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Efficient kit-free nucleic acid isolation uses a combination of precipitation and centrifugation separation methods

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Overview

Immunotherapy and gene therapy requires highly purified DNA to deliver a gene editing message, or plasmid, to the host cell. Kit free plasmid miniprep has been traditionally used to deliver high quality and high yield DNA. This method is accomplished by a combination of salts and isopropanol and the efficient precipitation of nucleic acids from solutions via centrifugation. It is high-throughput, cost efficient and it produces higher yields as there is no loss of material due to membrane or filter interaction.

Introduction

Immunotherapy is a therapeutic technique that reconditions the immune system to combat diseases. It requires retraining or reprogramming cells via treatment with direct genetic material or a biologics such as a virus gene delivery system.

The starting material, almost always, stems from production and isolation a plasmid containing the reprograming piece of DNA. Plasmid DNA isolation is a crucial step in producing high quality material for immunotherapy gene transfers. In addition to immunotherapy, many molecular biology techniques require highly purified and concentrated plasmid DNA.

Adenoviruses are in the forefront of genetic delivery systems in immunotherapy. Adenovirus-based immunotherapy has been evaluated and optimized to increase safety and enhance efficiency. In short, it starts with the generation of the recombinant baculoviruses expression vector, bacmid, from the host bacterial cells.

Here we describe the bacmid plasmid isolation using a traditional combination of salts and isopropanol and the efficient separation of nucleic acids from solutions via centrifugation (Figure 1).

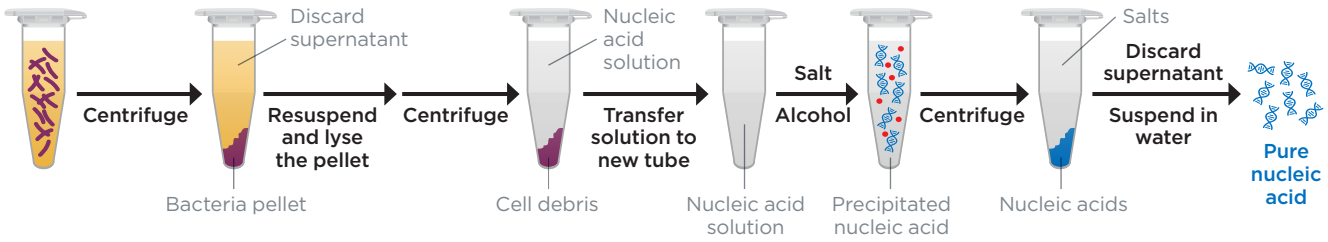

Figure 1: Schematic overview of an isopropanol precipitation of nucleic acids

What You Would Need

- Max Efficiency DH10Bac bacterial cell strain (cat. 10361-012), pFastBac-*gus* (cat. 91401), NanoDrop 8000 Spectrophotometer equipped with measurement software version 2.3.2 were purchased from Thermo Scientific.
- Microfuge 20R (P/N B30147) IVD model equipped with FA241.5P rotor were from Beckman Coulter Inc.

What You Would Do

Heat-shock Transformation

100 µL of bacteria was aliquot to a pre-chilled 15mL round bottom tube. 1 ng of pFastBac plasmid was added to the bacteria tube. Cells and plasmid mixture was incubated on ice for 30 minutes. Next, the tube was placed at 42°C for 45 seconds and immediately transferred to an ice-water bath for 2 minutes. 500 µL of SOC media was added to the tube and cells were allowed to recover for 4 hours at 37°C while shaking at 220 rpm. The cells were plated on conditioned LB Agar plate containing kanamycin (kan), gentamycin (gen), tetracycline (tet), and IPTG for selection. Positive colonies were observed after 48 hours.

Cell growth and harvest

One single positive colony was picked and resuspended in 5 mL of LB supplemented with kan/gen/tet/IPTG and allowed to grow at 37°C while shaking at 220 rpm. After 16 hours, the cells were spun on the Microfuge 20R equipped with FA241.5P rotor at 10,000 xg RCF for 2 minutes. The supernatant was discarded and the pellet was kept for the next step.

