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Molecular biology - Recombinant DNA Technology & restriction enzymes

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AUTHORS DISCLOSURE

The authors have no actual or potential financial conflicts of interest associated with this presentation.

This presentation is directed to the students registered on the 'Pharmaceutical Biotechnology' course at the School of Pharmaceutical Sciences, the Hashemite University, Jordan. Academic year 2024/2025.

LEARNING OBJECTIVES

لازم تكونو قادرين توصفو الخطوات لهاي ال techniques

موضوع ال ethical و ال safety كتير مهم لما نتعامل مع هاي ال technology
ومن أهم الأمور اللي رح تواجهكم لو شتغلتو في هاد المجال

دائما حاولوا فكروا خارج الصندوق وناقشو أفكاركم وابحثو عنها

By the end of this presentation, students will be able to:

1. Define recombinant DNA technology and explain the role of restriction enzymes and vectors.
2. Describe key steps involved in the rDNA process, including gene isolation, ligation, and transformation.
3. Describe how sticky and blunt ends influence the ligation process in recombinant DNA experiments.
4. Explain the principle of DNA extraction and purification methods used in rDNA technology.
5. Describe how recombinant proteins, such as insulin, are produced in a laboratory setting.
6. Examine the ethical and safety concerns associated with using rDNA technology.
7. Propose innovative applications of rDNA technology in addressing global challenges, such as food security or disease treatment.

INTRODUCTION – RECOMBINANT DNA TECHNOLOGY

من كلمة recombinant بنستنتج أنه ال DNA الناتج رح يكون معدل أو معاد تركيبه لاني جبت جين جديد وضفته عليه صار DNA معدل وراثيا

- **Recombinant DNA Technology** is a technology that uses **enzymes** to *cut* and *paste* together DNA sequences of interest.

ليش اعتبرنا artificial لانه صار عليه تدخل بشري

- The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources.
- Recombinant DNA technology is popularly known as **genetic engineering**.

في اسم تاني رح تلاقو يستخدم لنفس المعنى وهو ال genetic engineering

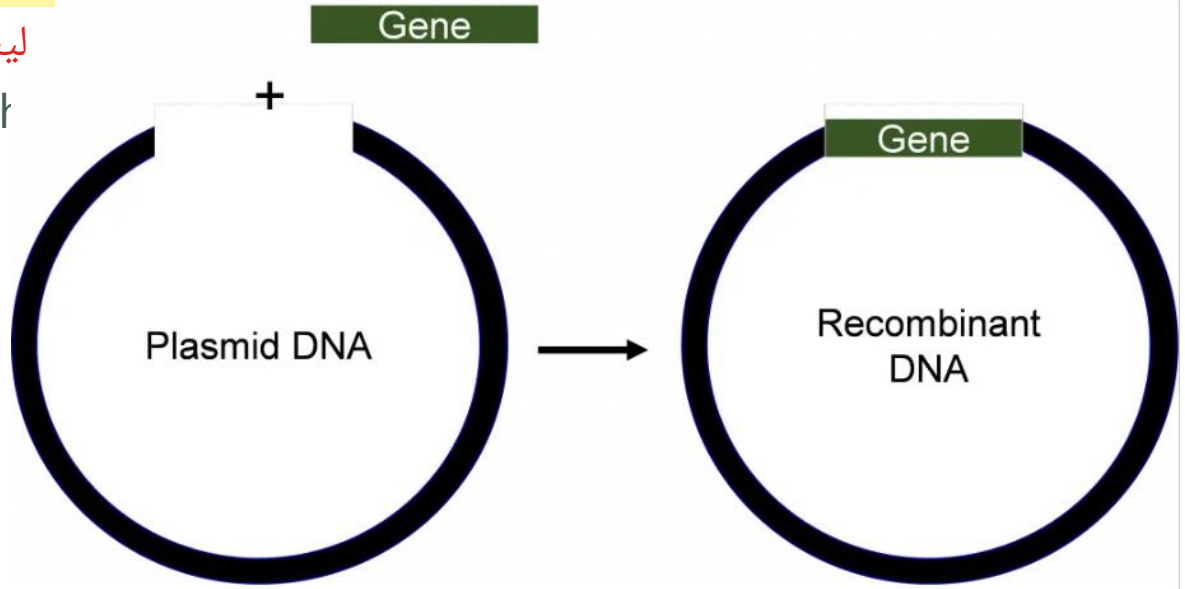


Fig: Recombinant DNA Technology

- The recombinant DNA technology emerged with the discovery of *restriction enzymes* in the year 1968 by Swiss microbiologist Werner Arber.

هاي ال technology كلها ما كانت رح تصير الا لما واحد من العلماء Werner arber اكتشف ال restriction enzymes

ال restriction enzymes الهم دور كتير مهم في هاي ال technology همة اللي بقصو الجين عشان ننقل من مكان لمكان

