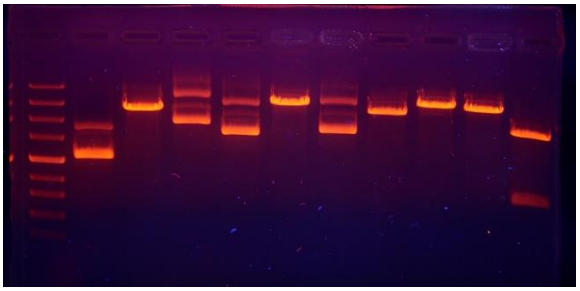
Working with DNA

Booklet 3: Restriction Enzyme Analysis Using Gel Electrophoresis



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Part A - Restriction Enzymes

1 a) In your own words, describe restriction enzymes.

_____

_____

_____

b) Identify at three applications (purposes) for using restriction enzymes.

_____

_____

_____

2. For each of the sequences below, a) identify the sites where the named restriction enzyme would cut. Then, b) identify the number of fragments and the length of each fragment.*

*For consistency, don't count the unpaired bases at the sticky ends. See the example.

Example sequence. Cut with EcoRI.

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

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

Number of fragments: *3* Size of each fragment: *12bp, 9bp, 4bp*

Sequence (i) Cut with EcoRI.

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

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

Number of fragments: Size of each fragment:

Sequence (ii) Cut with EcoRI.

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

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

Number of fragments: Size of each fragment:

Sequence (iii) Cut with PstI.

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

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

Number of fragments: Size of each fragment:

Part B - Analysing Plasmids with Restriction Enzymes and Gel Electrophoresis

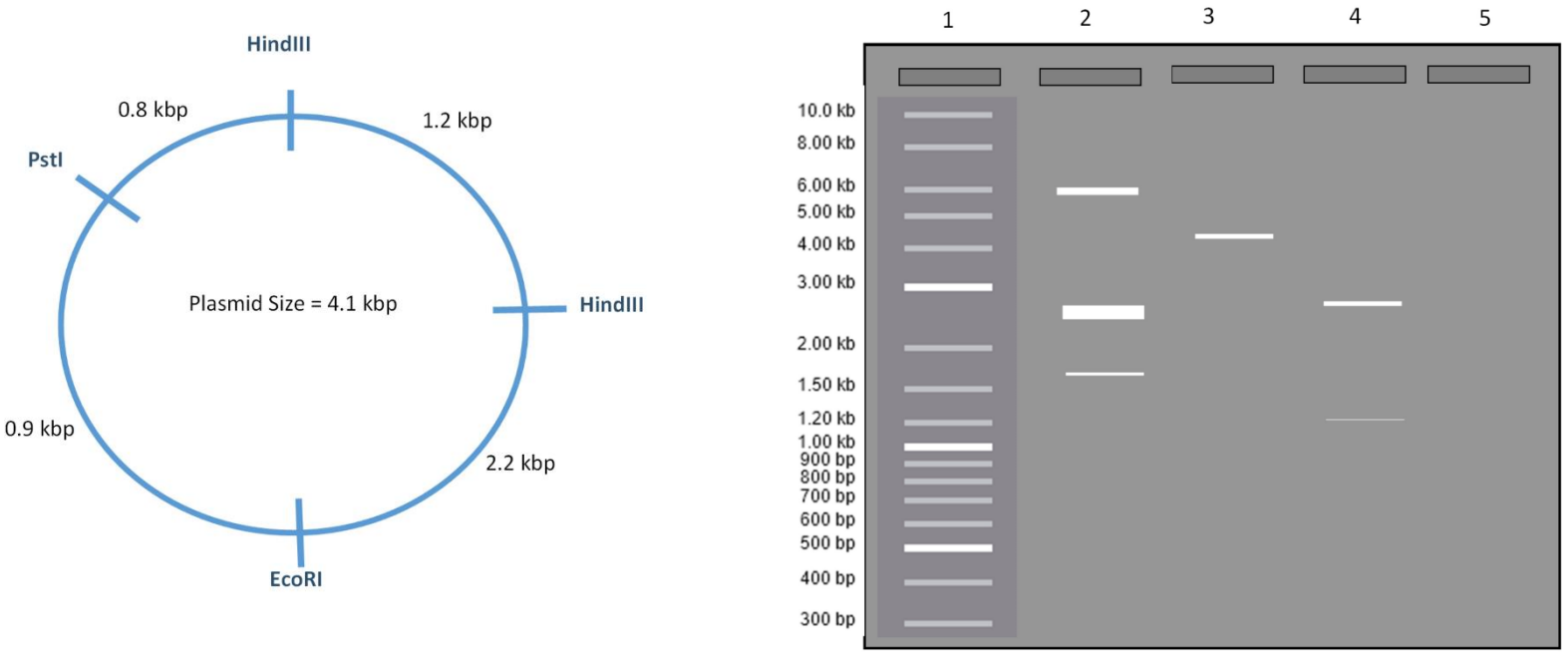

Figure 1: A plasmid with restriction enzyme sites is shown on the left. After the plasmid was extracted from bacterial cells using a mini-prep, the one sample of the plasmid was cut with restriction enzyme HindIII, and another sample was cut with EcoRI. The two samples, a standard DNA ladder (lane 1), and the uncut extracted plasmid (lane 2) were loaded into separate lanes on an agarose gel and the DNA was separated by gel electrophoresis. The water in which other samples were mixed was loaded in lane 5. A diagram of the resulting gel is shown on the right.

