The EMnetik System Enables Fast and Efficient DNA Purifications for Molecular Cloning Workflows

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INTRODUCTION

The EMnetik system is a semi-automated system for bead-based DNA cleanups and plasmid purifications. It's capable of processing 24 samples simultaneously and offers step-by-step on-screen guidance, negates the need to move samples on and off the manifold, and provides the consistency offered by automated mixing and separation.

Two gene assembly methods were used to prepare plasmids for genome editing. As part of this process, the EMnetik system was used to perform PCR cleanups and plasmid purifications alongside traditional column-based methods for comparison.

MATERIALS AND METHODS

Plasmids were assembled via GeneArt™ Gibson Assembly and GeneArt™ Type IIs Golden Gate Assembly. EMnetik PCR Cleanup kit and spin-column kit were used for DNA purifications and constructs were assessed for yield and purity via the NanodropTM. After transformations, selected colonies were grown overnight and subjected to plasmid purification via the EMnetik Plasmid preps and the spin-column based kit. The plasmid obtained via two purification methods were subsequently prepared for NGS using an Illumina Nextera XT DNA Library Preparation Kit, pooled, and sequenced on an Illumina MiSeq using a 2 × 151 paired-end sequencing protocol. Sample reads were mapped to the appropriate reference sequence to confirm the identity of the product. The quality of the sequences generated from each plasmid library was determined using CLC's QC for Sequencing Reads tool.

RESULTS

Gibson and Type IIs Golden Gate Assemblies

Concentration and purity metrics for the Gibson assembly reactions **(Table 1)** and Type IIs Golden Gate assembly reactions **(Table 2)** after cleanup with the EMnetik PCR Cleanup kit and spin-column kit. The EMnetik PCR Cleanup kit provided a greater DNA concentration (ng/µL) than the spin-column kit, as determined by Nanodrop™ in both assembly methods. Nanodrop™ determined 260/280 and 260/230 ratios varied by user and cleanup method and included some outlier reactions (red text) for both methods and users.

Assembly Method	PCR Cleanup Method	Operator	Sample	Concentration (ng/µL)	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
Gibson	EMnetik 24 PCR Cleanup	1	EM 1	9.7	2.5	1.8	9.6	3.2	1.8
			EM 2	9.9	2.7	0.6			
			EM 3	11.4	2.8	1.9			
		2	EM 4	9.6	2.1	2.1			
			EM 5	9.6	2.4	2.1			
			EM 6	7.4	6.9	2.3			
	Spin-column PCR Cleanup	1	SC 1	4.1	1.9	1.4	3.3	2.1	1.2
			SC 2	3.6	1.9	1.4			
			SC 3	3.6	1.9	1			
		2	SC 4	2.7	1.8	1.4			
			SC 5	2.8	3.4	1.1			
			SC 6	3.2	1.8	0.9			

Table 1 Gibson Assembly Reaction Purification Metrics.

Assembly Method	PCR Cleanup Method	Operator	Sample	Concentration (ng/µL)	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
Type IIs Golden Gate	EMnetik 24 PCR Cleanup	1	EM 7	13	3.6	3.1	10.8	9.3	2.8
			EM 8	14.2	8.2	3.3			
			EM 9	14.6	4.7	3			
		2	EM 10	8.2	14.3	2.6			
			EM 11	8.2	10	2.9			
			EM 12	6.5	15.2	2.1			
	Spin-column PCR Cleanup	1	SC 7	4.6	0.1	0.5	3.9	3.1	0.7
			SC 8	3.7	0.1	0.8			
			SC 9	3.7	0	1			
		2	SC 10	3.6	3.4	0.8			
			SC 11	3.6	11.3	0.7			
			SC 12	3.9	3.9	0.6			

Table 2. Type IIs Golden Gate Assembly Reaction Purification Metrics.

Plasmid Purification Results

Concentration and purity metrics for plasmids purified with either the EMnetik Plasmid Purification kit or spin-column kit are presented in Tables 3-4. For both users and assembly methods, the EMnetik Plasmid Purification kit produced similar concentrations compared to the spin-column plasmid purification kit. Purity metrics (260/280 and 260/230 ratios) were also relatively similar between both plasmid purification methods.

Assembly Method	Plasmid Purification Method	Operator	Sample	Concentration (ng/µL)*	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
Gibson	EMnetik 24 Plasmid Purification Kit	1	EM 1	8.5	1.8	1.4	8.7	1.9	1.6
			EM 2	10	1.9	1.4			
			EM 3	6.6	2	1.6			
		2	EM 4	8.8	1.8	1.5			
			EM 5	7.8	1.9	2.1			
			EM 6	10.4	1.8	1.5			
	Spin-column Plasmid Purification Kit	1	SC 1	8	1.8	1.8	7.7	1.8	1.8
			SC 2	6.7	1.8	1.9			
			SC 3	7	1.8	1.7			
		2	SC 4	8.4	1.8	1.7			
			SC 5	7.4	1.8	1.6			
			SC 6	8.7	1.9	1.9			

Table 3. Gibson Assembly Plasmid Purification Metrics.

