

APPLICATIONS OF RESTRICTION ENZYMES

(The Significance / Importance and Uses of Restriction Endonucleases in Biotechnology)



Restriction endonucleases (also called as molecular scissors) are a class of nuclease enzymes which cut the DNA strand at precise locations. They are specific endonuclease enzymes in the cells which first recognize the specific sequence (called restriction sites) within the DNA strand and cleave the phosphodiester backbone of the DNA at specific sites. The Nobel Prize in 1978 (in Physiology and Medicine) was shared by Werner Arber, Daniel Nathans and Hamilton Smith for the discovery of restriction enzymes and their applications in molecular genetics. Restriction enzymes are now an inevitable tool for the manipulation of DNA in various recombination studies both *in vitro* and *in vivo*. The main applications of restriction enzymes are:

1. Construction of Restriction Maps
2. Construction of DNA Fingerprints
3. Recombinant DNA Technology (rDNA Technology)

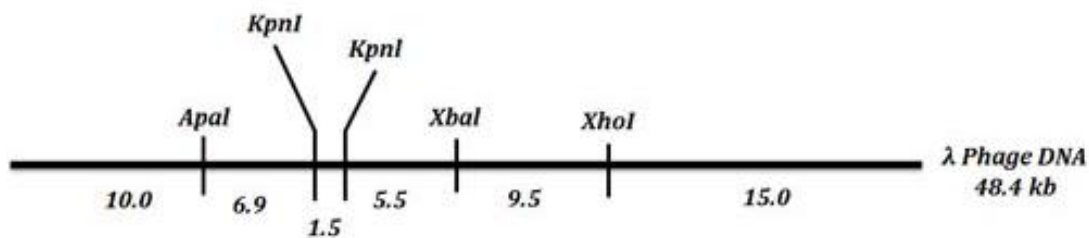
1. Construction of Restriction Maps:

- Restriction map: a diagram or map of DNA molecule of an organism that shows specific sites of cleavage (restriction sites).
- Construction of restriction maps was one of the first described uses of restriction enzymes.
- Restriction maps are used to identify the fragments of DNA which contain specific genes.
- The data from many restriction digests of a common DNA sample is combined to produce a complete and accurate restriction map.

- Restriction maps are also design and engineer cloning vectors and plasmids.

How to construct a Restriction Map?

- ❖ Take the sample DNA in 'x' number of vials.
- ❖ 'x' is the number of restriction enzymes that we have (example 5).
- ❖ Treat the sample DNA with the specific restriction enzyme separately.
- ❖ After the restriction digestion, run the DNA sample on a 2% agarose gel along with DNA marker for the separation of DNA fragments.
- ❖ On agarose gel, the smallest fragment moves fastest and found at the bottom of the gel.
- ❖ Then find out the size of each fragment and draw a map according to the size of the fragments and the total size of the intact DNA as in the following figure.



