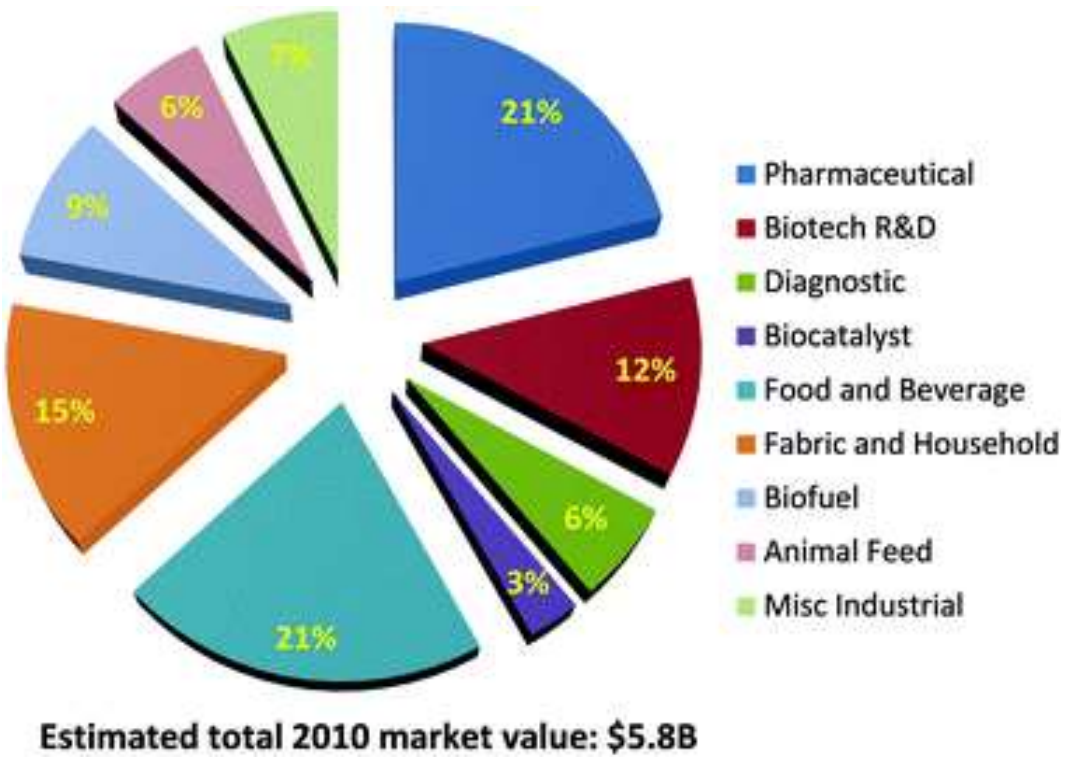
Applications of Biotechnology: Industry



Estimated total 2010 market value: $5.8B

DNA Technology

Genetic Engineering (Recombinant DNA Technology)

DNA Sequencing, PCR, and DNA finger printing

Human Genome Project

Dolly and Golden Rice

DNA CLONING

- **DNA cloning is a technique for reproducing DNA fragments.**
- **It can be achieved by two different approaches:**
  - **cell based**
  - **using polymerase chain reaction (PCR).**
- **a vector is required to carry the DNA fragment of interest into the host cell.**

Tools used in Recombinant DNA Technology

1. **Enzymes**

   - cut and join DNA fragments from different organisms

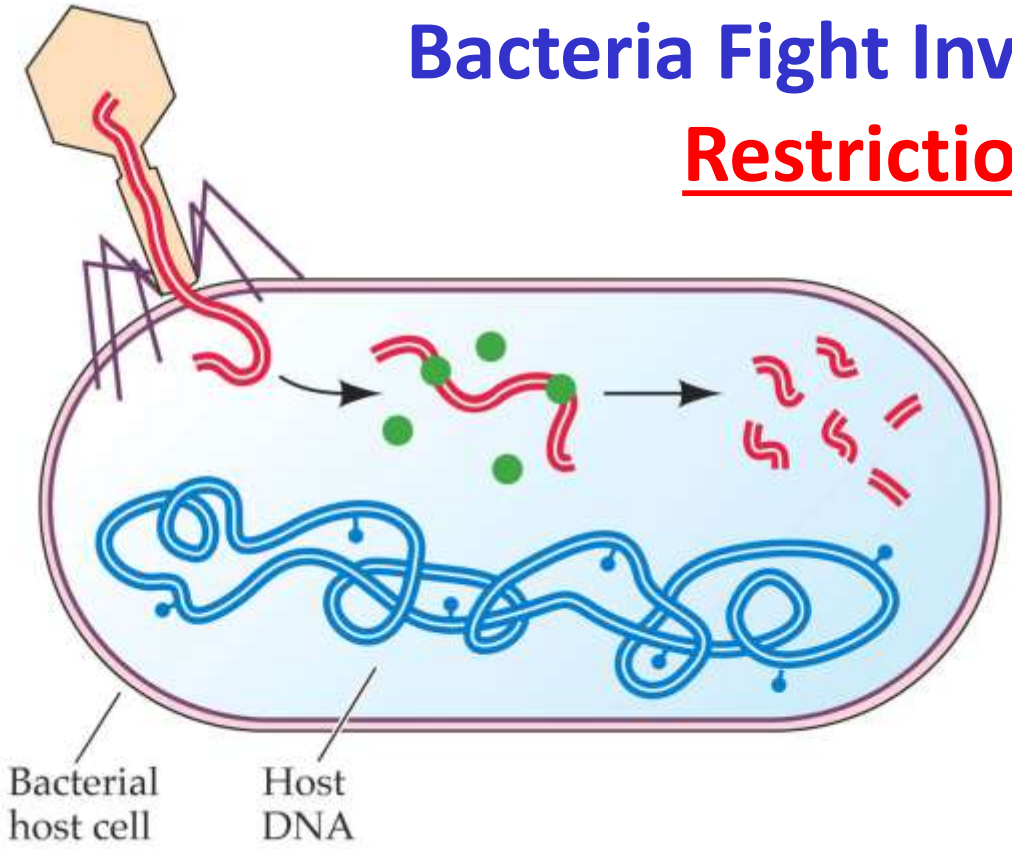
2. **Vectors**

   - carry foreign DNA fragments in to host cells

3. **Hosts**

   - cells allow to propagate Recombinant DNA (r-DNA)

Bacteria Fight Invading Viruses with Restriction Enzymes

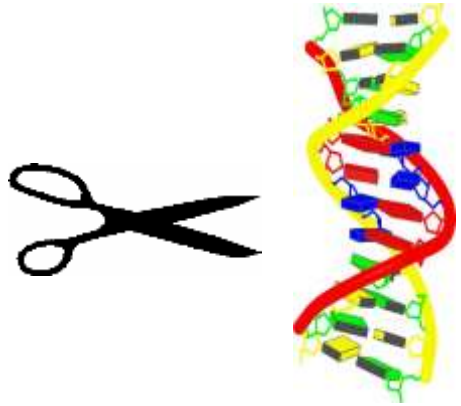


- **The enzymes cut the bonds between the 3'hydroxyl of one nucleotide, and the 5' phosphate of the next.**

