Golden Gate Assembly

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1 Introduction

Pending...

2 Protocol

2.1 Reaction System

As an example, here, we use BsaI (type IIs restriction enzyme) in this reaction system. Below is an typical reaction system which is 20 μL.

Components	Volume
Fragments	40 fmol or 100 ng ea. (1)
BsaI	10 U (2 μL)
T4 ligase	5 U (0.5 μL) (2)
10× T4 Ligase Buffer	2 μL
ddH_2O	Up to 20 μL

Table 1: Golden Gate reaction system

Note:

(1) When do $1 \sim 2$ insertions reaction, it is convenient adding 100 ng of each fragment into reaction. If performing multiple (> 3 insertions) ligation, we recommend adding 40 fmol of each fragment.

(2) If assembling more than 3 fragments, high concentration of T4 ligase is necessary. I recommend 20 U (for NEB CEU, 1 U = 200 CEU).

(3) For convenience, we often prepare 10 μL reactions and the volume of each enzyme is 1 μL.

2.2 Ligation Procedure

If performing one fragment insertion ligation, incubating reaction system 30 min at 37 °C. When insertions are more than 2, we recommend using thermocycler: 37 °C for 2 min, the 16 °C for 3 min, repeat for 10 - 100 cycles, depending on the number of insertions (10 cycles per insertion). Following, the reaction is heated to 50 °C for 5 min, which is for digesting non-specific ligation events, completely. Last, the reaction is incubated 5 min at 80 °C for inactivating the reaction enzyme. The 10 μL reaction is transformed to 50 μL chemically competent cell. Then stalled cell in sawed ice for 30 min, and allowed to recover for an hour in SOC medium.

3 Design

3.1 Primer Design

The flank sequences of primer for introducing enzymatic cutting site into double sites of insertions are collected in Table 2.

Enzyme Name	Sequence
BsaI	GCATTAGGTCTCCNNNN
BbsI	AAGTGCGAAGACCANNNN
BfuAI	GGCAATACCTGCGTGANNNN
BtgZI	CGAATGGCGATGTTGTACTGCCNNNN
SapI	GGAATCGCTCTTCCNNN
BsmBI	ATAGCGCGTCTCCNNNN

Table 2: Flank Sequence of Primer, the underlined nucleotides are denoted as the enzyme recognition sequence, and the sequence before recognition sequence is protection sequence, and the base N is denote as the sticky end.