Practical Biotechnology

Lab 7

Isolation of Plasmids

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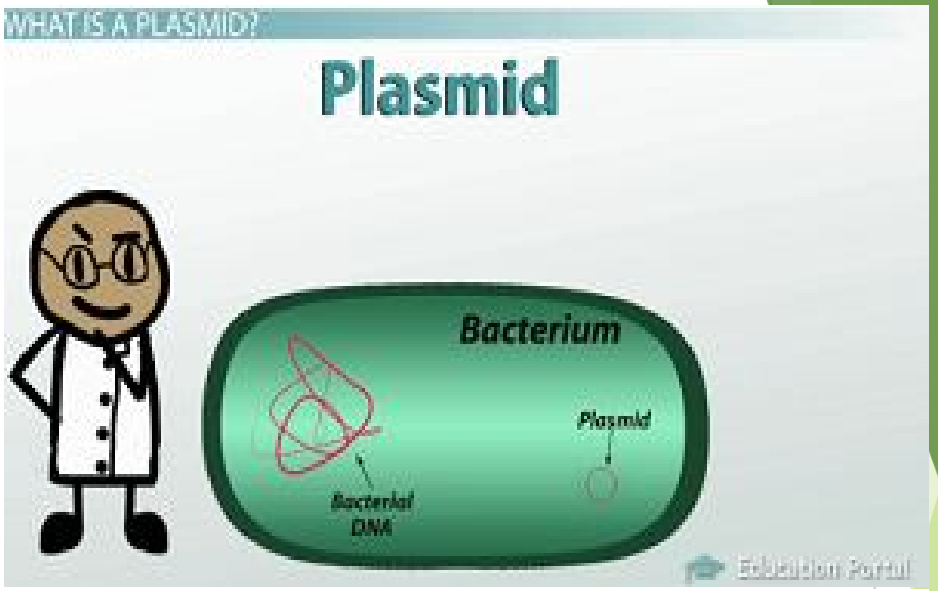
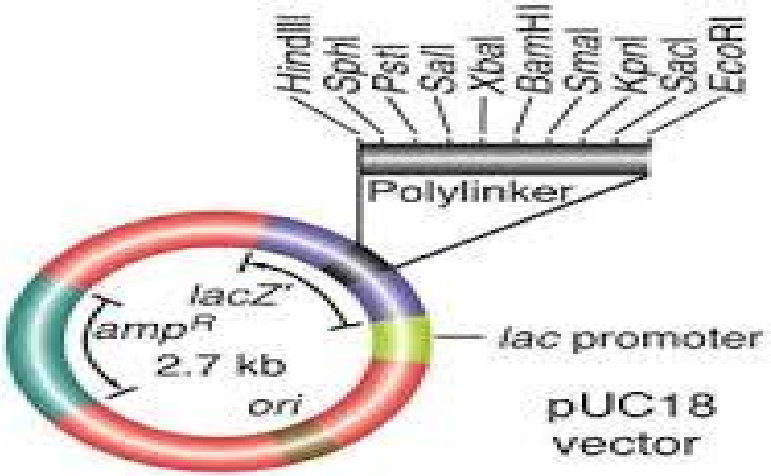
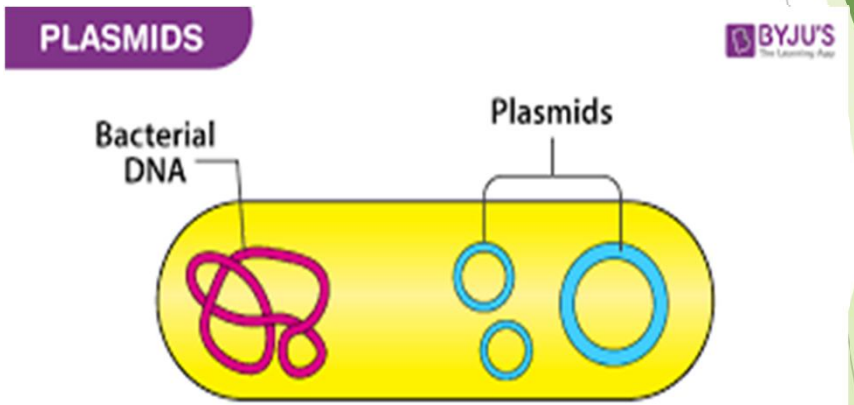




plasmid

A plasmid is a small extrachromosomal DNA molecule within a cell , can replicate independently.

