NEB® Golden Gate Assembly Kit (BsaI-HF®v2)

NEB #E1601S/L

20/100 reactions

Version 2.0_2/20

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The NEB Golden Gate Assembly Kit (BsaI-HFv2) Includes

Important Note: Upon arrival, store the kit components at –20°C.

NEB Golden Gate Enzyme Mix (BsaI-HFv2)
Contains an optimized mix of BsaI-HFv2 and T4 DNA Ligase.

pGGAselect Destination Plasmid

Provides the vector backbone for assemblies.

T4 DNA Ligase Buffer (10X)
Contains buffer components optimal for BsaI-HFv2 digestion and ligation of DNA

Required Materials Not Included:

User-defined inserts
Competent cells
Other materials for transformation

Introduction

New England Biolabs now offers two specificities for Golden Gate Assembly; this assembly kit (NEB #E1601) for BsaI-directed assemblies, and a new assembly kit (NEB #E1602) for BsmBI-directed assemblies. This allows flexibility in choosing an appropriate kit based on the presence of internal sites in assembly components for any one Type IIS restriction enzyme. For both kits, optimization of enzyme amounts /ratios, buffer composition and soak/cycling parameters yields a kit of unparalleled functionality in terms of efficiency (number of transformants) and fidelity (% correct assemblies).

This assembly kit contains an optimized mix of BsaI-HFv2 and T4 DNA Ligase. BsaI-HFv2 has been engineered by NEB and outperforms BsaI in Golden Gate Assemblies. Together these enzymes can direct the assembly of multiple inserts/modules and also single insert/library generation cloning with single insert(s) using the Golden Gate approach. Also provided is the pGGAselect destination plasmid, which provides a backbone for your assembly. This versatile destination construct has flanking recognition sites in the correct orientation for BsaI-directed assemblies, and also BsmBI- and BbsI-directed assemblies, enabling the destination plasmid to conveniently be used with all three of the most commonly used Type IIS restriction enzymes used for Golden Gate Assembly. It features convenient restriction enzyme sites for subcloning, and has T7/SP6 promoter sequences to enable *in vitro* transcription.

The efficient and seamless assembly of DNA fragments, commonly referred to as Golden Gate Assembly (1,2), had its origins in 1996, when for the first time it was shown that multiple inserts could be assembled into a vector backbone using only the sequential (3) or simultaneous (4) activities of a single Type IIS restriction enzyme and T4 DNA Ligase.

Type IIS restriction enzymes bind to their recognition sites but cut the DNA downstream from that site at a positional, not sequence-specific, cut site. Thus, a single Type IIS restriction enzyme can be used to generate DNA fragments with unique overhangs. As an example, BsaI has a recognition site of GGTCTC(N1/N5), where the GGTCTC represents the recognition/binding site, and the N1/N5 indicates the cut site is one base downstream on the top strand, and five bases downstream on the bottom strand. Assembly of digested fragments proceeds through annealing of complementary four base overhangs on adjacent fragments. The digested fragments and the final assembly no longer contain Type IIS restriction enzyme recognition sites, so no further cutting is possible. The assembly product accumulates with time.

While particularly useful for multi-fragment assemblies such as Transcription Activator Like Effectors (TALEs)(5) and TALEs fused to a FokI nuclease catalytic domain (TALENs)(6), the Golden Gate method can also be used for cloning of single inserts and inserts from diverse populations that enable library creation, and multi-site mutagenesis involved in directed evolution (7).

A technical note describing Golden Gate Assembly initiatives at NEB and typical results for assemblies differing in complexity is available on our website www.neb.com/GoldenGate.

Please note that while general descriptions regarding Golden Gate Assembly use the BsaI nomenclature, this kit and protocols feature the specific engineered form optimized for Golden Gate Assembly, BsaI-HFv2.

