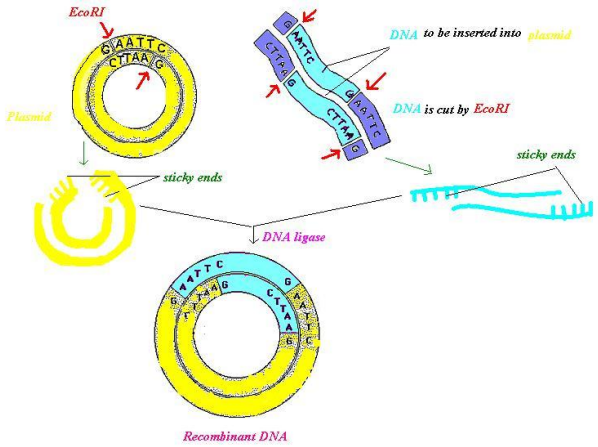


Recombinant DNA (rDNA) technology

Ms. Amal Al-Ayedh

Learning Objectives:

- Genetic modification, and recombinant DNA technology.
- Identify the types of plasmid.
- Identify the roles of a clone and a vector in making recombinant DNA.
- Define *restriction enzymes*, and outline how they are used to make recombinant DNA.
- The properties of vectors.
- Describe the use of plasmid and viral vectors.

Introduction

- **Plasmid DNA** is circular form that include origin sequences (ori) needed for replication in bacterial cells.

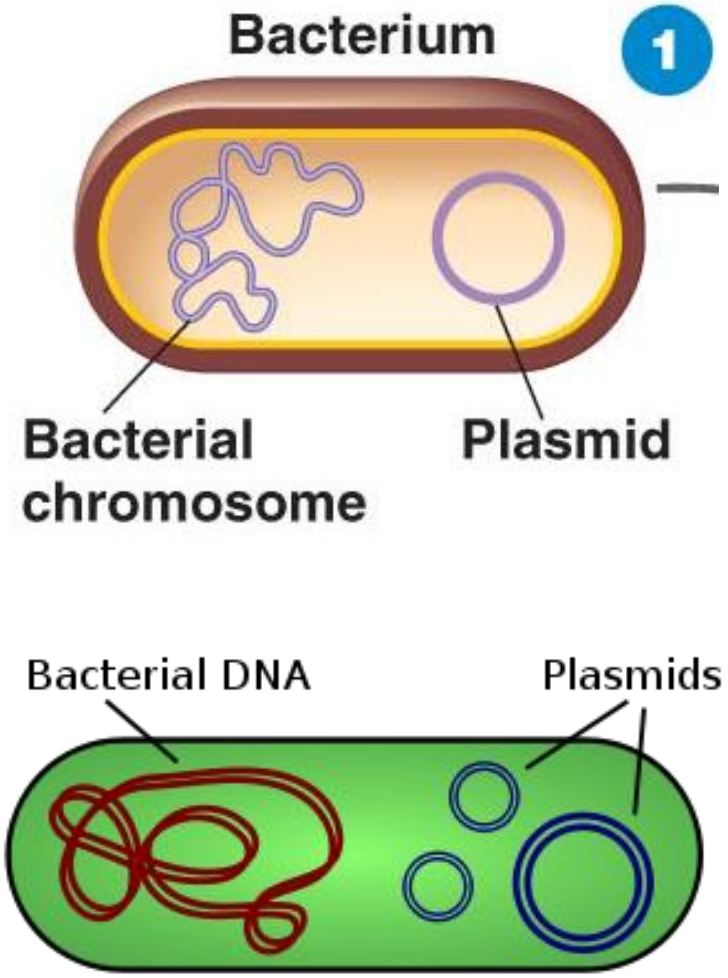


Figure: Illustration of a bacterium with plasmid enclosed showing chromosomal DNA and plasmids. http://en.wikipedia.org/wiki/Plasmid

Types of plasmids

1- Resistance plasmids (R) : These are the most widespread and well studied group of plasmids conferring resistance to antibiotics and various other growth inhibitors.

Examples: *Staphylococcus aureus, Streptococcus* and *Enterococcus*

2- Colicins

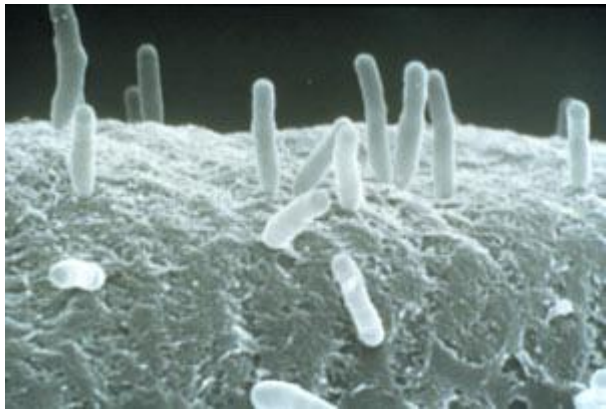
- These plasmids carry genes that confer ability to the host bacterium to kill other bacteria by secreting **bacteriocins,** a type of proteins.

