Lysate Direct PhyTip® Columns for Plasmid DNA Purification

Performance Features:

- No filtration or centrifugation of lysate required
- Consistent purification of up to 15 µg
- Transfection-ready, sequencing-ready
- 96 samples at a time or 12 at a time
- Suitable for downstream assays
- Fast and fully automated, easy-to-use system

Introduction

The pace of innovation from life science researchers is constantly pushing the limits of available tools. While technologies such as high throughput construct screening, crystallography and DNA sequencing have advanced, techniques for performing plasmid DNA minipreps have remained fairly unchanged. At the same time greater emphasis is being placed on utilizing automated liquid handling systems to adapt traditional plasmid DNA purification formats like filter plates and magnetic beads to higher throughput, but with limited success. The major challenge to these approaches lies in the complexity of the lysed sample. Current methods require removal of precipitates and debris from bacterial cell lysate prior to DNA binding step by centrifugation or vacuum filtration and 'careful transfer' (Fig. 1).

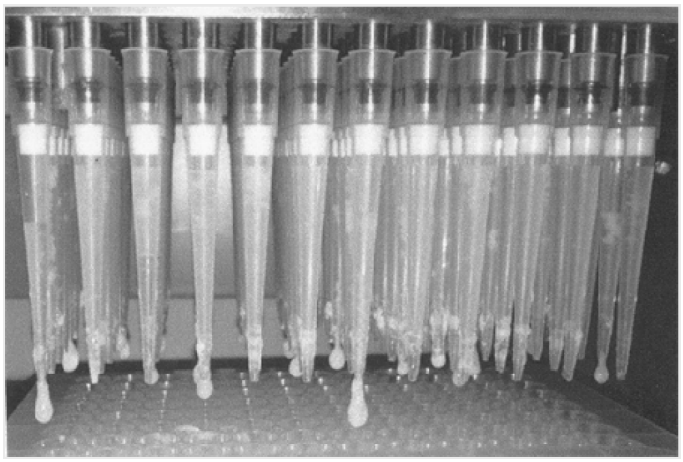


Figure 1. The problem with automated minipreps: Following manufacturer's procedures, a 96-well plate miniprep is carried out starting with resuspension, cell lysis and neutralization steps. Subsequent attempts at using liquid handling automation to transfer plasmidcontaining supernatant to magnetic beads or vacuum filter plate resulted in clogged pipette tips, inconsistent fluid transfer and transfer of precipitants.

Centrifugation is difficult to automate and requires human intervention. Filtration and transfer methods often result in clogged pipet tips, transfer of precipitates to filters, inconsistent fluid flow, and cross contamination between samples. Such obstacles to high throughput have made currently available methods for automating plasmid DNA minipreps less than satisfactory.

PhyNexus has developed a solution for obtaining reproducible automated plasmid DNA minipreps in a truly walk-away fashion. Lysate Direct PhyTip® Column technology consists of a purification column in a pipet tip format. These novel purification columns are composed of a pipet tip containing plasmid DNA binding resin and a thin, inert frit screen at the end of the tip to retain the resin (Fig. 2A).

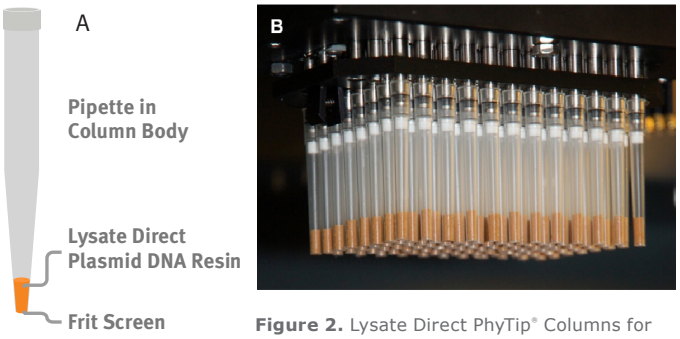


Figure 2. Lysate Direct PhyTip® Columns for Plasmid DNA Purification

The unique design of the column allows for DNA binding directly from cell lysates in the presence of precipitates and debris without sample clearing. By eliminating the need for centrifugation or filtration, Lysate Direct PhyTip® Columns for Plasmid DNA Purification enable utilization of liquid handing automation found in high throughput laboratories to easily purify up to 96 samples in parallel (Fig. 2B).

