Genetic Engineering

- Manipulation of an organism's genome
- Can range from changing one base pair (A-T or C-G), deleting a whole region of DNA, or introducing an additional copy of a gene
- Extracting DNA from an organism's genome and combining it with the DNA of another individual
- Used to enhance or modify the characteristics of an individual organism

An Overview of Genetic Engineering

Agricultural application (Bt Cotton, Genetically modified sweet corn)

Application in Dairy, Poultry Farms etc (Growth hormones to increase production of cow milk)

Environmental application (*Pseudomonas putida*, *an oil eating bacteria)*

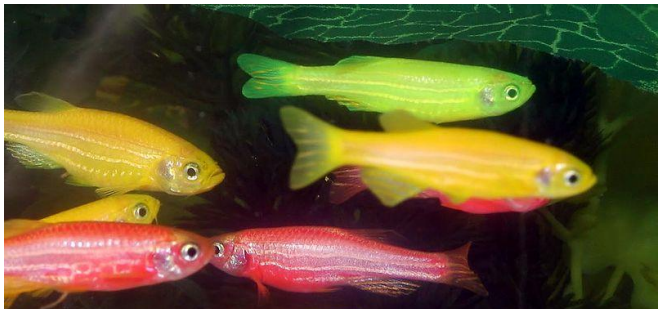
Therapeutic application (Human insulin, many growth hormones, etc)

Genetically modified Product

Flavr Savr (also known as CGN-89564-2; pronounced "flavor saver"), a genetically modified tomato

Glow Fish: Zebra fish with green fluorescent protein (GFP) and others

Wild type zebra fish



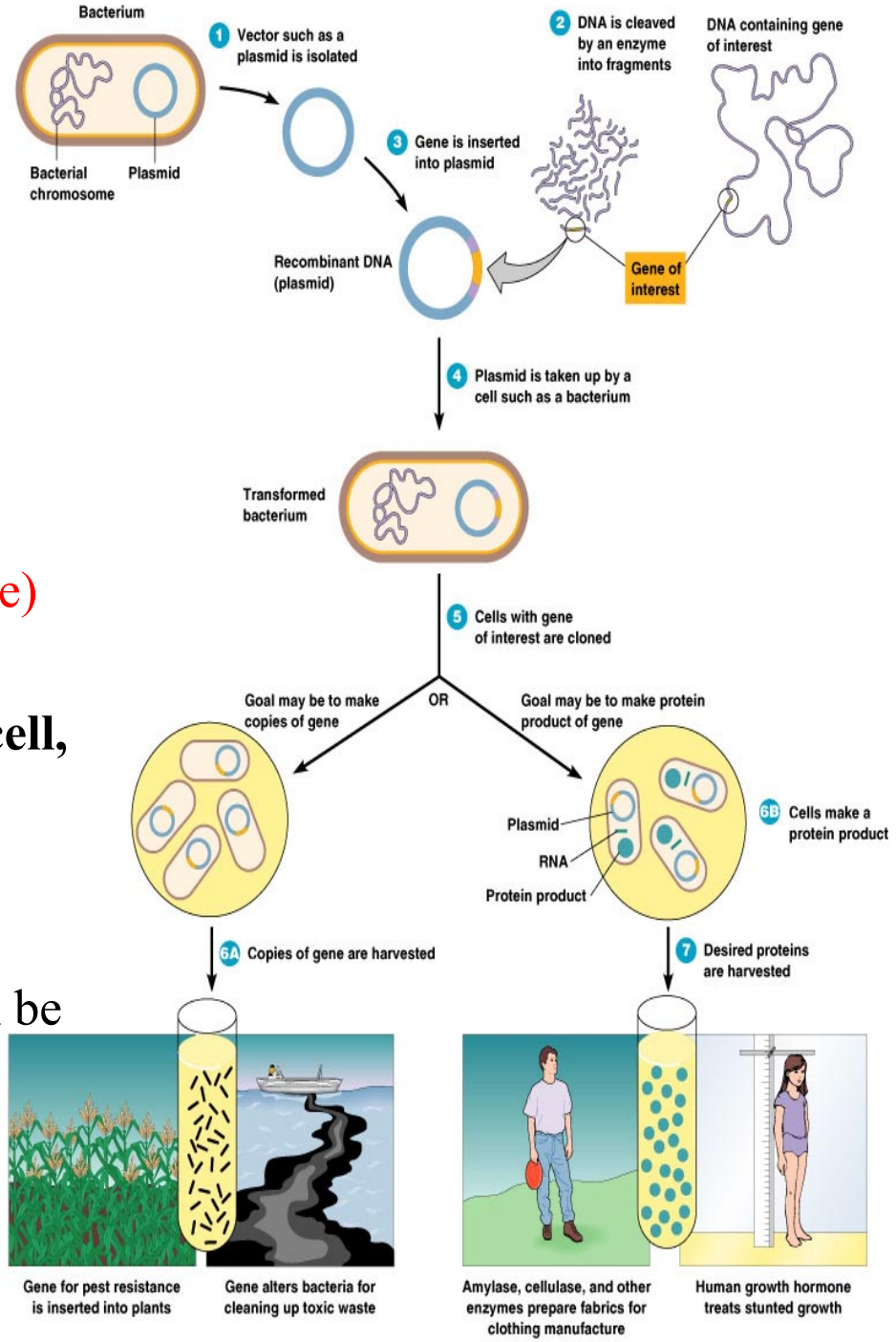
Glow fish (genetically modified fish)

