Cornell Institute for Biology Teachers

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Title: **DNA Profiling – Paternity Testing**

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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).

Teacher Information

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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.

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 dry 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 out)		# 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 below, 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 and certainly within a day 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

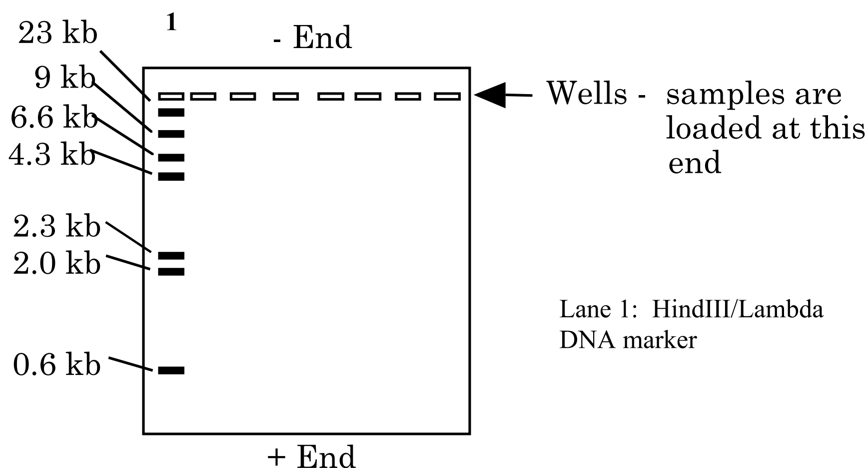
Answers to Pre-lab Questions

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.

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 "-" reactions, the reactions that omit the restriction enzyme. If the "+" 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.

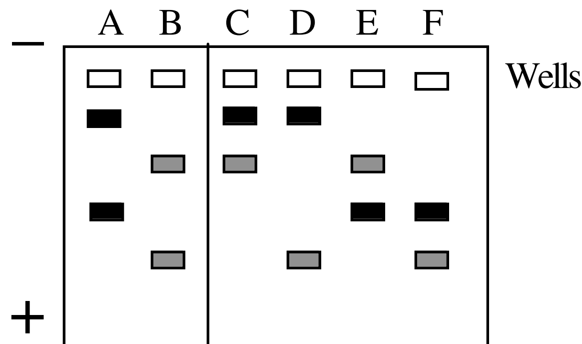
3. What probably happened to very large and very small DNA fragments?

   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 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).

6. 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

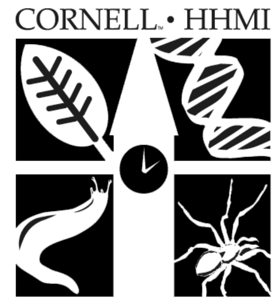
- 2.1 – Explain how the structure and replication of genetic material result in offspring that resemble their parents
  - c. Hereditary information is contained in genes…A human cell contains many thousands of different genes in its nucleus.
  - e. In sexually reproducing organisms, the new individual 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 typically contributed by each parent.

DNA Profiling

Student Laboratory Exercise



In this lab, your lab group will be acting as doctors performing a paternity test for one of two couples. You will use a restriction digest and gel electrophoresis to analyze DNA samples from two different cases (number 3 and 4).

Two students from the four-student group will determine whether Mother 3's baby (Baby 3) was fathered by Suspect father 3. These two students will have the following three DNA samples:

- one from Mother 3 (sample M3)
- one from Suspect father 3 (sample S3)
- one from Baby 3 (sample B3)

Two students from the four-student group will determine whether Mother 4's baby (Baby 4) was fathered by Suspect father 4. These two students will have the following three DNA samples:

- one from Mother 4 (sample M4)
- one from Suspect father 4 (sample S4)
- one from Baby 4 (sample B4)

