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పునఃసంయోజక DNA పరిజ్ఞానం

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STEPS IN RECOMBINANT DNA TECHNOLOGY

పునఃసంయోజక DNA పరిజ్ఞానం

1. Isolation of Genetic Material
2. Restriction Enzyme Digestion
3. Amplification Using PCR
4. Ligation of DNA Molecules
5. Insertion of Recombinant DNA Into Host
6. Isolation of Recombinant Cells

1. Isolation of Genetic Material

➢The first step in rDNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.

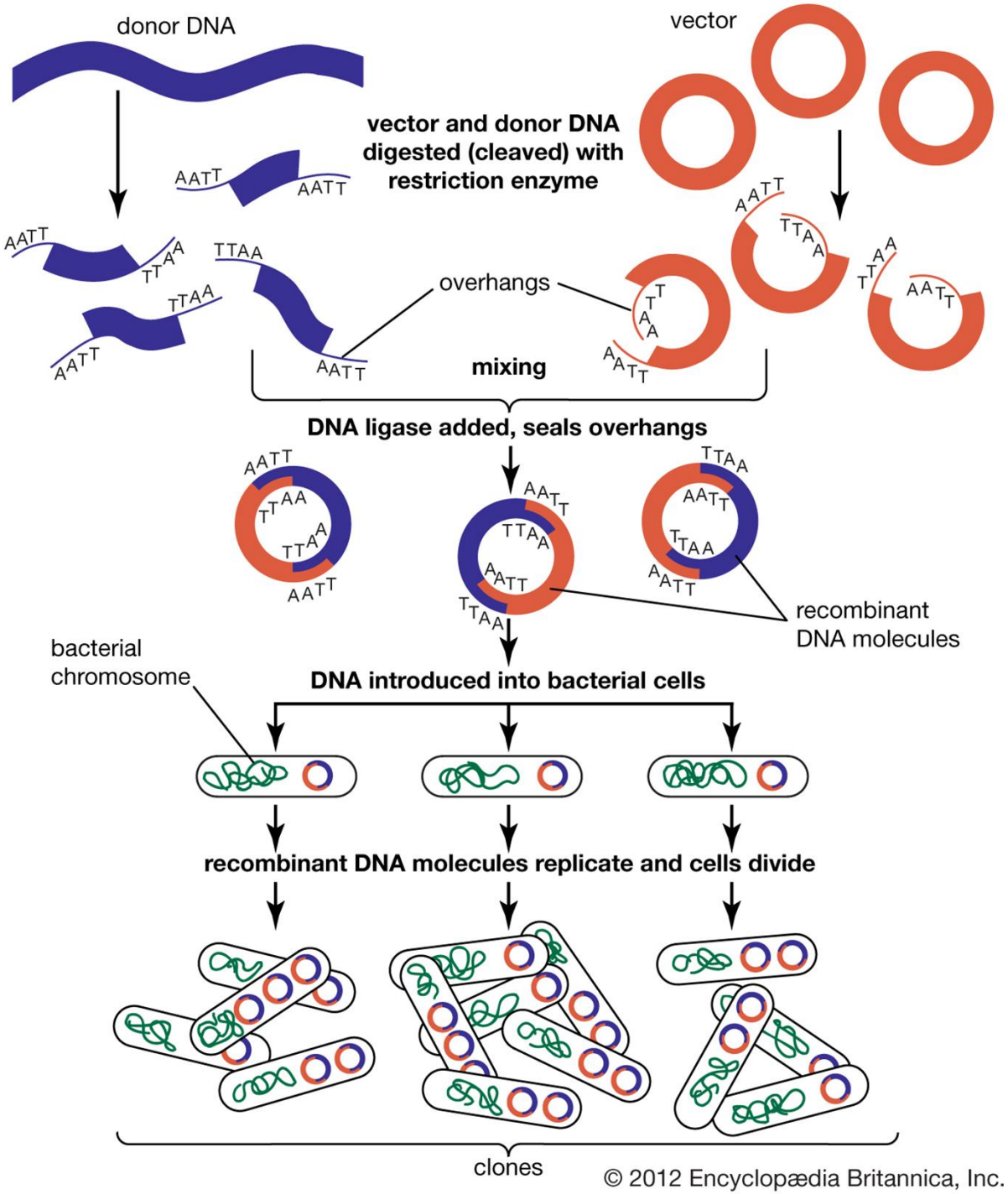
➢Since DNA exists within the cell membrane along with other macromolecules such as RNA, polysaccharides, proteins, and lipids, it must be separated and purified which involves enzymes such as lysozymes, cellulase, chitinase, ribonuclease, proteases etc.

➢Other macromolecules are removable with other enzymes or treatments. Ultimately, the addition of ethanol causes the DNA to precipitate out as fine threads. This is then spooled out to give purified DNA.

