Superior Cloning Performance with SGI-DNA Gibson Assembly® kits

Introduction

Molecular cloning techniques have evolved rapidly over the last decade, particularly with the development, adoption, and refinement of seamless cloning strategies that allow for the scarless insertion of DNA fragments into a vector. In addition to leaving insert sequence fully intact, seamless cloning technologies offer the added advantage of being faster than traditional restriction enzyme digest-based cloning. One seamless cloning strategy in particular, Gibson Assembly® seamless cloning, has been extensively embraced by the life science community, as evidenced by over 1200 citations of the manuscript (1) originally describing the technique.

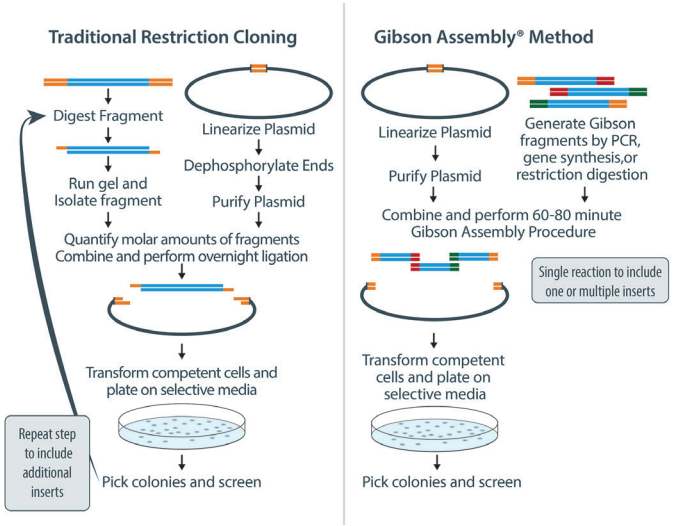

Figure 1. The Gibson Assembly Method is faster and more efficient than traditional cloning. Traditional restriction cloning using compatible restriction endonucleases requires 1–2 days of preparative steps to generate cloning ends on the insert and plasmid. Typically, only one insert can be ligated into the plasmid at a time. Generating longer inserts usually requires multiple rounds of restriction and ligation. The Gibson Assembly Method allows for several inserts to be simultaneously assembled in a single reaction that takes only ~1 hour, allowing for the rapid generation of very large constructs. The Gibson Assembly Method requires a linearized vector and 20–80 bp sequence overlaps at the ends of the DNA elements to be assembled. Overlap sequences are intrinsic to the construct(s) and plasmid, eliminating the need for specific restriction sites.

Because of its ease-of-use and efficiency, the Gibson Assembly method is ideally suited for routine cloning. In addition to the straightforward cloning of a single insert with a single vector, the Gibson Assembly method is the ideal choice for complex assembly projects, such as the simultaneous assembly of multiple inserts with a vector, as depicted in Figure 1. The Gibson Assembly method offers substantial time savings for multiple-insert assembly projects, which would typically require multiple rounds of traditional restriction enzyme digest-based cloning.

Gibson Assembly was first developed by Daniel Gibson and colleagues at the J. Craig Venter Institute. In the ensuing years, Daniel Gibson and his team have been refining and improving the technique and reagent formulations at SGI-DNA. The results of their expertise and years of development are available commercially as the SGI-DNA Gibson Assembly HiFi 1-Step and Ultra kits. Although several other seamless cloning kits are commercially available, only the SGI-DNA Gibson Assembly kits use the precise reagent formulation invented and further refined by Dr. Daniel Gibson.

Evaluating commercially available seamless assembly kits

To evaluate the performance of SGI-DNA Gibson Assembly HiFi 1-Step and Ultra kits in multi-fragment assembly reactions, five 800 bp fragments were assembled into an 8 kb vector using the HiFi or Ultra kit and three other commercially available

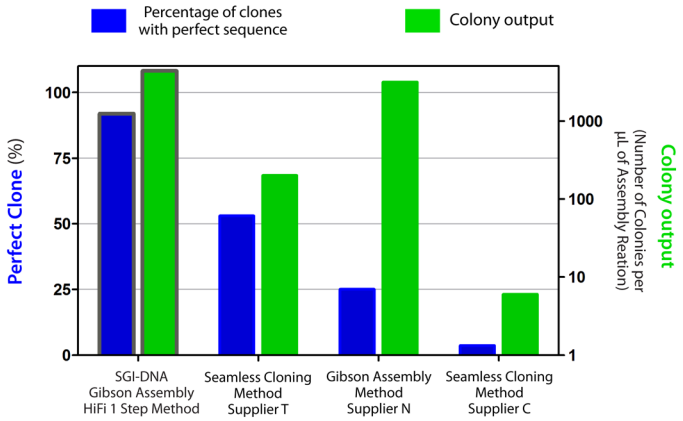

Figure 2. SGI-DNA Gibson Assembly HiFi 1 Step kit exhibits superior results. Five 800 bp fragments were assembled into an 8 kb vector using the HiFi 1 Step kit and three other commercial kits, here called Supplier T, N, or C. The assembly protocol (including relative amounts of DNA used in the assembly reaction) supplied by the manufacturer was followed for each respective kit. Following assembly and transformation, the number of colonies was counted and normalized to the volume of the assembly reaction used for transformation. Twelve colonies were randomly selected for colony PCR. Only positive colonies were utilized for sequencing. The percentage of clones containing perfect sequence is shown in the figure above. Data are presented as the mean value from two assembly experiments performed in triplicate.