Biotage®

Methods

E. coli cells were harvested by centrifugation and bacterial cell pellets were resuspended, lysed, and precipitated by mixing using standard pipet tips (Fig. 3). Following the precipitation step, the plasmid DNA was immediately captured on Lysate Direct PhyTip® Columns directly from the cell lysates. Twelve cycles of back and forth flow were performed to allow binding of plasmid DNA to resin in the presence of precipitants. Columns were washed with three separate aliquots of wash buffer using two cycles of back and forth flow each. Columns were air dried on a vacuum manifold. Lastly, plasmid DNA was eluted from the PhyTip® column with one cycle of back and forth flow.

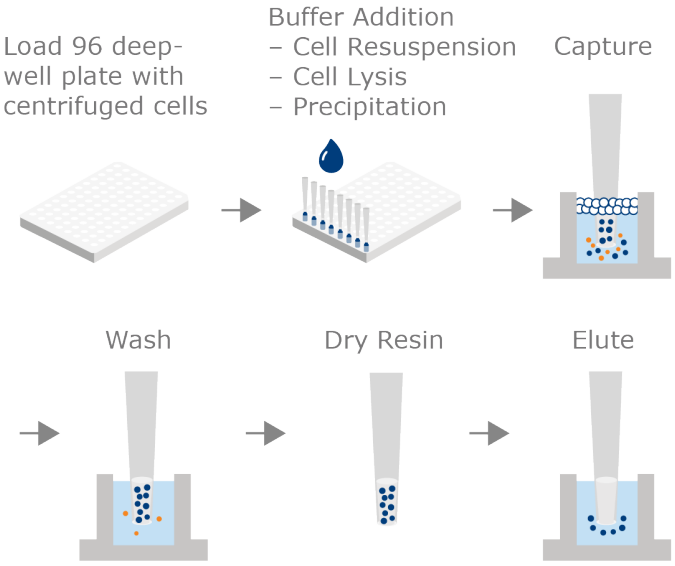
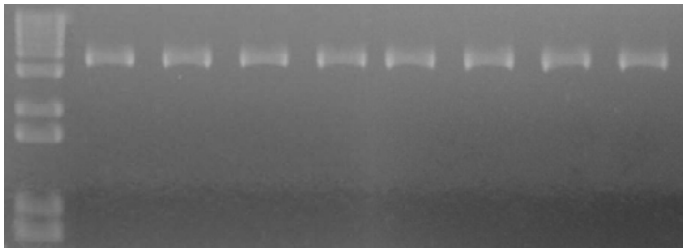


Figure 3. Schematic of sample processing with Lysate Direct PhyTip® columns. Buffers are pre-aliquoted into 96-well blocks for processing. Liquid handling instrumentation is used to move columns from position to position and perform pipetting functions.

Results

Lysate Direct PhyTip® Columns produce consistent purity and high yields. Eight cultures were inoculated with single bacterial colonies transformed with pCR4-TOPO® plasmid. Cultures were grown for 16 hours with shaking at 37 ºC. The cultures were processed as described and analyzed by UV spectroscopy and agarose gel electrophoresis (Fig. 4). Yields were consistent, averaging 19 µg recovered with 6% CV. DNA was visualized on an agarose gel and purity measured by A260/A280 and A260/A230 ratios, which were in acceptable ranges.



Sample	Yield	Conc	A260/A280	A260/A230
1	18.5	201	1.97	2.12
2	19.4	208	1.98	2.02
3	18.5	204	1.95	1.97
4	16.5	188	1.97	2.12
5	17.7	195	1.95	2.00
6	19.1	206	1.94	1.96
7	19.5	206	1.96	1.96

Figure 4. Analysis of plasmid DNA purified with Lysate Direct PhyTip® Columns. DNA quality was qualitatively assessed by agarose gel analysis of non-linearized plasmid (pCR4-TOPO) purified using Lysate Direct PhyTip® Columns. Purified samples were run on a 2% agarose gel and stained with ethidium bromide. Lanes 2-9: 1 µL loaded from eight independent purifications. Lane 1:400 ng of 1 Kb Plus ladder.

