Gibson Assembly®

CLONING GUIDE

2ND EDITION

RESTRICTION DIGEST-FREE, SEAMLESS CLONING

Applications, tools, and protocols for the Gibson Assembly® method:

- **Single Insert**
- **Multiple Inserts**
- **Site-Directed Mutagenesis**

SGIDNA
A Synthetic Genomics, Inc. Company

#DNAMYWAY
sgidna.com/gibson-assembly

Foreword

The Gibson Assembly method has been an integral part of our work at Synthetic Genomics, Inc. and the J. Craig Venter Institute (JCVI) for nearly a decade, enabling us to synthesize a complete bacterial genome in 2008, create the first synthetic cell in 2010, and generate a minimal bacterial genome in 2016. These studies form the framework for basic research in understanding the fundamental principles of cellular function and the precise function of essential genes. Additionally, synthetic cells can potentially be harnessed for commercial applications which could offer great benefits to society through the renewable and sustainable production of therapeutics, biofuels, and biobased textiles.

In 2004, JCVI had embarked on a quest to synthesize genome-sized DNA and needed to develop the tools to make this possible. When I first learned that JCVI was attempting to create a synthetic cell, I truly understood the significance and reached out to Hamilton (Ham) Smith, who leads the Synthetic Biology Group at JCVI. I joined Ham's team as a postdoctoral fellow and the development of Gibson Assembly began as I started investigating methods that would allow overlapping DNA fragments to be assembled toward the goal of generating genome-sized DNA. Over time, we had multiple methods in place for assembling DNA molecules by *in vitro* recombination, including the method that would later come to be known as Gibson Assembly.

What we were attempting was simply not possible with restriction enzyme / ligation-based cloning and other technologies. Since the development and implementation of the Gibson Assembly method, I no longer use traditional restriction enzyme-based cloning. There simply are no benefits to using restriction methods for gene assembly. Gibson Assembly is faster and more robust. With the commercialization of Gibson Assembly by SGI-DNA, this technology is readily available to all research labs. Previously, a major technical bottleneck was in obtaining a large construct. Now, anyone has the ability to build large DNA constructs. If you can easily build constructs 100 kb in size that can constitute entire biological pathways or even an entire bacterial genome, it changes your approach. The question is no longer how, but what, to build.

This guide contains useful information for new and experienced Gibson Assembly users alike, compiling some of the uses and downstream applications of the Gibson Assembly method and providing an overview and technical resource for the field of synthetic biology. Included are historical perspectives and overviews of some recent uses of Gibson Assembly cloning in the literature. Protocols, tips, and FAQs in this guide will assist users in experimental design and maximize opportunities for success. A section of this guide walks users through SGI-DNA's free primer design tool to ensure simple and optimal primer design. Additionally, a recently developed variation presented in this guide, Gibson Assembly Primer-Bridge End (PBnJ) Cloning, enables users to assemble fragments without homologous overlaps, adding to the flexibility of the method.

Ultimately, Gibson Assembly is a tool. It is the implementation of that tool that opens the door of innumerable possibilities.

Daniel G. Gibson, Ph.D.
Vice President, Synthetic Genomics, Inc.
Associate Professor, J. Craig Venter Institute
Inventor of Gibson Assembly
La Jolla, CA

Contents

Historical Perspective

Key Discoveries Enabling Molecular Cloning

1949–1977

	Key Discoveries & Breakthroughs	Notable Awards & Commercial Ventures	Key Authors
1949	**Bacterial host "Restriction" described**	**Nobel Laureates:** Salvador Edward Luria and Renato Dulbecco	*Salvador Edward Luria and Renato Dulbecco* [1]
1963	**Demonstration of joining DNA by cohesive sites**		*Allan Campbell* [2]
1972	**Generation of recombinant DNA**	**Nobel Laureate:** Paul Berg	*David Jackson, Robert Symons, and Paul Berg* [3]
1972	**First synthesis of a complete gene**	**Nobel Laureate:** Har G. Khorana	*Har G. Khorana et al* [4]
1974	**Eukaryotic genes cloned into bacteria**	**Co-founder of Genentech:** Herbert Boyer	*John F. Morrow,... Herbert Boyer et al* [5]
1975	**Isolation of restriction enzymes**	**Nobel Laureates:** Daniel Nathans and Hamilton O. Smith	*Daniel Nathans and Hamilton O. Smith* [6]
1977	**Improved DNA sequencing techniques**	**Nobel Laureates:** Walter Gilbert and Frederick Sanger	*Allan M. Maxam and Walter Gilbert* [7]*; Frederick Sanger et al* [8]
1977	**Discovery of RNA splicing**	**Nobel Laureates:** Richard J. Roberts and Phillip A. Sharp	*Susan M. Berget, Claire Moore, & Phillip A. Sharp* [9]*; Louise T. Chow et al.... Richard J. Roberts* [10]

Figure 1. (A) Key Discoveries Enabling Molecular Cloning, 1949–1977.

Historical Perspective

Key Discoveries Enabling Synthetic Biology

1987–2016

	Key Discoveries & Breakthroughs	Notable Awards & Commercial Ventures	Key Authors
1987	PCR described	**Nobel Laureate**: Kary B. Mullis	*Kary B. Mullis & Fred A. Faloona*[11]
1995	First complete sequence of a free-living organism	**Co-founders of Synthetic Genomics, Inc.:** J. Craig Venter and Hamilton O. Smith	*Robert D. Fleischmann et al.... Hamilton O. Smith, J. Craig Venter*[12]
2001	First draft of human genome sequence		*John D. McPherson, et al.*[13] *Eric S. Lander, et al.*[14] *J. Craig Venter, et al.*[15]
2008	First NGS-sequenced human genome	**Founder of 454 Life Sciences Corporation:** Jonathan M. Rothberg	*David A. Wheeler et al.... Jonathan M. Rothberg*[16]
2008	DNA Synthesis of complete bacterial genome		*Daniel G. Gibson et al.*[17]
2009	Gibson Assembly® cloning developed	**Head of DNA Technology, Synthetic Genomics, Inc.:** Daniel G. Gibson	*Daniel G. Gibson et al.*[18]
2010	Creation of first synthetic bacterial cell	**The J. Craig Venter Institute, at the forefront of Genomics Discovery**	*Daniel G. Gibson et al.*[19]
2016	Minimal genome design and synthesis		*Clyde A. Hutchison III et al... Daniel G. Gibson, J. Craig Venter*[20]

Figure 1. (B) Key Discoveries Enabling Synthetic Biology, 1987–2016.

Gibson Assembly® Cloning

Overview

The Gibson Assembly® method is a cloning procedure that allows the cloning of two or more fragments without the need for restriction enzyme digestion or compatible restriction sites. Instead, user-defined overlapping ends are incorporated into the fragments to allow the seamless joining of adjacent fragments.

This innovative approach to creating both simple and complex constructs was first published in 2008 by Daniel Gibson and colleagues[17]. Since that time, the Gibson Assembly® method has been cited in over 3000 peer-reviewed publications. Due to its many advantages over traditional restriction enzyme cloning, Gibson Assembly® cloning is rapidly becoming the preferred method for cloning DNA into plasmids and bacterial artificial chromosomes (BAC) in many laboratories.

The Gibson Assembly® method can be used to rapidly clone multiple DNA fragments into any vector in one hour or less without the use of restriction enzymes. By designing DNA fragments with homologous overlapping ends, users of the Gibson Assembly® method can create DNA constructs in a single round of cloning. The method is initiated by combining DNA fragments with the Gibson Assembly® Master Mix. The master mix enzyme cocktail mediates strand chew back, exposing a single strand which allows for annealing of the terminal homologous overlap sequences. Annealing of the homologous overlap sequences is followed by extension and ligation to yield an assembled product (see Figure 2 on page 7). Seamless assembly can be readily applied to both routine cloning and large and complex cloning projects.

Types of Gibson Assembly® Kits

Daniel Gibson and his team at SGI-DNA have further refined and optimized the Gibson Assembly® process resulting in three different types of Gibson Assembly® Kits:

- **Gibson Assembly® HiFi 1-Step Cloning Kit, available as a 2X or 4X master mix**
- **Gibson Assembly® Ultra Cloning Kit**
- **Gibson Assembly® Site-Directed Mutagenesis Kit**

The HiFi and Ultra kits are ideal for the assembly of plasmids and BAC constructs. While both kits enable the cloning of multiple fragments, the numbers of fragments, the recommended sizes of the fragments, and the specifics of the workflow are different.

The **Gibson Assembly® HiFi 1-Step** method allows for the assembly of up to 5 different fragments ranging from 500 bp to 32 kb using an isothermal process. As implied by the name, the HiFi 1-Step process is performed in a single step. The Gibson Assembly® HiFi 1-Step Kit is available in two formats– a 2X master mix or a 4X high concentration (HC) master mix. The 4X **Gibson Assembly HiFi HC 1-Step** kit requires only 2.5 µL of master mix per 10 µL reaction making it ideal for assemblies using less concentrated DNA samples. An overview of the HiFi 1-Step method with either the 2X or 4X format is shown on the left side of Figure 2 on page 7. To perform the HiFi 1-Step method, fragments and a vector with appropriate overlapping ends are combined with the master mix and incubated at 50°C. After 1 hour of incubation, the fully ligated constructs are ready to transform into competent cells.

The **Gibson Assembly® Ultra** kit is optimal for more complex constructs (up to 15 fragments) or for constructs incorporating fragments from 100 bp to 100 kb. An overview of this method is shown on the right side of Figure 2 on page 7. The Ultra kit utilizes a robust two-step reaction that requires two separate additions of master mix and different incubation temperatures. While the Gibson Assembly® Ultra process requires additional hands-on time and has slightly longer incubation times than the Gibson Assembly® HiFi 1-Step process, some researchers prefer the Ultra approach because of its robustness and its ability to accommodate a wide range of fragment sizes. A comparison of the two workflows is shown in Figure 2. Differences between the specifications for each kit are shown in Table 1 on page 9.

Gibson Assembly® HiFi 1-Step and Ultra Kits Overview

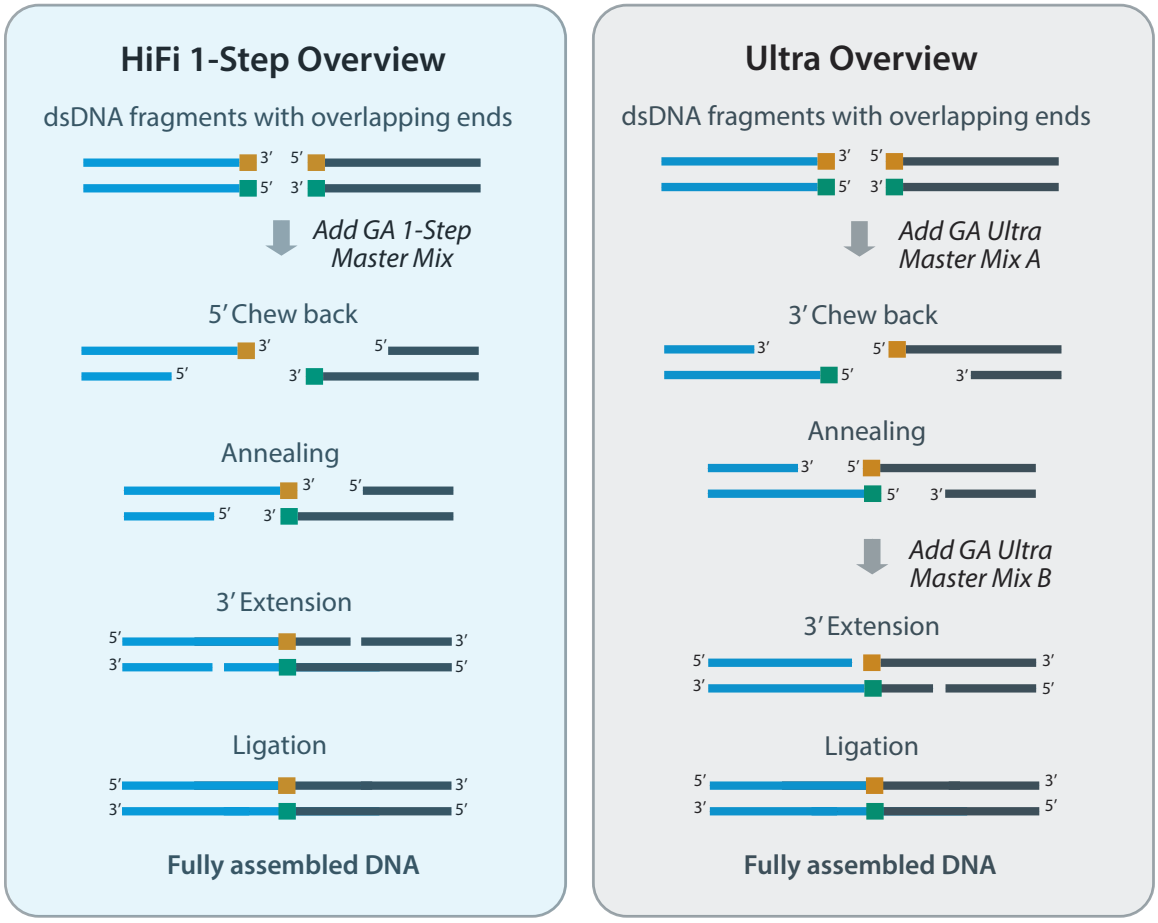


Figure 2. Overview of the Gibson Assembly® Cloning Methods. DNA fragments containing homologous overlapping ends are assembled in 60 minutes with the HiFi 1-Step Kit or in 80 minutes with the Ultra Kit.