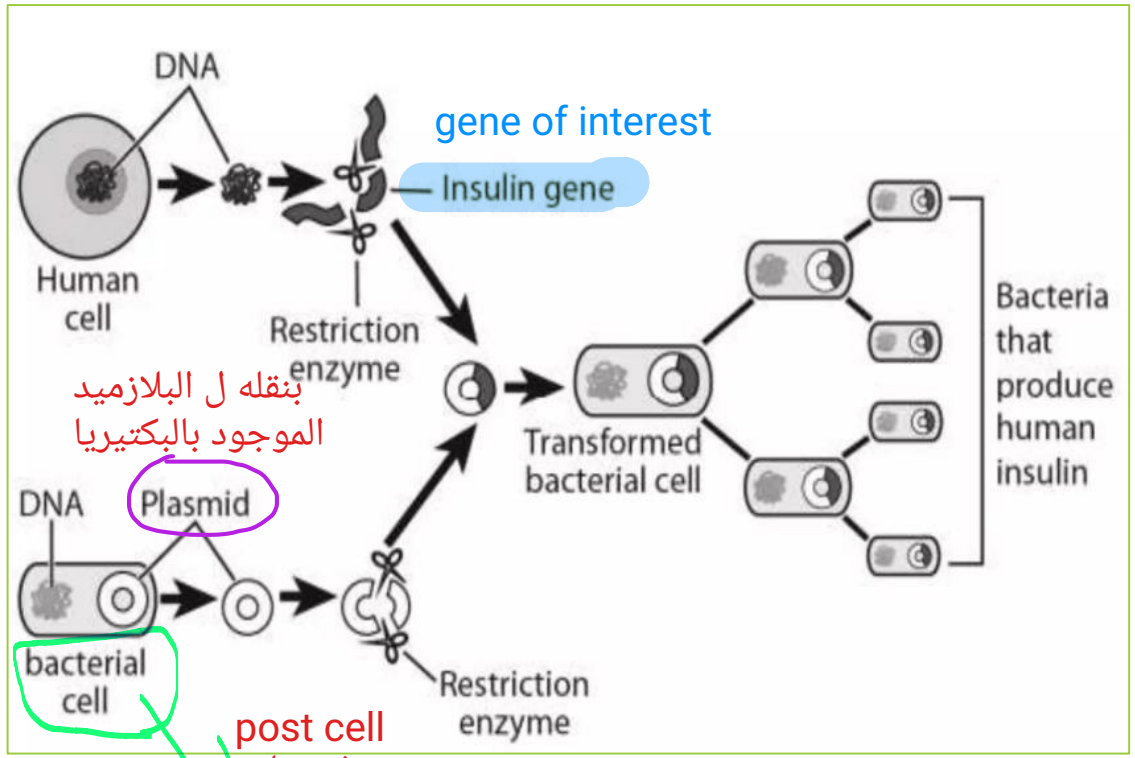
Process Of Recombinant DNA Technology

عشان نفهم اكتر شو ال recombinant DNA technology لازم نفهم الخطوات المتضمنة بالعملية

ونحتاج لنسخه نفس فكرة لما بدك تشتغل chemical reaction بتحتاج لتركيز كافي نفس الاشي بعملية ال recombinant

Step 3?

- Step-1. ***Isolation*** of Genetic Material. اول خطوة بعملها اني بشتغل على ال genatic material ولازم ال genetic material تطلع برا الخلية حتى اقدر اشتغل عليها فبفصل ال genetic material عن الخلية
  - The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.
- Step-2. ***Cutting*** the gene at the recognition sites.
  - The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called 'restriction enzyme digestions'. فصلت ال gene of interest وعملتله cutting الخطوة التالتة بعمله amplification (يعني اعمل منه نسخ) قبل نقله ل New DNA
- Step-3. ***Amplifying*** the gene copies through Polymerase chain reaction (PCR).
  - It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using restriction enzymes.
- Step-4. ***Ligation*** of DNA Molecules. بنقل ال DNA ل new genatic material
  - In this step of Ligation, the joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.
- Step-5. ***Insertion*** of Recombinant DNA Into Host. اخر خطوة برجع البلازميد ل ال host cell
  - In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once the recombinant DNA is inserted into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.



ال بروتين الناتج عن العملية بسمي ال recombinant protein

BASIC PRINCIPLE OF RECOMBINANT DNA TECHNOLOGY

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- The principle of recombinant DNA technology encompasses a series of systematic steps that facilitate the manipulation and analysis of genetic material.

- This technology enables researchers to isolate, modify, and transfer specific genes, playing critical and potential roles related to genetic functions and applications in various fields such as medicine and agriculture.

لما نحكي عن خطوات ال recombinant DNA من زاوية تانية في sub application زي ال gene cloning
من أهم ال application with in ال recombinant DNA

- The following points outline the foundational steps involved in this technology.

البكتيريا بعد ما دخلنا عليها ال recombinant DNA عشان احصل على كميات من البكتيريا كافية لإنتاج كميات كافية من ال protein of interest زي الأنسولين بالمثال السابق عن طريق (recombinant protein) ال{ cloning }

