Bacterial transformation (contd)

Methods of transformation in laboratory

In bacteria, **artificial competence** can be induced in laboratory by procedures that involve making the cell passively permeable to DNA by exposing it to conditions that do not normally occur in nature.

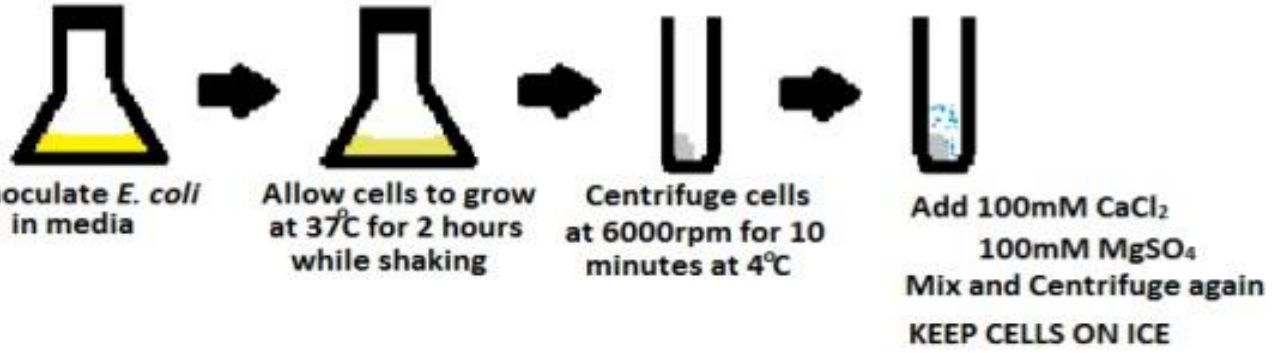
Chemical treatment

- Typically the cells are incubated in a solution containing **divalent cations (often $CaCl_2$)** under **cold conditions**, before being exposed to a **heat pulse (heat shock).**

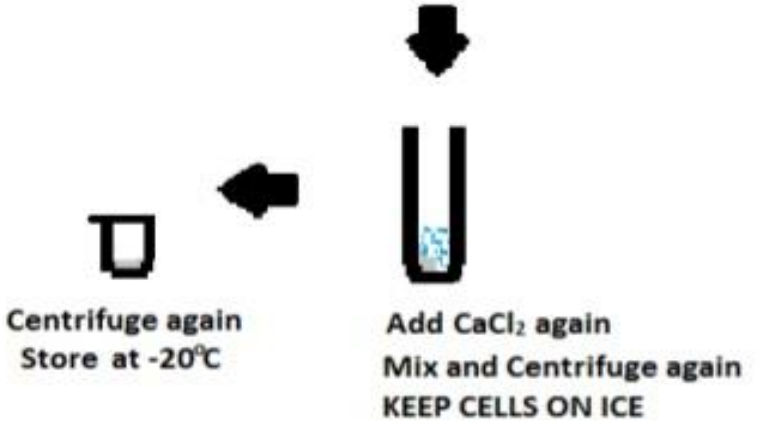
- Calcium chloride/Magnesium chloride **partially disrupts the cell membrane**, which allows the recombinant DNA enter the host cell.

- Cells that are able to take up the DNA are called **competent cells.**

Preparation of Competent Cells



CALCIUM CHLORIDE TREATMENT –
KEEP CELLS COLD!



- The surface of bacteria such as *E. coli* is **negatively charged** due to phospholipids and lipopolysaccharides on cell surface, and the DNA is also negatively charged.

