Transformation

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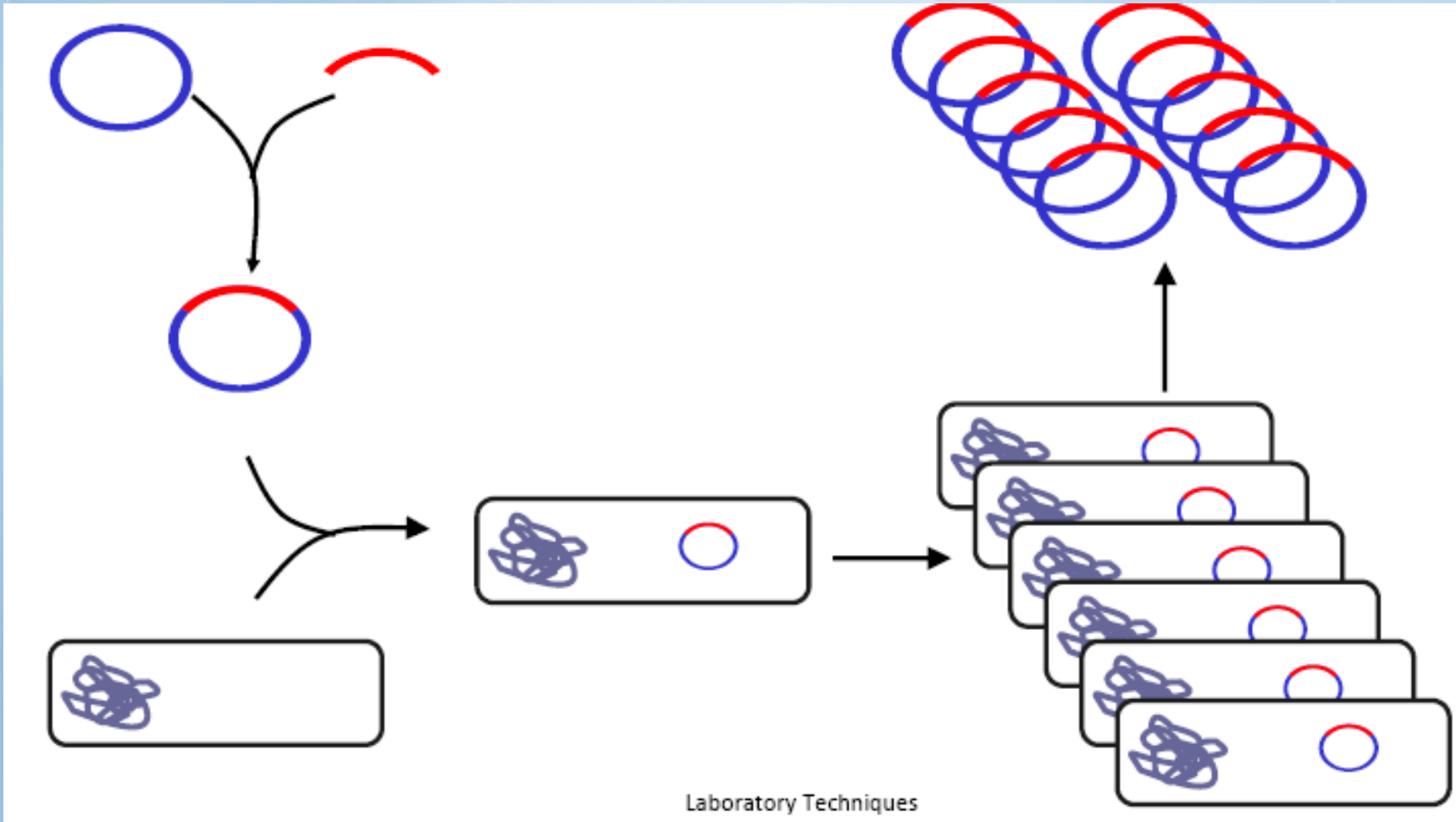
DNA Cloning Overview



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 phaga
- 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

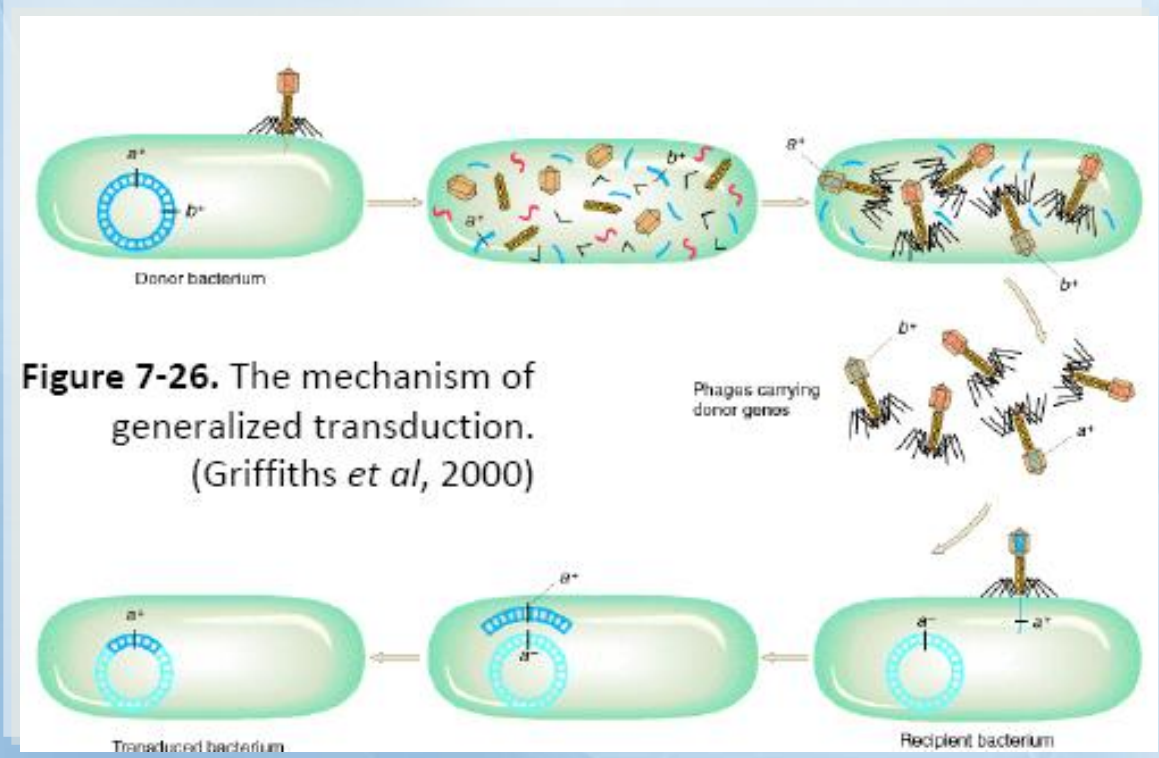
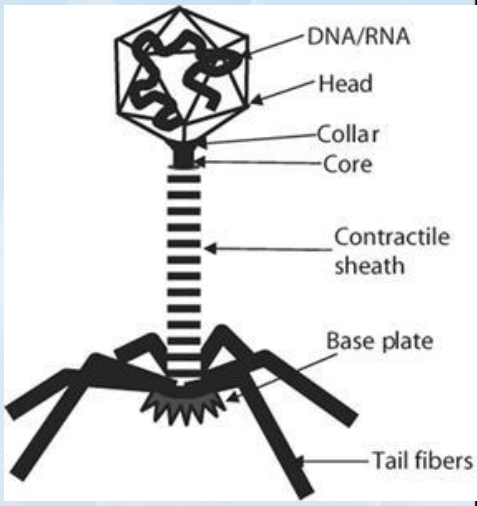


