Golden-Gate-Assembly

Adapted from: Golden-Gate-Assembly with NEB Golden-Gate Mix

Use NEB Tm Calculator to calculate the Tm of the primers for your PCR.

Aim of the experiment

This experiment can be used to insert specific DNA sequences into a backbone of choice. Insertion of different insert parts at the same time can be performed.

Materials

- Golden-Gate-Assembly Mix (NEB, Germany)
- 10x Golden-Gate-Buffer (NEB, Germany)
- DNA of interest (backbone [75-100 ng] + inserts [2:1 molar ratio in comparison to backbone])
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)

Procedure

1. To a PCR tube add following reagents:

Table 1: Mix for Golden-Gate reaction

Volume (μl)	**Chemicals**
1	Golden-Gate-Assembly Mix
2	10x Golden-Gate-Buffer
75-100 ng	Backbones
2:1 molar ratio	Inserts
fill up to 20	nf H_2O

2. Transfer tube to a Thermocycler and the fitting program:

Table 2: Thermocycling conditions

Insert number	**Suggested Protocol**
1-4 Inserts	37 $^{\circ}C$ for 1 hour
5-10 Inserts	30 x (37 $^{\circ}C$, 1 min $\rightarrow$ 16 $^{\circ}C$, 1 min) $\rightarrow$ 55 $^{\circ}C$ for 5 min
11-20 Inserts	30 x (37 $^{\circ}C$, 5 min $\rightarrow$ 16 $^{\circ}C$, 5 min) $\rightarrow$ 55 $^{\circ}C$ for 5 min

Accompanying Protocols

1. PCR (Before Golden-Gate-Assembly inserts have to be amplified by PCR to get higher concentrations. Further it gives the possibility to add the cutting sides for the Type IIb restriction enzyme.)
2. Transformation