EXPERIMENT NO: 9 (A)

DATE:

DNA ISOLATION

1. Bacterial culture was pelleted.
2. Pellet was washed twice with TE (pH 8.0)
3. 500µl of TE buffer was added.
4. Cells were lysed by adding 50µl of 20% SDS (tubes were invert mixed).
5. 50µl of proteinase k(20µg/ml) was added.
6. Incubated at 37 c for 1 hour.
7. 500µl of equal volume of phenol.Choloroform mixture was added and centrifuged for 2 min at 5000rpm.
8. Aqueous tap phase was separated and 10µl of 3M sodium acetate was added.
9. After mixing 60µl of isopropanol was added (allowed the DNA to dissolve).
10. Tubes were spin for a few minutes supernant discarded and pellet dissolved in 50-100 µl TE buffer.

MATERIAL REQUIRED:

1. TE BUFFER:
   i. 10mM Tris Hcl pH 7.4
   ii. 1mM EDTA pH 8.0

2. TE BUFFER STOCK:
   i. Tris Hcl 1M stock pH 7.4
   ii. EDTA 0.5 M stock pH 8.0

3. WORKING-RT:
   i. 1ml of 1M Tris Hcl
   ii. 200µl of 0.5 M EDTA
   iii. 98.9 ml of water.