

Fast Recovery of Plasmid and Fosmid DNA in a 96-Well Plate for High-Throughput Sequencing

Dilara Begum and Fred Hyde, EPICENTRE Biotechnologies

Introduction

The speed, time, and quality of nucleic acid obtained are critical factors for any nucleic acid purification method, to ensure successful downstream applications. EPICENTRE's DirectLysis Plasmid96 DNA Purification Kit and DirectLysis Fosmid96 DNA Purification Kit were designed to expedite template preparation for high-throughput sequencing of multiple-sized libraries at 40 kb, 8-10 kb, and 3-5 kb. The DirectLysis protocol can be used to isolate DNA from plasmids (either single- or high-copy) up to 40 kb. The protocol uses direct lysis of an overnight culture and eliminates the need for centrifugation of the culture and resuspension of the pellet, thus speeding up the preparation and reducing cross-contamination. The optimized protocol requires only 200 μ l of culture, to which lysis buffer is added, followed by neutralization and clearing using a filter plate that does not bind DNA. The procedure has been successfully used to isolate and sequence DNA from metagenomic CopyControl™ fosmid libraries and high-copy pUC vector-based clones.

Methods and Results

Overview

A schematic overview of the DirectLysis 96 protocol for DNA isolation is presented in Figure 1. The protocol offers several advantages over conventional alkaline lysis-based methods:

- Direct lysis of overnight culture, without harvesting or resuspension of the cell pellet.
- Expedited preparation of sequencing templates in a 96-well format.
- Inoculation of culture in a 200- μ l volume eliminates the need for 2-ml, deep-well growth plates.
- DNA yield from 200 μ l of culture is sufficient for multiple applications, including restriction mapping, PCR, sequencing and *in vitro* transcription.

Quality of plasmid and fosmid DNA

Plasmid and fosmid DNA quality was evaluated by 1% agarose gel electrophoresis of both supercoiled DNA and by restriction endonuclease digestion with the enzyme *Not I* (fosmid DNA only). The high purity of the fosmid and plasmid DNA preparations are demonstrated in Figs. 2-4. The gel analysis shows high-quality small plasmid preparation with almost no host-cell chromosomal DNA and only a low level of nicked, open-circular DNA. The fosmid DNA purification also yielded relatively low levels of chromosomal DNA.

Sequencing of plasmid and fosmid DNA

Plasmid and fosmid DNAs were sequenced using ABI BigDye™ 3.1 reagents on an ABI PRISM® 3730 Sequencer. Sequencing of fosmids and plasmids purified with the DirectLysis Fosmid96 Kit and DirectLysis Plasmid96 Kit yielded excellent reads of at least 750 bases (Figs. 5 and 6) when using the sequencing primers described.

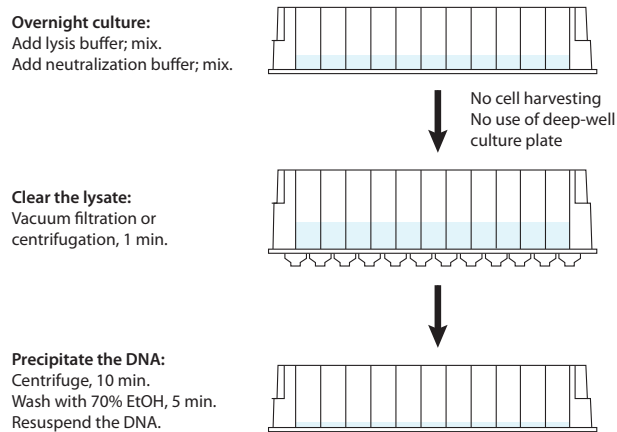


Figure 1. Overview of the Direct Lysis Plasmid/Fosmid 96 purification process.

Sequencing of fosmids and plasmids purified with the DirectLysis Fosmid96 Kit and DirectLysis Plasmid96 Kit yielded excellent reads of at least 750 bases.

