Gibson Assembly®

CLONING GUIDE

RESTRICTION DIGEST-FREE, SEAMLESS CLONING

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

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

#DNAMYWAY
sgidna.com/gibson-assembly

Foreword

Gibson Assembly 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.

By 2004, JCVI was already 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 Gibson Assembly, 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 Gibson Assembly 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 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 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

Foreword 2

Historical Perspective 4
- A Brief History of Molecular Cloning and Synthetic Genomics 4

Gibson Assembly® Cloning 5
- Overview 5
- Types of Gibson Assembly® Kits 5

Applications 8
- Simple Cloning: One insert with one vector 8
- Assembly of Multiple Fragments 9
- Site-Directed Mutagenesis 10
- Using the Gibson Assembly® Method for Library Construction 12
- Using the Gibson Assembly® Method for Shotgun Cloning 13

Advantages of Gibson Assembly® Cloning 14
- Gibson Assembly® Method Advantages 14
- Gibson Assembly® Method and Other Cloning Approaches 15

Designing Homologous Overlaps 16
- Primer Design 16
- Online Tool for Designing Gibson Assembly® Primers 18

Variations of Gibson Assembly® Cloning 23
- Gibson Assembly® PBnJ™ Cloning 23
- Gibson Assembly® PBnJ™ Seamless Joining 24
- Gibson Assembly® PBnJ™ 3′ Overhang Extension 25
- Gibson Assembly® PBnJ™ Sequence Insertion Cloning 26

Appendix A: Protocols 27
- PCR Amplification of DNA Fragments — Before Starting the Gibson Assembly® Reaction 27
- Gibson Assembly® HiFi 1-Step Kit 28
- Gibson Assembly® Ultra Kit 29
- Gibson Assembly® Site-Directed Mutagenesis Kit 30

Appendix B: FAQs 32
- General Gibson Assembly® Cloning Questions 32
- Questions about Primers 32
- Questions about Inserts 33
- Questions about Vectors 33
- Homologous Overlap Region Questions 33
- Procedural Questions 34
- Troubleshooting and Optimization 35

Appendix C: Gibson Assembly® Citations 36

Appendix D: References 37

Ordering Information 38

Historical Perspective

A Brief History of Molecular Cloning and Synthetic Genomics

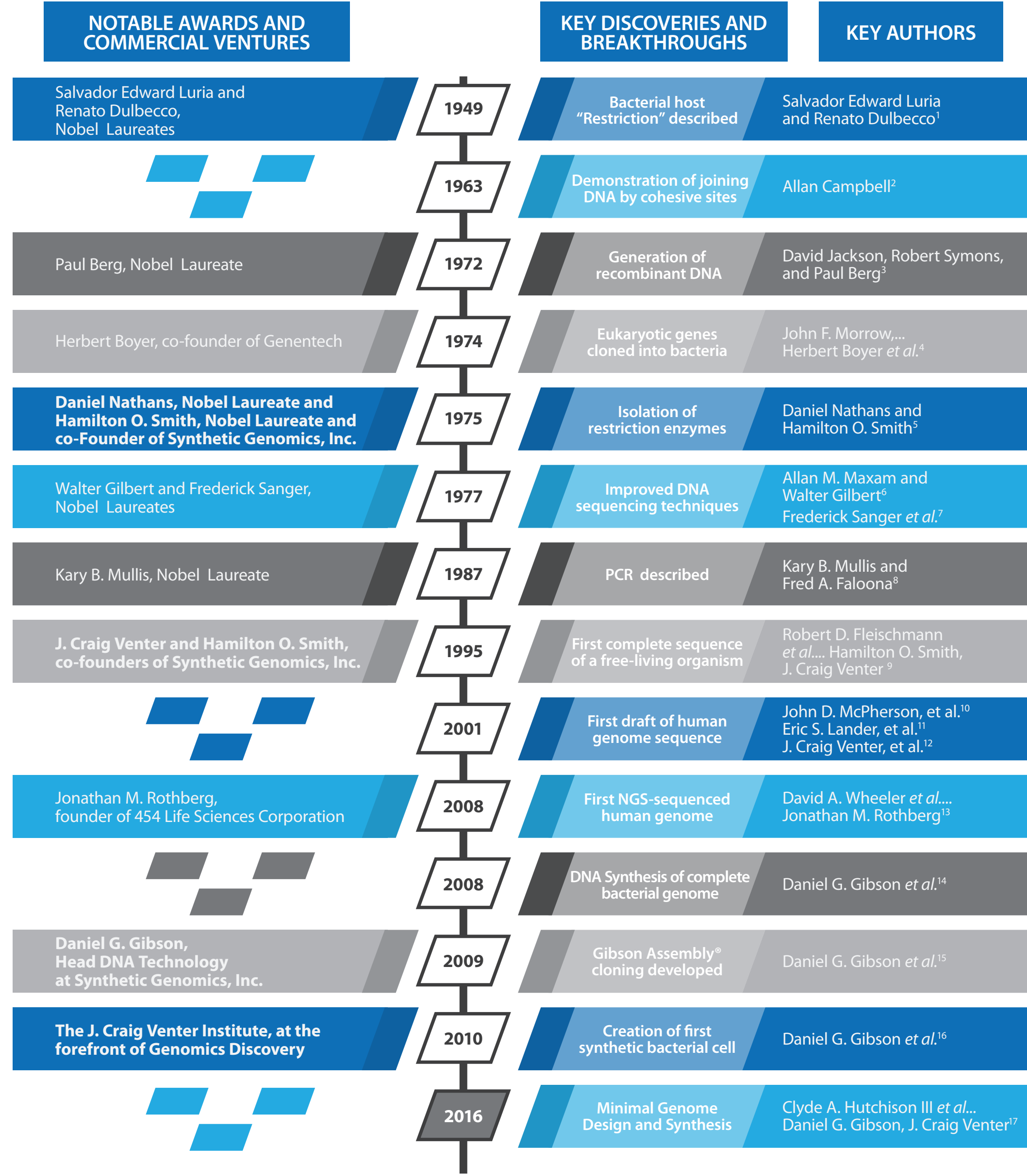


Figure 1. A Brief History of Molecular Cloning and Synthetic Genomics. The nonlinear timeline above shows some of the key historical discoveries and breakthroughs in molecular cloning and synthetic biology. The founding of relevant and notable companies, as well as the Nobel Prizes awarded for pivotal discoveries, are also noted.

Gibson Assembly® Cloning

Overview

The Gibson Assembly® method is a recently developed cloning procedure (see Figure 1) that allows the cloning of any two fragments without the need for restriction enzyme digestion or compatible restriction sites. This innovative approach to creating both simple and complex constructs was first published in 2008 by Daniel Gibson and colleagues[14]. Since that time, the Gibson Assembly® method has been cited in over 1,200 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 a vector in about one hour without the use of restriction enzymes. By designing DNA fragments with overlapping ends, users of the Gibson Assembly® method can create DNA constructs in a single round of cloning containing multiple inserts using virtually any vector. The Gibson Assembly® cloning method is initiated by combining DNA fragments with the Gibson Assembly® Master Mix. The master mix enzyme cocktail initiates 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 6). Seamless assembly using the Gibson Assembly® method 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 Gibson Assembly® Kits:

- **Gibson Assembly® HiFi 1-Step Cloning Kit**
- **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 these kits both enable the cloning of multiple fragments, the numbers of fragments, the recommend sizes of the fragments, and the specifics of the workflow are different (see Table 1 on page 6 for an overview).

The **Gibson Assembly® HiFi 1-Step** method allows for the assembly of up to 5 different fragments using an isothermal process. As implied by the name, the HiFi 1-Step process is performed in a single step. An overview of this method is shown on the left side of Figure 2 on page 6. To perform the HiFi 1-Step method, fragments and a vector with appropriate overlapping ends are combined with the mastermix and incubated at 50°C. After 1 hour of incubation, the fully ligated constructs are ready to transform into competent cells. The Gibson Assembly® HiFi 1-Step kit is simple to use and ideal for assembling up to 5 unique fragments ranging in size from 500 bp to 32 kb.

For the creation of more complex constructs with up to 15 fragments, or for constructs incorporating fragments from 100 bp to 100 kb, the **Gibson Assembly® Ultra** kit is recommended. An overview of this method is shown on the right side of Figure 2 on page 6. 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 kit because of its robustness and its ability to accommodate a wide range of fragment sizes. A comparison of the two workflows is shown in Figures 2 and 3 and the differences between the specifications for each kit is shown in Table 1.

