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Purification of Plasmid DNA by Miniprep

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We use this protocol and it's working

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Abstract

Extraction of Plasmid DNA by Miniprep PureLink T M Quick Plasmid Miniprepa Kits

Materials

- PureLink T M Quick Plasmid Miniprep Kits
- Resuspension Buffer (R3)
- RNasa
- Lysis Buffer (L7)
- Precipitation Buffer (N4)
- Wash Buffer (W9)
- Wash Buffer (W10)
- Buffer TE
- Centrifugation columns in recollection tubes
- Overnight LB-culture
- Eppendorf Tubes of 1.6 mL
- Etanol 96-100%

1 Centrifuge 1-5 mL of the overnight LB-culture. Remove all medium.

2 Add 250 µL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous

3 Add 250 µL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate the tube at room temperature for 00:05:00 . 5m

4 Add 350 µL Precipitation Buffer (N4). Mix immediately by inverting the tube, or for large pellets, vigorously shaking the tube, until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × g for 00:10:00 10m

5 Load the supernatant from step 4 onto a spin column in a 2-mL wash tube. Centrifuge the column at 12,000 × g for 00:01:00 . Discard the flowthrough and place the column back into the wash tube. 1m

6 Add 500 µL Wash Buffer (W10) with ethanol to the column. Incubate the column for 00:01:00 at room temperature. Centrifuge the column at 12,000 × g for 00:01:00 . Discard the flowthrough and place column back into the wash tube 2m

7 Add 700 µL Wash Buffer (W9) with ethanol to the column. Centrifuge the column at 12,000 × g for 00:01:00 . Discard the flowthrough and place the column into the wash tube. Centrifuge the column at 12,000 × g for 00:01:00 . Discard the wash tube with the flowthrough 2m

8 Place the Spin Column in a clean 1.5-mL elution tube. Add 75 µL of preheated TE Buffer (TE) to the center of the column. Incubate the column for 00:01:00 at room temperature. 1m

9 Centrifuge the column at 12,000 × g for 2 minutes. The elution tube contains the purified plasmid DNA. Discard the column. Store plasmid DNA at 4°C (short term) or store the

DNA in aliquots at −20°C (long term).