Lecture for Monday

Dr. Prince

Welcome Back!

I hope you studied for today's test

Chapter 12

DNA Technology and Genomics

Lecture by Dr. Prince

DNA and Crime Scene Investigations

DNA is unique to an individual and can be used to prove their presence in a location

In the example used in your book, DNA was used to solve the murder of two victims

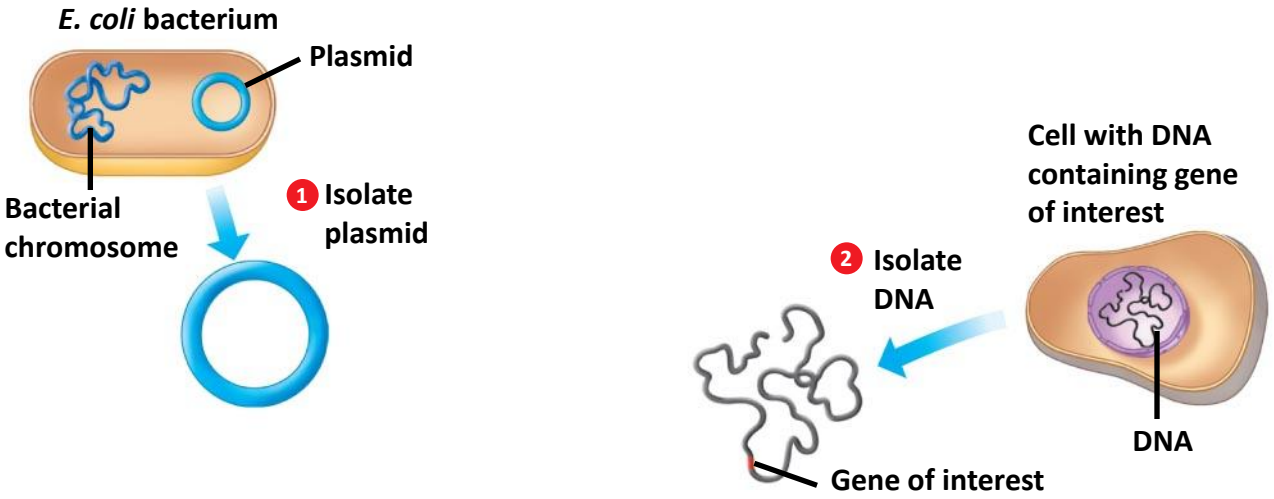
GENE CLONING

Genetic Engineering

- Genes or “information” can be copied, combined, transferred, and even manipulated for practical purposes.
- Copying genes also known as Gene cloning is just that making multiple copies of the same gene.
- **Recombinant DNA** is DNA from two different sources combined or “recombinant”
- One is the gene that will be cloned and the another is the vector.
- Bacterial Plasmids are often used as vectors.

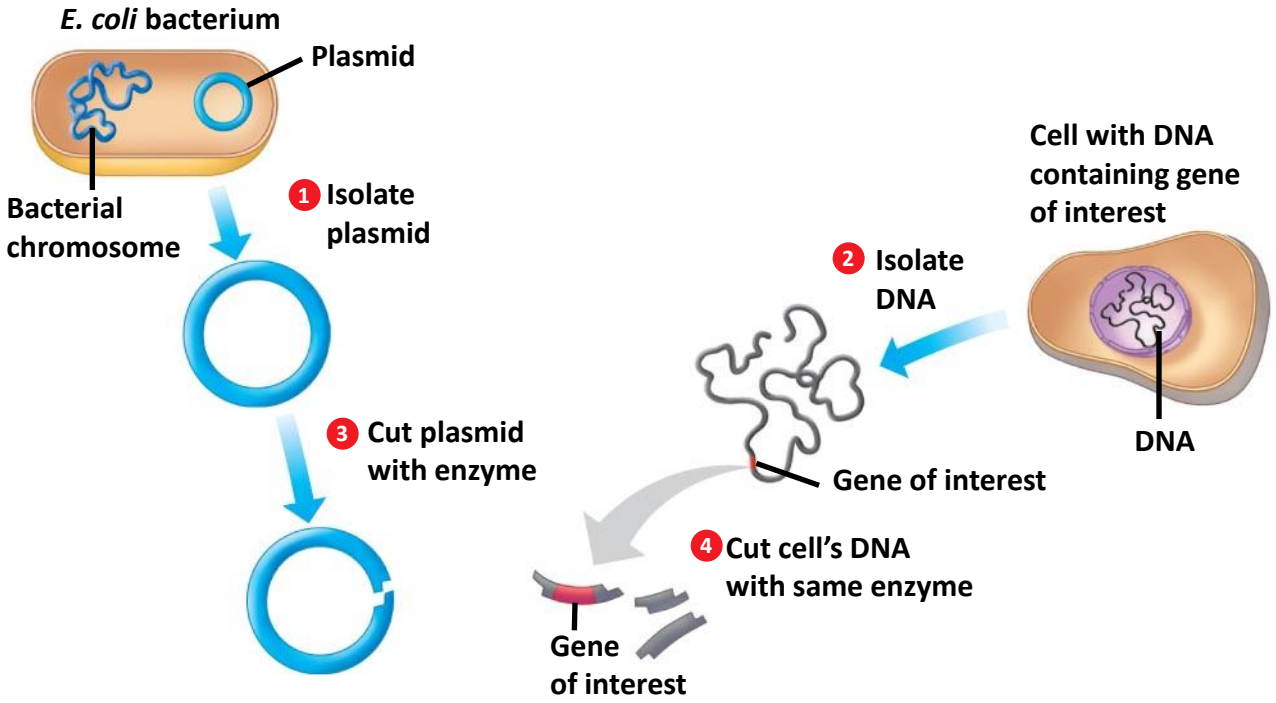
Gene Cloning

- Steps in cloning a gene
  1. Plasmid DNA is isolated
  2. DNA containing the gene of interest is isolated
  3. Plasmid DNA is treated with restriction enzyme that cuts in one place, opening the circle
  4. DNA with the target gene is treated with the same enzyme and many fragments are produced
  5. Plasmid and target DNA are mixed and associate with each other
  6. Recombinant DNA molecules are produced when **DNA ligase** joins plasmid and target segments together
  7. The recombinant DNA is taken up by a bacterial cell
  8. The bacterial cell reproduces to form a **clone** of cells



