DNA Mapping & Recombinant DNA Technology

Dr. Kalsoom Tariq

Learning Objectives:

- What is DNA/Gene mapping?
- What is recombinant DNA technology?
- An Overview of Recombinant DNA Technology
- Tools for Genetic engineering including enzymes,
- Vectors & Hosts for DNA recombinant technology
- Methods to Insert the naked DNA into a host cell
- Blue-white screening system
- Screening for the desired Gene
- Copying the genetic material of interest - PCR
- Applications of recombinant DNA technology

What do we know?

1. **Mutation & Recombination:** Genetic mutation and recombination provide a diversity of organisms.

   – The process of natural selection allows the growth of those best adapted to a given environment.

2. **genetic modification**: Microorganisms can exchange genes in a process of natural DNA recombination.

DNA or Gene mapping

Mapping - determining the location of elements within a genome, with respect to identifiable landmarks.

Types of mapping…with tools/resources utilized

- ❑ **Genetic mapping – linear description of DNA markers/genes on a given chromosome with closely placed markers being inherited together more often.**
  - linkage mapping
  - pedigree
  - polymorphic markers
- ❑ **Physical mapping – physical location on the chromosome, relating more towards exact positioning of gene elements.**
  - cytogenetic mapping
  - somatic cell mapping
  - radiation hybrid mapping
  - restriction mapping - *PFGE, BAC contigs, sequencing*
- ❑ **Comparative mapping**
  - **gene sequences**
  - **databases**
  - **DNA chips**

GENOME/GENETIC MAPS - Graphic representation of the relative positions of genes on a DNA sequences.

What does genome-mapping tell us?

- According to the Human Genome Project, there are estimated to be over 20,500 human genes. **Genome** refers to an organism's complete set of DNA, which includes all its genes and **mapping these genes** simply means finding out the location of these genes in a chromosome.

- In humans, each cell consists of 23 pairs of chromosomes for a total of 46 chromosomes, which means that for 23 pairs of chromosomes in each cell, there are roughly 20,500 genes located on them.

- Genome mapping, therefore, essentially means figuring out the **location of a specific gene** on a particular region of the chromosome and also determining the location of and **relative distances between other genes** on that chromosome.

- Significantly, genome mapping enables scientists to gather evidence if a disease transmitted from the parent to the child is **linked to one or more genes**. Furthermore, mapping also helps in determining the **particular chromosome** which contains that gene and the **location of that gene** in the chromosome.

GENE MAPPING VERSUS GENE SEQUENCING

GENE MAPPING	GENE SEQUENCING
Charting of the positions of genes on a DNA molecule or chromosome and the distance, in linkage units or physical units between genes	Process of ascertaining the sequence of nucleotides in a segment of DNA
Identifies the landmarks of the genome	Spells out the sequence of nucleotides in the genome
Outcome is less detailed	Outcome is fully detailed
A cheap method, which is less time-consuming	An expensive method, which takes time

Recombinant DNA Technology:

- **Recombinant DNA technology** is genetic engineering ,which involves artificial modification of the genetic constitution of a living cell, by introduction of foreign DNA through experimental techniques.
- The technique involves the **splicing of DNA** by restriction endonucleases, **preparation of chimeric DNA molecule**, followed by **cloning** for the production of large number of identical target DNA molecules .
  - **Recombinant DNA** - DNA that has been artificially manipulated to combine genes from **two different** sources.
  - **Genetic engineering -** human manipulation of an organism's genetic material in a way **that does not occur under natural conditions.**

An Overview of Recombinant DNA Technology

1. **Gene of interest (DNA)** is isolated

   (DNA fragment)

2. **A desired gene** is inserted into a DNA molecule - **vector**

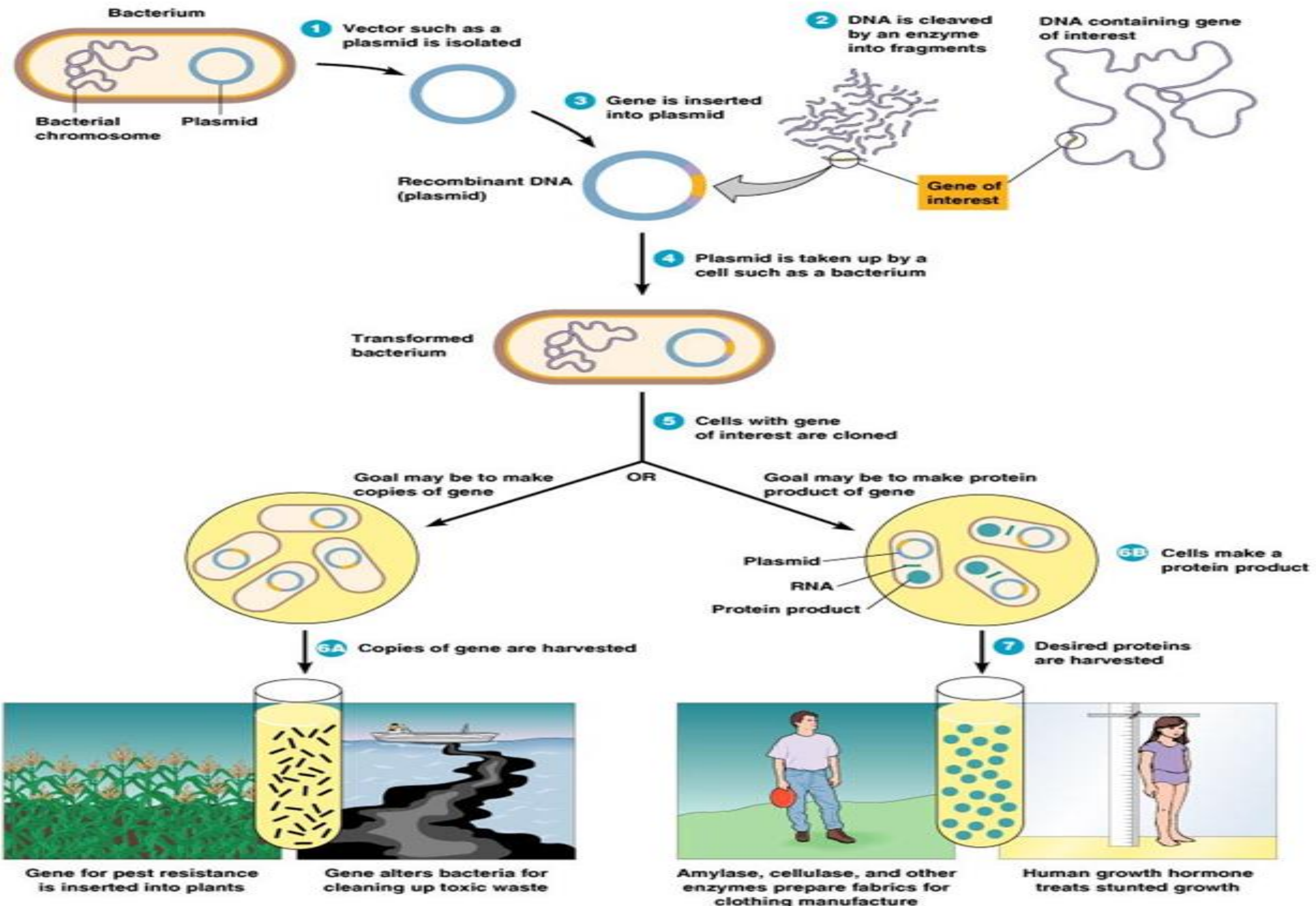
   (plasmid, bacteriophage , a viral genome or a cosmid)

