بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 9

Recombinant DNA & DNA cloning

Written by : DST

Reviewed by : NST member

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: المتحقق كونه ووجوده، المألوه المعبود بحق، الرب الملك الذي لا شك فيه، الكامل في أفعاله وصفاته، فكلامه حق وقضاؤه حق ووعده حق وشرعه حق.

الورود: ورد في القرآن (١٠) مرات.

الشاهد: ﴿ فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ﴾ [طه:١١٤].

اضغط هنا لشرحٍ أكثر تفصيلًا

What is DNA cloning?

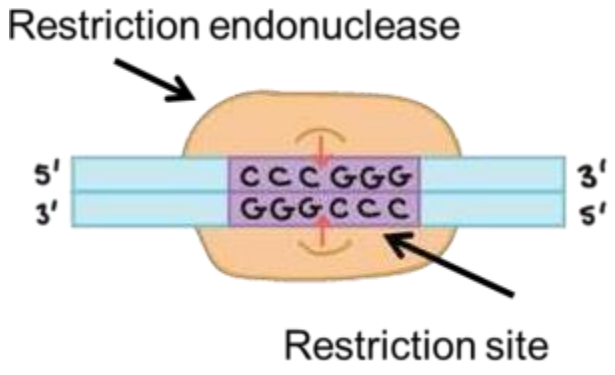
Bacteria are commonly used for cloning, but yeast and human cells can also be utilized by culturing them in laboratories.

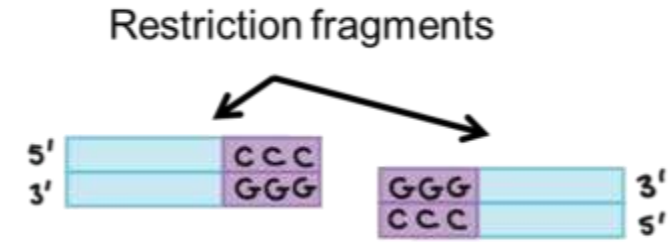
- DNA cloning is a technique that allows for:
  - amplifying a DNA segment into many, many copies in a biological system.
  - expressing a gene inside a biological system such as bacteria, human cells grown in labs, animals, or even the human body as a whole.
- It usually involves:
  - The formation of a recombinant DNA composed of a vector (a carrier of the gene or the DNA segment of interest; usually a bacterial plasmid) and a gene that encodes a protein or a non-coding RNA using restriction endonucleases.
  - Insertion into the cell(s).

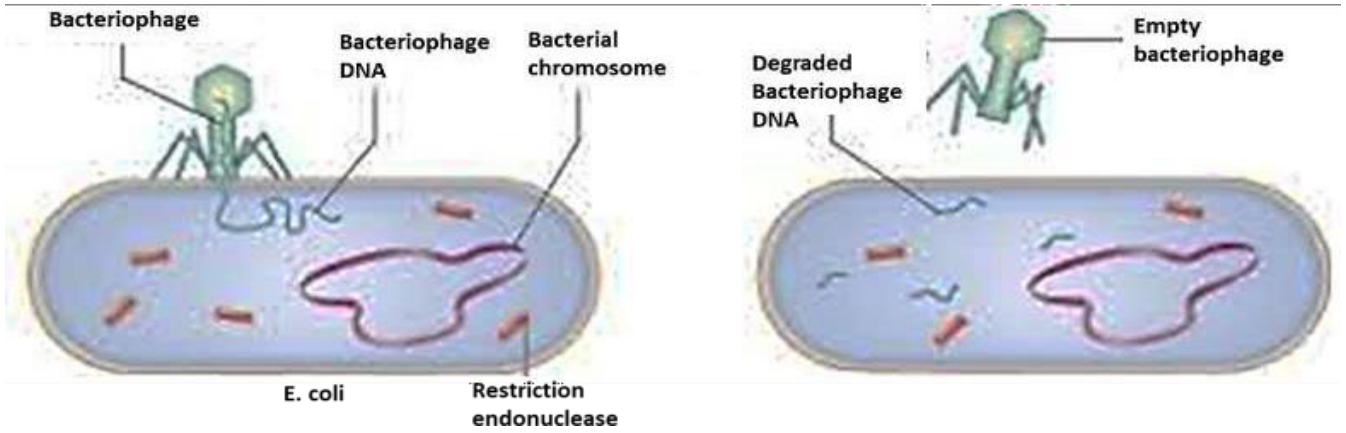
Recombinant DNA is a DNA molecule formed by combining genetic material from different sources, such as human and bacterial DNA. Restriction endonucleases are used to cut DNA at specific sites to create these molecules.

Restriction endonucleases

- Endonucleass are enzymes that degrade DNA within the molecule.
- Restriction endonucleases: Bacterial enzymes that recognize and cut (break) the phosphodiester bond between nucleotides at specific sequences (4- to 8-bp restriction sites) generating restriction fragments.







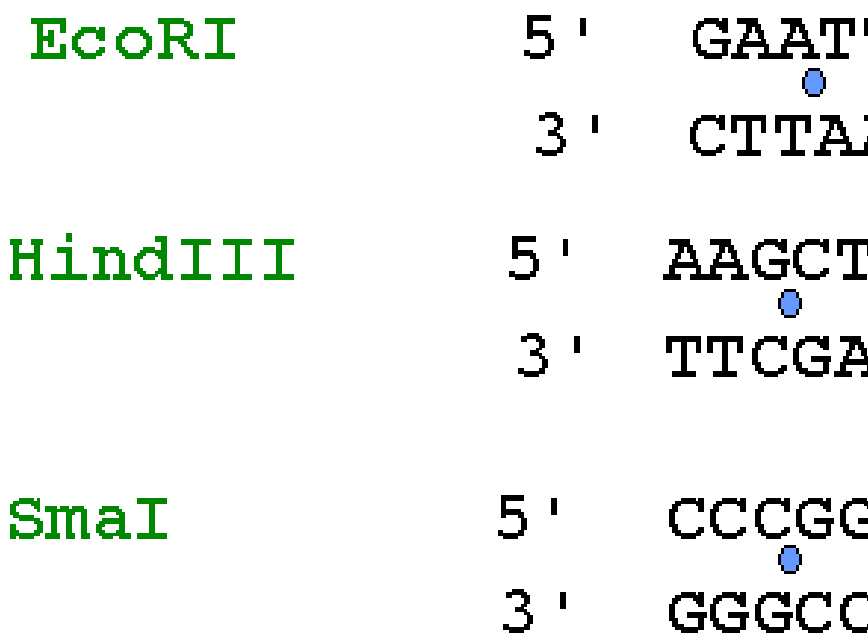
The natural role of endonucleases in bacteria is to cleave bacteriophage DNA, preventing bacteriophages from taking control of bacterial cells.

Humans have harnessed these enzymes for use in genetic cloning

Palindromic sequences

- The sequences recognized by restriction endonucleases—their sites of action— Palindromic sequences which read the same from left to right as they do from right to left (on the complementary strand).

Restriction sites which are palindromic sequences for each enzyme:

