PAPER RFLP
TEACHER GUIDE

Paper = DNA
Scissors = Restriction Enzyme
Desktop = Electrophoresis

NOTE: There are TWO versions of this activity – one where the students write their own sentences (to represent DNA segments) on graph paper, and another where they are given paragraphs about elephants (pages 38-40) to cut into strips. The graph paper version is more direct and takes less time, but the elephant-paragraph version allows for more discussion.

MATERIALS:
Graph paper (1/4 inch, half sheet)
Or
Elephant paragraphs
Scissors
Tape or glue

OBJECTIVES:

- To understand how DNA is analyzed in forensics, diseases, paternity, species comparison, ancient DNA, mutations, and preparing a DNA fragment for recombinant DNA work.
- To understand the basic principle of how endonucleases (restriction enzymes) are used to fragment DNA into smaller pieces.
- To introduce and explain RFLP's—Restriction Fragment Length Polymorphisms, an understanding of which will be critical for the upcoming lab on elephant DNA and ivory.

Getting Started: Students get instruction sheet, paper paragraph or graph paper, scissors, tape and cut out their paragraphs into strips, taping them end-to-end into one long continuous statement.

In **Part 1**, students should find it quite easy to see that they have a different banding pattern because they have a different sentence (DNA) than other students. They should relate this to the concept that different DNA cut with the same enzyme results in different banding patterns.

In **Part 2,** students should notice that their banding pattern has changed because the second sentence is not identical. One sentence has an addition or a deletion of a word, perhaps adding a restriction site or making one band considerably longer, therefore changing the banding pattern.

Be sure to direct the students toward the concept of POLYMORPHISMS:

RFLP'S AND POLYMORPHISMS

RFLP's are "Restriction Fragment Length Polymorphisms"

RFLP's are the structural variations in DNA between alleles and can be used as genetic markers to map genes to specific locations on a chromosome, to diagnose disease or as in this simulation of DNA "fingerprinting".

Polymorphisms are differences in a DNA sequence because of mutations (changes such as single base pair changes, deletions, insertions, repetitions and substitutions) in the sequence. Polymorphisms are used by scientists as DNA markers.

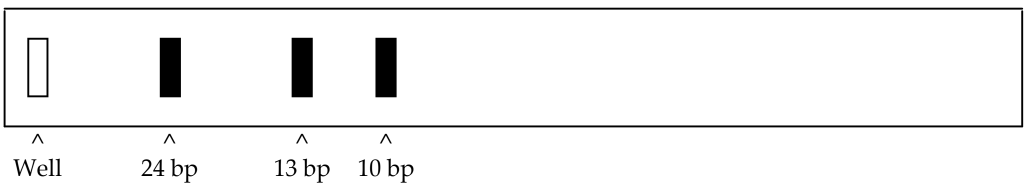
When DNA is cut into fragments by restriction enzymes, the polymorphisms will cause the DNA to be cut in different fragments.   When the DNA is electrophoresed, there will be new banding pattern. Every living organism (except exact clones/identical twins) has harmless polymorphisms that make their banding pattern unique.

EXAMPLE: RFLP due to an 8bp repetitive DNA insertion

DNA SAMPLE A

```
5'            Bam HI                                   Bam HI             3'
G GATAG\GATCCCACACACAGAAAGCGATCG\GATCCATGATAGC

C CTATCCTAG\GGTGTGTGTCTTTCGCTAGCCTAG\GTACTATCG
3'     10 bp                    24 bp                       13 bp        5'
```