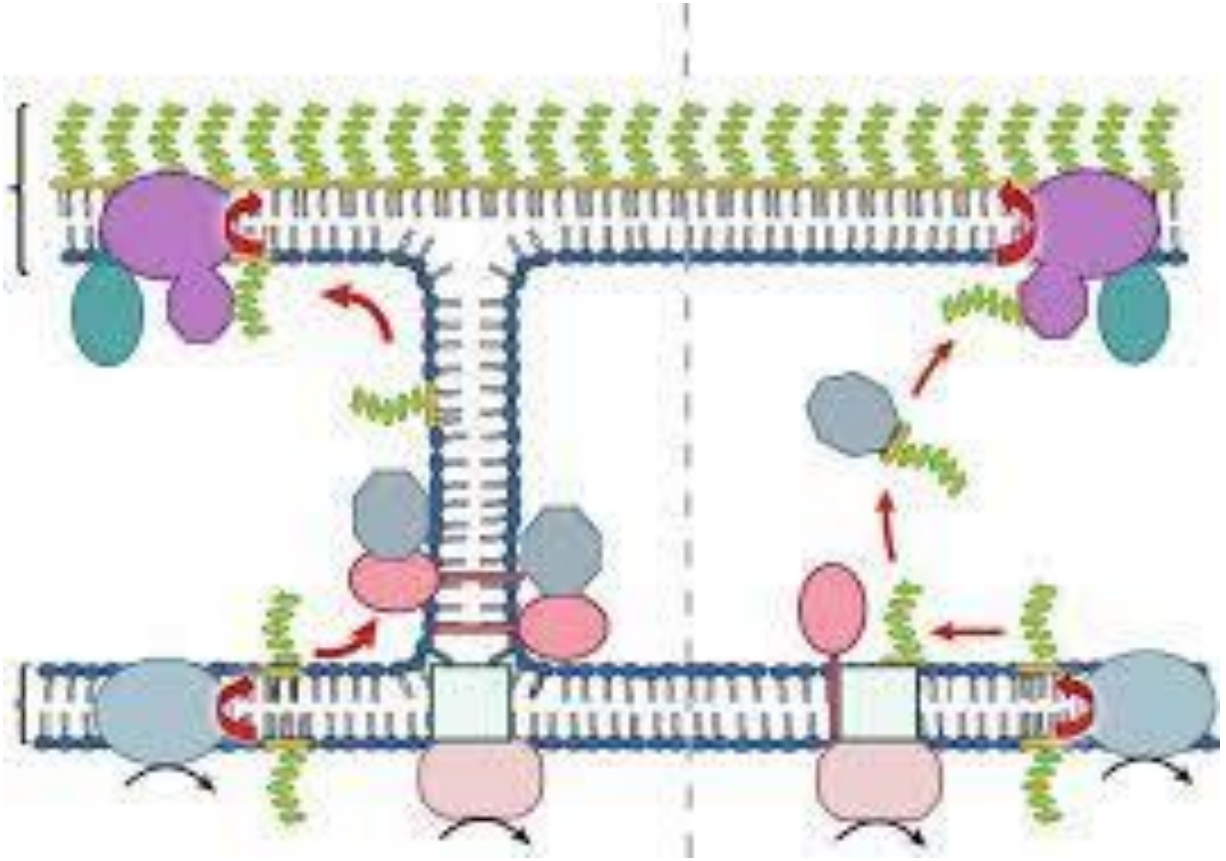
- It has been found that growth of **bacteria** in **20 mM Mg** reduces the number of **protein-to-lipopolysaccharide bonds** which allow **DNA molecules** to adhere to the cell surface.

- They also **increase the membrane fluidity**, facilitating transformation by reducing viscosity of the lipid bilayer of a cell membrane.

- DNA entry into *E. coli* cells is through channels known as **zones of adhesion or Bayer's junction**, with a typical cell carrying as many as **400** such zones.

- The cytoplasmic membrane and outer membrane are joined **Bayer's junctions**. In these regions, the outer surface of the cytoplasmic membrane is **continuous** with the inner surface of the outer membrane creating pores that vary in diameter from 25 to 50 nm.