Background Information

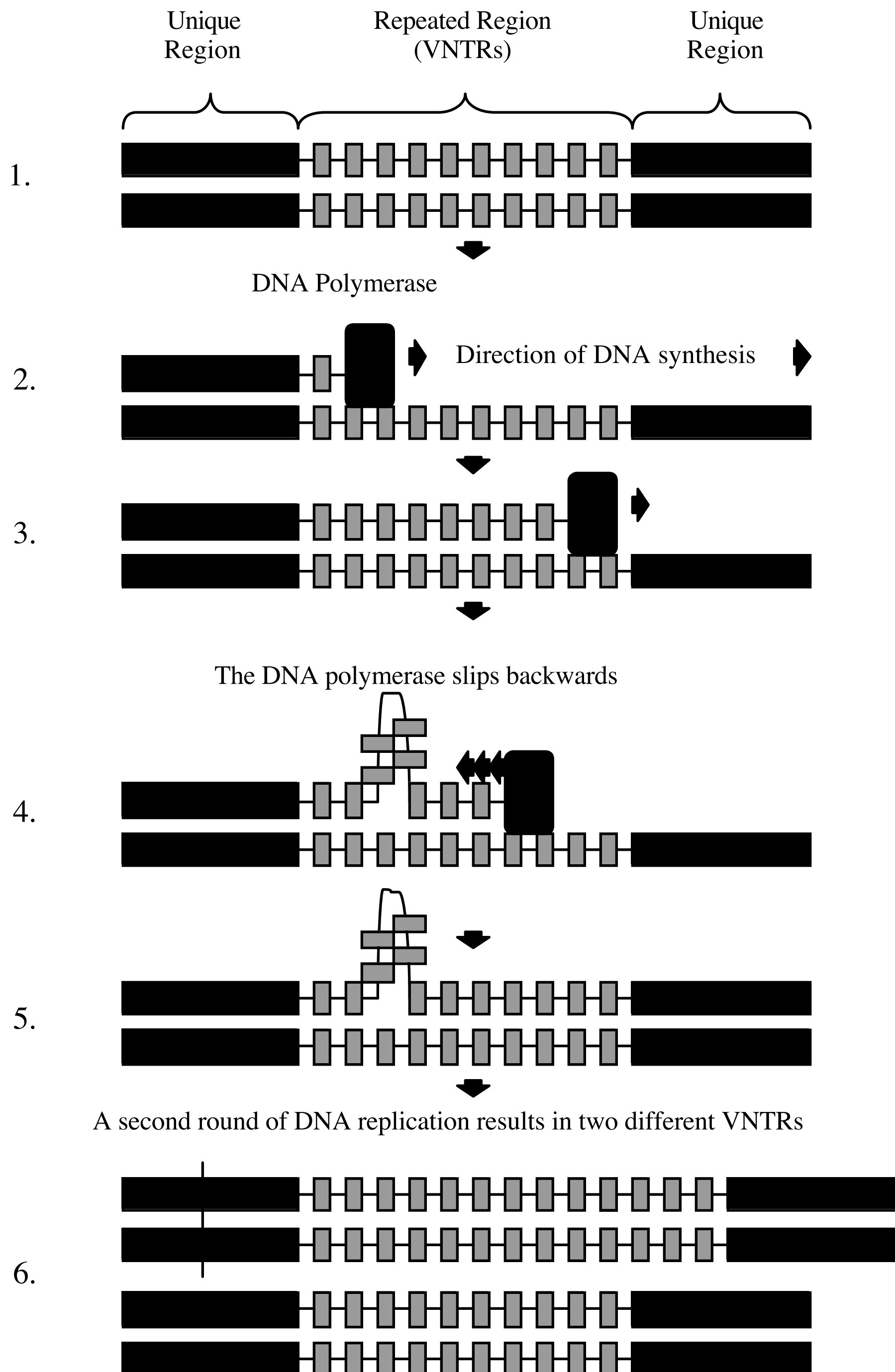
(Note: Definitions of words printed in bold can be found in a glossary located at the back of this handout).

Deoxyribonucleic acid (DNA) is the master molecule that programs all living processes. DNA molecules are composed of four chemical units, **nucleotides**, bonded in different linear arrangements in different individuals. An arrangement of DNA that specifies the blueprint for a polypeptide or an RNA product is called a **gene**.

In living cells, DNA molecules are surrounded by proteins and organized into **chromosomes**. Each chromosome contains one very long and thin molecule of DNA. It is possible to isolate chromosomal DNA from most organisms. This long molecule can be "cut" (digested) into small pieces by incubating it in the presence of **restriction enzymes**. Restriction enzymes are used in the laboratory to cut DNA molecules at specific sequences and generate fragments of various sizes. Agarose **gel electrophoresis** is used to determine the size of these DNA fragments. An **agarose** gel is like a complex maze with very tiny channels. DNA has a negative charge and will move toward the positive pole in an electrical field. Short pieces of DNA navigate more easily through the maze and therefore move a greater distance. An indicator dye is added to the DNA digest prior to injecting it into the gel so that the researcher can tell when the small fragments have run the length of the gel. Otherwise, the DNA would either be run off the positive end of the gel or would not move far enough into the gel. The DNA itself will not be visible until a stain is added. Some stains are visible under normal light, while others fluoresce under UV light.