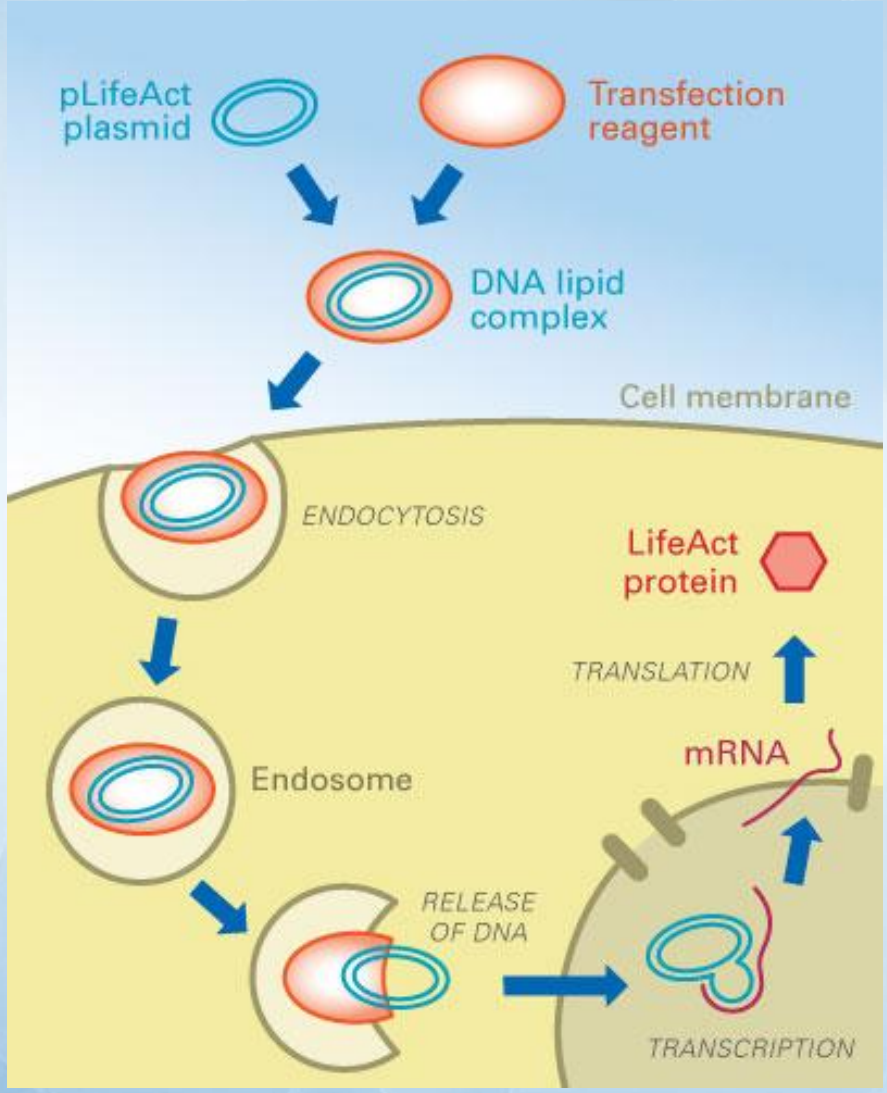
Figure 7-26. The mechanism of generalized transduction. (Griffiths *et al*, 2000)



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

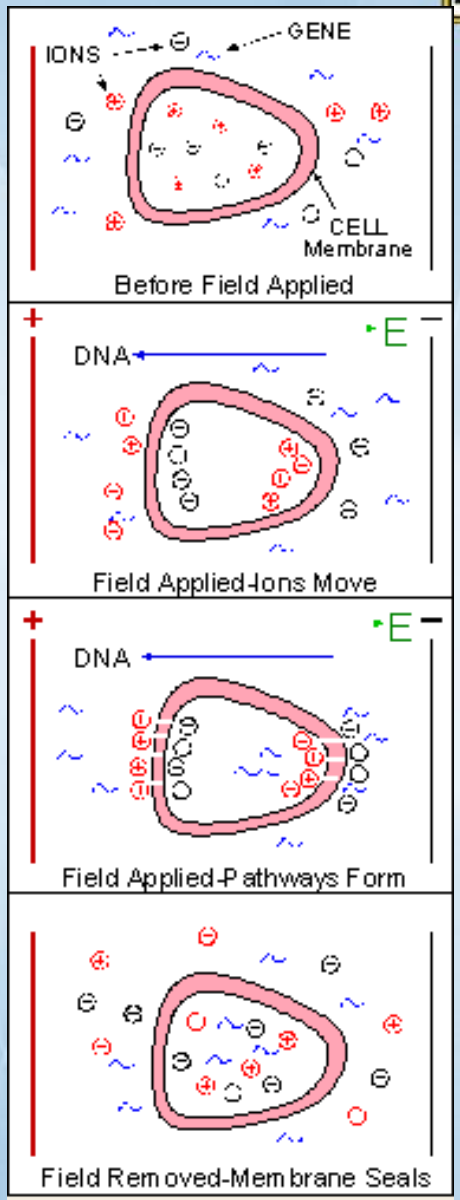
Transfection

Transfection is frequently carried out by mixing a cationic lipid with the material to produce liposomes, which fuse with the cell plasma membrane and deposit their cargo inside.