Gibson Assembly® Site-Directed Mutagenesis Kit

SGI-DNA has developed a Gibson Assembly® Site-Directed Mutagenesis (SDM) kit to enable the incorporation of multiple targeted mutations into a construct. Using this single kit solution, it is possible to add any combination of substitution, insertion, or deletion mutations in a single round of mutagenesis and cloning. Starting with either linear or circular DNA templates, single base pair point mutations, insertions of up to 40 bp, or deletions of any size can be made. The inherent flexibility of this approach lends itself to small and large constructs encompassing both single and multiple insert assemblies of fragments from 500 bp to 5.5 kb.

Site-directed mutagenesis is a two-part protocol. First, using appropriately designed primers, targeted changes are made and overlaps for Gibson Assembly® cloning are generated through PCR amplification. Second, fragments with specific insertions, deletions, or substitutions are joined using the Gibson Assembly® process. To simplify the design of PCR primers to generate mutated fragments containing homologous overlapping ends, the SGI-DNA SDM Primer Design Tool is freely available. Visit **sgidna.com/sdm-tool** to design primers for your mutagenesis experiment.

Make Multiple Targeted Mutations with Gibson Assembly® SDM

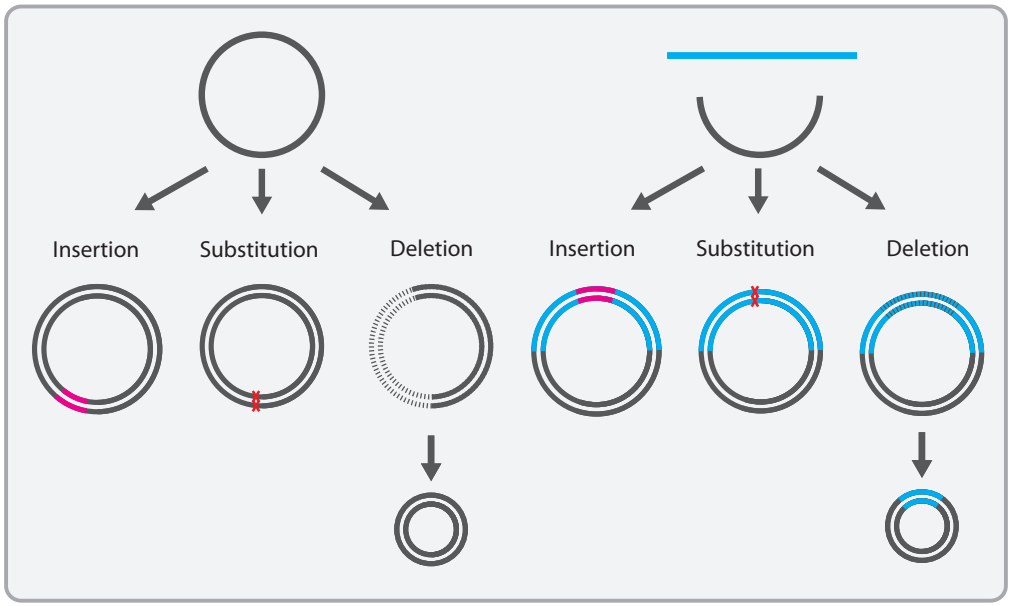


Figure 3. The Gibson Assembly® Site-Directed Mutagenesis Kit is a one kit solution for a wide variety of site-directed mutagenesis applications. Starting material may be a circular vector containing a gene-of-interest or a linear DNA fragment that will be cloned into a vector during assembly.

Features of the Gibson Assembly® Cloning Kits

Feature	HiFi 1-Step Kit	Ultra Kit	Site-Directed Mutagenesis Kit
Hands-on time	<5 minutes	<6 minutes	<40 minutes
Total reaction time	60 minutes	80 minutes	~4–6 hours
Number of steps	1	2	3
Fragment size range	500 bp to 32 kb	100 bp to 100 kb	500 bp to 5.5 kb
Cloning efficiency	>90%	>95%	95% single site 55% five site
Fragments per reaction	up to 5	up to 15	up to 5
Maximum construct size	100 kb (multi-stage reactions)	1000 kb (multi-stage reactions)	25 kb (multi-stage reactions)

Table 1. Gibson Assembly kits selection chart. Both the Gibson Assembly® HiFi 1-Step and Gibson Assembly® Ultra methods enable the cloning of multiple fragments. The Gibson Assembly® Site-Directed Mutagenesis kit allows multiple mutations to be made in a single round of cloning.

Applications

The Gibson Assembly® method may be leveraged for numerous applications, including assembly of single fragments, multiple fragments, site-directed mutagenesis, library construction, and shotgun cloning. These applications are discussed in the following sections.

Simple Cloning: One insert with one vector

To perform Gibson Assembly® cloning, dsDNA fragments with 20 to 40 bp overlapping ends are generated. The insert(s) and vector DNA are combined with Gibson Assembly® reagents and incubated. During incubation, the Gibson Assembly® reagents mediate the generation of compatible ends, annealing, extension, and ligation to create a fully assembled, seamless DNA construct.

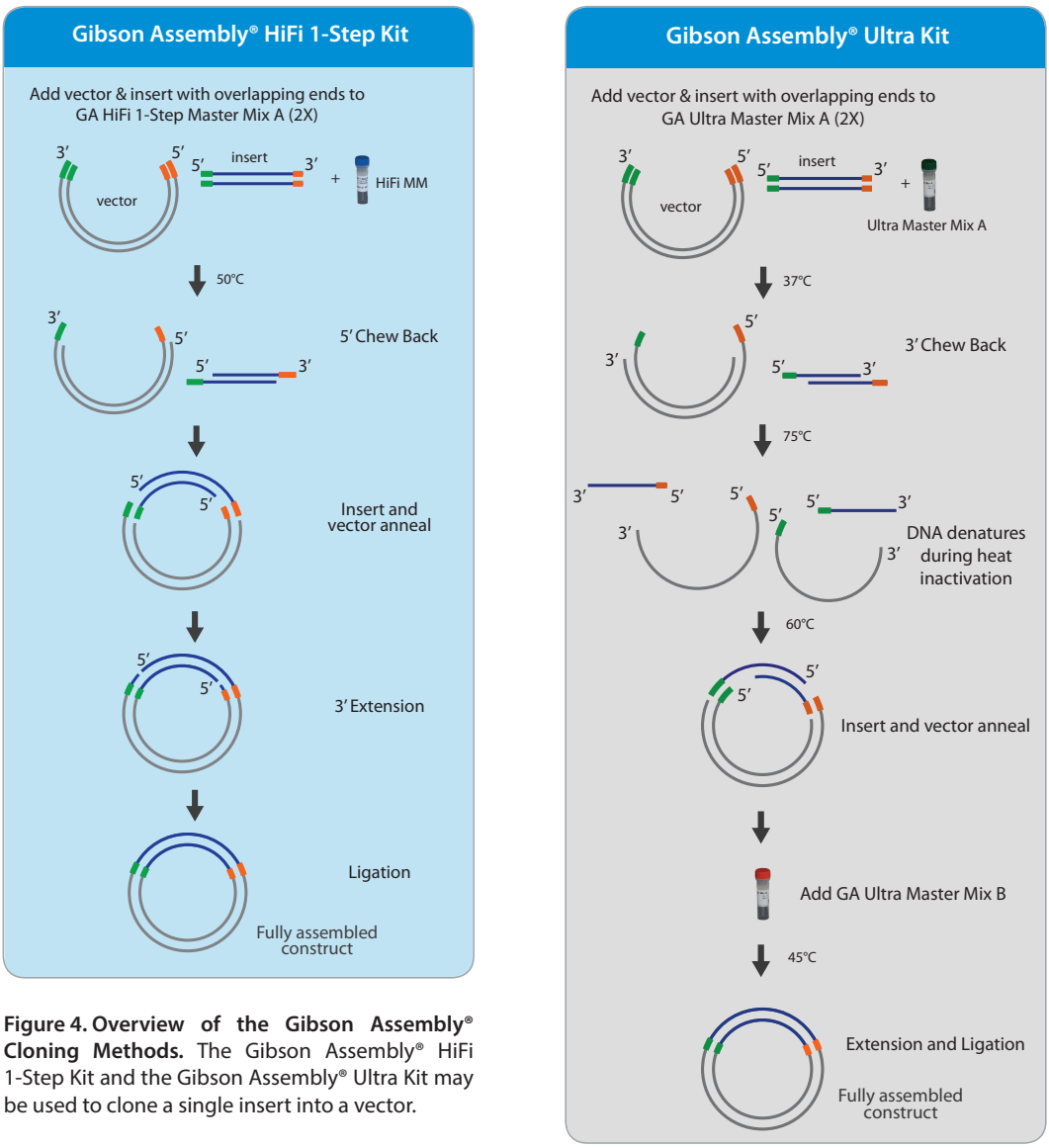


Figure 4. Overview of the Gibson Assembly® Cloning Methods. The Gibson Assembly® HiFi 1-Step Kit and the Gibson Assembly® Ultra Kit may be used to clone a single insert into a vector.

Assembly of Multiple Fragments

The Gibson Assembly® method can be used for the simultaneous assembly of multiple inserts and offers substantial time savings for these types of projects, which typically require multiple rounds of traditional restriction enzyme digest-based cloning. Cloning multiple fragments using traditional cloning methods can prove challenging. With restriction enzyme-based cloning, identifying appropriate enzyme recognition sites may not be possible, necessitating multiple subcloning steps. Cloning blunt-end fragments generated by PCR or restriction enzyme digestion is especially inefficient and may require additional screening and selection to confirm the insert orientation. In addition, with multi-fragment restriction enzyme cloning, the ligation of large and small fragments in a single reaction is often inefficient, favoring the ligation of smaller fragments. The Gibson Assembly® method overcomes these challenges through the efficient assembly of multiple fragments of varying sizes in a single round of cloning, generated without reliance on compatible restriction enzyme recognition sites.

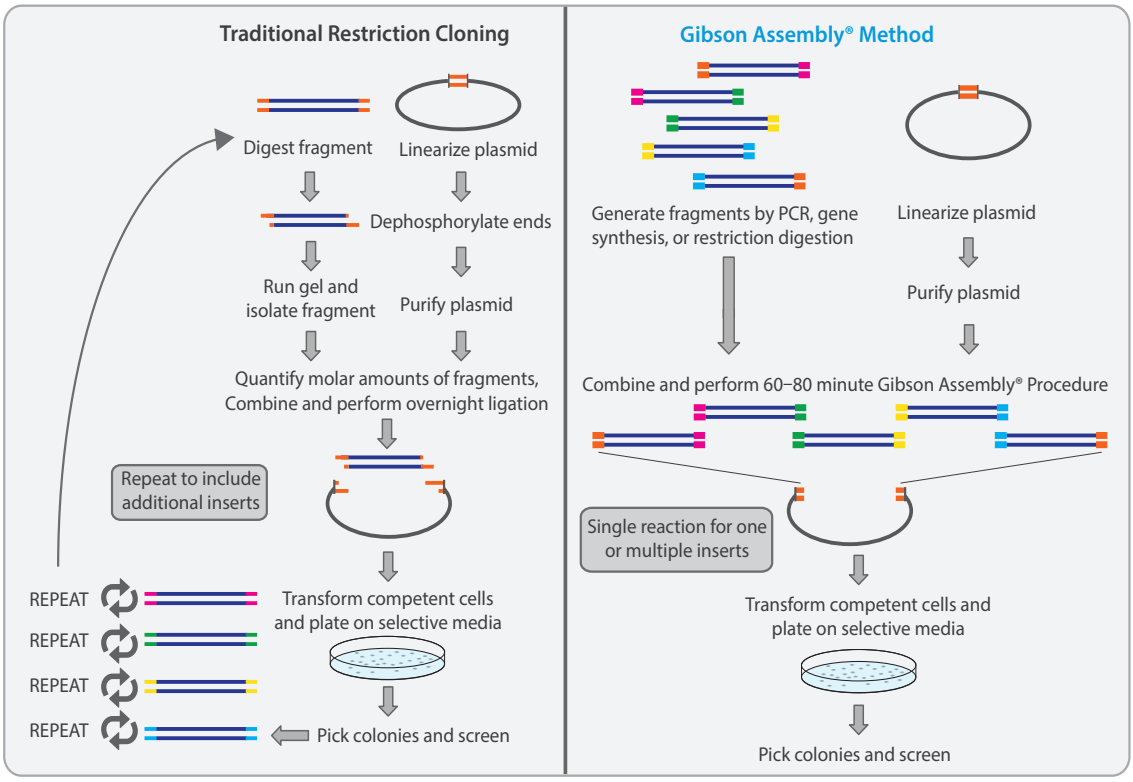


Figure 5. The Gibson Assembly® method is faster and more efficient than traditional cloning. Traditional restriction cloning using compatible restriction endonucleases requires 1–2 days of preparative steps to generate cloning ends on the insert and plasmid. Typically, only one insert can be ligated into the plasmid at a time. The Gibson Assembly® method allows for several inserts to be simultaneously assembled in a single reaction that takes approximately 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.