If you determined the nucleotide sequence of each of your chromosomes (an enormous task) and compared it to one of your classmates, much of it would be the same. There are, however, regions that are highly variable (e.g., **variable number tandem repeats, VNTRs**). Because the DNA in these regions can vary from one individual to another, it can be used as a form of identification similar to a fingerprint. This type of evidence has become commonplace in courtrooms in cases involving paternity, rape, murder, etc.

VNTRs are formed through an error in DNA replication (see figure below). DNA is copied by an enzyme called DNA polymerase. DNA polymerase reads one strand of DNA and fills in the corresponding nucleotide sequence to make a second strand (2 & 3). In a small number of cases, the newly synthesized DNA strand can slip (4). When this happens with a non-repetitive DNA sequence, there is only one way that the two strands can "zip" back together. In the case of **VNTRs**, though, there are many ways that the repeated sequence can come together. One of the strands of DNA can loop out, which will result in chromosomes with two differently sized **VNTRs** after the next round of DNA replication (5 & 6).



One of the tools that molecular biologists rely on is the use of **restriction enzymes**. Restriction enzymes are proteins isolated from bacteria. They protect the bacteria from attacks from bacterial viruses by cutting the viral DNA. Molecular biologists use restriction

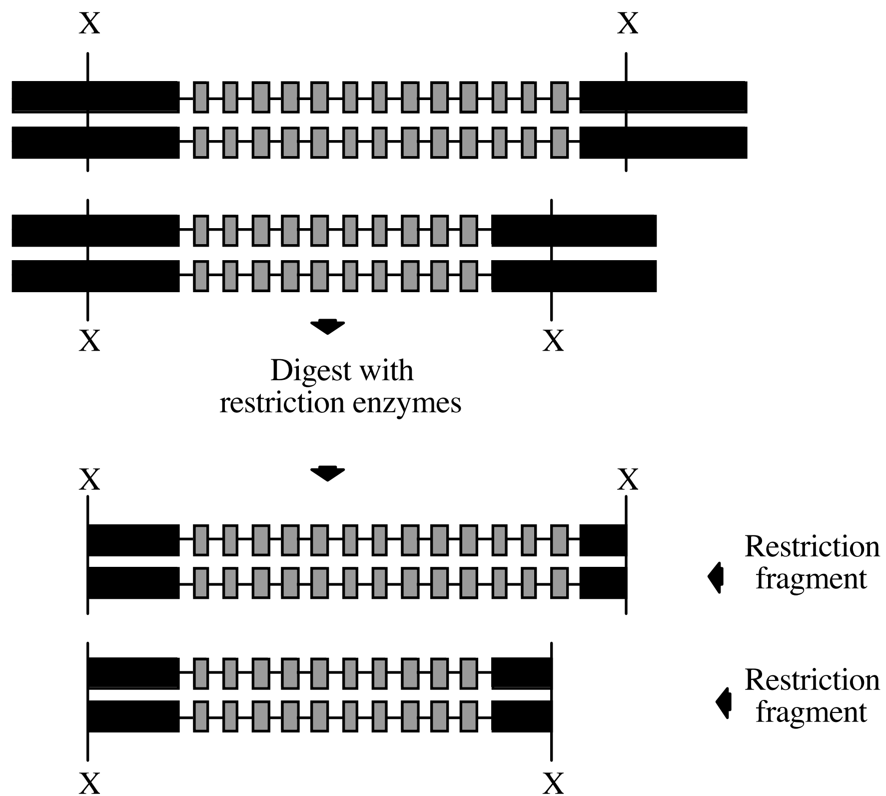
enzymes because they recognize unique DNA nucleotide sequences and cut the DNA at that site. The sequences recognized by restriction enzymes are known as **restriction sites**. Restriction sites usually range from four to eight basepairs in length and are usually palindromic sequences. Palindromic sequences are sequences that are the same on both strands of the DNA, though in the opposite order.

```
5'-ACGCGT-3'
3'-TGCGCA-5'
```

Notice the sequence of **both** strands, when read 5' to 3', is ACGCGT. Since the strands run in opposite directions, this sequence can basepair with itself.

