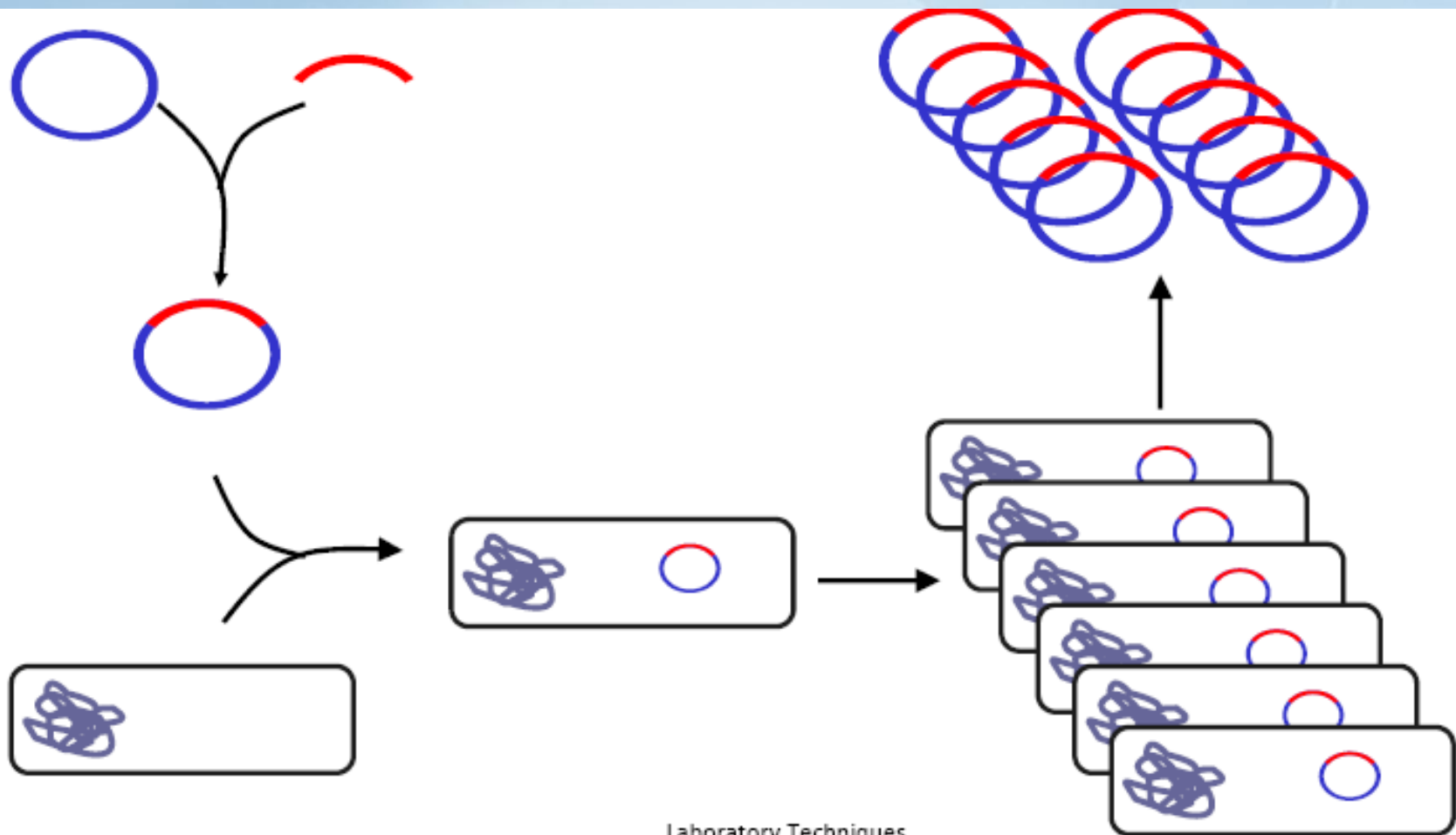


# Transformation

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# DNA Cloning Overview



# Introduction to cell

A. Cell type: Bacteria, Mammalian cells, insect cells

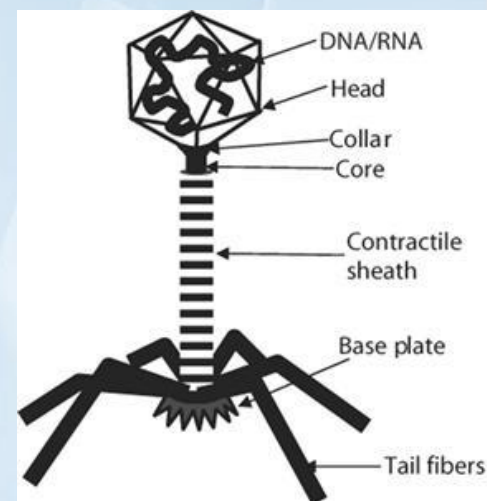
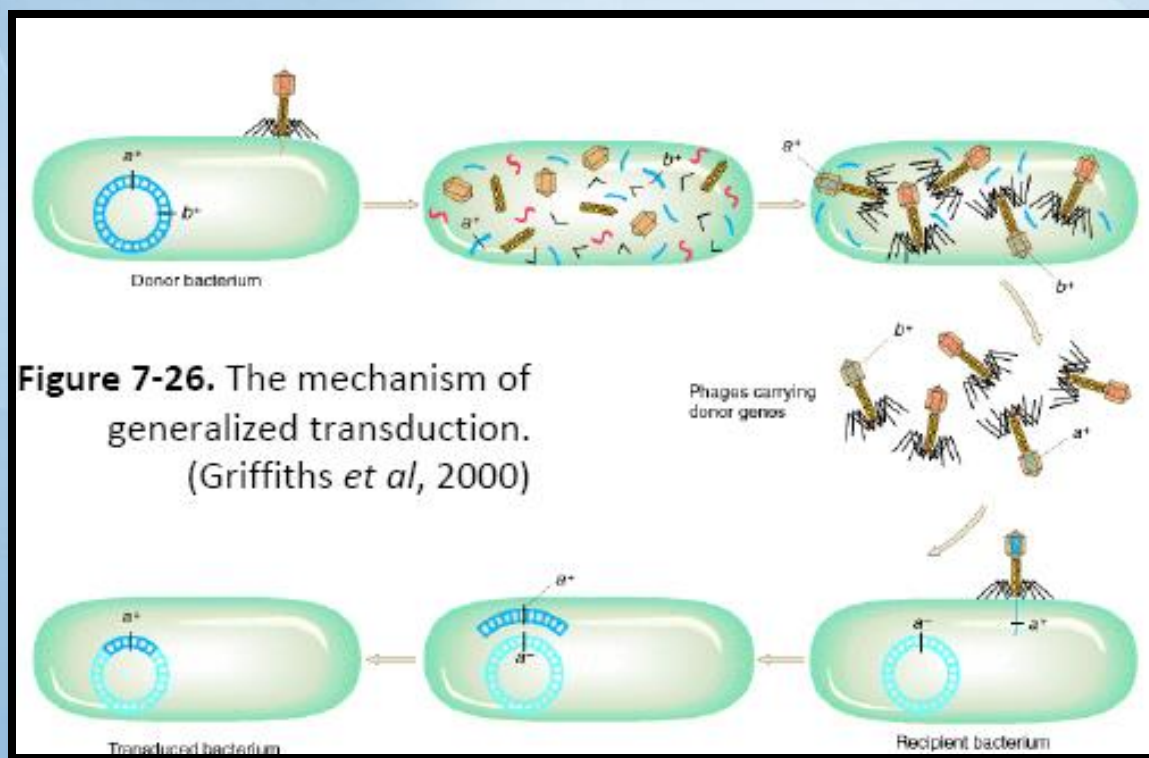
B. Condition of Cells:

- Easy and fast growth
- Non pathogen
- Suitable for plasmid or phage
- Express and secret protein