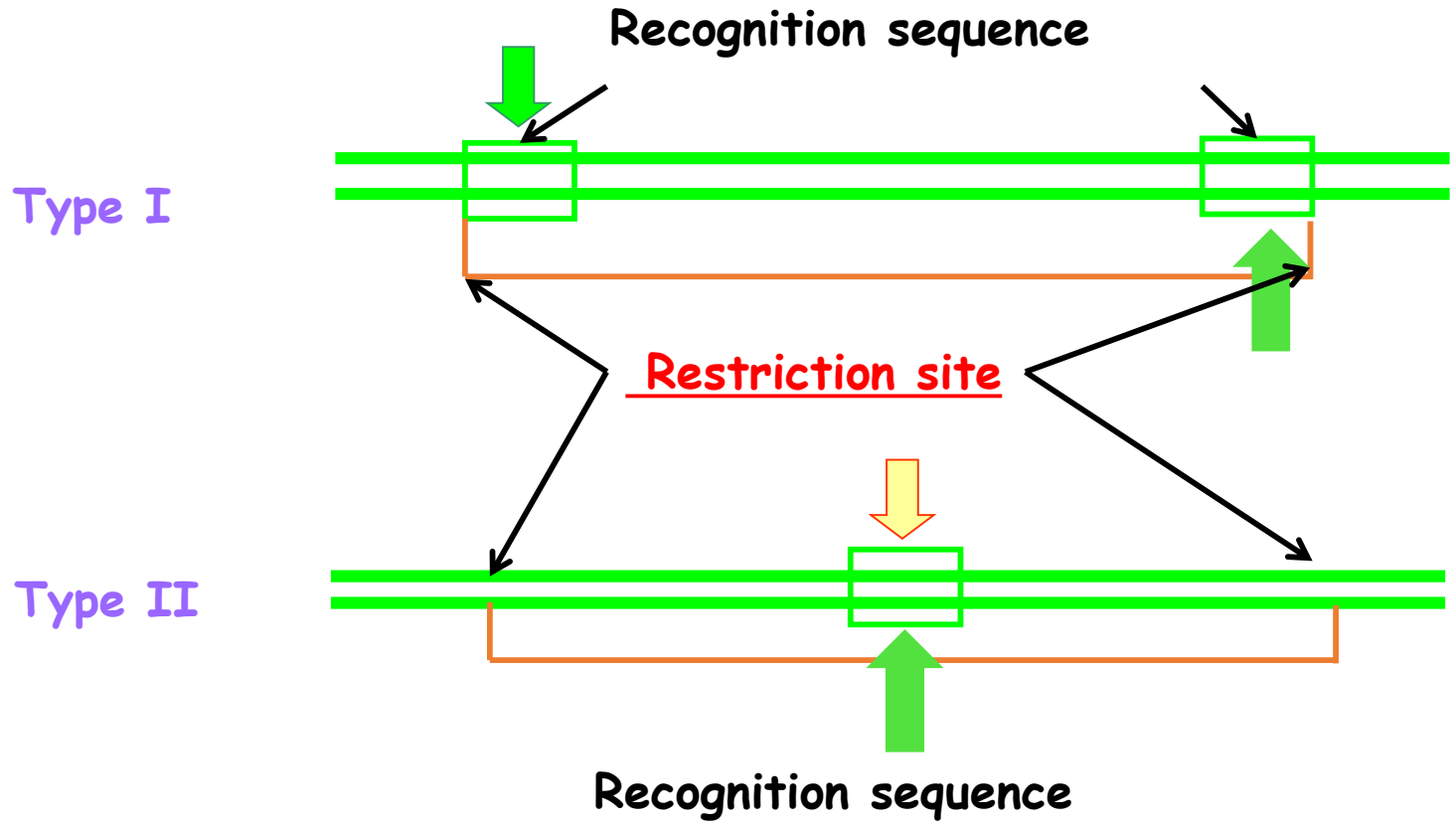
- **There are many such enzymes, each of which recognizes and cuts a specific sequence of bases, called a recognition sequence or restriction site (4 to 6 base pairs long).**

Restriction Endonucleases

Source : Bacteria



Types

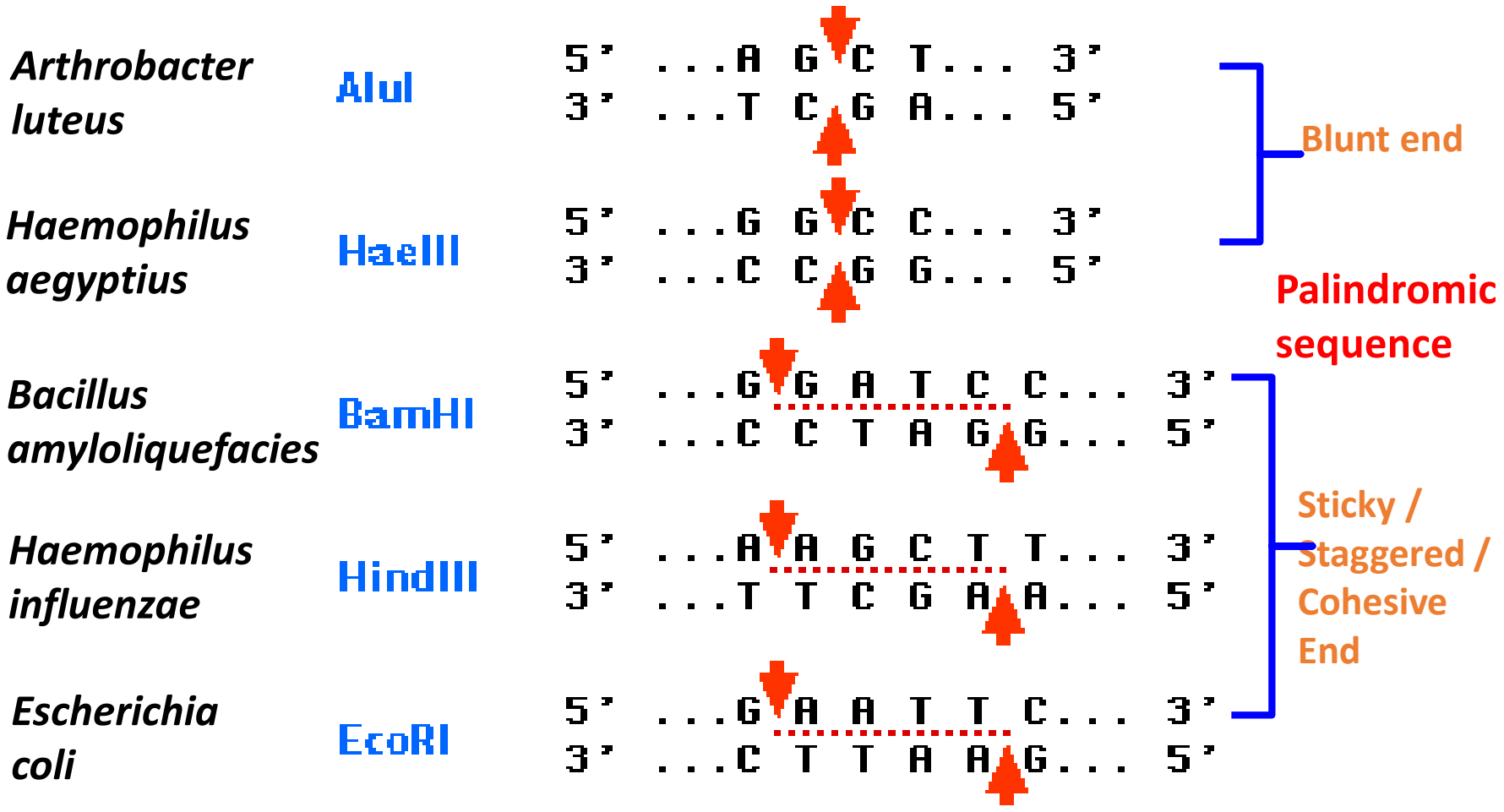


- Recognition: Enzymes identify specific palindromic sequences in DNA.
- Cleavage: Enzymes cleave the phosphodiester bond between nucleotides, resulting in fragments.
- Recognition sequences are typically 4-8 base pairs long and often palindromic (reads the same forward and backward).
- Examples:
- **EcoRI:** GAATTC
- **HindIII:** AAGCTT

