Athar Batch

Genetics

Lecture: 25

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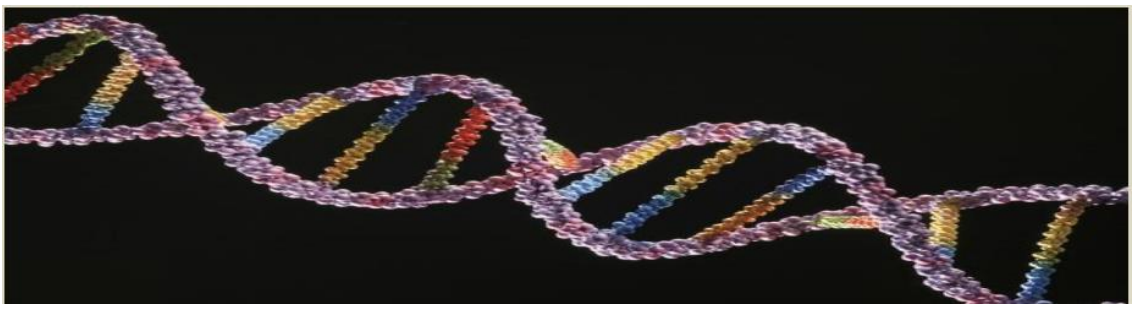
النادي الطبي

LECTURE 25



Recombinant DNA Technology

By
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Recombinant DNA technology

- **In the early 1970s, biochemists at stanford university showed that genetic traits could be transferred from one organism to another.**

- **In this experiment, the DNA of one microorganism recombined with the inserted DNA sequence of another, and thus had been edited to exhibit a very specific modification.**

- The DNA of an organism and the DNA of another organism are recombined with each other → one hybrid molecule from two different sources.

- **The actual editing, or insertion process, is painstaking, as it involves manipulating incredibly tiny pieces of incredibly tiny organisms.**

العملية هاي شاقة جدا لأننا بنتعامل مع جزيئات صغيرة جدا لكائنات حية دقيقة

Important definitions

- **The recombinant DNA: the term given to the fused human gene DNA and the bacterial (plasmid) DNA.**
- **Plasmid : the extrachromosomal DNA present in some bacterial cells and normally gives the bacterial cells the power to resist the action of antibiotics.**
- **The recombinant DNA molecule is also known as hybrid or chimeric molecule.**

Plasmid: circular and double stranded DNA.

Recombinant DNA = hybrid DNA = chimeric DNA

Recombinant DNA technology

- **It is genetic engineering which causes artificial modifications of genetic constitution of a living cell by introduction of foreign DNA through experimental techniques.**

- **The techniques involves:**
  - **Splicing of DNA by restriction endonucleases.**
  - **Preparation of chimeric molecules.**
  - **Cloning of large number of identical target DNA molecules**

Tools of recombinant DNA technology

Restriction endonucleases

Vectors or vehicle DNA

Passenger DNA (foreign DNA)

Hosts: they include Bacterial-animal or plant cells

مثلا لو بدنا نشتغل على ال Human gene

رح نجيب Human gene و DNA من مصدر آخر و بعدين ندمجهم مع بعض بعدين نحقنهم في خلية حية

تسمى بال Host cell زي الخلية البكتيرية او الخلية النباتية او خلية حيوانية فلما تتكاثر هاي الخلايا رح يضل

ال DNA ينقسم و هيك بكون كثرت ال Hybrid gene و عملت منه نسخ كبيرة منه

بهاي الحالة ال Passenger DNA هو ال Human gene و ال Vector هو ال Plasmid

Restriction endonucleases

They are important class of DNA endonucleases that recognize specific sequence of bases in DNA (restriction sites) and have the ability to cleave DNA molecules at these sites so they serve as molecular scissors.

Endonucleases: break down the polynucleotide chain from the inside.

- Restriction sites: restriction endonucleases التسلسل اللي بتقطع عنده ال
- Each restriction endonuclease can recognize it specific restriction site.

They are found in a wide range of bacteria, their function is to recognize and cleave foreign DNA and so prevent or restrict the infecting virus "bacteriophages" (so the name restriction). They are called endonucleases because they cut in the middle of the polynucleotide chain.

- تستخدمهم البكتيريا كوسيلة دفاع من الفايروسات

- **The cell's own DNA is protected from cleavage by these restriction enzymes by methylation as bacteria that contain these enzymes also contain a DNA methylase enzyme that methylates the cytosine bases of the bacterial DNA at the restriction site rendering the bacterial DNA resistant to the action of the restriction endonuclease.**

- The bacterial DNA may have a sequence of nucleotides that is the same for the viral sequence in the restriction site, but the sequence of nucleotides in the bacterial cell is protected by methylation process.

