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 25 μl total volume assembly reaction mixture as follows:

			linearized vector backbone (100 ng)
+			each additional assembly piece (to equimolar with backbone)
+	5	μl	5X HF Phusion Reaction Buffer
+	1	μl	10 mM dNTPs
+	0.75	μl	DMSO
+	0.5	μl	2U/μl Phusion Polymerase
+	_____		dH_20 to
	25	μl	

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

30	sec	@ 98 C	1	cycle
10	sec	@ 98 C }		
30	sec	@ 55 C }	1 to 15	cycle(s)**
length* (kb) x 15	sec	@ 72 C }		
10	min	@ 72 C	1	cycle

*The total length of the assembled product (in kb)

**The number of repeated cycles should exceed the number of assembly pieces

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.