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.

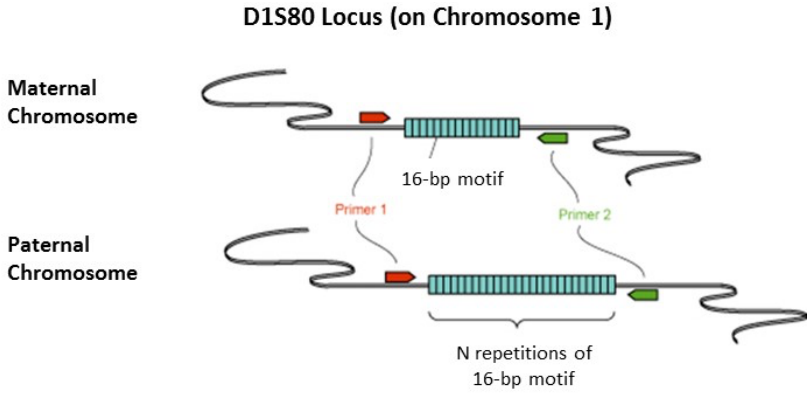
VNTRs are predominantly found in non-coding regions of DNA, and therefore their length does not usually bear any functional importance (though there are reported exceptions). The highly repetitive nature of VNTRs can lead to a relatively high frequency of errors in DNA replication or crossing over. When 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 assorts 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.

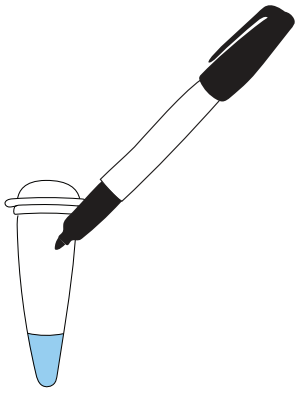
Read how miniPCR thermocyclers are used by real forensic scientists in the field using this miniPCR Case Study.
https://www.minipcr.com/case-studies/forensic-dna-analysis-evidence/

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.

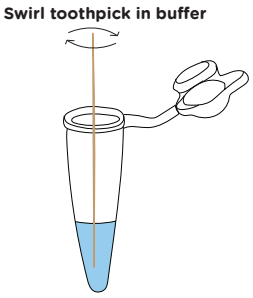
DNA extraction



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 µl 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.

4. **Cap your tube**

5. **Incubate your tube for 10 minutes at 95°C**
   - You can use a miniPCR® 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.

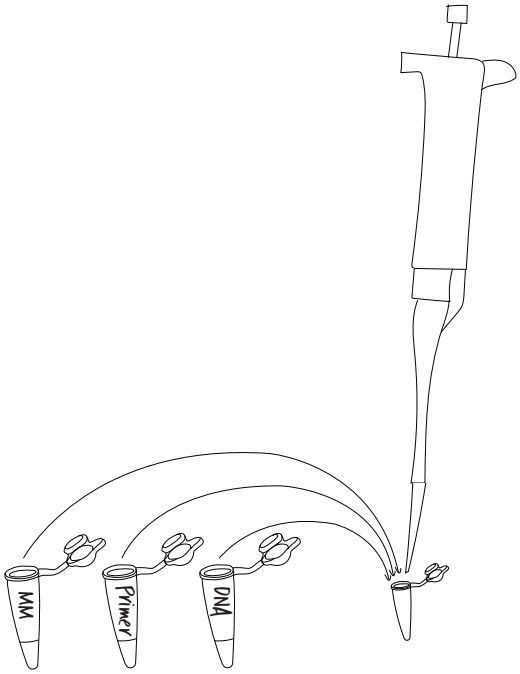
PCR setup

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
TOTAL VOLUME	26 µl

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

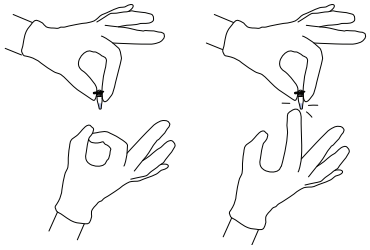


Use a micropipette to add each of the reagents. Remember to change tips at each step!

