

RECOMBINANT DNA TECHNOLOGY

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Some slide material from Dr Walaa

Intro

- **Biotechnology:** “method by which a living organism or its parts are used to change or to incorporate a particular character to another living organism”.
- **Genetic recombination** is the exchange of information between two DNA segments
 - This is a common occurrence within the same species
 - **Recombinant DNA technology:** Artificial means, when a gene of one species is transferred to another living organism (AKA ***genetic engineering***)

Applications of recombinant DNA technology

- Quantitative preparation of biomolecules (e.g. hormones)
- Eliminate risk of contamination when producing vaccines (e.g. Hep BsAg)
- Specific probes for diagnosis of diseases
- **Gene therapy:**
 - Application of recombinant technology
 - Normal genes could be introduced into the patient → genetic diseases can be cured

Specific Probes for Diagnosis of Diseases

- Antenatal diagnosis of genetic diseases (e.g. single gene defects as CF by taking cells from fetus)
- To identify viral/ bacterial DNA in suspected blood/ tissue samples
- To demonstrate virus integration in transformed cells
- To detect activation of oncogenes in cancer
- To identify mutations in genes and for pedigree analysis: point mutations, deletions, insertions and rearrangements (e.g. Sickle cell disease)

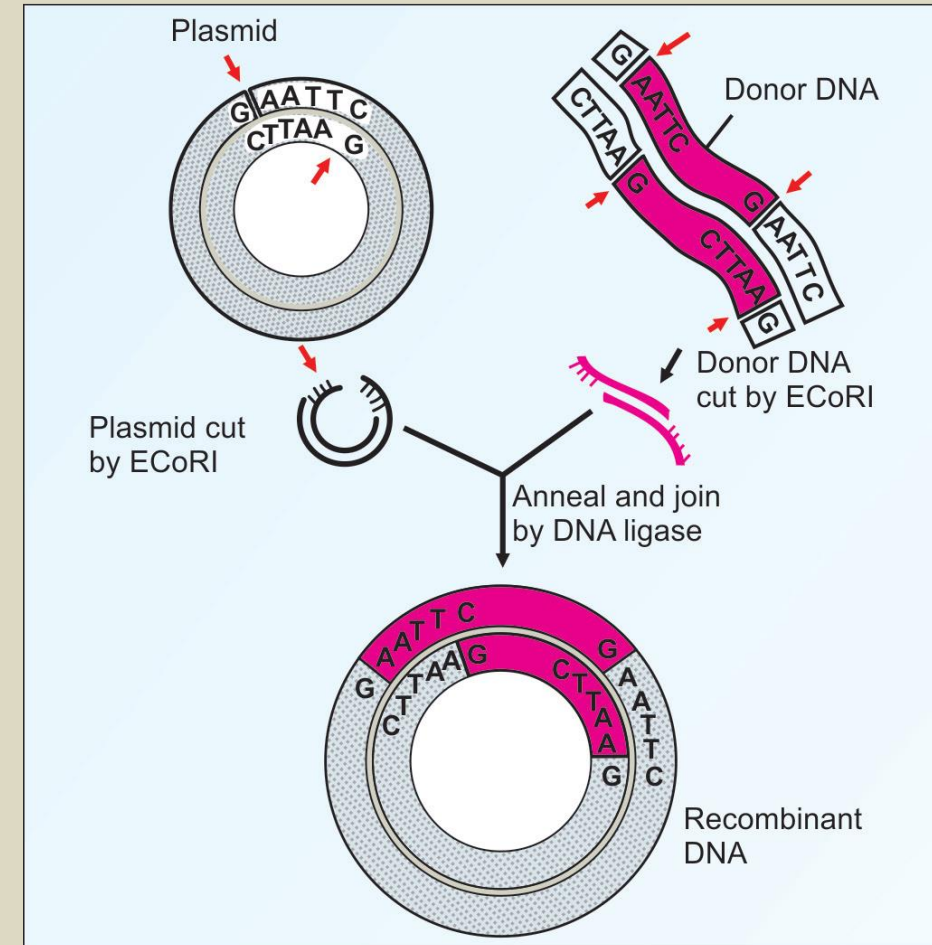
Tools of recombinant DNA technology

Restriction endonucleases

Vectors or vehicle DNA

Passenger DNA (foreign DNA)