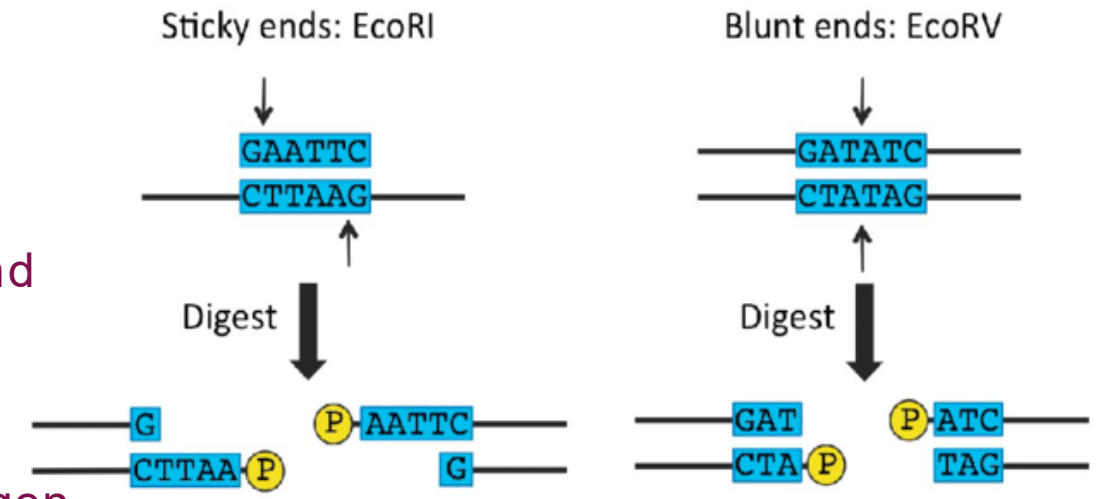


Types of cuts by restriction endonucleases

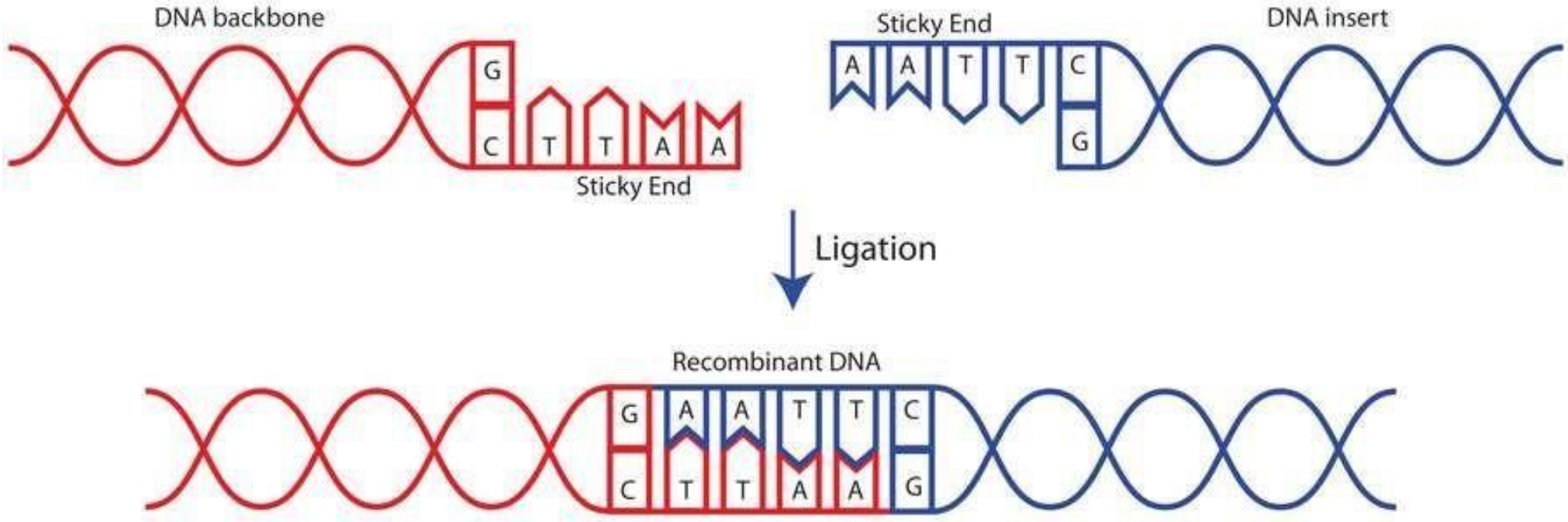
- Staggered (off-center): enzymes cut the two DNA strands at different positions generating sticky or cohesive ends.
- The DNA restriction fragments would have short single-stranded overhangs at each end.

- Blunt: enzymes cut at the same position on both strands giving blunt-ended fragments.
  - The cut occurs in the middle of the restriction site

- The cut occurs not in the center rather on the sides of the restriction site
- They are called cohesive ends because they are complementary to each other.
- Remember these cohesive or sticky ends can temporarily bind through reversible hydrogen bonds, so the two strands can separate.
- In order to have the two strands connected to each other again you need to form a phosphodiester bond and a hydrogen bond between both fragments (between A and G, T and A).

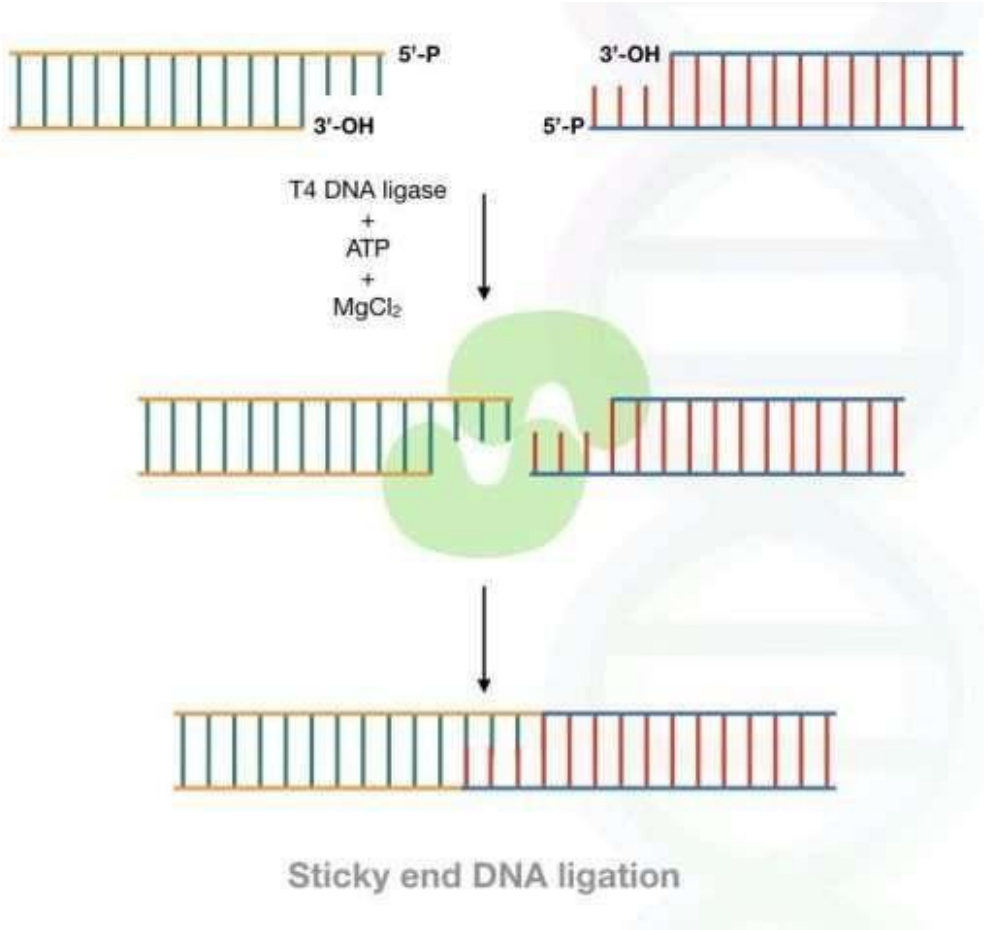


Zoom into the sticky ends



If two DNA pieces from different sources are cut with EcoRI, they produce restriction fragments with cohesive **(complementary)** ends. These ends pair through-> hydrogen bonds, and DNA ligase forms-> phosphodiester bonds, resulting in recombinant DNA.

DNA ligase

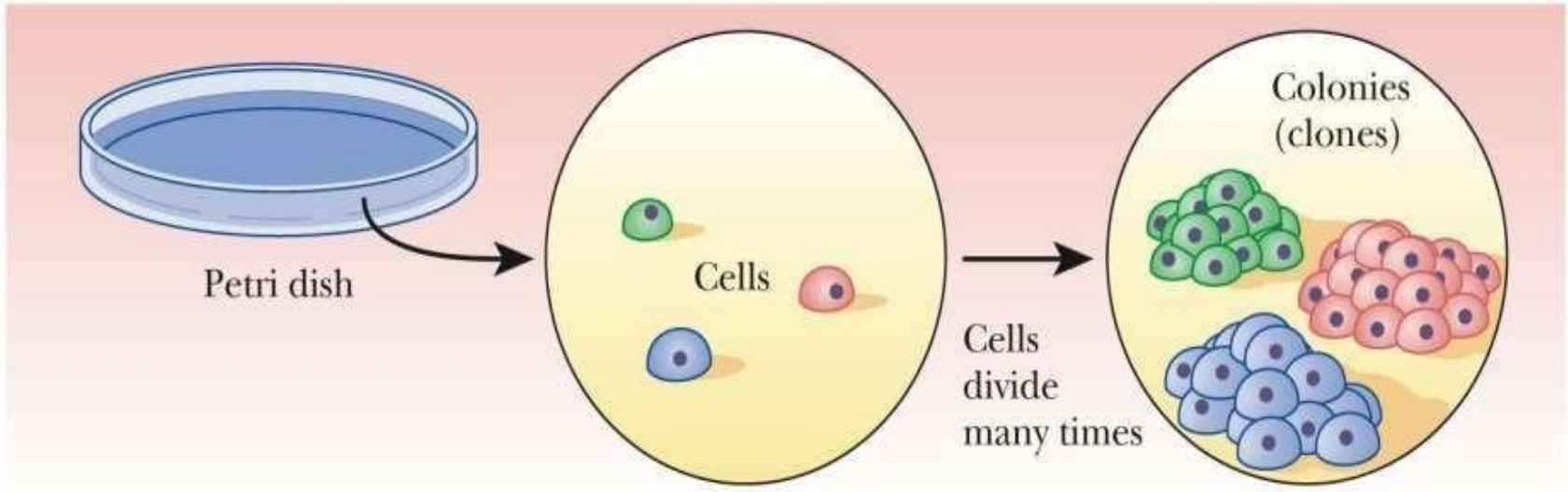


It covalently joins DNA ends (example, restriction fragments) by catalyzing the ATP-dependent formation of permanent phosphodiester bonds between the 3'-hydroxyl group of one strand and the 5'-phosphate end of another strand.