Questions

1. According to data from the gel, determine whether the gel electrophoresis was successful. Refer to evidence from the gel to support your answer.

2. According to data from the gel, determine whether the DNA extraction was successful. Refer to evidence from the gel to support your answer.

3. Out of EcoRI and HindIII, infer which restriction enzyme was used to cut the sample in a) lane 3 and b) lane 4. Refer to evidence in the gel to support your answer.

4. Analyse the evidence in lane 4 to identify the relationship between the length and mass of DNA.

5. Suggest a possible reason for loading only water into lane 5.

Part C - Restriction Enzyme Digest and Gel Electrophoresis of pGLO

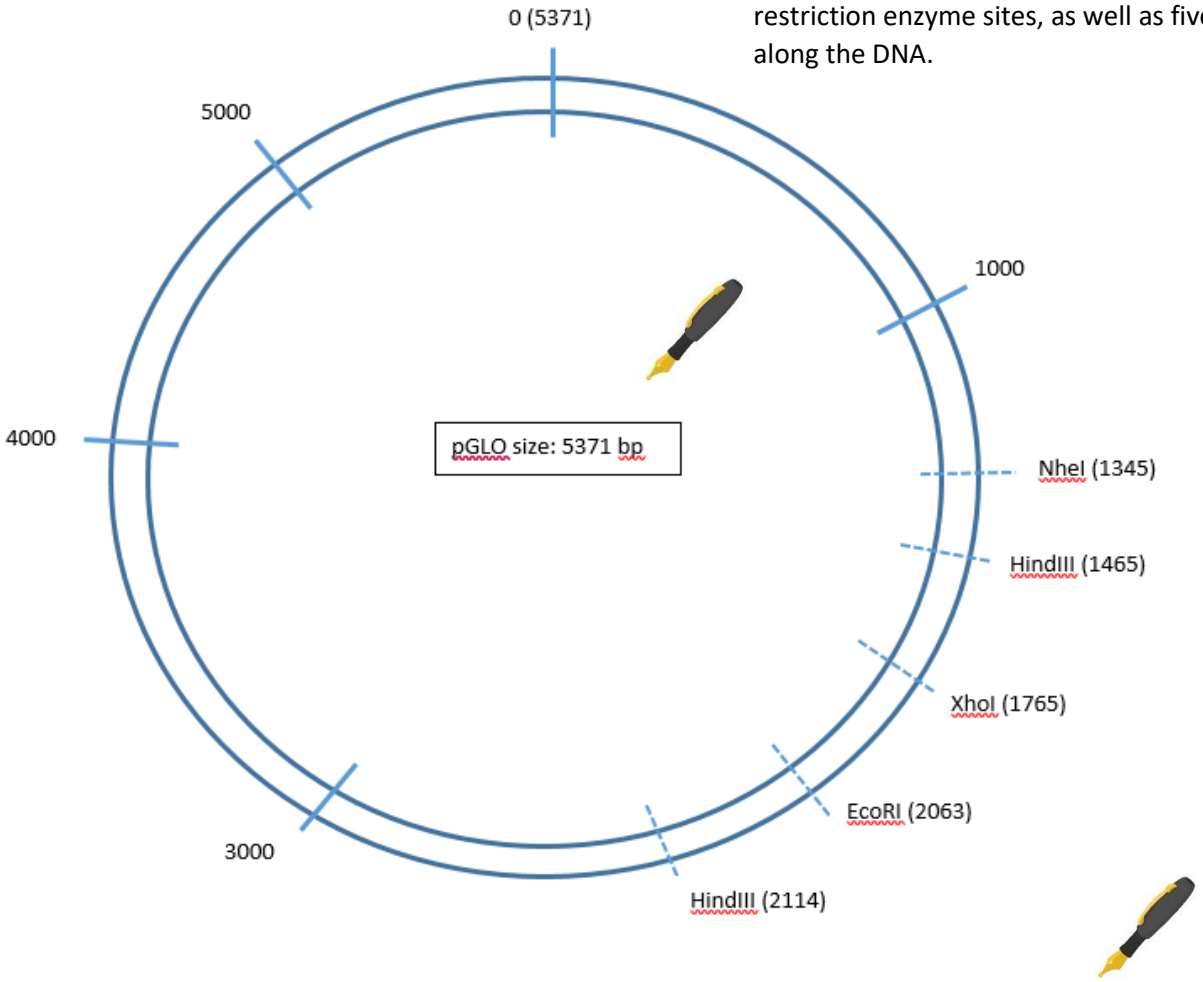

Figure 2: A map of pGLO showing only a selection of restriction enzyme sites, as well as five distance points along the DNA.

Five different restriction enzyme digests will be performed. For each of these listed below, use figure 2 to predict the number of linear DNA fragments, and the size of each.