**Hosts: they include
Bacterial-animal or plant cells**



Restriction endonucleases

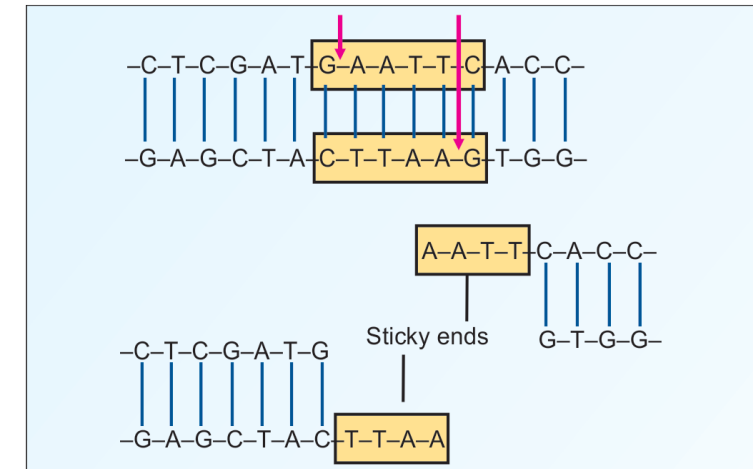
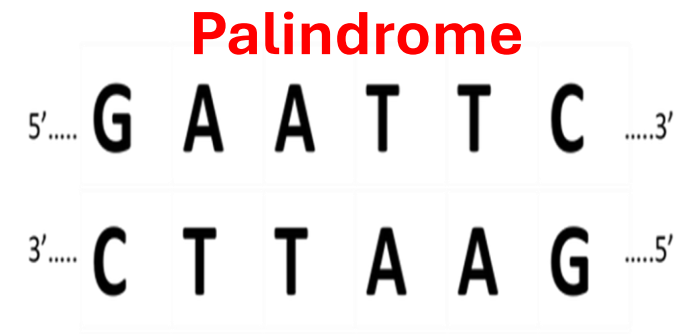
- In order to transfer a gene, it is to be first selectively split from the parent DNA
 - This is usually achieved by restriction endonucleases (**molecular scissors**)
- Restriction endonucleases are named after the species & strains of bacteria and the order of discovery
 - For example, the enzyme Eco RI is isolated from *Escherichia coli* RY13 strain

TABLE 48.1: Specificity of restriction enzymes (The arrows show the site or cut by the enzyme)

Enzyme	Source of enzyme	Specific sequence identified by the enzyme
Eco RI	<i>Escherichia coli</i> RY 13	G $\uparrow$ AATT C C TTAA $\downarrow$ G
Hind III	<i>Haemophilus influenzae</i> Rd	A $\uparrow$ AGCT T T TCGA $\downarrow$ A
Taq I	<i>Thermus aquaticus</i>	T $\uparrow$ CG A A GC $\downarrow$ T
Hpa I	<i>Haemophilus parainfluenzae</i>	GTT $\uparrow$ AAC CAA $\downarrow$ TTG

Restriction sites

- Restriction endonucleases have specific recognition sites where they cut the DNA
 - There are more than 800 enzymes now available commercially
- These enzymes recognize specific sequence with **palindrome** arrangement
- **Palindrome** in Greek means “to run backwards”
 - It is similar to a word that reads backwards or forwards similarly, e.g. “madam”- usually 4, 6 or 8 bases
 - AKA inverted repeat sequences, which means the nucleotide sequence in 5' to 3' direction is the same in both strands
- The resultant DNA cuts could have either sticky (usually) as more effective ligase or blunt ends
 - Restriction endonucleases may also produce blunt ends