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**.

Figure 9.1.2


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
Transformed bacterium
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 Desired proteins are harvested
Gene for pest resistance is inserted into plants
Gene alters bacteria for cleaning up toxic waste
Amylase, cellulase, and other enzymes prepare fabrics for clothing manufacture
Human growth hormone treats stunted growth

Tools of Recombinant DNA Technology

- The various biological tools used to bring about genetic manipulations are:
- a. Enzymes: including Restriction endonucleases & ligases.
- b. Passenger DNA: Foreign DNA (insert DNA fragment) which is passively transferred from one cell to another cell or organ is known as passenger DNA **(Foreign DNA)**
- c. Vector or vehicle DNA: The DNA which acts as the carrier is known as the vector or vehicle DNA

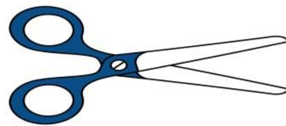
Tools for Genetic engineering

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

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
*Hind*III	*Haemophilus influenzae*	A↓A G C T T T T C G A↑A

Restriction Enzymes

- Fragments of DNA produced by the same restriction enzyme will spontaneously join by **base pairing**.

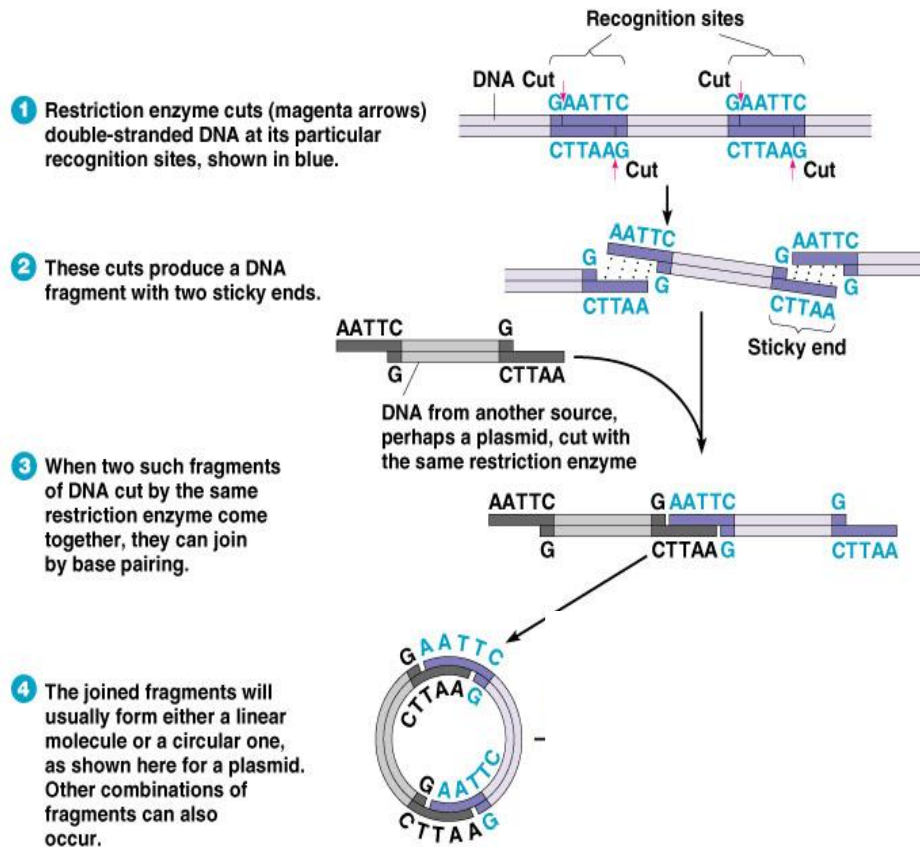
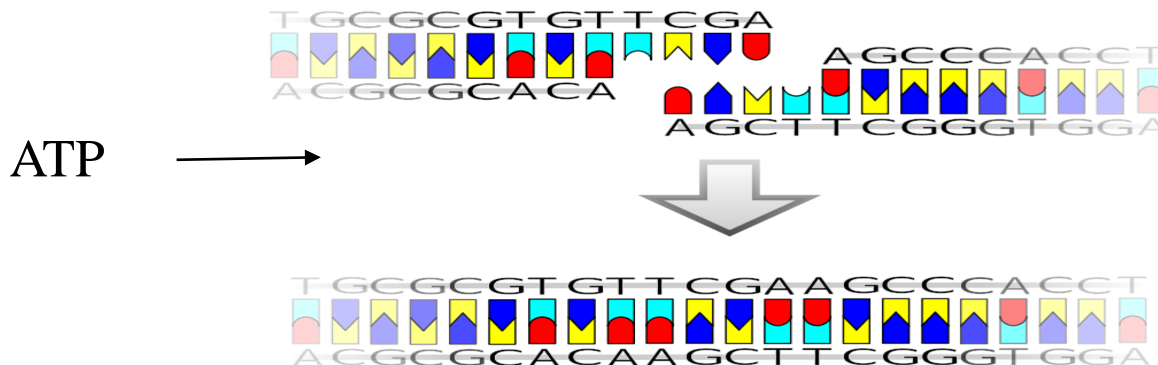


