Golden Gate Assembly

1. Set up assembly reactions as follows:

Reagent	Negative Control	Assembly Reaction
pGGA Destination Plasmid*, 75 ng/µl	1ul	1ul
Inserts (user provided): - if precloned** - if in amplicon form***	–	75 ng each plasmid 2:1 molar ratio (insert : vector; pGGA = 2,174 bp; 75 ng = 0.056 pmol)
T4 DNA Ligase Buffer (10X)	2ul	2ul
NEB Golden Gate Assembly Mix	1 – 2ul****	1 – 2ul
Nuclease-free H_2O	to 20ul	to 20ul

* or user provided.
** 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 and BsaI restriction sites at both ends of the amplicon and in the proper orientation.
**** For assemblies $\leq$ 10 inserts, use 1 µl : for assemblies $\geq$ 10 inserts, use 2 µl.

Note: Negative controls are not routinely done for assembly reactions, but are described for first time users.

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 2-4 inserts	37°C, 1 hr → 60°C, 5 min
For 5-10 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

In reactions with longer inserts (5-10 inserts), we modified the protocol as follows:

(37°C, 2 min → 16°C, 2 min) x 30 → 60°C, 10 min

Product: NEB® Golden Gate Assembly Kit (BsaI-HF®v2) (E1601)

Protocol from New England Biolabs
https://international.neb.com/protocols/2018/10/02/golden-gate-assembly-protocol-for-using-neb-golden-gate-assembly-mix-e1601