Cloning

- Cloning means that you make several copies of one thing.
- A clone is a genetically identical population, whether of organisms, cells, viruses, or DNA molecules.
- Every member of the population is derived from a single cell, virus, or DNA molecule.

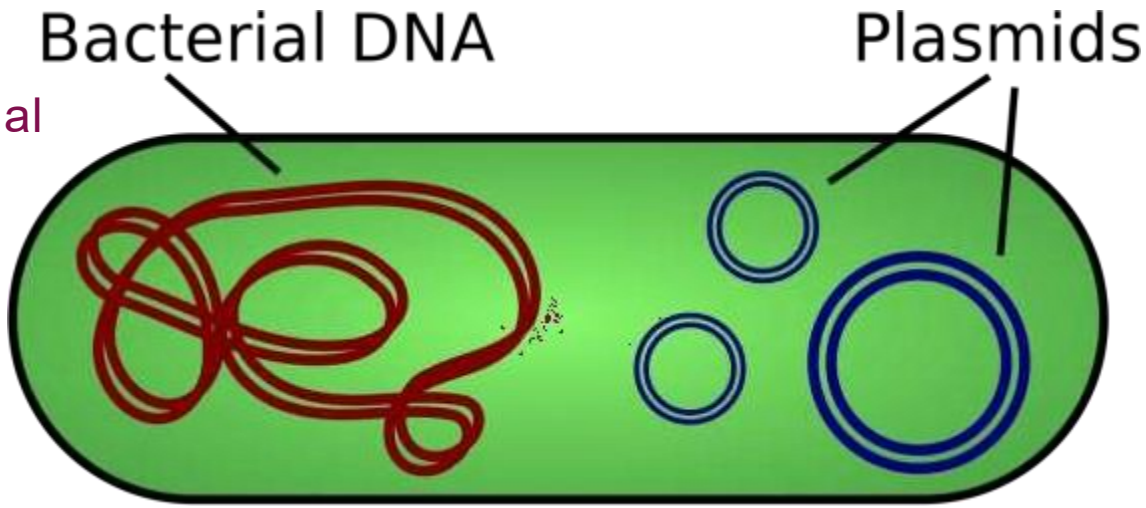
- Bacterial cells can be grown on dishes, where each forms a clone. Each clone consists of cells that are identical to one another and to the original cell.
- In human cloning, a copy of a person is made with identical DNA to the original individual.



Using plasmids as vectors

Vectors: carriers of the DNA that we want to clone

- Bacterial plasmids are considered excellent vectors that are used for cloning (cloning vectors) or expression (expression vectors).
- These are natural bacterial extra chromosomal circular DNA that is not part of the main circular DNA chromosome of the bacterium.
- A plasmid exists as a closed circle and replicates independently of the main bacterial genome.



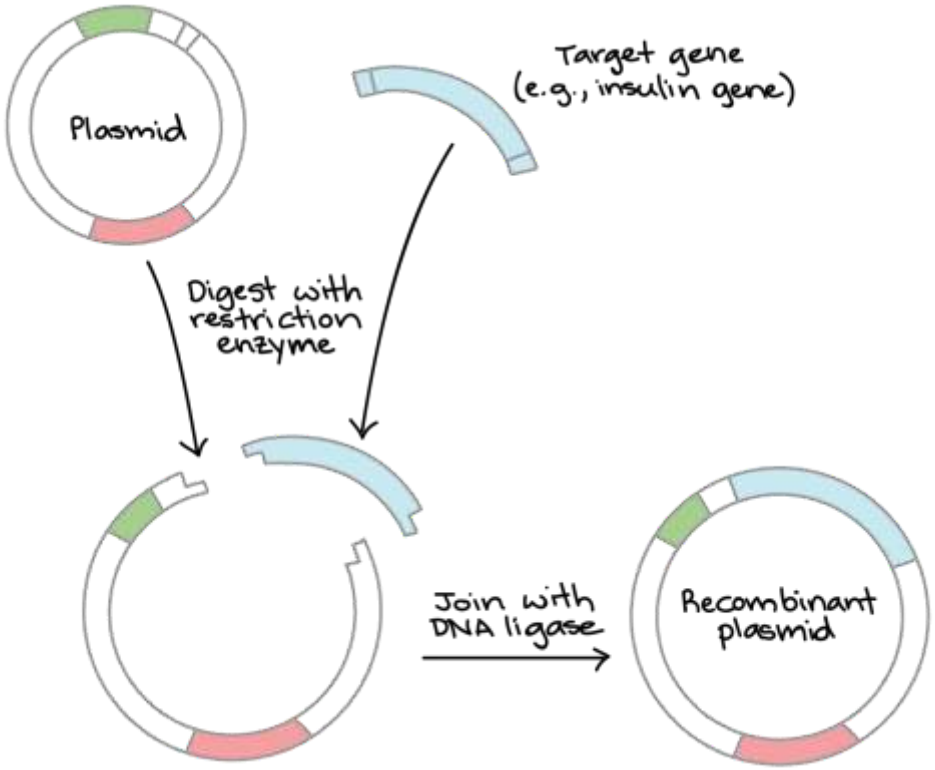
- Main genetic material one copy only

- Plasmids naturally carry genes that provide advantages to bacteria, such as resistance to viruses or antibiotics.

- A single bacterial cell can have multiple copies of plasmids.
- They can also be genetically engineered for specific purposes.

How do we clone a DNA molecule?

- a DNA fragment of interest is inserted into a DNA carrier (called a vector) that can be replicated.
- The resulting DNA molecule is what is known as a recombinant DNA molecule.
- The procedure is known as recombinant DNA technology, which is part of genetic engineering.



Further explanation in the next slide

How do we clone a DNA molecule?

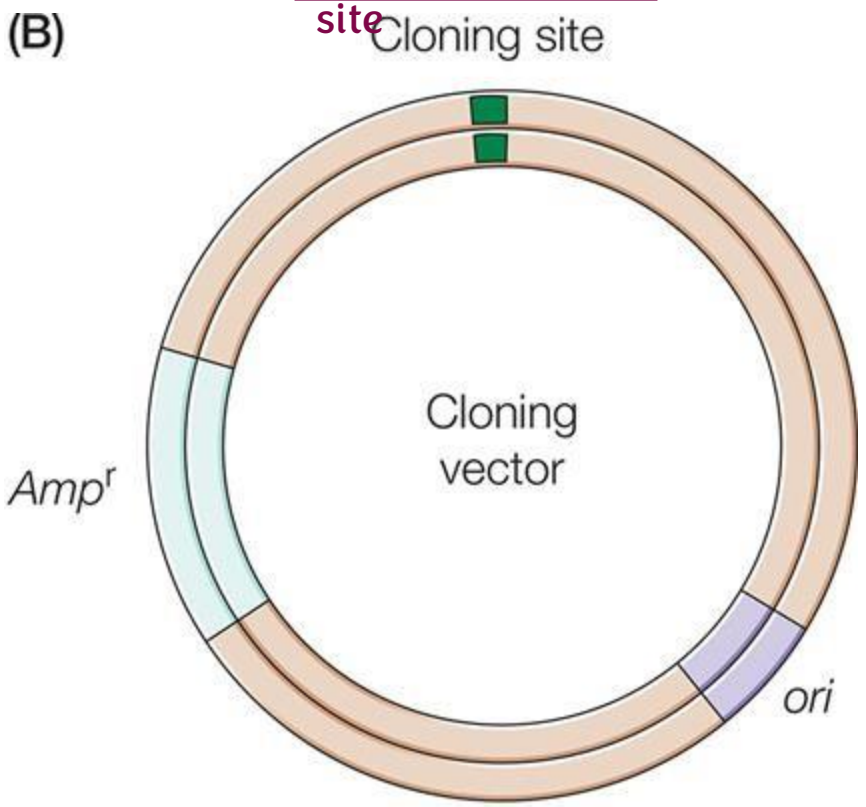
We take these plasmids and make them function as vectors (carriers of target gene) and create recombinant DNA as follows:

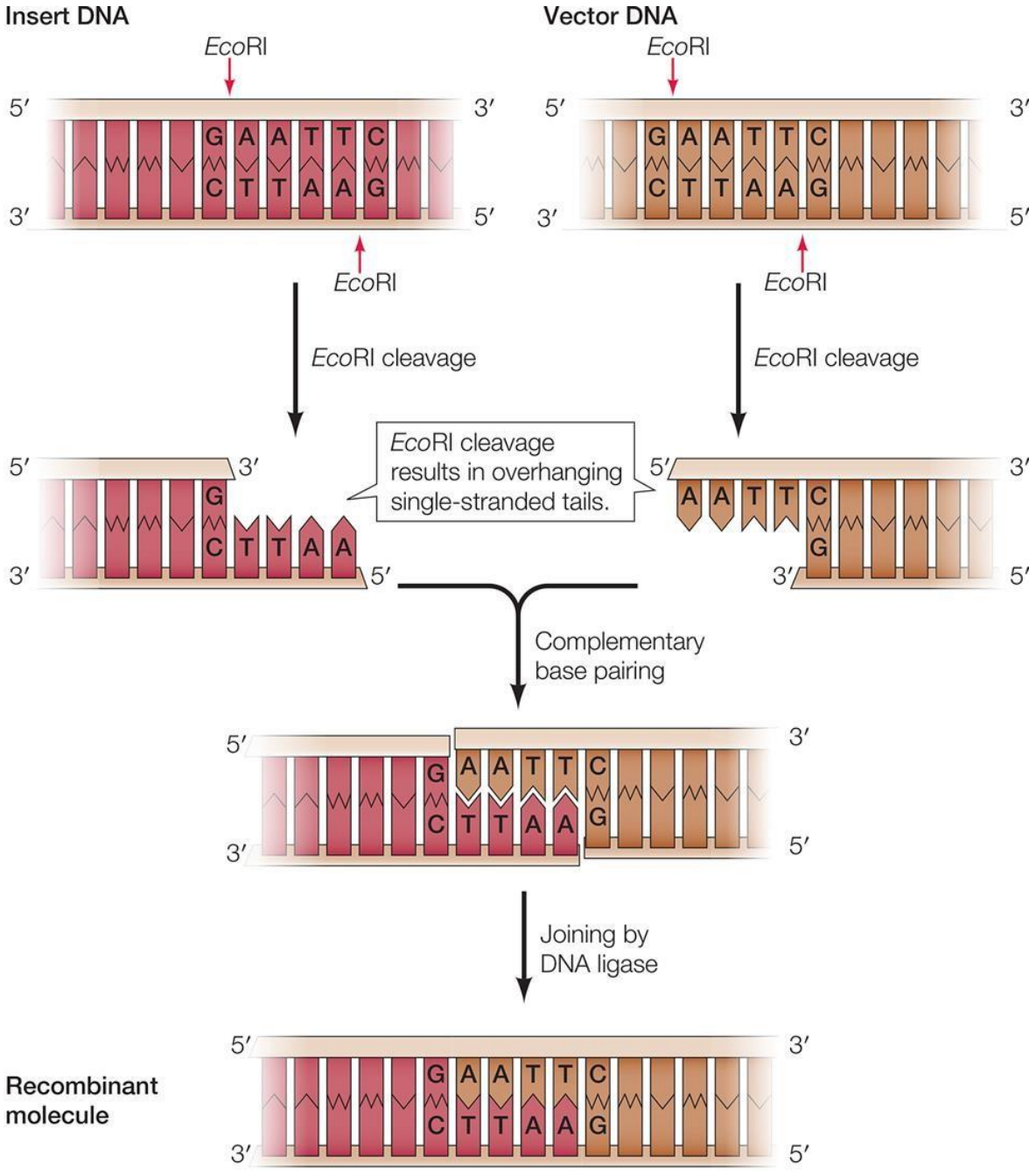
- We take the plasmids and open them with a restriction enzyme
- We take the target DNA, and we add the **same** restriction endonuclease, now both have cohesive ends that hydrogen bond to each other
- We combine them with each other using a DNA ligase, a recombinant DNA is formed (now the target DNA is permanently part of the plasmid).

Features of plasmid cloning vectors

- Plasmid cloning vectors must have the following three components:
  - Their own origin of replication (OriC) that allows them to replicate independently of the bacterial chromosome. They need just one OriC
  - A selectable gene such as an antibiotic resistance gene that allows for selecting for/against the cells that have them.
- A restriction site that allows for insertion of the DNA segment of interest into the plasmid. Using a restriction endonuclease that recognizes the site

= restriction site





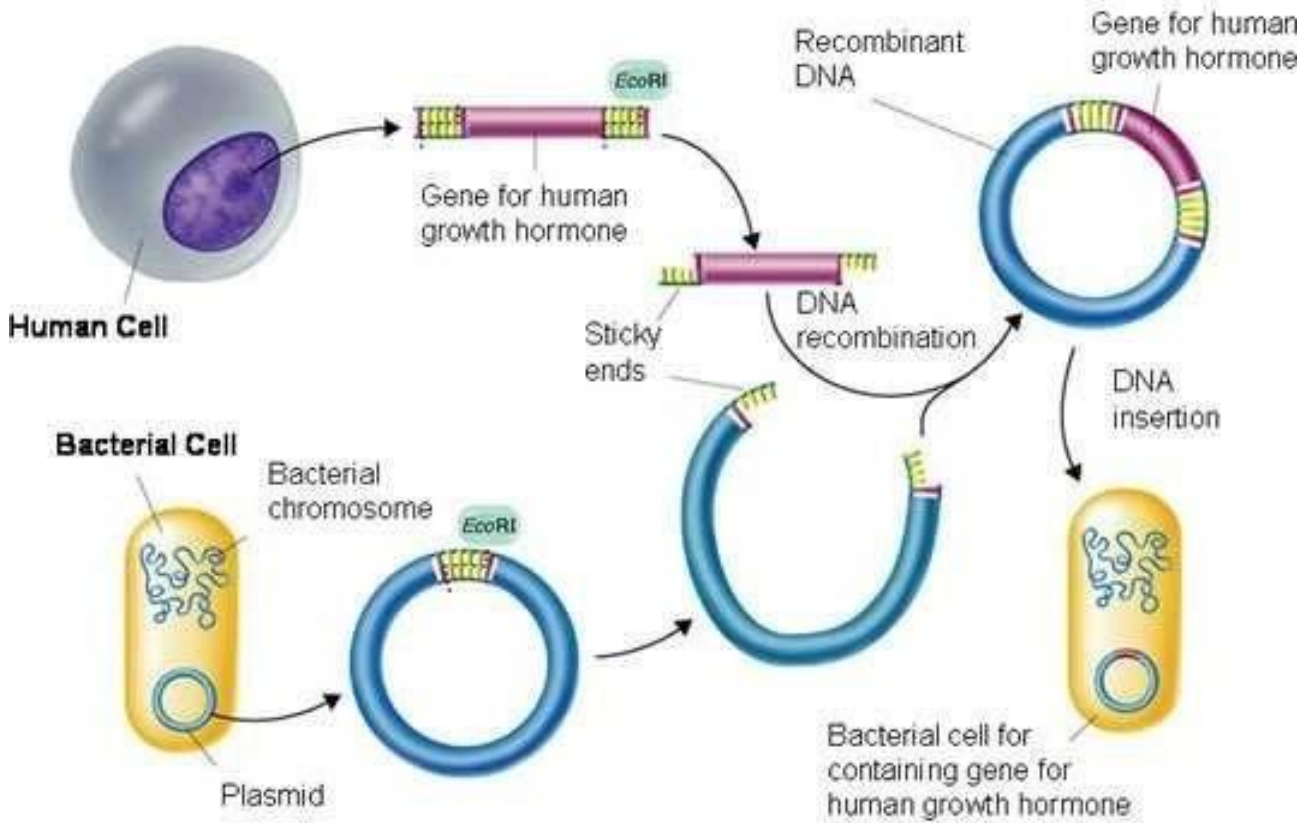
Creation of a recombinant DNA

- Insert and vector DNAs are digested with a restriction endonuclease (such as EcoRI), which cleaves at staggered sites leaving overhanging single-stranded tails.