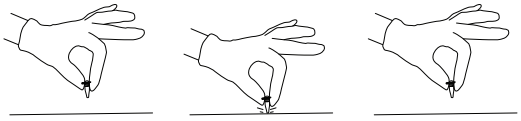
3. . **Cap the tubes and ensure the reagents mix well**
   - You may flick each tube with your fingers to ensure proper mixing.
   - Gently tap tubes on your bench to collect liquid at the bottom.

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.

Flick to mix



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 (m) 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.

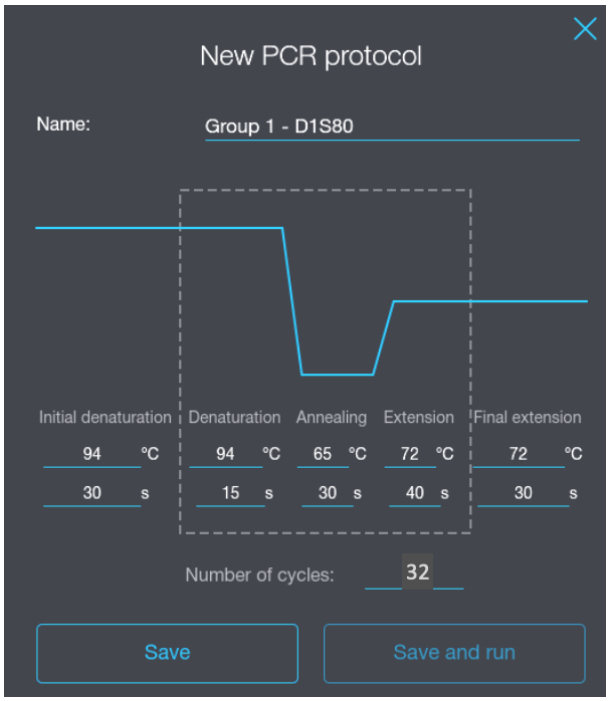
4. **While in the "Library" window, click the ⊕ button to create a new protocol**
   - Button is located in the upper right hand corner of the window.

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

6. **Enter a name for the protocol; for example: "D1S80 Lab"**

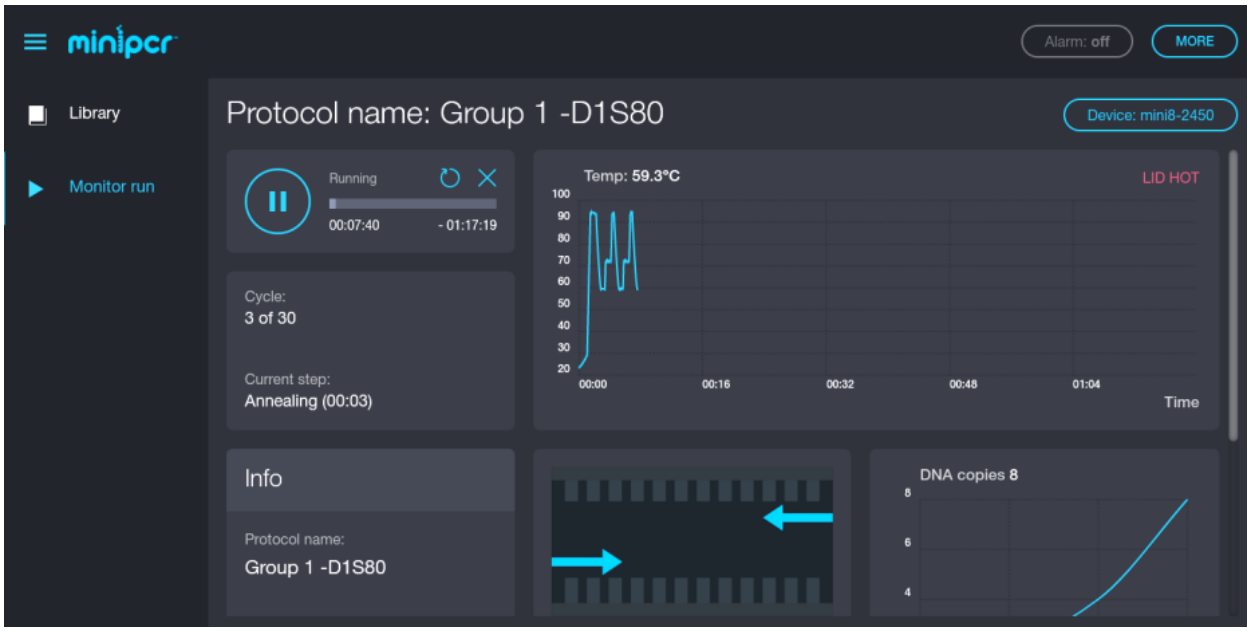
7. **Enter the PCR protocol parameters:**
   - Initial denaturation 94°C, 30 sec
   - Denaturation 94°C, 15 sec
   - Annealing 65°C, 30 sec
   - Extension 72°C, 40 sec
   - Number of cycles 32
   - Final extension 72°C, 30 sec

