Gel electrophoresis: sort and see the DNA

Pre-class activity

1. How does the process of gel electrophoresis separate DNA fragments?

   It uses an electric current to separate different sized molecules of DNA in a porous sponge-like matrix.

2. What is the purpose of the agarose gel?

   To separate the different sized fragments of DNA.

3. What is the purpose of adding blue "tracking" dye to the DNA samples?

   It makes it easier to load the samples and visually track the migration of DNA through the gel.

4. Explain why DNA has an overall negative charge.

   Phosphate groups in the DNA backbone carry negatively-charged oxygen molecules giving the phosphate-sugar backbone of DNA an overall negative charge.

5. Why is the fact that DNA has a negative charge so important in the gel electrophoresis process?

   The negatively charged DNA can be pulled toward the positive field of the gel.

6. Explain how an agarose gel can separate DNA fragments of different lengths.

   Smaller fragments move faster, and therefore further, than larger fragments as they snake through the gel.

7. What is the purpose of ethidium bromide?

   Ethidium bromide is a dye used by scientists to see where the DNA fragments are located in the gel. It binds to the DNA and glows when illuminated with UV light.

8. Why is a marker used when running the fragments through the gel?

   A marker contains DNA fragments of known size. Markers are run in every gel for comparison with the unknown fragments in other gel lanes.

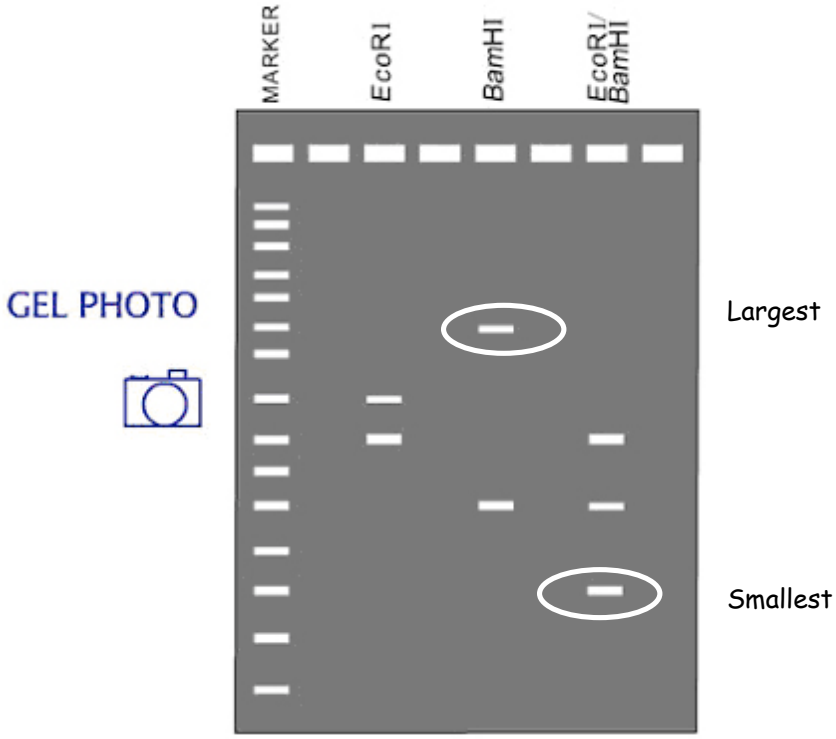
9. What is a restriction map?

   It shows where restriction enzymes cut the original piece of DNA.

10. On the gel picture below,

(a) circle the smallest fragment produced by a restriction enzyme and label it "smallest."

(b) circle the largest fragment produced by a restriction enzyme and label it "largest."



11. In one or two sentences, summarize the technique of gel electrophoresis. *Student answers*

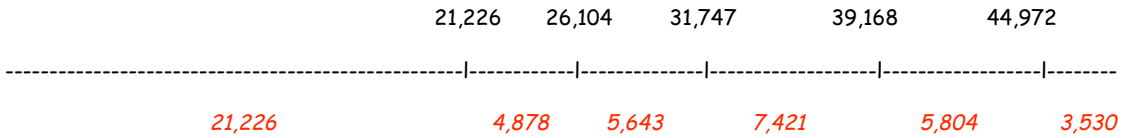
DNA restriction fragment size chart

*Eco*RI	*Hind*III	*Bam*HI	*Nco*I	*Bmr*I	*Stu*I
21,226	23,130	16,841	19,329	18,170	19,044
7,421	9,416	7,233	16,380	14,083	12,434
5,804	6,557	6,770	4,572	7,054	7,888
5,643	4,361	6,527	4,254	4,641	6,995
4,878	2,322	5,626	3,967	4,554	1,519
3,530	2,027	5,505			604
	564				18
	125				

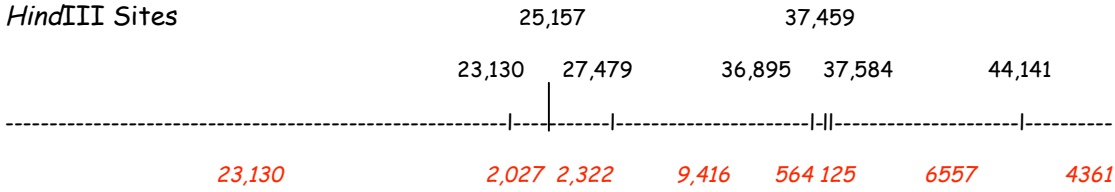
Restriction maps of the linear λ genome

Lambda (λ)

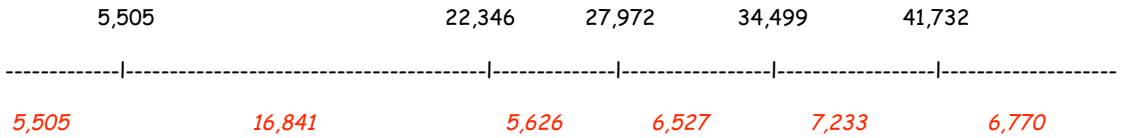
*Eco*RI Sites



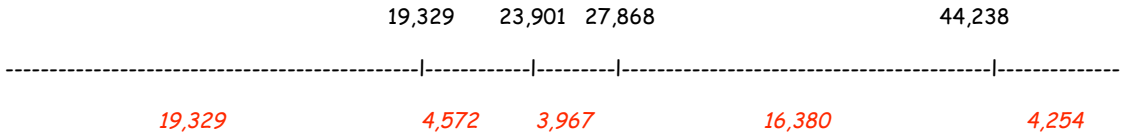
*Hind*III Sites



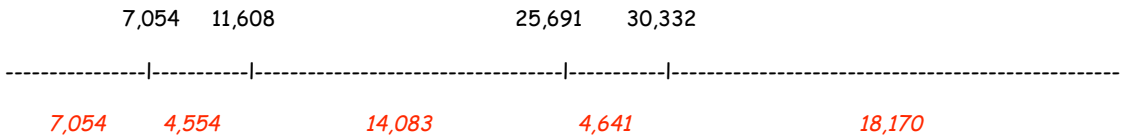
*Bam*HI Sites



*Nco*I Sites



*Bmr*I Sites



*Stu*I Sites

ANSWERS

DNA fingerprints

Marker	EcoRI	HindIII	BamHI	NcoI	BmrI	StuI
(50,000)						
(30,000)						
(20,000)						
(15,000)						
(10,000)						
(5,000)						
(2,500)						
(1,000)						