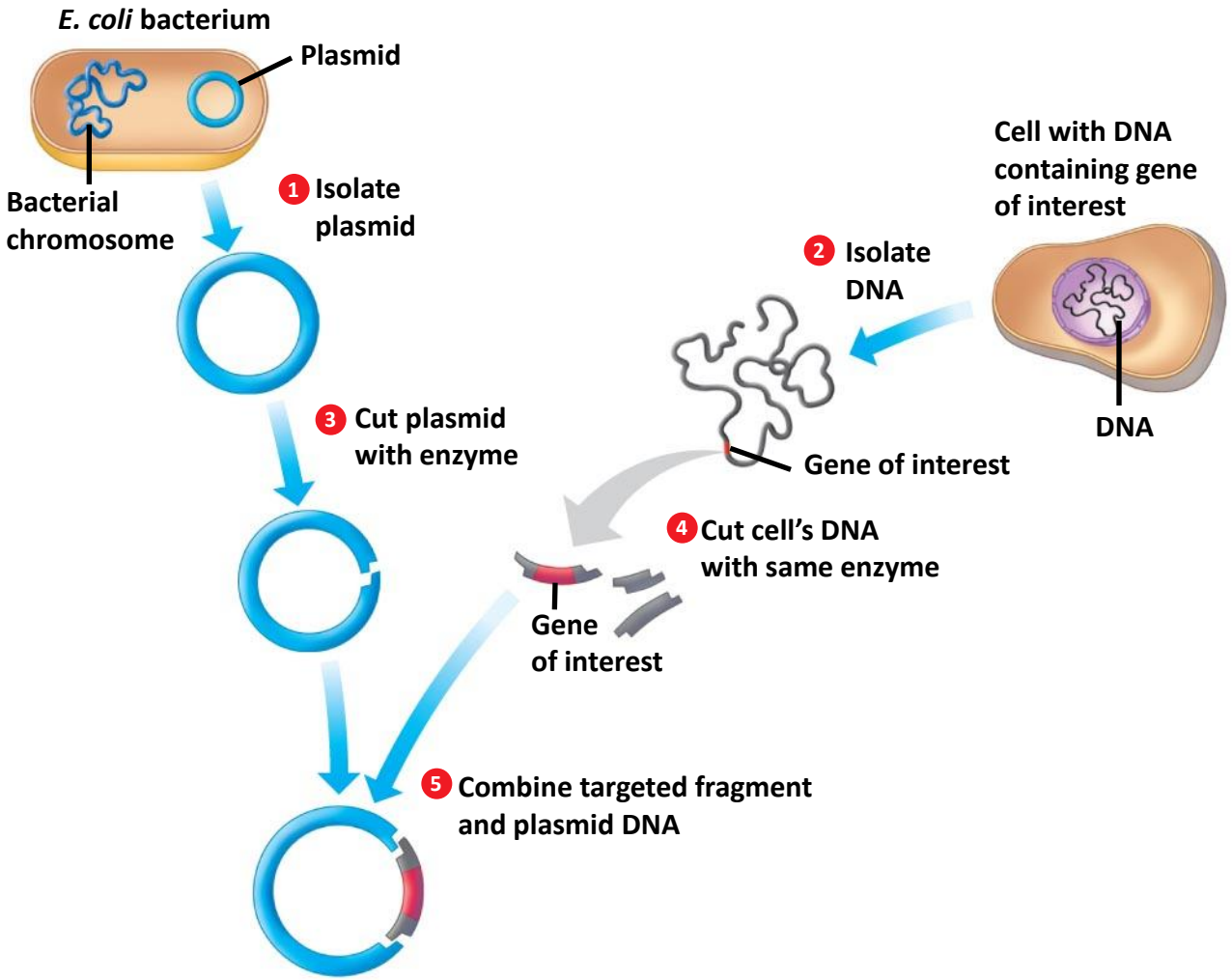
Plasmid DNA is isolated

DNA containing the gene of interest is isolated

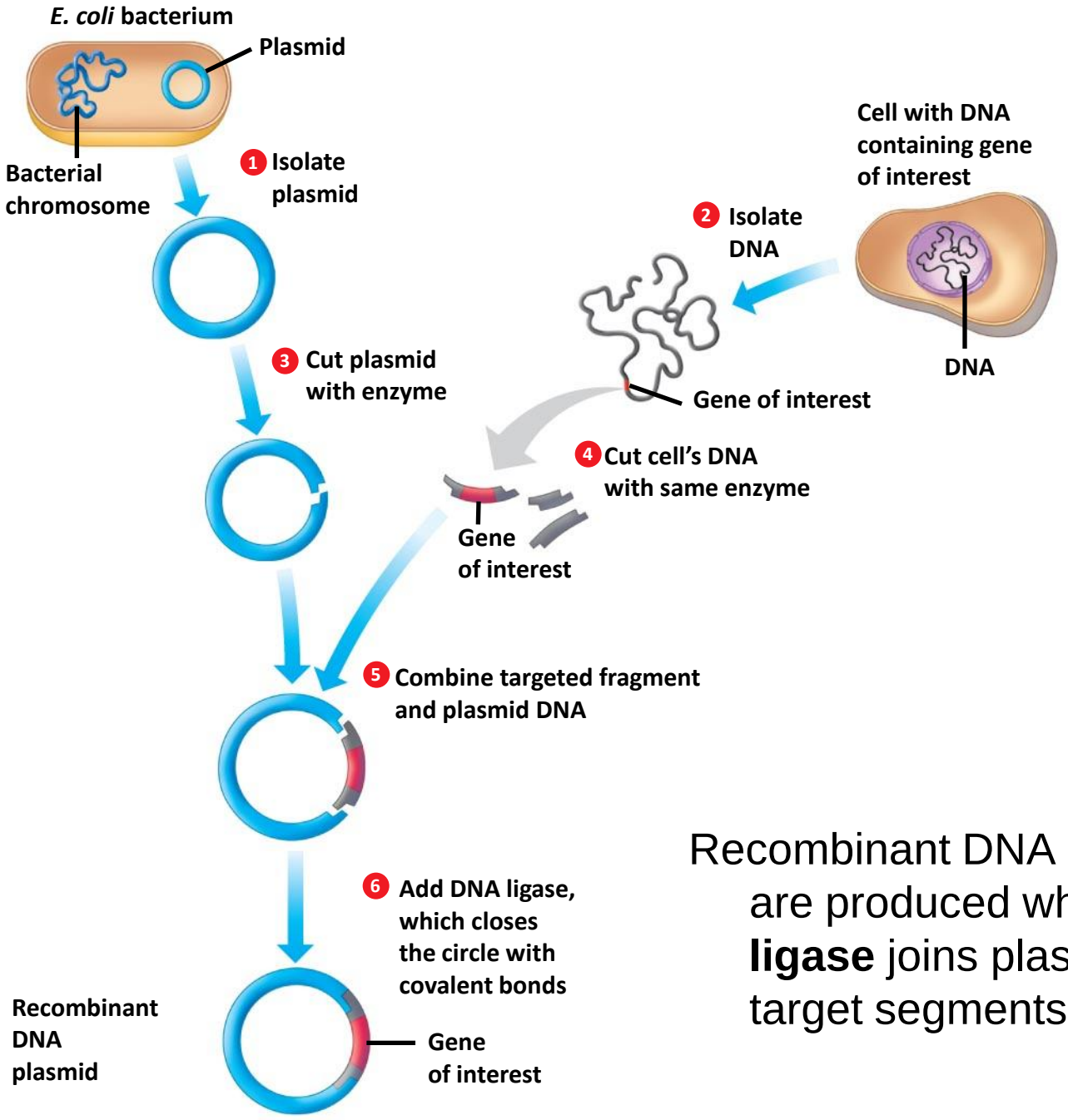


Plasmid DNA is treated with restriction enzyme that cuts in one place, opening the circle