Plasmid Isolation

Plasmid isolation consists of five steps: cell lysis, debris clearing, nucleic acid precipitation, nucleic acid isolation and resuspension. All solutions were prepared ahead of time and stored at corresponding working temperature. The Microfuge 20R equipped with FA241.5P rotor was used in all centrifugation steps.

1. Cell Lysis. First, the cells were brought into solution with 500 µL of 25 mM Tris-HCl (pH 8), 50 mM glucose, 10 mM EDTA buffer supplemented with 1% RNAse A. The tube was inverted five times followed by addition of 500 µL of alkaline lysis buffer containing 0.2 N NaCl and 1% (w/v) SDS. The NaOH is important to break down the cell walls but most importantly, it denatures DNA, an important step in separation. SDS denatures the cell proteins. The tube was inverted five times and incubated at room temperature for 5 minutes. 500 µL of 5M potassium acetate solution was added to the mixture to renature and stabilize plasmid DNA. This is a selective step that separates only small plasmid DNA from host cell genomic DNA. The mixture is incubated on ice for 10 minutes
2. Debris clearing. To separate the desired plasmid from contaminants such as host DNA, proteins and cellular debris, the sample is spun at 15,000 xg for 10 minutes at room temperature. The nucleic acid solution was transfered to a new tube.
3. Nucleic acid precipitation. To separate nucleic acid from solution, 800 µL of isopropanol was added to the mixture. The tube was incubated on ice for 10 minutes. This step precipitated the DNA in the pellet.
4. Nucleic acid isolation. The desired plasmid DNA need to be separated from impurities by centrifugation. The sample was spun at 15,000 xg RCF at room temperature for 15 minutes. The DNA pellet was rinsed in 500 µL of ice-cold ethanol and air-dried for 10 minutes.
5. Resuspension. The dry DNA pellet was resuspended in 70 µLTE buffer or water and analyzed for concentration (and quality)

What you would expect

A spectrophotometer equipped with a measurement software was used. 2 µL of sample was measured in the NanoDrop 8000 Spectrophotometer. 5mL of bacteria yield 332.1 ng/ µL†. The spectra (Figure 2) displays a typical pattern for nucleic acids, with 260/280 ratio of 2.1 and a 260/230 ratio of 1.8.

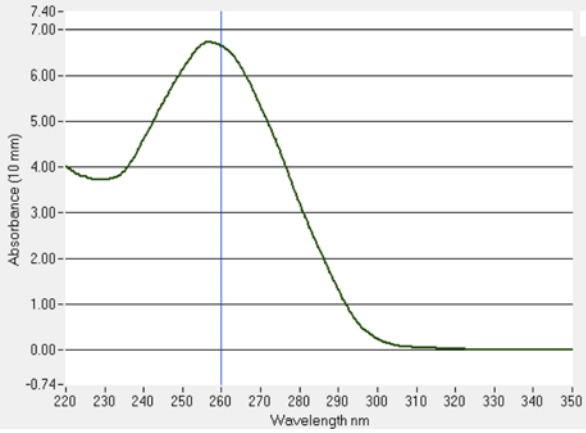

Figure 2: Nucleic acid spectra of bacmid plasmid.

Conclusion

Traditional plasmid isolation is efficient and economical. Apart from buffer preparation using standard laboratory chemicals, the precipitation-based DNA isolation method needs only a centrifuge. The results obtained from membrane-free and filter-free DNA purification (Figure 2) provide the workflow with adequate plasmid concentration to produce high quality recombinant adenovirus viral particles. The initial culture can also be optimized to scale up to 500 µg of plasmid, if desired, without the need to add significant cost by purchasing commercial kits. Furthermore, alternative centrifugation techniques can also provide a solution for large scale DNA isolation. Ultracentrifugation of DNA in CsCl gradients produces large amounts of highly purified DNA for immunotherapy purposes or any downstream application.

References

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