Cell-free Synthetic Biology

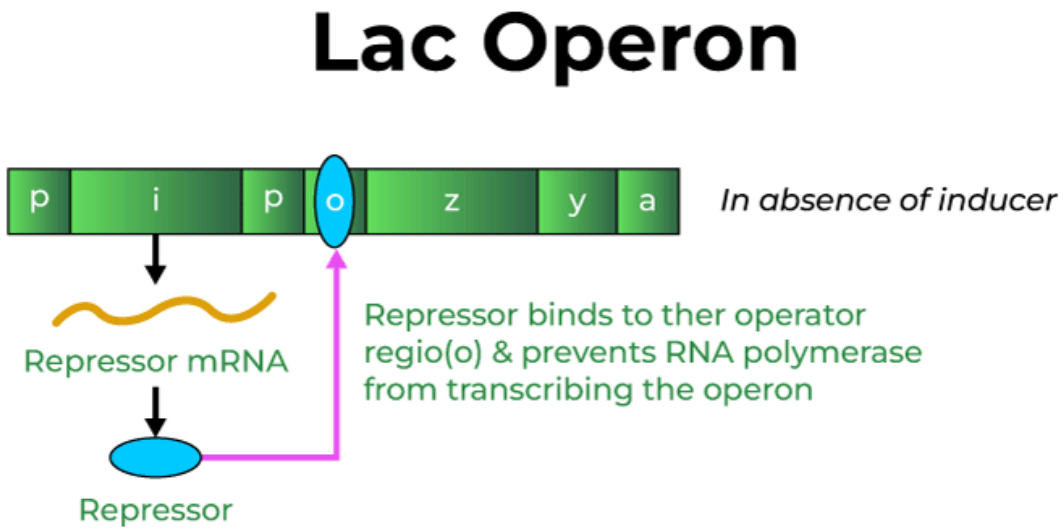
EE-490J

03.10.2023

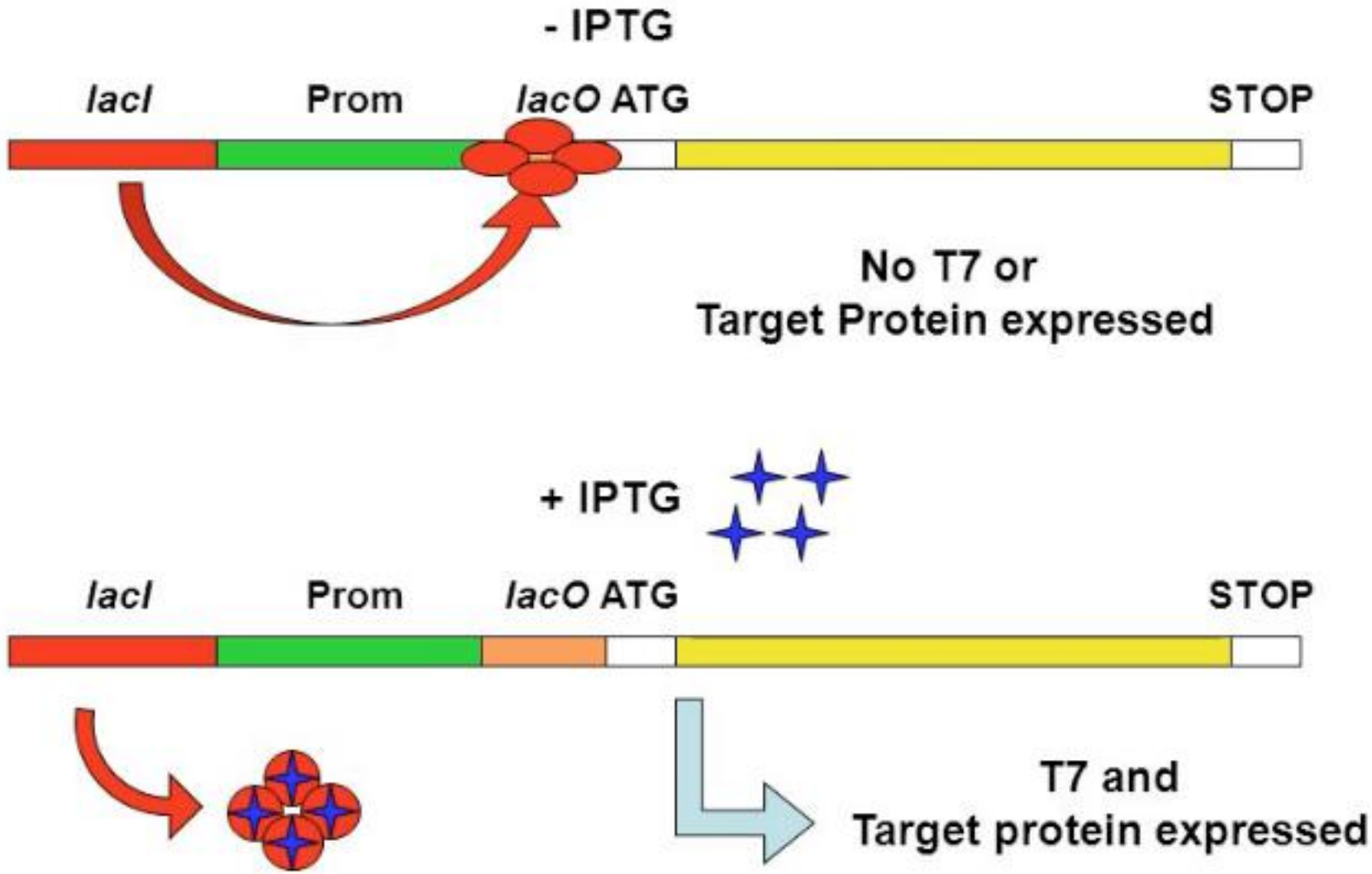
Objectives

- How to regulate bacterial gene expression?
  - Lac operon
  - IPTG induction
- How to introduce plasmid into cells
  - Bacterial Transformation
- How to cut a piece of DNA and visualize ?
  - Restriction enzymes
  - Electrophoresis