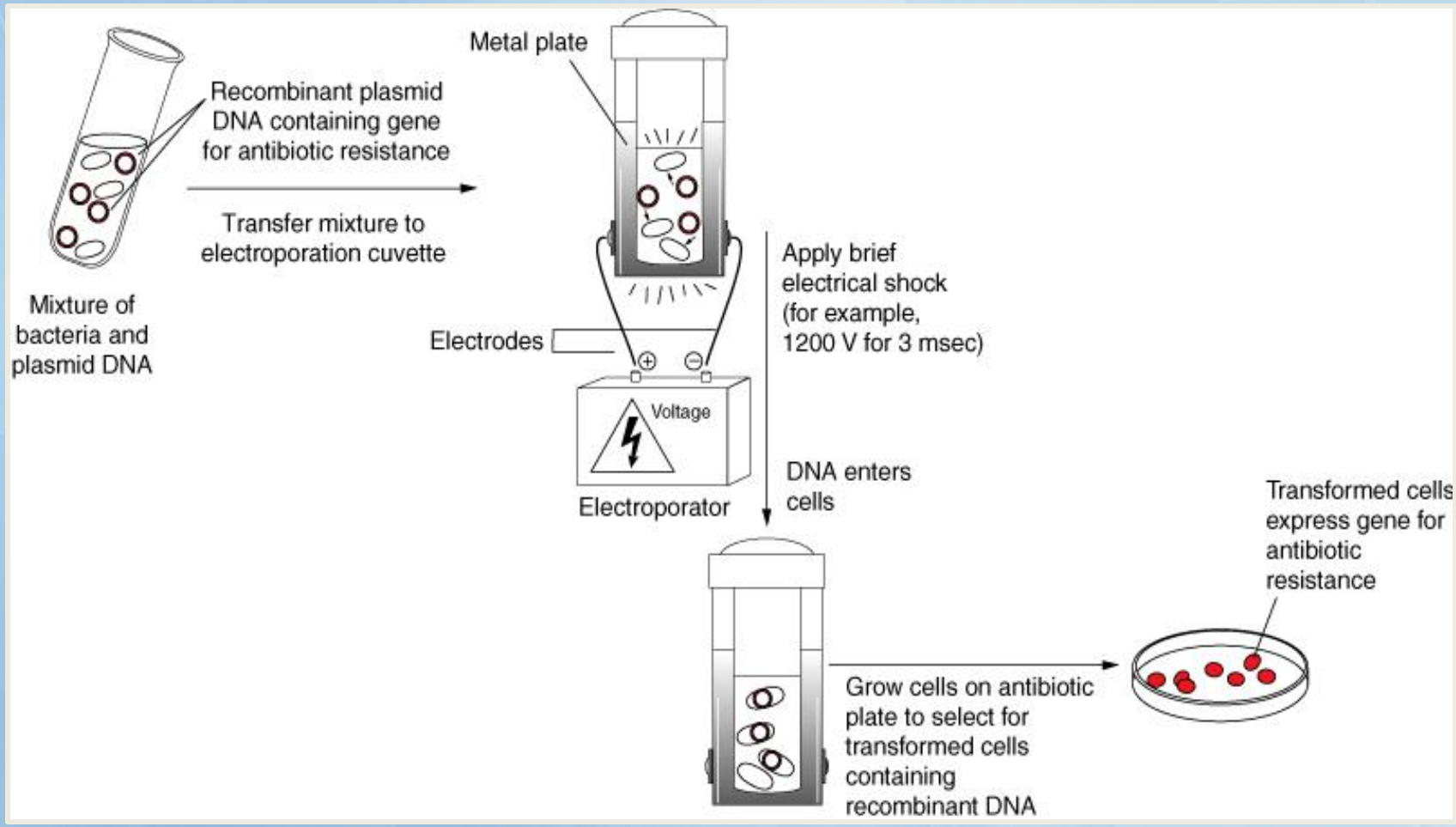


Electroporation

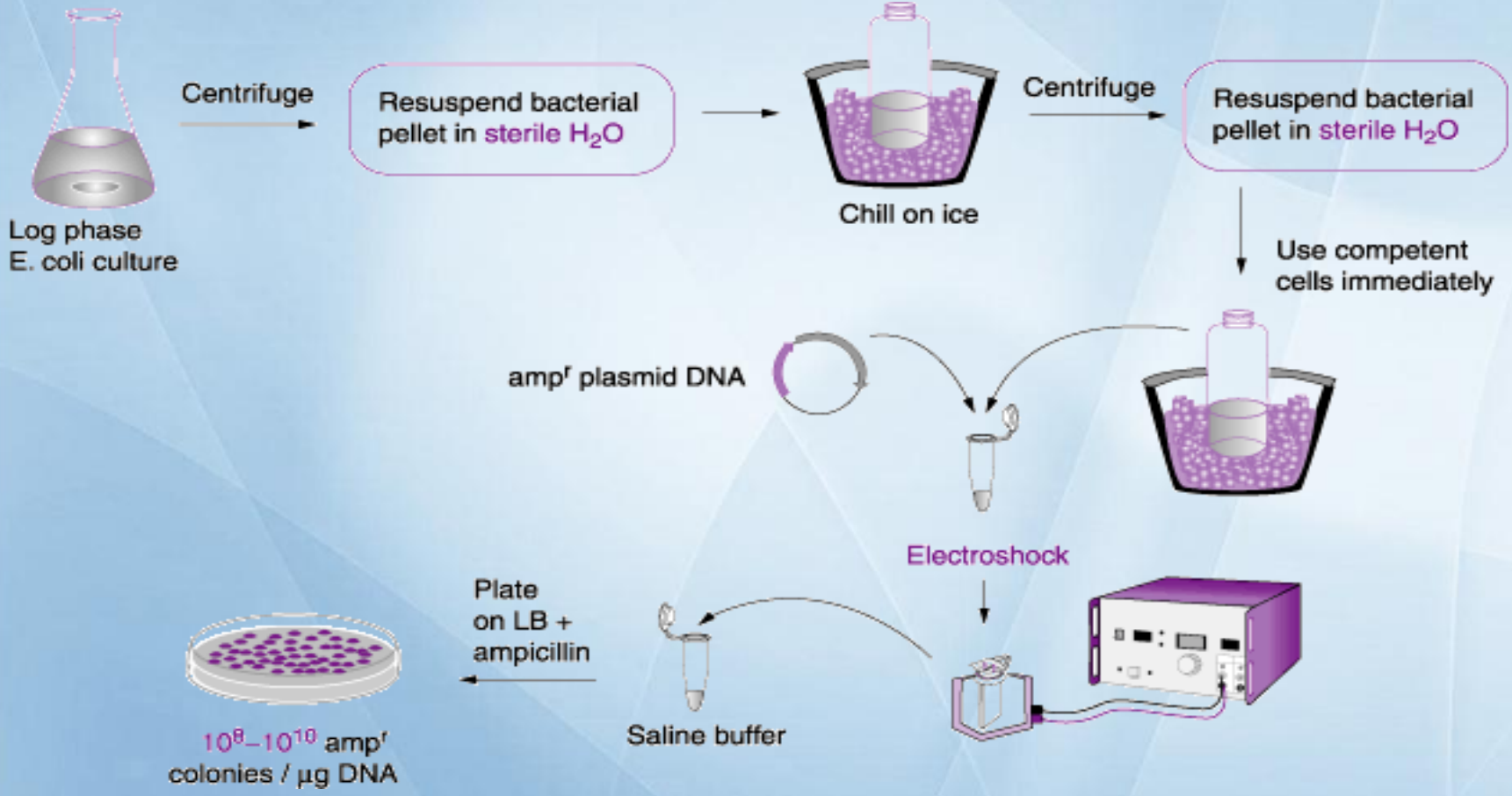
- if host cell has cell walls, enzymes are used to dissolve the walls, leaving only a protoplast (cell without walls)
- Foreign DNA is introduced via ELECTROPORATION--protoplasts are exposed to a short electrical pulse which opens transient membrane channels through which DNA can pass
- transformed cells 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



