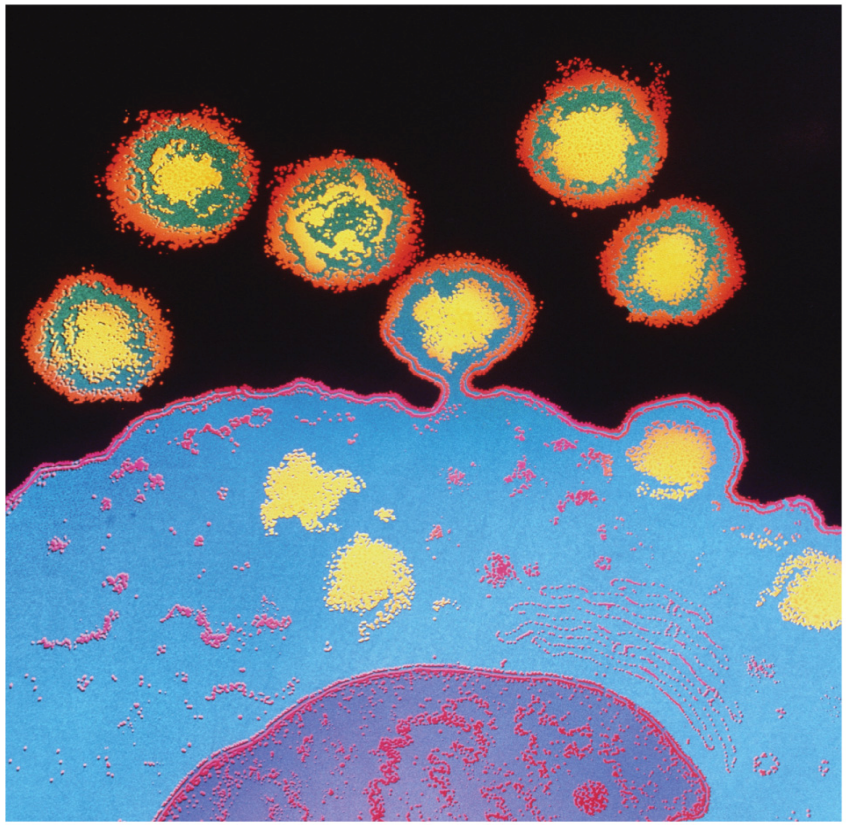
Chapter 9

Biotechnology and DNA Technology



Biotechnology and Recombinant DNA

- **Biotechnology (formally known as genetic engineering)**:
  - the use of microorganisms, cells, or cell components to make a product
  - Eg. = Foods, antibiotics, vitamins, enzymes, hormones
- **Recombinant DNA (rDNA) technology**:
  - insertion of new genes or modification of existing genes to produce desired proteins

Recombinant DNA

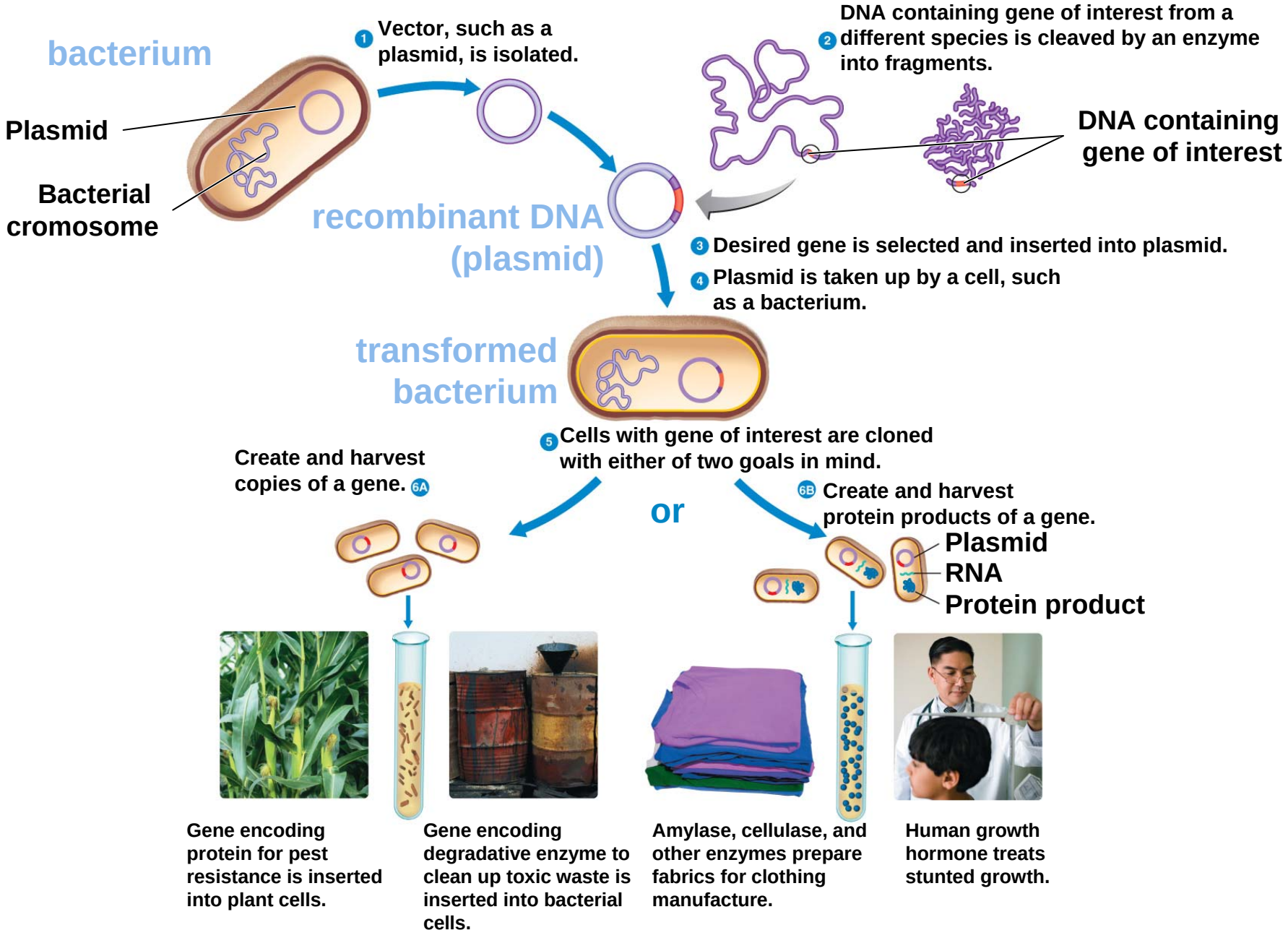
- Closely related micro organisms can exchange genes via “natural recombination”

- Genes can be transferred among “unrelated species” via laboratory manipulation = rDNA

Biotechnology and Recombinant DNA

- **Vector**:
  - DNA “segment” capable of self-replicating
  - Used to carry the desired gene into a new cell
- **Clone**:
  - Population of cells arising from a cell infected by vector
  - Each new cell within clone carries the new gene

A Typical Genetic Modification Procedure.



Some Pharmaceutical Products of rDNA

TABLE 9.2 Some Pharmaceutical Products of rDNA

Product	Comments
α-Glucosidase	Produced by genetically modified mammalian cells to treat Pompe disease
Antitrypsin	Assists emphysema patients; produced by genetically modified sheep
Bone Morphogenic Proteins	Induces new bone formation; useful in healing fractures and reconstructive surgery; produced by mammalian cell culture
Cervical Cancer Vaccine	Consists of viral proteins; produced by *S. cerevisiae* or by insect cells
Colony-Stimulating Factor	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 VII	Treatment of hemorrhagic strokes; produced by mammalian cell culture
Factor VIII	Treatment of hemophilia; improves clotting; produced by mammalian cell culture
Interferon	
IFN–α	Therapy for leukemia, melanoma, and hepatitis; produced by *E. coli* and *S. cerevisiae* (yeast)
IFN–β	Treatment for multiple sclerosis; produced by mammalian cell culture
IFN–γ	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 *E. coli*
Influenza Vaccine	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 OKT3 **Muromonab-CD3**	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 *Komagataella pastoris* (yeast)
Taxol	Plant product used for treatment for ovarian cancer; produced in *E. coli*
Tissue Plasminogen Activator	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*
Veterinary Use	
Canine Distemper Vaccine	Canarypox virus carrying canine distemper virus genes
Feline Leukemia Vaccine	Canarypox virus carrying feline leukemia virus genes

