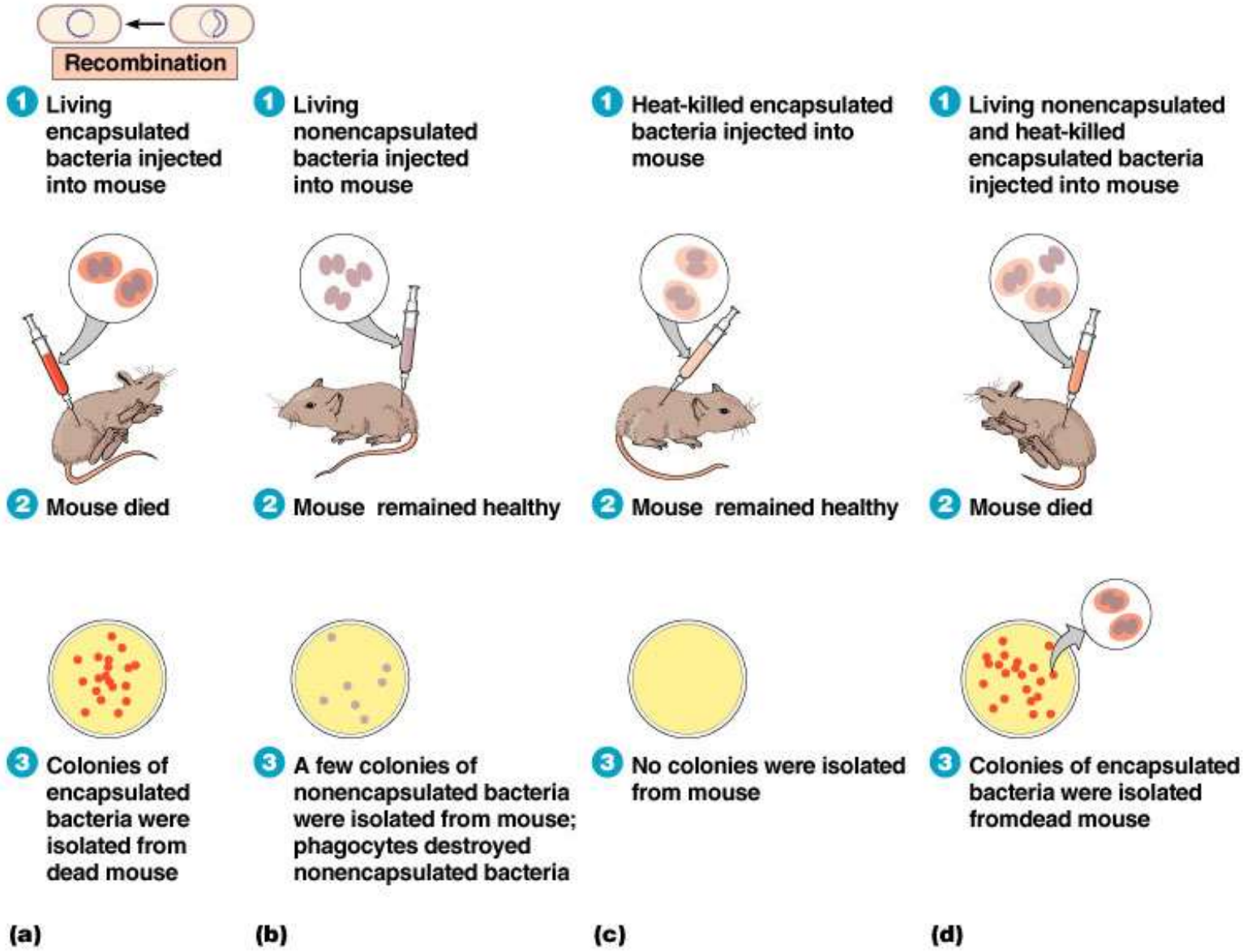
Microbiología

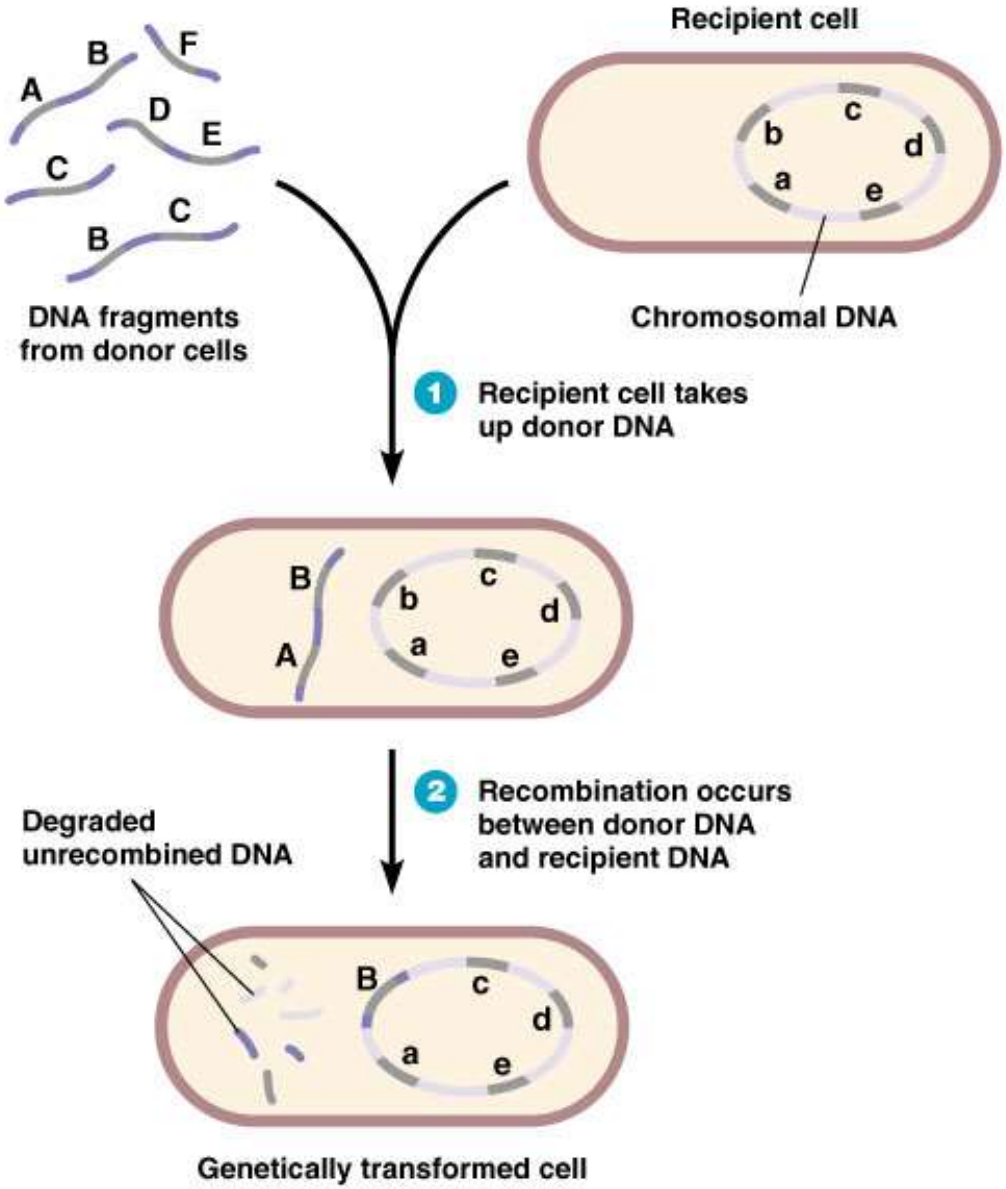
Transferencia y manipulación de genes

Transformación

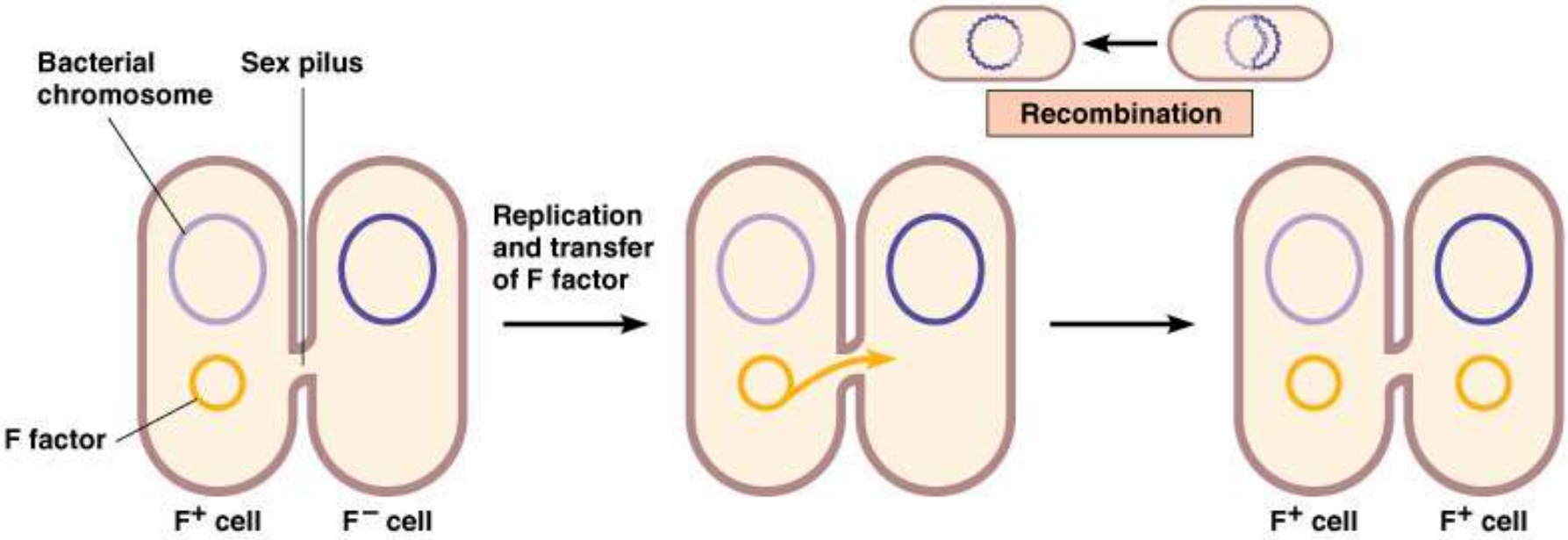


El experimento de Griffith, llevado a cabo en 1928

Recombination

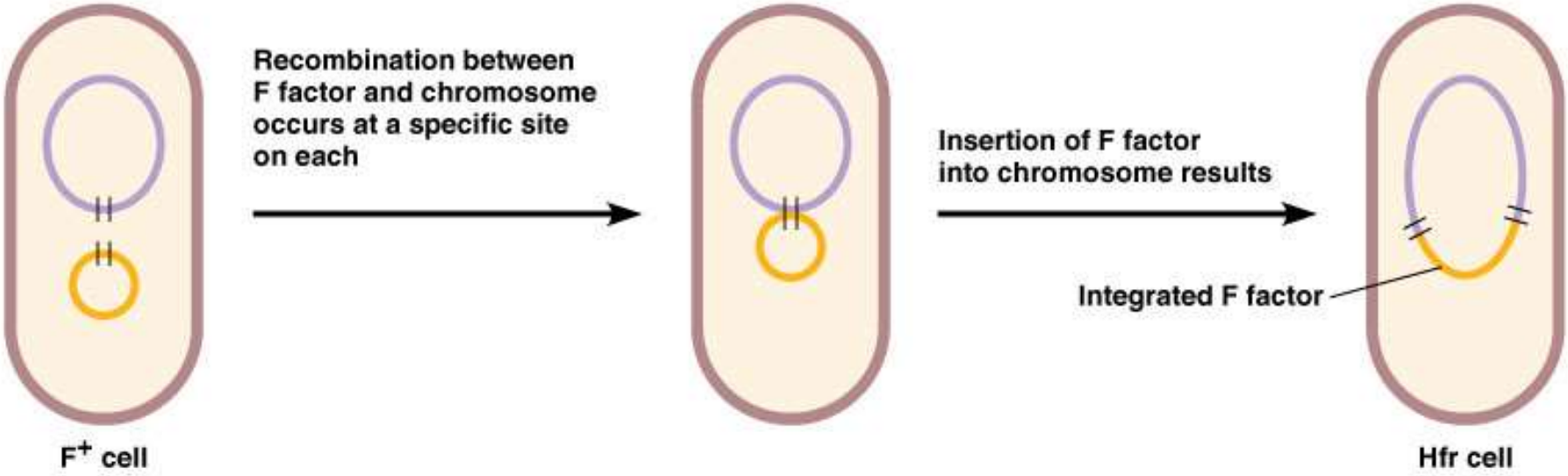


Conjugación



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