Vectors

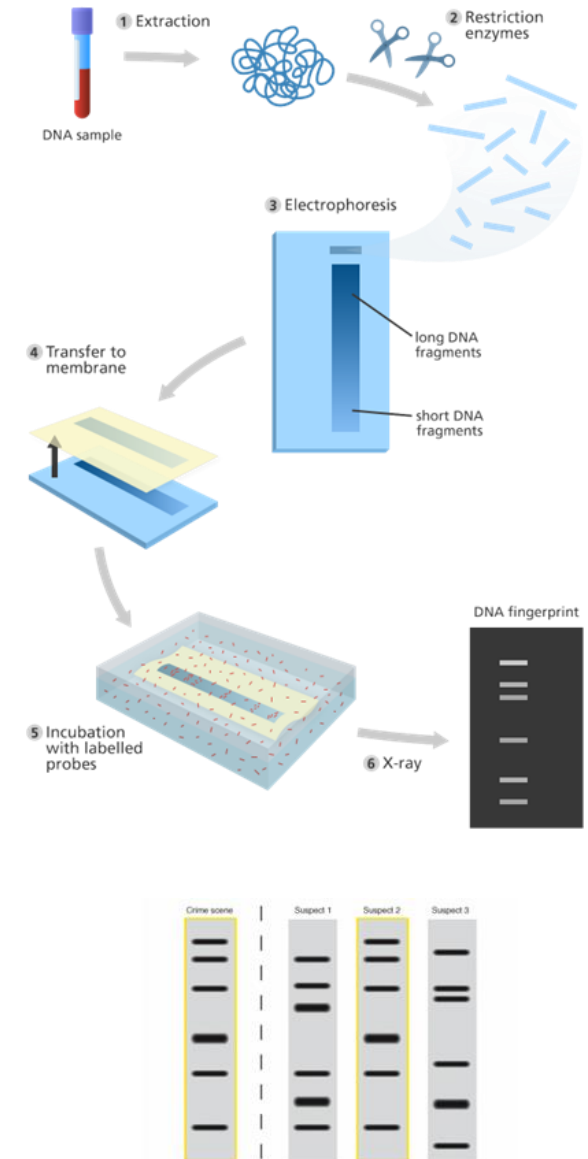
- In order to introduce the human gene into bacteria, at first, the gene is transferred to a carrier, known as a vector
- Most commonly used vectors are **plasmids**
 - Plasmids are circular double-stranded DNA molecules seen inside bacteria
 - In nature, plasmids confer antibiotic resistance to host bacteria
- Plasmids replicate independent bacterial DNA
 - Foreign DNA could be incorporated into them by using specific RE
 - They have a selectable marker to trace them after insertion (antibiotic resistance)

Types of vectors

- **Plasmids** can accept only about 6–10 kbp long foreign DNA
- If a DNA segment of 10–20 Kbp is to be introduced, bacteriophages may be the vectors of choice
 - **Bacteriophage**: a virus that infects and replicates within a bacterium
 - The lambda phage DNA is linear, 40% of genome is nonessential → in this region, a large fragment of foreign DNA may be introduced
- **Cosmid** vectors (artificially constructed cloning vectors)
 - Cosmids are plasmids that contain DNA sequences (cos sites) required for packaging of lambda DNA into phage.
 - Can take upper bigger fragments (20–50 kbp length)

