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

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

 linearized vector backbone (100 ng)

 + each additional assembly piece (to equimolar with backbone)

 + 1.5 ml 10X NEB T4 Buffer

 + 0.15 ml 100X BSA\*

 + 1 ml *Bsa*I

 + 1 ml NEB T4 Ligase, 2 million cohesive end units / mL

 +  dH20 to

 15 ml

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