Cornell Institute for Biology Teachers

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Title:	**DNA Profiling – Paternity Testing**
Authors:	Jim Blankenship, Cornell University, Ithaca, NY, Mary Colvard, Cobleskill High School, Cobleskill, NY (Original lab 1993). Revised by: Robert Suran and Mike Darwin Yerky, Cornell University, Ithaca, NY
Appropriate Level:	Honors and Advanced Placement Biology.
Living Environment	1 – Analysis, Inquiry and Design: 3 – Analyzing Observations: 3.2; 4 – Content: 2 – Inheritance: 2.1e, 2.1f, 2.1g, 2.1h, 2.2c; 4 – Continuity of life: 4.1b.
Abstract:	Students will cut DNA with restriction enzymes. The DNA fragments will be separated electrophoretically on an agarose gel. The results will simulate a DNA profile. Students can learn how this type of evidence is prepared and interpreted.
Time Required:	This experiment will take about five standard periods to complete. Alternatively, it can be done in one day as an in-school field trip. See the Teacher Section for more suggestions.
Special Needs:	CIBT DNA electrophoresis kit and additional equipment (see Teacher Section for more info). CIBT PowerPoint presentation included.

Teacher Information

Contents

Background Information

DNA profiling (also called DNA fingerprinting) is widely used in criminal and legal cases where DNA samples are available to determine identity or parentage. DNA may be extracted from relatively small samples of cells, such as a blood stain the size of a nickel (about two drops) or a semen stain the size of a dime. When performed under properly controlled conditions and interpreted by an experienced forensic scientist, such profiling can link a suspect to a particular incident with compelling accuracy or completely exonerate a suspect. This simulation activity allows students to work through the theory of DNA profiling and to grapple with some analytical and ethical questions. It can be used to reinforce basic concepts such as base pairing in DNA as well as teach the principles of restriction enzyme digestion, gel electrophoresis, and probe hybridization.

At the DNA level individual people are about 99.9% identical; they differ on average in 1 out of 1000 base pairs. Some of these differences are in genes that lead to the visible differences between us. Some of the differences, however, are in "junk" DNA, which is DNA that is not transcribed into RNA. The most variable sequences known are tandem repeated sequences; the basic unit of a repeat is usually a sequence of 2 to 300 base pairs. In tandem repeats, each unit has the same orientation (e.g., CATCAT). Different repeated sequences appear in different places in the genome. In each case, what is variable is the **number of copies** of the sequence in an allele. So for example, if the repeated sequence were CAT, in one allele there might be 3 copies [CATCATCAT] whereas another allele might have 7 copies [CATCATCATCATCATCATCAT]. These are known as **V**ariable **N**umber **T**andem **R**epeats, or **VNTRs**. In a given population there may be dozens or even hundreds of different alleles. Of course, every individual has only two alleles, one on each of the homologous chromosomes, each of which was inherited from one parent. Since there are so many alleles in a population, most people are heterozygous for alleles of any given VNTR. Because of the great variability in alleles of VNTRs, if one examines enough different VNTRs (6 to 12) in a given person, one can put together a molecular picture or "DNA profile" or "DNA fingerprint" of that person. For example, this can be used for identification of tissue left at the scene of a crime (semen from a rape victim), or for paternity testing, in which case the VNTR alleles in the child that are not present in the mother must have come from the biological father. Population studies must be done to determine the frequency of each allele in the population. Using that information, calculations may be made to determine the chance that a random person in the population would have the same alleles as the suspect or alleged father.

A DNA profile usually involves isolating DNA from a few cells, cutting the DNA with restriction enzymes and resolving the fragments on the basis of size by agarose gel electrophoresis. The pattern of bands that results from such a treatment is incredibly complex; however, it is possible to transfer these DNA fragments to a filter paper and to bathe them in a solution containing a single stranded DNA probe that is complementary to a VNTR. The radioactive probe will bind to the band(s) of DNA on the filter that contains VNTR sequences. The filter is placed on X-ray film to visualize the result.

