

## Isolation Of Plasmid DNA Using FIBERLite® F65L- 6 x 13.5 mL Rotor

Owen Mitch Griffith, Ph.D

Self-generating cesium chloride gradients form faster in fixed angle and vertical/near vertical tube rotors than in swinging bucket rotors. There may be various reasons why researchers prefer one rotor instead of another (e.g. available rotors, size of tube, tube angle orientation, rotor capacity). In previous studies, the above separation method has been performed using a variety of rotors, both fixed angle and vertical tube.(1) For this separation, a FIBERLite Centrifuge carbon fiber fixed angle rotor was preferred instead of the metallic vertical or near vertical tube rotor. Three main reasons below are identified for purifying of bacterial plasmids from cleared lysates with Carbon Fiber fixed angle rotors.

1. FIBERLite Centrifuge rotors are lightweight, accelerate faster, and have better temperature control during lengthy runs when compared to metallic rotors. They are also non-corroding when alkaline solutions are used in them.
2. At the end of the centrifugation process, the RNA pellet is confined to a small "button" at the bottom of the tube. In the vertical/near vertical tube rotors the RNA sediment spreads all along the centrifugal wall of the centrifuge tube which can contaminate the separated DNA zones in the gradient during solution reorientation.
3. The gradient in fixed angle rotors extends over a greater radial distance and encompasses a greater range of densities than either vertical or near vertical tube rotors. This means that the accuracy in making up a solution of predetermined density of cesium chloride can be relaxed in fixed angle rotors to some extent.

### Sample Preparation

A cleared lysate was prepared from a 500 ml culture of E.Coli carrying the pBR322 plasmid. For each 8.9 ml OptiSeal polycarbonate tube used for the centrifugation process, 6.1 g cesium chloride was added to 6.4 ml of cleared lysate. To this cesium chloride solution, 0.3 ml of 10mg/mL ethidium bromide was added. The final cesium chloride concentration was 1.55 g/mL (4.4 M).

### Centrifugation Conditions

<b>Technique</b>	Isopycnic Density gradient
<b>Ultracentrifuge</b>	Beckman L8-80M
<b>Rotor</b>	F65L 6 x 13.5mL
<b>Centrifuge Tubes</b>	OptiSeal polycarbonate (Beckman Part# 361623)
<b>Speed</b>	55,000 rpm
<b>Acceleration</b>	Rapid
<b>Deceleration</b>	Slow (Profile 2)
<b>Temperature</b>	20 °C
<b>Time</b>	12 - 14 hour



**FIBERLite F65L-6x13.5 mL  
rotor**

### Sample Recovery

After the centrifuge tubes were removed from the rotor, the separated DNA zones were visible by long wave UV light. The zones were removed by tube puncturing with a 22-gauge hypodermic needle and syringe. Figure 2 below shows the tube puncturing technique for recovery of the sample zone. The chromosomal DNA zone was found at a density of 1.53 g/mL (refractive index of 1.384) and the plasmid DNA zone was found at a density of 1.57 g/mL (refractive index of 1.387).

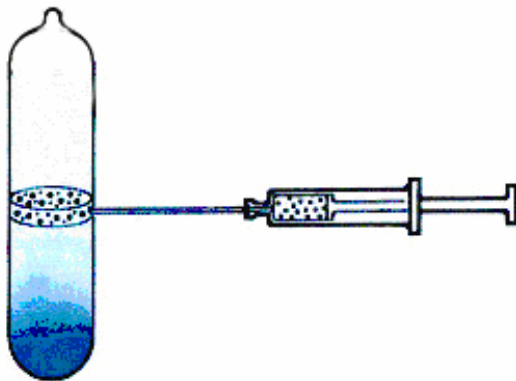


Figure 2 Sample Recovery After Centrifugation

### **For Further Information:**

Contact us at:

FIBERLite Centrifuge, Inc.  
422 Aldo Ave.  
Santa Clara, CA 95054  
408-988-1103 Tel  
408-988-1196 Fax  
[www.piramoon.com](http://www.piramoon.com)

### References:

1. Griffith, O.M. Density Gradient Separations in Vertical Tube, Near Vertical Tube, FIBERLite Fixed Angle and Swinging Bucket Rotors: A Comparative Study. Applications Article 11342. FIBERLite Piramoon Technologies, Santa Clara CA. 95054 (2007)