Golden Gate Assembly Protocol for using NEBridge® Golden Gate Assembly Kit (BsmBI-v2) (NEB #E1602)

Materials

- Gel Loading Dye, Purple (6X), no SDS

Materials Required but not Supplied

NEBridge® Golden Gate Assembly Kit (BsmBI-v2)

- User-defined inserts
- Competent cells
- Other materials for transformation

Overview

Use this protocol for seamless cloning and assembly of multiple DNA fragments using the engineered Type IIS restriction enzyme BsmBI-v2 optimized for Golden Gate Assembly. It can be used for directed assembly of multiple inserts/modules and single insert/library generation cloning with single insert(s) using the Golden Gate approach.

Negative controls are not routinely done for assembly reactions but are described here for first-time users. This protocol uses the destination plasmid, pGGAselect, but others may be selected using the DNA Sequences and Maps Tool, which contains sequence files, vector maps, and cut sites.

Protocol

1. Set up assembly reactions as follows:

REAGENT	ASSEMBLY REACTION
pGGAselect Destination Plasmid*, 75 ng/µl	1 µl
Inserts (user provided): - if precloned** - if in amplicon form***	75 ng each plasmid 2:1 molar ratio**** (insert:vector backbone; pGGAselect = 2,155 bp; 75 ng = 0.05 pmol)
T4 DNA Ligase Buffer (10X)	2 µl
NEBridge Golden Gate Enzyme Mix (BsmBI-v2)	1–2 µl *****
Nuclease-free Water	to 20 µl******

** Or user provided. Consider using the DNA Sequences and Maps Tool to select destination plasmids*
*** Precloned inserts must possess BsmBI restriction sites at both ends of the insert sequence and in the proper orientation.*
**** Amplicon inserts must possess 5´ flanking bases (6 recommended) and BsmBI restriction sites at both ends of the amplicon and in the proper orientation.*
***** The NEBiocalculator® Tool can be used for molar calculations.*
****** For assemblies ≤ 10 inserts, use 1 µl; for assemblies > 10 inserts, use 2 µl.*
******* 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	42°C, 5 min (cloning) or 42°C, 1 hr (library preparation) → 60°C, 5 min
For 2–10 inserts	(42°C, 1 min → 16°C,1 min) x 30 → 60°C, 5 min
For 11–20+ Inserts	(42°C, 5 min → 16°C, 5 min) x 30 → 60°C, 5 min

To learn more about performing complex assemblies, visit NEBridge Golden Gate Assembly.

Resources:

- Learn more about NEBridge for Golden Gate Assembly
- NEBridge Golden Gate Assembly Tool
- DNA Sequences and Maps Tool
- NEBioCalculator Tool
- NEBridge Ligase Fidelity Tools
- Applications of Ligase Fidelity Data & Tools
- Breaking through the Limitations of Golden Gate Assembly