number of repeats in this region can vary from 14 to 41, with different individuals having different numbers of repeats. On either side of this sequence are conserved sequences of DNA that are complimentary to the primers we will use for PCR. By running products of the PCR amplification on a gel we can separate the 27 possible alleles by size. Each individual has two alleles, which will appear as one or two bands, depending on whether the individual is homozygous or heterozygous for the locus. D1S80 is one of several VNTR loci that are actually used by forensic scientists today in criminal cases and paternity suits as well as other situations which require human identification.

**Lab Exercises:**

**Isolation of Cheek Cell DNA**
*(view pipetting demo video on course website)*

Each bench should have four 15 ml orange-capped tubes containing a 0.9% saline solution, eight 1.5 ml tubes, paper cups, a bottle of Chelex beads, pipetmen and tips.

1. Pour the saline solution into your mouth and swish around for 10 seconds.
2. Expel the saline solution into a paper cup.
3. Pour the saline back into the orange-capped tube and cap tightly.
4. **With TF supervision:** Place the tube in a centrifuge with other class samples. Make sure the centrifuge is balanced and then spin the tubes for 10 minutes.
5. There should be a cell pellet at the bottom of the tubes. Carefully pour off the supernatant and save the cell pellet.
6. Resuspend the Chelex beads by rapidly pipetting the solution back and forth with a micropipet. Before the beads settle, draw off 500 µl of the suspension and transfer it to the tube containing your cell pellet. Chelex beads bind positive ions, effectively inactivating enzymes.
7. Resuspend the cells by pipetting in and out several times. Make sure there are no visible cell clumps.
8. Pipet 500 µl of the resuspended cell sample to a labeled 1.5 ml tube.
9. Place your new sample tube in a 100°C heat block for 10 minutes. Heating lyases the cells, releasing DNA, protein, and other cellular contents.
10. **With TF supervision:** Place your sample tube in a balanced microcentrifuge and spin for 30 seconds.
11. Transfer 200 µl of the supernatant to a clean, labeled 1.5 ml tube.
12. Proceed directly to PCR.

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**PCR Reaction**

Each bench should have an ice bucket, pipetmen, and tips.

1. Fill the bench ice bucket with crushed ice (pack it down so that shards won’t get knocked into open tubes and possibly contaminate your sample).
2. Each student will receive a PCR tube containing a Ready-To-Go PCR Bead, which already contains buffer, MgCl₂, deoxynucleotides, and the *Taq* polymerase (in sort of “suspended animation”). The bead should be dry and roll around freely. Label your tube with your initials.
3. Use micro-pipettors to add these volumes of reagents to the tube:

   - Primer Mix 22.5 µl
   - Cheek DNA 2.5 µl
4. Pipet up and down to dissolve the Ready-To-Go PCR bead. Close PCR tube and place your sample in a balanced micro-centrifuge and spin for 30 seconds.
5. Immediately after the spin, place your reaction tubes in the Thermal Cycler. Your TF will set the Thermal Cycler to stay at 4°C until everyone has added their tubes to the machine.
6. Your TF will prepare a tube with Primer Mix, DNA, and a Ready-to-Go PCR bead. The DNA sample is known to be good and the D1S80 locus can be amplified from this DNA using these primers. The sizes of the fragments generated are known. This serves as the positive control.
7. Your TF will also prepare a tube with Primer Mix, PCR bead, and water instead of DNA. This serves as the negative control.
8. When the section is ready, your TF will start the D1S80 PCR Reaction program. The program will last about 1.5 hours.
9. When the program is finished, your TF will store your samples at -20°C until gel electrophoresis can be performed.
Gel Electrophoresis and VNTR Analysis
(execute during week of Restriction Enzyme Lab)

NOTE: Ethidium bromide is a mutagen. Gloves should always be worn when using ethidium bromide or handling gels containing ethidium bromide. Ethidium bromide should not be placed down the sink or in the regular trash.

1. Your group will be given a precast 2.5% agarose gel with ethidium bromide already present in the gel. Ethidium bromide is a mutagen, so wear gloves when loading the gel. Your group should decide on, and record, the order of adding samples to the gel; reserve the first lane for running a sample of marker DNA (100 bp ladder).

2. Load all 25 μl of each reaction into your designated lanes and 20 μl of the 100 bp ladder marker sample into the first lane.