Key Words in Genetic Engineering

- Gene of interest: DNA segment that is to be inserted or deleted
- Plasmid: a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA (commonly found in bacteria and may provide antibiotic resistance)
- Vector: DNA molecule (such as plasmid) used as a vehicle to carry gene of interest (or foreign genetic material) into another cell where it can be expressed
- Transformation: Transfer of gene of interest in to a host cell (may be bacteria) where it can be maintained as well as expressed.
- Clone: Organisms carrying identical genes

An Overview of Genetic Engineering

1. **Gene of interest (DNA)** is isolated
   (DNA fragment)
2. **A desired gene** is inserted into a DNA molecule - **vector**
   (plasmid, bacteriophage or a viral genome)
3. **The vector** inserts the DNA into **a new cell,** which is grown to form a **clone**.
   (bacteria, yeast, plant or animal cell)
4. Large quantities of the **gene product** can be harvested from **the clone**.



Tools for Genetic engineering

1. Restriction Enzymes

- **Naturally produced by bacteria – restriction endonucleases**
  - **Natural function** - destroy bacteriophage DNA in bacterial cells
  - Cannot digest host DNA with methylated C (cytosine)

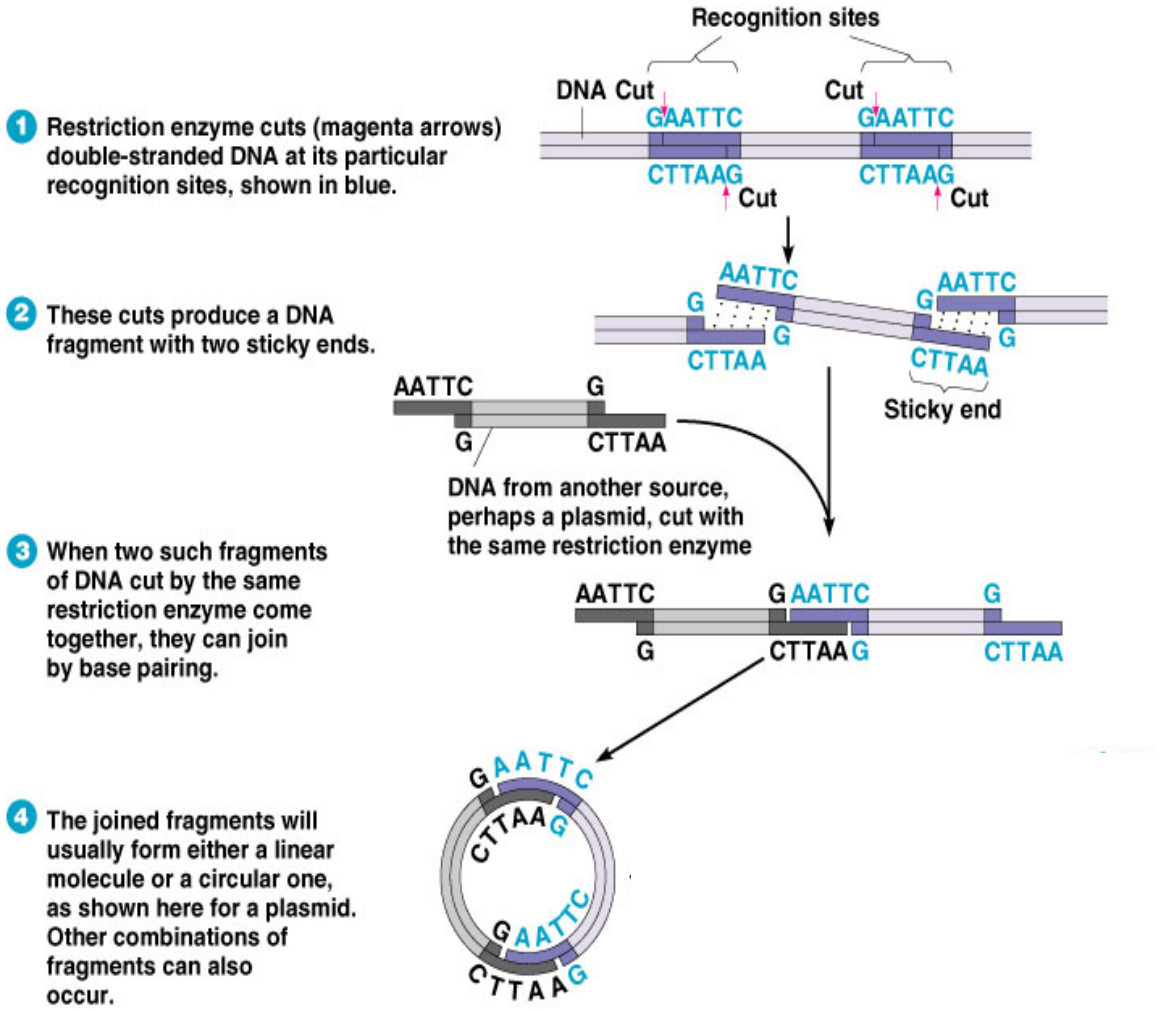
- **A restriction enzyme**
  - **Substrate –DNA -**recognizes one particular nucleotide sequence in DNA and **cuts** the DNA molecule (breaks down the bond between two nucleotides)

