Column-free plasmid miniprep

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Abstract

This is a protocol for plasmid minipreps that does not involve any columns and uses commonly accessible reagents.

While it doesn't result in highly pure DNA, it can provide a sufficient quantity of plasmid for sequencing, restriction analysis, preparative digests.

Beware of the large amount of RNA that co-purifies with plasmid DNA (it acts as an efficient carrier during the nucleic acid precipitation step). This contamination will make it impossible to determine DNA concentration from standard 280-nm absorbance readings.

Credits: I first learned this protocol from the old-style protocols for bacmid purification using the Bak-to-Bac system (Invitrogen).

In that system, the large (>50 kb) bacmid molecules were deemed too fragile for the Qiagen spin columns.

Citation: Vladimir Vigdorovich Column-free plasmid miniprep. protocols.io

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Guidelines

Qiagen plasmid purification buffers (from OpenWetWare.org)

Buffer P1

- * 50 mM Tris-HCl pH 8.0
- * 10 mM EDTA
- * 100 ug/ml RNaseA

The buffer and RNaseA can also be ordered from Qiagen separately (catalog numbers 19051 and 19101).

Note: LyseBlue reagent (1000X = 43 mg/ml thymolphthalein in ethanol)

Buffer P2

- * 200 mM NaOH
- * 1% SDS

Buffer P3 (not for spin columns, but for Qiatips, midi, maxi, giga kits; N3 contains the equilibration buffer, while P3 does not)

* 3.0 M potassium acetate pH 5.5

Materials

- ✓ Buffer P1 by Contributed by users
- ✓ Buffer P2 by Contributed by users
- ✓ Buffer P3 19053 by Contributed by users
- ✓ Isopropanol by Contributed by users
- ✓ 1.5 mL Eppendorf tubes by Contributed by users
- nuclease-free water by Contributed by users

Protocol

Culture growth

Step 1.

Grow an overnight 3-mL *E. coli* culture in LB with appropriate antibiotics. Preferably, in round-bottom snap-cap culture tubes suitable for centrifugation (in the next step).



3 µl Additional info:

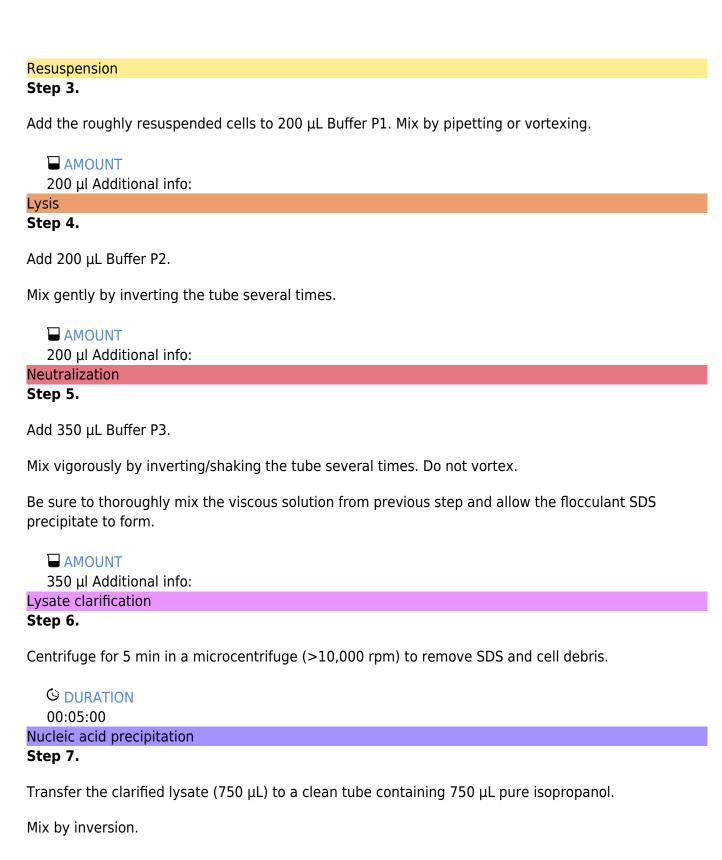
Culture harvesting

Step 2.

Spin down at 4,000 rpm in a table-top centrifuge for 15 min. Discard supernatant and vortex pellets to resuspend in residual culture medium.

O DURATION

00:15:00



AMOUNT

750 µl Additional info:

Nucleic acid isolation

Step 8.

Centrifuge for 5 min in a microcentrifuge (>10,000 rpm) to pellet nucleic acids.

© DURATION

00:05:00

Nucleic acid pellet cleanup

Step 9.

Wash the nucleic acid pellet with 500 μ L of 70% ethanol.

Gently dislodge the nucleic acid pellet from the side of the tube by shaking.

■ AMOUNT

500 µl Additional info:

Nucleic acid pellet cleanup: salt removal

Step 10.

Centrifuge for 5 min in a microcentrifuge (>10,000 rpm) to pellet nucleic acids.

O DURATION

00:05:00

Nucleic acid pellet cleanup: ethanol removal

Step 11.

Gently pipette off the ethanol and air-dry the nucleic acid pellet.

If you have a SpeedVac centrifuge, use it for 2 min.

Don't overdry the pellets, as that will cause problems with resuspension.

Nucleic acid pellet resuspension

Step 12.

Dissolve the pellet in 50 µL nuclease-free water.