Table 1: DNA fragments produced in each restriction digest (reaction).

	Enzyme	Number of fragments	Size of each fragment
A	EcoRI + NheI		
B	EcoRI		
C	NheI		
D	XhoI		
E	HindIII		

On an agarose gel, a sample of each restriction enzyme reaction (A-E) will be placed with loading dye. The dye will help to visualise the DNA samples move down the gel from positive (+) to negative (-). It will also help the DNA samples sink into the wells at the top of the gel.

There will be 8 wells loaded in the gel.

Lane 1: ladder

Lane 2: sample A

Lane 3: sample B

Lane 4: sample C

Lane 5: sample D

Lane 6: sample E

Lane 7: pGLO alone

Lane 8: water

On figure 3, use your predictions from table 1, and the standard in lane 1, to draw the DNA fragment at the place where you think it will move to after electrophoresis.

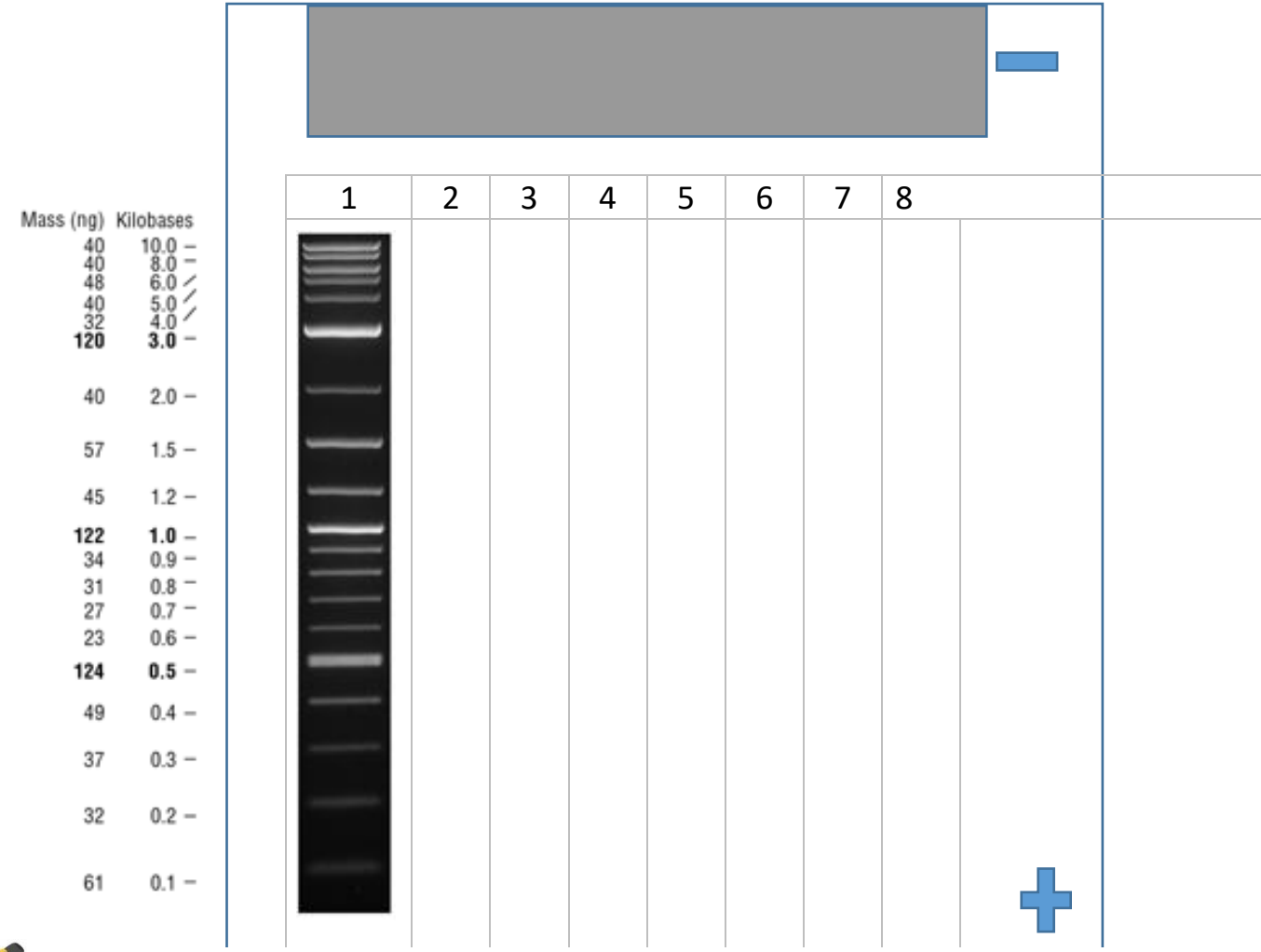

Figure 3: Diagram of an agarose gel on which DNA fragments have been separated based on size. Lane 1 shows a standard, containing fragments of known sizes. These sizes are marked on the left in kilobases (kb). The brightness of the bands represents their mass, which is in nanograms (ng).

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