LS2203 Lab Report

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1 Isolation of Plasmid DNA using alkaline lysis

1.1 Principle

The most common method of plasmid extraction is Alkaline Lysis. The principle is using an alkaline buffer using a detergent like sodium dodecylsulphate(SDS) and a strong base like sodium hydroxide(NaOH) to break cells open. Then the purification of plasmid DNA is done on the basis of differential denaturation of chromosomal and plasmid DNA using Alkaline lysis to seperate the two types of DNA.

1.2 Materials Used

We are using three solutions named creatively Solution I, II and III. The compositions of the Solutions are given below:

- **Solution 1:**
  50 mM glucose($C_6H_{12}O_6$), 25 mM Tris HCl(pH 8.0), 10 mM EDTA(pH 8.0). This prepared solution is autoclaved for 15 minutes at 10 lb/in2 on a liquid cycle and stored at 4° C.
  - Glucose : maintains osmotic pressure in the cells.
  - Tris HCl: functions as a chemical to keep a stabel pH.
  - EDTA : Acts as a chelating agent to chelate Mg^{2+} ions to keep DNAse from damaging plasmid DNA
- **Solution 2:**
  0.2 NaOH(freshly diluted from 10N stock) and 1% SDS(sodium deodecylsulphate).
  - NaOH: denatures the DNA.
  - Tris HCl: functions as a chemical to keep a stabel pH.
  - EDTA : Acts as a chelating agent to chelate Mg^{2+} ions to keep DNAse from damaging plasmid DNA