Application Note: ANCFGLYNXSUPERDNA 1012

DNA Preparation Using the Thermo Scientific Sorvall LYNX Superspeed Centrifuge Series

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Introduction

Today's molecular biologist relies on the plasmid, a closed, circular, double stranded form of DNA, that is propagated in bacteria. Many different protocols can produce plasmid DNA in large quantities that are sufficiently pure for general cloning, enzyme digestion, sequencing, cellular transfection, *in vitro* transcription/ translation, protein expression and other purposes. For instance, DNA can be isolated in cesium chloride gradients, but this method requires an ultracentrifuge and utilizes ethidium bromide, which must be handled with care.1 Alternatively, gravity or spin column kits effectively purify plasmid DNA in superspeed and microcentrifuges. This brief highlights Thermo Scientific Sorvall superspeed centrifuges and rotors that can be used in concert with the commercially available DNA preparation kits and also provides a low-cost method to obtain plasmid DNA in large-scale (about 1 mg) without using a kit.

Procedures

PROTOCOL 1: Cost-Efficient, Large-Scale DNA Plasmid Prep Using Sorvall Superspeed Centrifuges for the Entire Process

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This procedure describes the isolation of plasmid DNA using a floor model superspeed centrifuge from start-to-finish. The Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge employs a diverse array of rotors and can accommodate a wide variety of applications.

1. Using the appropriate antibiotic selection, grow 500 mL or more of bacterial culture (usually overnight at 37 °C, with shaking).

2. Divide culture by pouring into 250 mL bottles. Pellet bacteria by performing centrifugation in a Sorvall® LYNX 6000 superspeed centrifuge with a Thermo Scientific Fiberlite carbon fiber rotor (such as the Fiberlite® F14-6x250y, F12-6x500 LEX, F10-4x1000 LEX, or F9-6x1000 LEX rotor) with the following parameters: 6,000 x g for 15 min at 4 °C.

Figure 1. Thermo Scientific Fiberlite large capacity carbon fiber rotors.

Solutions Needed
Buffer A: 50 mM Tris-HCl, pH 8.0; 10 mM EDTA
Buffer B: 0.2 M NaOH; 1% SDS
Buffer C: 3 M Potassium Acetate, pH 5.5
Isopropanol
70% Ethanol
RNAase A stock solution: 10 mg/mL
PEG/NaCl solution: 20% PEG 8000, 2.5 M NaCl or 13% PEG 8000, 1.6 M NaCl
Phenol, Tris buffered to pH 8.0
Chloroform
10 mM Tris or TE
3 M Sodium acetate, pH 4.5-5.5

Table 1. Solutions for DNA preparation

Figure 2. Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge.

3. Decant supernatant and thoroughly resuspend bacteria in one of the bottles with 15 mL buffer A. Transfer bacteria to the other bottle and resuspend the combined pellets.

Note: There should be no “clumps” remaining.

4. Add 15 mL buffer B, mix by inversion 5-6 times and let sit at room temperature for 5 min.

Note: Solution should become more clear and viscous.

5. Add 15 mL buffer C, mix by inversion 5-6 times.

Note: A heavy precipitate will form; swirl to break up precipitate.

6. Centrifuge at 20,000 x g for 20 min at 4 °C.

7. Pour supernatant through cheese cloth and collect in a clean bottle.

Note: An additional spin for 10 min may pellet any excessive debris not cleared in the first spin.

8. Add an equal amount of isopropanol to the bottle and swirl gently to mix. Perform centrifugation with the following parameters: 18,000 x g for 30 min at 4 °C.

Note: Use a lab pen to mark the outer edge of the bottle before centrifugation to help locate the somewhat clear and translucent pellet (also applies to subsequent steps).

9. Carefully remove the supernatant. Add >50 mL of 70% ethanol to the 250 mL bottle and agitate to resuspend and wash the DNA. Perform centrifugation with the following parameters: 18,000 x g for 10 min at 4 °C.

10. Remove all the supernatant and dry the pellet in a vacuum dessicator for 30-60 min.

Note: Over-drying the pellet can prevent effective DNA resuspension in subsequent steps.

11. Dissolve the pellet in a total of 2 mL TE containing RNAase at a final concentration of 100 µg/mL.

Note: If dissolved in higher volumes, the DNA may be too dilute to effectively precipitate in subsequent steps.

12 Transfer to a 15 mL or similar volume tube and incubate at 37 °C for 1 hr.

13. Precipitate the DNA for 30 min on ice with 0.5 volume 20% PEG 8000, 2.5 M NaCl or with one volume of 13% PEG 8000, 1.6 M NaCl.

14. Perform centrifugation in the Thermo Scientific Fiberlite F14-14x50cy rotor with 15 mL conical adapters or in the Thermo Scientific A21-24x15c rotor with the following parameters: 20,000 x g for 20 min at 4 °C.

15. Carefully remove all the supernatant.

Note: The pellet may be very translucent and smeared along the outer edge of the tube. It is critical to remove all the PEG.

16. Dissolve the DNA in 700 µL of TE or 10 mM Tris and transfer to a high performance 1.5 mL micro-centrifuge tube.

17. Add an equal volume (700 µL) of phenol, vortex 30 sec and perform centrifugation in the Thermo Scientific Fiberlite F27-48x1.5 rotor with the following parameters: 19,000 x g for 5 min at 4 °C.

18. Remove the upper aqueous phase and transfer to another high performance microtube containing 350 µL phenol and 350 µL chloroform.

19. Vortex and perform centrifugation with the following parameters: 19,000 x g for 5 min at 4 °C.

20. Perform a final extraction in 700 µL chloroform.

Note: Repeating the phenol/ chloroform extraction may increase DNA purity. A back extraction once with 700 µL of TE can increase yield.