C. Method: Transformation, Transfection, Electroporation etc

# Transduction

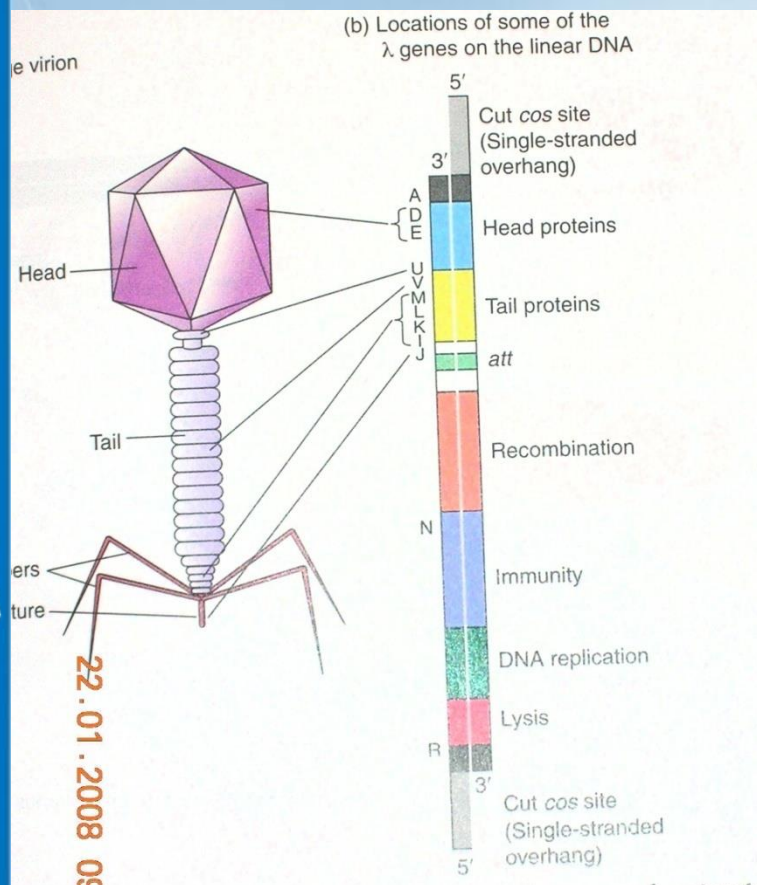
- A phage virus attaches to bacterial cell and transfers its DNA into the bacterium



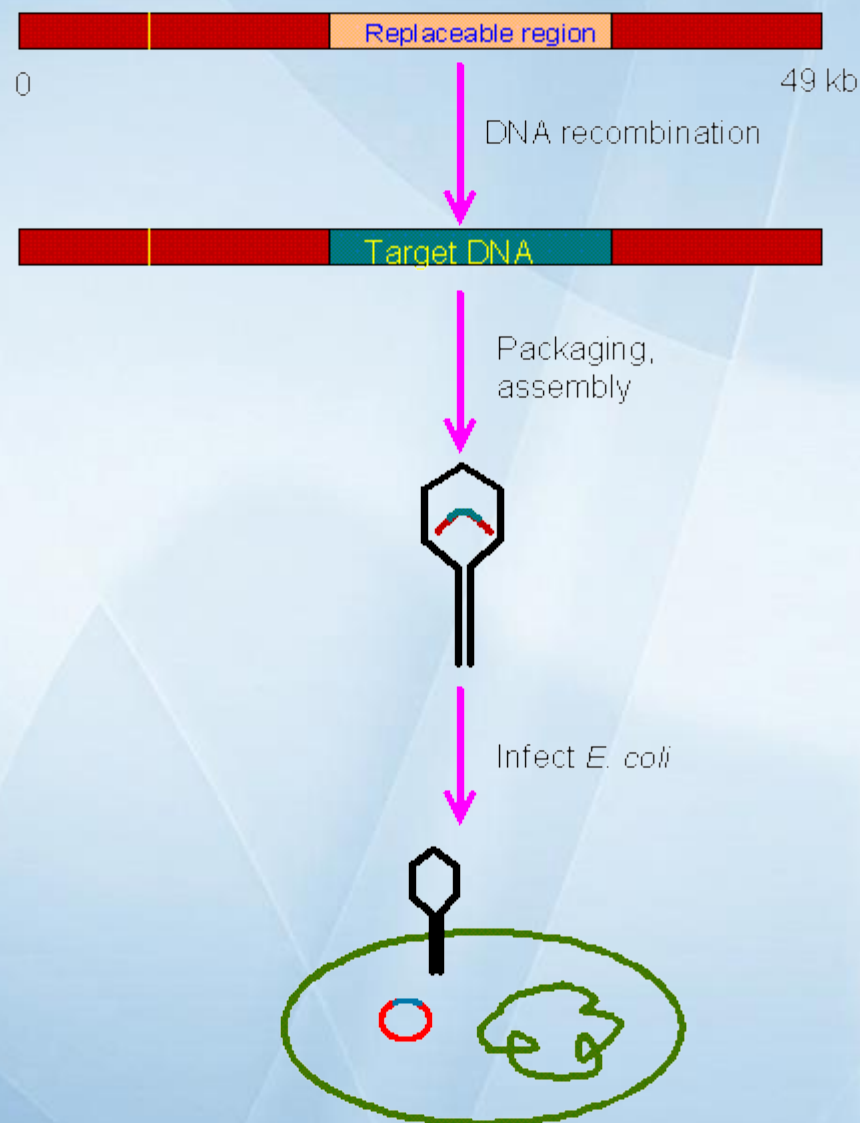
- if they are "loaded" with desired foreign DNA and allowed to infect target host cells