Site-Directed Mutagenesis

Site-directed mutagenesis (SDM) allows for specific, intentional, targeted changes to DNA sequences. These DNA alterations can be designed to impact the regulatory elements of a gene, RNA intermediates, or encoded proteins. SDM studies allow researchers to examine the biological activities of nucleic acids and proteins and are often used for protein engineering. For gene function studies, SDM allows for a targeted, rational approach. Similarly, rationally designed proteins generated by SDM can be engineered to have improved or unique properties that make the engineered protein suitable for a specific application. For laboratories interested in generating point mutations, insertions, or deletions, the Gibson Assembly® SDM kit is a "one kit solution" providing a rapid and highly efficient approach to incorporate multiple targeted mutations in both circular and linear templates.

Gibson Assembly® Site-Directed Mutagenesis of a Circular Template

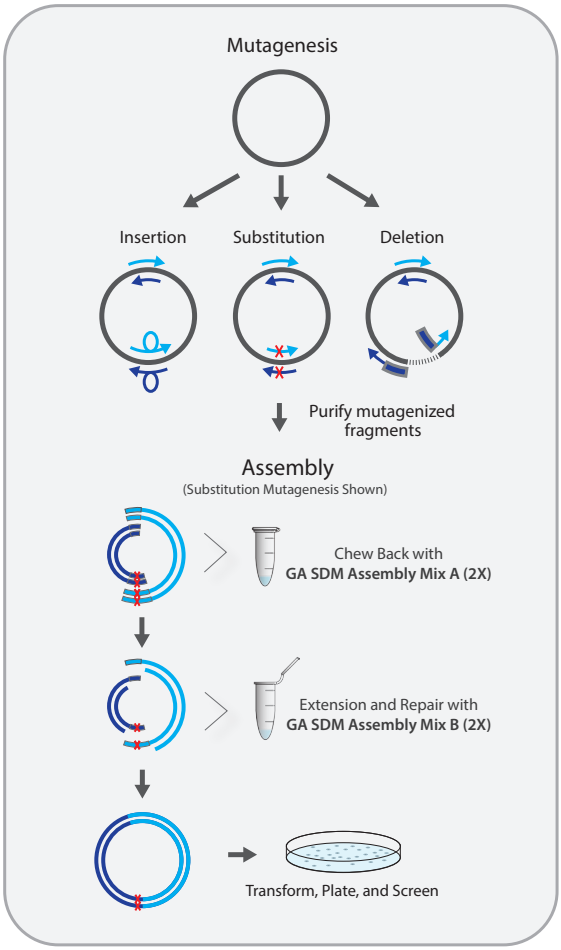


Figure 6. Overview of Gibson Assembly® SDM of a circular template. Following mutagenesis, DNA fragments of various lengths are uniformly assembled using complementary overlaps between fragments.

sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

Gibson Assembly® SDM of a Linear Template

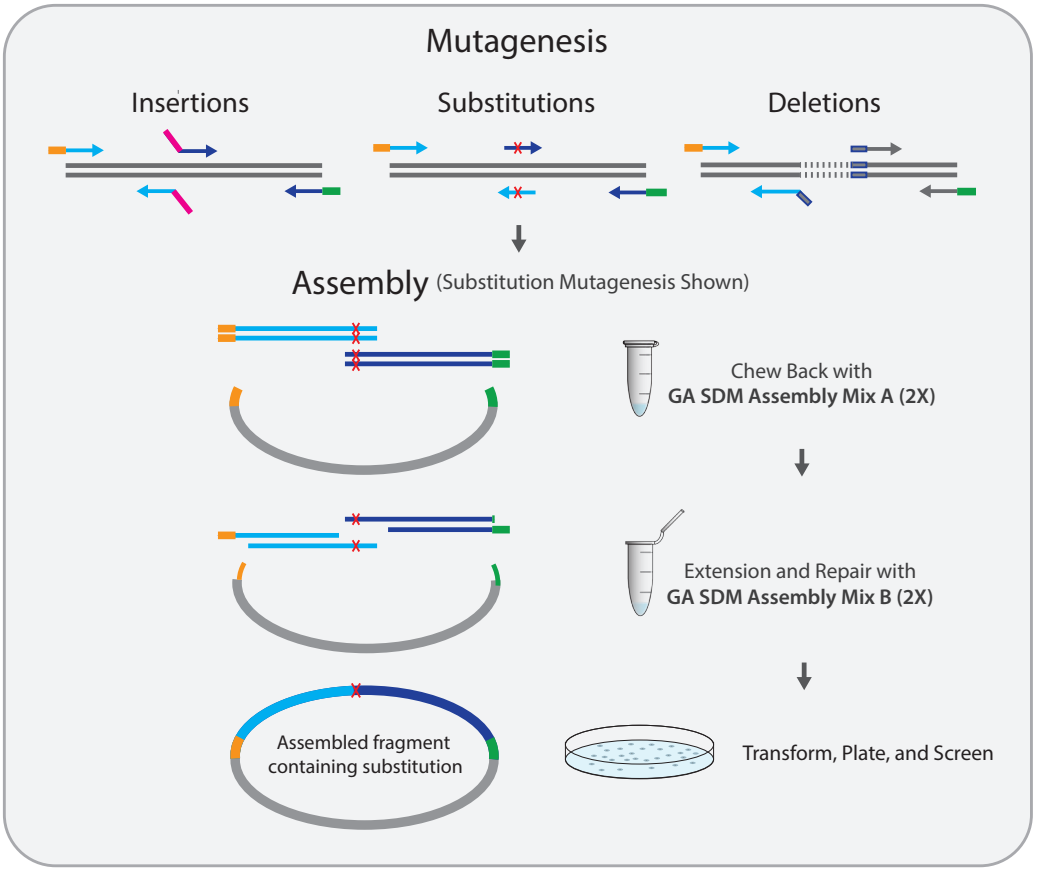


Figure 7. Overview of Gibson Assembly® Site-Directed Mutagenesis of a linear template. Following mutagenesis, DNA fragments of various lengths are uniformly assembled using complementary overlaps between fragments.

Using the Gibson Assembly® Method for Library Construction

The highly efficient and robust Gibson Assembly® method is ideal for library construction applications. Examples of Gibson Assembly®-compatible libraries include, but are not limited to, NNK libraries for scanning functional motifs or combinatorial site libraries for testing genetic elements.

To prepare a library vector for Gibson Assembly® cloning:

1. Linearize the vector and identify 20–40 bases from each end.
2. Add these 20–40 nucleotides to the primers used for library amplification, creating homologous overlap regions for assembly.
3. PCR-amplify the library to introduce the intended variation and add homologous overlap regions to the library inserts.

   Note: Alternatively, a pool of custom synthesized double-stranded DNA fragments (*e.g.,* **DNA Tiles™** error-corrected dsDNA fragments) with compatible ends can be used. These fragments are ready for library construction and can be cloned directly into the library vector, thereby avoiding steps 2 and 3.
4. Seamlessly assemble the prepared library with vector using Gibson Assembly® Cloning.

Library Vector and Insert Preparation for Gibson Assembly® Cloning

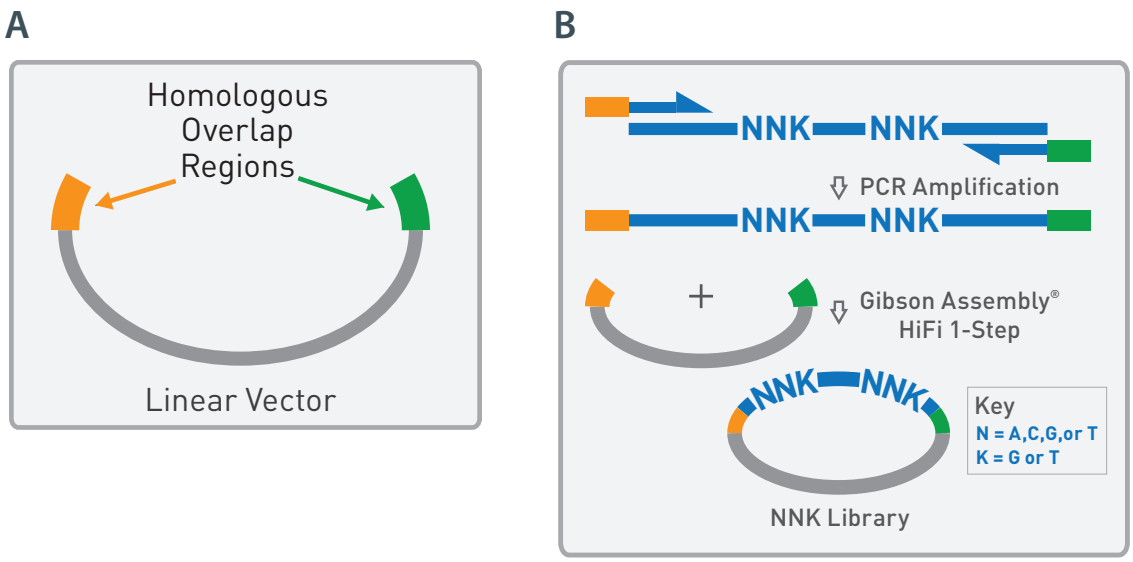


Figure 8. Preparing the Library Vector (A) and Library Insert (B) for Gibson Assembly® Cloning.
(A) To prepare the library vector, linearize the library vector and identify 20 bases from each end. Add these 20–40 nucleotides to the primers used in library amplification to create homologous overlap regions for assembly. **(B)** To prepare the library for Gibson Assembly® Cloning, amplify the library using primers containing vector overlap. Assemble the amplified library with the vector using the Gibson Assembly® HiFi 1-Step kit.

Using the Gibson Assembly® Method for Shotgun Cloning

Shotgun cloning is another high-throughput cloning application that makes use of multiple DNA fragments with the same homologous ends to simultaneously clone a pooled mixture of heterogeneous DNA fragments with a single vector. Combining a single round of 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.

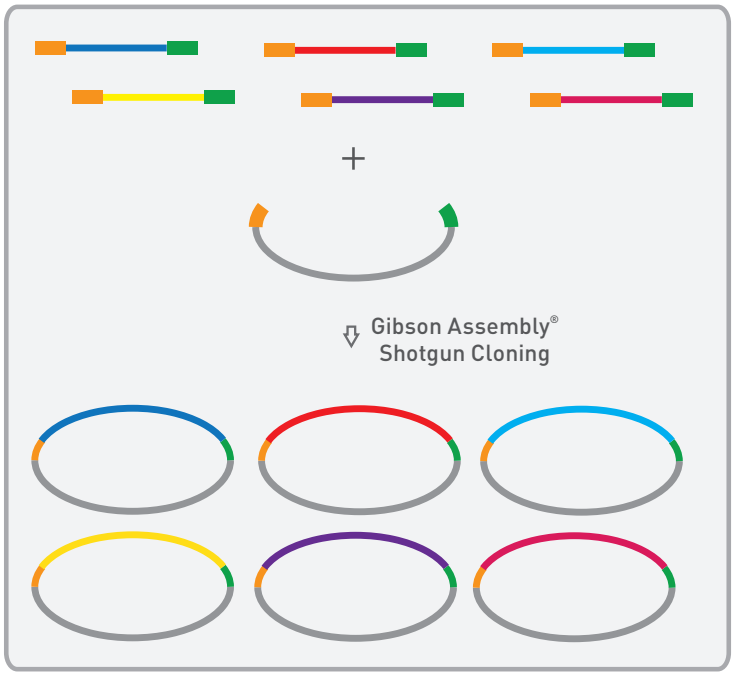


Figure 9. Gibson Assembly® Shotgun Cloning. Similar sized DNA fragments may be shotgun cloned in parallel. In order to allow for assembly, DNA fragments can be synthesized or PCR amplified with primers containing regions of homology to a single cloning vector. Following PCR amplification, DNA fragments contain termini with homologous overlaps to the vector, allowing for seamless and efficient cloning using the Gibson Assembly® method.