seamless cloning kits. Assembly reactions were performed according to each manufacturer's protocol, including the relative amounts of insert and vector DNA used in the assembly reaction. Data are presented as the mean of two experiments performed in triplicate. Results are presented in Figures 2 (HiFi 1Step Kit) and 3 (Ultra Kit).

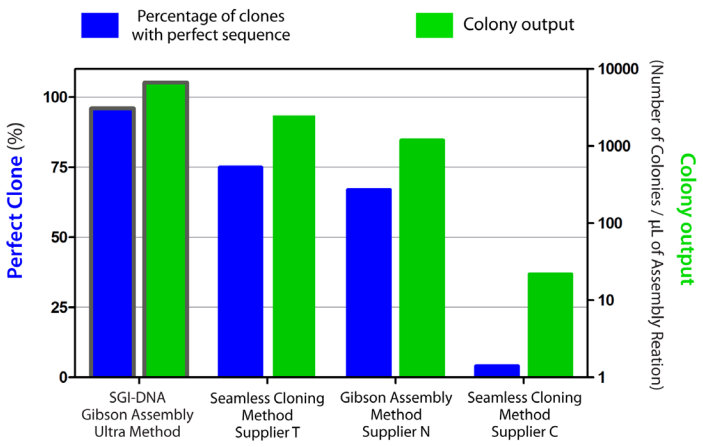

Figure 3. SGI-DNA Gibson Assembly Ultra kit exhibits superior results. Five 800 bp fragments were assembled into an 8 kb vector using the Ultra kit and three other commercial kits, here called Supplier T, N, or C. The assembly protocol (including relative amounts of DNA used in the assembly reaction) supplied by the manufacturer was followed for each respective kit. Following assembly and transformation, the number of colonies was counted and normalized to the volume of the assembly reaction used for transformation. Twelve colonies were randomly selected for colony PCR. Only positive colonies were utilized for sequencing. The percentage of clones containing perfect sequence is shown in the figure above. Data are presented as the mean value from two assembly experiments performed in triplicate.

Results and Summary

Compared to the other commercially available seamless cloning kits used for these 5-fragment assembly experiments, the SGI-DNA HiFi 1-Step and Ultra kits exhibit the highest colony output and sequence accuracy. As shown in Figure 2, the Gibson Assembly HiFi 1-Step Kit delivers the highest percentage of perfect clones– on average, 92% of analyzed clones exhibited perfect sequence. The Gibson Assembly Ultra Kit also delivers the highest percentage of perfect clones (96%) in comparison to the other kits used for multi-fragment assembly reactions (Figure 3). Additionally, like the HiFi 1-Step kit, the Ultra kit exhibits superior colony output. The HiFi 1-Step and Ultra kits also demonstrate similar robust performance when used for single insert cloning, exhibiting the highest cloning efficiency of all kits tested (data not shown).

When selecting a cloning strategy, many options are available. To achieve fast, accurate, and efficient results, SGI-DNA Gibson Assembly HiFi 1-Step and Ultra kits are optimal. The HiFi 1-Step Kit achieves fast assembly (1 hour reaction at a single temperature) and is recommended for assemblies with ≤5 fragments. The Ultra kit is recommended for more complex assemblies of up to 15 fragments, achieving assembly in 1 hour and 20 minutes. The Ultra Kit is also compatible with a broad range of DNA fragment sizes (100 bp – 100 kb). Key features of the kits are shown in Table 1. The crucial advantage of both the HiFi 1-Step and Ultra kits is the ability to clone genes of interest seamlessly, quickly, and accurately.

Table 1. Key Features of SGI-DNA Gibson Assembly Kits.

Feature	Gibson Assembly® HiFi 1 Step Kit	Gibson Assembly® Ultra Kit
Reaction Time	60 minutes	80 minutes
Number of Steps	1	2
Fragment Size Range	500 bp to 32 kb	100 bp to 100 kb
Cloning Efficiency	>90%	~96%
Fragments Per Reaction	up to 5	up to 15
Maximum Construct Size	100 kb (multi-stage reactions)	1 Mb (multi-stage reactions)
Key Advantages	• Quick and easy • Clone up to 5 fragments in a single reaction • Proof-reading polymerase reduces chance of mutations at cloning junctions	• Robust and efficient • Clone up to 15 fragments in a single reaction • Suitable for small and large fragments

Reference

1. Gibson, D.G. et al. 2009. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat. Methods 6: 343–345.

To learn more about Gibson Assembly kits, visit **sgidna.com/pages/gibson-assembly-reagents** or email **info@sgidna.com**.

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