Bacterial gene regulation



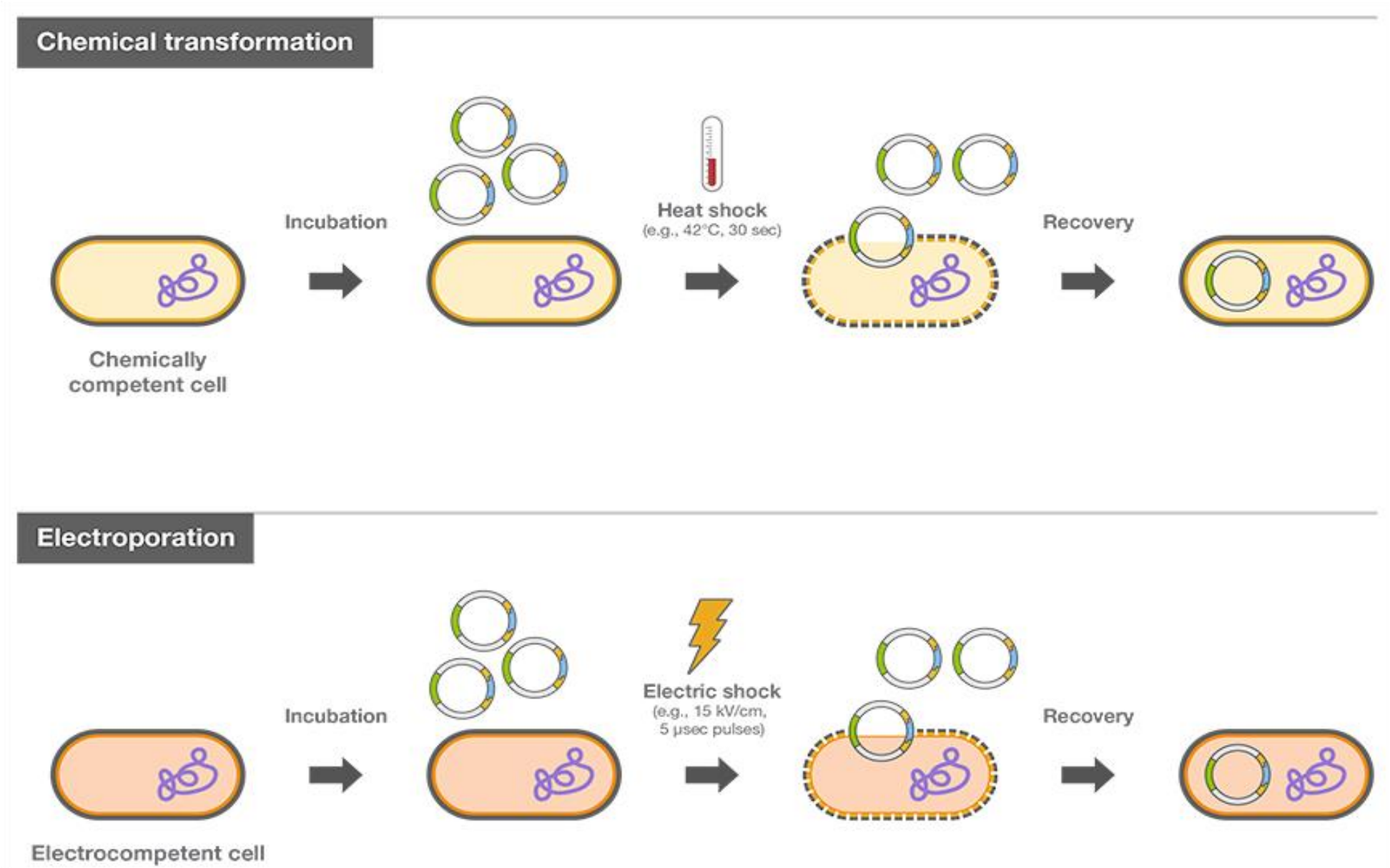
IPTG induction



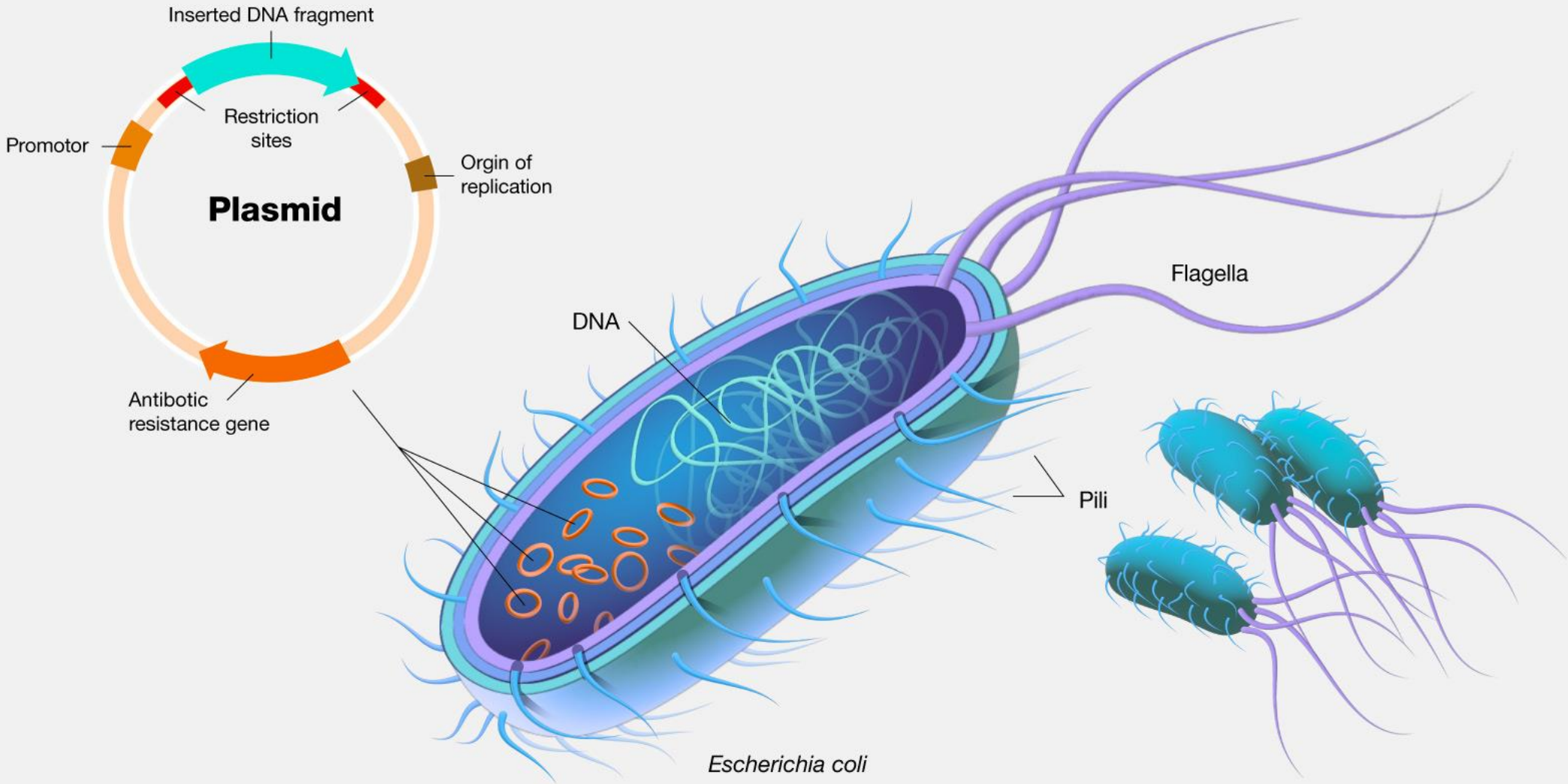
Bacterial Transformation

- Bacterial transformation is the process of introducing foreign DNA (plasmids) into bacterial cells.
  - Heat shock
  - Electroporation
  - Chemical methods
- Selection :Antibiotic resistance
- BL21 DE3 strain