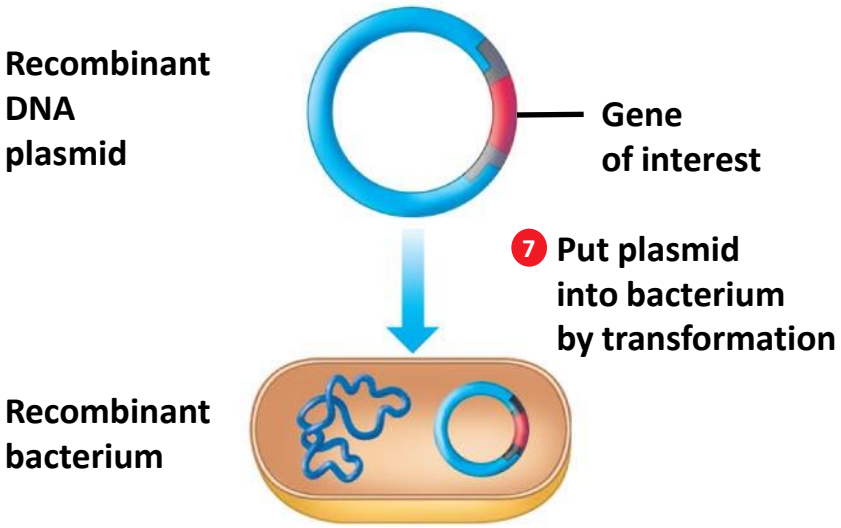
DNA with the target gene is treated with the same enzyme and many fragments are produced



Plasmid and target DNA are mixed and associate with each other

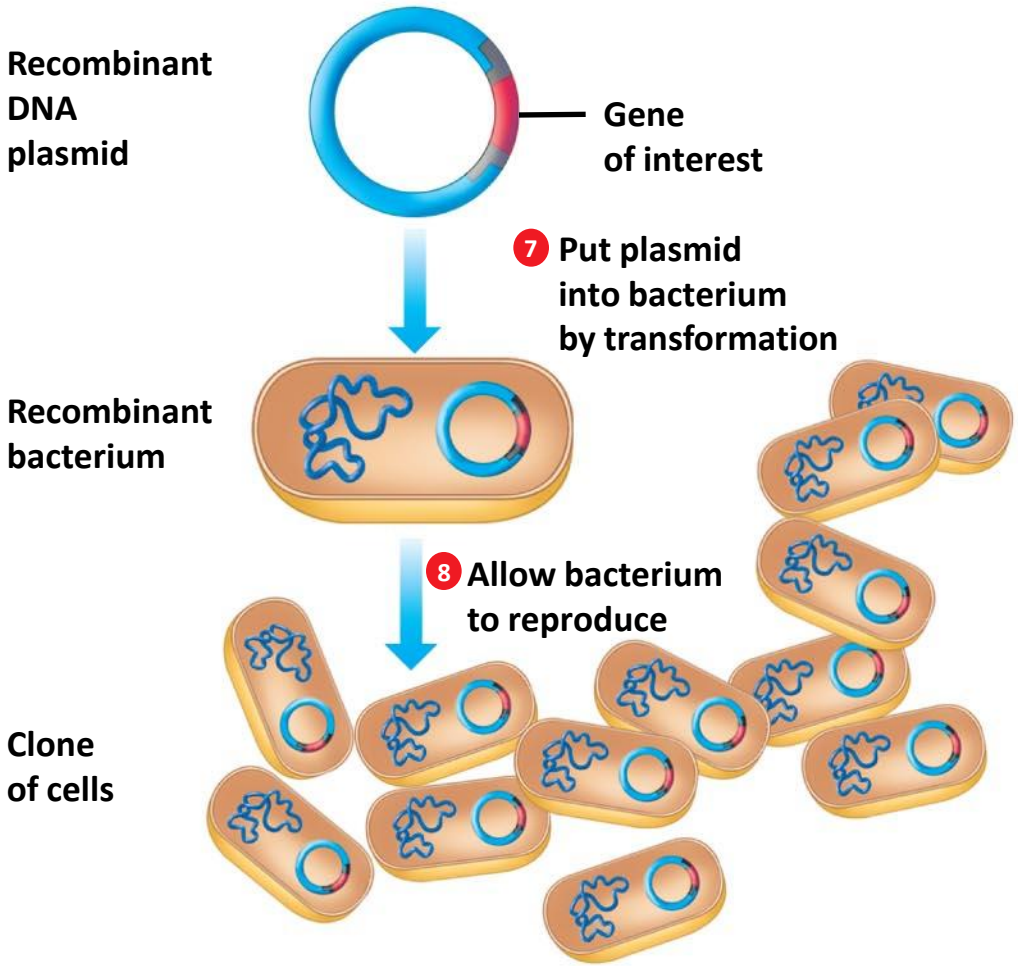


Recombinant DNA molecules are produced when **DNA ligase** joins plasmid and target segments together



The recombinant DNA is taken up by a bacterial cell

The bacterial cell reproduces to form a **clone** of cells



Examples of gene use

Genes may be inserted into other organisms

Recombinant DNA plasmid

Gene of interest

7 Put plasmid into bacterium by transformation

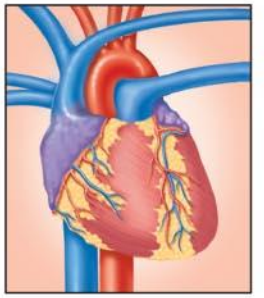
9 Genes or proteins are isolated from the cloned bacterium

