



Practical Genetic Engineering 2025-2026

By
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Lab 1

Introduction

Genetic Engineering

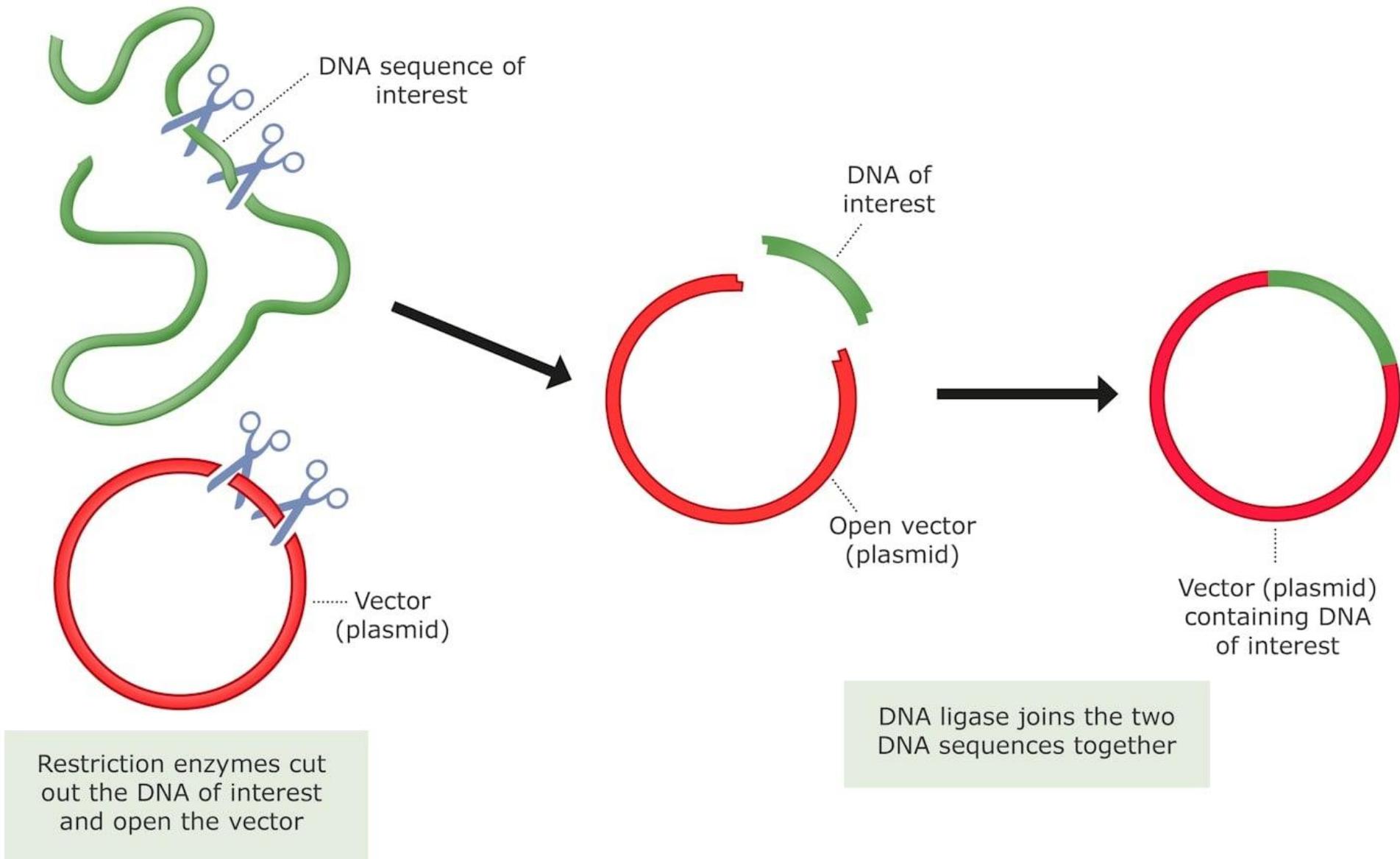
It is also called **Recombinant DNA technology** or **gene manipulation**. It refers to the process of direct manipulation of an organism's genome by manually transferring new DNA genes to an organism across species (prokaryotes & eukaryotes) to change one or more of its characteristics. The goal is to add one or more new traits that are not already found in that organism. Example of genetically engineered (transgenic) organisms including:

- 1 One example of a transgenic microorganism is the bacterial strain that produces human insulin.
- 2 Plants with resistance to some insects, plants that can tolerate herbicides, and crops with modified oil content.

