Pure Midiprep Plasmid DNA Purification

Plasmids are circular, extrachromosomal DNA molecules that act as carriers, or vectors, for a specific DNA segment in molecular biology. Bacteria are employed to reproduce plasmids, allowing your desired DNA to be mass-produced. The method by which researchers acquire plasmids from bacteria is known as plasmid purification.

Purifying plasmid DNA from bacterial cells is a critical step in cloning. Bacterial cells are lysed during plasmid purification, which liberates DNA and other biological components from the cell wall. After removing cellular components, the DNA-containing lysate is treated to eliminate impurities and separate the plasmid DNA from the genomic DNA.

The plasmid DNA extracted is the same quality as that produced by purification with two passes through cesium chloride gradients—the most stringent approach for DNA purification. High-quality DNA can be purified in less than twenty minutes for transfections and other applications. There is no need for extra processes to remove impurities such as RNA, proteins, or endotoxins. Furthermore, the protocol's exclusion of phenol, chloroform, ethidium bromide, and cesium chloride reduce exposure to and disposal of hazardous compounds.

Principle & workflow (Fig.1)

BcMag™ Pure Midiprep Plasmid DNA Purification uses weak anion exchange beads to quickly purify plasmid DNA. The beads allow for plasmid DNA binding directly. This approach uses typical cell resuspension, alkaline lysis, and neutralization processes and allows plasmid DNA to bind directly to the beads. A single bead wash removes impurities such as RNA, proteins, metabolites, and other low molecular weight molecules. Unique wash buffers ensure that salts, proteins, RNA, and other biological components are eliminated, allowing concentrated, highly pure DNA to be eluted. The ultrapure plasmid DNA is subsequently eluted with a high-salt buffer. The DNA is desalted, concentrated with an isopropanol precipitation process, and centrifuged. The entire treatment takes roughly twenty minutes to complete.

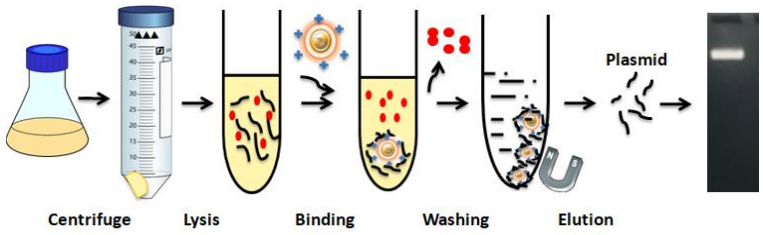

Fig1. Principle and workflow of midiprep plasmid purification

Feature and benefits

- Simple protocols- no columns or filters required.
- Quick results- purify plasmid DNA in less than twenty minutes.
- No Phenol/chloroform extractions
- Low endotoxin
- Quality - DNA is suitable for most downstream applications.
- Scalable - easily adjusts for sample size and automation.
- Reproducible results

Products	Catalog # AY-101	Catalog # AY-102
BcMag™ Pure Midiprep Plasmid DNA Purification	5ml	10ml

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Specificities	
Composition	weak anion exchange beads
Magnetization	~45 EMU/g
Type of Magnetization	Superparamagnetic
Beads concentration	2.0 g/ml
Concentration	50mg/ml (ultrapure water)
Binding Capacity	~500µg DNA / ml beads
Storage	Store at 4°C upon receipt

Protocol

Materials Required

A. Buffer Composition

- Material Required:
- BcMag™ Plasmid DNA Magnetic Beads.
- TE Buffer: 10mM Tris.HCL PH8.0 1mM EDTA
- Isopropanol (Sigma cat# 19516)
- P1 (Resuspension buffer): 50mM Tris.HCl, pH 8.0, 10mM EDTA, 100ug/ml RNase A (Store at 4°C after addition of RNase A)
- P2 (lysis buffer): 200mM NaOH, 1% SDS (w/v)
- P3 (Neutralization buffer): 3.1M Potassium Acetate, pH 5.5 (Store at 4°C)
- P4 (Equilibration buffer): 750mM NaCl, 50mM MOPS, pH 7.0, 15% isopropanol (v/v), 0.15% Triton X-100 (v/v)
- P5 (Wash buffer): 1.0 M NaCl, 50mM MOPS PH 7.0, 15% isopropanol(v/v)
- P6 (Elution buffer): 2.5 M NaCl, 50mM Tris.HCl, pH8.5, 15% isopropanol (v/v)

B. Equipments

a. Magnetic Rack (for manual operation)

Based on sample volume, the user can choose one of the following magnetic Racks: BcMag Rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag Rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ Rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)

- Corning 430825 cell culture flask for large-scale purification (Cole-Parmer, Cat#EW-01936-22)
- Mini BlotBoy 3D Rocker, fixed speed, small 10" x 7.5" platform w/ flat mat (Benchmark Scientific, Inc. Cat# B3D1008) or compatible

Procedure

** The protocol is based on a 50 ml bacterial culture with a high copy plasmid. However, the protocol can be properly scaled up or down based on bacterial culture volume.

1. Bacterial lysis

a. Transfer 50ml overnight cell culture to a centrifuge tube, centrifuge for 10 minutes at rpm 6000, and remove the supernatant.

b. Completely resuspending bacterial pellets with 4ml of P1 buffer, add 4ml of P2 buffer mix gently by inverting and incubating at room temperature for 5 min.

c. Add buffer 4ml of P3, mix gently by inverting, centrifuge at 12000-16000 rpm for 10 min at room temperature, and carefully transfer the supernatant. (If necessary, filter the supernatant through six layers of cheesecloth) to a fresh tube.