Restriction map/ DNA fingerprint

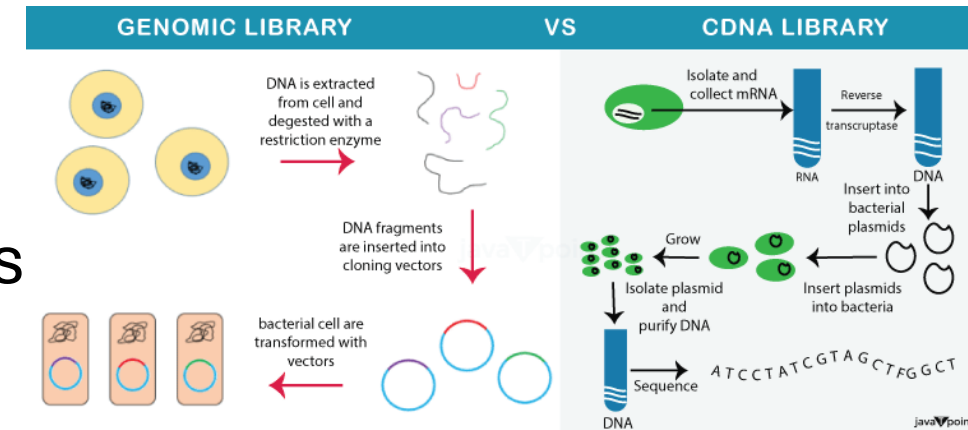
- If a piece of DNA from a species is made to react with a specific RE
 - → array of cut pieces will be produced, these are called restriction fragments.
- By electrophoretic separation of overlapping fragments → a restriction map can be created
- The study of the restriction map using suitable probes of short tandem repeats (STR)
 - → “DNA fingerprint” which is characteristic of each individual/organism



Procedure of DNA Recombination

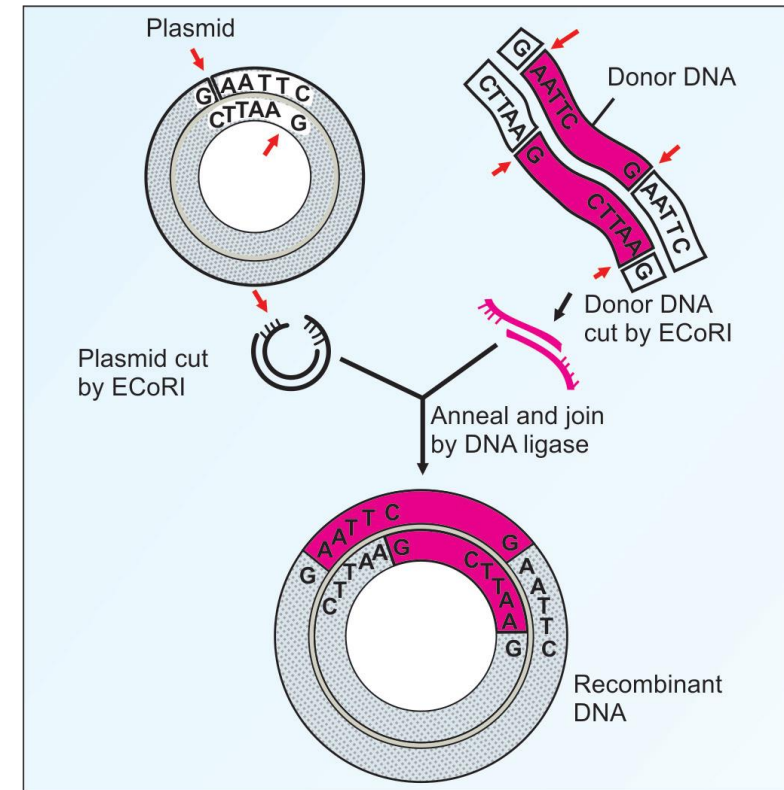
- Isolation of a specific gene from the human DNA is a laborious process
- This is solved by preparation of **cDNA** (complementary DNA)
 - It is easier to start the work with mRNA, because there is selective expression of a particular gene in certain tissue
 - For example, insulin mRNA will be abundant in the beta cells of

• Another advantage of using the mRNA is that RNA will have only coding sequences since introns are already removed by splicing process