- Such as : ***Bacillus thuringiensis (BT)***

3- Virulence plasmid

- For example, Ti-plasmids of Agrobacterium tumefaciens induce crown gall disease of angiospermic plants.

- (Ti) : Tumor Inducing gene



Agrobacterium tumefaciens **cells attached to a plant cell.**

4- degradative plasmids

-possess genes to code enzymes that degrade unusual substances such as toluene (aromatic compounds), pesticides and sugars (lactose).

- plasmid of Pseudomonas putida is an example.

Recombinant DNA (rDNA) Technology

- **rDNA technology** involves cloning DNA by cutting & pasting DNA from different sources
- **Restriction enzymes & DNA ligases** are important enzymes for this process
- **DNA ligases** join together adjacent DNA fragments

Recombinant DNA

- **Recombinant DNA (rDNA) technology**: Insertion or modification of genes to produce desired proteins.

- **Recombinant DNA technology** provides a means to transplant genes from one species into the genome of another.

- Technique in which strands of DNA from different sources, are spliced (by restriction enzymes) and put together in a plasmid, to form DNA for a new life form.

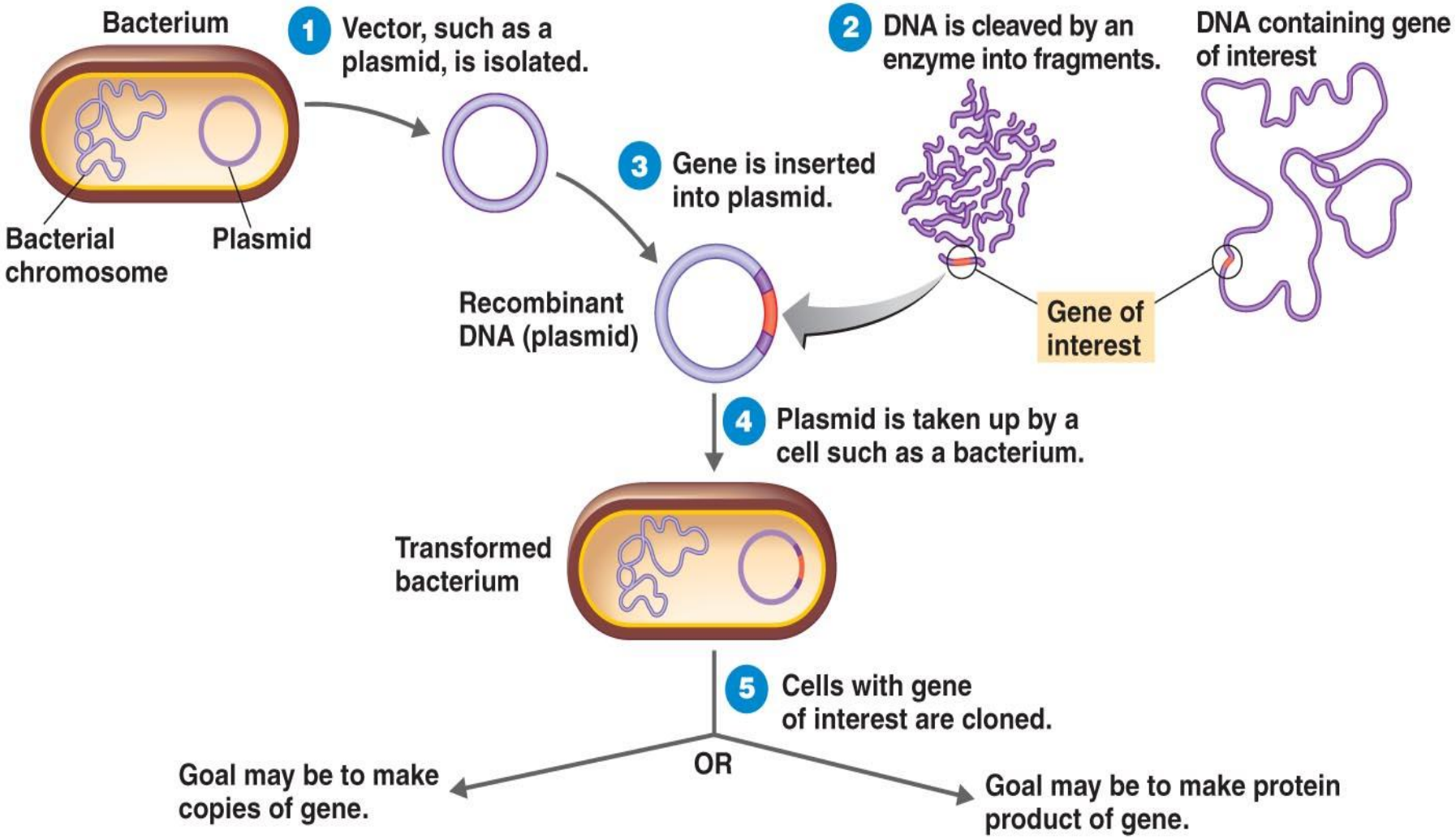
Recombinant DNA

- **Vector**: Self-replicating DNA used to carry the desired gene to a new cell.
- Shuttle Vectors: Plasmids that can exist in several different species.
- Plasmids and viruses can be used as vectors.
- A Plasmid vector used for cloning.
- **Clone**: Population of cells arising from one cell, each carries the new gene.

