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Alkaline Lysis Plasmid Purification

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We use this protocol and it's working

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Abstract

Plasmid DNA purification by alkaline lysis is a widely used molecular biology technique for isolating plasmid DNA from *Escherichia coli* cultures. This method leverages the unique structural properties of plasmid DNA to separate it effectively from chromosomal DNA and cellular debris, yielding high-quality plasmid suitable for downstream applications such as cloning, sequencing, PCR, and transfection.

Guidelines

- Use sterile techniques to avoid contamination that may degrade plasmid DNA.
- Adjust reagent volumes proportionally according to the culture volume (mini, midi, or maxi prep).
- Mix buffers and cell suspensions gently by inversion rather than vortexing to prevent shearing of genomic DNA.
- Keep all buffers and samples cold when indicated to maintain plasmid stability and reduce nuclease activity.
- Always include RNase A in the resuspension buffer to remove RNA contamination.
- Perform centrifugation steps at recommended speeds and temperatures to ensure efficient separation of plasmid DNA.
- Validate plasmid DNA quality and concentration post-prep by spectrophotometry or gel electrophoresis.

Materials

Resuspension Buffer

- 50 mM Tris base
- 10 mM EDTA
- 100 µg/ml of RNAse (optional)
- pH 8.0

Lysis Buffer

- 200 mM NaOH
- 1% SDS

Neutralization Buffer

- 3M Potassium acetate
- pH 5.0

Elution Buffer

- 10 mM Tris base
- 1 mM EDTA
- pH 8.0

Isopropanol

Ethanol

Sterile Water

Shaking incubator

Laboratory centrifuge

Safety warnings

- Sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS) are caustic reagents: wear gloves, protective eyewear, and lab coat when handling.
- Avoid vigorous vortexing or shaking after lysis buffer addition to reduce shearing of chromosomal DNA that contaminates plasmid preps.
- Do not exceed recommended incubation times during lysis to prevent irreversible denaturation of plasmid DNA.
- Handle pellets gently during washing and resuspension to avoid loss of plasmid DNA.
- Dispose of bacterial waste and chemical reagents according to institutional biosafety and chemical safety guidelines.

Before start

- Prepare all buffers fresh or ensure they are properly stored and equilibrated to appropriate temperatures before starting.
- Pre-cool centrifuges, tubes, and neutralization buffer to 4°C or on ice as required.
- Grow *E. coli* cultures overnight in LB medium with appropriate antibiotics to reach stationary phase (14–16 hours, 37°C, shaking).
- Confirm culture density to avoid overloading the lysis step, which can reduce purity and yield.
- Have all materials including pipettes, filter tips, tubes, and reagents ready and labeled for efficient workflow.

Bacterial culture

1 Inoculate *E. coli* harboring the plasmid into LB broth with appropriate antibiotic.

- Mini prep: 2-5 mL
- Midi prep: 25-100 mL
- Maxi prep: 250-1000 mL

2 Incubate Overnight at 150 rpm, 37°C

Bacterial harvesting

2m

3 Centrifuge

2m

- Mini prep: 12000 x g, 4°C, 00:01:00
- Midi prep: 10000 x g, 4°C, 00:10:00
- Maxi prep: 10000 x g, 4°C, 00:10:00

4 Discard superntatant completely.

Resuspension

5 Resuspend cell pellet in resuspension buffer:

- Mini prep: 250 µL
- Midi prep: 4 mL
- Maxi prep: 10 mL

6 Mix gently by pipetting until cells are completely resuspended.

Alkaline lysis

4m

7 Add lysis buffer to resuspended cells:

- Mini prep: 250 µL
- Midi prep: 4 mL
- Maxi prep: 10 mL

8 Mix gently by inverting the tube 4-6 times. Avoid vortexing to prevent shearing genomic DNA.

9 Incubate at Room temperature for 00:04:00. Do not exceed 5 minutes to prevent irreversible denaturation. 4m

Neutralization 10m

10 Add ice-cold neutralization buffer
- Mini prep: 350 µL
- Midi prep: 6 mL
- Maxi prep: 15 mL

11 Mix gently by inverting until a thick withe precipitate forms.

12 Incubate on ice for 00:10:00 to enhance precipitate formation. 10m

Lysate clearing.

13 Centrifuge
- Mini prep: 12000 x g, 4°C, 00:10:00
- Midi prep: 10000 x g, 4°C, 00:15:00
- Maxi prep: 10000 x g, 4°C, 00:15:00

14
- Carefully transfer the clear supernatant to a fresh tube without disturbing the pellet.

Precipitation of plasmid DNA 30m

15 Add ice-cold isopropanol
- Mini prep: 250 µL
- Midi prep: 4 mL
- Maxi prep: 10 mL

16 Mix by gentle inversion.

17 Incubate at -20 °C for 00:30:00 30m

18 Centrifuge 45m
- Mini prep: 12000 x g, 4°C, 00:15:00
- Midi prep: 10000 x g, 4°C, 00:15:00
- Maxi prep: 10000 x g, 4°C, 00:15:00

19
- Discard supernatant gently without disturbing pellet.

Wash DNA pellet 5m

20 Add 1 mL of [M] 70 % (v/v) ice-cold ethanol.

21 Centrifuge
- Mini prep: 12000 x g, 4°C, 00:15:00
- Midi prep: 10000 x g, 4°C, 00:15:00
- Maxi prep: 10000 x g, 4°C, 00:15:00

22
- Remove ethanol carefully and air-dry pellet for 00:05:00 . Avoid over-drying. 5m

Resuspension 5m

23 Resuspend DNA pellet TE buffer or nuclease-free water.
- Mini prep: 50-100 µL
- Midi prep: 500-2000 µL
- Maxi prep: 5-20 mL

24
- Incubate at 37 °C or Room temperature for 00:05:00 until fully dissolved. 5m

Protocol references

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