Figure 9.2

Tools for Genetic engineering
2. Ligase

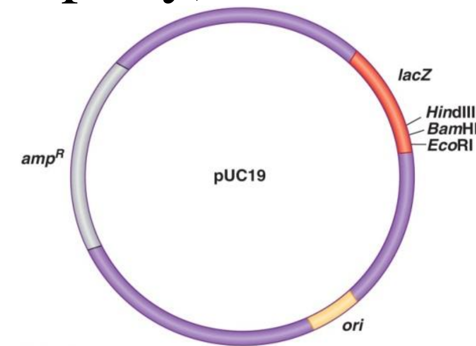
- **DNA ligase** is a enzyme that can link together DNA strands that have double-strand breaks (a break in both complementary strands of DNA).
  - Naturally DNA ligase has applications in both **DNA replication** and **DNA repair .**
  - Needs ATP
- DNA ligase has extensive use in molecular biology laboratories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



Tools for Genetic engineering

3. Vectors

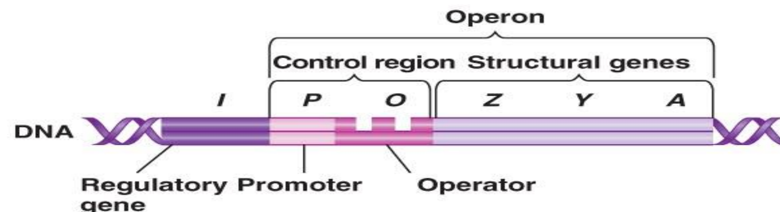
- **Vectors** - small pieces of DNA used for cloning (the gene to be inserted into the genetically modified organism must be combined with other genetic elements in order for it to work properly)
- **Requirements of the Vector**



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

2. Cloning site (site for recognition of restriction nucleases)

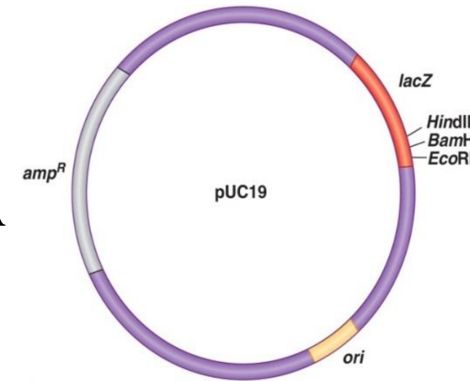
3. Promoter (and operator) - to support the gene (new DNA) expression in the host



4. Selectable marker – vector contains the genes for antibiotic resistance

5. Proper size

Vectors



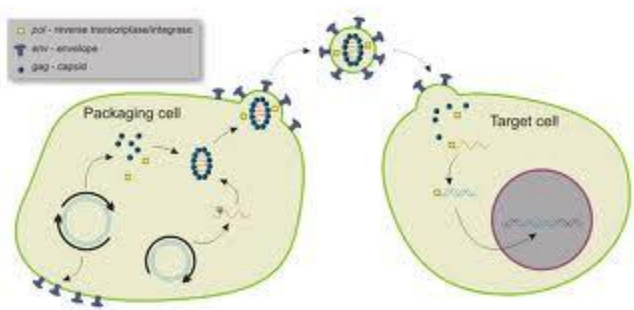
1. Plasmid vectors

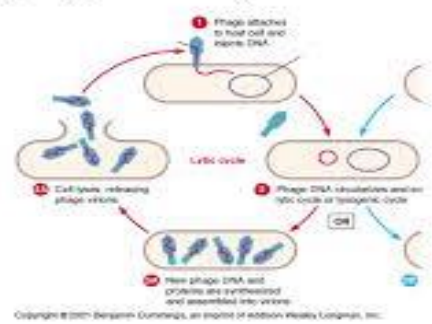
- Plasmids are **self-replicating circular** molecules of DNA
- Encode antibiotic resistance (selection marker)

  and can accept short DNA pieces about 6 to 10 kb long.

2. Viral vectors - **retroviruses, adenoviruses and herpes viruses**

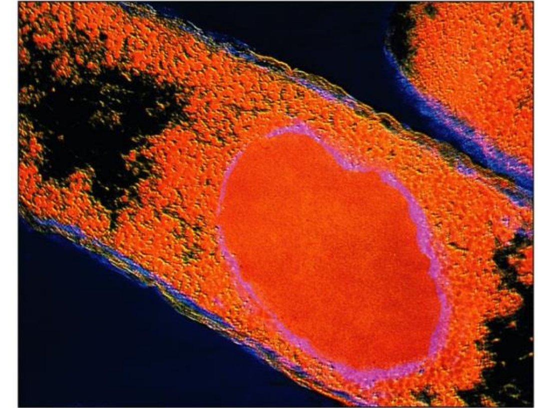
- Accept much larger pieces of DNA,
- they can accept DNA fragments 10 to 20 kb long





3. Cosmids: These are specialized plasmids(of λ phages), can accept very large DNA fragments 35 to 50kb.

Hosts for DNA recombinant 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.

Methods to Insert the naked DNA into a host cell

1.Transformation

* treatment make cells competent to accept foreign DNA ($CaCl_2$ make pores in cell membrane)

2. Electroporation

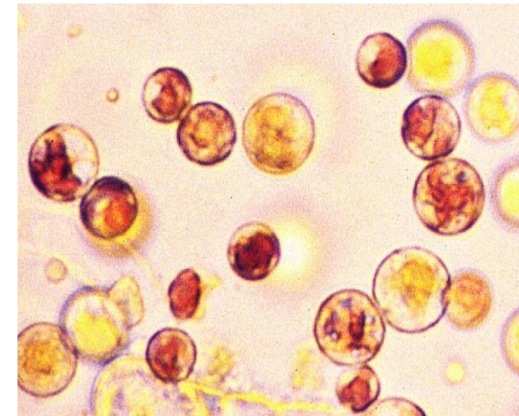
*use electrical current to form microscopic pores in the membranes of cell

3. Protoplast fusion

– yeast, plants and algal cells

4. Microinjection

5. Gene gun



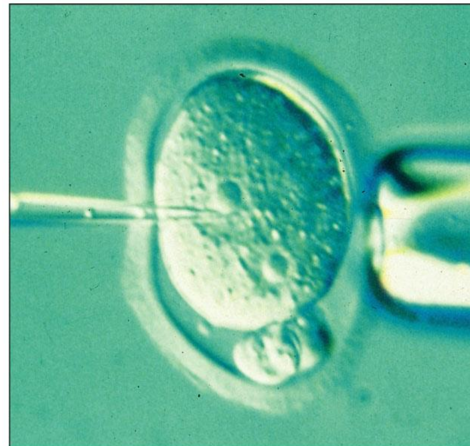


Figure 9.5b

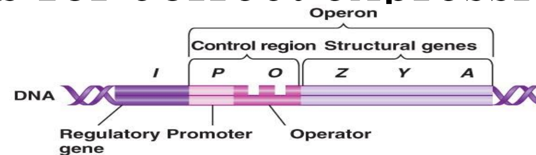
Recombinant DNA technology - Cloning

A process of producing genetically modified organisms

A multi-step process.