Some Agriculturally Important Products of rDNA Technology

TABLE 9.3 Some Agriculturally Important Products of rDNA Technology

Product	Comments
AGRICULTURAL PRODUCTS	
Bt cotton and Bt corn	Plants have toxin-producing gene from *Bacillus thuringiensis;* toxin kills insects that eat plants.
Genetically modified tomatoes , raspberries	Antisense gene blocks pectin degradation, so fruits have longer shelf life.
Pseudomonas fluorescens bacterium	Has toxin-producing gene from insect pathogen *B. thuringiensis;* toxin kills root-eating insects that ingest bacteria.
Pseudomonas syringae, ice-minus bacterium	Lacks normal protein product that initiates undesirable ice formation on plants.
Rhizobium meliloti bacterium	Modified for enhanced nitrogen fixation.
Round up (glyphosate)-resistant crops	Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.
ANIMAL HUSBANDRY PRODUCTS	
Bovine growth hormone (bGH)	Improves weight gain and milk production in cattle; produced by *E. coli.*
Porcine growth hormone (pGH)	Improves weight gain in swine; produced by *E. coli.*
Transgenic animals	Genetic modification of animals to produce medically useful products in their milk.
OTHER FOOD PRODUCTION PRODUCTS	
Cellulase	Enzyme that degrades cellulose to make animal feedstocks; produced by *E. coli.*
Chymogen	Causes formation of milk curds in cheese-making; produced by *Aspergillus niger.*

Selection and Mutation

- **Selection**: culture a naturally occurring microbe that produces the desired product
- **Mutation**: mutagens cause mutations that might result in a microbe with a desirable trait
- **Site-directed mutagenesis**: change a specific DNA code to change a protein
- Select and culture a microbe with the desired mutation

Restriction Enzymes

- Naturally occurring enzymes in bacteria
- RE cut specific sequences of DNA
- Purpose is to destroy bacteriophage DNA that infect bacterial cells
- Bacteria protects its genes from RE by adding methyl groups to the bacteria's cytosine nucleotides
- Prevents RE from digesting bacterial DNA

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

The role of a restriction enzyme in making recombinant DNA.

1. Restriction enzyme cuts (red arrows) double-stranded DNA at its particular recognition sites, shown in blue.

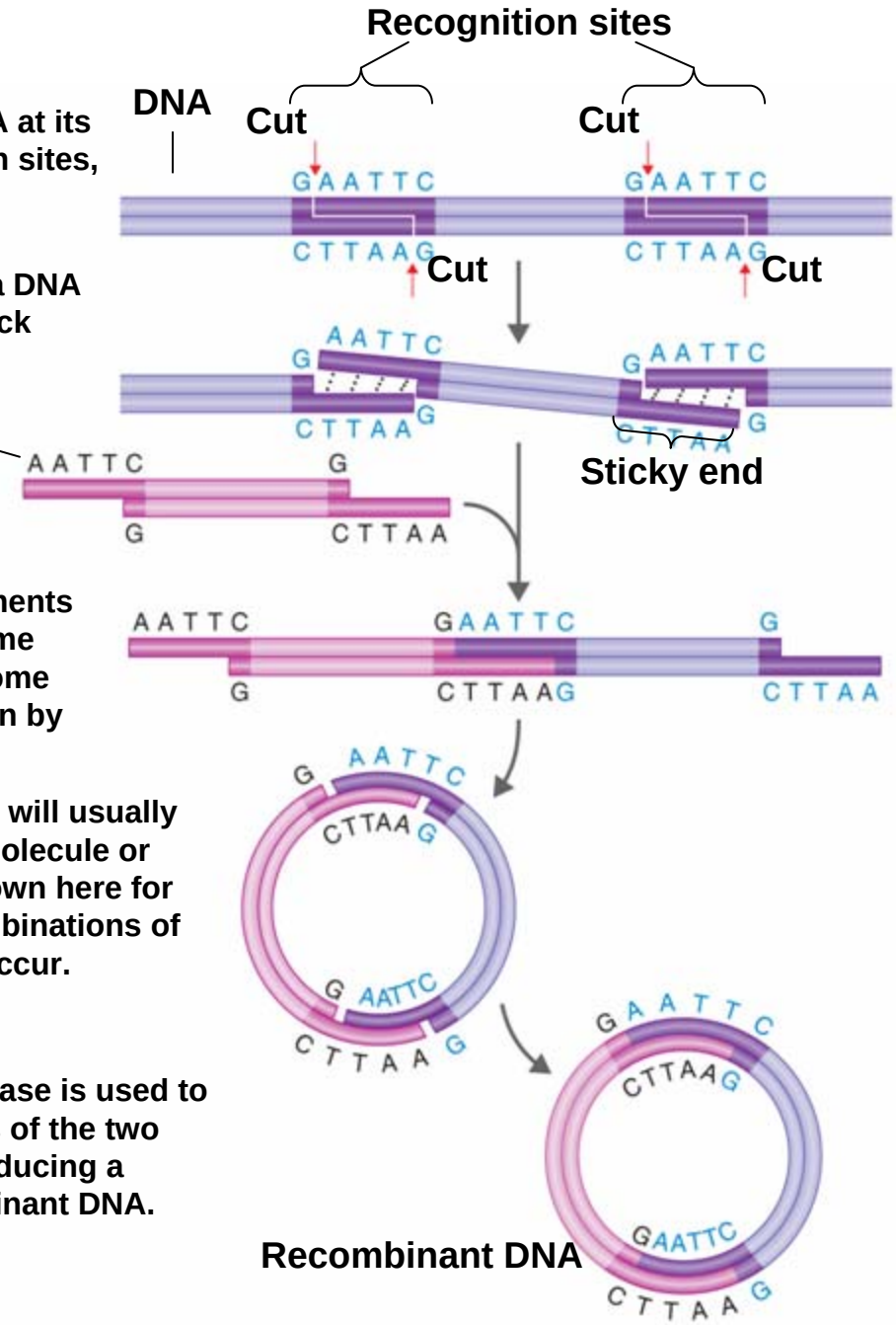
2. These cuts produce a DNA fragment with two stick ends.

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.

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.

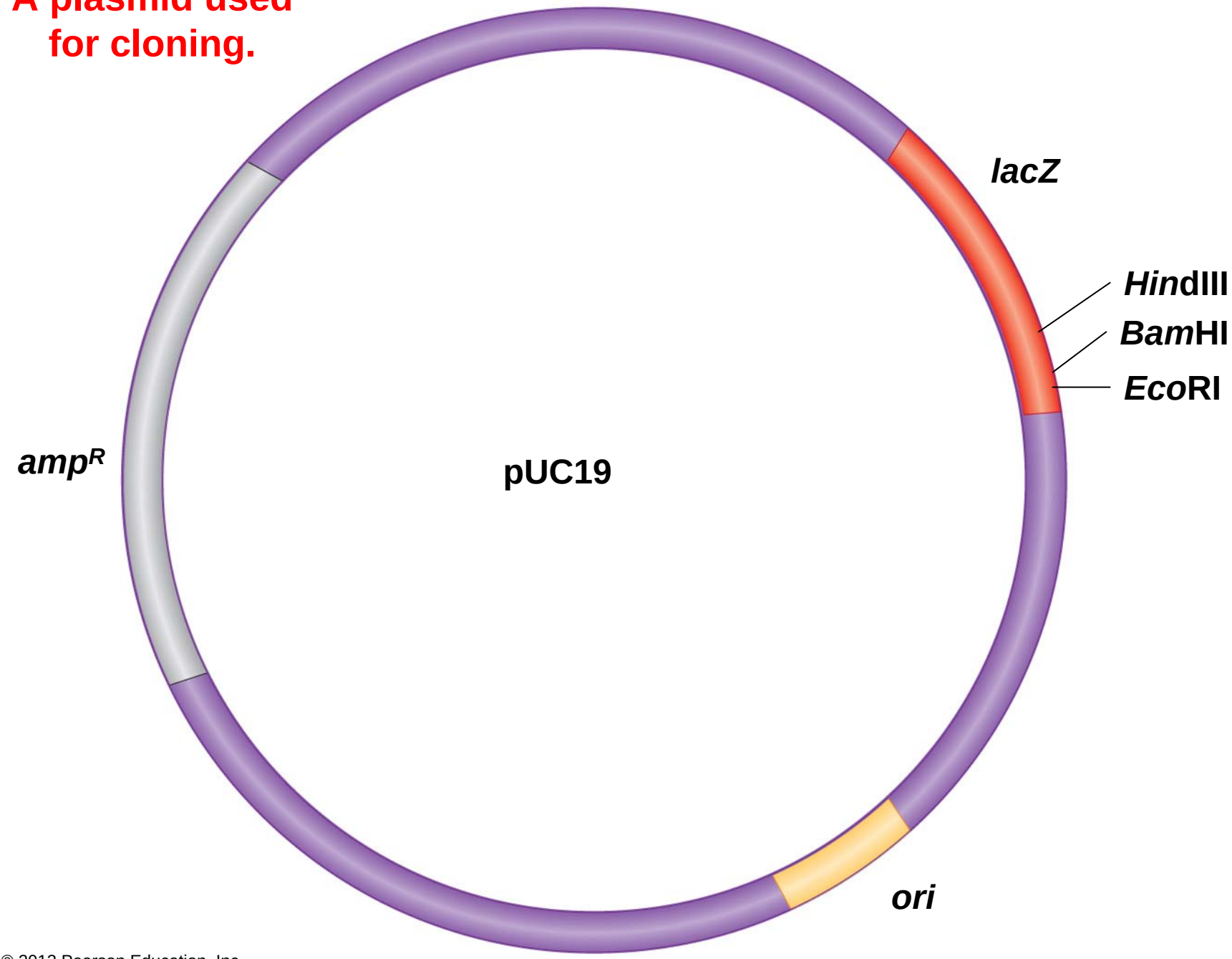
5. The enzyme DNA ligase is used to unite the backbones of the two DNA fragments, producing a molecule of recombinant DNA.