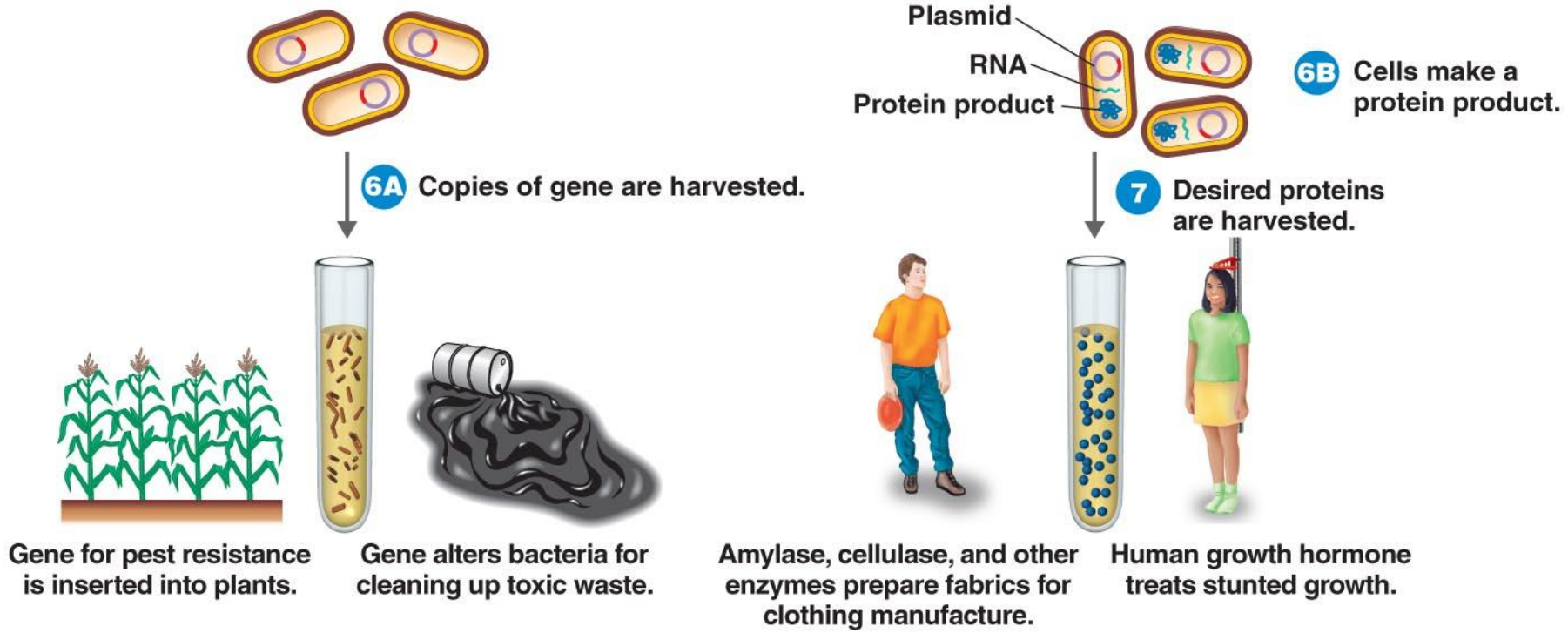
Cloning Vectors

- Plasmid;
- Phage;
- Cosmid;
- Shuttle;
- Yeast Artificial Chromosomes (YACs)
- Bacterial Artificial Chromosomes (BACs)

A Typical Genetic Modification Procedure



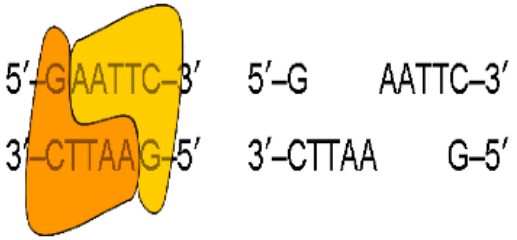
A Typical Genetic Modification Procedure



Restriction Enzymes

Restriction enzymes are DNA-cutting enzymes that are found in *bacteria*. They are also called endonucleases.

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



How do they work?

