

**ZYMO RESEARCH***The Beauty of Science is to Make Things Simple®*

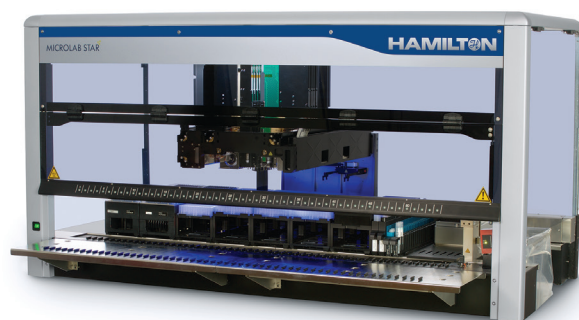
Automated Pellet-Free Plasmid Purification on the Hamilton Microlab® STAR™

High-throughput, magnetic bead-based plasmid DNA purification of high quality endotoxin-free DNA using the Zyppy™-96 Plasmid MagBead Miniprep.

Introduction

The success of plasmid DNA extraction can be highly variable, differing from one manual operator to the next and typically requires long centrifugation times. This is a time-consuming procedure and increases the risk for low quality DNA. The Zyppy™ procedure allows for high-throughput automation and requires no centrifugation or pelleting of cells. The technology features a modified alkaline lysis system that allows for the direct lysis of *E. coli*.

Sample variability is greatly reduced by using the Microlab STAR and the resulting endotoxin-free and high quality DNA is ready for restriction endonuclease digestion, ligation, PCR, transformation, sequencing, etc.



Materials and Methods

E. coli culture was grown overnight at 37 °C. After culturing, 675 µl of cells were used as input samples for plasmid DNA extraction using Zymo Zyppy-96 Plasmid MagBead Miniprep (Cat. No. D4100). Twenty-four cell cultured samples were processed manually and another 24 samples were processed using the Microlab STAR.

The Microlab STAR used was configured with 8 Independent Pipetting Channels, Autoload (optional), CO-RE® 96 Multi-Probe Head (MPH), CO-RE Grippers, Hamilton Heater Shaker (HHS), ZR-96 MagStand (Cat. No. P1005), and all required tips and reagent carriers.

The DNA concentration was analyzed using Thermo Scientific NanoDrop™ 2000 UV-Vis Spectrophotometer. The uniquely formulated Deep Blue Lysis Buffer was added directly to bacterial cultures with no centrifugation necessary. After neutralization, lysate was cleared using Mag-Clearing Beads. The supernatant was extracted by the CO-RE 96 MPH using capacitance Liquid Level Detection (cLLD) to ensure no cellular debris was transferred. Mag-Binding Beads were added and the DNA-bound beads were washed, dried, and eluted.

Purified plasmid DNA (pGEM-3Zf(+)) was digested with HindIII for one hour at 37 °C. Both undigested and digested samples were separated in a 1.0% agarose gel (Figure 4, page 3).

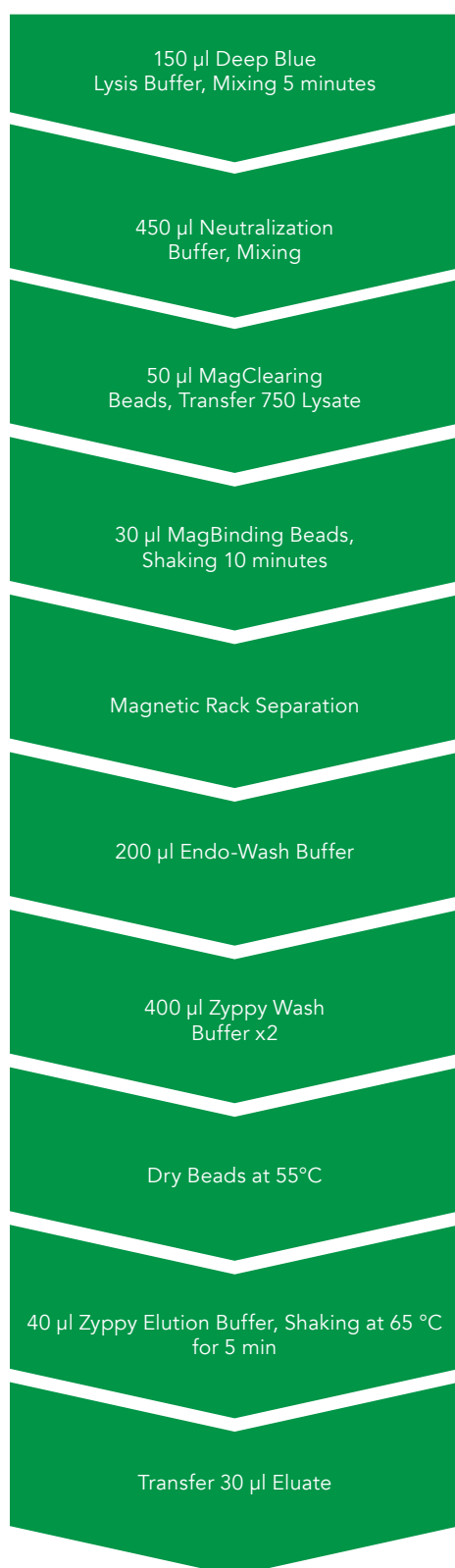


Figure 1: Zyppy-96 Plasmid MagBead Miniprep workflow.

Results and Discussion

High Yields and Quality with Automation

DNA concentration, recovered volume, and yields from replicate DNA samples were compared between 24 manually processed samples and 24 automated processed samples. The results in Figure 2 indicate that automation has improved sample yield and higher recovery concentration than that of manual processing. The automated processed samples have an average yield 50.86 ± 9.04 ng and the manual processed samples an average yield 43.55 ± 4.40 ng.