Advantages of Gibson Assembly® Cloning

Gibson Assembly® Method Advantages

Feature	Advantage
Faster than traditional cloning—assembly in 60–80 minutes	Saves time over traditional cloning methods. Gibson Assembly® cloning can be completed: • In a single, 1-hour isothermal incubation with the Gibson Assembly® HiFi 1-Step Kit or the Gibson Assembly® HiFi HC 1-Step Kit. • In a robust, two-step, 80-minute reaction with the Gibson Assembly® Ultra Kit.
No need to rely on compatible restriction sites	• Join virtually any two fragments. • Simplifies construct design by removing the requirement for compatible restriction enzyme recognition sites.
Efficient, single-round cloning of one or many fragments	Seamless, multi-fragment, directional cloning helps avoid the need for multiple rounds of cloning and screening. • One to 5 fragments may be cloned at once using the Gibson Assembly® HiFi 1-Step isothermal, single-tube method. • One to 15 fragments may be simultaneously cloned using the highly robust Gibson Assembly® Ultra two-step approach.
Can be performed with nanogram quantities of input DNA	Build constructs when only limited amounts of starting material are available.
Clone fragments from 100 bp to 100 kb	Capable of assembling a wide range of fragment sizes for simple and complex cloning projects.
High cloning efficiencies	Obtain larger numbers of colonies with full-length clones.
Proof-reading polymerase included in the master mix	Minimizes incorporation errors at cloning junctions to yield a high percentage of error-free constructs.

Table 2. Gibson Assembly® advantages.

Gibson Assembly® Method and Other Cloning Approaches

Cloning Method Comparison

Type of cloning	Gibson Assembly® Kit	Restriction enzyme digest	T/A (PCR fragment cloning)	Recombination-based (Gateway™ cloning)	Type IIS (Golden Gate)	Ligation independent cloning (LIC)
Methodology	Relies on homologous overlap sequences	Restriction enzyme digestion	Single base overhang	Requires vectors and inserts with recombination sequences (att sites)	Type IIS restriction enzymes cut outside recognition site	T4 DNA polymerase 3'→5' exonuclease overhang creation
Efficiency	>90%	Variable	Variable	Up to 95%	Variable	Variable
Insertion site after cloning	Seamless	May leave a scar or seam	Single base insertion	att sites remain as part of insert/ construct	Seamless	Seamless
Clone multiple fragments in a single round?	Yes	No	No	Yes (limited)*	Yes (limited)	Yes (limited)
Use any vector?	Yes	Yes	No	Yes (att vectors only)†	Yes	Yes
Directional?	Yes	Experiment dependent	No	Yes, with appropriate vector	Experiment dependent	Yes

* A multi-fragment approach is possible, with limitations. For example, three fragment cloning requires a separate kit and protocol. Cloning with more than 3 fragments is not possible using this method.

† It is possible to convert other vectors to Gateway™ vectors, with vector modification. Non-Gateway™ vectors must be modified in order to be compatible with the Gateway™ system.

Table 3. Gibson Assembly® Kits offer many advantages over other cloning approaches.

Designing Homologous Overlaps

Primer Design

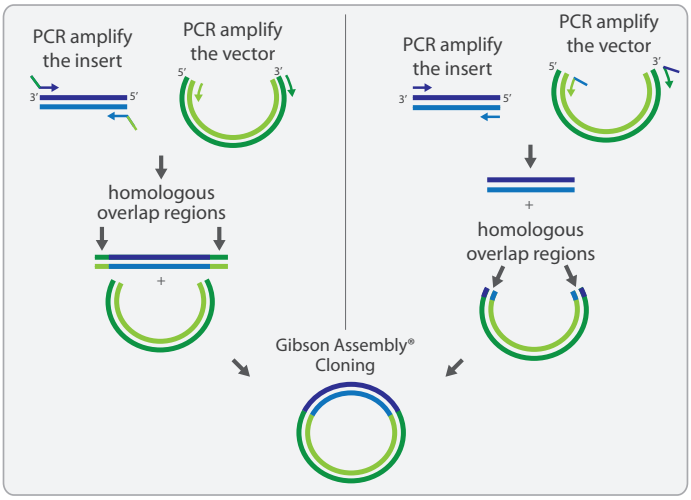
Designing optimal homologous overlap regions for Gibson Assembly® cloning is critical to a successful assembly reaction. This section covers the basics of Gibson Assembly® primer design and should be helpful as you create your Gibson Assembly® design strategies. The free online tool at **sgidna.com/gibson-assembly-primers** is also available to assist with primer design. More information about the online tool can be found on page 20.

To assemble an insert with a vector, a 20–40 bp homologous region must be added to the ends of a standard-sized fragment. The sequence of this homologous region can correspond to the vector (by adding overlap to insert as shown in the left panel of Figure 10) or the insert (by adding overlap to vector as shown in the right panel of Figure 10). Alternatively, the overlap can be split between the vector and insert ends (not shown). If multiple inserts are being added to the vector, overlapping ends are incorporated into each adjacent fragment.

Design Strategies for Gibson Assembly® Seamless Cloning

Inserts with overlapping ends can be generated by PCR as shown in Figure 10, below. Alternatively, overlapping double-stranded error-corrected fragments can be made synthetically by a service provider, such as SGI-DNA (e.g., **DNA Tiles™**).

Add overlap to the insert	Add overlap to the vector
If PCR amplifying the insert is preferred, add sequence homologous to the vector to the ends of the insert	If PCR amplifying the vector is preferred, add sequence homologous to the insert to the ends of the linearized vector



When to Use	When to Use
• For small inserts (< 2 kb) • Using one vector for cloning different inserts, such as shotgun cloning or preparing combinatorial libraries	• For small vectors (< 8 kb) • When inserts are large or difficult to amplify • Using one insert to shuttle between different vectors

Figure 10. Adding homologous overlap regions to inserts or vectors prior to Gibson Assembly® cloning. Homologous overlaps may be added to the insert (shown on the left panel) or to the vector (shown on the right panel) or split between the insert and vector (not shown).

sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

Tips for Designing Primers to Generate Homologous Overlaps

Careful primer design is required to properly prepare DNA fragments for assembly. For optimal primer design, use the following tips and guidelines.

- The ideal length of the overlap region depends on the number and length of the fragments in the assembly reaction (see Table 4, below).
- For higher order assembly, longer overlap regions will result in higher efficiency.
- Avoid tandem repeats, homopolymers, high secondary structure, and GC content below 40% or above 60%.
- It may be necessary to optimize PCR amplification reactions when using PCR primers with long homologous overlap regions.
- Adding a restriction enzyme site to the primers between the overlap region and the sequence-specific segment enables subsequent release of the insert from the vector. In this case, be certain that the restriction enzyme site introduced in the primers is not also present within the insert.

Suggested Length of the Overlap Region: Inserts

Number of Inserts	Insert Size							
	0.1–0.5 kb	0.5–2 kb	2–5 kb	5–8 kb	8–10 kb	10–20 kb	20–32 kb	32–100 kb
1	20 bp	30 bp	30 bp	40 bp	40 bp	80 bp	80 bp	80 bp
2	30 bp	30 bp	40 bp	40 bp	40 bp	80 bp	80 bp	80 bp
3	40 bp	40 bp	40 bp	40 bp	40 bp	80 bp	80 bp	—
4	40 bp	40 bp	40 bp	40 bp	40 bp	80 bp	—	—
5	40 bp	40 bp	40 bp	40 bp	40 bp	—	—	—
6	40 bp	40 bp	40 bp	40 bp	40 bp	—	—	—
7	40 bp	40 bp	40 bp	40 bp	—	—	—	—
8	40 bp	40 bp	40 bp	40 bp	—	—	—	—
9	40 bp	40 bp	40 bp	—	—	—	—	—
10	40 bp	40 bp	40 bp	—	—	—	—	—
11	40 bp	40 bp	40 bp	—	—	—	—	—
12	40 bp	40 bp	40 bp	—	—	—	—	—
13	40 bp	40 bp	—	—	—	—	—	—
14	40 bp	40 bp	—	—	—	—	—	—
15	40 bp	40 bp	—	—	—	—	—	—

Key

Gibson Assembly® HiFi 1-Step Kit Recommended

Gibson Assembly® Ultra Kit Recommended

— = Not recommended

Table 4. Suggested kits and overlap lengths for different numbers of inserts and sizes.

Online Tool for Designing Gibson Assembly® Primers

The Gibson Assembly® Primer Design Tool (GAP Tool) is a free online tool developed to assist in designing primers for the assembly of DNA fragments. Primers designed with the online tool can be used for DNA assembly with the Gibson Assembly® HiFi 1-Step Kit and the Gibson Assembly® Ultra Kit. For assistance with mutagenesis primer design for the Gibson Assembly® Site-Directed Mutagenesis kit, a separate tool is available at **sgidna.com/sdm-tool**.

Using the Gibson Assembly® Primer Design Tool

The Gibson Assembly® Primer Design Tool is available at
sgidna.com/gibson-assembly-primers

To use the GAP Tool, input the vector and fragment sequences and indicate how DNA fragments will be prepared. DNA fragments can be prepared using PCR amplification, restriction digestion, or synthesis (e.g., SGI-DNA **DNA Tiles™**). The GAP Tool designs primers that add 30 to 40 base pairs of homologous sequence at the end of adjacent fragments. Output primer sequences from the tool can be used to amplify fragments for assembly into circular constructs using the Gibson Assembly® method. The homologous sequences allow for scarless DNA assembly using the Gibson Assembly® HiFi 1-Step and Ultra Kits.

Fragment Properties for GAP Tool Use

Although the Gibson Assembly® method can be used to assemble DNA fragments from 100 bp to 100 kb, the GAP Tool has been optimized for use with DNA fragments up to 10 kb. For assistance with the assembly of products larger than 10 kb or to design primers for complex assembly reactions, contact technical services at **techservices@sgidna.com**.

For fragments <5.5 kb, the GAP Tool is capable of generating primers for 1–15 fragments. For fragments >5.5 kb, optional subassembly primers will be generated by the GAP Tool to enable efficient PCR amplification. For assistance with primer design for the assembly of multiple, large fragments, contact technical services at **techservices@sgidna.com**.

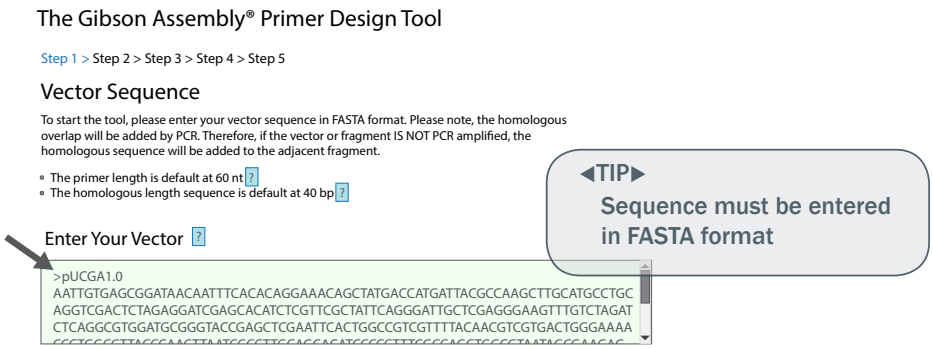
sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

Gibson Assembly® Primer Design Tool Example

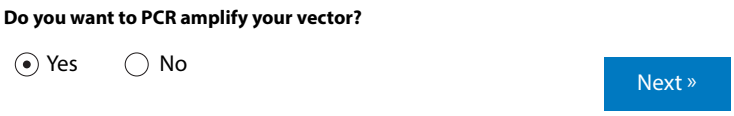
The following example demonstrates using the GAP Tool for designing Gibson Assembly® primers for inserting a single DNA fragment into a plasmid cloning vector. The following example shows the *Kan*^{R} gene assembled into the pUC19-derived vector, pUCGA 1.0.

Using the Gibson Assembly® Primer Design Tool

1. Enter the vector sequence in FASTA format.

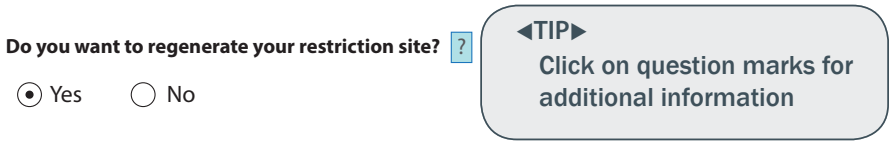


2. Indicate if vector will be PCR-amplified and Press Next.



3. Indicate if insertion site is by position or restriction enzyme digest and Press Next.

4. Indicate whether restriction enzyme site should be regenerated.



5. Indicate number of restriction enzyme sites.

Designing Homologous Overlaps

Online Tool for Designing Gibson Assembly® Primers

6. Enter restriction enzyme(s) and press Scan Sites.

Vector 3′ End ?

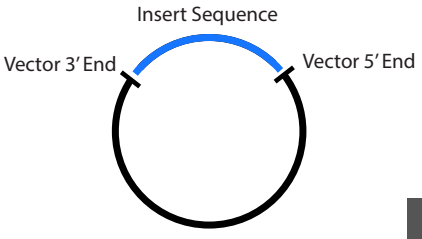
Restriction Site

XbaI

Vector 5′ End ?

Restriction Site

BglII



Scan Sites ?

7. Identify site of insertion.

Select Site

...

Please select a restriction site position.

Select Site

139 - 144

8. Select the appropriate site from the drop-down menu and Press Next.

Select Site

...