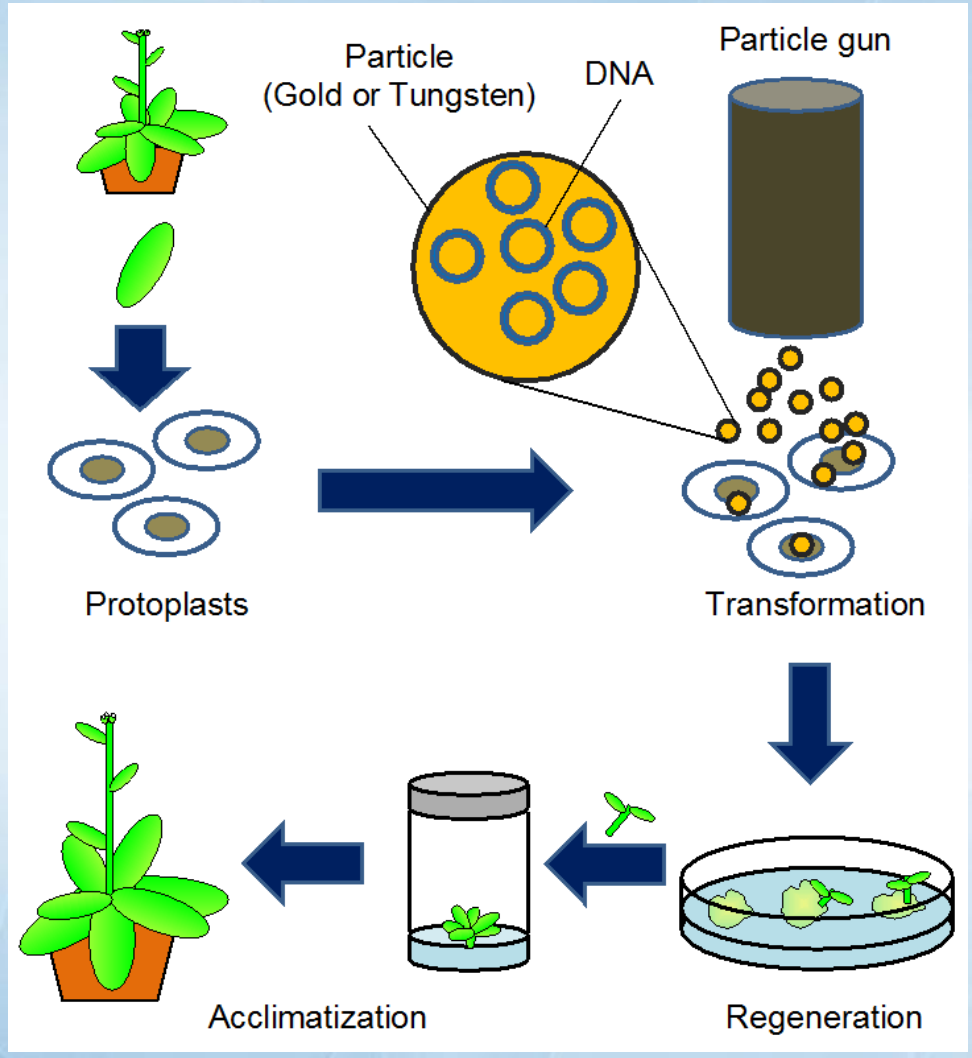
ELECTROPORATION



BIOLISTICS

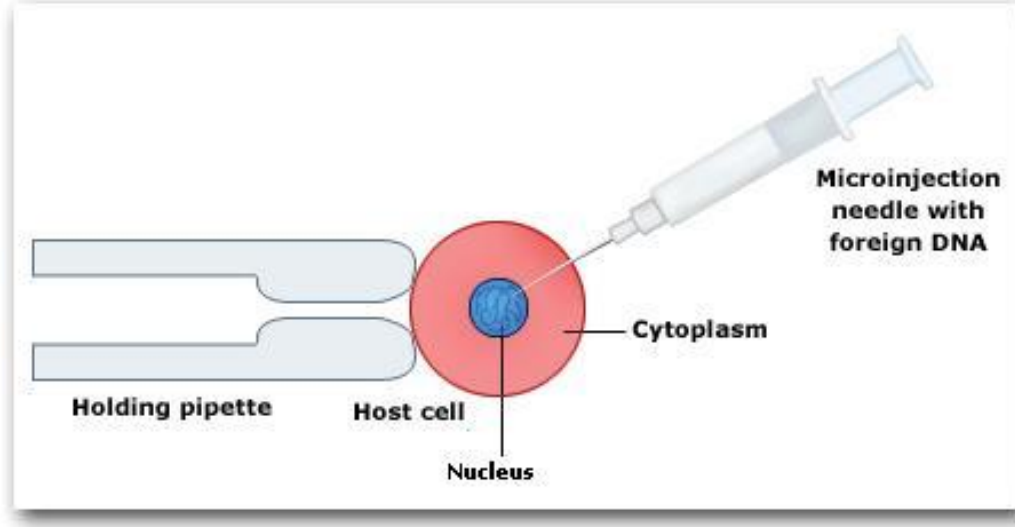
- BIOLISTICS is the process of bombarding cells with microscopic projectiles (usually gold) and coated with DNA

- This technique is promising for use in live organisms

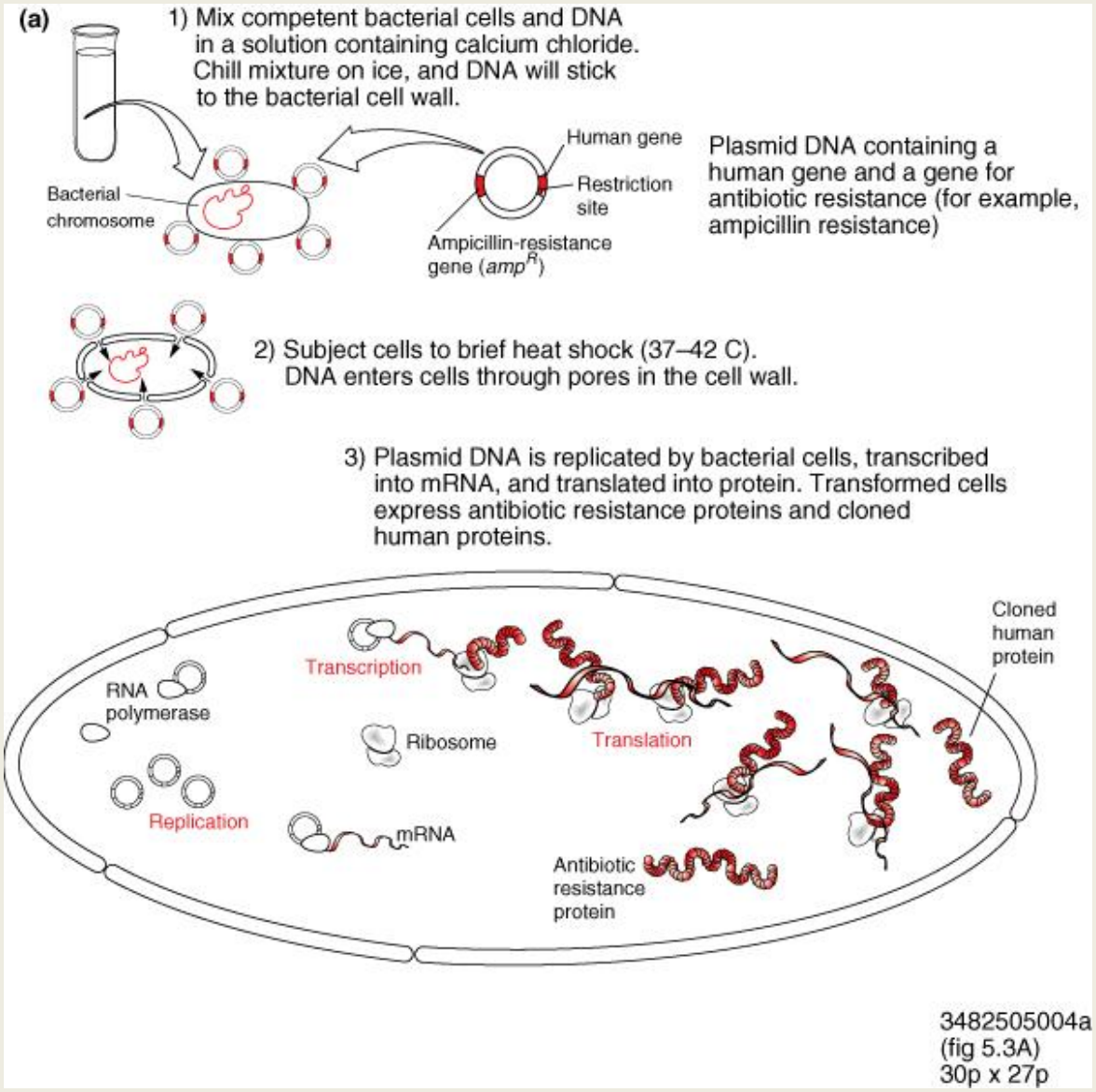


MICROINJECTION

- 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.



