guide: Preparatory activities

**Kit is sized for up to 32 students.** We recommend working in 8 lab groups of 4 students. Each student will test one human DNA sample (their own).

### A. DNA Extraction

- For each group, label and dispense 4 PCR tubes (200  $\mu$ L tubes), each containing:
  - **X-Tract™ DNA Extraction Buffer**                      **50  $\mu$ L** (1 per student)

### B. PCR set up

- Thaw tubes containing D1S80 Primer Mix and Crime Scene DNA samples by placing them on a rack or water bath at room temperature
- For each lab group of 4 students, label and dispense three 1.7 ml tubes:
  - **2X Blue PCR Master Mix**                                      **60  $\mu$ L**
  - **D1S80 Primer Mix**    **60  $\mu$ L**
- Each lab group will additionally need the following supplies:
  - Flat-headed toothpicks for cheek cell sampling
  - Micropipette, 2-20  $\mu$ L range
  - Disposable micropipette tips and a small beaker or cup to dispose them
  - 4 PCR tubes (thin walled, 200  $\mu$ L microtubes)
  - Permanent marker (fine-tipped)

### C. PCR programming and monitoring

- Ensure lab benches are set up with a miniPCR™ machine and power supply
- PCR product is stable after amplification at room temperature for several days. For long term storage, move tubes to a freezer

### D. Gel electrophoresis

- 2% Agarose gels can be poured in advance of the second class period
  - Pre-poured gels can be stored at room temperature, in a sealed container or wrapped in plastic wrap, and protected from light
- For each lab group of 4 students, label and dispense three 1.7 ml tubes:
  - **DNA ladder**    **12  $\mu$ L**
  - **Crime Scene DNA Control Sample**                      **20  $\mu$ L**

### E. Size determination and interpretation

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

## 4. Scenario overview

This lab is presented as a hypothetical crime where DNA evidence has been collected. Included in the lab is a sample of “Crime Scene DNA” that will be used by students to compare to their own DNA sample. Students whose DNA does not match the “Crime Scene DNA” can rule themselves out as suspects. It is possible that some students will match the DNA sample provided. These students cannot rule themselves out and are considered possible suspects.

### **The crime:**

Yesterday, after class, a half-consumed bottle of water was found at one of the lab benches in your classroom. As eating and drinking in the lab is strictly prohibited, you, the teacher, stepped into crime solving mode. You swabbed the mouth of the bottle to collect a DNA sample. You then purified the DNA and used PCR to amplify the D1S80 region of chromosome 1.

To eliminate themselves as suspects, all students in your classes must now amplify their own D1S80 region. If a student's D1S80 alleles do not match the criminal's they may be eliminated as a suspect. A student whose DNA matches the criminal must be considered as a possible suspect.

## 5. Background

### D1S80, a Variable Number Tandem Repeat Region

Variable number tandem repeats (VNTRs) are regions in a genome that contain short stretches of DNA (6 to about 100 base pairs) repeated a number of times. The number of repeats in a particular VNTR can vary from individual to individual, and between chromosomes within an individual. In this way, VNTRs are one source of genetic polymorphism (variation), and can be used as markers for personal identification as well as in the study of inheritance, genetic diversity, population genetics, and genetic disorders.

VNTRs are predominantly found in non-coding regions of DNA, and therefore their length does not usually bear any functional importance (though there are reported exceptions). The highly repetitive nature of VNTRs can lead to a relatively high frequency of errors in DNA replication or crossing over. When these mutations occur, they add or remove repeats thus changing the length of the VNTR. The result is that there are many different versions (alleles) of any one VNTR in the population, each with a different number of repeats. This variation means that we usually inherit different alleles from each parent, making most people heterozygous for a given VNTR. This variation also means that any two randomly selected people will rarely have matching alleles for their two copies of a VNTR. When we analyze two people for more than one VNTR, the likelihood of a random match decreases multiplicatively.

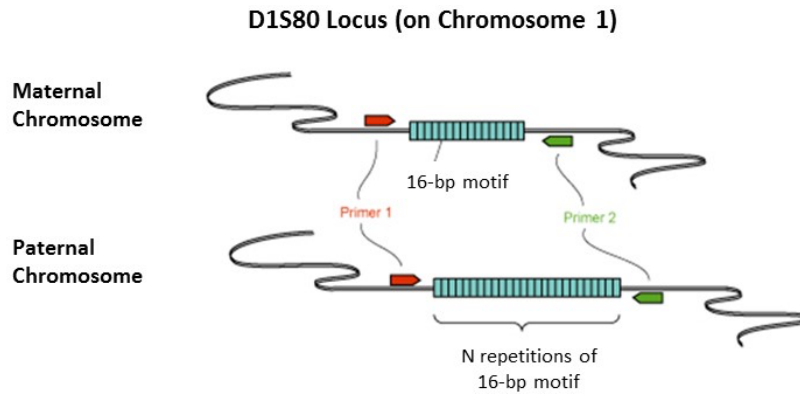
D1S80 is a VNTR region located on human chromosome 1 and consists of a 16-base pair-long repeat unit. Most people have between 14 and 41 copies of this repeat, resulting in D1S80 alleles with repeat regions of 224 to 656 base pairs in length. The most common alleles are comprised of 18 and 24 repeat units (288 and 384 base pairs).