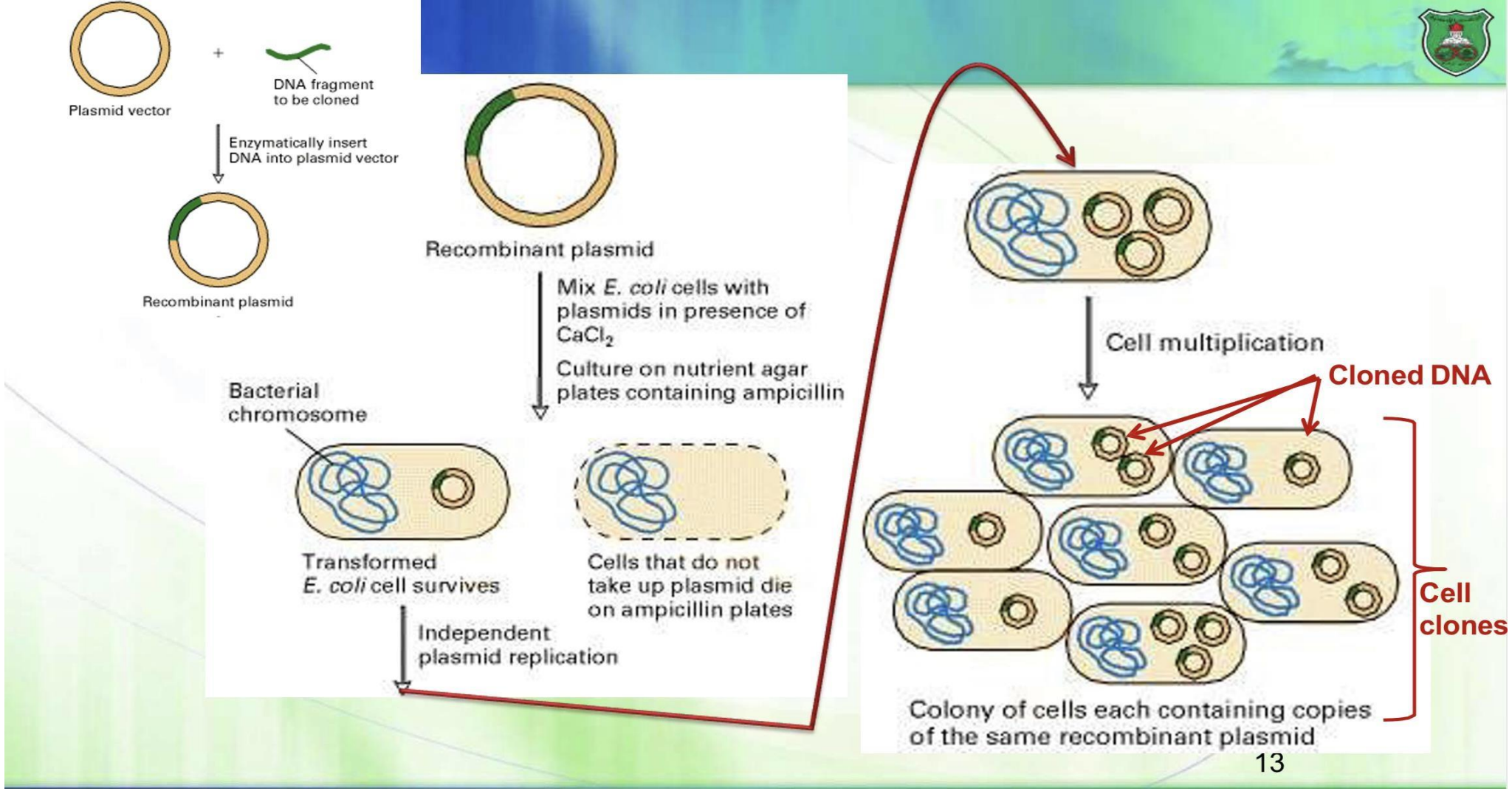
- Insert and vector DNAs can then associate by complementary base pairing by hydrogen bonds, and covalent joining of the DNA strands by DNA ligase yields a recombinant molecule.

The making of a recombinant DNA

- Both DNA fragments (the DNA to be cloned and a vector) are cut by the same restriction endonuclease that makes DNA fragments with same sticky-ends hybridize (anneal) to each other, when mixed.
- A DNA ligase is added to "close" the plasmid.



After creating recombinant DNA, the bacterial cell is transformed; meaning that we insert the plasmid into the bacterium.



- Now, we have bacterial cells with antibiotic-resistant plasmids and bacterial cells without the plasmids.
- We add ampicillin to the bacterial cells, the ones without the plasmid would die as they are not resistant to it, while the bacterial cell with the plasmid would survive.
- We can grow the bacteria that survived by inoculation and allowing them to grow overnight ending up with millions of cells after 16-18 hours, each bacterial cell will contain at least one plasmid.

DNA libraries

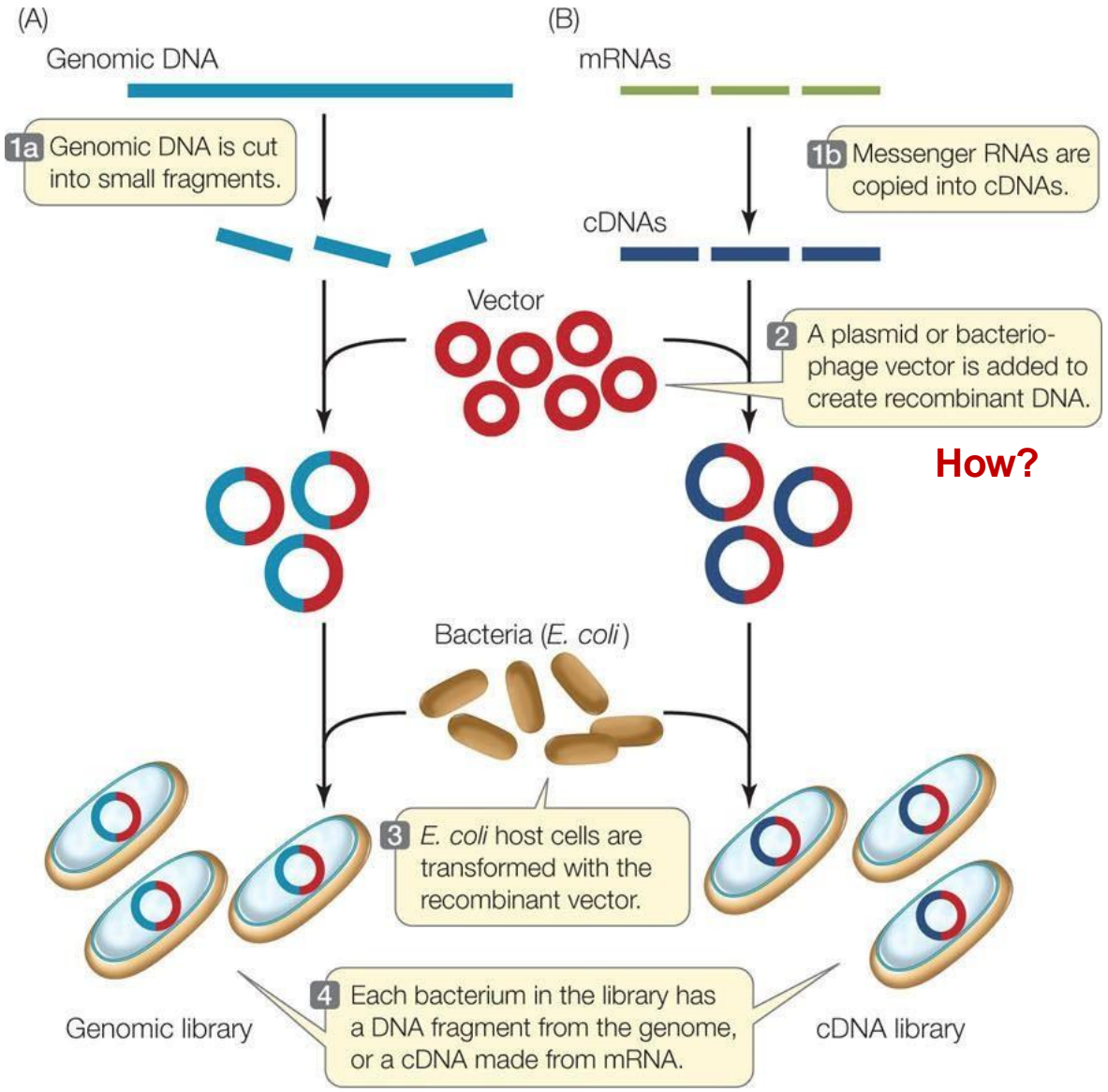
A DNA library functions like a traditional library, where each plasmid contains a different piece of DNA. These plasmids can be stored in various ways, such as in fridges or organized on shelves. The key idea is that, if you want to study a specific gene, you can locate the plasmid of interest in the exact storage location, ensuring easy and efficient access.