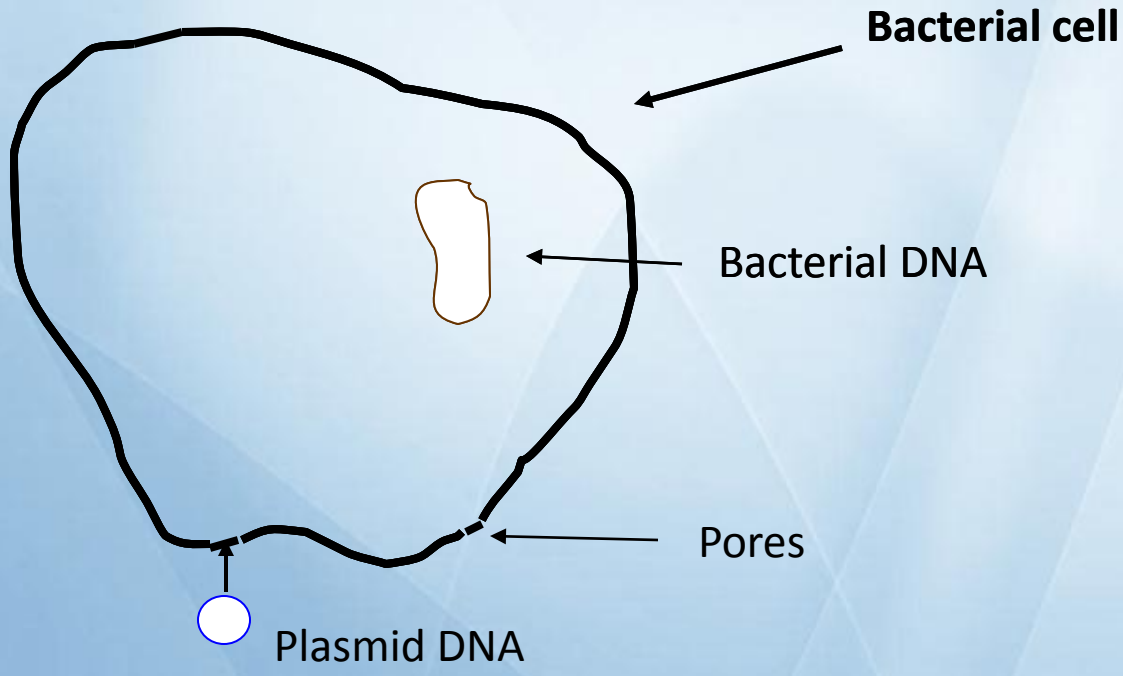
Transformation



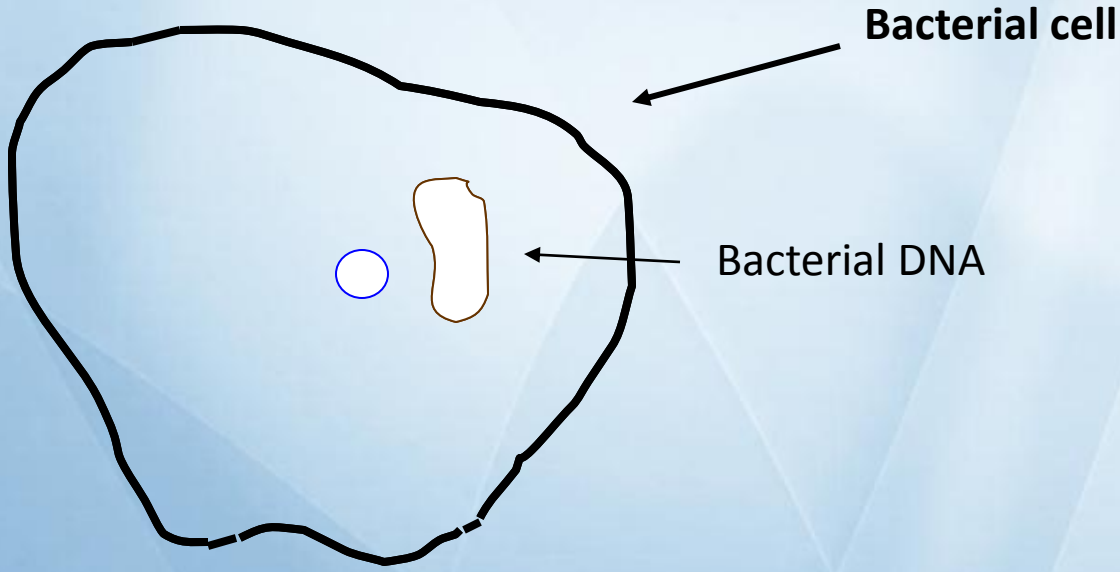
Bacterial Growth

Transformation

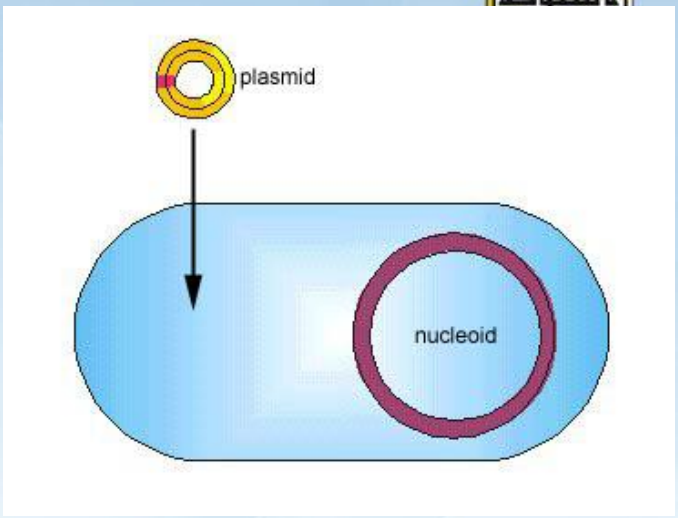
Put the $CaCl_2$



Bacterial Growth



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
- Δ*(lacZ)* M15 is the alpha acceptor allele needed for blue-white screening with many lacZ based vectors.

F – Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) 169 *rec*A1 *end*A1 *hsd*R17 (rK–, mK+) *pho*A *sup*E44 λ– *thi*-1 *gyr*A96 *rel*A1

Competent cells

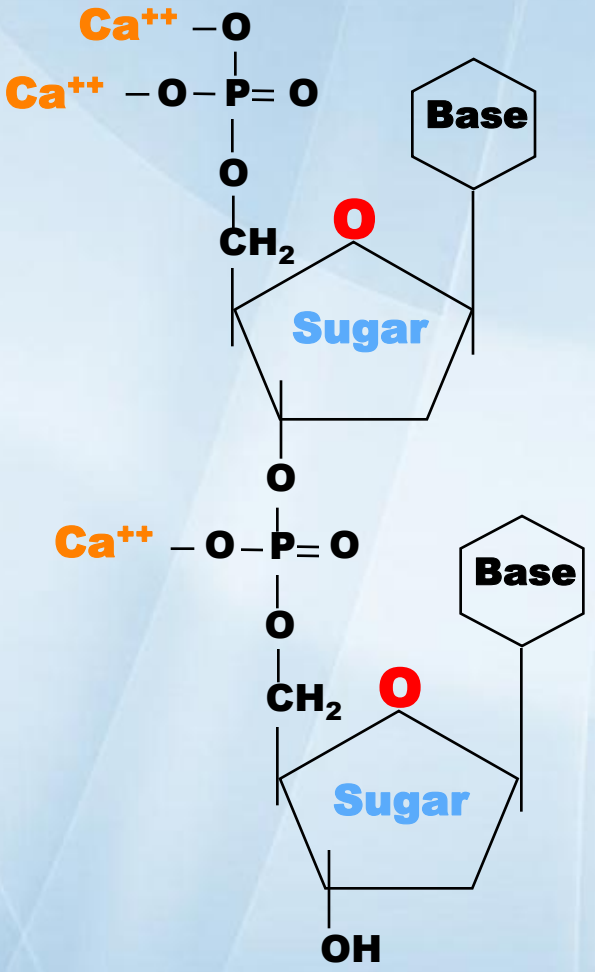
Prepare bacterial cells for transformation of plasmid.