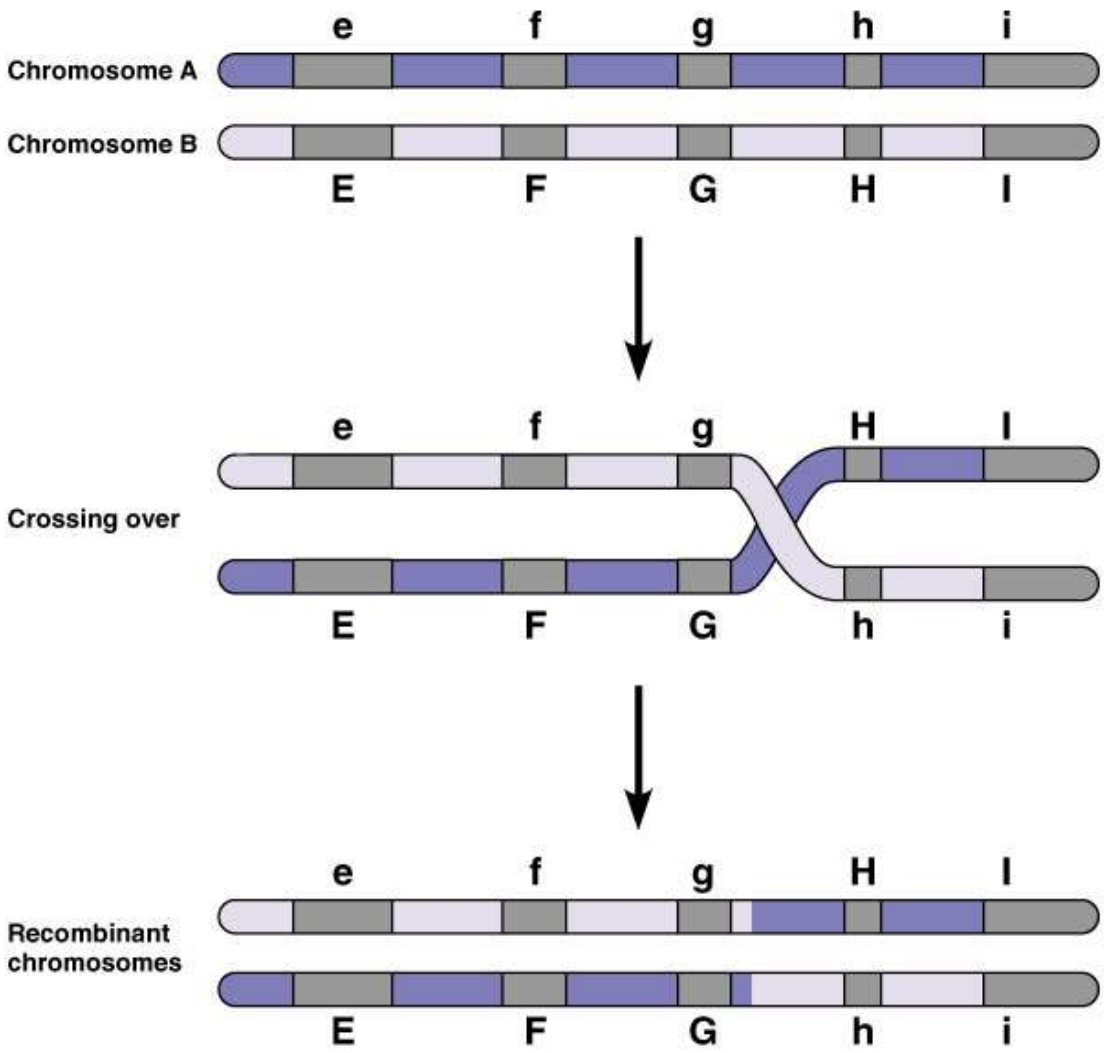
Conjugación



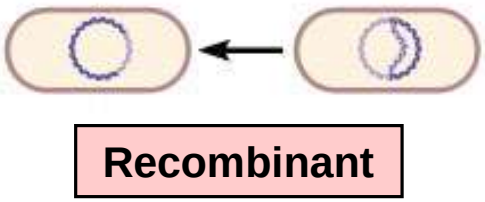
(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

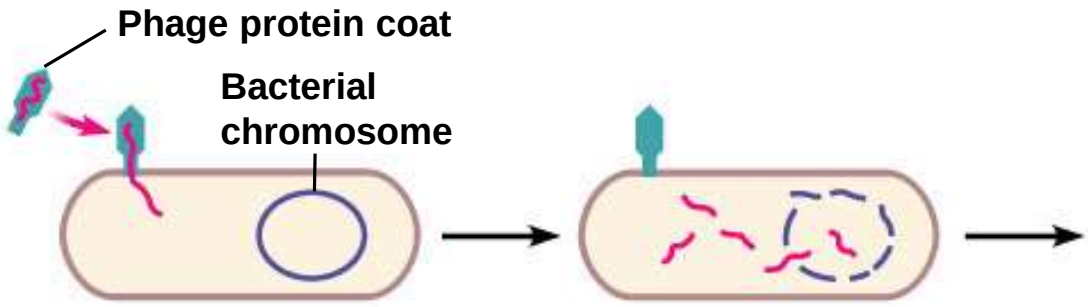
Recombinación Genética

- Exchange of genes between two DNA molecules
  - Crossing over occurs when two chromosomes break and rejoin



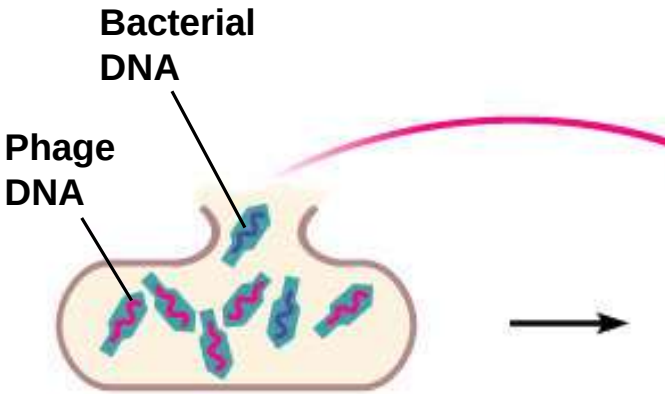