Genomic vs. cDNA libraries

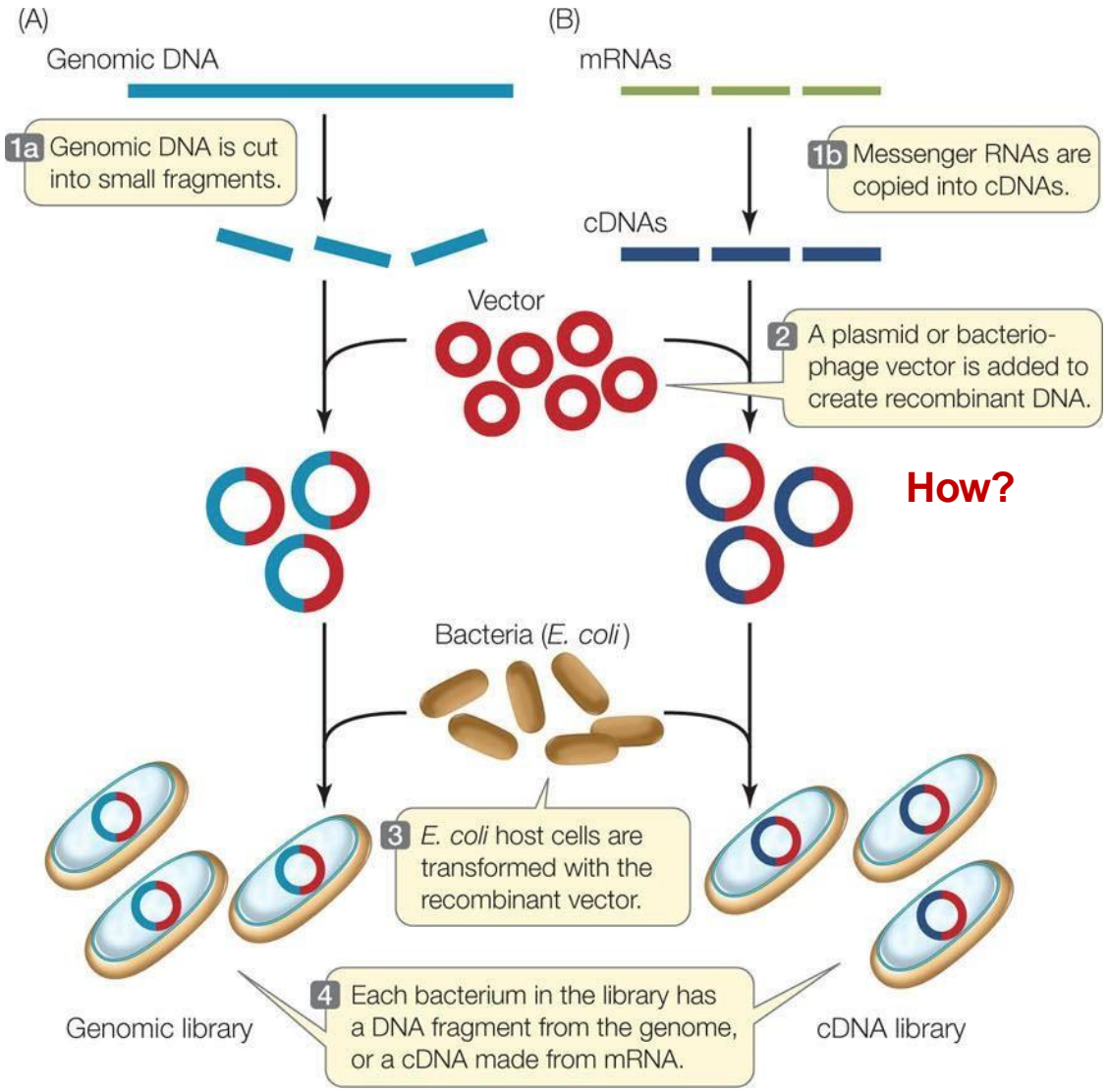
There are two different types of libraries:
Genomic and **cDNA libraries**

Genomic library:

- Genomic DNA is the complete DNA set from a cell or a group of cells. It is first cut into fragments using restriction endonucleases and inserted into plasmids, which serve as vectors. Each DNA fragment is inserted into a plasmid, forming recombinant DNA molecules.
- These plasmids are then introduced into bacteria through transformation, resulting in each bacterial cell carrying a unique DNA fragment.
- Finally, researchers identify and isolate bacteria containing the desired DNA fragment for further study.



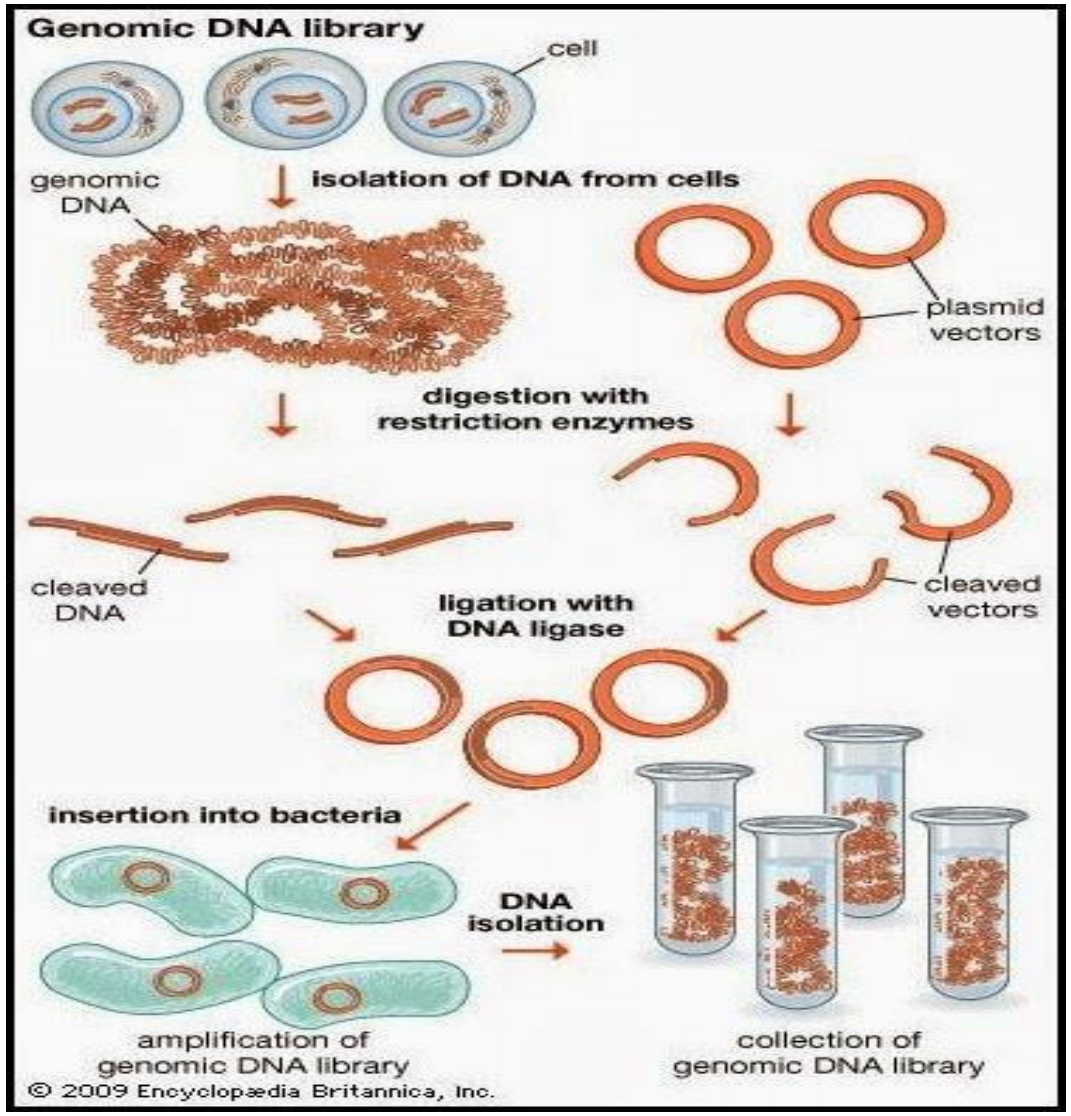
Genomic vs. cDNA libraries



cDNA libraries:

- cDNA libraries are made from mRNA, representing expressed genes.
- To create such a library, mRNA is extracted from cells and converted into cDNA (complimentary DNA) using reverse transcriptase, viral enzyme that synthesizes DNA from RNA.
- Each mRNA is transformed into cDNA fragments, which are processed with restriction enzymes and inserted into plasmids, forming recombinant DNA, each carrying a distinct gene.
- These plasmids are introduced into bacteria through transformation, resulting in each bacterial cell carrying a unique gene.
- By knowing what each clone contains, researchers can then identify, grow, and isolate the desired gene for further study.