Most DNA molecules found in nature are much too large to be analyzed without breaking them down into smaller pieces. Restriction enzymes are used to cut DNA into manageable pieces. Since restriction enzymes cut DNA at specific sequences, they will cut DNA molecules with the same sequence at the same sites, which will result in DNA fragments of the same size. On the other hand, if there is a different amount of DNA sequence between the restriction sites, the resulting DNA pieces will be of different length.

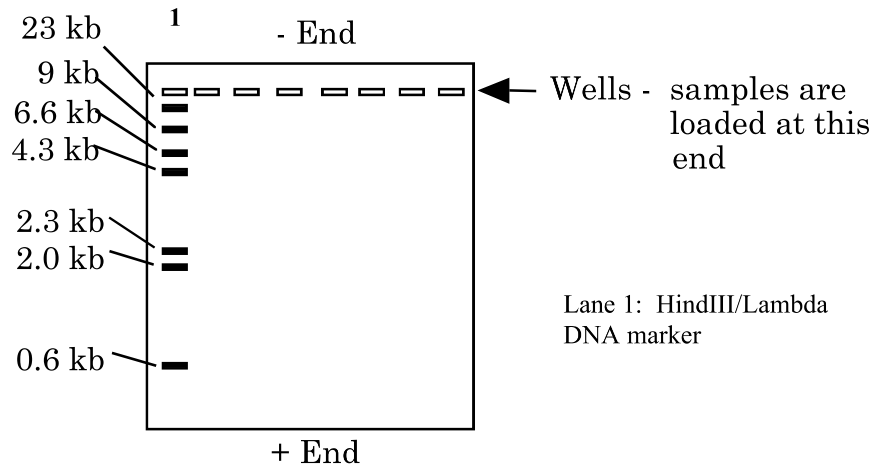
For example, look at the figure on the next page. If restriction enzymes were used to cut at the sites marked by "X", they would cut out a smaller piece of DNA from the top DNA strand than they would from the lower DNA strand. Sites where cutting with restriction enzymes would result in different lengths of DNA between different members of a population, such as at VNTRs, are called **restriction fragment length polymorphisms** (**RFLP**s).



Pre-Lab Questions

1. What is a gene?

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?



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?

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?

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?

Precautions

1. DNA samples and enzymes should be stored in the freezer between laboratory periods. At room temperature, enzyme activity will be rapidly lost and plasmid DNA may be degraded by contaminating enzymes from your hands. **Enzymes should be kept in the freezer right up until use (or kept on ice while being used).**

2. **To avoid cross contamination of reagents, use a fresh sterile micropipettor tip for each transfer.**

3. Collect all plasmid-contaminated micropipette tips, microfuge tubes, etc., into a specially marked beaker. Your teacher will dispose of these.

4. When you have finished working with the plasmid DNA, wash your hands and bench surface with soapy water.

5. Wear gloves when touching the gel after you add the GelStar® to the gel.

Digestion of DNA with Restriction Enzymes

In order to analyze your DNA samples, it is necessary to digest them with the restriction enzyme HindIII. HindIII is an enzyme that cuts DNA specifically at the sequence AAGCTT.

1. Discuss with your group how you want to set up your experiment before you begin. Record the DNA digests that you want to set up in the chart below. The number of wells in the gel limits the number of samples that you will be able to analyze. Because of this, each pair of students will be able to do a **maximum** of six samples. It is also important to take into consideration what controls you should do for this experiment. Note: your controls or "-" tubes should contain the same total volume as your non-control or "+" tubes.

2. Each digest (non-control or "+") should contain:
   - Distilled water:............ 12 µl
   - Buffer: ....2 µl
   - DNA sample: ....5 µl
   - HindIII........................1 µl

Sample:	M3-/M4-	B3-/B4-	S3-/S4-	M3+/M4+	B3+/B4+	S3+/S4+
Distilled water						
Buffer						
Case 3 or 4 DNA samples						
HindIII (enzyme)						

3. Each team of 2 students gets 6 microfuge tubes and labels them on the lid according to their case number.

4. **Use a P20 micropipettor to add chemicals to each tube as detailed in the table you made above. Pipette each component directly into the bottom of the tube.** You can make a check mark on the table as you add a component to help you keep track of your additions.

5. Place all six tubes in the microcentrifuge and spin for 5 seconds to make sure that all the liquid is combined in the bottom of the tube.