...
80 - 85
136 - 141

Select Site

139 - 144

aattgtgagcggataacaatttcacacaggaaacagctatgaccatgattacgcaagcttgcatgcctgcaggtcgactctagaggatcgag
cacatctcgttcgctattcagggattgctcgagggaagtttgtctagatctcaggcgtgatgcgggtaccgagctcgaattcactggccgtcgt
tttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagccatccctttcgccagctggcgtaatagcgaagag

9. Indicate number of inserts (from 1-15).

Step 1 > Step 2 > Step 3 > Step 4 > Step 5

3. Your Insert Input

Insert sequences ?

Enter the insert sequence as GATC in text format. For multiple fragments, enter each sequence in the order of assembly in the boxes below. For example, if 3 insert sequences are provided, the tool will design primers to assemble the inserts in a 5′ to 3′ direction as insert-1, insert-2, insert-3.

How many insert sequences? ?

1

sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

10. Copy and paste insert sequence.

Input Sequences

ATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTA
AATTCCAACATGGATGCTGATTTATATGGGTATAAATGGG

TIP▶
Enter Insert Sequence in plain text format using GATC bases only

11. Indicate method of insert preparation and press Next.

How is this Insert Prepared?

(•) PCR ( ) RE Digest ( ) RE Digest

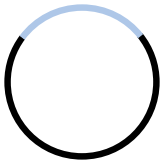
« Previous Next »

12. View Results.

Step 1 > Step 2 > Step 3 > Step 4 > Step 5

4. Your Results

The graphic circular map mimics the final assembled construct. Vector is displayed in black and inserts are displayed in color. Sequence information provides complete assembled construct with vector sequence in lowercase and the insert sequences in uppercase with highlight color. Primers will be designed between each junction to create fragments for Gibson Assembly. Please read the warning as primer design is based on the sequence information. The success of assembly is subject to many considerations. Some sequence elements such as repetitive motifs, GC flux, or highly homologous sequences may interfere with assembly. Primers generated with these sequences may require additional design to increase the assembly efficiency.



◀TIP▶
Insert sequence is in UPPER CASE

Vector sequence is in lower case

Sequence Information (Vector sequence in lowercase, Insert sequence in UPPERCASE)

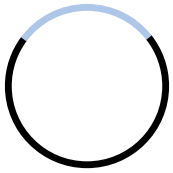
aattgtgagcggataacaatttcacacaggaaacagctatgaccatgattacgccaagcttgcatgcctgcaggtcgactctagaATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCAACATGG
ATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAG
CGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGA
TCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTT
AACAGCGATCGCGTATTTCGTCTTGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAA
ATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAAT
CGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATGTGAATAAATTGCAGT
TTCATTTGATGCTCGATGAGTTTTTCTAAagatctcaggcgtggatgcgggtaccgagctcgaattcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcacatccccc
tttcgccagctggcgtaatagcgaagaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatggcgcctgatgcggtattttctccttacgcatctgtgcggtatttcacaccgcatatggtgcactctcagtacaa
tctgctctgatgccgcatagttaagccagccccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatca
ccgaaacgcgcgagacgaaagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtttctagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgt
atccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaag
atgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtattgac
gccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggc
caacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccatacccaaacgacgagcgtgacaccacgatgcctgtag
caatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatct
ggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactg
attaagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcctttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcaga
ccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcag
agcgcagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactc
aagacgatagttaccggataaggcgcagcggtcgggctgaacgggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggag
aaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcagg
ggggcggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgc
agccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgca
acgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtgg

13. View Summary

Step 1 > Step 2 > Step 3 > Step 4 > Step 5

4. Your Summary

The final design of the assembled construct is displayed in cartoon map. Below are the primers provided by SGI Gibson Assembly Primer design tool. For your convenience, the primer information can be download as Excel file or PDF summary. Additional information regarding the Gibson Assembly® HiFi 1-Step Kit or the Gibson Assembly® Ultra Kit can be found in SGI-DNA website.



Primer Sequence (Annealing sequence in lowercase, Homologous sequence in UPPERCASE)

NAME	TEMP	SEQUENCE
MyInsert1_F1	58.2	atgagccatattcaacggga
MyInsert1_R1	79.2	GTGAATTCGAGCTCGGTACCCGCATCCACGCCTGAGATCTttagaaaaactcatcgagca
vector_F1	66.2	tcaggcgtggatgcgggtac

14. Download PDF or Excel spreadsheet summary to save primer sequences and T_m.

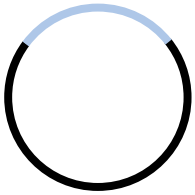
Gibson Assembly® Primer Design Summary

Date: 2017-01-01

Vector: pUCGA1.0

Inserts: 1

Graphic display of the assembled construct



Primer Output

Primer Name	Restriction Site	Primer Sequence	Tm(°C)
MyInsert1_F1	N/A	atgagccatattcaacggga	58.2
MyInsert1_R1	N/A	GTGAATTCGAGCTCGGTACCCGCATCCACGCCTGAGATCTttagaaaaac tcatcgagca	79.2
vector_F1	N/A	tcaggcgtggatgcgggtac	66.2
vector_R1	N/A	CGCGGCCTCGACGTTTCCCGTTGAATATGGCTCATTCTAGagtcgacctg caggcatgca	82.5

Vec: Vector; F:forward primer; R: reverse primer; Ins: insert
Primer sequence: lowercase indicates gene annealing sequence.
UPPERCASE indicates homologous overlap sequence.

Variations of Gibson Assembly® Cloning

Gibson Assembly® PBnJ™ Cloning

Primer-Bridge End Joining™ (PBnJ™) is a novel modification of Gibson Assembly® Cloning that seamlessly joins fragments without homologous overlaps. Because Gibson Assembly® PBnJ™ Cloning does not rely on overlapping ends to join adjacent fragments, generating overlap regions by PCR prior to assembly is not necessary. To assemble non-overlapping ends, Gibson Assembly® PBnJ™ Cloning utilizes single primers or primer pairs, typically with phosphorothioate-modified 3′ ends. This novel application of Gibson Assembly® cloning offers increased flexibility and eliminates PCR amplification steps.

Gibson Assembly® PBnJ™ Cloning Applications include:

- Assembling large fragments with non-homologous ends
- Assembling fragments that are difficult to PCR amplify
- Assembling parts from a library without introducing PCR-mediated errors
- Editing (adding or deleting) sequences at junctions based on primer design
- Generating unique 3′ overhangs of desired lengths for standard cloning

Overview of Gibson Assembly® PBnJ™ Cloning

Gibson Assembly® PBnJ™ Cloning relies on the stepwise activities of the Gibson Assembly® Ultra Kit, followed by the Gibson Assembly® HiFi 1-Step Kit (see Figure 11 on page 26). For Gibson Assembly® PBnJ™ Cloning, instead of designing PCR primers to generate homologous overlap regions, a primer pair is used to bridge non-homologous overlap regions. The primer pairs can contain phosphorothioate-modified 3′ ends, which protect the primer from 3′ exonuclease chew back activity during assembly. After template chew back, the primers anneal to the non-overlapping, exposed template sequence, which is later extended and ligated by the 5′ → 3′ polymerase activity of the Gibson Assembly® HiFi 1-Step Master Mix. For a more detailed discussion on how to perform the PBnJ method, refer to the technical application note "**Gibson Assembly® Primer-Bridge End Joining (PBnJ™) Cloning**," available on the SGI-DNA website.

Some Applications of Gibson Assembly® PBnJ™ Cloning

Gibson Assembly® PBnJ™ Cloning	Overview	Description
Seamless Joining with a Primer Pair	Gibson Assembly® PBnJ™ Cloning is accomplished using non-overlapping fragments assembled with the Gibson Assembly® Ultra Kit, followed by the Gibson Assembly® HiFi 1-Step Kit. A primer pair bridging two targeted fragments contains phosphorothioate-modified 3′ ends.	See Figure 11 on page 26
3′ Overhang Extension	A single primer containing phosphorothioate-modified bases at the 3′ terminus is designed to generate a 3′ overhang extension.	See Figure 12 on page 27
Sequence Insertion Cloning	Gibson Assembly® PBnJ™ Sequence Insertion Cloning can be used to insert sequence between adjoining fragments during assembly. Phosphorothioate-modified primers bridge the fragments to be joined and create the insertion.	See Figure 13 on page 28

Table 5. Gibson Assembly® PBnJ variations.

Gibson Assembly® PBnJ™ Seamless Joining

The following illustration outlines the steps of Gibson Assembly® PBnJ™ Seamless Joining at a single junction. The technique can also be used to join multiple DNA fragments to build a circular vector. Gibson Assembly® PBnJ™ Cloning relies on the stepwise activities of the Gibson Assembly® Ultra Kit, followed by the Gibson Assembly® HiFi 1-Step Kit. A primer pair is used to bridge non-homologous overlap regions of unrelated, nonhomologous DNA fragments. The primer pair contains phosphorothioate-modified 3′ ends, which protect from 3′ exonuclease chew back activity mediated by Gibson Assembly® Ultra Master Mix A. After template chew back, the primers anneal to the non-overlapping, exposed template sequence. The primer sequence is extended during incubation with Gibson Assembly® Ultra Master Mix B, leaving nonhomologous 3′ single strand overhangs. The DNA fragments are then incubated with Gibson Assembly® HiFi 1-Step Master Mix, which mediates 5′ chew back, allowing for annealing of the nonhomologous bridge region of the primer to the template DNA. Strand extension and ligation by the 5′→3′ polymerase activity of the Gibson Assembly® HiFi 1-Step Master Mix yields the final assembly product.

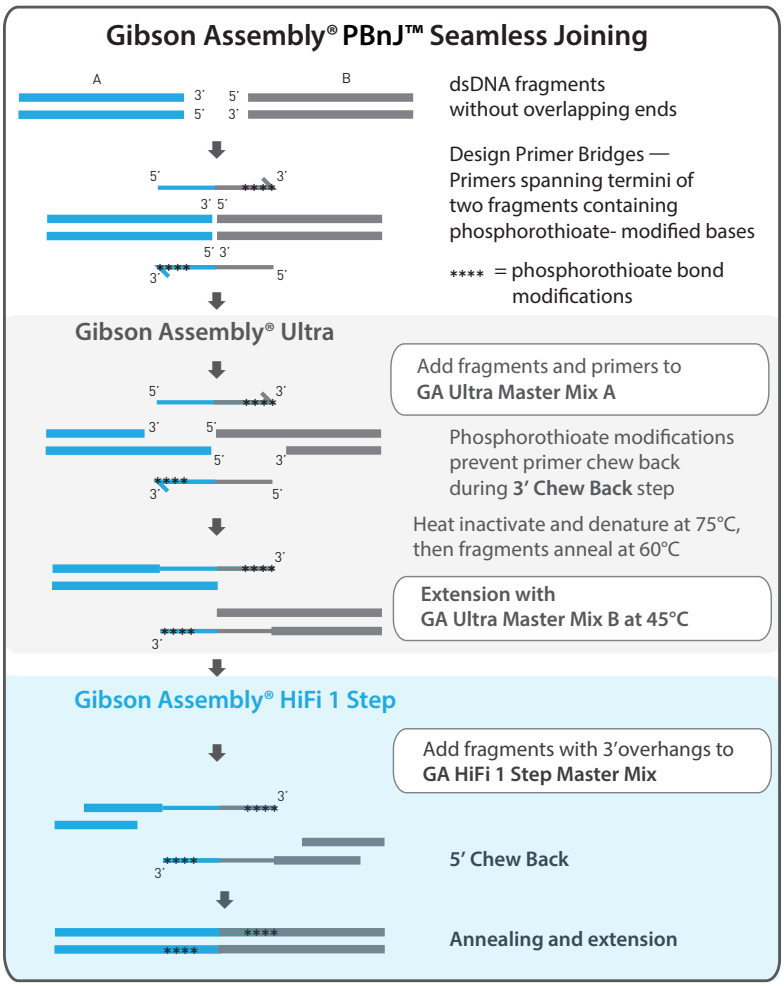


Figure 11. Overview of Gibson Assembly® PBnJ™ Seamless Joining. Gibson Assembly® PBnJ™ seamless joining is accomplished using non-overlapping fragments assembled with the Gibson Assembly® Ultra Kit, followed by the Gibson Assembly® HiFi 1-Step Kit.

sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

Gibson Assembly® PBnJ™ 3′ Overhang Extension

Using only the master mixes provided in the Gibson Assembly® Ultra kit, PBnJ™ cloning may also be used to create a DNA fragment containing a single strand 3′ overhang. The following figure demonstrates this usage, which is initiated by designing a single primer containing phosphorothioate-modified bases at the 3′ terminus.