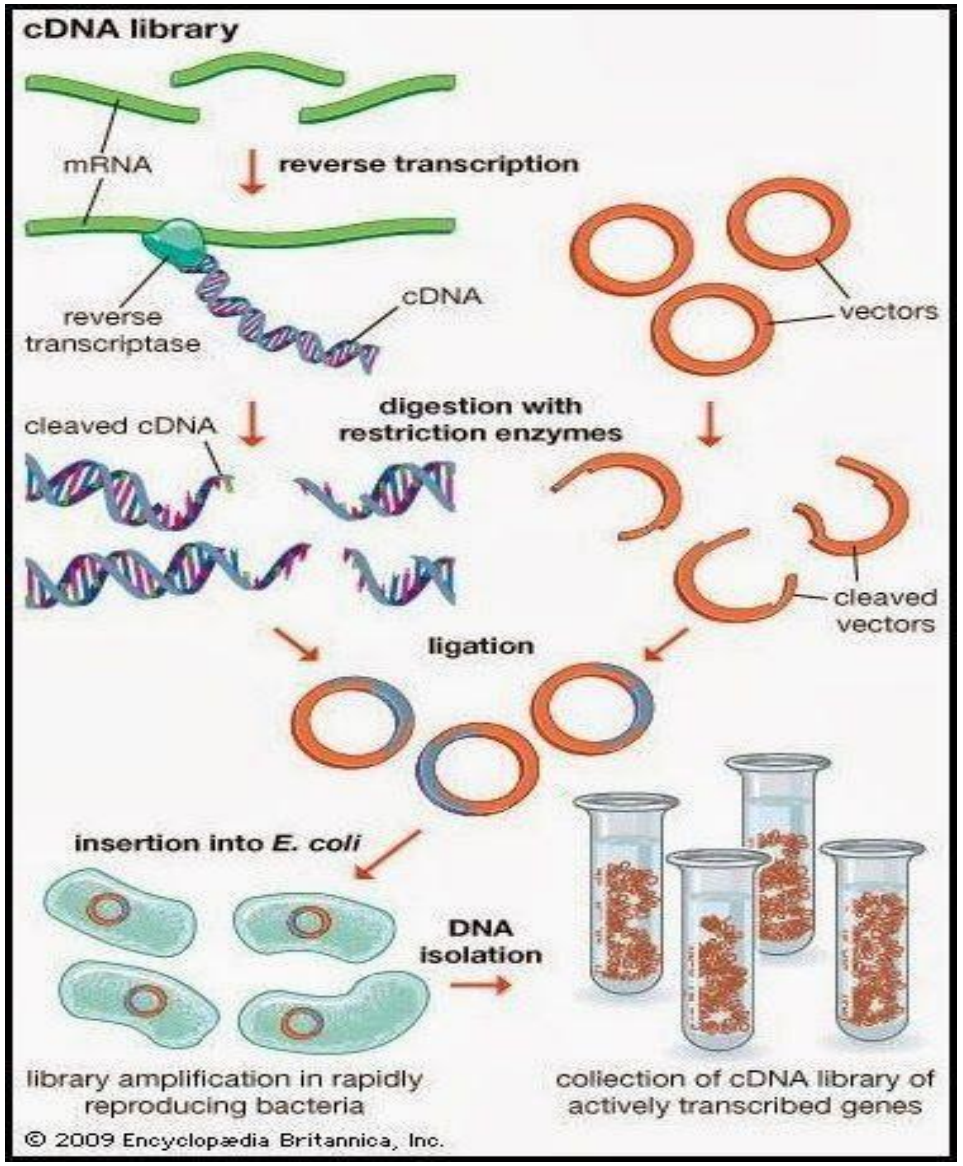
Genomic vs. cDNA libraries



- Genomic DNA is used to create a genomic library, which is digested with restriction enzymes (the same enzymes used to cut plasmid DNA) and ligated into plasmids.

- This process generates DNA molecules carrying fragments of genomic DNA of different sizes.
- Next, these plasmids are transformed into bacterial cells, and each clone carries a unique genomic DNA fragment that is different from other cells.

Genomic vs. cDNA libraries



In a cDNA library, mRNA is converted into complementary DNA (cDNA) using reverse transcriptase.

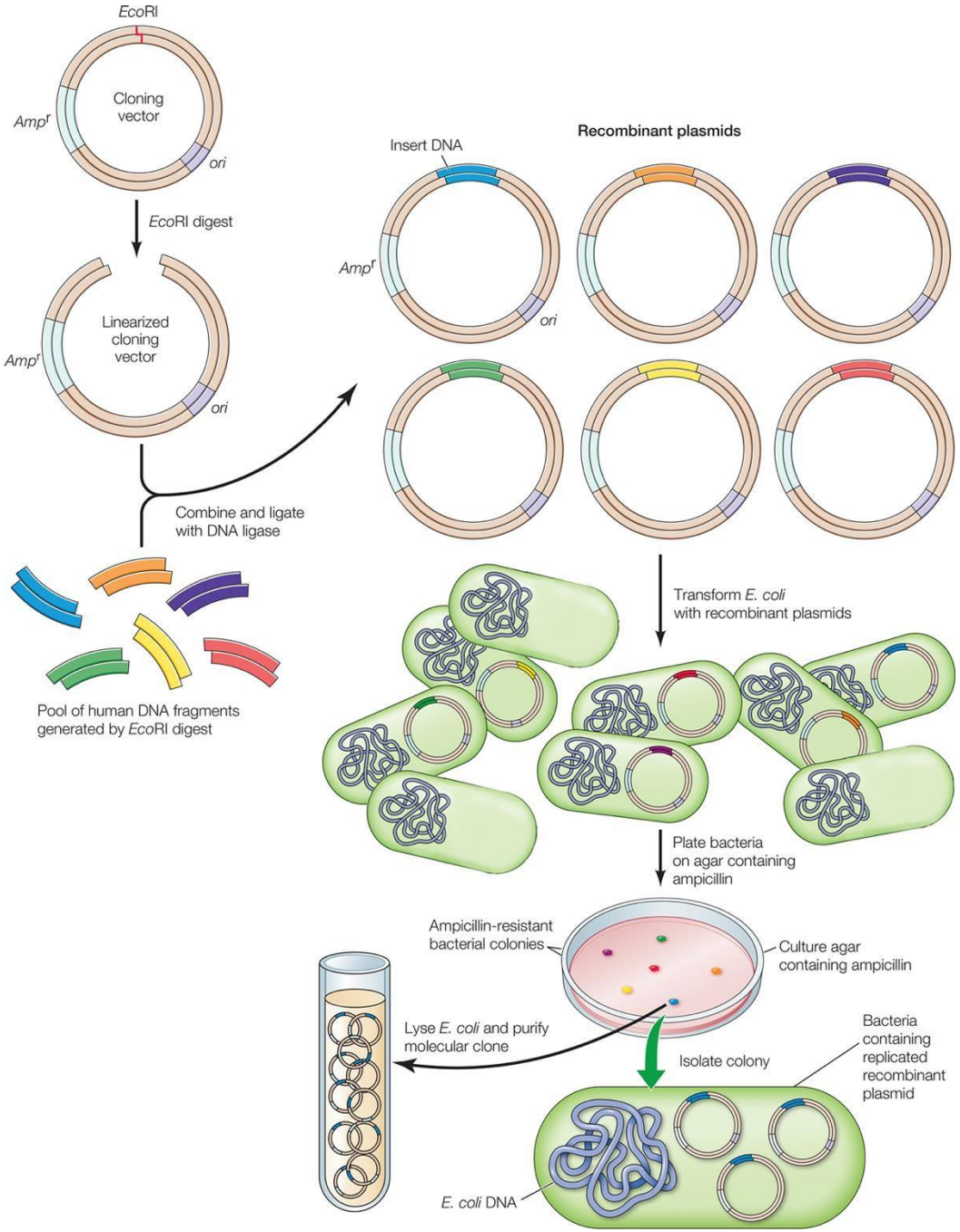
The cDNA is then inserted into plasmids to create recombinant DNA molecules.