sticky ends **blunt ends**

- Prepackaged kits are available for rDNA techniques

Restriction Enzymes: How it Works?

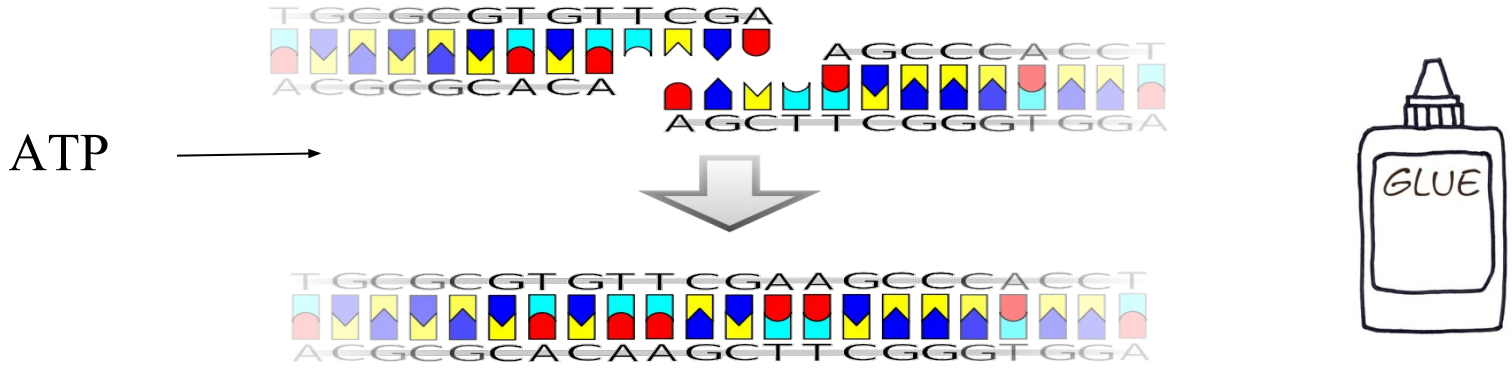
- Fragments of DNA produced by the same restriction enzyme will spontaneously join by **base pairing**.
- Each of the DNA strands will have a break



