

# 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



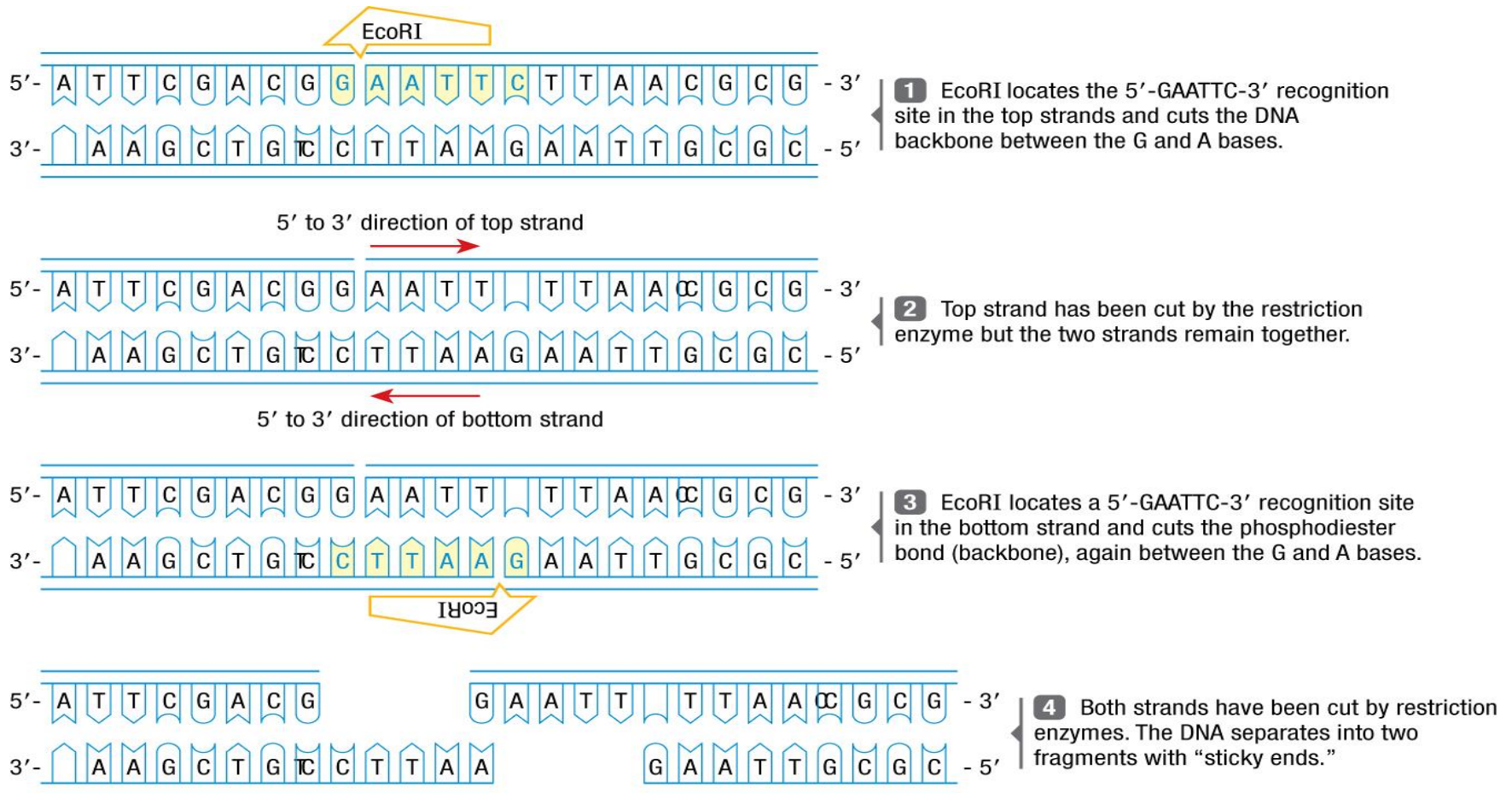
- Molecular biologists have used their laboratory tools to cut, join, and replicate DNA.
- The ability to manipulate DNA makes it possible for scientists to treat specific DNA sequences as modules and move them from one DNA molecule to another, forming ***recombinant DNA***.
- Recombinant DNA technology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

