**BCH 462 Lab# 1**

**Lab (1) Plasmid isolation and Purification**

**Materials and reagent preparation:**

**1-Harvesting of bacteria from culture:**

Generally bacterial cells containing the plasmid are grown in a liquid media. Therefore, it is essential to separate the bacterial cells from the culture medium by **centrifugation**. 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 resuspension buffer.)

**2-Suspension of bacterial pellet in resuspension buffer:**

-By using alkaline lysis solution I: [contains: 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.

**3-Lysis of bacteria:**

-By using alkaline lysis solution II: [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.

**4-Neutralization of lysate:**

-By using alkaline lysis solution III: [ contains potassium acetate and acetic acid]

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 association of both the strands. Sodium dodecyl sulfate (SDS) reacts with potassium acetate and form insoluble potassium dodecyl sulfate (KDS).

**Protocol:**

1. **isolation and purification of plasmid following the steps shown below:**

1. Centrifuge the bacterial samples at 4°C, maximum speed for 5 minutes, using microcentrifuge device.
2. After centrifugation, remove the medium by aspiration, leaving the bacterial pellet as dry as possible.
3. Resuspend each bacterial pellet in 100 µl of ice cold (alkaline lysis solution I) then vortex vigorously.
4. Add 200 µl of freshly prepared (alkaline lysis solution II) to each bacterial suspension. Invert the tube rapidly 5 times. Incubate the tubes on ice for 1 min.
5. Add 150 µl ice cold (alkaline lysis solution III) to each microcentrifuge tube. Invert the tubes 3-5 times. Incubate the tubes on ice for 3-5 minutes.
6. Centrifuge the bacterial lysate at maximum speed for 2 minutes.
7. Transfer the supernatant to a new labeled microcentrifuge tube.
8. On each tube, add 2 volumes of 95% Ethanol. Vortex and allow the tubes to stand at room temperature for 2 minutes.
9. Centrifuge at maximum speed for 5 minutes.
10. Remove the supernatant by gentle aspiration.
11. Stand the tubes in an inverted position over a paper towel to allow all fluid to drain away.
12. Add 20 µl of 70% Ethanol; then invert the closed tube several times.
13. Centrifuge at maximum speed for 5 minutes.
14. Remove the supernatant by gentle aspiration.
15. Remove any beads of ethanol from the sides of the tube. Leave tube open at room temperature until residual ethanol has evaporated.
16. Dissolve the pellet in 25-50 µl sterile water or TE buffer and vortex the solution gently for few seconds.

The DNA plasmid can be stored at -20 °C.

**Results:**

**B. Nucleic acid quantification and purity assessment.**

Using the nanodrop device:

➢ Concentration of plasmid DNA (ng/µl) = ………………………….

➢ Plasmid purity [A260/A280] = ………………………….