pUCGA 1.0
Quick Reference Manual

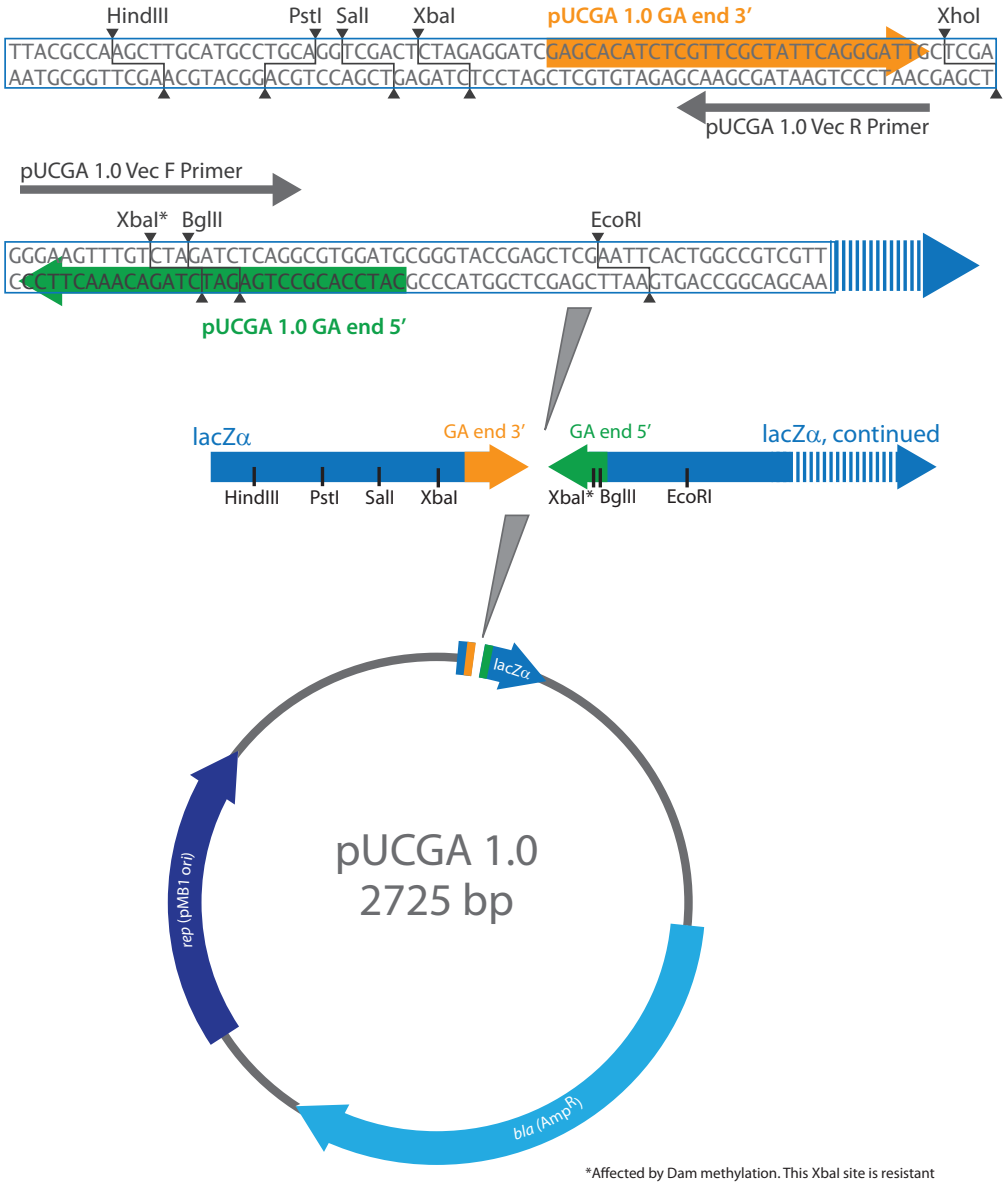
For additional product information and a detailed protocol, visit **sgidna.com/gibson-assembly**