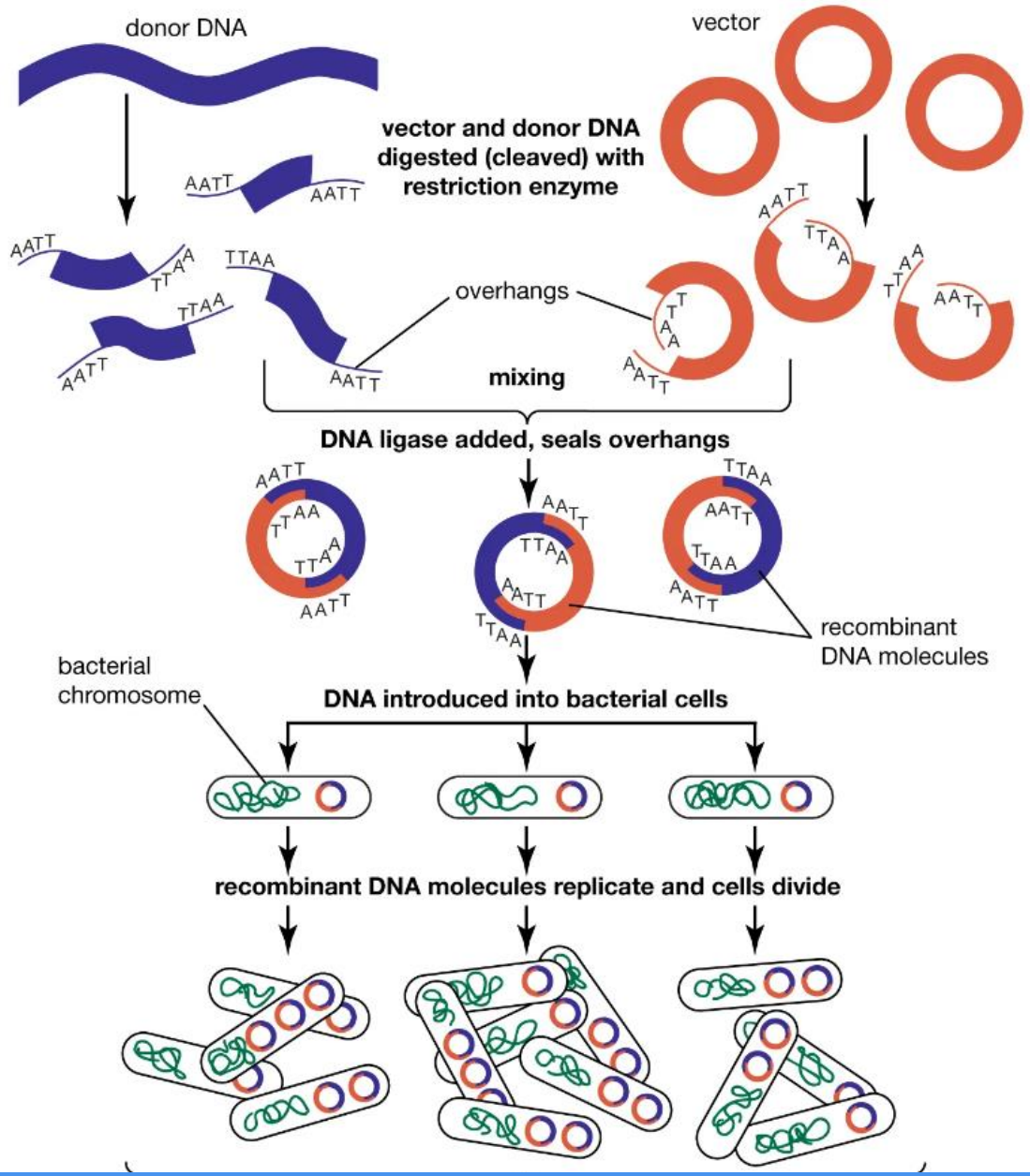
عملية ال cloning technically زي فكرة زراعة الخلية بنعمل culture for recombinant bacteria to produce clones كل خلية بتزرعها رح تصير تنقسم وتعطي عدد كبير من الخلايا كل بكتيريا رح تنتج clones بالنهاية رح يكون في number of colonies

1. GENE CLONING AND DEVELOPMENT OF RECOMBINANT DNA

- **Gene Cloning**: This is a specific application of recombinant DNA technology. It refers to the process of making multiple copies of a particular gene (*making clones, colonies!).*
- This initial step involves isolating the gene of interest (GOI) from the source organism.
  - Finding a specific gene in a DNA – finding a needle in a haystack!
- The target DNA is then ligated into a suitable vector, such as a plasmid, to create recombinant DNA.
- This recombinant DNA can replicate independently within a host cell, allowing for the generation of multiple copies of the desired gene.

ما يميز هذه العملية

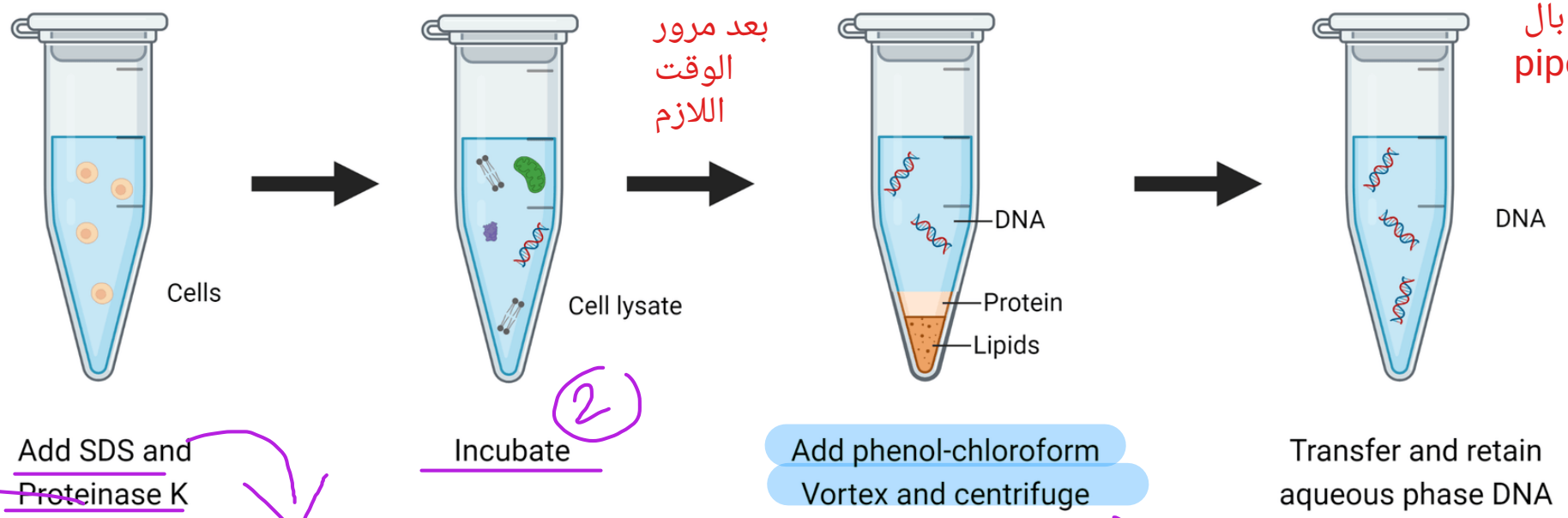
  - The members of a clone are genetically identical, because cell replication produces identical daughter cells each time.



