1) Measure the DNA concentration (ng/μl) of each assembly piece.

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

			linearized vector backbone (100 ng)
+			each additional assembly piece (to equimolar with backbone)
+	1.5	μl	10X NEB T4 Buffer
+	0.15	μl	100X BSA*
+	1	μl	*Bsa*I
+	1	μl	NEB T4 Ligase, 2 million cohesive end units / mL
+	_____		dH_20 to
	15	μl	

NOTE: It is ***essential*** to use a High Concentration Ligase

* *Bsa*I is only 10% active at 37 C without the addition of BSA.

3) Perform the assembly reaction in a thermocycler as follows:

either (following Engler 2009):

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

or, alternatively (modified from Engler 2008)

1	hour	@ 37 C	1 cycle
5	min	@ 50 C }	
5	min	@ 80 C }	1 cycle

NOTE: If any of the assembly pieces contain an internal *Bsa*I site(s), it would be first preferable to silence the internal *Bsa*I site(s) through point mutation(s); a second option, if the internal *Bsa*I site overhang(s) are not cohesive with the other assembly overhangs, is to adjust the thermocycling parameters to terminate after a ligation step (e.g. skip the final cycle at 50 and then 80 C).

4) Transform 5 μl of the assembly reaction into 100 μl of competent *E. coli* and/or run a diagnostic agarose gel to check for successful assembly.