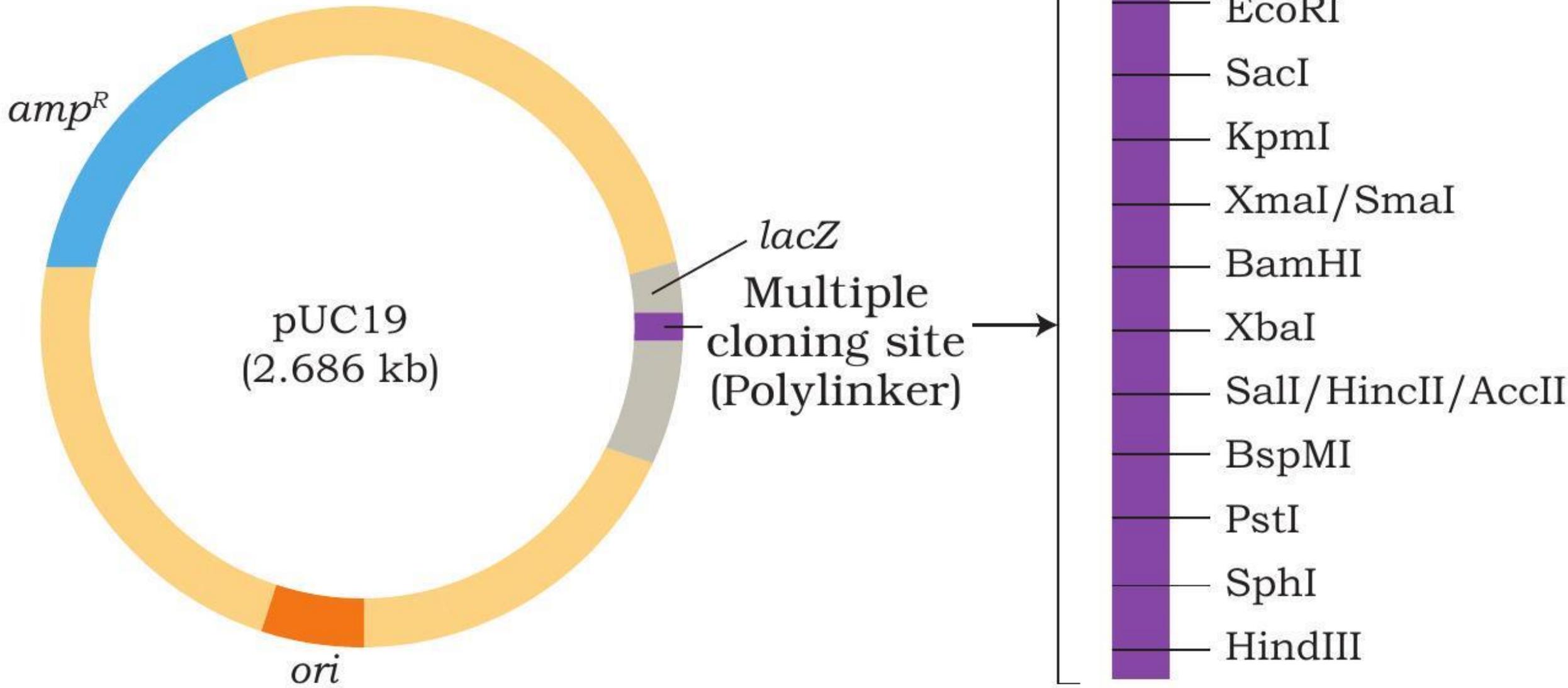
Gene cloning steps

Cutting and ligation

1. Determine the specific gene/s encoded to specific trait/s belong to some an organism and cutting (Isolating) by using **Restriction Enzymes**.
2. Amplification of genetic material (specific gene) for many copies by using **PolymeraseChain Reaction (PCR)**.
3. Gene has ligated with vector's genome by **ligase** enzyme to produce new constructed vector(plasmid that contains naked gene) called **recombinant molecule**. The vectors must be restricted by same restriction enzyme.