➢A palindromic sequence is a sequence of nitrogen bases in a double stranded DNA or RNA, which when read from 5' end to 3' end is same as that on the complementary strand read from 3'end to 5' end

example 5′ _____ *GAATTC* _____ 3′

3′ _____ *CTTAAG* _____ 5′



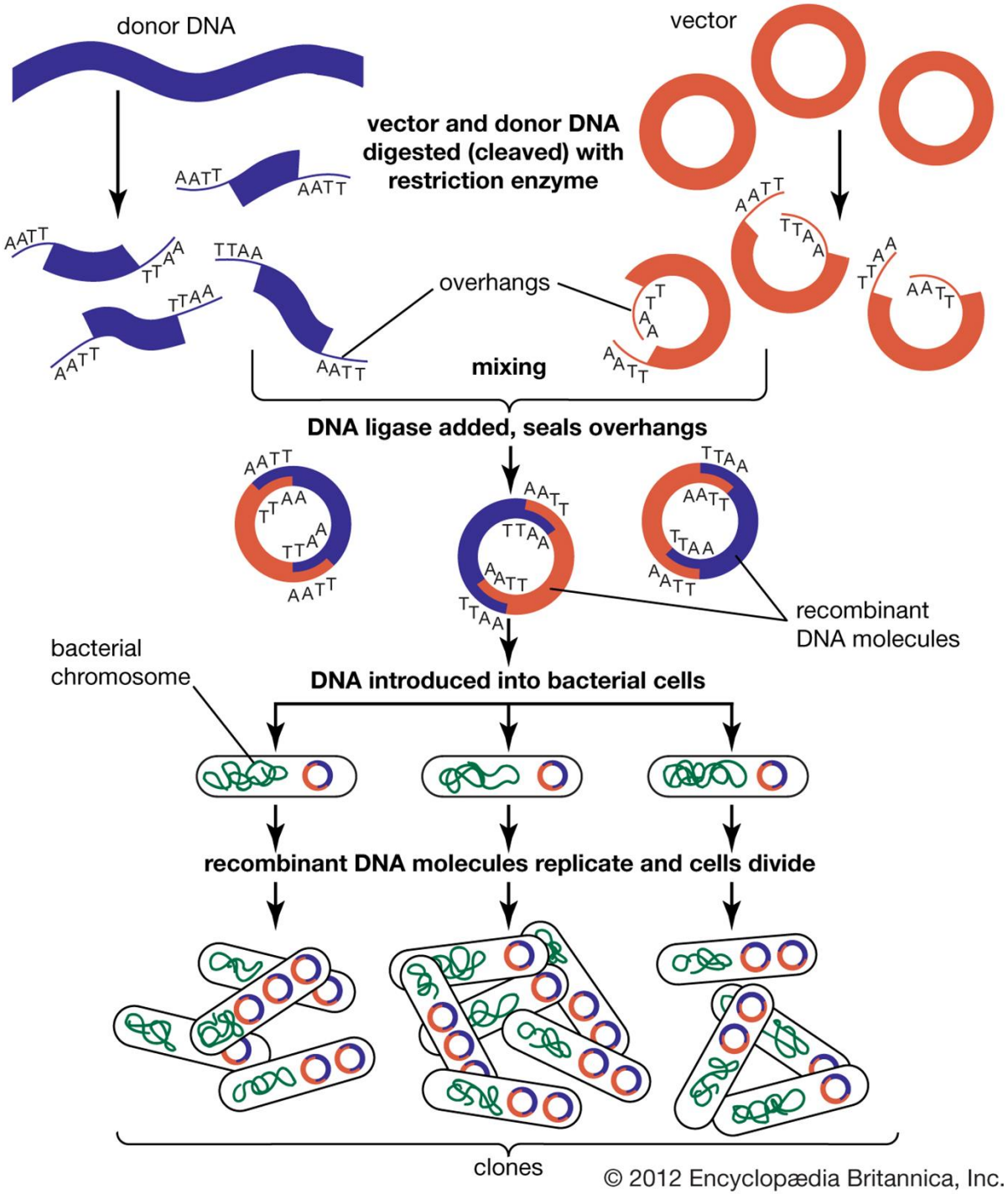
2. Restriction Enzyme Digestion

Restriction enzymes act as **molecular scissors** that cut DNA at specific locations. These reactions are called '**restriction enzyme digestions'.**

They involve the incubation of the purified DNA with the selected restriction enzyme, at conditions optimal for that specific enzyme.

The technique 'Agarose Gel Electrophoresis' reveals the progress of the restriction enzyme digestion.

This technique involves running out the DNA on an agarose gel. On the application of current, the negatively charged DNA travels to the positive electrode and is separated out based on size. This allows separating and cutting out the digested DNA fragments.
The vector DNA is also processed using the same procedure.



