Golden Gate Cloning

Golden Gate is the cloning methods used by our team for the preparation of our various plasmids. Using several steps, always including a new plasmid backbone allowed us to easily obtain different versions of our plasmids e.g. to test which of our two primers leads to the higher expression rates. For the different steps of the Golden Gate, different restriction enzymes were used to create the desired sticky ends. For this project the restriction enzyme BsaI was used to create plasmids, using the backbone 1. For our backbone 2 versions, the restriction enzyme BbsI was chosen. Since the backbone 2 step can be skipped, if only one expression cassette is needed in the complete plasmid, two different restriction enzymes were used for the creation of plasmids, using the backbone 3 versions. If the backbone 3 was created directly out of the backbone 1, the restriction enzyme BbsI was used, whereas the restriction enzyme BsaI was taken, if the backbone 3 was built out of backbone 2 versions.

For each sample a reaction, according to the table below, and a negative control are prepared in PCR-tubes.

Golden Gate Reaction/sample	
	Volume [µL]
Insert 1 (40nM)	1,0
Insert 2 (40nM)	1,0
Insert 3 (40nM)	1,0
Further inserts can be added if needed	
Backbone (40nM)	1,0
CutSmart Buffer	2,0
ATP (20 mM)	2,0
Restriction enzyme	1,0
T4 Ligase (1:10)	2,5
dH2O or TRIS	*Depending on the number of inserts, fill to a total of 20µL*
total	20,0

The PCR-tubes were transferred to a thermocycler to go through the following temperature program.

Thermocycler	
5 min	37° C
2.5 min	16° C
45 repeats	
5 min	50° C
10 min	80° C