Plasmid DNA Separation Fixed Angle and Vertical Rotors in the Thermo Scientific Sorvall MTX and MX Plus Micro-Ultracentrifuge Series

Key Words

Sorvall MTX Micro-Ultracentrifuge, Sorvall MX Plus Micro-Ultracentrifuge, Plasmid DNA Separation, Single-step Run, Multi-step Run, Gradient Ultracentrifugation, Cesium Chloride, Cesium Trifluoroacetate, Chloroplast DNA

Introduction

Plasmid DNA is widely used as a vector in molecular cloning experiments and as gene delivery material during studies of protein function. Many methods exist for plasmid DNA isolation using fixed angle and vertical rotors in Thermo Scientific Sorvall MTX and MX Plus micro-ultracentrifuges. In recent years, more plasmid isolation procedures have used vertical rotors because the short path length (maximum sedimentation distance = centrifuge tube diameter), large surface area, and high starting g force decrease the total run time and improve DNA banding quality. Several examples of plasmid DNA purification are given to demonstrate the performance of various rotors.

Rotors and Protocol Summary

A variety of fixed angle and vertical rotors have been used to purify plasmid DNA. The rotor specifications are listed in Table 1 and a summary of centrifugation protocols are listed in Table 2.

Figure 1. Thermo Scientific Sorvall MX Plus Micro-Ultracentrifuge.

Thermo Scientific Rotor	Max Speed (rpm)	Max RCF (x g)	K Factor	Max Radius (cm)	Capacity (places x mL)	Tube Angle
S150-AT	150,000	899,744	6.1	3.58	8 x 2	30
S120-AT2	120,000	649,826	8.2	4.04	10 x 2	30
S100-AT4	100,000	540,628	15.9	4.84	6 x 3.5	30
S100-AT6	100,000	603,180	17.6	5.40	8 x 5.1	30
S80-AT3	80,000	414,630	23.1	5.80	8 x 8.3	30
S120-VT	120,000	500,237	7.8	3.11	8 x 2	0

Table 1. Thermo Scientific Micro-Ultracentrifuge Rotor Specifications.

Procedures

PROTOCOL 1: Using the Thermo Scientific S150-AT Fixed Angle Rotor

Reduce Separation Time of Plasmid DNA with the S150-AT Fixed Angle Rotor

When separating plasmid DNA from *E. coli.* with a homogeneous solution of cesium chloride (CsCl) and

Thermo Scientific Rotor	Plasmid Protocol	Gradient Density (g/mL)*	Rotor Speed (rpm)	Run Time (hrs)	Temp (°C)	Acceleration Setting	Deceleration Rate
S150-AT	5 Step Run	1.55	150,000 – 80,000	2.5 total	20	9	7
S120-AT2	Homogenous Step Run	1.57	120,000 – 85,000	3.5 total	20	N/A	N/A
S120-AT2	Pre-formed Two Layer Gradient	1.39 1.75	120,000	2.0	20	N/A	N/A
S120-AT2	Homogenous	1.57	120,000	12.0	20	N/A	N/A
S100-AT4	Homogenous	1.57	85,000	>16.0	20	9	7
S100-AT6	Homogenous	1.55	70,000	16.0	20	9	7
S80-AT3	4 Step Run	1.55	80,000 – 60,000	12.0 total	20	9	7
S80-AT3	Homogenous	1.55	70,000	16.0	20	9	7
S120-VT	Homogenous	1.57	120,000	2.5	20	9	7
S120-VT	Homogenous	2.0 (Cesium trifluoroacetate)	120,000	3.0	20	9	7

Table 2. Conditions of Centrifugation.
*All gradients are cesium chloride (CsCl) unless otherwise noted.

ethidium bromide (EtBr), ultracentrifugation for at least 3.5 hours in a fixed angle rotor was necessary. However, this separation time has been shortened to 2.5 hours by using the S150-AT fixed angle rotor in a Sorvall® MTX or MX Plus micro-ultracentrifuge, which delivers a maximum speed of 150,000 rpm and a maximum RCF of 901,000 x g.

