*Genetic Engineering laboratory protocol*

**Plasmid DNA Isolation (Mini-Prep Method) by alkaline lysis method**

1. Prepare 3 ml of overnight cultures for plasmid DNA isolation.

2. Grow a single *E.coli* colony carrying a recombinant plasmid for 14-18 hr in LB medium containing specific antibiotic, at 37 °C with shaking at 180 rpm.

3. Harvest the culture and resuspend the cells in 100 µl of solution I.

4. Lyse the cells with 200 µl of freshly prepared solution II. Mix the contents thoroughly but gently by inversion of the tube.

5. Add 150 µl of ice cold solution III incubate on ice for 5 min followed by centrifugation at 12000 g for 10 min at 4 °C. This will differentially precipitated Bacterial genomic DNA and bacterial cell debris.

6. Transferred the supernatant to a fresh tube and add equal volume of phenol/chloroform (1:1 v/v; here chloroform means chloroform/isoamylalcohol (24:1 v/v). Mix the resulting mixture gently by inversion. To separate the phases, centrifugation at 12,000 g for 10 min at 4 °C.

7. Aspirate out the aqueous phase in a fresh tube and add 2 volumes of ethanol to precipitate the plasmid DNA and incubate at room temperature for 10 min.

8. Precipitate the plasmid DNA by centrifugation at 12,000g for 10 min at 4 °C. Wash the DNA pellet with 70% ethanol, air-dried and resuspend in the required amount of nuclease-free water.

**Solutions for Plasmid Isolation**

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| **Solution I** | 50 mM Glucose ,<br>25 mM Tris.HCl,<br>10 mM EDTA (pH 8.0) |
| **Solution II** | 0.2 N NaOH, 1% SDS |
| **Solution III** | 5 M potassium acetate,<br>pH adjusted to 4.8 with acetic acid |