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

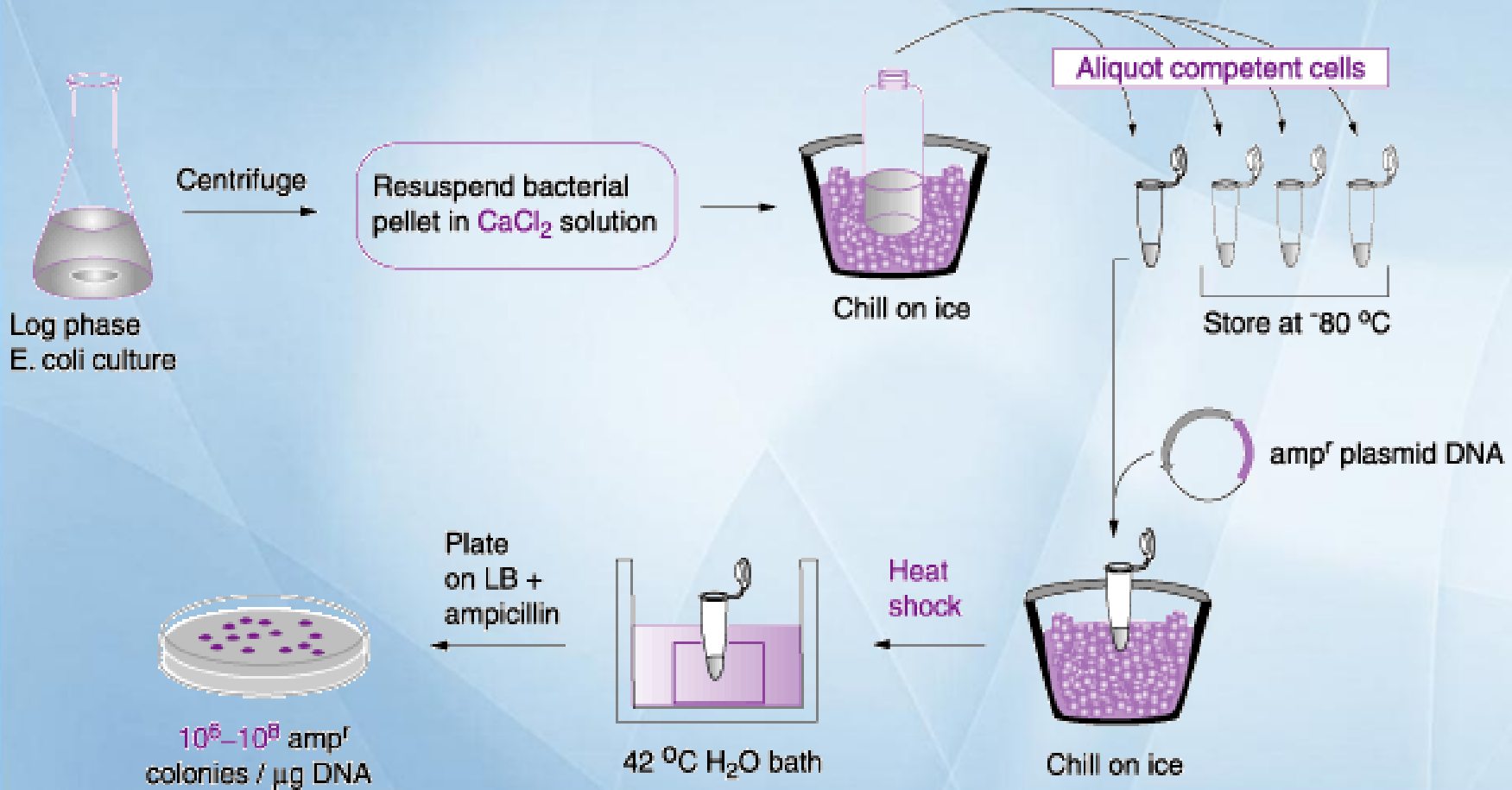
Animation

Reasons for Performing Each Transformation Step?

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



CHEMICAL TRANSFORMATION WITH CALCIUM CHLORIDE

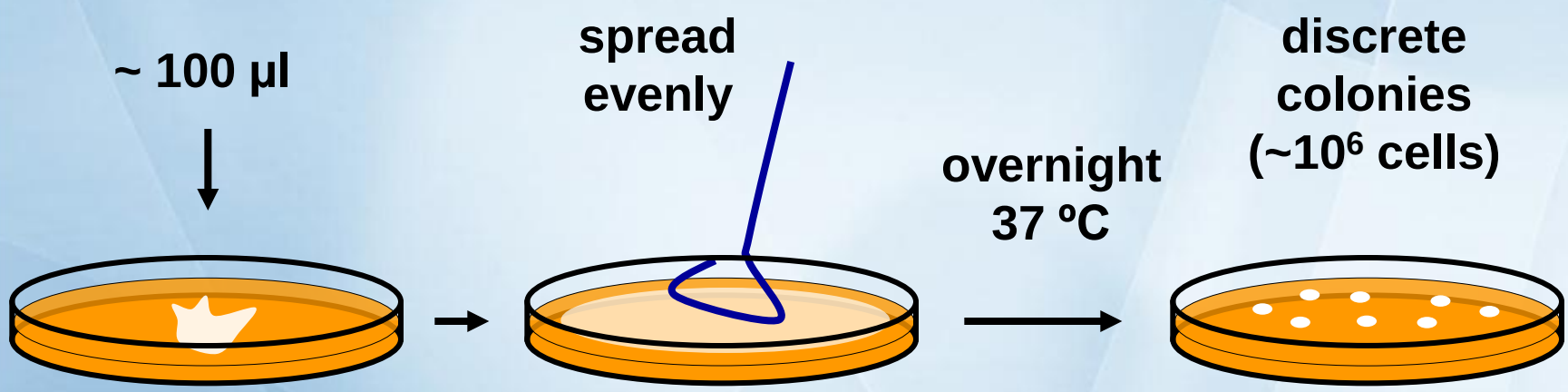


It’s not over until the morning after…

Transformation is not an end in itself

Transformed bacteria are plated on agar

- With appropriate selection (antibiotics)