Procedure

1. Mix the following:
   a. 1.4 mL *E. coli* JM109 including plasmid (pUC19) DNA sample
   b. 1.35g CsCl in TE buffer (pH 8.0) (ρ: 1.55 g/mL) with 40 µL EtBr (10 mg/mL)
2. Insert into a 2 mL polyallomer Re-Seal® (PN 45246) tube. If the tubes cannot be completely filled, supplementary liquid [CsCl (1 g) dissolved in TE buffer, pH 8.0 (1 mL)] should be added. Seal tubes with plug (PN 45307) and crown (PN 45306).
3. Using the automatic step-mode perform centrifugation in the Sorvall MTX or MX Plus micro-ultracentrifuge series as follows:

Speed (rpm)	Time (mins)
150,000	80
130,000	25
120,000	10
100,000	10
80,0000	25
Total	150

Conclusion

The multi-step operation described in this brief decreases speed gradually to prevent CsCl from crystallizing. Rapid separation of plasmid DNA was achieved with high quality (Figure 1). No crystallization resulted, and the two bands of DNA were well separated.

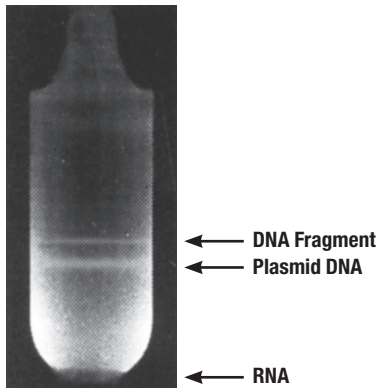


Figure 2. Plasmid DNA Separation after Centrifugation.

PROTOCOL 2: Using the Thermo Scientific S120-AT2 Fixed Angle and S120-VT Vertical Rotors

Plasmid DNA Separation Using the S120-AT2 and S120-VT Rotors with Multiple Protocols

A CsCl/EtBr gradient is often used to separate plasmid DNA during ultracentrifugation. DNA binds differential amounts of EtBr resulting in two DNA species of different buoyant densities. These are linear DNA of bacterial origin and circular plasmid DNA. The buoyant densities in the EtBr/CsCl medium are 1.50 g/mL and 1.55 g/mL respectively. Using a CsCl density gradient, two distinctive bands are formed using ultracentrifugation. The difference in their buoyant density is 0.05 g/mL. The resolution between these two DNA bands depends on the CsCl gradient profile at equilibrium. Higher speeds save run times and yield steeper gradients. In an extremely steep gradient, the DNA bands would overlap and affect DNA purity. In addition, a steep gradient might precipitate CsCl at the high density area when saturated. The latter is a safety concern because solid CsCl has a density of 4 g/mL, which can result in tube

leakage, sample loss and rotor failure. The following describes multiple methods for plasmid DNA separation using the S120-AT2 and S120-VT rotors with a Sorvall MTX or MX Plus micro-ultracentrifuge.

Procedure

1. Mix the following:
   a. DNA sample
   b. CsCl in TE buffer (pH 8.0) (ρ: 1.57 g/mL) with 40 µL EtBr (10 mg/mL)
2. Fill centrifuge tubes to capacity and overlay with supplementary liquid [CsCl (1 g) dissolved in TE buffer, pH 8.0 (1 mL)] if needed.
3. Perform centrifugation in the Sorvall MTX or MX Plus micro-utracentrifuge series as follows:

Protocol*	CsCl Density (g/mL)	Speed (rpm)	Time (hrs)
Homogenous Step Run	1.57	120,000 85,000	2.5 1.0
Pre-formed Two Layer Gradient	1.39 1.75	120,000	2.0
Homogenous Single Run	1.57	120,000	12.0
S120-VT Homogenous Single Run	1.57	120,000	2.5

*S120-AT2 rotor unless otherwise indicated

Conclusion

The best overall performance with good band separation of 4 mm and a short run time of 2.5 hrs is achieved using the Thermo Scientific S120-VT rotor at 120,000 rpm (Table 3). The S120-AT2 rotor 12 hr run produces a steeper gradient than the S120-VT rotor 2.5 hr run (Figure 2). The shallow gradient in the S120-VT rotor enhances the resolution of the DNA bands. In addition, significant time savings in the purification of plasmid DNA are achieved. Also, similar gradients are obtained with a homogenous density sample 12.0 hr run and a two layered sample 2.0 hr run in a S120-AT2 rotor (Figure 3). There is a significant time savings using a pre-formed two layer gradient however, the DNA bands obtained are usually fuzzy and the purity of plasmid DNA is compromised.