DNA isolation - extraction

بنقدر نعمل isolation ل DNA وال procedure كتير سهلة الدكتورة حبت تحطها حتى نستفيد منها practically
حتى افصل ال DNA بتبع هاي الخطوات :
اول اشي بجيب عينة الخلايا بضيف cell suspended in aquase solvent عليهم SDS و protein kinase

في مشكلة وجدو أنه الخلية في المراحل الأخيرة لما تبلش بدها تموت بسبب ال surfactant بتصير تهاجم حالها عن طريق البروتينات الموجودة فيها ف حتى امنع البروتينات تكسر ال DNA بضيف ال proteinase K



بعد مرور الوقت اللازم

بسحب ال DNA بال pipette

وظيفة ال SDS هو surfactant بيكسر ال cell تحديد ال cell membrane
بعد ما تكسر ال cell membrane رح تطلع ال DNA لبرا في ال solutions

إضافة organic phase بعمل ترسيب ل protein and lipids وبطلع ال DNA
ال aquase phase لانه عليه شحنة

عشان اعمل cutting لازم اول احدد موقع الجين

HOW TO LOCATE THE GENE OF INTEREST?!

- To locate a specific gene of interest (GOI) in a DNA sample, scientists use a combination of techniques that allow them to target, identify, and isolate that gene with precision. Here's how it's generally done:

1. Identify the Sequence or Features of the Gene of Interest

✓ First, researchers need to have some information about the GOI, such as part of its DNA sequence, or at least some of its functional features (like protein it codes for).

✓ If they know the sequence, they can design specific **primers** or **probes** that match the gene's unique regions.

2. Polymerase Chain Reaction (PCR)

- PCR is one of the most common methods for locating and amplifying a GOI if its sequence is known.
- **Primers**: Short, single-stranded DNA sequences are designed to match the sequences flanking the GOI. They're complementary to the beginning and end of the gene, allowing the DNA polymerase enzyme to only amplify that specific region.
- PCR selectively amplifies the GOI, producing millions of copies of it, making it easy to detect in a DNA sample.
- This technique is highly specific and allows scientists to locate and isolate the GOI even in a large DNA sample.

3. Hybridization with a Gene Probe

4. DNA Sequencing and Bioinformatics

5. Screening a DNA Library

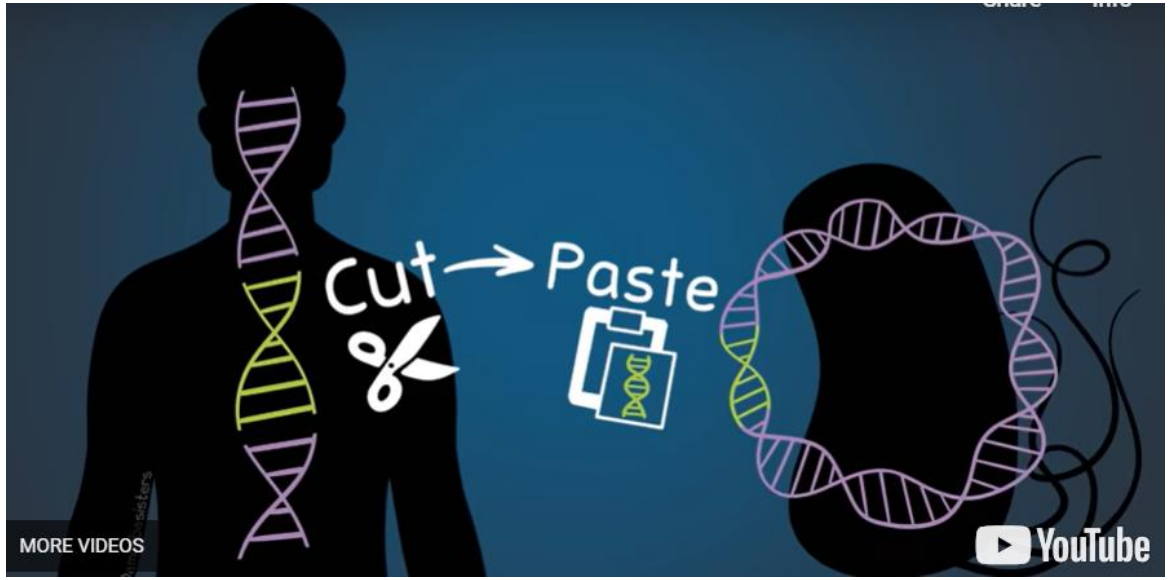
6. Fluorescent In Situ Hybridization (FISH)

- **FISH** is a technique often used to locate a gene's position on a chromosome within a cell.

