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

2) Add 1 μg of each assembly piece (including the linearized vector backbone) to a separate 20 μl chew-back reaction mixture as follows:

	1	μg	assembly piece
+	0.1	μl	5 U/μl T4 DNA polymerase
+	2	μl	10X Promega ligase buffer
+	_______		$dH_2 0$ to
	20	μl	

3) Incubate the chew-back reactions at room temperature for 30 minutes (optimal for 20 bp overhangs). Arrest the chew-back with the addition of 2 μl 10 mM dCTP, and place on ice.

4) On ice, add 100 ng of the chewed-back linearized vector backbone (still in the chew-back reaction mixture) and equimolar amounts of the other chewed-back assembly pieces (also still in their respective chew-back reaction mixtures) to a 15 μl total volume assembly reaction mixture as follows:

			linearized vector backbone (100 ng)
+			each additional assembly piece (to equimolar with backbone)
+	_______		1X Promega ligase buffer to
	15	μl	

4) Incubate the assembly reaction at 37 C for 30 minutes, and then place on ice.

5) 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.