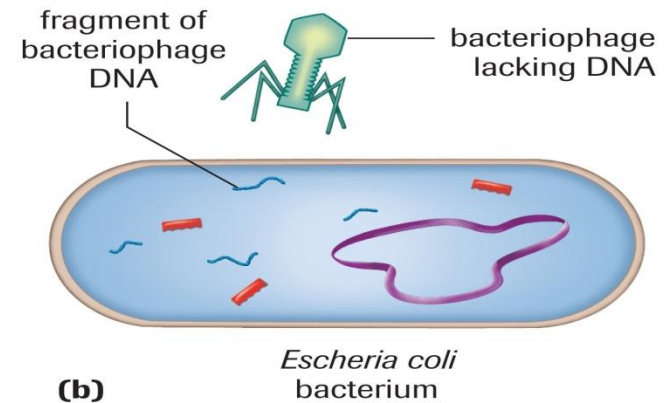
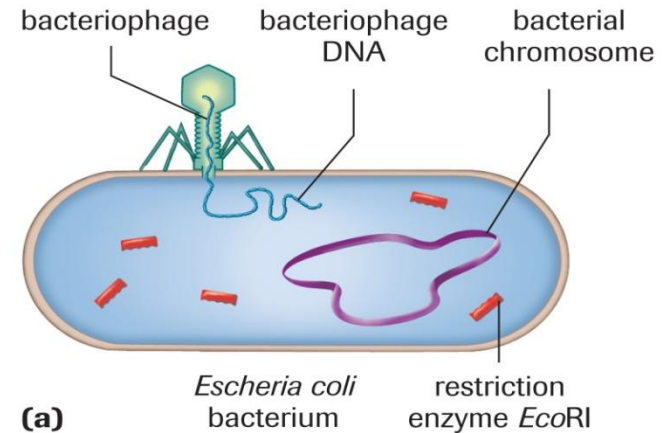


Enzyme name	Recognition site	End type	Outcome after restriction enzyme digestion	
EcoRI	5'-GAATTC-3' 3'-CTTAAG-5'	sticky	5'-G 3'-CTTAA	AATTC-3' G-5'
XhoI	5'-CTCGAG-3' 3'-GAGCTC-5'	sticky	5'-G 3'-GAGCT	TCGAG-3' C-5'
HindIII	5'-AAGCTT-3' 3'-TTCGAA-5'	sticky	5'-A 3'-CAGGT	TCGAC-3' G-5'
SmaI	5'-ACCCGGGT-3'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

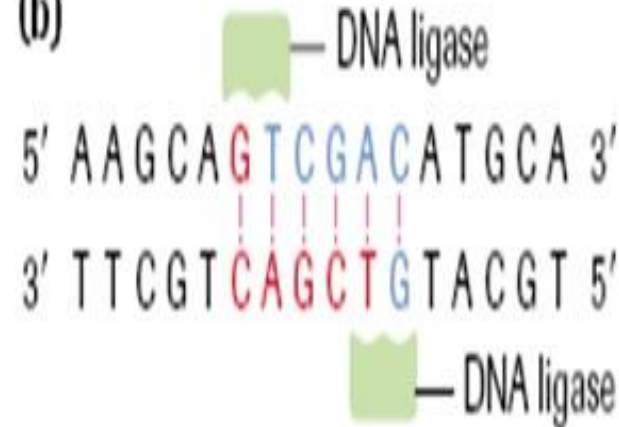


- **DNA Ligase** is an enzyme used to join together sticky ends.
- **T<sub>4</sub> DNA Ligase** is a second form of the enzyme that is used to join together blunt ends.

(a)



(b)