Thermo Scientific Rotor	Plasmid Protocol	DNA band separation (mm)
S120-AT2	Homogenous Step Run	3.0
S120-AT2	Pre-formed Two Layer Gradient	2.0
S120-AT2	Homogenous Single Run	2.5
S120-VT	Homogenous Single Run	4.0

Table 3. DNA Separation using Plasmid Preparation Protocols.

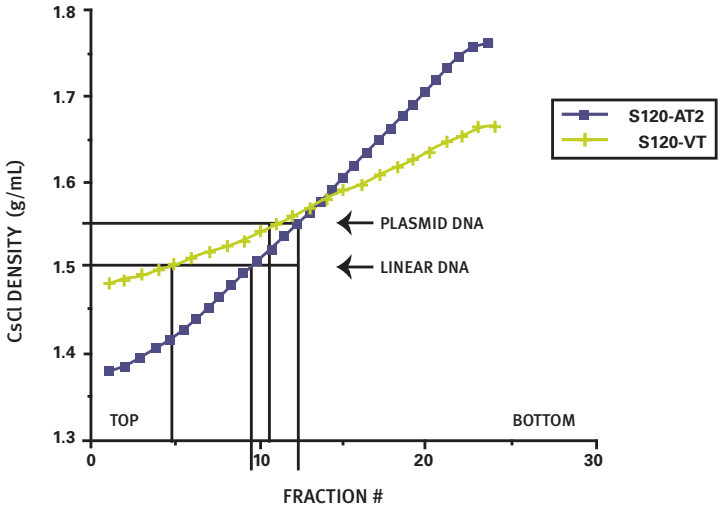


Figure 2. CsCl Density Gradient vs. Tube Fraction Number (S120-AT2 run at 120,000 rpm, 20 °C for 12 hr. and S120-VT run at 120,000 rpm, 20 °C for 2.5 hr.).

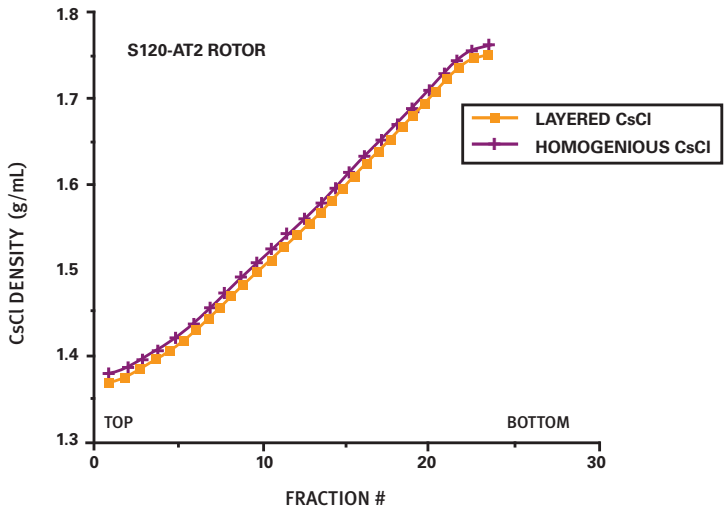


Figure 3. CsCl Density Gradient vs. Tube Fraction Number (Homogeneous CsCl Density Run at 120,000 rpm, 20 ° C for 12 hr. and Two Layer CsCl Density Run at 120,000 rpm, 20 ° C for 2 hr.).

PROTOCOL 3: Using the Thermo Scientific S120-VT Vertical Rotor

Separation of Plasmid DNA with Cesium Trifluoroacetate and the S120-VT Vertical Rotor: Reduced Ethidium Bromide Concentration Enhances User Safety

The procedure using CsCl and EtBr is most commonly used in the separation of plasmid DNA with a preparative ultracentrifuge. However, the concentration of EtBr, a highly potent mutagenic substance, must be relatively high, from 0.1 to 1.0 mg/mL, and reductions in this amount are desirable. Therefore, pursuant to the procedure set forth by Andersson *et al.*, this experiment was performed using cesium trifluoroacetate (CsTFA) in place of CsCl[1, 2].

