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

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

1 mg assembly piece

+ 0.1 ml 5 U/ml T4 DNA polymerase

+ 2 ml 10X Promega ligase buffer

+  dH20 to

20 ml

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 ml 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 ml 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 ml

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

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