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

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Abstract

We introduce a new high-throughput protocol for fast isolation of plasmid and fosmid DNA, ideally suited for sequencing genomic libraries and other applications. The protocol can be used to isolate plasmid DNA sized up to 40 kb, from either high-copy or single-copy clones. T4 endonuclease uses a direct lysis of an overnight culture, and eliminates the need for centrifugation of the culture and resuspension of the pellet, steps which are cumbersome in high-throughput and automated formats. DNA isolation requires only 200 µl of culture, thus eliminating the need for expensive 2-ml deep-well culture plates. The protocol has been successfully used to isolate and sequence DNA from metagenomic fosmid libraries, as well as high-copy, pUC-based clones. Unlike cosmids, fosmids are maintained at single copy in an E. coli host. Using the CopyControl™ System, fosmids that contain the OriV origin of replication can be induced to a higher copy number per cell. The DNA yield from an induced CopyControl 40 kb fosmid is sufficient for six to eight sequencing reactions, whereas the DNA isolated from a high-copy pUC-based clone is sufficient for approximately 50 sequencing reactions. The purified DNA can also be used for restriction digestion.

Methods

Overview

A schematic overview of the protocol for DNA isolation is presented in Fig. 1. The protocol offers several advantages over current methods:

- Direct lysis of the overnight culture, without harvesting or resuspension of the cell pellet.
- The rapid protocol expedites preparation of sequencing templates in a 96-well plate.
- Inoculation of culture in a 200-µl volume eliminates the need for 2-ml, deep-well growth plates.
- DNA yield from 200 µl of culture is sufficient for multiple applications.
- DNA from a single preparation of a 40-kb fosmid is sufficient for six to eight sequencing reactions.

Results

2-6 Kbp Plasmid DNA

Fig. 2. Examination of the quality of plasmid DNA. DNA was isolated from 200-µl overnight cultures of randomly picked clones from a shotgun library (2-4 kb) in a 96 well plate using the DirectPlasmid DNA Kit. A 5-µl aliquot of each 50-µl prep was analyzed on a 1% agarose gel in 1X TAE buffer for 2 hours. Lane M, Supercoiled DNA Ladder; lanes 1-3, supercoiled plasmid DNA.

40 Kbp Fosmid DNA

Fig. 3. Examination of the quality of fosmid DNA. DNA was isolated from 200-µl overnight cultures of randomly picked pCC1 Fosmid clones from a metagenomic fosmid library in a 96 well plate using the DirectPlasmid DNA Kit. A 5-µl aliquot of each 50-µl 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.

Not I Restriction Digestion of Fosmid DNA

Fig. 4. Not I digestion of fosmid DNA isolated from randomly chosen clones verifies a 40-kb insert size. Lane M, 1-µl base ladder; lane 1, Not I control insert; lanes 2-29, Not I digested fosmid DNA.

Sequencing of Plasmid DNA

Not I digestion of plasmid DNA isolated from a single preparation verifies a 2-6 Kbp insert size. Sequencing was performed using 2 µl of DNA isolated from a plasmid and M13 forward primer, and analyzed on an ABI 3730 Sequencer in the UWBC DNA Sequencing and Synthesis Facility.

Sequencing of Fosmid DNA

Not I digestion of fosmid DNA isolated from an autoinduced culture of randomly picked clones verifies a 40-kb insert size. Sequencing was performed using 4 µl of DNA isolated from an autoinduced fosmid DNA. Sequencing was performed using pCCR10 primer, and analyzed on an ABI 3730 Sequencer in the UWBC DNA Sequencing and Synthesis Facility.

Conclusions

The direct lysis procedure for plasmid and fosmid DNA provides a rapid and convenient method for 96-well DNA preparation. It results in high yields that enable multiple sequencing reactions from a single preparation of a plasmid or a 40-kb fosmid from as little as 200 µl of culture. The resulting DNA is suitable for sequencing and restriction digests.