6. Incubate at 37°C for 30 minutes in the incubator block. The timing of this step is not critical. The 1µl of HindIII enzyme that you add is enough to cut most of the DNA in your samples in 30 minutes, but it will not hurt your samples if they incubate for a longer period of time.

Preparation of Agarose Gel

1. The group of students that is to prepare the 0.5 X TBE buffer will need to add 200 ml of distilled water to the bottle of TBE powder (USB #70454). **Mix thoroughly.** This is now a 10X concentration of the TBE buffer. Pour 100 ml of the 10x TBE into a 2-liter beaker or Erlenmeyer flask. Carefully add 1900 ml of distilled water and mix until fully dissolved. This is enough buffer for two gels. Share the extra buffer with another group of students. If only 1 liter of 0.5X TBE is to be prepared, then use 50 ml of the 10X concentrate and 950 ml of distilled water.

2. Each Investigatory group (4 students) will need to prepare a gel. Each pair of students in the Investigatory group will load their samples on to this gel.

    a. Weigh out 1.0 gram of agarose and add it to 100 ml of 0.5X TBE buffer in a 250 ml Erlenmeyer flask.

    b. Place a magnetic stir bar into the flask and heat the flask on a stirring hot plate until the agarose particles are fully dissolved. The solution will have to boil for several minutes for this to occur. If a magnetic hot plate isn't available, heat the materials in a 250 ml or 500 ml beaker and **stir frequently**. The agarose solution will look clear when the agarose is completely dissolved. Alternatively, a microwave oven can be used for this purpose if it is available. If a microwave is used, microwave on high for 1 minute, then swirl the flasks. If the agarose is not completely dissolved, microwave for a few seconds longer until it is.

    c. Once the agarose is fully dissolved remove the flask from the hotplate, insert a thermometer, and allow the solution to cool to about 75°C.

3. a. While the agarose is cooling, the next step is to prepare the casting tray. Place the casting tray in the gel running apparatus so that the open ends of the casting tray are facing the sides of the gel running apparatus. The gaskets should make a seal with the sides of the gel box. To prevent leaking, make sure that the gaskets haven't popped out of their grooves when you have pushed the casting tray in the gel box.

b. Add 2 µl of GelStar® to 100 ml of the cooled agarose before pouring the gel. **Wear gloves** when handling the GelStar® stain.

c. Pour the cooled solution into the tray to a height of approximately 0.8 to 1 cm.

d. After the gel is poured, place the comb so that it fits in the slots that are closest to the end of the gel-casting tray. There are two sets of slots in the gel casting tray, one close to the end of the tray and one in the middle of the tray. **Do not** use the slots in the middle of the tray. You will notice that the green comb has teeth on both sides. One set of teeth is slightly thicker than the other. Position the comb so that the thicker teeth are facing down into the gel.

e. Leave the gel undisturbed for 20 minutes until the agarose becomes firm and opaque.

4. a. When the gel has cooled, carefully lift the gel casting tray and turn it 90˚. The gel should be positioned so that the comb/wells are by the negative (black) terminal of the gel box. There should be a piece of red tape marking the negative terminal on the bottom of the gel box.

   b. Add 0.5X TBE buffer to the gel chamber until the level reaches the black fill line. The gel should be fully covered by about 2 - 3 mm of buffer.

   c. Remove the comb by gently **pulling it straight upward**. If the gel moves during this process, gently push it back into place using the blunt end of a pipette tip. You will load your samples into the slots created by the comb.

Loading of the Gel

1. Each pair of students will load 7 of the 20 wells in the gel (you will have 6 extra wells). In addition to your six samples, your teacher will also give you a sample of DNA size standard. This size standard is actually the genome of a virus, bacteriophage λ (lambda) that has been previously digested with HindIII into fragments of known length (see the figure in Prelab Question 2 for the size of the HindIII/λ fragments). Remove the restriction digests from the incubator.

2. Use a P20 micropipette to add 5 µl 5X loading dye to each sample. The loading dye is a mixture of an indicator dye with a dense molecule called glycerol. Place all six of the tubes into the microcentrifuge and spin for 5 seconds to make sure that all the liquid is combined in the bottom of the tube.