#### Mutation vs Genetic Polymorphism:

In this lab, we use *mutation* to refer to a change in a DNA sequence. Mutations can occur in individual cells due to copying errors in replication, errors in crossing over during meiosis, or even rearrangements of chromosomes. The changes in a DNA sequence that are introduced by a mutation can vary significantly in their effect on an organism. Often, they are deleterious and will quickly be removed from a population by natural selection. Other changes to the DNA are neutral and have no effect on the organism; they may remain in a population and spread through stochastic processes like genetic drift. Occasionally, a change in DNA sequence will be beneficial; these mutations will tend to spread in a population propelled by natural selection.

We refer to *genetic polymorphisms* when there is more than one sequence variant, or allele, present in a population for a particular gene or locus. A new variant first arises through mutation, and if that new sequence spreads through the population it is called a genetic polymorphism. Genetic polymorphisms exist on the population level. An individual that has more than one version of a gene or DNA sequence is called a heterozygote. Heterozygotes can only exist in a population that has a genetic polymorphism.





PCR amplification and gel electrophoresis can be used to establish the length of a person's D1S80 alleles. To amplify the D1S80 allele, primers are used that bind just outside the region of repeats. PCR will then copy the entire set of repeats. When the PCR products are run on an electrophoresis gel they will differ in size depending on how many repeats are present in the D1S80 allele. The actual length of product analyzed on the gel will include the repeats plus the additional length of the PCR primers and any DNA region flanking the repeats. The additional length of the PCR product in this lab is about 180 base pairs.

## Use of DNA as an identifier in forensics

Every person has a unique DNA sequence; it is what makes one person different from every other person. For this reason, DNA is in many ways the best, most accurate way of identifying an individual. The problem lies in that the vast majority of DNA between any two people is identical. Approximately 99.6-99.9% of DNA between any two people is the same.

The most comprehensive way to identify the genetic differences between individuals would be to sequence large regions of an individual's genome – identifying every possible difference in their DNA. This is not practical for many reasons, most notably cost and time required. Therefore, when using DNA to identify individuals, it is important to first identify regions of DNA that hold high levels of variability between individuals. VNTRs are just such regions.

Because the variation in VNTRs affects the length of the DNA, scientists can use techniques that detect DNA length differences to identify variability between individuals relatively easily and inexpensively. Common VNTR analysis techniques are PCR and DNA electrophoresis (gel or capillary), or other methods.

Normally when trying to make a genetic match of individuals, several variable regions are compared simultaneously. This is because when looking at any one VNTR locus, there is a reasonable likelihood that any two people's alleles may match by chance. While finding two people who match in their alleles at any one VNTR locus may be somewhat likely, the combined probability of matching many different regions simultaneously is extremely unlikely, even for related individuals. When each region investigated is on a different chromosome and therefore assort independently in meiosis (a founding principle of

Mendelian genetics), the probabilities of matching at each locus are also independent. This is where the extremely low probabilities of making a false match come from. In modern crime databases the theoretical probability of getting a false match, matching many loci just by chance, is reported to be as low as one in a quintillion.

In forensics today, the use of VNTRs has generally been replaced by identification through the use of STRs (short tandem repeats). They consist of repetitive units of DNA that are 2-13 base pairs long, instead of 6-100 base pairs like VNTRs. Otherwise, the use of VNTR and STR analysis is basically the same. Both look at length variation of a highly polymorphic region and, by looking at many of these regions simultaneously, increase the likelihood that any particular DNA profile will be unique. The FBI and other law enforcement agencies standardized the use of DNA analysis in personal identification by choosing 20 STR loci that are used in all forensics investigations. Now, because all DNA analysis is done in the same way on the same variable regions, the FBI, different states, and different law enforcement agencies across the country can all compare DNA in the same uniform way. The database used to house and organize all these data is called the Combined DNA Index System, or CODIS. When you hear on the news or on a crime TV show that a DNA sequence is being compared to a criminal database to look for a match, this set of 20 STRs is what they are talking about.

It is important to remember that, while DNA evidence has become the gold standard for forensics identification, it is not perfect. While the normally reported chance of a false match in the CODIS system is less than one in a quintillion, that number only refers to two randomly selected individuals. Two related individuals, or individuals from a genetically distinct ethnic group, will be more likely to share alleles (though an exact match is still highly unlikely). Much more problematic is attempting to match DNA to a complex sample. While comparing DNA evidence collected from one individual to evidence that also contains a single individual (as you will do in this lab) is fairly straightforward, often DNA samples are mixed from several individuals. Imagine, for example, if in today's lab 2 people had drunk from the same water bottle. Every locus investigated will now be represented by alleles from two people. It may be impossible to separate the DNA from the two individuals, making analysis more difficult. These problems are not insurmountable, but they can lessen the overall reliability of DNA evidence and require highly trained technicians to interpret the data. Lastly, contamination of samples will often create the biggest problems. Because of the pervasiveness of DNA in the environment and the power of PCR amplification, very small sources of contamination can lead to false positives. To combat this problem, technicians often run multiple negative controls in their analyses. In the end, as is often the case, the evidence is only as good as the technician doing the analysis.

## Today's lab

While STRs are currently used in most forensics analyses, they present some problems for classroom investigation. Because STRs consist of such short, repeated segments, they are usually analyzed by capillary electrophoresis, a procedure that uses highly specialized equipment. Capillary electrophoresis operates on the same principle as traditional gel electrophoresis, but can resolve size differences of just a few base pairs. A major downside, however, can be the cost and complexity of the equipment. This tends to relegate the use of such equipment to highly specialized laboratories.

Today we will use the D1S80 locus, a VNTR found on chromosome 1. Because the size differences regularly found in the D1S80 locus are on the order of dozens or hundreds of base pairs, regular classroom gel electrophoresis equipment can be used to analyze your data. Students should note that the VNTR analysis done in this lab is based on the exact same principles as STR analysis, and can be used for DNA identification in the same way. The main divergences from actual forensic analysis are that your size differences may be larger than some STR differences, and that we will only analyze one locus instead of the 20 usually used for criminal forensics data.