- This method works very well for **circular plasmid DNA.**



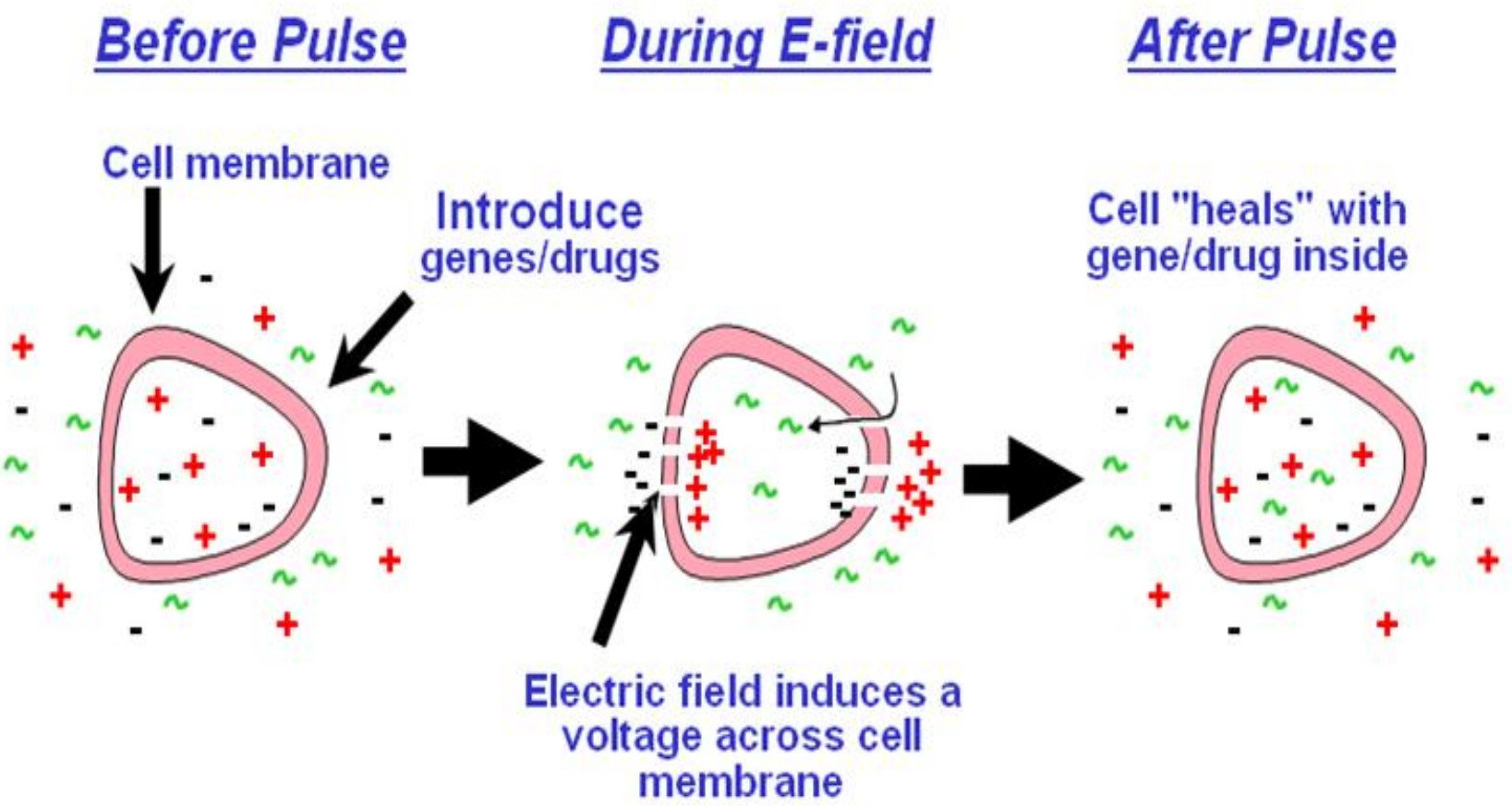
Bayer’s junction

- It is suggested that exposing the cells to **divalent cations in cold condition** may also **change or weaken the cell surface structure**, making it more permeable to DNA.

- The **heat-pulse is thought to create a thermal imbalance** across the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.