Tools for Genetic engineering

2. Ligase

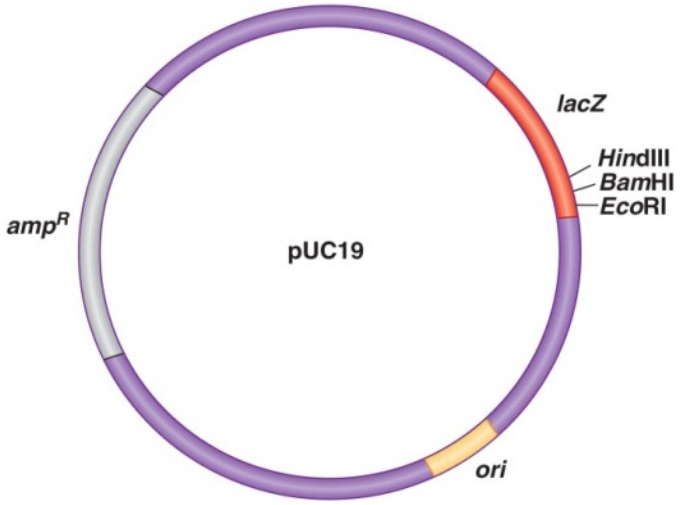
- **DNA ligase** is an enzyme that can link together DNA strands that have double-strand breaks (a break in both complementary strands of DNA).
  - Naturally DNA ligase has applications in both **DNA replication** and **DNA repair .**
  - Needs ATP
- DNA ligase has extensive use in molecular biology laboratories for **genetic recombination experiments**



Tools for Genetic engineering

3. Plasmids

<u>Vectors</u> - Small pieces of circular DNA used for cloning



<u>Requirements of the Vector</u>

1. Self-replication - able to replicate in the host (independent origin of replication)

2. Cloning site -(region containing multiple restriction sites)

3. Promoter (and operator) - to support the expression of insert DNA (i.e. gene of interest) in the host.

4. Selectable marker – antibiotic resistance (Ampicillin resistant)

5. Proper size- for easy handling

Hosts for Recombinant DNA Technology

1. **Bacteria**
   - ***E. coli*** - used because is easily grown and its genomics are well understood.
   - Gene product is purified from host cells

2. **Yeasts - *Saccharomyces cerevisiae***
   - Used because it is easily grown and its genomics are known
   - May express eukaryotic genes easily
   - Continuously **secrete** the gene product.
   - Easily collected and purified

3. **Plant cells and whole plants**
   - May express eukaryotic genes easily
   - Plants are easily grown - produce plants with new properties.

4. **Mammalian cells**
   - May express eukaryotic genes easily
   - Harder to grow
   - Medical use.