Conclusions

The direct lysis procedure for plasmid and fosmid DNA provides a rapid and convenient method for 96-well DNA preparation. The DNA yield from 200 μ l of an autoinduced CopyControl™ 40-kb fosmid culture is sufficient for 3-4 pairs of end-sequencing reactions, and DNA isolated from a pUC-based clone provides enough DNA for 50 sequencing reactions. The sequencing reactions from both plasmid and fosmid result in average read lengths of 750 bases when analyzed on an ABI PRISM® 3730 96-well capillary sequencer.

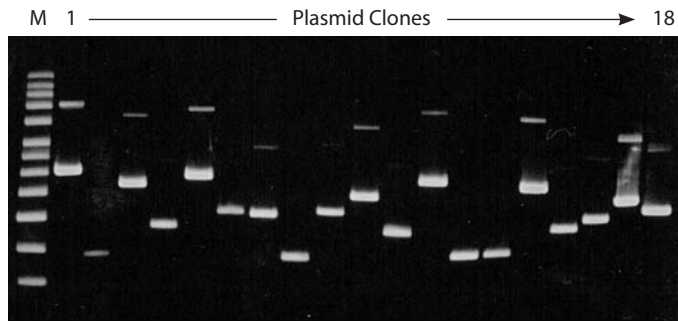


Figure 2. Examination of the quality of plasmid DNA. DNA was isolated from 200- μ l overnight cultures of randomly picked clones from a shotgun library (2-6 kb) in a 96-well plate using the DirectLysis Plasmid96 Kit. A 5- μ l aliquot of each 50- μ l prep was analyzed on a 1% agarose gel in 1X TAE buffer for 2 hours. Lane M, Supercoiled DNA Ladder; lanes 1-18, supercoiled plasmid DNA.

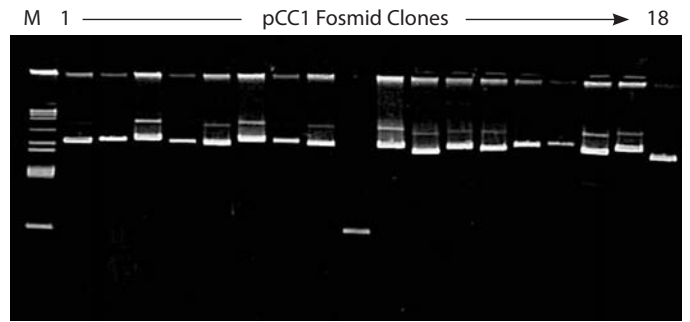


Figure 3. Examination of the quality of fosmid DNA. DNA was isolated from 200- μ l overnight, autoinduced cultures of randomly picked pCC1TM Fosmid clones from a metagenomic fosmid library in a 96-well plate using the DirectLysis Fosmid96 Kit. A 5- μ l aliquot of each 25- μ l prep was analyzed on a 1% agarose gel in 1X TAE buffer for 2 hours. Lane M, BAC Tracker Supercoiled DNA Ladder; lanes 1-18, supercoiled fosmid DNA.

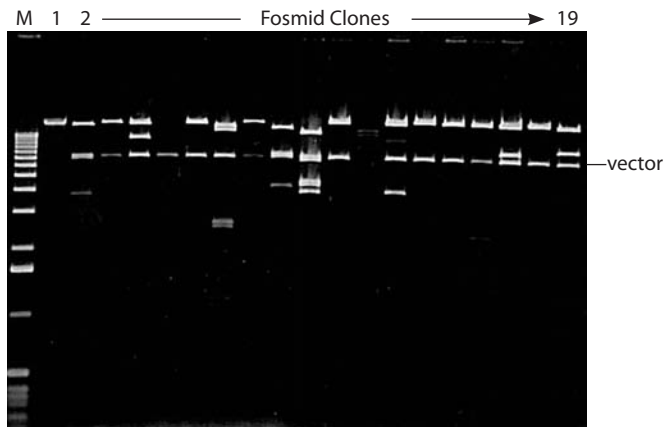


Figure 4. *Not* I digestion of fosmid DNA isolated from randomly chosen clones verifies a 40-kb insert size. Lane M, Kilobase ladder; lane 1, 40-kb Control Insert; lanes 2-19, *Not* I-digested fosmid DNA.

