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

| CsCl : 3.35g   |
| Sample : 3.61ml |
| Ethydiyum Bromide (10mg/ml) : 50µl |

\[
\text{CsCl/TE} \ (\rho = 1.55g/cm^3) : \text{about 0.5m}
\]

Fig.1 Before centrifugation

Chloroplast DNA

Fig.2 After centrifugation

Conditions
(1) Condition of ultracentrifugation

<table>
<thead>
<tr>
<th>Rotor</th>
<th>Speed (rpm)</th>
<th>Time (h)</th>
<th>Temp. (°C)</th>
<th>Acc. mode</th>
<th>Dec. mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>S100AT6 fixed-angle rotor</td>
<td>70,000</td>
<td>16</td>
<td>20</td>
<td>&quot;9&quot;</td>
<td>&quot;7&quot;</td>
</tr>
</tbody>
</table>

(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 \(^2\))

\[\rightarrow\] Add 5 volumes of buffer A.

\[\rightarrow\] centrifugation

\[\rightarrow\] CF8DL refrigerated centrifuge, 2,500rpm, 15min, 4˚C, 50ml volume tube.

\[\rightarrow\] pellet

\[\rightarrow\] Wash the pellet for 2 or 3 times with 20-30ml of buffer A.

\[\rightarrow\] washed pellet

\[\rightarrow\] Resuspend 2ml of buffer A and add pronase.

\[\rightarrow\] incubation

\[\rightarrow\] Incubate 37˚C, 2h and then room tepm. 2min.

\[\rightarrow\] mix

\[\rightarrow\] Mix gently for 15min.

\[\rightarrow\] incubation

\[\rightarrow\] Incubate 4˚C for 2-3 hours.

\[\rightarrow\] centrifugation

\[\rightarrow\] CF8DL refrigerated centrifuge, 2,500rpm, 15min, 4˚C.

\[\rightarrow\] supernatant (crude DNA solution)

\[\rightarrow\] ultracentrifugation

\[\rightarrow\] Fixed-angle rotor S100AT6, 70,000rpm, 16h, 20˚C.

\[\rightarrow\] 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 ENZYMOLGY, 118, 167 - 186 (1986).
2) himac APPLICATION No.61 (1997).