Chap.3 Doc.4 Detection of genetic polymorphism

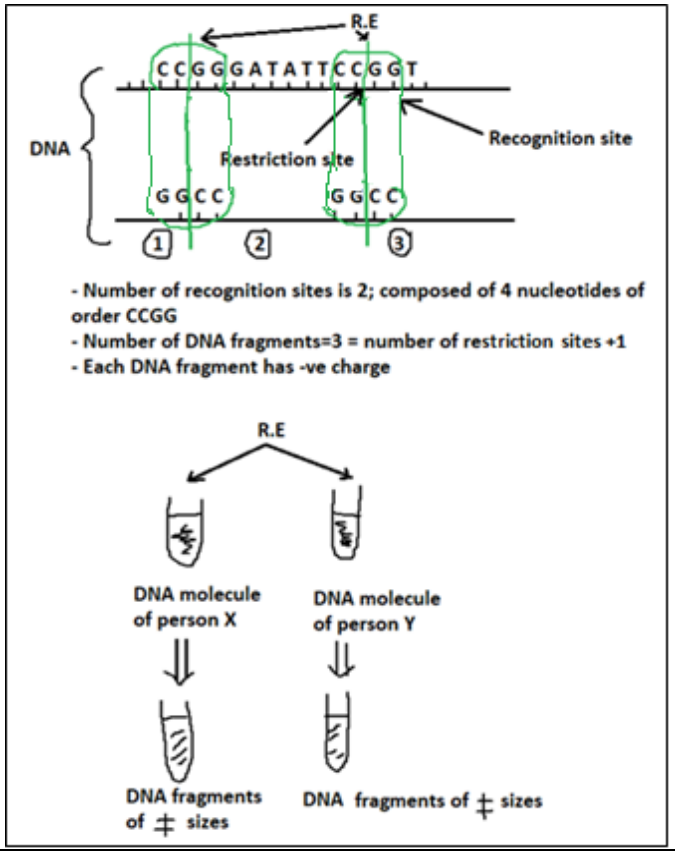
1. Restriction enzymes
2. Gel electrophoresis
3. RFLP

1. Restriction enzymes (R.E)(Doc.a p.64) :

Restriction enzyme is a biological scissor used to cut DNA molecule into DNA fragments of different sizes.

- Restriction enzymes are naturally produced by bacteria

Aim of restriction enzymes defend themselves against invading bacterial viruses by cutting the viral DNA into pieces.



-Characteristics of R.E:

Every R.E cuts DNA molecule in a particular site called "**Restriction site**". This restriction enzyme recognizes and cuts at the restriction site a particular nucleotide sequence (**Recognition site**) which is composed of specific number of nucleotides in a specific order that ranges from 4 nucleotides to 8 nucleotides.

***Restriction site** **is** the place on a DNA molecule where a restriction enzyme acts.

*A **restriction fragment** is a DNA fragment resulting from the cutting of a DNA strand by a **restriction** enzyme

***Recognition site**:A nucleotide **sequence**—composed typically of 4, 6, or 8 nucleotides--that is **recognized** by a **Restriction** enzyme.