1. **Isolating** and **copying** the genetic material of interest (DNA fragment).

2. **Building a construct** (recombinant DNA - vector and desired gene) containing all the genetic elements for correct expression.



3. **Inserting** the vector into the **host organism**, directly through injection or transformation.

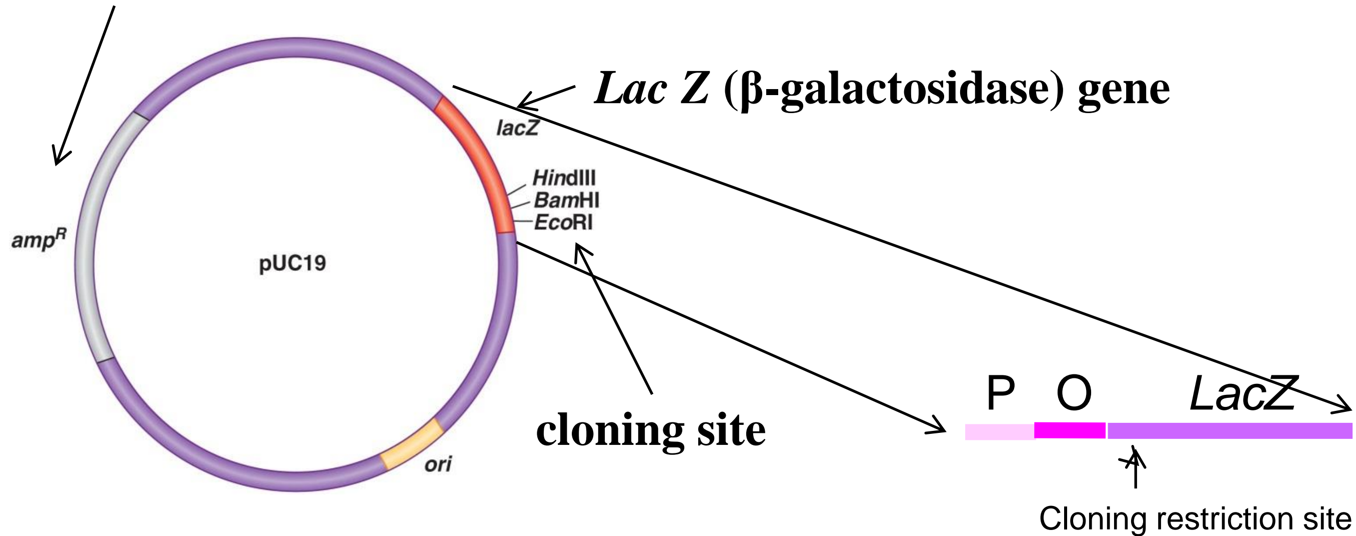
4. **Selecting** the cells expressing that gene by growing under positive selection (of an antibiotic or chemical) – **clone** .

5. Growing successfully the clone (transformed organisms).

Blue-white screening system

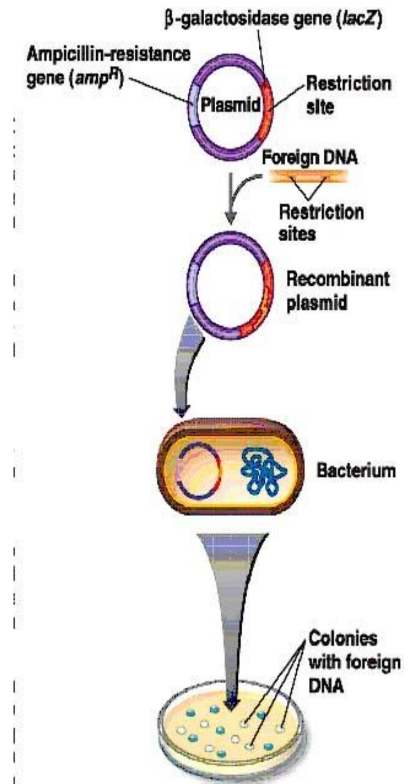
1. Plasmid vector contains the genes for:

amp^R^ (ampicillin resistance)



- The cloning site (restriction enzymes site) is inserted into the β-galactosidase gene.
- Cloning the desired gene at that site destroys β-galactosidase gene.

Blue-white screening system



2. The vector is then transformed into host competent cell (bacteria).

- Host is **sensitive to ampicillin**
- Host is **β-galactosidase negative** (do not carry LacZ gene)

3. The transformed cells are grown in the presence of:

- **ampicillin.**
- **X-gal** – substrate for β-galactosidase
  - a colourless modified galactose sugar
  - When metabolized by β-galactosidase form an insoluble product (5-bromo-4 chloroindole) which is bright **blue,** and thus functions as an indicator

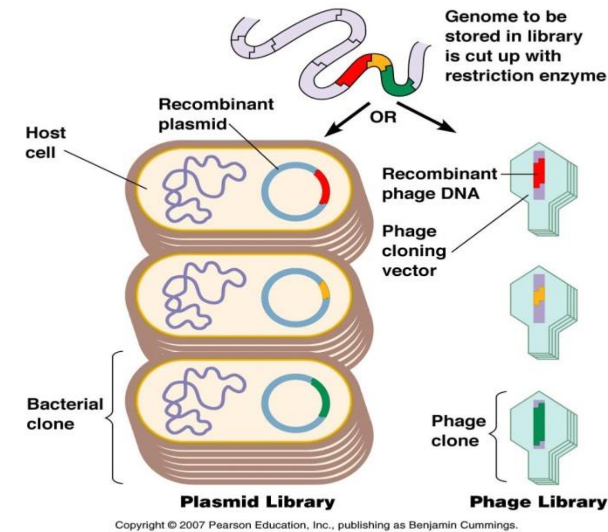
4. Results

- Clones lacking the vector will not grow.
- Clones containing the vector without the new gene will be **resistant to ampicillin, able to metabolized X-gal and** will **be blue.**
- Clones containing the recombinant vector will be **resistant to ampicillin** and **unable to hydrolyze X-gal** (white colonies).

- If the ligation was successful, the bacterial colony will be white; if not, the colony will be blue.