Read how miniPCR thermocyclers are used by real forensic scientists in the field using this miniPCR Case Study.

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

Learn more about DNA fingerprinting using DNAdots – a free resource from miniPCR.

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





# Laboratory guide



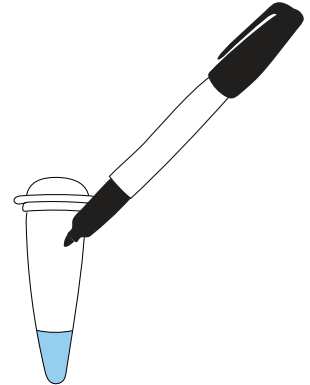
Protective gloves and eyewear should be worn for the entirety of this experiment.

## DNA extraction

For best results, don't eat or chew gum for ~20 minutes prior to cheek cell collection

- 1. You should receive a tube with 50  $\mu$ l extraction buffer in it**  
**Label the tube with your initials**

- Label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off.



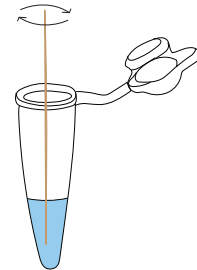
- 2. Collect cheek cells**

- Gently scrape the inside of your cheek 3-4 times with a flat-end toothpick.
- It shouldn't hurt.

- 3. Transfer cheek cells to tube with buffer**

- Dip the end of the toothpick with your cheek cells in the X-Tract™ buffer.
- Swirl the toothpick to dislodge the cells.
- Dispose of the toothpick.

Swirl toothpick in buffer



- 4. Cap your tube**

- 5. Incubate your tube for 10 minutes at 95°C**

- You can use a miniPCR<sup>®</sup> thermocycler in Heat Block mode, a water bath, or other heat block.
- Note: If using a heat block or water bath, weigh down the lids of your tubes so they don't pop open.

- 6. Remove your tube from heat and immediately use DNA extract for PCR**

- Note: The DNA extract is stable for up to two hours, but the sooner you use it for PCR the better.



## PCR setup

### 1. Label a PCR tube (200 µl tube) with your initials followed by "P" for PCR

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

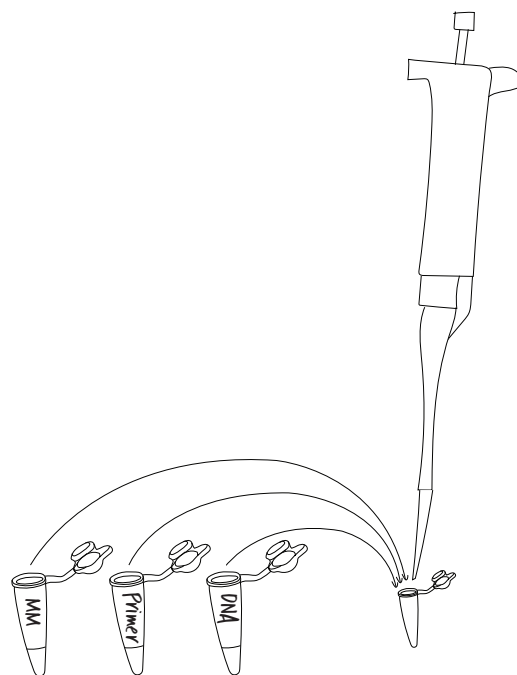
### 2. Add PCR reagents to your labeled PCR tube

- Change pipette tips between samples to prevent contamination.

Reagent	Volume
2X EZ PCR Master Mix™	12 µl
D1S80 Primer Mix	12 µl
Student DNA sample	2 µl
<b>TOTAL VOLUME</b>	<b>26 µl</b>

Note: EZ PCR Master Mix™ contains:

- Taq DNA polymerase • dNTPs
- PCR buffer with Mg<sup>2+</sup> • Gel loading dye

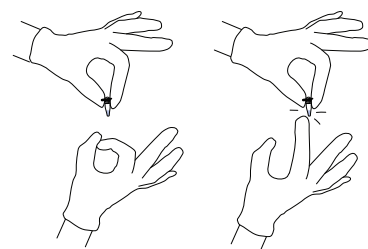


Use a micropipette to add each of the reagents. Remember to change tips at each step!

### 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






## PCR programming

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 7).

### 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 button to create a new protocol

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

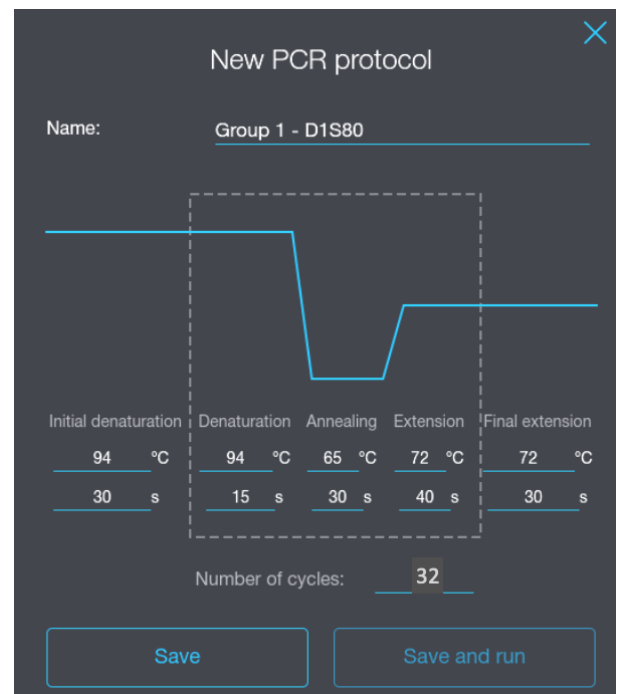
### 5. Select “PCR” from the drop-down menu

### 6. Enter a name for the protocol; for example: “D1S80 Lab”

### 7. Enter the PCR protocol parameters:

- Initial denaturation 94°C, 30 sec
- Denaturation 94°C, 15 sec
- Annealing 65°C, 30 sec
- Extension 72°C, 40 sec
- Number of cycles 32
- Final extension 72°C, 30 sec

Note: The “Heated lid” slider should be in the on position.