Cutting of DNA!

لما بدي اعمل cutting بدي مقص ومقص الخلية هو ال restriction enzymes

- Into large fragments by mechanical shearing
- Restriction enzymes are the scissors of molecular genetics.



Restriction enzymes

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- Restriction enzymes are endonucleases (Endo (inside), nuclease(cuts nucleic acid), which catalyze the cleavage of the phosphodiester bonds within both strands of DNA.

يحفز ال cleavage

- They require Mg2+ for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the point of cleavage.

بتبلش

بتنتهي

لازم يكون في mg+2 في ال environment سواء
كان ال restriction داخل الخلية أو خارجها

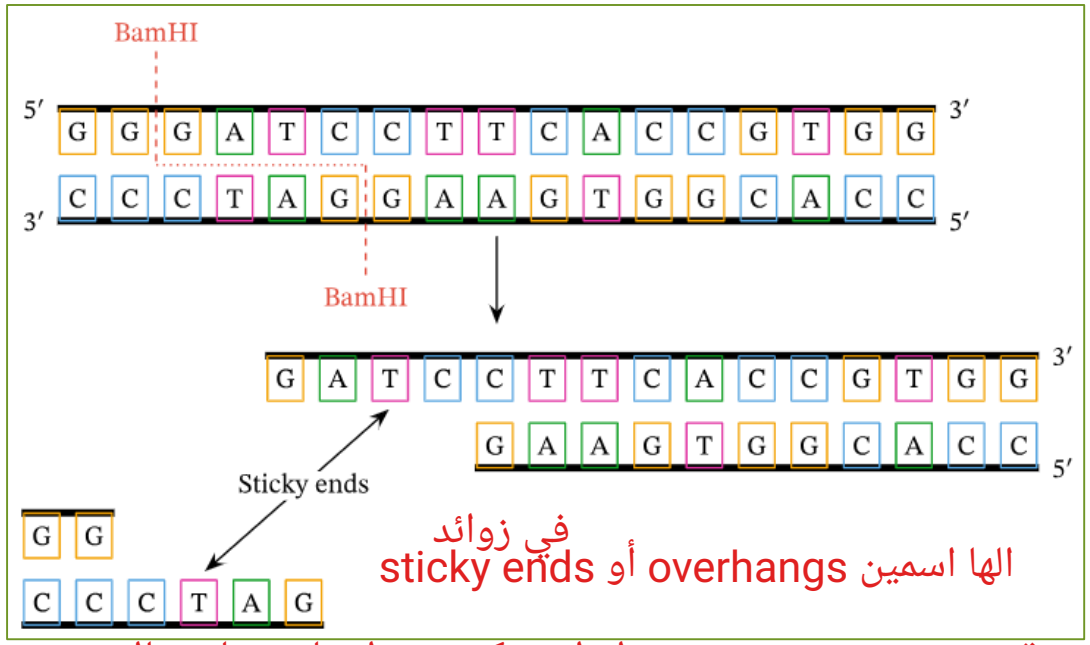
Restriction enzymes – BIOLOGICAL FUNCTION

ال restriction مش موجودة بال human cell
موجودة في ال bacteria cell بتستفيد منها البكتيريا للدفاع عن نفسها

- Restriction enzyme is part of the cell's restriction-modification system in bacteria.
- The phenomenon of restriction modification in bacteria is a small scale immune system for protection from infection by foreign DNA.
- Bacteria can protect themselves only after foreign DNA has entered their cytoplasm (as bacteriophages).

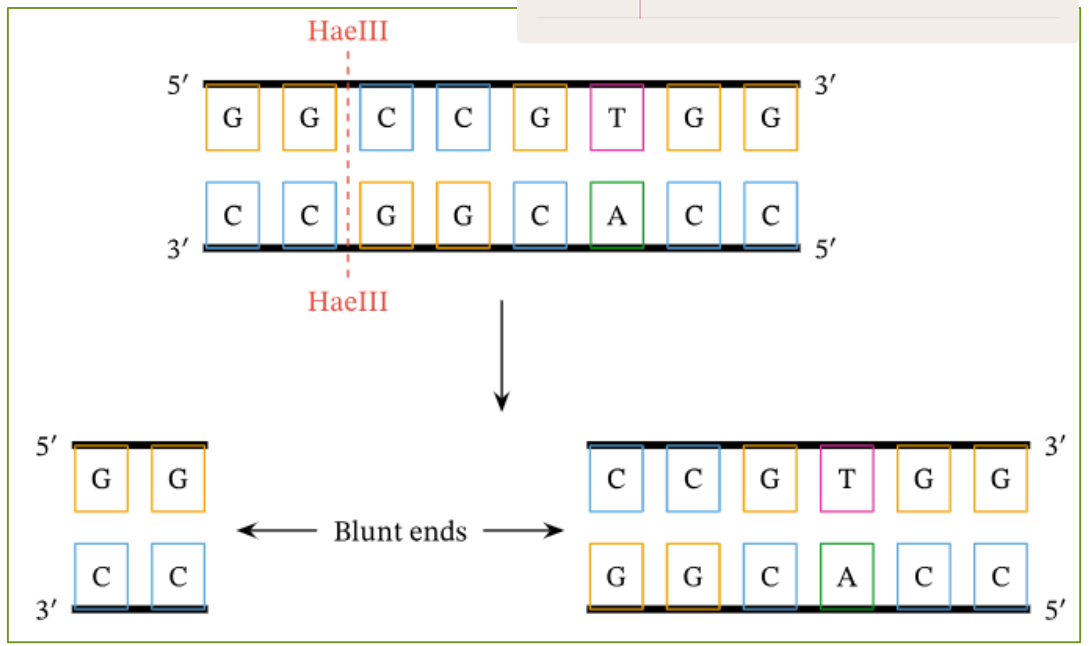
ال DNA لما يصير له
بصير cutting أو restriction
ب two way حسب نوع ال
restriction enzymes
وحسب ال goal اللي بدي اوصله

DNA can be cut by restriction enzymes in two different ways!