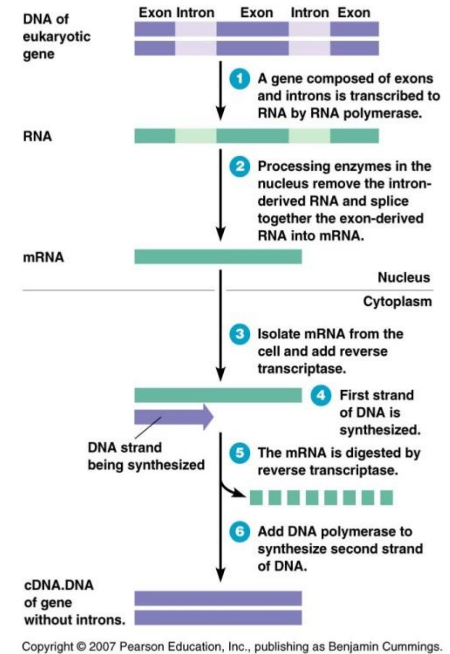
Obtaining DNA – gene of interest

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



2. cDNA library (complementary DNA) is made from mRNA by **reverse transcriptase** (enzyme found in retroviruses)

- Intron free DNA



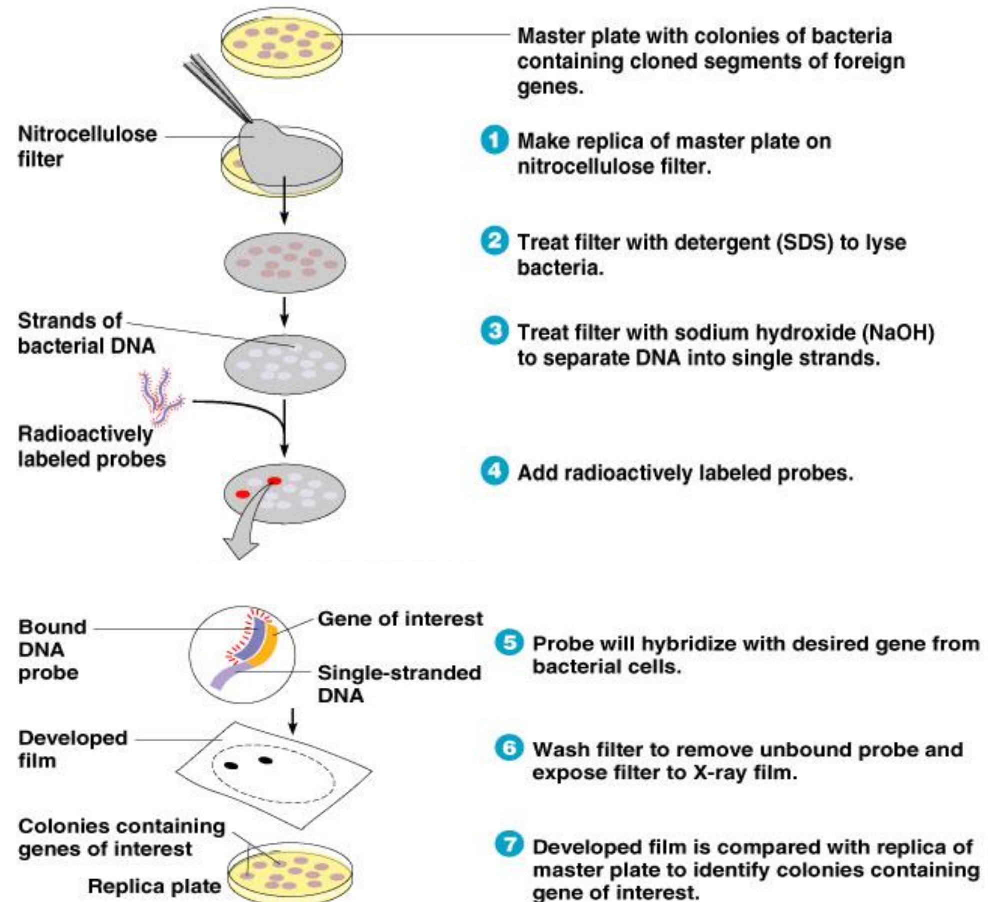
Screening for the desired Gene

- Identify the particular cell that contains the specific gene of interest (Presence of the vector with correct gene of interest)

- A short piece of labeled DNA called a **DNA probe** can be used to identify clones carrying the desired gene.
  - Radioactive labeled
  - Fluorescent labeled

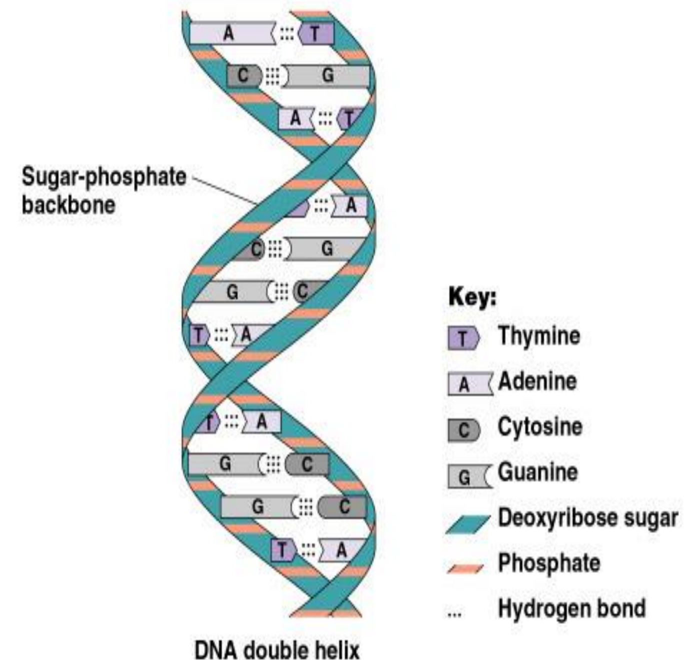
Labeled DNA probe (^{32}P or fluorescence)

5' * AGGCTTGTACTTTGGCGG 3'



Copying the genetic material of interest - PCR

- **Polymerase Chain Reaction** (**PCR**)
  - A reaction to make multiple copies of a piece of DNA **enzymatically**
- **Polymerase** – enzyme is **DNA polymerase** from *Thermus aquaticus* – **Taq polymerase**
  - Taq's optimum temperature for activity is 75-80°C
  - Can replicate a 1000 base pair strand of DNA in less than 10 seconds at 72°C
- **Chain** – chain of cycles of multiplication