**New PCR protocol**

Name: Group 1 - D1S80

Initial denaturation	Denaturation	Annealing	Extension	Final extension
94 °C	94 °C	65 °C	72 °C	72 °C
30 s	15 s	30 s	40 s	30 s

Number of cycles: 32

**Save** **Save and run**

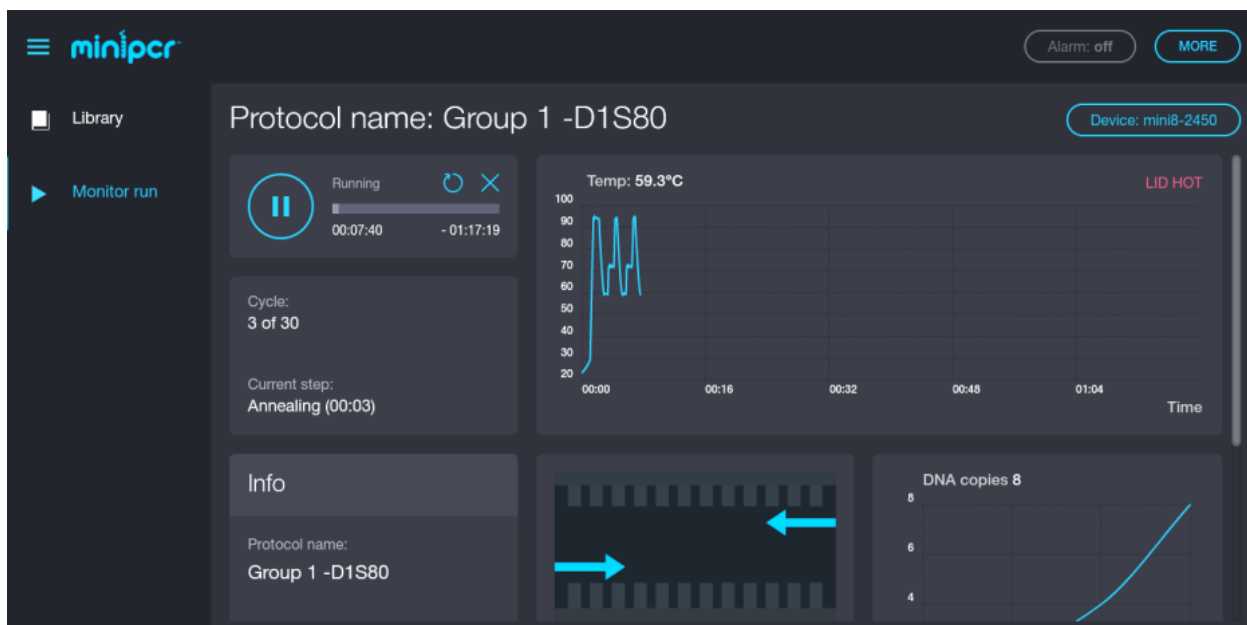


## 8. Click “Save and run” to start the protocol

- If connected to more than one machine, choose 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 protocol 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 70 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.

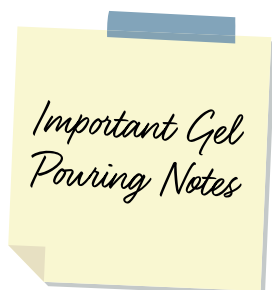


# Laboratory guide



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 stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need one lane per student, plus one lane for ladder and one lane for the control sample.

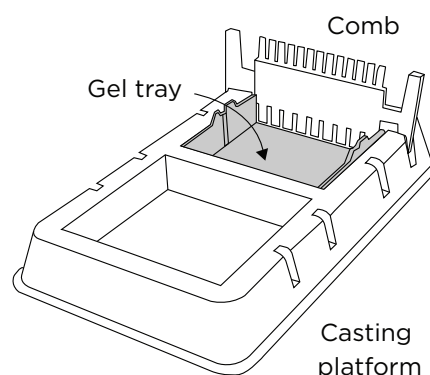
These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

### 1. Prepare 1X 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/](http://www.minipcr.com/agarose-gel/)



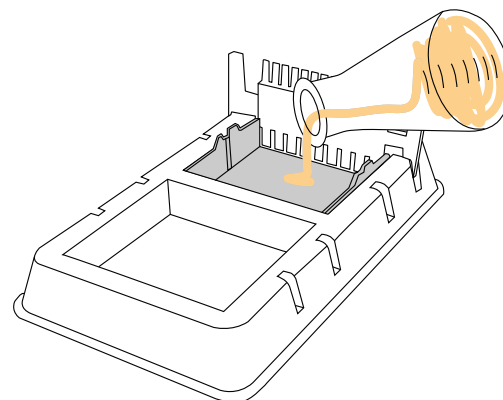


**4. Pour the agarose solution into the prepared casting platform with a gel tray and comb**

- 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.





