Gibson Assembly® HiFi 1 Step and Ultra Kits are Compatible with Multiple Electrocompetent and Chemically Competent Cells

Christine Chen, Ph.D., Synthetic Genomics, Inc., La Jolla, CA

Abstract

The Gibson Assembly® method is an easy-to-use, robust, seamless cloning method that allows for efficient cloning of multiple DNA fragments simultaneously. Gene constructs assembled with Gibson Assembly® are often introduced into *E. coli* for propagation and maintenance. In this practical guide, we analyzed the transformation efficiencies of eight commercially available competent cells: three electrocompetent cells and five chemically competent cells, with genes constructed using the Gibson Assembly® method. Here, we demonstrate that Gibson Assembly® constructs can be successfully transformed into a wide variety of competent cells. Although transformation was successful in some cases with Gibson Assembly® reaction mixture percentages as high as 8% of the total transformation reaction mixture volume, the highest transformation efficiencies were generally achieved using lower volumes of assembly reaction mixtures. Successful transformation was achieved with all chemically competent and electrocompetent cells when the Gibson Assembly® reaction comprised 2% of the transformation reaction.

Introduction

Electrocompetent and chemically competent *E. coli* cells are common transformation hosts for constructs prepared using the Gibson Assembly® seamless cloning method. Here we test the effect of the enzyme Master Mixes from SGI-DNA Gibson Assembly® Kits on several commercially available competent cells in order to asses optimal parameters for achieving highest transformation efficiencies. Chemically competent cells with genotypes identical to electrocompetent cells tested in this study exhibit different tolerances to the enzyme Master Mixes. Methodology differences used to render the cells competent may account for observed transformation efficiency divergences.

As shown in Table 4, the baseline transformation of the competent cells analyzed in this study varied widely, ranging from 140,000–6,900,000 CFU/ng (assessed by transformation of pUC19 only). A two-fragment assembly of pUC vector and a 1.5 kb insert was used to evaluate the effect of the assembly reaction mixture on transformation efficiency in the different competent cells. Competent cells with higher transformation efficiencies generally exhibited higher overall success, demonstrating the importance of selecting competent cells with a high transformation efficiency for transformation of Gibson Assembly® HiFi 1 Step or Ultra constructs.

Materials and Methods

Ten ng of 2.7 kb vector and 25 ng of 1.5 kb insert were assembled using the Gibson Assembly® HiFi 1 Step Kit (SGI-DNA, Cat. No. GA 1100) or Gibson Assembly® Ultra Kit (SGI-DNA, Cat. No. GA 1200). To asses transformation efficiency, increasing ratios of Gibson Assembly® reaction mixture (v/v) were transformed into competent cells. Transformation of chemically competent cells consisted of adding the Gibson Assembly®/DNA mixture to 50 µL of competent cells, followed by incubation of the Master Mix/DNA mixture on ice for 30 minutes, heat shock at 42 °C for 30 seconds, and recovery in 1 mL SOC for 1 hour at 37 °C. Cells were then plated on LB plates with 100 µg/mL carbenecillin. Transformation of electrocompetent cells consisted of adding the Gibson Assembly®/DNA mixture to 30 µL of competent cells. Competent cells were incubated with the Master Mix / DNA mixture for 1 minute on ice prior to electroporation. Settings with the BioRad Gene Pulser system were 1200–2000 V, 25 µF, and 200 Ω in a 0.1 cm cuvette, and recovery was in 1 mL SOC for 1 hour at 37 °C. Cells were then plated on LB plates with 100 µg/mL carbenecillin. Final transformation efficiency is calculated per ng of vector.

Results

	Gibson Assembly® Transformation Efficiency (CFU/ng vector) using Chemically Competent Cells									
	EPI300™		DH10B™		NEB 5-α		DH5α		XL 10-Gold	
Percentage of Assembly Reaction (Per Total Volume of Transformation Reaction)	HiFi 1 Step	Ultra	HiFi 1 Step	Ultra	HiFi 1 Step	Ultra	HiFi 1 Step	Ultra	HiFi 1 Step	Ultra
2%	10,200	22,400	20,000	45,600	220,000	406,000	75,000	402,000	120,000	336,000
4%	9000	46,800	700	22,800	207,000	264,000	10,000	4,000	126,000	56,000
8%	18,400	23,600	0	5,500	108,000	34,500	0	0	43,500	2,000

Table 1. The Gibson Assembly® HiFi 1 Step Kit and Gibson Assembly® Ultra Kits are compatible with multiple chemically competent cells. The table above gives the Transformation Efficiency in CFU/ng from experiments using increasing volumes of an assembly reaction to transform various chemically competent cells. Transformation efficiency is calculated from the colony output normalized with the amount of vector used in the two-fragment assembly reaction. Refer to Table 4 for details about the methods and chemically competent cells analyzed in this study.

	Gibson Assembly® Transformation Efficiency (CFU/ng vector) using Electrocompetent Cells					
	EPI300™		DH10B™		NEB 5-α	
Percentage of Assembly Reaction (Per Total Volume of Transformation Reaction)	HiFi 1 Step	Ultra	HiFi 1 Step	Ultra	HiFi 1 Step	Ultra
1%	875,000	250,000	20,000	70,000	585,000	805,000
2%	528,000	472,000	52,000	84,000	800,000	406,000

Table 2. The Gibson Assembly® HiFi 1 Step Kit and Gibson Assembly® Ultra Kits are compatible with electrocompetent cells. The table above gives the Transformation Efficiency in CFU/ng from experiments using increasing percentages of an assembly reaction to transform various electrocompetent cells. Transformation efficiency is calculated from the colony output normalized with the amount of vector used in the two-fragment assembly reaction. Refer to Table 4 for details about the methods and electrocompetent cells analyzed in this study.