Figure 1. Overview: Assembly Protocol of Golden Gate Assembly

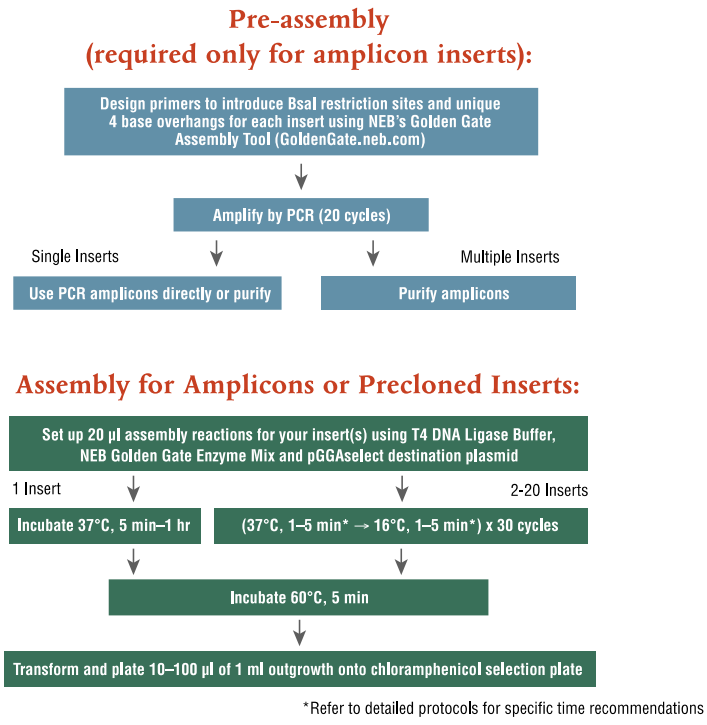
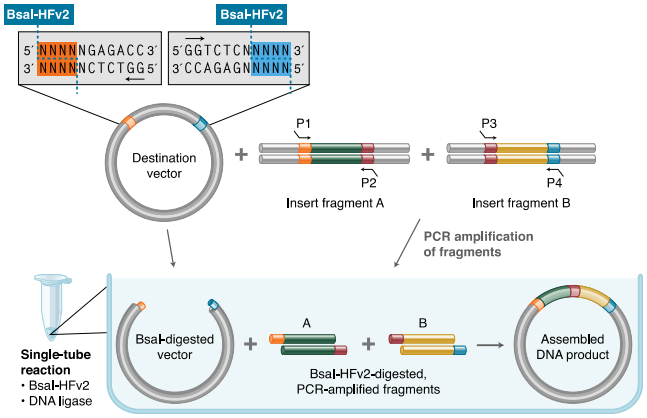


Figure 2. Golden Gate Workflow

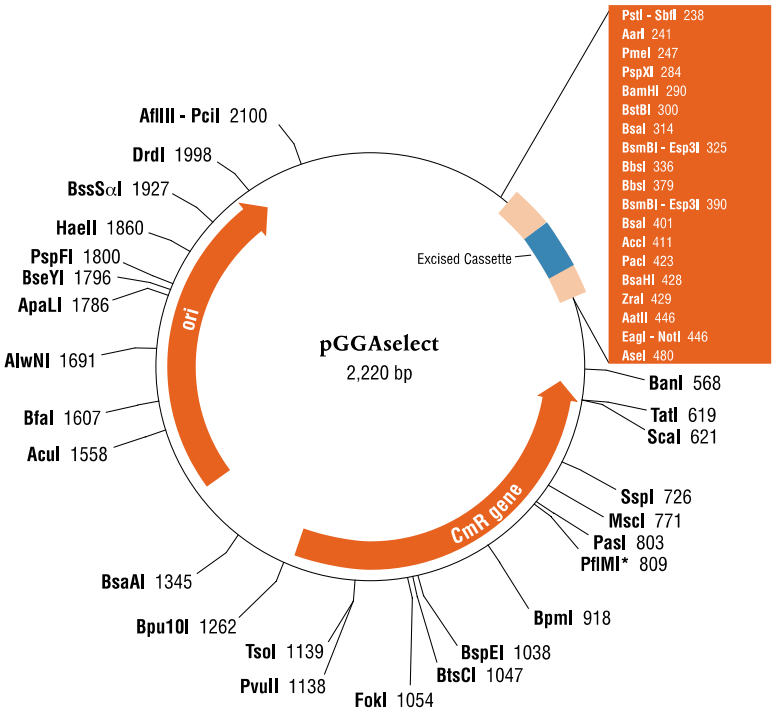


In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI-HFv2 (GGTCTC), added to both ends of a dsDNA fragment. After digestion, these sites are left behind, with each fragment bearing the designed 4-base overhangs that direct the assembly.

