Application Note:
ANCFGGPMXPQ-0509

High-Throughput Maxi-Prep Using the New Thermo Scientific General Purpose Centrifuge with Fiberlite Rotors

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KEY WORDS

- Maxi-Prep
- DNA Isolation Kit
- High Quality Plasmid DNA Isolation
- General Purpose Centrifuge
- Fiberlite Rotors

Introduction

A maxi-prep is used to isolate plasmid (or cosmid) DNA from bacteria, normally *Escherichia coli (E. coli)*. Various procedures exist for the isolation and characterization of plasmid DNA. Generally, procedures involve using an overnight culture of cells, harvesting cell material, lysing and removing the majority of the cells contents and finally recovering plasmid DNA by precipitation.

One traditional DNA purification technique used over the years is Phenol/Chloroform extraction[1], separation of DNA from histones and other soluble proteins. Other traditional techniques isolate plasmid DNA from crude lysate, including alkaline, boiling, and Thermo Scientific Triton mediated lysis[2].

More recently, less time consuming and less hazardous methods for the isolation of plasmid DNA from large scale volumes have been developed. Commercial plasmid DNA preparation kits are available from many life science manufacturers, such as Qiagen, Inc., Sigma-Aldrich, Inc., Clontech and Bio-Rad Laboratories, Inc. These kits use a column format with a matrix that binds the DNA.

The procedures can be conducted using the new Thermo Scientific general purpose benchtop centrifuge series and the high speed Thermo Scientific Fiberlite F15-8x50c rotor (24,000 x g) or the high capacity Fiberlite® F13-14x50c rotor (17,000 x g). Using our equipment, isolation of plasmid DNA is performed in significantly less time (less than 45 minutes) resulting in increased throughput and enhanced productivity.

This application brief describes a procedure for isolation of plasmid DNA in a large scale using the new Thermo Scientific general purpose benchtop centrifuge and Fiberlite F15-8x50c or F13-14x50c rotors for reduced spin times (less than 45 minutes) during the preparation of plasmid DNA with the Plasmid Maxi Kit[3].

Thermo Scientific general purpose centrifuge with Fiberlite F13-14x50c Rotor

Thermo Scientific Fiberlite F15-8x50 Rotor

Procedure

Using the new Thermo Scientific general purpose centrifuge and Fiberlite F15-8x50c or F13-14x50c rotor, the general method for plasmid maxi-prep using a Qiagen® kit can be performed as described briefly in the following procedure.

As reported in the published Qiagen Handbook, the precipitation of the crude lysate containing the genomic DNA, proteins, cell debris and SDS was carried out by using Buffer P3.

This Qiagen procedure has been modified for the purpose of this application note. The lysate including the plasmid DNA was transferred into the Thermo Scientific Nunc 50 mL conical tubes instead of loading the crude lysate directly into the Qiagen column as described in the procedure.

Plasmid DNA maxi-preps using Qiagen Kit

1. Place the tubes in the Fiberlite F15-8x50c (or F13-14x50c) rotor.
2. Spin tubes in the Fiberlite F15-8x50c rotor at the max speed 24,400 x g for 30 min at 4 °C (or in F13-14x50c at 17,000 x g for 40 minutes at 4 °C); then the Qiagen protocol was followed.

Note: This centrifugation step allows removal of the particulates from the crude lysate allowing faster and more consistent use of the gravity inserted into Qiagen columns; this simplifies the collection of purified plasmid DNA. Also, with the Fiberlite rotor, samples are spun up to maximum speeds, without damaging tubes, and removing the time consuming task of sample transfers.

3. Collect the plasmid DNA from the cleared lysate and elute it from a Qiagen column.
4. Precipitate the DNA with 90% ethanol at room temperature.
5. Centrifuge at 24,400 x g for 10 min.
6. Carefully decant the supernatant without disturbing the recovered plasmid DNA pellet; all anionic salts remain in the aqueous liquid.
7. Wash the plasmid DNA pellet twice with 70% ethanol.
8. Centrifuge at the same conditions and air-dry the pellet for 5 min and re-dissolve in a suitable volume of buffer.
9. Resuspend the pellet in TE buffer pH 8.0.

Note: Fiberlite conical tube rotors with disposable conical tubes can be used at the maximum speeds.

Results

The plasmid DNA was crudely checked for concentration and purity using agarose gel electrophoresis against known standard molecular weight markers (see Figure 1).

Reliable results with good yields of pure plasmid DNA (good 260/280 ratios) were obtained using the new general purpose centrifuge and Fiberlite F15-8x50c rotor with Qiagen's Plasmid Maxi Kit. These are typical results that can be obtained using this procedure.

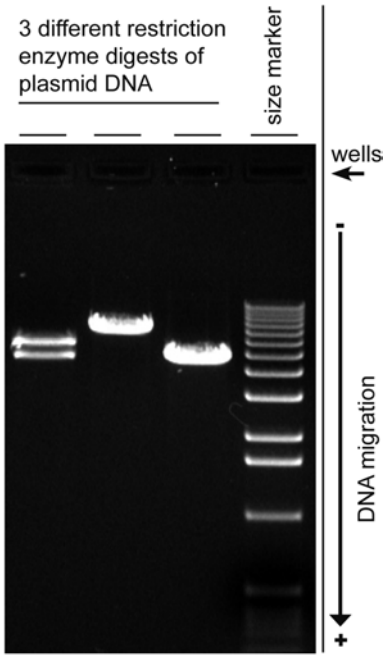

Figure 1: Plasmid DNA is isolated from bacteria culture using new Thermo Scientific general purpose centrifuge and Fiberlite F15-8x50c rotor. Plasmid restriction digests are run on a 1% w/v agarose gel stained with ethidium bromide. The DNA size marker is a commercial 1 kbp ladder. The direction of DNA migration and position of the wells is noted.

With this exceptional new Thermo Scientific centrifugation equipment, large quantities of high quality plasmid DNA can be extracted for further work, e.g. subcloning, transformations, verification by digestion/sequencing, PCR, southern blots etc.

Conclusion

The powerful new Thermo Scientific centrifuge, Fiberlite rotor and Nunc tube combination allows acceleration of total sample processing.

Carbon fiber rotors provide the opportunity to spin Nunc 50 mL tubes up to the maximum speed of the rotor, 24,400 x g with the Fiberlite F15-8x50c or with the Fiberlite F13-14x50c rotor's unique high capacity processing, 17,000 x g.

In conclusion, the new Thermo Scientific general purpose centrifuge enables time savings, either by extremely fast sample processing with the Fiberlite F15-8x50c rotor or by increased capacity with the Fiberlite F13-14x50c rotor.

References

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3. Qiagen Plasmid maxi (and midi) kit. Qiagen Inc., Valencia, CA, Handbook, 3rd Edition. 2005.

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