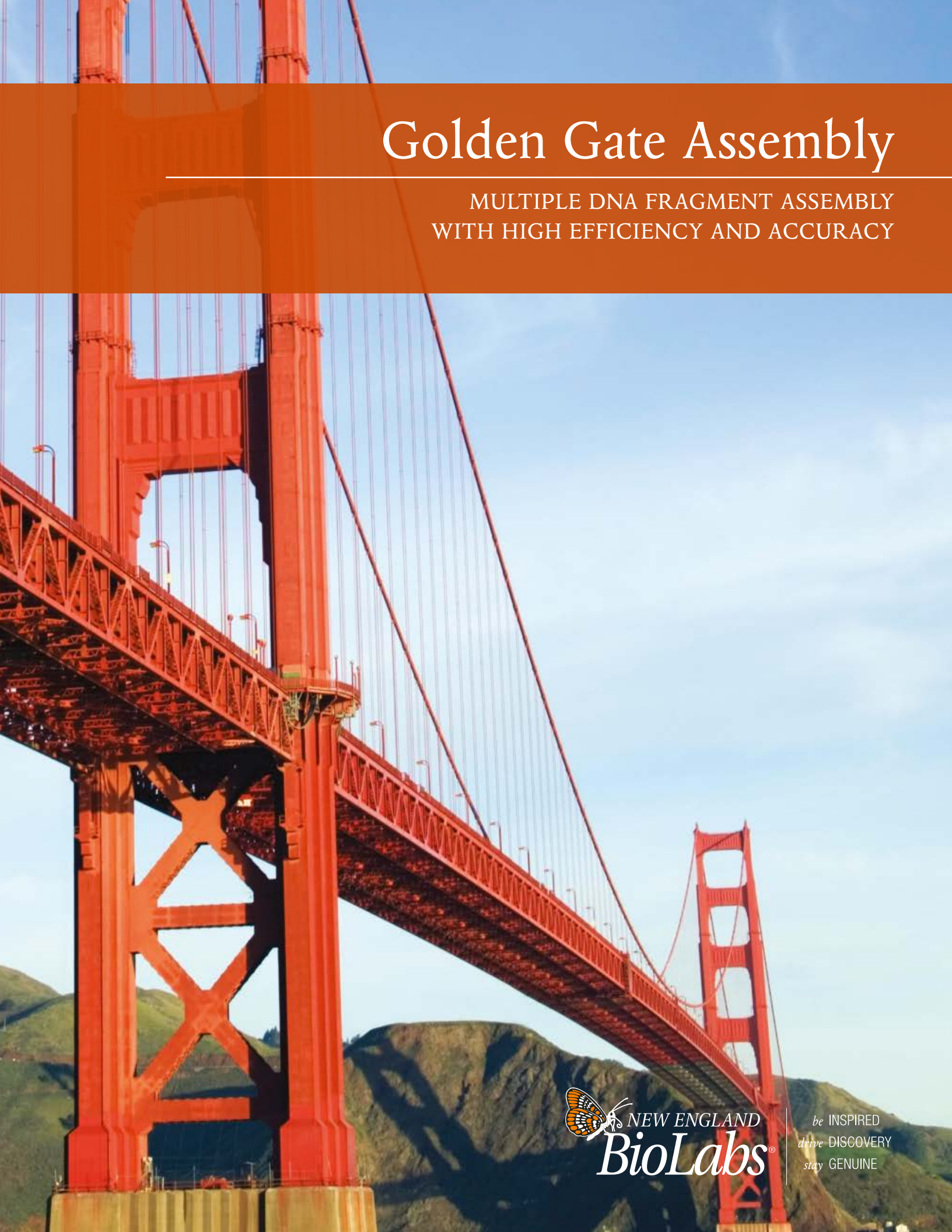


Golden Gate Assembly

MULTIPLE DNA FRAGMENT ASSEMBLY WITH HIGH EFFICIENCY AND ACCURACY

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

Push the limits of Golden Gate Assembly

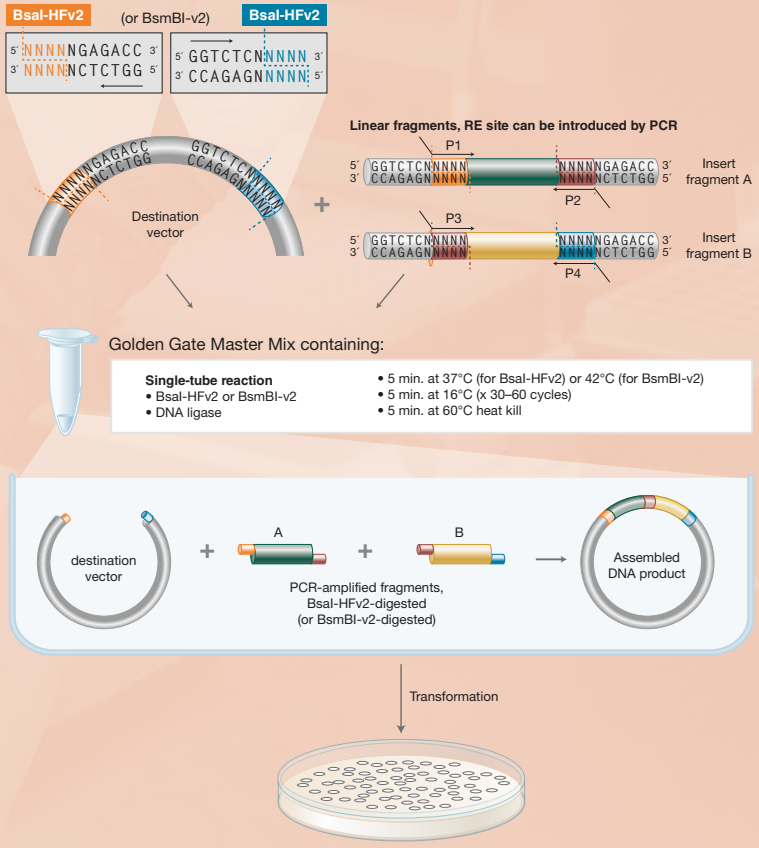
Golden Gate Assembly is a molecular DNA assembly technique that utilizes simultaneous digestion with Type IIS restriction enzymes (which cleave outside their non-palindromic recognition sequence) and ligation by a DNA ligase to enable the scarless, ordered assembly of multiple fragments. (1,2)

With constant advances in both the development of new enzymes and research on maximizing enzyme functionality (e.g., ligase fidelity), NEB is the industry leader in pushing the limits of Golden Gate Assembly and related methods.

Advantages:

- Clone seamlessly, with no scars remaining after assembly
- Perform single insert cloning in just 5 minutes using our fast protocols
- Generate libraries with high efficiencies
- Assemble multiple fragments (2-20+) in order, in a single reaction
- Experience high efficiency, even with regions of high GC content and areas of repeats
- Use with a broad range of fragment sizes (<100 bp to >15 kb)

GOLDEN GATE ASSEMBLY WORKFLOW
FOR BOTH SIMPLE AND COMPLEX ASSEMBLIES:



Golden Gate assembly utilizes a Type IIS restriction enzyme (REase), which cleaves outside of its non-palindromic recognition sequence and T4 DNA Ligase in a simultaneous, single-tube reaction. Inserts and vectors are designed to place the Type IIS recognition site distal to the cleavage site. Cut sites can be introduced by PCR primers, if needed. During the reaction, the Type IIS REase removes the recognition sequence from the assembly with each fragment bearing the designed 3- or 4-base complementary overhangs that direct the assembly. The fragments anneal, T4 DNA Ligase seals the nicks, and the final construct accumulates over time. Cycling between optimal restriction and ligation temperature further enhances the Golden Gate efficiency. Golden Gate Assembly can be used for ordered assembly of 2–20+ fragments simultaneously.

Explore and Discover

Download* the NEB Augmented Reality (AR) app and enjoy videos, tutorials and immersive experiences by scanning the icons.

How does Golden Gate Assembly work?

*see back cover for details

FEATURED PRODUCTS:

Type IIS Restriction Enzymes used in Golden Gate Assembly

Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence. Type IIS enzymes commonly used in Golden Gate Assembly are listed below. NEB currently offers over 45 Type IIS restriction enzymes.

Please visit **www.neb.com** for comprehensive table.