3. Loading Tips:
   a. Be careful not to punch the pipet tip through the bottom of the gel.
   b. Gently depress the pipettor button to slowly expel the sample into the appropriate well. Because of the loading buffer, the reaction solution will sink to fill the well; it is heavier than the buffer. Be extremely careful that it doesn't leak into a neighboring well.

4. Run the gel at 120 V for 1.5 to 2 hours. You may check the progress of your DNA fragments by periodically viewing with the handheld UV light source. Stop the gel when the 100 bp fragment in the ladder is at the bottom of the gel. Your TF will give you a photograph of the gel, which should be included in the results section of your lab notebook.

Data Analysis:

1. Examine the photograph of the stained gel containing your sample and those from other individuals. Photographs of gels are historically oriented with the sample wells at the top. First, ascertain whether or not you can see a diffuse band of primers at the same position in each lane toward the bottom of the gel (these may have “run off the end of the gel” and may not always be visible). These are unincorporated primers and “primer dimers” formed by the amplification of overlapping primers. Excluding primers, examine each lane of the gel:
   a. There should be no bands in the lanes of samples that did not contain cheek DNA. Presence of amplification products indicates contamination (or possibly a gel loading error).
   b. There should be amplification products visible in the lanes of samples that contained cheek DNA. A lack of bands usually results from an error during sample preparation, such as losing the cheek cell pellet or failing to resuspend Chelex beads prior to transferring solutions between test tubes. One visible band probably indicates homozygosity at the D1S80 locus (other possibilities are that the two alleles are so similar in size that they cannot be resolved in this gel system or that a larger allele has failed to amplify efficiently). Two bands indicate heterozygosity at the D1S80 locus. Often the larger allele amplifies less efficiently and appears less intense than the smaller one.

2. If a lane has more than two bands, the two brightest bands are likely the true alleles. Additional bands may occur when the primers bind nonspecifically to chromosome loci other than D1S80, or when an amplification product acts as a primer out of register with the multiple repeats of the locus.

3. Estimate the size of the DNA fragments by comparing their positions to the molecular weight DNA ladder of size markers (the 100 bp ladder is pictured at left). Present this information in a table. If you choose to be more precise, use the procedure described in the Restriction Enzyme lab to generate a standard curve and then use the curve to determine the size of alleles on your gel. Notice that the 500 and 100 base pair bands in the 100 bp ladder are brighter than the other bands, allowing for orientation. You may not be able to see all of the bands at the top or bottom of the DNA ladder on your gel.

4. Estimate the size of the alleles. The primers used in this experiment are each 30 bases long. The 5' end of the forward primer binds 115 base pairs from the start of the first repeat. The 5' end of the reverse primer binds 30 base pairs from the end of the last repeat. Using this information, determine the size of the alleles (which includes just the repeat region). How many 16 base pair repeats are in each allele? Include this information in a table.

5. Determine the number of different alleles represented among your classmates. Allele frequency is the number of times a particular allele occurred out of the total number of alleles observed. Using your class data, calculate the allele frequencies of the alleles found in your section population.

Discussion:

1. When the PCR was run, why did we include positive and negative controls? What is the purpose of each, and what did your results tell you about the quality of your experiment?

2. Population studies have identified 27 different alleles at the D1S80 locus. Allele frequencies for the North American population are given in the chart at the end of this protocol. About 90% of individuals are heterozygous. Compare your section data to what is known about the North American population. Comment on the number of alleles, the range of allele sizes, the allele frequencies, and the degree of heterozygosity at the D1S80 locus. How do the data sets differ and what might be the reason for any discrepancies?

3. What is your genotype at the D1S80 locus? Express the alleles in terms of the number of repeats in each allele. (If your amplification did not work, describe the possible reasons.)

4. The genotype frequency is the number of times a particular genotype occurs out of the total number of genotypes sampled. Expected genotype frequencies can be calculated using the allele frequencies—assuming that the locus is at Hardy-Weinberg equilibrium, the genotype frequency for a combination of two alleles is twice the product of the two allele frequencies (2pq). The genotype frequency for a homozygote is the square of the allele frequency (p²). Using the allele frequencies in the chart below, calculate your own genotype frequency. Calculate a “1 in…” number by taking the inverse of your genotype frequency.

5. Based on your results, do you think this procedure could be used to link a suspect with a crime or establish a paternity relationship? Why or why not?