Golden Gate Assembly

Introduction

This protocol describes the cloning technique of Gold Gate Assembly.

Tips for optimisation of Golden Gate Assembly: https://www.neb.com/en-gb/tools-and-resources/usage-guidelines/technical-tips-for-optimizing-golden-gate-assembly-reactions.

Tools to check the fidelity of overhang ligations: https://ligasefidelity.neb.com/

Materials

- DNA insert/plasmid
- 10x T4 DNA Ligase buffer
  - NEB
- T7 DNA Ligase
  - NEB
- BsaI-HF or BsmBI
  - Both X U/ml from NEB
- PCR tubes
- MilliQ Water
- Thermocycler

Procedure

1. Calculate DNA insert : plasmid volume for 3:1 ratio

   - The amount of vector plasmid for successful ligation ≅ is 27-30 fmol.
   - To change the ligation ratio to 2:1, 5:1 or 7:1, alter the multiplier of 3 for the insert's 'ng required' column.

DNA insert : plasmid volume calculations for 3:1 ratio:

	DNA	Name	No. of bases (kb)	[DNA] (ng/µl)	ng required	Volume required (µl)	Normalised volume required (µl)
1	Vector backbone	pSB1C3-T7-LacZ	2.791	187.65	51.575412	0.2748489848	0.3
2	Insert 1	VcCDA_NC_002505_His	1.404	10	77.8343372784	7.7834337278	7.8
3	Insert 2	SmChiA_WU_His	1.8	10	99.7876118954	9.9787611895	10.0
4	Insert 3	SmChiA_BBa_Wag	1.806	10	100.1202372684	10.0120237268	10.0
5							
6	insert 4	cda chia 1	1.365	10	75.672272354	7.5672272354	7.6
7		cda chia 2	1.823	10	101.0626758252	10.1062675825	10.1
8							
9		chia cda 1	1.761	10	97.625546971	9.7625546971	9.8
10		chia cda 2	1.427	10	79.1094012082	7.9109401208	7.9
11							
12							

NOTE:

- Green sections can be edited or values inserted

- Equations:

moles dsDNA (mol) = mass of dsDNA (g)/((length of dsDNA (bp) x 615.96 g/mol/bp) + 36.04 g/mol)

required mass insert (g) = desired insert/vector molar ratio x mass of vector (g) x ratio of insert to vector lengths

2. Prepare the reaction mixture:

- If using small volume or multiple clones make a master mix.

Golden Gate Reaction Mixture

	Components	Volume (µl)
1	T4 DNA Ligase buffer	2
2	Insert : Plasmid	3:1
3	BsaI-HF v2	1
4	T4 DNA Ligase	1
5	MilliQ Water	Add up to 20
6	Total Volume:	20

Enzyme final concentrations:

- BsaI-HFv2 = 1 U/µl
- T4 DNA ligase = 20 U/µl

3. Incubate reaction mixture in a thermocycler with the following program:

25-30 cycles, can be increase to **45-65**:

a) 37 °C for 1.5 min (Restriction Enzyme)

b) 16 °C for 1.5 min (T4 DNA ligase)

Then, 60 °C for 5 min (Heat inactivation)

Hold temperature of 4 °C

NOTE:

- Increasing temperature cycling can increase the formation of unwanted concatemers.
- Reducing ligation concentration or time (1-2 min) can help reduce concatamerisation.
- Also, typically, increasing the insert to the background can help reduce concatamerisation.