DNA SAMPLE B

```
5'          Bam HI                                        Bam HI            3'
G GATAG\GATCCCACACACACACACACACAGAAAGCGATCG\GATCCATGATAGC

C CTATCCTAG\GGTGTGTGTGTGTGTGTCTTTCGCTAGCCTAG\GTACTATCG
3'     10 bp                      32 bp                        13 bp        5'
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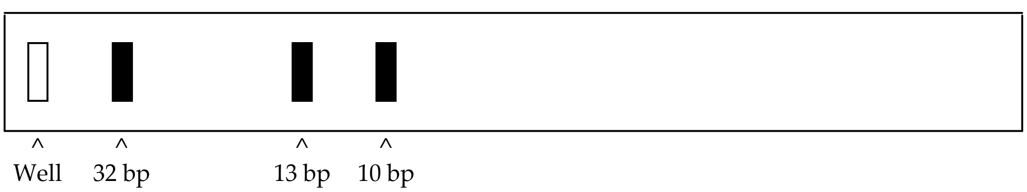

RFLP LEARNING ASSESSMENT
CHECKING FOR UNDERSTANDING
TEACHER GUIDE

One of the simplest ways to collect large amounts of DNA for analysis is from cells found in the elephant's dung. This is exactly what Wasser and Comstock and other scientists use for their DNA analysis of elephant families and individuals.

Below, you are given two sections of DNA that represent segments from elephants. Using what you know about BamHI, RFLPs and electrophoresis, **cut the following DNA segments, and tape/glue the pieces (according to how they would electrophorese) to the picture of the gel on the following page. Answer the questions.**

Dung Pile X

T A A A **G G A T C C** C C T A G C T A **G G A T C C** T A T A T A C C A T A T A C G A G
A T T T **C C T A G G** G G A T C G A T **C C T A G G** A T A T A T G G T A T A T G C T C

Dung Pile Y

T A A A **G G A T C C** C C T A T A T A C C C G A G G T A C C A **G G A T C C** T A G T C
A T T T **C C T A G G** G G A T A T A T G G G C T C C A T G G T **C C T A G G** A T C A G

RFLP ASSESSMENT **NAME** ______________________

PERIOD ______ **DATE** __________

Dung Pile X	Dung Pile Y
	GATCCCCTATATACCCGAGGTACCAG GGGATATATGGGCTCCATGGTCCTAG
GATCCTATATACCATATACGAG GATATATGGTATATGCTC	
GATCCCCTAGCTAG GGGATCGATCCTAG	
	GATCCTAGTC GATCAG
TAAAG ATTTCCTAG	TAAAG ATTTCCTAG

1. Compare and contrast the banding patterns between X and Y.
   Both samples show three bands. The smallest band is the same in both samples, but they differ in the sizes of the other bands.
2. How many restriction sites does each linear DNA sample have? *X has 2, Y has 2*
3. Explain why the two dung piles have different DNA banding patterns using the concept of RFLPs.
   They have different patterns because the inherited DNA sequences are different, and the restriction sites may be in different places. Consequently, when BamHI cuts the linear DNA, the fragments vary in size, and therefore move different distances through the gel.
4. If the elephants are sisters, what might we expect to see in their RFLP patterns?
   They might have patterns that are very similar because they are related and would have the same parents. However, the patterns would not appear exactly the same because each individual has their own unique polymorphisms, and will show their own unique banding pattern.
5. Predict what you would expect to see if the elephants are NOT related?
   They might have some bands in common because they are the same species, but many bands would be different because they have different parents.

PAPER RFLP

Paper = DNA
Scissors = Restriction Enzyme
Desktop = Electrophoresis

OBJECTIVES*:* To help you understand how DNA is analyzed for forensics, diseases, paternity, species comparison, ancient DNA, mutations, and preparing a DNA sequence for recombinant work. Keep in mind, this simulation is meant to give you the basic idea of how fragments of DNA can be separated into pieces and studied.

MATERIALS: A half sheet of 1/4inch graph paper, scissors and tape (or glue).

GET READY: (ISOLATING YOUR PAPER DNA)

1. On your half sheet of graph paper, write a sentence that only you will know. Put each letter or punctuation mark in a square, and use a square for a space between each word. The sentence should be no more than two lines in length.
2. Make a second copy of your sentence, but in this one, make a change by adding or omitting a word. Try to make this sentence still make sense like: "My dog has fleas" and "My dog has many fleas."
3. Using scissors cut the **first** sentence into strips and carefully tape or glue the ends together so that it is one long strip. Now, this sentence pieced together end-to-end will be used to represent a unique strand of DNA that might be isolated from a tissue sample.

PART 1

THE RESTRICTION DIGEST: Cut up the DNA into fragments

Endonucleases occur naturally in most bacteria, and act as the bacteria's defense system to "restrict" the growth of invading viruses by breaking apart the virus' DNA. Scientists have learned to isolate these "restriction enzymes" and use them to cut desirable DNA into smaller pieces. When these pieces are loaded into the well of an agarose gel and electrophoresed, the fragments will move through the gel at different rates and become separated.