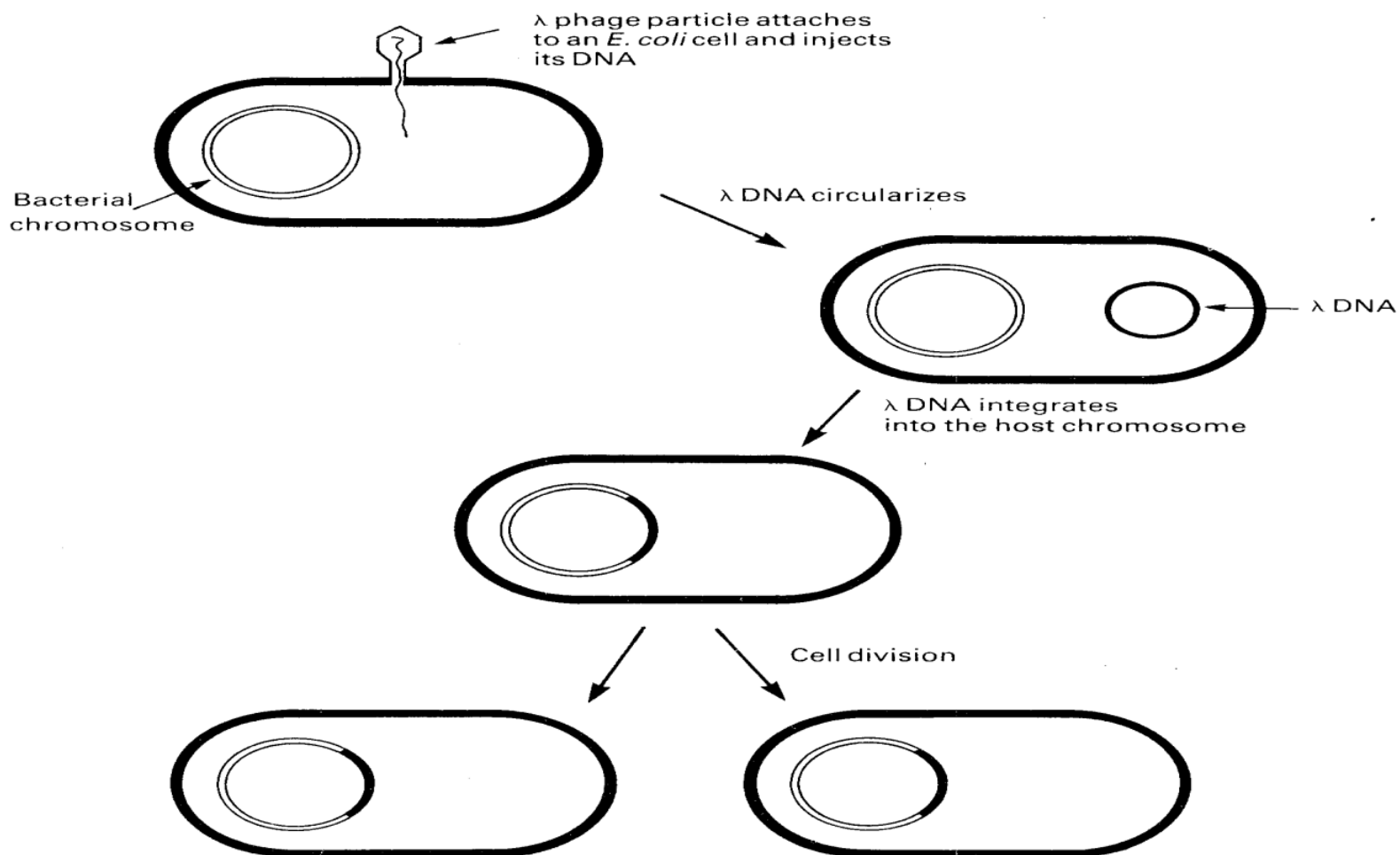


# BACTERIOPHAGE LAMBDA



$\lambda$ -Phage genome

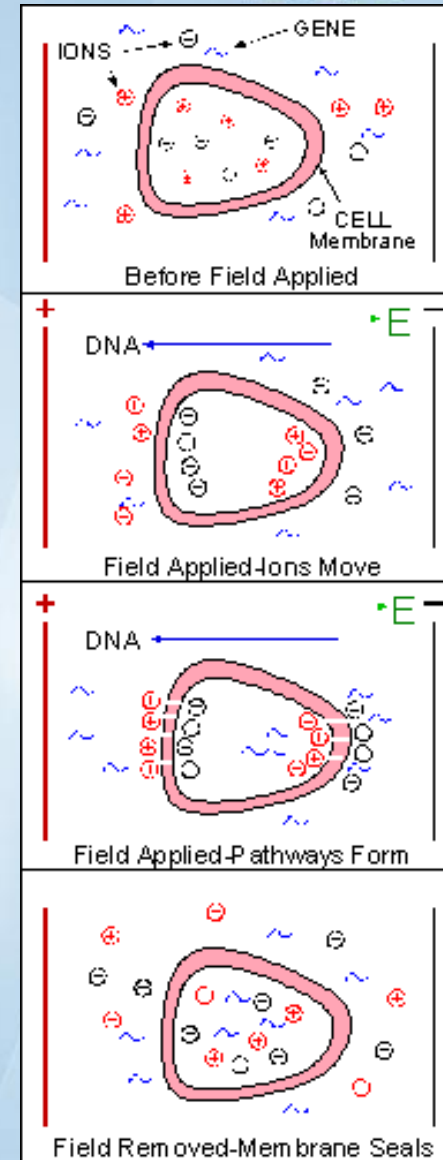




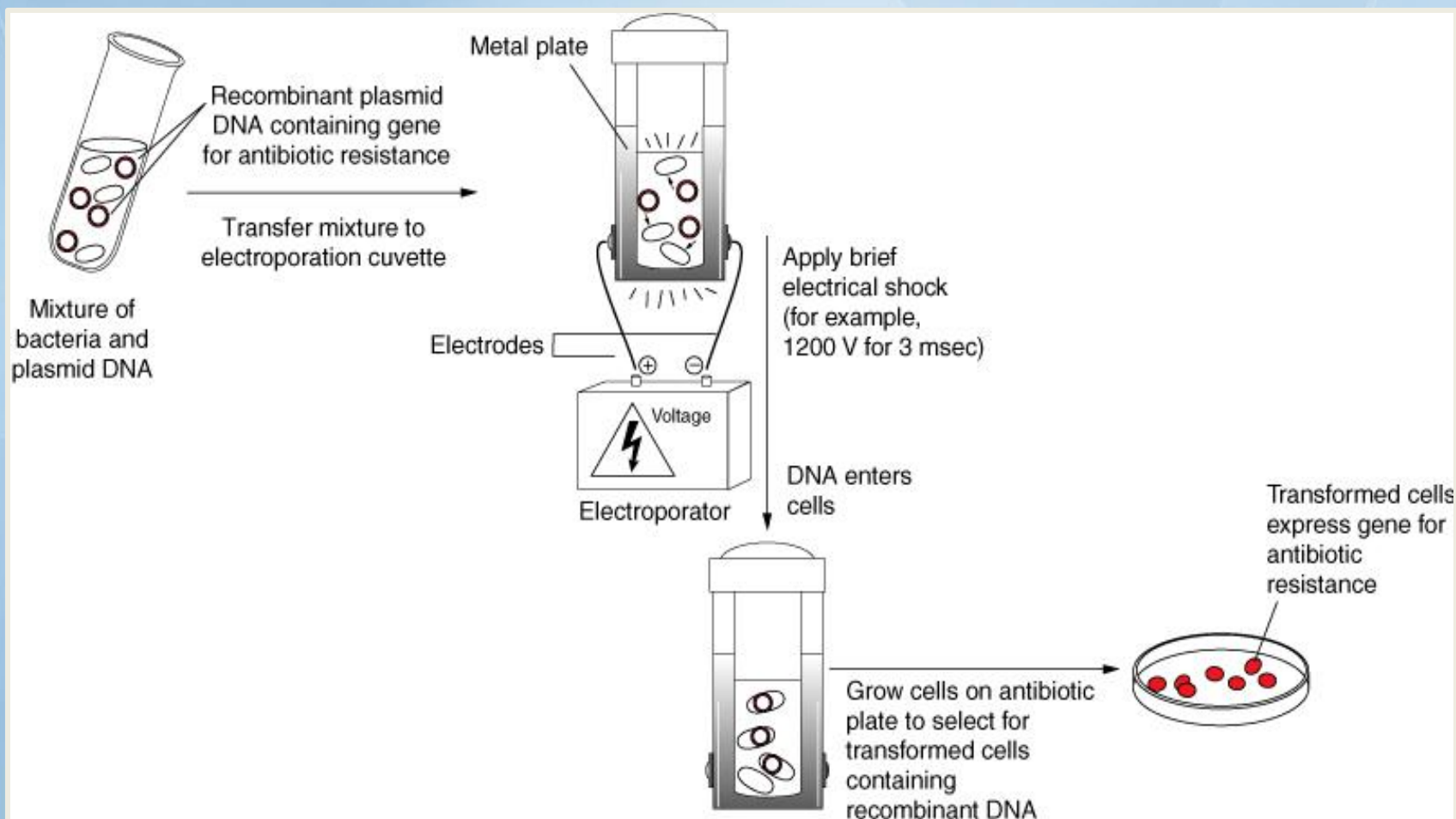
# Electroporation



- If host cell has cell walls, enzymes are used to dissolve the walls, leaving only a protoplast (cell without walls)
- Foreign DNA is introduced via **ELECTROPORATION**--protoplasts are exposed to a short electrical pulse which opens transient membrane channels through which DNA can pass
- transformed cells can then be cultured in media that allows re-formation of cell walls and normal growth into a whole organism (plants, fungi, some protists).
- Animal cells lack cell walls, and so are easily transformed via electroporation.