Procedure

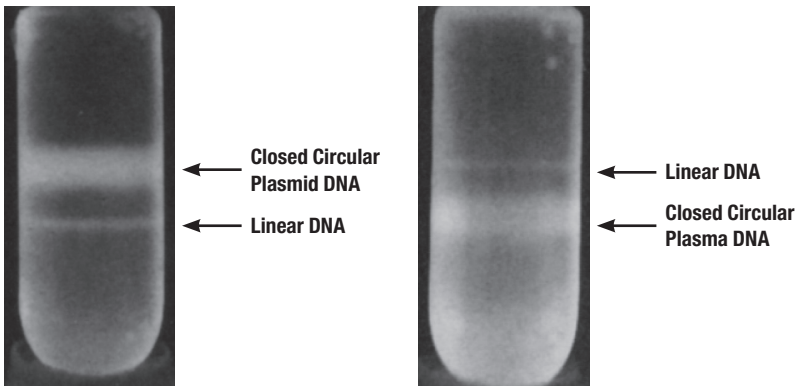
1. Mix the following:
   a. 0.10 mL DNA sample (treated according to alkaline-SDS method[3])
   b. 1.06 mL cesium trifluoroacetate (ρ: 2.0±0.05 g/mL) with 20 µL EtBr (0.1 mg/mL) to 0.64 mL of TE buffer solution (10mM Tris-HCl, 1mM EDTA, pH 8.0)

2. Place into 2.0 mL polyallomer Re-Seal (PN 45246) tubes. If the tubes cannot be completely filled, supplementary liquid consisting of TE buffer solution and cesium trifoluoroacetate in the proportion of 1.0 mL to 1.7 mL.

3. Perform centrifugation in the Sorvall MTX or MX Plus micro-ultracentrifuge series with the following parameters: 120,000 rpm for 3 hrs at 20 °C.

Conclusion

With cesium trifluoroacetate, the buoyant density of closed circular DNA and linear DNA are 1.60 g/mL and 1.65 g/mL, respectively, which renders unnecessary the addition of EtBr[1]. Because of this, EtBr is needed only to permit recognition of each type of DNA when the centrifugation tube is illuminated under a UV lamp. After centrifugation, this allows extraction of a band of closed circular plasmid DNA. For this purpose, it was recognized that 1 µg/mL of EtBr, which is 1/100th to 1/1000th of the conventional amount, is sufficient. In this case, the relationship between the closed circular plasmid DNA and the band of linear DNA is the opposite of that obtained when CsCl and EtBr are used: the upper band, which is of lower density, is closed circular plasmid DNA (Figure 4).



Using Cesium Trifluoroacetate
Ethidium bromide: 1 µg/mL (final)
S120-VT vertical rotor,
120,000 rpm, 3 hrs, 20 °C

Using Cesium Chloride
Ethidium bromide: 100 µg/mL (final)
S120-VT vertical rotor,
120,000 rpm, 3 hrs, 20 °C

Figure 4. Results of Separation of Plasmid DNA.

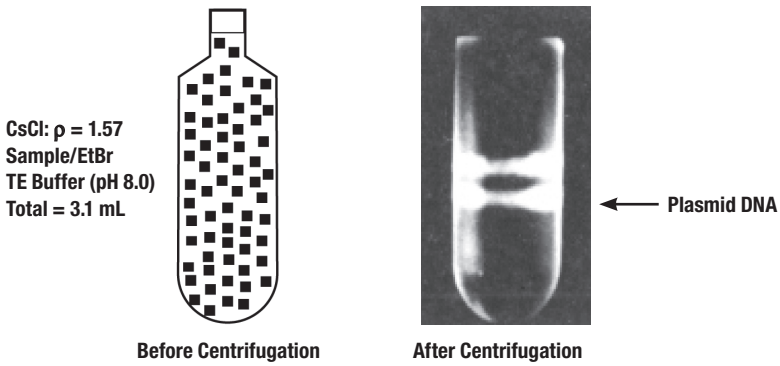


Figure 5. Results of Centrifugation in a Thermo Scientific S100-AT4 Rotor.

