Golden Gate Cloning

- Add 100 ng of the linearized vector backbone and equimolar amounts of the other assembly pieces to 15 µl total volume assembly reaction mixture as follows

Linearized vector backbone (100ng)		
+		each additional assembly piece (to equimolar with backbone)
+	1,5 µl	10X NEB T4 Puffer
+	0,15 µl	100XBSA*
+	1 µl	BsaI
+	1 µl	NEB T4 Ligase,2 million cohesive end units/mL
+		dH_2O to
_____		
	15 µl	

NOTE: It is essential to use a High Concentration Ligase

* BsaI is only 10 % active at 37 °C without the addition of BSA.

- Perform the assembly reaction in a thermocycler as follows: (Engler 2009)

3	min	@ 37 °C }
4	min	@ 16 °C} 25 cycles
5	min	@ 50 °C}
5	min	@ 80 °C} 1 cycle

- Transform into competent *E.coli*

Protocol generously provided by the lab
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