Types of Gibson Assembly® Kits

Gibson Assembly® HiFi 1-Step and Ultra Kits Overview

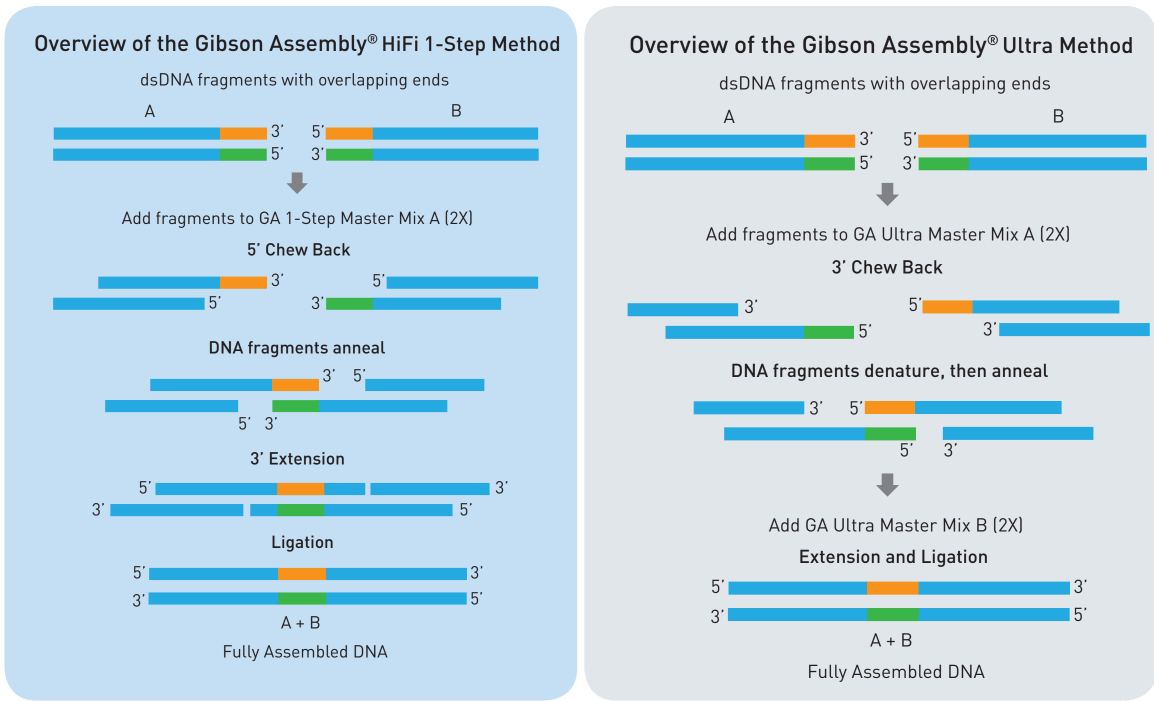


Figure 2. Overview of the two Gibson Assembly® Cloning Methods. DNA fragments containing homologous overlapping ends are assembled in 60 minutes with the HiFi 1-Step Kit or in 80 minutes with the Ultra Kit.

Highlights of the Gibson Assembly® Cloning Kits

Feature	Gibson Assembly® HiFi 1-Step Kit	Gibson Assembly® Ultra Kit
Reaction Time	60 minutes	80 minutes
Number of Steps	1	2
Fragment Size Range	500 bp to 32 kb	100 bp to 100 kb
Cloning Efficiency	>90%	>95%
Fragments Per Reaction	up to 5	up to 15
Maximum Construct Size	100 kb (multi-stage reactions)	1 Mb (multi-stage reactions)

Table 1. Specifications of the Gibson Assembly® HiFi 1-Step and Ultra Kits.

Gibson Assembly® Site-Directed Mutagenesis Kit

SGI-DNA has developed a Gibson Assembly® Site-Directed Mutagenesis (SDM) kit to join multiple, mutagenized DNA fragments. The inherent flexibility of this approach lends itself to small and large constructs and encompasses both single and multiple insert assemblies, and it can be applied to linear or circular templates. Insertions of up to 40 bp and deletions of any size are efficiently generated with the Gibson Assembly® SDM kit. A significant advantage of the Gibson Assembly® SDM kit over other mutagenesis approaches is that it allows for the incorporation of up to 5 insertions, deletions, or nucleotide changes in a single round of mutagenesis.

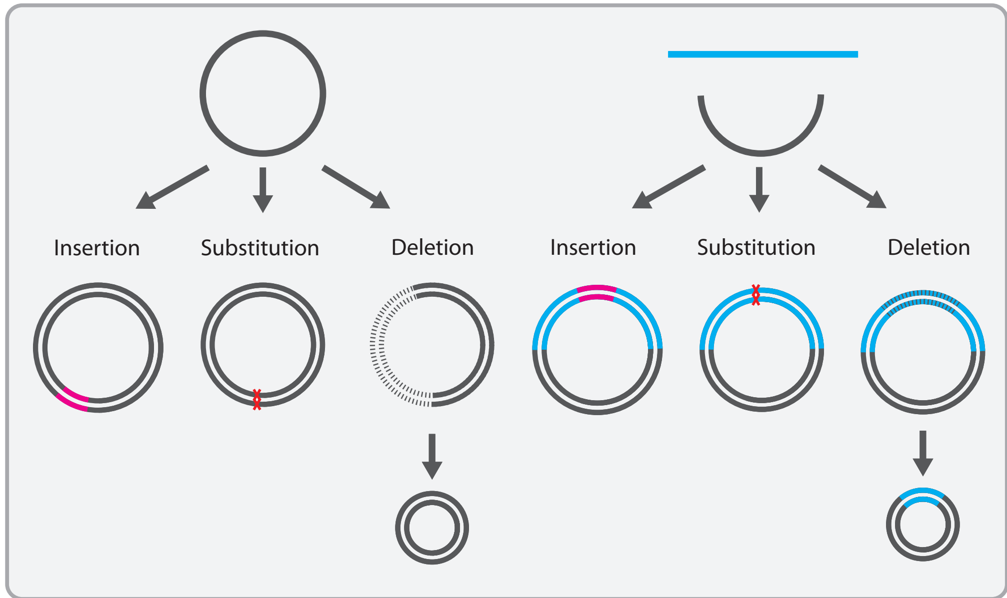


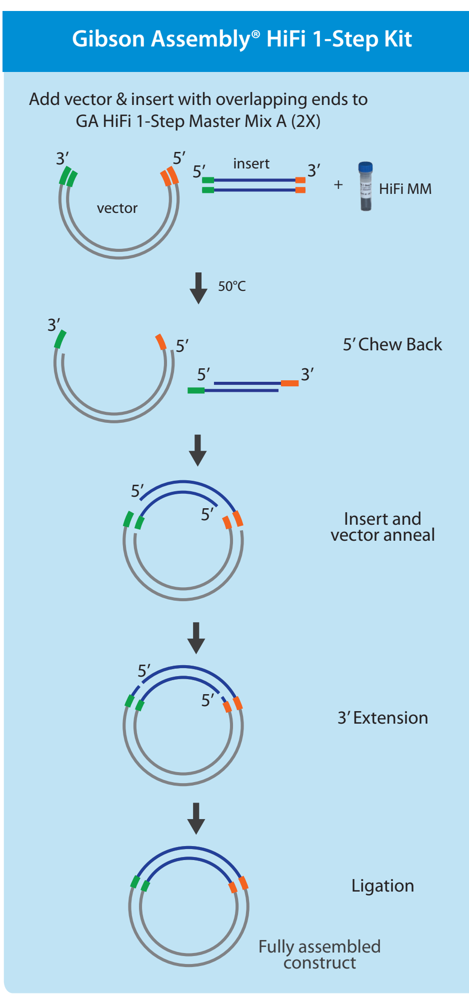
Figure 3. The Gibson Assembly® Site-Directed Mutagenesis Kit is the one kit solution for a wide variety of site-directed mutagenesis applications. Starting material may be a circular vector containing a gene of interest or the starting material may be a linear DNA fragment that will be cloned into a vector during assembly. Regardless of starting material, the kit is capable of producing up to 5 mutations in a single round of mutagenesis and assembly. Possible mutations include insertions up to 40 bp long, substitutions, and deletions of any size.

Applications

The Gibson Assembly® method may be leveraged for a number of 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.



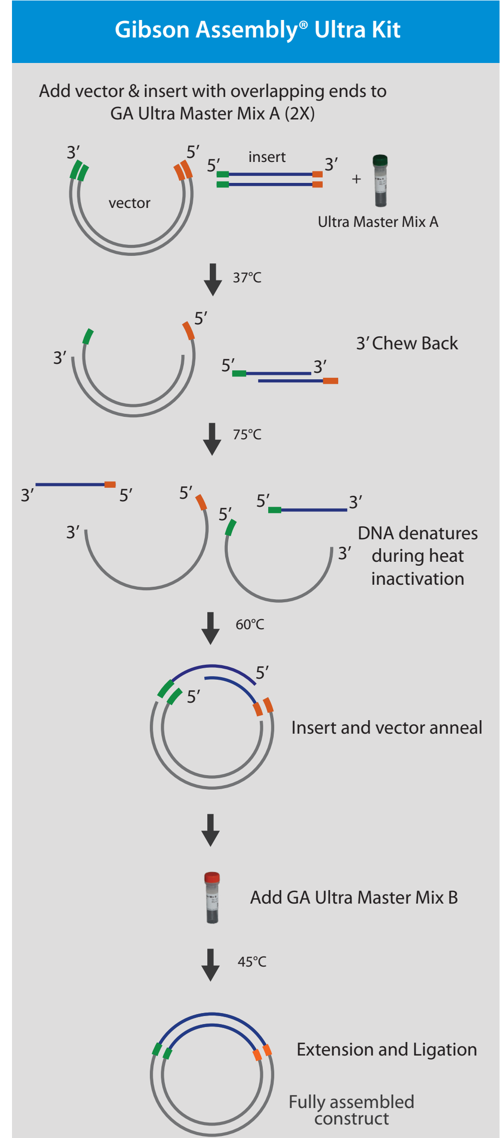


Figure 4. Overview of the Gibson Assembly® Cloning Methods. The Gibson Assembly® HiFi 1-Step Kit and the Gibson Assembly® Ultra Kit may be used to clone a single insert into a vector.

