VNTR Analysis:
The Science Behind DNA Fingerprinting
Student Materials

Background........................................................................................................................................... 2
Pre-Lab Questions.................................................................................................................................. 6
Lab Protocol............................................................................................................................................ 7
Data Collection Worksheet...................................................................................................................... 10
Post-Lab Questions and Analysis............................................................................................................ 11
VNTR Analysis

Background

We all know that no two people have the same DNA (with the exception of identical twins), but did you know that even people that appear very different, are actually very similar at the genetic level? We all share 99.9% of the same DNA. This similarity makes using DNA to identify people a little tricky, but not impossible. Although DNA from different individuals is nearly identical, there are regions on every chromosome that contain small differences or polymorphisms that make each of us unique. For example, in any given sequence of DNA a single nucleotide may vary between two individuals. This type of polymorphism is known as a single nucleotide polymorphism or SNP. Genetic variation can also be caused by differences in the number of repeated DNA sequences at specific locations (loci) in the genome. These repeated sequences come in various sizes, some are short – 2 to 10 base pairs (bp) long – and produce Short Tandem Repeats (STRs) (See Figure 1 below for examples). Other repeated sequences can be longer – 11 to 100 bp long – and produce longer repetitive segments known as Variable Number of Tandem Repeats (VNTRs). Polymorphisms such as STRs and VNTRs generally occur within the estimated 90% of human genome that does not encode for proteins. However, some polymorphisms may disrupt a gene and result in a disease or altered phenotype.

Figure 1. Short Tandem Repeats (STRs)

One copy of STR:

5’ ATTCAGGTACCGGAATCGATGCGATCGATGGCCATATA 3’
3’ TAAAGCTCATGCC ATTACCCGTTATAT 5’

Four copies of STR:

5’ ATTCAGGTACCGGAATCGATGCGATCGATGGCCATATA 3’
3’ TAAAGCTCATGCC ATTACCCGTTATAT 5’

Figure 2. Variable Number of Tandem Repeats (VNTRs).

One copy of VNTR:

5’ ATTTGGCCAGGGCTTAAATTCCGATTGG 3’
3’ TAAACCGGTCCCGGAATTTAAGGGTAACC 5’

Four copies of VNTR:

5’ ATTTGGCCAGGGCTTAAATTCCGATTGG 3’
3’ TAAACCGGTCCCGGAATTTAAGGGTAACC 5’

Whether the repeated sequence is short or long, the number of times the sequence is repeated will vary from person to person. Every person has only two copies (alleles) of each repeated region: one copy on the chromosome that is inherited from their mother and one copy on the homologous chromosome from their father. Because STR and VNTR regions are inherited alleles, they are perfect for forensic or familial testing, determining cultural or ethnic heritage, identifying immigration patterns of different populations and archeological discoveries.

Let’s consider a VNTR found on chromosome 1 that is commonly used in human identification called D1S80. At the D1S80 locus (location) 29 different alleles have been identified in human populations.
consisting of between 14 and 41 repeats of a 16 bp repeat sequence. Using primers specific to the regions flanking the D1S80 locus, scientists can perform PCR (polymerase chain reaction) to amplify a single copy of the repeat region to generate multiple copies (see Figure 3).

**Figure 3.** Forward and reverse primers are designed to flank the D1S80 locus and amplify that region on the maternal and the paternal chromosomes.

Billions of copies of each of the two original D1S80 alleles in any individual’s DNA can be synthesized using PCR. These copies contain the same number of VNTRs present in the original DNA and can be studied to determine an individual’s genotype or DNA profile. Let’s look at how PCR works using the diagrams below.

**Step 1: Denaturation.** The template DNA must be separated into single-stranded DNA molecules by increasing the temperature to 94 °C. The high temperature disrupts the hydrogen bonds between the complementary base pairs.

**Step 2: Annealing.** The reaction temperature is decreased to a temperature between 50 and 70 °C. This temperature, called the annealing temperature, is determined by the sequence of the primers and is set to optimize primers binding (annealing) to the complementary sequences on the template DNA. Because primers will be incorporated into each new molecule, the primers are present in vast excess. The high concentration also increases binding of primers to the template.
**Step 3: Extension.** The reaction temperature is increased to the optimal temperature for the DNA polymerase being used in the reaction. There are several thermostable DNA polymerases used in PCR, and each has a slightly different optimal temperature. At this temperature, the DNA polymerase adds nucleotides to the 3’ end of the primers in a manner that is complementary to the template strand. In a reaction mixture, there is an abundance of polymerase so all the template strands are copied simultaneously.