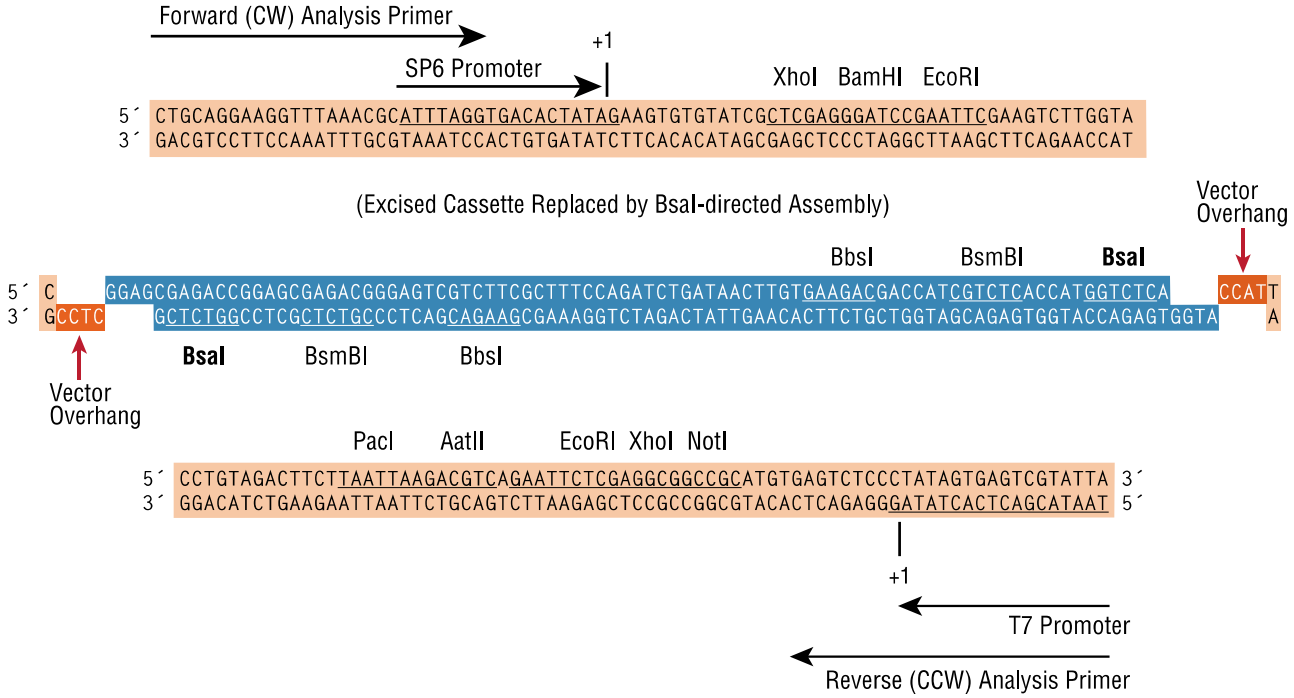
pGGAselect Destination Plasmid

pGGAselect is a 2,220 bp cloning vector useful for Golden Gate Assembly.

The plasmid contains two BsaI, BsmBI and BbsI restriction sites; digestion with BsmBI releases an 87 bp fragment and a 2,133 bp vector backbone fragment to receive your insert or assembly.