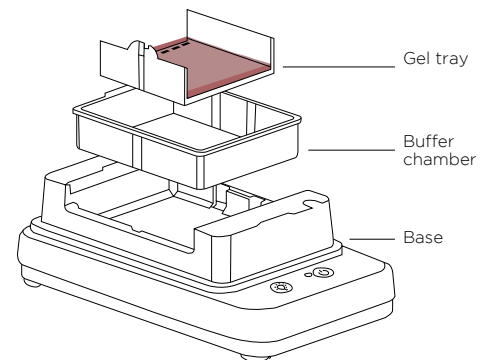
Protective gloves and eyewear should be worn for the entirety of this experiment.

## 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.

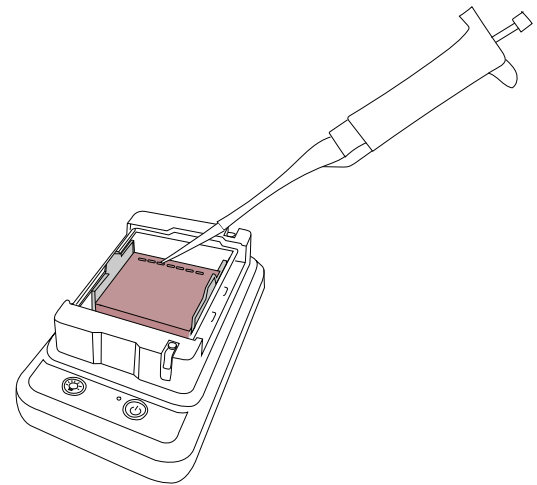
### 1. Place the gel tray containing your gel in the buffer chamber

- 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

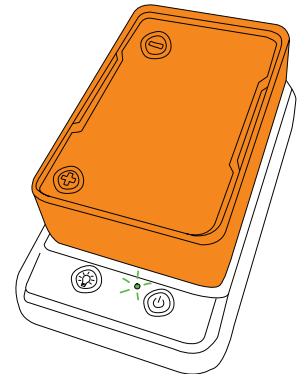
- **Lane 1:** 10 µl DNA Ladder
- **Lane 2:** 15 µl Student 1 PCR product
- **Lane 3:** 15 µl Student 2 PCR product
- **Lane 4:** 15 µl Student 3 PCR product
- **Lane 5:** 15 µl Student 4 PCR product
- **Lane 6:** 15 µl Crime Scene DNA Sample

Note: Change pipette tips between samples to prevent contamination.



#### 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 electrodes of the lid should be aligned with the metal leads on the 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.

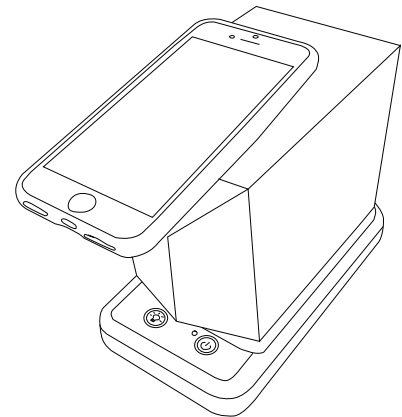
#### 6. Conduct electrophoresis for 20-30 minutes

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

## Gel electrophoresis – Visualizing results

#### 1. Press the “light bulb” 💡 button to turn on the blueGel™ transilluminator

- For best viewing, dim 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 the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.



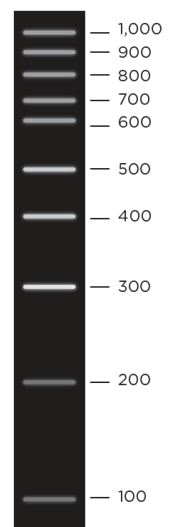
#### 2. Ensure that there is sufficient band separation in the 400-700 bp range

- Run the gel longer if needed to increase resolution.