They are most commonly found as small circular, double-stranded DNA molecules in bacteria; however, plasmids are sometimes present in archaea and eukaryotic organisms.



Functions of Plasmids

Since plasmids are so small, they usually only contain a few genes with a specific function.

They may contain genes that enhance the survival of an organism, either by killing other organisms or by defending the host cell by producing toxins.

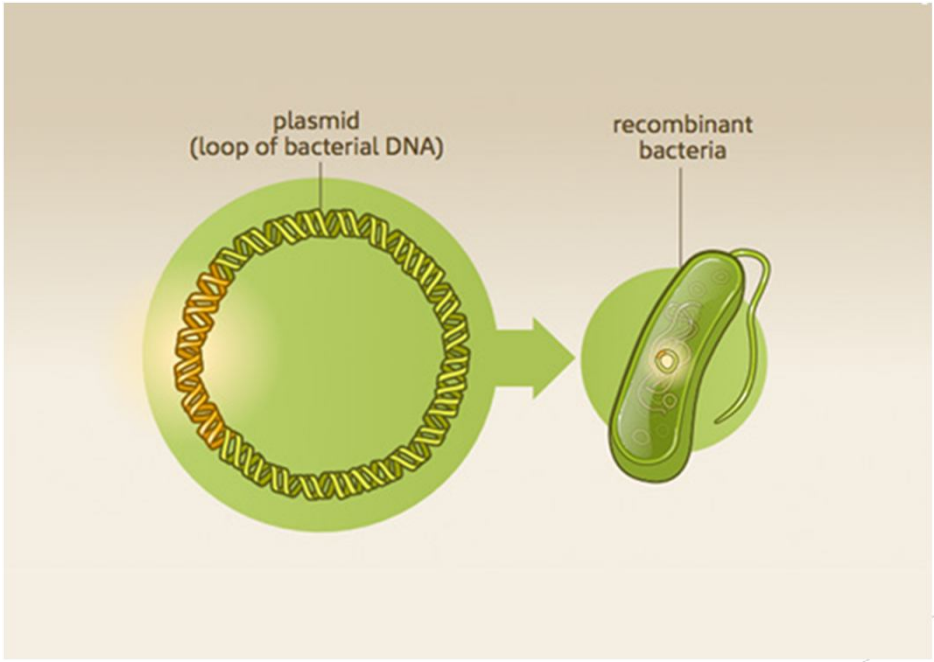
Some plasmids facilitate the process of replication in bacteria.

Multiple plasmids can coexist in the same cell, each with different functions.

Specific Types of Plasmids

There are five main types of plasmids:

1. fertility F-plasmids
2. resistance plasmids
3. virulence plasmids
4. degradative plasmids
5. Col plasmids.



1. fertility F-plasmids :

contain transfer genes that allow genes to be transferred from one bacteria to another through conjugation.
F-plasmids are episomes, that can be inserted into chromosomal DNA. Bacteria that have the F-plasmid are known as F positive (F+), and bacteria without it are F negative (F–).

2. resistance plasmids :

Resistance or R plasmids contain genes that help a bacterial cell defend against environmental factors such as poisons or antibiotics.

