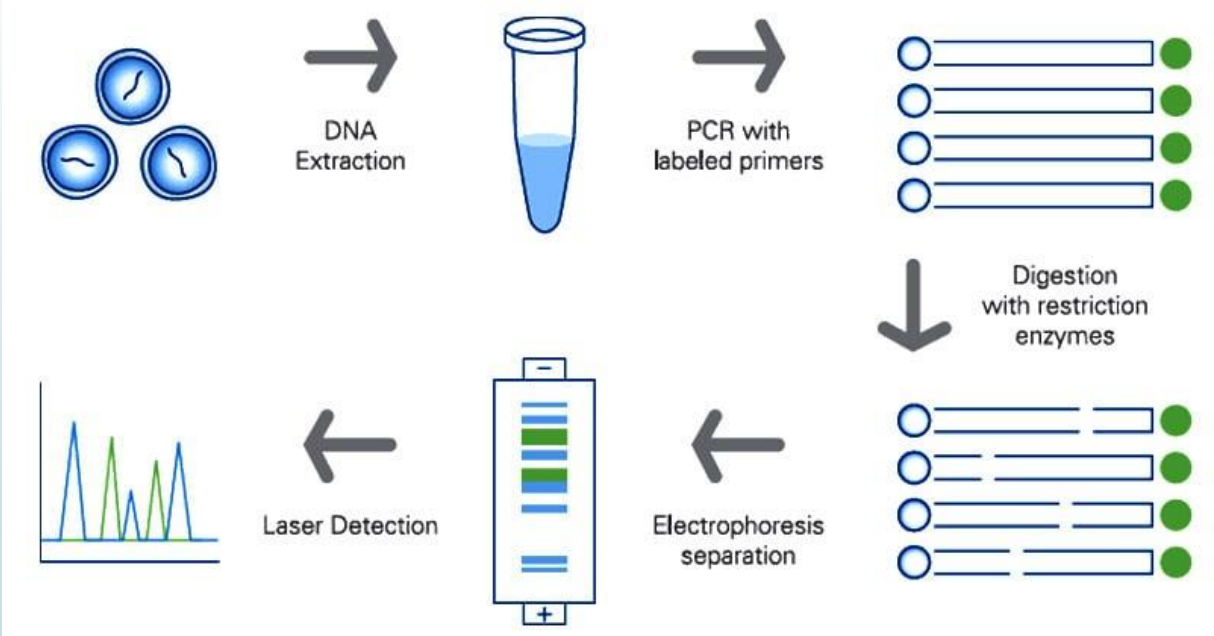
Chapter 3: Genetic Variation and Polymorphism

- Document 1: Mutations and the Environment
- Document 2: Mutation and Multiple Alleles
- Document 3: Polymorphic Genes in a Population
- **Document 4:** Detection of Genetic Polymorphism
- Document 5: Genetic Identity of Individuals

Document 4

Detection of Genetic Polymorphism



- Genetic polymorphism is responsible for the diversity of the individuals.
- ***How is the variation detected?***

- Genetic polymorphism can be detected by many different techniques:

  1- Gel electrophoresis

  2- **R**estriction **F**ragment **L**ength **P**olymorphism **(RFLP**)

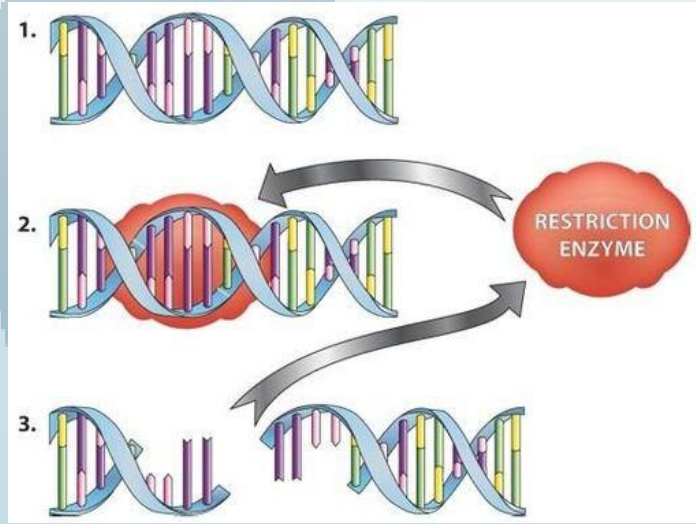
  → These techniques require the use of **Restriction Enzymes.**

