Next Generation Shotgun Cloning Using the Gibson Assembly® Method

Coupling Powerful Technologies for Substantial Cost and Time Savings

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Traditional cloning and sequencing methods can be laborious, expensive, and time-consuming techniques, especially when applied to large sample numbers. Even for the routine cloning of small sample sizes, however, many research laboratories have yet to discover the power, ease, and efficiency of the Gibson Assembly® method. First described by Dan Gibson at the J. Craig Venter Institute (JCVI) in 2009, the Gibson Assembly® method is a sequence-independent, seamless cloning method that offers many advantages over traditional cloning, most notably the ability to assemble multiple DNA fragments quickly, accurately, and efficiently in a single-tube reaction.

SGI-DNA, a Synthetic Genomics company, offers Gibson Assembly® reagent kits: the Gibson Assembly® HiFi 1 Step Kit can be used for the simultaneous assembly of up to 5 fragments and the Gibson Assembly® Ultra Kit can be used for the simultaneous assembly of up to 15 fragments. The Gibson Assembly® method can be leveraged for a variety of applications, including routine cloning, site-directed mutagenesis, and whole-genome synthesis.

Here, we discuss the results and advantages of coupling the Gibson Assembly® method with shotgun cloning and next generation sequencing. The combined technologies of the Gibson Assembly® method, shotgun cloning, and next generation sequencing constitute Gibson Assembly® next generation shotgun cloning.

Shotgun cloning and next generation sequencing methods offer significant time and cost savings relative to traditional (Sanger) sequencing methods and independent, repetitive cloning methods. Shotgun cloning drastically reduces sample number and next generation sequencing drastically reduces the amount of time required for high-throughput sample processing. Combining the highly-efficient, error-correcting Gibson Assembly® method with shotgun cloning and next generation sequencing harnesses the advantages of the combined technologies, yielding substantial time and resource savings in comparison to traditional methods.

Gibson Assembly® Next Generation Shotgun Cloning Workflow

Traditional cloning and sequencing methods require the processing of individual samples through the following steps:

Preparation of insert and vector DNA by PCR amplification or restriction enzyme digestion → Vector Dephosphorylation → Ligation Cloning → Transformation → Plating → Picking Colonies → Plasmid Preparation → Sequencing → Analysis → Construct Retrieval

The sequencing and cloning of *n* constructs (where *n* = the number of constructs) requires individually manipulating all *n* samples through the 10 workflow steps outlined above in *n* tubes (i.e. cloning 12 constructs requires handling 12 samples during every workflow stage). See *Figure 1A* for a schematic overview.

Combining Gibson Assembly® shotgun cloning with next generation sequencing is achieved by processing samples through the following steps:

Preparation of insert and vector DNA by PCR amplification → Gel Purification → The Gibson Assembly® Method → Transformation → Plating → Picking Colonies → Plasmid Preparation → Next Generation Sequencing → Analysis → Construct Retrieval

In the Gibson Assembly® next generation shotgun cloning workflow, samples are pooled prior to gel purification and processed in size-correlated batches. Therefore, to sequence and clone *n* samples using Gibson Assembly® shotgun cloning, *n* constructs will be individually PCR-amplified. Following amplification, however, samples are pooled. For convenience and processing using 96-well plates, pools are typically batched with 8 samples per batch. Because of batching, for the remaining workflow steps, instead of processing *n* samples, only *n*/8 sample batches are manipulated for each step, which translates into substantial reagent savings (*see Figures 1B and 2*).