Features within Sequence Flanking the Assembly Site



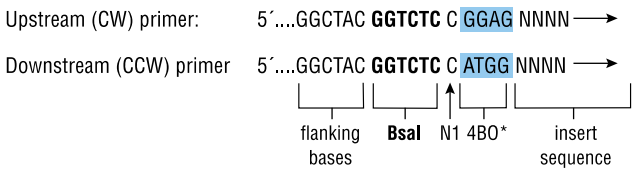
Insert Considerations:

Historically, Golden Gate inserts were precloned into plasmid constructs having flanking Type IIS restriction sites to generate the appropriate 4 base overhang sequences that guide the assembly. However, the use of amplicon inserts without precloning also supports efficient assembly levels and saves time. See below for specific recommendations for precloned inserts, and amplicon inserts for single insert cloning and multiple insert assembly:

A. Precloned Inserts: Precloning is always an option, and is superior for inserts < 250 bp or > 3 kb, or those containing repetitive elements that might accumulate errors during PCR amplification. The pMiniT 2.0 vector backbone used in the NEB PCR Cloning Kit (NEB #E1202/#E1203) is an excellent cloning option as the pMiniT 2.0 Vector backbone has no BsaI sites. Note that all sequences that will be part of the assembly must be flanked by correctly oriented BsaI restriction sites, facing towards the insert on the top and bottom strands.

B. Amplicon Inserts: The 5´ flanking bases and BsaI restriction enzyme recognition site are introduced through PCR primer design upstream and downstream of sequences to be assembled. In all cases, the 2:1 insert:vector backbone (2,133 bp for pGGAselect) ratio is suggested to achieve assembly efficiencies similar to that with precloned inserts. For molar calculations we recommend using the NEBioCalculator® (nebiocalculator.neb.com).

(a) Single Insert Cloning/Assembly Primer Design:



* 4 base overhang to allow annealing/ligating into pGGAselect vector backbone in CW orientation; for CCW assembly orientation, switch the 4 base overhang sequences in the 2 primers.

Single insert amplicons should be single specific PCR products, with no non-specific amplification or smearing present; if the amplicon is not present as a single product, optimize the PCR amplification.

While single insert amplicons can be used directly from PCR without purification under certain circumstances (see FAQ), it is always best to purify amplicons using spin columns such as the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). Use of these kits will result in purified, higher concentration DNA due to smaller elution volumes.

(b) Multiple Insert Assembly Primer Design. The first and last inserts will require the same four base overhangs diagrammed above for single insert assembly as these overhangs will ligate to the pGGAselect vector backbone. For all other inserts involved in the multiple insert assembly, we recommend using the NEB Golden Gate Assembly Tool (goldengate.neb.com) for the design of PCR primers to ensure the correct unique 4 base overhangs between inserts.

All amplicons should be single specific PCR products, with no non-specific amplification smearing present; if the amplicon is not present as a single product, optimize the PCR amplification.

All multiple inserts must be purified using spin columns as described above.

Detailed Protocols

Golden Gate Assembly Protocol:

1. Set up assembly reactions as follows:

REAGENT	ASSEMBLY REACTION
pGGAselect Destination Plasmid (1), 75 ng/µl	1 µl
Inserts (user provided): -if precloned (2) - if in amplicon form (3)	 75 ng each plasmid 2:1 molar ratio (4), (insert:vector backbone; pGGAselect = 2,133 bp; 75 ng = 0.05 pmol)
T4 DNA Ligase Buffer (10X)	2 µl
NEB Golden Gate Enzyme Mix (BsaI-HFv2)	1–2 µl (5)
Nuclease-free H_2O	to 20 µl (6)

(1) Or user provided.
(2) Precloned inserts must possess BsaI restriction sites at both ends of the insert sequence and in the proper orientation.
(3) Amplicon inserts must possess 5´ flanking bases (6 recommended) and BsaI restriction sites at both ends of the amplicon and in the proper orientation.
(4) The NEBiocalculator® Tool (nebiocalculator.neb.com) can be used for molar calculations.
(5) For assemblies ≤ 10 inserts, use 1 µl; for assemblies > 10 inserts, use 2 µl.
(6) Can be increased to 25 µl volume if required due to DNA component volumes; add additional 0.5 µl T4 DNA Ligase Buffer (10X).

