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 25 ml total volume assembly reaction mixture as follows:

linearized vector backbone (100 ng)

+ each additional assembly piece (to equimolar with backbone)

+ 5 ml 5X HF Phusion Reaction Buffer

+ 1 ml 10 mM dNTPs

+ 0.75 ml DMSO

+ 0.5 ml 2U/ml Phusion Polymerase

+  dH20 to

25 ml

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