These experiments are obviously excessively rigorous for the scope of a high school biology lab; however, it is possible to simulate the types of evidence that could be obtained by simply cutting samples of DNA with restriction enzymes, resolving the fragments on agarose gels, and detecting the DNA bands using a stain or fluorescent dye. For this experiment you will provide each pair of students with three different tubes of plasmid DNA. The plasmids have been selected because when they are cut with the restriction enzyme HindIII, some of the fragments will migrate at the same positions and some will migrate differently. In DNA profiling a small region of a very large amount of DNA is being studied. This makes it necessary to use a radioactive probe. In this experiment each tube contains a simple DNA molecule (a plasmid). Each student will cut enough DNA with restriction enzyme so that it can be visualized by adding a fluorescent dye to the gel, such as GelStar®. It is important to stress the differences between this experiment and an actual DNA fingerprinting experiment. However, the experiment, as written, is an exciting opportunity to work with DNA, electrophoresis, and to talk about a type of evidence that is being used in courtrooms. Where does this evidence come from? How reliable is it? The lab ties in nicely with general discussions of DNA and biotechnology. CIBT's lab *How Many CATs?* (Mayo, E. and A. Bertino) can be used to help illustrate many of these important points.

In this scenario your students will be acting as forensic investigators who are analyzing samples from two paternity tests. The class should be divided into investigatory groups. Each investigatory group will consist of 4 students; one pair will prepare samples for case number 3 while the other pair will prepare samples for case number 4. Both pairs will then run the samples on the same gel and determine if the suspected father could actually be the biological father.

Time Required

This experiment will take about five standard periods to complete. Use the first period practicing with the micropipettors and labeling tubes for the upcoming restriction digest. Use the second period setting up restriction digests and pouring agarose gels. After a half hour of incubation at 37°C (can be longer) the digests can be transferred to a freezer. When set, the gels can be thoroughly wrapped in saran wrap and placed in the refrigerator until needed. The third period is for adding dye to the samples and instructions as to how to load the samples onto the gel. The fourth period is for loading the gel for electrophoresis. Some students may be able to observe the beginning of the electrophoresis, while some will be loading their gels right to the end of the period. The fifth period is for photography and analysis of the gels.

A double period (without a break) for the second lab day makes for a smoother experience. Use some of the additional time in the first period for discussing background material. Use the remaining time of the double period (after the digests are set up and the gels poured) for much needed lecture/discussion. The ideal schedule would be a double period for Day 1, a double period for Day 2, and a single period for Day 3.

See Table on the following page.

CIBT DNA PROFILING LAB – TIME REQUIREMENTS	
PERIOD 1	Lab intro and pipette practice
PERIOD 2	1. Set up digestion tubes and begin incubation 2. Prepare gels (After digestion, the tubes can be kept in a freezer overnight, or for a few days. After solidifying, the gels can be wrapped in saran plastic or kept in the fridge **(NOT FREEZER)** in ziplock baggies)
PERIOD 3	1. Add loading dye to all tubes, in preparation for loading the gels 2. Loading gel instruction
PERIOD 4	Load the gels and start the gel box run (After running the gels for 90 minutes, the gels can be stored, in a very small amout of buffer, in the fridge, **(NOT FREEZER)** in ziplock baggies.
PERIOD 5	Analyze and take picture of the gels

Student Prerequisites

1. Students should be familiar with DNA structure and function.
2. Students should be familiar with classical Mendelian genetics.
3. Students should be familiar with the basic principles of gel electrophoresis.

Equipment and Supplies

Equipment and supplies included in the CIBT DNA Profiling kit:

- 4 gel boxes with trays, combs, and power cables
- 2 power supplies
- 8 micropipettors (2-20 µl)
- 1 micropipettor (20-200 µl) (for teacher use)
- 1 dry bath for 1.5 ml microcentrifuge tubes, with block
- 1 thermometer
- 4 microcentrifuge tube racks
- 1 microcentrifuge
- 4 boxes micropipette tips
- 2 bags 1.5 ml microcentrifuge tubes (at least 30 tubes)*
- 1 conical tube (10 g) agarose*
- 1 bottle TBE running buffer*
- 1 spare fuse for the power supplies
- 1 dark reader

*Amounts of these items are listed for 16 students (4 gels). More of these items will be sent if you are running more gels.

In a separate container you will be sent the frozen expendables. These items are shipped overnight on ice. **When these reagents arrive, put them in the freezer as soon as possible.** You will have to use the P200 and P20 pipettors to aliquot these reagents for each pair of students (amounts shown in third column below). We recommend centrifuging the stock tubes for a few seconds before aliquoting, as the contents may have been disturbed during shipping.