Assembly of Multiple Fragments

The Gibson Assembly® method can be used for the simultaneous assembly of multiple inserts and offers substantial time savings for multiple-insert assembly projects, which would typically require multiple rounds of traditional restriction enzyme digest-based cloning. Traditional cloning of multiple fragments with restriction enzyme digest-based cloning can prove challenging. Identifying appropriate restriction enzyme recognition sites may prove impossible, 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. Additionally, the ligation of large and small fragments in a single reaction is often inefficient, favoring the ligation of smaller fragments. Gibson Assembly® cloning overcomes these challenges through the efficient assembly of fragments of varying sizes, generated without reliance on compatible restriction enzyme recognition sites.

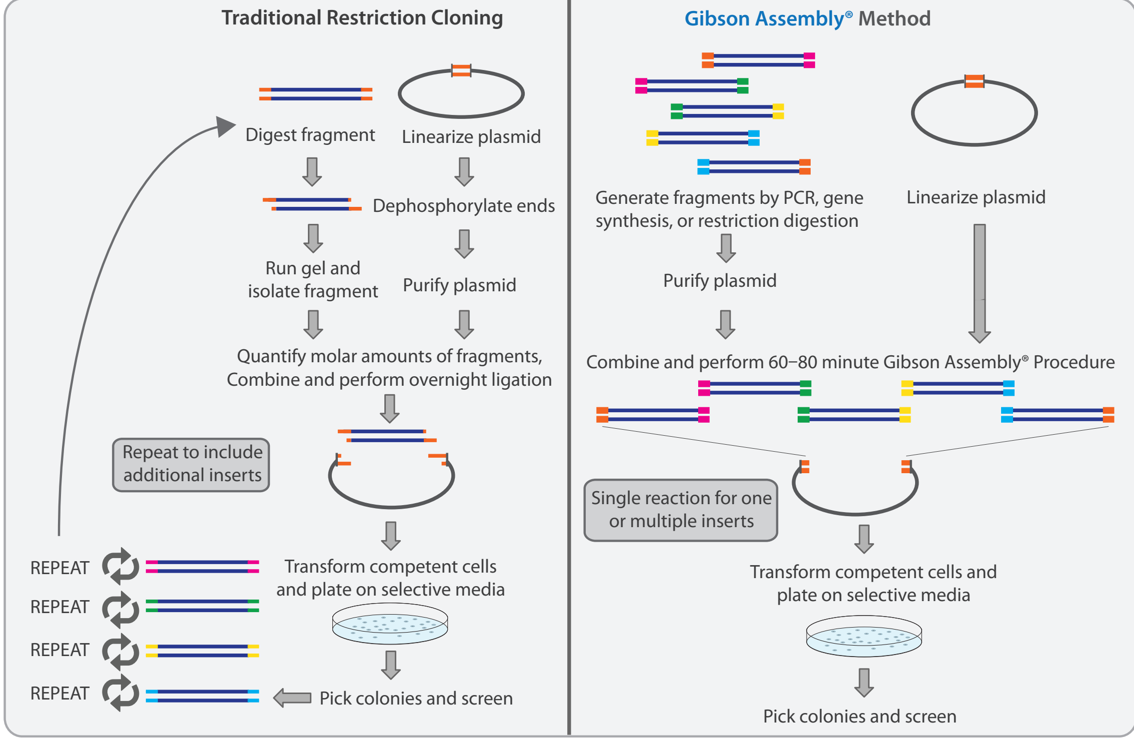


Figure 5. The Gibson Assembly® Method is faster and more efficient than traditional cloning. Traditional restriction cloning using compatible restriction endonucleases requires 1–2 days of preparative steps to generate cloning ends on the insert and plasmid. Typically, only one insert can be ligated 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 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 enable the examination of the biological activity of nucleic acids and proteins and may also be integral for protein engineering. For gene function studies, site-directed mutagenesis allows for a targeted, rational approach. Similarly, rationally designed proteins generated by site-directed mutagenesis can be engineered to have improved or unique properties that make the engineered protein suitable for a specific application. For laboratories interested in site-directed mutagenesis, 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

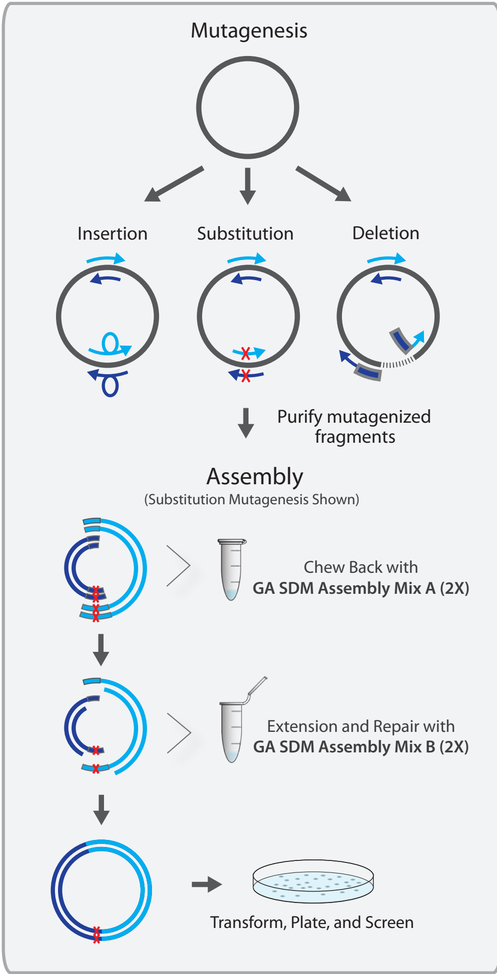


Figure 6. Overview of Gibson Assembly® SDM of a circular template. Following mutagenesis, DNA fragments of various lengths are uniformly assembled using complementary overlaps between fragments.

Gibson Assembly® Site-Directed Mutagenesis of a Linear Template

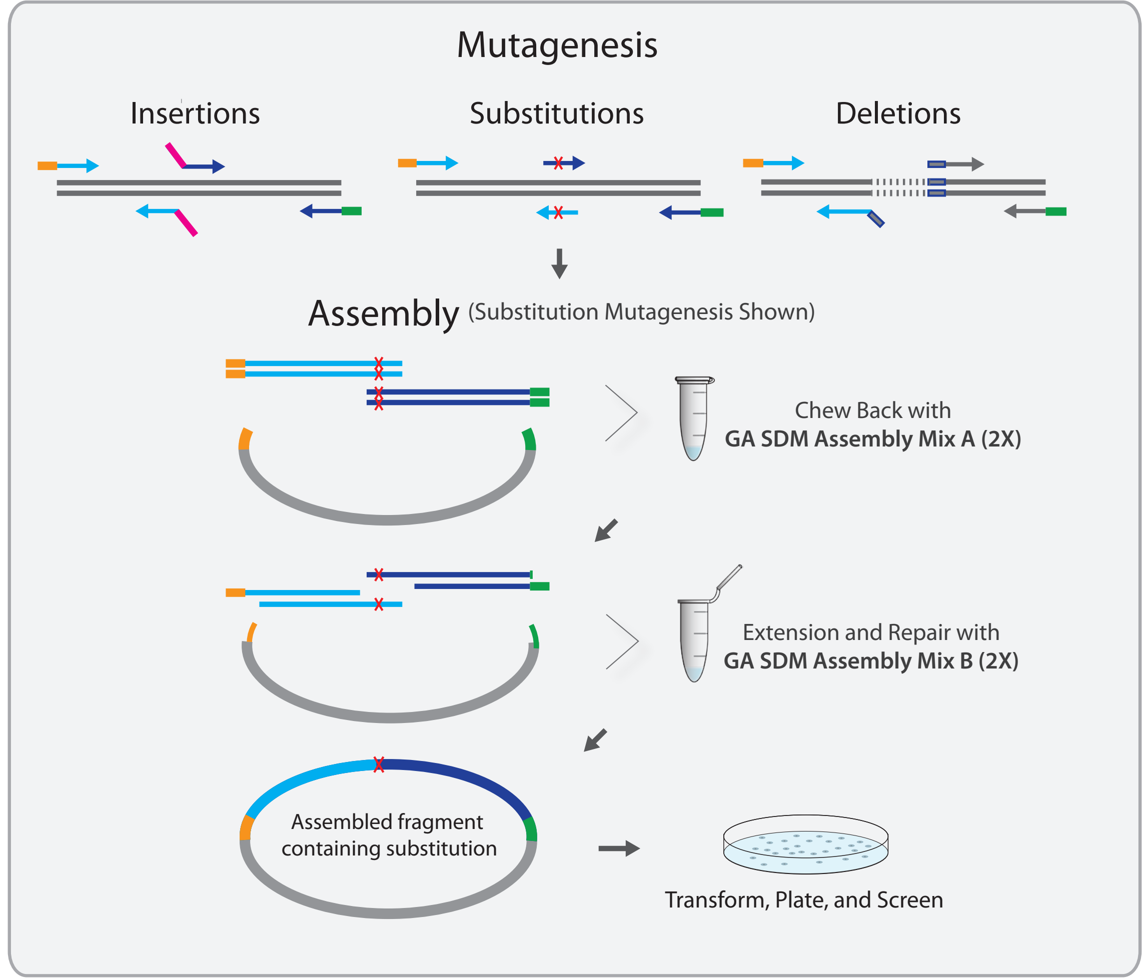


Figure 7. Overview of Gibson Assembly® Site-Directed Mutagenesis of a linear template. Following mutagenesis, DNA fragments of various lengths are uniformly assembled using complementary overlaps between fragments.