لو رجعت قربتهم ع بعض برجعو برتبطو او ممكن يرتبطو باشي تاني بال environment

One way is to cut DNA to leave **overhangs** of unpaired DNA based, or "**sticky**" ends (overhangs). They are called sticky ends because the DNA bases of each unpaired end have an affinity for each other based on complementary base-pairing rules.



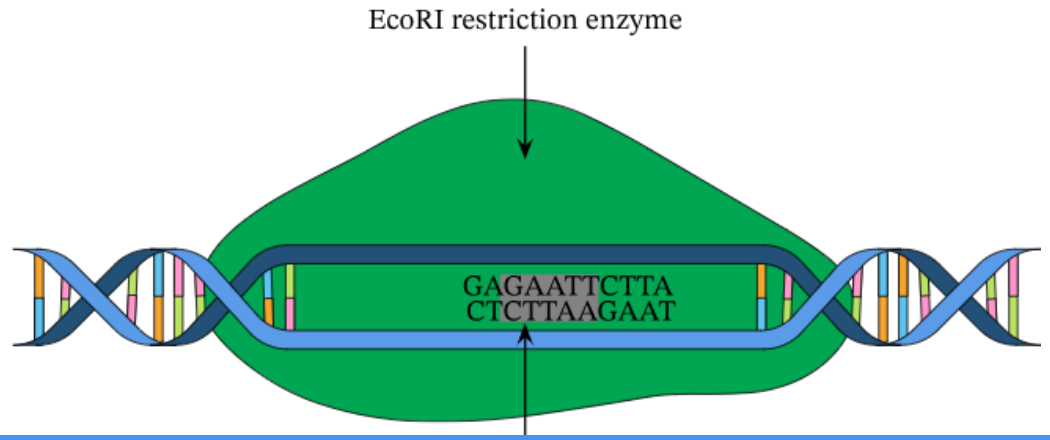
مافي زوائد تسمى blunt

Another way is to cut DNA to leave a "**blunt**" end in which there are no overhangs.

Recognition sequence

كيف ال restriction enzymes بعرف وين يروح يقص ؟ بكون في recognition sequence زي لابل

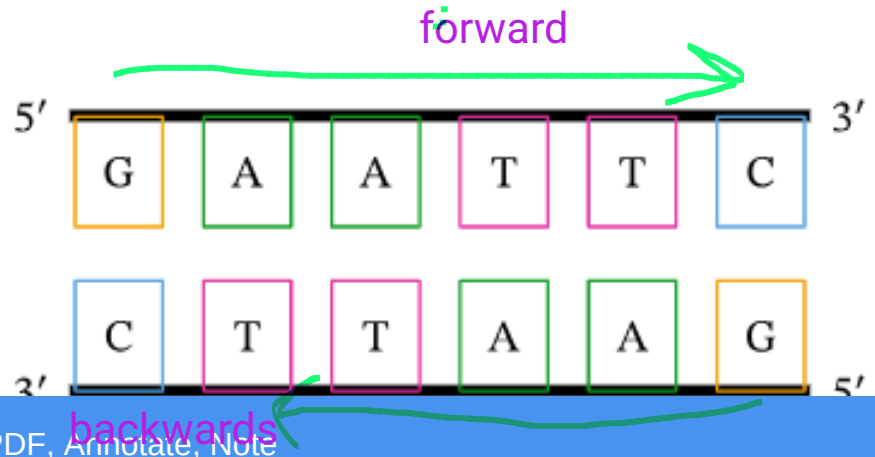
- The distinguishing feature of restriction enzymes is that they only cut at very specific sequences of bases. This specific DNA sequence is called **recognition sequence**.
- A recognition sequence is the specific sequence of DNA that a restriction enzyme recognizes and cleaves.
- Restriction enzymes are traditionally classified according to the subunit composition, cleavage position, sequence-specificity and cofactor requirements.
- A restriction enzyme requires a specific double stranded recognition sequence of nucleotides to cut DNA.
- Recognition sites are usually 4 to 8 base pairs in length.
- Cleavage occurs within or near the site.



Palindrome

recognition sequence نوع من أنواع ال

- A **palindrome** is a word or sequence that reads the same forward and backward (for example, "radar"). Palindromes are how restriction enzymes recognize their target sequences. They read the same when read in the 5′ to 3′ direction on both strands.
- The majority of restriction enzymes recognize and cut DNA at palindromic sequences. معظمهم
- The vast majority of restriction enzymes do recognize palindromic sequences, as this allows for the recognition of both strands of the DNA simultaneously. However, some restriction enzymes may target non-palindromic sequences, but these are relatively uncommon.