3. Amplification Using PCR

➢Polymerase Chain Reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme – **DNA polymerase** in vitro.

It helps to amplify a single copy or a few copies of DNA into **thousands to millions** of copies.

➢PCR reactions are run on 'thermal cyclers' using the following components:

Template – DNA to be amplified

Primers – small, chemically synthesized oligonucleotides that are complementary to a region of the DNA.

Enzyme – DNA polymerase

Nucleotides – needed to extend the primers by the enzyme.

➢The cut fragments of DNA can be amplified using PCR and then ligated with the cut vector.

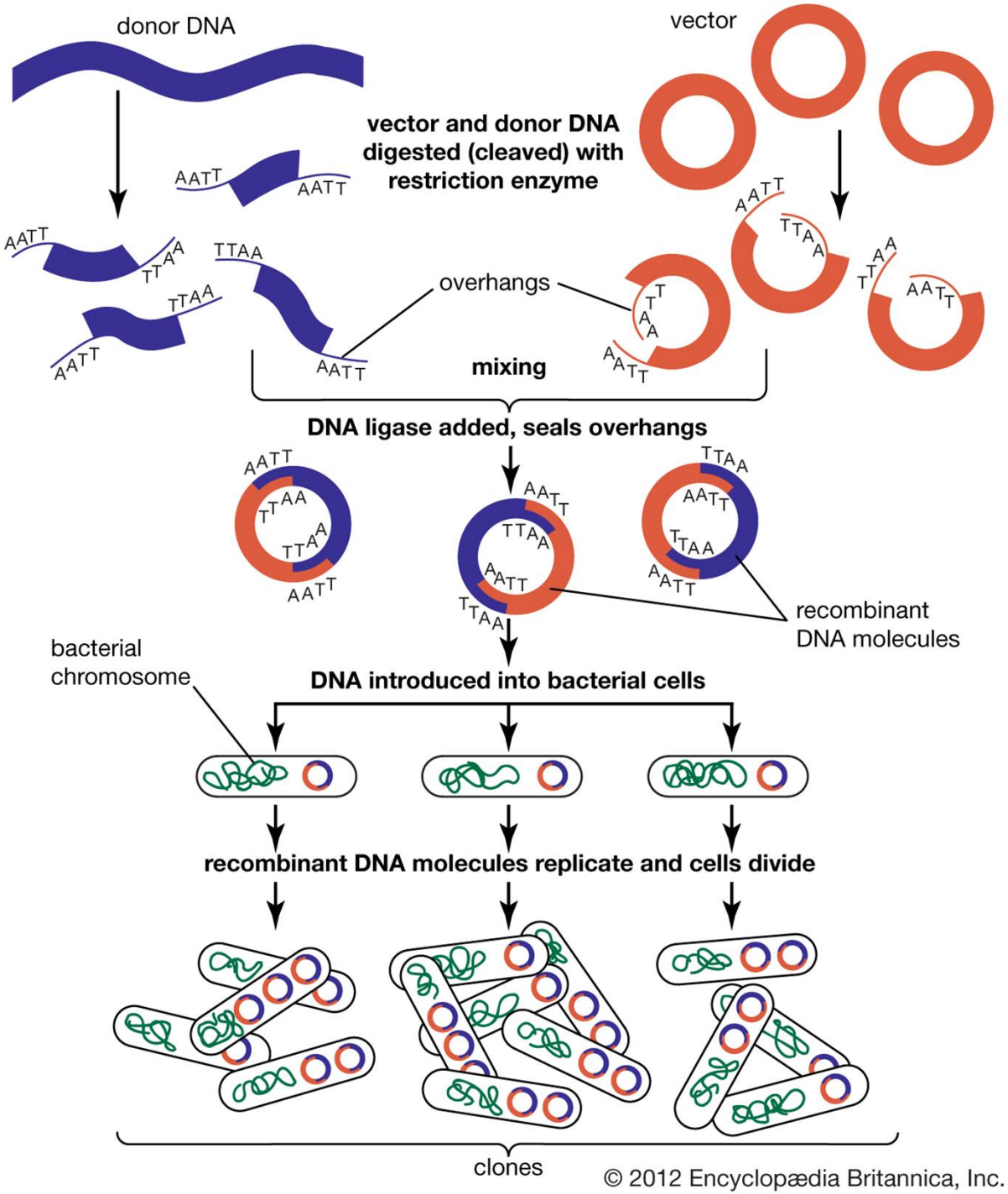
4. Ligation of DNA Molecules

➢The purified DNA and the vector of interest are cut with the same restriction enzyme. This gives us the cut fragment of DNA and the cut vector, that is now open.
The process of joining these two pieces together using the enzyme 'DNA ligase' is 'ligation'.

➢The resulting DNA molecule is a hybrid of two DNA molecules – the interest molecule and the vector.

