Isolation of plasmid DNA from E. coli (Alkaline lysis method)

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Abstract

Isolation of plasmid DNA from E. coli using the alkaline lysis method modified from Birnboim *et al.*, 1979.

This protocol is suitable for fast, cheap recovery of large amounts of plasmid, e.g. for cloning purposes or restriction analysis. However, the purity of plasmid is insufficient for sequencing.

Citation: Anna Behle Isolation of plasmid DNA from E. coli (Alkaline lysis method). **protocols.io** dx.doi.org/10.17504/protocols.io.gtbbwin
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Protocol

Buffers required

Step 1.

- Buffer P1:

  50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 mg/mL RNase A, (store at 4˚C)

- Buffer P2:

  0.2 M NaOH; 1 % (w/v) SDS

- Buffer P3:

  3 M Potassium acetate, pH 5.5

Inoculation

Step 2.

Inoculate 5 mL LB, supplemented with appropriate antibiotics, using a single colony. Grow over night at 37˚C.

Cell harvest

Step 3.

Centrifuge culture at maximum speed and RT for 1 min.

Cell lysis

Step 4.

Completely remove supernatant. Resuspend in 350 µL buffer P1.

Step 5.

Add 350 µL buffer P2. Gently mix by inverting (do not vortex!). Incubate for up to 5 min at RT. (Note: If incubated for too long, sheared genomic DNA fragments may contaminate the sample.)

DURATION
00:05:00

Step 6.

Add 400 µL buffer P3. Gently mix by inverting (do not vortex!).

Step 7.

Centrifuge for 10 minutes at RT and maximum speed

Step 8.

Carefully transfer supernatant to a fresh tube, with as little contamination of pelletted material as possible. If necessary, centrifuge the supernatant again (Step 7) to completely remove pellet

Isopropanol precipitation

Step 9.

Add 1 volume of isopropanol. Incubate on ice for at least 2 minutes. (Note: Incubation time can be increased to an hour, if required.)

DURATION
00:02:00

Step 10.

Centrifuge sample for 5 minutes at RT and maximum speed. Carefully remove supernatant without discarding the DNA pellet.

Step 11.

Wash pellet by adding 500 µL of 70 % ethanol (do not resuspend). Centrifuge sample for 5 minutes at RT and maximum speed.

Step 12.

Dry pellet in a thermoblock at 65 ˚C for 10 minutes.

DURATION

00:10:00

DNA recovery

Step 13.

Resuspend pellet in 50-100 µL pure water or buffer of choice (e.g. TE buffer).

Optional: Sample can be incubated at 65 ˚C to dissolve DNA more efficiently.

Warnings

The purity of the plasmid is insufficient for sequencing and therefore should be cleaned up beforehand using a spin column or chloroform/phenol extraction.