

DNA Preparation Using Thermo Scientific Sorvall Superspeed Centrifuges

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KEY WORDS

- Bacterial Pelleting
- DNA Preparation
- DNA Purification Kits
- Superspeed Centrifugation

Introduction

Today's molecular biologist relies extensively 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 Superspeed centrifuges from start-to-finish. The Thermo Scientific Sorvall RC6 Plus or Thermo Scientific Sorvall Evolution RC employs a diverse array of rotors and can accommodate a wide variety of applications.



Figure 1. Diverse Array of Accessories for Thermo Scientific Superspeed Centrifuges

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 in a Thermo Scientific FIBERLite F14S-6x250y carbon fiber rotor, Thermo Scientific SLA-1500 or SLA-1000 aluminum rotors by spinning at 6,000 x g for 15 min. at 4°C in a Sorvall® RC6™ Plus or Sorvall Evolution™ RC.
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. See Table 1 for a listing of required solution components.
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 250 mL bottle. Note: An additional spin for 10 min. may pellet any excessive debris not cleared in the first spin.



Figure 2. Thermo Scientific Sorvall RC6 Plus

8. Add an equal amount of isopropanol to the bottle and swirl gently to mix. Spin at 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. Centrifuge at 18,000 x g for 10 min.
10. Remove all the supernatant and dry the pellet in a vacuum desiccator for 30 min. to 1 hr. 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. Transfer to a 15 mL or similar volume tube and incubate at 37°C for 1 hr.
12. 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.
13. Centrifuge at 20,000 x g for 20 min. Note: Use appropriate adaptor to fit tube in rotor. Carefully remove all the supernatant. The pellet may be very translucent and smeared along the outer edge of the tube. It is critical to remove all the PEG.
14. Dissolve the DNA in 700 µL of TE or 10 mM Tris and transfer to a 1.5 mL micro-centrifuge tube.
15. Add equal volume (700 µL) of phenol, vortex 30 sec. and spin in a Thermo Scientific F-20/Micro or F22/Micro rotor for 5 min. at 19,000 x g. Remove upper aqueous phase and transfer to another tube

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 3. Large-scale Pelleting using Thermo Scientific Superspeed Centrifuges

16. Add 0.8 volume of isopropanol and 0.1 volume of 3M sodium acetate pH 4.5-5.5. Mix several times by inversion. Centrifuge in the F-20/Micro or F22/Micro rotor for 15 min. at 19,000 x g at 4°C.
17. Decant supernatant and wash DNA pellet with 1 mL of 70% ethanol.
18. Air dry pellet and dissolve in 500-1000 µL Tris or TE.
19. Measure OD₂₆₀ and OD_{260/280} to calculate DNA concentration and assess purity. DNA concentration in g/L = (OD₂₆₀)(50)(Dilution). OD_{260/280} below 1.8 suggests impurities (Double phenol/chloroform extraction can improve 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 RC6 Plus or Evolution RC. 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 1 L can be processed using our Superspeed centrifuges or bacterial culture pellets can be combined to maximize DNA yield.

Centrifugation at 6,000 x g for 10-15 min. is sufficient to pellet bacteria grown in a 500 mL culture. For smaller volume cultures, the time and speed for pelleting is reduced; centrifugation at 5,000 x g for 10 min. is sufficient for a 150 mL bacterial culture. Table 3 lists a selection of rotors that are compatible with Superspeed centrifuges and that will serve to accommodate small and large scale pelleting (up to 6 L) needs prior to DNA purification.

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.

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

Rotor	Capacity (place x mL)	Max Speed (rpm)	Max RCF (x g)
<i>Fixed-Angle Rotors – Carbon Fiber</i>			
F8S-6x1000y*	6 x 1000	8,500	15,810
F9S-4x1000y	4 x 1000	8,000	12,006
F14S-6x250	6 x 250	14,000	25,862
F12S-6x500y	6 x 500	11,000	20,449
F14S-6x250y	6 x 250	14,000	25,862
<i>Fixed-Angle Rotors – Aluminum</i>			
SLA-3000	6 x 500	11,000	20,450
SLA-1500	6 x 250	14,500	31,916
SLA-1000	4 x 250	16,500	35,793
<i>Swinging Bucket Rotors</i>			
SH-3000	4 x 750	4,700	4,579
	3 standard	4,700	3,709
	or 1 deepwell microplate		

Table 3. Rotors Available for DNA preparation in Thermo Scientific Sorvall Superspeed Centrifuges

*This rotor is only compatible with the Sorvall Evolution RC.

References

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