Types of Restriction Enzymes

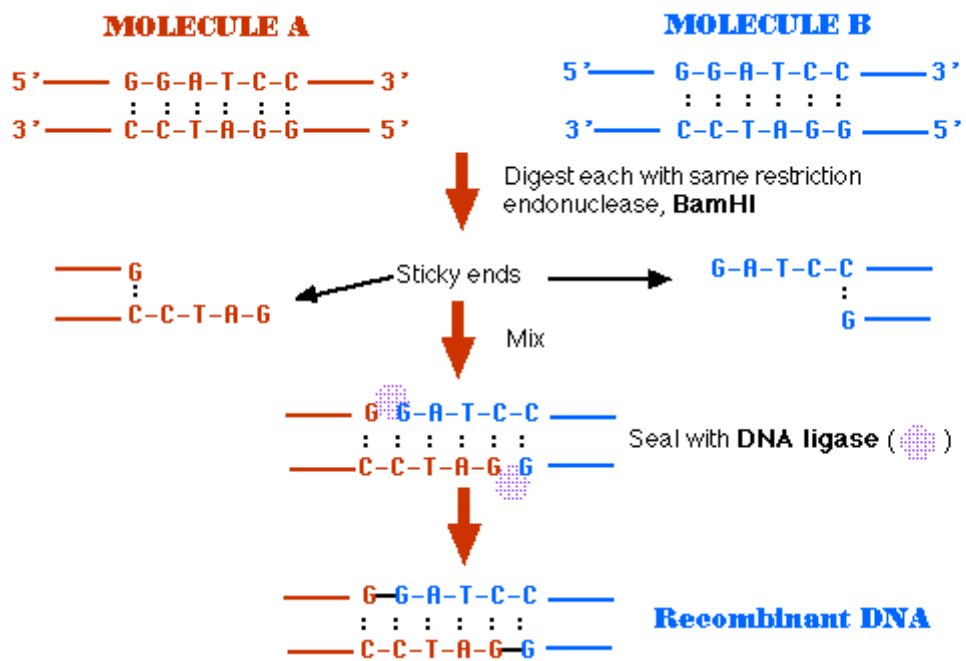
- **Type I:** Cuts DNA at random sites, requires ATP, complex structure.
- **Type II:** Cuts at specific sites, widely used in labs (e.g., EcoRI, HindIII).
- **Type III:** Cuts DNA a short distance from the recognition site, requires ATP but is less common in labs.
- Type II restriction enzymes can generate "sticky ends" (overhanging ends) or "blunt ends," crucial for DNA ligation.

