HITACHI

himac APPLICATION

No. 62 April 1997

Subject Separation of chloroplast DNA

Model Preparative Micro-Ultracentrifuge CS-GX series
Fixed-angle rotor S100AT6

Separation of chloroplast DNA from spinach by fixed-angle rotor S100AT6

It is known chloroplast and mitochondria have own DNA distinct from chromosomal DNA. Here we tried to separate spinach chloroplast DNA by ultracentrifugation. As a result it took over night (16 hours) to separate by CsCl-density gradient ultracentrifugation using preparative micro-ultracentrifuge CS-GX series and fixed-angle rotor S100AT6 (holding 5ml tube).

Results

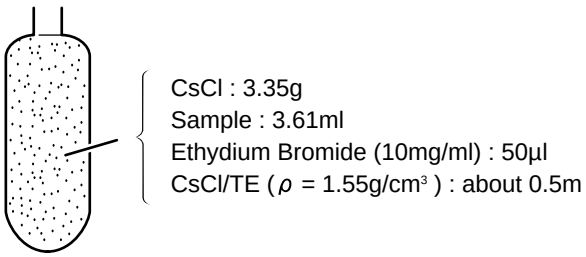

Fig.1 Before centrifugation

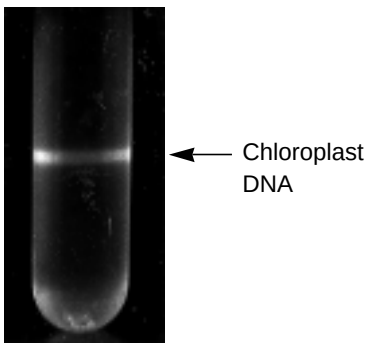

Fig.2 After centrifugation

Conditions

(1) Condition of ultracentrifugation

Rotor	Speed (rpm)	Time (h)	Temp. (˚C)	Acc. mode	Dec. mode
S100AT6 fixed-angle rotor	70,000	16	20	"9"	"7"

(2) Used tube : 5PA Re-seal tube*

*Registered trade mark of Seton Scientific Company.

(3) Sample preparation[1)]

5ml of chloroplast suspension (separated as described [2)])

↓ ← Add 5 volumes of buffer A.

centrifugation

↻↓ CF8DL refrigerated centrifuge, 2,500rpm, 15min, 4˚C, 50ml volume tube.

pellet

↓ ← Wash the pellet for 2 or 3 times with 20-30ml of buffer A.

washed pellet

↓ ← Resuspend 2ml of buffer A and add pronase.

incubation

↓ ← Incubate 37˚C, 2h and then room tepm. 2min.

mix

↓ ← Mix gently for 15min.

incubation

↓ ← Incubate 4˚C for 2-3 hours.

centrifugation

↻↓ CF8DL refrigerated centrifuge, 2,500rpm, 15min, 4˚C.

supernatant (crude DNA solution)

↓

ultracentrifugation

↻↓ Fixed-angle rotor S100AT6, 70,000rpm, 16h, 20˚C.

chloroplast DNA

Buffer A : 0.35M Sorbitol, 50mM Tris-HCl (pH8.0), 25mM EDTA-Na2.
Buffer B : 5% (w/w) Sodium N-lauroyl Sarcosinate, 50mM Tris-HCl (pH8.0), 25mM EDTA Na2.

Reference

1) METHODS IN ENZYMOLOGY, 118 , 167 - 186 (1986).
2) himac APPLICATION No.61 (1997).

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