PLASMIDS

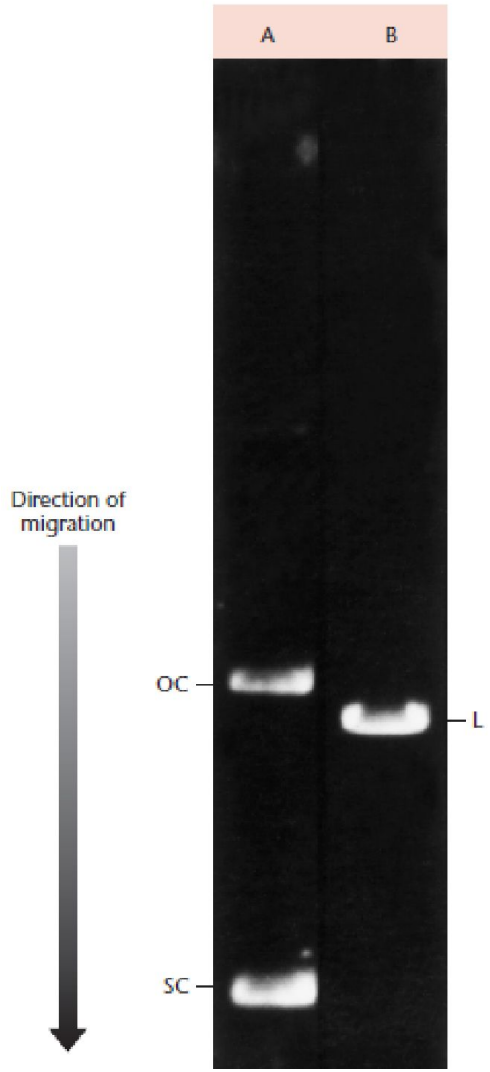
Plasmids

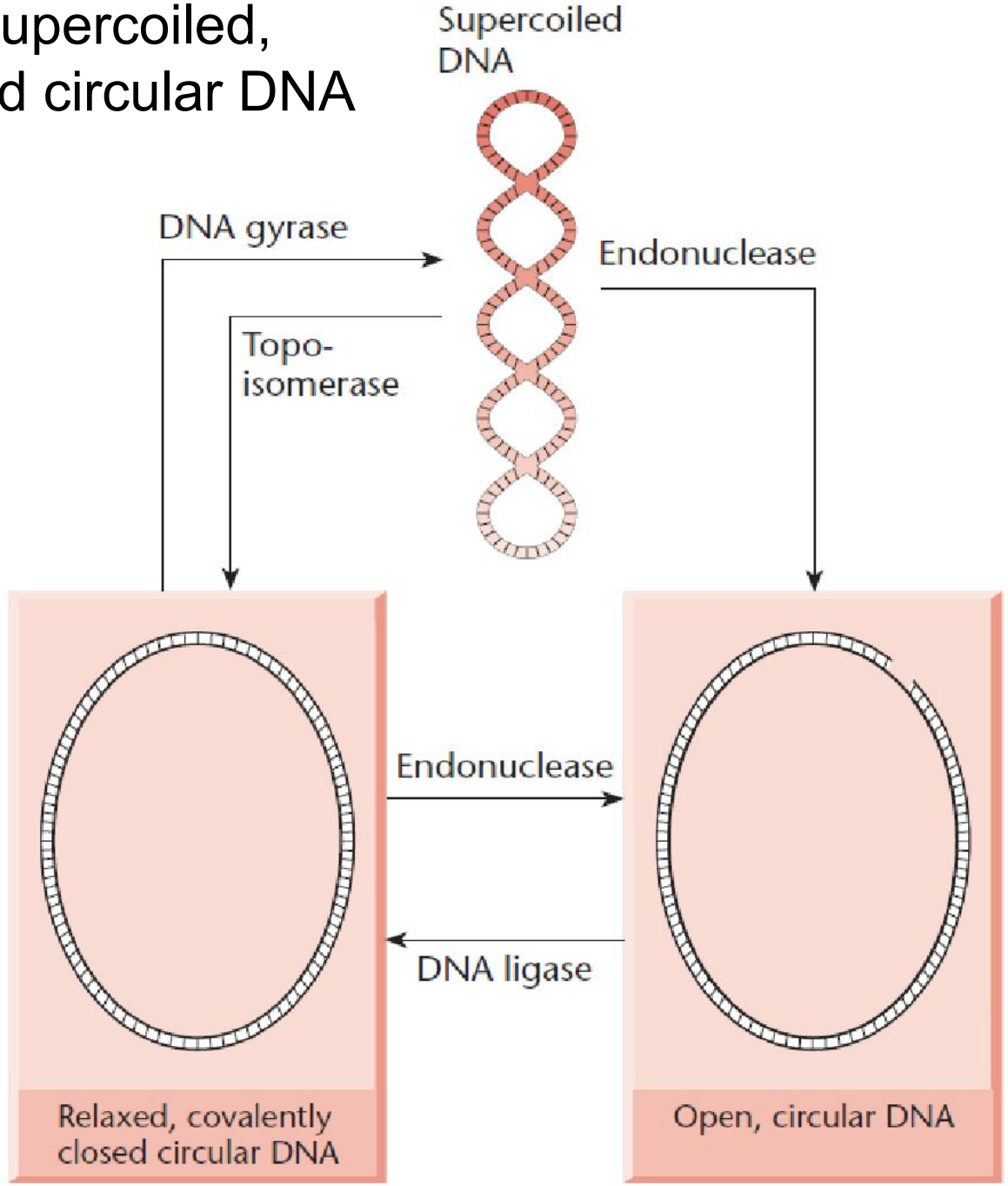
- Many DNA sequences in bacteria are mobile and can be transferred between individuals and among species.
- Plasmids are circular DNA molecules that replicate independently of the bacterial chromosome
- Plasmids often carry antibiotic resistance genes
- Plasmids are used in genetic engineering as gene transfer vectors