I. Restriction Enzymes (RE):

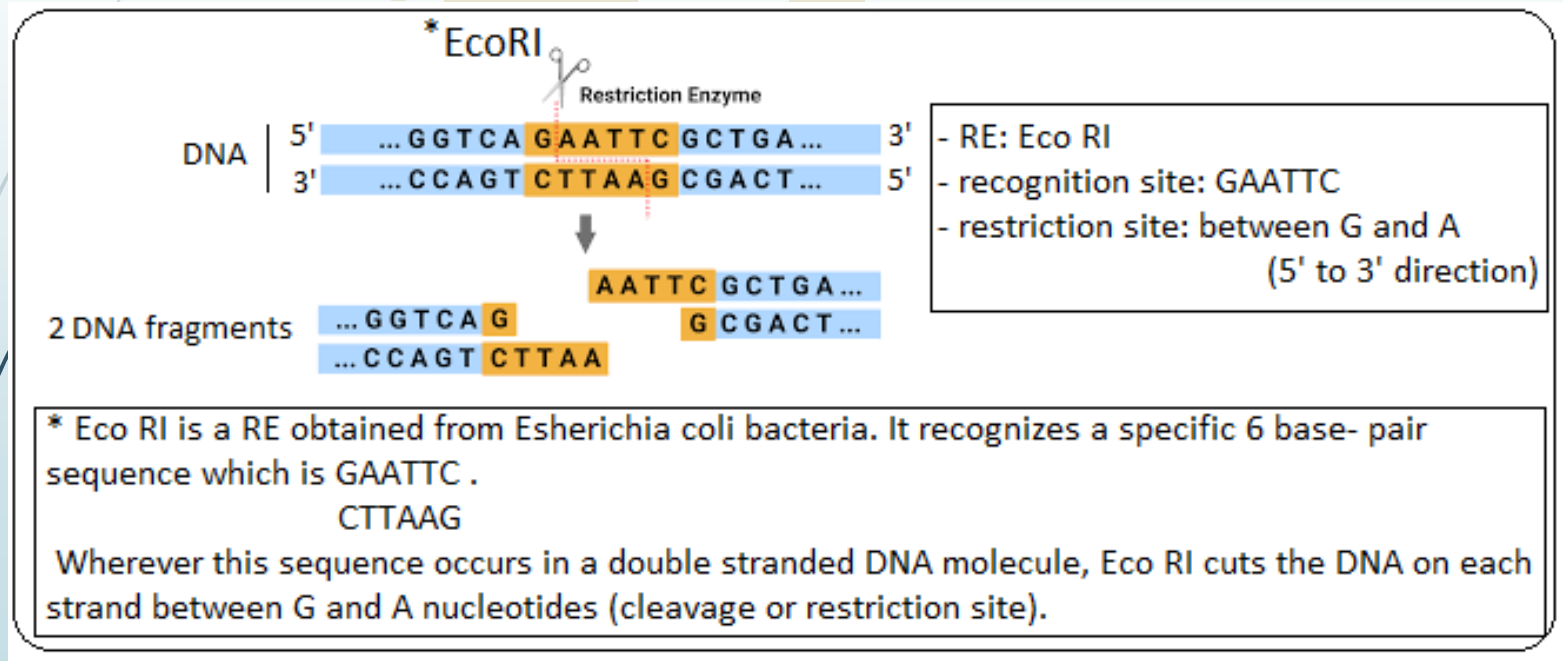
- They are biological scissors extracted from bacteria.

- RE are naturally produced by bacteria to defend themselves against invading viruses, by cutting viral DNA into pieces.

- There are many different types of RE that cut the DNA in a specific manner.



- Each RE recognizes a specific sequence of nucleotides called **recognition site** (usually is 4 to 8 base pairs) and cuts the 2 strands of DNA at a **specific site, called: cleavage site** or restriction site. RE cuts from 5' to 3' direction.



- Doc.a , p.64 shows examples of restriction enzymes.

restriction enzyme	source	recognition site	cleavage site
Hae III	Haemophilus aegyptius		
Eco RI	Escherichia coli		
Bam HI	Bacillus amyloliquefaciens		
Not I	Nocardia otitidis-cavarium		

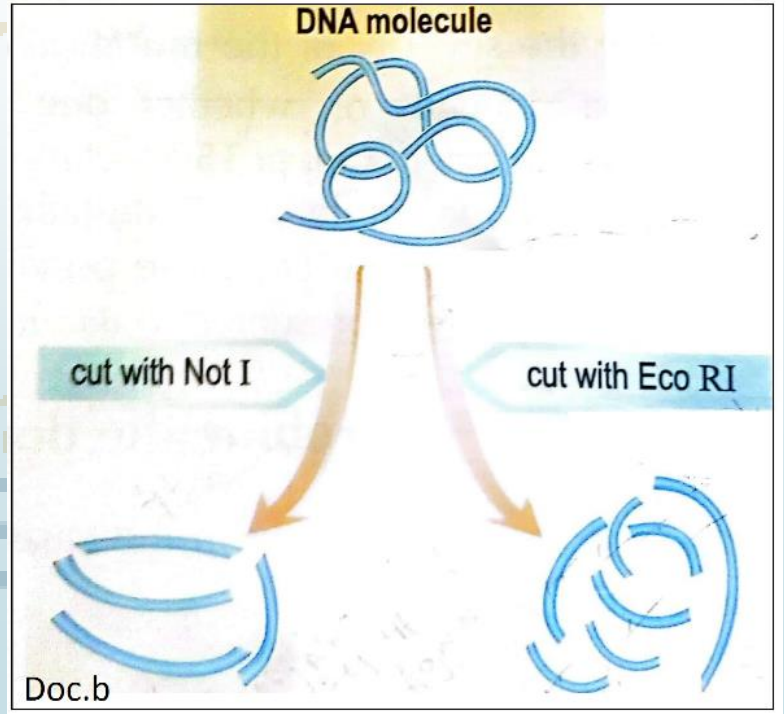
Doc.a *Examples of restriction enzymes. Cleavage sites on each strand are indicated by arrows. Usually, the recognition sequences are the same on both DNA strands, when read in the 5'-3' direction.*

- **Doc.b, p.64 shows cleavage of a DNA molecule by 2 different RE: Not I and Eco RI.**

The number of DNA fragments obtained = number of restriction sites + 1.