Preparation of Chimeric DNA Molecules

- A vector carrying a foreign DNA is called **chimeric DNA or hybrid DNA**
- A circular plasmid vector DNA is cut with a specific RE
 - If EcoRI is used → sticky ends are produced with TTAA sequence on one DNA strand, and AATT sequence on the other strand
 - Human DNA is also treated with the same RE → so that the same sequences are generated at the sticky ends of the cut piece
- Then the vector DNA and human cut-piece DNA are incubated together so that **annealing takes place**
- The sticky ends of both vector and human DNA have complementary sequences
 - → come into contact with each other
- **DNA ligase** enzyme is added → phosphodiester linkages between the vector and the insert molecules
 - → **chimeric DNA** is finally produced

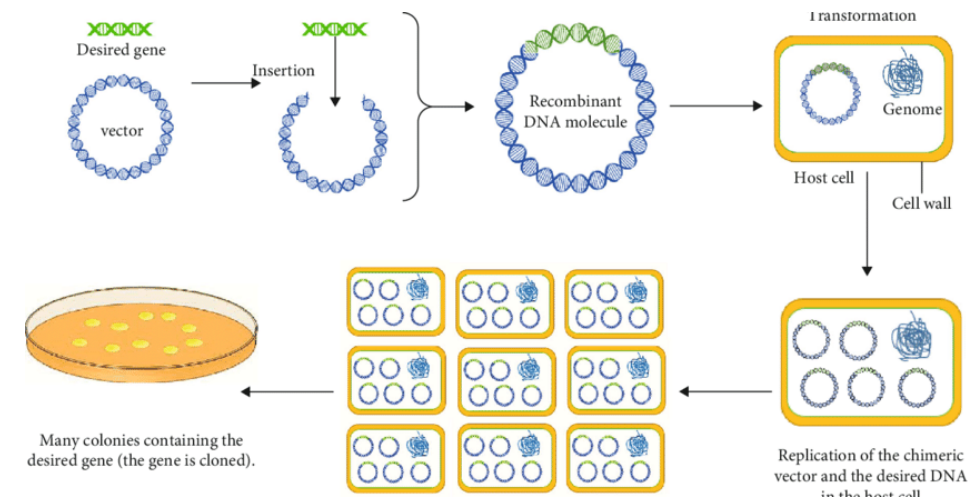


Step 2: Transfection of Vector into the Host

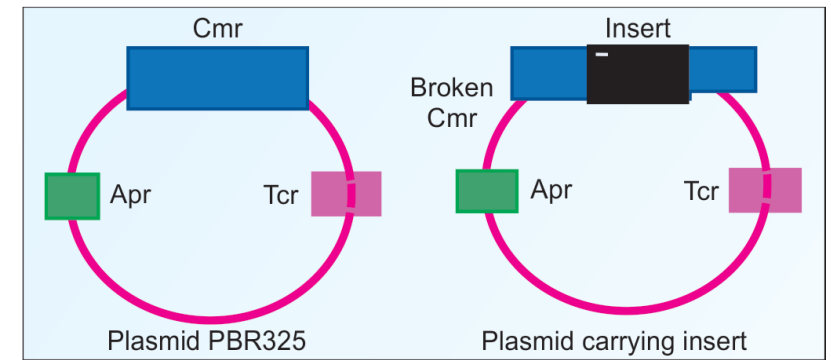
- The process by which plasmid is introduced into the host is called **transfection**
- Host E. coli cells and plasmid vectors are incubated in calcium
 - Ion channels are opened → the plasmid is taken into the host cell
- Now the host cells are allowed to grow in agar plates containing growth medium
- Only 5% of bacterial colonies contain the desired vector
 - Therefore, we have to select the desired colonies

