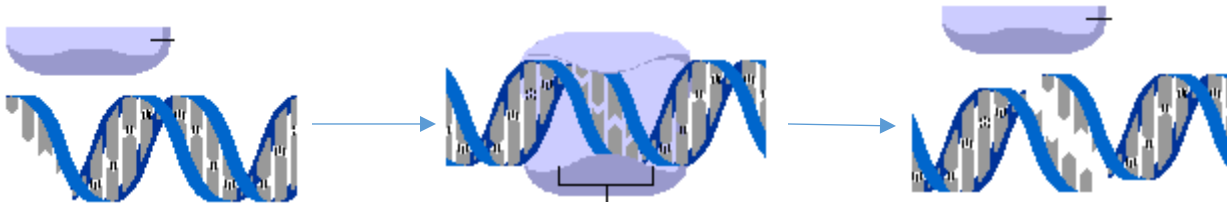


## Lab 9 Restriction Enzyme Analysis

[http://www.phschool.com/science/biology\\_place/labbench/lab6/concepts2.html](http://www.phschool.com/science/biology_place/labbench/lab6/concepts2.html)

- 1) Define restriction enzyme
- 2) Define recognition sequence
- 3) Label the images below **AND** explain how restriction enzymes work



- 4) What was the significance of the discovery of restriction enzymes?
- 5) What does gel electrophoresis do?

Click Next Concept

- 6) Define and give an example of a palindromic recognition sequence.

Click Next Concept

- 7) Define microliter
- 8) What is the symbol for microliter?

Click Next Concept

9) What determines the direction molecules move through gels?

10) What determines the rate molecules move through gels?

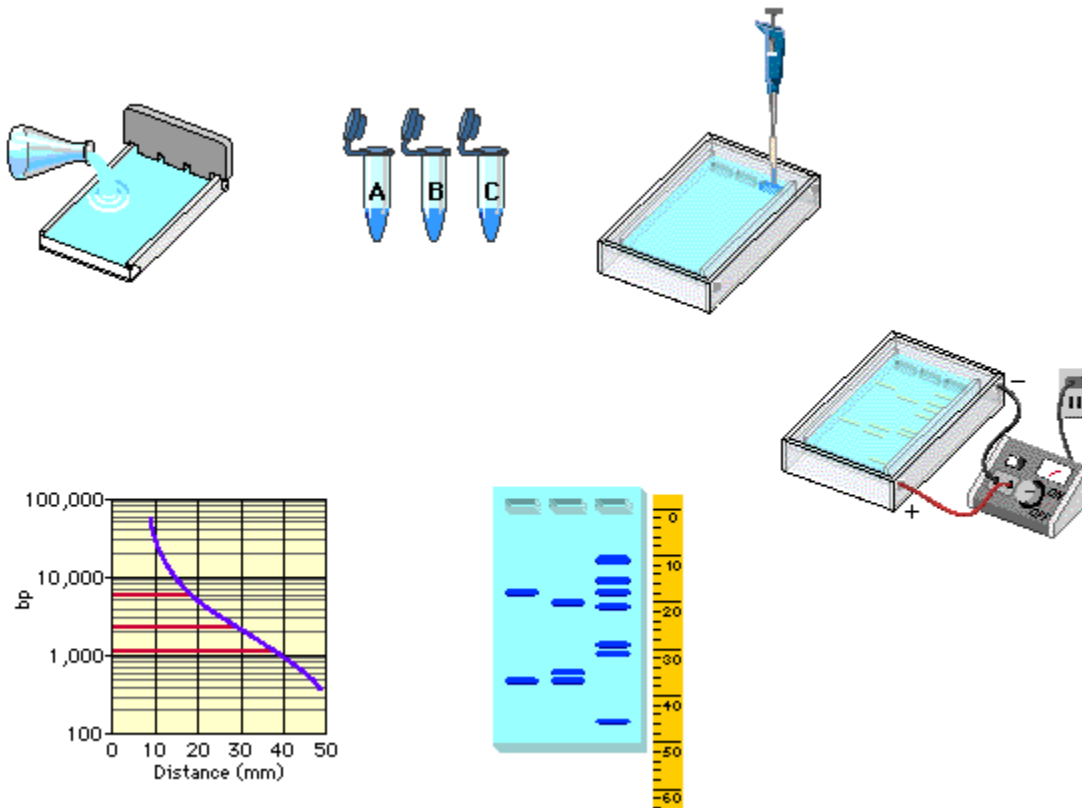
11) What direction does DNA move through a gel and why?