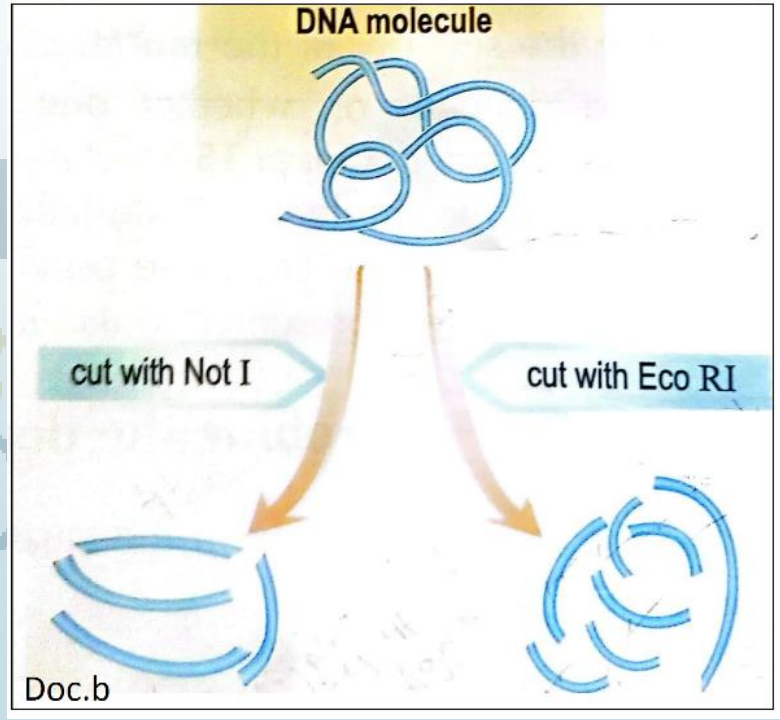
→ Determine the number of restriction sites of Not I and Eco R.I.

Since 4 DNA fragments were obtained upon cutting the DNA molecule with Not I, so the number of restriction sites is 3. Since 7 DNA fragments were obtained upon cutting the DNA molecule with Eco RI, so the number of restriction sites is 6.



Doc.b

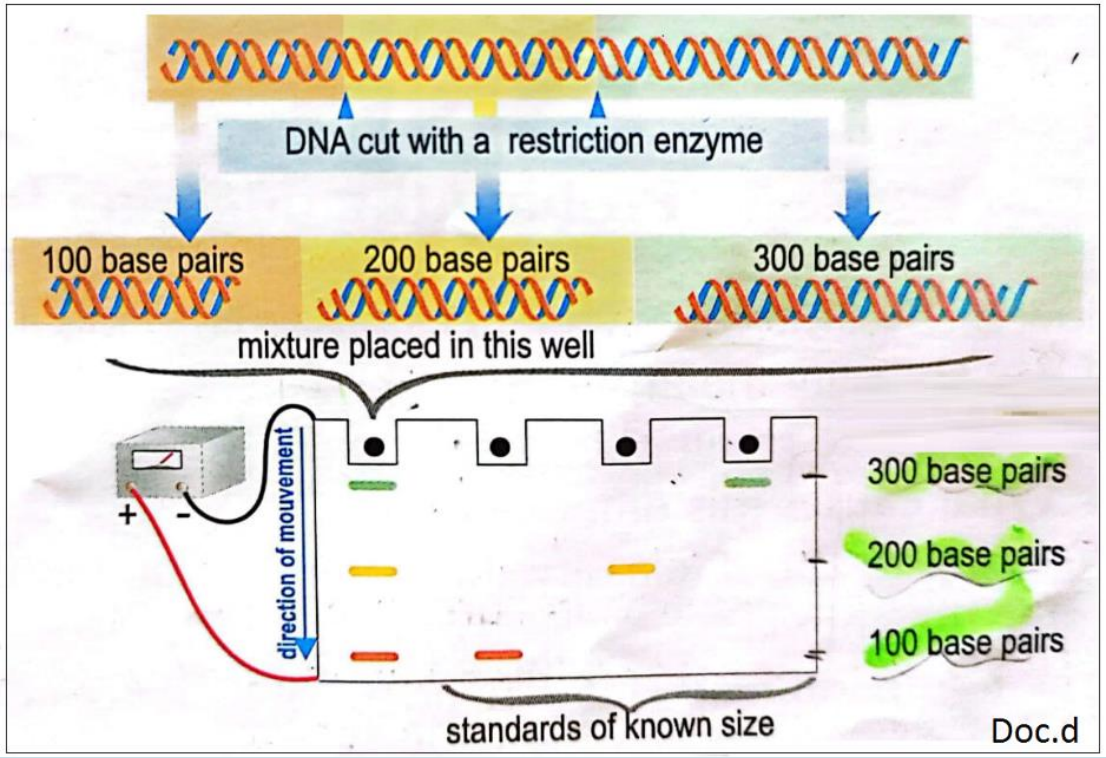
*If the same DNA molecule was cut with 2 different RE, the number and length of DNA fragments obtained with each RE will be different.