Transducción



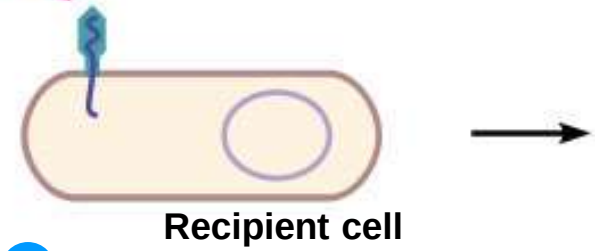


1 A phage infects the donor bacterial cell.

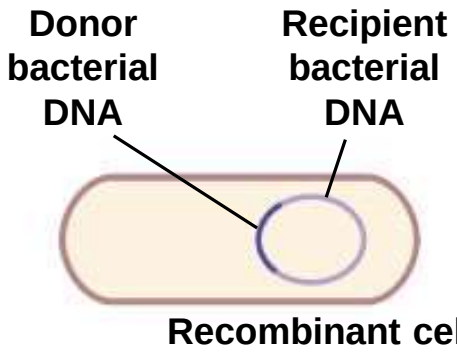
2 Phage DNA and proteins are made, and the bacterial chromosome is broken down into pieces.



3 Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. Then the donor cell lyses and releases phage particles containing bacterial DNA.