# Plasmids



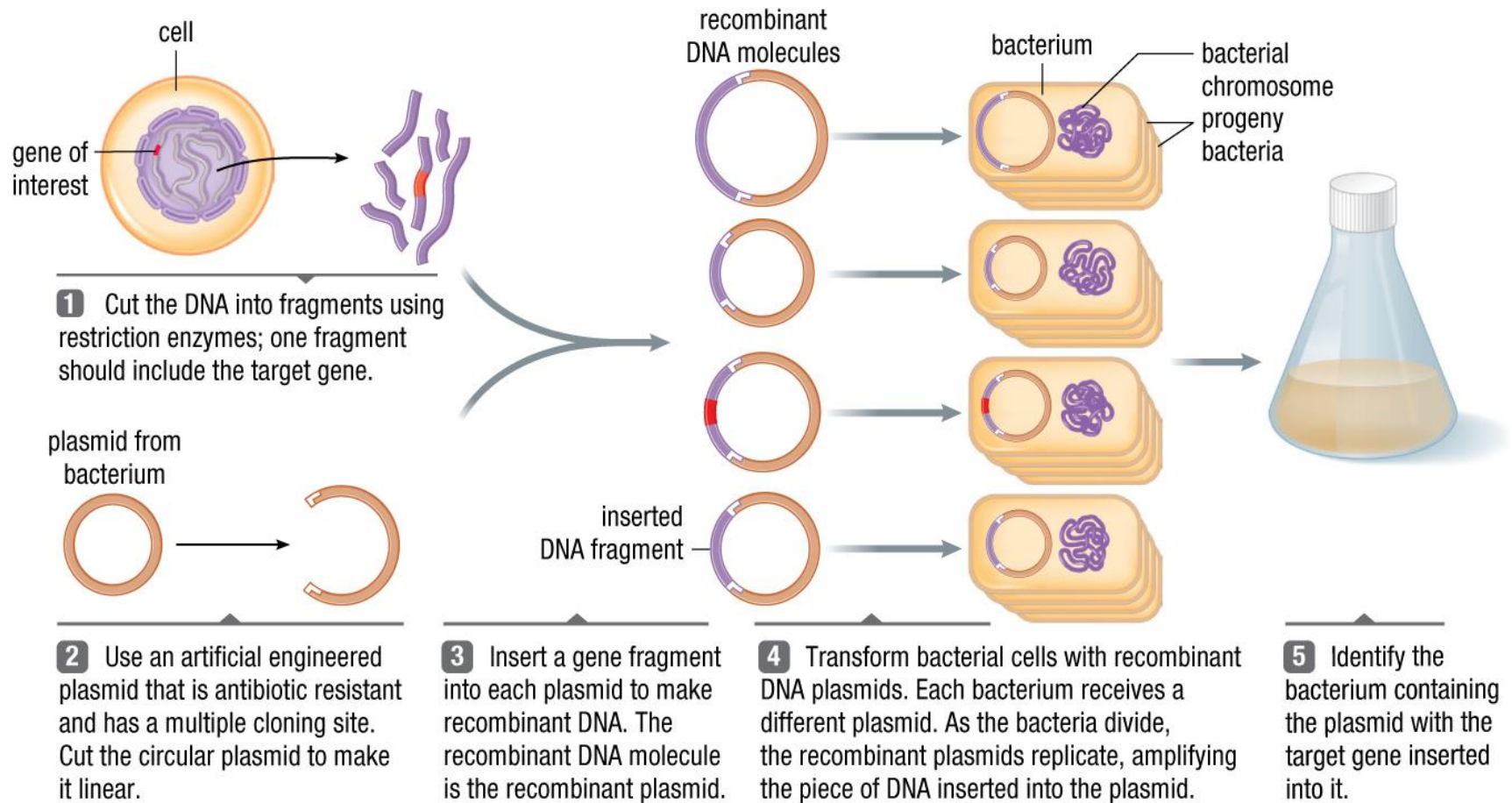
- ***Plasmids*** are small circular pieces of DNA that can exit and enter bacterial cells.
- Plasmids are not digested by restriction enzymes because there exists a mutual beneficial arrangement (endosymbiotic) between chromosomal DNA and the plasmid.

