Lesson 1 Restriction Digests of DNA Samples

1. How many **pieces** of DNA would result from this cut ? __2__
2. Write the **base sequence** of the DNA fragments on both the left and right side of the "cut".

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

3. What differences are there in the two pieces?

   Each fragment is a different size.

4. DNA fragment **size** can be expressed as the number of **base pairs** in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect].

   One fragment is short and one is long; also some bases are unpaired.

   a) The smaller fragment is__3__ base pairs (bp).

   b) What is the length of the longer fragment ? __11__

5. Consider the two samples of DNA shown below [single strands are shown for simplicity]:

   Sample #1: **C A G T G A T C T C G A A T T C G C T A G T A A C G T T**

   Sample #2: **T C A T G A A T T C C T G G A A T C A G C A A A T G C A**

   If both samples are treated with a restriction enzyme [recognition sequence **GAATTC**] then indicate the number of fragments and the size of each fragment from each sample of DNA.

   Sample # 1 **Sample # 2**

   # of fragments:__2__ # of fragments:__2__

   List fragment size in ascending order: largest ——> smallest

   Sample # 1 **Sample # 2**

   17 bp fragment **23 bp fragment**

   11 bp fragment **5 bp fragment**

Lesson 1 Restriction Digestion of DNA Samples

Observation Questions

1. Describe the samples of DNA (physical properties).

   The DNA samples are clear, colorless liquid samples.

2. Is there any observable difference between the samples of DNA?

   No. All samples appear similar.

3. Describe the appearance of the restriction endonuclease mix.

   The restriction enzymes appear to be clear, colorless liquids.

Lesson 1 Restriction Digestion of DNA Samples

Review Questions

1. Before you incubated your samples, describe any visible signs of change in the contents of the tubes containing the DNA combined with the restriction enzymes.

   DNA + EcoRI/PstI enzyme mix:

   No visible change apparent in the tubes.

2. Can you see any evidence to indicate that your samples of DNA were fragmented or altered in any way by the addition of EcoRI/PstI? Explain.

   No. No visible change is apparent in the tubes.

3. In the absence of visible evidence of change, is it still possible that the DNA samples were fragmented? Explain your reasoning.

   Yes. They may be chemically changed but the changes may not be visible. Enzymes may have cut the DNA.

4. **After a 24 hour incubation period**, are there any visible clues that the restriction enzymes may have in some way changed the DNA in any of the tubes? Explain your reasoning.

   No. No visible change is apparent in the tubes but the enzymes may have cut the DNA. The reactions are at the molecular level and too small to be seen.

Lesson 2 Agarose Gel Electrophoresis

Review Questions

1. The electrophoresis apparatus creates an electrical field [positive and negative ends of the gel]. DNA molecules are negatively charged. To which pole of the electrophoresis field would you expect DNA to migrate (+ or -)? Explain.

   Positive.

2. What color represents the negative pole?

   The negative pole (or cathode) is black. The positive pole (or anode) is red.

3. After DNA samples are loaded in wells, they are "forced" to move through the gel matrix. Which size fragment (large vs small) would you expect to move toward the opposite end of the gel most quickly? Explain.

   Smaller. There is less resistance to their movement through the gel matrix.

4. Which fragments are expected to travel the shortest distance [remain closest to the well]? Explain.

   Larger. There is more resistance to their movement through the gel matrix.

Post-Lab Thought Questions

1. What can you assume is contained within each band?

   DNA fragments.

2. If this were a fingerprinting gel, then how many kinds (samples) of DNA can you assume were **placed in each separate** well?

   One.

3. What would be a logical explanation as to why there is more than one band of DNA for each of the samples?

   The DNA must have been cut into fragments by restriction enzymes.

4. What probably caused the DNA to become fragmented?

   The chemical action of the restriction enzymes cutting at specific base sequences.

5. Which of the DNA samples have the same number of restriction sites for the restriction endonuclease used? Write the lane numbers.

   Lanes 2, 3, and 4 (CS, S1, and S2).

6. Which sample has the smallest DNA fragment?

   The sample in lane 5 (S3).

7. Assuming a circular piece of DNA (plasmid) was used as starting material, how many restriction sites were there in lane three? Please note that the starting material was a circular piece of DNA.

   Two sites that cut the sample into two fragments.

8. From the gel drawing on page 35, which DNA samples appear to have been “cut” into the same number and size of fragments?

   Lanes 2 and 4 (CS and S2).

