

## Isolation of Mini-Preps Of DNA Using Fiberlite<sup>®</sup> F21-48x1.5/2.0 mL Centrifuge Rotor

Owen Mitch Griffith, Ph.D

Small amounts of plasmid DNA (mini-preps) can be isolated from various individual bacterial colonies using the alkaline lysis method with FIBERLite<sup>®</sup> F21-48 x 1.5/2.0 ml tube rotor in the Universal Table Top centrifuge marketed by FIBERLite<sup>®</sup> Centrifuge Inc. This method was originally described by Birnboim and Doly(1) and later reported by Maniatis et al.(2)

These referenced reports indicate that the plasmids can be produced in sufficient quantities and purified by restriction endonuclease digestion, gel electrophoresis, Southern blotting or DNA sequencing. The individual bacterial colonies were identified by their ability to hybridize a given probe and can be rapidly characterized in more detail for subsequent procedures.

### Instrumentation

Micro Table Top Centrifuges have been used for many biological separations requiring low temperatures. In general, these instruments are not refrigerated and had to be placed in cold rooms to achieve the desired run temperature. In this study, the Universal Refrigerated Centrifuge marketed by FIBERLite<sup>®</sup> Centrifuge Inc. was used with the FIBERLite F21-48 x 1.5/2.0 mL carbon fiber rotor. (Fig. 1)

This rotor is capable of running 48 micro centrifuge tubes (1.5 mL). With these tubes the rotor will generate 26,000 x g at 15,000 rpm, the maximum rpm for the Universal Refrigerated centrifuge.

### Preparation and Procedure

1. Inoculate 2.5 mL of LB medium containing the appropriate antibiotic with a single bacterial colony. Incubate at 37° C overnight with vigorous shaking.
2. Transfer 1.5 mL of the culture into each 1.5 mL micro centrifuge. Centrifuge at 26,000 x g at 20° C for 3 min. Longer spin times will make it difficult to re-suspend the cells. Store the overnight culture at 4° C.
3. Completely re-suspend the pellet by vortexing in 100 ml of an ice-cold solution of 50 mM glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA, 4 mg/ml Lysozyme.
4. Store at room temperature for 5 min. The cap of the tube need not be closed during this period.
5. Add 200 ml of freshly prepared solution of 0.2 N NaOH, 1% SDS, to each tube. Close the tube, and rapidly mix the contents by inverting the tube rapidly two or three times. Do not vortex. Store on ice for 5 min.
6. Add 150 ml of ice-cold solution of potassium acetate, pH 4.8 (60 mL 5M K-acetate, 11.5 mL glacial acetic acid, 28.5 mL H<sub>2</sub>O). The solution is 3 M with respect to potassium and 5 M with respect to acetate. Close the cap of the tube and vortex it in an inverted position for 10 sec. Be sure mixing is complete. Store on ice for 5 min.
7. Centrifuge at maximum speed in the refrigerated Universal Centrifuge at 15,000 rpm /26,000 x g using the Micro-48 x 1.5 mL rotor for 10 min at 4° C. Transfer the supernatants to fresh tubes.



**Figure 1. Rotor Model: F21-48x1.5/2**





8. Add an equal volume of Tris-saturated phenol:chloroform (1:1) to deprotenize the mixture. Vortex. Centrifuge at 26,000 x g for 5 min at 4° C. Transfer the supernatants to fresh tubes.
  9. Add two volumes of ethanol at room temperature. Mix by vortexing. Keep the tube standing at room temperature for 2 min.
  10. Centrifuge at 26,000 x g / 15,000 rpm for 5 min at 20° C.
  11. Remove the supernatant. Place the tube in an inverted position on a paper towel to allow all of the fluid to drain away.
  12. Add 1 mL of 70% ethanol to wash the pelleted DNA. Centrifuge again at 26,000 x g / 15,000 rpm for 5 min at 20° C.
  13. Again remove the supernatant and vacuum dry the pelleted DNA.
  14. Re-suspend the pellet in 50 ml Tris - EDTA pH 8.0 (10 mM Tris-HCl, 1 mM EDTA), containing DNase - free RNase (20 mg/mL). Vortex briefly.
  15. Remove 10 ml of the mixture to new micro centrifuge tubes. Add 1.2 ml of the appropriate buffer and 1 unit of the desired restriction enzyme. Incubate for 1 - 2 at the appropriate temperature. Store the remainder of the solution at -20° C.
- Note: Mini-preps of plasmid DNA can be cleaved to completion with most restriction enzymes. However, batches of some enzymes do not work as well on mini-preps as on highly purified DNA. This problem can be avoided by increasing the amount of enzyme two-fold to three-fold and or by carrying out the digestion in larger reaction volumes (20 - 50 ml) so that any inhibitions of the DNA are diluted.*
16. Analyze the DNA fragments in the restriction digest by gel electrophoresis.

References:

1. Birnboim, H.C. and Doly, J. (1979), A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res.* 7: 1513
2. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

**For Further Information:**

Contact us at:

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