Manipulating and Cloning DNA

- Molecular biologists use various tools to complete a multitude of different projects.
- Laboratory tools may aid biologists in:
  - Investigating genetic disorders
  - Altering the genetic make-up of organisms
  - The production of useful products such as insulin
  - Analyzing DNA evidence in criminal investigations or issues of paternity
- ***Genetic engineering*** is the intentional production of new genes and alteration of genomes by the substitution or introduction of new genetic material.

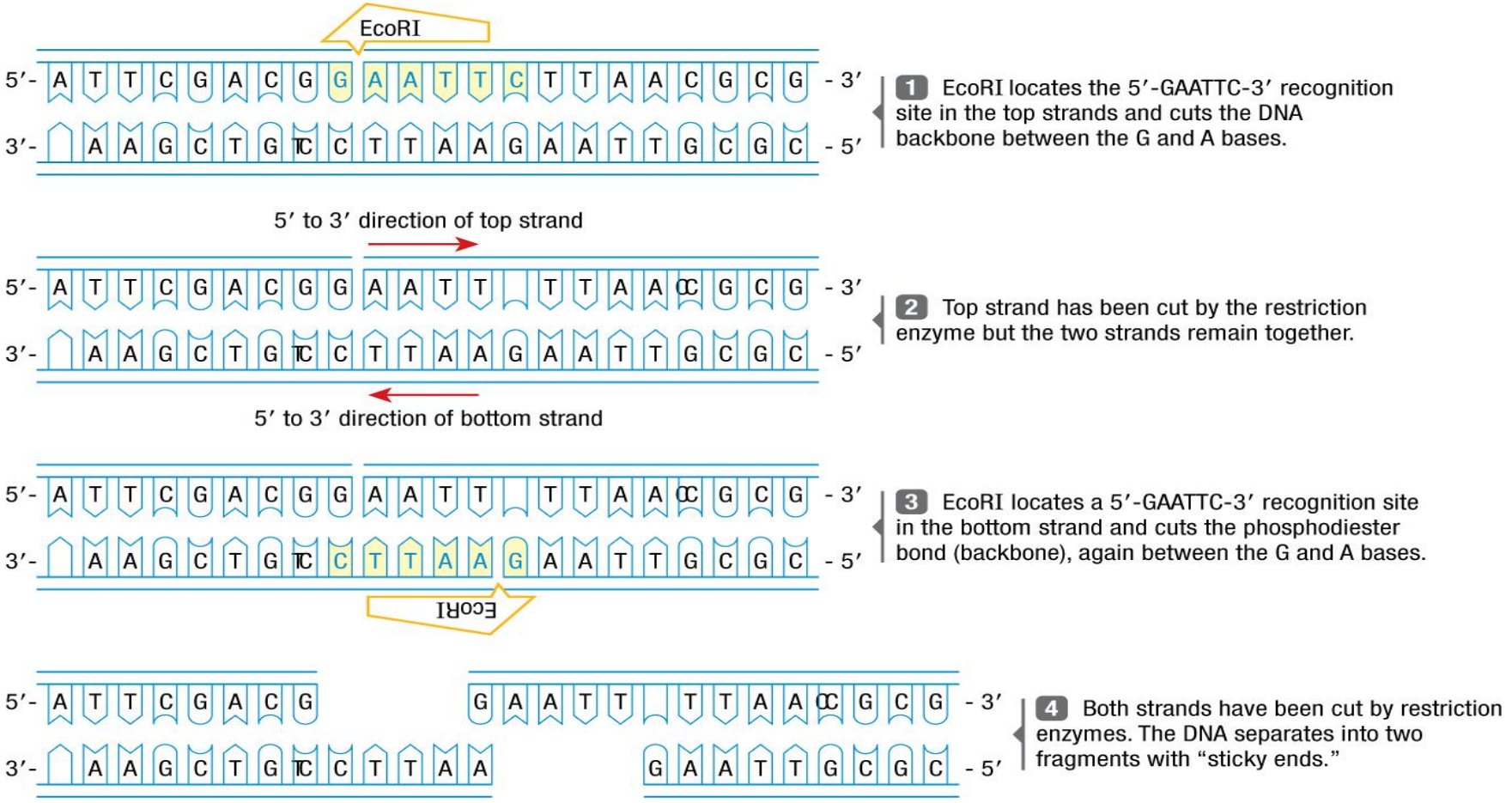
Recombinant DNA Technology

- Molecular biologists have used their laboratory tools to cut, join, and replicate DNA.
- The ability to manipulate DNA makes it possible for scientists to treat specific DNA sequences as modules and move them from one DNA molecule to another, forming ***recombinant DNA***.
- Recombinant DNA technology has enabled molecular biologists to analyze and alter genes and their respective proteins.