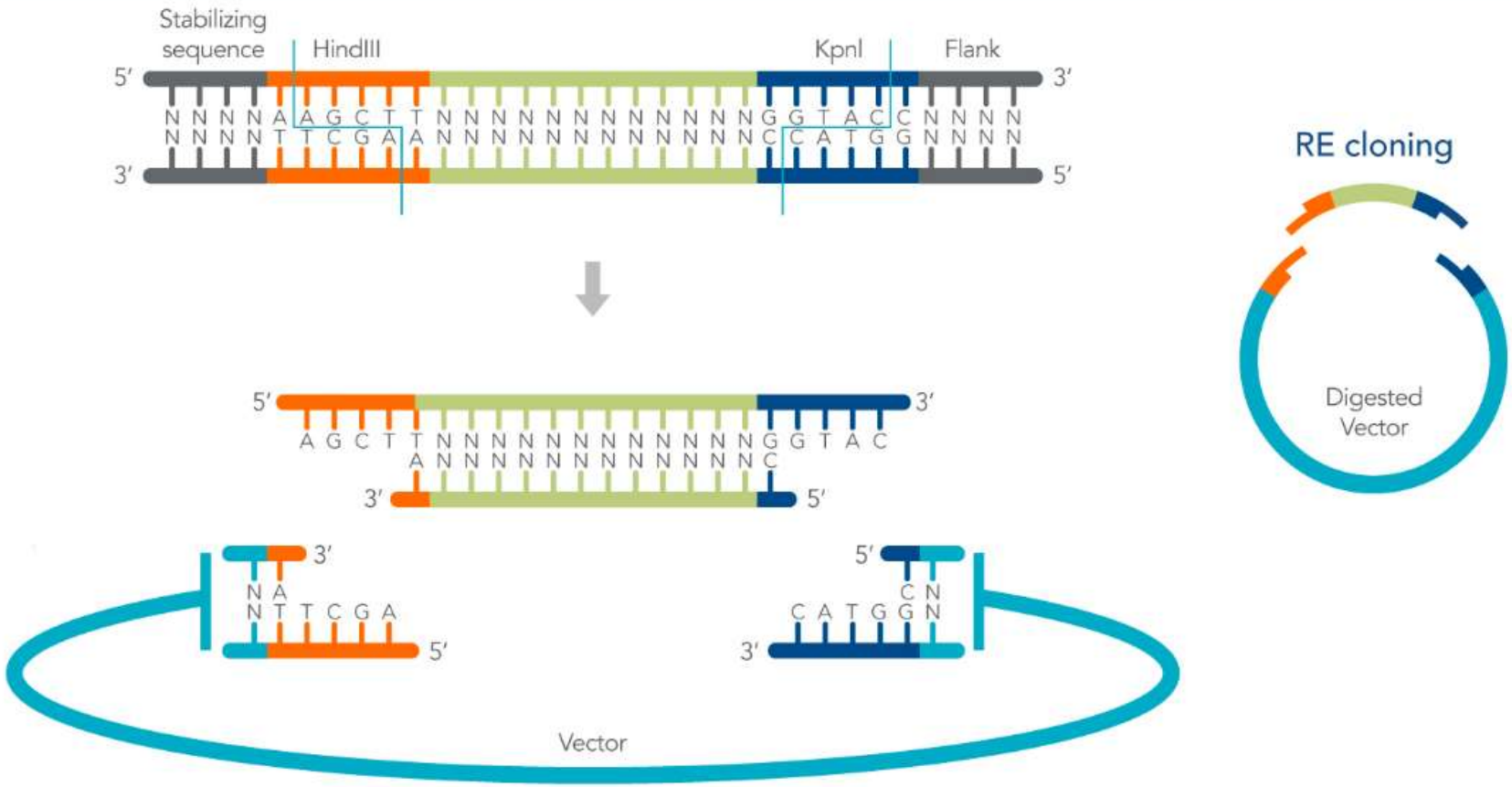
Restriction Endonucleases

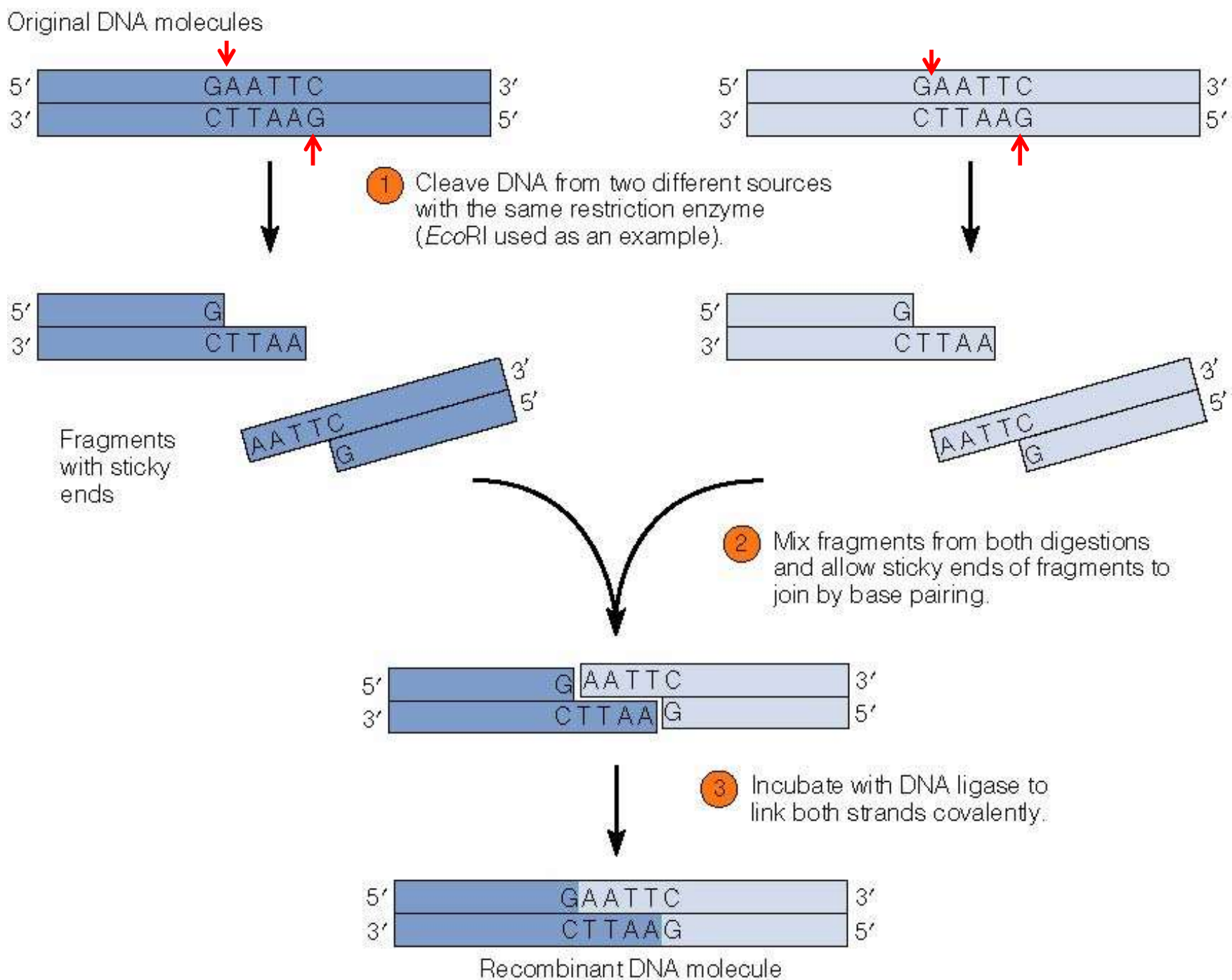


AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

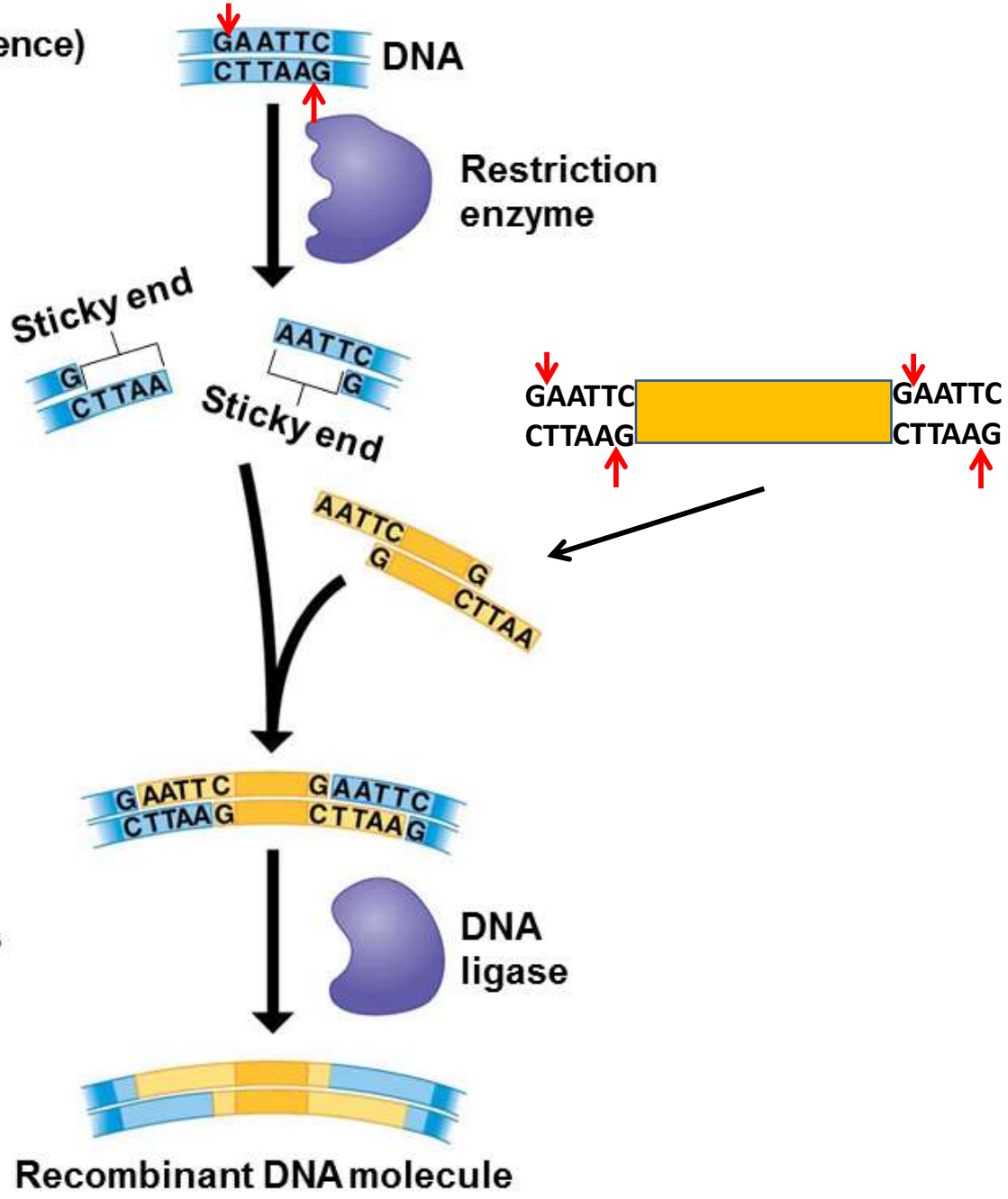


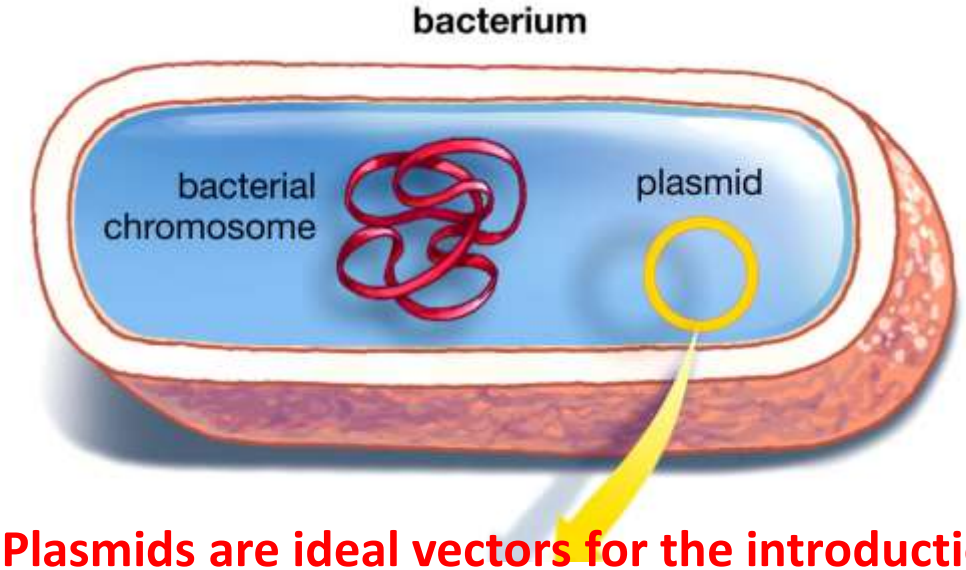



Original DNA molecules
5′ GAATTC 3′
3′ CTTAAG 5′
5′ GAATTC 3′
3′ CTTAAG 5′
1 Cleave DNA from two different sources with the same restriction enzyme (*Eco*RI used as an example).
5′ G
3′ CTTAA
5′ G
3′ CTTAA
Fragments with sticky ends
AATTC 3′
G 5′
AATTC 3′
G 5′
2 Mix fragments from both digestions and allow sticky ends of fragments to join by base pairing.
5′ G AATTC 3′
3′ CTTAA G 5′
3 Incubate with DNA ligase to link both strands covalently.
5′ GAATTC 3′
3′ CTTAAG 5′
Recombinant DNA molecule

Recognition site (recognition sequence) for a restriction enzyme

1. A restriction enzyme cuts the DNA into fragments.
2. A DNA fragment is added from another source.
3. Fragments stick together by base pairing.
4. DNA ligase joins the fragments into strands.