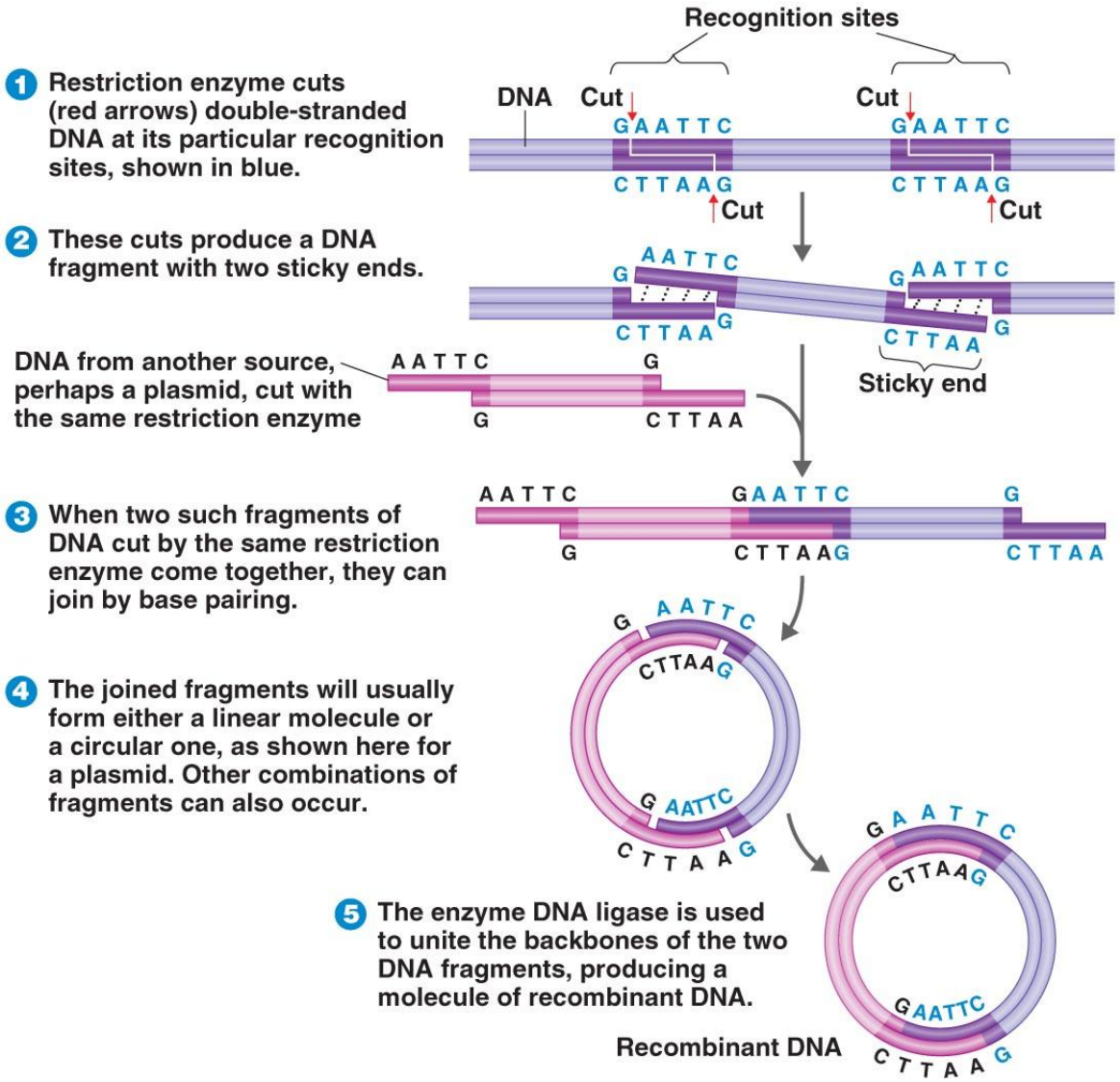
They cut DNA by cleaving *phosphodiester bonds* (in sugar-phosphate backbone) that join adjacent nucleotides.

- Usually a 4-base pair or 6-base pair cutter.

- Restriction sites are **palindromes** (reads same forward & backwards on opposite strands).

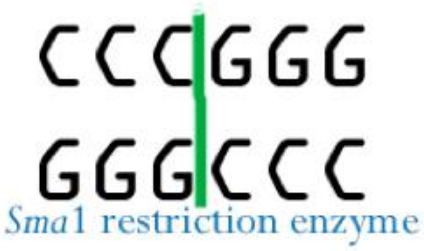
*Sma*1 restriction enzyme

Restriction Enzyme & Recombinant DNA



Restriction Enzymes

- **Blunt ends:** both strands are cut at the same position.



- **Sticky ends:** overhanging regions (3' or 5') are useful in cloning. They are complementary, thus anneal, DNA ligase can covalently link them.

