Biotechnology – Processes and Principles Flow Chart

Prepared by Mr. John Ebenezer

HEAT SHOCK METHOD

Making the bacterial cell competent to take up DNA

Treating Bacterial cell with a specific concentration of a divalent cation - Calcium

Increases the efficiency of Bacterial cell.
DNA can enter the bacteria through pores in their cell walls.

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Incubation of Bacterial cells + Recombinant DNA on ice

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Placing them briefly at 42°C (Heat shock)

↓

Placing them back on ice

Enables the bacteria to take up the recombinant DNA

Insertion of Recombinant DNA into the Host Cell

Recipient cells after making them **competent** to receive DNA

↓

Take up DNA present in its surrounding

↓

Recombinant DNA bearing **gene for resistance** to an antibiotic (e.g., ampicillin)

↓

Transferred into *E. coli* cells

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The host cells become **transformed** into ampicillin-resistant cells.

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Spread the transformed cells on agar plates containing ampicillin

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Only **Transformants grow**

↓

Non-Transformed cells will die

The ampicillin resistance gene in this case is used as a **selectable marker** in selecting the transformants

Use of Antibiotic Resistance Gene as Selectable Marker

The ligation of alien DNA is carried out at a restriction site present in one of the two **antibiotic resistance genes.**

Ligation of foreign DNA

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At the **Bam H I** site of tetracycline resistance gene in the vector **pBR322**

↓

The recombinant plasmids **lose tetracycline resistance due to insertion of foreign DNA**

Transfer of transformants growing on ampicillin containing medium

The recombinants grow in ampicillin medium

↓

Medium containing tetracycline

The recombinants do not grow in tetracycline medium

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But, **non-recombinants grow on** the medium containing **both the antibiotics.**

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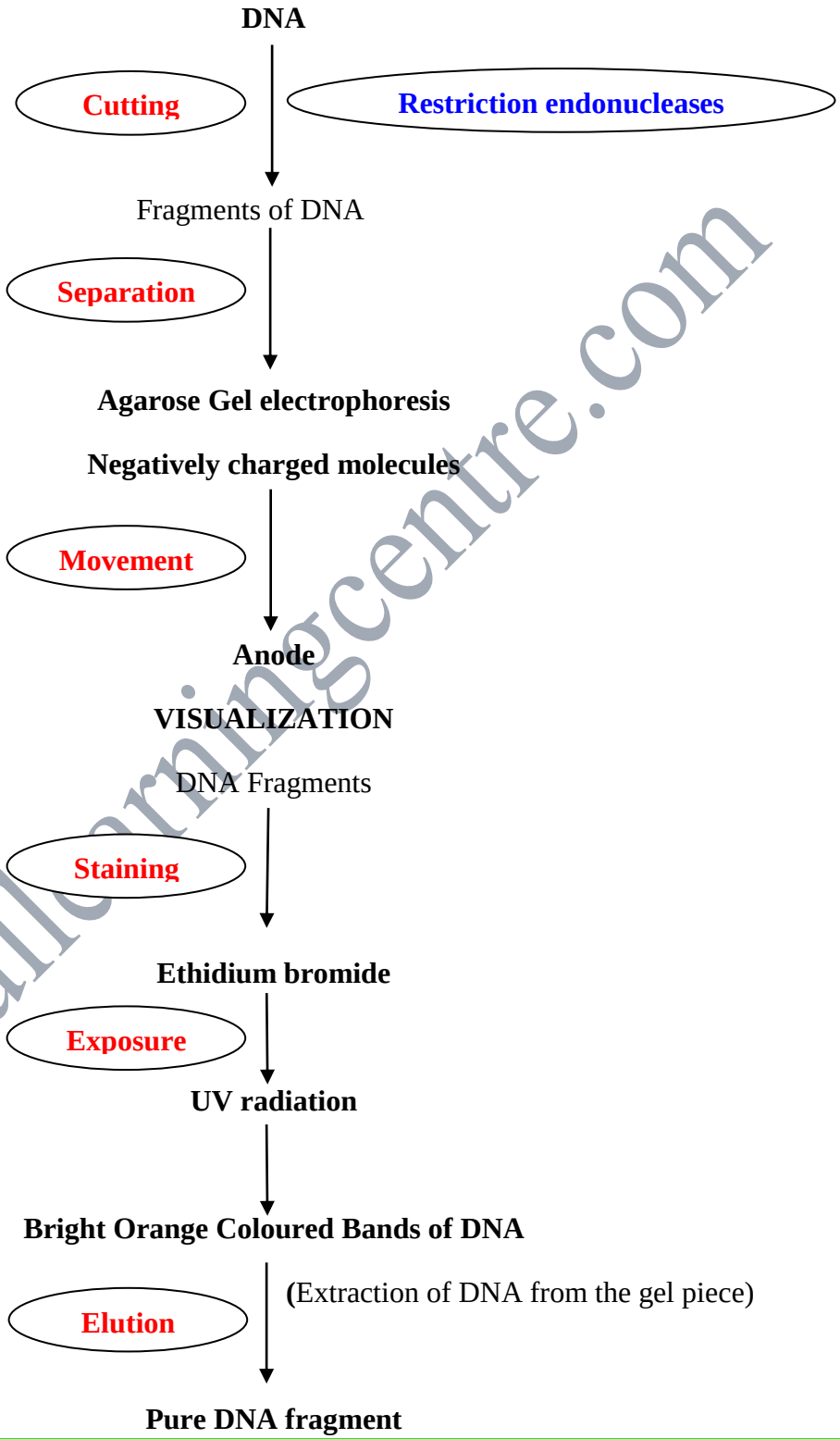
In this case, **ampicillin resistance gene helps in selecting the transformants**

