

DNA Midiprep

Overview:

Growth of plasmid containing bacteria for use in midiprep

Materials:

1. LB media (room temp)
2. Ampicilin, 1000X (100 mg/mml; 4°C or -20°C)
3. LB Amp Plates (4°C)
4. Midiprep Kit (Biorad; bench)

Proceedures:

1. Inoculate 1 mL LB AMP with bacteria containing plasmid of interest
2. Grow at 37°C in shaking incubator for several hours until turbid
3. Inoculate 50 mL LB + AMP w/ 100-250 uL (depending on turbidity) of fresh growth
4. Grow overnight.
 - a. You want log phase growth for beginning of midi-prep – begin midi ~ 9 AM
5. Do midi with 40 mL and follow kit directions
 - a. Exception: resuspend product in 500 uL **1 mM Tris, pH 8-8.5.**
6. A260/280 – ideal is 1.8; usually get 0.2-0.5 mg/mL DNA