Restriction Enzymes

- ***Restriction enzymes***, otherwise known as restriction endonucleases, are molecular scissors that can cut double-stranded DNA at a specific base-pair sequence.
- Each restriction enzyme recognizes a characteristic sequence of nucleotides that is known as its ***recognition site***.
- Recognition sites are usually *palindromic* in nature and consisting of four to eight nucleotides.
- When the restriction enzyme cuts the DNA molecule, the pieces it creates are known as ***restriction fragments***.

Restriction Enzymes at Work



Restriction Enzymes – Types of Cuts

- The ends of DNA fragments produced from a cut by different restriction enzymes differ and produce one of two end types:

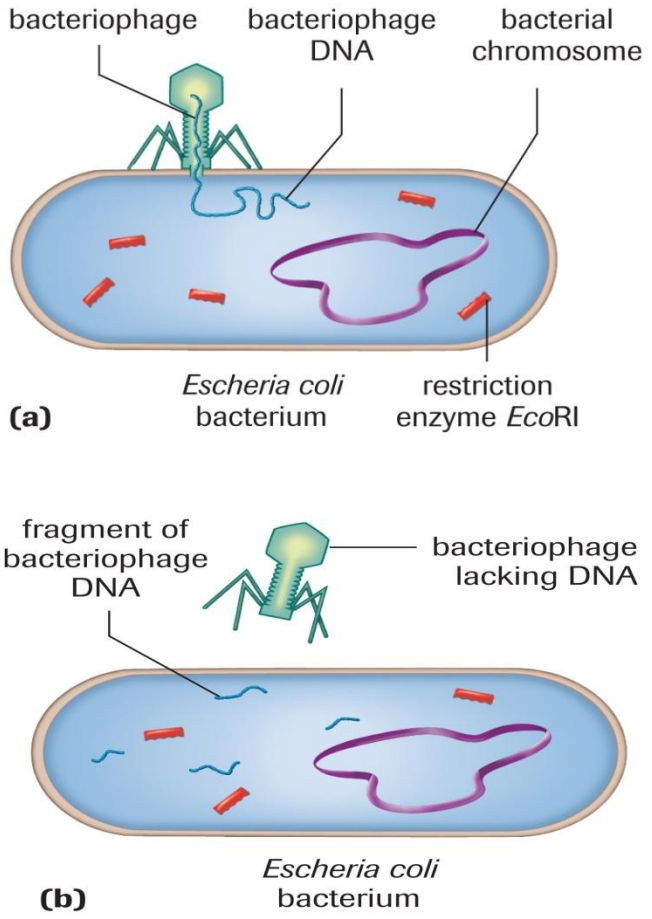
1. ***Sticky ends*** – the end that remains after restriction enzymes cut on a zigzag across a DNA strand; a sticky end of a DNA fragment can form hydrogen binds with complementary sticky ends on any DNA molecule that has been cut by the same enzyme..
2. ***Blunt ends*** – the end that remains after restriction enzymes cut straight across a DNA strand; a blunt end is more difficult than a sticky end to recombine with another strand.

A List of Restriction Enzymes and Their Respective Recognition Sites

Enzyme name	Recognition site	End type	Outcome after restriction enzyme digestion
EcoRI	5′-GAATTC-3′ 3′-CTTAAG-5′	sticky	5′-G AATTC-3′ 3′-CTTAA G-5′
XhoI	5′-CTCGAG-3′ 3'-GAGCTC-5′	sticky	5′-G TCGAG-3′ 3′-GAGCT C-5′
HindIII	5′-AAGCTT-3′ 3′-TTCGAA-5′	sticky	5′-A TCGAC-3′ 3′-CAGGT G-5′
SmaI	5′-ACCCGGGT-3′ 3′-TGGGCCCA-5′	blunt	5′-ACCC GGGT-3′ 3′-TGGG CCCA-5′

Naming Restriction Enzymes

- Restriction enzymes come from all types of bacterial cells.
- Restriction enzymes act as a crude immune system for its respective bacterium.
- Restriction enzymes are named according to the bacteria from which they originate.