2. Choose the appropriate assembly protocol:

INSERT NUMBER	SUGGESTED ASSEMBLY PROTOCOL
For 1 Insert	37°C, 5 min (cloning) or 37°C, 1 hr (library preparation) → 60°C, 5 min
For 2–10 Inserts	(37°C, 1 min → 16°C, 1 min) x 30 → 60°C, 5 min
For 11–20+ Inserts	(37°C, 5 min → 16°C, 5 min) x 30 → 60°C, 5 min

Transformation Protocol:

The following protocol is designed for NEB 10-beta Competent *E. coli* (High Efficiency, NEB #C3019), as this strain is highly efficient for the stable maintenance of large plasmids. For other strains (discussed further in the FAQ section) please refer to the protocol specific to the strain. If using electrocompetent cells, such as NEB 10-beta Electrocompetent *E. coli* (NEB #C3020), follow the protocol provided with the cells, which can also be found at https://www.neb.com/protocols/1/01/01/electroporation-protocol-c3020 .

For NEB 10-beta Competent *E. coli*:

1. Thaw a 50 µl tube of NEB 10-beta Competent *E. coli* cells on ice for 10 minutes.
2. Add 2 µl assembly reaction; gently mix by flicking the tube 4–5 times.
3. Incubate on ice for 30 minutes.
4. Heat shock at 42°C for 30 seconds.
5. Place on ice for 5 minutes.
6. Add 950 µl of room temperature NEB 10-beta/Stable Outgrowth Medium. Incubate at 37°C for 60 minutes, shaking vigorously (250 rpm) or using a rotation device.

Plating Protocol:

1. Warm LB agar plates containing chloramphenicol (for pGGAselect) or other appropriate antibiotic at 37°C.
2. Mix the cells thoroughly by flicking the tube and inverting, then spread 50 µl of a 1:5 dilution (single inserts) or 50–100 µl (multiple inserts) of the 1 ml outgrowth onto each plate.
3. Incubate the plate overnight at 37°C, or if desired, 24–36 hours at 30°C or 48 hours at 25°C.

Recommended Screening Protocols

The following are different ways to screen your assemblies:

1. Colony PCR screening using an appropriate DNA polymerase for amplification of the insert region. Analysis primers are not included with the Golden Gate Assembly Kit. We recommend the following oligos be custom ordered through any oligo synthesis provider for colony PCR screening or sequencing of pGGAselect-based assemblies:

   Forward (CW) primer 65 bp upstream from assembly point:
   5´-CTGCAGGAAGGTTTAAACGCATTTAGG-3´

   Reverse (CCW) primer 62 bp downstream from assembly point:
   5´-TAATACGACTCACTATAGGGAGACTC-3´

   Note: The suggested forward primer is identical to that recommended for assemblies using the previous destination plasmid pGGA. The reverse primer however is different and specific to the pGGAselect destination constructs.

   While many DNA polymerases are suitable for colony PCR, One*Taq*® DNA Polymerase (NEB #M0480) or One*Taq* Hot Start DNA Polymerase (NEB #M0481) is recommended. *Taq* DNA Polymerase (NEB #M0267) can also be used. For PCR of larger assemblies, LongAmp® *Taq* DNA Polymerase (NEB #M0323) or LongAmp Hot Start *Taq* DNA Polymerase (NEB #M0534) are strongly recommended.

2. Prepare plasmid mini-preps using the Monarch Plasmid Miniprep Kit (NEB #T1010) and map by using appropriate restriction endonucleases to confirm the correct assembly.

   Regardless of the screening protocol used, the correct assembly of insert(s) should always be confirmed by sequencing of the plasmid construct across the 4 base junctions and inserts. Note that larger assemblies will require internal assembly-specific primers to verify the full assembly sequence.

Frequently Asked Questions (FAQs)

For a complete list of FAQs, please visit the product page at www.neb.com

Q1. Why does the Golden Gate Assembly Mix now feature BsaI-HFv2 instead of BsaI?

A1: Research using more challenging 12- and 24- fragment assembly test systems showed the clear superiority of the recently re-engineered BsaI-HFv2 enzyme over BsaI. This is evident in efficiencies of assembly (number of transformants), accuracies of assembly (fidelity) and continued increases in assembly formation at higher than usual cycle numbers if desired. The mix is identical to the original except for the Type IIS restriction enzyme replacement.

Q2. What is the mechanism for Golden Gate Assembly?