Doc.b

II. Gel Electrophoresis

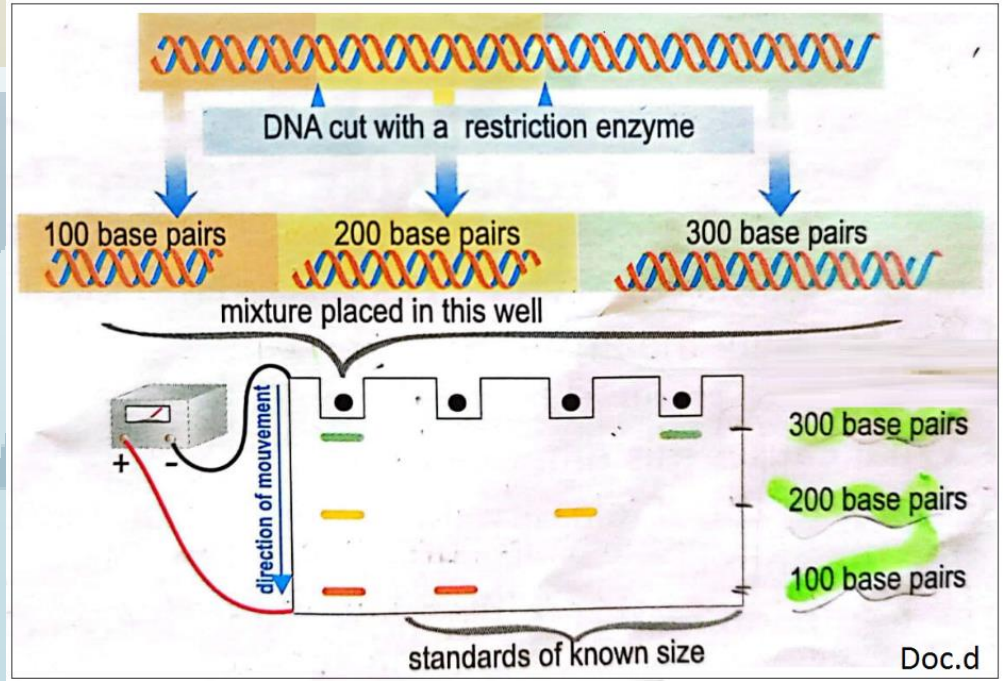
- Is a technique used to separate different DNA fragments on a gel according to their molecular weight in the presence of an electric current.



Be Smart ACADEMY

- DNA fragments are negatively (–ve) charged due to the presence of phosphate which is negatively charged.
- In gel electrophoresis, DNA fragments are attracted to the +ve pole of the gel, so they migrate from the –ve pole to the + ve pole.
- Small fragments migrate further than the large fragments.

- Doc.d p.65 shows gel electrophoresis of DNA fragments.



