Golden Gate Assembly with BsaI

Introduction

LucidChart Overviews | DNA | Combined

Procedures List

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

Product Manual

Materials

- NEB® Golden Gate Assembly Kit (BsaI-HF®v2)
  - T4 DNA Ligase Reaction Buffer
  - NEB Golden Gate Enzyme Mix (BsaI-HFv2)
  - pGGAselect DNA (need for negative control?)
- Backbone Vector
- Inserts
- Nuclease Free H_2O
- Time:
  - Variable (at least 65 minutes)

Procedure

1. Set up assembly reactions as follows:
   ** 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.

Table1

	A	B	C
1	**Reagent**	**Negative Control**	**Assembly Reaction**
2	**Backbone, 75 ng/µl**	1 µl	1 µl
3	**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)
4	**T4 DNA Ligase Buffer (10X)**	2 µl	2 µl
5	**NEB Golden Gate Assembly Mix**	1 - 2 µl***	1 - 2 µl***
6	**Nuclease-free H_2O**	to 20 µl	to 20 µl

2. Choose the appropriate assembly protocol:

Table2

	A	B
1	**Insert Number**	**Suggested Assembly Protocol**
2	**For 1 Insert**	37°C, 5 min (cloning) or 37°, 1 hr (library preparation) → 60°C, 5 min
3	**For 2-4 Inserts**	37°C, 1 hr → 60°C, 5 min
4	**For 5-10 Inserts**	(37°C, 1 min → 16°C, 1 min) x 30 → 60°C, 5 min
5	**For 11 - 20+ inserts**	(37°C, 5 min → 16°C, 5 min) x 30 → 60°C, 5 min