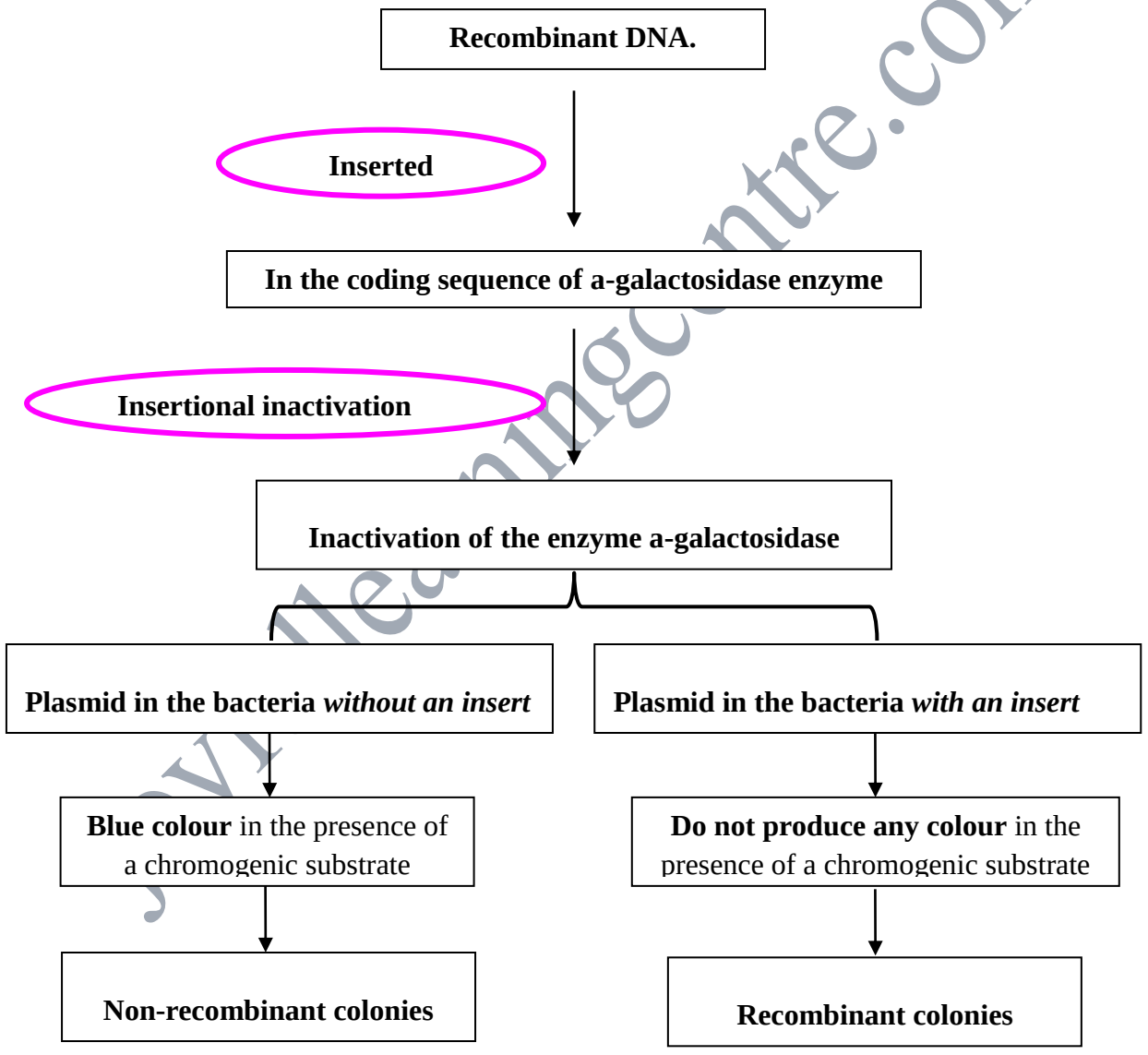
Tetracycline resistance gene gets inactivated due to insertion of alien DNA and helps in **selection of recombinants.**

The process of isolation of DNA fragments



Process of Selection of Recombinants from Non-Recombinants using Alternative Selectable Markers

Alternative selectable markers differentiate recombinants from non-recombinants on the basis of their ability to **produce colour in the presence of a chromogenic substrate.**



If the plasmid in the bacteria **has an insert,** it results into insertional inactivation of the a-galactosidase and the colonies **do not produce any colour**, these are identified as recombinant colonies.

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