Cat. #	Quantity
Direct Lysis Fosmid96 DNA Purification Kit	
FOS84596	5 x 96-well preps
Direct Lysis Plasmid96 DNA Purification Kit	
PLS84596	5 x 96-well preps

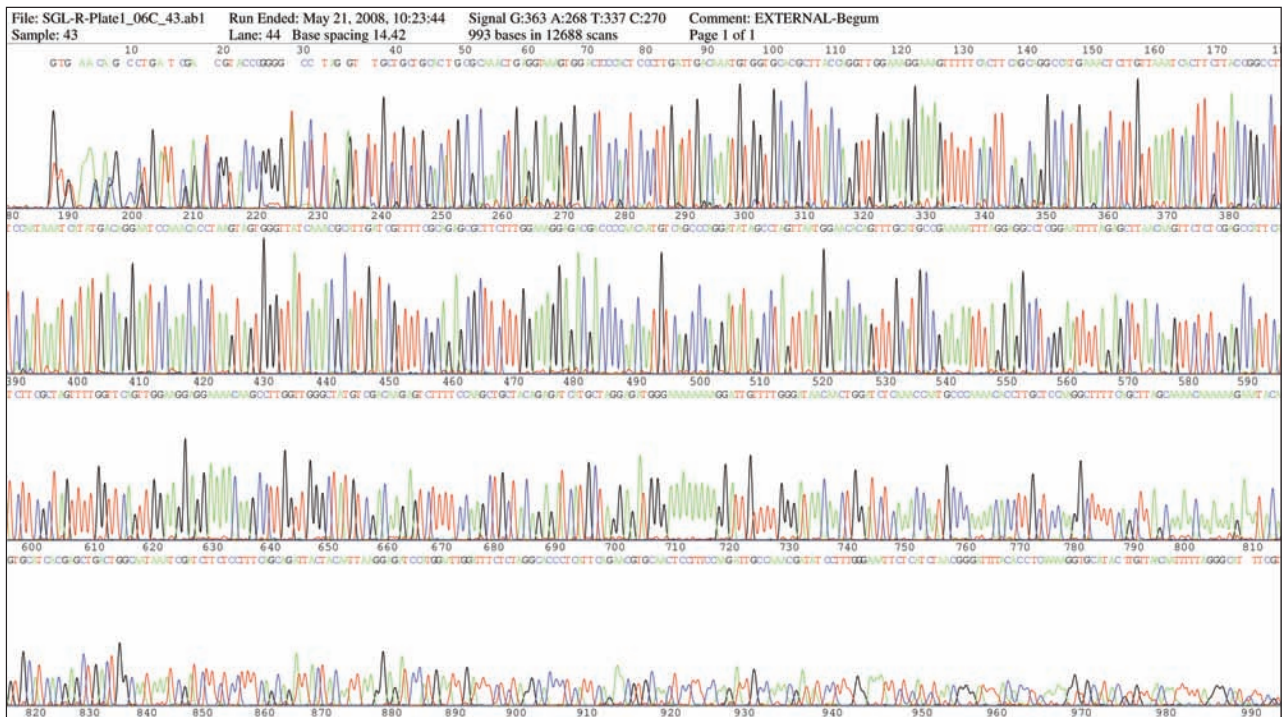


Figure 5. Sequence analysis confirms plasmid DNA quality. Sequencing was performed using 2 μ l of DNA isolated from a plasmid prep and M13 Forward primer, and was analyzed on an ABI PRISM[®] 3730 Sequencer in the UWBC DNA Sequencing and Synthesis Facility (Madison, WI).