Vectors

- Carry new DNA to desired cell
- DNA segments maybe inserted into a plasmids // plasmid then placed back into microbe to make new product
- Viruses can also be used as vectors
- **Shuttle vectors** // vectors capable of delivering DNA to several different species

A plasmid used for cloning.



Polymerase Chain Reaction (PCR)

- Laboratory technique which makes it possible to make multiple copies of a piece of DNA very rapidly // uses a promoter, enzymes, and heat // Used to:
  - Clone DNA for recombination
  - Amplify DNA to detectable levels
  - Sequence DNA
  - Diagnose genetic disease
  - Detect pathogens

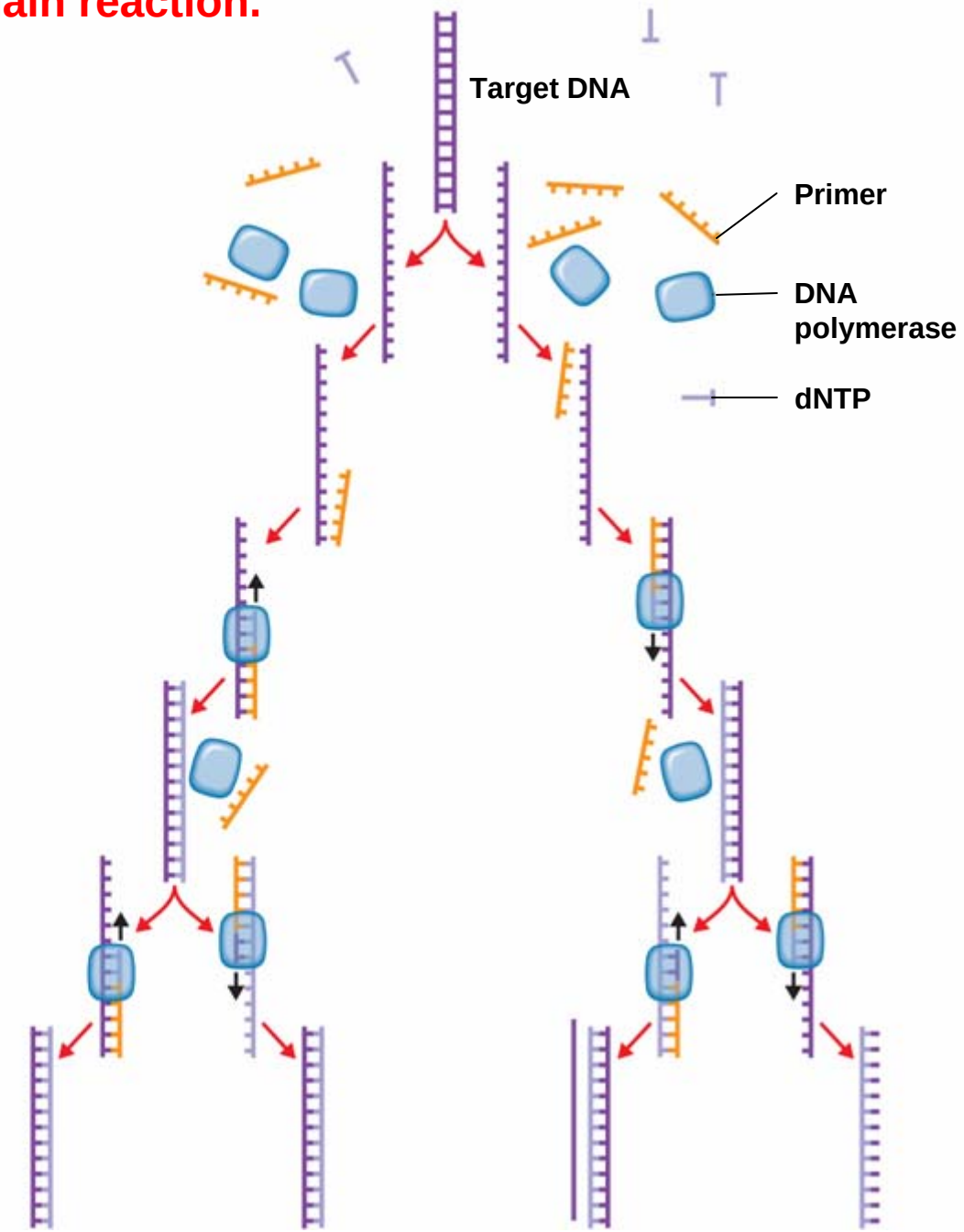
The polymerase chain reaction.

First cycle

1. Incubate target DNA at 94°C for 1 minute to separate the strands.
2. Add primers, nucleotides (deoxynucleotides, dNTP), 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; DNA polymerase copies the target DNA at this temperature.

Second cycle

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



The Human Genome Project

- Human DNA's nucleotides have been sequenced
- **Human Proteome Project** may provide diagnostics and treatments
- **Reverse genetics**: block a gene to determine its function

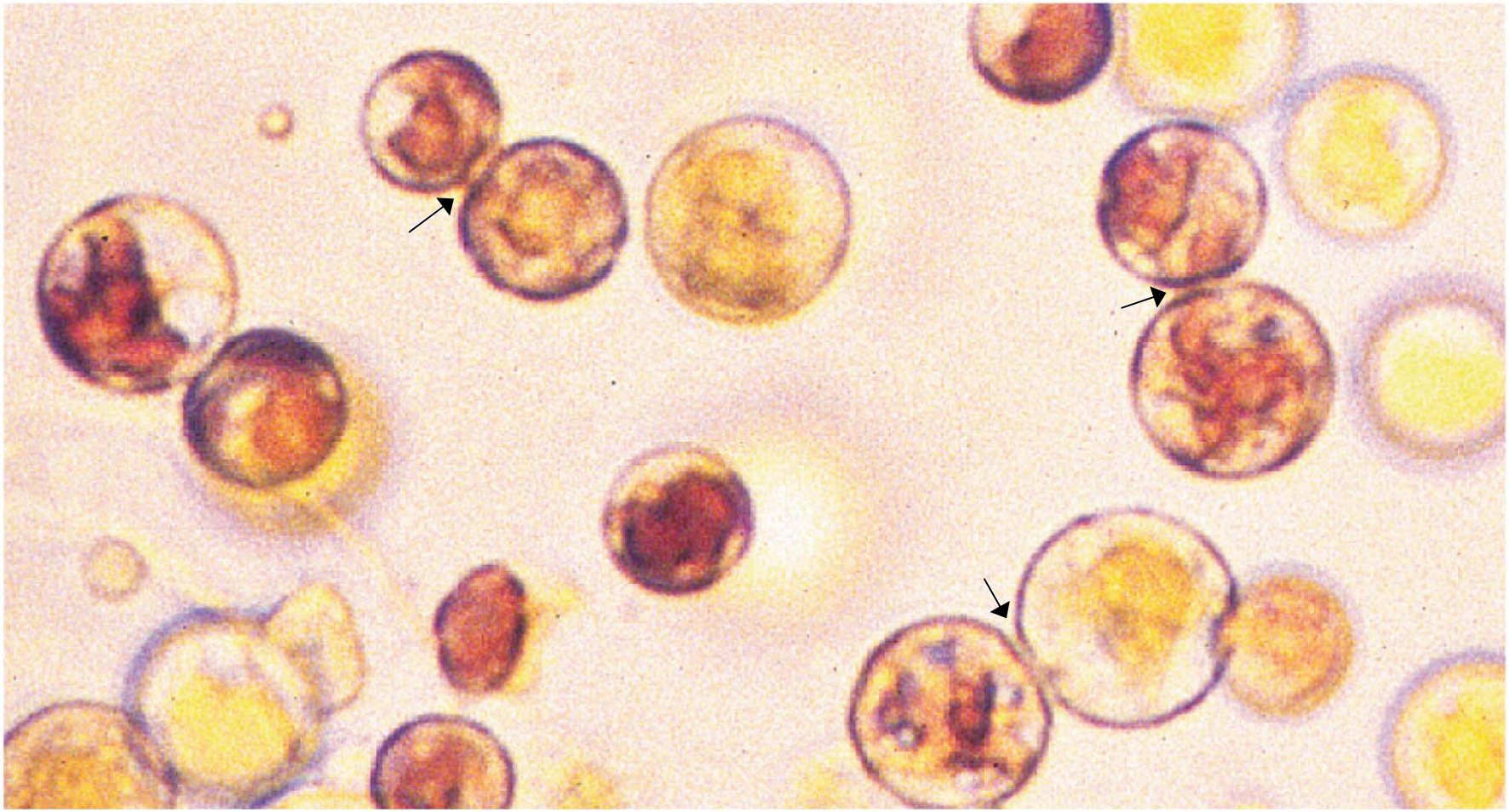
The Human Genome Project's Early Findings

- Human genome approximately 3 billion nucleotide pairs
- 20,000 to 25,000 genes
- Less than 2% of genes code for functional products
- Other 98% of genome = miRNA genes, viral remnants, repetitive sequences (short tandem repeats), introns, transposons, and chromosomal ends (telomeres).