Recombinant bacterium

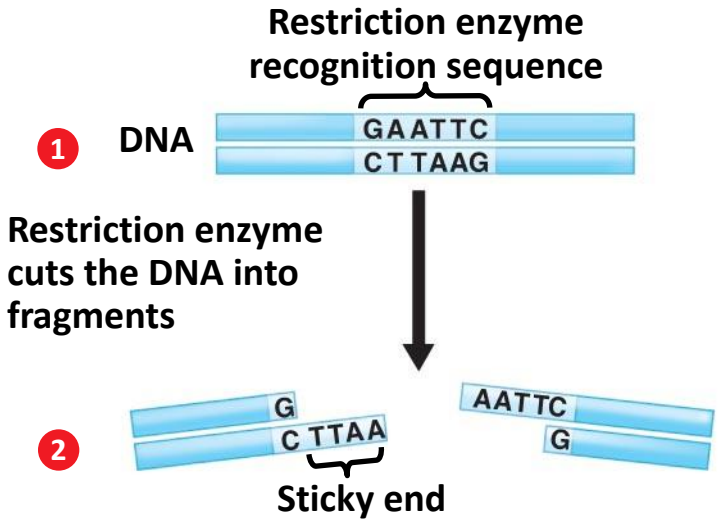
8 Allow bacterium to reproduce

Harvested proteins may be used directly

Clone of cells



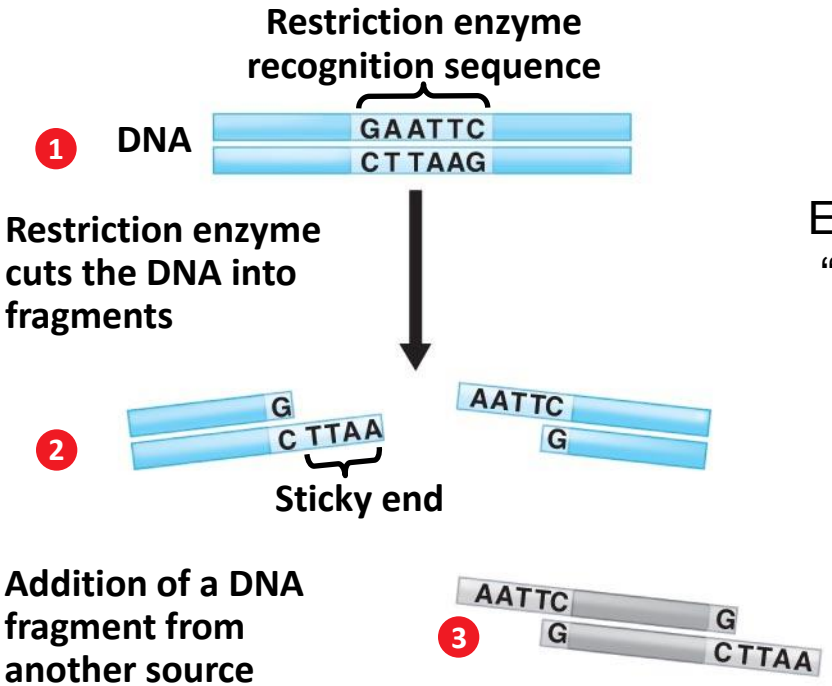
Examples of protein use



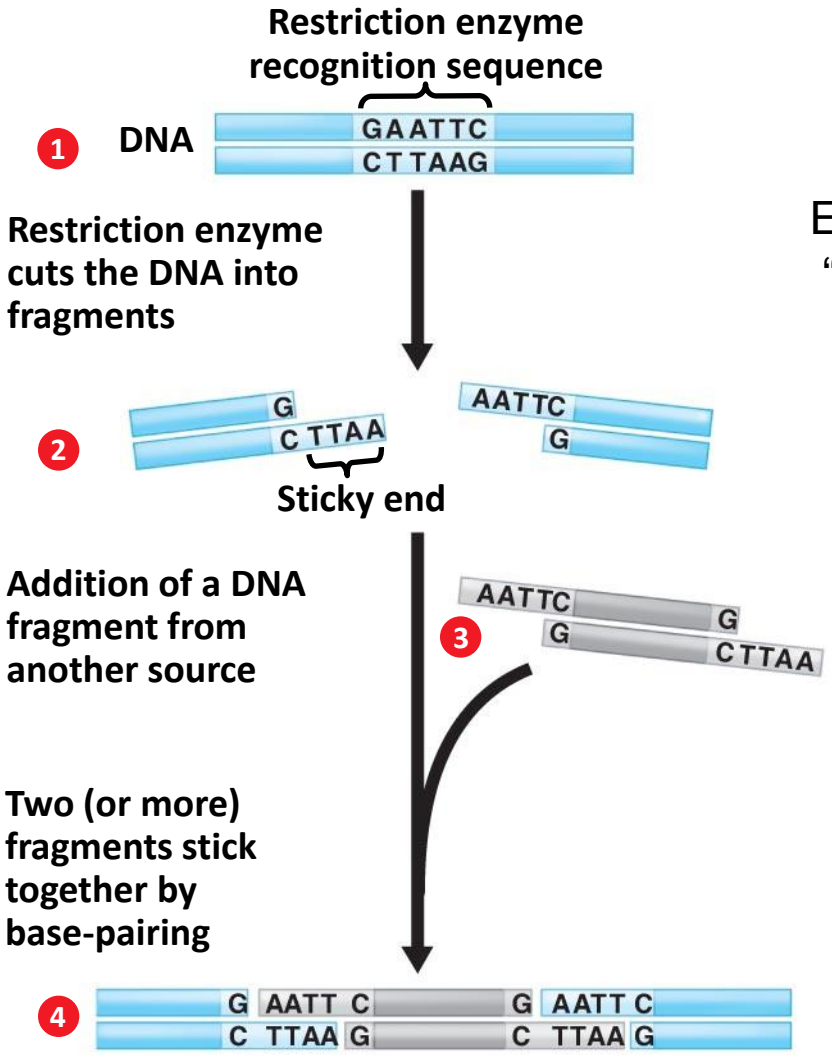
Enzymes are used to "cut and paste" DNA

There are many restriction enzymes that cut DNA at different sequences resulting is staggered cuts.

The staggered cuts are called restriction fragments with "sticky ends"

