Golden Gate Assembly Protocol for NEBridge® Ligase Master Mix (NEB #M1100)

Materials Required but not Supplied

NEBridge® Ligase Master Mix

- User-defined DNA fragments
- User-choice of NEB Type IIS restriction enzymes
- Competent cells
- Other materials for transformation

Overview

This protocol is designed for optimal Golden Gate assembly using NEBridge® Ligase Master Mix with any of the following NEB Type IIS restriction enyzmes: BbsI-HF®, BsaI-HF®v2, BsmBI-v2, Esp3I, PaqCI®, SapI, BspQI, or BspQI-HF®. The result is a seamless assembly of multiple DNA fragments. This method can be used for cloning of single inserts and library preparations and is ideal for the ordered assembly of multiple fragments (2-25+) in a single reaction.

Protocol

1. Based on assembly complexity, determine reaction component volumes (Table 1). The volume of Type IIS restriction enzyme (x µl) can be found in Table 2.

Table 1: Assembly Component Amounts

COMPONENTS	2 FRAGMENT OR 3–6 FRAGMENT ASSEMBLY	7+ FRAGMENT
NEBridge Ligase Master Mix	5 µl	10 µl
DNA Fragments*	0.05 pmol each	0.05 pmol each
Type IIS Restriction Enzyme	x µl	x µl
Nuclease-free Water	y µl	y µl
Total Reaction Volume	**15 µl**	**30 µl**

** Use NEBiocalculator® to calculate the mass of each DNA fragment*

Table 2: Suggested Type IIS Restriction Enzyme Amounts

ENZYME	2 FRAGMENT ASSEMBLY	3–6 FRAGMENT ASSEMBLY	>7+ FRAGMENT ASSEMBLY
BbsI-HF	1 µl (20 U)	1 µl (20 U)	1 µl (50 U)*

ENZYME	2 FRAGMENT ASSEMBLY	3–6 FRAGMENT ASSEMBLY	>7+ FRAGMENT ASSEMBLY
BsaI-HFv2	1 µl (20 U)	1 µl (20 U)	1 µl (20 U)
BsmBI-v2	3 µl (30 U)	3 µl (30 U)	6 µl (60 U)
Esp3I	2 µl (20 U)	3 µl (30 U)	4 µl (40 U)
PaqCI**	1 µl (10 U)	1 µl (10 U)	2.5 µl (25 U)
SapI	1 µl (10 U)	1 µl (10 U)	2 µl (20 U)
BspQI	1 µl (10 U)	1 µl (10 U)	2 µl (20 U)
BspQI-HF	1 µl (10 U)	1 µl (10 U)	2 µl (20 U)

** Use BbsI-HF (NEB #R3539M) (50 U/µl).*

*** Requires PaqCI activator (20 µM), 0.5 µl for 2 and 3–6 fragment assembly; 1.25 µl for 7+ fragment assembly.*

2. Set up a reaction in a microcentrifuge tube on ice. Mix DNA fragments (0.05 pmol of each) with nuclease-free water (y µl).

3. Add NEBridge Ligase Master Mix (5 µl or 10 µl) to DNA fragments and water. Gently mix by pipetting 3 times.

4. Add Type IIS restriction enzyme (x µl). Gently mix by pipetting 5 times.

5. Incubate for the recommended time and temperature (see Table 3).

Table 3: Suggested cycle times

	2 FRAGMENT ASSEMBLY		3–6 FRAGMENT ASSEMBLY	7+ FRAGMENT ASSEMBLY	
	SINGLE GENE CLONING	LIBRARY CONSTRUCTION		7–13 FRAGMENT	14+ FRAGMENT
BbsI-HF, BsaI-HFv2, BspQI-HF, Esp3I, PaqCI, SapI	37ºC for 15 min.	37ºC for 60 min.	30 cycles at 37ºC for 1 min. and 16ºC for 1 min.	30 cycles at 37ºC for 5 min. and 16ºC for 5 min.	60 cycles at 37ºC for 5 min. and 16ºC for 5 min.
BsmBI-v2, BspQI	15 cycles at 42ºC for 1 min. and 16ºC for 1 min.	30 cycles at 42ºC for 1 min. and 16ºC for 1 min.	30 cycles at 42ºC for 1 min. and 16ºC for 1 min.	30 cycles at 42ºC for 5 min. and 16ºC for 5 min.	60 cycles at 42ºC for 5 min. and 16ºC for 5 min.

6. End Soak: Incubate at 60°C for 5 minutes, before transformation.

7. Chill on ice.

8. Use 2 µl of the reaction to transform 50 µl of competent cells. If reaction will not be used immediately for transformation, store at -20°C.

Related Resources

- NEBridge® Ligase Master Mix Protocol Guidelines
- NEBioCalculator®

- NEBridge® Golden Gate Assembly Tool
- Getting Started with Golden Gate Assembly