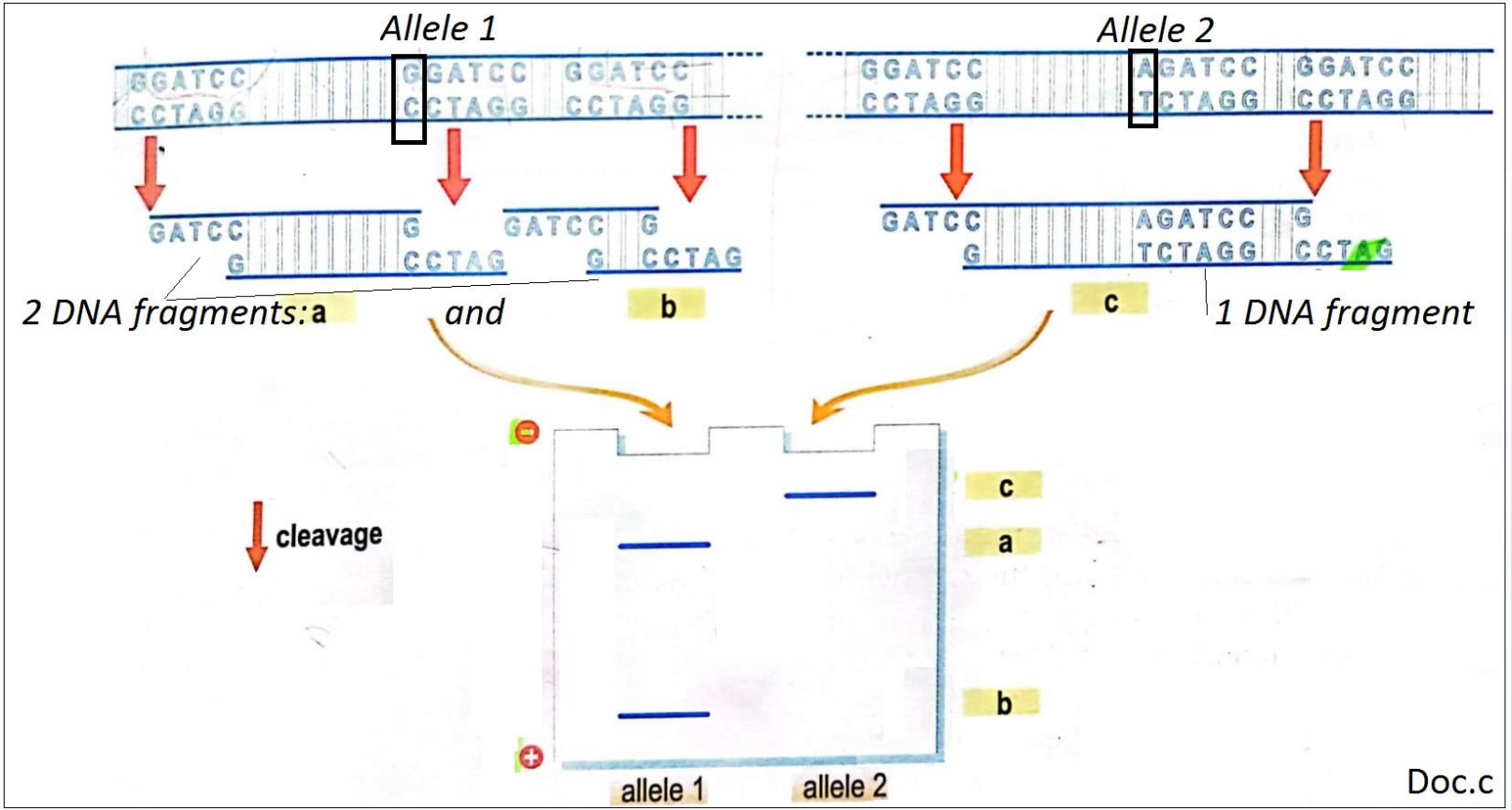
Be Smart ACADEMY

- **Procedure:**

- Cut the DNA molecule with RE to obtain DNA fragments.
- Place the fragments inside wells inside the gel.
- Connect to a source of electricity.
- DNA fragments migrate to the positive pole according to their size to form bands (small fragments migrate further towards the + ve pole).
- Add ethidium bromide to color the bands on the gel.
- Expose the bands to UV radiations where fragments can be seen.

→ The obtained pattern of bands is known as **restriction map.**

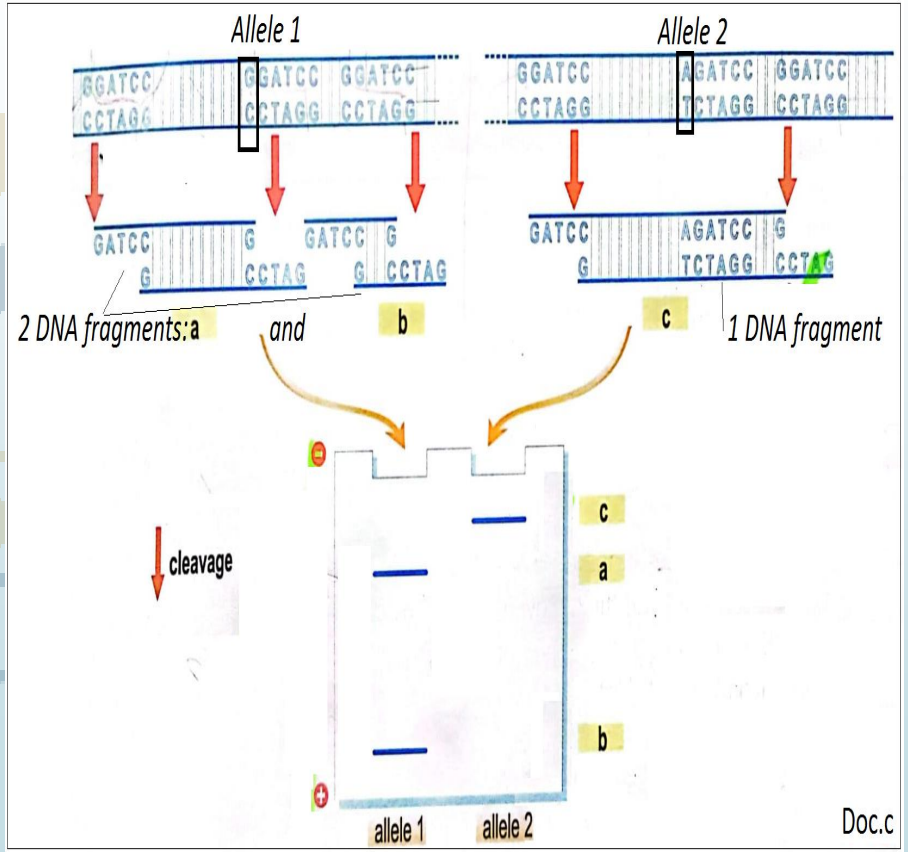
- Doc.c, p.65 (represented below) shows gel electrophoresis of two different alleles (Allele 1 and Allele 2) cleaved by the same restriction enzyme.



- Referring to Doc.c, answer the following questions:

1- Explain the results obtained in Doc.c.

