High-efficiency, low-cost transformation of Gibson Assembly® constructs

E. cloni® 10G Chemically Competent Cells exhibit high transformation efficiencies of plasmids generated on the BioXp™ 3200 system

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Abstract

Gibson Assembly® constructs may be prepared using SGI-DNA Gibson Assembly HiFi 1-Step and Ultra kits or by the automated cloning instrument, the BioXp™ 3200 system. Constructs generated manually by the kits or hands-free by the instrument are routinely transformed into EPI300 electrocompetent cells. In this study, we compared the relative transformation efficiency of EPI300 cells side-by-side with chemically competent *E. cloni*® 10G cells from Lucigen Corporation. For every construct analyzed in this study, 10G cells exhibited higher relative transformation efficiencies than EPI300 electrocompetent cells. Although chemically competent 10G cells exhibited higher transformation efficiencies than the electrocompetent EPI300 cells, our results indicate that Gibson Assembly constructs can be readily transformed with high efficiency into either of these competent cells.

Introduction

TransforMax™ EPI300™ Electrocompetent *E. coli* have been used successfully and extensively by Daniel Gibson and his team at the J. Craig Venter Institute and SGI-DNA for the transformation of Gibson Assembly constructs[1–3]. For this reason and because EPI300 cells are compatible with large, inducible clones, SGI-DNA has traditionally recommended electroporating Gibson Assembly constructs into EPI300 cells as the transformation method-of-choice following assembly.

Additionally, largely because of our extensive experience and historical success with EPI300 cells, we have recommended transforming constructs built on the SGI-DNA automated genomic workstation, the BioXp™ System, with EPI300 Electrocompetent *E. coli*.

For some Gibson Assembly and BioXp users, the lack of electroporation equipment and the expense of EPI300 cells have been barriers to their use. We have previously shown that the SGI-DNA Gibson Assembly HiFi 1-Step and Ultra Kits are compatible with multiple electrocompetent and chemically competent cells[4]. In this study, we compare *E. cloni* 10G cells, a commercially available high-efficiency chemically competent cell, head-to-head with EPI300 cells using constructs generated with the BioXp 3200 system.

Methods

TransforMax EPI300 Electrocompetent *E. coli* (Epicentre Cat. No. C300105) and *E. cloni* 10G chemically competent cells (Lucigen Cat. No. 60107) were acquired from vendors and stored at –80°C until use. Genotypes listed below are from each vendor's website.

EPI300 genotype: *F⁻ mcrA Δ(mrr-hsdRMS-mcrBC)Φ80dlacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara,leu)7697 galU galK λ⁻ rpsL (Str^R) nupG trfA dhfr*

E. cloni 10G genotype: *F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) endA1 recA1 Φ80dlacZΔM15 ΔlacX74 araD139 Δ(ara,leu)7697 galU galK rpsL nupG λ⁻ tonA (Str^R)*

TransforMax EPI300 electrocompetent cells have a transformation efficiency of $> 1 \times 10^{10}$ when used as directed. Lucigen high-efficiency *E. cloni* 10G chemically competent cells have a transformation efficiency of $> 1 \times 10^{9}$ when used as directed. For this study, experiments with the 10G cells were performed according to the manufacturer's protocol. EPI300 experiments were performed according to the procedure published in the BioXp manual. Differences between the procedure in the BioXp manual and the manufacturer's recommended procedure are outlined in Table 1.

Table 1. Differences between the EPI300 manufacturer's recommended procedure and the BioXp procedure.

Parameter	Epicentre Procedure	SGI-DNA BioXp Procedure
Electroporator	Eppendorf Multiporator®	Bio-Rad Gene Pulser® Xcell™ Electroporator
Voltage	2.5 kV	1.2 kV
Cuvette size	2 mm	1 mm
Note that the applied voltage across the cuvette gap is similar. This change is not expected to result in any significant performance difference in the transformation efficiency of EPI300 cells.		

The fully automated BioXp 3200 system was used to create constructs for transformation. First, 1416, 890, 660, and 430 bp inserts were generated on the BioXp 3200 system as BioXp Tiles. These Tiles were then cloned into a pUC-derived vector in an automated Gibson Assembly reaction during the same hands-free instrument run. Following this BioXp cloning run, products from the first four wells (A1, B1, C1 and D1) of the Recovery Plate were diluted 1:1 with water. One µL of diluted Gibson Assembled product was used to transform the cells as described. 10G cells were recovered in a final volume of 1000 µL of the provided Recovery Medium. EPI300 cells were recovered in a final volume of 1000 µL of SOC. The post-transformation recovery time was extended to 90 minutes. Two volumes (15 µL and 150 µL) of transformed cells were plated on LB plates containing 100 µg/mL carbenicillin, X-Gal, and IPTG. Plates were incubated overnight at 37°C. Colonies were counted and picked the following day.

