Golden gate assembly with SapI (Type IIs assembly)

Introduction

This protocol is made to assembly parts with TypeIIs enzyme SapI

Materials

- Reagens
  - T4 DNA ligase
  - 10X T4 DNA ligase buffer
  - SapI enzyme (NEB)
  - Reciever plasmid
  - DNA fragments with SapI 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 Type IIS 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 SapI	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 SapI	65 °C	20 min
6	4: Inactivation T4 ligase	85 °C	10 min
7	5: Hold	4 °C	Hold