DNA quality is suitable for DNA sequencing.

Plasmid DNA purified with Lysate Direct PhyTip® Columns was sent to Genscript for sequencing using the ABI 3730xl instrument. Good quality sequence data was obtained to 600 base pairs (Fig. 5).

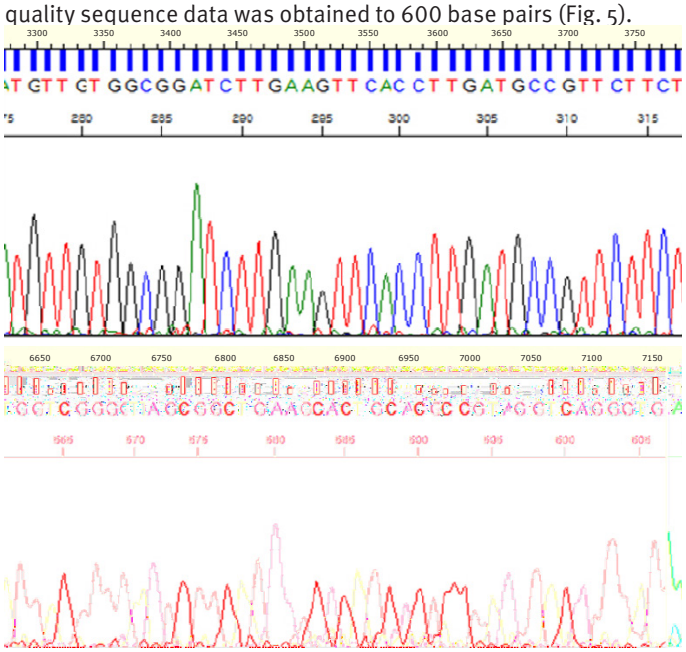


Figure 5. Sequencing Analysis with Lysate Direct PhyTip® Column purification method. Plasmid DNA purified with Lysate Direct PhyTip® Columns was sequenced by an ABI 3730xl instrument. Sequence was analyzed using Sequence Scanner version 1.0 software resulting in a 784 continuous read length with 754 of the bases with QV ≥ 20 was obtained. (Left) Sequence peaks from 275 to 336 bases are shown. The sequence peaks are very sharp with minimal background noise peaks. (Right) Sequence peaks from 560 to 606 bases. The peaks are still very sharp with slight increased background noise peaks which does not interfere with sequence read.