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



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.

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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)

Important Gel Pouring Notes

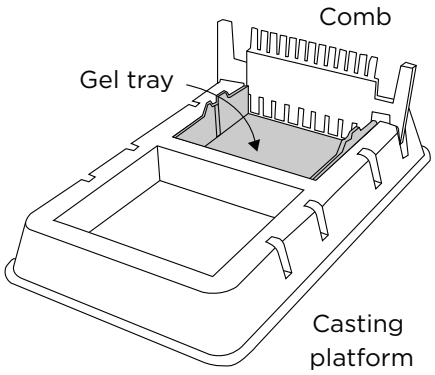
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

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**

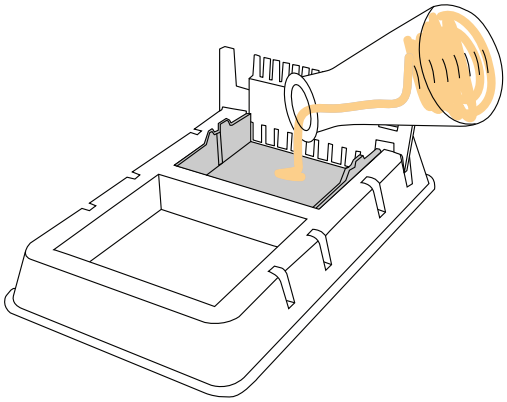
IMPORTANT NOTE: There are several ways to prepare agarose gels

- 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/

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).

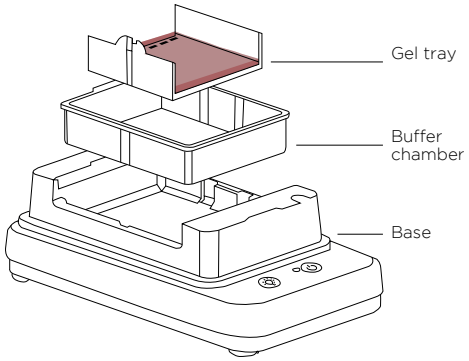
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



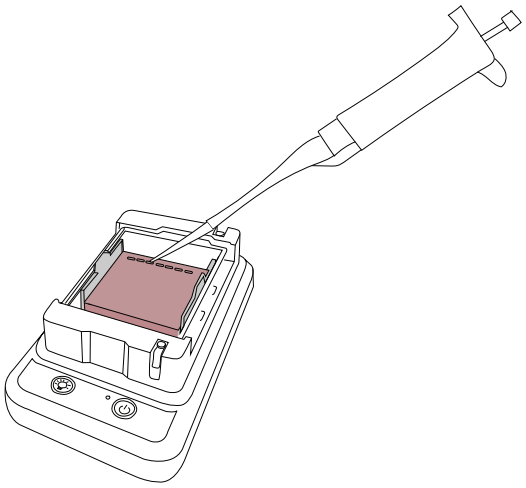
Protective gloves and eyewear should be worn for the entirety of this experiment.