DNA Ligase

- ***DNA Ligase*** is an enzyme used to join together sticky ends.
- ***T4 DNA Ligase*** is a second form of the enzyme that is used to join together blunt ends.

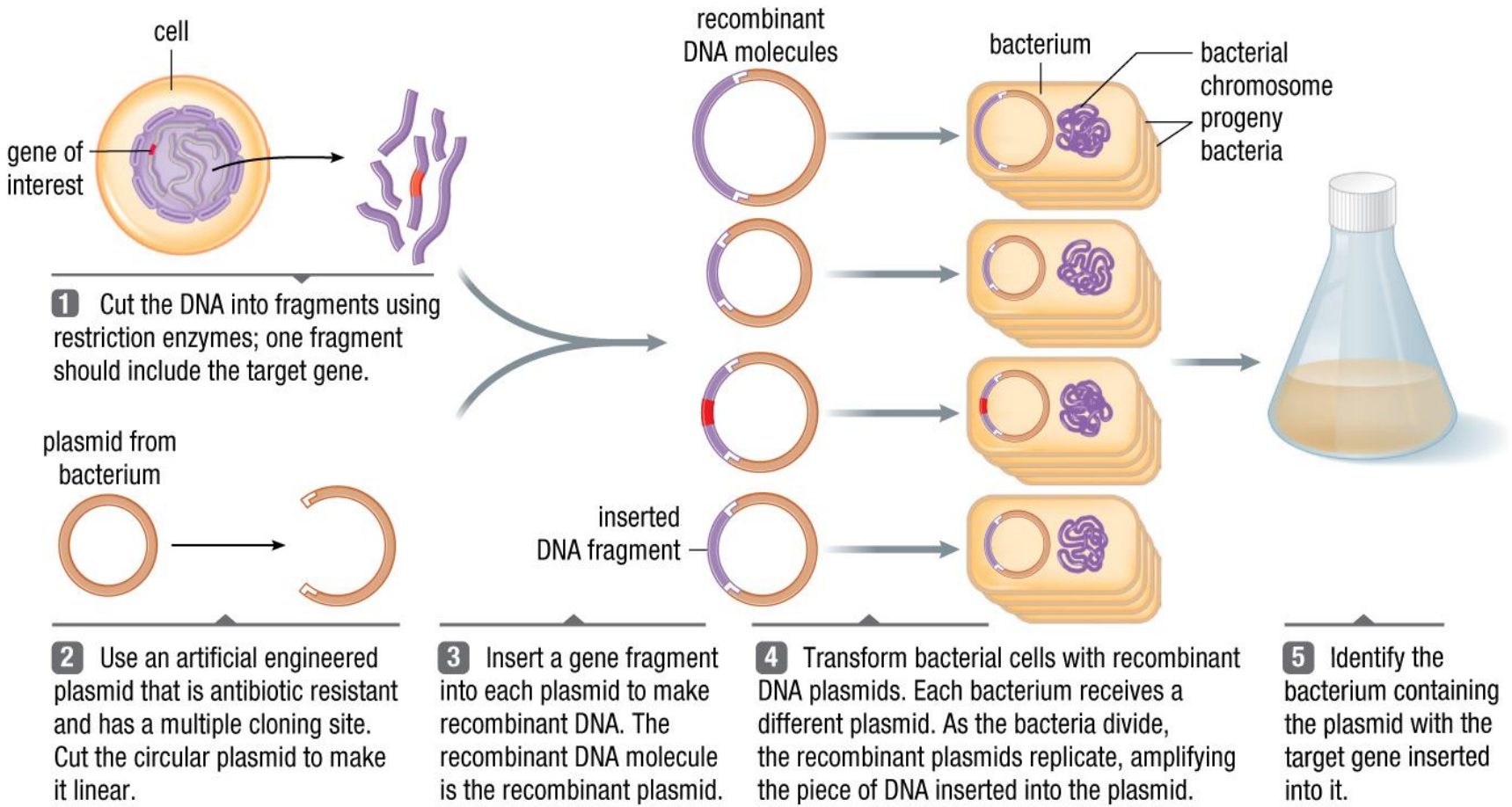
Plasmids

- ***Plasmids*** are small circular pieces of DNA that can exit and enter bacterial cells.
- Plasmids are not digested by restriction enzymes because there exists a mutual beneficial arrangement (endosymbiotic) between chromosomal DNA and the plasmid.