Transformation procedure

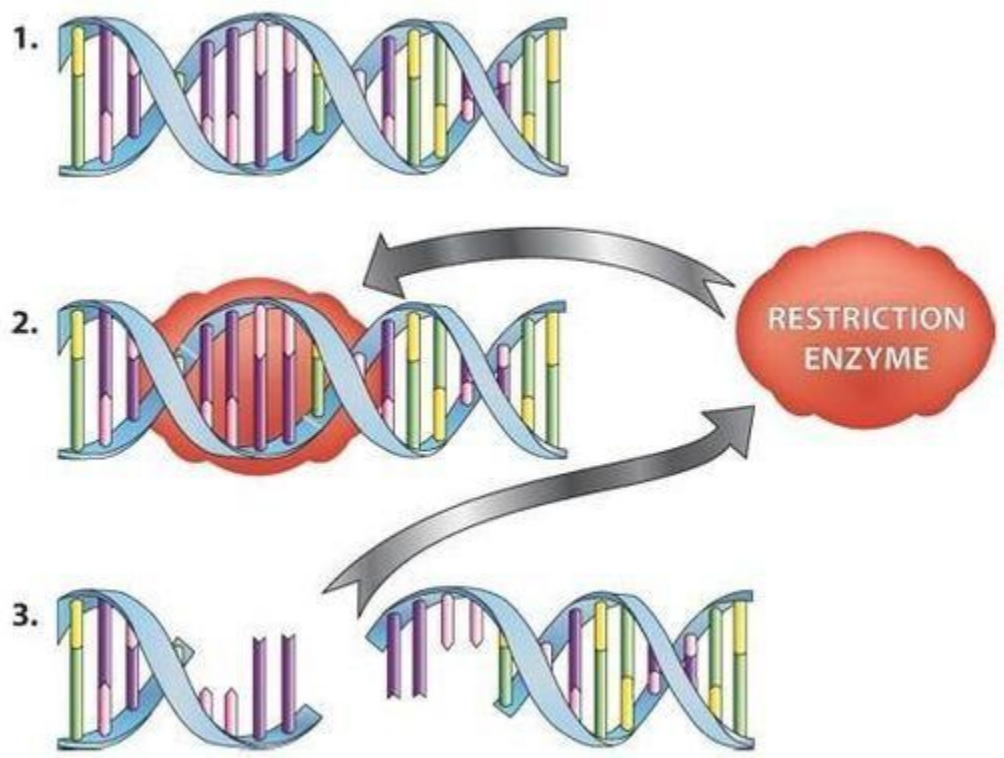


Plasmids


Inserted DNA fragment
Restriction sites
Promotor
Orgin of replication
Plasmid
Antibotic resistance gene
DNA
Flagella
Pili
Escherichia coli

Restriction Enzymes – Molecular Scissors

- These enzymes were discovered in bacteria.
- They help the bacteria destroy viral DNA.
- They cut between specific bases (letters) of the double stranded DNA molecule
- The DNA is then in multiple pieces
- The pieces are separated by gel electrophoresis for analysis

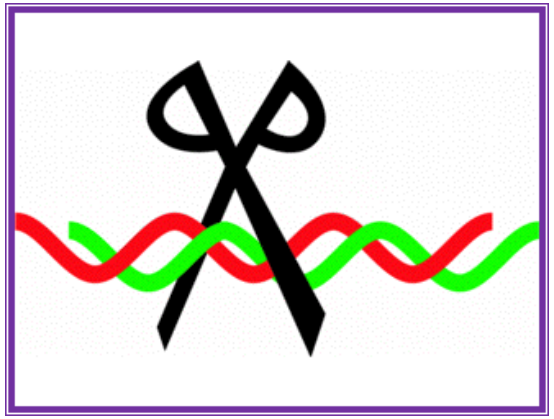


Enzymes are class of proteins

- Biological catalysts – found in all cells.