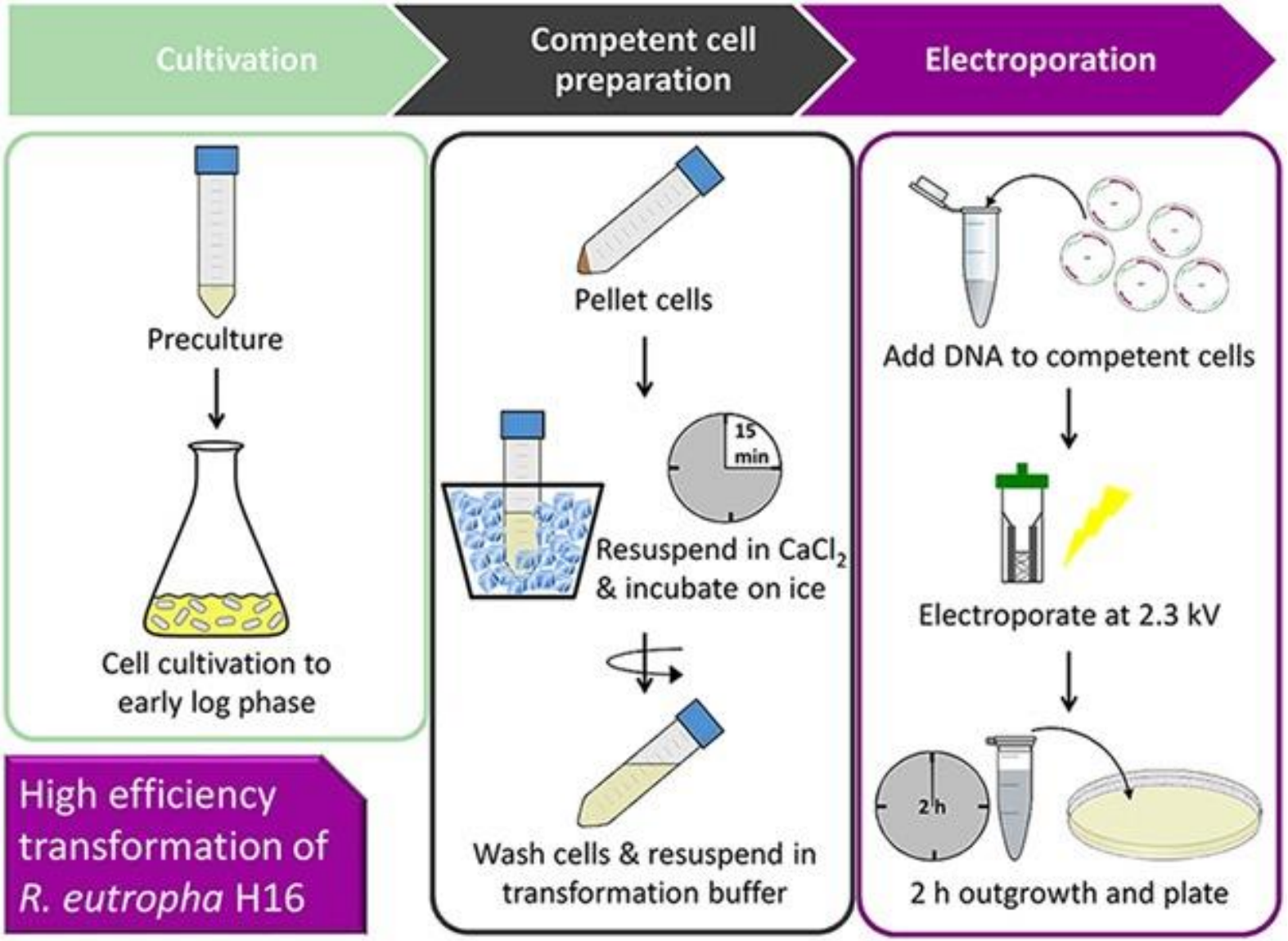
Electroporation

- Electroporation is another method of promoting competence.
- In this method the cells are **briefly shocked with an electric field** of 10-20 kV/cm, which is thought to **create holes in the cell membrane** through which the plasmid DNA may enter.
- After the electric shock, the holes are **rapidly closed** by the cell's **membrane-repair mechanisms**.
- **Attraction** between phospholipids allows cell membranes to repair breaks in the bilayer.


