Column-free plasmid miniprep

Vladimir Vigdorovich

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** dx.doi.org/10.17504/protocols.io.d7e9jd
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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).

AMOUNT
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.

DURATION
00:15:00

Resuspension

Step 3.

Add the roughly resuspended cells to 200 µL Buffer P1. Mix by pipetting or vortexing.

AMOUNT

200 µl Additional info:

Lysis

Step 4.

Add 200 µL Buffer P2.

Mix gently by inverting the tube several times.

AMOUNT

200 µl Additional info:

Neutralization

Step 5.

Add 350 µL Buffer P3.

Mix vigorously by inverting/shaking the tube several times. Do not vortex.

Be sure to thoroughly mix the viscous solution from previous step and allow the flocculant SDS precipitate to form.

AMOUNT

350 µl Additional info:

Lysate clarification

Step 6.

Centrifuge for 5 min in a microcentrifuge (>10,000 rpm) to remove SDS and cell debris.

DURATION

00:05:00

Nucleic acid precipitation

Step 7.

Transfer the clarified lysate (750 µL) to a clean tube containing 750 µL pure isopropanol.

Mix by inversion.

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 µL 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.

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.