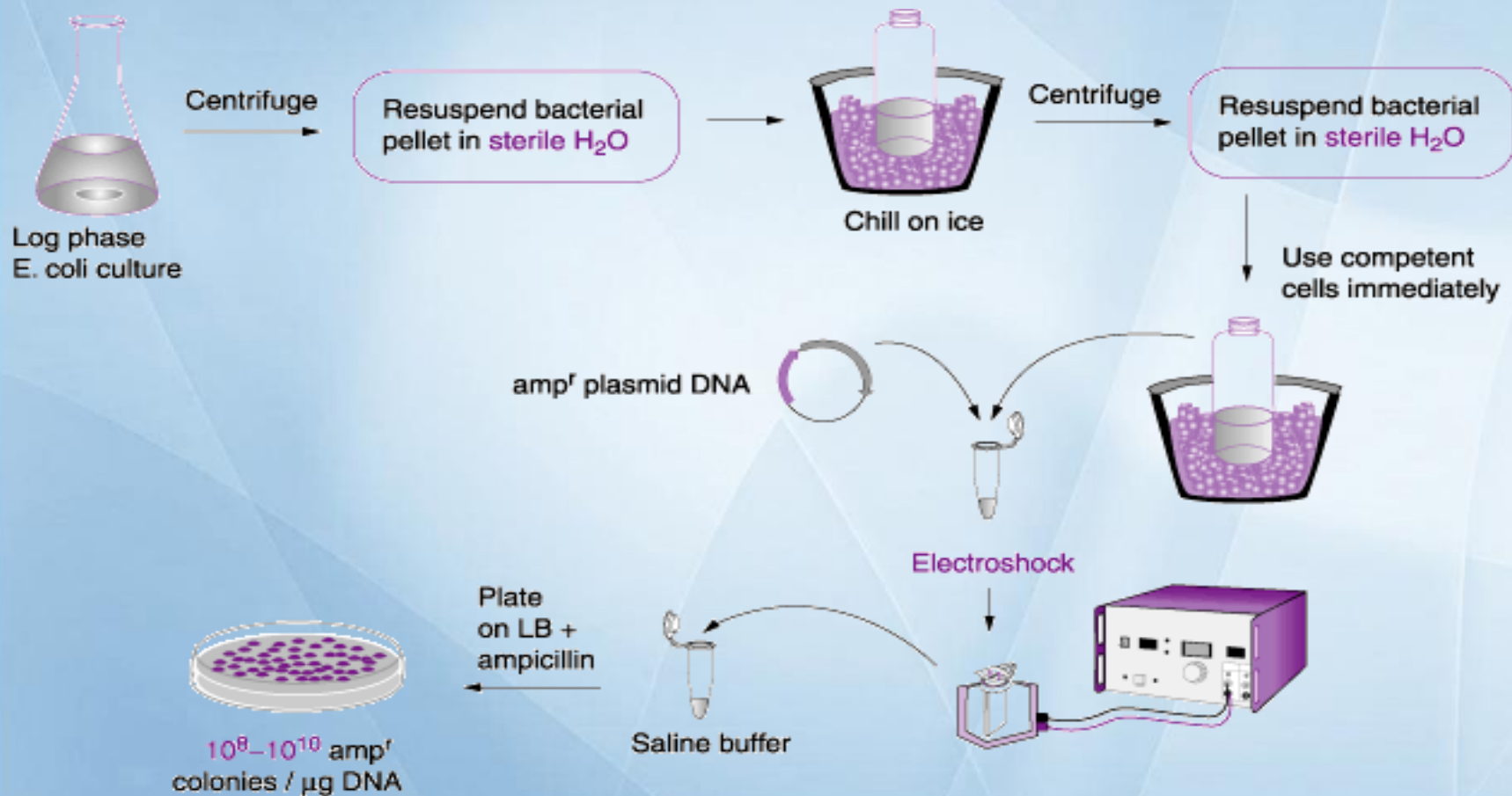


# Electroporation





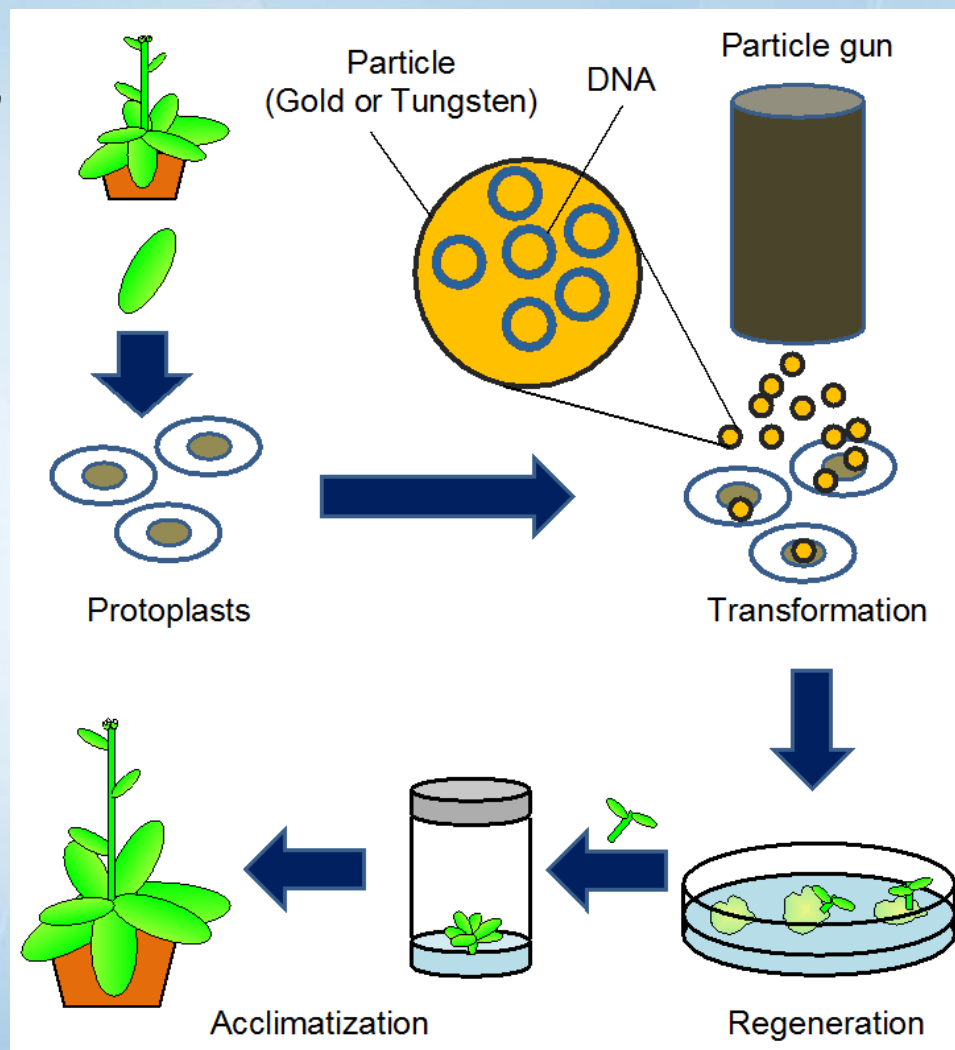
# ELECTROPORATION



# BIOLISTICS



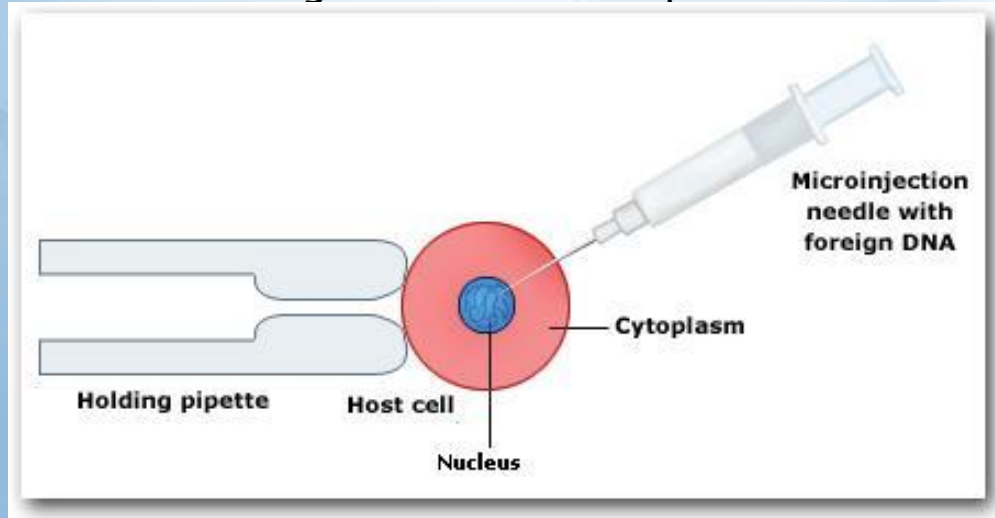
- BIOLISTICS is the process of bombarding cells with microscopic projectiles (usually gold) and coated with DNA
- This technique is promising for use in live organisms



