Technical Note:
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DNA Purification Using Thermo Scientific Superspeed Centrifuges and Thermo Scientific Fiberlite F13S-14x50cy rotor with the Qiagen® Plasmid Midi and Maxi Kits

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KEY WORDS

- Qiagen Plasmid DNA Kit
- Carbon Fiber Rotor
- Superspeed Centrifuge
- DNA Purification

Introduction

The Qiagen® Plasmid Midi and Maxi kits are designed for the preparation of up to 100-500 µg of high- or low-copy plasmid or cosmid DNA. The protocols within these kits begin with a modified alkaline lysis procedure, followed by DNA binding to Qiagen Anion-Exchange Resin under appropriate low-salt and pH conditions.[1] Next, molecular components such as RNA, proteins, dyes and low-molecular-weight impurities are removed by a medium-salt wash. The final steps include the elution of plasmid DNA in a high-salt buffer and concentration and desalting by isopropanol precipitation[1].

These protocols require a refrigerated centrifuge that is capable of g-force in excess of 20,000 x g. This technical note describes a modification to the Qiagen protocol designed to save researchers valuable time; the modified protocol uses Fiberlite™ carbon fiber rotors in Thermo Scientific superspeed centrifuges such as the Sorvall® RC-6™ Plus or Evolution® RC.

Fiberlite F13-14x50cy Carbon Fiber Rotor

Procedure

Follow the procedure specified in the Qiagen protocol handbook as described below[1]. Actual volumes will vary if the plasmid of interest is a low- or high-copy plasmid. (Please see handbook for specific details.)

Preparation of Crude Lysate (Protocol Steps 1-6)

1. Prepare starter bacterial culture containing gene of interest.
2. Prepare larger volume culture from starter culture.
3. Harvest cells at 6,000 x g for 15 min at 4°C. The volume of harvest may vary between 25 to 500 mL.
4. Resuspend pellet in Buffer P1.
5. Add Buffer P2. Invert and incubate at room temperature for 5 min.
6. Add chilled Buffer P3. Mix immediately, invert, and incubate on ice for 15 to 20 min.

Clearing of Lysate by Centrifugation and DNA Elution (Modification of Protocol Steps 7-15)

At this point, the crude lysate can be clarified using filtration or centrifugation. Removal of the particulates from the crude lysate by centrifugation simplifies the collection of plasmid DNA without the need for an extra filtration kit and allows the protocol to be conducted more efficiently.

7. Transfer lysate to a 50 mL conical tube, place in the Fiberlite F13S-14x50cy rotor and centrifuge in a Sorvall superspeed centrifuge at 25,000 x g for 30 min at 4°C.
8. Collect plasmid DNA from cleared lysate.
9. Elute DNA from a Qiagen column.
10. Precipitate DNA with 70% ethanol at room temperature.
11. Centrifuge at 25,000 x g for 10 min.
12. Decant supernatant carefully without disturbing the recovered plasmid DNA pellet.
13. Air dry the DNA pellet for 5 to 10 min and re-dissolve in a suitable volume of buffer.

Conclusion

In all the procedures, the Fiberlite F13S-14x50cy carbon fiber rotor and disposable conical tubes can be used at the maximum speeds in conjunction with the Qiagen Plasmid Midi and Maxi kits.

The ability of the Fiberlite rotor to spin the conical tubes at high g-force allows researchers to save valuable time and reduce costs since steps can be eliminated from the Qiagen protocol. The modified protocol can be conducted using one reliable centrifuge: the Thermo Scientific Sorvall Superspeed RC-6 Plus or Evolution RC.

References

1. Qiagen® (November 2005). Plasmid or cosmid DNA purification using Qiagen Plasmid Midi and Maxi Kits. *In Qiagen® Plasmid Purification Handbook*. 3rd ed. (pp. 19- 23).

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