Step 2: Cloning of Chimeric DNA

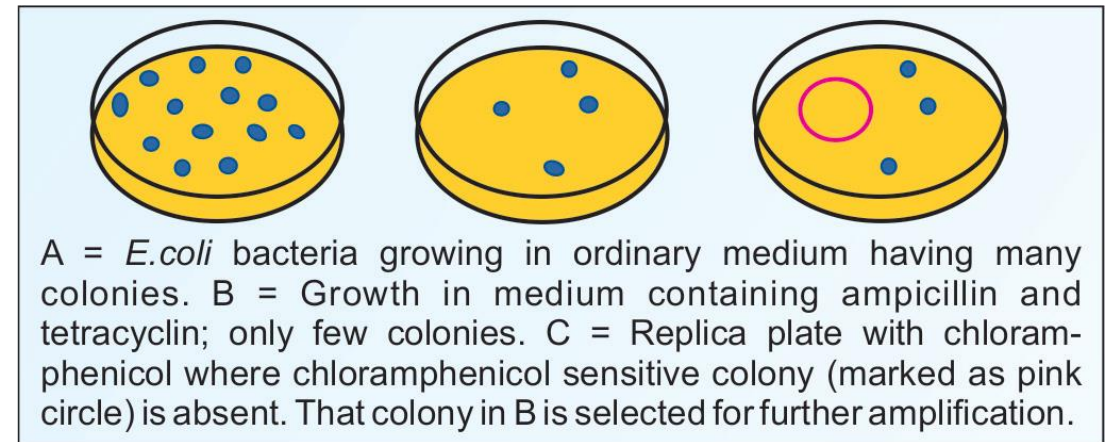
- The next step is **cloning**
- A clone is a large population of identical bacteria or cells that arise from a common ancestor molecule
- Cloning allows the production of a large number of identical DNA molecules
- The hybrid molecules are amplified by the cloning technique



Plasmid Carries Antibiotic Resistance Genes



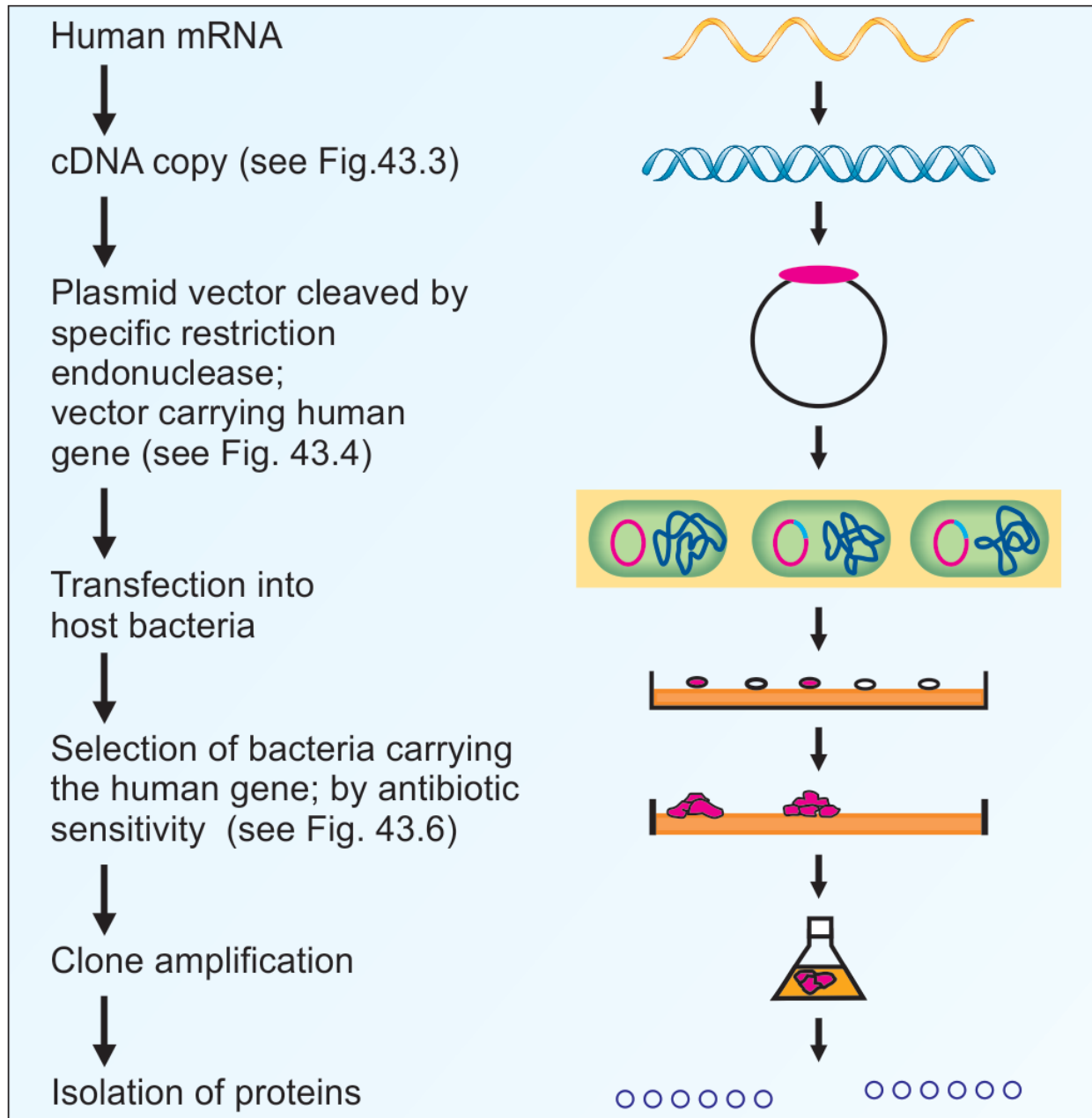
- Plasmid pBR-325 vector contains Apr (ampicillin resistance), Tcr (tetracycline resistance) and Cmr (chloramphenicol resistance) genes