# MICROINJECTION

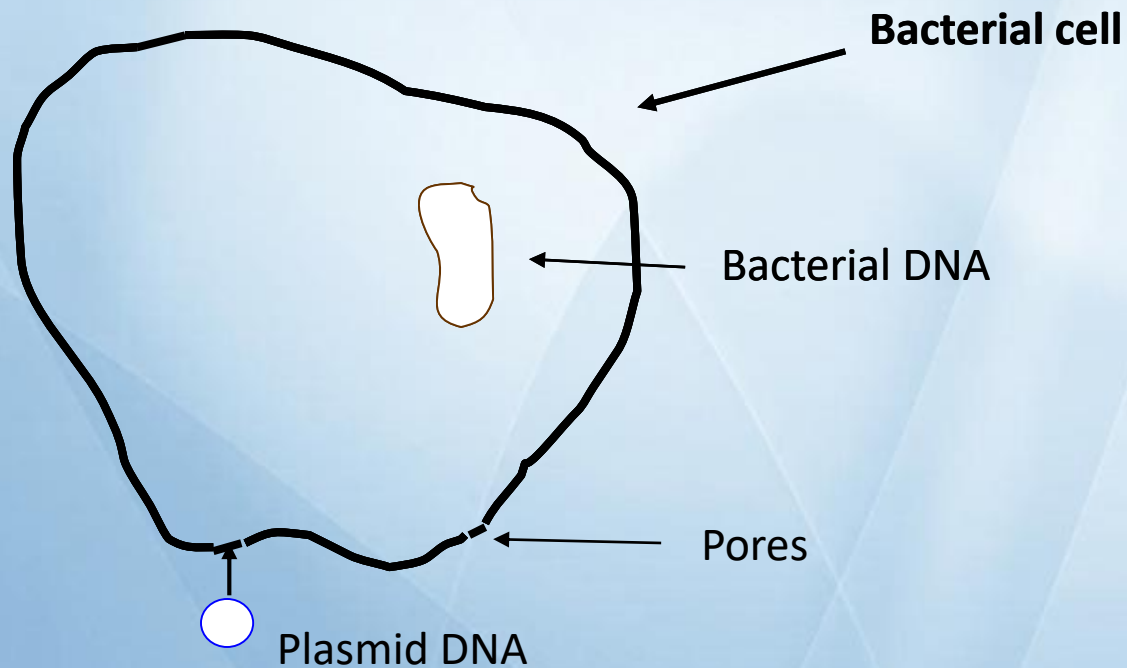


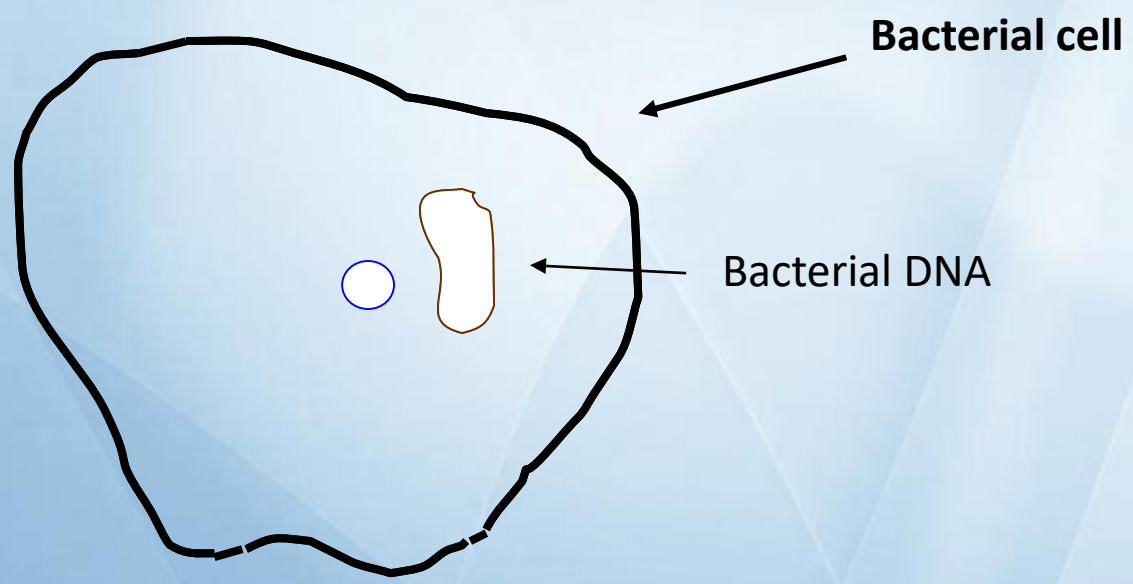
- Transgenic animal with an entirely genetically altered animal can be obtained via **MICROINJECTION**.
  - To generate a **transgenic animal**, foreign DNA must be inserted into a zygote or very early embryo.
  - DNA is injected directly into the nucleus of the cell with an extremely tiny pipette.
  - Once DNA transfer is accomplished, it is sometimes incorporated into the host cell chromosome
  - The transformed zygote/embryo can then be implanted into a surrogate mother for growth and development.



# Transformation

Put the  $\text{CaCl}_2$



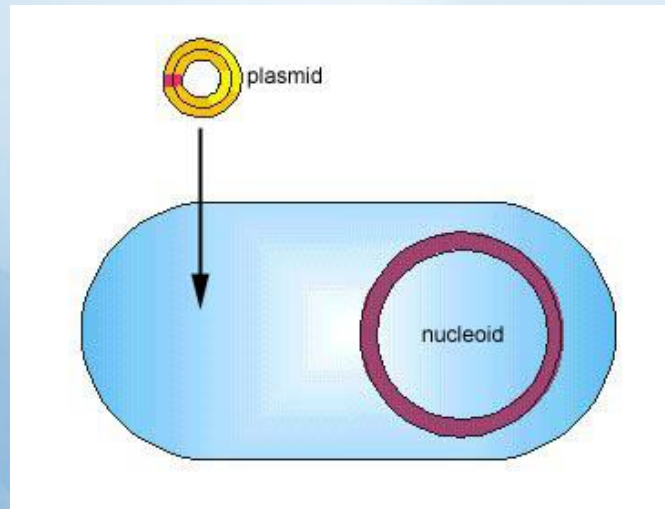




# Transformation

Choose a bacterial host.

- a. *E.coli* is a model organism.
  - i. Well studied
  - ii. No nuclear membranes
  - iii. Has enzymes necessary for replication





# DH5 alpha features

- Transforms with high efficiency.
- The endA1 mutation inactivates an intracellular endonucleases
- $\Delta(lacZ)$  M15 is the alpha acceptor allele needed for blue-white screening with many lacZ based vectors.

$F\Phi80 lacZ \Delta M15 \Delta(lacZYA-argF)$

169 *recA1 endA1 hsdR17* (rK<sup>-</sup>, mK<sup>+</sup>) *phoA supE44*

$\lambda$ - *thi-1 gyrA96 relA1*

# Competent cells

Prepare bacterial cells for transformation of plasmid.

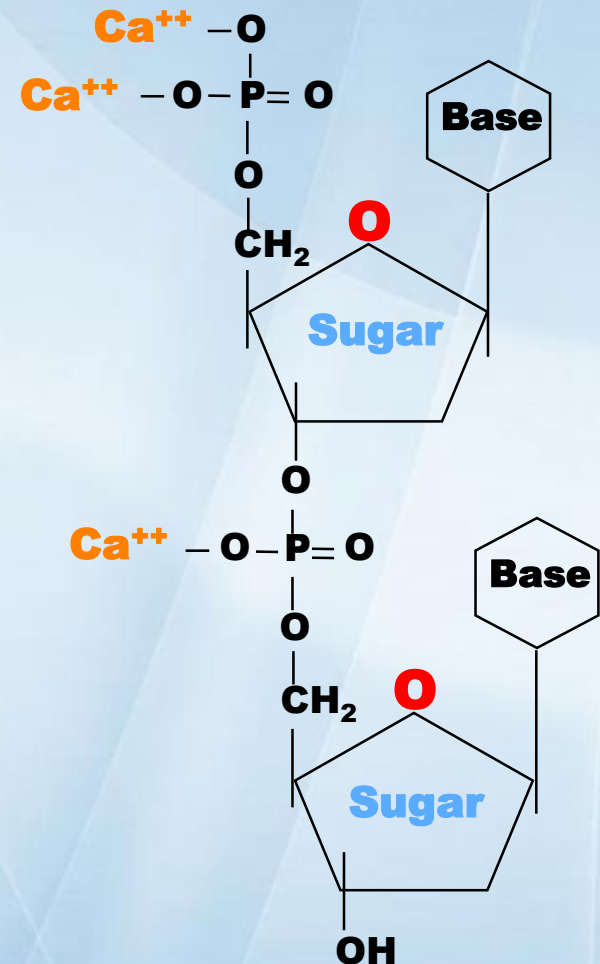
Treat with calcium chloride, which allows plasmid to pass through bacterial cell walls. This is the most common method.