Using the Gibson Assembly® Method for Library Construction

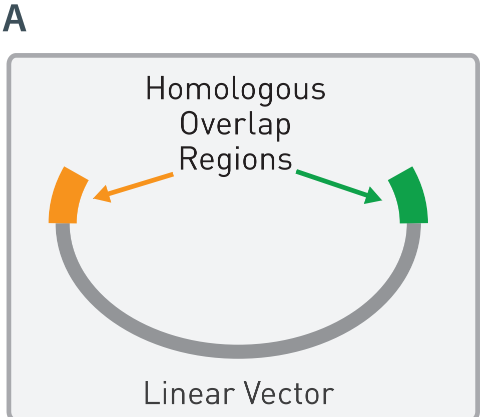
The highly efficient and robust Gibson Assembly® method is ideal for library construction applications. Examples of Gibson Assembly-compatible libraries include, but are not limited to, NNK libraries for scanning functional motifs or combinatorial site libraries for testing genetic elements.

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 in 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



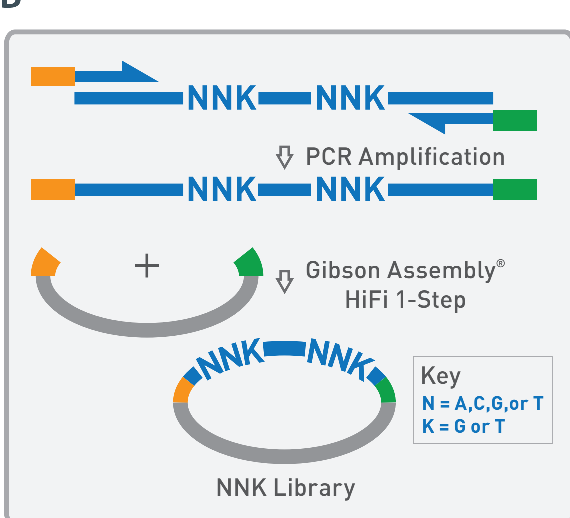


Figure 8. Preparing the Library Vector (A) and Library Insert (B) for Gibson Assembly® Cloning. (A) To prepare the library vector, linearize the library vector and identify 20 bases from each end. Add these 20–40 nucleotides to the primers used in library amplification to create homologous overlap regions 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 in comparison to traditional methods.

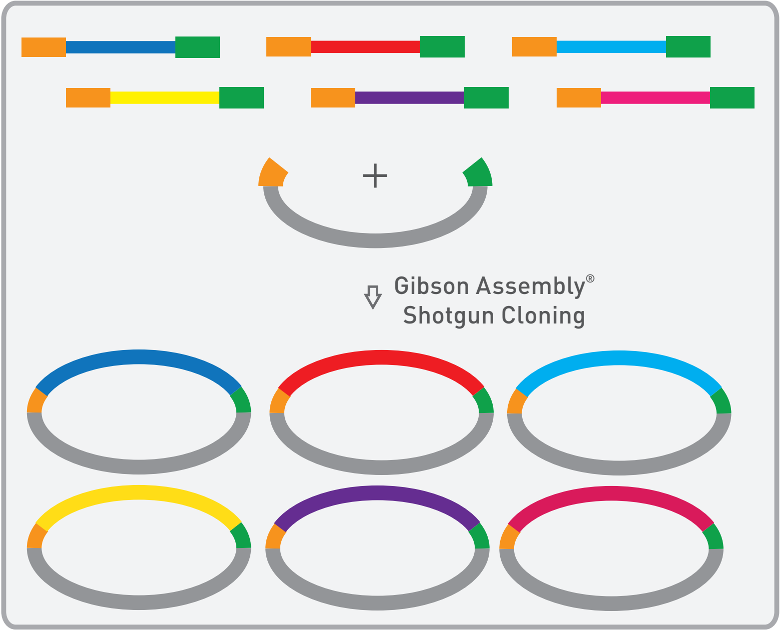


Figure 9. Gibson Assembly® Shotgun Cloning. Similar sized DNA fragments may be shotgun cloned in parallel. In order to allow for assembly, DNA fragments can be synthesized or PCR amplified with primers containing regions of homology to a single cloning vector. Following PCR amplification, DNA fragments 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	Cloning with the Gibson Assembly® method may be completed: • In a single isothermal incubation of 1 hour with the Gibson Assembly® HiFi 1-Step Kit. • In a robust, two-step reaction in 80 minutes with the Gibson Assembly® Ultra Kit.
No need to rely on compatible restriction sites	• Gibson Assembly® cloning is seamless and is achieved without the introduction of additional nucleotides. • Tedious and labor-intensive steps of searching for compatible restriction enzyme recognition sites are eliminated with the Gibson Assembly® method.
Efficient cloning of single or multiple fragments	Gibson Assembly® cloning can be used to directionally and seamlessly clone both single and multiple fragments in one round of cloning. • 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 two-step approach of the Gibson Assembly® Ultra Kit at multiple incubation temperatures.
Clone fragments from 100 bp to 100 kb	The Gibson Assembly® method and the design of the overlapping fragments allow for the assembly of both small and large fragments.
High cloning efficiencies	High cloning efficiencies translate to high rates of successfully identifying downstream full-length, error-free clones.
High percentage of error-free constructs	The incorporation of proof-reading polymerases and error repair technology in the SGI-DNA Gibson Assembly® mixes helps minimize the presence of errors at cloning junctions, enabling high-fidelity cloning.
Low substrate requirement	Efficient assembly may be accomplished with only nanogram quantities of starting material.

Gibson Assembly® Method and Other Cloning Approaches

Cloning Method Comparison

	Gibson Assembly® Kit Cloning	Restriction Enzyme Digest Cloning	T/A Cloning (PCR Fragment Cloning)	Recombination-Based Cloning (*e.g.* Gateway™ Cloning)
Methodology	**Relies on homologous overlap sequences**	Restriction enzyme digestion	Single base overhang	Requires vectors and inserts with recombination sequences (att sites)
Efficiency	**>90%**	Variable	Variable	Up to 95%
Insertion site after cloning	**Seamless**	May leave a scar or seam	Single base insertion	att sites remain as part of insert/ construct
Ability to clone multiple fragments in a single round of cloning?	**Yes**	No	No	Yes
Compatible with any vector?	**Yes**	Yes	No	Yes
Directional?	**Yes**	Maybe	No	Maybe

Table 2. Gibson Assembly® Kits offer many advantages over other cloning approaches.

Designing Homologous Overlaps

Primer Design

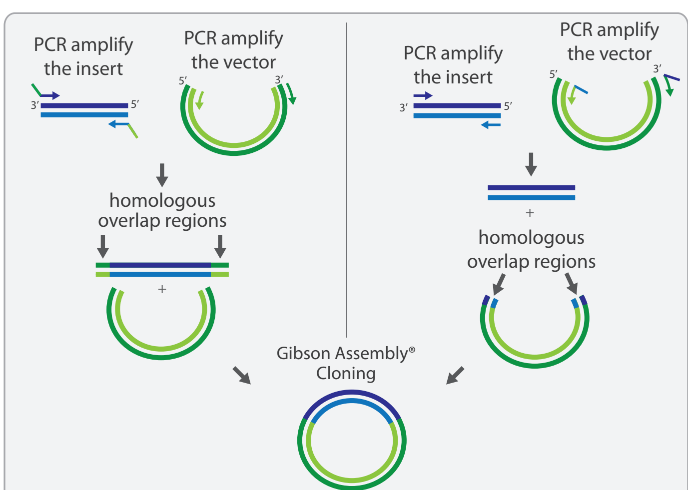
Designing optimal homologous overlap regions for Gibson Assembly® cloning is critical to a successful assembly reaction. This section covers the basics of Gibson Assembly® primer design and should be helpful as you create your Gibson Assembly® design strategies. The free online primer design tool at **sgidna.com/gibson-assembly-primers** is also available to assist with primer design (see page 18).