Catalog Numbers GA1020-05 and GA1020-100

pUCGA 1.0

Catalog number	Quantity	Volume	Concentration	Number of reactions	Storage temperature
GA1020-05	1 tube	5 µL	15 ng/µL	5 Reactions	–20°C
GA1020-100		100 µL		100 Reactions	

pUCGA 1.0 Vector Map and Sequence Information



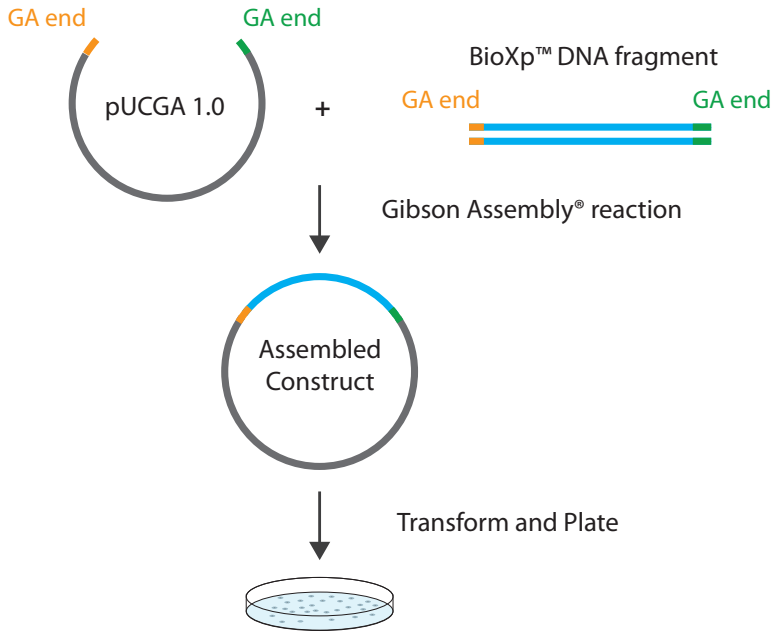
*Affected by Dam methylation. This XbaI site is resistant to cleavage unless DNA is isolated from dam– *E. coli*.

pUCGA 1.0 Vector Information

The pUCGA 1.0 Amp vector is a 2725 bp linearized vector containing 30-bp long GA ends. As shown in the vector map on the preceding page, the vector contains *bla* (amp^R, the ampicillin resistance gene), *lacZ* (the gene encoding the N-terminal fragment of β-galactosidase), and *rep* (the pMB1 ori site). The multiple cloning site (MCS) present within the *lacZ* gene contains the site of linearization between flanking GA ends.

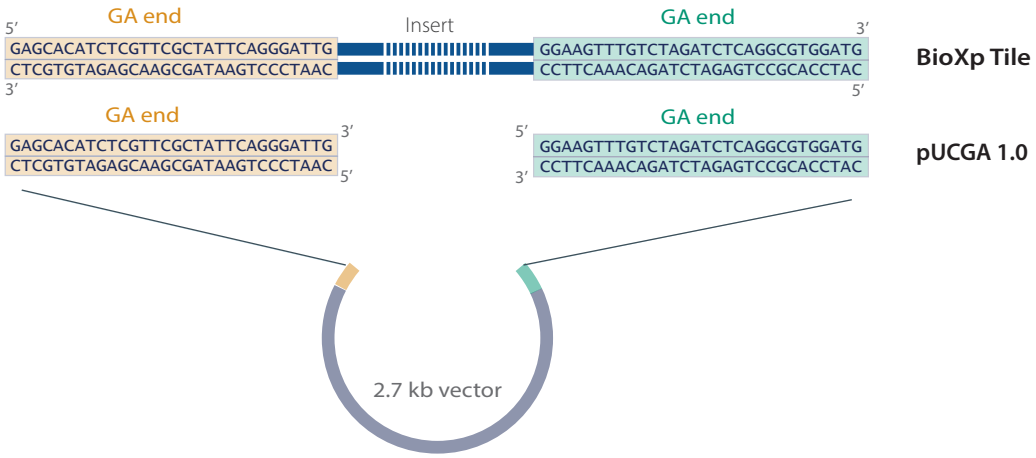
GA Ends

GA ends facilitate BioXp™ fragment assembly with the pUCGA 1.0 vector. As shown in the vector map illustration on the previous page, GA end sequences are present within the MCS of the *lacZ* gene. BioXp™ Tiles, GA ends generated from the SGI-DNA BioXp™ 3200 genomic workstation, include GA ends at the termini of the BioXp™ fragments. Because GA ends are homologous overlap regions present at the termini of both the pUCGA 1.0 vector and BioXp™ Tiles, GA ends, no further sequence modification of BioXp™ Tiles, GA ends is necessary for direct downstream assembly of these BioXp™ fragments with pUCGA 1.0 using the Gibson Assembly® method.