9. Based on your analysis of the example gel drawing on page 35, what is your conclusion about the DNA samples in the photograph? Do any of the samples seem to be from the same source. If so which ones? Describe the evidence that supports your conclusion.

   The DNA samples in lanes 2 and 4 (CS and S2) are from the same individual because they have identical restrictions sites that yield identical fragments.

	Lambda/HindIII size standard		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4***		Suspect 5***	
Band	Distance (mm)	Actual size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)
1	11.0	23,130	19.0	3,679	21.0	2,817**	21.0	2,817**	19.0	3,679	21.0	2,817**	21.0	2,817**
2	13.0	9,416	20.5	2,817**	23.5	1,191	25.0	1,700	20.5	2,817**	29.5	1,093	24.0	1,986
3	15.0	6,557	32.0	820	30.5	949	28.5	1,159	32.0	820			29.5	1,093
4	18.0	4,361*												
5	23.0	2,322												
6	24.0	2,027												

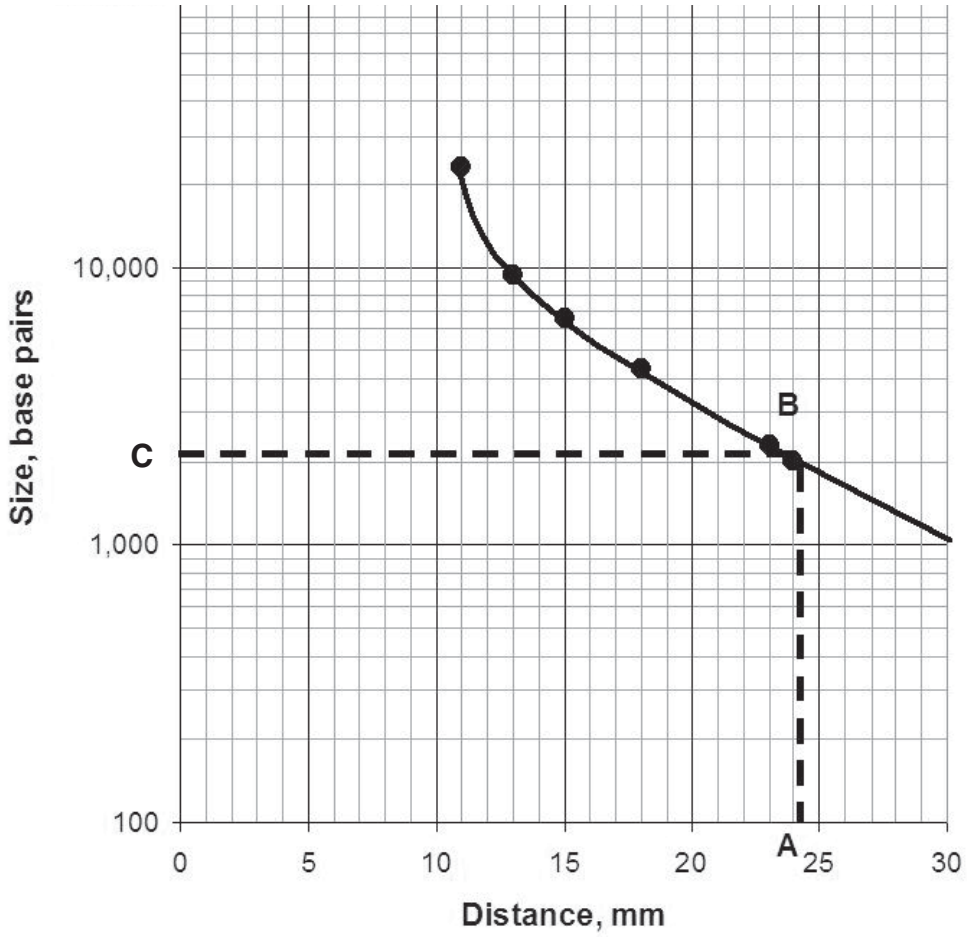
*This fragment may appear faint if the standards were not heated to 65 °C. Lamba HindIII digestion also generates bands of 564 and 125 bp that are usually too faint to see on a gel.

**The measured migration distance for these bands varies depending upon the thickness of the bands. The 2,817 bp bands in plasmids S4 and S5 are especially intense because they are actually two individual bands (2,817 bp and 2,838 bp) that are too close to be visibly separated.