Enzyme activity

Scanning

GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Recognition Sequence

GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Cleavage

GGACGCTAGCTGAT**G**
CCTGCGATCGACTA**CTTAA**

AATTCGCATCGGATCCGAATCCGCTCTTTCAA
GCGTAGCCTAGGCTTAGGCGAGAAAGTT

Naming of restriction enzymes

ال restriction enzymes
أنواع كتير لانه بقدر اجيبه
من اكتر من نوع من البكتيريا

- Restriction enzymes are named according to the organism from which they are isolated.
- This is done by using the first letter of the genus followed by the first two letters of the species and additional letter or number represent the strain or serotypes.
- Only certain strains or sub-strains of a particular species may produce restriction enzymes.
- Example of restriction enzymes

معنى أنه هاد اول انزيم تم
استخراجه من هاي البكتيريا

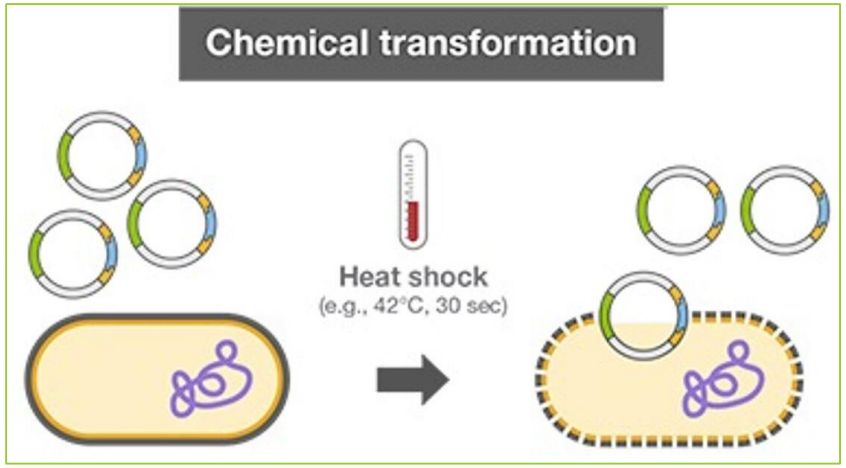
EcoRI	*Escherichia coli* R	G/AATTC
BamHI	*Bacillus amyloliquefaciens* H	G/GATCC
HindIII	*Haemophilus influenzae* Rd	A/AGCTT
PstI	*Providencia stuartii*	CTGCA/G
PmeI	*Psuedomonas mendocina*	GTTT/AAAC

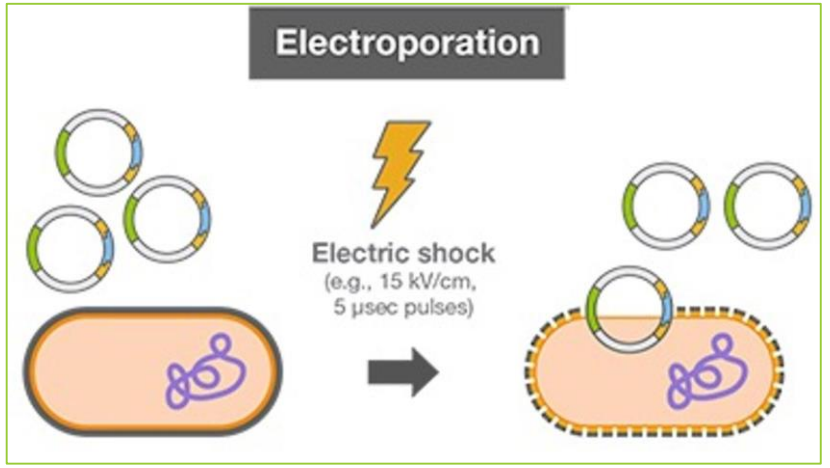
تدل على موقع
الفصل

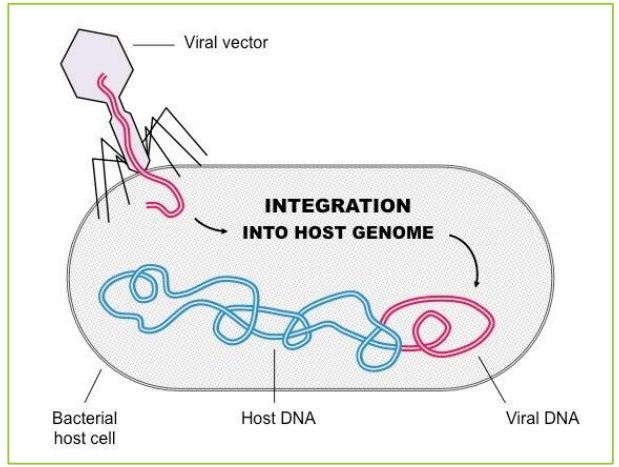
مش حفظ وين ال cutting
بصير بس نفهم شو معناها

2. Transfer of vector into the host

- After creating recombinant DNA, the vector must be introduced into a host organism. This process is known as ***transformation*** (in bacteria) or ***transfection*** (in eukaryotic cells).
- Various techniques, such as heat shock, electroporation, or viral vectors, can be employed to facilitate this transfer, ensuring the host cells can incorporate the recombinant DNA.