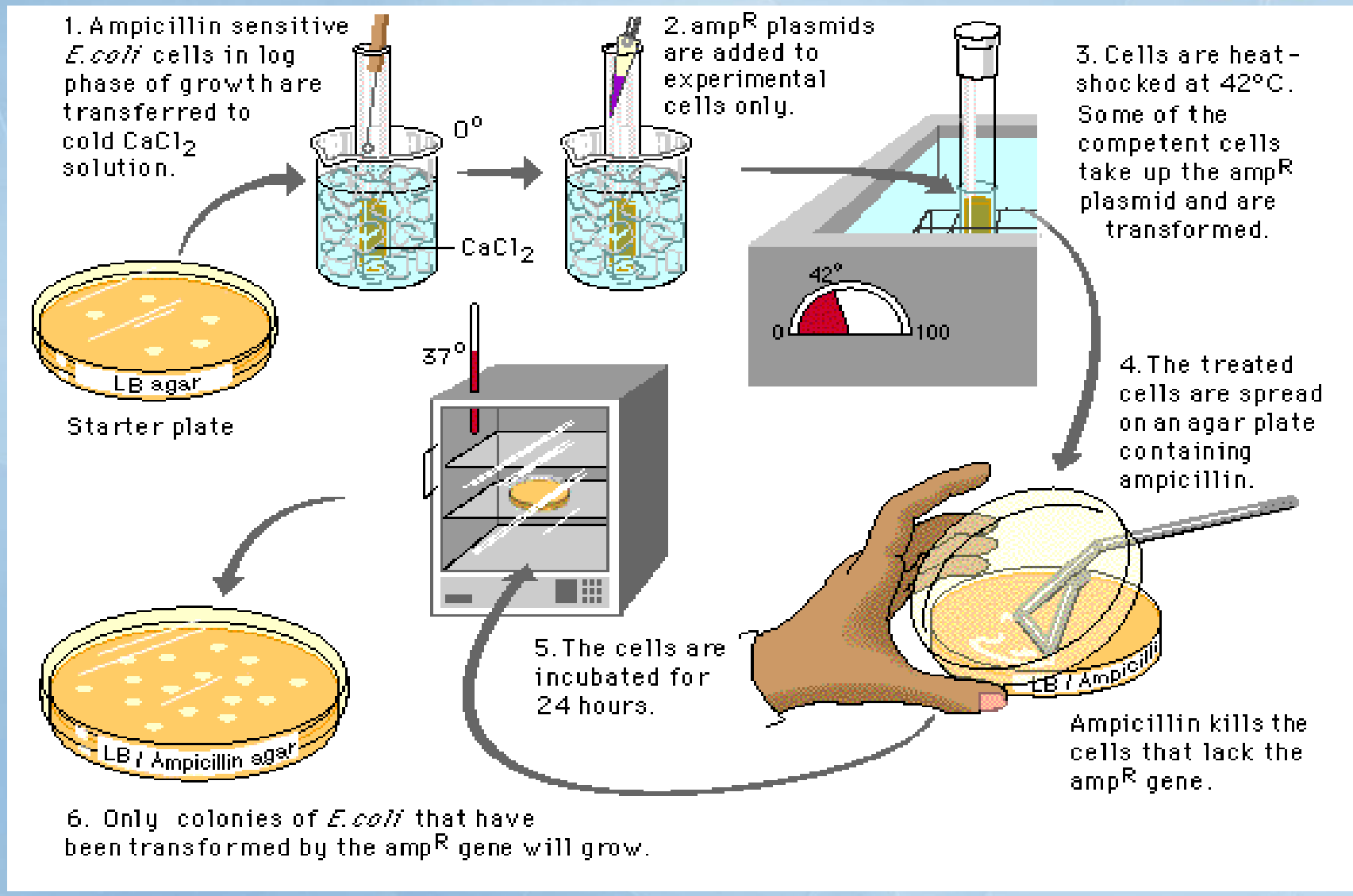


Methods must be optimized

-Too many transformants – can’t pick discrete clones

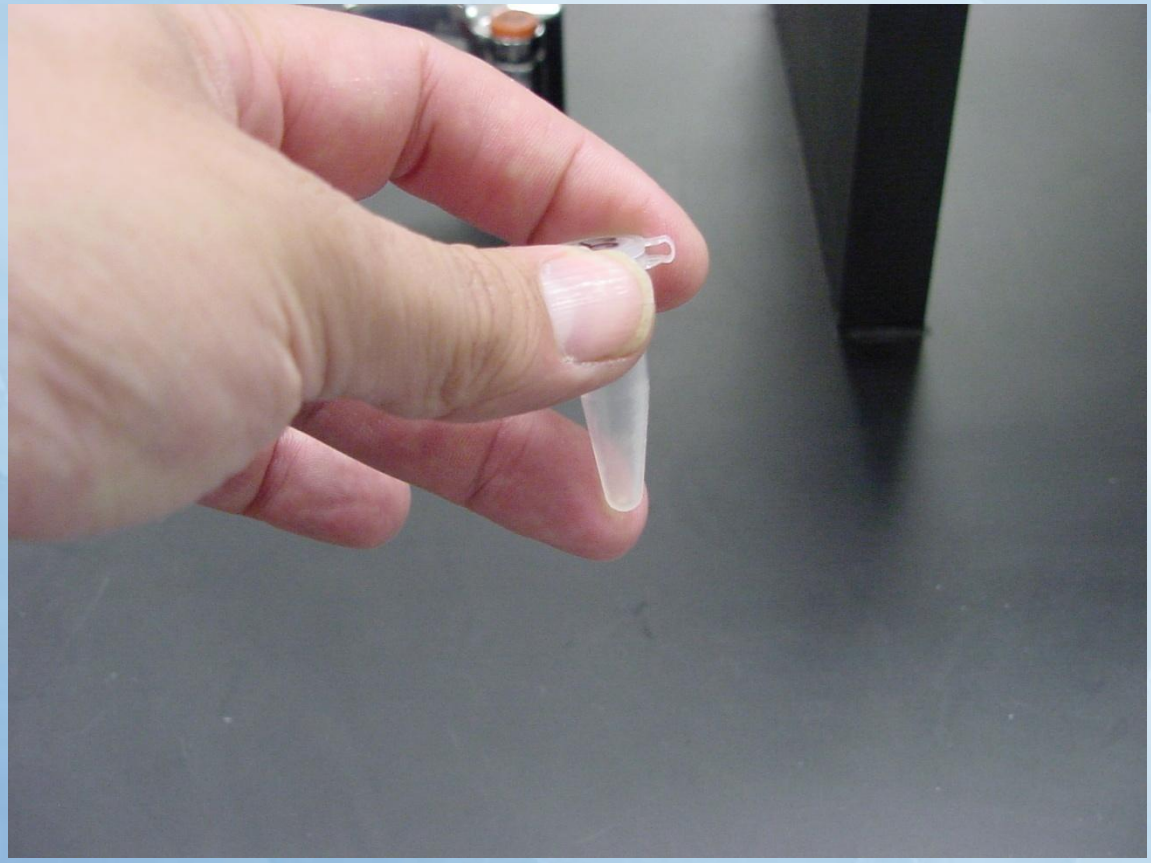
-Too few transformants – nothing to pick

Transformation Procedure



Recombinant Transformation

Finger flick tube “CC” to resuspend cells.

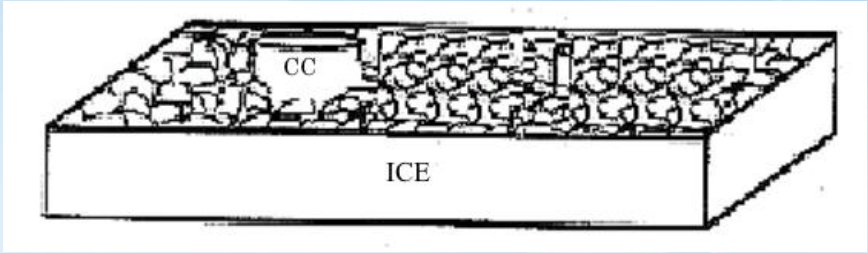


Recombinant Transformation

Open the tube of competent cells labeled “CC” and add 5 µl of the ligation solution “Lig” directly to the “CC” tube solution using a micropipettor and sterile tip. Close the tube.

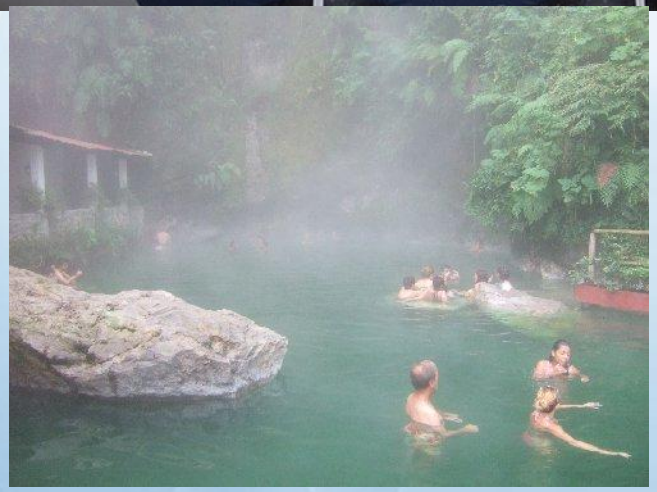
Recombinant Transformation

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 µl of sterile LB nutrient broth to the competent cell “CC” tube.

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

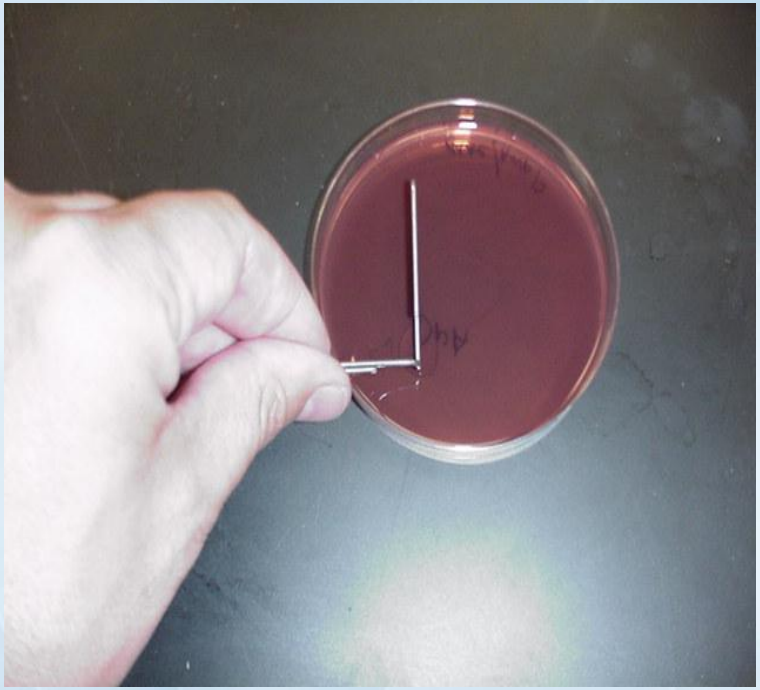
Recombinant Transformation

Incubate the mixture for 1 hours at 37^{o}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.