12) Describe the relative sizes of the DNA fragments in the different bands below



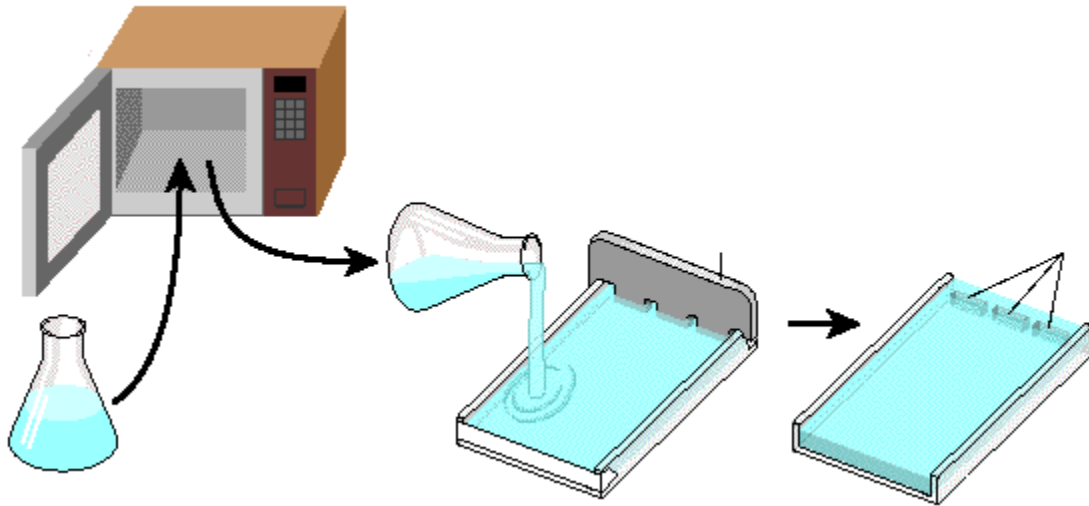
Click Next

13) Label the diagram below



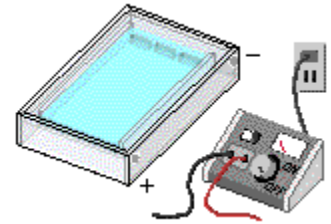
Click Next

14) Fill in the diagram below



15) What do you do after making the gel?

16) Which electrode end is DNA loaded in?



17) Explain why DNA is added to the end it is. You need to use an outside source to answer this question.

Click Next

18) What is the purpose of the dye?

19) Does the dye stain DNA?

20) What is a micropipette?

Click Next

21) Describe how to load a gel with DNA

Click Next and then Next again

22) Can you see the DNA?

23) What can you see?

Click Next

24) What is methylene blue used for?

25) Why do you repeatedly rinse the gel?

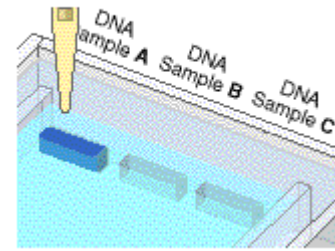
Click Next

26) How do scientists determine fragment sizes using gel electrophoresis?

27) What is the DNA cut with the restriction enzyme HindIII used for?