3. virulence plasmids :

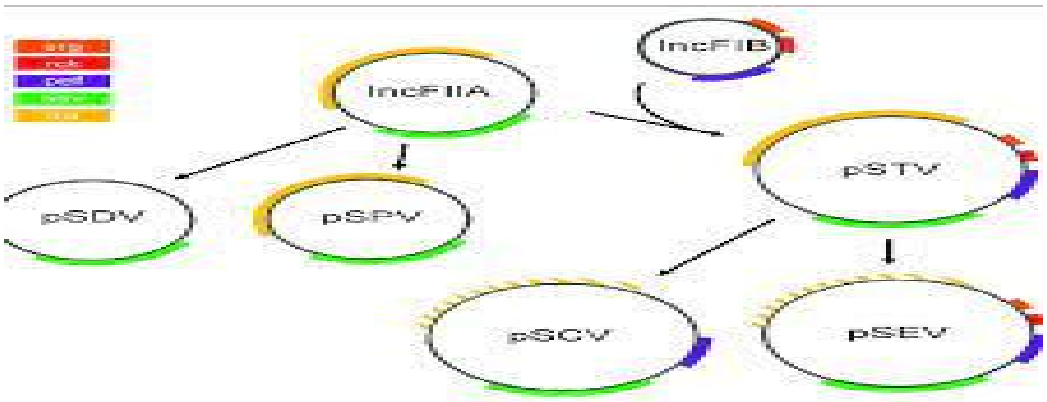
When a virulence plasmid is inside a bacterium, it turns that bacterium into a pathogen, which is an agent of disease. E. coli is found naturally in the human gut and in other animals, but certain strains of E. coli can cause severe diarrhea and vomiting Because it has several virulence plasmids.

4. degradative plasmids :

These plasmids contain genes for special enzymes that break down specific compounds. help the host bacterium to digest compounds that are not commonly found in nature, such as xylene and salicylic acid.

5. Col plasmids :

Col plasmids contain genes that make bacteriocins (also known as colicins), which are proteins that kill other bacteria and thus defend the host bacterium. Bacteriocins are found in many types of bacteria including *E. coli*, which gets them from the plasmid ColE1.



Applications of Plasmids

- Plasmids are used in genetic engineering to amplify, or produce many copies of certain genes.
- a plasmid is a type of vector. A vector is a DNA sequence that can transport foreign genetic material from one cell to another cell.
- Also, plasmids can be used to replicate proteins, such as the protein that codes for insulin, in large amounts.
- Cells may lack a specific protein if the patient has a hereditary disorder, Inserting a plasmid into DNA would allow cells to express a protein that they are lacking.

procedure

1. Culture *E. coli* with plasmid in LB media with antibiotic selective pressure, overnight on a shaker at 37°C.

2. Pellet 1.5 ml of bacterial culture in a microfuge tube by centrifuging for 2 minutes at 10,000 rpm.

3. Decant the supernatant and add 200 µl of the resuspension buffer. In order to resuspend the pellet you may have to vortex.

4. Add 250 µl of the lysis buffer, invert the tube 10 times to mix thoroughly. The solution should become clear and viscous.

5. Add 350 µl of the neutralization buffer, invert the tube 10 times or until a precipitate forms. The precipitate is a mixture of protein and chromosomal DNA.