Inserting Foreign DNA into Cells

- DNA can be inserted into a cell by:
  - **Electroporation**
  - **Transformation**
  - **Protoplast fusion**

Protoplast fusion.

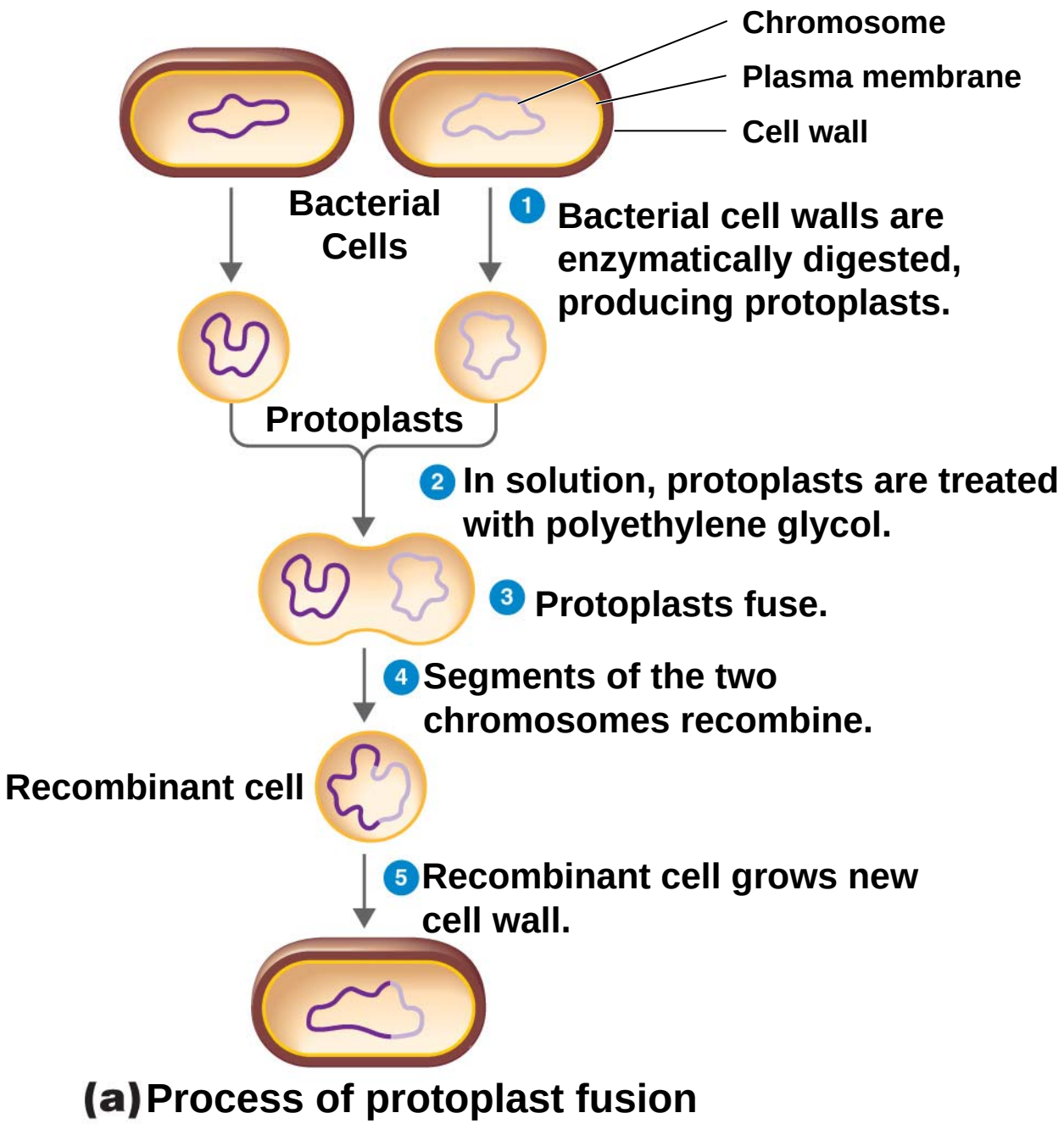


(b) Algal protoplasts fusing

LM

10 μm

Protoplast fusion.



(a) Process of protoplast fusion

Inserting Foreign DNA into Cells

- DNA can be inserted into a cell by:
  - **Gene gun**
  - **Microinjection**

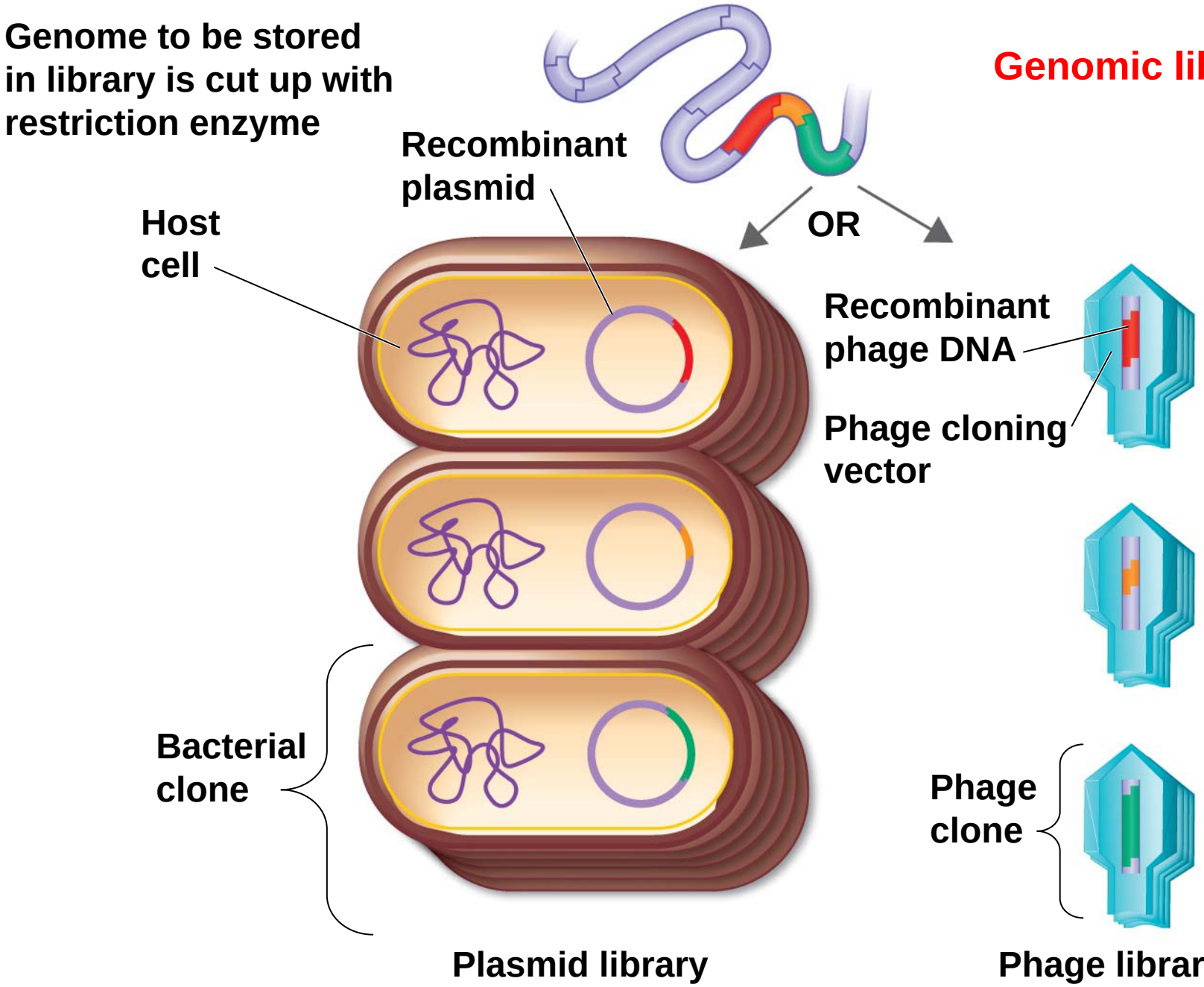
A gene gun, which can be used to insert DNA-coated “bullets” into a cell.

The microinjection of foreign DNA into an egg.

