Efficient Automated Extraction of Plasmid DNA in Medium Throughput

Authors: Sebastien Lambertucci[1], Nans Bodet[2], Jane Paget[2], Chris Hirst[1], Franziska Hausig-Punke[3], Andreas Kronberg[3] and Debra Conway[1]

[1]Analytik Jena UK Ltd., Translation & Innovation Hub Imperial College White City Campus, 84 Wood Lane, London W12 0BZ, Great Britain
[2]Promega France, 24 Chem. des Verrières, 69260 Charbonnières-les-Bains, France
[3]Analytik Jena GmbH+Co. KG, Konrad-Zuse-Str. 1, 07745 Jena, Germany

MAIN LEARNINGS

Purified plasmid DNA is required for a broad range of downstream applications. Analytik Jena's liquid handler CyBio FeliX can be used to isolate transfection-quality plasmid DNA using a customized protocol for Promega's Wizard MagneSil Tfx™ system. The fully automated workflow can process 96 samples in parallel and does not require centrifugation after removal of the cell pellets. Automation enables simple and fast results without compromising plasmid DNA yield and purity. This study highlights the effectiveness of the Promega MagneSil Tfx™ System and Analytik Jena's CyBio FeliX in the simple, rapid, and reliable isolation of transfection-quality plasmid DNA in a 96-well high-throughput format.

INTRODUCTION

The isolation of plasmid DNA is an essential molecular biology technique for many downstream applications. The multistep protocol is simple, but labor intensive and error prone. The Analytik Jena CyBio FeliX alleviates the need for manual pipetting and greatly speeds up the protocol's processing time while reducing variability. The use of Promega MagneSil® Paramagnetic Particles for lysate purification and DNA capture bypasses the need for centrifugation or a vacuum manifold, allowing DNA purification to be fully automated with the Promega Wizard MagneSil Tfx™ System on the Analytik Jena CyBio FeliX. In this method, after harvesting and alkaline lysis of bacterial cells, magnetic beads are used to clarify the lysate and separate plasmid DNA. Finally, plasmid DNA is present in nuclease-free water and can be used for transfection, cloning or other molecular biology methods.

METHODS & RESULTS

JM109 *E. coli* bacteria were transformed with pGL4.50 and cultured overnight in Terrific Broth Medium (TB) or Luria Broth Medium (LB). Bacterial cells from cultures were pelleted and the supernatent was discarded. Working plates with the plasmid extraction kit components were prepared and loaded onto the CyBio FeliX deck together with the required consumables according to the programmed automation script (scan QR-code to view the detailed Application Note).

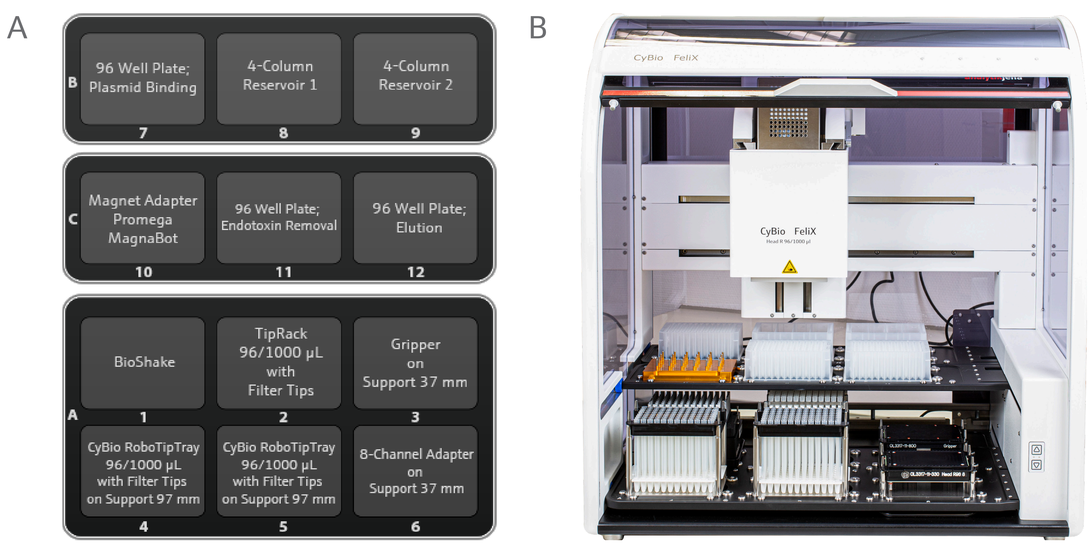

Figure 1 | Deck layout for plasmid DNA purification on the CyBio FeliX liquid handler using the Wizard MagneSil Tfx™ System. Reagents are dispensed by the instrument from 4-column reservoirs, with the exception of Endotoxin Removal Resin and Elution Buffer which are pre-dispensed manually in the indicated deep well plates. (A) Reagent, labware, consumable and accessory positions for implementing the Wizard MagneSil Tfx™ System kit on the CyBio FeliX liquid handler.
(B) CyBio FeliX instrument (Analytik Jena) with the 'CyBio FeliX Extraction Set', the MagnaBot® FLEX 96 Magnetic Separation Device (Promega) and Bioshake 3000-T elm (QInstruments) mounted with a Nunc® 2.0 mL heat plate adapter (QInstruments).