[Image of DNA polymerase adding nucleotides during extension]

These three steps constitute one PCR cycle. A typical PCR amplification repeats the three step cycle 25-35 times. The target DNA (the DNA flanked by the primers) is doubled during each cycle. Beginning with a single DNA molecule, 20 cycles can produce over a million copies on the D1S80 region. Each copy contains the same number of VNTR repeats present in the original DNA (see Figure 4).

The following schematic shows the first four cycles of PCR amplification. Notice that the targeted region of DNA is not produced until the third cycle of PCR and that as the number of cycles increases, the percentage of PCR products of the desired region also increases.

**Figure 4. PCR Amplification of a VNTR**
By comparing the PCR products with size standards that correspond to the known D1S80 alleles, the sizes of the PCR products can be determined, as well as the genotype of the individual tested (Figure 5). Homozygous individuals will have the same number of repeats at the D1S80 locus on the homologous chromosomes and will display a single band. More often, an individual will be heterozygous for the D1S80 locus. Heterozygous individuals have different number of repeats on their two different chromosome 1s and thus, two distinct bands of different lengths.

**Figure 5. Gel Electrophoresis of PCR Products**

Different alleles appear as distinct bands, each composed of billions of copies of the amplified allele. Lane 7 shows the DNA ladder or marker used to estimate the size of unknown fragments in lab. Lane 2 represents an individual who is homozygous D16; Lane 4 homozygous D18 individual; and Lane 5 represents an individual who is heterozygous (D18, D14).
VNTR Analysis
Pre-Lab Questions

Directions: After reading through the introduction and protocol for the VNTR lab, answer the questions below.

1. What is the difference between an STR and a VNTR?

2. How many times do you expect to find the D1S80 allele in your genome? Explain your answer.

3. Why do you have to do PCR on a DNA sample before you analyze the VNTRs?

4. Study the VNTR gel image below, and answer the following questions.

   a. Label the positive and negative ends of the gels, and draw an arrow showing the direction in which the DNA moved.

   b. In Lane 2, circle the band that represents the longer VNTR allele.

   c. Lane 4 has only one band. Why?

   d. This gel represents a single blended family – Lane 2 Dad, Lane 3 Mom and Lanes 4-6 children. Which of the children is from the mother’s first marriage? How do you know?
**VNTR Analysis**

**Lab Protocol**

**Introduction: The Lions Revenge**

July 3, 2018 – Rangers at the Sibuya Game Reserve in South Africa discovered what appeared to be human remains near a pride of lions and alerted the park’s antipoaching unit. A quick search of the area uncovered a high-powered rifle, gloves, wire cutters and the remains of a backpack with food, water and other supplies – classic provisions of rhino poachers. Rangers returned to the scene the following day to tranquilize the lions and comb the area more closely for clues. They found three pairs of boots and gloves, but only one skull and several bone fragments. Park officials speculate that at least three poachers entered the park in the early morning hours of July 2nd when dogs on patrol with the antipoaching unit reacted strangely to a commotion near the territory of the lions. Clearly, the poachers had walked into a pride of six lions and some, if not all had been killed. Forensic teams were called in to the park to investigate further.

**July 20, 2018** – Sister of a suspected poacher from Kasouga, a small town in the Eastern Cape of South Africa, reported her brother missing to local authorities. The unidentified woman told police that her brother had left home on July 1st to do a job in the neighboring city of Port Alfred but had not been heard from since. The local anti-poaching task force reported that the missing man was an associate of known members of a criminal network that the task force has been investigating for trafficking in rhino horns, ivory and other animal parts. Police collected hair and other samples from the missing man’s home and plan to run DNA samples against DNA collected from the bone fragments found in Sibuya Game Reserve earlier in July. If the DNA samples are a match, authorities might be able to identify at least one of the poachers killed by lions.

In today’s lab, you will be analyzing a single VNTR locus (Variable Number of Tandem Repeats) from three different DNA samples extracted from tissue found on/in bone fragments at the scene of the lion attack and one DNA sample extracted from tissue samples from the missing man’s home. Prior to today’s experiment, lab technicians have already extracted the DNA samples from the tissue and used PCR (polymerase chain reaction) to make billions of copies of the D1S80 locus that we are interested in studying. The primers used for the PCR recognize DNA sequences that flank the D1S80 locus and produce PCR products of varying sizes depending on the allele. You will be using agarose gel electrophoresis to separate the PCR products. By comparing the PCR products to a DNA ladder on the gel, you will try to determine if the missing man was one of the poachers killed in the game reserve.