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 homologous region sequence may be derived from the vector (by adding overlap to insert as shown below) or the insert (by adding overlap to vector as shown below). Alternatively, the overlap can be split between the vector and insert ends (not shown here). If multiple inserts are being added to the vector, one end of each adjacent fragment is incorporated or split between the fragments.

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).

Tips for Designing Primers to Generate Homologous Overlaps

If fragments will be generated by PCR instead of made synthetically (e.g., DNA Tiles™), careful primer design is required. The tips below and standard guidelines for designing effective PCR amplification primers should be used.

- The optimal length of the overlap region depends on the number and length of the fragments in the assembly reaction.
- For higher order assembly, longer overlap regions will result in higher efficiency.
- Avoid tandem repeats, homopolymers, high secondary structure, and extremely high or low GC content.
- 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	—	—	—	—	—	—

Table 3. Suggested kits and overlap lengths for different numbers of inserts and sizes.

Key

Gibson Assembly® HiFi 1-Step Kit Recommended
Gibson Assembly® Ultra Kit Recommended
— = Not recommended

Online Tool for Designing Gibson Assembly® Primers

The Gibson Assembly® Primer Design 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. The primers designed by the tool are derived from sequences provided by the user.

Using the Gibson Assembly® Primer Design Tool

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

To use the 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 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 Tool Use

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

For fragments less than 5.5 kb, the Tool is capable of generating primers for 1–15 fragments. For fragments >5.5 kb, optional subassembly primers will be generated by the 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**.

Gibson Assembly® Primer Design Tool Example

The following example demonstrates using the GAP Primer Design Tool for designing Gibson Assembly® primers for inserting the *Kan*^{R} gene 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.**
3. **Press Next.**

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

Vector Sequence

To start the tool, please enter your vector sequence in FASTA format. Please note, the homologous overlapped will be added by PCR reaction. Therefore, if the vector or fragment IS NOT PCR amplified, the homologous sequence will be added to the adjacent fragment.

- The primer length is default at 60 nt ?
- The homologous length sequence is default at 40 bp ?

Enter Your Vector ?

```
>pUCGA1.0
AATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCT
TGCATGCCTGCAGGTCGACTCTAGAGGATCGAGCACATCTCGTTCGCTATTCAGGGATTG
CTCGAGGGAAGTTTGTCTAGATCTCAGGCGTGGATGCGGGTACCGAGCTCGAATTCACTG
```

◀TIP▶ Sequence must be entered in FASTA format

Do you want to PCR amplify your vector?

(•) Yes ( ) No

Next »

4. **Input Vector Properties:**
   - A. Indicate if insertion site is by position or restriction enzyme digest.
   - B. Indicate whether restriction enzyme site should be regenerated.
   - C. Indicate number of restriction enzyme sites.

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

2. Your Vector Input

How would you like to select the site to place insert(s)? ?

( ) By Position (•) By Restriction

Do you want to regenerate your restriction site? ?

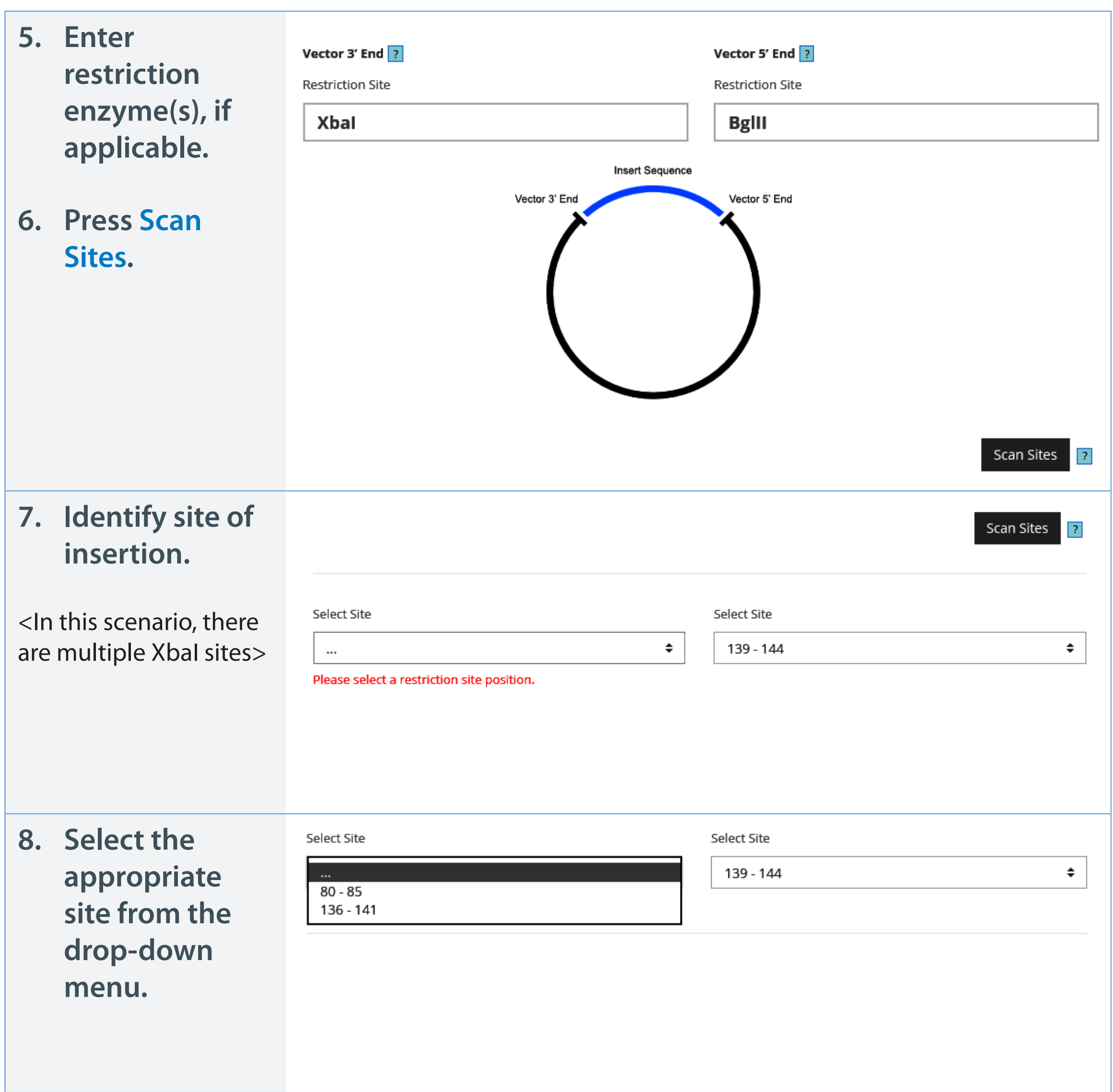
(•) Yes ( ) No

◀TIP▶ Click on question marks for additional information

How many restriction cut sites do you want? ?