- **Over 3,000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially. They are named according to the bacterial species from which they are isolated .**

- 600 restriction enzymes are sold by companies for experimental use.

> - **The first letter indicates the genus name , and the other two indicate the species name and a roman numeral indicates the order of discovery of an enzyme from that species. e.g. EcoRI was the first enzyme isolated from Escherichia coli (E.coli).**

- They are named according to the bacteria species that produce this enzyme.
- EcoR1→ E: *Escherichia*, co: *coli*, R: strain of the bacteria, 1: indicates that this enzyme is the first discovered restriction enzyme in this type of bacteria.

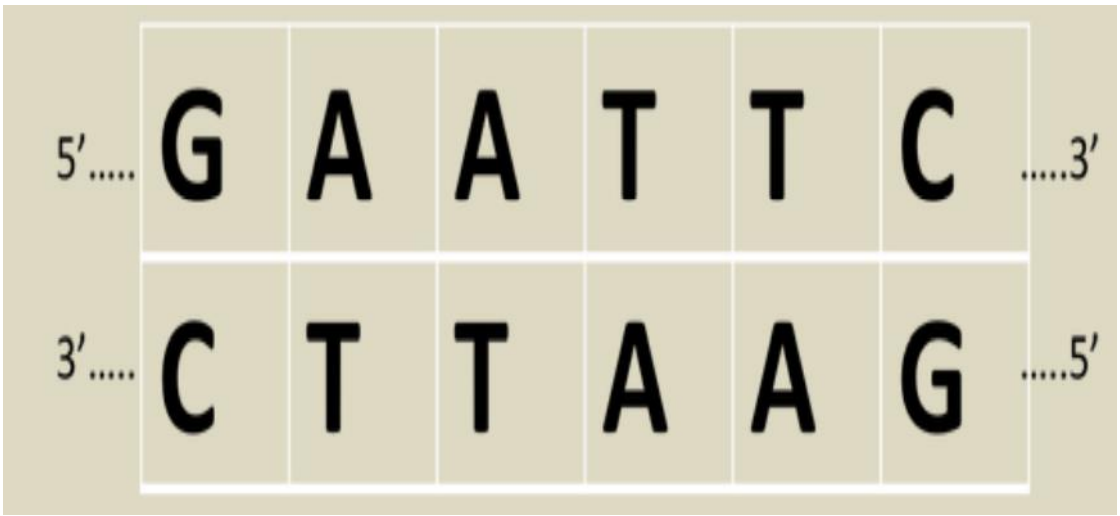
Derivation of the EcoRI name		
Abbreviation	**Meaning**	**Description**
E	*Escherichia*	genus
co	*coli*	specific species
R	RY13	strain
I	First identified	order of identification in the bacterium

Restriction sites

- **They are sequences of four, six, eight or rarely more bases with palindrome arrangement.**
- **Palindrome in Greek means "to run backwards". It is similar to a word that reads backwards or forwards similarly e.g. madam. These are also called inverted repeat sequences which means the nucleotide sequence in 5`to 3` direction is the same in both strands.**

- *Each restriction enzyme can recognize only one restriction site.*
- *Palindrome sequence: inverted repeat sequence (the reading of the sequence from 3` to 5` direction in one strand is the same for the reading from 3` to 5` direction in the other strand).*