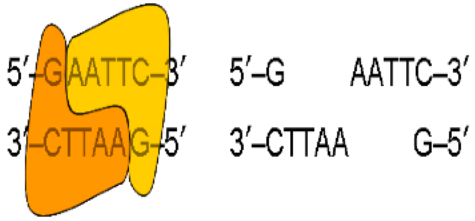
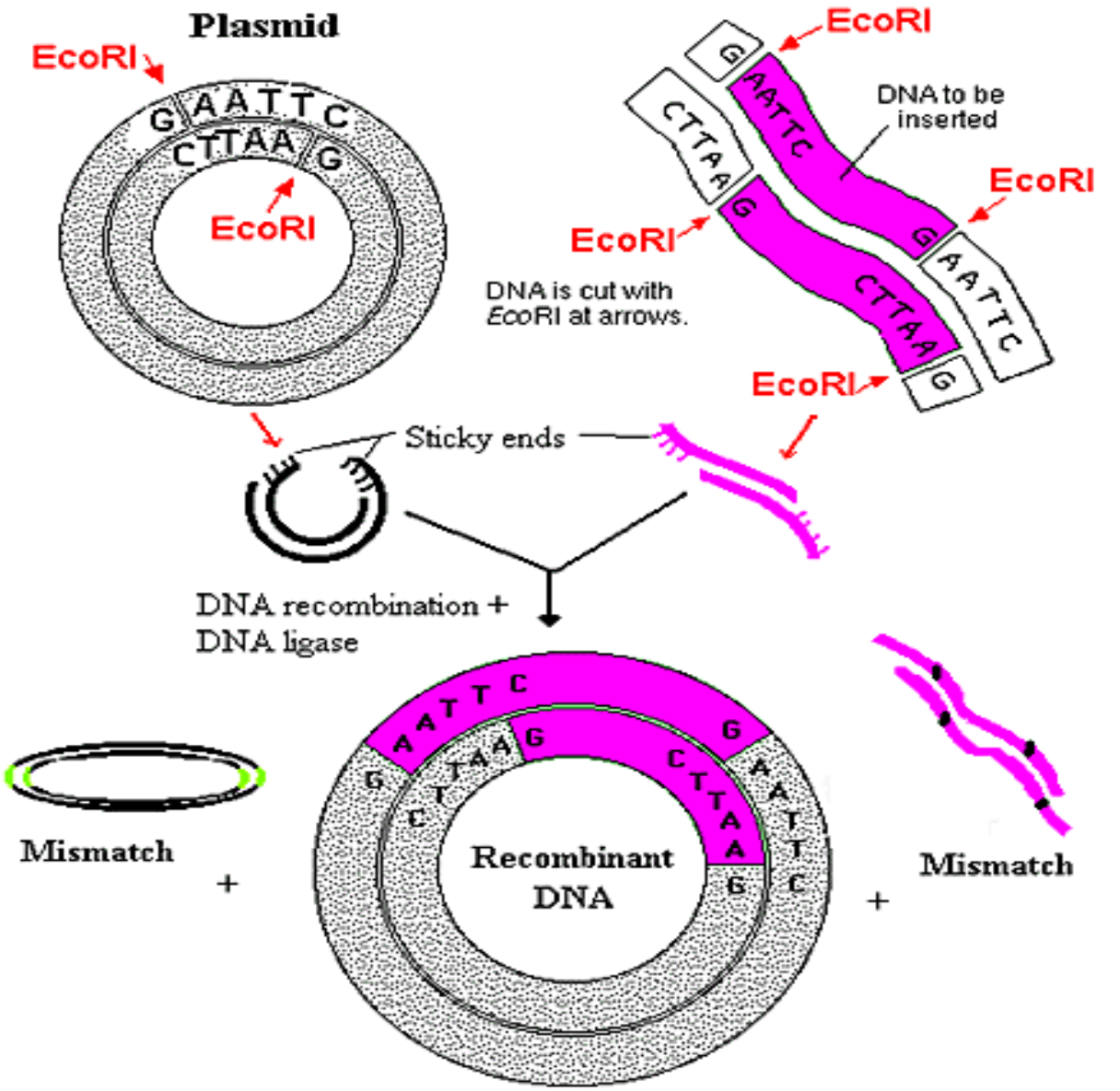


Table 9.1 Selected Restriction Enzymes Used in rDNA Technology

Enzyme	Bacterial Source	Recognition Sequence
*Bam*HI	*Bacillus amyloliquefaciens*	G↓G A T C C G C T A G↑G
*Eco*RI	*Escherichia coli*	G↓A A T T C C T T A A↑G
*Hae*III	*Haemophilus aegyptius*	G G↓C C C C↑G G
*Hin*dIII	*Haemophilus influenzae*	A↓A G C T T T T C G A↑A