***S4 and S5 DNA lanes may also contain a very faint band of 468 bp.

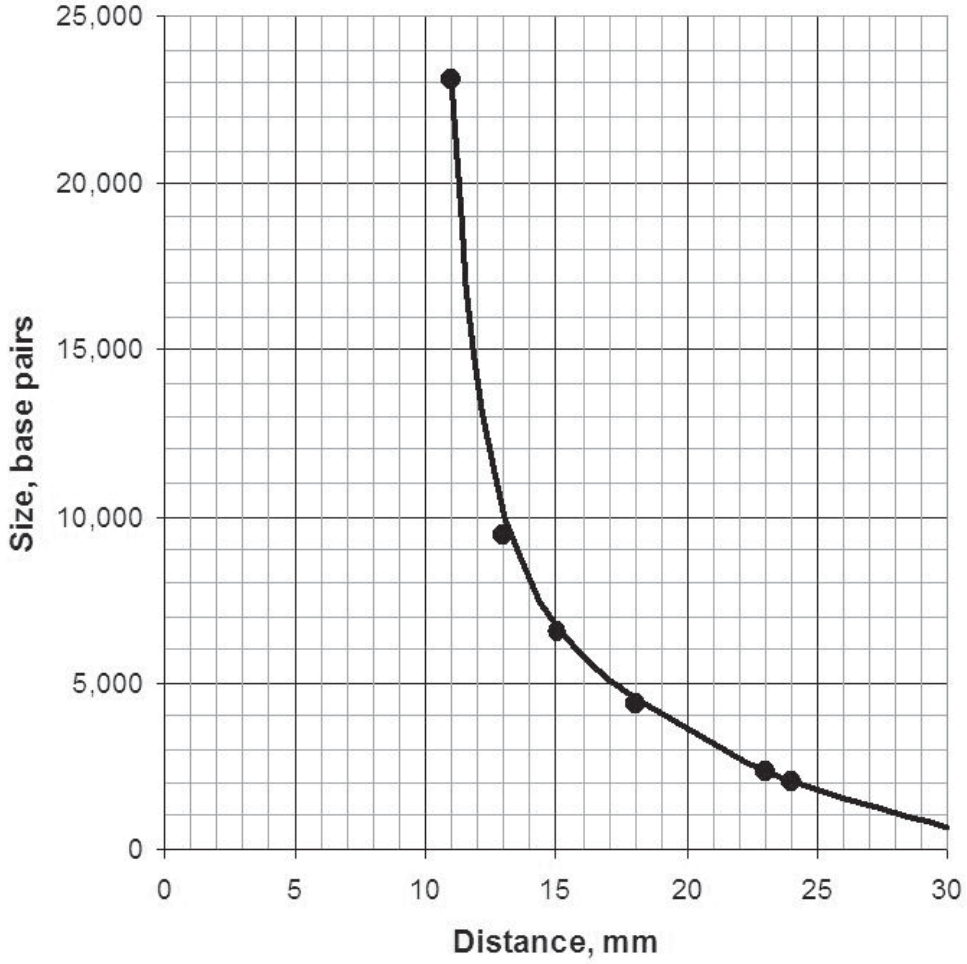
APPENDIX C

DNA Standard Band Migration



To estimate the size of any unknown crime scene or suspect fragment, you first need to determine the distances the specific fragment travelled. Locate the distance on the x-axis of your standard graph. For example, suspect 5, band 2 migrated 24 mm (A). From the 24 mm mark on the x-axis, read up to the standard line; when you intersect your standard curve, mark the spot with a shaded circle (B). Follow the intersect point over to the y-axis and determine where the graph line meets the y-axis this is the approximate size of the fragment (C). Therefore, suspect 5, band 2 is approximately 2,000 bp. Repeat this procedure for the crime scene and all suspects' fragments. As you determine the approximate fragment sizes, fill in the data in the data table.

Fingerprinting Standard Curve: Linear



Post Lab Activity: Interpretation of Results

1. What are we trying to determine? Restate the central question.

   We are trying to determine if samples of DNA that we were provided with are from the same individual or from different individuals.

2. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

   The number of fragmented samples will vary. They will have one band on the gel if the DNA was not cut.

3. What caused the DNA to become fragmented?

   The addition of restriction enzymes.

4. What determines where a restriction endonuclease will "cut" a DNA molecule?

   A special sequence of bases on the DNA called restriction sites.

5. A restriction endonuclease "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at that location?

   The restriction sites are identical.

6. Do any of your suspect samples appear to have EcoRI or PstI recognition sites at the same location as the DNA from the crime scene?

   The samples in lanes 2 and 5 match (CS and S3).

7. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.

   The CS and S3 samples appear to be identical. They both produce similar banding patterns on the gel.