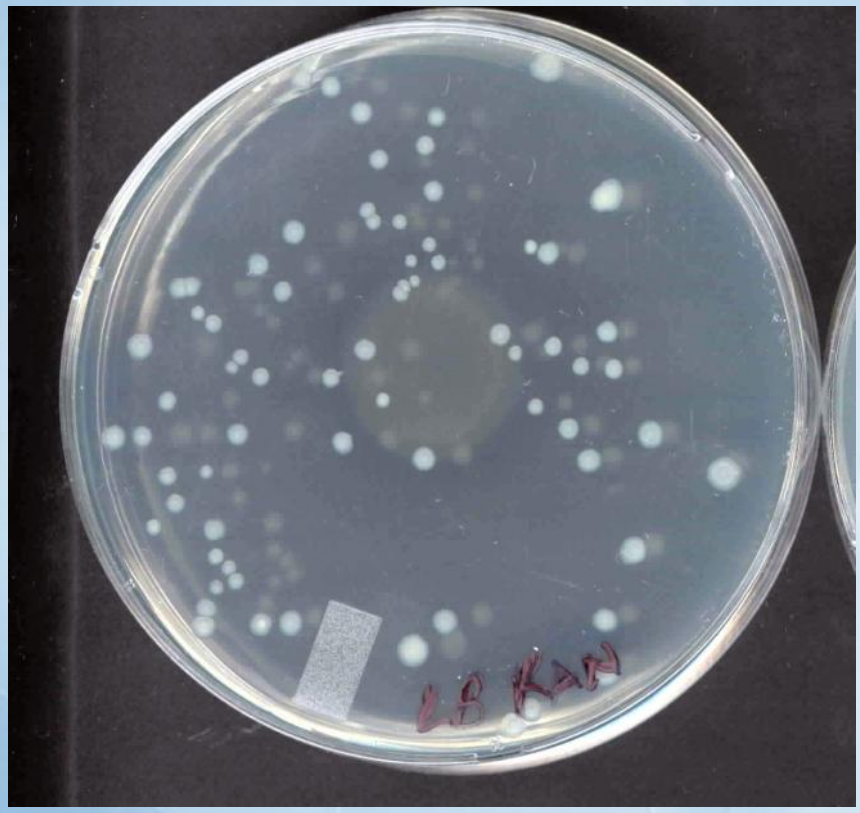


Recombinant Transformation

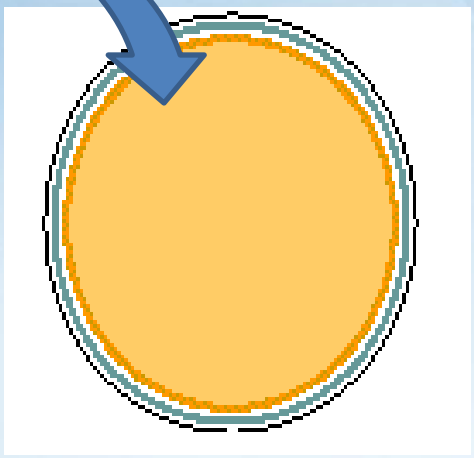
Incubate the plates (agar up) for 24 hours in a 37° C incubator.

Recombinant Transformation

Results

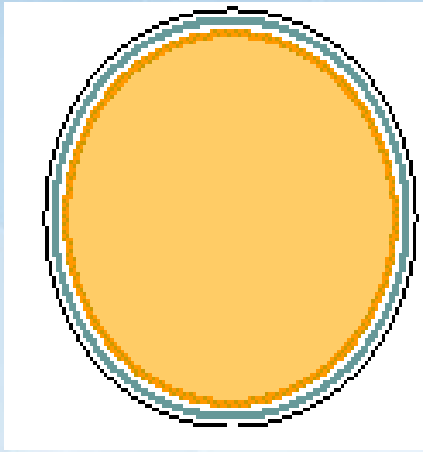


CC + No plasmid



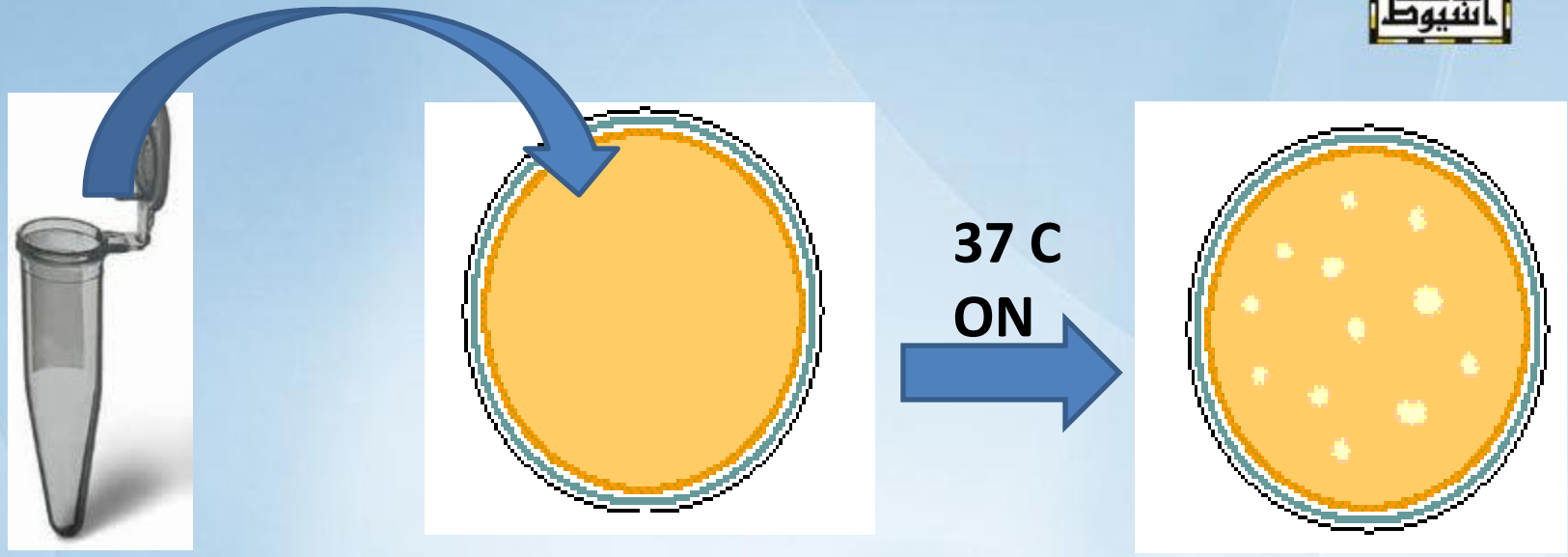
LB – Amp

37 C
ON



NO colonies

Negative control

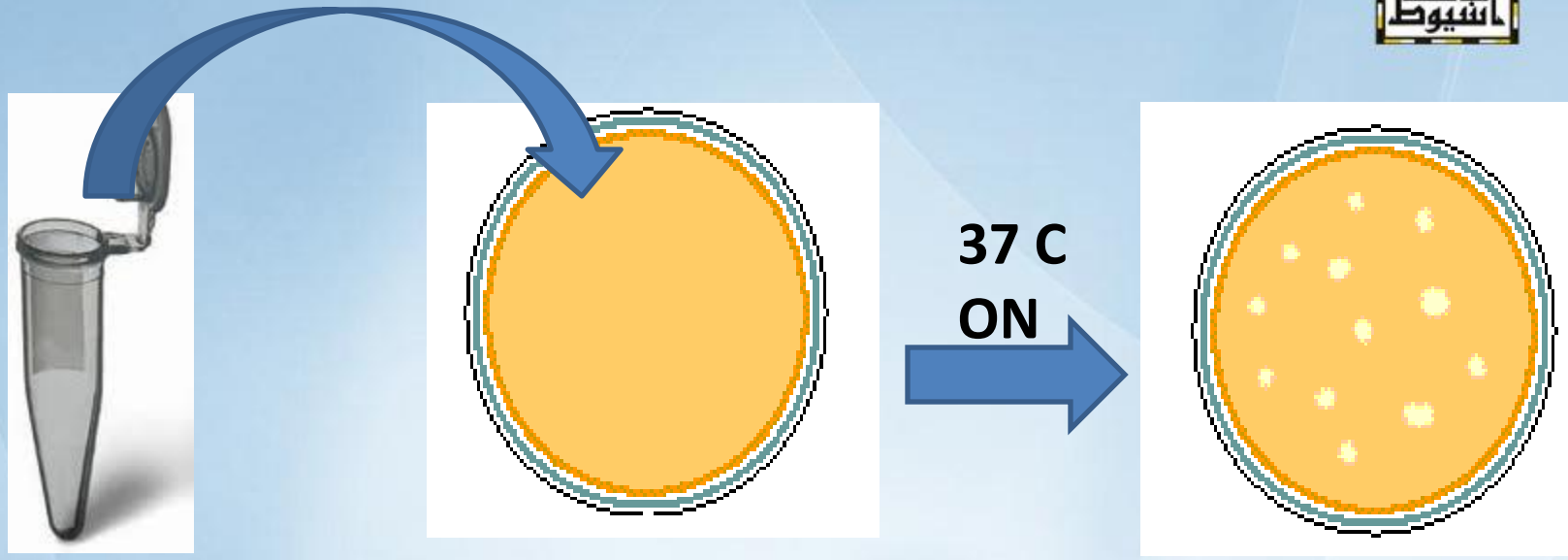


CC + empty plasmid

LB – Amp

growing colonies

Positive control



CC +
recombinant
plasmid

LB – Amp

growing
colonies