**Animation**

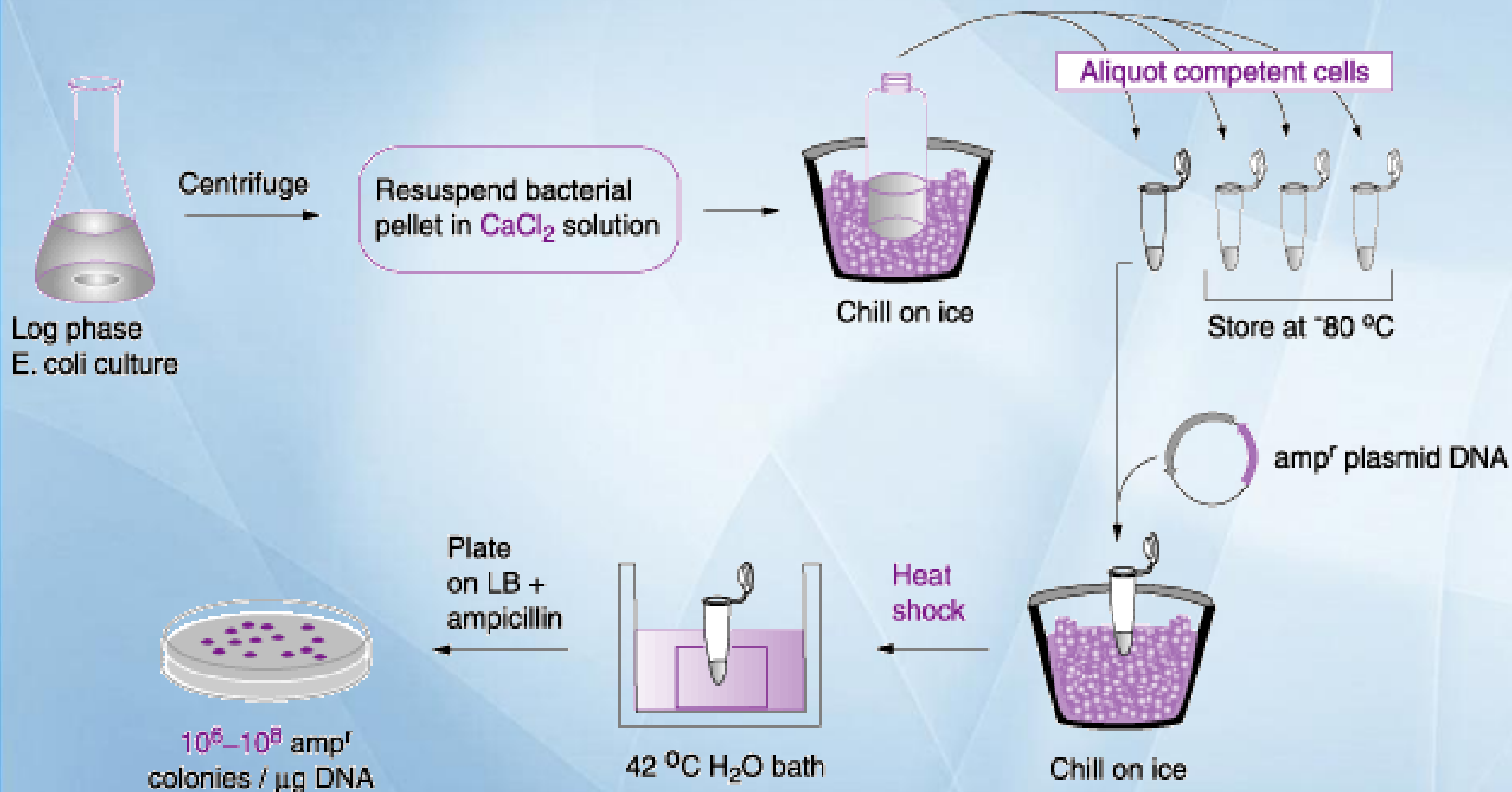
# Reasons for Performing Each Transformation Step?



Positive charge of  $\text{Ca}^{++}$  ions shields negative charge of DNA phosphates



# CHEMICAL TRANSFORMATION WITH CALCIUM CHLORIDE



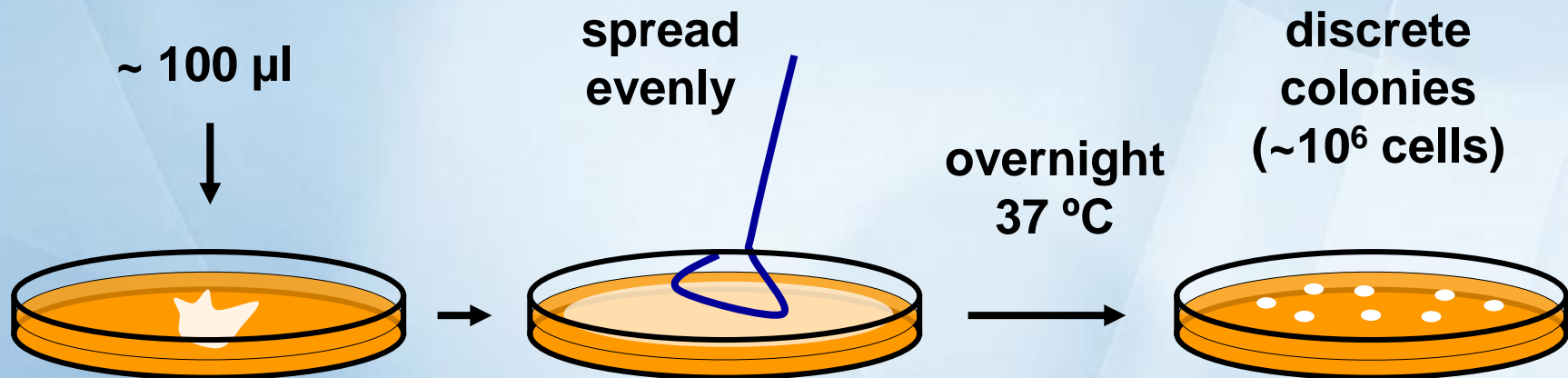


# It's not over until the morning after...

**Transformation is not an end in itself**

**Transformed bacteria are plated on agar**

- With appropriate selection (antibiotics)