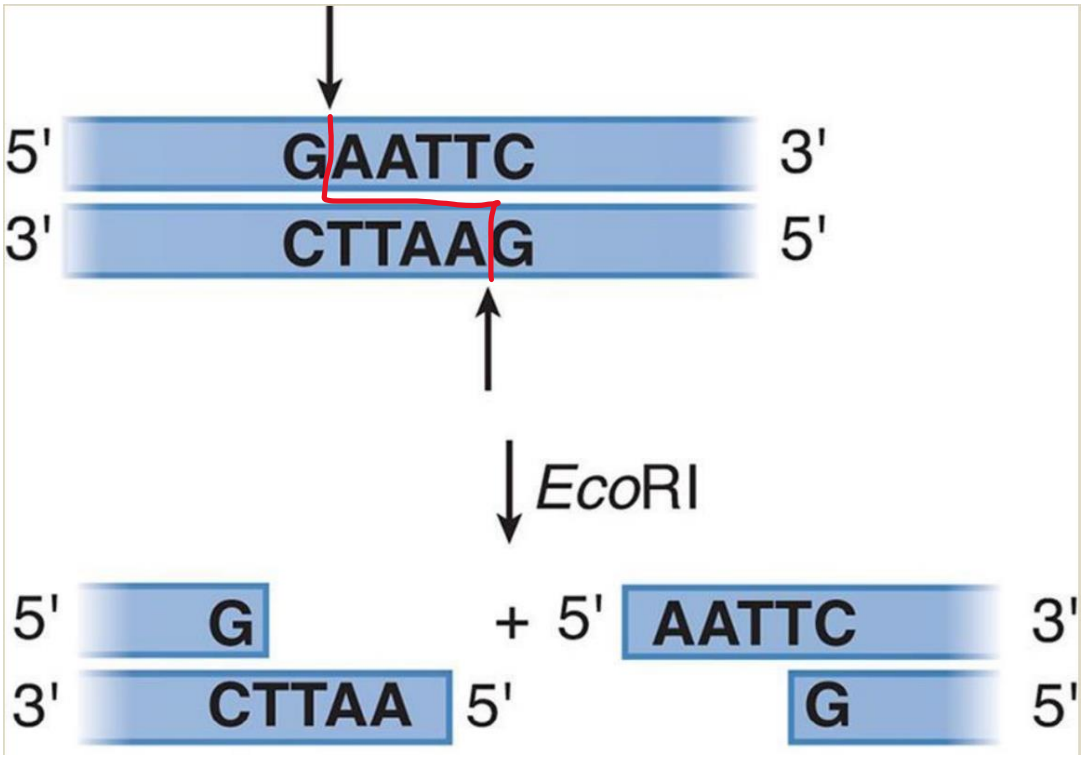
palindrome arrangement



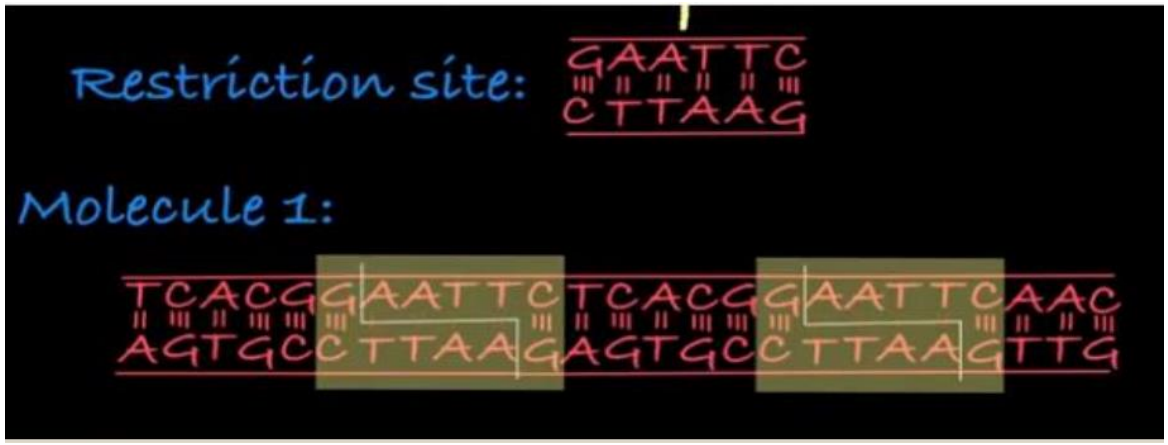
- **They cut either blunt end cut or sticky end cut.**
- **Blunt end cut cleaves both strands of DNA so as to leave no unpaired bases on either ends.**

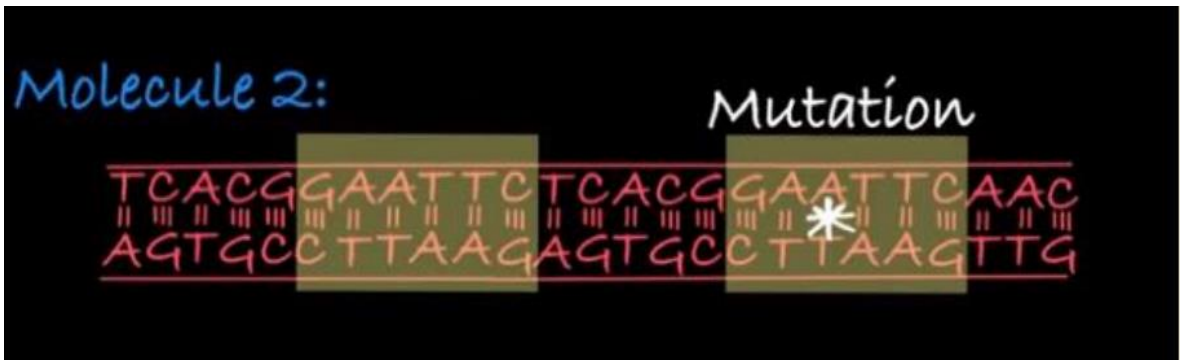
- *Blunt end: each base is paired with its complementary base, and there is no base unpaired.*