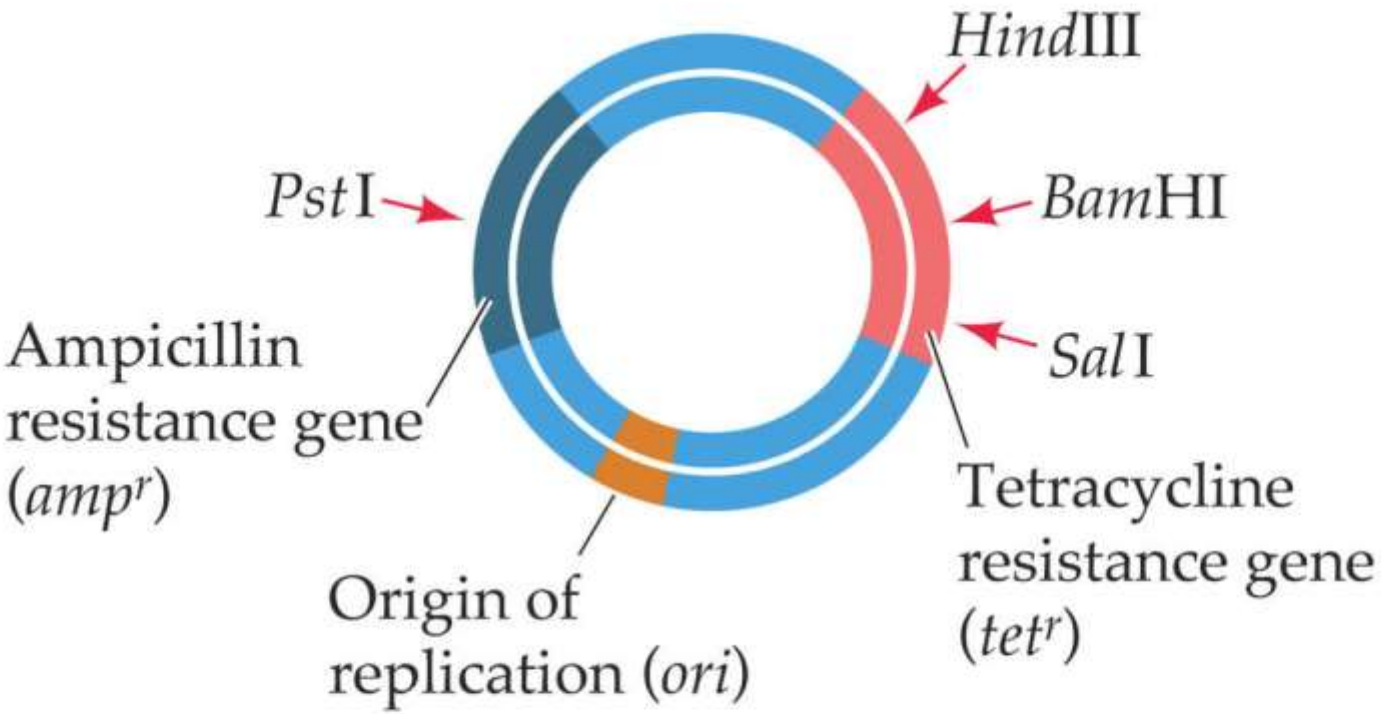
Vectors

Plasmids are ideal vectors for the introduction of r-DNA into bacteria.

- A plasmid is small and **can divide separately from the host's chromosome**.
- Each plasmid contains an **origin of replication** called a **replicon**, or **replication unit**.
- They often have **a restriction site, for a given restriction enzyme**.
- Cutting the plasmid at one site makes it a linear molecule with sticky ends.
- If another DNA is cut **with the same enzyme**, it is possible to insert the DNA into the plasmid.
- **Plasmids often contain antibiotic resistance genes**, which serve as **genetic markers.**

Vectors for Carrying DNA into Cells

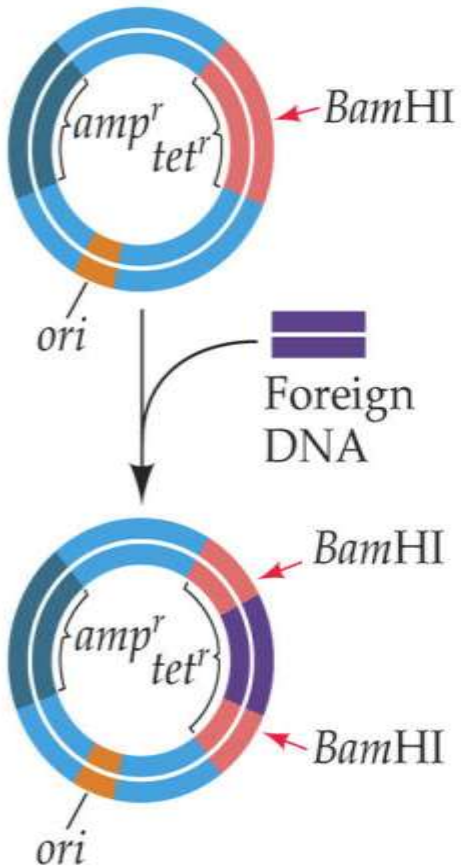
(*a*) Plasmid pBR322
Host: *E. coli*



↓ Recognition site for restriction enzymes

LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 16.5 Vectors for Carrying DNA into Cells (Part 1)