4 A phage carrying bacterial DNA infects a new host cell, the recipient cell.

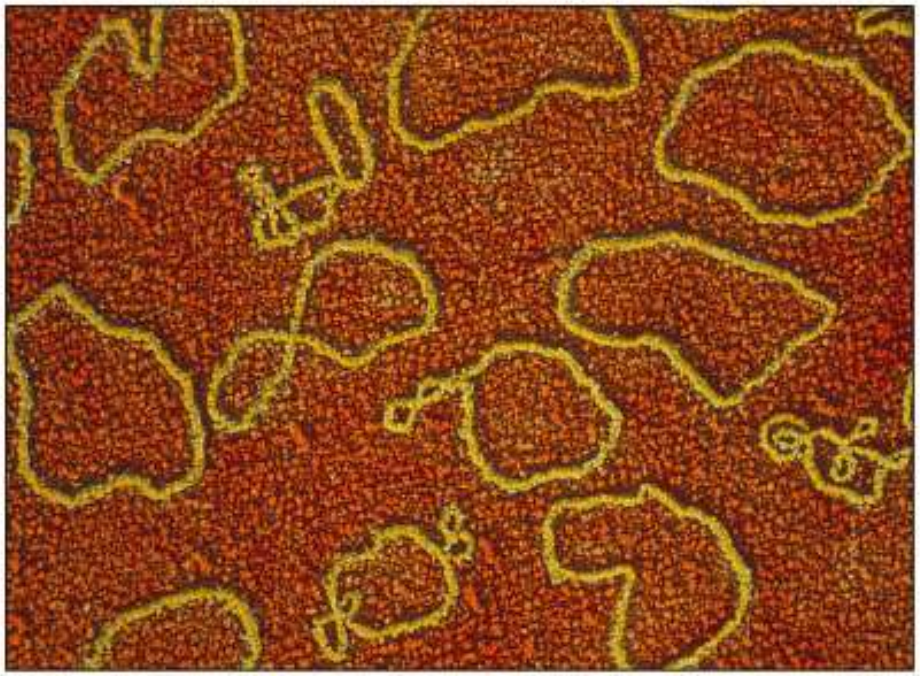


5 Recombinant can occur, producing a recombinant cell with a genotype different from both the donor and recipient cells.

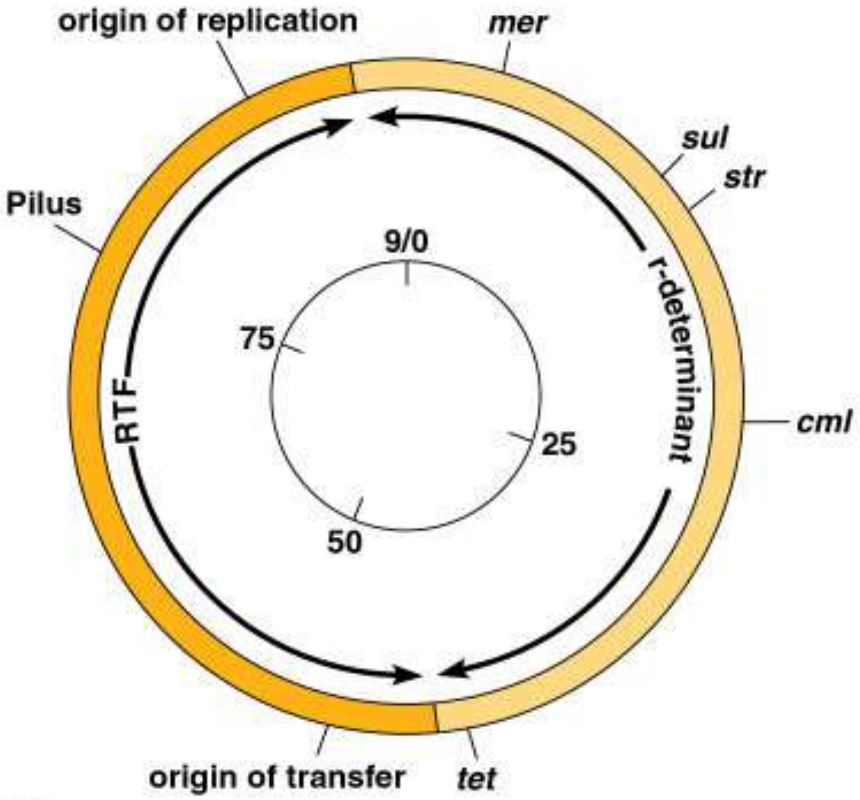
Plasmids

• Conjugative plasmid	Carries genes for sex pili and transfer of the plasmid
• Dissimilation plasmids	Encode enzymes for catabolism of unusual compounds
• R factors	Encode antibiotic resistance

Plasmids



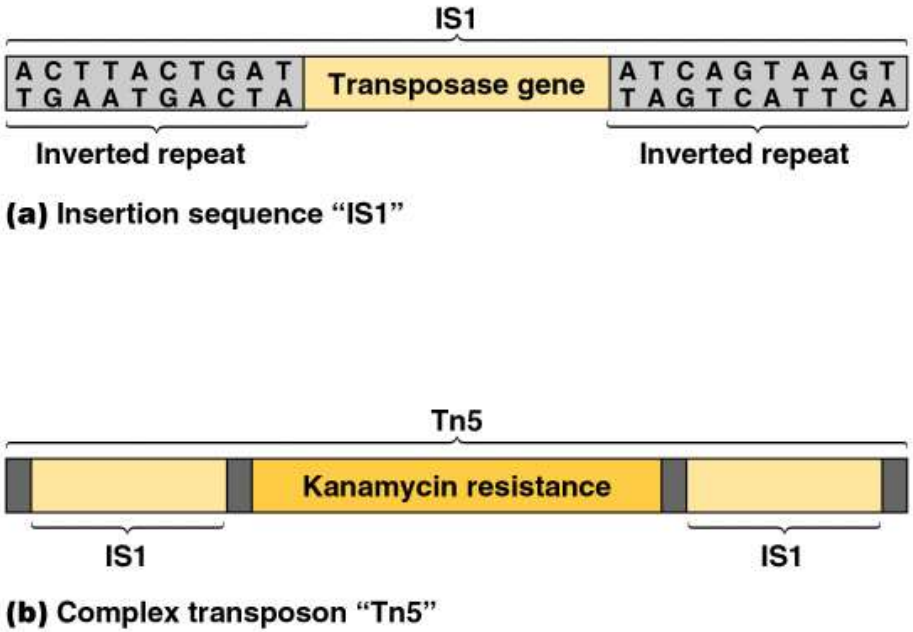
(a)


origin of replication
mer
sul
str
Pilus
9/0
75
25
50
RTF
r-determinant
cml
origin of transfer
tet

(b)

Transposons

- Segments of DNA that can move from one region of DNA to another
- Contain insertion sequences for cutting and resealing DNA (transposase)
- Complex transposons carry other genes



Mazorca de maíz mostrando granos con diferente distribución de su pigmentación causado por inserción de un transposón en el gen que determina la coloración de la aleurona