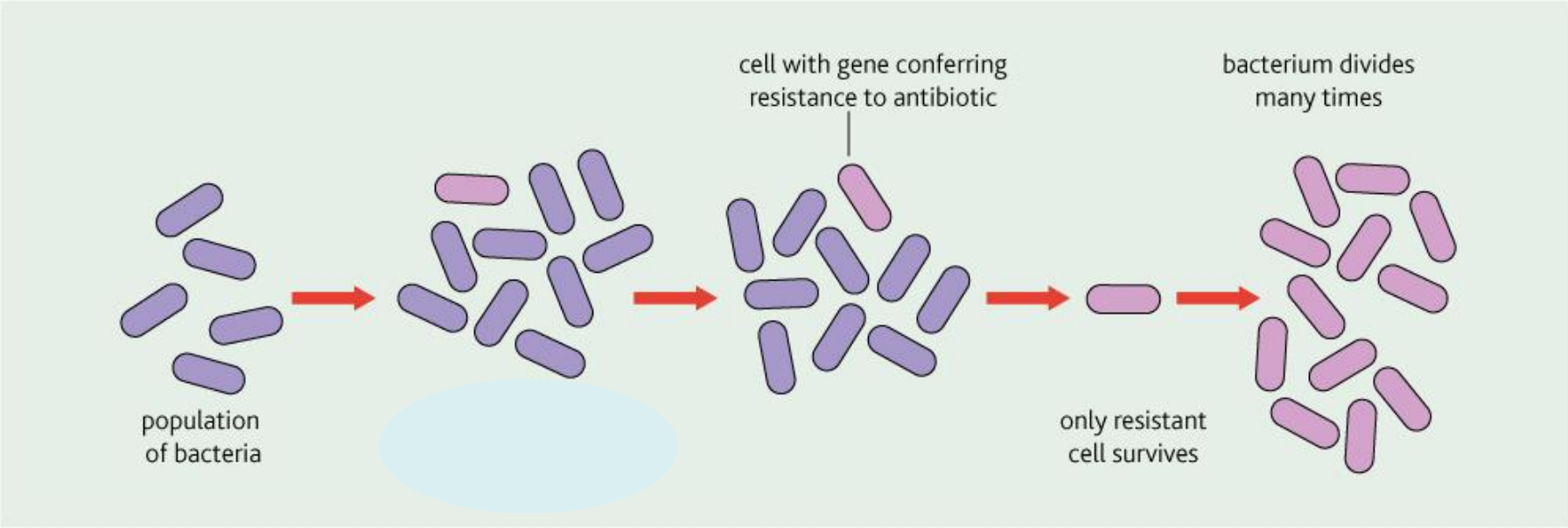






3. Selection of transformed cells

- Following the introduction of the recombinant DNA, it is crucial to identify and select the cells that have successfully incorporated the vector.
- This is typically achieved using selectable markers, such as antibiotic resistance genes, which allow transformed cells to survive in selective media while non-transformed cells are eliminated. The selected cells can then be expanded for further analysis.



4. Transcription And Translation Of The Inserted Gene

- Once the transformed cells are identified, the next step involves the expression of the inserted gene.

- This includes transcription, where the gene is transcribed into messenger RNA (mRNA), and translation, where the mRNA is translated into a functional protein.

5. Genetic analysis and sequencing

- Recombinant DNA technology also enables researchers to study the genetic makeup of organisms.
- By isolating and producing specific genes in large quantities, researchers can analyze genetic information through sequencing. Two primary methods are used:

  - **Expressed Tag Sequencing (ETS):** This method focuses on identifying expressed sequences (exons) that are translated into proteins.
  - **Sequence Annotation:** This approach includes the analysis of both exons and introns, providing a comprehensive view of the gene structure.

Applications Of Recombinant DNA Technology:

1. **Production of Therapeutic Proteins –** Enables large-scale production of proteins like *insulin* for treating diseases such as diabetes.
2. **Monoclonal Antibodies** – Developing targeted therapies for diseases like cancer using engineered antibody-producing cells.
3. **Genetically Modified Organisms (GMOs)**: Improves crop yield and pest resistance by introducing beneficial genes (e.g., Bt cotton).
4. **Model Organisms** – Creating transgenic animals (e.g., mice) to study human diseases and test potential therapies.
5. **Gene Therapy** – Correcting genetic disorders (e.g., SCID) by replacing faulty genes with functional ones.
6. **Personalized Medicine** – Studying genetic variations to customize treatments for improved efficacy and reduced side effects.
7. **Biotechnological Solutions** – Engineering microbes for sustainable applications like biofuel production or environmental cleanup

Ethical Considerations And Safety Concerns

1. **Biosafety Risks**:
   - Potential unintended release of genetically modified organisms (GMOs) into the environment, disrupting ecosystems.
   - Risk of engineered pathogens causing biohazards or misuse for bioterrorism.
2. **Genetic Privacy and Security**:
   - Possibility of unauthorized use of genetic data, raising concerns about consent and confidentiality.
   - Ethical dilemmas in genetic profiling and discrimination based on genetic information.
3. **Ethics of Genetic Modification**:
   - Moral concerns about altering the genetic makeup of organisms, including humans (e.g., germline editing).
   - Fear of creating "designer babies" or exacerbating social inequality.
4. **Impact on Natural Biodiversity**:
   - GMO crops potentially outcompeting native species, reducing biodiversity.
   - Risk of gene flow between GMOs and wild relatives leading to "superweeds."

Ethical Considerations And Safety Concerns

5. **Animal Welfare**:
   - Ethical issues in using animals for genetic modification experiments or creating transgenic species.
6. **Accessibility and Equity**:
   - Unequal access to the benefits of recombinant DNA technology between developed and developing nations.
   - High costs of therapies limiting availability for disadvantaged populations.
7. **Regulation and Oversight**:
   - Need for stringent guidelines and global cooperation to ensure safety and prevent unethical practices