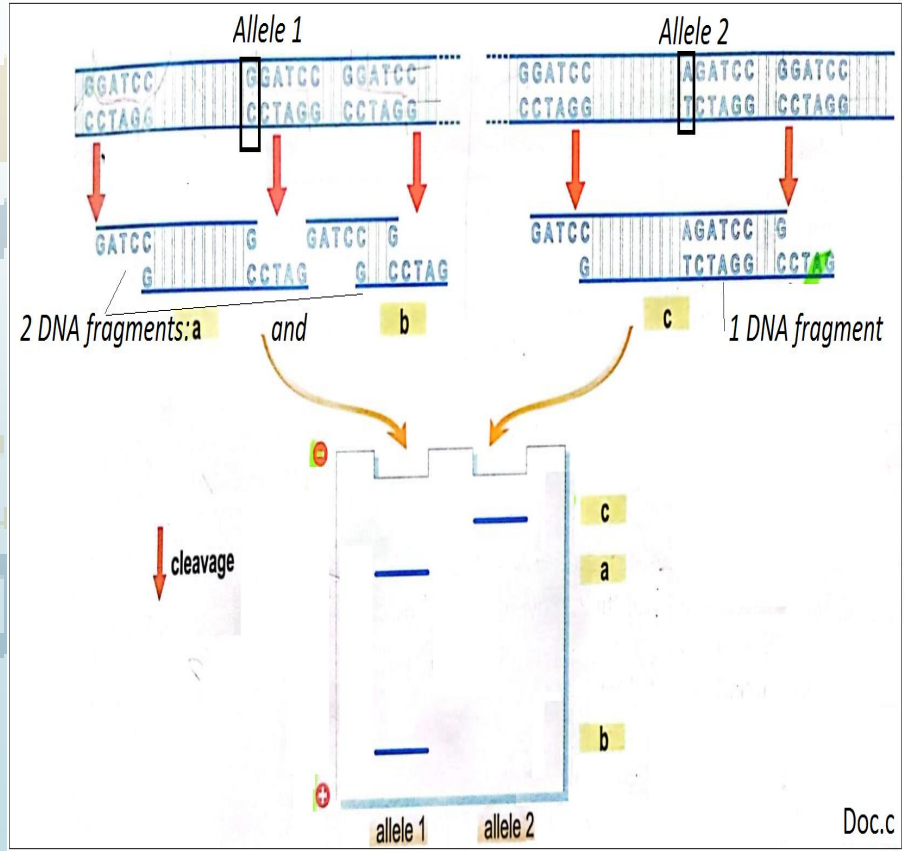
Allele 1 is cleaved (cut) by the restriction enzyme into 2 DNA fragments where 2 bands appear on gel electrophoresis. While, allele 2 cleaved by the same restriction enzyme, only 1 fragment was obtained and 1 band appears on gel electrophoresis. This is because alleles 1 and 2 don't have the same nucleotide sequence where 1 nucleotide was substituted in allele 2 (A instead of G). Thus the substitution mutation in allele 2 changed the recognition site of the RE which affects the number of DNA fragments and which inturn affects the number of bands on gel electrophoresis.

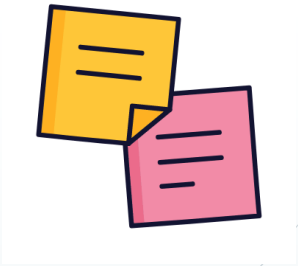


2- Classify the fragments according to their decreasing length. Justify your answer.

The order of fragments according to their decreasing length c-a-b. This is because the heavier DNA fragments (the largest in size) is the nearest to the well, and the lighter fragment (the smaller in size) move further from –ve to the + ve pole.

Migration of fragments occurs according to the weight.





- The base sequences of the DNA vary from one allele to another (for example the polymorphic genes of the ABO blood groups).
- If the mutation occurs within the recognition site, the restriction enzyme will not recognize this site (the mutation may add or delete a restriction site) , thus affecting the number and the length of the obtained fragments.
- 2 different alleles of the same gene will give different number and size of DNA fragments with the same RE.⇨ RE are used to show polymorphism.

- **Uses of Gel Electrophoresis:**

- To detect genetic mutations.
- Determine the real genotype of a person (pure or hybrid).
- Know if a gene is polymorphic.
- Paternity test.
- Forensic science.

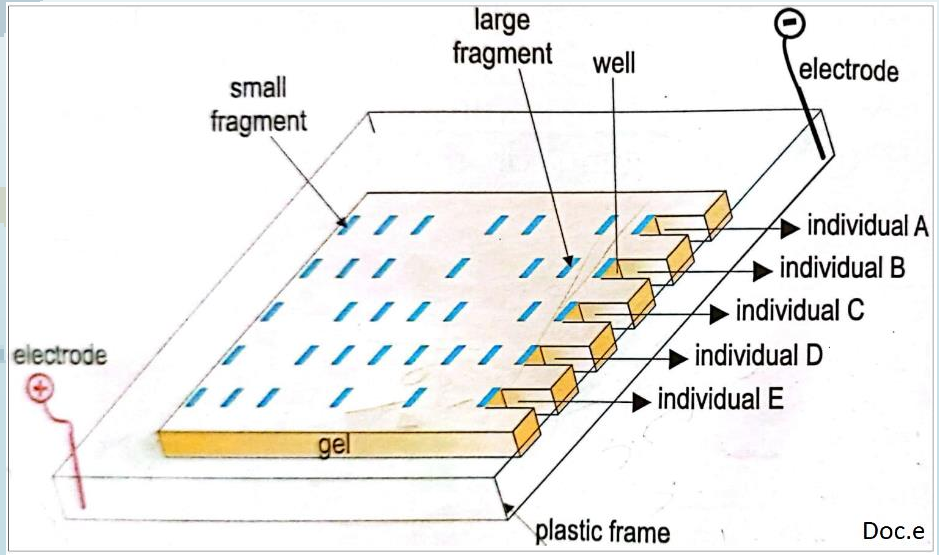
III. RFLP: Restriction Fragment Length Polymorphism

- Different individuals have different nucleotide sequence and alleles.

⇨ different location and number of restriction sites.

