Gibson Assembly

Adapted from: Gibson-Assembly with NEB 2x Gibson-Assembly Mix

Use NEB Tm Calculator to calculate the Tm of the primers for your PCR pre-Gibson-Assembly.

Aim of the experiment

This protocol can be used to insert specific DNA sequences into a backbone of choice. Further insertion of different insert parts at the same time can be done.

Materials

- 2x Gibson-Assembly Mix (NEB, Germany)
- DNA of interest (backbone [50-100 ng] + inserts [2-3 fold excess of backbone, up to 5 fold excess for fragments <200 bp])
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)
- Thermocycler

Procedure

1. To a 200 μl tube add the following reagents:

Table 1: Mix for Gibson-Assembly reaction

Volume (μl)	**Chemicals**
10	NEB 2x Gibson-Assembly Mix
50-100 ng	Backbones
2-3 fold excess	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**
2-3 Inserts	50 $^{\circ}C$ for 15 minutes
4-6 Inserts	50 $^{\circ}C$ for 60 minutes

Accompanying Protocols

1. PCR (Before Gibson-Assembly inserts have to be amplified by PCR to get higher concentrations.)
2. Transformation