- They speed up chemical reactions.
- Only act on specific substrates
- Enzymes are critical for a range of cellular processes including digestion, DNA replication, protein synthesis.

Restriction Enzymes - Purpose

Researchers rely on **restriction enzymes** to assist with many processes in laboratories around the world:



1. Making recombinant DNA and appraising success

- For research, medicine and agriculture

2. DNA profile analysis

- For disease diagnosis, paternity/family relationship testing, and forensics

Restriction Enzymes Sites

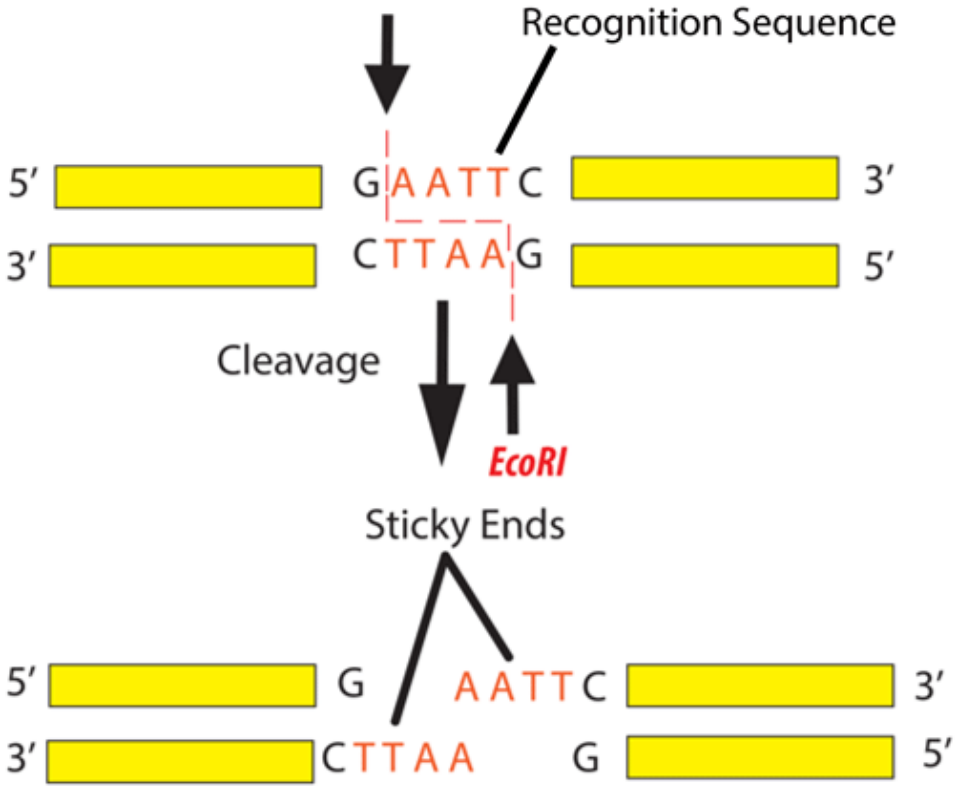
Specific restriction enzymes cut at specific DNA sequences.