DNA Profiling Reagents	Volumes supplied for 16 students (what's shipped for 4 gels)	Supply tubes for each pair of students (what teachers aliquot)		# of tubes needed for 4 gels each with one Case 3 and one Case 4 pair of students	Actual amounts of reagents used by each pair of students	
		Case 3	Case 4		Case 3	Case 4
Sterile H_2O	1000 µl	100 µl	100 µl	8	75 µl	75 µl
Buffer	150 µl	18 µl	18 µl	8	12 µl	12 µl
M3*	50 µl	12 µl		4	10 µl	
S3*	50 µl	12 µl		4	10 µl	
B3*	50 µl	12 µl		4	10 µl	
M4*	50 µl		12 µl	4		10 µl
S4*	50 µl		12 µl	4		10 µl
B4*	50 µl		12 µl	4		10 µl
HindIII	48 µl	6 µl	6 µl	8	3 µl	3 µl
Loading Dye	400 µl	40 µl	40 µl	8	30 µl	30 µl
HindIII λ DNA (STD)	45 µl	5 µl	5 µl	8	4 µl	4 µl
GelStar DNA Stain	15 µl	n/a (added by teacher)		n/a	2 µl per gel	

*Since DNA samples M3/S4, S3/B4, and B3/M4 are actually the same plasmids, they may be shipped in one tube containing enough for both samples (i.e., you may receive three tubes, labeled M3/S4, S3/B4, and B3/M4, each containing 100 µl of plasmid for 16 students).

Equipment and supplies NOT included in the CIBT DNA Profiling kit:

- Hot plate with a magnetic stirrer or a microwave oven for dissolving agarose (at least 1 per class)
- Two 2 liter flasks and 100 ml graduated cylinders for mixing the 0.5x TBE running buffer
- Four 250 ml flasks for melting the agarose
- 4 liters of distilled H_2O for making 0.5x TBE running buffer.
- 1 box latex gloves
- Waste containers
- Sharpie markers (it helps prevent errors if you label the aliquots you hand out to students with different colors while the students use black for their tubes)
- Ice (optional if freezer is nearby for storage of solutions until right before use)
- Containers for ice (optional if freezer is nearby)
- Semi-log paper or access to a computer graphing program

Technical Information

The following table shows the approximate band pattern you should see using these DNA samples cut with HindIII:

Sample	Plasmid	Size of Smaller Band	Size of Larger Band
Pair I (Case 3)			
Mother 3 (M3)	pTA 2.0	2.0 kb	3.9 kb
Suspected father 3 (S3)	pUC 1.4	1.4 kb	2.7 kb
Baby 3 (B3)	pTA 1.4	1.4 kb	3.9 kb
Pair II (Case 4)			
Mother 4 (M4)	pTA 1.4	1.4 kb	3.9 kb
Suspected father 4 (S4)	pTA 2.0	2.0 kb	3.9 kb
Baby 4 (B4)	pUC 1.4	1.4 kb	2.7 kb

Notes/Recommendations

- It is critical that the HindIII enzyme stay on ice or in the freezer until the students are ready to use it.
- We recommend not handing out the lambda DNA standard that is cut with HindIII until the students are ready to load their gels.
- If you are in a situation where you are preparing the gels for your students, you can prepare more than one gel at a time by scaling up the recipe (2.0 grams in 200 ml, etc.).
- Determine ahead of time which groups of students will prepare the 0.5x TBE buffer. One bottle of TBE powder when prepared will make 4 liters of 0.5X buffer, which is enough for 4 gels. The group of students that prepared the buffer will be able to share the extra buffer with another group of students.
- You might feel that your students will not have enough time to fill out the chart on page 8 of the student section (which shows how much of each solution they will mix together for each restriction digest). In this case, we have included the chart on the next page, which indicates the reactions that we suggest that you set up. Alternatively, you can have your students fill out the chart as homework before class.

	M3- (M4-)	B3- (B4-)	S3- (S4-)	M3+ (M4+)	B3+ (B4+)	S3+ (S4+)
Sterile water	13 μl	13 μl	13 μl	12 μl	12 μl	12 μl
Buffer	2 μl	2 μl	2 μl	2 μl	2 μl	2 μl
Case 3 or 4 DNA Samples	5 μl	5 μl	5 μl	5 μl	5 μl	5 μl
HindIII (enzyme)	0 μl	0 μl	0 μl	1 μl	1 μl	1 μl

- There should be a piece of red tape on the bottom of the gel box marking the negative terminal. This red tape has two basic functions: 1) It makes sure the gel is positioned the right way after it solidified (comb end right above the red tape, i.e., the negative pole); 2) Once the comb is removed, the darker red squares surrounded by the lighter tape (because the red tape shines through the opaque gel pinkish) indicate the wells and help the students find the wells when loading the samples on to the gel.