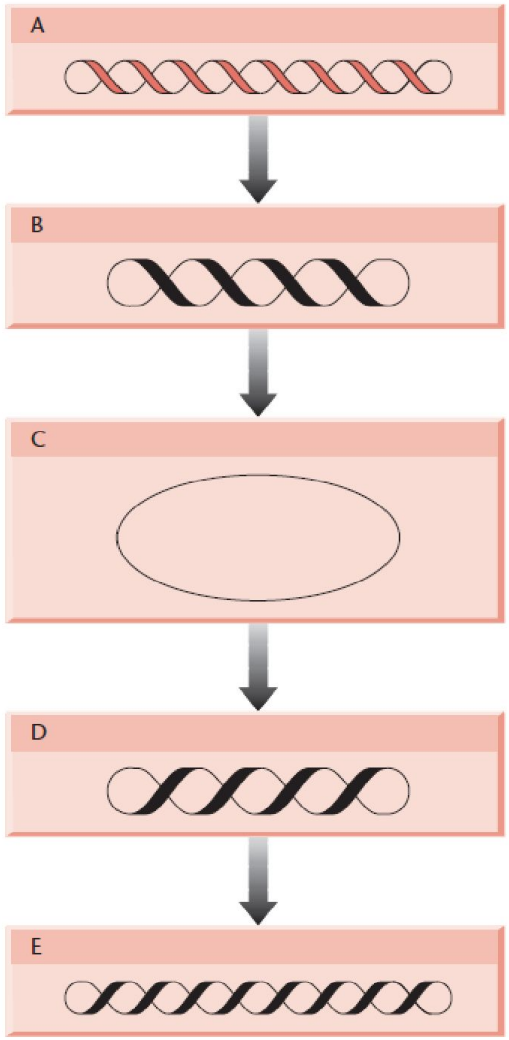
F factor and Conjugation

- F (fertility) factor is a conjugative plasmid transferred from cell to cell by conjugation
- F factor is an episome = genetic element that can insert into chromosome or replicate as circular plasmid
- The F plasmid is a low-copy-number plasmid ~100 kb in length, and is present in 1–2 copies per cell
- It replicates once per cell cycle and segregates to both daughter cells in cell division

The interconversion of supercoiled, relaxed covalently closed circular DNA and open circular DNA