Applications of recombinant DNA technology

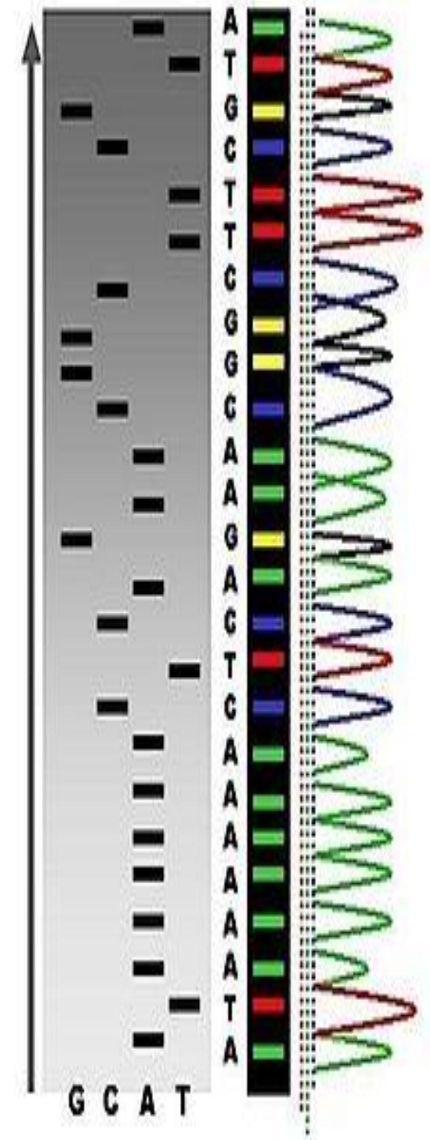
1. Scientific applications:

- **Many copies of** DNA can be produced
- Increase **understanding of DNA**
- Identify & cure **mutations** in DNA
- **Alter the phenotype** of an organism

- [**Bioinformatics** is the use of computer applications to study genetic data;
- **Proteomics** – proteomics is the study of a cell's proteins.
  - determination of all the proteins expressing in the cell.

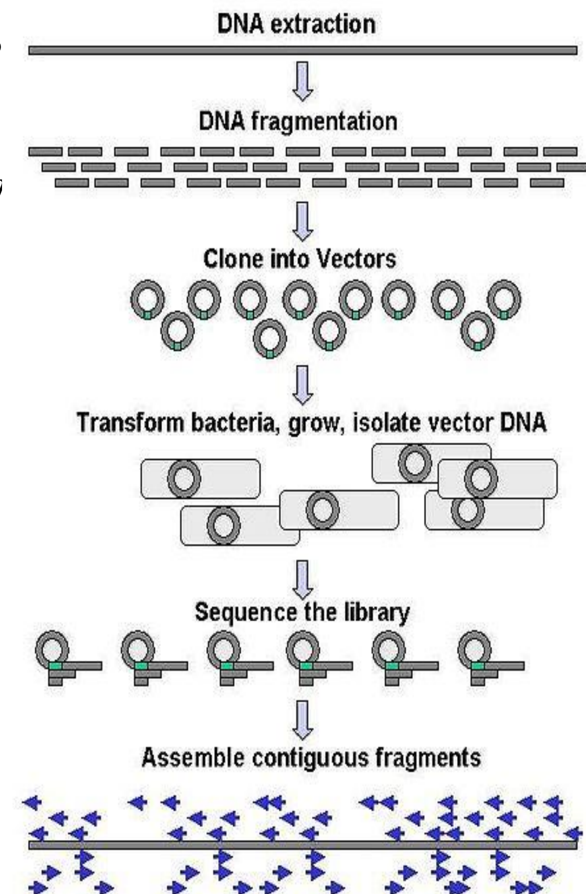
DNA sequencing

- **DNA sequencing** - is the process of determining the precise order of nucleotides within a DNA molecule
  (A, G, C and T in a molecule of DNA)



Applications of recombinant DNA technology

- **Shotgun sequencing -** Recombinant DNA techniques were used to map the human genome through the **Human Genome Project**
  - has 23 pairs of chromosomes (22 autosomal + X + Y)
  - with a total of approximately 3 billion DNA base pairs
  - containing an estimated 20,000–25,000genes
  - with only about 1.5-2% coding for proteins
  - the rest comprised by RNA genes, regulatory sequences, introns so-called junk DNA



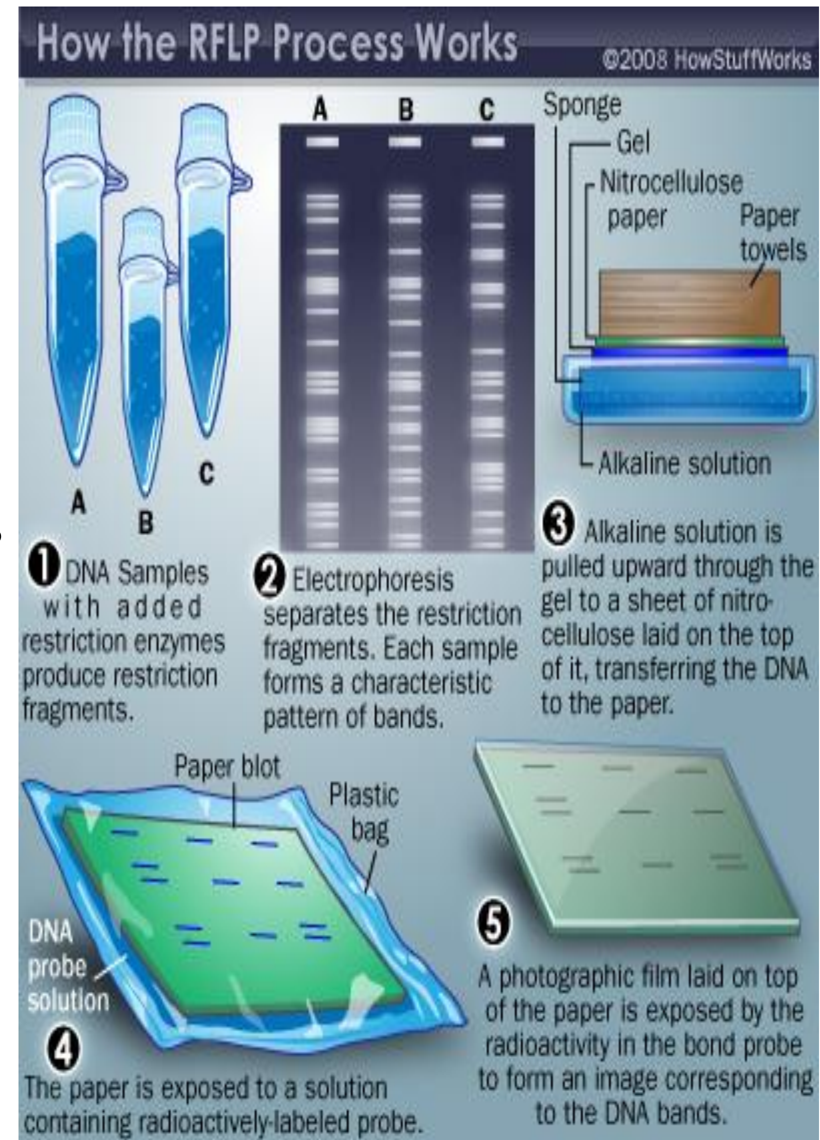
  - **This provides tool for diagnosis and possibly the repair of genetic diseases**

