BECKMAN COULTER
Life Sciences

Using the EMnetik System to Enable Fast and Easy DNA Cleanups and Plasmid 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, making it an excellent alternative to time-consuming and tedious column-based methods. The system also offers step-by-step on-screen guidance, negates the need to move samples on and off the manifold (preventing spills and sample mix-ups), and the consistency offered by automated mixing and separation.

The objective of this study was to evaluate the EMnetik system used for DNA cleanup in support of genome editing, and for plasmid purifications, including next-generation sequencing (NGS) of the plasmids prepared. To meet this objective, plasmids were prepared for genome editing using two different classic assembly methods: Gibson Assembly and Type IIs Golden Gate Assembly. As part of this process, the EMnetik system was used to perform PCR cleanups and plasmid purifications alongside traditional column-based methods for comparison.

Cleaned-up PCR reactions and plasmid preparations were analyzed to determine quantity, quality and compatibility with downstream assays. Two independent analysts performed these studies and provided user feedback, including overall impressions, turnaround time, hands-on time, touchpoints and difficulty level.

Study Design and Methods

The EMnetik system, including the EMnetik 24 microparticle processor (PN: C57784), was used for DNA cleanup steps required to assemble plasmids for transformation via GeneArt™ Gibson Assembly (PN:A46626) and GeneArt™ Type IIs Golden Gate Assembly (PN:A15916, A15917, A15918). Both methods were performed by two different analysts for one gene edit in triplicate, for a total of three assembly samples per method per analyst.

The gene edit selected for performing this evaluation was an exon 1 deletion of the hprt1 gene. For comparison the same study design was replicated using the EMnetik PCR Cleanup kit (PN: C68442) and spin-column cleanup kit. Prior to transformation, all assembly products were assessed for yield and purity via the Nanodrop™ spectrophotometer. After transformation, the number of colonies with the expected phenotype were counted.

PCR was used to screen colonies generated via Gibson Assembly and Type IIs Golden Gate Assembly, and one colony from each technical replicate processed with the EMnetik system and one colony from each technical replicate processed with the spin-column cleanup kit. The colonies were grown overnight and subjected to plasmid purification via the EMnetik Plasmid Purification kit (PN: C68445) or the spin-column based plasmid purification kit. Plasmid preps were then assessed for yield and purity via the Nanodrop™ spectrophotometer.

The plasmid obtained via two purification methods were subsequently prepared for NGS using an Illumina Nextera XT DNA Library Preparation kit (PN: FC-131-1024), then pooled and sequenced on an Illumina MiSeq using a 2 × 151 paired-end sequencing protocol. Following sequencing, the FASTQ reads for each plasmid library were analyzed for identity and quality using CLC Genomics Workbench software. Sample reads were mapped to the appropriate reference sequence (i.e., the vector with the edited gene) to confirm identity of the product. Quality of the sequences generated from each plasmid library was determined using CLC's *QC for Sequencing Reads tool.*

Results

Gibson Assemblies

Concentration and purity metrics for the Gibson assembly reactions after cleanup with the EMnetik PCR Cleanup kit and spin-column kit are presented in Table 1. For both users, the EMnetik PCR Cleanup kit provided a greater DNA concentration (ng/µL), as determined by Nanodrop™ spectrophotometer. Nanodrop™ spectrophotometer - 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.

PCR Cleanup Method	Operator	Sample	Concentration (ng/µL)	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
EMnetik 24 PCR Cleanup kit	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 kit	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.

Type IIs Golden Gate Assemblies

Concentration and purity metrics for the Type IIs Golden Gate assembly reactions after cleanup with the EMnetik PCR and spin-column kits are presented in Table 2. Nanodrop™ spectrophotometer -determined DNA concentration was higher for the EMnetik kit than the spin-column kit. However, accuracy of these concentrations may be adversely affected by the abnormal absorbance spectra determined by 260/280 and 260/230 ratios, which varied by user and cleanup method, and in most cases, differed significantly from ideal values for both methods and users.

PCR Cleanup Method	Operator	Sample	Concentration (ng/µL)	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
EMnetik 24 PCR Cleanup kit	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 kit	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.