PROTOCOL 4: Using the Thermo Scientific S100-AT4 Fixed Angle Rotor

Isolation of Plasmid DNA Using the S100-AT4 Fixed Angle Rotor

The S100-AT4 rotor is designed to accommodate 3.5 mL polyallomer tubes (PN 75000509), which are ideally suited for density gradient separations. A major advantage of performing a density gradient separation is the ability to physically isolate plasmid DNA. In general, higher rotor speeds result in steep density gradients and a poor separation between the bands. Therefore, for most DNA separations, centrifugation is performed at lower speeds to produce a shallow equilibrium gradient and more effectively separate the bands.

Procedure

1. Mix the following:
   a. 50 µg of pBR322 and 25 µg of lambda phage DNA
   b. 2.56 g CsCl (ρ: 1.57 g/mL) in 2.55 mL TE buffer (pH 8.0) with 33 µL EtBr (10 mg/mL)

2. Fill centrifuge tubes to capacity and overlay with supplementary liquid [CsCl (1 g) dissolved in TE buffer (1 mL)] if needed.

3. Perform centrifugation in the Sorvall MTX or MX Plus micro-ultracentrifuge series with the following parameters: 85,000 rpm overnight (>16 hrs) at 20 °C.

Conclusion

Following centrifugation, the desired plasmid band (lower) is typically collected using a syringe inserted through the wall of the centrifuge tube (Figure 5). To avoid contamination and/or sample loss, a significant separation between the two bands is necessary. When starting with samples uniformly distributed in a homogeneous solution in the S100-AT4 fixed angle rotor, the separations are typically run overnight at lower speeds. By setting the rotor speed to 85,000 rpm the density gradient range becomes more shallow than at full speed (data not shown); and the distance between the upper and lower bands increases.

PROTOCOL 5: Using the Thermo Scientific S100-AT6 Fixed Angle Rotor

Separation of Chloroplast DNA From Spinach Using the S100-AT6 Fixed Angle Rotor

It is well known that chloroplasts and mitochondria have DNA distinct from chromosomal DNA. Using the following protocol, we separated spinach chloroplast DNA by ultracentrifugation. The overnight separation (16 hrs) involved CsCl density gradient ultracentrifu-gation using a Sorvall MTX or MX Plus micro-ultracentrifuge series and the S100-AT6 rotor accommodating 5 mL polyallomer Re-Seal® (PN 45248) or Easy-Seal tubes (75000508).

Procedure

1. Add 5 volumes of Buffer A (0.35 M Sorbitol, 50 mM Tris-HCl (pH 8.0), 25 mM EDTA-Na_2) to 5 mL of chloroplast solution in a 50 mL conical tube.

2. Perform centrifugation in a Thermo Scientific general purpose or superspeed centrifuge with the following parameters: 2,500 rpm for 15 mins at 4 °C.

3. Remove supernatant and wash pellet 2 or 3 times with 20-30 mL Buffer A.

4. Resuspend pellet in 2 mL of Buffer A and add pronase.
5. Incubate at 37 °C for 2 hrs, and then room temp. for 2 mins.
6. Mix gently for 15 mins.
7. Incubate at 4 °C for 2-3 hrs.
8. Perform centrifugation in a Thermo Scientific general purpose or superspeed centrifuge with the following parameters: 2,500 rpm for 15 mins at 4 °C.
9. Harvest supernatant (crude DNA solution) and place into 5 mL polyallomer Re-Seal or Easy-Seal tubes and perform centrifugation in the Sorvall MTX or MX Plus micro-ultracentrifuge series with the following parameters: 70,000 rpm overnight (>16 hrs) at 20 °C.

Conclusion

Using this protocol, a distinct band forms representing chloroplast DNA after centrifugation (Figure 6).