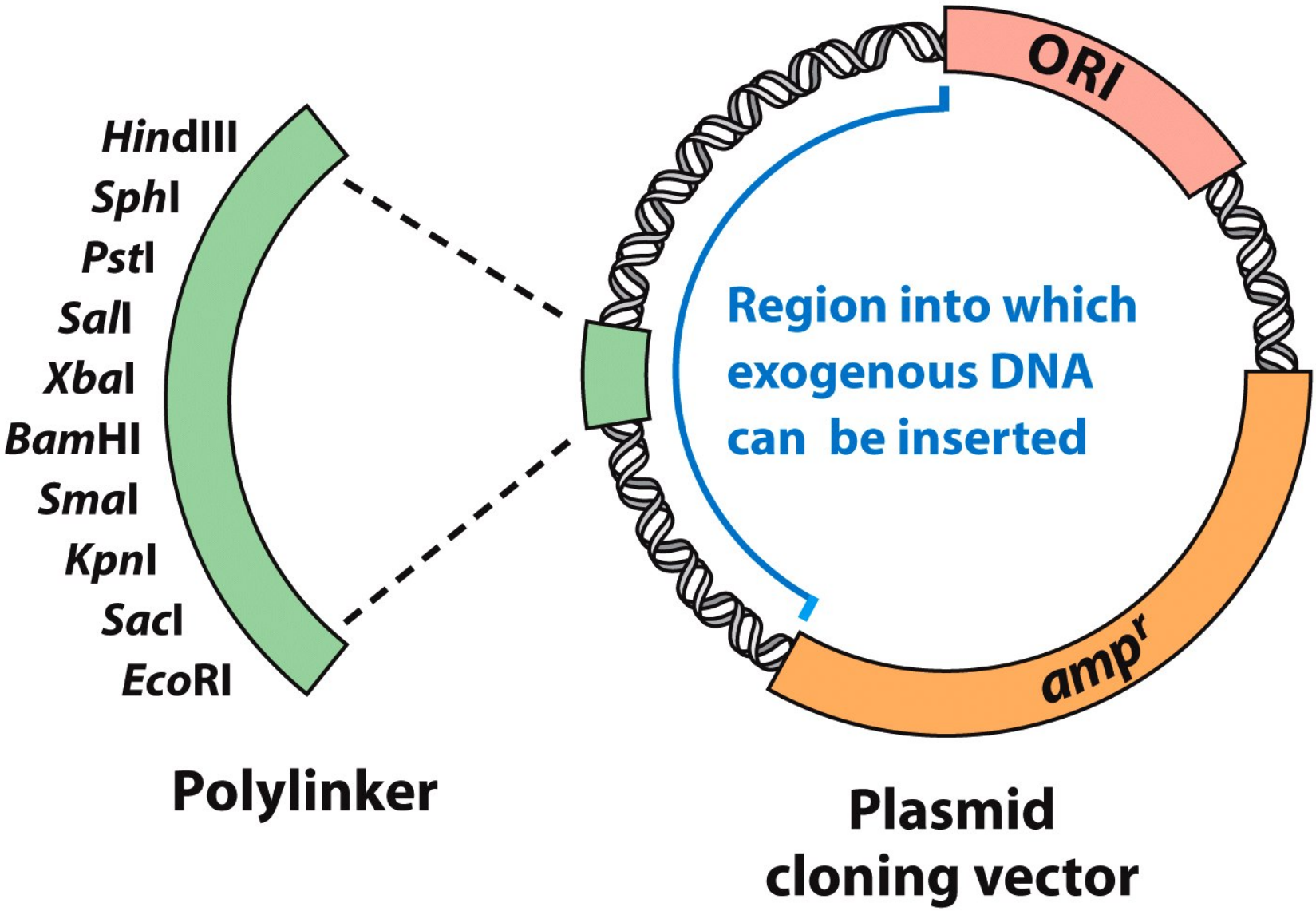


Effect of intercalation of ethidium bromide on supercoiling of DNA

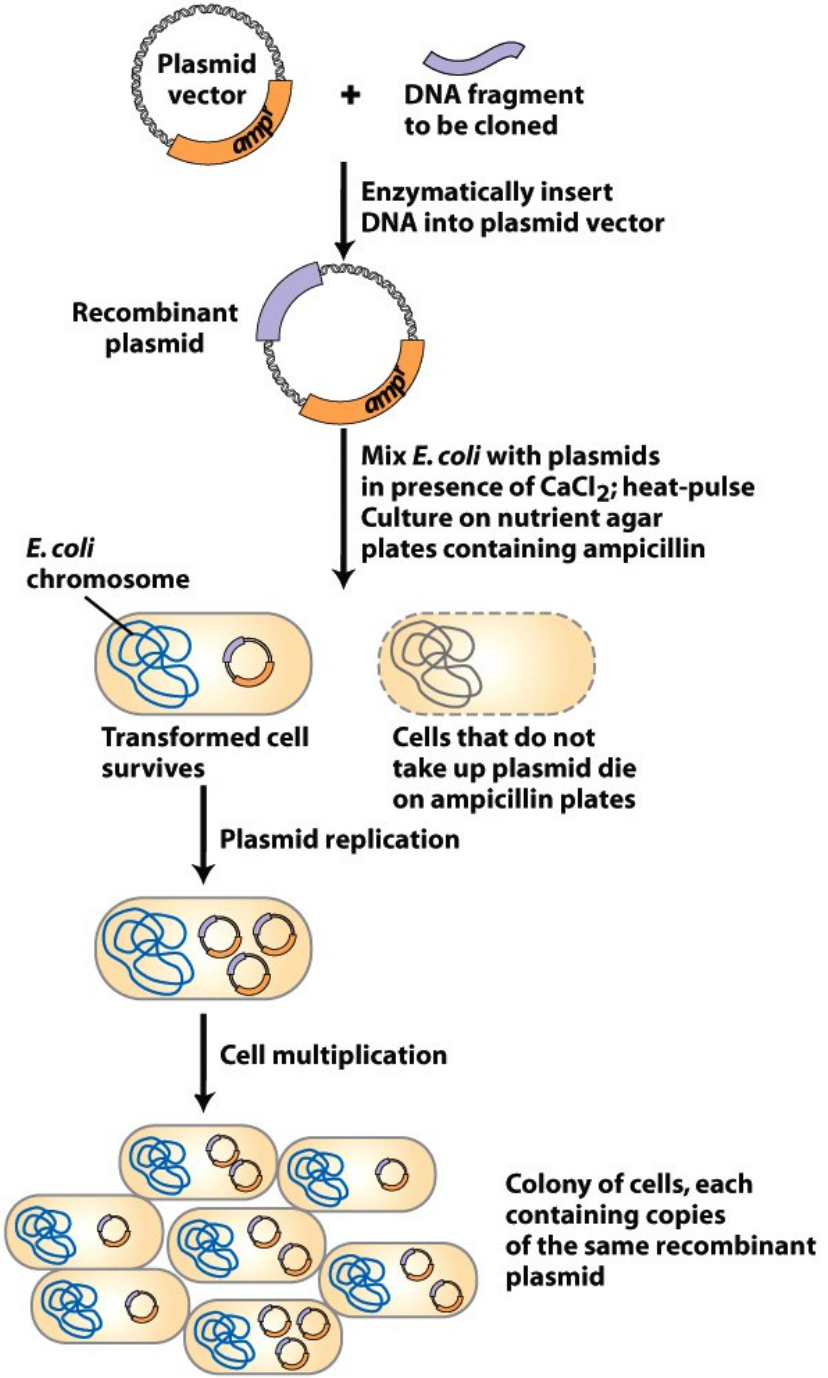
Table 4.1 Some phenotypic traits exhibited by plasmid-carried genes.