Obtaining DNA

- **Genomic libraries** are made of pieces of an entire genome stored in plasmids or phages

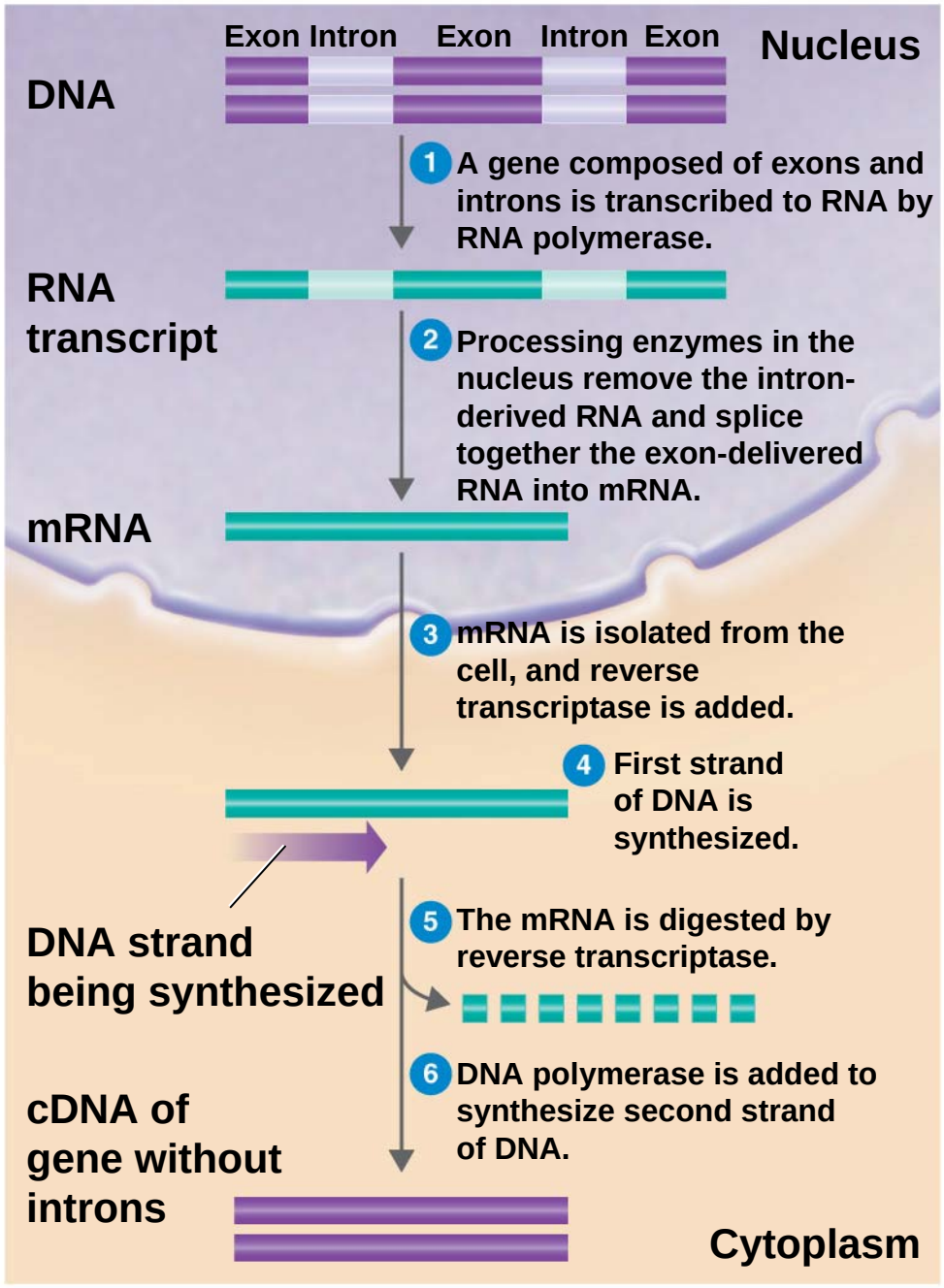
Genomic libraries.



Obtaining DNA

- **Complementary DNA (cDNA)** is made from mRNA by reverse transcriptase

Making complementary DNA (cDNA) for a eukaryotic gene.



Obtaining DNA

- **Synthetic DNA** is made by a DNA synthesis machine

A DNA synthesis machine.

Blue-white screening, one method of selecting recombinant bacteria.

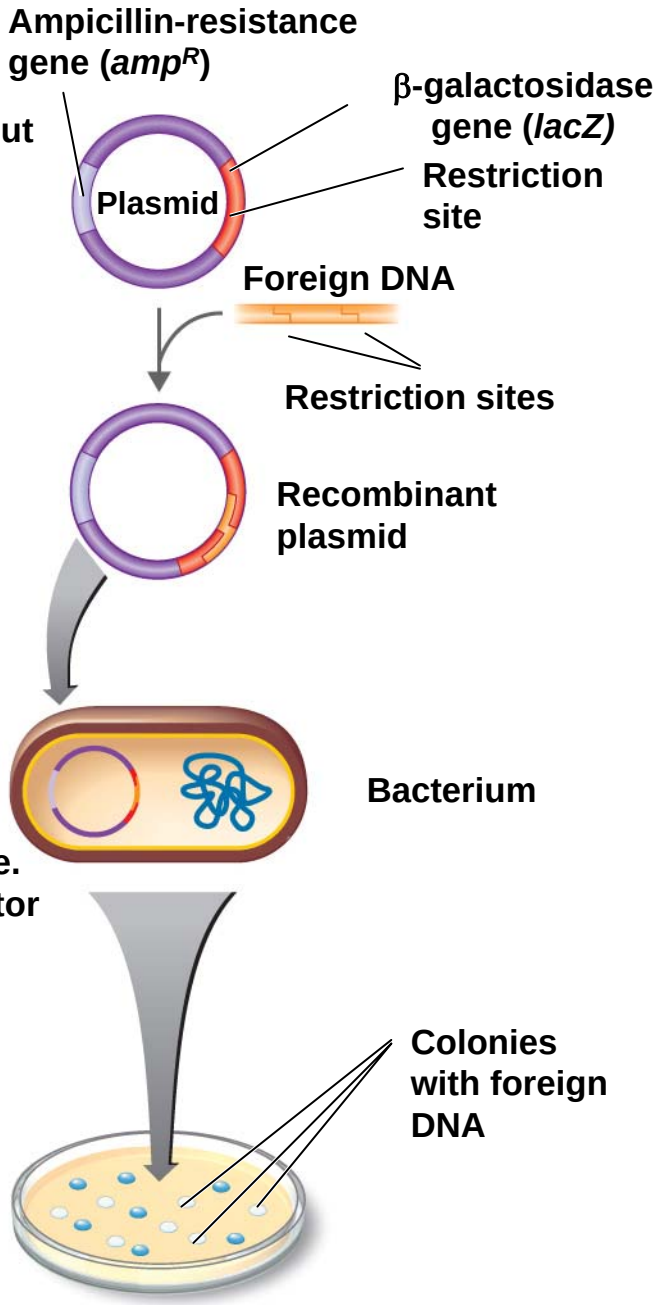
1. Plasmid DNA and foreign DNA are both cut with the same restriction enzyme. The plasmid has the genes for lactose hydrolysis (the *lacZ* gene encodes the enzyme β-galactosidase) and ampicillin resistance.

2. Foreign DNA will insert into the *lacZ* gene. The bacterium receiving the plasmid vector will not produce the enzyme β-galactosidase if foreign DNA has been inserted into the plasmid.

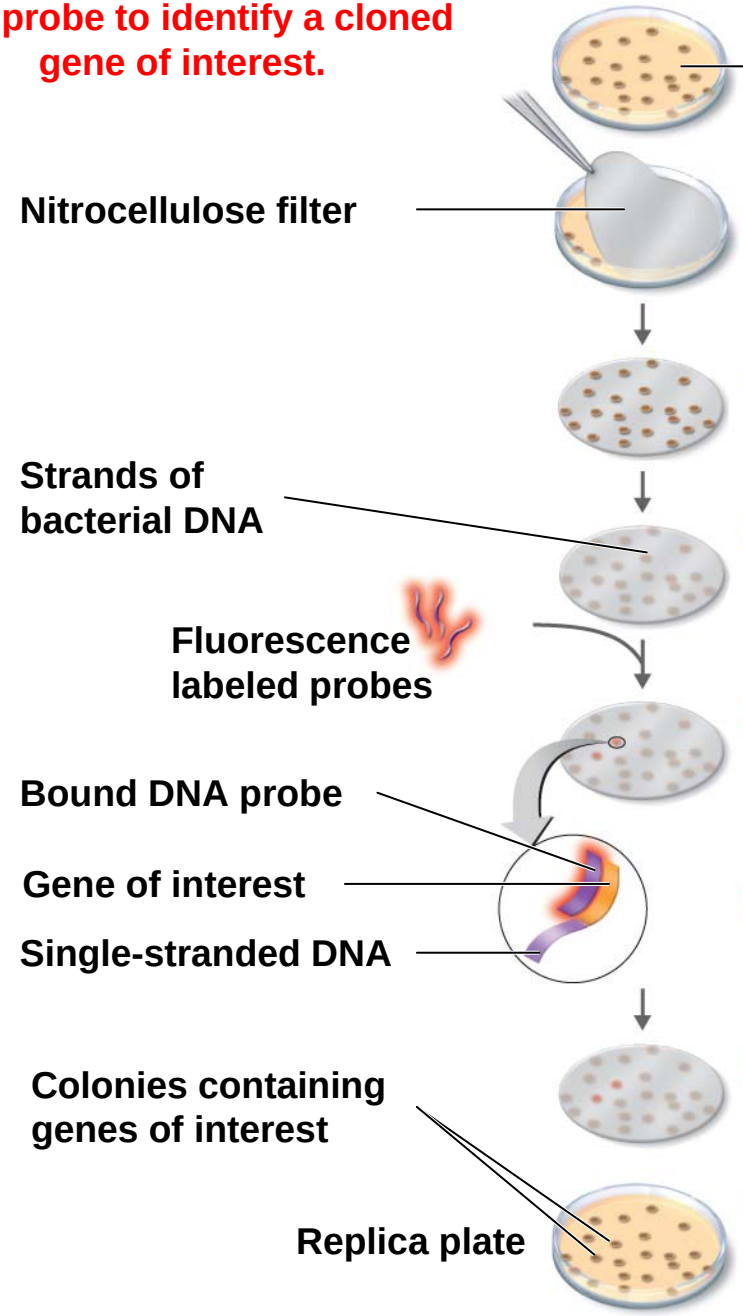
3. The recombinant plasmid is introduced into a bacterium, which becomes ampicillin resistant.