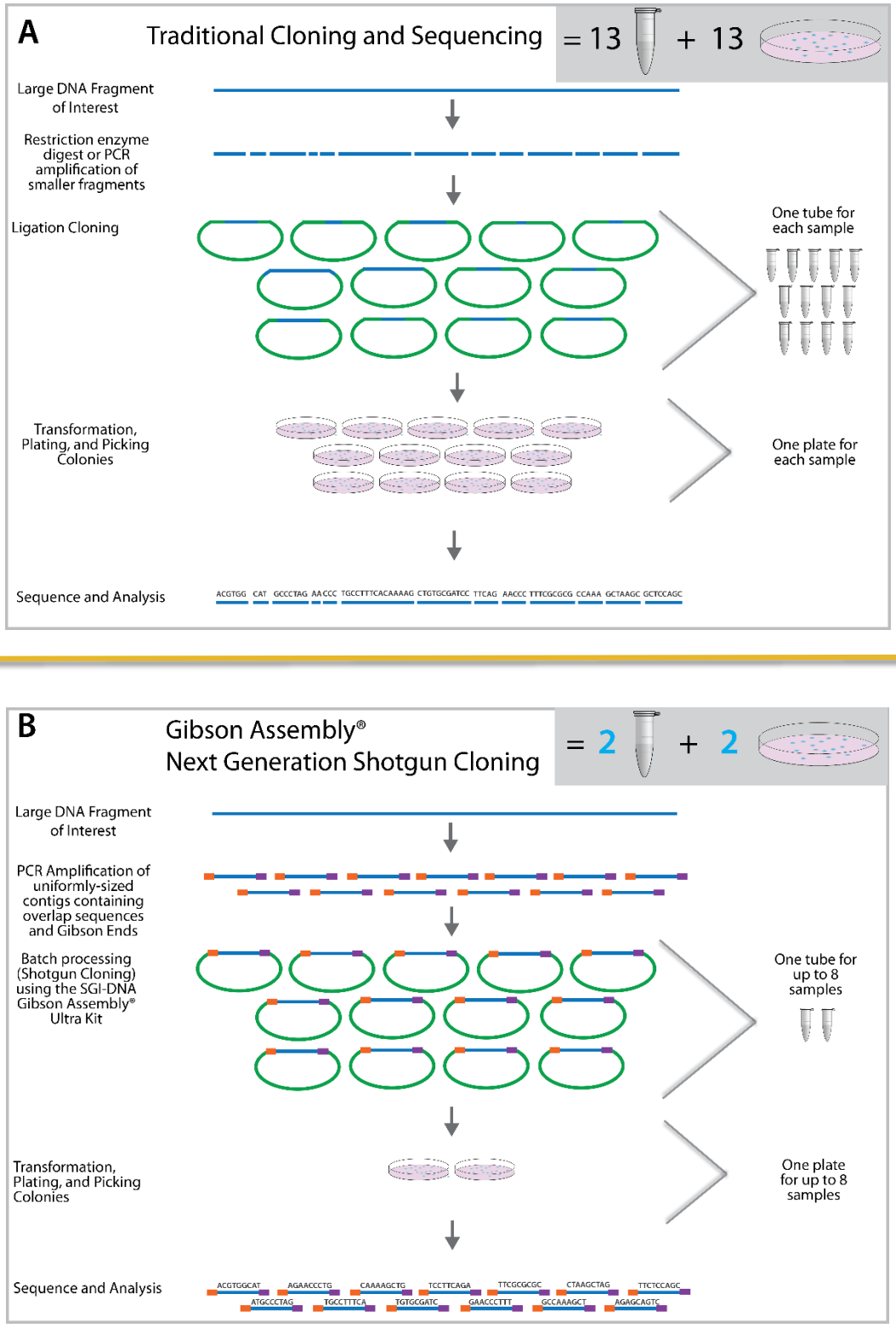

Figure 1. Comparison of (A) Traditional Cloning and Sequencing and (B) Gibson Assembly® Next Generation Shotgun Cloning.

Time and Reagent Savings

Shotgun cloning allows for batch processing of multiple samples simultaneously, which translates into reagent cost and time savings, and a reduction of tedious benchtop manipulations. As shown in *Figure 2*, savings become more pronounced with increasing sample size. For example, processing 96 samples using traditional methods requires processing 96 individual samples through every stage of the workflow. Processing 96 samples in batches of 8, using next generation shotgun cloning with the Gibson Assembly® Method, requires the processing of only 12 batched samples. Figure 2 illustrates the reagent savings per construct number when selecting Gibson Assembly® Shotgun Cloning instead of traditional cloning methods.

