Golden Gate Assembly Protocol for Using NEBridge® Golden Gate Assembly Kit (BsaI-HF®v2) (NEB #E1601)

Materials Required but not Supplied

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

- ユーザ定義のインサート
- コンピテントセル
- 形質転換用の他の試薬

Overview

Overview:

Use this protocol for seamless cloning and assembly of multiple DNA fragments using the engineered Type IIS restriction enzyme BsaI-HFv2 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; pGGAselect = 2133 bp; 75 ng = 0.05 pmol)
T4 DNA Ligase Buffer (10X)	2 µl
NEB Golden Gate Assembly Mix	1 - 2 µl*****
Nuclease-free H_2O	to 20 µl

* Or user provided. Consider using the DNA Sequences and Maps Tool to select destination plasmids.
** Precloned inserts must possess BsaI restriction sites at both ends of the insert sequence and in the proper orientation.
*** Amplicon inserts must possess 5´ flanking bases (6 recommended) and BsaI restriction sites at both ends of the amplicon and in the proper orientation.
**** The NEBiocalculator® can be used for molar calculations.
***** For assemblies < 10 inserts, use 1 µl: for assemblies > 10 inserts, use 2 µl.

2. Choose the appropriate assembly protocol:

INSERT NUMBER	SUGGESTED ASSEMBLY PROTOCOL
For 1 Insert	37°C, 5 min (cloning) or 37°, 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

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

Resources:

- Learn more about NEBridge for Golden Gate Assembly
- NEBBridge Golden Gate Assembly Tool
- NEBBridge Ligase Fidelity Tools
- DNA Sequences and Maps Tool
- The NEBiocalculator®
- Breaking through the Limitations of Golden Gate Assembly– The Co-Evolution of Test Systems, Engineered Enzymes and Understanding Ligase Fidelity
- Applications of Ligase Fidelity Data & Tools