PRODUCT	NEB #	SEQUENCE	SIZE
BbsI	R0539S/L	GAAGAC(2/6)	300/1,500 units
BbsI-HF	R3539S/L	GAAGAC(2/6)	300/1,500 units
BsaI	R0535S/L	GGTCTC(1/5)	1,000/5,000 units
BsaI-HFv2	R3733S/L	GGTCTC(1/5)	1,000/5,000 units
BsmBI-v2	R0739S/L	CGTCTC(1/5)	200/1,000 units
Esp3I	R0734S/L	CGTCTC(1/5)	300/1,500 units
BtgZI	R0703S/L	GCGATG(10/14)	100/500 units
SapI	R0569S/L	GCTCTTC(1/4)	250/1,250 units
BspQI	R0712S/L	GCTCTTC(1/4)	500/2,500 units

What users are saying:

"NEB has developed a reliable set of enzymes and design tools for Golden Gate Assembly that we use regularly with success. We have found the Ligase Fidelity Viewer particularly useful for screening overhang sets that are constrained by a pre-existing protein/DNA sequence. The thorough experimental basis of the tool and the availability of the underlying data are added bonuses."

– Dr. Glenna Foight,
Senior Scientist, Lyell Immunopharma

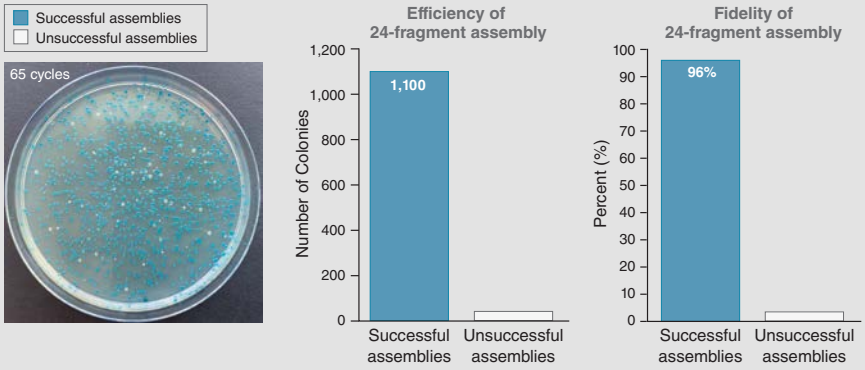
FEATURED KITS:

NEB® Golden Gate Assembly Kits (BsmBI-v2 or BsaI-HF®v2)

The absence of internal sites in a sequence determines the choice of which Type IIS restriction enzyme to drive the assembly. For your convenience, NEB now offers two kits for Golden Gate Assembly featuring BsaI-HFv2 or BsmBI-v2. Both kits incorporate digestion followed by ligation with T4 DNA Ligase into a single reaction, and can be used to assemble 2-20+ fragments in a single step.

PRODUCT	NEB #	SIZE
NEB Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 rxns
NEB Golden Gate Assembly Kit (BsaI-HFv2)	E1601S/L	20/100 rxns

COMPLEX GOLDEN GATE ASSEMBLY
WITH > 95% FIDELITY AND UNPRECEDENTED EFFICIENCY

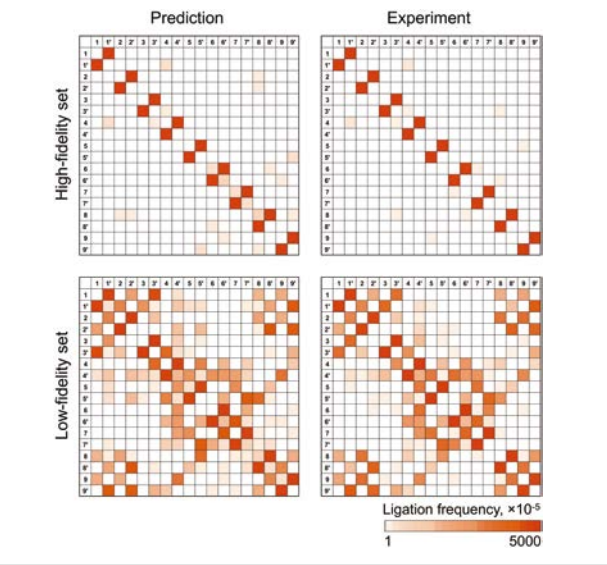


Using NEB Golden Gate Assembly Kit (BsmBI-v2), twenty-four fragment assemblies of a LacI/LacZ cassette were performed using the recommended cycling protocol for 11-20+ fragments, with an extension to 65 cycles. Successful assembly leads to functional LacI/LacZ gene product. Therefore, positive colonies will appear blue on β-Gal Blue/white selection plates. Plating 1/10 of the outgrowth from transforming 2 µl of the 25 µl assembly reaction resulted in 1,100 colonies with a 96% fidelity level, equivalent to 137,500 colonies per assembly reaction. This performance illustrates the stability of the enzyme mix that allows the option of cycling beyond the standard 30 cycle level if maximal assembly performance is desired.