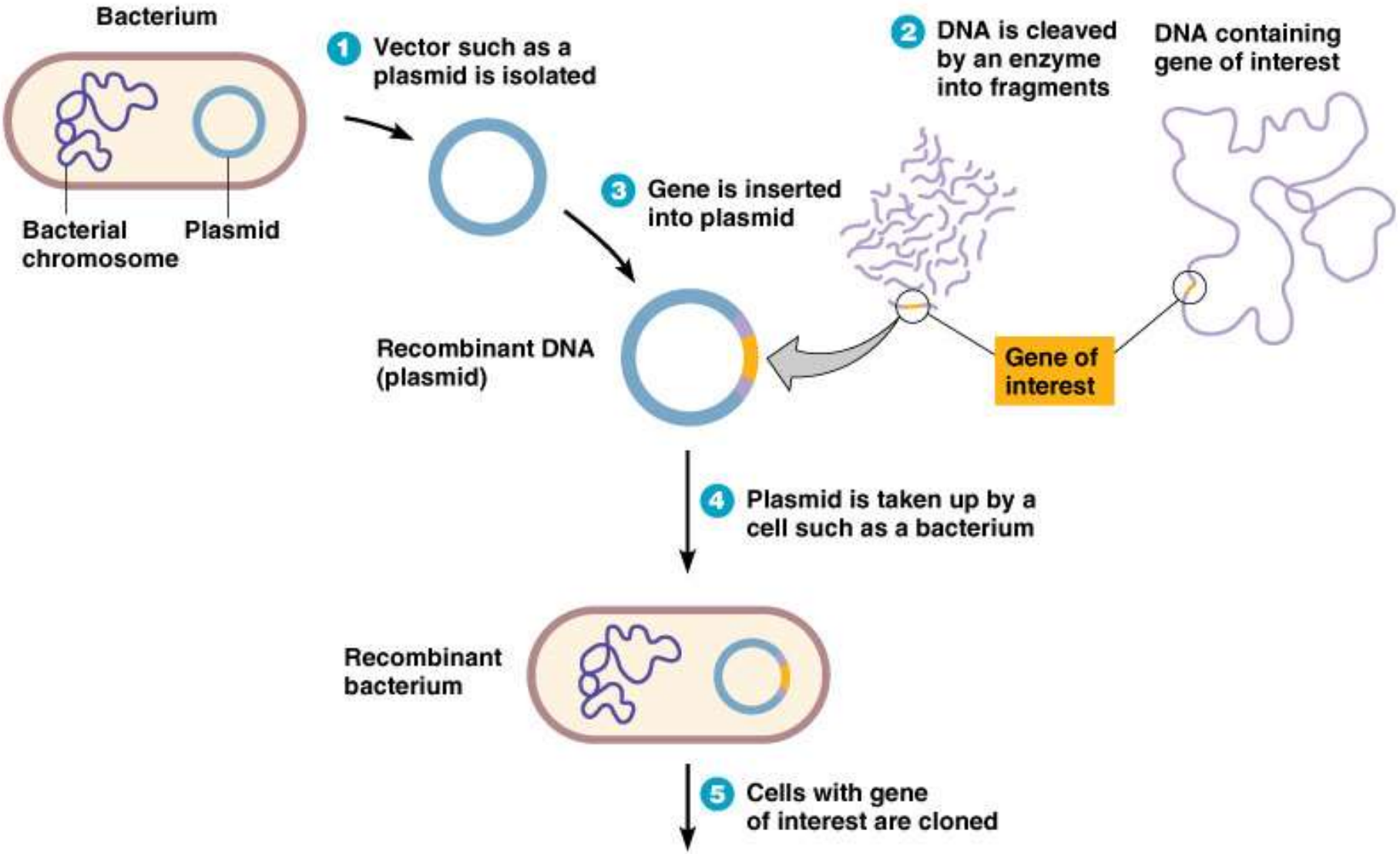
Microbiología

Capítulo 9b

Biotecnología y DNA Recombinante

Biotechnology and recombinant DNA technology

- Biotechnology:
  - The use of microorganisms, cells, or cell components to make a product
  - Foods, antibiotics, vitamins, enzymes
- Recombinant DNA Technology:
  - Insertion or modification of genes to produce desired proteins


Bacterium
Bacterial chromosome
Plasmid
1 Vector such as a plasmid is isolated
2 DNA is cleaved by an enzyme into fragments
DNA containing gene of interest
3 Gene is inserted into plasmid
Recombinant DNA (plasmid)
Gene of interest
4 Plasmid is taken up by a cell such as a bacterium
Recombinant bacterium
5 Cells with gene of interest are cloned