GA Ends Sequence

GA end sequences of the pUCGA 1.0 vector and an insert with homologous Gibson ends are shown below. The 5′ GA end of the pUCGA 1.0 vector is illustrated with green and the 3′ GA end of the vector is illustrated with orange. Reciprocally, the 5′ GA end of the insert is shown in orange and the 3′ insert GA end is green.



Cloning Fragments without GA ends into pUCGA 1.0 with the Gibson Assembly® Method

To use the Gibson Assembly® method to clone fragments that do not contain GA ends into pUCGA 1.0, you will first add homologous overlap sequences (in the form of GA ends) to your fragment(s) of interest. Add GA end 3′ sequence to the forward primer and GA end 5′ sequence to the reverse primer (as shown below). Amplify your fragments to add the GA ends to the fragments prior to assembly.

Fragment Forward Primer: 5′-GAGCACATCTCGTTCGCTATTCAGGGATTGnnnnnnnnnnnnnnnnnn-3′

Fragment Reverse Primer: 5′-CATCCACGCCTGAGATCTAGACAAACTTCCnnnnnnnnnnnnnnnnnn-3′

where n = fragment-specific sequence

Gibson Assembly® Kits

Use either the Gibson Assembly® HiFi 1-Step Kit or the Gibson Assembly® Ultra Kit for BioXp™ fragment assembly with pUCGA 1.0. By default (i.e., for most applications), we recommend using the Gibson Assembly® Ultra Kit (Cat. GA1200) for the assembly reaction. Alternatively, you may use the Gibson Assembly® HiFi 1-Step Kit (Cat. GA1100). If you use the Gibson Assembly® HiFi 1-Step Kit to clone BioXp™ fragments, we recommend gel purifying the fragments prior to performing the assembly reaction.

See **sgidna.com** for details and ordering information.

Protocols

Guidelines for Assembly

- Use approximately 10–40 ng of each DNA fragment in equimolar amounts:

Fragment size	Amount	pmol
≤1 kb	20–40 ng	0.04
1–5 kb	10–25 ng	0.008–0.04

- Use 1–1.5 µL of pUCGA 1.0 in the assembly reaction.

- For best results, we recommend using the Gibson Assembly® Ultra Kit for BioXp™ fragment assembly. The Gibson Assembly® HiFi 1-Step Kit is also compatible with pUCGA 1.0 cloning of BioXp™ fragments. If you use the Gibson Assembly® HiFi 1-Step Kit to clone BioXp™ fragments, gel purify the BioXp™ fragments prior to performing the assembly reaction to increase the cloning efficiency.

Gibson Assembly® Ultra Procedure (Recommended Procedure)

1. Thaw GA Ultra Master Mix A (2X) on ice.

2. Combine the DNA fragments with pUCGA 1.0 and nuclease-free water in PCR tubes to a total volume of 5 µL according to the following table:

Component	Amount	Volume
DNA fragment(s)	10–40 ng	1–3.5 µL
pUCGA 1.0	15–23 ng	1–1.5 µL
Nuclease-free water	—	to 5 µL

3. **Vortex the thawed master mix immediately before use.**

4. In a 0.2 mL PCR tube on ice, combine 5 µL of the DNA fragment/pUCGA 1.0 vector mixture and 5 µL of GA Ultra Master Mix A (2X). Mix the reaction by pipetting.

5. Vortex and spin down all reactions.

6. Transfer assembly reaction tubes to a thermocycler and run the following conditions (1 cycle):

3′ end Chew Back	37°C for 5 minutes
Inactivation	75°C for 20 minutes
Slowly Cool	0.1°C/second to 60°C
Anneal	60°C for 30 minutes
Slowly Cool	0.1°C/second to 4°C

7. Thaw the GA Ultra Master Mix B (2X) on ice and **vortex the thawed master mix immediately before use.**

8. While keeping the tubes on ice, add 10 µL of GA Ultra Master Mix B (2X) to the reactions from step 6. Mix the reaction by pipetting.

9. Incubate the reactions using the following conditions:

Repair	45°C for 15 minutes

10. After the incubation is complete, store reactions at –20°C or proceed to transformation.

11. (Optional) Analyze assembly reactions with agarose gel electrophoresis of 5–10 µL of the reaction on a 0.8–2% agarose gel. A high molecular weight smear is indicative of a successful assembly reaction.