- If you plan on taking photographs of the gels, please make sure to do so as soon as possible after the gel has run its course. It's best to turn the flash off and mount the camera on a tripod. Within hours after the gel run, the DNA bands will begin to diffuse and become fuzzy. After photographing, the gel can be disposed of in the regular trash.

Answers to Student Questions

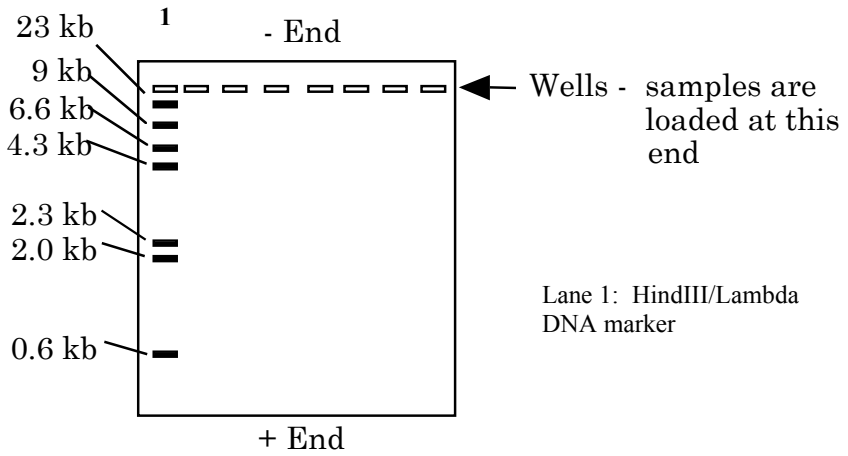
Answers to Pre-lab Questions:

1. What is a gene?

   A gene is a segment of DNA that specifies the information required to build a polypeptide or an RNA molecule (like tRNA or rRNA).

2. What force causes samples to move in electrophoresis? In the case of agarose gel electrophoresis of DNA fragments, why do the DNA molecules move? Look at the structure of DNA in your biology textbook. What charge does DNA have? In which direction will it move? Label the proper orientation of the (+) and (-) electrodes on the diagram below. Why do pieces of different size move at different rates?

   In electrophoresis, a sample is placed in an electrical field; this causes charged molecules to move toward the pole of opposite polarity. DNA is negatively charged due to the phosphodiester linkages between nucleotides and it moves toward the positive electrode. In gel electrophoresis, the electrical force pulls charged molecules through the mesh-like gel. Larger molecules encounter more difficulty passing through the mesh and therefore migrate at a slower rate.



3. What do you expect to observe during the course of the electrophoresis?

   a. What will happen to the indicator dye while the gel is running?

   b. Will you be able to see the DNA fragments as the gel is being run? Why or why not?

      During electrophoresis you will see at least one colored band. This is an indicator dye that was added to the samples to help you to monitor the progress of the run. The DNA fragments will not be visible until the GelStar® gel is viewed with a special viewer (Dark Reader).

4. How can the size of a DNA fragment be determined on the basis of the distance that it has moved? (Hint: What information do the molecular weight markers provide?)

   In order to determine fragment size, molecular weight markers must be loaded on the same gel. These are DNA fragments of known size. In agarose gel electrophoresis, the distance migrated is inversely proportional to the log of the size. By relating the migration distance for each fragment to its size it is possible to generate a standard curve. The standard curve can be used to determine the size of other fragments on the same gel.

5. Why do we incubate these reactions at 37˚C? Where does the restriction enzyme come from? What does that imply about our incubation temperature?

   Restriction enzymes are isolated from bacteria, many of which live in or on the human body. Because of this, bacterial enzymes work best at human body temperature, which is 37˚C.