1. The scissors represent the endonuclease. Use them to cut the DNA sentence after every letter "a."

THE ELECTROPHORESIS: Separate the fragments by size

2. Now, turn the sentence fragments over--printed side down-- and arrange them from largest (on your left) to the smallest (on your right).
3. Big fragments do not electrophorese very well—they get "stuck" in the gel, whereas little fragments are able to move great distances. Now, walk around the room, and observe other students' banding patterns and compare yours to theirs by looking at
    - the SIZE of the fragments
    - the NUMBER of fragments (bands) that show up on the tabletop "gel."

CLASS DISCUSSION: PART 1

4. Did anyone in the room have the same banding pattern as you? Why or why not?

PART 2

FINDING POLYMORPHISMS

1. Leave your first set of strips on the table, and repeat the process with your **second** copy—attaching the ends together into one long strip.
2. Now cut the second strip again after every letter "a," line them up from largest (on your left) to smallest, right next to your previous set of fragments.

CLASS DISCUSSION: PART 2

3. Describe HOW your second banding pattern is different from your first banding pattern, even though you cut the strips with the same endonuclease. What caused the differences between these two patterns?

PAPER RFLP
ELEPHANT PARAGRAPHS

Paper = DNA
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OBJECTIVES: To help you understand how DNA is analyzed for forensics, diseases, paternity, species comparison, ancient DNA, mutations, and preparing a DNA sequence for recombinant work. Keep in mind, this simulation is meant to give you the basic idea of how fragments of DNA can be separated into pieces and studied.

MATERIALS: A page with two copies of the same (similar) paragraph, scissors and tape (or glue).

GET READY: Isolating your paper DNA

1. Your class is provided with three different paragraphs that represent different DNA samples. Choose one of the three paragraphs. You will notice that you have two copies of the same (similar) paragraph.
2. Using scissors cut the first paragraph into strips (keep the strips in order), and carefully tape the ends together so that it is one long strip. Treat spaces and punctuation in the paragraph as if they are letters too. Make the strip as neat as possible. Now, this paragraph pieced together end-to-end will be used to represent a strand of DNA that might be isolated from a tissue sample.

PART 1

THE RESTRICTION DIGEST: Cut up the DNA into fragments

Endonucleases occur naturally in most bacteria, and act as the bacteria's defense system to "restrict" the growth of invading viruses by breaking apart the virus' DNA. Scientists have learned to isolate these "restriction enzymes" and use them to cut desirable DNA into smaller pieces so that when these pieces are loaded into the well of an agarose gel and electrophoresed, the fragments will move through the gel at different rates, and therefore become separated.

1. The scissors represent the endonuclease. Use them to cut the DNA-paragraph between every "ea" (not "ae").

THE ELECTROPHORESIS: (Separate the fragments by size)

2. Now, turn the sentence fragments over--printed side down-- and arrange them from largest (on your left) to the smallest (on your right).
3. Big fragments can not electrophorese very well—they get "stuck" in the gel, whereas little fragments are able to move great distances. Now, walk around the room, and determine other tabletop "gels" that have the same banding pattern as yours by:
   - Comparing the SIZE of the fragments
   - Comparing the NUMBER of fragments (bands) that show up on the tabletop "gel"

CLASS DISCUSSION: PART 1

4. Was it easier to INCLUDE other groups as matching yours--or was it easier to EXCLUDE groups?

5. Was your banding pattern an EXACT match to another group? What might account for differences?

PART 2

Finding Polymorphisms

1. Now repeat the process with the second paragraph. Prepare it into strips, tape the ends together, and then cut between every "ea" combination. Lay out your fragments on your tabletop, and compare your second banding pattern with your first banding pattern.

CLASS DISCUSSION: PART 2

2. Describe HOW your second banding pattern is different from your first banding pattern.
