ApplicationForum

A Practical Guide for Introducing Gibson Assembly® Gene Constructs into 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 the 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 tested three commercially available chemically competent cells with Gibson Assembly® reaction mixtures. Similar to traditional ligation-dependent cloning methods, salt and enzymes present in the Gibson Assembly® reaction mixture can be detrimental to competent cells. Although transformation was successful in some cases with percentages as high as 10% of the Gibson Assembly® reaction mixture, highest transformation efficiencies were generally achieved using lower volumes of the reaction mixture. Based on our results, we recommend using up to 2% of the Gibson Assembly® reaction as a starting point when transforming chemically competent cells with Gibson Assembly® constructs.

Materials and Methods

Chemically Competent Cells: TransforMax™ EPI300™ Chemically Competent *E. coli* (Epicentre, Cat. No. C300C105), One Shot® MAX Efficiency™ DH10B™ T1 Phage-Resistant Cells (Life Technologies, Cat. No. 12331 013), NEB 5-α Competent *E. coli* (New England BioLabs, Cat. No. C2987). Assembly Kits: Gibson Assembly® HiFi 1 Step Kit (SGI-DNA, Cat. No. GA 1100) and Gibson Assembly® Ultra Kit (SGI-DNA, Cat. No. GA 1200).

Ten pg of pUC19 supercoiled plasmid was mixed with the Gibson Assembly® Master Mixes from the HiFi or Ultra kits and the total volume was brought to 5 µL with 10 mM Tris-HCl, pH 8.0. The Gibson Assembly®/DNA mixture was then added to 50 µL competent cells for transformation. The percentages shown in the graphs are the v/v ratio of total Gibson Assembly®/DNA mixture to competent cell volume. Graphs show the Transformation Efficiency (TE) normalized with pUC19 only control.

Results

Figure 1. The effect of the Gibson Assembly® HiFi 1 Step Master Mix (A) and Gibson Assembly® Ultra Master Mixes (B) on the Transformation Efficiency (TE) of chemically competent cells

Summary

Similar to transforming cells with ligation reactions, transformation with Gibson Assembly® Master Mixes negatively impacts the survival of competent cells. A 10-fold reduction in transformation efficiency over control DNA alone can be expected when introducing Gibson Assembly® reaction mixtures. To achieve high transformation efficiency, it is important to use minimal amounts of the Gibson Assembly® reaction mixtures for transformation of competent cells. Our data indicate that the cells exhibit different tolerances to the reaction mixtures. Some cells, such as DH10B, exhibit higher sensitivity to the Gibson Assembly® Master Mixes than other commercially available competent cells. When first using Gibson Assembly®, or when using a new type of competent cell, we recommend transforming 2% of the Gibson Assembly® reaction mixture as a starting point. For low efficient assembly reactions, plating the competent cells at a higher volume rather than increasing the amount of assembled product used in the transformation reaction will lead to improved results.

For full and expanded details of this study, please visit www.sgidna.com/gibson_applications.