Assembly Method	Plasmid Purification Method	Operator	Sample	Concentration (ng/µL)*	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
Type IIs Golden Gate	EMnetik 24 Plasmid Purification Kit	1	EM 7	8.8	2.4	1.1	26.4	2.2	1.7
			EM 8	9.6	2.4	1			
			EM 9	10.2	2.3	1.1			
		2	EM 10	55.4	1.9	2.3			
			EM 11	42.8	2	2.3			
			EM 12	31.7	2	2.3			
	Spin-column Plasmid Purification Kit	1	SC 7	5.3	2.1	1.6	9.7	1.9	1.8
			SC 8	5.1	2	1.5			
			SC 9	4.2	2.4	1.7			
		2	SC 10	14.1	1.9	1.9			
			SC 11	14.3	2	1.9			
			SC 12	15.1	0.9	2			

Table 4. Type IIs Golden Gate Assembly Plasmid Purification Metrics.

*For comparison, Nanodrop™ spectrophotometer readings were normalized to reflect the quantity of plasmid DNA from 1/3 of the bacteria cell lysate.

Transformations

The number of colonies with the expected phenotype that resulted from plating 100 µL of each culture transformed with Gibson and Type IIs Golden Gate Assembly reactions cleaned up using the EMnetik 24 PCR Cleanup kit are presented in Figure 1 and Figure 2, respectively. In Figure 2, operator 2's transformation efficiency was much higher than operator 1's. This indicates that the poor transformation efficiency observed for Operator 1's reactions are likely due in part to an issue with the operator's transformation methodology rather than something specific to the cleanup method.

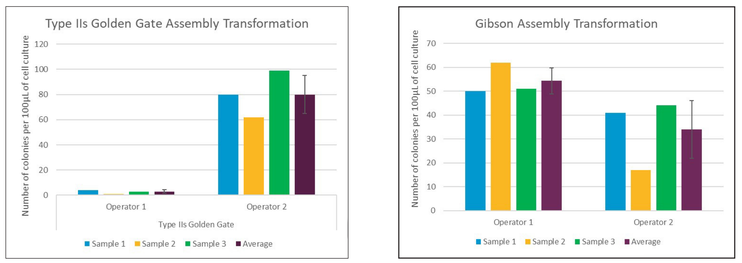

Figure 1. Number of colonies following transformation of Gibson assembly **(left)** and Type IIs Golden Gate Assembly **(right)** DNA purified using the EMnetik PCR Cleanup kit. The purple bar is the average colony counts from the operator. The error bars are the standard deviation of the average from the individual operators.

Next-Generation Sequencing (NGS) Results

Both plasmid purification methods yielded abundant and high-quality NGS reads **(Table 5)**. Read-mapping to the reference sequences confirmed that the correct plasmid assemblies were produced (100% coverage), and a very high depth of coverage was obtained for all samples. The average depth of coverage, percentage of reads mapped to the plasmid, and percentage of reads with average PHRED quality scores ≥ 30 were similar for both plasmid purification methods and assembly types, confirming the suitability of the EMnetik system for the preparation of assembled plasmids for gene editing.

Assembly Method	Plasmid Purification Method	Operator	Sample	Individual Sample Metrics			
				% of plasmid covered	Average depth of plasmid coverage	% of reads mapped to plasmid	% reads with average PHRED score ≥ 30
Gibson	EMnetik 24 Plasmid Purification Kit	1	EM 1	100%	79,669	97%	91%
			EM 2	100%	38,428	95%	88%
			EM 3	100%	68,151	96%	86%
		2	EM 4	100%	42,764	98%	91%
			EM 5	100%	82,235	99%	90%
			EM 6	100%	63,725	99%	93%
	Spin-column Plasmid Purification Kit	1	SC 1	100%	95,192	99%	92%
			SC 2	100%	56,989	99%	82%
			SC 3	100%	35,042	99%	91%
		2	SC 4	100%	57,139	99%	85%
			SC 5	100%	66,238	99%	87%
			SC 6	100%	74,013	100%	91%
Type IIs Golden Gate	EMnetik 24 Plasmid Purification Kit	1	EM 7	100%	114,173	97%	92%
			EM 8	100%	79,238	98%	94%
			EM 9	100%	96,909	99%	88%
		2	EM 10	100%	164,874	97%	94%
			EM 11	100%	135,816	97%	93%
			EM 12	100%	122,958	99%	88%
	Spin-column Plasmid Purification Kit	1	SC 7	100%	98,760	100%	94%
			SC 8	100%	80,495	100%	93%
			SC 9	100%	108,650	100%	94%
		2	SC 10	100%	113,190	100%	94%
			SC 11	100%	139,268	100%	94%
			SC 12	100%	118,508	100%	92%

Table 5.

CONCLUSION

The EMnetik PCR Cleanup kit combined with the EMnetik 24 microparticle processor outperformed the traditional column-based PCR cleanup method in terms of yield when used to clean up DNA from Gibson and Type IIs Golden Gate assembly protocols, but due to the variance in 260/280 ratios when using both protocols with the different operators, the two different methods can be considered to perform comparably. EMnetik Plasmid Purification kit performed comparably to the spin-column method when assessing the quantity and quality of purified plasmids. The NGS results were satisfactory and similar for both EMnetik system and spin-column kits. To find out more https://becls.co/emnetik24.

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