A2: Assembly utilizes two simultaneous enzymatic activities in a single reaction, Type IIS restriction endonuclease digestion and T4 DNA Ligase ligation. With optimized buffer components and enzyme ratios, a single reaction containing a destination plasmid and inserts (PCR amplicons or precloned) will result in ligation of inserts in the correct order and the accumulation of assembled product over time. The final assembly has none of the chosen Type IIS recognition sites, rendering the assembly inert to further digestion. For more information, view our online tutorial at www.neb.com/goldengate

Q3. Which kit from NEB should I use for Golden Gate Assembly—this BsaI-HFv2 kit (NEB #E1601) or the BsmBI-v2 kit (NEB #E1602)?

A3: It depends on whether there are any internal sites for these enzymes in your insert sequences. Since internal sites need to be eliminated by site-directed mutagenesis, choose the kit based on the Type IIS restriction enzyme that has no, or the fewest, sites in your insert sequences. If your sequences have neither BsaI nor BsmBI sites, either can be used as both support even 24 fragment assemblies. However the BsmBI-v2 kit (NEB #E1602) supports the highest complex assembly performance yet developed at NEB both in terms of efficiency (number of transformants) and fidelity (% correct assemblies).

Q4. If there are internal BsaI and BsmBI sites in my insert sequences, can I use either kit?

A4: Either use site-directed mutagenesis to eliminate the internal sites, screen your sequences for the absence of other Type IIS restriction sites that could allow an alternative Type IIS restriction enzyme to be used such as BbsI, SapI/BspQI or BtgZI (building your assembly reactions using individual restriction enzyme and T4 DNA Ligase stocks), or consider another assembly approach such as NEBuilder® HiFi DNA Assembly if the assembly will involve 5 or less inserts.

Q5. What affects the efficiency of Golden Gate Assembly?

A5: Single insert cloning is significantly more efficient than multiple insert cloning. Assembly efficiency decreases as the number of fragments increases. The presence of repetitive sequences in an insert will also decrease efficiency. For inserts < 250 bp or > 3 kb, precloning will increase efficiency. Lastly, the normal restrictions on overall plasmid size to allow stable maintenance in *E. coli* apply to Golden Gate Assemblies. Efficiencies are highest with assembled product plasmid constructs ~ 10-12 kb. Larger sized completed assemblies can be made but will require larger numbers of colonies to be screened for the correct full length assembled products. For experimental examples of complexity vs. efficiency, refer to the Golden Gate Assembly Technical Note on our website www.neb.com/GoldenGate.

Q6. Why do many of the published Golden Gate Assembly articles feature precloned inserts as opposed to inserts generated by PCR?

A6: Precloned inserts allow stable storage of inserts while using amplicon inserts saves time. Stable storage of amplicon inserts is important and is best in a buffered solution. Single insert cloning/assemblies can use unpurified amplicons but will result in lower performance than if purified, while multiple insert amplicons should be purified, for example, by spin column protocols. We recommend the Monarch® PCR and DNA Cleanup Kit (5 µg, NEB #T1030S). For long term storage at –20°C, store DNA in 10 mM Tris (pH 8.5), 1 mM EDTA (TE) or short-term storage in 10 mM Tris (pH 8.5), 0.1 mM EDTA (modified TE). EDTA at these levels will not significantly lower the 10 mM $MgCl_2$ present in the T4 DNA Ligase Buffer used for assembly reactions.

Q7. Using purified amplicons directly without precloning seems much easier, but is the assembly efficiency decreased?

A7: No. While in general DNA is more stable in circular form than in linear form due to the absence of free ends, amplicons are a viable and easy way to build assemblies as long as they have been purified and are stored in the appropriate buffer (see FAQ #5). The suggested 2:1 molar ratio of amplicon inserts : destination vector backbone brings the assembly efficiency to that of precloned inserts (using 75 ng of each plasmid) for most assemblies.

Q8. Why is Golden Gate Assembly also used for single insert cloning?

A8: While Golden Gate is normally used for insert assemblies of 5–10 or more fragments, it also allows easy and highly efficient cloning of single inserts following the provided directions. Golden Gate can also be used with diverse single insert populations for library preparations and directed evolution requiring multiple site mutagenesis.

Q9. Can PCR amplicons be used directly in single insert (cloning) assembly reactions without purification?