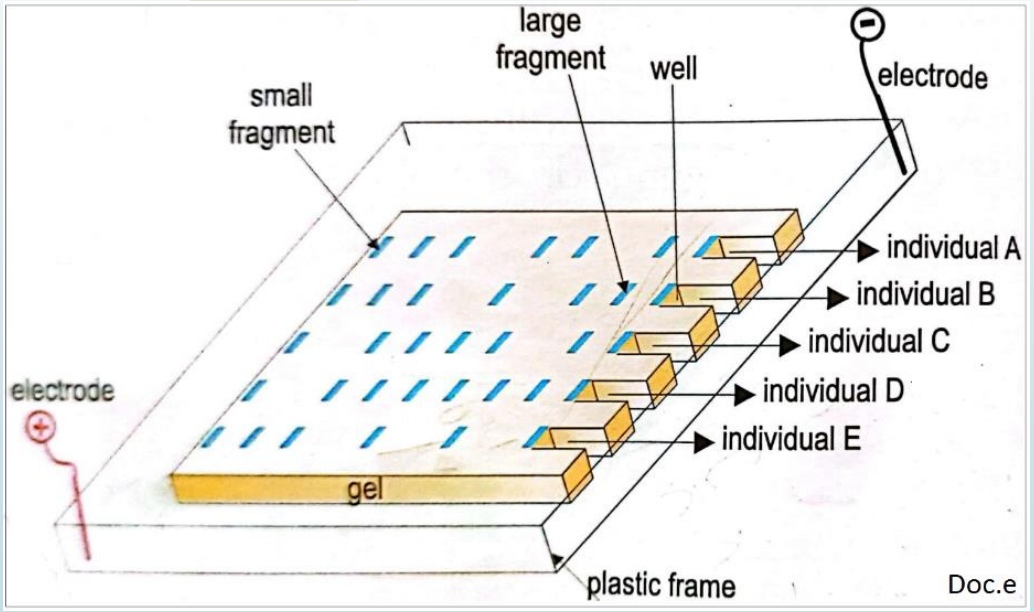
⇨ different restriction maps will be obtained when the DNA of different individuals is cut with the same RE.

⇨ RFLP is obtained, Doc e p.66.



Doc.e

- RFLP is a difference in restriction maps of 2 individuals.
- It is used to detect genetic polymorphism between different individuals.



“The restriction map is independent of gene function”. Explain this statement.

RFLP is independent of the gene function, since a restriction map depends on differences in restriction sites of the DNA in both coding and non-coding regions regardless of the gene function.

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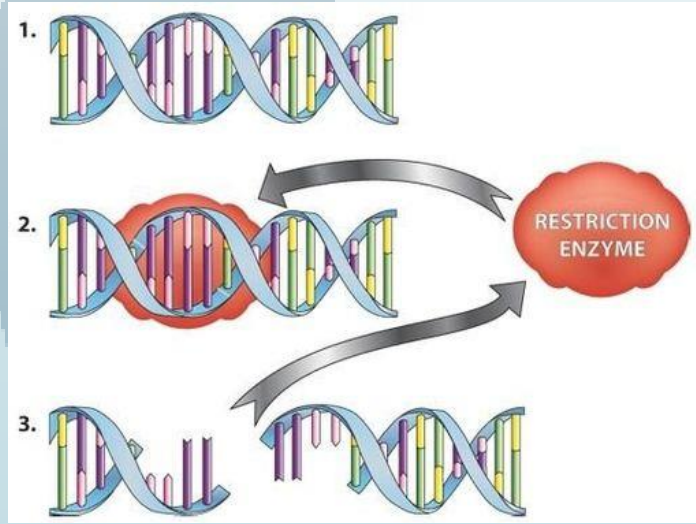

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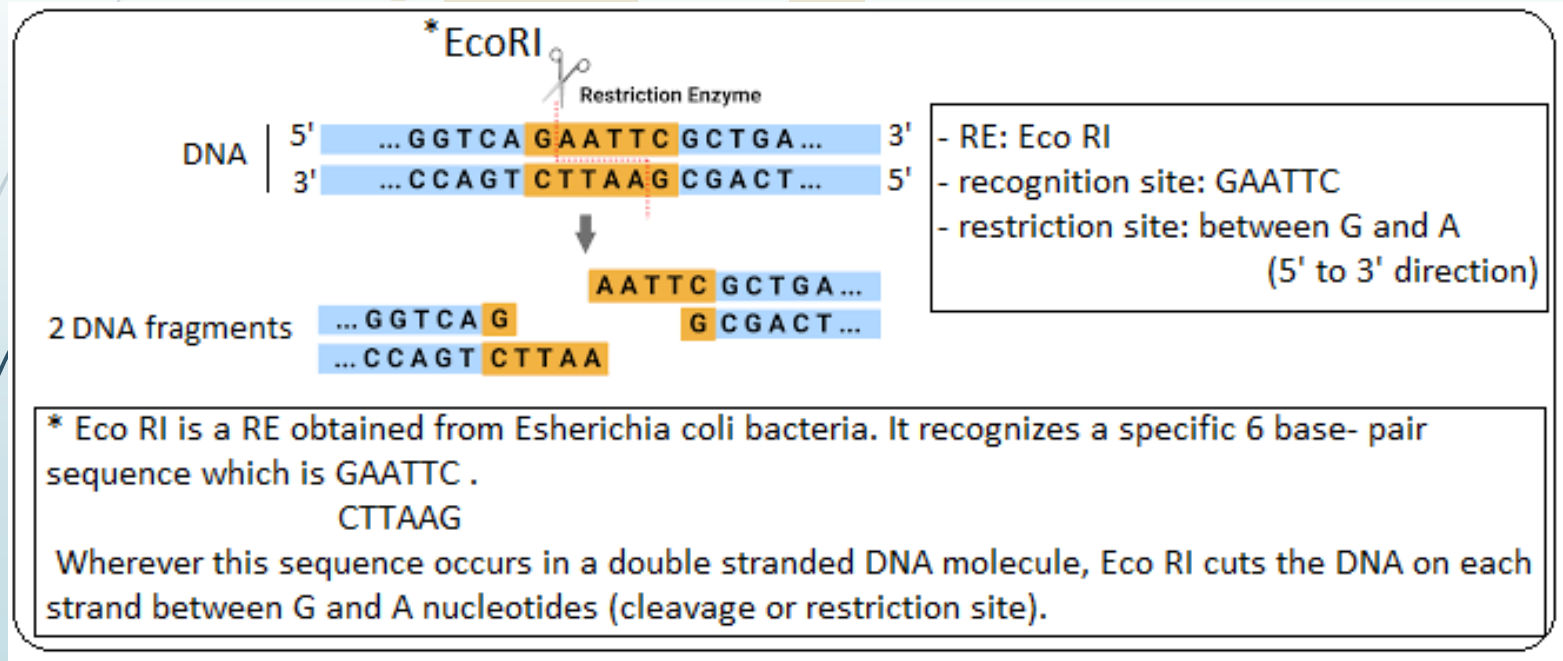

