Plasmid Preparation Procedure (By Hand)

Introduction

Plasmid Preparation Procedure (by hand) is used only when necessary.

Materials

- **Solutions**
- TENS (make fresh each time) To 4.5 mL TE add: 250 λ 10% SDS 250 λ 2N NaOH
- Sodium Acetate 3.0M; pH 5.5
- TE with 10ug/mL of RNAse A

Procedure

Main Procedure

1. Spin 1.5 mL of overnight culture for 30 seconds in microfuge.
2. Aspirate off all but 100 λ of the supernatant and resuspend the pellet by vortexing.
3. Add 300 λ of TENS and mix by inversion for 3-5 minutes. The solution should become viscous.
4. Add 150 λ of sodium acetate and vortex. A fine white precipitate should form.
5. Centrifuge for 2.5 minutes at 10K.
6. TRANSFER the supernatant to a clean tube and add 2 volumes (1 mL) of room temperature EtOH.
7. Vortex and pellet DNA by centrifugation for 2-5 minutes at 10K.
8. Wash pellet with 70% ethanol and allow the pellet to dry.
9. Resuspend the pellet in 30 λ of TE with RNAseA (with a P100).
10. Digest 5-10 λ as usual.