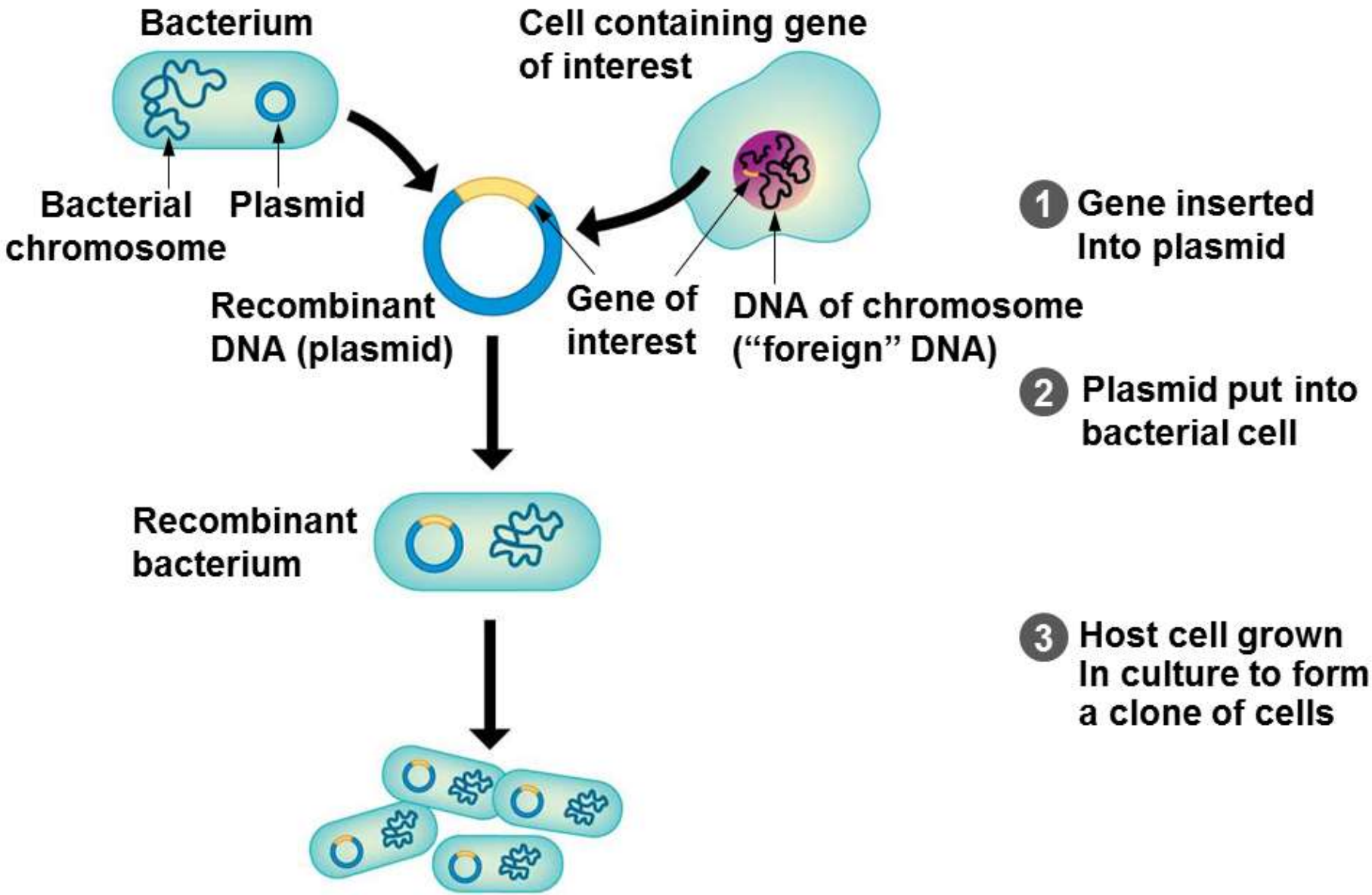
RESEARCH METHOD



DNA taken up by *amp*^{s} and *tet*^{s} *E. coli*	Phenotype for ampicillin	Phenotype for tetracycline
None	Sensitive	Sensitive
Foreign DNA only	Sensitive	Sensitive
pBR322 plasmid	Resistant	Resistant
pBR322 recombinant plasmid	Resistant	Sensitive

LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 16.6 Marking Recombinant DNA by Inactivating a Gene

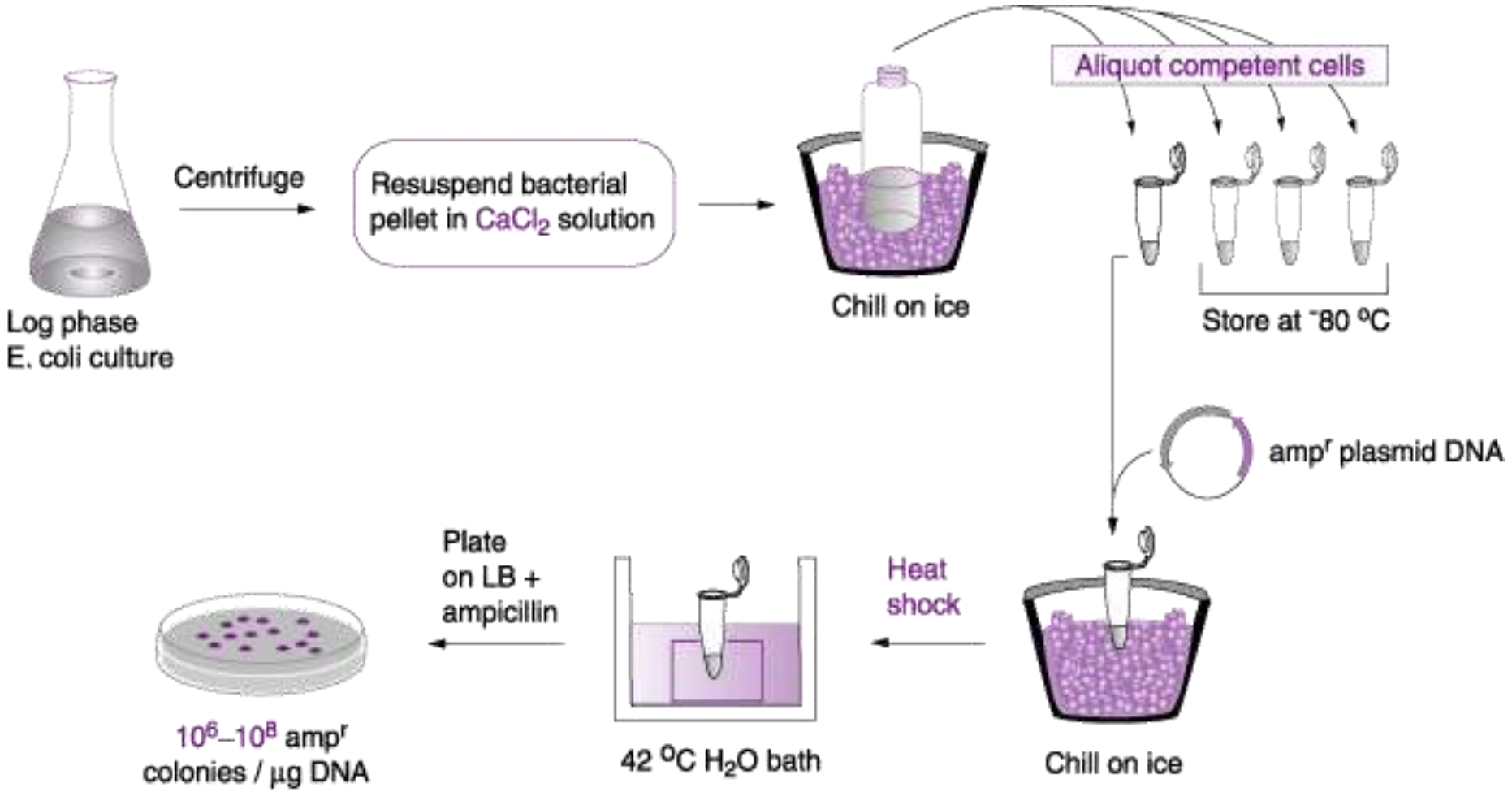
Recombinant DNA Technology



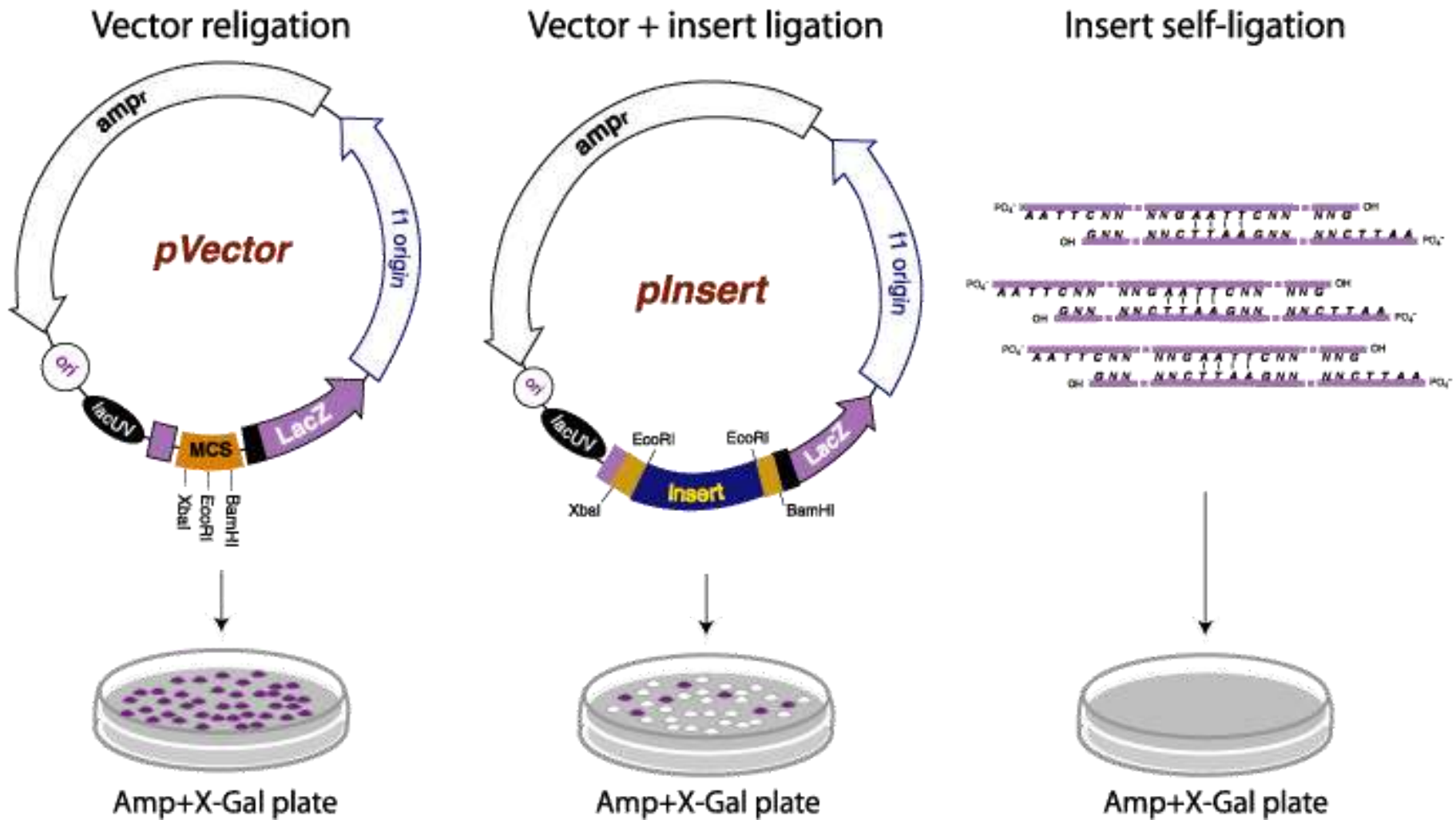
STEP 4. TRANSFORMATION OF LIGATION PRODUCTS