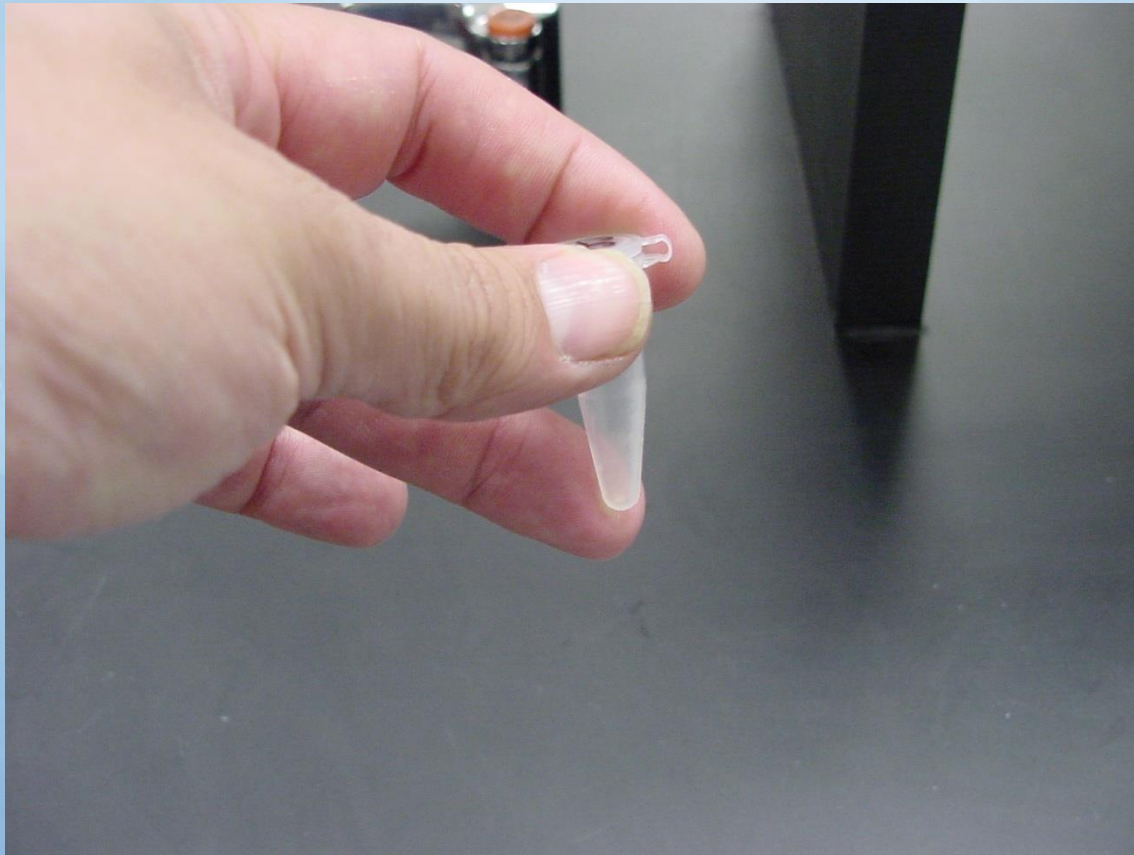


**Methods must be optimized**

- Too many transformants – can't pick discrete clones
- Too few transformants – nothing to pick

# Recombinant Transformation

Finger flick tube "CC" to resuspend cells.



# Recombinant Transformation

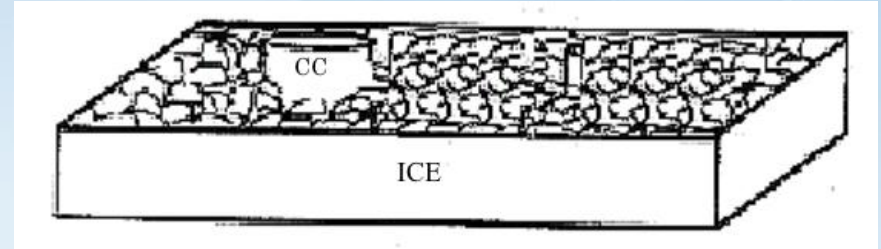
Open the tube of competent cells labeled "CC" and add 5  $\mu$ l of the ligation solution "Lig" directly to the "CC" tube solution using a micropipettor and sterile tip. Close the tube.





# Recombinant Transformation

Place the tube on ice for 20 minutes.



# Recombinant Transformation

Remove the tube from the ice and immediately hold it in a 42°C water bath for 90 seconds. Place the tube directly back on ice for 1 minute.





# Recombinant Transformation

Use a sterile pipette to add 500  $\mu$ l of sterile LB nutrient broth to the competent cell "CC" tube.

Close the tube. Mix by tipping the tube and inverting it gently.



# Recombinant Transformation

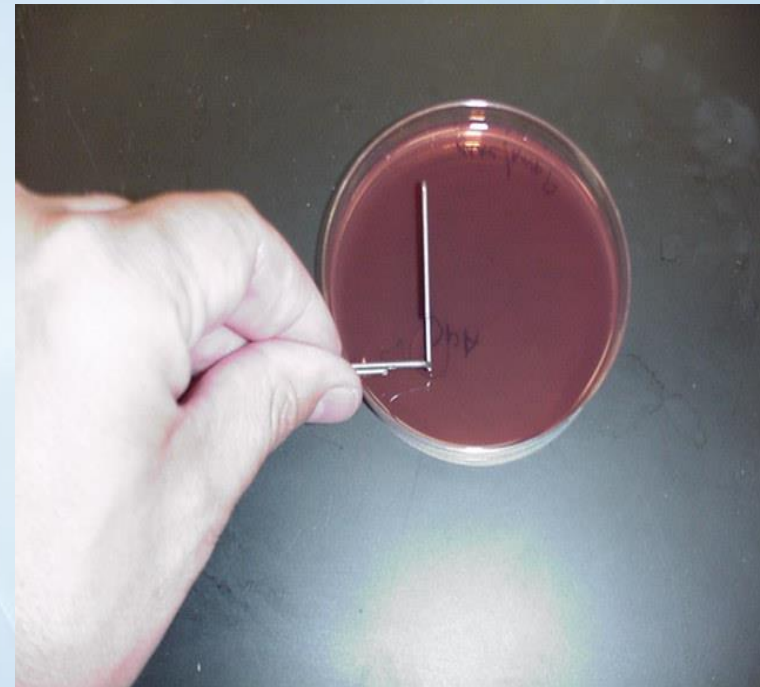
Incubate the mixture  
for 1 hours at 37°C  
with shaking



# Recombinant Transformation

Use a fresh sterile paper clip to spread the liquid evenly across the surface of each plate.

Be careful not to touch the part of the paper clip that comes in contact with the agar.



# Recombinant Transformation

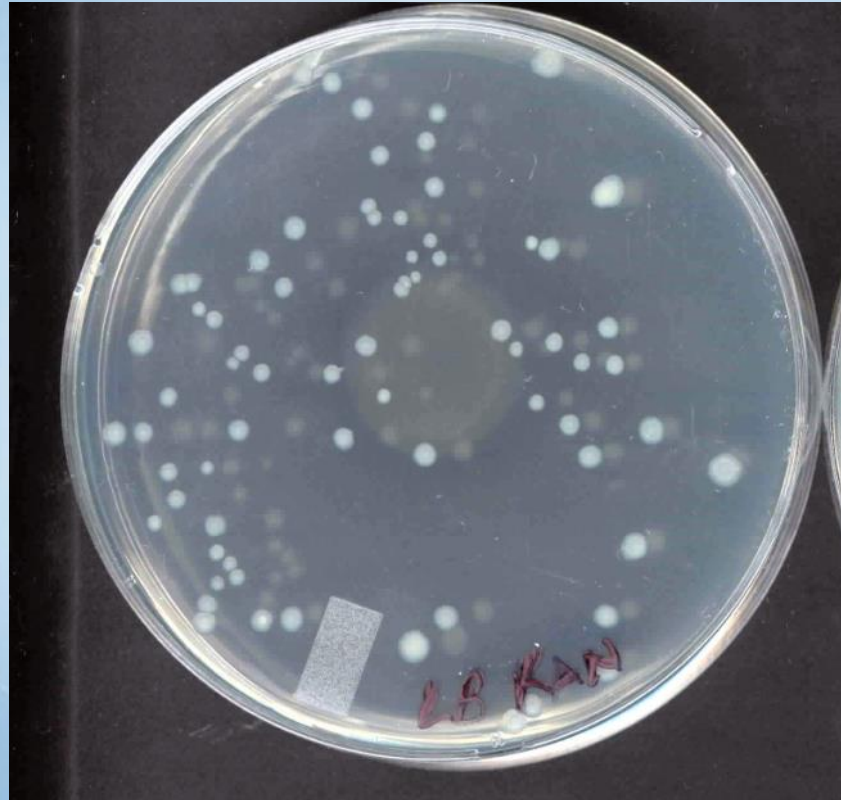
Incubate the plates (agar up) for 24 hours in a 37° C incubator.