PROTOCOL 6: Using the Thermo Scientific S80-AT3 Fixed Angle Rotor

Separation of Plasmid DNA with a Single-Step and Multi-Step Centrifuge Run with the S80-AT3 Fixed Angle Rotor

The S80-AT3 rotor is capable of processing a 66.4 mL sample (8 x 8.3 mL). This brief describes 2 protocols for the separation of plasmid DNA (single and multi-step) using the S80-AT3 rotor and the Sorvall MTX or MX Plus micro-ultracentrifuge series.

Procedure

1. Mix the following:
   a. DNA sample (treated according to alkaline-SDS method[3])
   b. 5.4 g CsCl (ρ: 1.55 g/mL) with 160 µL EtBr (10 mg/mL) to 5.6 mL of TE buffer solution (10mM Tris-HCl, 1 mM EDTA, pH 8.0)
2. Place into 8 mL polyallomer Re-Seal tube (PN 45597). If the tubes cannot be completely filled, supplementary liquid [CsCl (1 g) dissolved in TE buffer (1 mL)] if needed] should be added. Seal the tube with a plug and crown.

Protocol	Speed (rpm)	Run Time (hrs)	Temp. (°C)	Acceleration Setting	Deceleration Setting
Single-Step	70,000	16	20	9	7
Multi-Step	80,000	5	20	9	7
	70,000	5			
	65,000	1			
	60,000	1			

3. Perform centrifugation in a Sorvall MTX or MX Plus micro-ultracentrifuge series with the following conditions:

Conclusion

The multi-step method with this large volume rotor shortens the separation time of plasmid DNA significantly from the single-step method and still allows for good band resolution (Figure 7). However, both protocols are efficient for plasmid DNA isolation.

References and Notes

1. K.Andersson, R.Hjorth, Plasmid, 13, 78-80 (1985).
2. Commercially available as “Cs-TFA” from Pharmacia LKB Biotechnology.
3. Separation may not succeed where treatment is performed using the boiling method (See Reference 1).

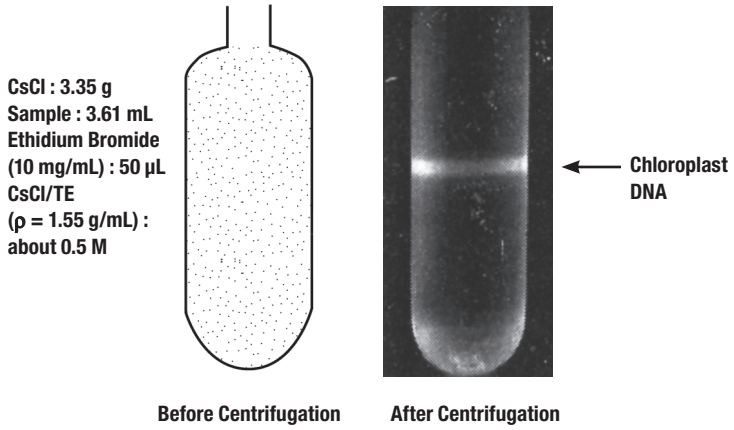


Figure 6. Chloroplast DNA Separation.

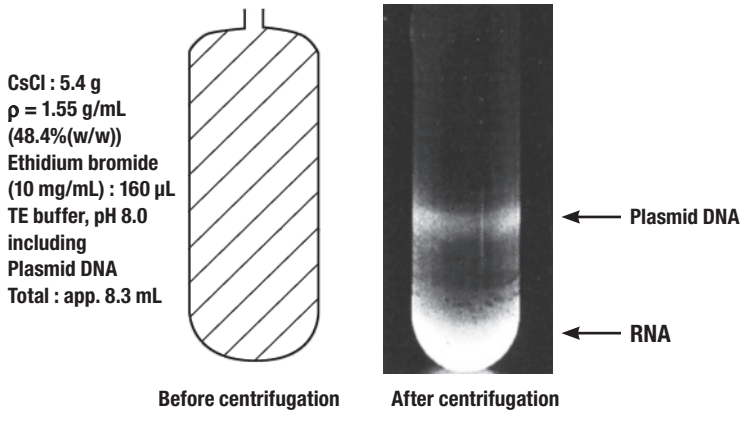


Figure 7. Results of Separation of Plasmid DNA.

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