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.

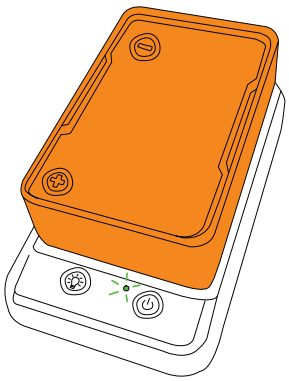


2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

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.

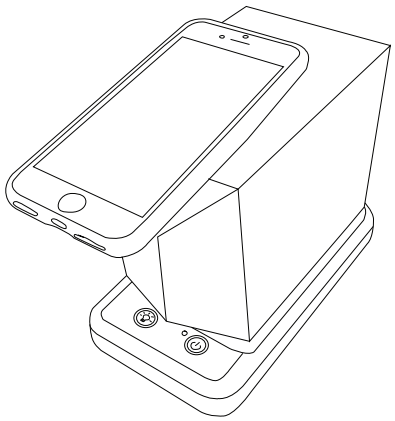
4. **Place the orange cover on the blueGel™ electrophoresis system**
   - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
   - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative 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 littl 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

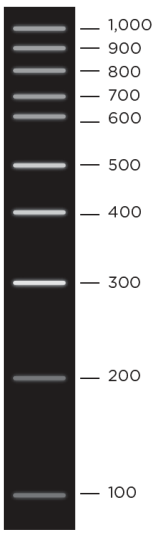


1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
   - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
   - Gels may be viewed at the end of the run or periodically throughout the run.
   - If the image appears hazy, wipe 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.)