#### 3. Document your results

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

100 bp Ladder



## 7. Study questions

## Questions before experimental set-up

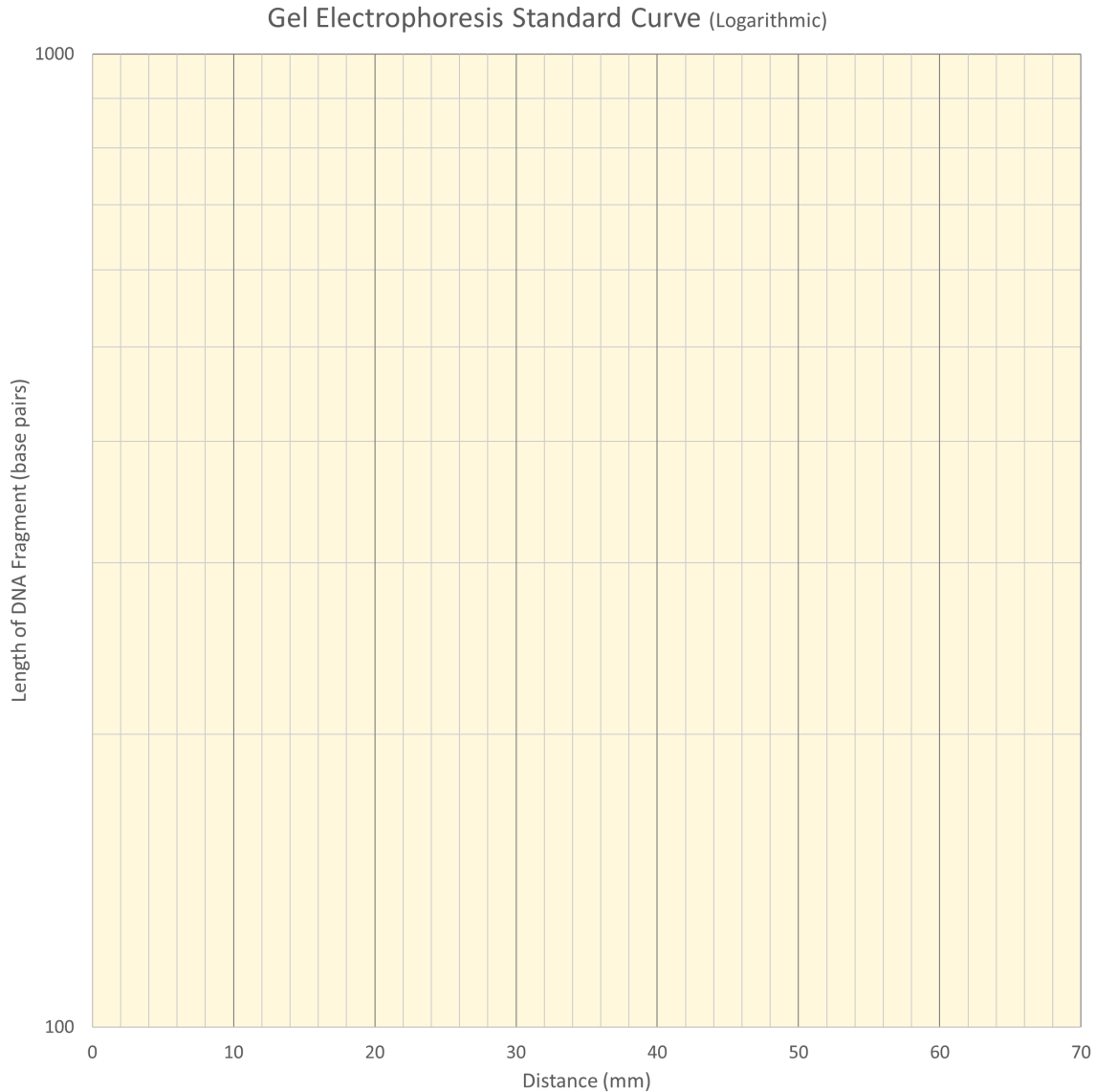
1. What are VNTRs?
2. Why can VNTRs be used in personal identification?
3. Why do police use twenty different regions to test someone's identity?
4. If you were able to analyze the D1S80 alleles from two people, what would you expect to see if the two individuals were biological siblings? You may want to use a Punnett square to justify your answer.
5. What would you expect to see if one individual were the parent of the other?

6. Looking at data like this, is it easier to rule someone out as a suspect or to determine that a person is guilty?
7. If you have a D1S80 allele with 32 repeats, how big a segment of DNA do you actually expect to see on your gel?

## Questions after gel electrophoresis and visualization

1. **By comparing your DNA bands to the ladder, can you estimate the size of the two fragments on your DNA sample?** (Note: Identifying the precise size of your DNA band is difficult. Use your best estimate for size by comparing your band to the DNA ladder. You should be able to resolve sizes within 30-50 base pairs.)
  
2. **Using the sizes you estimated in question number 1, can you estimate how many repeats you have in each of your alleles? Remember that the sizes you estimated include the length of your primers and flanking regions (180 bp) and that D1S80 repeats are 16 base pairs long.**
  
3. **Do you or anyone else have only one band instead of two? What does this mean?**
  
4. **How do your D1S80 alleles compare to the “Crime Scene DNA” alleles? Can you rule yourself out as a suspect?**
  
5. **Compare your alleles to other students’ alleles on your gel. Does anyone match at both alleles? Does anyone match at only one allele?**

## Estimating the size of your band using a standard curve.



1. Using a metric ruler, measure in millimeters the distance from the edge of the well to the center of each band in your DNA ladder. (It can be easier to do this on a screen after taking a picture.)
2. Plot each point on the graph above. The X axis is the distance traveled by each band measured in millimeters. The Y axis is the size of the band in the DNA ladder. (See *E. Size determination and interpretation* for band sizes.)
3. Connect your points to make a curve/line.

### Estimating unknown band size:

4. Measure the distance each of your D1S80 bands traveled from the well. This is the X axis value for your band.
5. Use the line that you drew in step 3 to estimate the size of the D1S80 bands.







## 8. Student-centered investigations and extension activities

### Extension 1: Genetic privacy discussion

Personal identification using DNA can now be done using extremely small amounts of DNA, such as the amount collected from a used water bottle. As a result, a person's DNA can be easily collected and analyzed without their consent.

- Research what laws exist to limit the use of genetic analysis of individuals for personal identification. A good place to start is the "Privacy in Genomics" page of the National Human Genome Research Institute: <https://www.genome.gov/27561246/privacy-in-genomics/>
- Write a persuasive essay on where the line should be drawn for use of genetic identification. Who should be allowed to collect DNA samples? Who can be compelled to give a sample of DNA? What samples should be stored? When should data be expunged?
- Conduct a debate between students. On one side assign the idea that law enforcement should be able to collect DNA at will; after all, it will be used to solved crimes that otherwise may go on unsolved. On the other side assign the idea that law enforcement use of DNA should be extremely limited because collecting DNA is a breach of personal privacy and people should have control of how and when their own DNA is used.

