Golden Gate Assembly

This protocol allows for Type IIS restriction enzymes (such as BsaI-HFv2 or BsmBI-v2) and T4 DNA ligase to join multiple DNA fragments together in a single reaction

Materials

- BsaI
- BSA
- T4 ligation buffer (NEB)
- T4 ligase (NEB)
- Milli Q Water DNA

Equipment

- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- PCR tubes
- Pipettes and pipette tips (0.5 - 10, 2-20 µL)

Meta Assembly Mix (for 50 µL)

Component	Volume (µL)
T4 ligation buffer	15
T4 ligase	10
BsaI	10
BSA	1.5
Milli-Q water	13.5

Golden Gate Reaction (for 8 µL)

Component	Volume (µL)
Meta Assembly Mix	2
Linearised plasmid	1
Milli-Q water	3

Thermocycling

The thermocycler, which is set as follows:

Step	Time	Temperature	Cycles
Initial denaturation	5 min	37 °C	
Ligation	2 min	16 °C	30×
Digestion	2 min	37 °C	
Final ligation	5 min	16 °C	
Heat inactivation	10 min	70 °C	
Hold	∞	4 °C	

Gibson Assembly

This protocol allows efficient joining of multiple overlapping DNA fragments in a single-tube isothermal reaction

Materials

- Amplified fragments (>500 bp recommended)
- NeBuilder HiFi DNA assembly Master Mix (NEB M5520A)
- Milli Q Water DNA

Equipment

- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- PCR tubes
- Pipettes and pipette tips (0.5 - 10, 2-20 µL)

Protocol

1. Calculate, for each fragment, the amount of ng required, using the NEBioCalculator from New England Biolabs (Moles to Mass). For the calculation, use 0.05 pmol
2. Set up a 20 µLreaction with al the fragments, 10 µLof HiFi Master Mix and fill in the rest with MiliQ water
3. Use the following setting:

Thermocycling

The thermocycler, which is set as follows:

Step	Time	Temperature	Cycles
Step 1	15 min	50 °C	1×
Hold	∞	16 °C	