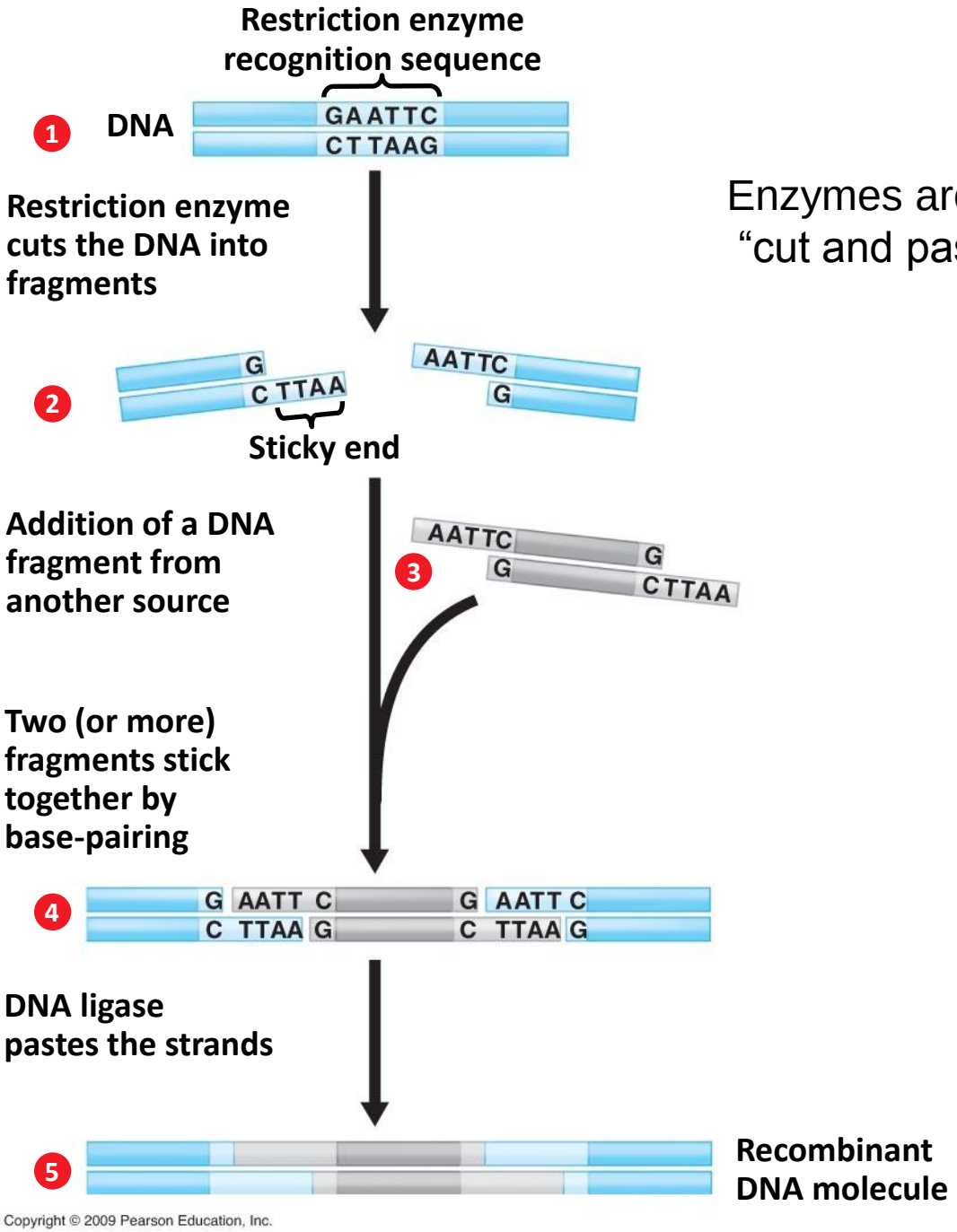


Enzymes are used to "cut and paste" DNA



Enzymes are used to "cut and paste" DNA

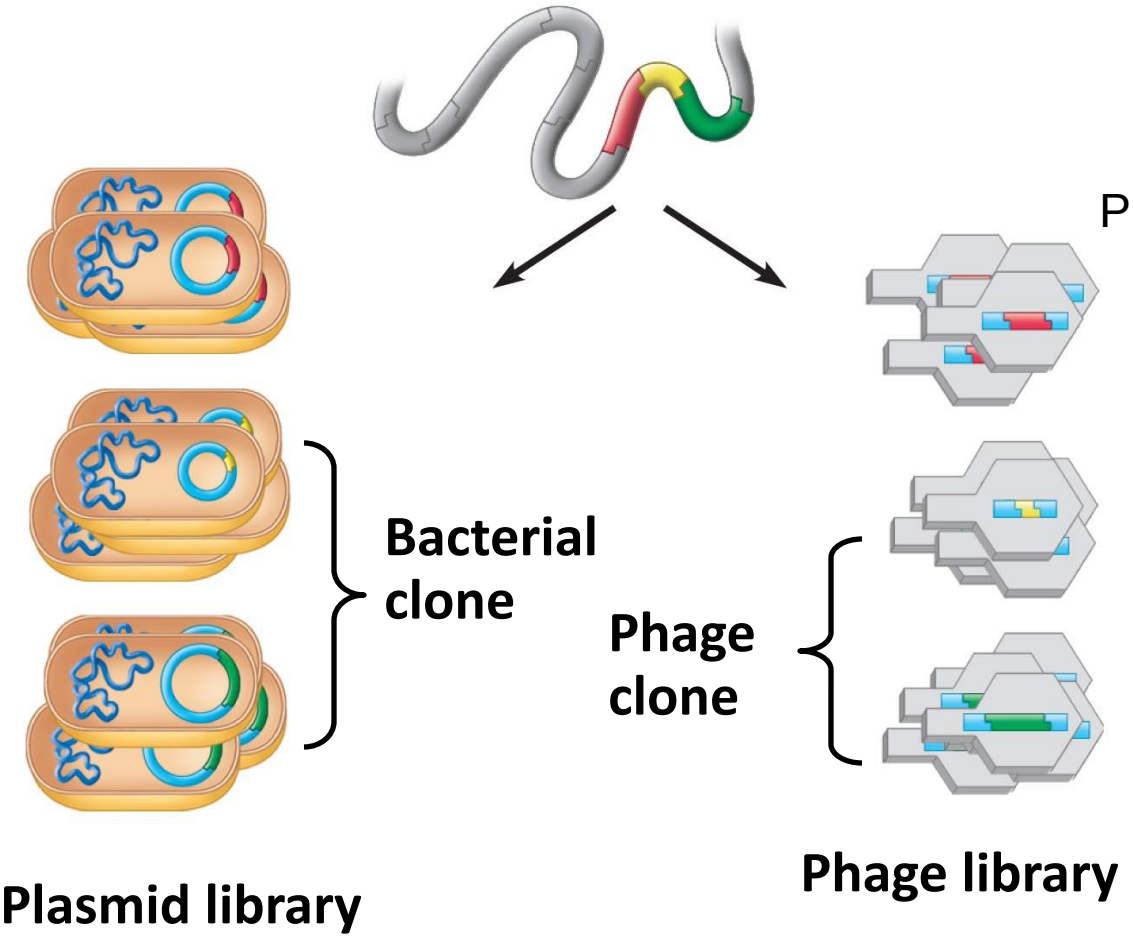
DNA ligase joins DNA fragments together



Enzymes are used to "cut and paste" DNA

Genes can be organized into genomic libraries

Plasmid library: genomic DNA is carried by plasmids



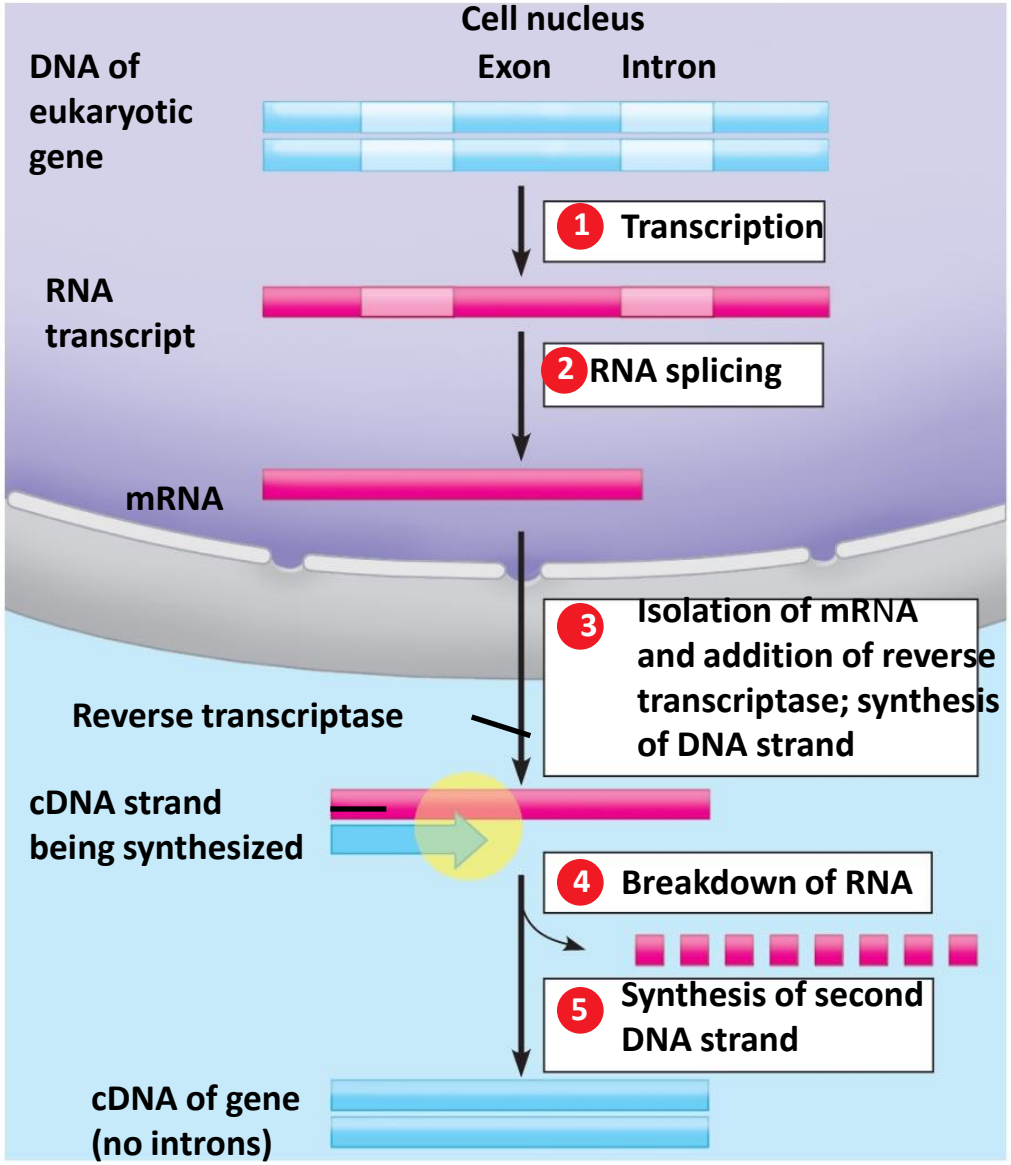
Phage library: genomic DNA is incorporated into bacteriophage DNA