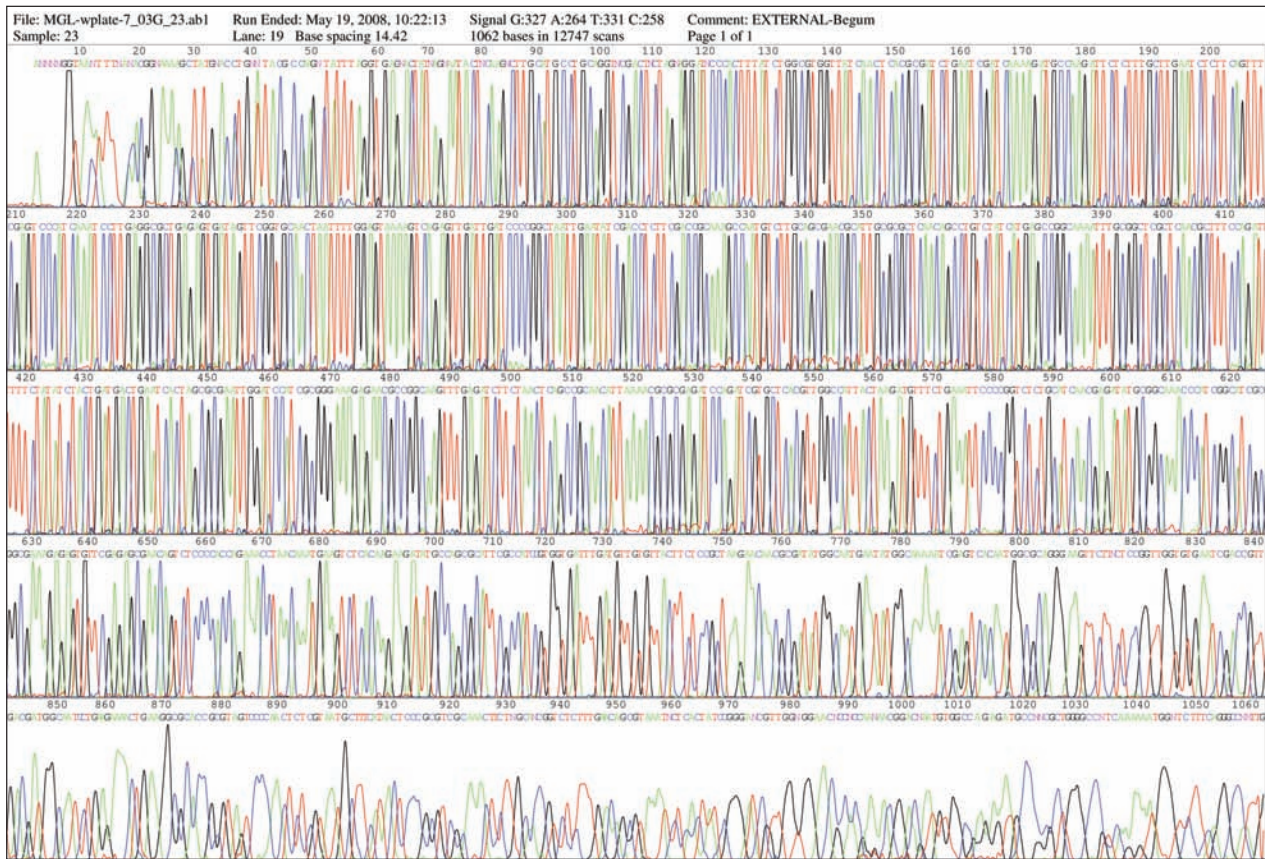


Figure 6. Sequence analysis confirms fosmid DNA quality. Sequencing was performed using 4 μ l of DNA isolated from an autoinduced pCC1 fosmid prep using pCC1Fos R primer, and analyzed on an ABI PRISM[®] 3730 Sequencer in the UWBC DNA Sequencing and Synthesis Facility (Madison, WI).

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