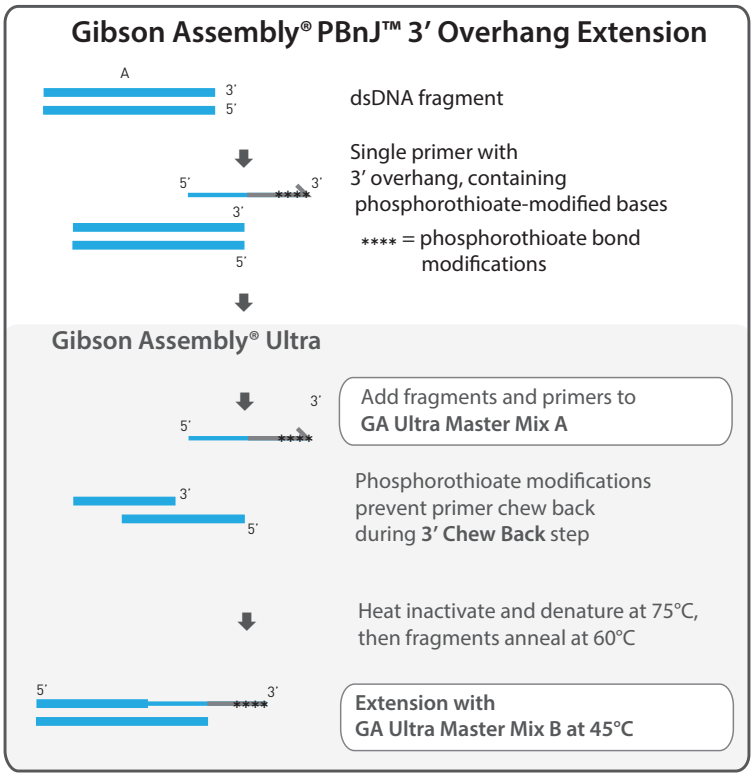


Figure 12. Overview of Gibson Assembly® PBnJ™ 3′ Overhang Extension. Gibson Assembly® PBnJ™ 3′ Overhang Extension is accomplished with a single phosphorothioate-modified primer and the Gibson Assembly® Ultra Kit. Extension of the 3′ overhang is initiated by combining a single phosphorothioate-modified primer, Gibson Assembly® Ultra Master Mix A and a DNA fragment-of-interest. The Gibson Assembly® Ultra procedure yields a DNA fragment containing a 3′ overhang.

Gibson Assembly® PBnJ™ Sequence Insertion Cloning

Gibson Assembly® PBnJ™ Cloning can also be used to add DNA sequence between non-overlapping fragments during assembly. This modification called Gibson Assembly® PBnJ™ Sequence Insertion Cloning is outlined in the figure below. This technique is accomplished using only the master mixes from the Gibson Assembly Ultra® kit and can be applied to many types of downstream applications, such as mutagenesis studies, promoter or enhancer studies, and large-scale genome modification studies.

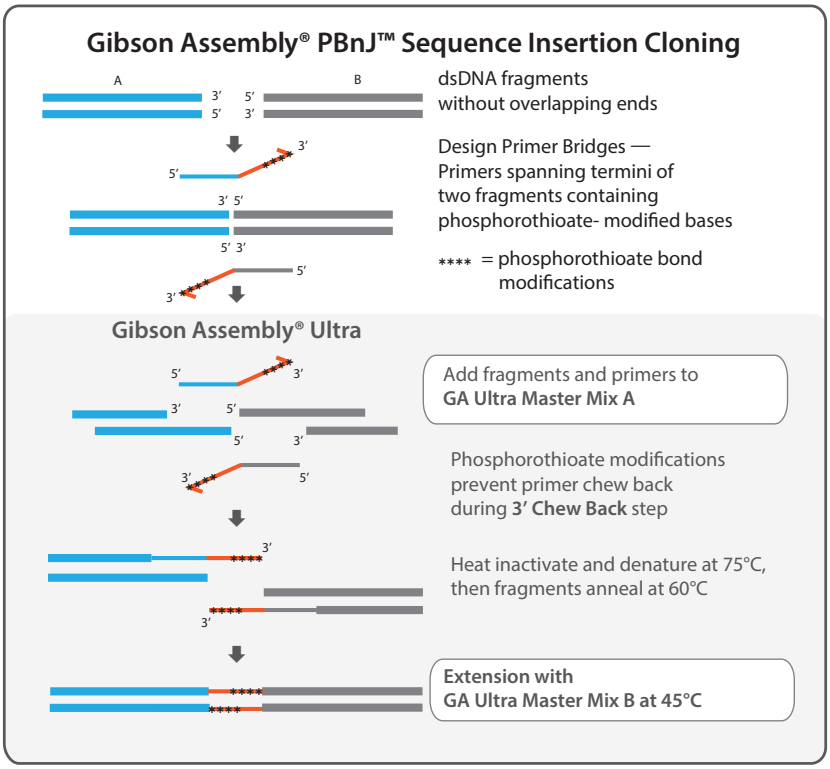


Figure 13. Overview of Gibson Assembly® PBnJ™ Sequence Insertion Cloning. Gibson Assembly® PBnJ™ Sequence Insertion Cloning adds sequence between adjoining fragments during assembly. The assembly reaction is initiated by combining phosphorothioate-modified primers, Gibson Assembly® Ultra Master Mix A, and the template DNA fragments. The orange portion of the primer represents the inserted sequence.

sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

Appendix A: Protocols

PCR Amplification of DNA Fragments — Before Starting the Gibson Assembly® Reaction

Homologous overlaps can be added to input fragments by PCR amplification of a DNA template using primers designed with the Gibson Assembly® Primer Design Tool (see page 20). You can use the following basic amplification conditions to generate the input fragments with homologous overlapping ends. We recommend using a high-fidelity polymerase, such as Phusion® DNA Polymerase, and reducing the number of PCR cycles used during amplification to minimize the potential for the introduction of amplification errors. After PCR amplification, analyze the fragments on a gel to verify the presence of fragments of the expected size. If multiple bands are present, consider gel extraction before proceeding to the Gibson Assembly® method. For best results, we suggest performing a clean-up of the PCR reaction to remove dNTPs, enzymes, and buffer components in all cases.

PCR Preparation of DNA fragments for Gibson Assembly® cloning

1. Prepare Reaction

Component	Volume
Insert or Vector DNA (100 pg/µL – 1 ng/µL in TE)	0.5 µL
10 µM Forward Primer	2.5 µL
10 µM Reverse Primer	2.5 µL
10 mM dNTPs	1 µL
5X Phusion HF Buffer	10 µL
Phusion® DNA Polymerase (2 U/ µL)	0.5 µL
Nuclease-free Water	33 µL
Total	**50 µL**

2. Thermocycle

Step	Temperature	Duration	Number of cycles
Initial denaturation	98°C	30 seconds	1 cycle
Amplification	98°C Primer T_m (60°C to 70°C) 72°C	10 seconds 20 seconds 30 seconds per kb	25–30 cycles
Final extension	72°C	5 minutes	1 cycle
Hold	4°C	—	1 cycle

Calculating the amount of DNA to use in a Gibson Assembly® reaction

Gibson Assembly® HiFi 1-Step and Ultra reactions

For a typical Gibson Assembly® HiFi 1-Step or Ultra reaction, combine 25–50 ng of vector with approximately 10–300 ng of insert. For best results, we recommend balancing the molar ratio of the DNA fragments. For fragments >1 kb, use an equimolar ratio. For DNA fragments ≤1 kb, we recommend using a 5-fold molar excess of insert. To precisely determine the pmol or ng of DNA for a given size fragment, use the following formulas:

pmol DNA = [ng DNA/(660 x # of bases)] x 1000

ng of DNA = [pmol DNA x (660 x # of bases)]/1000

Refer to the following table for approximate pmol of DNA for a given fragment size and amount.

Fragment size	ng of DNA	pmol of DNA
0.5 kb	20 ng	0.061
	40 ng	0.121
1 kb	10 ng	0.015
	25 ng	0.038
5 kb	10 ng	0.003
	25 ng	0.008
8 kb	25 ng	0.005
	50 ng	0.009
10 kb	25 ng	0.004
	50 ng	0.008
15 kb	50 ng	0.005
	100 ng	0.010
20 kb	50 ng	0.004
	100 ng	0.008
30 kb	50 ng	0.003
	100 ng	0.005

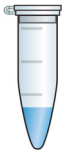
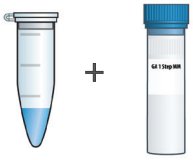
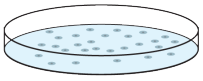
Gibson Assembly® SDM reactions

For Gibson Assembly® SDM, use approximately 100 pg – 30 ng of template DNA in the mutagenesis reaction. For the assembly reaction that follows the mutagenesis reaction, recommended DNA amounts can be found in the **Gibson Assembly® Site-Directed Mutagenesis Kit Instructions**.

Gibson Assembly® HiFi 1-Step Kit

Gibson Assembly® HiFi 1-Step— Quick Protocol

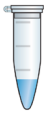
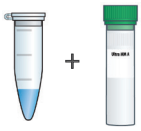
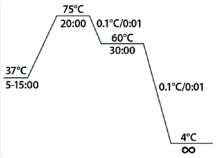
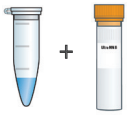
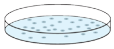
For a detailed protocol, visit **sgidna.com/hifi_kit**

1. **Thaw**		Thaw GA HiFi 1-Step Master Mix on ice.
2. **Combine DNA**		Combine insert and vector DNA to a total volume of: **5 µL** for the **2X** kit *or* **7.5 µL** for the **HC 4X** kit
3. **Vortex**		Vortex the master mix.
4. **Combine Master Mix and DNA**		On ice, combine: **5 µL DNA** and **5 µL Master Mix** for the **2X** kit *or* **7.5 µL DNA** and **2.5 µL Master Mix** for the **HC 4X** kit. Mix well and briefly centrifuge.
5. **Incubate**		Incubate at **50°C** for **1 hour**.
6. **Transform**		Store reactions at –20°C or proceed to transformation.

Gibson Assembly® Ultra Kit

Gibson Assembly® Ultra— Quick Protocol

For a detailed protocol, visit **sgidna.com/ultra_kit**

1. Thaw		Thaw Gibson Assembly® (GA) Ultra Master Mix A on ice.
2. Combine DNA		Combine insert and vector DNA to a total volume of **5 µL.**
3. Vortex		Vortex GA Ultra Master Mix A (MM A) and briefly centrifuge.
4. Combine MM A and DNA		On ice, combine **5 µL of DNA** with **5 µL of MM A.** Mix well and briefly centrifuge.
5. Incubate		37°C for 5–15 min → 75°C for 20 min → 0.1°C/sec to 60°C → 60°C for 30 min → 0.1°C/sec to 4°C
6. Thaw		Thaw GA Ultra Master Mix B on ice.
7. Vortex		Vortex GA Ultra Master Mix B (MM B) and briefly centrifuge.
8. Add MM B		On ice, combine **10 µL of the reaction** with **10 µL of MM B.**
9. Incubate		Incubate at **45°C** for **15 minutes.**
10. Transform		Store reactions at –20°C or proceed to transformation.

sgidna.com | CustomerService@sgidna.com
1-855-474-4362 (North America) or 1-858-228-4115 (outside North America)

Gibson Assembly® Site-Directed Mutagenesis Kit

Gibson Assembly® SDM Kit— Quick Protocol

For a detailed protocol, visit **sgidna.com/sdm-kit**

1. Thaw

Thaw GA SDM PCR Mix on ice.

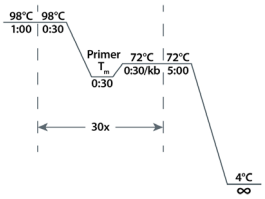
2. Vortex and Quick Spin

Vortex and briefly centrifuge GA SDM PCR Mix.

3. Prepare Reaction

Component	Volume
GA SDM PCR Mix (2X)	12.5 µL
dsDNA template	χ µL
SDM Forward Primer (5 µM)	2.5 µL
SDM Reverse Primer (5 µM)	2.5 µL
PCR water	7.5–χ µL
Total	**to 25 µL**

4. Introduce Mutations



98°C for 1 min →

98°C	10–30 sec	30 Cycles	→
Primer T_m	30 sec		
72°C	30 sec per kb		

72°C for 5 min → 4°C

5. Analyze

Gel electrophoresis of 5 µL.

6. Purify

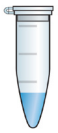
Column or gel purify DNA.

7. Thaw

Thaw GA SDM Assembly Mix A on ice.

Continued on the following page

8. Combine DNA



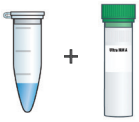
Combine 1 to 5 SDM fragment(s) and vector DNA to a total volume of 5 µL:

Fragment	Size	Amount	Insert:Vector Molar Ratio
Insert	≤ 1 kb	10–40 ng	1:1 to 5:1
	1–5.5 kb	10–25 ng	1:1 to 2:1
Vector	2–10 kb	25 ng	—

9. Vortex

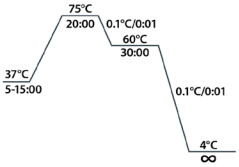
Vortex GA SDM Assembly Mix A.

10. Combine SDM Mix A and DNA



On ice, combine **5 µL of DNA** with **5 µL of GA SDM Assembly Mix A.** Mix well and briefly centrifuge.

