Golden Gate Assembly with BsmBI

Introduction

LucidChart Overviews | DNA | Combined

Procedures List

Golden Gate Assembly Protocol for using NEB Golden Gate Assembly Kit (BsmBI-v2) (NEB #E1602)

Product Manual

Materials

- NEB® Golden Gate Assembly Kit (BsmBI-v2)
  - T4 DNA Ligase Reaction Buffer
  - NEB Golden Gate Enzyme Mix (BsmbI-v2)
  - 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 BsmBI restriction sites at both ends of the insert sequence and in the proper orientation. **Amplicon inserts must possess 5´ flanking bases (6 recommended) and BsmBI restriction sites at both ends of the amplicon and in the proper orientation. ***The NEBiocalculator® Tool (nebiocalculator.neb.com) can be used for molar calculations. ****For assemblies ≤ 10 inserts, use 1 µl; for assemblies > 10 inserts, use 2 µl.*
   ******Can be increased to 25 µl volume if required due to DNA component volumes; add additional 0.5 µl T4 DNA Ligase Buffer (10X)*

Table1

	A	B
1	**Reagent**	**Assembly Reaction**
2	**Backbone, 75 ng/µl**	1 µl
3	**Inserts (user provided):** **- if precloned*** **- if in amplicon form****	75 ng each plasmid 2:1 molar ratio*** (insert : vector backbone; pGGA = 2,155 bp; 75 ng = 0.05 pmol)
4	**T4 DNA Ligase Buffer (10X)**	2 µl
5	**NEB Golden Gate Enzyme Mix (BsmBI-v2)**	1–2 µl (5)****
6	**Nuclease-free H_2O**	to 20 µl (6)*****

2. Choose the appropriate assembly protocol:

Table2

	A	B
1	**Insert Number**	**Suggested Assembly Protocol**
2	**For 1 insert**	42°C, 5 min (cloning) or 42°C, 1 hr (library preparation) → 60°C, 5 min
3	**For 2–10 inserts**	(42°C, 1 min → 16°C,1 min) x 30 → 60°C, 5 min
4	**For 11–20+ Inserts**	(42°C, 5 min → 16°C, 5 min) x 30 → 60°C, 5 min