**Materials:** check your workstations to make sure all supplies are present before beginning the lab.

<table>
<thead>
<tr>
<th>Student Workstation:</th>
<th>Common Workstation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 tubes</td>
<td>1X electrophoresis buffer</td>
</tr>
<tr>
<td>1 tube</td>
<td>UV or blue light source</td>
</tr>
<tr>
<td>1 tube</td>
<td>centrifuge (optional)</td>
</tr>
<tr>
<td>Tissue DNA samples with 12 μL of DNA</td>
<td></td>
</tr>
<tr>
<td>Suspect DNA with 12 μL of DNA</td>
<td></td>
</tr>
<tr>
<td>DNA Ladder with 12 μL 100bp Quick Load DNA ladder</td>
<td></td>
</tr>
<tr>
<td>agarose gel (2.0%) with DNA stain</td>
<td></td>
</tr>
<tr>
<td>p20 micropipette with tips</td>
<td></td>
</tr>
<tr>
<td>microcentrifuge rack</td>
<td></td>
</tr>
<tr>
<td>waste container</td>
<td></td>
</tr>
<tr>
<td>gel electrophoresis unit with power supply</td>
<td></td>
</tr>
</tbody>
</table>
Procedures:

1. Find the tubes containing DNA from the 3 samples from the game park (Tissue A, B, C) and the Suspect DNA, as well as the tube of 100 bp DNA ladder (labeled Ladder). Loading Dye has already been added to the samples and the ladder.

2. Check to make sure that there are no bubbles or droplets on the sides of the tubes. If needed, quickly spin the tubes in a centrifuge to get all the samples and the ladder to the bottom.

   Tip: If you do not have a centrifuge, you can gently tap the tubes on the lab bench to collect the contents in the bottom of the tube.

3. Prepare or obtain a 2.0% agarose gel with DNA stain and 1X electrophoresis buffer as instructed by your teacher.

4. Assemble the gel box and position it where you can let it run without moving it.

   Reminder: Check to make sure that the gel tray is in the correct orientation with the wells closest to the negative electrode.

5. Using your p20 micropipette, load 10 μL of the ladder into a well in the gel and record the location of the well in Table 1 below.

6. Using your p20 micropipette and a clean tip, load 10 μL of each sample into a separate well of the gel, following the directions in Table 1.

   Caution: Don’t forget to use a new tip for each sample.

Table 1. Location of samples in the gel

<table>
<thead>
<tr>
<th>Lane</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Ladder</td>
<td>Tissue A</td>
<td>Tissue B</td>
<td>Tissue C</td>
<td>Suspect</td>
<td></td>
</tr>
</tbody>
</table>

7. Run your samples as instructed by your teacher or until the dye front is two-thirds of the way down the gel.

   Reminder: If your gel unit has a blue light, do not leave it on while running the gel.

8. While the gel is running calculate the size of the PCR products that you expect to see for six of the more common alleles. Record them in Table 2 on the next page.

9. Once your gel is finished running, examine the location of the bands and complete the data collection worksheet.
Table 2. Alleles and PCR Product Size

Remember – The D1S80 is a 16 bp segment that is usually repeated 14 to 41 times in the genome. There is a 146 bp constant within each product: 23 bp (forward primer) + 23 bp (reverse primer) + 100 bp flanking primer regions.

For example: D14 = (14 repeats x 16 bp) + 146 bp = 370 bp

<table>
<thead>
<tr>
<th>Allele</th>
<th>Number of Repeats</th>
<th>Length of PCR Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D14</td>
<td>14</td>
<td>370</td>
</tr>
<tr>
<td>D16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>D29</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>D35</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>D38</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>D41</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>
**VNTR Analysis**  
**Data Collection Worksheet**

**Directions:** After completing the VNTR Analysis lab, answer the questions below.

1. On the image below draw what you see after gel electrophoresis. Refer to the ideal image of the 100bp Ladder to the right when determining the size of the visible bands.

   ![100 bp DNA Ladder visualized on a 1.3% TAE agarose gel.]

2. Determine the genotypes of each of your subjects and write them below. Then share your data with the class.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Estimated Band Lengths (bp)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
VNTR Analysis
Post-Lab Questions and Analysis

Directions: After completing the VNTR lab, answer the questions below.

1. What are the two techniques used to create a DNA profile in this experiment? What function does each perform? (Hint: One technique was performed before today's lab.)

2. What is a DNA ladder? What is its function?

3. Analyze your gel image and the genotype chart you created. From the data you collected, can you determine if the missing man was one of the poachers killed in Sibuya Reserve? Why or why not?

4. Park rangers believe that three poachers were killed by the lions. Based on your data, can you confirm that three poachers were killed by the lions? Why or why not?