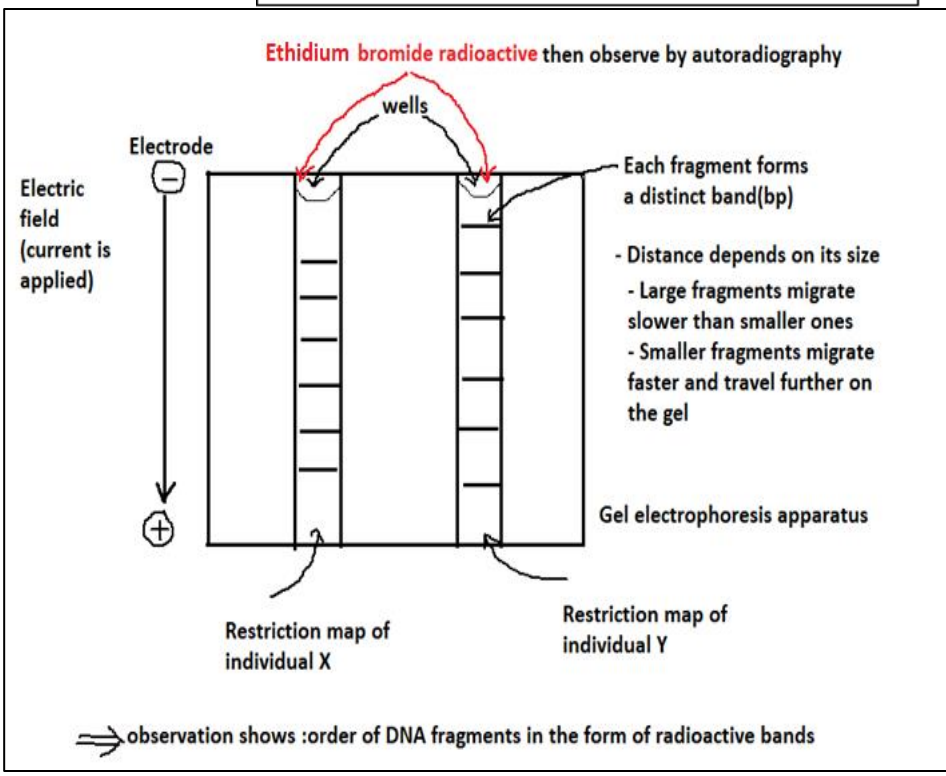
- If the recognition sequence or recognition site is submitted to mutation, then the restriction enzyme can no more recognize and cut this sequence.

2. Gel Electrophoresis

Gel: colloid substance

Electro: electric current

Separation of DNA fragments of DNA molecule



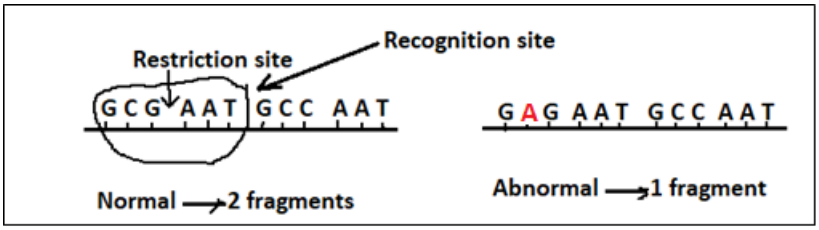
Gel electrophoresis allows to:

- Detect the mutant allele
- Determine the real genotype

*Note: Ethidium bromide is a dye that binds to DNA fragments and fluoresces under UV light.

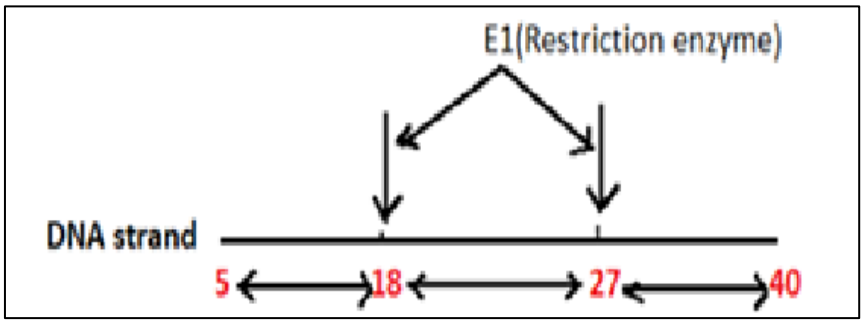
- **Restriction map** : is an ordered DNA fragment: coded fragments and non- coded fragments of a particular person.(It is the generated pattern of bands)

- If mutation occurs in the recognition site, Restriction enzyme doesn't recognize the mutant site because the order of nucleotides differ.



Application:E1 cuts the DNA strand in 2 sites at position 18 and position 27

1.Determine the number of DNA fragments and their sizes.



