SYNTHETIC GENOMICS®

DNA library construction using Gibson Assembly®

Since its introduction 6 years ago, the Gibson Assembly method has been widely adopted as a preferred cloning method. Here we describe an application of Gibson Assembly beyond routine cloning: assembly in DNA library construction. Advantages of using Gibson Assembly, specifically the SGI-DNA Gibson Assembly HiFi 1 Step kit, in library construction include speed, efficiency, scarless assembly with vector and versatility.

The seminal manuscript describing the Gibson Assembly method[1] has been cited more than 1,000 times since its publication in 2009 (on average, nearly every other day). Gibson Assembly is faster than traditional cloning, includes fewer steps and reagents, and is scarless. Applications of Gibson Assembly include site-directed mutagenesis, assembly of large DNA fragments (up to 100 kb) and library construction, described in further detail here.

SGI-DNA, a Synthetic Genomics, Inc., company, has developed Gibson Assembly HiFi 1 Step and Ultra kits for assembly and cloning applications. In addition to offering DNA assembly kits, SGI-DNA offers custom DNA services such as synthesis, sequencing, cell engineering and library construction (more information can be found at http://sgidna.com). Key advantages of custom synthesis of gene variant libraries include precise and efficient library design. For quick results or for the construction of complex libraries, custom library synthesis may be the preferred route. Alternatively, for routine DNA library projects, the ideal choice may be to engineer DNA and utilize Gibson Assembly for cloning and screening.

In this Application Note, we describe some of the types of DNA libraries that are optimal targets for Gibson Assembly cloning and screening (**Table 1**), and we give an example of DNA library construction and assembly using the Gibson Assembly HiFi 1 Step kit. The methodology describing the use of Gibson Assembly for library construction may be applied generally for the construction of any DNA library.

Library construction with the Gibson Assembly HiFi 1 Step kit: design

Any gene variant library may be designed for assembly using Gibson Assembly. Homologous overlap regions between library fragments and the vector are essential for assembly. For example, a gene variant library may be constructed for assembly as described in the following paragraphs.

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Table 1 | Examples of Gibson Assembly–compatible gene variant libraries

Library type	Methodology	Uses
Alanine scan	Substitute individual amino acids with alanine at every position	Understand amino acid residues critical to protein function, interaction and shape
Antibody	Introduce targeted mutations in the complementarity determining regions of the variable domains of antibody genes	Create a high-diversity synthetic antibody library or improve existing antibody functionality (i.e., specificity, immunogenicity, affinity, expression or aggregation)
Combinatorial	Examples include site-saturated NNK or NNS libraries where each targeted amino acid is encoded by a degenerate codon	Examine a small region, or multiple small regions, of a protein in combination, instead of examining a single site

Vector preparation: At the site of linearization, identify 20 bases at the 5′ end and 20 bases at the 3′ end to use as homologous overlap regions (**Fig. 1**). Add these sequences to the primers used in PCR amplification of the library template.

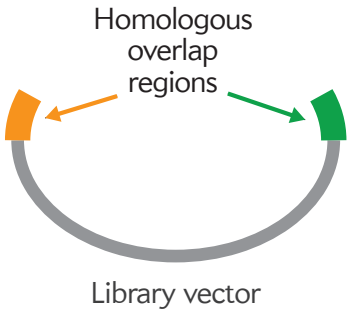

Figure 1 | Library vector. Linearize the library vector and identify 20 bases from each end. Add these 20 nucleotides to the primers used in library amplification to create homologous overlap regions for assembly.

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Library preparation: PCR-amplify the library to introduce the intended variation and simultaneously add homologous overlap regions to the library inserts (**Fig. 2**). After library and vector preparation, library fragments are quickly, conveniently and seamlessly assembled using the Gibson Assembly HiFi 1 Step kit.

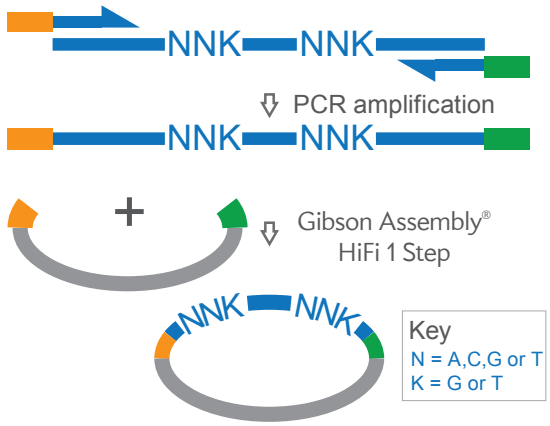

Figure 2 | Library preparation. Amplify the library using primers containing vector overlap, then assemble the amplified library with the vector using the Gibson Assembly HiFi 1 Step kit.

Library construction with the Gibson Assembly HiFi 1 Step kit: example

We have successfully generated various gene variant libraries and assembled library fragments into vectors using the method outlined above. One of the libraries we generated is an NNK library, in which any nucleotide—A, C, G or T—may be present in the first two positions of a targeted codon (N), and only G or T may be present in the third position (K). For our study, we simultaneously targeted two amino acid positions on a 138–base pair fragment. After library amplification, we performed assembly using the Gibson Assembly HiFi 1 Step kit. Library fragments were incubated with vector at 50 °C for only 60 min and then transformed into *Escherichia coli*. Sanger sequences were obtained for 34 clones. Twenty-nine of the 34 sequenced clones contained a perfect sequence and the intended NNK variation (**Fig. 3**). We have successfully applied similar strategies to other types of gene variant libraries described in **Table 1** (data not shown).

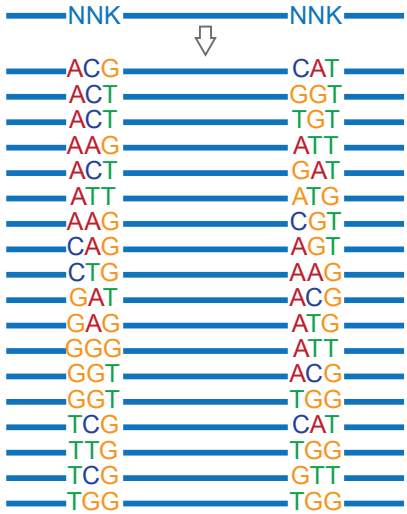

Figure 3 | NNK library-sequence variability for the first 18 screened clones. More than 85% of clones contained a perfect sequence with the expected NNK variation.

Conclusions

- Gibson Assembly is a powerful tool with broad applications beyond routine cloning.
- Gene variant libraries are optimal templates for library cloning using Gibson Assembly.
- We have demonstrated ease of use and successful cloning of NNK library fragments using the Gibson Assembly HiFi 1 Step kit.

Complete product information and additional resources are available at http://www.sgidna.com.

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