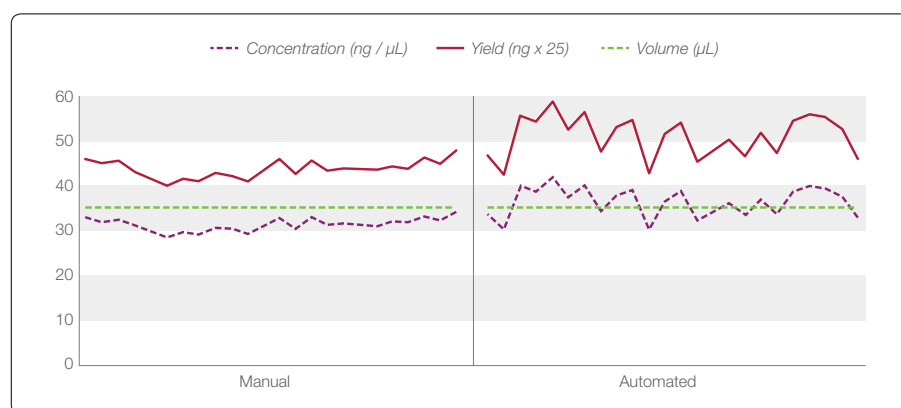


Figure 2: Comparison between manual and automated (Microlab STAR) sample processing.

Improved Purity with Automation

DNA purity was compared between 24 manually processed samples and 24 automated processed samples using absorption ratio of A260/280. The automated processed samples have an average purity (A260/280) of 1.85 ± 0.04 and the manually processed samples an average purity (A260/280) of 1.78 ± 1.12 .

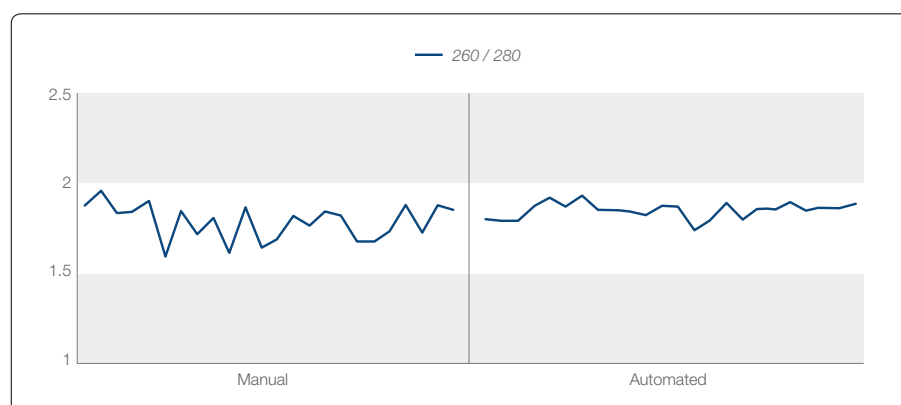


Figure 3: Purity comparison between manual and automated (Microlab STAR) sample processing.

Plasmid Size Confirmation

The undigested samples show super-coiled plasmid, while the digested samples show the linearized 3,197 bp fragment, single band in each digested lane.

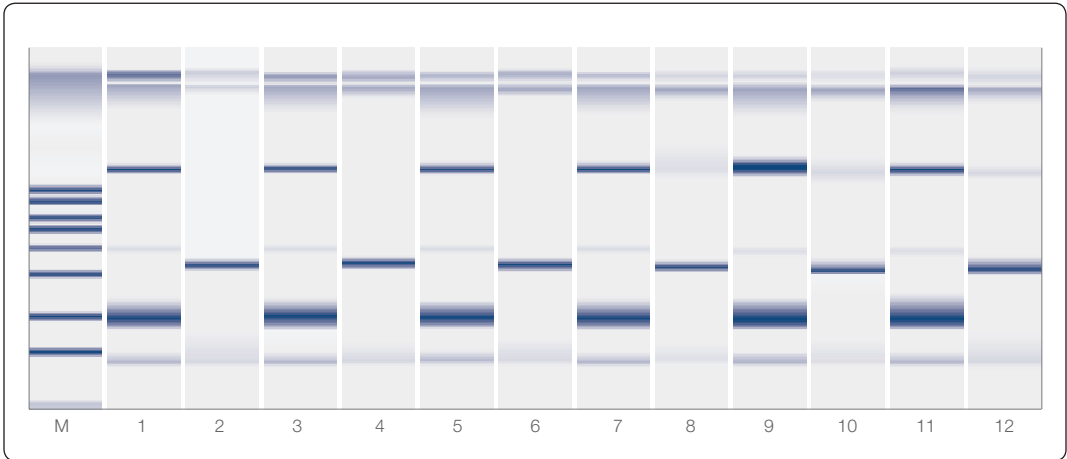


Figure 4: Restriction endonuclease digestion of plasmid DNA. Both undigested (odd lanes) and digested (even lanes) samples were separated in a 1.0% agarose gel.

Conclusions

Samples processed using the Zyppy-96 Plasmid MagBead Miniprep with the Microlab STAR exhibit better performance compared to manual pipetting techniques and methods. This is shown by the consistently higher yield and purity in automation, eliminating any sample deviation by human operator error and greatly reducing variability. This pellet-free extraction yields high-quality endotoxin-free DNA for an easy and straightforward high-throughput plasmid purification.

Product	Cat. No.	Size
Zyppy-96 Plasmid MagBead Miniprep	D4100	2 x 96 preps
	D4101	4 x 96 preps
	D4102	8 x 96 preps