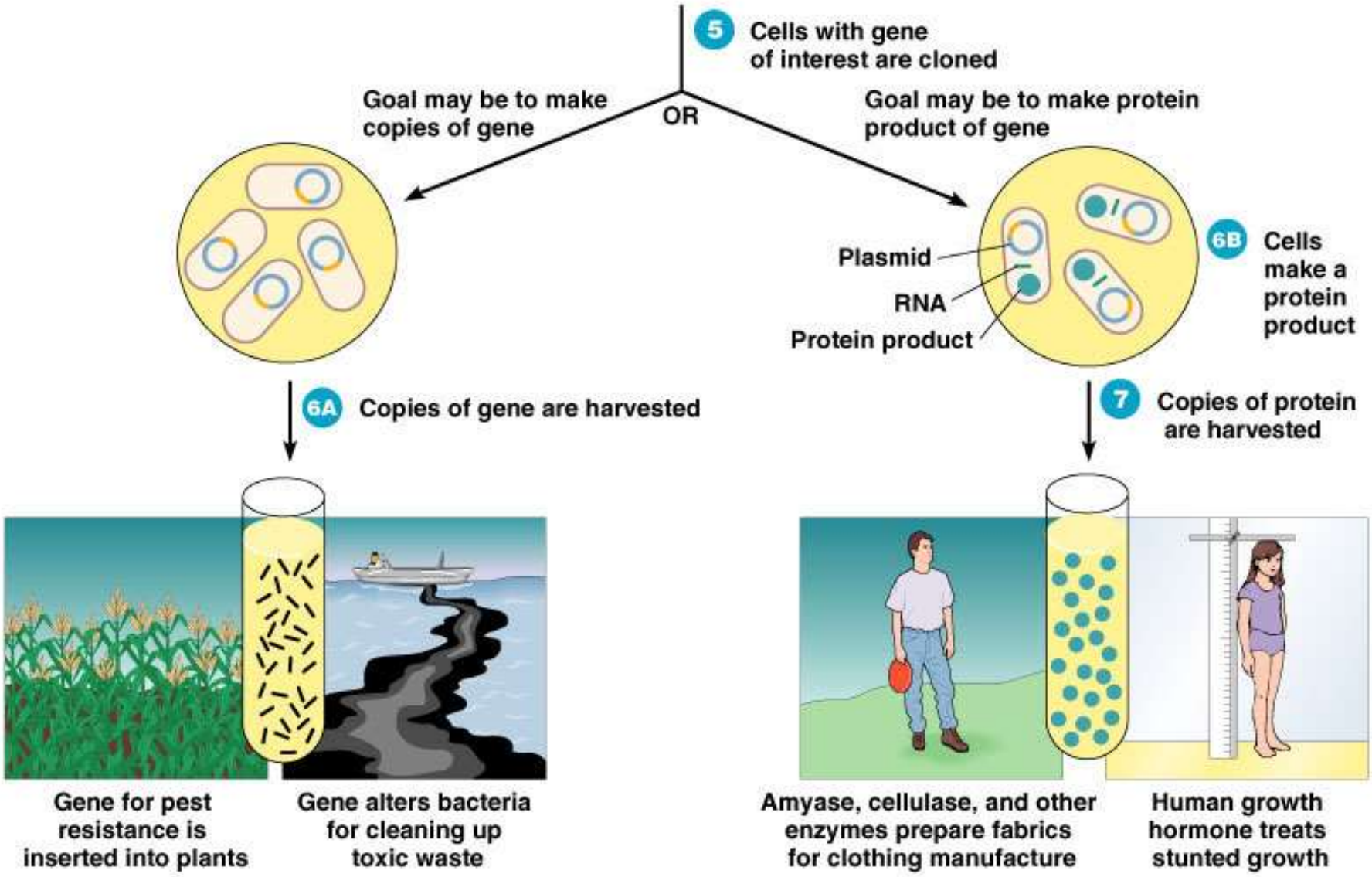

5 Cells with gene of interest are cloned
Goal may be to make copies of gene
OR
Goal may be to make protein product of gene
Plasmid
RNA
Protein product
6B Cells make a protein product
6A Copies of gene are harvested
7 Copies of protein are harvested
Gene for pest resistance is inserted into plants
Gene alters bacteria for cleaning up toxic waste
Amyase, cellulase, and other enzymes prepare fabrics for clothing manufacture
Human growth hormone treats stunted growth

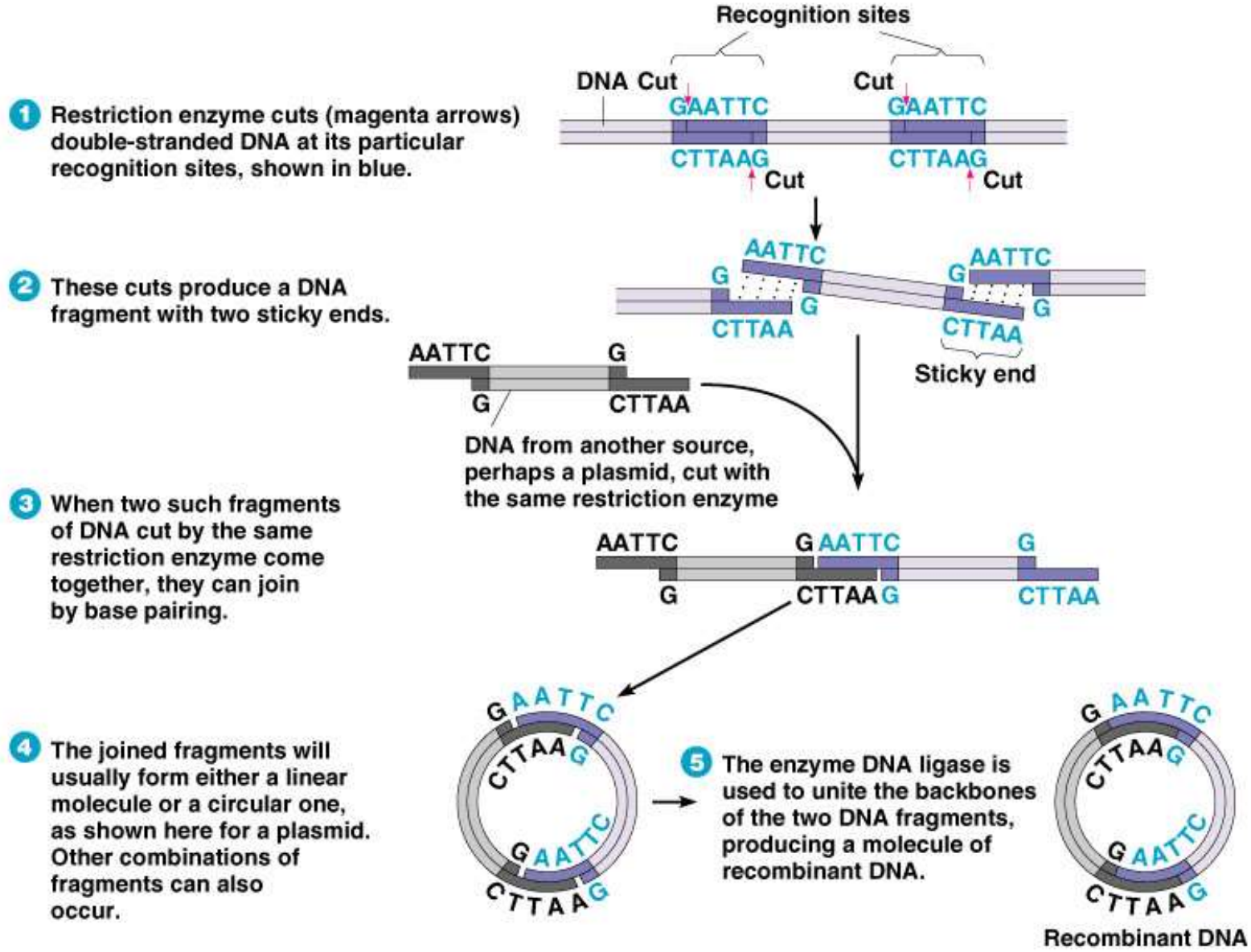
TABLE 9.1	**Some Pharmaceutical Products of Genetic Engineering**
Product	**Comments**
Alpha-interferon	Therapy for leukemia, melanoma, and hepatitis; produced by *E. coli* and *Saccharomyces cerevisiae* (yeast).
Antitrypsin	Assists emphysema patients; produced by genetically modified sheep.
Beta-interferon	Treatment for multiple sclerosis; produced by mammalian cell culture.
Bone morphogenic proteins	Induces new bone formation; useful in healing fractures and reconstructive surgery; produced by mammalian cell culture.
Colony-stimulating factor (CSF)	Counteracts effects of chemotherapy; improves resistance to infectious disease such as AIDS; treatment of leukemia; produced by *E. coli* and *S. cerevisiae*.
Epidermal growth factor (EGF)	Heals wounds, burns, ulcers; produced by *E. coli*.
Erythropoietin (EPO)	Treatment of anemia; produced by mammalian cell culture.
Factor VIII	Treatment of hemophilia; improves clotting; produced by mammalian cell culture.
Gamma-interferon	Treatment of chronic granulomatous disease; produced by *E. coli*.
Hepatitis B vaccine	Produced by *S. cerevisiae* that carries hepatitis-virus gene on a plasmid.
Human growth hormone (hGH)	Corrects growth deficiencies in children; produced by *E. coli*.
Human insulin	Therapy for diabetes; better tolerated than insulin extracted from animals; produced by *Escherichia coli*.