4. Foreign DNA will insert into the *lacZ* gene. The bacterium receiving the plasmid vector will not produce the enzyme β-galactosidase if foreign DNA has been inserted into the plasmid.

5. Only bacteria that picked up the plasmid will grow in the presence of ampicillin. Bacteria that hydrolyze X-gal produce galactose and an indigo compound. The indigo turns the colonies blue. Bacteria that cannot hydrolyze X-gal produce white colonies.



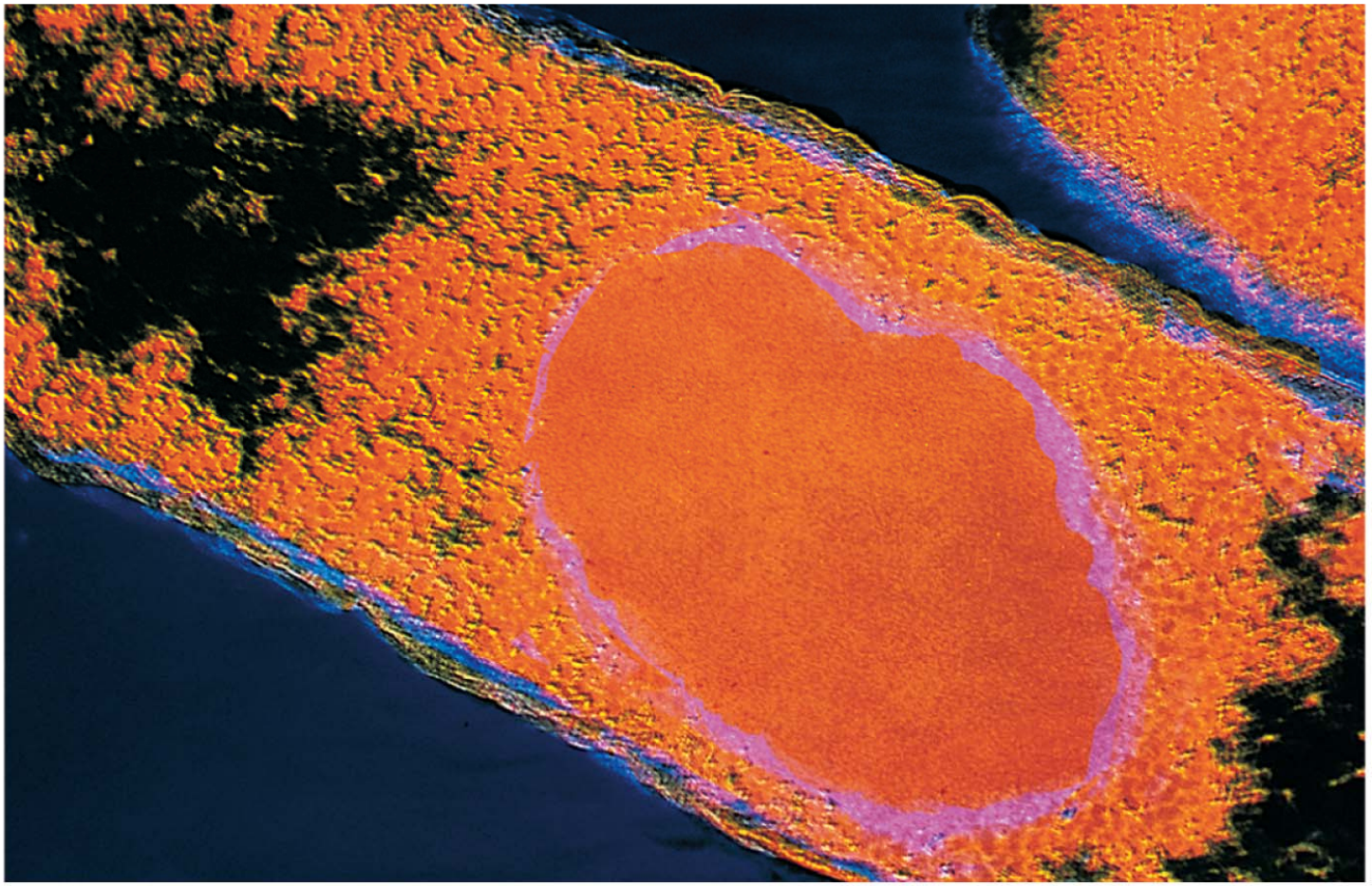
Colony hybridization: using a DNA probe to identify a cloned gene of interest.



Making a Product

- *E. coli*
  - Used because it is easily grown and its genomics are known
  - Need to eliminate endotoxin from products
  - Cells must be lysed to get product

***E. coli* genetically modified to produce gamma interferon, a human protein that promotes an immune response.**



Making a Product

- ***Saccharomyces cerevisiae* //** Used because it is easily grown and its genomics are known // May express eukaryotic genes easily

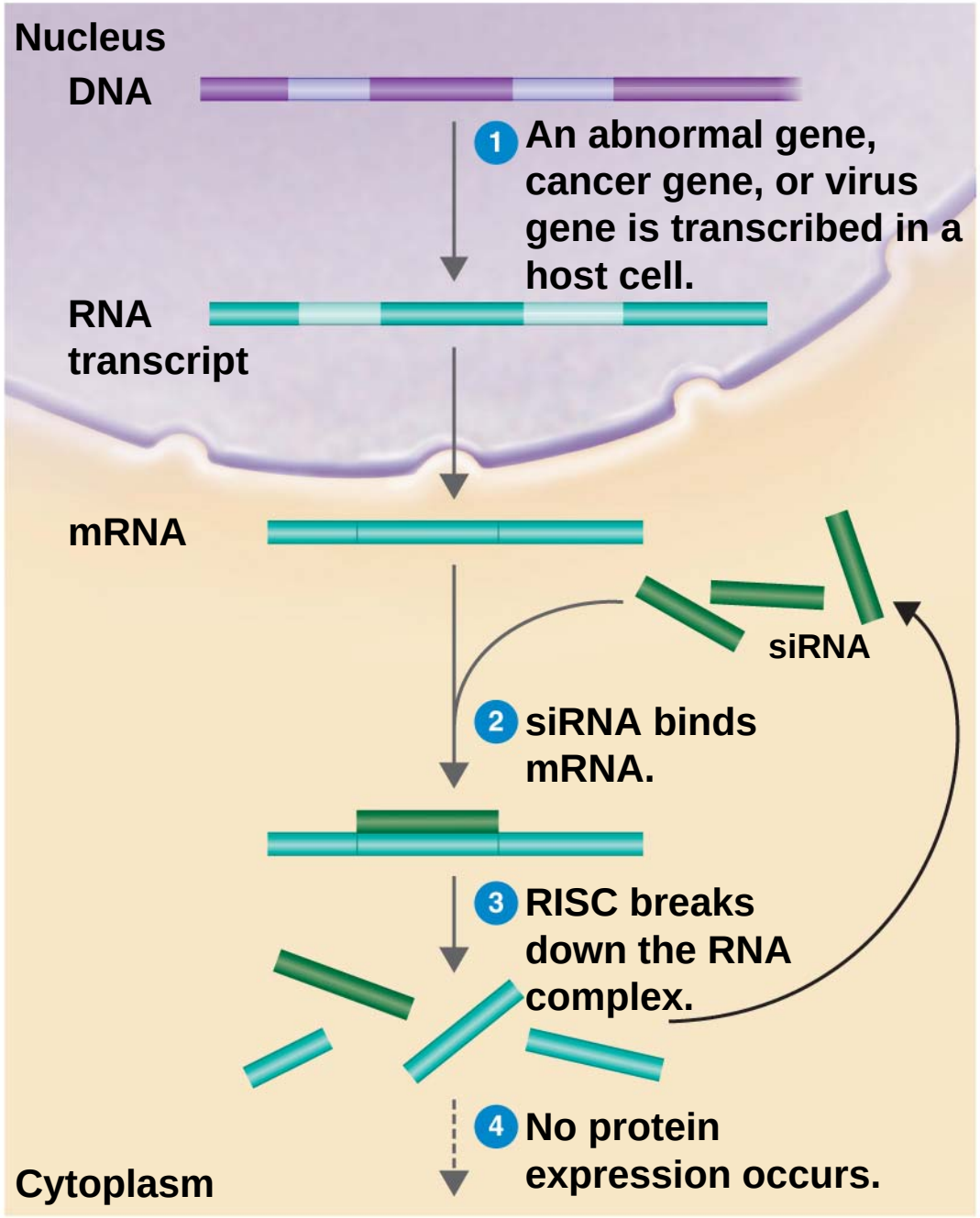
- **Plant cells and whole plants //** May express eukaryotic genes easily // Plants are easily grown