Before Pulse
During E-field
After Pulse
Cell membrane
Introduce
genes/drugs
Cell "heals" with
gene/drug inside
Electric field induces a
voltage across cell
membrane

- The transformation efficiency using the **$CaCl_2$** method **decreases with plasmid size**, and **electroporation** therefore may be a **more effective method** for the uptake of **large plasmid DNA.**

- Cells used in electroporation should be prepared first by washing in cold double-distilled water to **remove charged particles** that may create sparks during the electroporation process.


Cultivation
Competent cell preparation
Electroporation
Preculture
Cell cultivation to early log phase
High efficiency transformation of R. eutropha H16
Pellet cells
15 min
Resuspend in CaCl2 & incubate on ice
Wash cells & resuspend in transformation buffer
Add DNA to competent cells
Electroporate at 2.3 kV
2 h
2 h outgrowth and plate

Yeast

- Most species of yeast, including *Saccharomyces cerevisiae*, may be transformed by **exogenous DNA** in the environment.

- Several methods have been developed to facilitate this transformation at high frequency in the lab.

- Yeast cells may be treated with **enzymes** to degrade their cell walls, yielding **spheroplasts**.

- These cells are **very fragile** but take up foreign DNA at a **high rate.**

- Exposing intact yeast cells to **alkali cations** such as those of caesium or lithium allows the cells to take up **plasmid DNA**.

Spheroplasts have two membranes as compared to protoplasts which have a single membrane.

Plants

- **Agrobacterium-mediated transformation** is the **easiest and most simple** plant transformation.

- Plant tissue (often leaves) are cut into **small pieces**, e.g. 10x10mm, and soaked for 10 minutes in a **fluid containing suspended *Agrobacterium*.**

- The bacteria will attach to many of the plant cells **exposed by the cut.**

- The plant cells secrete **wound-related phenolic compounds** which in turn act to **upregulate the virulence operon** of the *Agrobacterium* to facilitate intake.

- The **transferred DNA (called T-DNA)** is piloted to the plant cell nucleus by **nuclear localization signals**.

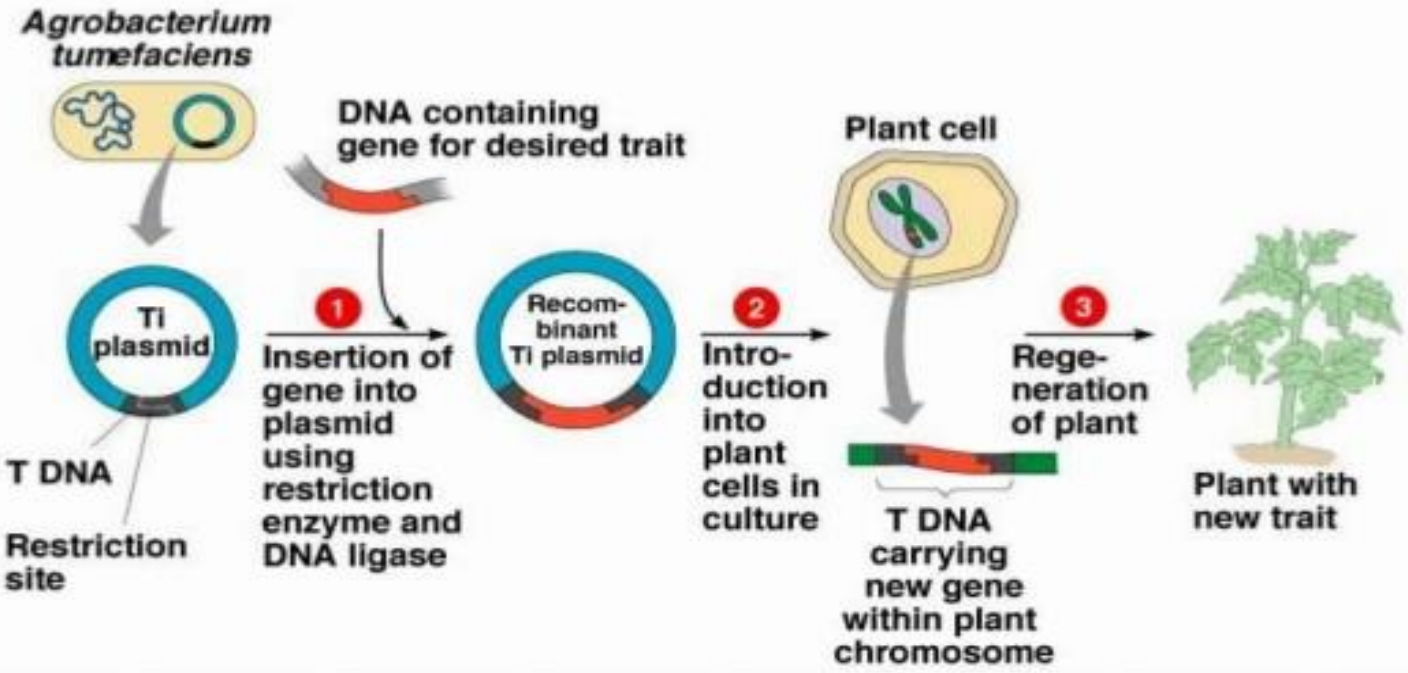
- Assuming that a selection marker (such as an antibiotic resistance gene) was included in the T-DNA, the transformed plant tissue can be **cultured on selective media** to produce shoots.

