Technical Note:
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Rapid Isolation of Small Volume Plasmid DNA Using the New Thermo Scientific 1-Liter General Purpose Centrifuge with Fiberlite Rotor

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KEY WORDS

- Plasmid DNA Isolation
- Bacterial Pelleting
- 1-Liter General Purpose Centrifuge
- Fiberlite F21-48x2 Rotor

Introduction

Mini-preparation of plasmid DNA is a rapid, small-scale isolation of plasmid DNA from bacteria. A variety of procedures exist for the isolation and characterization of plasmid DNA. Generally, procedures involve growing the organism, harvesting cell material, lysing cells using a detergent solution with or without lysozyme, precipitating cell debris and chromosomal material then recovering plasmid DNA by precipitation. One traditional DNA purification method in use is based on the alkaline lysis method[1]. Plasmids of high purity must be obtained to be digested with restriction endonucleases and to be used in the process of molecular cloning to analyze DNA function.

Thermo Scientific Fiberlite F21-48x2 Rotor

Thermo Scientific 1-Liter General Purpose Benchtop Centrifuge

A High Throughput Solution

The new Thermo Scientific one-liter general purpose centrifuge, together with the Thermo Scientific Fiberlite F21-48x2 rotor, offer a unique solution for any facility performing separations using microvolume samples. The one-liter general purpose centrifuge with the 48-place carbon fiber rotor can increase the speed by spinning samples up to 25,055 x g resulting in shorter run times and double the throughput of a standard tabletop microcentrifuge.

The following describes a procedure for isolation of plasmid DNA using the new general purpose benchtop centrifuge with the Fiberlite® F21-48x2 rotor. By increasing the capacity of the standard microcentrifuge, more DNA preparations can be processed at one time, allowing more downstream experiments quicker. This is a great solution for research labs, clinical labs, forensics labs and any facility performing microvolume separations.

Procedure

Using the one-liter general purpose centrifuge and Fiberlite F21-48x2 rotor, the general method for plasmid mini-prep using alkaline lysis can be performed as follows.

PROTOCOL: Classic Plasmid DNA mini-preps such as Alkaline Lysis Procedure

1. Inoculate 2.5 mL of LB medium containing the appropriate antibiotic with a single bacterial colony. Incubate at 37 °C overnight with vigorous shaking.
2. Transfer 1.5 mL of the bacteria culture into each microcentrifuge tube. Centrifuge at the maximum speed 25,055 x g for 4 min at 20 °C.
3. Completely resuspend the pellet by vortexing in 100 µL of ice-cold solution of 50 mM glucose, 25 mM Tris-HCL pH 8.0, 10 mM EDTA and 4 mg/mL Lysozyme. Store at room temperature for 5 min.
4. Add 200 µL of freshly prepared solution of 0.2 N NaOH, 1% SDS to each tube. Close the tube and mix the contents by inverting the tube two or three times. Do not vortex. Store on ice for 5 min.
5. Add 150 µL of ice-cold solution of potassium acetate, pH 4.8 (60 mL 5M K-acetate, 11.5 mL glacial acetic acid, 28.5 mL water). The solution is 3M with respect to potassium and 5M with respect to acetate. Close the cap of the tube and vortex it in an inverted position for 10 seconds. Store on ice for 5 min.

6. Centrifuge at maximum speed for 11 min at 4 °C. Transfer the supernatant to a clean tube.
7. Add an equal volume of Tris-saturated phenol:chloroform (1:1) to deproteinize the mixture. Mix by vortexing. Centrifuge at the maximum speed for 5 min at 4 °C. Transfer the supernatant to a new tube.

Note: Addition of phenol/chlorofrom can dissolve and denature proteins, like DNase. This is especially important if the plasmids are to be used for enzyme digestion. Otherwise, smearing may occur in enzyme restricted form of plasmid DNA.

8. Add two volumes of ethanol at room temperature. Mix by vortexing. Keep the tube standing at room temperature for 2 min.
9. Centrifuge at the maximum speed for 5 min at 20 °C.
10. Remove the supernatant. Place the tube in an inverted position on a paper towel to allow all of the fluid to drain away.
11. Add 1 mL of 70% ethanol to wash the pelleted DNA. Centrifuge again at 25,055 x g for 5 min at 20 °C.
12. Again remove the supernatant and dry the pelleted DNA.
13. Re-suspend the pellet in 50 µL of Tris-EDTA pH 8.0 (10 mM Tris-HCL, 1mM EDTA) containing DNase-free RNase (20 mg/mL). Vortex briefly.

Restriction Digest of DNA

Move 10 µL of the mixture to new microcentrifuge tube. Add 1.2 µL of the appropriate buffer and 1 unit of the desired restriction enzyme. Incubate 1-2 minutes at the appropriate temperature.

The DNA can be crudely checked for concentration and purity using agarose gel electrophoresis against known standards.

A typical plasmid DNA yield of a miniprep is 20 to 30 µg depending on the bacteria strain.

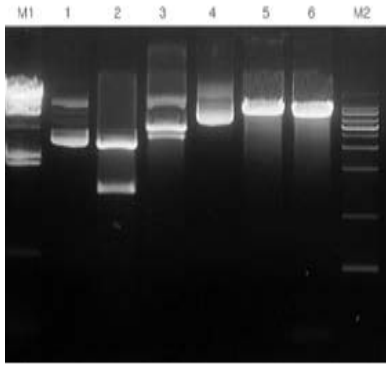

Figure 1: Plasmid DNA is isolated from bacteria culture using the new one-liter general purpose centrifuge and the Fiberlite F21-48x2 rotor. DNA is separated on a 1 % agarose gel ethidium bromide-stained. Lane 1 and 8 contain molecular weight markers; lanes 2-7 contain samples.

Alternative Method

Commercial plasmid DNA preparation kits are available from many life science manufacturers, such as Qiagen, Inc. or Sigma-Aldrich, Inc. These techniques are less time consuming however, some procedures may still require the use of the traditional methods of plasmid DNA isolation, such as the separation of different DNA isotopes, i.e. heavy (P^{32}) and light DNA.

Conclusion

High yield, high purity DNA was obtained from bacteria culture using the new one-liter general purpose centrifuge and its 48-place Fiberlite rotor. DNA prepared in this way is ready to be digested with restriction endonucleases and has been used to analyze the DNA function.

The end-user can be ensured that the combined use of the general purpose centrifuge and the Fiberlite F21-48x2 rotor will shorten their run times and double the throughput of a standard tabletop microcentrifuge making it a cost-effective solution for research laboratories today.

References

1. Maniatis, T., Fritsch, E.F. and Sambrock, J. 1982. Molecular Cloning. A Laboratory Manual. 488. Cold Spring Harbor Laboratory. USA: 86-96.
2. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Birnboim HC, Doly J Nucleic Acids Res. 1979 Nov 24;7(6):1513-23.

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