3. Before you start loading, organize your samples in the correct loading order in your tube rack (see below). It is important that you do this before you start loading your gel, since if you take too long when loading the gel, your samples will diffuse out of their slots. It also is helpful if one team loads their DNA standard in one of the middle lanes and the other loads it in one of the outside lanes. This asymmetry will allow you to tell if you

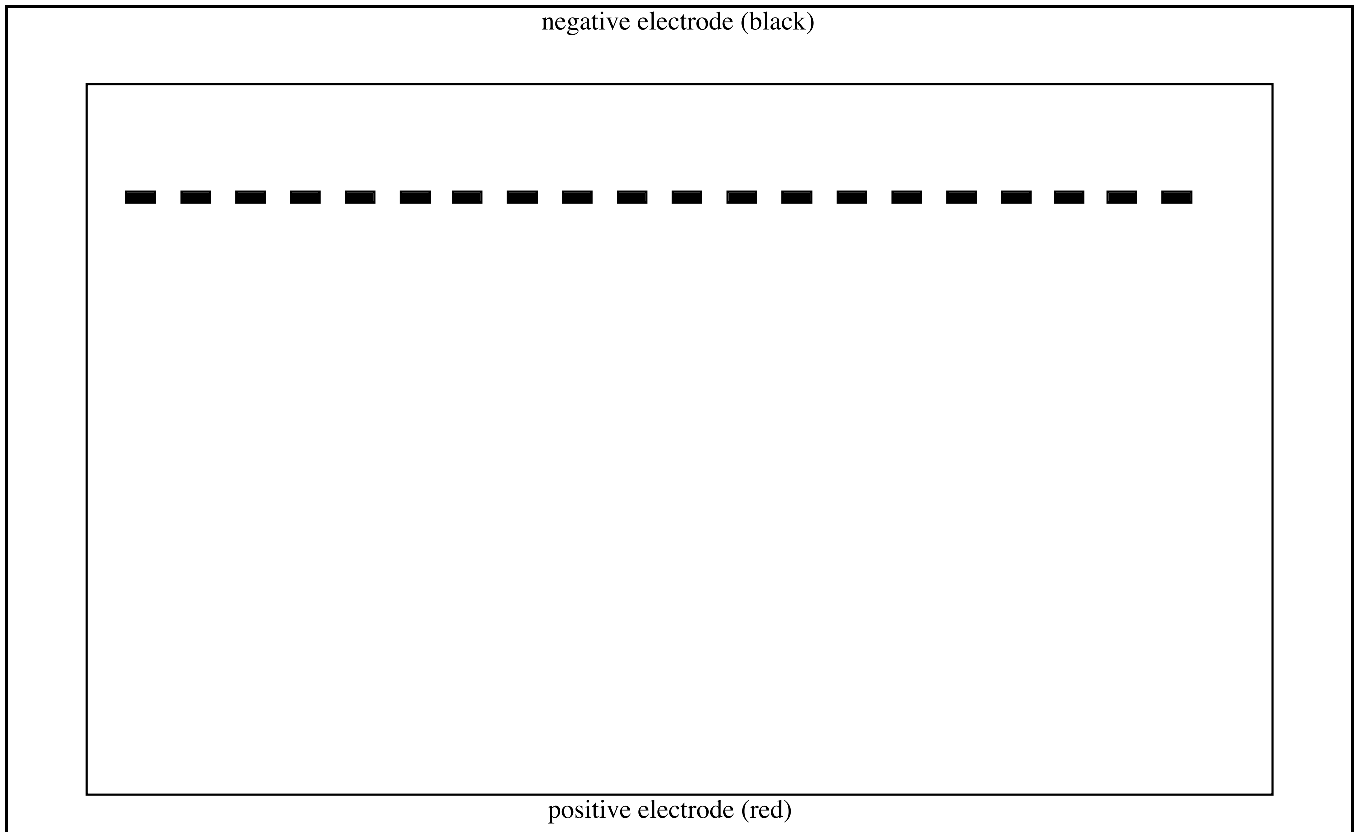
have flipped the gel over. Record the order in which you are loading your samples and any deviations from the default order on the gel diagram (next page).