A9: Yes, as long as insert volume is 1 µl or less, although efficiencies will be decreased. Most Type IIS restriction enzymes used for Golden Gate Assembly generate 5´-four base overhangs that can be filled-in by the carryover DNA polymerase used in PCR when using unpurified amplicons, producing blunt ends. This will lead to nonspecific assembly. For single insert cloning/assembly, the ligase successfully competes with the carryover DNA polymerase such that unpurified PCR amplicon inserts can be used but will result in lower assembly performance. For multiple insert Golden Gate assemblies, purify the amplicons and if non-specific products are present, optimize the PCR or gel purify.

Q10. Why do assembly reactions end with a 5 minute, 60°C incubation step?

A10: The final incubation step at 60°C favors Type IIS restriction enzyme cutting, in the absence of DNA ligation. Digesting any uncut or cut/religated destination plasmid still present in the assembly reactions reduces background.

Q11. How can I minimize PCR-generated errors in my amplicon inserts?

A11: Use a high-fidelity DNA polymerase and avoid over-amplification. We recommend Q5® High-Fidelity DNA Polymerase formulations for maximal fidelity (NEB #M0491, #M0493), which is also available in Master Mix format (NEB #M0492, #M0494). Also, use the minimum number of cycles required to generate the amount of DNA required for assembly; this is usually 20 cycles or less.

Q12. Can the Golden Gate Assembly reactions be scaled down?

A12: Except for very complex, multi-insert assembly reactions, the reactions can be scaled down 2-3 fold if input volumes allow. This can be done by scaling down the reaction volume and components proportionately, or keeping the 20 µl reaction volume but using 2-3 fold less of each DNA component. In this latter case plate a larger amount of outgrowth to compensate.

Q13. What is an appropriate negative control for Golden Gate Assembly?

A13: Golden Gate assembly protocols do not usually call for a negative control. However if desired, a “no insert(s) added” reaction can be used.

Q14. How many cycles are optimal?

A14: Our enhanced enzyme stability allows more cycles than the traditional 30 cycles if larger numbers of transformants are desirable for complex assemblies or single insert library generation. For both our original BsaI-HFv2-based assembly kit and our BsmBI-v2-based assembly kit, efficiency increases dramatically from 30 cycles to 60-65 cycles, with no loss of fidelity.

Q15. Can I use other competent *E. coli* strains than NEB 10-beta? Can I use subcloning efficiency cells?

A15: Yes, other cell strains can be used, but large assemblies will require strains known to maintain large plasmid stability, such as NEB 10-beta competent *E. coli* (High Efficiency, NEB #C3019) or NEB Stable Competent *E. coli* (High Efficiency, NEB #C3040). NEB Stable Competent *E. coli* are also recommended for inserts containing repeat/unstable elements. For smaller assemblies other strains such as NEB 5-alpha Competent *E. coli* (High Efficiency, NEB #C2987), NEB Turbo Competent *E. coli* (High Efficiency, NEB #C2984) or NEB T7 Express Competent *E. coli* (High Efficiency, NEB #C2566) can also be used. Subcloning efficiency cells will result in lower transformation levels and should not be used for multi-component assemblies.

Golden Gate Assembly Tips

1. Use of the NEB Golden Gate Assembly Tool (GoldenGate.neb.com) is strongly recommended; this tool will check insert sequences for internal BsmBI sites and design primers to amplify your inserts for Golden Gate Assembly. The primers will feature 6 bases at the 5´ end flanking the BsmBI recognition site, the recognition site itself, plus the 4-base overhangs that determine correct annealing and ligation of the inserts. All overhangs will automatically be designed as non-palindromic (to eliminate self insert ligations), unique, and in the correct orientations to ensure correct assembly.

2. Research at NEB has led to an increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs will result in greater accuracy (8). This ligase fidelity information can be used in conjunction with the NEB Golden Gate Assembly Kit (BsmBI-v2) to achieve high efficiency and accurate complex assemblies. Please visit www.neb.com/GoldenGate for more information.