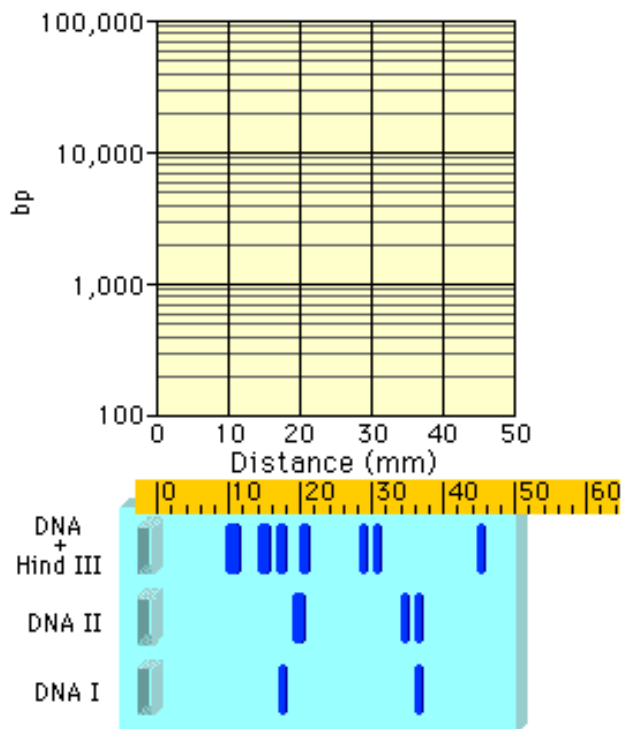
Click Next

## Filling the Wells

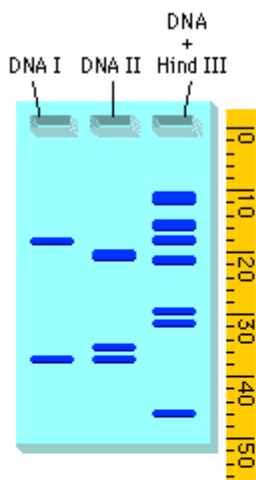


Graph a standard curve based on the know fragment lengths produced by Hind III

## Making a Standard Curve



28) Fill in the data table below



Actual Base Pairs (bp)	Measured Distance (mm)
23,130	<input type="text"/>
9416	<input type="text"/>
6557	<input type="text"/>
4361	<input type="text"/>
2322	<input type="text"/>
2207	<input type="text"/>
564	<input type="text"/>

[check answer](#)

Click Next

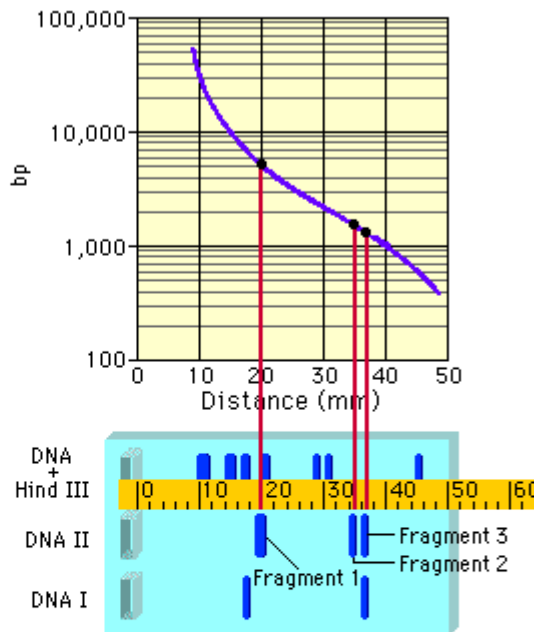
29) What is a standard curve and what is it used for?

Click Next

30) Answer the problem below

### Practice Problem 1

1. Find the distance migrated for each unknown fragment size of the sample DNA II.
2. Locate this distance on the graph and follow the line to its intersection with the standard curve.
3. Read the number of base pairs in the unknown fragment from the graph. Record your answers in the table below.

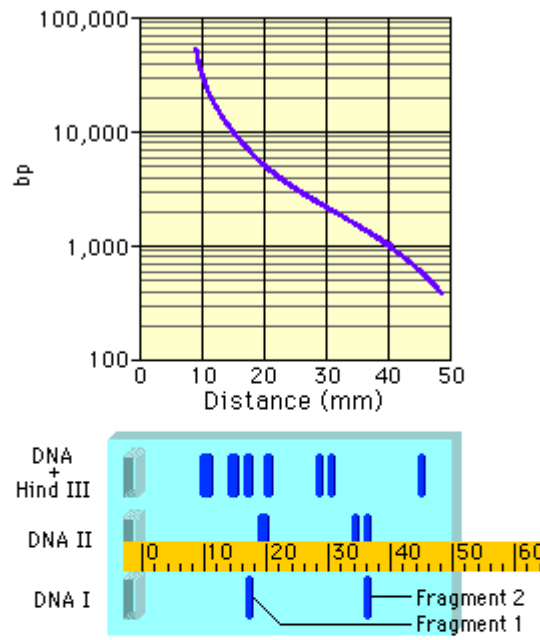


	Distance Migrated (mm)	Interpolated Fragment Size (in base pairs)
Fragment 1	<input type="text"/>	<input type="text"/>
Fragment 2	<input type="text"/>	<input type="text"/>
Fragment 3	<input type="text"/>	<input type="text"/>

Click Next

31) Answer the problem below

### Practice Problem 2



What are the two fragment sizes in DNA I?

