Alkaline lysis method :

* A typical isolation procedure by alkaline lysis have the following steps.
1. Harvesting of baceria from culture
2. Suspension of bacterial pellet in resuspension buffer
3. Lysis of bacteria
4. Neutralization of lysate
5. Clearing of lysate
6. Recovery of plasmid from cleared lysate
7. Washing of plasmid DNA pellet
8. Storage of Plasmid DNA

**Harvesting of baceria from culture**

Generally bacterial cells containing the plasmid are grown in a liquid media.

 Therefore it is essential to separete the bacterial cells from the culture medium. by centrigugation. Centrifugation speed is optimized in such a way that it results in accumulation of all the bacterial cells in a form of pellet (pellet should be loose enough to be resuspended easily in resuspention buffer.)

**Suspension of bacterial pellet in resuspension buffer**

**By using alkaline lysis solution 1 ( glucose – tris PH=8 – EDTA PH =8 )**

Glucose is required to make the solution isotonic.

EDTA chelate the divalent cations which are released upon bacterial lysis. Divalent cations are required for many enzymatic reactions.(EDTA action results in inactivation of many enzymes which may harm plasmid DNA.)

Tris.Cl acts as a buffering agent

**Lysis of bacteria**

 **By using alkaline lysis solution 11 contains Sodium dodecyl sulfate (SDS) and NaOH.**

SDS is a detergent which solubilizes the phospholipid and denatures protein components of the cell membrane, leading to lysis and release of the cell contents. High alkaline condition due to NaOH denatures the plasmid and genomic DNA.

**Neutralization of lysate:**

* **By using alkaline lysis solution 111** Addition of neutralization solution (Acidic potassium acetate) bring the lysate pH back to normal, resulting in precipitation of protein and genomic DNA. Both plasmid and genomic DNA renatures upon addition of neutralization buffer. While plasmid DNA renatures in correct conformation due to its circular and covalent nature, therefore, remains in the solution, genomic DNA precipitates due to random asociation of both the strands. Sodium dodecyl sulfate (SDS) reacts with potassium acetate and form insoluble potassium dodecyl sulfate (KDS).
1. Remove 1.5 ml aliquot of the culture to a microcentrifuge tube. Repeat for the second culture into a second microcentrifuge tube. Make sure that the tubes are labeled.
2. Centrifuge at 4ºC, maximum speed for 30 seconds in a microfuge. Label the unused portions of the original culture and store at 4ºC.
3. After centrifugation, remove the medium, leaving the bacterial pellet as dry as possible.
4. Resuspend each bacterial pellet in 100 µl of ice cold Alkaline Lysis Solution I. Vortex vigorously.
5. Add 200 µl of freshly prepared Alkaline Lysis Solution II to each bacterial suspension. Invert the tube rapidly 5 times. Do not vortex! Store the tube on ice.
6. Add 150 µl ice cold **Alkaline Lysis Solution III** to each microfuge tube. Invert the tube 3 to 5 times. Incubate tubes **on ice** for 3 to 5 minutes.
7. Centrifuge the bacterial lysate at maximum speed, 4ºC for 2 minutes. Transfer the supernatant to a fresh labeled tube.
8. Add 2 volumes of ethanol at room temperature. Vortex and allow tubes to stand at room temperature for 2 minutes
9. Centrifuge at maximum speed , 4ºC for 5 minutes. Orient the microfuge tubes so that the plastic hinges point outwards. The precipitate will collect on inside surface of the tube furthest from the center of rotation.
10. Remove the supernatant.
11. Stand the tube in an inverted position over a paper towel to allow all fluid to drain away.
12. Add 1ml 70% ethanol, invert the closed tube several times. Centrifuge at maximum speed, 4ºC for 5 minutes.
13. Remove the supernatant.
14. Remove any beads of ethanol from the sides of the tube. Leave tube open at room temperature (upright position) until residual ethanol has evaporated (5 to 10 minutes).
15. Dissolve the pellet in 25 µl sterile water or TE and vortex the solution gently for a few seconds. The DNA can be stored at -20ºC.