- The shoots are then **transferred to a different medium** to promote root formation.

- Once roots begin to grow from the **transgenic shoot**, the plants can be transferred to soil to complete a normal life cycle (make seeds).

Nuclear localization signals (NLS) are generally short peptides that act as a signal fragment that mediates the transport of proteins from the cytoplasm into the nucleus.

Agrobacterium mediated Transformation.



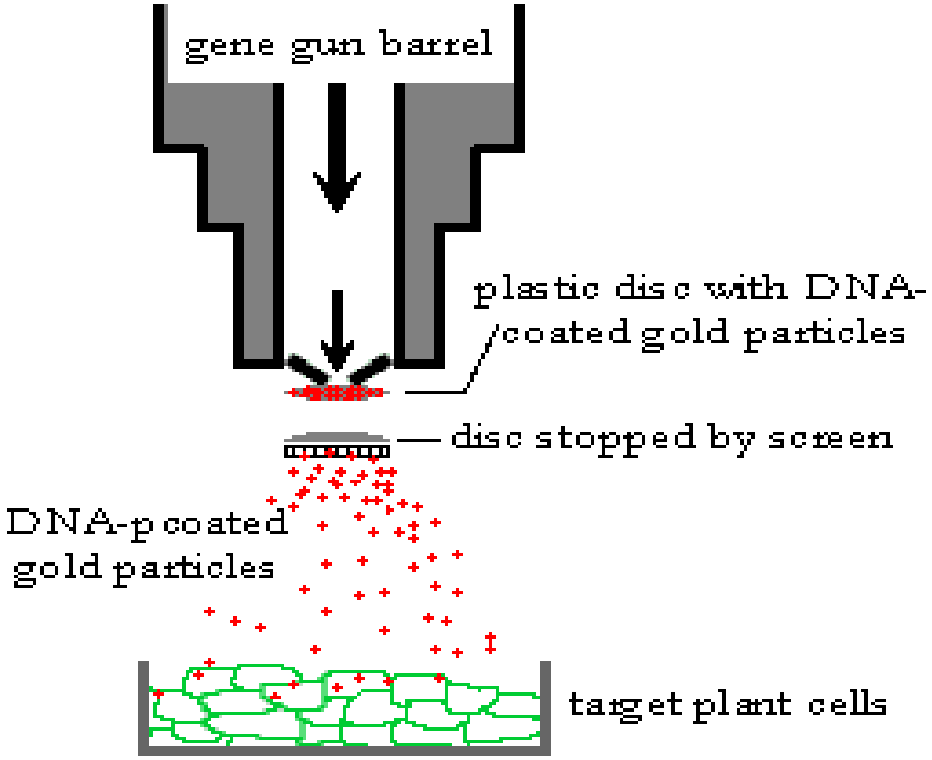
Vector-less methods

Gene gun:

- Also referred to as **particle bombardment**, **microprojectile bombardment**, or **biolistics**.
- Particles of **gold or tungsten are coated with DNA** and then **shot into young plant cells or plant embryos**.
- Some genetic material will **stay in the cells** and transform them.

