In his Name

Work Sheet 3
Documents 4 and 5

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Exercise 1: Genetic application of restriction enzymes:

A. Bacteria are unicellular microorganisms. Yet those micro-organisms might be infected by viruses. However, bacteria are capable of defending themselves against those viruses by bacterial enzymes that are used to destroy the viral DNA. These enzymes are found in bacteria and provide a **defense mechanism** against invading viruses. These enzymes selectively cut up foreign viral DNA in a process called restriction; meanwhile, host DNA is protected. That's why those enzymes are called **restriction enzymes.**

A **restriction enzyme** is an enzyme that cuts DNA at specific recognition sequences known as restriction sites. To cut DNA, all restriction enzymes make two incisions (cuts), one through each strand of the DNA double helix.

Ever since different restriction enzymes are identified and isolated from different bacteria, they have been used to help in genetic studies.

As any other enzyme, restriction enzymes are specific to their substrates. They recognize specific sequences of DNA nucleotides known as restriction sites and cleave (cut) the DNA at those sites.

Document 1 shows some of those enzymes and their corresponding restriction sites.

Enzyme	Recognition site	Cleavage site
Hae III	CCGG	C|CGG
Eco RI	GAATTC	G|AATTC
Bam HI	GGATCC	G|GATCC
Not I	GCGGCCGC	G|CGGCCGC

Document 1

1. Pick out from the above text:

a) The way used by bacteria to defend themselves against viruses.

b) The definition of a restriction enzyme.

Given the following DNA molecule that corresponds to a certain gene.

	1				5				9				14				20				25				30				35
coding	A	A	A	C	C	C	G	G	A	T	C	C	T	C	G	G	T	A	G	A	A	T	T	C	T	C	C	G	G	A	T	T	A	A	G
Non-coding	T	T	T	G	G	G	C	C	T	A	G	G	A	G	C	C	A	T	C	T	T	A	A	G	A	G	G	C	C	T	A	A	T	T	C

2. Pick out from document 1, the enzymes that can be used to cut this DNA molecule. Justify

3. For every enzyme you picked, give the number of DNA fragments obtained after cleavage.

The given gene is subjected to mutations by substitution at the following nucleotides 14, 16 and 21. These nucleotides are replaced by G, T and T respectively. The mutant allele formed causes a disease.

	1				5				9				14				20				25				30				35
coding	A	A	A	C	C	C	G	G	A	T	C	C	T	**G**	G	**T**	T	A	G	A	**T**	T	T	C	T	C	C	G	G	A	T	T	A	A	G
Non-coding	T	T	T	G	G	G	C	C	T	A	G	G	A	**C**	C	**A**	A	T	C	T	**A**	A	A	G	A	G	G	C	C	T	A	A	T	T	C

4. Pick out from document 1, the enzymes that can be used to cut this DNA molecule and determine the number of DNA fragments obtained after the use of each enzyme.

5. If you are to choose an enzyme that allows you to distinguish the normal allele from the mutant one, which enzyme would you choose? Justify.

6. If we use Eco RI enzyme. Determine the size of the fragments obtained in each case. (Given that, the size of a DNA fragment is measured by base pairs).

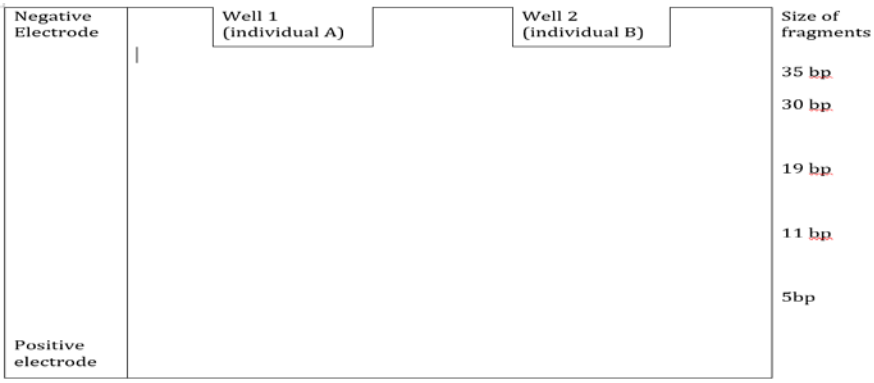
B. In molecular genetics, after the enzymes are used to cleave the DNA, the fragments obtained are allowed to travel on a gel placed in an electric field. With the DNA fragments being negatively charged, they will travel from the negative electrode to the positive electrode. But not all fragments migrate the same distances, the distance covered by every fragment is related to its size; the smaller the fragment the bigger the distance.

7. Pick out from part B:

a) The direction of migration of DNA fragments and the cause of this migration.

b) The relation between the distance migrated by DNA fragments and the size of these fragments.

8. Knowing that the mutant allele of this gene is recessive and the normal one is dominant, construct the restriction maps of the 2 individuals A & B based on the following figure:
A: Normal individual that carries the mutant allele
B: Affected by the disease



C. Hae III is used to prepare restriction fragments of both alleles. The fragments are then separated by gel electrophoresis. The DNA fragments are blotted to a filter paper then incubated in the presence of a DNA probe with the sequence **GGAC** in order to make hybridization. Bands are then detected by autoradiography.

9.

a) Give the name of the technique described above.

b) Define hybridization.

c) Indicate the necessary treatment that should precede hybridization.

d) Draw the results of the autoradiography done after the usage of Hae III and the hybridization with the above probe of the DNA fragments. Justify

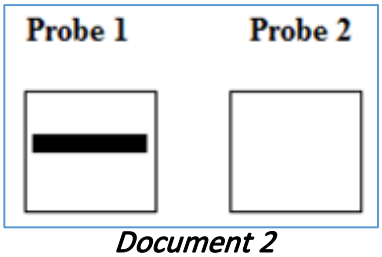
e) Is this method able to detect the genetic polymorphism of this gene? Justify

10. An individual is tested to determine whether he is affected with the disease related to the mutant allele or not. His DNA is cleaved using Hae III. And then incubated with one of the following probes every time:

Probe 1: **ACC AAT CTA AA**

Probe 2: **AGC CAT CTT AA**

The autoradiography after hybridization reveals the results represented in document 2.



Document 2

a) Which allele does each of the above probes hybridize?

b) Give the genotype and phenotype of this individual. Justify

11. We are interested in locating the gene related to this disease on a chromosome.

a) Give the name of the technique used for this purpose.

b) Briefly describe the procedure of this technique.