Antibiotic resistance
Antibiotic production
Degradation of aromatic compounds
Haemolysin production
Sugar fermentation
Enterotoxin production
Heavy-metal resistance
Bacteriocin production
Induction of plant tumours
Hydrogen sulphide production
Host-controlled restriction and modification

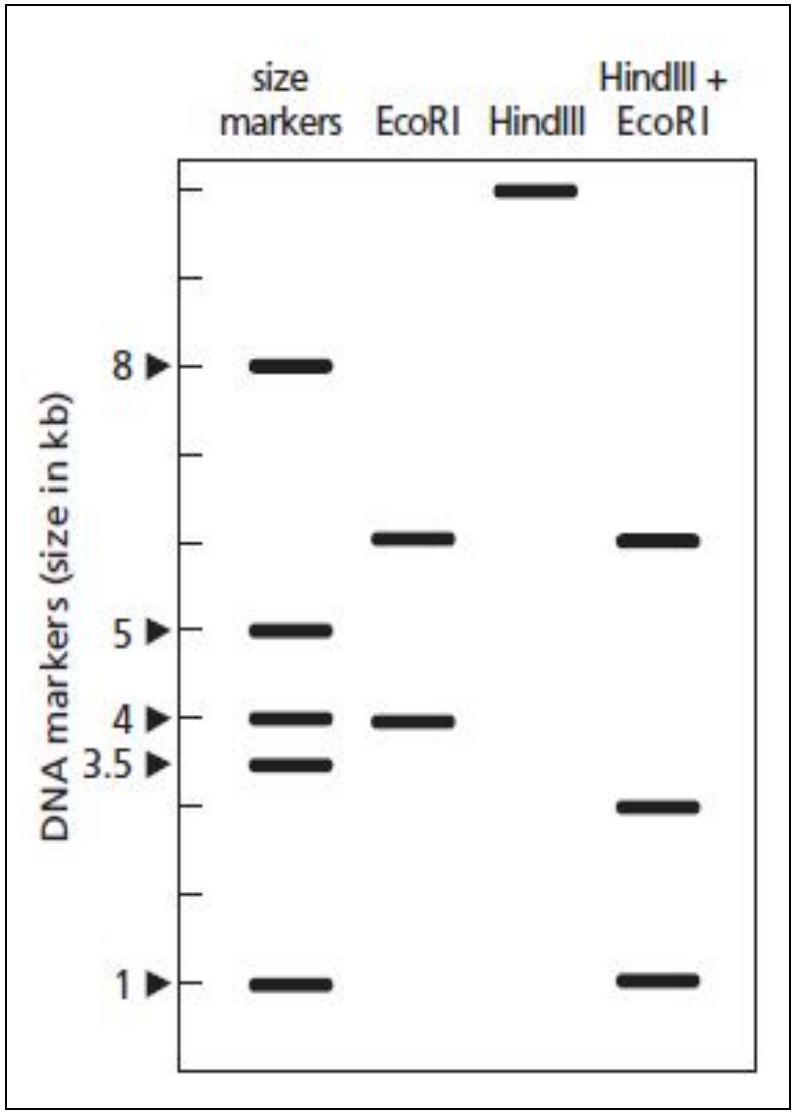
Cloning of a Gene



Cloning and Transformation of a Gene



Problem-1



A molecule of double-stranded DNA was cleaved with restriction nucleases, and the resulting products were separated by gel electrophoresis (adjacent figure). DNA fragments of known sizes were electrophoresed on the same gel for use as size markers (leftmost lane). Using the size markers as a guide, estimate the length of each restriction fragment obtained. From this information, **construct a map of the original DNA molecule indicating the relative positions of all the restriction enzyme cleavage sites.**

Thank You