### Extension 2: VNTR consensus sequence analysis

The VNTR locus D1S80 (GenBank sequence accession number [D28507](#)) has a 16-base pair core repeat. The smallest described human allele contains 13 repeats, while the largest alleles contain up to 72 repeats. In addition to variation in the number of repeats, there is also variation (polymorphism) in DNA sequence among the repeats. While the predominant core repeat region has been characterized as the nucleotide sequence 5' **GGAGGACCACCAGGAA** 3', the range of described sequence variation is illustrated by the figure below (from Balamurugan *et al.* *The Scientific World Journal* (2012): 917235.)

Repeat type	Sequence															
Type A	T	C	A	-	C	-	-	-	-	A	-	-	-	-	-	-
Type B	A	C	A	-	-	-	-	-	-	A	-	-	-	-	-	-
Type C	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
Type D	-	-	A	-	-	-	-	-	-	-	-	-	A	-	-	-
Type E	-	-	A	-	-	-	-	-	-	A	-	-	-	-	-	-
Type F	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-
Type G	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-
Type H	G	A	G	G	A	C	C	A	C	C	G	G	C	A	A	G
Type I	-	-	-	-	-	-	-	-	-	-	A	-	G	-	-	-
Type J	-	-	-	A	-	-	-	-	-	-	A	-	G	-	-	-
Type K	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-
Type L	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-
Type M	-	-	-	-	-	-	-	-	-	-	A	-	G	-	G	-
Type N	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-
Type O	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-
Type P	-	-	A	-	-	-	-	-	-	-	-	-	A	-	G	-
Type Q	-	-	A	-	-	-	-	-	G	-	-	-	A	-	-	-
Type R	-	G	-	A	-	-	-	-	-	-	A	-	G	-	-	-
Type S	-	-	A	-	-	-	-	-	-	-	A	-	G	-	-	-
Type T	-	-	A	-	-	-	-	-	-	-	A	-	-	-	-	-
Type U	-	-	A	-	-	-	-	-	-	-	A	-	A	-	-	-

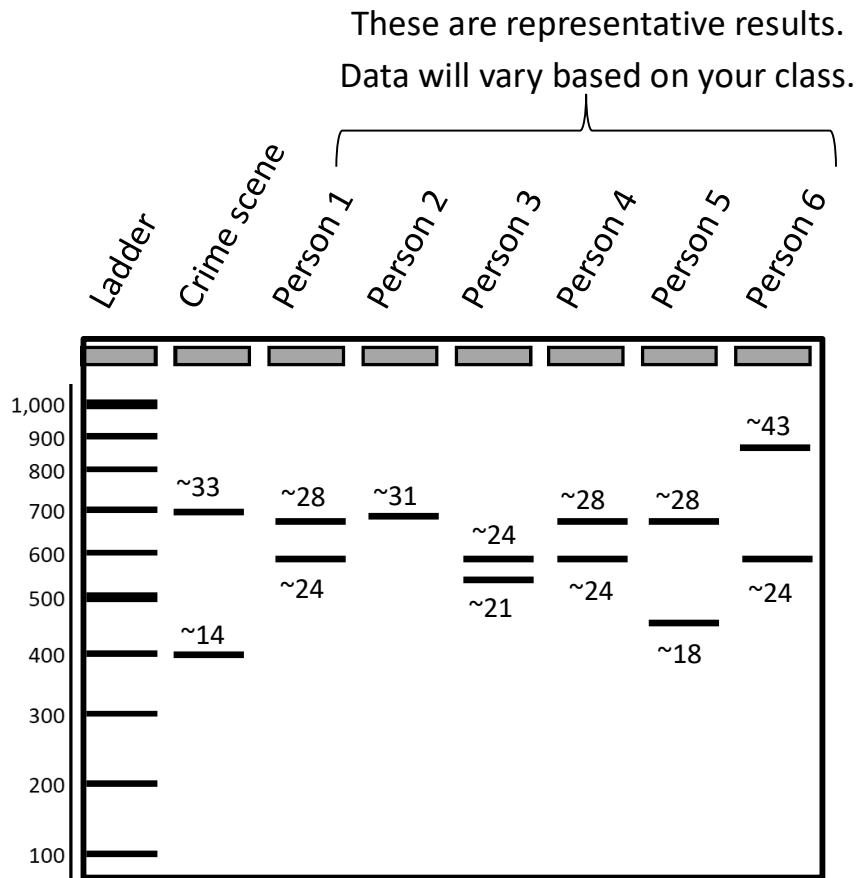
You now know that the D1S80 allele is polymorphic primarily due to variation in the number of repeat units, but also due to sequence variation among repeats.

- With this information, can you identify the 22 repeats in the D1S80 allele displayed below? (Hint: we've underlined one of the 16-nucleotide repeats as a starting point)

5'CCCACGCGCCAGCACTGCATTGAGATAAGCGCTGGCTCAGTGTCAGCCCAAGGAAGACAGACCACAGGCAAGG  
AGGACCACCGGAAAGGAAGACCACCGGAAAGGAAGACCACAGGCAAGGAGGACCAC  
CGGCAAGGAGGACCACCAGGAAGGAGGACCACCAGCAAGGAGGACCACCAGCAAGGAGGACCACCAGGAAGG  
AGGACCACCAGGAAGGAGGACCACCGGCAAGGAGGACCACCAGGAAGGAGAACCACCAGGAAGGAGGACCAC  
CGGCAAGGAGGACCACCAGGAAGGAGAACCACCAGGAAGGAGGACCACCAGGAAGGAGGACCACCAGGAAGG  
AGGACCACTGGCAAGGAAGACCACCGGCAAGCCTGCAAGGGGCACGT3'

- Suppose you are a crime scene expert being presented with electrophoresis results showing that two different forensic samples yielded identical D1S80 alleles (by DNA length, or number of repeats). What additional study can you perform to establish unambiguously that these two samples are in fact a perfect molecular match? (Hint: Think of a modern genetics technique that can yield more detailed information about those DNA molecules.)