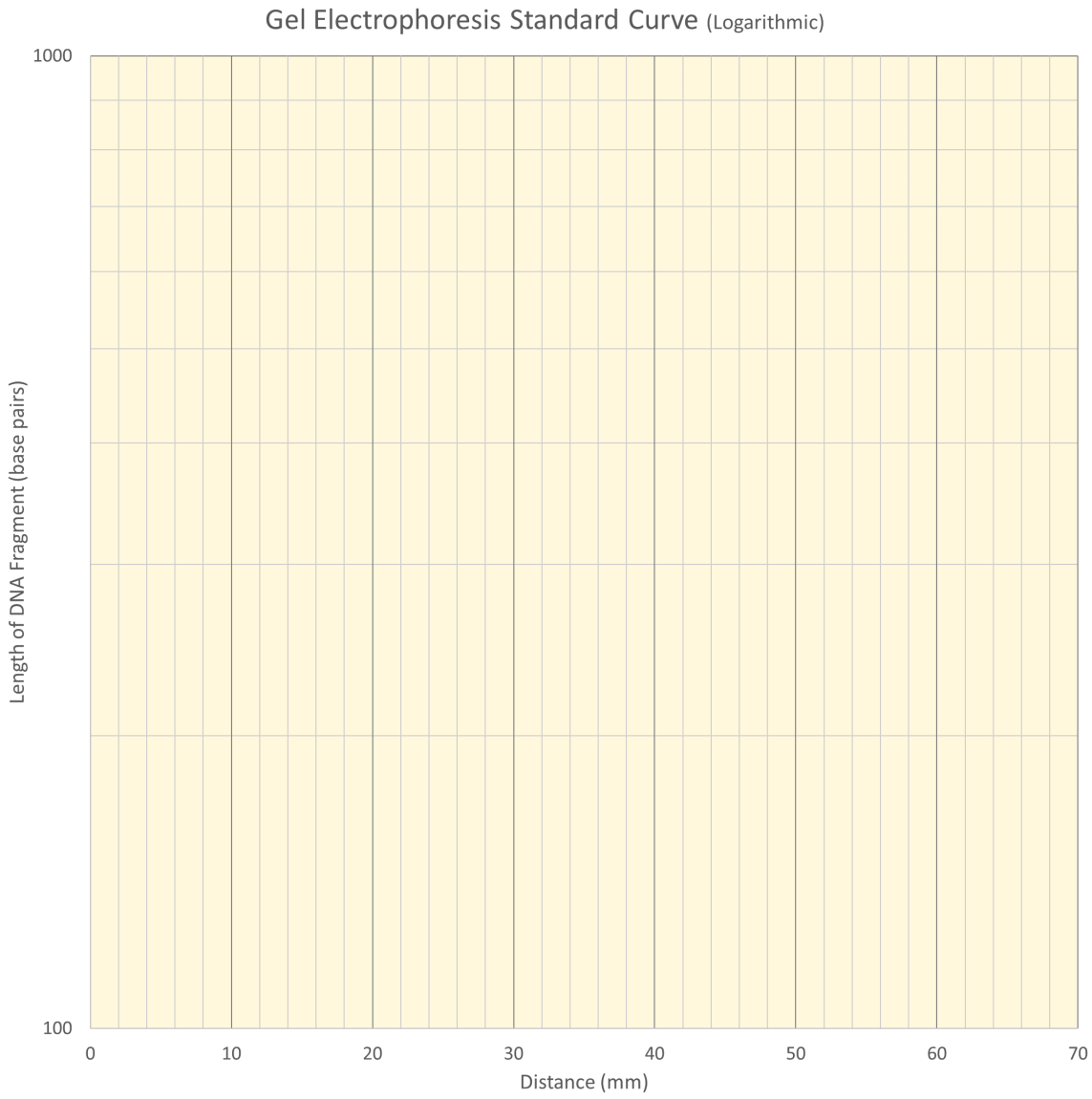
2. **Using the sizes you estimated in question number 1, can you estimate how many repeats you have in each of your alleles? Remember that the sizes you estimated include the length of your primers and flanking regions (180 bp) and that D1S80 repeats are 16 base pairs long.**

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.

Questions after compiling Class Data

Compile the number of repeats found in each student in your class. A formatted spreadsheet for this task is available at miniPCR.com.

1. **Looking at your class data, does it appear that any two individuals have the same alleles for D1S80? Explain why this would be relevant for forensics analysis. (Remember that band size is an estimate so allow for minor variation between individuals due to lack of precision in size estimation)**

2. **Do any alleles appear to be more common than others? What does this say about the probability of have matching alleles with another person?**

3. **causing genetic polymorphisms in the D1S80 loci are relatively common compared to the coding regions of genes. Considering, however, that the 18 and 24 allele repeats tend to segregate at significantly higher frequencies than other alleles, how frequently do you expect mutations to actually occur? Nearly every generation? Spread out occasionally over human history? Some other amount?**

Calculating probabilities

1. **Assume that the 18 repeat allele represents approximately 25% of all alleles in the population. What is the probability that an individual will have at least one 18 repeat allele?**

2. **Assume that the 18 repeat represents approximately 25% of total alleles and the 24 repeat represents 35% of total alleles. What is the chance of being an 18/24 repeat heterozygote.**

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	(Consensus)
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'CCCACGCGCCAGCACTGCATTCAGATAAGCGCTGGCTCAGTGTCAGCCCAAGGAAGACAGACCACAGGCAAGG
AGGACCACCGGAAAG**GAAGACCACCGGAAAG**GAAGACCACCGGAAAGGAAGACCACAGGCAAGGAGGACCAC
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.)