Figure 3. High speed 15 mL and 50 mL conical tube rotors for the Sorvall LYNX 6000 superspeed centrifuge.

Company	Product Line	Capabilities
Bio-Rad® Laboratories	Quantum Prep® Plasmid Purification Kits	Mini-, midi-, and maxi-prep
Clontech®	Nucleobond® Kits	Mini-, midi-, and maxi-prep
Clontech	NucleoSpin® Kits	96 well, mini-, midi-, and maxi-prep
MO BIO Laboratories	UltraClean™ Kits	Endotoxin-free, 96 well, mini-, midi-, and maxi-prep
QIAGEN®	QIAprep® Spin Miniprep Kit	96 well, mini-prep
QIAGEN	QIAGEN Plasmid Kits and QIAfilter Plasmid Kits	Endotoxin-free, mini-, midi-, maxi-, and mega-prep
Sigma-Aldrich®	GenElute™ Plasmid Kit	Endotoxin-free, mini-, midi-, maxi-, giga-, and mega-prep

Table 2. Plasmid DNA preparation kits.

21. Precipitate the DNA by the addition of 0.8 volume of isopropanol and 0.1 volume of 3M NaoAc, pH 4.5-5.5. Mix several times by inversion. Perform centrifugation in the Fiberlite F27-48x1.5 rotor with the following parameters: 19,000 x g for 15 min at 4 °C.

22. Decant supernatant and carefully wash the DNA pellet with 1 mL of 70% EtOH.

23. Air dry pellet and dissolve in 500-1000 µL Tris or TE.

24. Measure the OD_{260} and $OD_{260/280}$ and calculate DNA concentration (DNA concentration in g/L = (OD_{260})(50)(Dilution)) and assess purity.

Note: An $OD_{260/280}$ below 1.8 suggests impurities. Double phenol/chloroform extraction can improve the ratio.

PROTOCOL 2: Using Superspeed Centrifuges for Plasmid DNA Preparation with Commercially Available Kits

Protocol 1 describes the process for plasmid DNA preparation without using a commercially available kit. Table 2 lists many commercially available kits for plasmid DNA preparation that call for the use of a centrifuge
with high speed and large volume capabilities, such as the Sorvall LYNX 6000 superspeed centrifuge. The initial steps in DNA preparation involves the pelleting of bacteria in which the plasmid of interest has been propagated. Individual bacterial cultures with volumes up to 6 L can be processed using the Sorvall LYNX 6000 superspeed centrifuge. Centrifugation at 6,000 x g for 10-15 min at 4 °C is sufficient to pellet bacteria grown in a 5 mL or larger culture. For smaller volume cultures,

Thermo Scientific Rotor	Capacity (place x mL)	Max Speed (rpm)	Max RCF (x g)
Fixed Angle Rotors			
Fiberlite F9-6x1000 LEX	6 x 1000	9,000	17,568
Fiberlite F10-4x1000 LEX	4 x 1000	10,500	20,584
Fiberlite F12-6x500 LEX	6 x 500	12,000	24,471
Fiberlite F14-6x250	6 x 250	14,000	30,240
Swinging Bucket Rotors			
BIOFlex HC	4 x 1000	5,500	7,068
BIOFlex HC with adapter 75004253	4 x 500	5,500	7,068
BIOFlex HC with adapter 75007305	8 x 250	5,500	7,068
BIOFlex HS	4 x 400	7,000	10,025

Table 3. Rotors available for DNA preparation in the Sorvall LYNX 6000 superspeed centrifuge.

Thermo Scientific Rotor	Capacity (place x mL)	Max Speed (rpm)	Max RCF (x g)
Fixed Angle Rotors			
Fiberlite F14-14x50cy	14 x 50	14,000	33,746
Fiberlite F14-14x50cy with adapter 010-0378	14 x 15	14,000	33,746
A21-24x15c	24 x 15	21,500	63,049
Swinging Bucket Rotors			
TH13-6x50 with adapter 75007322	6 x 50	13,100	30,314

Table 4. High speed 15 mL and 50 mL conical tube rotors and adapters available for DNA preparation in the Sorvall LYNX 6000 superspeed centrifuge.

the time and speed for pelleting is reduced; centrifugation at 5,000 x g for 10 min at 4 °C is sufficient for a 150 mL bacterial culture. Table 3 lists a selection of rotors the Sorvall LYNX 6000 superspeed centrifuge that will serve to accommodate small and large scale pelleting (up to 6 L) needs prior to DNA purification.

Following the initial pelleting of bacterial culture, DNA purification steps require centrifugation at >15,000 x g. For ease of use and to ensure the integrity of samples, the use of sterile 15 mL to 50 mL conical tubes can be desired, however, these conical tubes are typically limited to <7,000 x g. The unique Thermo Scientific conical tube rotors and conical tube adapters allow for the use of disposable sterile conical tubes at RCFs up to 63,409 x g. Table 4 lists a selection of rotors and adapters for the Sorvall LYNX 6000 superspeed centrifuge that will serve to accommodate the forces required by most commercial kits.

Conclusion

This application brief describes the wide variety of superspeed offerings provided by Thermo Fisher Scientific to accommodate the needs of molecular biologists during plasmid DNA preparation. Thermo Scientific equipment accommodates small and large volume bacterial cell pelleting and offers efficient, versatile, lightweight, and reliable rotors.

References

1. Noles, S.R., Schwartz, M.W., Fischer, R.A., Martin, R., Schneider, W., Jagadeeswaran, P., Rao, K.J. 2008. Traditional Methods for CsCl Isolation of Plasmid DNA by Ultracentrifugation.

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