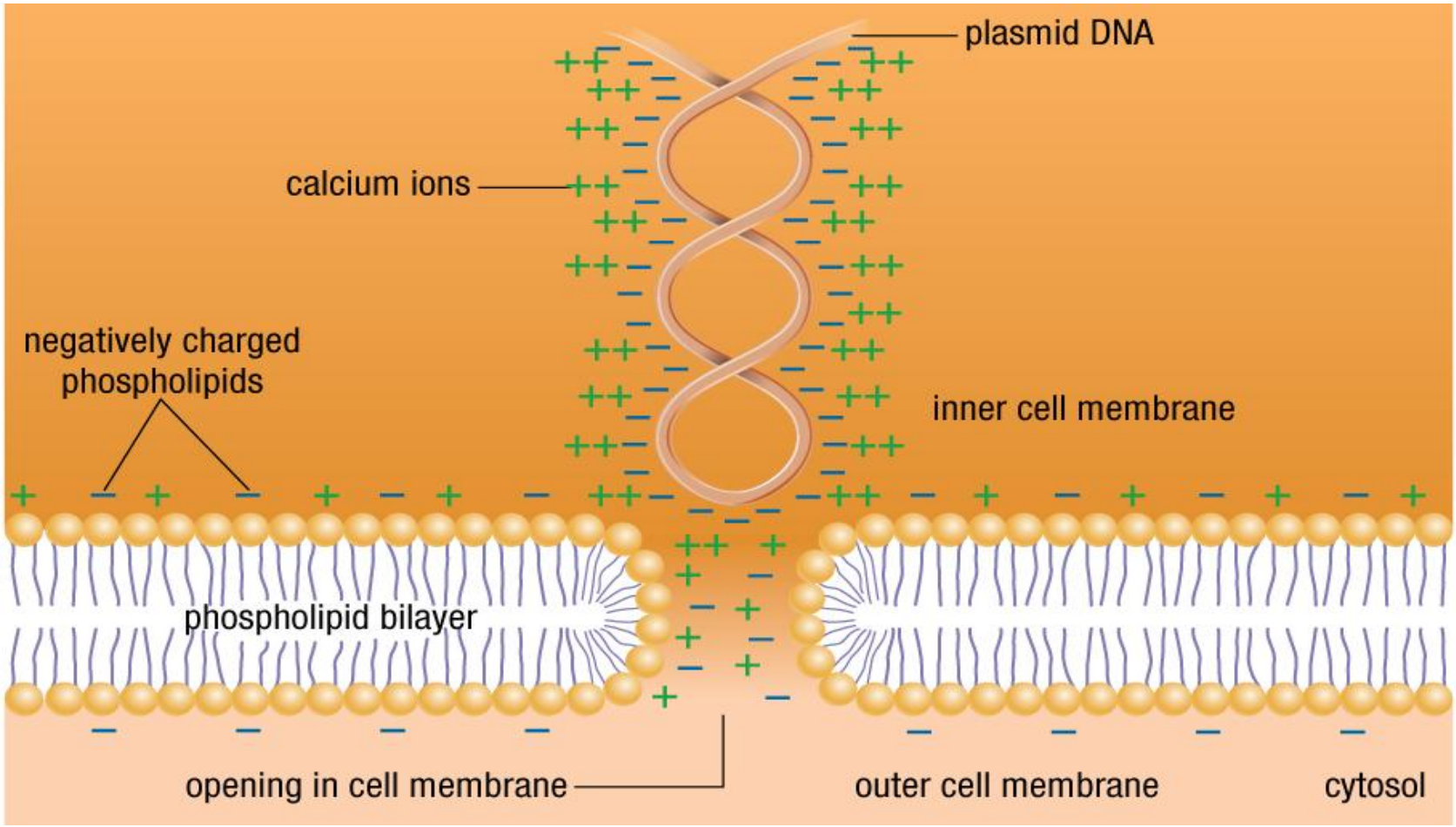
Plasmids

- A cell that is able to take up foreign DNA is called a ***competent cell***.
- A plasmid that has been designed to be a vehicle for transferring foreign genetic materials into a cell is called a ***vector***.
- The plasmid ***copy number*** refers to the number of plasmids of a specific type within a cell.

A Look at Cloning DNA Fragments



Transformation & Cell Competency



Hybridization