Each plasmid may contain a gene or gene fragment.

Finally, we transform bacterial cells with these plasmids, creating a cDNA library.

(You can read this on your own ,it's the same basic idea, but written in different figure)

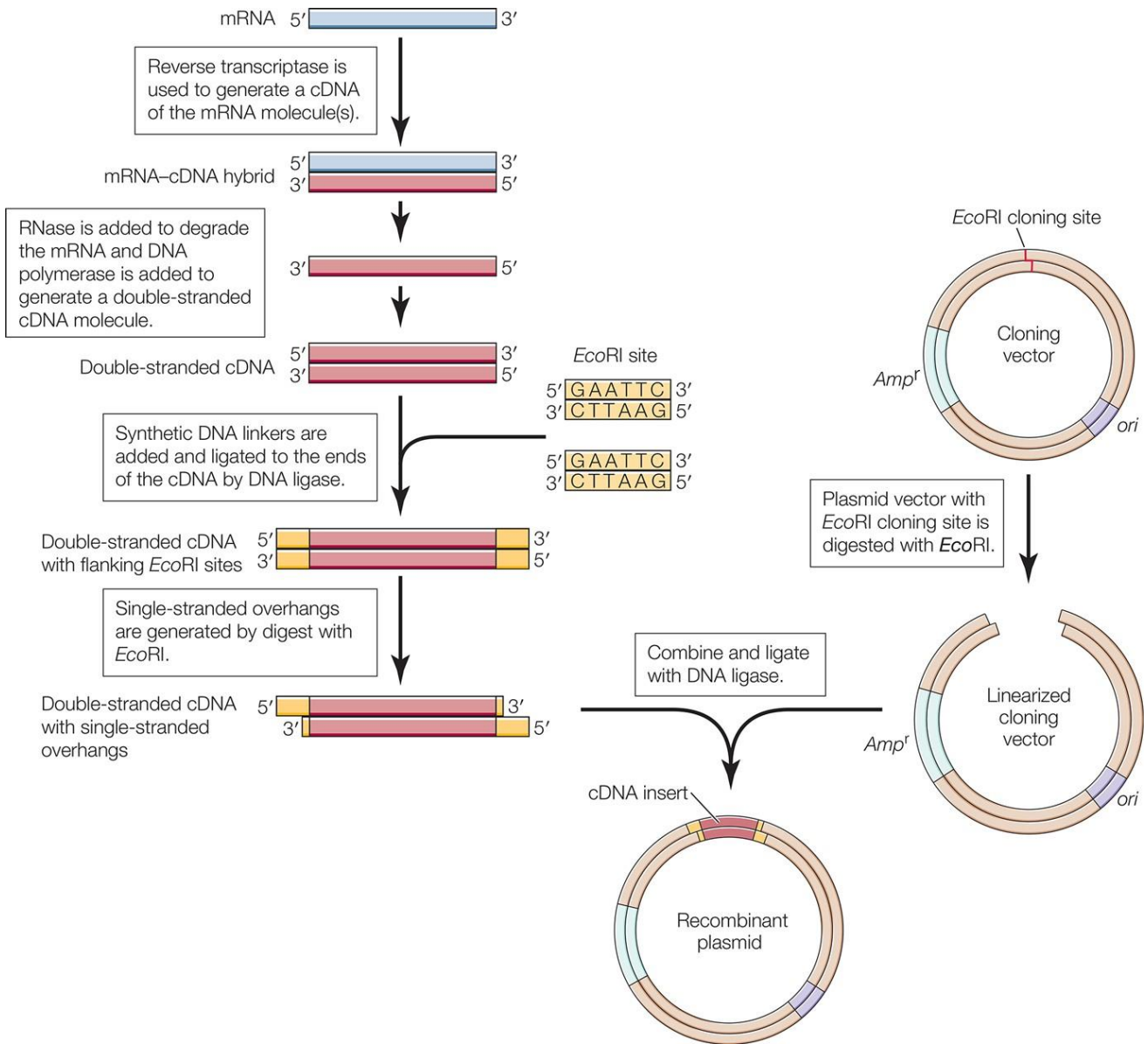
Creation of a genomic library



- A genome is cleaved by the same restriction endonuclease as that used for the vector.
- Each fragment is ligated into a vector.
- Bacterial cells are transformed with the plasmid vector with each cell having one plasmid DNA.
- Each cell can grow into millions of cells and each cell can make multiple copies of every plasmid ending up with billions of copies of plasmid with each plasmid having a specific DNA fragment.

Creation of a cDNA library

(Look at the next slide)



- Messenger RNAs are isolated and reverse transcribed by reverse transcriptase into a cDNA molecule that is replicated by DNA polymerase to form a double-stranded cDNA.
- Synthetic linkers containing a restriction site are ligated to the ends of the cDNAs and then digested with the restriction endonuclease to form overhangs.
- The cDNAs are then cloned into a plasmid.

#What are the differences between a cDNA library and a genomic library? Notice that with the genomic DNA, we take everything, so we can have pieces that can represent genes, exons, introns, centromeres, telomeres, promoter-proximal elements and enhancers (everything).

With a cDNA library, we're only capturing the expressed genes. If I take genomic DNA from nerve cells or epithelial cells (skin cells) from a person, and I extract and cut the DNA using restriction enzymes in the lab, I can use it to create recombinant DNA or generate a genomic library. These genomic libraries will be identical because they contain the same DNA sequence. However, if I create a cDNA library from **nerve cells**, it will be **quite different** from a cDNA library made from **skin cells** because these **different cells express different genes.**

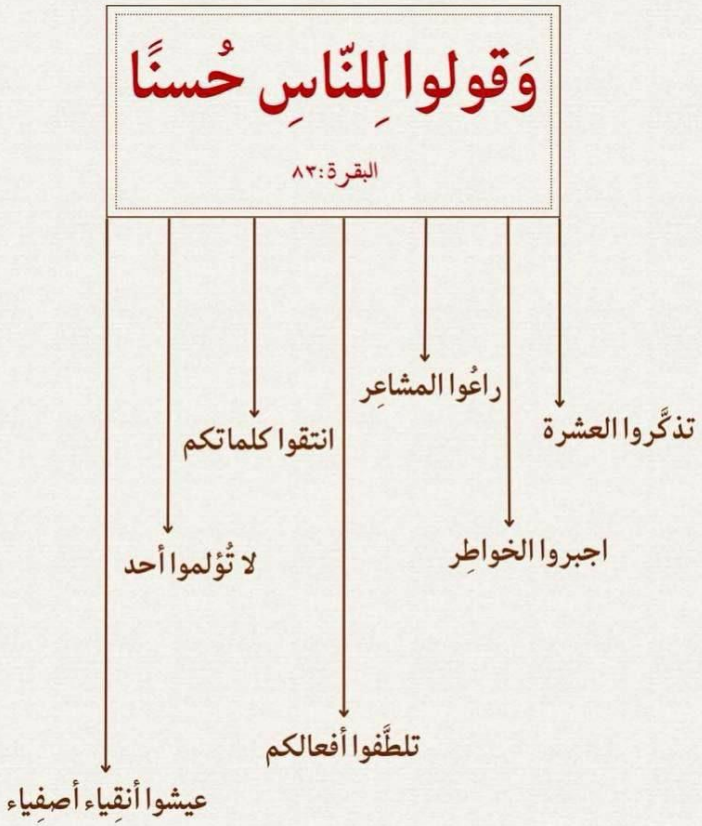
We have approximately 20,000 protein-coding genes. For example, 5,000 genes might be expressed in nerve cells, while 6,000 genes may be expressed in skin cells. These libraries are not identical—some genes, like histone and actin genes, may overlap because they are widely expressed, but others are **cell-specific**. For instance, genes coding for neurotransmitters are expressed in nerve cells, whereas genes for enzymes involved in pigment synthesis are specific to skin cells. Therefore, cDNA libraries differ based on the cell type from which they are derived.

Continued

- Another key point is that cDNA libraries contain only exons. They do not include promoters because promoters are not transcribed into mRNA.
- cDNA libraries are generated from mRNA molecules, specifically representing protein-coding genes. Since the cDNA is synthesized from processed mRNA templates, it excludes non-coding regions such as introns, promoters, enhancers, and other regulatory elements. Therefore, cDNA libraries consist solely of sequences corresponding to exons.

رسالة من الفريق العلمي:

عن النبيّ صلّى الله عليه وسلّم : حَرُم على النارِ كلُّ هيّنٍ ليّنٍ سهلٍ قريبٍ من الناسِ.



فهذا منهج الأنبياء وأخلاق الصَّالحين.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			