Golden gate assembly with BsaI (Type IIs assembly)

Introduction

This protocol is made to assembly parts with TypeIIs enzyme BsaI HF v2. These parts will most probably have the standard MoClo overhangs. Made by PHS, adapted from http://2017.igem.org/wiki/images/e/e8/T--Evry_Paris-Saclay--protocol--pdf--gate.pdf

Materials

Reagens

- T4 DNA ligase
- 10X T4 DNA ligase buffer
- BsaI HF v2 enzyme (NEB)
- Reciever plasmid (BsaI compatible like pGIA2P2o)
- DNA fragments with BsaI overhangs
- MilliQ water

Materials

- PCR tubes
- 10uL pipette tips
- Thermocycler

Procedure

Setting up the reaction

1. In a PCR tube, mix the following:
   - 0.5 µL of T4 DNA Ligase
   - 2 µL of 10X T4 DNA Ligase Buffer
   - 0.5 µL of BsaI HF v2 restriction enzyme
   - 100 ng of receiver plasmid
   - Equimolar amounts of inserts
   - MilliQ for a total volume of 20 µL.
2. Mix gently
3. Place the tube on a thermocycler

Thermocycler program

Table1

	A	B	C
1	**Step**	**Temp**	**Time**
2	1: Activation of BsaI HF v2	37 °C	5 min
3	2: Activation of T4 ligase	16 °C	5 min
4	**Repeat step 1 & 2 for 25 cycles**		
5	3: Inactivation BsaI HF v2	65 °C	20 min
6	4: Inactivation T4 ligase	85 °C	10 min
7	5: Hold	4 °C	Hold