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Purification of Plasmid DNA by Miniprep

Georgina Elisa Diego Macías[1], Ana Belem García González[1], Irán Alessandra Chaparro Rodríguez[1], Jair Alexis Gardea Sáenz[1]

[1]Tecnologico de Monterrey Campus Chihuahua

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G Georgina Elisa Diego Macías

ABSTRACT

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

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MATERIALS TEXT

- 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 2m

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× 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

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).

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