3. What caused the differences between these two patterns?

An elephant herd depends heavily on the knowledge and experience of the leader, who is an older female between 40 and 60 years old. During periods of drought she may be the only elephant in the herd who remembers the location of a distant water hole, where the richest food sources are and how to avoid hazards. As the matriarch, she leads the herd in times of danger, deciding whether to threaten, attack or retreat.

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Elephants greet each other with joyful trumpeting, vocal rumblings and ear-flapping. They twist their trunks together in a kind of "hug" and clack their tusks together in celebration. When threatened the herd forms a circle facing outward and the babies crowd into the center or stand beneath their mothers. The sight and sound of a herd of angry, trumpeting elephants usually frightens off predators such as lions and wild dogs.

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Terrifically strong and yet highly tactile and sensitive, an elephant's trunk is more versatile than a human hand. Baby elephants do not know how to use their trunks instinctively and have a lot to learn. With much practice and instruction from their mothers, they learn to eat and drink, mudsplash and dust, to lift and push objects, use branches and stones as tools, dig holes in the sand to find water, smell and vocalize or simply scratch an itch.

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Polymorphisms are differences in a DNA sequence because of mutations (changes) in the sequence. These changes can be single base pair changes, deletions, insertions, repetitions, and substitutions.

When DNA is cut into fragments by restriction enzymes, the polymorphisms will cause the DNA to be cut in different fragments. When the DNA is electrophoresed, there will be a different banding pattern. Every living organism (except exact clones/identical twins) has harmless polymorphisms that make their banding pattern unique.

Endonucleases (restriction enzymes) were discovered in the late 1960's and were found to be the natural weapons of bacteria to protect themselves against invading viruses. Some viruses have endonucleases as well, and use them to cut into a host cells DNA in order to insert their own. Endonucleases are named according to when and where they were isolated: BamHI came from genus *Bacillus*, species *amyloliquifaciens*, strain H and was the first (I) one isolated. Bam cuts DNA where ever it finds a palindrome of GGATCC, and cuts between the two Gs.

EXAMPLE: RFLP due to an 8bp repetitive DNA insertion

DNA SAMPLE A

5' Bam HI Bam HI 3'

G G A T A G \ G A T C C C A C A C A C A G A A A G C G A T C G \ G A T C C A T G A T A G C

C C T A T C C T A G \ G G T G T G T G T C T T T C G C T A G C C T A G \ G T A C T A T C G

3' 10 bp 24 bp 13 bp 5'

^ Well ^ 24 bp ^ 13 bp ^ 10 bp

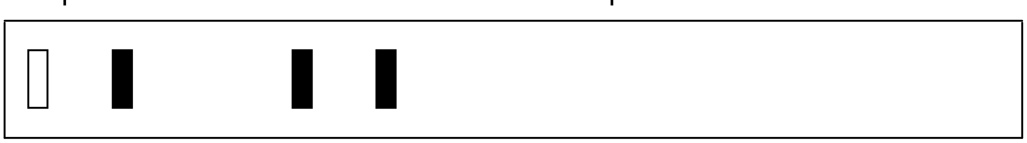
DNA SAMPLE B

5' Bam HI Bam HI 3'

G GATAG\GATCCCACACACACACACACAGAAAGCGATCG\GATCCATGATAGC

C CTATCCTAG\GGTGTGTGTGTGTGTGTCTTTCGCTAGCCTAG\GTACTATCG

3' 10 bp 32 bp 13 bp 5'



^ Well ^ 32 bp ^ 13 bp ^ 10 bp

RFLP LEARNING ASSESSMENT
CHECKING FOR UNDERSTANDING:

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Below, you are given two sections of DNA that represent segments from elephants. Using what you know about BamHI, RFLPs and electrophoresis, **cut the following DNA segments, and tape/glue the pieces (according to how they would electrophorese) to the picture of the gel on the following page. Answer the questions.**

Dung Pile X

T A A A G G A T C C C C T A G C T A G G A T C C T A T A T A C C A T A T A C G A G
A T T T C C T A G G G G A T C G A T C C T A G G A T A T A T G G T A T A T G C T C

Dung Pile Y

T A A A G G A T C C C C T A T A T A C C C G A G G T A C C A G G A T C C T A G T C
A T T T C C T A G G G G A T A T A T G G G C T C C A T G G T C C T A G G A T C A G

RFLP ASSESSMENT **NAME** ____________________________

PERIOD ______ **DATE** _________

Dung Pile X	Dung Pile Y

1. Compare and contrast the banding patterns between X and Y.

2. How many restriction sites does each DNA sample have? X____ Y____

3. Explain why the two dung piles have different DNA banding patterns using the concept of RFLPs.

4. If the elephants are sisters, what might we expect to see in their RFLP patterns?

5. Predict what you would expect to see if the elephants are NOT related?