- When the foreign DNA is inserted, the resistance against chloramphenicol is lost
 - This insertional inactivation of Cmr gene is the marker for hybrid DNA

Expression vectors

- Cells containing an appropriate chimeric plasmid are cultured then the plasmids are isolated from host cells
 - bacteria are lysed and the hybrid plasmids are isolated
 - → treated with the same restriction enzyme to release the foreign DNA
- E. coli carrying the vector with the insert is allowed to grow, without any protein inhibitors
- Such a vector carrying the foreign gene, which is translated into a protein, is called **expression vector**
- The human proteins can be harvested from the bacterial culture



Box. 48.1. Proteins produced by recombinant DNA technology

1. Recombinant human insulin
2. Recombinant human growth hormone (HGH, somatotropin) and other human hormones (e.g. FSH)
3. Recombinant blood clotting factor VIII and other clotting factors (Factor IX, tPA, hirudin)
4. Recombinant hepatitis B vaccine, HPV vaccine, etc.
5. Cytokines and growth factors (interferon, interleukins, etc.)
6. Monoclonal antibodies and other related products (rituximab, trastuzumab, etc.)
7. Recombinant enzymes (acid α glucosidase [myozyme], α L iduronidase [aldurazyme])
8. Recombinant HIV protein for HIV ELISA testing
9. Herbicide and insect resistant crops
10. Other products: Bone morphogenic protein (BMP), albumin, fibrinolytic and thrombolytic agents, etc.

DNA methylation

- DNA methylation is a biological process by which methyl groups are added to the DNA molecule
- Methylation can change the activity of a DNA segment without changing the sequence
- When located in a gene promoter, DNA methylation typically acts to repress gene transcription
- In mammals, DNA methylation is essential for normal development and is associated with a number of key processes including
 - genomic imprinting
 - X-chromosome inactivation
 - repression of transposable elements
 - Aging
 - carcinogenesis