3. NEB has developed ligase fidelity tools to facilitate the design of high-fidelity Golden Gate Assemblies:

    - Ligase Fidelity Viewer-visualize overhang ligation preferences
    - GetSet™ – predict high-fidelity junction sets
    - SplitSet™ – split DNA sequence for scarless high-fidelity assembly

    All tools are available at neb.com/research/nebeta-tools

4. Standard Golden Gate protocol suggests using 30 cycles, alternating between restriction and cutting. BsaI-HFv2 and T4 DNA Ligase however are very stable, allowing cycling up to 60 cycles, with high efficiency and fidelity. Consider whether your workflow would be enhanced by adding more cycles.

5. For precloning of inserts, we recommend using the NEB PCR Cloning Kit as the kit's pMiniT 2.0 vector backbone has no BsaI sites present.

6. While BsaI and BsaI-HFv2 are blocked by overlapping dcm methylation (methylation at the C5 position of cytosine in the sequences CCAGG or CCTGG), this is usually not an issue for Golden Gate Assembly. Commonly used destination vectors are designed to avoid upstream CC(A or T) bases in front of the BsaI GGTCTC recognition site that would create an overlapping dcm methylation site.

Specifications

A 20 µl reaction containing T4 DNA Ligase Buffer (1X), 75 ng pGGAselect (Golden Gate destination plasmid, Cam^R), 75 ng each of 5 plasmids carrying fragments of a gene encoding *laclZ* and 1 µl Golden Gate Enzyme Mix (BsaI-HFv2) containing T4 DNA Ligase and BsaI-HFv2 is incubated for 30 cycles of 37°C for 1 minute, 16°C for 1 minute, then at 60°C for 5 minutes.

Successfully assembled fragments result in *laclZ* gene in pGGAselect vector and yield blue colonies on an IPTG/Xgal/Chloramphenicol plate.

Transformation of T7 Express Competent *E. coli* (High Efficiency) (NEB #C2566) with 2 µl of the assembly reaction yields greater than 250 colonies and > 80% blue colonies when 5% of the outgrowth is spread on an IPTG/Xgal/Chloramphenicol plate and incubated overnight at 37°C.

References

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2. Engler, C. et al. (2009) *PLoS ONE*, 4: e5553.
3. Lee, J.H. et al. (1996) *Genetic Analysis: Biomolecular Engineering*, 13; 139–145.
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6. Christian, M. et al. (2010) *Genetics*, 186, 757–761.
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8. Potapov, V. et al. (2018) *ACS Synth. Biol.*, 7, 2665–2674.

Ordering Information

NEB #	PRODUCT	SIZE
E1601S/L	NEB Golden Gate Assembly Kit (BsaI-HFv2)	20/100 reactions

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
N0550S/L	Quick-Load® Purple 1 kb Plus DNA Ladder (0.1-10.0 kb)	125-250 gel lanes
M0494S	Q5 Hot Start High-Fidelity 2X Master Mix	100 reactions
M0493S	Q5 Hot Start High-Fidelity DNA Polymerase	100 units
N0447S	Deoxynucleotide (dNTP) Solution Mix	8 µmol of each
E0554S	Q5 Site-Directed Mutagenesis Kit	10 reactions
E0552S	Q5 Site-Directed Mutagenesis Kit (without competent cells)	10 reactions
C3019H	NEB 10-beta Competent *E. coli* (High Efficiency)	20 x 0.05 ml/tube
C2987H	NEB 5-alpha Competent *E. coli* (High Efficiency)	20 x 0.05 ml/tube
C2984H	NEB Turbo Competent *E. coli* (High Efficiency)	20 x 0.05 ml/tube
C3040H	NEB Stable Competent *E. coli* (High Efficiency)	20 x 0.05 ml/tube
C3020K	NEB 10-beta Electrocompetent *E. coli*	6 x 0.1 ml/tube
C2566H	NEB T7 Express Competent *E. coli* (High Efficiency)	20 x 0.05 ml/tube
E1202S	NEB PCR Cloning Kit	20 reactions
E1203S	NEB PCR Cloning Kit (without competent cells)	20 reactions
B1500S/L	Nuclease-free Water	25/100 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	9/18
2.0	Updated to new manual format. Changed the pGGA vector to pGGAselect vector. Updated protocols and FAQs.	2/20

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