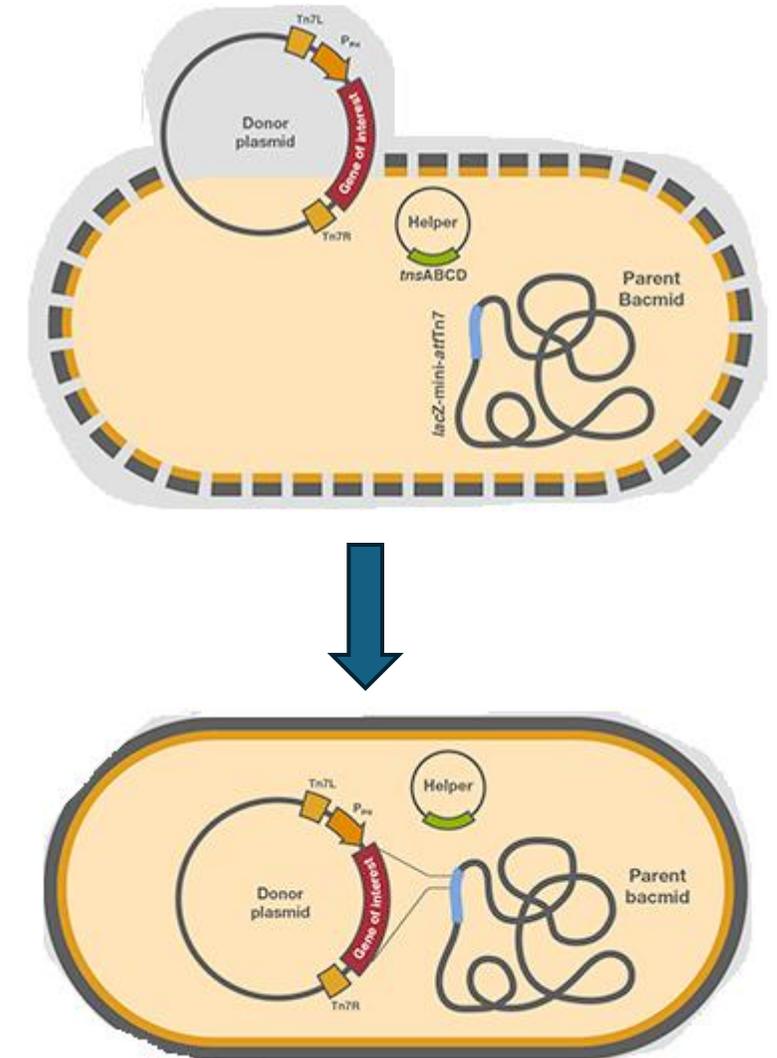
Cloning vector plasmid map



Bacterial transformation:

- The recombinant plasmid now needs to be introduced into a suitable host organism (*Escherichia coli*), by **transformation**.
- **How to make *E. coli* cells competent (able to take up foreign DNA)?**
 - ❖ **CaCl₂ Treatment:** This allows the DNA to get closer to the cell surface.
 - ❖ **Heat Shock:** Heating 42°C for 30-60 seconds to destruct the cell membrane to uptake the DNA .
 - ❖ The **CaCl₂** effectively neutralizing these charges and reducing the repulsion.

host cells such as *E.coli* BL21 or *E.coli* DH5α



Finally, identification the positive clones that carrying specific genes by different methods such as hybridization of DNA or RNA with chemical or radiochemical probs.

Gene expression in host:

the successfully transformed colonies grow up in suitable medium again and induced thiogalactopyranoside (IPTG) to expression the specific trait (protein/s) then harvested, checked and purified.