Advances in Ligase Fidelity

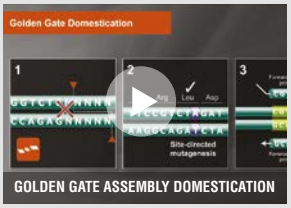
Research at NEB has led to an increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict overhang sets with improved fidelity (3). This research has enabled complex fragment assemblies with high efficiency and >90% accuracy. More information can be found in the NEB publication, *Comprehensive Profiling of Four Base Overhang Ligation Fidelity by T4 DNA Ligase and Application to DNA Assembly* (3) or in our webinar, *Fidelity and Bias in End-Joining Ligation: Enabling complex, multi-fragment Golden Gate DNA Assembly.* We also encourage you to try our free Ligase Fidelity Tools.

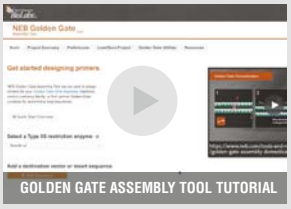
All of these are accessible at **www.neb.com/GoldenGate**.



Predicted versus observed fragment linkages in Golden Gate assembly of HF (high-fidelity) and LF (low-fidelity) 10-fragment assemblies. The intensity of the color corresponds to the number of instances of that junction observed in a Pacific Biosciences SMRT sequencing experiment, normalized to 100,000 total junctions. Predicted frequencies of junctions are based on the fidelity library data generated for the four-base overhang substrate ligated with T4 DNA ligase at 25°C for 18 hours. The experimental observations shown are for assembly of the 10-fragment HF and LF sets with Golden Gate Assembly Mix (BsaI-HFv2), 37°C, 5 minutes/16°C, 5 minutes, 30 cycles.

Visit **www.neb.com/GoldenGate** to learn more and view related videos





Expanded "assembly standards" for MoClo, GoldenBraid 2.0 and Other Modular Golden Gate Assembly Methods

MoClo (and GoldenBraid 2.0) uses 3 levels of successive assembly. The community has agreed upon a set of common standard overhangs for each level. Utilizing gathered ligase fidelity information, NEB has expanded each level of assembly overhangs without sacrificing fidelity. The expanded sets are:

Expanded MoClo Standardized Assembly Overhangs*

- Level 0 (Basic parts):
  `ACAT, TTGT, ACTG, GCTA, CCCA, AATA, ATTC, GTGA, CGCC, AAGA, AAAC, AACG, CTGC, GACC, AAGA, AAAC, AACG, CTGC, GACC, CTAA, ACCC, TACA, GGAA, CAAG, AGAG`
  (93% fidelity)
- Level 1 (Transcriptional units):
  `GGAG, TACT, CCAT, AATG, AGGT, TTCG, GCTT, GGTA, CGCT, GAAA, TCAA, ATAA, GCGA, CGGC, GTCA, AACA, AAAT, GCAC, CTTA, TCCA`
  (92% fidelity)
- Level 2 (Multigene constructs):
  `TGCC, GCAA, ACTA, TTAC, CAGA, TGTG, GAGC, GGGA, CGTA, CTTC, ATCC, ATAG, CCAG, AATC, ACCG, AAAA, AGAC, AGGG, TGAA, ATGA`
  (95% fidelity)

* These standardized assembly overhangs are part of the MoClo Toolkit referenced in this paper: *A modular cloning system for standardized assembly of multigene constructs.* Weber, E., Engler, C., Gruetzner, R., Werner, S., Marillonnet, S., *PLOS ONE.* 2011 Feb 18;6(2):e16765. doi: 10.1371/journal.pone.0016765. PubMed PMID 21364738.

References

1. Engler, C., Kandzia, R., and Marillonnet, S. (2008) *PLoS ONE* 3, e3647.
2. Engler, C., et al. (2009) *PLoS ONE* 4, e5553.
3. Potapov, V. et. al. (2018) *ACS Synth. Biol.* 7,1, 2665-2674.

Golden Gate Assembly Workflow...

Find the best assembly strategy/ TypeIIS REase → **Introduce desired cut sites into fragments by PCR** (design PCR primers using NEB Golden Gate Assembly tool) → **Perform single-tube Golden Gate Assembly reaction** → **Cycle rxn mix at optimal cutting and ligation temperatures** → **Heat kill reaction and transform/ plate** → **Pick positive colonies**

... And Eleven Tips For Optimizing Your Golden Gate Assembly Reactions

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your Golden Gate Assembly experiment.

