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:

$$\begin{align*}
1 \ \mu g \ \text{assembly piece} \\
+ 0.1 \ \mu l \ 5 \ U/\mu l \ T4 \ DNA \ polymerase \\
+ 2 \ \mu l \ 10X \ Promega \ ligase \ buffer \\
+ \text{dH}_2O \ \text{to} \\
20 \ \mu l
\end{align*}$$

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:

$$\begin{align*}
\text{linearized vector backbone (100 ng)} \\
+ \text{each additional assembly piece (to equimolar with backbone)} \\
+ \text{1X Promega ligase buffer to} \\
15 \ \mu l
\end{align*}$$

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.