11. Chew Back and Annealing



37°C for 5–15 min → 75°C for 20 min → 0.1°C/sec to 60°C → 60°C for 30 min → 0.1°C/sec to 4°C

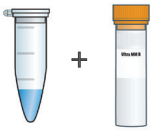
12. Thaw

Thaw GA SDM Assembly Mix B on ice.

13. Vortex

Vortex and briefly centrifuge.

14. Add SDM Mix B

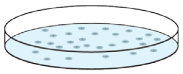


On ice, combine **10 µL of reaction** with **10 µL of GA SDM Assembly Mix B.**

15. Extension and ligation

Incubate at **45°C** for **15 minutes.**

16. Transform



Store reactions at –20°C or proceed to transformation.

Appendix B: Transformation

Transformation Guidelines

Gibson Assembly® constructs can be successfully transformed into a wide variety of electrocompetent or chemically competent high efficiency (>1 x 10^8 cfu/µg DNA) competent cells. We have had success using the following competent cells for transforming Gibson Assembly® products:

- *E. cloni* 10G chemically competent cells (Lucigen Cat. No. 60107)
- TransforMax™ EPI300™ Electrocompetent *E. coli* (Lucigen Cat. No. EC300110)

Use 2% (v/v) of the assembly reaction per transformation (e.g. 1 µL of the assembly reaction per 50 µL of high efficiency competent cells).

Plating Guidelines

We recommend preparing two plates for each transformation and plating two different volumes of the transformation mixture. See the following table for recommendations based on the number of fragments in the assembly reaction. These guidelines are for high-efficiency competent cells and are based on a 1 mL transformation. The plating volume in the following table is the fraction of transformation reaction plated per the total transformation mixture.

Number of fragments	Plating volume	Example	Average number of colonies
1–2	1/50	2 µL* and 20 µL*	>100
3–5	1/10	10 µL* and 100 µL	
> 5 (Ultra Kit only)	1/2	100 µL and 500 µL†	>50

* When plating <100 µL of the transformation mixture, add additional recovery media to allow cells to spread out across the agar plate.

† When plating >100 µL of the transformation mixture, spin down the reaction and reduce the volume to 100 µL before plating.

Table 6. Plating Guidelines.

Calculating Cloning Efficiency

Following transformation, you may want to calculate the cloning efficiency. For the positive control included in SGI-DNA Gibson Assembly® kits, white colonies indicate successful assembly with the insert; blue colonies indicate the absence of insert and vector assembly. For the positive control and other assembly reactions using blue-white screening, calculate the cloning efficiency using the following formula:

Cloning efficiency (CE, %) = Number of white colonies/ Total colonies x 100

We typically observe positive control cloning efficiencies >90%. Colony output is dependent on several factors, including the transformation efficiency of the competent cells used. Note that low colony output is not necessarily indicative of low cloning efficiency. If you observe fewer colonies than expected, it is still possible that your error-free clone is present. Unlike other kits and cloning methods, the SGI-DNA formulation is designed to generate a high percentage of error-free clones, reducing the need to screen and sequence a large numbers of colonies.

Appendix C: Expected Results

Gel Electrophoresis Following an Assembly Reaction

To troubleshoot or to evaluate the success of the assembly reaction, perform gel electrophoresis with 50% of the assembly reaction on a 0.8–2% agarose gel. The images below show successful assembly reactions with the Gibson Assembly® HiFi 1-Step kit.

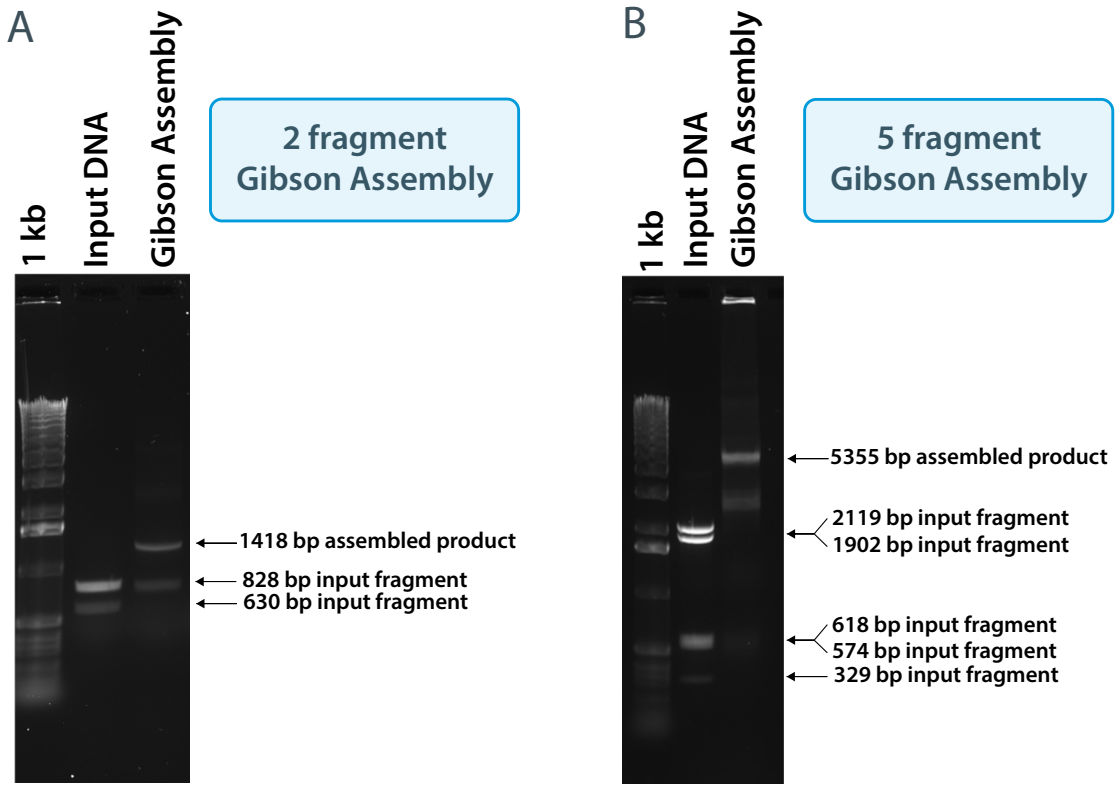


Figure 14. Successful Gibson Assembly® reactions analyzed using gel electrophoresis. (A) Two PCR fragments and **(B)** Five PCR fragments were assembled using the HiFi 1-Step kit. Following assembly, an aliquot from each completed reaction was loaded onto a 0.8% agarose gel with a 1 kb molecular marker. The Input DNA lane contains the DNA fragments prior to assembly. The assembled 1418 bp fragment (2-fragment assembly) and 5355 bp fragment (5-fragment assembly) are clearly visible in the Gibson Assembly reaction lanes.

Data Courtesy of Dr. Manoj Rajaure, Laboratory of Sankar Adhya, Center for Cancer Research, National Cancer Institute, Bethesda, MD

Appendix D: FAQs

General Gibson Assembly® Cloning Questions

1. What are the advantages of this method?

   - The Gibson Assembly® method enables one-step assembly of small and large DNA constructs, using overlapping oligonucleotides or dsDNA fragments as starting material.
   - Because it is not dependent on restriction enzyme sites, this method can be used to insert DNA fragments into any position of a linearized (restriction-digested or PCR-amplified) vector.
   - The Gibson Assembly® method is a seamless method leaving no problematic scars at the junctions in your DNA fragment.
   - The Gibson Assembly® method is much faster than traditional cloning methods.
   - Resulting DNA product may be used immediately for transformation, PCR, or rolling circle amplification.
   - The Gibson Assembly® method may be used to construct genes, genetic pathways, as well as genomes, and it has the capability of cloning multiple inserts into a vector simultaneously.
   - The Gibson Assembly® method can be used to perform site-directed mutagenesis to make simultaneous DNA sequence changes including insertions, deletions, and substitutions.
   - Multiple DNA fragments can be assembled simultaneously in a single reaction.

2. Can I PCR-amplify the assembled product?

   Yes. Because a covalently joined DNA molecule is produced, it may be PCR-amplified. In addition, if the final product is circular, it may be used in rolling circle amplification with φ29 polymerase.

3. Can ssDNA oligonucleotides be combined and assembled with dsDNA fragments?

   Yes. However, the optimal concentration of each oligonucleotide should be empirically determined. As a starting point, use 45 nM of each oligonucleotide. Keep in mind that oligonucleotides >90 bases may have secondary structures that interfere with assembly.

4. Can I assemble linear fragments without a vector?

   Yes, as long as the two extreme ends of the linear construct do not share homology to the ends of any internal fragments. We have not tested assembly of linear fragments for constructs >10 kb.

Questions about Primers

1. When using PCR to generate dsDNA fragments with Gibson Assembly® overlaps, are PAGE or HPLC purified oligonucleotide primers required? What if I want to anneal two single-stranded oligonucleotides to create my fragments?

   While you can use PAGE or HPLC purified oligonucleotides, it is not a requirement. Standard desalted primers can be used in either case.

Appendix D: FAQs

Questions about Inserts

1. How large of a DNA fragment can I assemble?

   The Gibson Assembly® HiFi 1-Step Master Mix has been used to assemble DNA fragments greater than 1 Mbp with multi-step assemblies. Assembled products as large as 300 kb DNA fragments have been successfully transformed into *E. coli*, which is the approximate upper limit for cloning into *E. coli*.

2. How many fragments can I assemble at once?

   The number of fragments that may be assembled is dependent on the length and the sequence of the DNA fragments. With the HiFi 1-Step Kit, we recommend assembling five or fewer inserts into a vector at once in order to reliably produce a clone with the correct insert. For assembly using >5 fragments, we recommend using the Ultra Kit. Table 4 on page 19 provides guidance on the numbers and sizes of fragments that can be assembled in a single round using either the HiFi 1-Step method or the Ultra two-step method.

3. Will this method work to assemble repetitive sequences?

   Yes. Design DNA fragments that incorporate the repetitive sequences internally (not at the overhanging ends). This strategy will ensure that each DNA fragment has a unique overlap and will be assembled in the correct order. The repetitive sequence can also be internalized in the first stage of a two-stage assembly strategy. If having the repetitive sequence at the ends is unavoidable, the correct DNA molecules may still be produced at lower efficiencies. Alternatively, longer overlaps that increase the uniqueness of the fragments being joined can be used.

4. Can ≤ 200 bp dsDNA fragments be assembled by this method?

   Yes, the Gibson Assembly® Ultra kit allows for the assembly of inserts as small as 100 bp. For optimal results, use a ≥ 5-fold molar excess of these smaller fragments relative to the vector.

Questions about Vectors

1. Do I have to prepare my vector using PCR?

   While vectors can have overlapping regions added by PCR, you are not required to prepare your vector by PCR. Cloning vectors can be linearized by restriction enzyme digest. Blunt ends, 5′ overhangs, and 3′ overhangs are all compatible with Gibson Assembly® cloning without further modification. If you are using vector prepared with a restriction enzyme digestion, you will need to add homologous overlap sequences to the insert. Alternatively, you may use Gibson Assembly® PBnJ™ cloning (see page 25).

Homologous Overlap Region Questions

1. What are the shortest overlaps that can be used with this method?

   As a starting point, we recommend using 40 bp overlaps when assembling dsDNA and 20 bp overlaps when assembling ssDNA oligonucleotides. Short overlaps can result in lower efficiency cloning. The ideal overlap length depends on fragment length and the numbers of fragments being assembled. Table 4 on page 19 provides more specific guidance.

2. What are the longest overlaps that can be used with this method?

   The kits are optimized for the assembly of DNA molecules with ≤80 bp overlaps.

Procedural Questions

1. How should I store a Gibson Assembly® kit?

   We recommend storing the kits at –20°C. It is important to store Gibson Assembly® kits at a stable temperature (*i.e.,* not in a frost-free freezer) and in a location where the kit will not be subject to temperature shifts (*e.g.,* do not store the kits in the door of an upright freezer). To have the most consistent and robust performance during the storage interval we suggest that you subject your Gibson Assembly® kit or master mix to no more than 5 freeze-thaw cycles. If you believe that you will require more than 5 freeze-thaw cycles to use your product, we suggest that you aliquot the master mix.

2. What is the recommended starting concentration of insert and vector DNA?

   For DNA fragments <1 kb, the concentration should be >40 ng/µL. With 25–50 ng of vector, the recommended range is 10–300 ng of each DNA fragment in equimolar amounts or in vector: insert ratios of 1:5. Generally, 1:5 is recommended for smaller fragments.

3. I have a limited amount of DNA. What is the lowest amount of DNA I can use?

   One of the advantages of the HiFi 1-Step kit is that small amounts of DNA may still be assembled efficiently. For fragments <1 kb, 20–40 ng of DNA can be used.

4. Can longer or shorter incubation times be used?

   Yes, for the HiFi 1-Step Kit, the assembly reaction has been optimized for a 1 hour, 50°C incubation. However, extended incubation times (e.g., 2–16 hours) have been shown to improve cloning efficiencies in some cases. Alternatively, for the assembly of ≤3 fragments, 15 minutes has been shown to be sufficient. Reaction times less than 15 minutes are not recommended. Incubation times for the Ultra kit have been optimized for use with a wide number and range of fragment sizes, and we recommend using the incubation periods included in the protocol.