Discussion

As shown above, Gibson Assembly® reactions are compatible with a wide variety of competent cells. The transformation of all competent cells analyzed in this study was successful to varying degrees, suggesting that any high efficiency competent cell may be used for transformation of Gibson Assembly® reactions from the HiFi 1 Step and Ultra Kits. Highest transformation efficiencies of assembled constructs were attained using electrocompetent cells (comparing Tables 1 and 2). Although any high efficiency competent cell may be used for transformation, we recommend Epicentre electrocompetent TransforMax™ EPI300™ *E. coli* (Epicentre, Cat No. EC300110) or NEB 5-α Electrocompetent *E. coli* (New England BioLabs, Cat. No. C2989K) for transforming Gibson Assembly® HiFi 1 Step and Ultra constructs because of the high observed transformation efficiencies (Table 2). EPI300™ cells offer the added advantage of compatibility with large, inducible clones. For routine Gibson Assembly® cloning, any high efficiency competent cell may be used.

Optimal transformation efficiencies may be determined empirically for each assembly reaction and each competent cell type. As a starting point, we recommend transforming the competent cells of your choice with the Gibson Assembly®/DNA mixture that equates to 2% of the total transformation reaction mixture volume. For low efficient assembly reactions, we recommend plating at a higher volume rather than increasing the amount of assembled product used in the transformation reaction. Adding more assembled product does not necessarily translate into higher colony output.

Conclusion

- Gibson Assembly® reactions are compatible with the transformation of a wide variety of high efficiency competent cells.
- To achieve highest transformation efficiency, it is important to use minimal amounts of the Gibson Assembly® reactions for transformation of competent cells.
- Guidelines for the transformation of Gibson Assembly® HiFi 1 Step or Ultra constructs into various competent cells are outlined in the following table.

Gibson Assembly® HiFi 1 Step Kit Transformation Guidelines				
Percentage of Assembly Reaction (Per Total Volume of Transformation Reaction)	Use For	Chemically Competent	EPI300™ Cells	Other Electrocompetent
1.5%	Optimal transformation efficiency for EPI300™ cells	—	Dilute Assembly Reaction 1:5. Use 2.5 µL of diluted reaction.	—
2%	Recommended Starting Point	1 µL of Assembly Reaction	—	Dilute Assembly Reaction 1:5. Use 3.5 µL of diluted reaction.

Table 3. Practical guidelines for transforming Gibson Assembly® HiFi 1 Step reactions with various competent cells. The table above gives the recommended Assembly Reaction volumes for transformation, based on a total cell volume of 50 µL for chemically competent cells and 30 µL for electrocompetent cells. The percentage of assembly reaction is the amount of assembly reaction per total volume of transformation reaction.

Gibson Assembly® Ultra Kit Transformation Guidelines				
Percentage of Assembly Reaction (Per Total Volume of Transformation Reaction)	Use For	Chemically Competent	EPI300™ Cells	Other Electrocompetent
2%	Recommended Starting Point	1 µL of Assembly Reaction	—	Dilute Assembly Reaction 1:5. Use 3.5 µL of diluted reaction.
3%–8%	Optimal transformation efficiency for EPI300™ cells	—	Use 1–2.5 µL of Assembly Reaction	—

Table 4. Practical guidelines for transforming Gibson Assembly® HiFi 1 Step and Gibson Assembly® Ultra reactions with various competent cells. The table above gives the recommended Assembly Reaction volumes for transformation, based on a total cell volume of 50 µL for chemically competent cells and 30 µL for electrocompetent cells. The percentage of assembly reaction is the amount of assembly reaction per total volume of transformation reaction.

Reference Materials

Type of Competent Cell	Product	Vendor	Cat. No.	Transformation Procedure	Baseline Transformation Efficiency
Chemical	TransforMax™ EPI300™ *E. coli*	Epicentre/Illumina	C300C105	42°C heat shock 30 sec.	1.40×10^8
Chemical	One Shot® MAX Efficiency™ DH10B™ T1 Phage-Resistant Cells	Life Technologies/ Thermo Fisher Scientific	12331-013	42°C heat shock 30 sec.	2.66×10^8
Chemical	NEB 5-α Competent *E. coli*	New England BioLabs	C2987I	42°C heat shock 30 sec.	1.20×10^9
Chemical	One Shot® MAX Efficiency® DH5α™-T1^R Competent Cells	Life Technologies/ Thermo Fisher Scientific	12297-016	42°C heat shock 30 sec.	1.20×10^9
Chemical	XL 10-Gold Ultracompetent cells	Agilent Technologies	200314	42°C heat shock 30 sec.	1.68×10^9
Electrocompetent	TransforMax™ EPI300™ *E. coli*	Epicentre/Illumina	EC300110	1200 V, 25 µF, 200 Ω	6.90×10^9
Electrocompetent	ElectroMAX™ DH10B™ T1 Phage-Resistant Competent Cells	Life Technologies/ Thermo Fisher Scientific	12033-015	1200 V, 25 µF, 200 Ω	8.00×10^8
Electrocompetent	NEB 5-α Electrocompetent *E. coli*	New England BioLabs	C2989K	1200 V, 25 µF, 200 Ω	5.65×10^9

Table 5. Competent cells and experimental conditions used in this study. The Baseline Transformation Efficiency in this study is the Transformation Efficiency in CFU/µg for each competent cell using pUC19 in the absence of Gibson Assembly® Master Mixes.