( ) 1 (•) 2

Online Tool for Designing Gibson Assembly® Primers



5. Enter restriction enzyme(s), if applicable.

6. Press Scan Sites.

7. Identify site of insertion.

<In this scenario, there are multiple XbaI sites>

8. Select the appropriate site from the drop-down menu.

9. Enter the insert properties and sequence:

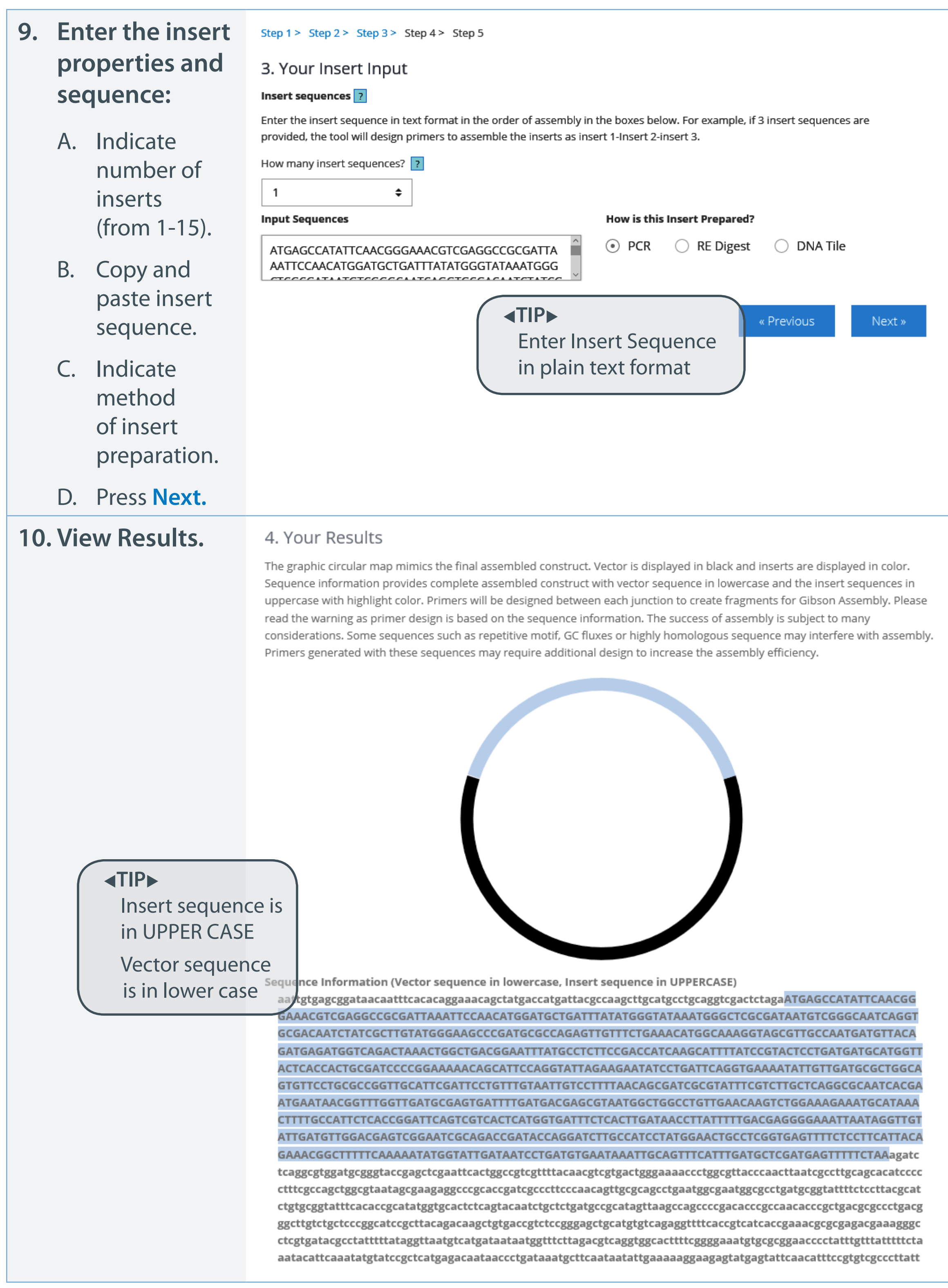
A. Indicate number of inserts (from 1-15).