Default Order:

1. M3- (uncut mother DNA - case 3)
2. B3- (uncut baby DNA - case 3)
3. S3- (uncut suspect DNA - case 3)
4. M3+ (HindIII cut mother DNA - case 3)
5. B3+ (HindIII cut baby DNA - case 3)
6. S3+ (HindIII cut suspect DNA - case 3)
7. HindIII cut λ DNA (size standard provided by your teacher)
8. M4- (uncut mother DNA - case 4)
9. B4- (uncut baby DNA - case 4)
10. S4- (uncut suspect DNA - case 4)
11. M4+ (HindIII cut mother DNA - case 4)
12. B4+ (HindIII cut baby DNA - case 4)
13. S4+ (HindIII cut suspect DNA - case 4)
14. HindIII cut λ DNA (size standard provided by your teacher)

4. Load 10 µl of each sample into the wells of the gel. The glycerol in the loading dye will make your samples dense so they will sink to the bottom of the slots in the agarose gel. Load your samples into the gel according to the default order. If you make a mistake in loading, make sure to note it on the diagram. Load **only 4 µl** of the DNA standard into the wells.

5. Place the cover on the apparatus and connect the leads to the power supply. Electrophorese at 180 - 195 volts for ~90 minutes or until the blue indicator dye is near the bottom of the gel. Gels can be run overnight at lower voltage (try 20 volts for 18 hours).



Viewing the Gel

1. Wearing gloves, gently transfer the gel to the Dark Reader for viewing. Place the orange filter screen over the gel. Turn on the dark reader.

2. Scientific results need to be documented in permanent form. A photograph of the gel could be used as evidence in the courtroom (the gel itself would break down over time). We recommend using a digital camera to take a photograph of the gel. We have found that digital cameras work best with a dark room and with the camera flash turned off. We also recommend using a ring stand and a clamp or a tripod to steady the camera.

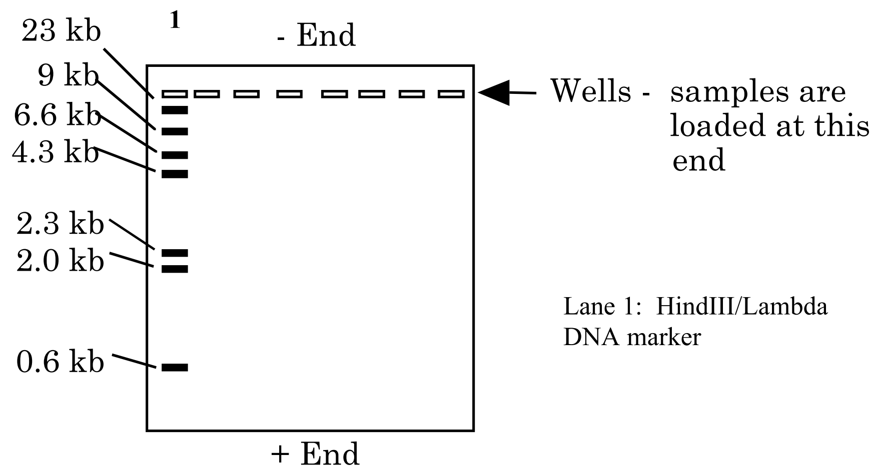
3. Once the pictures are taken, you can add labels to each of the lanes using programs such as Photoshop™ or PowerPoint™, or by hand on the paper print out.

Results

To interpret the size of each of your HindIII λ bands compare the spacing between the stained bands visible on your gel to the figure given below. The figure gives the expected banding pattern for the size standard and the size of each fragment (the smallest band may not be visible on your gel).

a. From your gel photo (or from the gel itself) measure the distance from the well to each of the bands.

b. For each of the 6 (or 7) bands, plot the distance migrated from the well on the X-axis and the size of the DNA fragment on the log scale (Y-axis). You can plot this using a piece of semi-log paper or using a computer graphing program (Note: If using a computer graphing program, make sure that the Y axis is set to a log scale). Connect the points with a smooth curve. This is a standard curve and can be used to determine the size of the linear fragments in samples treated with restriction enzymes for that particular gel.



c. For each sample compare your controls to the sample to which you added HindIII restriction enzyme. Do they look similar or different? If they look the same, there was a problem with the restriction enzyme and it did not cut the DNA properly. Hopefully this will not be the case and you can proceed to analyze the evidence.

d. If the samples are cut, you should see two bands in each lane. These bands should look different from those in the lanes containing no restriction enzymes. Human beings have 23 pairs of chromosomes. Each band corresponds to the same region in each of the homologous chromosomes in a pair. To determine the identity of the father compare the banding pattern of the potential father and the mother with the banding pattern of the child. What do you see? Are there any matching DNA fingerprints? If so, what do they mean?

Name: ________________________________ Date: ___________________

Post-Lab Questions

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

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.

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

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

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 of these possibilities to appear at the same frequency?

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

Glossary 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**).