Applications of recombinant DNA technology

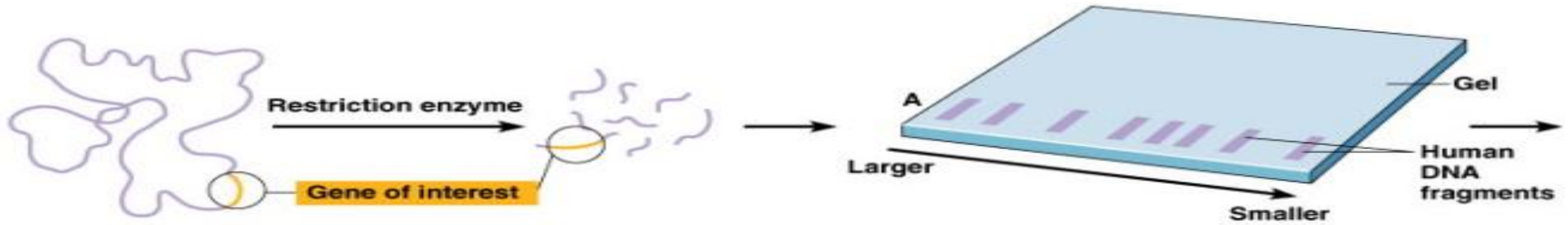
2. Diagnose genetic disease

- **RFLP analysis** (Restriction fragment length polymorphism)
  - DNA profiling involved restriction enzyme digestion, followed by gel electropheresis

  & Southern blot analysis
- **Southern blotting** is used for detection of a specific DNA sequence in DNA sample
  - **DNA probes** can be used to quickly identify the desired DNA.

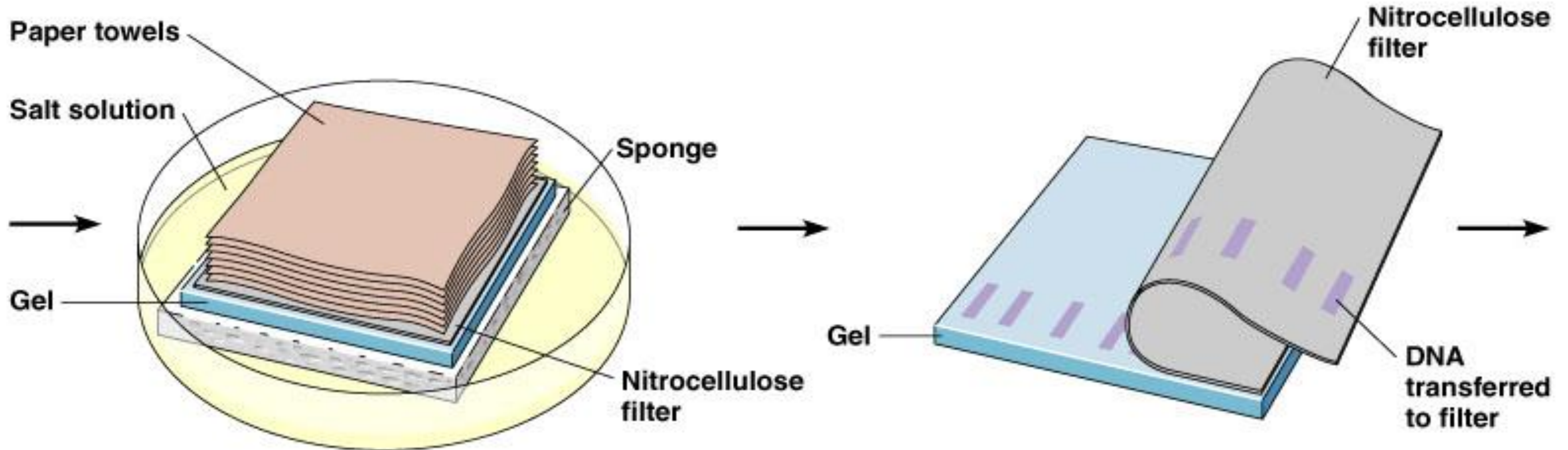


Southern blotting



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.



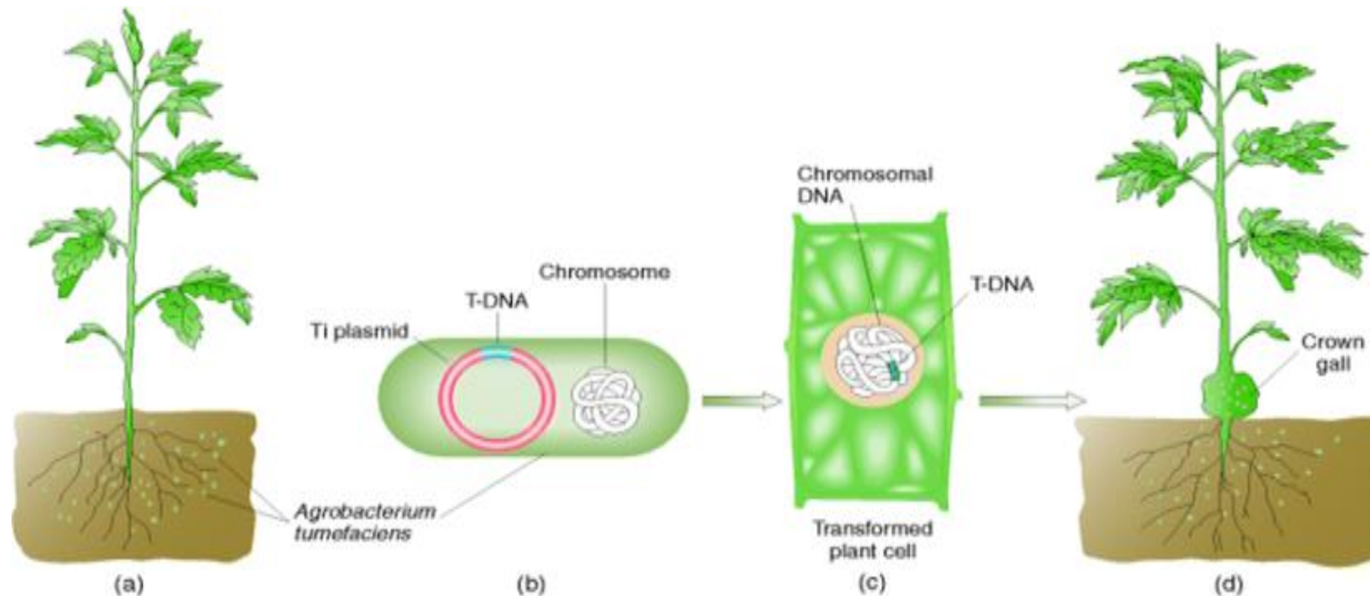
3. The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.