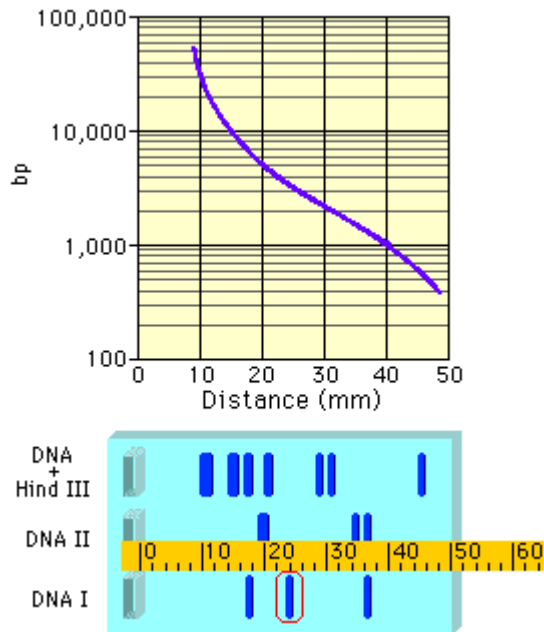
	Distance Migrated (mm)	Interpolated Fragment Size (in base pairs)
Fragment 1	<input type="text"/>	<input type="text"/>
Fragment 2	<input type="text"/>	<input type="text"/>

[check answer](#)

Click Self-quiz

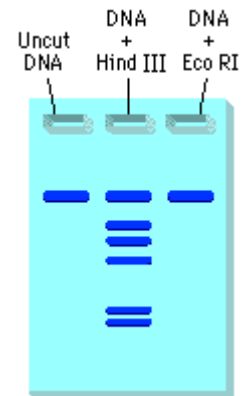
32) Which of the following statements is correct? (write down the correct statement)

33) How many base pairs is the fragment circled in red below?



34) An instructor had her students perform a lab beginning with setting up their own restriction enzyme digests. One team of students had results that looked like those to the right.

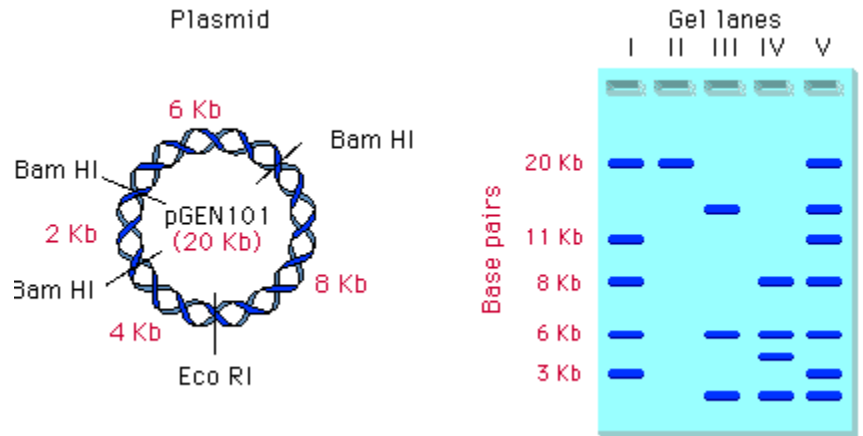
What is the most likely explanation for these results?





Below is a plasmid with restriction sites for BamHI and EcoRI. Several restriction digests were done using these two enzymes either alone or in combination. Use the figure to answer questions 4–6.

**Hint:** Begin by determining the number and size of the fragments produced with each enzyme. "kb" stands for kilobases, or thousands of base pairs.



35) Which lane shows a digest with BamHI only?

36) Which lane shows a digest with EcoRI only?

37) Which lane shows the fragments produced when the plasmid was incubated with both EcoRI and BamHI?

38) A restriction enzyme acts on the following DNA segment by cutting both strands between adjacent thymine and cytosine nucleotides

.....TCGCGA.....    .....AGCGCT.....

Which of the following pairs of sequences indicates the sticky ends that are formed?

39) A segment of DNA has two restriction sites—I and II. When incubated with restriction enzymes I and II, three fragments will be formed—a, b, and c. Which of the following gels produced by electrophoresis would represent the separation and identity of these fragments?