2. Plasmid purification

a. Vigorously shake the bottle until the magnetic resins become homogeneous and transfer 1 ml of beads to a fresh tube.

Note: Do not allow the resins to sit for more than 3 minutes before dispensing. Resuspend the magnetic beads every 3 minutes.

b. Place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack. Add ten bead-bed volumes of P4 buffer and mix the beads by pipetting or vortex. Again, place the tube on the magnetic Rack for 1-3 minutes and remove the supernatant while the tube remains on the Rack.
c. Mix the beads with cell lysate (step1c), incubate on Mini BlotBoy 3D Rocker with continuous rotation for 5-10 minutes at room temperature, and place the tube on a Rack for 1 minute until it becomes clear, and then remove supernatant.
d. Wash the magnetic bead with 6ml of P5 buffer three times using a magnetic Rack.
e. Add 1ml elution buffer to the magnetic beads and completely resuspend the beads by pipetting up and down 15-20 times. Place the tube on a Rack for 1-3min and transfer the supernatant to a new tube.

3. Plasmid precipitation

a. Add 700ul isopropanol, mix well, incubate at room temperature for 2 minutes, and centrifuge at 14000-16000 rpm for 10 minutes at room temperature.
b. Carefully remove the supernatant, wash pellets with 600ul of 70% ethanol, centrifuged at 14000-16000 rpm for 4 minutes,
c. Carefully remove the supernatant and air dry the pellet for 5-10 minutes. (**Do not overdry the pellet. Or the pellet is difficult to be resolved).
d. Resuspend the pellet with the desired amount of TE buffer. (**Run 2-3 ul of plasmid solution on 1% agarose gel to check the quality. If RNA is present, add RNAse A to completely remove the RNA)

Plasmid Purification Frequently Asked Questions

What cultural conditions do you suggest?

Cultures should be inoculated from a single colony and cultivated for the duration of the culture in the presence of an appropriate antibiotic. Sub-culturing from starter cultures is possible, but the outcomes may vary. Cells should be harvested as the culture progresses from the logarithmic to stationary phase to ensure that the maximum amount of DNA is present with minimal cell lysis. A single colony can be used to inoculate a 5-10 ml culture cultured at 37°C for 12-16 hours in normal cloning processes with medium to high-copy plasmids. 1-3 ml of this culture should give 3-6 µg of a high copy plasmid or 1-2µg of a medium copy plasmid. Lower copy plasmids will result in lower yields, which can be addressed by processing larger culture volumes while simultaneously scaling the P1-B6 buffers or by concentrating the purified DNA.

What factors influence plasmid DNA recovery?

Some factors can impact plasmid recovery both before and during the prep. Plasmid copy number, plasmid size, insert toxicity, host strain, antibiotic selection, growth media, and culture conditions can influence final plasmid recovery. Choose high copy plasmids when appropriate and keep the total size under 10kb for higher yields. Larger plasmids (up to 25 kb) can be isolated, although yields are lower. Maintaining antibiotic selection during growth ensures that the plasmid is not lost and that the culture is not overwhelmed by a faster-growing population of cells without the plasmid. Proper aeration and growth temperature ensure logarithmic cell growth with little cell lysis.

What should I do while utilizing a low-copy plasmid?

Increasing the number of intact cells containing the plasmid by ensuring that the culture is cultivated with optimum aeration and removed before major cell lysis occurs. Recovery of low-copy plasmids is always lower than that of high-copy plasmids. Using more cells can compensate for part of the shortage but follow the parameters to ensure alkaline lysis is efficient and the bead is not overwhelmed with cellular components.

Which cultural media do you suggest?

Rich media, such as 2X YT or Terrific Broth, can be employed. However, they often result in much greater cell densities at saturation. As a result, when using rich media, the amount of culture treated must be reduced to avoid insufficient alkaline lysis and column overloading due to greater biomass. We recommend growing E. coli in LB (Luria-Bertani) media.

Which E. coli strain(s) do you recommend?

Most common laboratory cloning strains can be utilized as hosts for plasmid propagation for miniprep purification. Thermofisher offers suitable strains such as DH5α (Thermo Scientific #18258012) or DH10B (Thermo Scientific # EC0113).

Are the plasmids recovered with the beads devoid of endotoxin?

Although weak anion exchange beads and buffers remove the vast majority of endotoxin from the sample, the DNA recovered cannot be guaranteed to be endotoxin-free. We have successfully transfected robust cell lines with plasmid DNA. While some manufacturers may claim that their minipreps kit is "endotoxin-free," there is usually some quantifiable endotoxin present.

What factors influence my (A260/A280) after plasmid miniprep?

This ratio usually reflects the relative purity of the eluted DNA during a plasmid miniprep by comparing absorbance at 260nm, where nucleic acids have absorbance maxima, to 280nm, where proteins have absorbance maxima. Inadequate neutralization or washing may allow an extra protein to remain attached to the matrix and elute with the DNA. Please adhere to the guidelines. Furthermore, utilizing the supplied elution buffer to elute the sample and blank the spectrophotometer ensures that there will be no shift in the 260/280

Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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