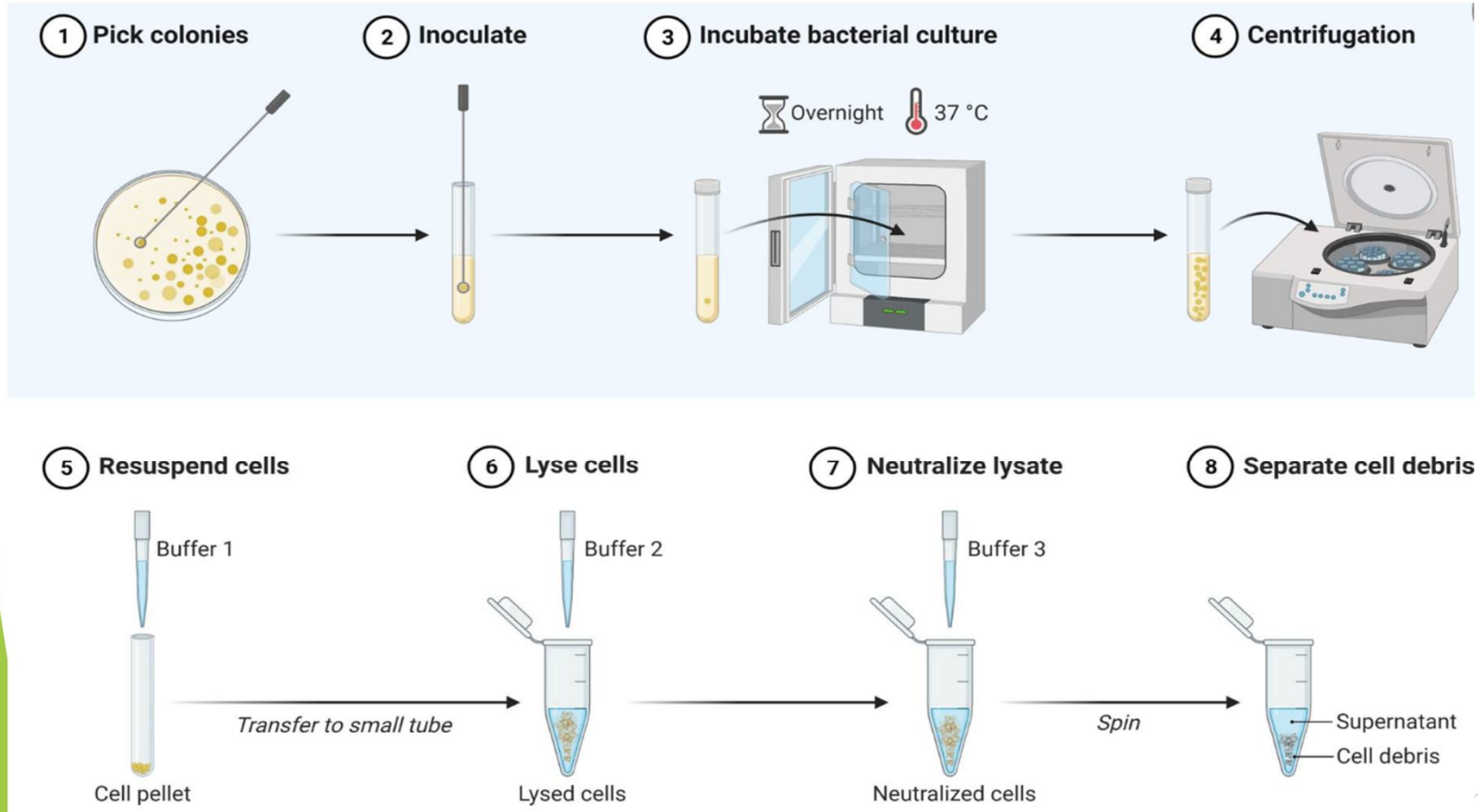
6. Centrifuge the tube for 10 minutes at 10,000 rpm. Transfer the supernatant to a microfuge tube and add 0.7 isopropanol. Incubate at -20°C for 15 minutes.

