Overlap Extension Protocol

Step 1

Reagent	Amount (µl)
Q5® High-Fidelity 2X Master Mix	12.5
Forward primer 10µM	1.25
Reverse primer 10µM	1.25
Template DNA	1
MilliQ water	9

Step 2

Reagent	Amount
Q5® High-Fidelity 2X Master Mix	12.5
Template DNA	1 picomol of one BioBrick + 1 picomol of one BioBrick
MilliQ water	17.7

Step 3

Reagent	Amount
Q5® High-Fidelity 2X Master Mix	25
Forward primer 10µM	5
Reverse primer 10µM	5
Template DNA	10 from step 2
MilliQ water	10

Thermocycling conditions

STEP	TEMP	TIME
Initial Denaturation	98°C	30s
25 Cycles (Denaturation, Annealing, Extension)	98°C Ta°C 72°C	10 seconds 30 seconds 30 seconds/kb

Final Extension	72°C	2 minutes
Hold	4°C	

For step 1 and 3, 25 or 30 cycles are used. In step 2, there are only 15 cycles.

The NEB Tm calculator (https://tmcalculator.neb.com/#!/main) was used to determine Ta for the primers with Q5® High-Fidelity 2X Master Mix. Depending on the step, there were different parts that annealed.