Gibson Assembly Transformations

The number of colonies with the expected phenotype that resulted from plating 100 µL of each culture transformed with assembly reactions cleaned up using the EMnetik 24 PCR Cleanup kit are presented in Figure 1. All reactions produced 17-62 colonies per 100 µL plated.

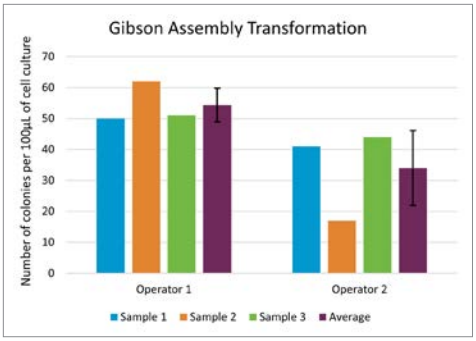

Figure 1. Number of colonies following transformation of Gibson assembly DNA purified using the EMnetik PCR Cleanup kit. The purple bar is the average colony counts from the operator. The error bars are the standard deviation of the average from the individual operators.

Type IIs Golden Gate Assembly Transformations

The number of colonies with the expected phenotype that resulted from plating 20 µL of each culture transformed with assembly reactions cleaned up using the EMnetik 24 PCR Cleanup kit are presented 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.

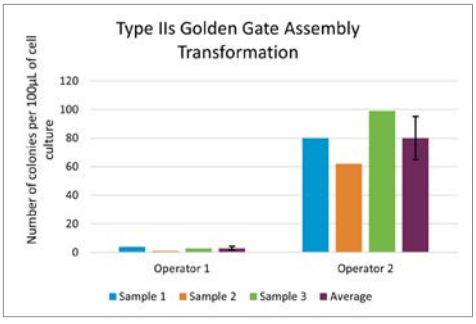

Figure 2. Number of colonies following transformation of Type IIs Golden Gate Assembly DNA purified using the EMnetik PCR Cleanup kit. The purple bar is the average colony counts from the operator. The error bars are the standard deviation of the average from the individual operators.

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.

Plasmid Purification Method	Operator	Sample	Concentration (ng/µL)*	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
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.

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

Plasmid Purification Method	Operator	Sample	Concentration (ng/µL)*	260/280	260/230	Average Concentration (ng/µL)	Average 260/280	Average 260/230
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.

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. 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 suitability of the EMnetik system for preparation of assembled plasmids for gene editing.

Assembly Method	Plasmid Purification Method	Operator	Sample	Individual Sample Metrics				Averages Per Sample Type			
				% Plasmid covered	Depth of plasmid coverage	% Reads mapped to plasmid	% Reads PHRED ≥ 30	% Plasmid covered	Depth of plasmid coverage	% Reads mapped to plasmid	% Reads PHRED ≥ 30
Gibson	EMnetik 24 Plasmid Purification kit	1	EM 1	100%	79,669	97%	91%	100%	62,495	98%	90%
			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%	100%	64,102	99%	88%
			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%	100%	118,995	98%	91%
			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%	100%	109,812	100%	93%
			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. NGS Metrics for Plasmid Products.

Conclusions

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 cleanup 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.

The study also surveyed feedback from two users regarding the EMnetik system on aspects of usability, e.g., simplicity, turnaround time, touchpoints and hands-on time. The users noted that the EMnetik system required significantly less hands-on time and touchpoints, and that they appreciated the simplicity of the semi-automated system.

Feedback on overall turnaround time relative to the column-based method differed between the two protocols. Users reported that turnaround time for the EMnetik PCR Cleanup kit combined with the EMnetik 24 microparticle processor was comparable to the competing column-based kit when a small number of samples are processed per run, but significantly faster as the number of samples increased.

Users felt that the EMnetik Plasmid Purification kit was typically a little slower, although this could be partially due to greater familiarity with the column-based protocol over the EMnetik system. However, users also noted that they would still recommend both the EMnetik PCR Cleanup kit and EMnetik Plasmid Purification kit due to the simplicity of using the semi-automated system, especially the reduced hands-on time and number of touchpoints.

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