- **Mammalian cells //** May express eukaryotic genes easily // Harder to grow

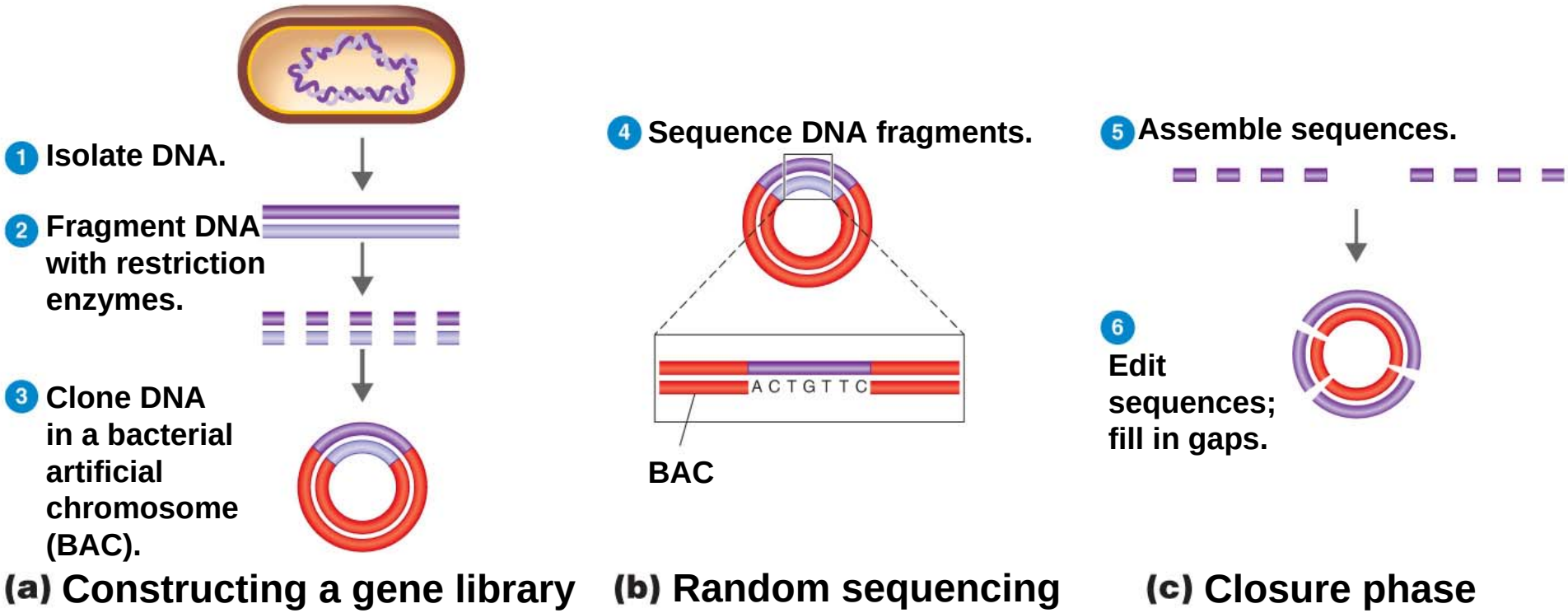
Therapeutic Applications

- **Human enzymes** and other proteins
- **Subunit vaccines**
- Nonpathogenic viruses carrying genes for pathogen's antigens as **DNA vaccines**
- **Gene therapy** to replace defective or missing genes

Gene silencing could provide treatments for a wide range of diseases.



Shotgun sequencing.



Scientific Applications

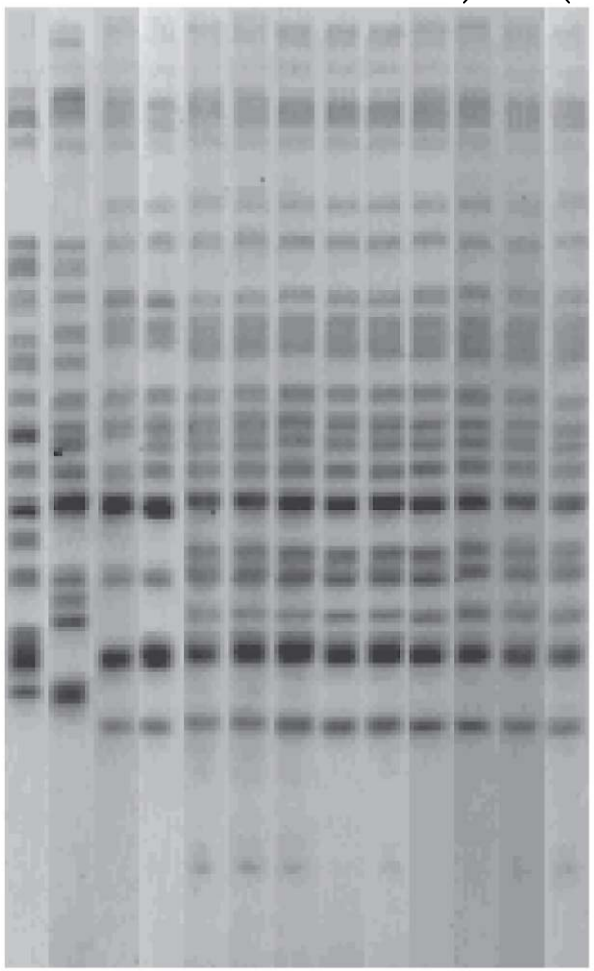
- Understanding DNA
- Sequencing organisms’ genomes
- DNA fingerprinting for identification

DNA fingerprints used to track an infectious disease.

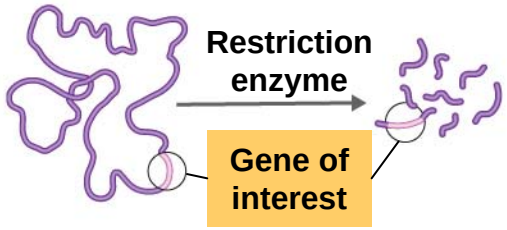
E. coli **isolates from patients whose infections were not juice related**

E. coli **isolates from patients who drank contaminated juice**

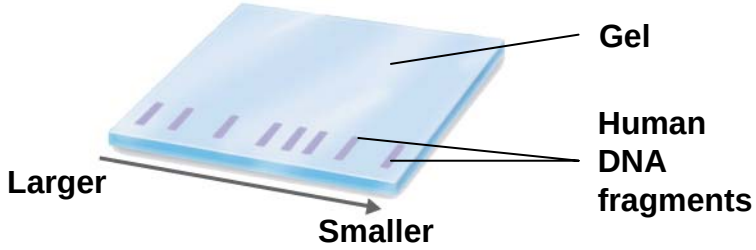
Apple juice isolates



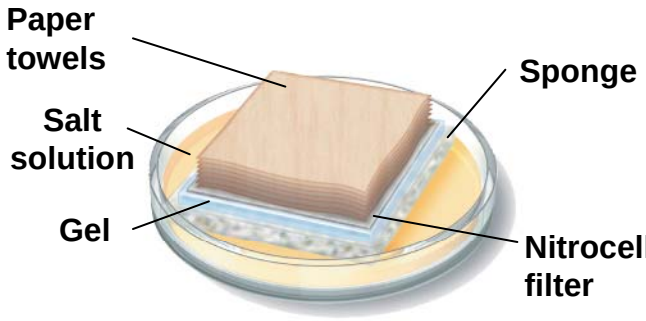
Southern blotting.



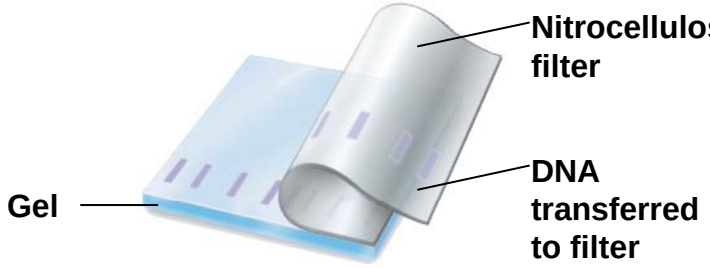
1. DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.