5. Do I need to inactivate restriction enzymes following vector digestion?

   Restriction enzyme inactivation is only necessary if the insert contains the restriction site recognized by the restriction enzyme used for vector linearization.

6. Is it necessary to gel-purify restriction fragments or PCR products?

   While gel purification helps to remove extraneous bands and helps you more accurately quantify the amount of DNA present, generally, this is not necessary. A cleanup kit or a standard phenol-chloroform extraction followed by ethanol precipitation is sufficient.

7. To ensure that my clone is error-free, what are your sequencing recommendations?

   SGI-DNA Gibson Assembly® kits and master mixes include a proofreading DNA polymerase to minimize the potential of an incorrect base being inserted during the extension reaction.

   To ensure that your insert is error-free, we suggest sequencing the entire insert and about 500 bp of the vector at the insert/vector junction. If PCR was used to prepare the vector, you may want to consider sequencing the entire vector.

Troubleshooting and Optimization

1. How can I reduce the number of vector-only background colonies?

   To significantly reduce the background of unwanted vector-only colonies, use a PCR-derived vector rather than a vector produced using a restriction enzyme digest. If the number of background colonies continues to be a problem, purify the PCR-amplified vector from an agarose gel following electrophoresis. A vector prepared by a double digestion or phosphatase treatment may also reduce the number of background colonies if a vector produced by restriction enzyme digestion is used.

2. What should I do if my assembly reaction yields no colonies, a small number of colonies, or clones with the incorrect insert size, following transformation into *E. coli*?

   - Ensure that you are plating multiple volumes of the assembly reaction onto multiple LB plates with appropriate antibiotics.
   - Assemble and transform the positive control provided in the kit. The successful cloning of the positive control will demonstrate that the assembly mixture is functional and the transformation conditions are suitable.
   - Analyze the reaction on an agarose gel. An efficient assembly reaction will show assembled products of the correct size and the absence or reduction of the starting substrate DNA fragments (see Figure 14 on page 36).
   - Check the design of the overlapping DNA fragments.
   - If the amount of vector is increased, be sure to use appropriately balanced molar amounts of input fragments. See "Calculating the amount of DNA to use in a Gibson Assembly® reaction" on page 30 for further details.
   - Consider whether the cloned insert may be toxic to *E. coli* and whether a low-copy vector, such as a BAC should be used.

3. I want to clone a large continuous piece of genomic DNA. It is larger than the suggested sizes for your kit. What suggestions do you have for cloning a large piece of genomic DNA?

   For large DNA fragments, we often break them into ~10 kb sections. Fragments of this size can be generated using appropriate restriction enzymes or Cas9 endonuclease with an appropriately designed guide RNA. For this approach, we use our XactEdit™ Cas9 enzyme. After obtaining the set of ~10 kb fragments, ~80 bp overlaps are added using PCR. For the amplification of large fragments, we suggest a highly processive, proof-reading RNA polymerase such as Phusion®, Q5®, or KOD Extreme™. The fragments can then be the joined by the Gibson Assembly® method. If multiple large fragments need to be joined (e.g. more than four 10 kb fragments), we suggest performing multistage assemblies.

4. The fragments I would like to assemble have some repeated elements in them. What guidance do you have to improve the cloning efficiency using fragments that have a high percentage of similarity?

   There are a number of strategies you can consider:

   - Design the overlaps such that the repetitive sequences are buried within each fragment as much as possible.
   - Use the Ultra kit with a 1–2 minute chew-back at 37°C instead of the recommended 5-minute chew-back.
   - To generate a full-length fragment, use the assembly mixture as a template for PCR with primers corresponding to the ends of the insert. If the amplification is successful, the insert can be assembled with the vector using the Gibson Assembly® method.
   - If none of these work, run the assembly reaction on a gel to see if laddering is occurring. To determine which region(s) might not be assembling, join pairs of adjacent regions with Gibson Assembly® and analyze the reaction on a gel. This can help identify if regions are not being effectively joined.

Appendix E: Selected Citations

Year	Description	How Gibson Assembly® Cloning enabled this research
2010	Design, synthesis, assembly, and creation of the first synthesized genome and cell[19]	Dan Gibson led the J. Craig Venter Institute efforts to synthesize two complete bacterial genomes. Those projects resulted in the creation of the first synthetic bacterial cell and development of the Gibson Assembly® method.
2012	Gibson Assembly® cloning of promoter inputs and promoter outputs to study genetic circuits in *Escherichia coli*[21]	Gibson Assembly® cloning was the method of choice for plasmid construction in this directed evolution study.
2013	Using Gibson Assembly® cloning to generate Cre recombinase mutants that were screened for improved accuracy[22]	Gibson Assembly® cloning was the method of choice for the assembly of Cre mutants and libraries.
2013	Site-directed mutagenesis followed by Gibson Assembly® cloning to study neuronal fluorescent calcium sensors[23]	Gibson Assembly® cloning was the method of choice for the assembly of ultra-sensitive protein calcium sensor mutants with lentiviral constructs following site-directed mutagenesis.
2013	Genetic engineering of *Streptococcus pneumoniae* with CRISPR-associated (Cas) nuclease Cas9 and Gibson Assembly® cloning[24]	Gibson Assembly® cloning was one of the methods used to assemble the genomic DNA of a strain of *Streptococcus pneumoniae* for targeted genome editing studies.
2015	Combining CRISPR/Cas9 and the Gibson Assembly® method for seamless cloning into any vector of any size[25]	After linearizing a 22 kb plasmid with the CRISPR/Cas9 technique, a 783-bp insert was assembled with the linearized plasmid using the Gibson Assembly® method.
2015	Single-step method to clone long genomic sequences (up to 100 kb) using Cas9 nuclease and Gibson Assembly® cloning[26]	Gibson Assembly® cloning of Cas9-Assisted Targeting of CHromosome segments reduced the amount of time and resources required to clone large genomic sequences.
2016	Design and Synthesis of a Minimal Genome[20]	The Gibson Assembly® HiFi 1-Step kit was used to generate 7 kb cassettes for bacterial cloning.
2016	Rapid gene assembly for the parallel expression of large protein subunits[27]	The Gibson Assembly® method was used to assemble multigene expression constructs.

Table 7. Descriptions of recent key publications citing Gibson Assembly® Cloning.

Appendix F: References

1. Luria SE, Dulbecco R. Genetic recombinations leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles. Genetics. 1949 Mar;34(2):93.
2. Campbell AM. Episomes. Advances in Genetics. 1963 Dec 31;11:101-45.
3. Jackson DA, Symons RH, Berg P. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coli*. Proceedings of the National Academy of Sciences. 1972 Oct 1;69(10):2904-9.
4. Khorana HG, Agarwal KL, Büchi H, Caruthers MH, Gupta NK, Klbppe K, Kumar A, Ohtsuka E, RajBhandary UL, Van de Sande JH, Sgaramella V. CIII. Total synthesis of the structural gene for an alanine transfer ribonucleic acid from yeast. Journal of Molecular Biology. 1972 Dec 28;72(2):209-17.
5. Morrow JF, Cohen SN, Chang AC, Boyer HW, Goodman HM, Helling RB. Replication and transcription of eukaryotic DNA in *Escherichia coli*. Proceedings of the National Academy of Sciences. 1974 May 1;71(5):1743-7.
6. Nathans D, Smith HO. Restriction endonucleases in the analysis and restructuring of DNA molecules. Annual Review of Biochemistry. 1975 Jul;44(1):273-93.
7. Maxam AM, Gilbert W. A new method for sequencing DNA. Proceedings of the National Academy of Sciences. 1977 Feb 1;74(2):560-4.
8. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences. 1977 Dec 1;74(12):5463-7.
9. Berget SM, Moore C, Sharp PA. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. Proceedings of the National Academy of Sciences. 1977 Aug 1;74(8):3171-5.
10. Chow LT, Gelinas RE, Broker TR, Roberts RJ. An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. Cell. 1977 Sep 1;12(1):1-8.
11. Mullis KB, Faloona FA. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods in Enzymology. 1987 Dec 31;155:335-50.
12. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science. 1995 Jul 28;269(5223):496-512.
13. McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J, Sekhon M, Wylie K, Mardis ER, Wilson RK, Fulton R et al. A physical map of the human genome. Nature. 2001 Feb 15;409(6822):934-41.
14. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R et al. Initial sequencing and analysis of the human genome. Nature. 2001 Feb 15;409(6822):860-921.
15. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD et al. The sequence of the human genome. Science. 2001 Feb 16;291(5507):1304-51.
16. Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X et al. The complete genome of an individual by massively parallel DNA sequencing. Nature. 2008 Apr 17;452(7189):872-6.
17. Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C et al. Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. Science. 2008 Feb 29;319(5867):1215-20.
18. Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA, Smith HO. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nature Methods. 2009 May 1;6(5):343-5.
19. Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C et al. Creation of a bacterial cell controlled by a chemically synthesized genome. Science. 2010 Jul 2;329(5987):52-6.
20. Hutchison CA, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, Pelletier JF et al. Design and synthesis of a minimal bacterial genome. Science. 2016 Mar 25;351(6280):aad6253.
21. Moon TS, Lou C, Tamsir A, Stanton BC, Voigt CA. Genetic programs constructed from layered logic gates in single cells. Nature. 2012 Nov 8;491(7423):249-53.
22. Eroshenko N, Church GM. Mutants of Cre recombinase with improved accuracy. Nature Communications. 2013 Sep 23;4:2509.
23. Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature. 2013 Jul 18;499(7458):295-300.
24. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature Biotechnology. 2013 Mar 1;31(3):233-9.
25. Wang JW, Wang A, Li K, Wang B, Jin S, Reiser M, Lockey RF. CRISPR/Cas9 nuclease cleavage combined with Gibson assembly for seamless cloning. BioTechniques. 2015 Apr 1;58(4):161-70.
26. Jiang W, Zhao X, Gabrieli T, Lou C, Ebenstein Y, Zhu TF. Cas9-Assisted Targeting of CHromosome segments CATCH enables one-step targeted cloning of large gene clusters. Nature Communications. 2015 Sep 1;6:8101.
27. Weissmann F, Petzold G, VanderLinden R, Brown NG, Lampert F, Westermann S, Stark H, Schulman BA, Peters JM. biGBac enables rapid gene assembly for the expression of large multisubunit protein complexes. Proceedings of the National Academy of Sciences. 2016 May 10;113(19):E2564-9.

Ordering Information

Gibson Assembly® product ordering information			
Product	**Product type**	**Number of reactions**	**Catalog number**
HiFi 1-Step (2X)	Kit	5	GA1100-S
	Kit	10	GA1100-10
	Kit	50	GA1100-50
	Master Mix only	10	GA1100-10MM
	Master Mix only	50	GA1100-50MM
HiFi HC 1-Step (4X)	Kit	5	GA1100-4XS
	Kit	10	GA1100-4X10
	Kit	50	GA1100-4X50
	Master Mix only	10	GA1100-4X10M
	Master Mix only	50	GA1100-4X50M
Ultra	Kit	5	GA1200-S
	Kit	10	GA1200-10
	Kit	50	GA1200-50
	Master Mix only	10	GA1200-10MM
	Master Mix only	50	GA1200-50MM
Positive Control	Control	—	GA1000-05C
Site-Directed Mutagenesis	Kit	5	GA2100-S
		10	GA2100-10

Bulk packaging prices are available.
Inquire at CustomerService@sgidna.com.

Learn more and order Gibson Assembly® kits at **sgidna.com/gibson-assembly**.

Download an electronic version of this guide and learn about all the ways you can get **#DNAMYWAY** at **sgidna.com/dnamyway**.

TransforMax™ EPI300™ are trademarks of Epicentre Technologies Corporation. Gateway™ is a trademark and Phusion® is a registered trademark of Thermo Fisher Scientific. Q5® is a registered trademark of New England BioLabs. KOD Extreme™ is a trademark of Merck KGaA, Germany.
Synthetic Genomics® and Gibson Assembly® are registered trademarks and SGI-DNA™, DNATiles™, PBnJ™, and XactEdit™ are trademarks of Synthetic Genomics Inc.
Excel is a registered trademark of Microsoft Corporation.
Gibson Assembly® US Patent Nos. 7,776,532, 8,435,736, and 8,968,999.

© 2017 Synthetic Genomics Inc. All rights reserved. GACG101417

SGI-DNA offers a complete set of synthetic genomic solutions to advance your research.

Learn more at **sgidna.com**

#DNAMYWAY

SGI-DNA
11099 North Torrey Pines Rd, Suite 150
La Jolla, CA 92037

SGIDNA
A Synthetic Genomics, Inc. Company

www.sgidna.com
North America: 1-855-474-4362
Outside North America: 1-858-228-4115

Copyright © 2017 SGI-DNA, Inc. All rights reserved. All noted trademarks are the property of Synthetic Genomics, Inc. and/or its affiliates. GA092117

#DNAMYWAY

sgidna.com/gibson-assembly