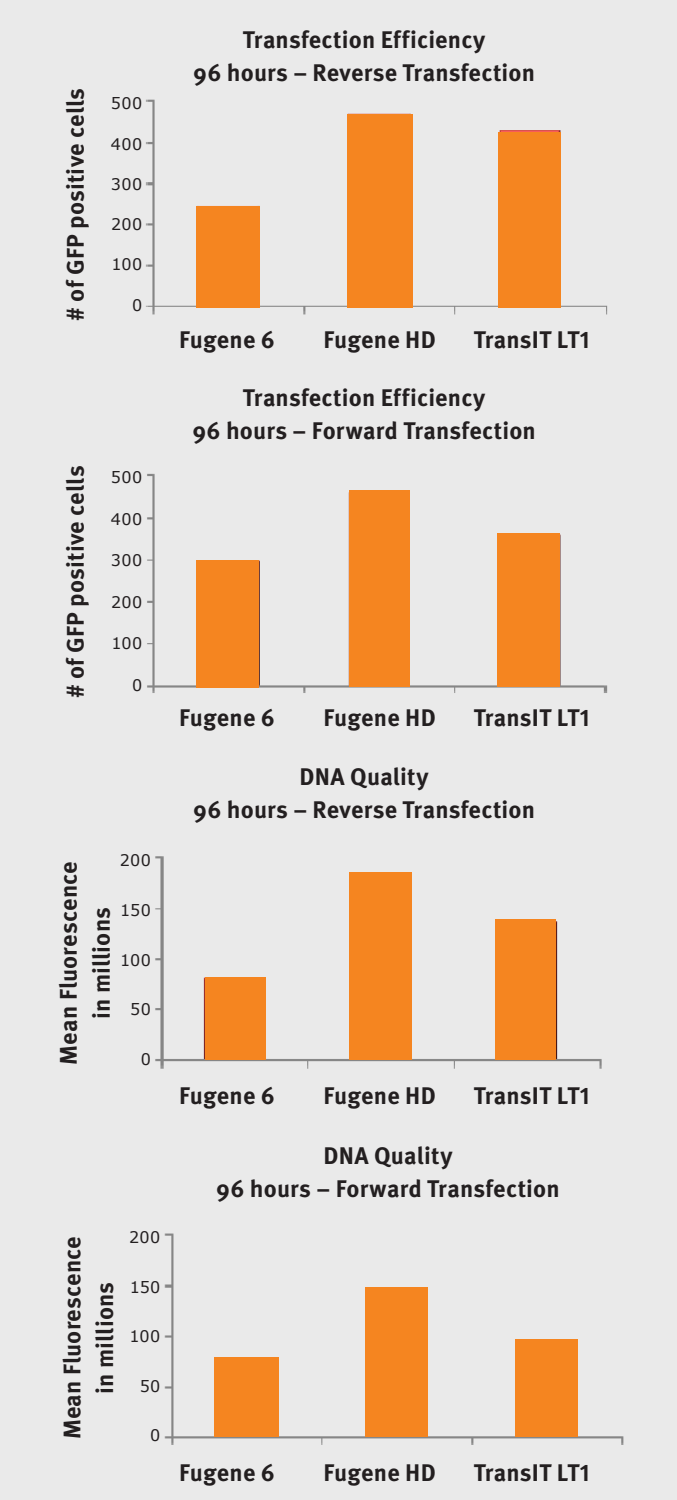


Figure 6. Transfection Efficiency Analysis of DNA from the Lysate Direct PhyTip® Column purification method. 50 ng of plasmid DNA was transfected to COS7 cells using three different transfection reagents (Fugene 6, Fugene HD, and TransIT LT1). Transfections were carried out as per manufacturers' suggested protocols. Ninety-six hours after transfection, GFP positive cells were counted and mean fluorescence calculated for each method using IncuCyte instrument.

****Data courtesy of K.** Billeci

The quality of purified plasmid DNA is transfection grade.

Plasmid DNA encoding GFP was purified using Lysate Direct PhyTip® columns and then tested for transfection efficiency. The quality of the purified plasmid was demonstrated by transfection into COS7 cells using three different transfection reagents (Fig. 6). Additionally, a DNA Quality assay was performed using the resulting expression of GFP.

Conclusion

Currently available systems for automated plasmid DNA purification are not reliable or reproducible due to the inherent issues with clearing the complex sample present after lysis and neutralization of bacterial cells. Automated methods rely on removing the cell debris, precipitated proteins, and genomic DNA away from the supernatant prior to purification. However, these particulates cannot be removed effectively without a centrifugation step, therefore requiring manual intervention. Even when careful pipetting and vacuum filter plate methods are used in lieu of centrifugation, other problems that arise as discussed in the introduction and Figure 1. Lysate Direct PhyTip® Columns provide an alternative, fully automated, high throughput solution for performing rapid DNA minipreps. Plasmid DNA is captured on to the resin at the end of the PhyTip® columns without the need for removal of debris and precipitates. Thus, no centrifugation step is required and the entire method is easily adapted for complete walk-away automated workflow. Using this method, ~19 µg of clean plasmid DNA was reproducibly purified and shown to be compatible with sequencing and mammalian transfection. Lysate Direct PhyTip® Columns for Plasmid DNA Purification are available for use with several mainstream robotic systems including Tecan, Hamilton, Beckman, Perkin Elmer, and Dynamic Devices.

Literature Number: AN137

Biotage®