Subsequent extraction was done automatically. Repeated purifications were performed (n=23). Concentrations and absorbance ratios (A260/A280 and A260/A230) were determined by spectrophotometry (Figure 2A-B). Plasmid size was visualized by gel electrophoresis (Figure 2C).

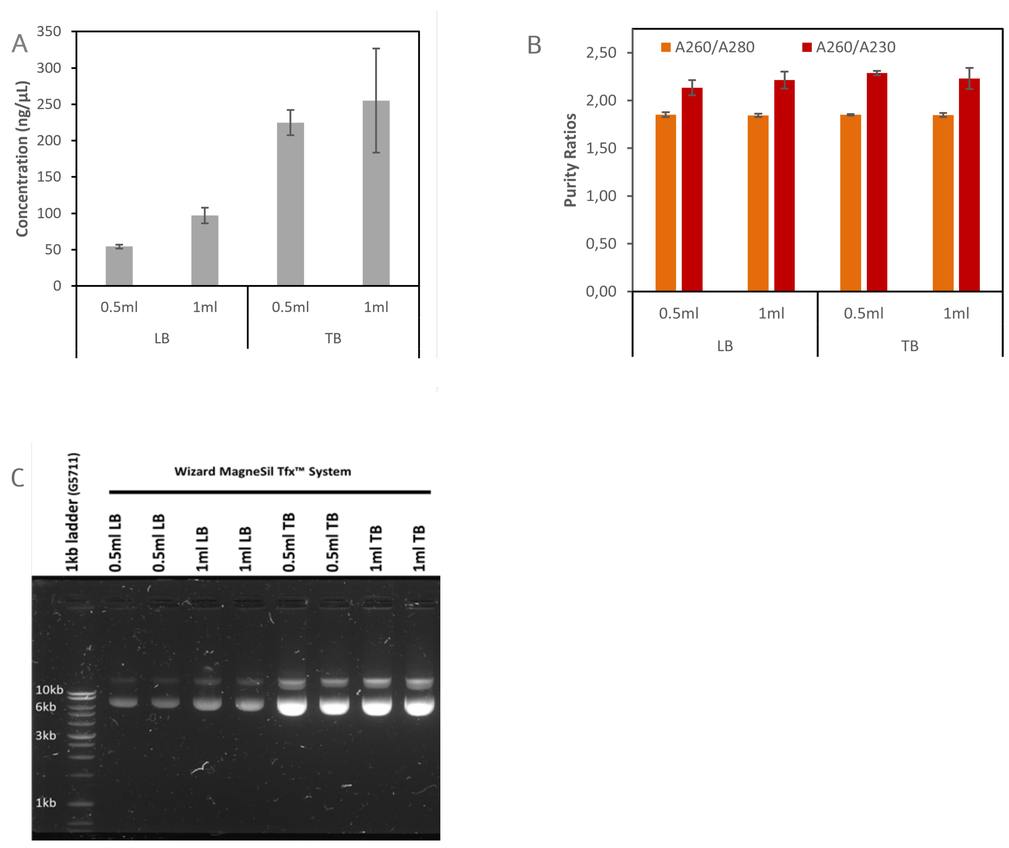

Figure 2 | Concentrations, purity and agarose gel electrophoresis of plasmid DNA purified from bacteria using the Wizard MagneSil Tfx™ System on the CyBio FeliX liquid handler. Plasmid DNA was purified from both 0.5 mL and 1 mL overnight cultures in either TB or LB medium. (A) Plasmid DNA concentrations and (B) A260/A280 and A260/A230 absorbance ratios were measured spectrometically. The average values ± standard deviations of n=23 replicate extractions are shown. (C) 10 µL of purified plasmid (0.5 mL or 1 mL of overnight bacterial cultures in either LB or TB medium, as indicated) were analyzed using a 0.8% agarose gel. A 1kb DNA Ladder was included as a size standard.

Plasmid DNA was successfully purified from both 0.5 mL and 1 mL LB and TB bacterial cultures, respectively. As expected, extraction from the TB culture resulted in a higher plasmid yield, which can be attributed to the higher density of the overnight culture. The absorbance-purity ratio and gel electrophoresis showed that the purified plasmid DNA was of high quality and purity.

CONCLUSION

In this study, we present an optimized approach for plasmid DNA extraction using Promega's Wizard MagneSil TfxTM paramagnetic particles and Analytik Jena's CyBio FeliX liquid handler to create a fully automated protocol for medium-throughput screening applications. The Analytik Jena CyBio FeliX automates the simple and rapid isolation of transfection-quality plasmid DNA using the Promega MagneSil Tfx™ System in a high-throughput 96-well format without compromising plasmid DNA yield and purity. The automated workflow is centrifugation-free after cell pellet removal.

For more information check out the detailed Application Note.

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