## 9. Expected results



180 base pairs of each PCR product is from DNA amplification outside the repeat.

To calculate an estimate for the number of repeats:

$$\# \text{ Repeats} = \frac{\text{Fragment Length} - 180}{16}$$

The crime scene DNA sample has bands at ~700 bp and ~400 bp

$$\# \text{ repeats} = (700 - 180) / 16 \approx 33 \text{ repeats}$$

$$\# \text{ repeats} = (400 - 180) / 16 \approx 14 \text{ repeats}$$

## 10. Additional resources

### Additional teacher resources

Allele Frequency Database for the D1S80 Locus

[http://alfred.med.yale.edu/alfred/recordinfo.asp?condition=loci.locus\\_uid=%27LO000333J#sites](http://alfred.med.yale.edu/alfred/recordinfo.asp?condition=loci.locus_uid=%27LO000333J#sites)

NCBI GenBank database – D1S80 sequence <http://www.ncbi.nlm.nih.gov/nuccore/D28507>

Federal Bureau of Investigation: Combined DNA Index System:

<http://www.fbi.gov/about-us/lab/biometric-analysis/codis>

### Selected publications of interest:

Balamurugan K, Tracey M L, Heine U, Maha G C, and Duncan G T (2012) Mutation at the human D1S80 minisatellite locus. Scientific World J : 1-8

Butler J (2015) The future of forensic DNA analysis. Philosophical Transactions of the Royal Society of London B: Biological Science: 370(1674)

## 11. Learning goals and skills developed

### Student Learning Goals:

- Understand the basic structure of DNA and its role in genetic inheritance
- Comprehend how DNA is passed from parent to offspring
- Understand that PCR is a technique for amplifying specific DNA sequences
- Learn about the existence of genetic polymorphisms, particularly in the highly variable VNTR regions of the human genome
- Learn about the application of DNA polymorphisms in personal identification and forensics
- Learn about VNTR analysis in the study of inheritance, human genetics, and genetic diversity
- Discuss ethical issues in human identification via DNA analysis

### Scientific Inquiry Skills:

- Students will create hypotheses and make predictions about results
- Students will compare experimental results to their predictions
- Students will generate graphics and tables to present their results
- Students will make conclusions about their hypothesis based their experimental results
- Students will follow laboratory safety protocols

### Molecular Biology Skills:

- Micropipetting
- DNA extraction (from cheek cells)
- PCR set-up and execution
- Preparation of agarose gels
- Agarose gel electrophoresis
- VNTR analysis
- Staining, visualization, and molecular weight analysis of DNA fragments

## 12. 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-PS1-4.	Develop a model to illustrate that the release or absorption of energy from a chemical reaction system depends upon the changes in total bond energy.
HS-PS1-5.	Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs.
HS-PS2-4.	Use mathematical representations of Newton's Law of Gravitation and Coulomb's Law to describe and predict the gravitational and electrostatic forces between objects.
HS-PS2-6.	Communicate scientific and technical information about why the molecular-level structure is important in the functioning of designed materials.

### 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.
RST.11-12.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks; analyze the specific results based on explanations in the text.
RST.11-12.7	Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.
RST.11-12.9	Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.
WHST.9-12.1	Write arguments focused on discipline-specific content.
WHST.9-12.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.

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WHST.9-12.7	Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation.
WHST.11-12.8	Gather relevant information from multiple authoritative print and digital sources, using advanced searches effectively; assess the strengths and limitations of each source in terms of the specific task, purpose, and audience; integrate information into the text selectively to maintain the flow of ideas, avoiding plagiarism and overreliance on any one source and following a standard format for citation.
WHST.9-12.9	Draw evidence from informational texts to support analysis, reflection, and research.
SL.11-12.4	Present claims and findings, emphasizing salient points in a focused, coherent manner with relevant evidence, sound valid reasoning, and well-chosen details; use appropriate eye contact, adequate volume, and clear pronunciation.
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.





# Ordering information

To order miniPCR® Forensics Lab kits, you can:



Call (781)-990-8PCR



email us at [orders@minipcr.com](mailto:orders@minipcr.com)



visit <https://www.minipcr.com>

**miniPCR® Forensics Lab (catalog no. KT-1009-01) contains the following reagents:**

- 2X EZ PCR Master Mix, Load Ready™
- D1S80 Lab Primer Mix
- Crime Scene DNA Control Sample
- X-Tract™ DNA Extraction Buffer
- 100 bp DNA Ladder, Load Ready™

Materials are sufficient for 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

- Agarose (electrophoresis grade)
- Fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®)
- Gel electrophoresis buffer (e.g., 1X TBE)
- Distilled or deionized water (to dilute TBE buffer concentrate)

**Note:** Agarose, DNA stain, and TBE buffer are available at [minipcr.com](http://minipcr.com) as part of the Learning Lab Companion Kit (KT-1510-01)

## 14. About miniPCR Learning Labs™

This Learning Lab was developed by the miniPCR™ 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 2-hour PCR-based experiment that recapitulates a real-life biotechnology application (in this case, VNTR analysis), 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 a simplified DNA extraction protocol suited for classroom use. We also use a VNTR instead of an STR as in most forensic analyses. The D1S80 VNTR region is well-studied and the PCR parameters (cycling conditions and primers) have been thoroughly tested.

We follow a proven model of experimental design<sup>1</sup> 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.

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<sup>1</sup> 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