

Plasmid Minipreps

Kits...



Mini-Prep:

A rapid, small scale method of obtaining or retrieving plasmid DNA (plasmid DNA + foreign/inserted DNA) from bacterial cells.

2 General Variations:

1. Boiling Method
2. Alkaline Lysis Method (With a modification to collect plasmids)

Overall Goals:

- To lyse cells using Alkaline Lysis Method
- To precipitate out proteins and bacterial DNA using Sodium Acetate
- To separate plasmid from solution with a column.
- To freeze plasmid DNA for further study

Important Solutions and Their Purpose:

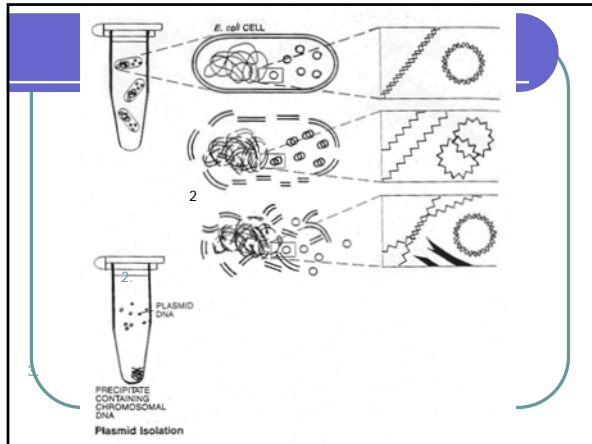
- **Solution 1: GTE (Glucose Tris EDTA):**
 - **Tris** is a buffer that works at physiological pH (pH = 7.4).
 - **EDTA** binds divalent cations in the lipid bilayer, thus weakening the cell membrane and also protecting DNA from DNAses by removing their cofactors.
- **Solution 2: SDS/NaOH (Sodium dodecylsulfate + Sodium Hydroxide) - Alkaline Lysis Solution:**
 - The detergent SDS(Soap) dissolves the lipid component of the cell membrane (plasma membrane), as well as cellular proteins
 - The NaOH denatures the chromosomal and plasmid DNA into single strands (ssDNA). The intact circle of plasmid DNA remain interwined.

Important Solutions and Their Purpose:

- **Solution 3: KOAc (Potassium Acetate + Acetic Acid):**
 - The Acetic Acid returns the pH to neutral, allowing DNA strands to renature. The large, disrupted chromosomal DNA strands can not rehybridize perfectly, but instead collapse into a partially hybridized tangle.
 - At the same time, the Sodium Acetate precipitates the SDS from the cell suspension along with proteins and lipids with which it has associated. The renatured chromosomal DNA is trapped in the SDS/Lipid/Protein precipitate. Only smaller plasmids DNA and RNA molecules escape the precipitate and remain in solution.

Important Solutions and Their Purpose:

Solution 4: Elution Solution. This low salt buffer will cause the plasmid to release from the column and come off in the spin.



Step by Step

- 1. Spin the culture and remove LB broth
- 2. Resuspend in GTE
- 3. Lyse in Soap and Base (SDS/NaOH)
- 4. Neutralize to renature the plasmids
- 5. Spin to remove Genomic DNA and cell debris.
- 6. Transfer Supernatant to column and spin
- 7. Wash by spinning with Wash buffer
- 8. Elute and Collect Plasmids