- The process of transferring exogenous DNA into cells is call “transformation”
- There are basically two general methods for transforming bacteria. The first is a chemical method utilizing CaCl2 and heat shock to promote DNA entry into cells.
- A second method is called electroporation based on a short pulse of electric charge to facilitate DNA uptake.

CHEMICAL TRANSFORMATION WITH CALCIUM CHLORIDE



STEP 5. GROWTH ON AGAR PLATES



STEP 5

- **Blue colonies** represent Ampicillin-resistant bacteria that contain pVector and express a functional **alpha fragment** from an intact LacZ alpha coding sequence.

  White colonies represent Ampicillin-resistant bacteria that contain pInsert and do **not** produce LacZ alpha fragment

Blue-white screening

Blue-white screening is a technique used in molecular biology, particularly in the cloning of DNA. It helps researchers identify bacteria that have successfully taken up a plasmid containing a DNA insert.

1.Plasmid Construction: A plasmid (a small, circular piece of DNA) is constructed with an antibiotic resistance gene and a gene that produces a blue pigment, often derived from the *lacZ* gene (alpha portion of beta galactosidase gene). When bacteria (typically *E. coli carrying the omega portion of* beta galactosidase) are transformed with this plasmid, those that take up the plasmid will survive in an antibiotic-containing environment.
2.Transformation: The plasmid is introduced into the bacteria through a process called transformation. Some bacteria will take up the plasmid, while others will not.
3.Culturing: The transformed bacteria are plated on agar that contains an antibiotic and a substrate (like X-gal) that will help indicate whether the *lacZ* gene is active.

4.Blue and White Colonies:

- **Blue Colonies**: If the *lacZ* gene is intact and functional, the bacteria will produce the blue pigment, resulting in blue colonies. This indicates that the plasmid does not have a DNA insert (the gene is complete).
- **White Colonies**: If the plasmid contains an insert that disrupts the *lacZ* gene, the bacteria will not produce the blue pigment and will appear white. This is what researchers want, as it indicates that the plasmid has taken up the desired DNA insert.

5.Selection: Researchers can then select the white colonies for further study, as these are more likely to contain the DNA of interest.

This method is an effective way to streamline the identification of successful clones in molecular cloning experiments.

TERMS USED IN CLONING

- **DNA recombination.**

  The DNA fragment to be cloned is inserted into a vector.
- **Transformation.**

  The recombinant DNA enters into the host cell and proliferates.
- **Selective amplification.**

  A specific antibiotic is added to kill *E. coli* without any protection. The transformed *E. coli* is protected by the antibiotic-resistance gene
- **Isolation of desired DNA clones**

CLONING VECTORS

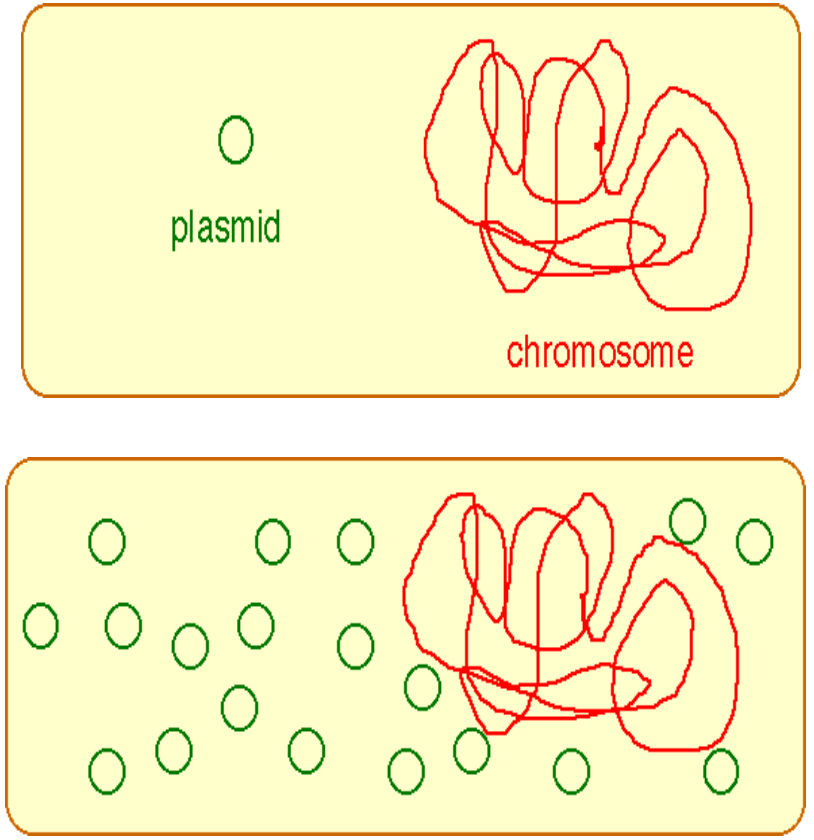
- **Cloning vectors** are DNA molecules that are used to "transport" cloned sequences between biological hosts and the test tube.