- **Sticky end cut leaves unpaired ends which are called cohesive ends, sticky ends, or staggered ends.**



- Sticky ends: unpaired bases at the end.





- If there is a mutation in the restriction site, restriction enzymes cannot recognize the restriction site.
- We can detect if there is a mutation in particular gene.

نعمل Restriction لل 2 Alleles للجين ولو مثلا هاد الجين يعطينا 3 قطع بشكل طبيعي و احد الاليلات اعطانا قطعتين فقط بعد القطع نكتشف انه فيه Mutation

- **Blunt end of the DNA fragment are ligated at a low efficiency than those with complementary sticky end, while the sticky end of the DNA fragment facilitates the ligation of amplified DNA into cloning vector.**

Restriction map

- **If a piece of DNA from a species is made to react with a specific restriction enzyme, a characteristic array of cut pieces will be produced , this is called a restriction map. These fragments can be isolated by electrophoresis.**

- *Restriction map is used to identify the restriction sites for a restriction enzyme.*
- *Restriction endonucleases are used to extract specific gene.*

vectors

- **In order to introduce the human gene into bacteria, at first, the gene is transferred into a carrier, known as a vector.**

- Vectors are the carriers for the extracted human gene to form the hybrid molecule.

- **Vectors show the following essential features:**
- **They are able to replicate.**
- **They must contain a site for insertion of target DNA.**
- **They could be inserted into the host cell**
- **They have a selectable marker to trace them after insertion.**

- Replication of the vector is important to make many copies of the hybrid gene.
- The selectable marker is important to make sure that it is inserted inside the host cell.

Commonly used vectors

- **(1) plasmids:**

➢ **They are bacterial extra chromosomal circular double stranded DNA.**

➢ **They replicate independent of bacterial DNA.**

➢ **Foreign DNA "small pieces from 6-10 Kbp" could be incorporated in plasmid by using specific restriction endonuclease.**

- **Plasmids usually carry one or more of antibiotic resistance genes "which are utilized as selectable markers". i.e. a method of selection of cells containing recombinant DNA molecule as growth in presence of antibiotic, only the bacteria containing the plasmid will grow.**

مثلا لو ال plasmid يحتوي على resistance gene لل Ampicillin

لو حقنت ال human gene اللي ماسك فيه الPlasmid في Bacterial cell فممكن هاي البكتيريا تعمل Uptake لهاد ال Hybrid gene و ممكن لا ... طيب كيف بدنا نعرف؟

بنحط ال bacteria في وسط يحتوي على Ampicillin فإذا عاشت البكتيريا يعني عملت Uptake للـ Hybrid gene و البكتيريا اللي ماتت ما بتكون اخذته

(2) Bacteriophage :

- **Is a virus that infects and replicates within a bacterium.**
- **Plasmids can accept only about 6-10 kbp long foreign DNA. If a DNA segment of 10-20 kbp is to be introduced, bacteriophages may be the vectors of choice.**

One Kb=1000 nocleotides base sequence.

- **(3) Cosmids :** *(artificially constructed cloning vectors)*

❖**They are plasmid that also contain some portion of bacteriophage DNA . They can take up still bigger fragments of DNA "up to 50 Kbp".**

ما بين ال plasmid و ال Bacteriophage

Preparation of Chimeric DNA Molecules

- **Chimera is the Greek mythological monster with a lion's head, goat's body and serpent's tail.**
- **A vector carrying a foreign DNA is called <u>Chimeric DNA</u> or <u>Hybrid DNA</u> or <u>Recombinant DNA.</u>**

Chimera:

فسميناه بهاد الاسم نسبة لهاد الوحش الاسطوري الهجين

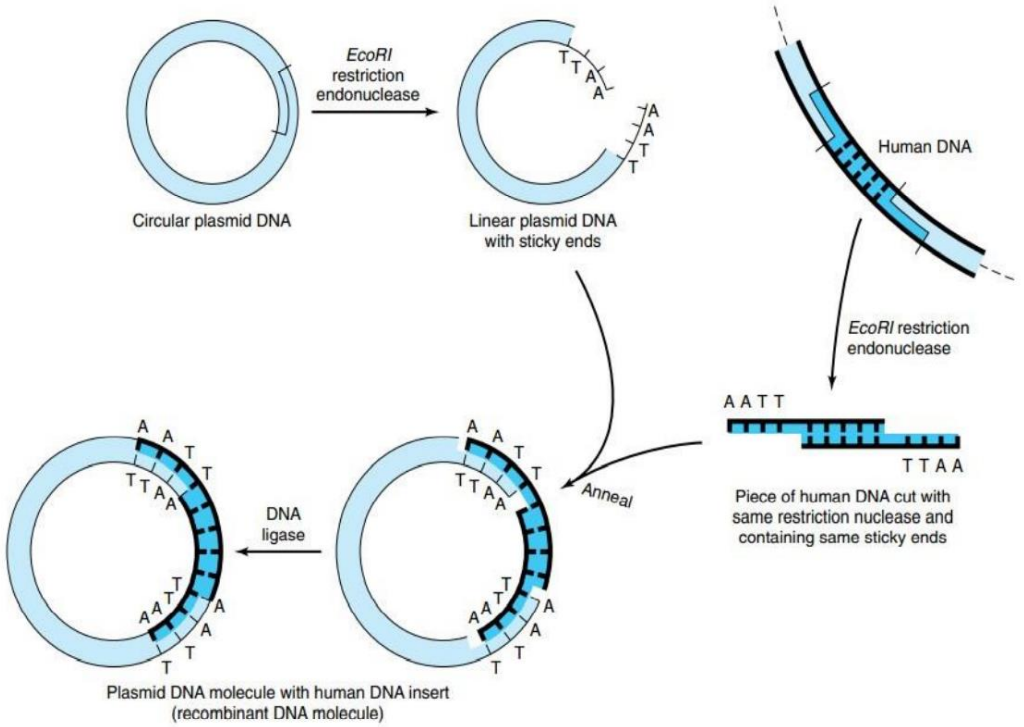
i. **A circular plasmid vector DNA is cut with a specific restriction endonuclease (RE). If EcoRI is used, sticky ends are produced with TTAA sequence on one DNA strand, and AATT sequence on the other strand**

- **ii. The human DNA is also treated with the same RE, so that the same sequences are generated on the sticky ends of the cut piece.**

لازم يكونوا ال *Sticky ends* لل *Human DNA* و الVector نفس الشي

- **iii. Then the vector DNA and human cut-piece DNA are incubated together so that annealing takes place. The sticky ends of both vector and human DNA have complementary sequences, and therefore they come into contact with each other.**

- **iv. Then <u>DNA ligase</u> enzyme is added, which introduces phosphodiester linkages between the vector and the insert molecules. Thus the chimeric DNA is finally produced.**



Cloning of Chimeric DNA

- **A clone is a large population of identical bacteria or cells that arise from a common ancestor molecule.**
- **Cloning allows the production of a large number of identical DNA molecules. The hybrid molecules are amplified by the cloning technique.**
- **DNA cloning is an in vivo DNA amplification.**

- *Clone:* مستعمرة
- *In vivo DNA amplification:* كثرنا ال DNA داخل خلايا حية
- *In vitro DNA amplification: using machineries to amplify the DNA.*

- **Only 5% of bacteria colonies contain the desired vector, so we have to select the desired colonies.**
- **The bacterial host cell containing the recombinant vector can be selected if the vector contains an antibiotic resistance genes.**
- **Bacteria without vector die in the presence of antibiotic medium.**

Isolation of cloned foreign DNA or its protein product:

Cells containing an appropriate chimeric plasmid are cultured then the plasmids are isolated from host cells (the bacteria are lysed and the hybrid plasmids are isolated) and treated with the same restriction enzyme to release the foreign DNA.

If the host cells are grown under conditions that permit the production of protein produced from target DNA, then the protein of interest can be isolated.

- We use recombinant DNA technology for:
  1. study of the gene and identification of the sequence of nucleotides in that gene.
  2. To make the gene express itself (production of proteins). An example is the gene that produces insulin, so that we can get human insulin

- **Hundreds of human proteins are now being synthesized by the recombinant technology.**

- **Recombinant human insulin is now available in market. Other useful products produced include; interferons, hepatitis B antigen and growth hormone.**