PACKING AND SET-UP LIST

STUDENTS WILL BE DIVIDED INTO:
WORKING GROUPS OF 4-5
…AND THEN TEAM GROUPS OF 2-3

- **Items the teacher needs to provide are noted in RED**
- **Items provided by the CIBT kit are noted in BLACK**

ITEMS THE TEACHER MUST PROVIDE:

(fill in the blank with the number that your *largest* class requires)

- ___ Waste containers, 1 per group of 2 students
- Box of gloves for adding Gelstar/handling the gels
- ___Heating plates, with magnetic stir (1 plate holds 2-3 250 ml flasks)
- ___ 250 ml flasks with magnetic stir bars (1 per gel)
- Magnetic wand or large tweezers, to take the magnetic stirrers out of the 250 ml flasks
- ___ 2L flasks (only half the groups of 4-5 get one)
- ___ 1L flasks (the other half of the groups of 4-5 get one)
- ___ 100 ml graduated cylinders (1 per group of 4-5)
- ___ small paper cups or weigh boats (1 per group of 4-5)
- Electronic scale
- ___ Kimwipes (1 box per group of 4-5)
- ___ permanent markers (1 per person if possible)
- ___ Parafilm squares (1 per group of 2-3)
- ___ Eppendorf tubes with colored water (1 per group of 2-3)
- Distilled water

For each working group of 4-5 students:

- 1 - 250 ml Erlenmeyer flask with magnetic stir bar
- 1 - 100 ml graduated cylinder
- 1 small paper cup or weigh boat
- 1 box of Kimwipes
- 1 2L flask with funnel **OR** 1 1L flask without funnel
- 100 ml of TBE solution
- 1 gel box
- 1 power supply (the power supply will be shared with another group of 4-5, so there will be 2 gel boxes per power supply)
- 1 baggie of Eppendorf tubes

For each team group of 2-3 students:

- 1 waste container
- 2 Eppendorf tubes with colored water
- 1 square of Parafilm
- 2 permanent markers
- 1 micropipette tip box
- 1 microtube rack

For the class:

- 1 centrifuge
- 1 dry bath
- Electronic scale
- 1 dark reader
- Agarose
- Hot plates

FIGURE 1: One working station, showing materials required by a working group of 4-5 students (divided in two team groups of 2-3 students each).

Answers to Post-Lab Questions

1. What controls did your group decide to do in this reaction? What information will you get from the controls?

   The control reactions are the "-" (minus) reactions, the reactions that omit the restriction enzyme. If the "+" (plus) reactions end up looking like the "-" reactions, it indicates that the DNA was not cut by the restriction enzyme, and thus we are unable to interpret the results of the "+" lanes.

2. Use a piece of semi-log paper or a computer-graphing program to generate a standard curve as described above. Use the standard curve to determine the size of the fragments generated when each sample of DNA was digested with restriction enzymes.

   From the gel photo (or stained gel) measure the distance in millimeters from the well to each of the bands in the molecular weight markers. Obtain a piece of 3 cycle semi-log graph paper. Label the bottom axis the distance migrated. The units on this axis will be millimeters. Label the Y-axis molecular weight. Here the units will be kilo basepairs (kb). To label the Y-axis put a 0.1 by the lower most line. Count ten divisions and label this line 1.0 (this line should be approximately one third of the way up the page). Count up ten more divisions and label this line 10.0, and ten more to get to the 100.0 line. Notice that the scale gets smaller and smaller as the size gets larger and larger. That is what makes the log scale. The paper is called semi-log because it is linear on one axis (the X-axis) and logarithmic on the other. The standard curve should be a smooth curve. Draw a "best fit curve." Do not just connect the points. Sometimes it is somewhat sigmoidal.

3. What probably happened to very large and very small DNA fragments?

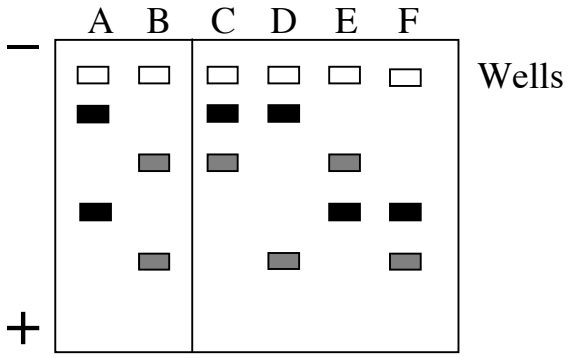
   Large molecules (above a certain size) fail to enter the gel, small molecules (below a certain size) travel unhindered through the gel and will run off the bottom.

4. How many bands did you expect to see in each lane? What does it mean if you saw only one?

   You expect to see one or two bands in each lane. Usually a person is heterozygous for alleles at these loci and two bands will be seen, each corresponding to one allele. It is, however, possible that both the father and mother could have the same, or similarly sized, allele/alleles in a given region and that the child could inherit these alleles. This would result in a single band in the child's sample.

