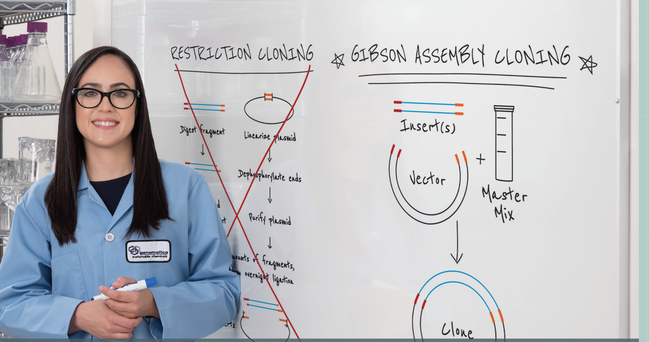

"SGI-DNA'S GIBSON ASSEMBLY® CLONING REDUCES THE TIME TO CREATE CONSTRUCTS THAT ADVANCE OUR BIOENGINEERING PROJECTS" *Stephanie Culler, Ph.D.* | Sr. Research Scientist at Genomatica

DNA MY WAY™ | Gibson Assembly® CLONING

Get DNA your way. Generate seamless plasmid DNA or BAC clones without restriction digestion or DNA scars.

Gibson Assembly® cloning, developed by the J. Craig Venter Institute and Synthetic Genomics, allows for insertion of DNA fragments into virtually any vector without the need for compatible restriction sites. Join any two fragments to generate seamless constructs in your desired vector.

- Significantly faster than traditional cloning methods
- Avoid time-consuming subcloning
- Insert 1–15 fragments in a single round of cloning in ~1 hour
- Achieve transformation efficiencies of up to 95%

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Gibson Assembly Cloning Guide

Download the Gibson Assembly® Cloning Guide at **sgidna.com/dnamyway**

To learn more about Genomatica, visit www.genomatica.com

Genomic solutions for advancing scientific discovery

At SGI-DNA, a Synthetic Genomics Inc. company, we are committed to leveraging our unique and proprietary DNA technologies into scientific solutions for accelerating and advancing research. Our ever-expanding suite of reagents, services, and instrumentation systems are fueling novel and groundbreaking approaches for basic synthetic biology research, as well as biomedical and industrial applications. We are committed to converting scientific breakthroughs into innovative products and services for scientists worldwide. From the world's first DNA printer to next-generation cloning and protein expression systems, our cutting-edge products and services are advancing scientific discovery.

GIBSON ASSEMBLY® CLONING METHOD

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"I USED THE BIOXP™ SYSTEM TO BUILD GENES TO CLONE AND EXPRESS 49 TRANSCRIPTION FACTORS FROM A MARINE DIATOM."

Sarah R. Smith, Ph.D.
Scripps Institution of Oceanography and J. Craig Venter Institute

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The **BioXp™ 3200 System** can help you develop new molecular biology workflows through automated DNA assembly.

Based on technologies developed at the J. Craig Venter Institute and Synthetic Genomics, the **BioXp™ 3200 System** enables you to build and clone DNA fragments into any vector with hands-free automation.

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GIBSON ASSEMBLY® CLONING METHOD

The Gibson Assembly method has quickly become an invaluable tool for molecular cloning workflows due to its flexibility and efficiency. Learn how to go from DNA fragments to complex plasmid constructs.

GIBSON ASSEMBLY ENZYMES

DNA Exonuclease: Chews back dsDNA to expose either 3' or 5' ssDNA overhangs, which are complementary to an overlapping region between inserts
DNA Polymerase: Fills in gaps between annealed fragments
DNA Ligase: Seals nicks in the final dsDNA construct

DNA ASSEMBLY 101

The Gibson Assembly method allows for the cloning of multiple fragments directly into any vector. This method does not require the use of restriction enzymes. Instead, user-defined overlapping ends are incorporated into fragments to allow seamless joining of adjacent fragments. Through a series of enzymatic steps, overlapping regions are created, annealed, and ligated over the course of an hour, resulting in a final covalently-joined double-stranded (ds) DNA product.

DESIGNING INPUT dsDNA

Design primers corresponding to your fragment that also include 30-40 bp homology with your adjacent fragment or vector.

Tips for designing primers:

1. The bonds between G and C nucleotides are stronger than A and T bonds. For optimal annealing, include a run of GC residues at the ends of your primers.
2. G and C nucleotides should make up 40%–60% of each primer sequence to maintain a similar melting temperature.
3. When submitting your primer sequence, be sure the reverse primer is flipped and written as 5' to 3'.

NO OVERLAPPING ENDS? NO PROBLEM.

With the Primer-Bridge End Joining (PBnJ™) method, you won't have to rely on sequence homology between adjacent DNA fragments. With Gibson Assembly PBnJ cloning, a single primer or primer pair with phosphorothioate-modified bases (PS) is used to "bridge" together the ends of DNA fragments. The PS modifications prevent primer chew back during the 3' chew back step.

Use the following table to determine the amount of input DNA required for each fragment size

Fragment size	0.5 kb	1.0 kb	5 kb	8 kb	10 kb	15 kb	20 kb	30 kb
Amount DNA	20 ng	25 ng	25 ng	50 ng	50 ng	50 ng	50 ng	50ng
pmol DNA	0.061	0.038	0.008	0.009	0.008	0.005	0.004	0.003

Or precisely determine the amount of pmol or ng of DNA using the following formulas

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

Quickly assemble up to 5 fragments **1**

DNA Assembly: 1-Step Method

Use the 1-step method for the assembly of up to 5 fragments between 500bp to 32 kb.

Assemble 5 to 15 fragments **2**

DNA Assembly: 2-Step Method

Use the 2-step method for DNA fragments between 100 bp to 100 kb OR for the assembly of 5 to 15 fragments.

OUTPUT dsDNA:

The fully assembled dsDNA is ready for direct use in a variety of downstream molecular biology applications, such as PCR, rolling-circle amplification (RCA), and direct transformation.

A CLOSE-UP OF THE GIBSON ASSEMBLY METHODS

1 GIBSON ASSEMBLY ONE-STEP METHOD

2 GIBSON ASSEMBLY TWO-STEP METHOD

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