TABLE 9.1 **Some Pharmaceutical Products of Genetic Engineering** (continued)

Product	Comments
Influenza vaccine	Trial vaccine made from *E. coli* or *S. cerevisiae* carrying virus genes.
Interleukins	Regulate the immune system; possible treatment for cancer; produced by *E. coli*.
Monoclonal antibodies	Possible therapy for cancer and transplant rejection; used in diagnostic tests; produced by mammalian cell culture (from fusion of cancer cell and antibody-producing cell).
Orthoclone®	Monoclonal antibody used in transplant patients to help suppress the immune system, reducing the chance of tissue rejection; produced by mouse cells.
Prourokinase	Anticoagulant; therapy for heart attacks; produced by *E. coli* and yeast.
Pulmozyme® (rhDNase)	Enzyme used to break down mucous secretions in cystic fibrosis patients; produced by mammalian cell culture.
Relaxin	Used to ease childbirth; produced by *E. coli*.
Superoxide dismutase (SOD)	Minimizes damage caused by oxygen free radicals when blood is resupplied to oxygen-deprived tissues; produced by *S. cerevisiae* and *Pichia pastoris* (yeast).
Taxol	Plant product used for treatment for ovarian cancer; produced in *E. coli*.
Tissue plasminogen activator (Activase®)	Dissolves the fibrin of blood clots; therapy for heart attacks; produced by mammalian cell culture.
Tumor necrosis factor (TNF)	Causes disintegration of tumor cells; produced by *E. coli*.

Restriction Enzymes

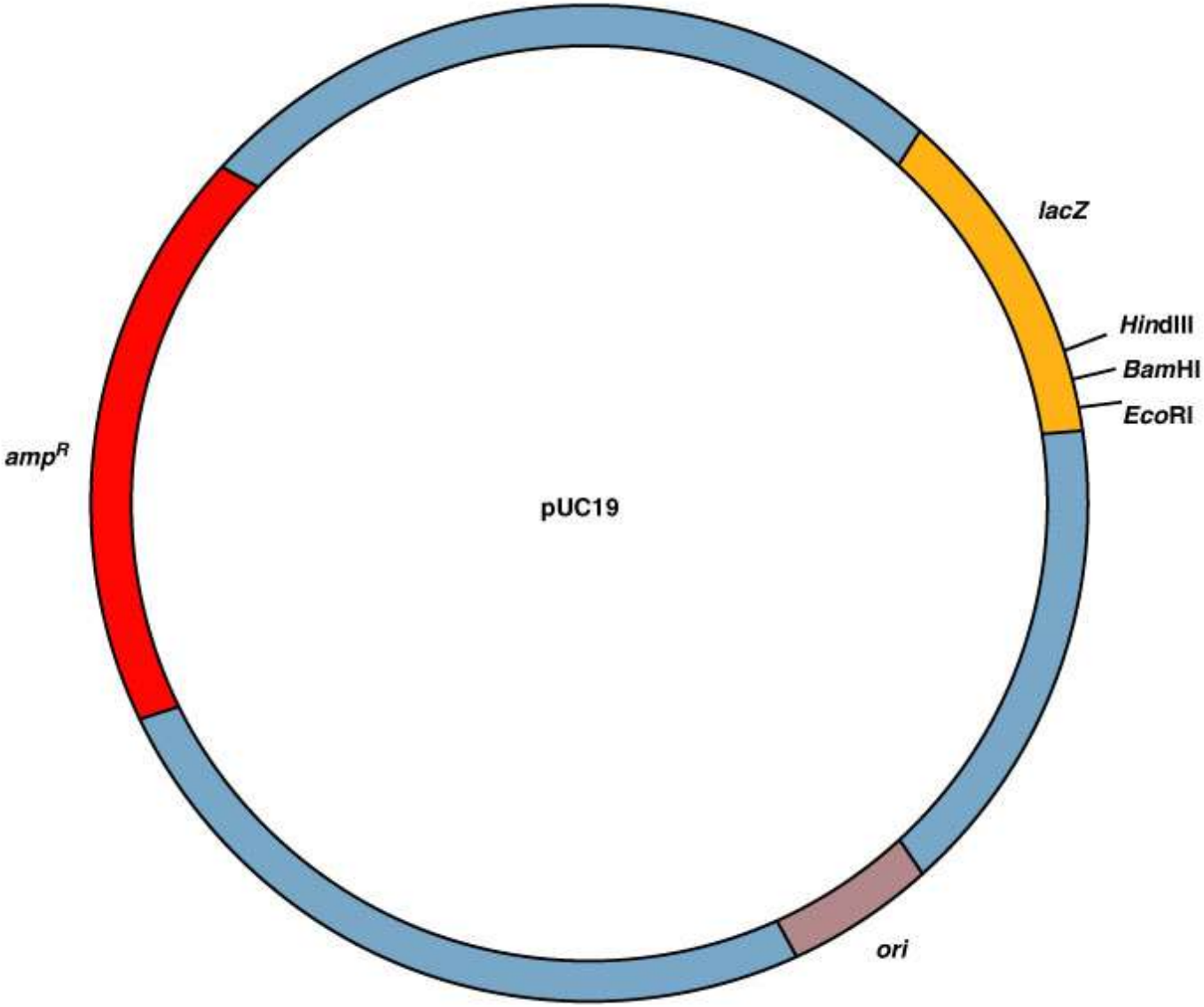
- Cut specific sequences of DNA
- Destroy bacteriophage DNA in bacterial cells
- Cannot digest (host) DNA with methylated cytosines


1 Restriction enzyme cuts (magenta arrows) double-stranded DNA at its particular recognition sites, shown in blue.
Recognition sites
DNA Cut
Cut
GAATTC
CTTAAG
Cut
Cut
2 These cuts produce a DNA fragment with two sticky ends.
AATTC
G
G
CTTAA
Sticky end
AATTC
G
G
CTTAA
DNA from another source, perhaps a plasmid, cut with the same restriction enzyme
3 When two such fragments of DNA cut by the same restriction enzyme come together, they can join by base pairing.
AATTC
G
G
CTTAA
G AATTC
G
CTTAA
4 The joined fragments will usually form either a linear molecule or a circular one, as shown here for a plasmid. Other combinations of fragments can also occur.
5 The enzyme DNA ligase is used to unite the backbones of the two DNA fragments, producing a molecule of recombinant DNA.
Recombinant DNA

Vectors

- Carry new DNA to desired cell
- Vectors can exist in several different species
- Plasmids and viruses can be used as vectors

