Maxi Prep

The Maxi preparation is a method of extracting and purifying plasmid DNA. This protocol can be used for the precipitation of plasmid DNA from an 100 ml *E.coli* culture.

Materials

- Sol I
  - 50 mM Glucose(-monohydrate)
  - 10 mM EDTA
  - 5 mM Tris
  - H20
- Sol II
  - 0.5 M NaOH
  - 20% SDS
  - H20
- Sol III
  - 5M Potassium Acetate (60 ml)
  - Acetic Acid (11.5 ml)
  - H20 (to 100 ml)
- 100% Isopropanol
- Sodium Acetate (M??)
- 70% EtOH
- Tris-HCL (M??)

Procedure

- Centrifuge 100 ml over night culture (2 min, 6000 rpm, RT)
- Discard supernatant
- Resuspend pellet in 5 m Sol I, then incubate for 10 min at RT
- Add 10 ml Sol II and mix the contents thoroughly by gently inverting the vessel several times
- Store the vessel on ice for 10 min
- Add 7.5 ml ice-cold Sol III and mix the contents by inverting the vessel several times
- Store the vessel on ice for 10 min
- Centrifuge the suspension for 10 min, 6000 rpm, 4°C
- Transfer the supernatant into new vessel, without transferring parts of the pellet
- Add one tenth of the supernatant volume of sodium acetate and 1 volume of isopropanol (pre-chilled on ice)
- Mix contents by inverting the tube several times
- Store the vessel on ice for 10 min
- Centrifuge the suspension at 6000 rpm for 20 min at 4°C
- Discard the supernatant
- Wash the pellet with 500 ul 70% EtOH by centrifuging it for 10 min at 6000 rpm, 4°C
- Dry the pellet at 37°C
- Dissolve DNA in 400 ul Tris-HCL buffer