QIAprep 96 Turbo miniprep for yeast plasmid DNA

Method Overview

This method describes yeast plasmid DNA isolation using the QIAprep 96 Turbo miniprep kit.

Materials and Equipment:

Yeast colonies containing assembled gene cluster plasmids
Selective media
2 mL deep-well blocks
Zymolyase, 1000U or 2000U (Zymo Research E1004/5)
QIAprep Turbo miniprep kit (Qiagen 27173/27191/27193)
RNase A (Qiagen 19101)
Foil plate seals (Fisher Scientific 07-200-684, Corning Costar 6570)
Air permeable membrane plate seals
1 mL multichannel pipette
96-well plate and block shaking incubator
QIAvac 96 Vacuum manifold (Qiagen 19504)
Vacuum regulator, helpful but not essential (Qiagen 19530)

Protocol

After step 8, refer to Qiagen QIAprep miniprep handbook

1. Pick yeast colonies into 1.5 mLs of selective media in a 2 mL deep well block.
2. Grow for 2 days at 30˚C with shaking.
3. Mix 75 uL of yeast culture with 75 uL of 50% glycerol. Seal with foil. Store at -80˚C.
4. Centrifuge culture block at 2800 g for 10 – 20 minutes.
5. Decant supernatant, seal block, and freeze pellets at -20˚C for at least 1 overnight.
6. Make Qiagen buffer P1 containing 1000 U of Zymolyase per 96 wells and and extra 0.1 mg/mL RNase A. Resuspend Zymolyase in storage buffer before

adding to buffer P1.

7. Resuspend cells in 250 µL Qiagen buffer P1 containing RNase A **0.2** mg/mL and 1000 U of Zymolyase per 96-wells.

8. Incubate at 37˚C with gentle shaking for at least one hour. Usual incubation time is 2 – 3 hours.

9. Continue with Qiagen QIAprep 96 Turbo miniprep protocol from step 2, except mix with a 1 mL multichannel pipette, not by inversion. Elute into a PCR plate or other inexpensive and convenient to store 96-well plate. Seal, and store at -20 until ready to use.