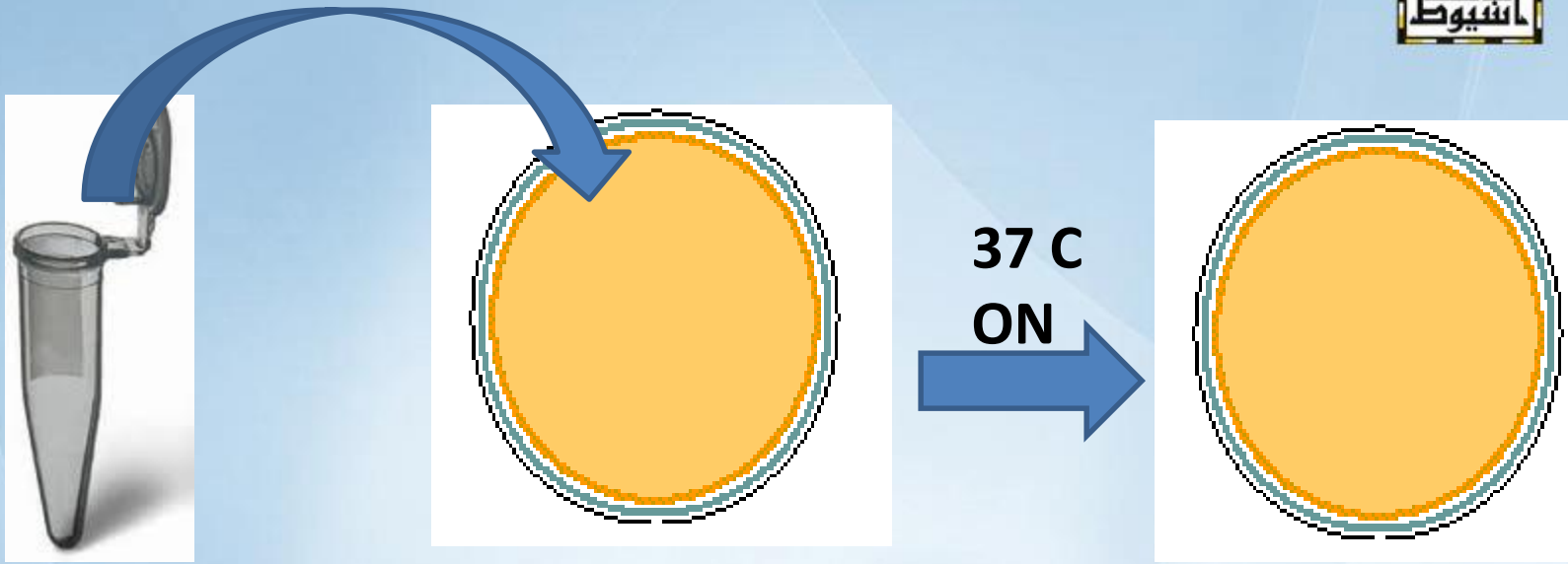


# Recombinant Transformation

## Results





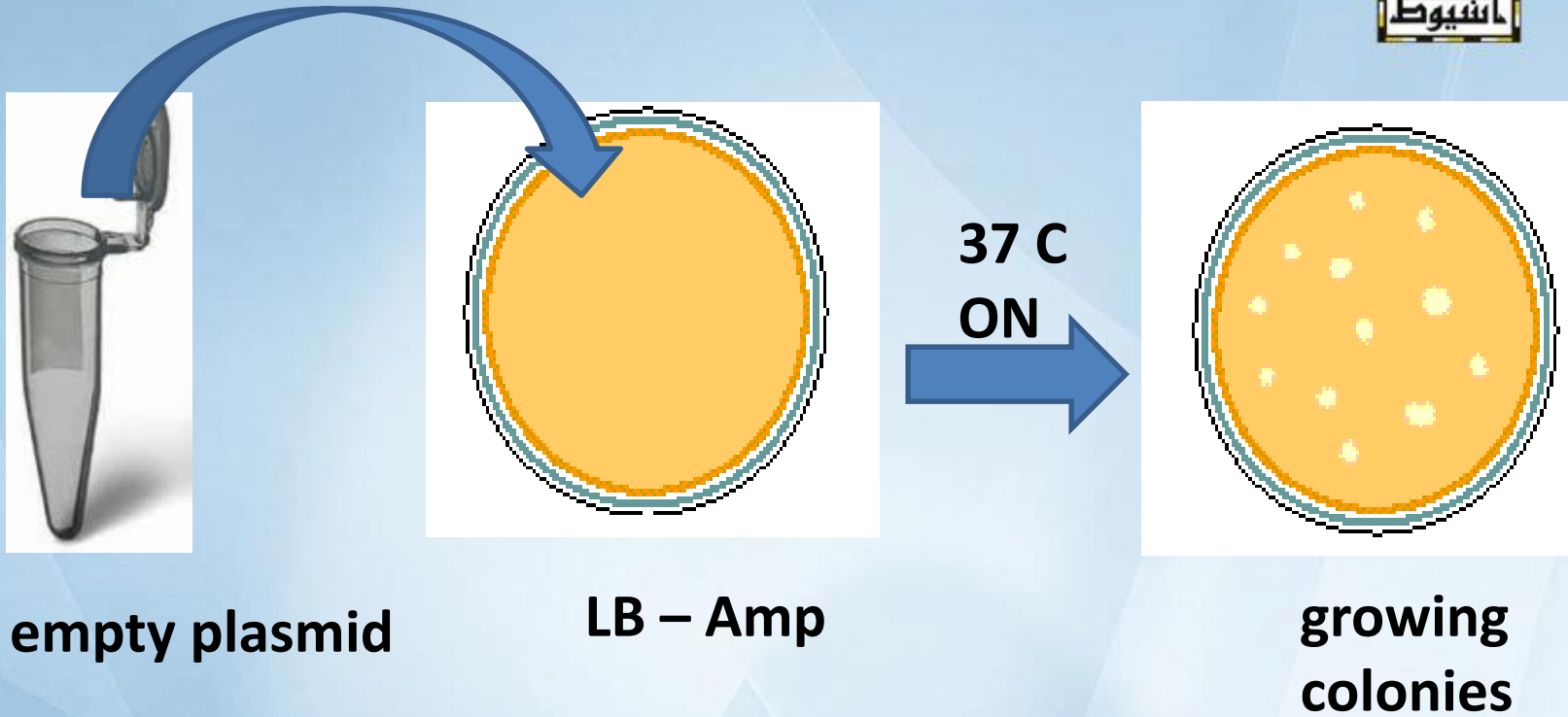


**CC + No plasmid**

**LB – Amp**

**NO colonies**

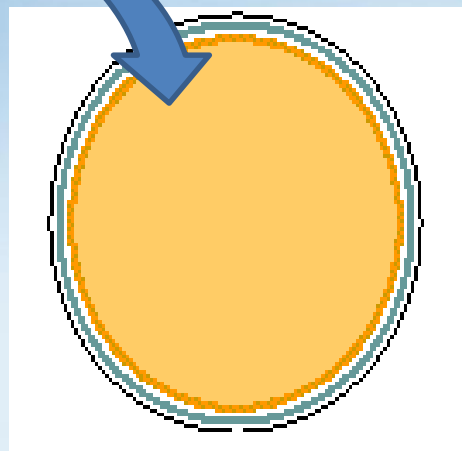
# Negative control



# Positive control

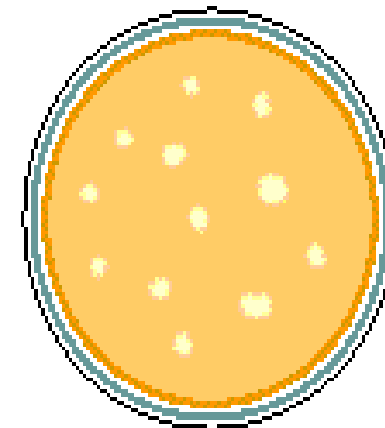


**CC +  
recombinant  
plasmid**



**LB – Amp**

**37 C  
ON**



**growing  
colonies**

# Transformation Procedure