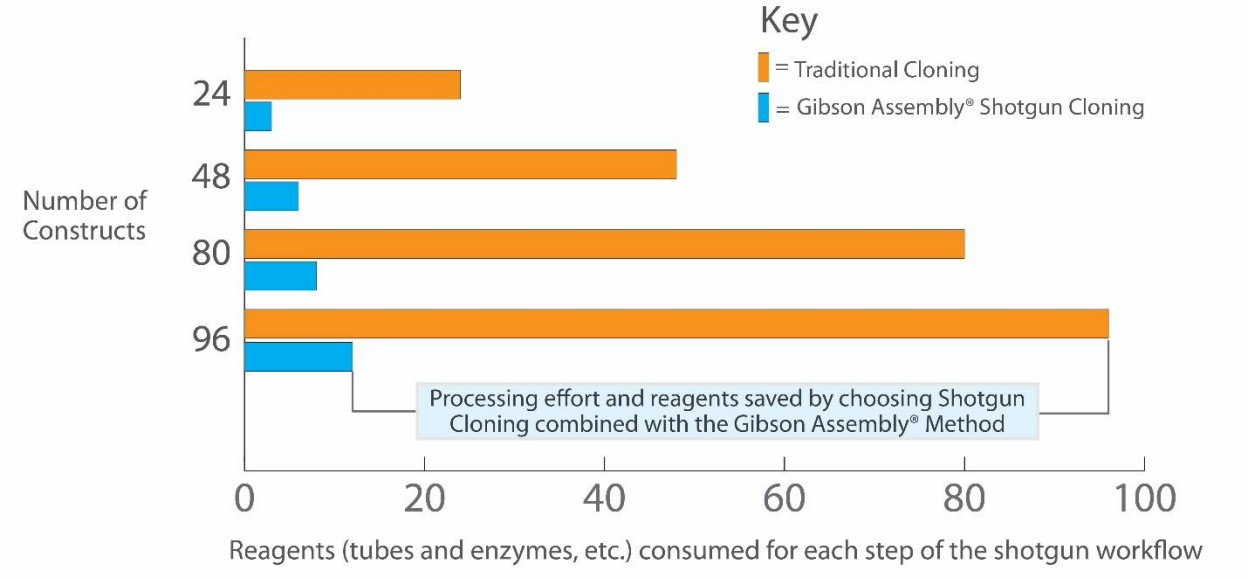

Figure 2. Shotgun Cloning using the Gibson Assembly® Method offers substantial reagent savings and reduced processing effort in comparison to traditional cloning methods.

The Efficiency and Accuracy of the Gibson Assembly® Method – A Case Study

Eliminating time-consuming processing steps for multiple samples translates into reagent and cost savings. However, those savings are only truly beneficial if the methodology used is efficient and accurate, such that sample-reprocessing is avoided. SGI-DNA has combined DNA synthesis using the Gibson Assembly® Ultra Kit with shotgun cloning to achieve highly efficient and accurate results.

Figure 3 shows ~90% first pass recovery from an experiment combing Gibson Assembly® cloning of 95 constructs using the Gibson Assembly® Ultra Kit with next generation shotgun sequencing. For this experiment, 95 constructs were PCR-amplified, pooled according to size, gel-purified, and assembled with the Gibson Assembly® Ultra Kit. Assembled constructs were then transformed into competent cells and 96 colonies from each of the 12 pooled fragment plates were screened by sequencing. The graph shows the percentage of recovered constructs from each pool. The overall first-pass recovery rate for all 95 constructs was 89.5%, and as shown in *Figure 3*, 9 out of the 12 pools demonstrated a 100% success rate. Additionally, the remaining 10 constructs were recovered during a second round of assembly and sequencing, demonstrating a 100% success rate after two rounds of assembly using the Gibson Assembly® Ultra Kit.

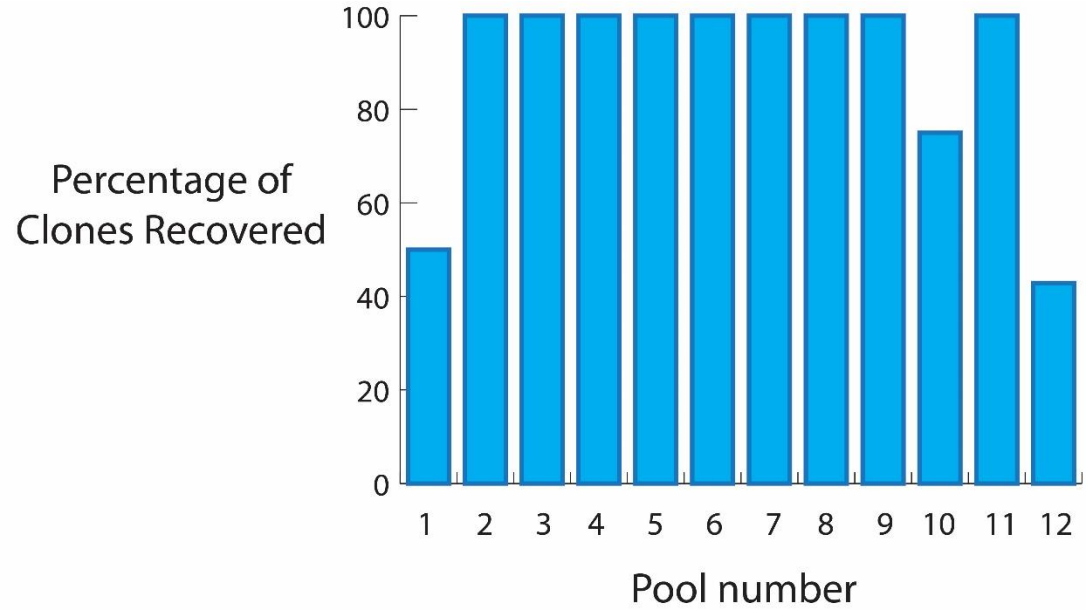

Figure 3. Results demonstrating 89.5% first-pass recovery of 95 constructs combining the Gibson Assembly® Ultra Kit with shotgun cloning.

Conclusion

The Gibson Assembly® method is a powerful and efficient DNA synthesis and cloning tool. SGI-DNA has developed two formats of the assembly method (the HiFi 1 Step Kit and the Ultra Kit), as well as an automated BioXp™ 3200 System for DNA fragment generation. These tools can help advance molecular biology research in many applications, including shotgun cloning, as shown here, but also when combined with other applications as well, including the creation of antibody libraries and genome editing.

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