5. Draw a hypothetical gel. Label the positive and negative ends and the wells. Draw in a hypothetical banding pattern for two parents. Now draw in all possible arrangements of bands that could occur in their offspring. Would you expect each one of these possibilities to appear at the same frequency?

   The negative end of the gel is the end closest to the wells. The positive charge is positioned at the far end of the gel. This pulls the DNA molecules through the gel. Take each band from the mother and combine with each band from the father to get the four combinations. The gel above shows a sample answer. If lanes A and B are DNA samples from the parents, lanes C, D, E, and F show all the possible combinations that offspring could have. The frequency of each would be expected to be the same (1:1:1:1).



5. Could the suspected father be the biological father of the baby in your paternity test? Why or why not?

In Case 3, the suspected father cannot be ruled out as the biological father of the baby, since they share the DNA fragment that is not shared between the baby and the mother. In case 4, the baby and suspected father do not share a DNA fragment, so it would be impossible for the suspected father to actually be the biological father of the baby.

DNA Profiling

New York State Learning Standards

Standard 1: Inquiry Analysis and Design

Key Idea 3: The observations made while testing proposed explanations, when analyzed using conventional and invented methods, provide new insights into natural phenomena.

3.2- Apply statistical analysis techniques when appropriate to test if chance alone explains the results.

Standard 4: Content

Key Idea 2: Organisms inherit information in a variety of ways that result in continuity of structure and function between parent and offspring

2.1 – Explain how the structure and replication of genetic material result in offspring that resemble their parents

c. Hereditary information is contained in genes…A human cell contains many thousands of different genes in its nucleus.

e. In sexually reproducing organisms, the new individual receives half of the genetic information from its mother and half from its father.

f. In all organisms the coded instructions for specifying the characteristics of the organisms are carried in DNA, a large molecule formed from subunits….

2.2 – Explain how the technology of genetic engineering allows humans to alter genetic makeup of organisms.

c. Different enzymes can be used to cut, copy and move segments of DNA.

Key Idea 4: The continuity of life is sustained through reproduction and development.

4.1 – Explain how organisms, including humans, reproduce their own kind.

b. …Other organisms reproduce sexually with half the genetic information

Glossry of Terms

Agarose - A sugar purified from seaweed. Agarose can be dissolved by heating above 100°C. When molten agarose cools and solidifies, the gel can be used for separating DNA fragments on the basis of size.

Aliquot - A portion of a total amount of a substance. Usually refers to a unit of volume.

Chromosome - A molecule of **DNA** complexed with proteins.

Deoxyribonucleic acid (DNA) - The genetic material of the cell. A long polymer composed of a mixture of four different types of **nucleotides**.

Electrophoresis - The process whereby substances are separated from one another by exposure to an electrical field.

Endonuclease - An enzyme that cuts **DNA** into smaller fragments.

Gel electrophoresis - A type of **electrophoresis** where samples are separated in a Jello-like substance. In gel electrophoresis, samples are separated on the basis of size.

Loading dye - A solution that contains glycerol and an indicator dye. When added to your **DNA** the glycerol causes it to become dense so that it is easy to load onto the gel. Loading dyes usually contain bromphenol blue, an indicator dye. Bromphenol blue is a small negatively charged molecule. Unlike the **DNA**, it can be observed during **electrophoresis** and can be used to monitor the run.

Microliter - 10^{-6} liter.

Micropipettor - A laboratory device that enables volumes in the **microliter** range to be measured at very high precision.

Nucleotide - The chemical repeating unit of **DNA**.

Polymorphic - Many different forms are found in a population.

Restriction enzyme - An enzyme that cuts **DNA** fragments at specific **nucleotide** sequences.

Restriction Fragment Length Polymorphism (RFLP) - Different individuals may vary in the presence or absence of sites where **restriction enzymes** cut or may have more or less **DNA** in between two given **restriction enzyme** cut sites. If this region of **DNA** is analyzed by **electrophoresis**, the result will be different in either case and therefore **polymorphic.**

Variable Number Tandem Repeats (VNTRs) - Some regions of **DNA** are composed of **DNA** sequences that repeat one after another. Different individuals often have differing numbers of repeats. If their **DNA** is cut with an enzyme that cuts outside of the repeated region, the pattern on an agarose gel will be different (**RFLP**)