Gibson Assembly® HiFi 1-Step Method (Alternate Procedure)

1. Gel-purify DNA fragments for assembly.
2. Thaw GA 1-Step Master Mix (2X) on ice.
3. Combine your DNA fragments with pUCGA 1.0 and nuclease-free water in PCR tubes to a total volume of 5 µL according to the following table:

Component	Amount	Volume
DNA fragment(s)	10–40 ng	1–3.5 µL
pUCGA 1.0	15–23 ng	1–1.5 µL
Nuclease-free water	—	to 5 µL

4. **Vigorously vortex the master mix for 15 seconds immediately before use** after it is thawed.
5. In a tube on ice, combine 5 µL of the DNA fragment/pUCGA 1.0 vector mixture and 5 µL of GA 1-Step Master Mix (2X). Mix the reaction by pipetting up and down.
6. Vortex and spin down all reactions.
7. Incubate the reactions at 50°C for 1 hour.
8. After the incubation period, store reactions at –20°C or proceed to transformation.
9. (Optional) Analyze the assembly reaction with electrophoresis of 5–10 µL of the reaction on a 0.8–2% agarose gel.

Transformation: Chemically Competent Cells

We recommend transformation with *E. cloni* 10G chemically competent cells (Lucigen Cat. No. 60107) or TransforMax™ EPI300™ Electrocompetent *E. coli*. (Lucigen Cat. No. EC300110). If you use competent cells other than the recommended cells, follow the transformation protocol provided with the competent cells. Use cells with a transformation efficiency ≥1×10^9 CFU/µg pUC19.

Because some of the ingredients in the buffer mix can negatively impact the survival of some competent cells, we recommend diluting the assembly reaction before performing the transformation. Dilute Ultra assemblies up to 2-fold and dilute HiFi assemblies up to 5-fold. You may need to empirically determine the optimal level of dilution, depending on the type of cells used.

1. Pre-chill 15 mL disposable polypropylene culture tubes (17 x 100 mm, one tube for each transformation reaction).
2. Thaw chemically competent cells on ice for 5–15 minutes. Mix gently.
3. Add 40 µL of thawed, chemically competent cells to each cold tube.
4. While keeping tubes on ice, add 2 µL of the diluted assembly reaction to each cold tube of competent cells. Mix gently by stirring with the end of a pipette tip (do not pipet up and down).
5. Incubate the cells and DNA on ice for 30 minutes. Do not mix.
6. Heat shock the cell/DNA mixture in a 42°C water bath for 45 seconds.
7. Return tubes to ice for 2 minutes.
8. Add 950 µL room temperature recovery media to the cells in the culture tube.
9. Incubate the tubes with shaking at about 250 rpm for 90 minutes at 37°C to allow the cells to recover.
10. Proceed to "Plating Procedure" on page 6.

Transformation Procedure for Electrocompetent EPI300™ Cells (Alternate Procedure)

1. Prepare 15 mL snap cap tubes with 1 mL SOC per tube. Place tubes on ice for 10 minutes.
2. Chill electroporation cuvettes on ice.
3. Pipet 30 µL of EPI300™ cells directly between the slit of the cuvettes on ice (one cuvette per reaction).
4. Add 2 µL of the diluted assembly reaction to the cells in the cuvette. Mix by pipetting up and down gently two times.
5. Incubate cuvette on ice for one minute.
6. Gently tap cuvette on a benchtop two times to make sure all contents are at the bottom of the cuvette in between the slit.
7. Insert the cuvette into a BioRad Electroporator or equivalent, and press PULSE. Pulse Settings for EPI300™ cells are 1200 V, 25 uF, 200 Ω, 0.1 cm cuvette.
8. During the pulse (≈2 seconds), remove 800 µL SOC from a pre-chilled 1.5 mL tube (step 1). Immediately add the SOC to the cuvette after the pulse.
9. Mix the cells and SOC by pipetting up and down. Add the mixture back into the tube containing the remaining SOC.
10. Incubate the cells for 1 hour at 37°C with shaking at 200 rpm.
11. Proceed to "Plating Procedure".

Plating Procedure

1. Pre-warm LB plates containing 100 µg/mL carbenicllin in an incubator upside down for 10–15 minutes.
2. After the incubation, plate 1/2–1/50 of the transformation reaction (2–500 µL of 1 mL) onto LB agar plates with appropriate antibiotics. See "Recommended Plating Volume" for more information.
3. Incubate plates at 37°C upside down, overnight.
4. Pick colonies for screening.

Recommended Plating Volume

Plate two plates: one low and one high volume

Number of fragments	Plating volume	For example, we normally plate...*
1–2	1/50	2 µL and 20 µL
3–5	1/10	10 µL and 100 µL
> 5	1/2†	100 µL and 500 µL

*Based on a 1000 µL transformation mixture
†Spin down the reaction before plating

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

Additional product information and resources are available at sgidna.com