For example:

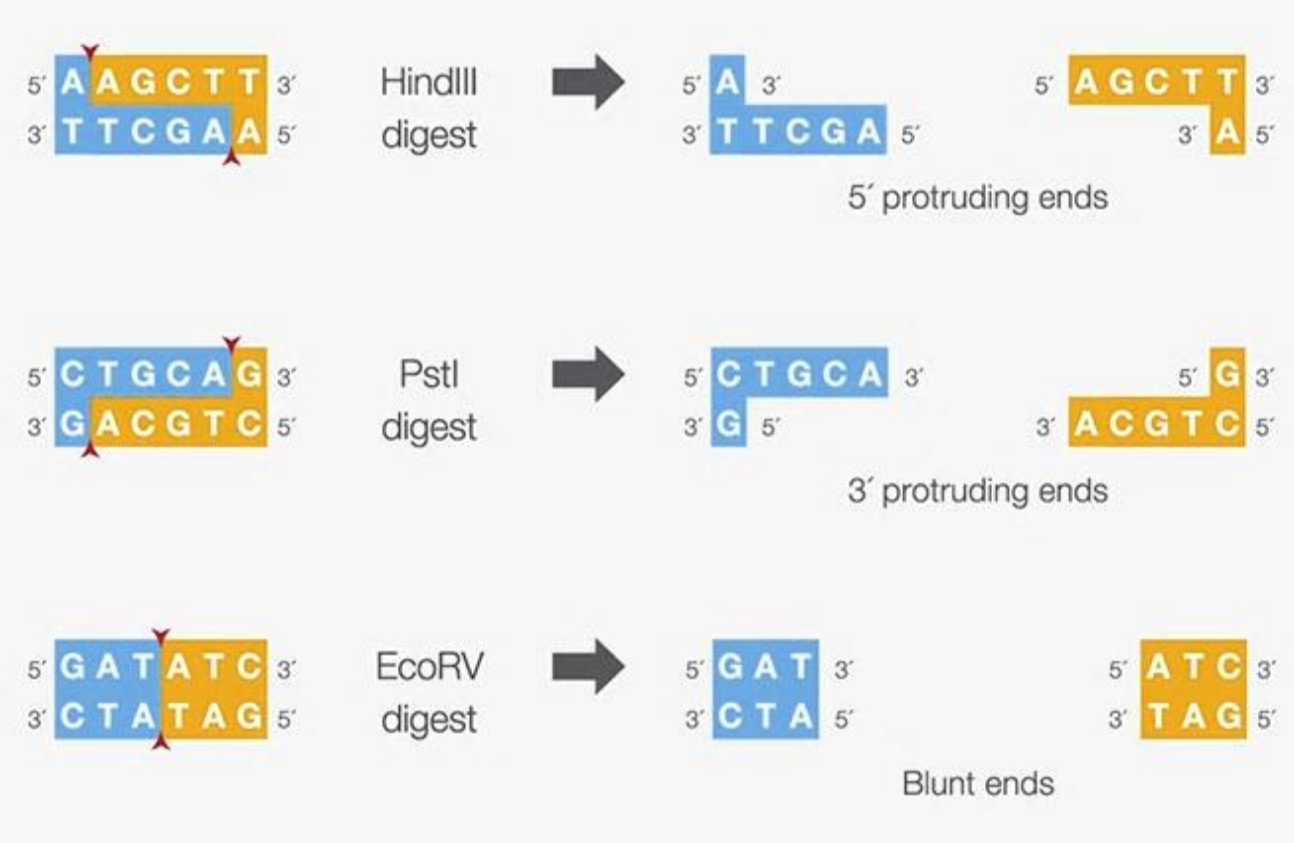
EcoRI is an enzyme that cuts at the following sequence: GAATTC

EcoRI was discovered in ***E. co****li* bacteria.

