Automated CosMCPrep Plasmid Preparation Kit on the Biomek i7 Hybrid Workstation

Kelly Marshall[1], Frank Lawrence[1], Sudha Savant[1],

[1] Beckman Coulter Life Sciences, Indianapolis, IN.

Introduction

Synthetic biology is a rapidly growing, multidisciplinary field of biotechnology which addresses challenges in areas of agriculture, medicine, and environmental conservation. It utilizes principles of genetic engineering to modify or create genetic material in different organisms. Liquid handling systems minimize human errors, reduce hands-on time, and increase throughput and reproducibility. Molecular cloning can benefit from utilizing these high-throughput platforms by making gene assembly more efficient for applications such as genetically modified crops, antibody discovery, proteomics, and oligo/gene synthesis. Plasmid purification is an integral part of the clone screening process.

The plasmids purified using this workflow can be used for downstream applications such as sequencing, cloning, transformations and transfections.

Advantages of automation on Biomek i7 Hybrid Workstation with paramagnetic SPRI bead-based reagents

- 1200µL Multichannel head with 1-1000µL pipetting capability allows for quick washing and transfer of sample
- Span-8 pod with disposable tips allows for flexible reagent addition and accessing 24- and 96-well labware
- Independent 360° rotating gripper with offset fingers moves plates around deck and onto integrated BioShake
- Guided Labware Setup (GLS) shows graphically where to place labware and what volumes are required in each labware
- Integrated BioShake allows for elimination of tip mixing
- Use of paramagnetic reagents based on SPRI technology for DNA binding eliminates need for vacuum filtration, can be scaled easily and is adapted to variable input volumes
- Minimized human errors and consistently reproducible results

Materials and Methods

The CosMCPrep Kit enables a plasmid purification procedure based on SPRI paramagnetic bead-based technology. It uses a single protocol to purify a variety of high and low copy number templates while maintaining the flexibility to support both manual and automated processing. Table 1 provides times for the different processing steps for the automated method.

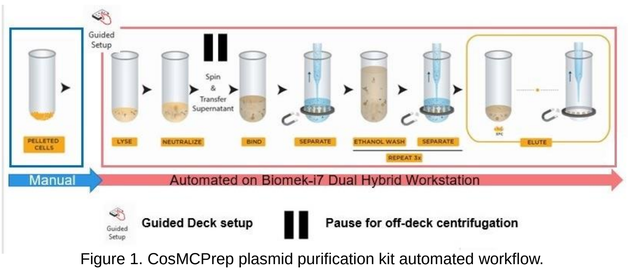

Figure 1. CosMCPrep plasmid purification kit automated workflow.

Here we demonstrate data from two extraction experiments. The first is a 96-sample automated plasmid purification starting with 1 mL of culture. For this demonstration we grew three different types of plasmids carrying bacteria overnight with shaking at 37°C. Each of the bacterial cultures was split into four columns of the plate.

To demonstrate scalability of the reagents for varying input volumes, we processed 48 samples (2 sets of 24) starting with 1, 2, 5 and 10 mL cultures. The 1 mL and 2 mL cultures were processed based on the CosMCPrep standard protocol. The 5 mL and 10 mL cultures were processed using scaled-up volumes of the CosMCPrep reagents.

1 and 2 mL cultures were pelleted and processed in 96-well plates. The 5 and 10mL cultures were pelleted in 24-well plates and then resuspended and transferred to a 96 well plate with the Span-8 pod for processing.

Kit Type	CosMCPrep Plasmid Purification Kit	
Sample Number	**24**	**96**
Instrument Setup Time	10 mins	10 mins
(a) Pre-centrifugation (until neutralization)	5 mins	10 mins
(b) Post-neutralization processing	19 mins	36 mins
Method Run Time (a+b)	24 mins	46 mins
Total Time* (with off-deck centrifuge)	54 mins	1 hour 16 mins

*Total timing estimates includes 20 min centrifugation at 3000 x g.

Table 1. Estimated run times for purifying 24 or 96 plasmid preps with the CosMCPrep Plasmid Purification kit on the Biomek i7 Hybrid Automated Workstation. .

Results

For the first experiment, the average yields of the 3 different plasmid types were 10.38 µg, 24.95 µg and 29.04 µg. The average A260/280 across 96 samples was 1.85 and for A260/230 was 2.3 (Fig 2 A-C). There were no plate-edge or column effects observed in any of the runs (Fig 2 D).

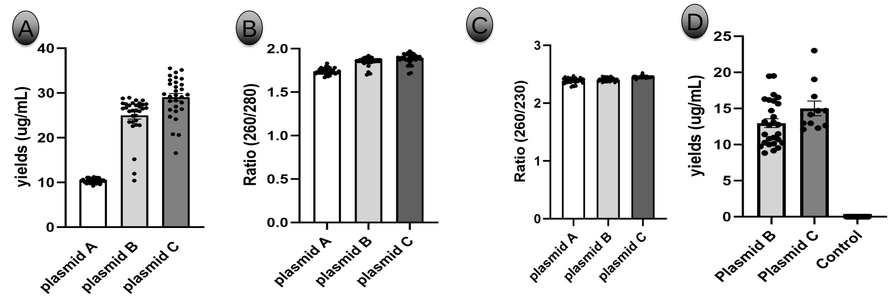

Figure 2. QC for 96 plasmids purified by automation. (A) Yields in µg/mL for the 32 replicates of each Plasmid A, Plasmid B and Plasmid C. (B) 260/280 ratios and (C) 260/230 ratios for the 32 replicates of each Plasmid A, Plasmid B and Plasmid C. (D) Yields in µg/mL for the 32 replicates of Plasmid B, 16 samples of Plasmid C and 48 of the negative control.

For the second experiment, the results of a scale-up show the 1 mL culture had yield of 5.7, 260/280 of 1.8 and A260/230 of 2.2. The 2 mL culture had yield of 11.6, 260/280 of 1.9 and a 260/230 of 2.2. The 5 mL culture had yield of 46.3, 260/280 of 2.0 and a 260/230 of 2.3. The 10 mL culture had yield of 125.4, 260/280 of 2.0 and a 260/230 of 2.4.

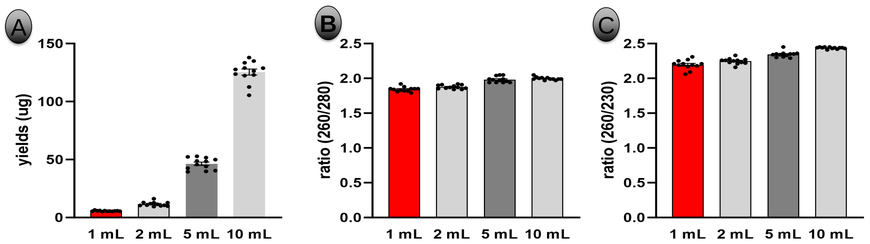

Figure 3. QC for 48 plasmids purified by automation. (A) Yields in µg/mL for the 12 replicates of each 1, 2, 5, and 10 mL Culture. (B) 260/280 ratios and (C) 260/230 ratios for the 12 replicates of 1, 2, 5, and 10 mL Culture.

Conclusion

In summary, we have demonstrated automated plasmid purification on the Biomek i7 workstation which can be easily scaled to sample input volumes. The automated method increases reproducibility of the assays and reduces user hands-on time and the chance for handling errors.

2025-GBL-EN-107193-v1