Results

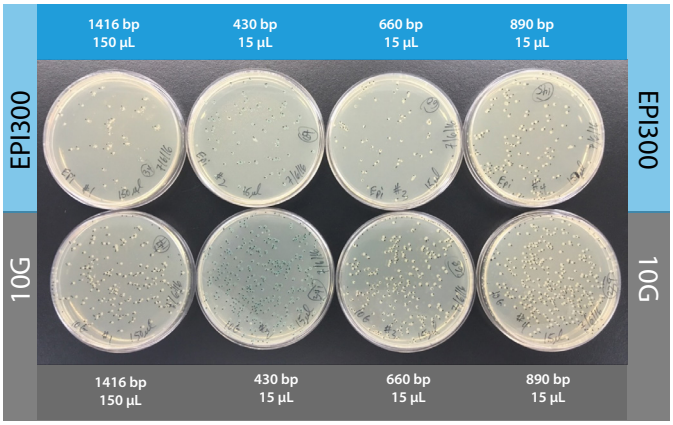

Figure 1. More transformants are visible on 10G plates in a side-by-side comparison of EPI300 and 10G transformants. Plates on the top row are EPI300 transformants. Plates on the bottom row are 10G transformants. Insert size and the volume of the reaction plated are indicated above (EPI300) or below (10G) the plates.

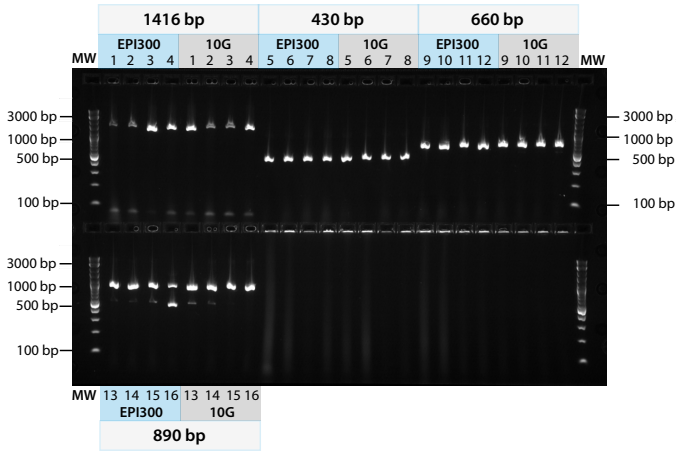

Figure 2. An insert of the expected size is evident in all colonies analyzed by colony PCR. Four colonies from each plate shown in Figure 1 were picked and used for colony PCR. Inserts were PCR-amplified using GA forward and reverse primers. MW denotes the lanes with a molecular weight marker: 2-Log DNA Ladder from New England BioLabs.

Table 2. 10G competent cells exhibit higher relative transformation efficiencies.

Insert Size	Volume plated (µL)	Number of Colonies		Fold Difference* (10G/EPI300)
		EPI300	10G	
1416 bp	15	4	25	6.25
	150	34	147	4.32
430 bp†	15	69	341	4.94
660 bp†	15	60	268	4.47
890 bp†	15	145	339	2.34

*The true transformation efficiency cannot be calculated because there is no method to determine the amount of full-length product from a Gibson Assembly reaction. These results are comparative and are based on the same amount of DNA from the same Gibson reaction from the same BioXp run.

†The 150 µL plates of the 430, 660, and 890 bp-inserts contained too many colonies to count.

Conclusion and Other Considerations

These results demonstrate that 10G chemically competent cells work as well or better than EPI300 electrocompetent cells for transforming product from a Gibson Assembly reaction.

The genotypes of these two commercially available cell lines are nearly identical. EPI300 cells contain additional elements allowing for single-copy plasmid propagation with the oriV origin of replication under normal growth conditions and propagation of high-copy number plasmids under inducible conditions. This feature of EPI300 cells might be advantageous to stably clone large or toxic genes.

The cost per reaction using the Lucigen 10G cells is about half the cost per transformation when compared to EPI300 cells. Additionally, chemically competent cells are amenable to high-throughput cloning without the added cost of an electroporation device.

References

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