The resulting pieces of DNA are called “restriction fragments.”



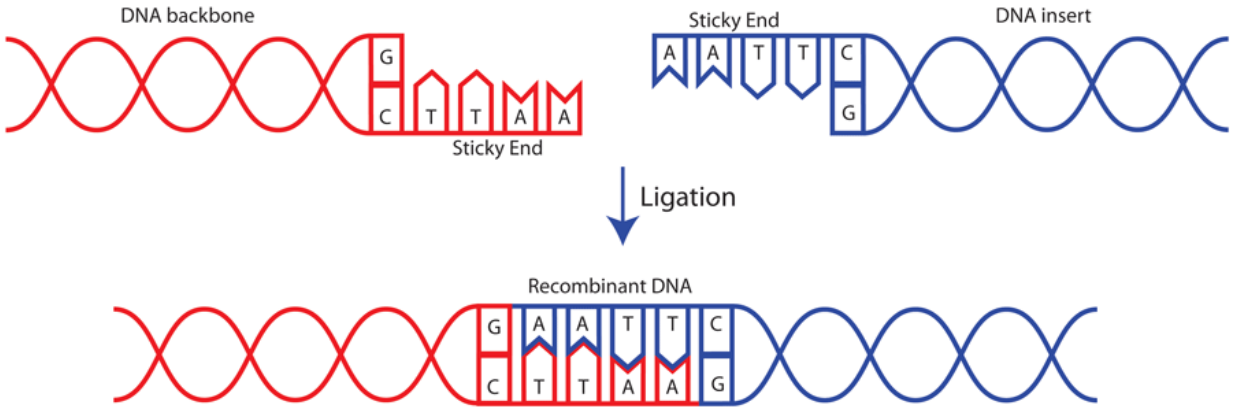
Many restriction enzymes



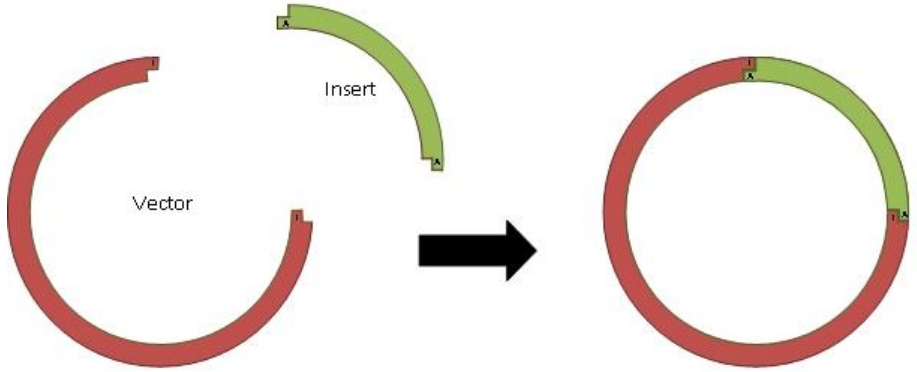
- HindIII was discovered in ***H. influenza***
- PstI was discovered in ***P. stuartii***
- *EcoRV was discovered in **E. coli***

  Protruding ends are also called "sticky" ends.

Restriction fragments: sticky ends



https://www.addgene.org/protocols/dna-ligation/



Purpose: making recombinant DNA

When you cut two separate molecules of DNA with the same restriction enzyme, the fragments will have matching sticky ends.
This is how recombinant DNA is created.

Agarose gel electrophoresis

- Separate and visualize DNA of different sizes
- Negatively charged DNA moves towards positive terminal
- Agar concentration determines the porosity of the matrix gel