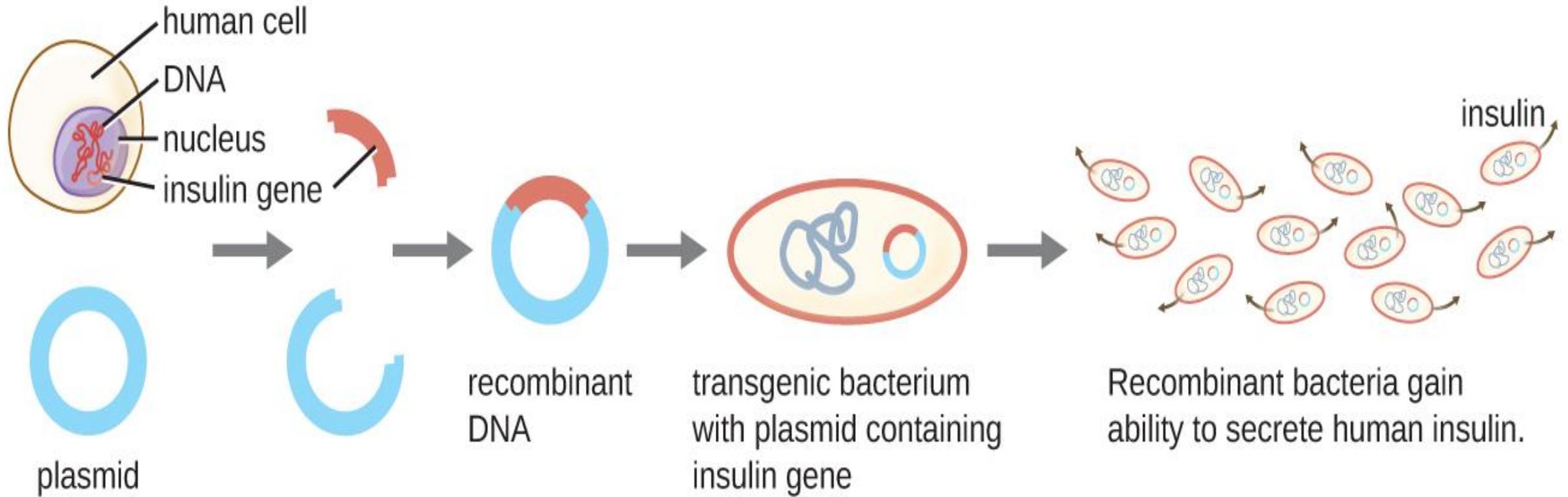


Figure:- Recombinant DNA technology is the artificial recombination of DNA from two organisms. In this example, the human insulin gene is inserted into a bacterial plasmid. This recombinant plasmid can then be used to transform bacteria, which gain the ability to produce the insulin protein.

Benefits of recombinant DNA

- 1)Development of drug synthesis
- 2)Vaccines synthesis
- 3)Improve nutrition & food safety improvement
- 4)Stronger plants & higher crop yield
- 5)Healthier farm animals
- 6)Environmental benefits

Types of vectors:

The most commonly used vectors are:

Plasmids (They are most found in some bacteria as small circular, double-stranded DNA

molecules separated from a chromosomal DNA and replicate independently.

Plasmids often carry genes that may benefit the survival of the organism such as antibiotics resistance, toxin production, producing pigments...etc. The Plasmids are most common used as vector because they are small enough to manipulate experimentally and furthermore, they will carry extra that integrated into them.

1) Viruses

2) Yeast cells.

DNA Extraction:

Although the order of nitrogen bases is what determines DNA's instructions, or genetic code, so the DNA sequences contain the information (gene or genes) required for building organism. During molecular techniques, the extraction of DNA is the first step to preformed cloning, DNA must be isolated from the organism and purified in order to prevent interaction with other molecules such as RNA, proteins, polysaccharides...etc.. DNA can be extracted by different methods.

Boiling method:

Boiling method used for extraction of genomic DNA from bacteria (G-ve) as follow:

- 1)The overnight cultured bacteria harvested in 1 ml of Tris-EDTA (TE) buffer and centrifuged at 12000 g for 2-5min.
- 2)The Pellet was re-suspended in 100 μ l of sterile distilled water.
- 3)The mixture boiled at 100°C for 10 min and then cooled on ice for 10 min.
- 4)The mixture re- centrifuged at 10000 g for 5 min., and then the supernatant is stored (contain bacterial DNA) at - 20 °C until use.

Q: What is the gene with Sticky end and Blunt end, and why it happened?