1 Check your sequences

Always check your assembly sequences for internal sites before choosing which Type IIS restriction endonuclease to use for your assembly. While single insert Golden Gate assembly has such high efficiencies of assembly that the desired product is obtainable regardless of the presence of an internal site, this is not true for assemblies with multiple inserts. Options include choosing a different Type IIS restriction enzyme to direct your assembly, or eliminating internal sites through mutagenesis. The Q5® Site-Directed Mutagenesis Kit (NEB #E0554) and the NEB web tool NEBaseChanger work well for this purpose. Alternately, a junction point can be created at the internal site's recognition sequence.

2 Orient your primers

When designing PCR primers to introduce Type IIS restriction enzyme sites, either for amplicon insert assembly or as an intermediate for pre-cloning the insert, remember that the recognition sites should always face inwards towards your DNA to be assembled. Consult the Golden Gate Assembly Kit manuals for further information regarding the placement and orientation of the sites.

3 Choose the right plasmid

Consider switching to the pGGAselect Destination Plasmid for your Golden Gate assembly. This versatile new destination construct is included in all Golden Gate Assembly kits and can be used for Bsa-HFv2, BsmBI-v2 or BbsI directed assemblies. It also features T7 and SP6 promoter sequences flanking the assembly site, and has no internal BsaI, BsmBI or BbsI sites. The pGGAselect plasmid can also be transformed into any *E. coli* strain compatible with pUC19 for producing your own plasmid preparation if so desired.

4 Choose the right buffer

T4 DNA Ligase Buffer works well for Golden Gate Assembly with both BsaI-HFv2 and BsmBI-v2. However, alternate buffers would be NEBuffer 1.1 for Bsa-HFv2 and NEBuffer 2.1 for BsmBI-v2, as long as supplemented with 1 mM ATP and 5-10 mM DTT.

5 Increase your complex assembly efficiency by increasing the Golden Gate cycling levels

T4 DNA Ligase, BsaI-HFv2 and BsmBI-v2 are very stable and survive extended cycling protocols; an easy way to increase assembly efficiencies without sacrificing fidelity is to increase the total cycles from 30 to 45-65, even when using long (5-minute) segments for the temperature steps.

6 Make sure your plasmid prep is RNA-free

For pre-cloned inserts/modules, make sure your plasmid prep is free of RNA to avoid an over-estimation of your plasmid concentrations.

7 Avoid primer dimers

For amplicon inserts/modules, make sure your PCR amplicon is a specific product and contains no primer dimers. Primer dimers, with Type IIS restriction endonuclease sites (introduced in the primers used for the PCR reactions), would be active in the assembly reaction and result in mis-assemblies.

8 Avoid PCR-induced errors

Do not over-cycle and use a proofreading high fidelity DNA polymerase, such as Q5 DNA High-Fidelity Polymerase.

9 Decrease insert amount for complex assemblies

For complex assemblies involving >10 fragments, pre-cloned insert/modules levels can be decreased from 75 to 50 ng each without significantly decreasing the efficiency of assembly.

10 Carefully design EVERY insert's overhang

An assembly is only as good as its weakest junction. 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 improved accuracy. This ligase fidelity information can be used in conjunction with the NEB Golden Gate Assembly Kits (BsaI-HFv2 or BsmBI-v2) to achieve high efficiency and accurate complex assemblies. Please use the free NEB Golden Gate Assembly Tool to design primers for your Golden Gate Assembly reactions, predict overhang fidelity or find optimal Golden Gate junctions for long sequences. When working with complex assemblies (>20 fragments), refer to the ligase fidelity tools on the NEBeta Tools site.

11 Check for a sequence error if your assembly becomes non-functional

Be aware that occasionally a pre-cloned insert/module can become corrupted by an error during propagation in *E. coli*, usually a frameshift due to slippage in a run of a single base (e.g., AAAA) by the *E. coli* DNA Polymerase. This should be suspected if previously functional assembly components suddenly become nonfunctional.

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Featured Tools

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For help designing primers, try the new **NEB Golden Gate Assembly Tool** at **GoldenGate.neb.com**

Try our ligase fidelity tools for the design of high-fidelity Golden Gate assemblies at **neb.com/research/nebeta-tools**

- Ligase Fidelity Viewer™ (v2) – Visualize overhang ligation preferences
- GetSet™ – Predict high-fidelity junction sets
- SplitSet™ – Split DNA sequence for scarless high-fidelity assembly

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