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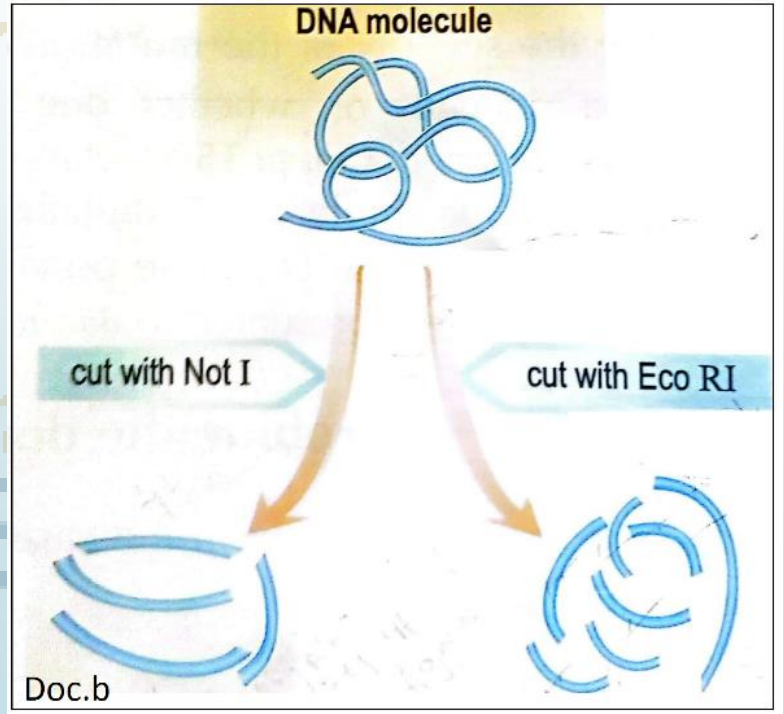

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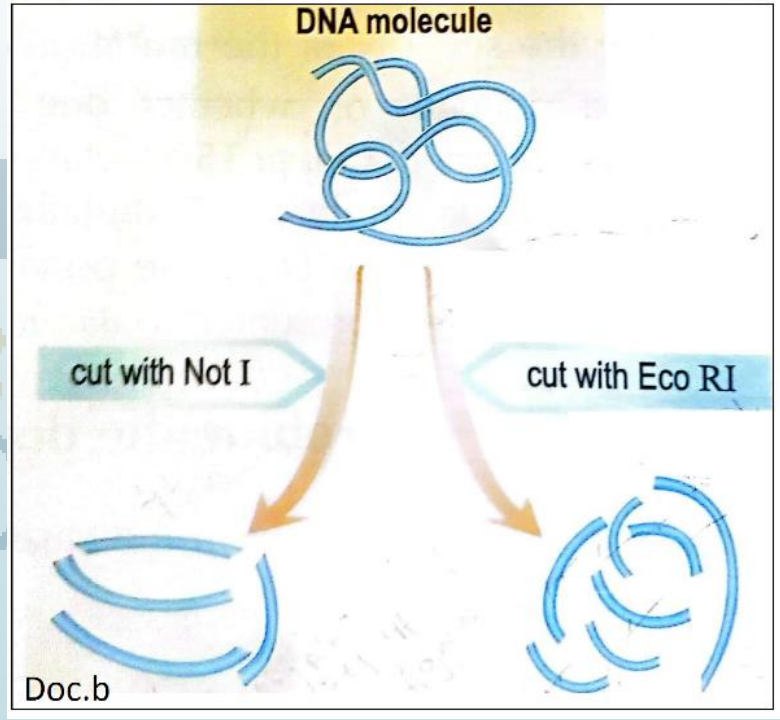

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