Golden Gate Assembly

This protocol is the "Golden Gate Assembly Protocol for Using NEB® Golden Gate Assembly Kit (BsaI-HF®v2) (E1601)" from New England BioLabs.

Protocol

1. Set up assembly reactions as follows:

REAGENT	NEGATIVE CONTROL	ASSEMBLY REACTION
pGGAselect Destination Plasmid*, 75 ng/µl	1 µl	1 µl
Inserts (user provided): - if precloned** - if in amplicon form***	–	75 ng each plasmid 2:1 molar ratio (insert : vector; pGGAselect = 2155 bp; 75 ng = 0.05 pmol)
T4 DNA Ligase Buffer (10X)	2 µl	2 µl
NEB Golden Gate Assembly Mix	1 - 2 µl****	1 - 2 µl
Nuclease-free H_2O	to 20 µl	to 20 µl

* 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 ≤ 10 inserts, use 1 µl : for assemblies ≥ 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