The number of DNA fragments is 3
The size of DNA fragments:
Length of 1st DNA fragment: 13 bps (18 -5)
Length of 2nd DNA fragment: 9 bps (27-18)
Length of 3rd DNA fragment: 13 bps(40 -27)

2.If ,at position 18, there was a mutation by substitution, determine the effect of this mutation on the number of DNA fragments and their sizes.

If mutation occurs at position 18, then E1 (the restriction enzyme) doesn't recognize the new site; then there will be cutting only at position 27 and the number of DNA fragments would be 2 and the sizes of DNA fragments would be:
Length of 1st DNA fragment: 22 bps(27-5)
Length of 2nd DNA fragment: 13 bps(40 -27)

3. **Restiction Fragment Length Polymorphism (RFLP)Doc.e p.66**
   The difference between restriction maps of individuals is called Restriction Fragment Length Polymorphism (RFLP). It ensures that individuals are different at the level of their DNA(both coding regions or expressed regions :1% of DNA and non-coding regions:99%of DNA which don't undergo transcription and have no function in gene expression nor in phenotype).
   Thus each individual has a unique restriction map indicating that each individual has a unique DNA.
   Probing the activity p.66
   1. GAATTC=6 times , because the cleavage of the bacterial DNA by EcoRI restriction enzyme at the sequence GAATTC has yielded 7 fragments.
      GCGGCCGC=3 times, because the cleavage of the same molecule by Not I restriction enzyme at the GCGGCCGC sequence has yielded 4 fragments. Note: Number of restriction fragments = number of cuts +1
   2. a. Alleles 1 and 2 don't have the same nucleotide sequence; allele 1 is cleaved by the restriction enzyme into 2 fragments, as shown as 2 bands in the electrophoresis,whereas allele 2 is not cleaved because only one band is shown in the electrophoresis.

b. This difference between both alleles of the same gene is due to a mutation that occurred in allele 2,which has changed the recognition site of the restriction enzyme.

3. Referring to doc.d, the heavier(the longest) DNA fragments are the nearest to the well, whereas the shorter and faster fragments travel further on a gel. Therefore the fragments in Doc.c are in the following order: c,a,b.

4. A restriction map depends on differences in restriction sites of the DNA, and these are distributed throughout the genome, in both coding and non-coding regions. Thus, when comparing RFLP of 2 organisms, the genotype is assessed and not the phenotype.

Thus, with restriction maps, one assesses the genome, i.e coding and non-coding sequences, rather than only coding sequences of the gene that express the phenotype.

- Explain how the restriction enzymes distinguish normal allele from mutant alleles (parg. 2)
If the recognition sequence of a certain enzyme is submitted to a mutation then this enzyme can no more recognize and cut this sequence. If a RE is used to cut 2 different alleles of the same gene, then the yield will be different. This is because the 2 alleles have different base sequence and thus the no of recognition sequences of this RE will be different in the two alleles. Therefore, the no of cleavage sites will vary as well as the no of DNA restriction fragments

- What is the difference between the recognition site and the cleavage site?
The recognition site is a double sequence of nucleotides having definite length (base pairs) and recognized by the restriction enzyme. However the cleavage site is a specific position of cutting in the recognition site

Note: Our DNA is made of both coding and non-coding regions.
• Any mutation at the level of the coding regions usually affect the phenotype.
• The non-coding regions are repetitive and abundant sequences in the DNA and have no function in gene expression neither in phenotype. Any mutation at the level of the non-coding regions do not affect the phenotype.
• Cuttings in the whole DNA will produce for each individual unique banding patterns on the gel which is called restriction map.
• The difference in restriction maps between two individuals is called a restriction fragment length polymorphism or RFLP.
• So the aim of RFLP is to ensure the fact that individuals are different at the level of their DNA in both coding and non-coding regions.

- justify the following statement: the restriction map is independent of gene function
A restriction map depends on differences in restriction sites of the DNA, and these are distributed throughout the genome, in both coding and non-coding regions. Thus, when comparing RFLP's of the two organisms, the genotype is assessed and not the phenotype. Thus, with restriction maps one assesses the genome, i.e. coding and non-coding sequences, rather than only coding sequences of the gene that express a phenotype.