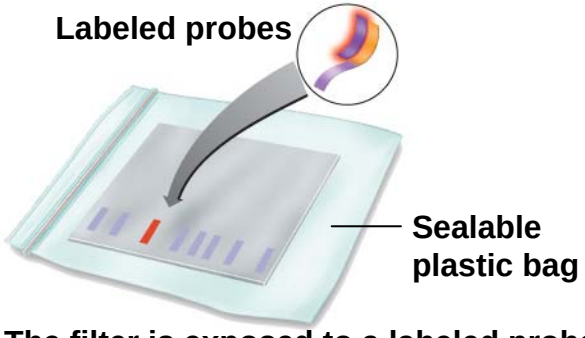
2. The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



3. The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels by capillary action.



4. This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.



5. The filter is exposed to a labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.

6. The fragment containing the gene of interest is identified by a band on the filter.

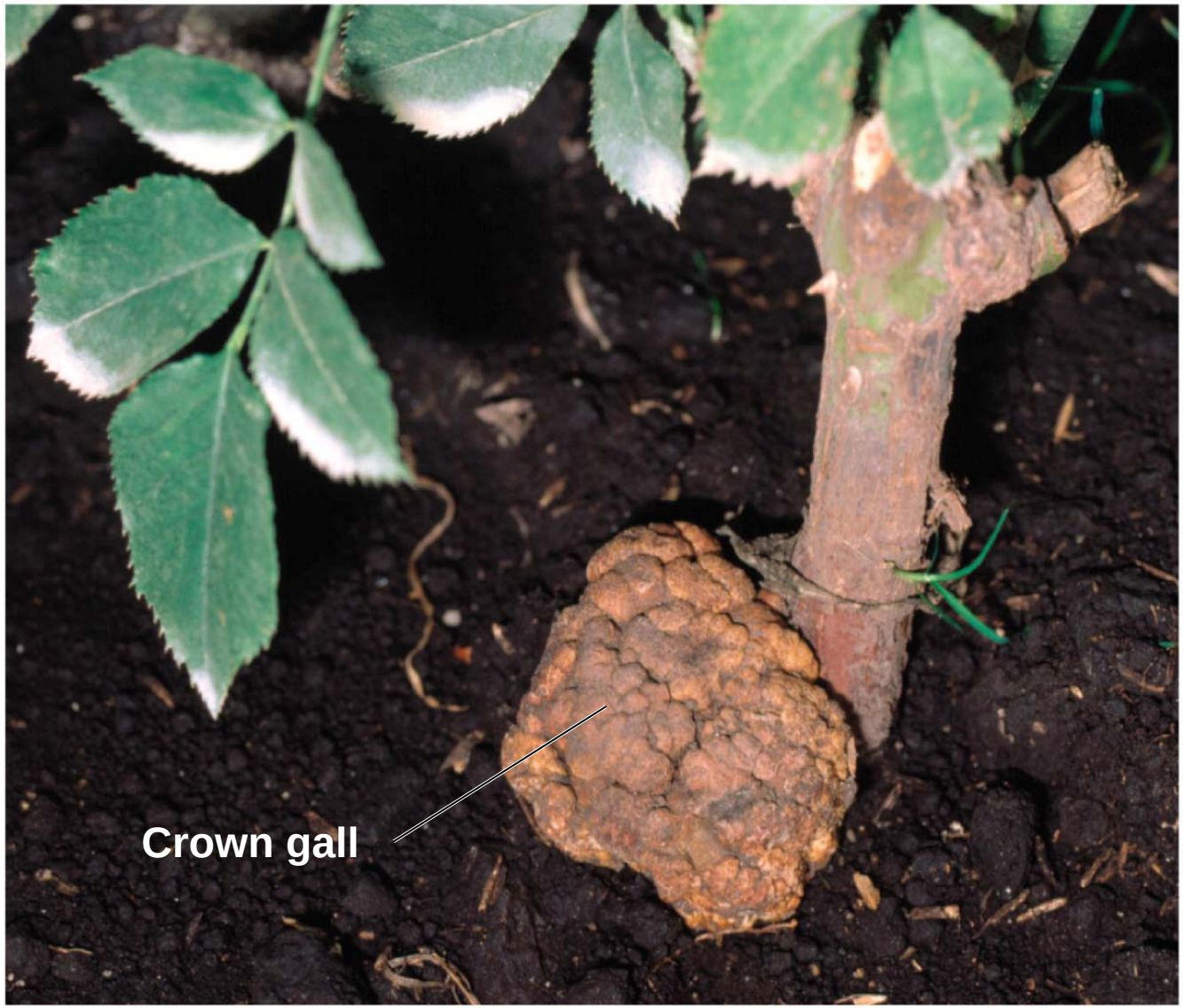
Forensic Microbiology

- PCR
- Primer for a specific organism will cause application if that organism is present
- **Real-time PCR**: newly made DNA is tagged with a fluorescent dye; the levels of fluorescence can be measured after every PCR cycle
- **Reverse-transcription (RT-PCR)**: reverse transcriptase makes DNA from viral RNA or mRNA

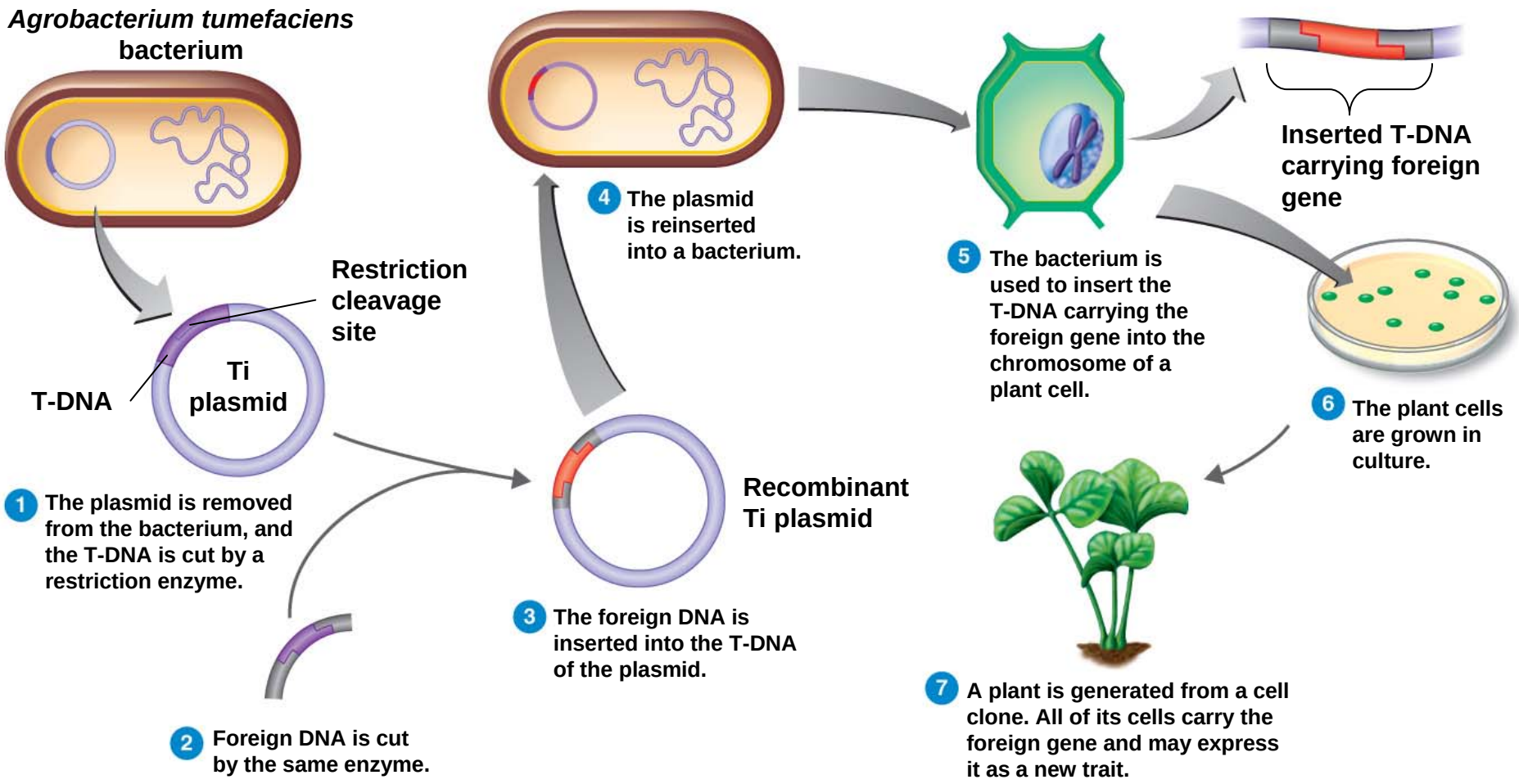
Forensic Microbiology

- Differs from medicine because it requires:
  - Proper evidence collection
  - Establishing chain of custody
    - Rape conviction
    - Tracing HIV to a physician who injected it
    - Anthrax in U.S. Mail

Crown gall disease on a rose plant.



Using the Ti plasmid as a vector for genetic modification in plants.



Safety Issues and Ethics of Using rDNA

- Need to avoid accidental release
- Genetically modified crops must be safe for consumption and for the environment
- Who will have access to an individual’s genetic information?