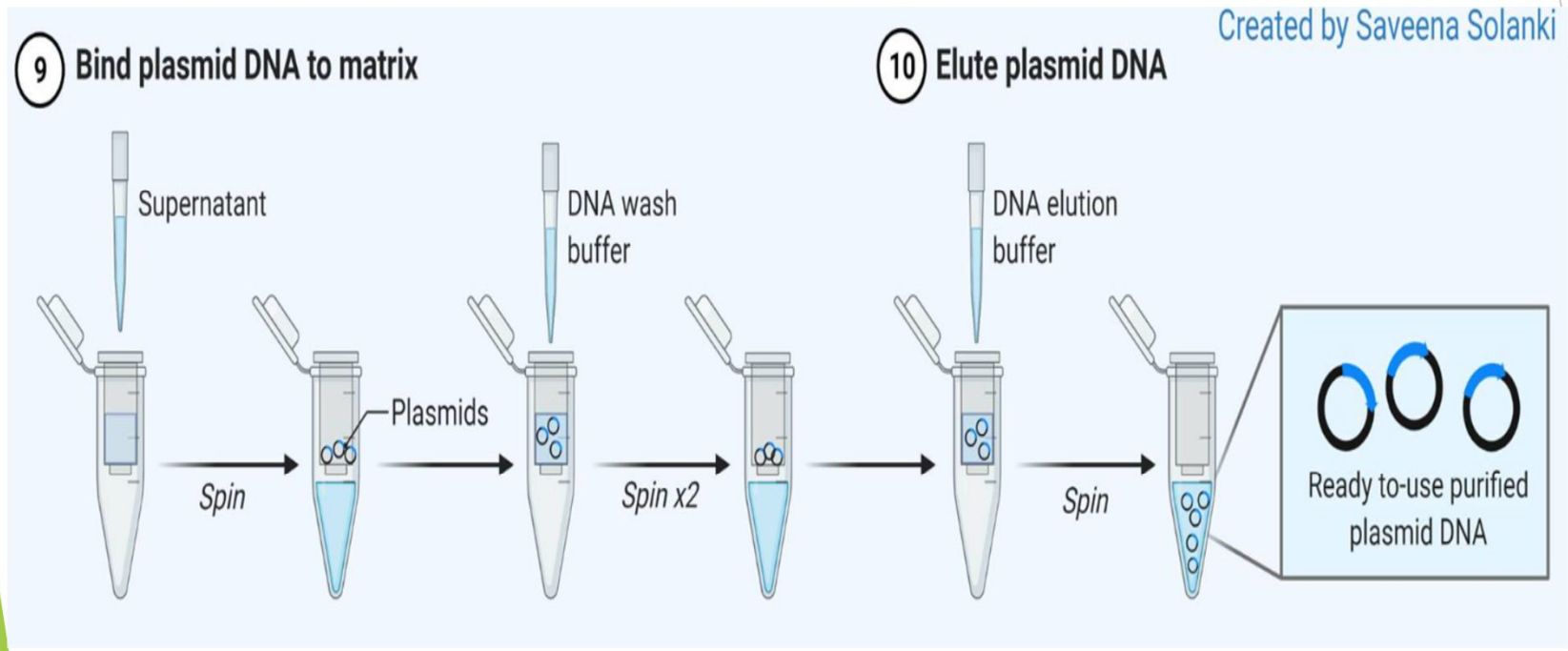
7. Transfer the solution to a spin column.
8. Centrifuge the spin column for 1 minute at 7,000 rpm. Discard the flow through.