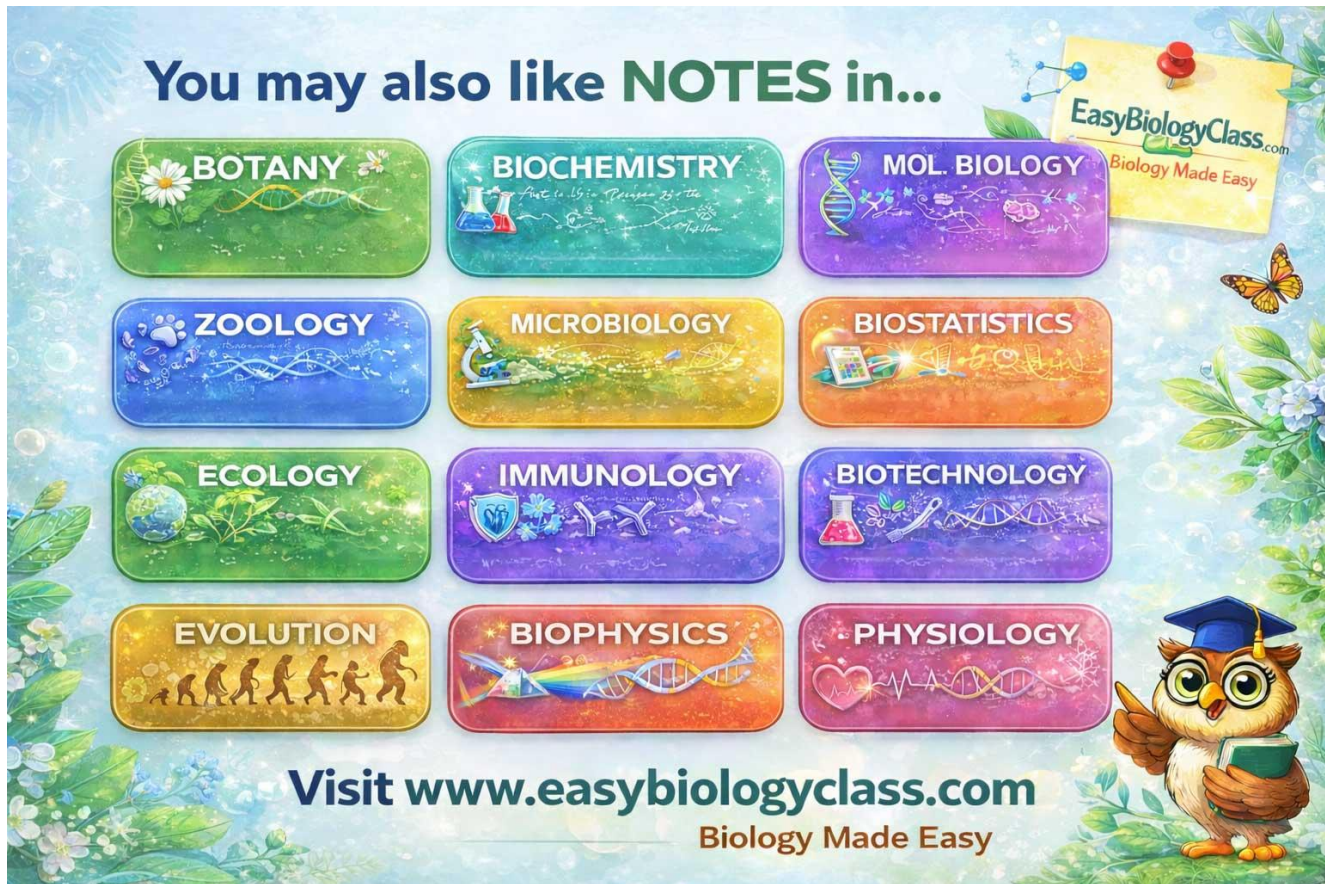
Restriction Map of Bacteriophage λ



2. Construction of DNA Fingerprints:

- DNA fingerprinting is a forensic technique used to identify individuals based on the variations in their DNA sequences.
- Many methods are now available for DNA fingerprinting and the most accurate one is DNA sequencing based methods.
- Restriction Enzymes also can be used to construct DNA fingerprints.
- DNA fingerprinting with restriction enzymes is actually an extended version of DNA restriction maps.
- The principle of DNA fingerprinting is that the different strains or species of DNA sample will have slightly different restriction maps.
- This difference in the restriction maps is because of their difference in the DNA sequences.

- Since restriction enzymes are sequence specific in their action, the restriction maps obtained will also show considerable variations depending on the extent of DNA sequence differences.
- The regions of DNA in an organism that are highly variable on restriction digestion generate unique DNA fingerprints.



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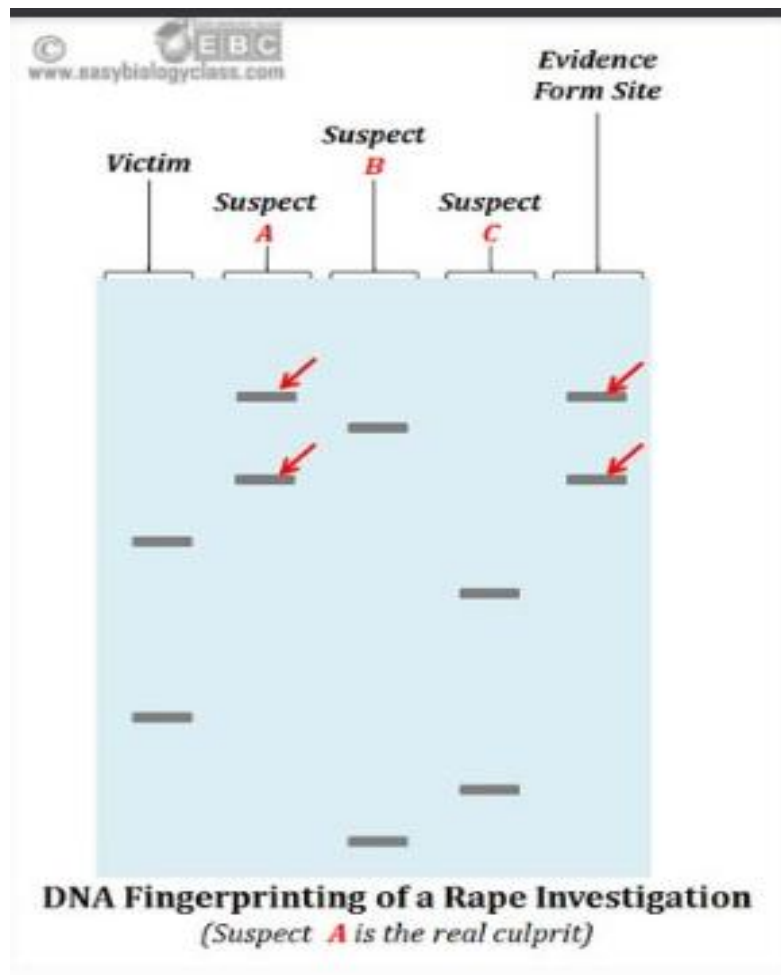
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- This feature is utilized in DNA fingerprinting for the identification of mutations or variations within in the genome of a population.
- Variation in the DNA fingerprints can be used to identify individuals in a large heterogenous population.
- Such DNA fingerprints are extensively used in solving paternity disputes, identification of suspects in forensic sciences etc.