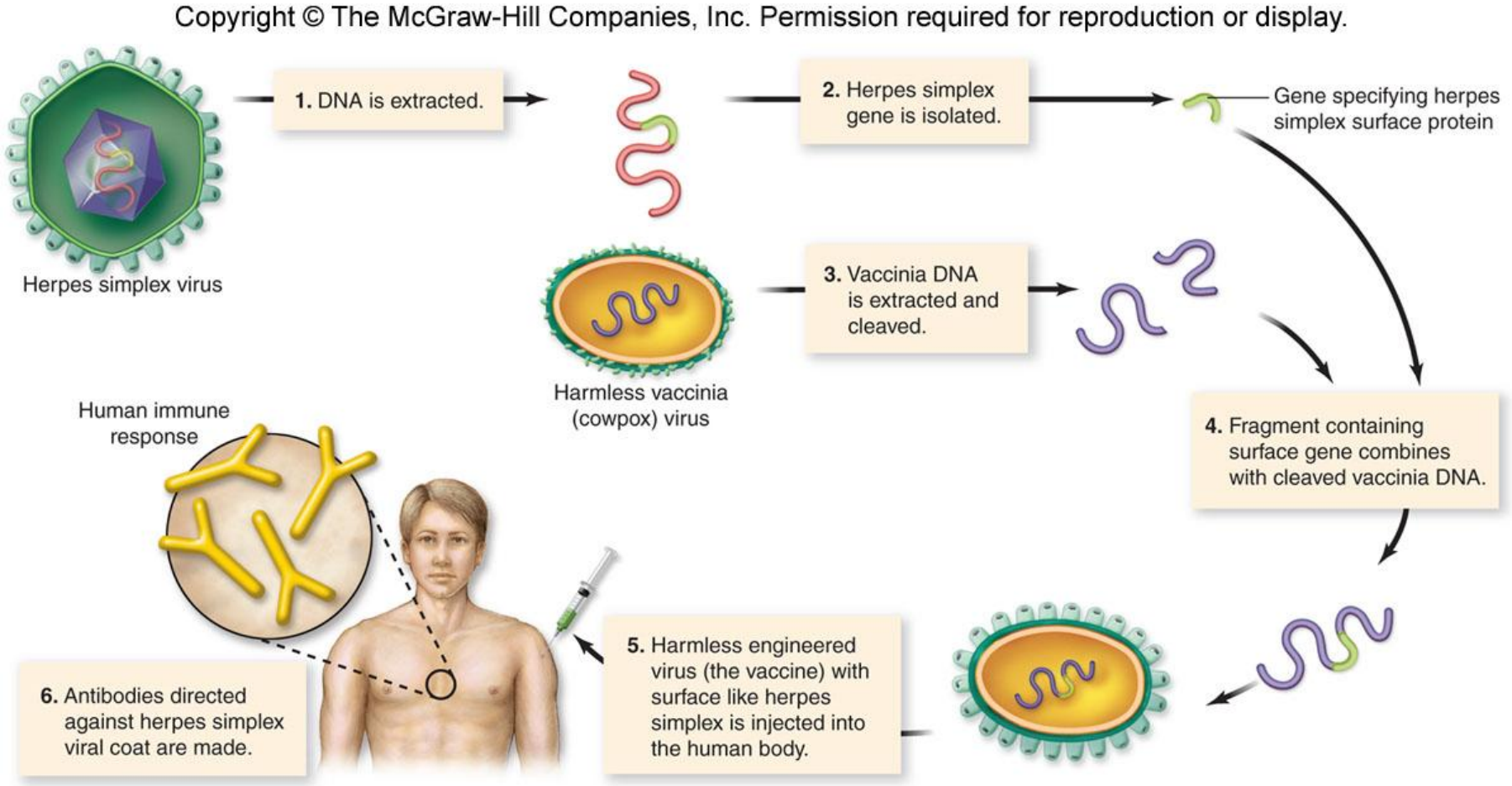


Inserting a DNA Sample into a Plasmid

Medical Applications

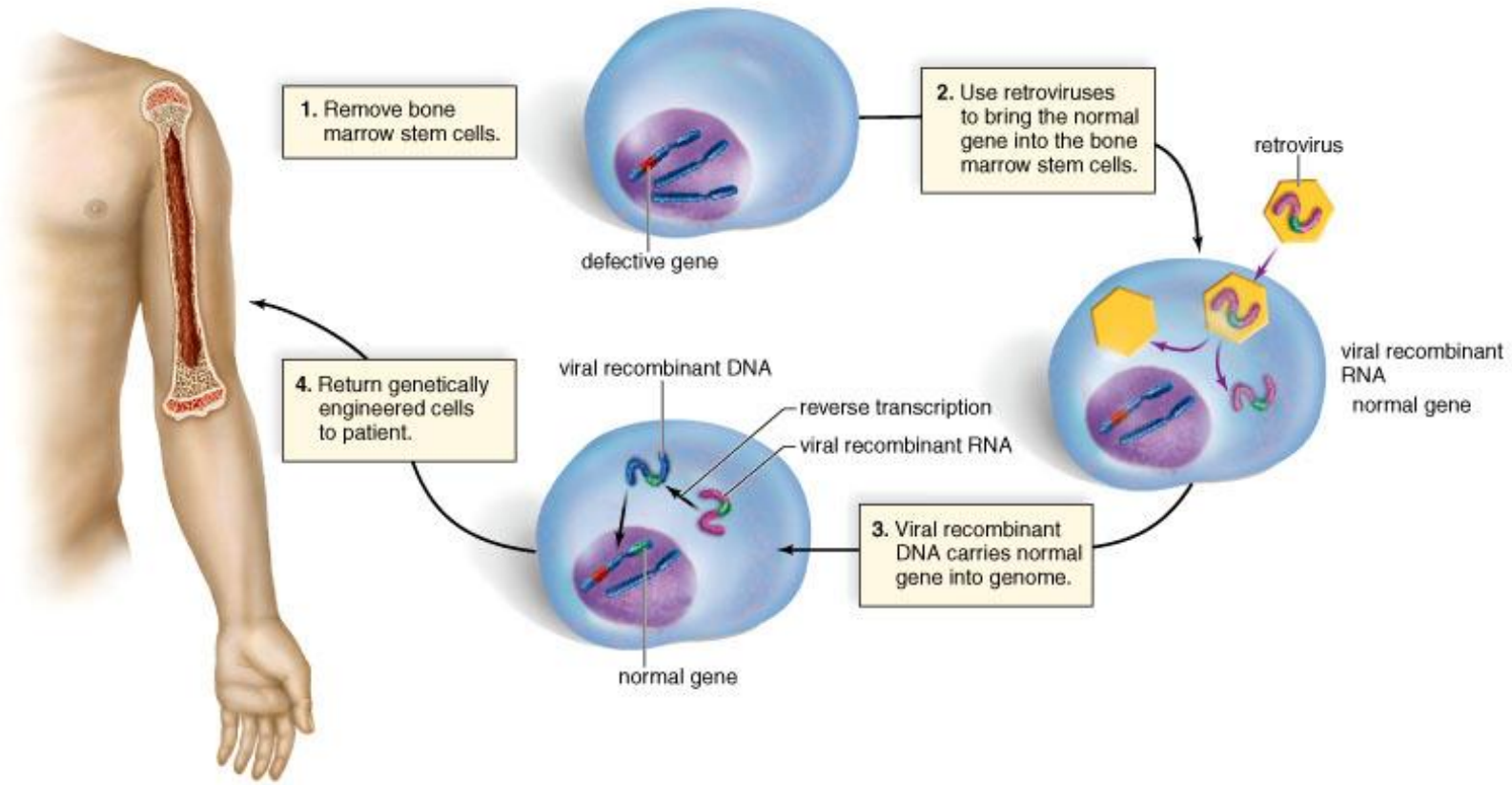
- The insertion of genetic material into human cells for the treatment of a disorder

Recombinant DNA Vaccines?



Strategy for a subunit vaccine for herpes simplex

Gene Therapy



Treatment of SCID (severe combined immunodeficiency). SCID affects the maturation of immune cells that develop in bone marrow. SCID sufferers lack the enzyme ADA (adenosine deaminase).