9. Add 400 µl of the wash buffer and centrifuge for 1 minute at 7,000 rpm. Discard the flow through. Repeat this step.

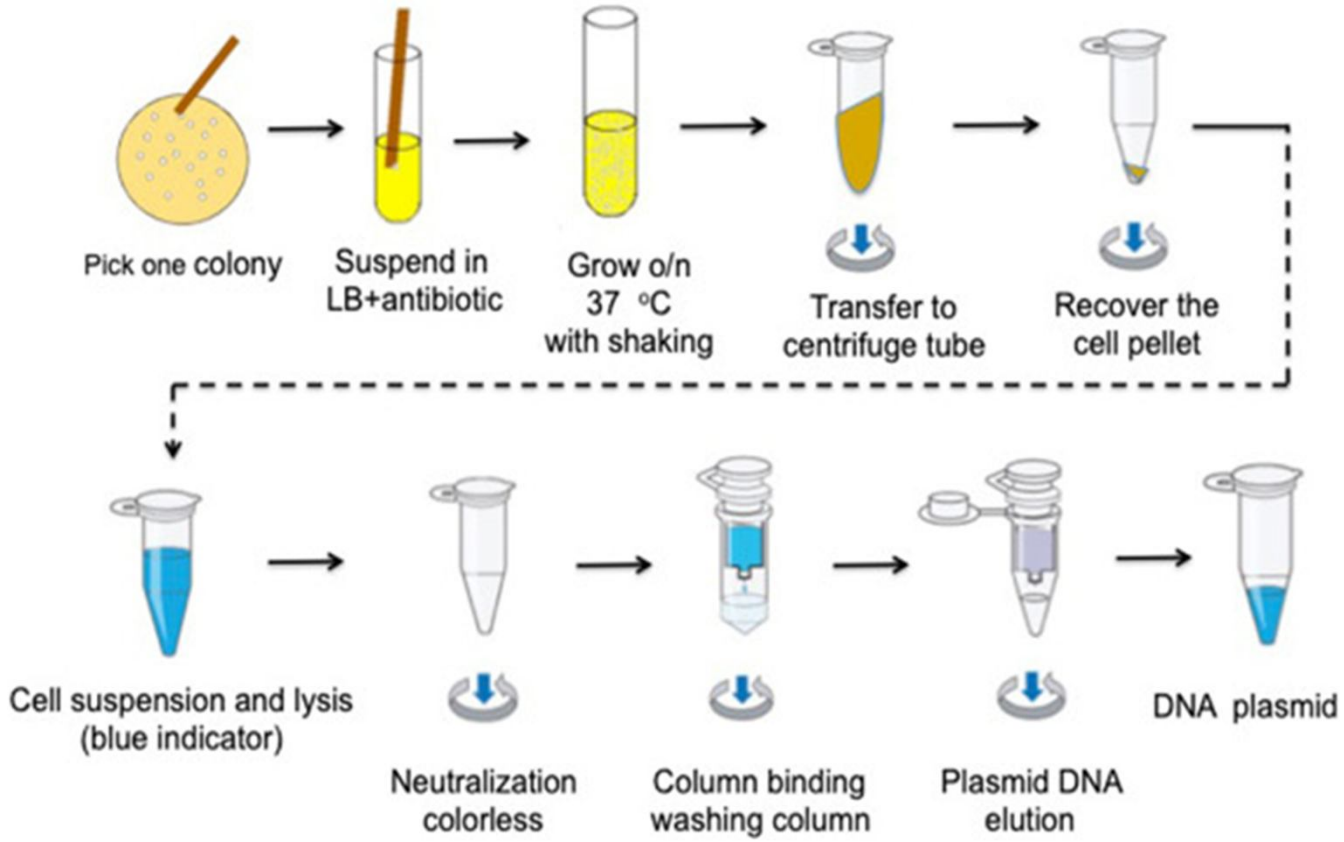
10. Centrifuge for an additional 2 minutes at 10,000 rpm to remove residual wash buffer.

11. Transfer the column to a clean microfuge tube. Add 50 µl of elution buffer and centrifuge for 1 minute at 10,000 rpm.


1 Pick colonies
2 Inoculate
3 Incubate bacterial culture
Overnight
37 °C
4 Centrifugation
5 Resuspend cells
Buffer 1
Cell pellet
Transfer to small tube
6 Lyse cells
Buffer 2
Lysed cells
7 Neutralize lysate
Buffer 3
Neutralized cells
Spin
8 Separate cell debris
Supernatant
Cell debris


Created by Saveena Solanki
9 Bind plasmid DNA to matrix
10 Elute plasmid DNA
Supernatant
Spin
Plasmids
DNA wash buffer
Spin x2
DNA elution buffer
Spin
Ready to-use purified plasmid DNA

Thank you for listening


Pick one colony
Suspend in LB+antibiotic
Grow o/n 37 °C with shaking
Transfer to centrifuge tube
Recover the cell pellet
Cell suspension and lysis (blue indicator)
Neutralization colorless
Column binding washing column
Plasmid DNA elution
DNA plasmid