- This method also allows **transformation of plant plastids** (membrane bound organelles).

- The transformation efficiency is **lower than in Agrobacterium-mediated transformation**, but **most plants can be transformed** with this method.

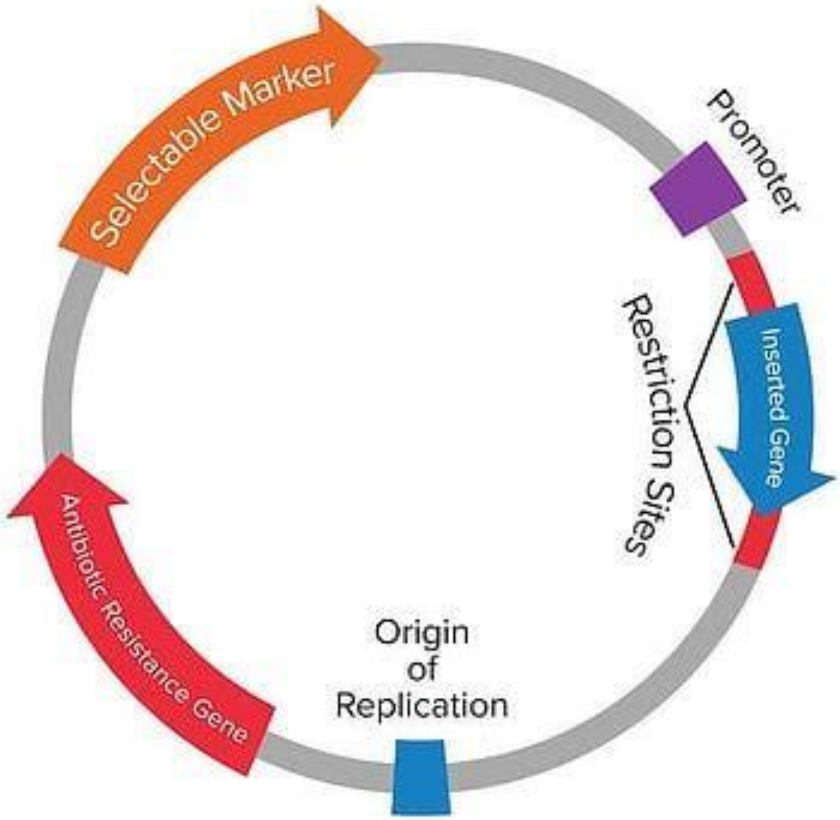


Uses of artificial transformation

- The artificially induced competence in bacteria allow bacteria such as *Escherichia coli* to be used as a convenient host for the **manipulation of DNA** as well as **expressing proteins,** e.g. insulin.

- Typically **plasmids** are used for transformation in *E. coli*.

- In order to be stably maintained in the cell, a plasmid DNA molecule must contain an **origin of replication**, which allows it to be replicated in the cell independently of the replication of the cell's own chromosome.


Selectable Marker
Promoter
Restriction Sites
Inserted Gene
Antibiotic Resistance Gene
Origin
of
Replication

- The efficiency with which a competent culture can take up exogenous DNA and express its genes is known as **transformation efficiency** and is measured in **colony forming unit (cfu) per µg DNA** used.

- A transformation efficiency of **1×10^8 cfu/µg** for a small plasmid like **pUC19** is roughly equivalent to **1 in 2000 molecules** of the plasmid used being transformed.