How to construct a DNA fingerprint?

Here we use the DNA sample from a forensic investigation (a rape case)

- ❖ DNA is isolated from (1) the victim, (2) the evidence (such as from semen, blood or hairs) and (3) the suspect(s).
- ❖ Then the DNA sample is digested with a specific restriction enzyme.
- ❖ Fragments after restriction digestion are separated on an agarose gel.
- ❖ After electrophoresis, the DNA fragments are blotted (transferred) to a nylon membrane by Southern blotting technique.
- ❖ The bound DNA is then denatured and then treated with radioactively labeled probes.
- ❖ The labeled DNA probes hybridize specifically to the restriction fragments on the nylon membrane that are derived from this region.
- ❖ The labeled fragments are then identified by autoradiography.
- ❖ A diagrammatic representation of the autoradiogram is given **below**.



- ❖ From the results it is clear that the suspect 'A' is the actual criminal in the investigation.

- ❖ In order to confirm the results, different restriction enzyme and corresponding DNA probes can be used.
- ❖ Advantage of DNA fingerprinting in forensic science:
 - Exact identification of culprit with molecular evidence.
 - By the combination of PCR techniques, even the tiny DNA sample obtained from the site of crime can be used for generating DNA fingerprints.

3. Recombinant DNA Technology (rDNA Technology)

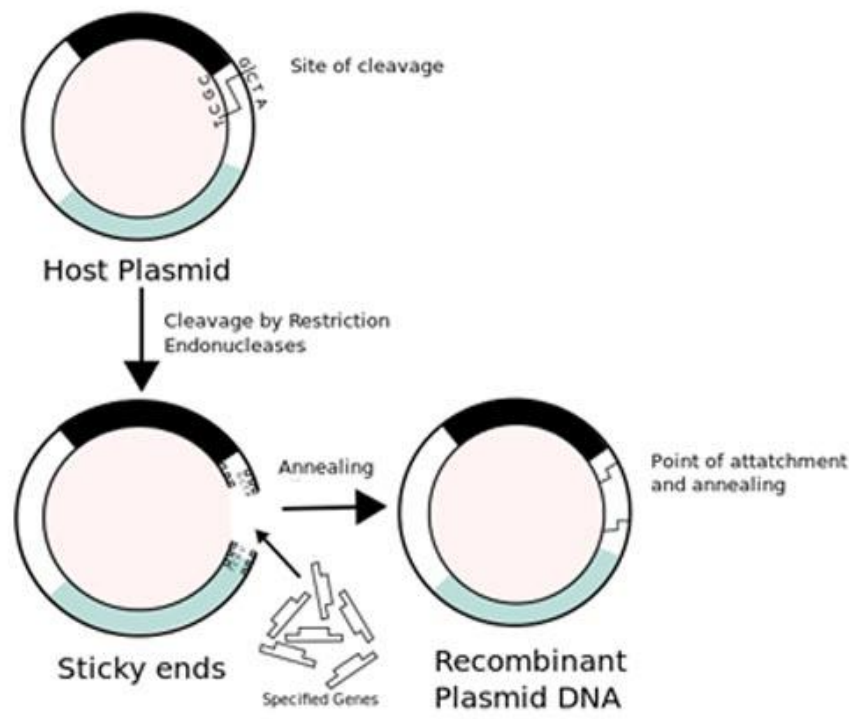
- Recombination technology is an artificial technique in the creation of recombined DNA molecules of different organism by joining or recombining the fragments of DNA generated by restriction enzyme treatment.
- The first recombinant DNA was produced by Stanley N. Cohen and Herbert Boyer in 1973.
- In their experiment, they combined two plasmids; pSC-101 and pSC-102 (each with two separate antibiotic resistant genes) and the newly created recombined DNA were incorporated into *E. coli*.
- The pSC-101 contains the gene for tetracycline resistance.
- The pSC-102 contains the gene for kanamycin resistance.
- The transformed bacteria after recombination show resistance to both these antibiotics.
- Many diverse techniques are now available in recombinant DNA technology.
- However, the basic recombinant technology is very simple as explained below:



Steps in recombinant DNA technology

- ❖ The first step is the extraction total DNA of the organism which contains the desired gene of interest.
- ❖ Next step is the generation of fragments of the above DNA with a suitable restriction enzyme.
- ❖ Then the DNA fragment containing the gene of interest is inserted to cloning vector to create a recombinant or chimeric DNA. The cloning vector can be a plasmid, bacteriophage, virus or small artificial chromosomes such as YAS and BAC.
- ❖ After cloning, introduce the recombined vector into a host cell such as bacterium (*E. coli*). Most of the cloning vectors contain sequences that allow them to replicate autonomously within the competent host cell.
- ❖ Then induce the express the gene of interest in the host cell to produce desired product.
- ❖ The product can be extracted from the medium using suitable downstream processing techniques.

Construction of a Recombinant DNA



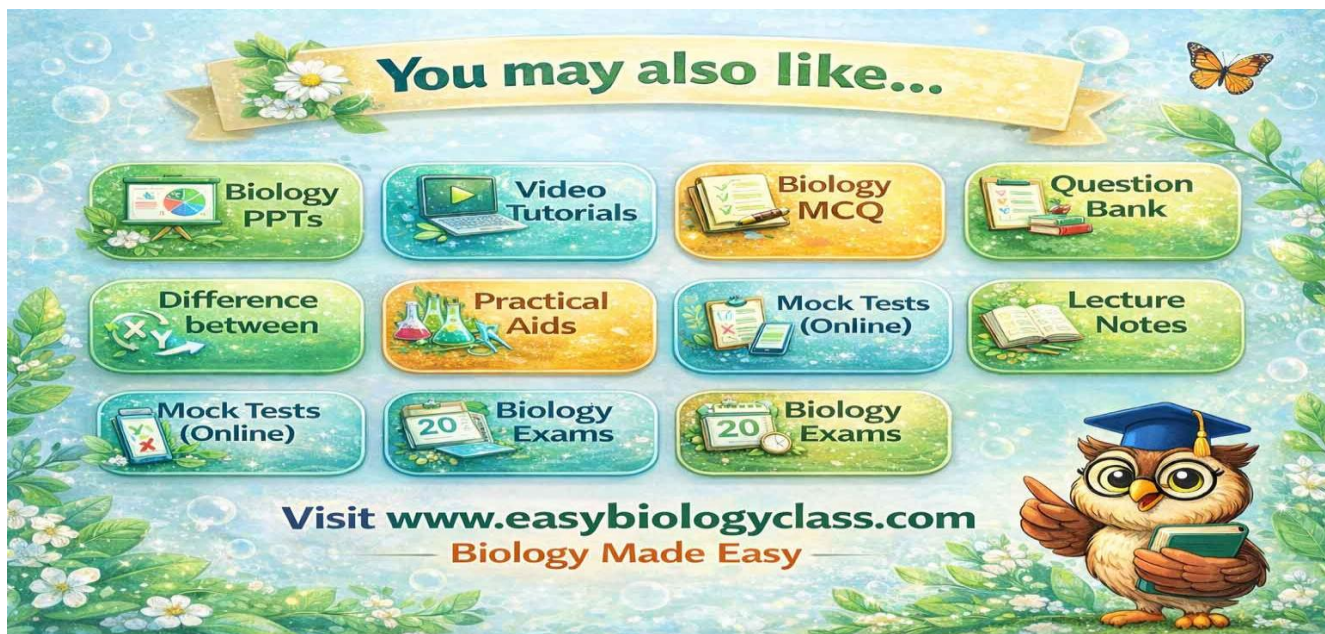
Restriction site in the cloning vectors

- All the cloning vectors possess a unique cloning site.

- This cloning site contains the target sequence of specific restriction endonuclease to allow site specific insertion of foreign DNA.
- The commonly used plasmid vectors possess several such restriction sites at the cloning site and such a site in the vector is called multiple cloning site or poly-linker.
- Restriction endonucleases used in recombination technology produce DNA fragments with single stranded over hangs at their 3' or 5' ends.
- These overhangs are called sticky ends or staggered ends.
- These sticky overhangs can transiently base pair with complementary sticky ends of the desired DNA fragments very easy and specifically.
- Thus, in a simplest kind of recombination, a single restriction enzyme is used to cut the cloning vector and the gene of interest.
- Due to the similarity in sequence complementarity, use of single restriction enzyme can cause two problems: (1). Ligation of the vector without insertion and (2) the orientation of inserted gene of interest get inverted.
- ❖ The use of two separate restriction enzymes can overcome this problem.

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