B. Copy and paste insert sequence.

C. Indicate method of insert preparation.

D. Press **Next.**

10. View Results.



11. Download Primer Information

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
```

Download Primers:

- Excel Spreadsheet (csv)
- PDF Summary

« Previous

12. Download PDF or Excel spreadsheet summary to view primer sequences and T_m.

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 fragment ends for assembly, steps involved in generating vector or fragment overlap regions by PCR prior to assembly are not necessary. 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 with the elimination of the inherent limitations of PCR.

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 24). For Gibson Assembly® PBnJ™ Cloning, instead of designing primers to generate homologous overlap regions, a primer pair is used to bridge non-homologous overlap regions. The primer pairs 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 nonoverlapping, exposed template sequence, which is later extended and ligated by the 5′ → 3′ polymerase activity of the GA HiFi 1-Step Master Mix.

Some Variations of Gibson Assembly® PBnJ™ Cloning

Gibson Assembly® PBnJ™ Cloning	Overview	Description
Seamless Joining with a Primer Pair	Gibson Assembly® PBnJ™ Cloning is accomplished using nonoverlapping 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 24
Variation	**Overview**	**Description**
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 25
Sequence Insertion Cloning	Gibson Assembly® PBnJ™ Sequence Insertion Cloning can be used to insert sequence between adjoining fragments during assembly. A pair of phosphorothioate-modified primers bridging fragments to be joined is utilized to create the insertion.	See Figure 13 on page 26

Gibson Assembly® PBnJ™ Seamless Joining

The following illustration outlines the steps of Gibson Assembly® PBnJ™ Seamless Joining. The illustration shows the assembly at a single junction, but the technique can be used with linearized inserts and vectors for assembly at multiple junctions to form 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 nonoverlapping, 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.

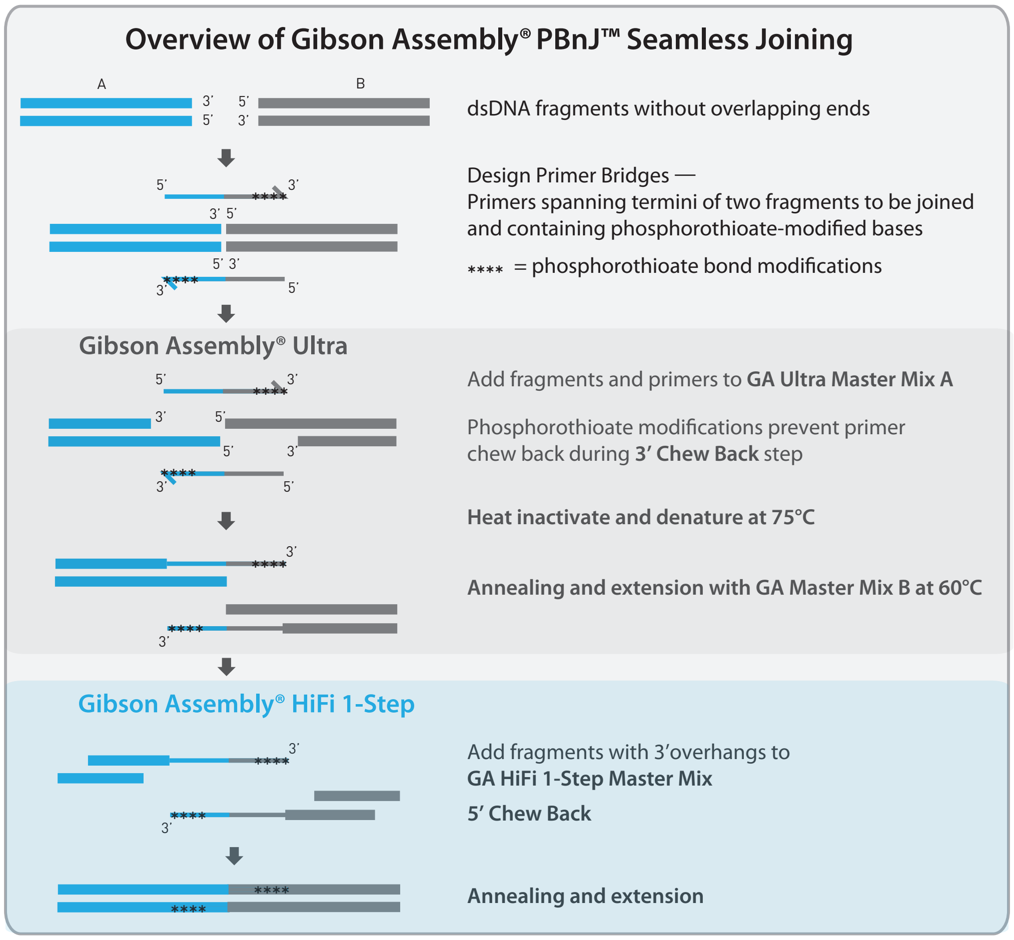


Figure 11. Overview of Gibson Assembly® PBnJ™ Seamless Joining. Gibson Assembly® PBnJ™ seamless joining is accomplished using nonoverlapping fragments assembled with the Gibson Assembly® Ultra Kit, followed by the Gibson Assembly® HiFi 1-Step Kit.

Gibson Assembly® PBnJ™ 3' Overhang Extension

Gibson Assembly® 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 .

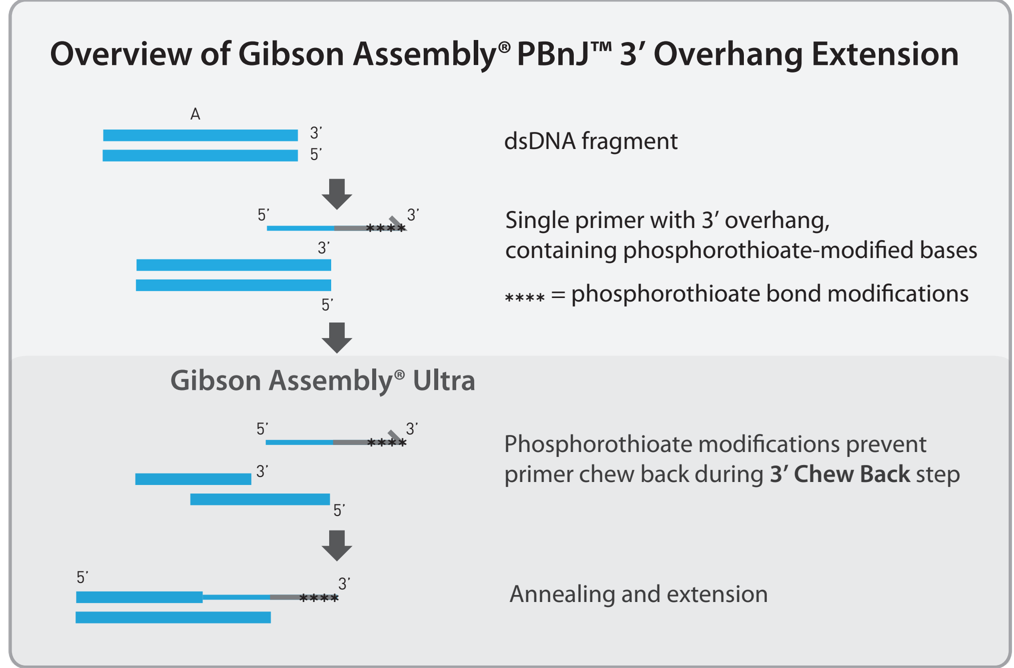


Figure 12. Overview of Gibson Assembly® PBnJ™ 3' Overhang Extension. Gibson Assembly® PBnJ™ 3' Overhang Extension is accomplished with a single phosphorothioate-modified primer and the Gibson Assembly® Ultra Kit. Extension of the 3' overhang is initiated by combining a single phosphorothioate-modified 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 utilized to add DNA sequence between non-overlapping fragments during assembly. This technique could be useful for many types of downstream experiments such as mutagenesis studies, promoter or enhancer studies, and large-scale genome modification studies. This modification called Gibson Assembly® PBnJ™ Sequence Insertion Cloning, is outlined in the figure below.

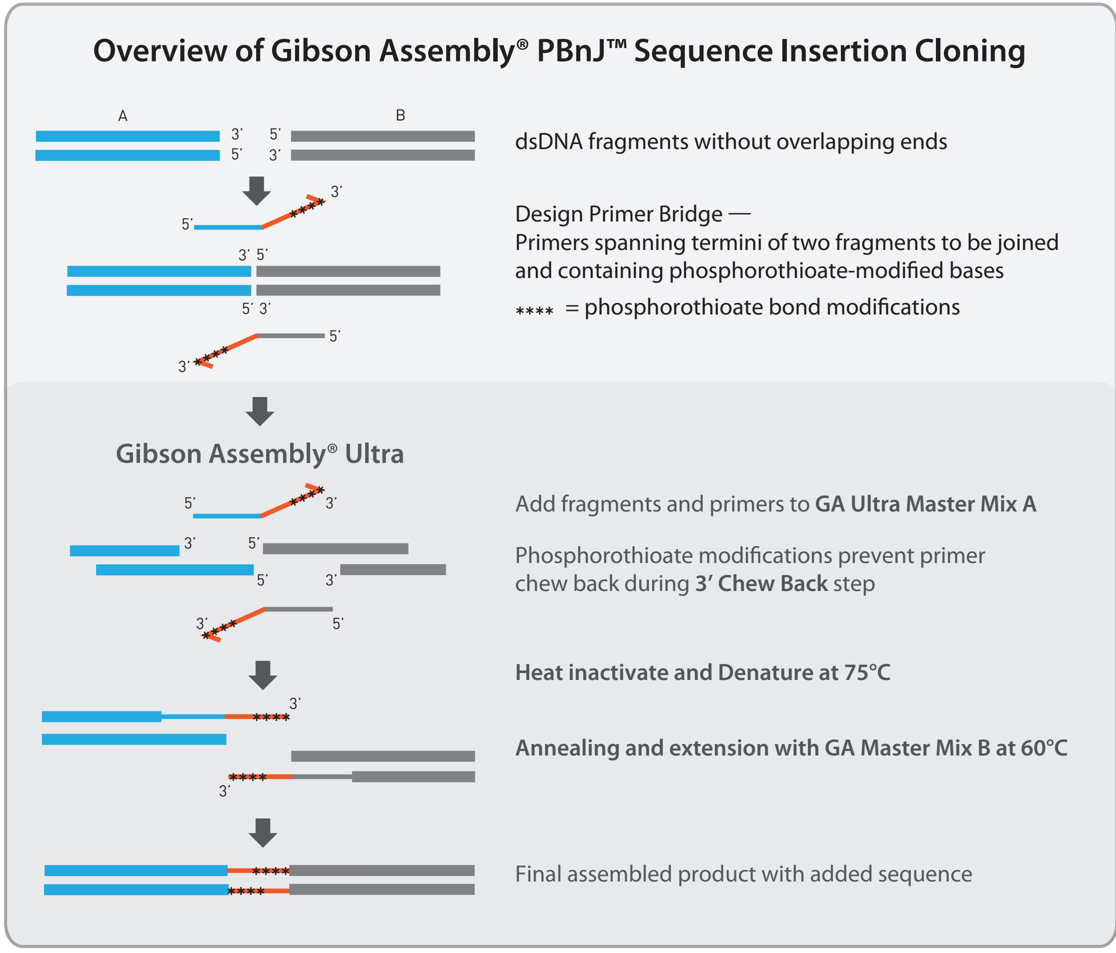


Figure 13. Overview of Gibson Assembly® PBnJ™ Sequence Insertion Cloning. Gibson Assembly® PBnJ™ Sequence Insertion Cloning adds sequence between adjoining fragments during assembly. The assembly reaction is initiated by combining phosphorothioate-modified primers, Gibson Assembly® Ultra Master Mix A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

Appendix A: Protocols

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

To add homologous ends to DNA fragments, SGI-DNA recommends 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.

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

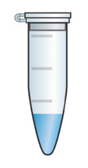
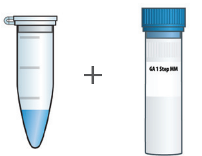
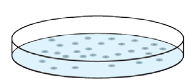
Step	Temperature	Duration	Number of Cycles
Initial Denaturation	98°C	30 seconds	1 Cycle
Amplification	98°C	10 seconds	25–30 Cycles
	Primer T_m (60°C to 70°C)	20 seconds	
	72°C	30 seconds per kb	
Final Extension	72°C	5 minutes	1 Cycle
Hold	4°C	—	1 Cycle

Gibson Assembly® HiFi 1-Step Kit

Use approximately 10–100 ng of each DNA fragment (including the cloning vector) in equimolar amounts.

Fragment size	Amount	pmol	Note
≤1 kb	20–40 ng	0.04	For DNA fragments ≤1 kb, we recommend using a 5-fold molar excess of insert.
1–5 kb	10–25 ng	0.008–0.04	
5–8 kb	25 ng	0.005–0.008	
8–20 kb	25–100 ng		
20–32 kb	100 ng	0.005	

Gibson Assembly® HiFi 1-Step— Quick Protocol

Step		Instruction
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**.
3. Vortex		Vortex the master mix.
4. Combine MM and DNA		On ice, combine **5 µL DNA** and **5 µL Master Mix**. Mix well and perform a quick spin.
5. Incubate		Incubate at **50°C** for **1 hour**.
6. Transform		Store reactions at –20°C or proceed to transformation.

Gibson Assembly® Ultra Kit

Use approximately 10–300 ng of each DNA fragment (including the cloning vector) in equimolar amounts.

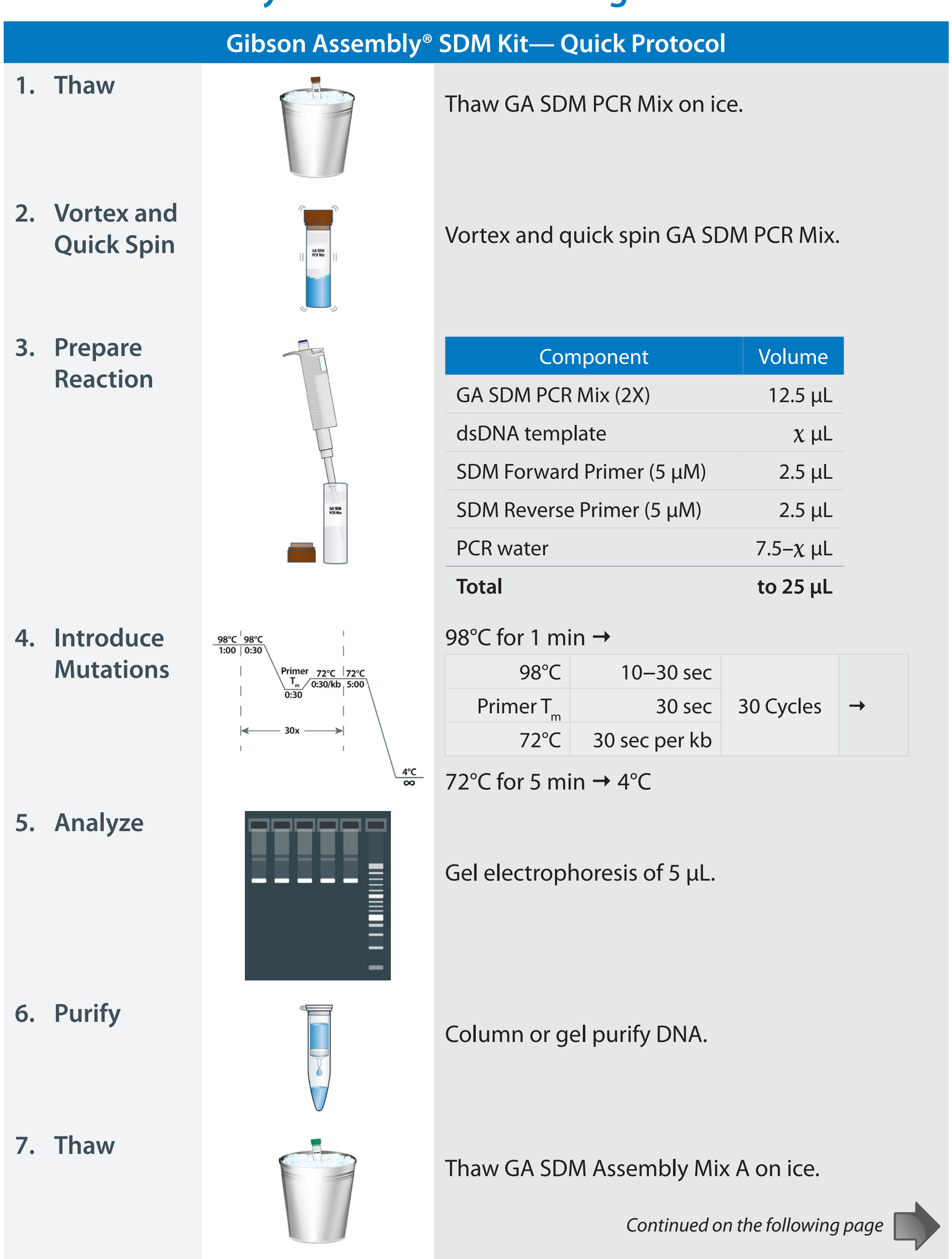
* For DNA fragments ≤1 kb, we recommend using a 5-fold molar excess of insert.

Fragment size	Amount	pmol
≤1 kb	20–40 ng*	0.04
1–5 kb	10–25 ng	0.008–0.04
5–8 kb	25 ng	0.005–0.008
8–20 kb	25–100 ng	
20–32 kb	100 ng	~0.008
32–100 kb	100–300 ng	0.005

Gibson Assembly® Ultra— Quick Protocol

Step	Instruction
1. Thaw	Thaw 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 perform a quick spin.
4. Combine MM A and DNA	On ice, combine **5 µL of DNA** with **5 µL of MM A.** Mix well and perform a quick spin.
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 perform a quick spin.
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.

Gibson Assembly® Site-Directed Mutagenesis Kit



Gibson Assembly® SDM Kit— Quick Protocol

1. Thaw

Thaw GA SDM PCR Mix on ice.

2. Vortex and Quick Spin

Vortex and quick spin 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

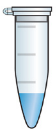
Column or gel purify DNA.

7. Thaw

Thaw GA SDM Assembly Mix A on ice.

Continued on the following page

8. Combine DNA



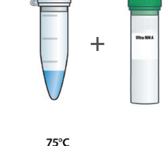
Combine 1 to 5 SDM fragment(s) and vector DNA to a total volume of 5 µL:

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

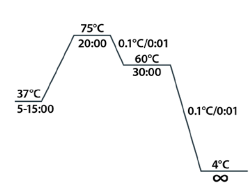
Vortex GA SDM Assembly Mix A.

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 perform a quick spin.

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

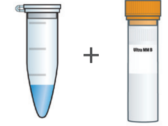
12. Thaw

Thaw GA SDM Assembly Mix B on ice.

13. Vortex

Vortex and quick spin.

14. Add SDM Mix B

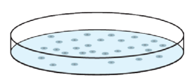


On ice, combine **10 µL of reaction** with **10 µL of GA SDM Assembly Mix B.**

15. Extension and ligation