# Plasmids

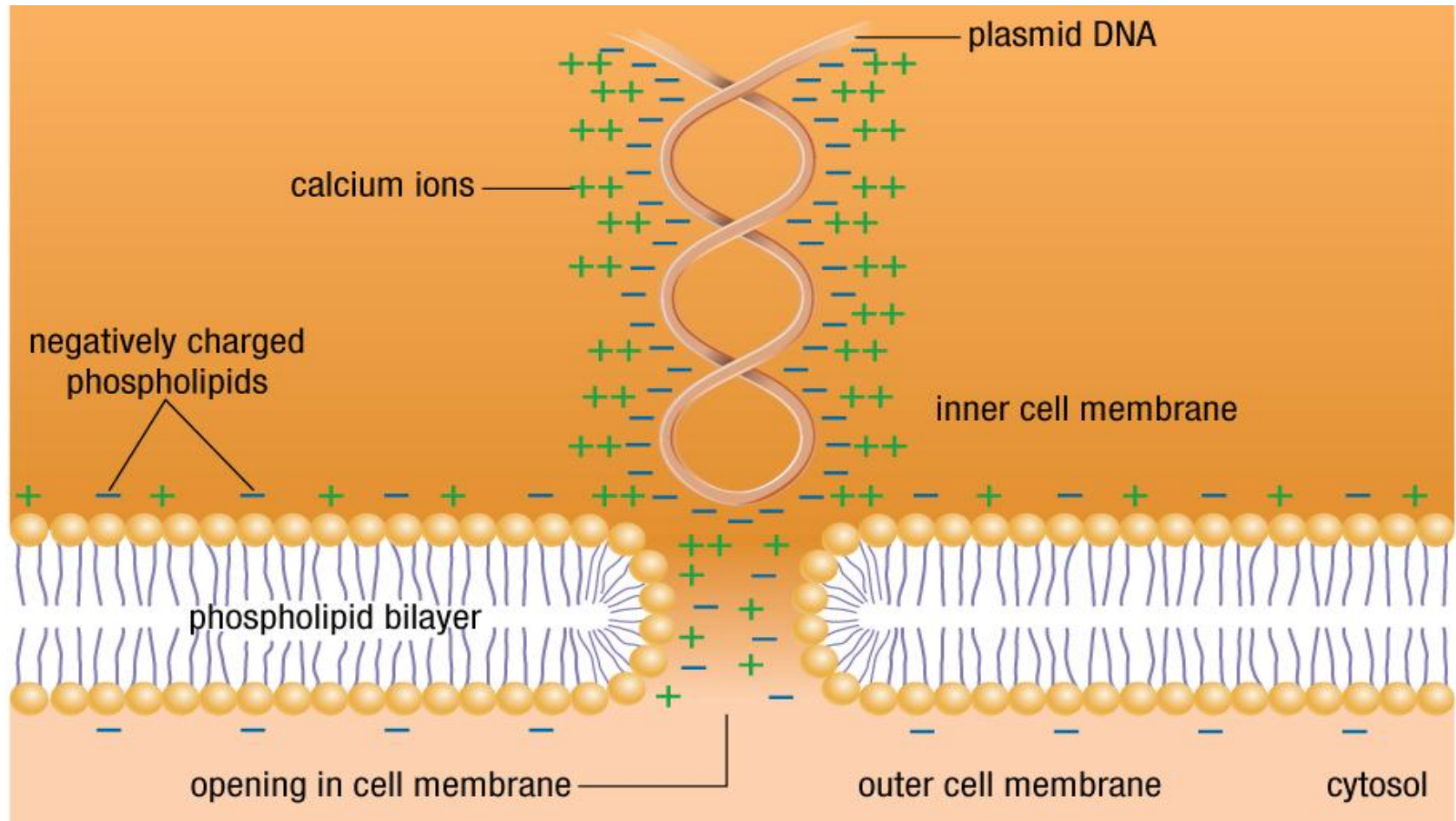


- A cell that is able to take up foreign DNA is called a ***competent cell***.
- A plasmid that has been designed to be a vehicle for transferring foreign genetic materials into a cell is called a ***vector***.
- The plasmid ***copy number*** refers to the number of plasmids of a specific type within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

# A Look at Cloning DNA Fragments



# Transformation & Cell Competency



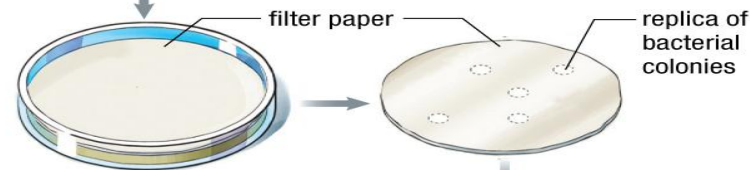
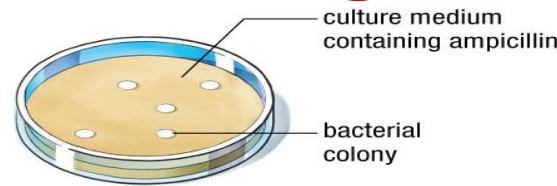
# Hybridization

**1** Bacteria containing plasmids are grown on a culture medium containing ampicillin. Only those bacteria that picked up a plasmid with the ampicillin-resistance gene will survive and grow into a colony.

**2** A special filter paper laid on the bacterial plate picks up cells from each bacterial colony. A replica of the plate is made.

**4** A radioactive-labelled probe complementary to the target gene (DNA or RNA) is incubated with the single-stranded DNA on the filter paper. The radioactive probe hybridizes (forms base pairs) only with the target gene. Excess probe is washed off.

**5** The filter is placed against film which is exposed wherever the radioactive probe has hybridized. The position of any radioactive spot is correlated to the original plate. This colony is isolated and used to produce large quantities of the target gene.

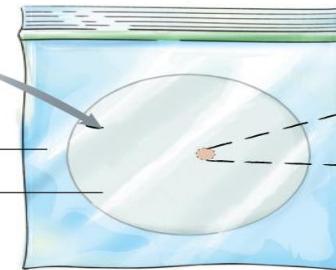


labelled single-stranded DNA probe for the gene of interest



bag

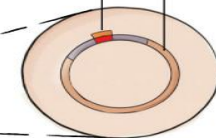
filter



**3** The filter paper is treated to cause the bacteria to break open. The double-stranded DNA is denatured to single-stranded DNA, which remains stuck to the filter paper in the same position as the colony it came from.

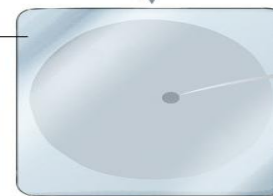
labelled probe (single stranded)

plasmid DNA (single stranded)

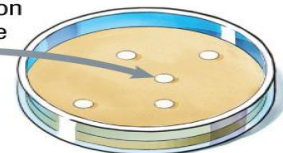


Hybridization has occurred between the labelled probe and the plasmids released from the bacteria in this colony. The hybridization is detected in subsequent steps.

developed photographic film



corresponds to one colony on master plate



original master plate