Revealing the Secrets of the Nucleus from mRNA

Reverse transcriptase produces a DNA strand from mRNA

Complementary DNA (**cDNA**) is used to clone eukaryotic genes

DNA polymerase produces the second DNA strand



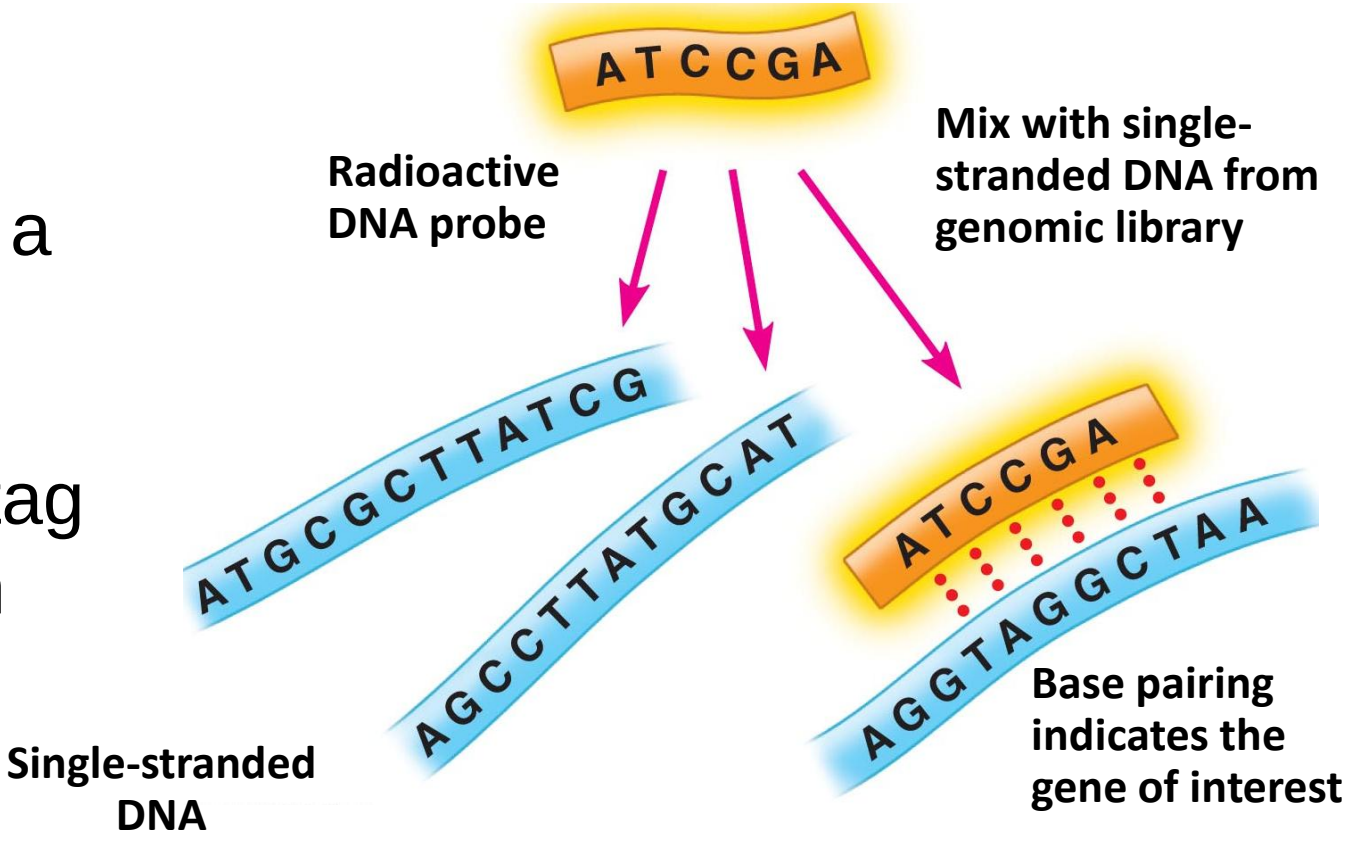
Smaller size is easier to handle

Allows expression in bacterial hosts

Nucleic acid probes are used to find genes

- **Nucleic acid (DNA or RNA) probes** bind to a portion of the gene of interest by base pairing.