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

16. Transform



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

Appendix B: 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.
    - It 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 reactions 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 assembly 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. I would like to produce overlapping dsDNA fragments by PCR. Do I need to use PCR primers that have been purified by PAGE or HPLC?

    No. Standard, desalted primers may be used.

2. I would like to assemble ssDNA oligonucleotides into dsDNA fragments. Do I need to use oligonucleotides that have been purified by PAGE or HPLC?

    No. Standard, desalted primers may be used.

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 at once 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.

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. For optimal results, use these fragments in ≥ 5-fold molar excess with the Ultra Kit.

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. To enable Gibson Assembly® cloning using a vector prepared with a restriction digest, the homologous overlap sequences would need to be added to the insert.

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.

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.

Appendix B: FAQs

Procedural Questions

1. How should I store a Gibson Assembly kit?

   Store Gibson Assembly® kits in a constant temperature freezer (*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. 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 very 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 following the provided procedural incubation periods.

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?

   Generally, this is not necessary. A cleanup kit or a standard phenol-chloroform extraction followed by ethanol precipitation is sufficient.

7. Is there anything unique about TransforMax™ EPI300™ Electrocompetent *E. coli* cells that make them ideal for this work?

   We have successfully used the Gibson Assembly® method in combination with a variety of chemically competent and electrocompetent *E. coli* cells. However, TransforMax™ EPI300™ Electrocompetent *E. coli* consistently produce the greatest number of clones and the highest efficiency of the correct insert in comparison to the other competent cells we tested.

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 background 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 must be 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*?

   - 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 disappearance of the starting substrate DNA fragments.
   - Check the design of the overlapping DNA fragments.
   - Consider whether the cloned insert may be toxic to *E. coli* and whether a low-copy vector, such as a BAC, should be used.

Appendix C: Gibson Assembly® Citations

Year	Description	How Gibson Assembly® Cloning enabled this research
2010	Design, synthesis, assembly, and creation of the first synthesized genome and cell[16]	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*[18]	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[19]	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[20]	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[21]	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	Single-step method to clone long genomic sequences (up to 100 kb) using Cas9 nuclease and Gibson Assembly® cloning[22]	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[17]	The Gibson Assembly® HiFi 1-Step kit was used to generate 7 kb cassettes for bacterial cloning.

Table 4. Descriptions of recent key publications citing Gibson Assembly® Cloning.

Appendix D: 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. 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.
5. Nathans D, Smith HO. Restriction endonucleases in the analysis and restructuring of DNA molecules. Annual Review of Biochemistry. 1975 Jul;44(1):273-93.
6. Maxam AM, Gilbert W. A new method for sequencing DNA. Proceedings of the National Academy of Sciences. 1977 Feb 1;74(2):560-4.
7. 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.
8. 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.
9. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science. 1995 Jul 28;269(5223):496-512.
10. McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J, Sekhon M, Wylie K, Mardis ER, Wilson RK, Fulton R. A physical map of the human genome. Nature. 2001 Feb 15;409(6822):934-41.
11. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R. Initial sequencing and analysis of the human genome. Nature. 2001 Feb 15;409(6822):860-921.
12. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD. The sequence of the human genome. Science. 2001 Feb 16;291(5507):1304-51.
13. Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X. The complete genome of an individual by massively parallel DNA sequencing. Nature. 2008 Apr 17;452(7189):872-6.
14. Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C. Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. Science. 2008 Feb 29;319(5867):1215-20.
15. 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.
16. Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C. Creation of a bacterial cell controlled by a chemically synthesized genome. Science. 2010 Jul 2;329(5987):52-6.
17. Hutchison CA, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, Pelletier JF. Design and synthesis of a minimal bacterial genome. Science. 2016 Mar 25;351(6280):aad6253.
18. 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.
19. Eroshenko N, Church GM. Mutants of Cre recombinase with improved accuracy. Nature Communications. 2013 Sep 23;4:2509.
20. Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature. 2013 Jul 18;499(7458):295-300.
21. 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.
22. 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.

Ordering Information

Product Ordering information			
Product	**Product Type**	**Number of Reactions**	**Catalog Number**
Gibson Assembly® HiFi 1-Step	Kit	5	GA1100-S
	Kit	10	GA1100-10
	Kit	50	GA1100-50
	Master Mix only	10	GA1100-10MM
	Master Mix only	50	GA1100-50MM
Gibson Assembly® 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
Gibson Assembly® Site-Directed Mutagenesis Kit	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.
Synthetic Genomics® and Gibson Assembly® are registered trademarks and SGI-DNA™, DNATiles™, and PBnJ™ are trademarks of Synthetic Genomics Inc.
Excel is a registered trademark of Microsoft Corporation.

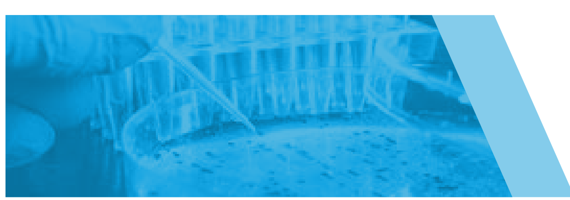
Gibson Assembly® US Patent Nos. 7,776,532, 8,435,736, and 8,968,999.

© 2016 Synthetic Genomics Inc. All rights reserved. GACG053116

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

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

Copyright © 2016 SGI-DNA, Inc. All rights reserved. All noted trademarks are the property of Synthetic Genomics, Inc. and/or its affiliates. GAG052416

#DNAMYWAY
sgidna.com/gibson-assembly