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

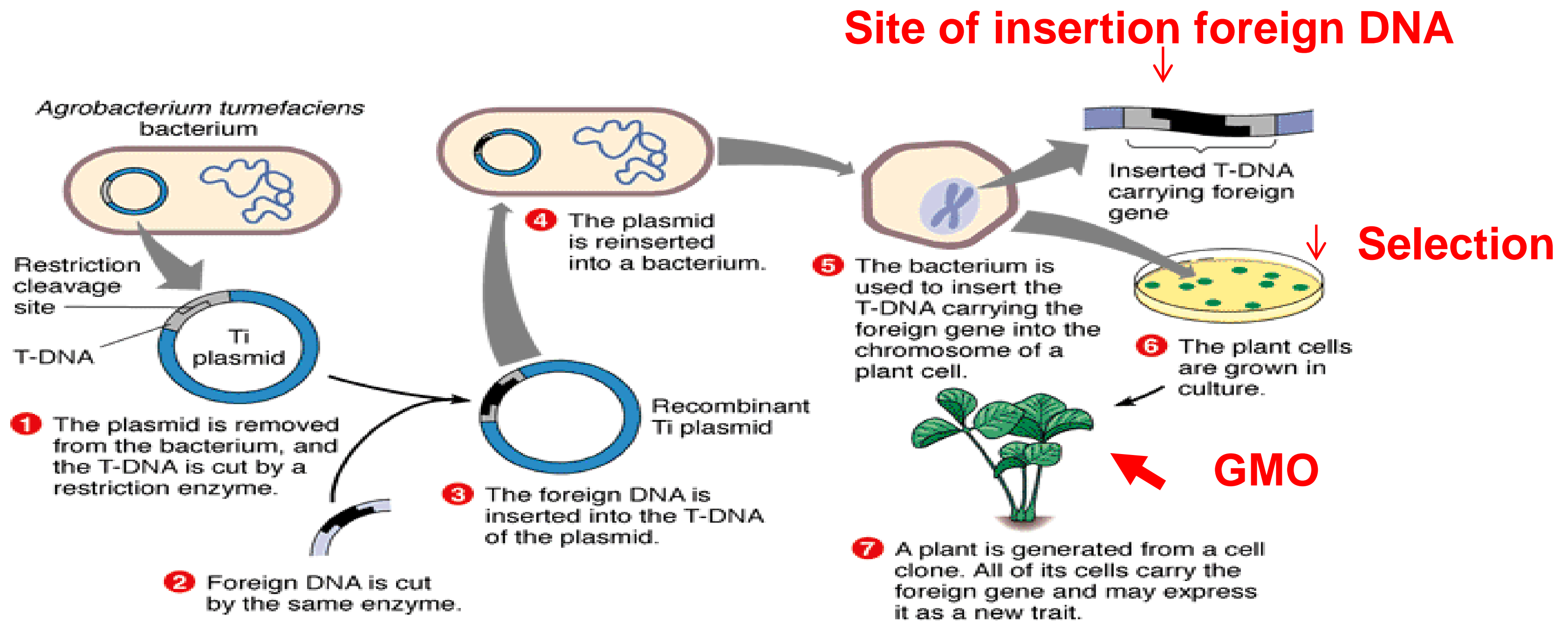
Applications of recombinant DNA technology

3. Agricultural Applications

- Cells from plants with desirable characteristics can be cloned to produce many identical cells, then can be used to produce whole plants from which seeds can be harvested.
- Some bacteria can transfer genes to unrelated species
  - *Agrobacterium tumefaciens* - a plant pathogen
  - Cause tumors in plants
  - **Natural genetic engineer**



Genetic engineering manipulation

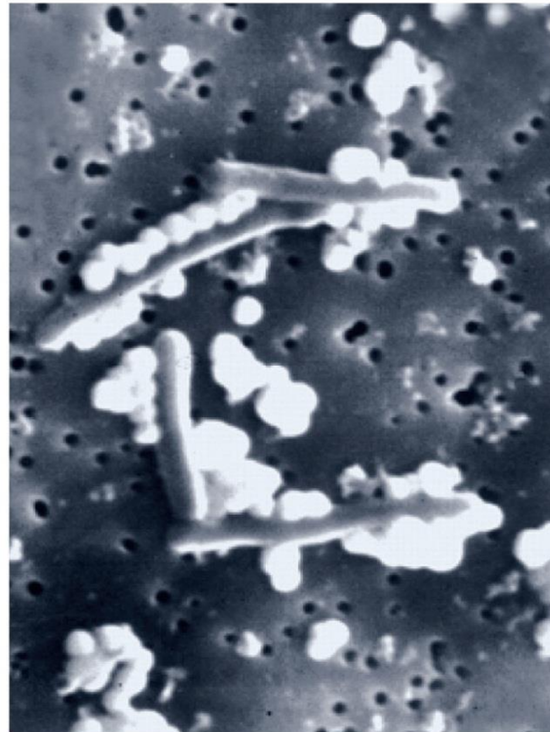


- Genes for resistance to herbicide glyphosate, Bt toxin, and pectinase suppression have been engineered into crop plants.
- Genetically modified *Rhizobium* has enhanced nitrogen fixation.
- Genetically modified *Pseudomonas* is a biological insecticide that produces a toxin.

Applications of recombinant DNA technology

4. Nanotechnology

- Bacteria can make molecule-sized particles
  - *Bacillus* cells growing on selenium form chains of elemental selenium



Applications of recombinant DNA technology

5. Therapeutic Applications

- Produce human proteins – hormones and enzymes
  - Insulin
  - hGH
  - INFα, INFβ and INFγ
- **Vaccines**
  - **Cells** and **viruses** can be modified to produce a pathogen's surface protein
    - Influenza
    - Hepatitis B
    - Cervical cancer vaccine
- **Gene therapy** can be used to cure genetic diseases by **replacing the defective or missing gene**.

THANK YOU