Vectors



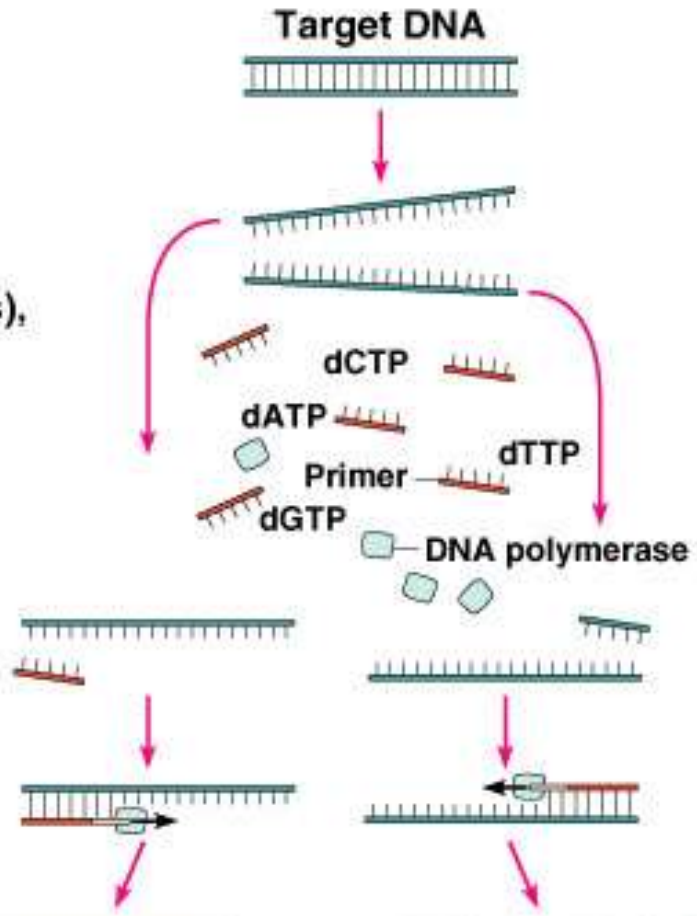
Polymerase Chain Reaction (PCR)

- To make multiple copies of a piece of DNA enzymatically
- Used to
  - Clone DNA for recombination
  - Amplify DNA to detectable levels
  - Sequence DNA
  - Diagnose genetic disease
  - Detect pathogens

PCR

First cycle

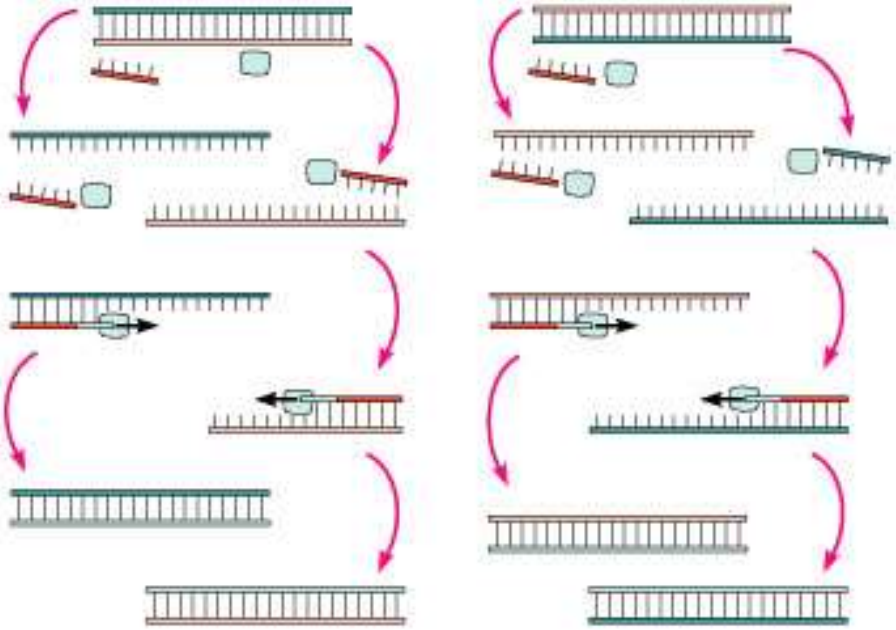
1. Incubate target DNA at 94°C for 1 minute to separate the strands.
2. Add primers, nucleotides (deoxynucleotides), and DNA polymerase.
3. Primers attach to single-stranded DNA during incubation at 60°C for 1 minute.
4. Incubate at 72°C for 1 minute; during this time, two copies of target DNA are formed.



PCR

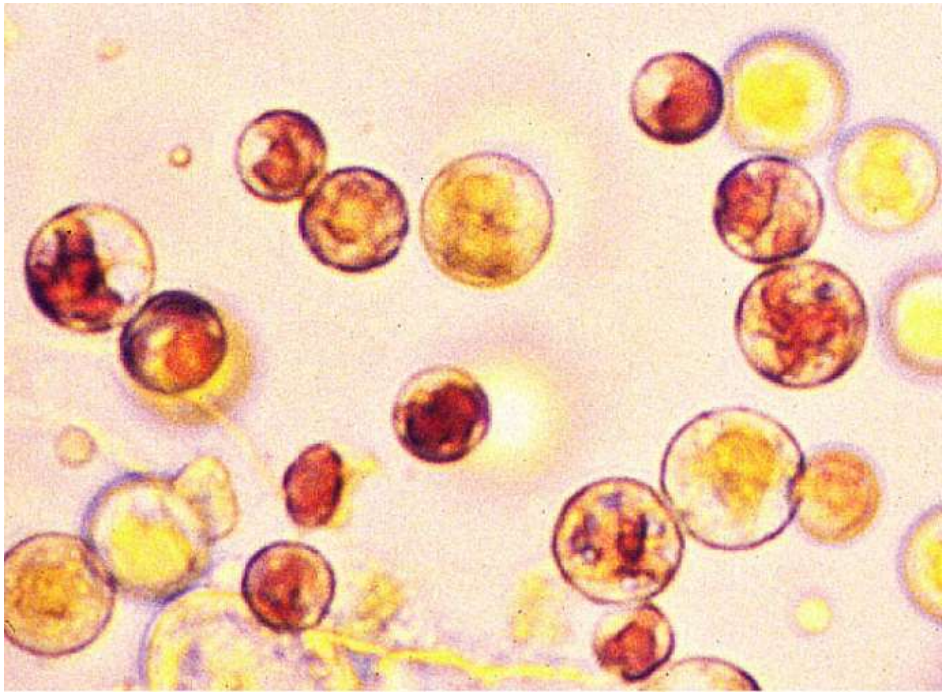
Second cycle

5 Repeat the cycle of heating and cooling to make two more copies of target DNA.



DNA can be inserted into a cell by:

- Transformation
- Electroporation
- Protoplast fusion



DNA can be inserted into a cell by:

- Microinjection
- Gene gun

Gen gun