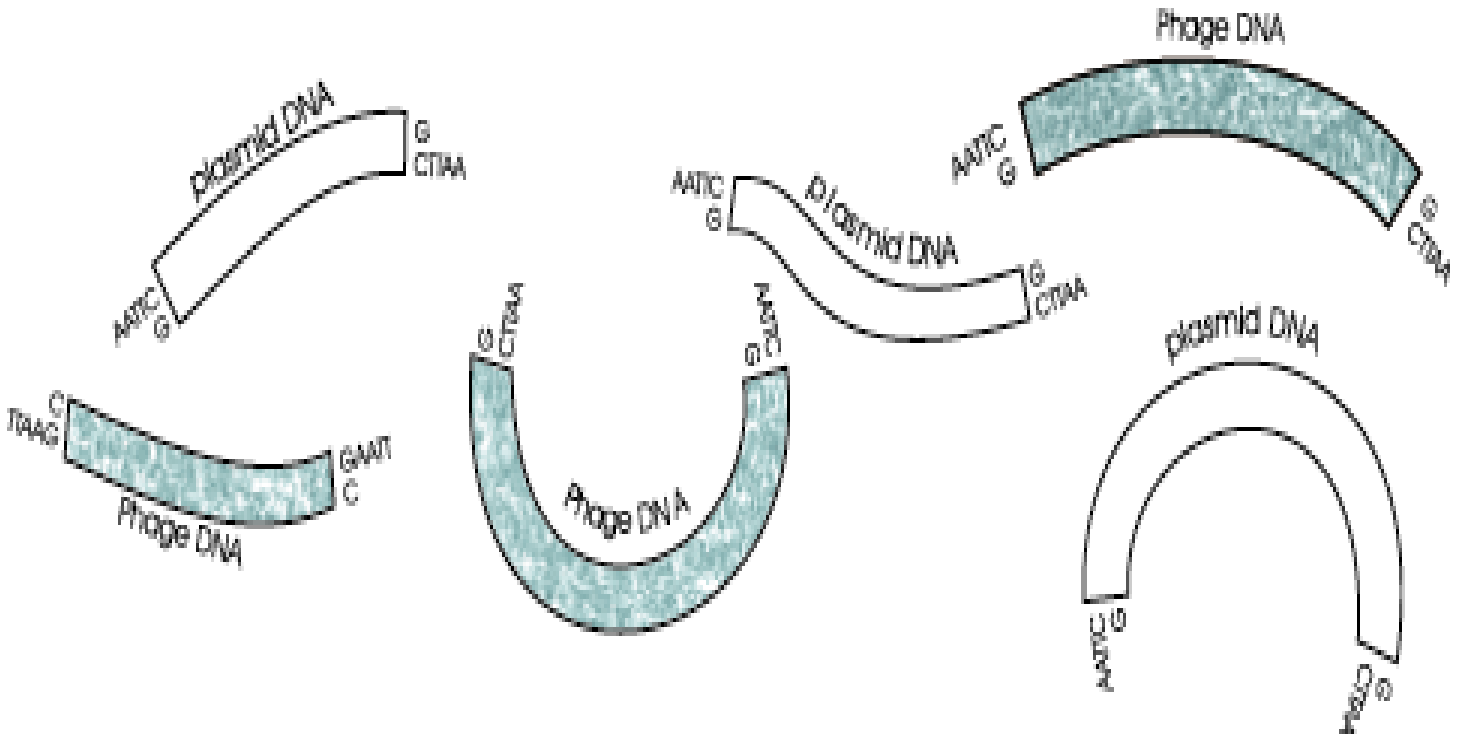
TABLE 12-6 SOME PROTEIN PRODUCTS OF RECOMBINANT DNA TECHNOLOGY

Product	Made In	Use
Human insulin	*E. coli*	Treatment for diabetes
Human growth hormone (HGH)	*E. coli*	Treatment for growth defects
Epidermal growth factor (EGF)	*E. coli*	Treatment for burns, ulcers
Interleukin-2 (IL-2)	*E. coli*	Possible treatment for cancer
Bovine growth hormone (BGH)	*E. coli*	Improving weight gain in cattle
Cellulase	*E. coli*	Breaking down cellulose for animal feeds
Taxol	*E. coli*	Treatment for ovarian cancer
Interferons (alpha and gamma)	*S. cerevisiae; E. coli*	Possible treatment for cancer and viral infections
Hepatitis B vaccine	*S. cerevisiae*	Prevention of viral hepatitis
Erythropoietin (EPO)	Mammalian cells	Treatment for anemia
Factor VIII	Mammalian cells	Treatment for hemophilia
Tissue plasminogen activator (TPA)	Mammalian cells	Treatment for heart attacks and some strokes

Human insulin produced by bacteria

Exercise:

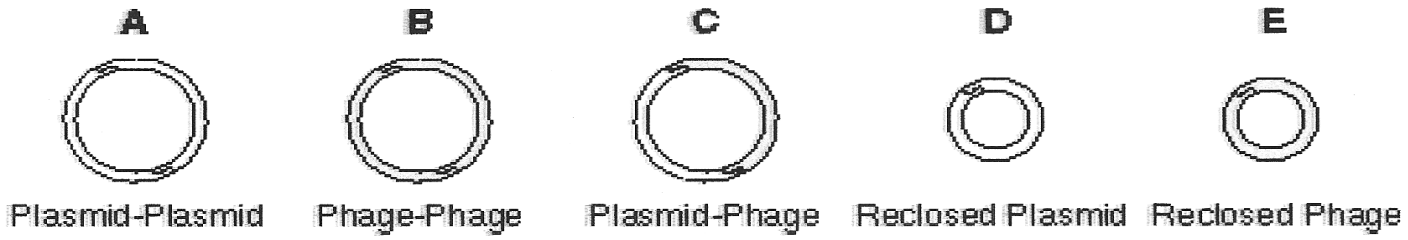
If pUC18 plasmids that have been cut with *EcoR*I are mixed with fragments of phage DNA that have also been cut with *EcoR*I, there are several ways the DNA pieces may join together. Color the cut plasmids and the phage DNA fragments illustrated below with two different colors to represent DNA from the two different sources:



Now, use the space below to draw 5 circles that show the five possible ways circular pieces of DNA can be formed by joining the ends of either one or two of the DNA fragments above. Note that the 2 ends of one piece of DNA can join to form a circle, or two different pieces of DNA can join to make a circle. Color the circles using the same colors that you used above to distinguish between plasmid DNA and phage DNA. Label your 5 drawings A through E.

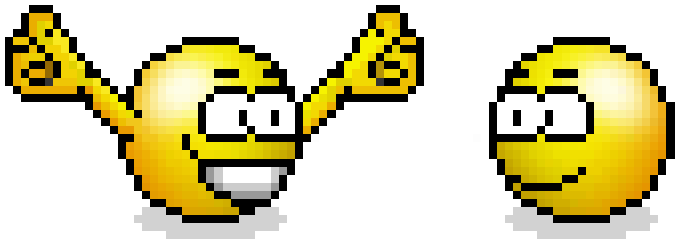
Which of your drawings (A-E) above represent a recombinant plasmid? _____
Which drawings represent plasmids that are not recombinant? _____
Which drawings represent circular pieces of DNA that do not include a plasmid? _____

The linearized plasmids and the phage DNA fragments are combined. Complementary sticky ends allow the pieces of DNA to anneal in five possible combinations.



Note that only combination “C” contains **recombinant DNA. Recombinant DNA is made by joining DNA** from 2 different sources, in this case, the plasmid and the phage. All the other combinations (A, B, D, and E) contain **non-recombinant DNA (DNA from one source only.)**

Thank you

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