- Probes are labeled with a radioactive isotope or fluorescent tag for detection



GENETICALLY MODIFIED ORGANISMS

Why recombine DNA?

- Cells containing recombinant DNA can be used to mass-produce gene products (proteins)
- Variable types of hosts
  - **Prokaryotic host: *E. coli***
    - Produce eukaryotic proteins that do not require post-translational modification
    - Advantages in gene transfer, cell growth, and quantity of protein production
  - **Eukaryotic hosts**
    - **Yeast: *S. cerevisiae***
      - Can produce and secrete complex eukaryotic proteins
    - **Mammalian cells in culture**
      - Can attach sugars to form glycoproteins
    - **"Pharm" animals**
      - Will secrete gene product in milk

DNA technology and medicine

- Diagnosis and treatment of disease
  - Testing for inherited diseases
  - Detecting infectious agents such as HIV

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

DNA technology and medicine

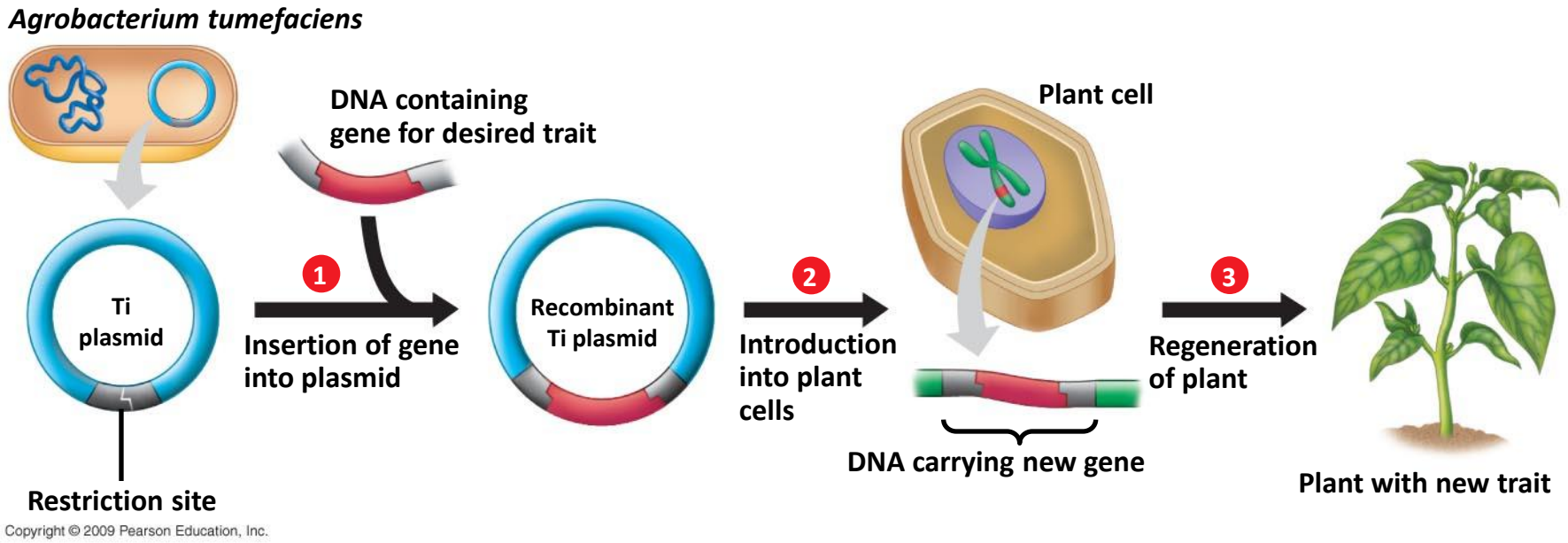
- Advantages of recombinant DNA products
  - Identity to human protein
  - Purity
  - Quantity

Genetically modified organisms and agriculture

- **Genetically modified** (**GM**) organisms contain genes introduced by artificial means if the gene is from a different species the organism is said to be Transgenic.