Selection and screening in plasmid transformation

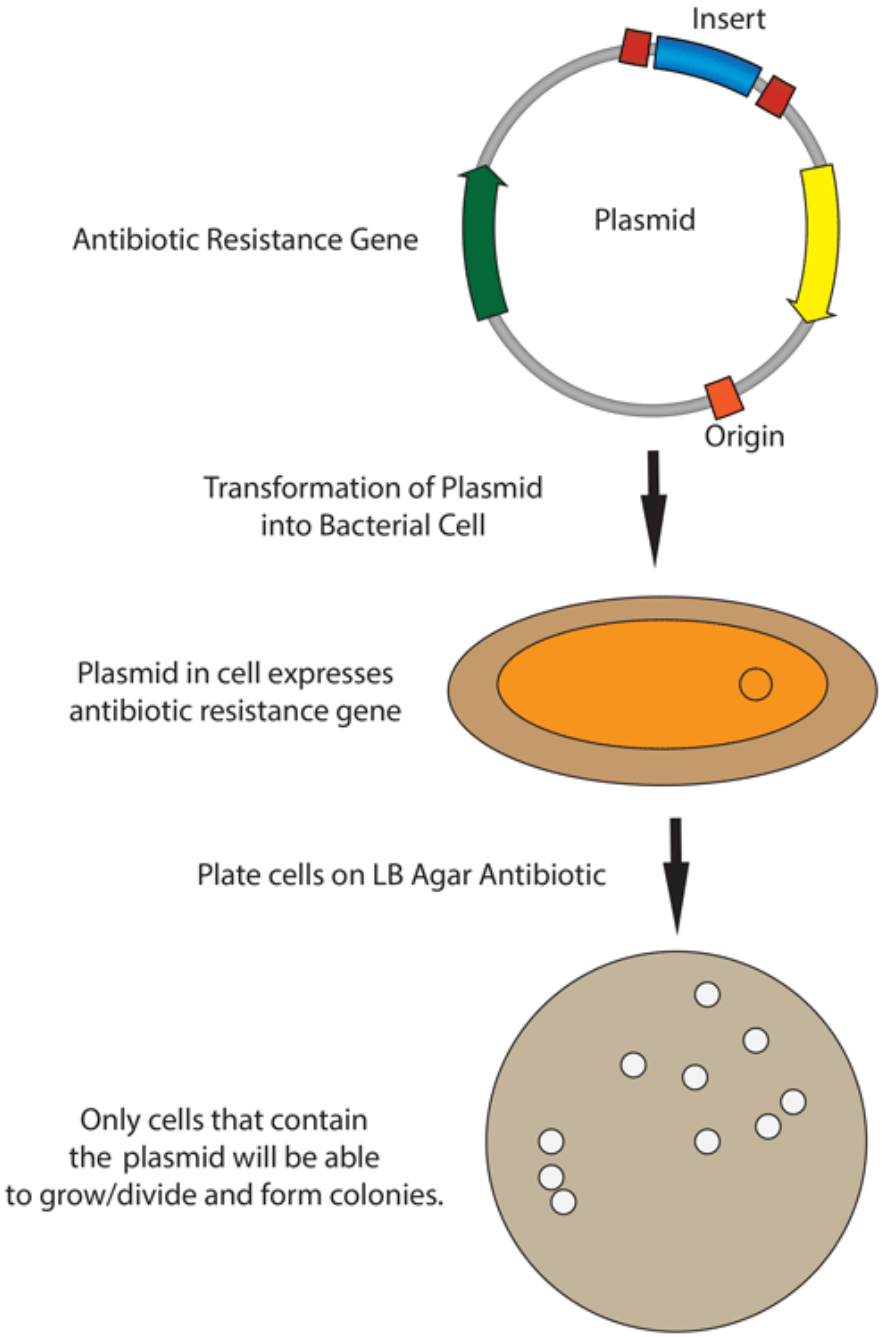
- Because transformation usually produces a mixture of **relatively few transformed cells** and an **abundance of non-transformed cells**, a method is necessary to **select** for the cells that have acquired the plasmid.

- The plasmid therefore requires a **selectable marker** such that those cells without the plasmid may be killed or have their growth arrested.

- **Antibiotic resistance** is the most commonly used marker for prokaryotes.

- The transforming plasmid **contains a gene that confers resistance to an antibiotic** that the bacteria are otherwise sensitive to.

- The mixture of treated cells is cultured on media that contain the antibiotic so that **only transformed cells are able to grow**.


Insert
Antibiotic Resistance Gene
Plasmid
Origin
Transformation of Plasmid into Bacterial Cell
Plasmid in cell expresses antibiotic resistance gene
Plate cells on LB Agar Antibiotic
Only cells that contain the plasmid will be able to grow/divide and form colonies.


Thank You!