➢ In the terminology of genetics this intermixing of different DNA strands is called recombination.

➢Hence, this new hybrid DNA molecule is also called a **recombinant DNA molecule** and the technology is referred to as the **recombinant DNA technology**.



5. Insertion of Recombinant DNA Into Host

➢In this step, the recombinant DNA is introduced into a recipient host cell mostly, a bacterial cell. This process is 'Transformation'.

➢Bacterial cells do not accept foreign DNA easily. Therefore, they are treated to make them 'competent' to accept new DNA. The processes used may be thermal shock, Ca^{++} ion treatment, electroporation etc.

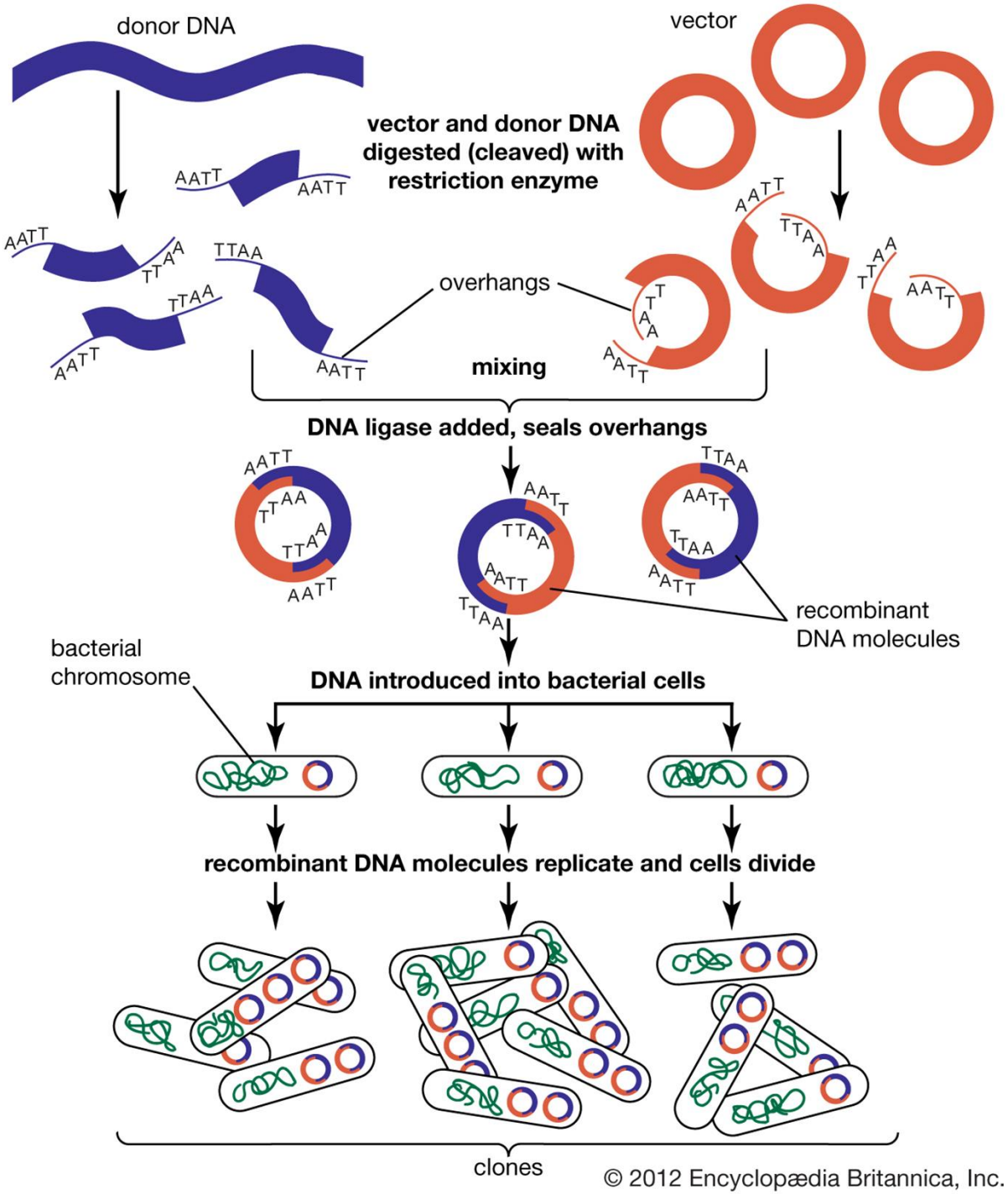
6. Isolation of Recombinant Cells

➢The transformation process generates a mixed population of transformed and non-trans- formed host cells.

➢The selection process involves filtering the transformed host cells only.

➢For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.

➢For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When **pst1 (Providencia stuartii) RE** is used, it will knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.



Thank you