- GM animals
  - Improved qualities
  - Production of proteins or therapeutics

Genetically modified organisms and agriculture

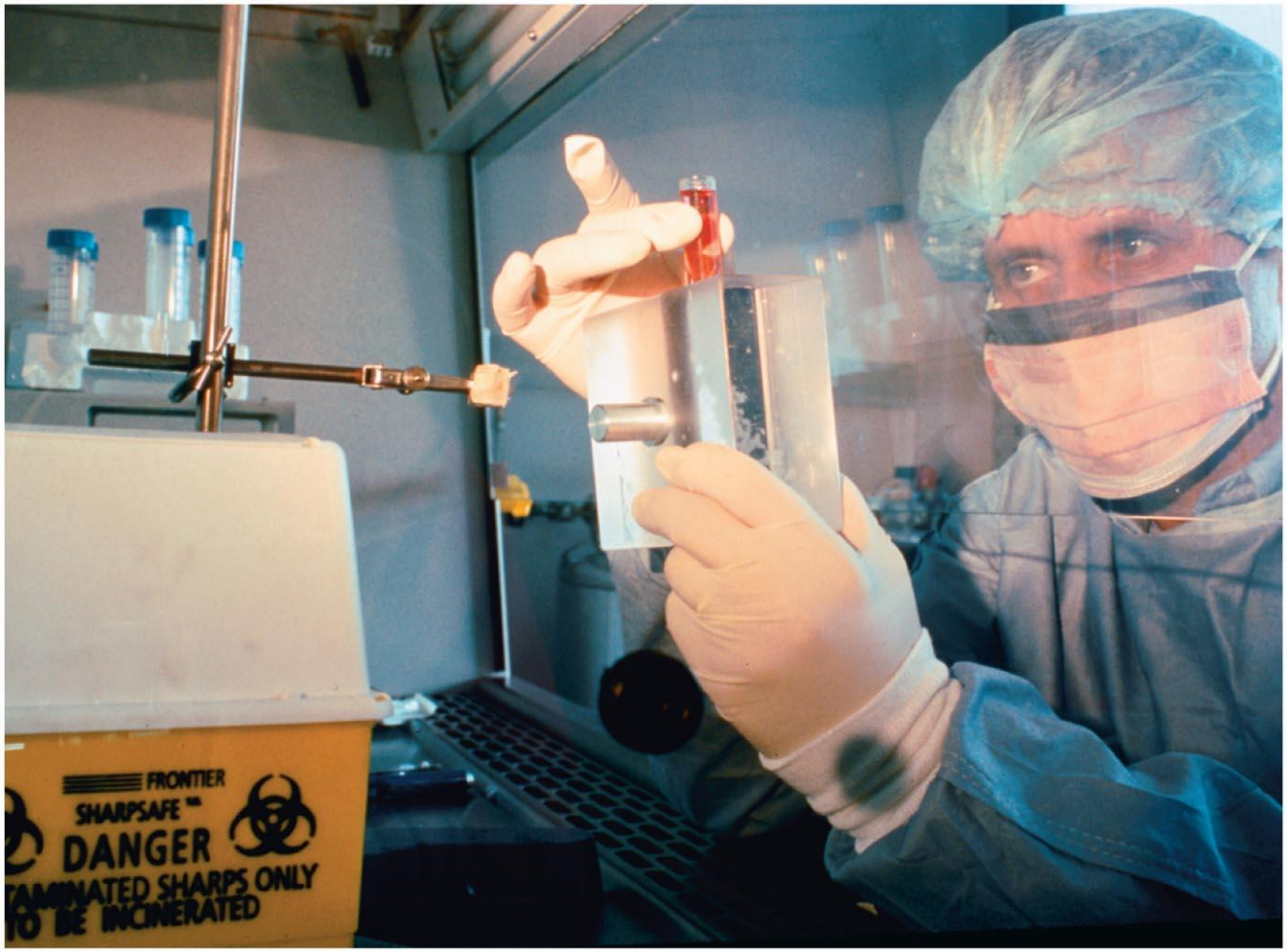


GM plants

- Resistance to herbicides
- Resistance to pests
- Improved nutritional profile

So if we can do it what is the problem?

The possibility of new pathogens



So if we can do it what is the problem?

Concerns related to GM organisms

- **Because you cannot fully control the reproduction of GM organisms they could introduce allergens into the food supply**
- **May spread genes to closely related organisms creating hybrids with native plants**

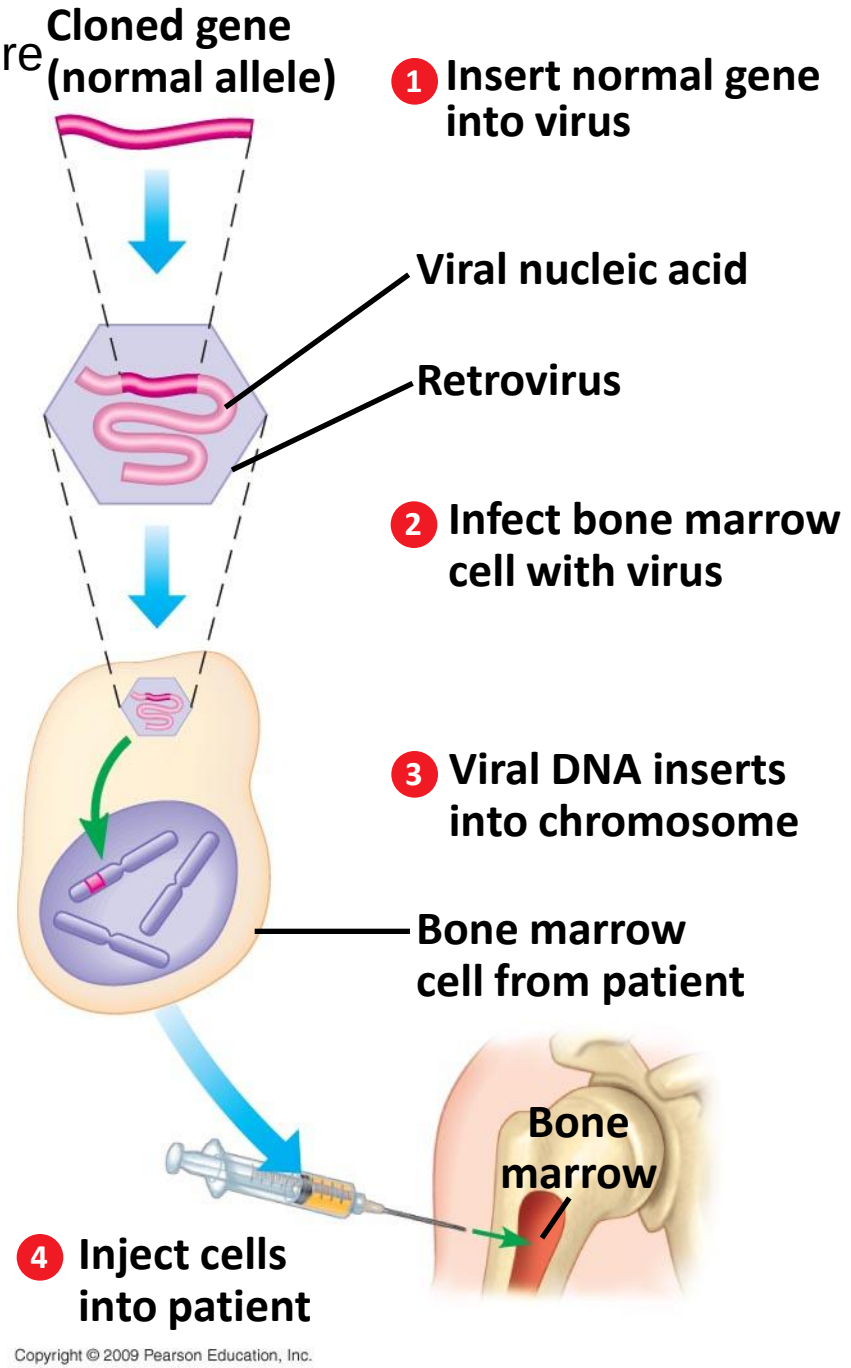
Gene therapy has the potential for treating many of today's chronic medical problems

- **If a disease is due to a defective, non functional gene or a missing gene then Gene therapy holds the possibility of being an effective treatment.**

One possible procedure

Clone the functional allele and insert it in a retroviral vector

Use the virus to deliver the gene to an affected cell type from the patient, such as a bone marrow cell



Viral DNA and the functional allele will insert into the patient's chromosome

Return the cells to the patient for growth and division

Challenges with Gene Therapy

- SCID (severe combined immune deficiency) was the first disease treated by gene therapy
  - First trial in 1990 was inconclusive
  - Second trial in 2000 led to the development of leukemia in some patients due to the site of gene insertion
- Challenges
  - Safe delivery to the area of the body affected by the disease
  - Achieving a long-lasting therapeutic effect
  - Addressing ethical questions