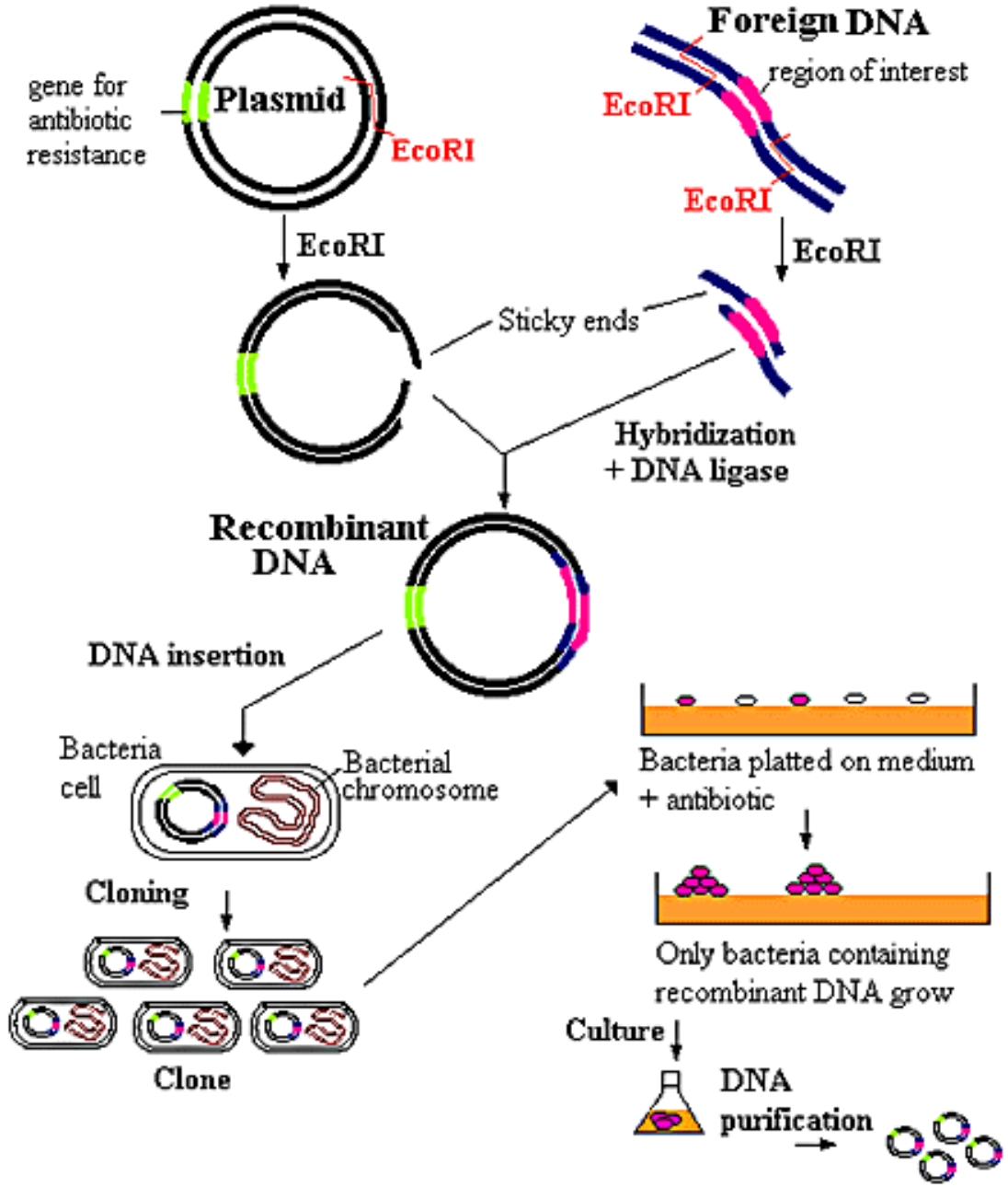
Cloning vectors share four common properties:

1. Ability to promote autonomous replication.
2. Contain a genetic marker (usually dominant) for selection.
3. Unique restriction sites to facilitate cloning of insert DNA.
4. Minimum amount of nonessential DNA to optimize cloning.

PLASMIDS

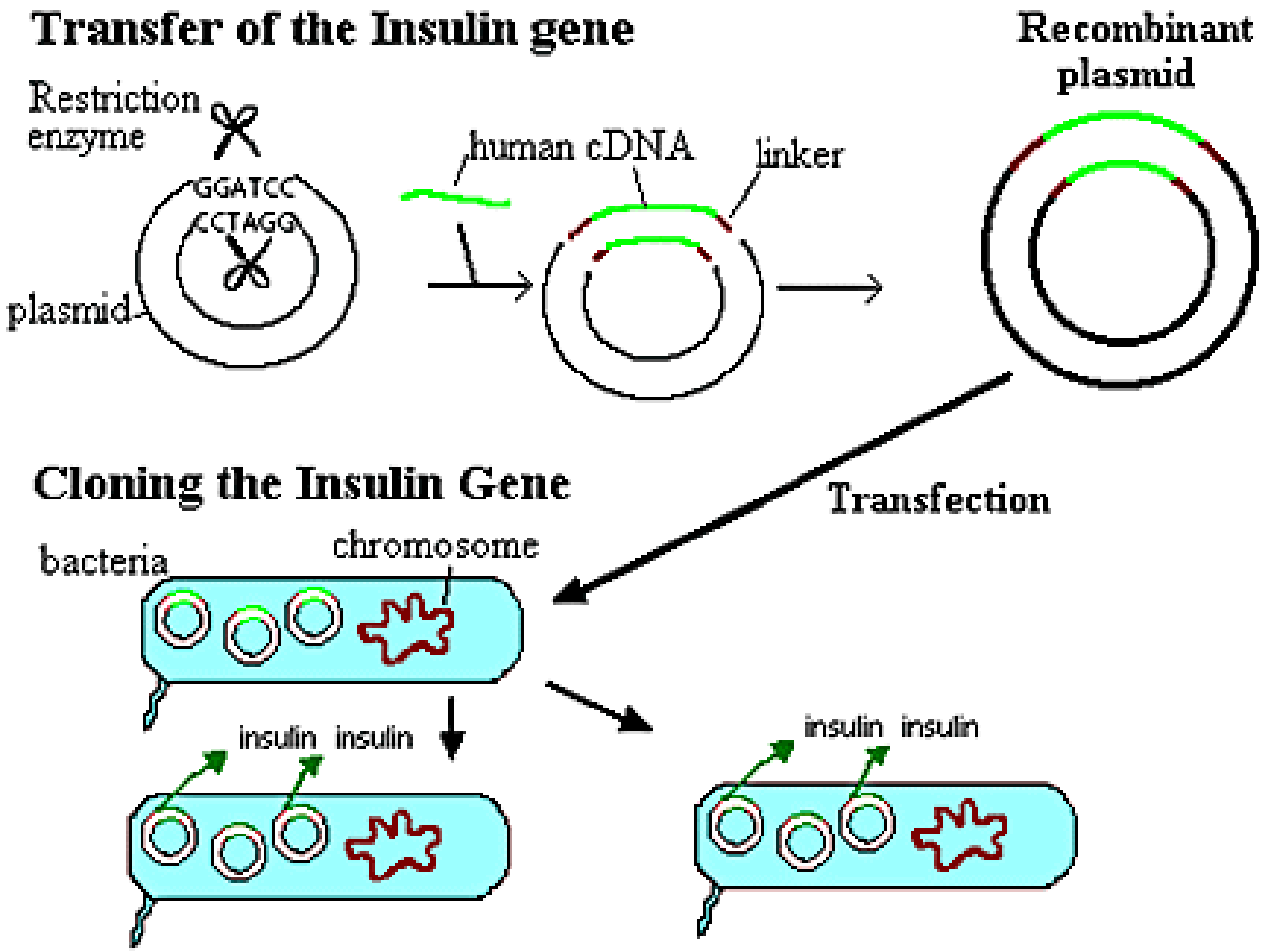
- Bacterial cells may contain extra-chromosomal DNA called plasmids.
- Plasmids are usually represented by small, circular DNA.
- Some plasmids are present in multiple copies in the cell





Cloning into a plasmid

Pharmaceutical Applications: Humulin



Transfer and cloning of the Insulin gene

Herbert Boyer and Stanley Cohen

Pharmaceutical Applications

- **Humulin is human insulin produced by genetically modified bacteria.**
  - In humans, insulin is a protein normally made by the pancreas.
  - Because human insulin is not readily available, diabetes was historically treated using insulin from cows and pigs.
  - In 1978, scientists working at a biotechnology company chemically synthesized DNA fragments and linked them to form the two genes that code for the two polypeptides that make up human insulin.

Pharmaceutical Applications

- DNA technology is used to produce medically valuable molecules, including
  - human growth hormone (HGH)
  - the hormone erythropoietin (EPO), which stimulates production of red blood cells, and
  - vaccines, a harmless variant or derivative of a disease-causing microbe—such as a bacterium or virus—that is used to prevent an infectious disease.
  - Genetically modified whole animals are also used to produce drugs.

Recombinant DNA Technology

Clone of cells containing the gene of interest

Some uses of genes

Genes for cleaning up toxic waste

Genes may be inserted into other organisms.

The gene and protein of interest are isolated from the bacteria.

Harvested proteins may be used directly

Some uses of proteins

Protein for “stone-washing” jeans

Gene for pest resistance

Protein for dissolving clots