Protocol PCR

For the amplification of double-stranded DNA (Biobrick Parts) PCR was performed, as recommended by iGEM (http://parts.igem.org/Primers/Catalog).
The reagents (listed in table 1) were combined in a master mix. The recipe was calculated for one more reaction than needed and distributed to the respective PCR reaction tubes. The DNA template was added last to the reaction tubes. All steps were performed on ice.

Table 1 – Reagents and respective concentrations used in a single PCR experiment.

Component	50 µl	Final concentration
5X Q5 Reaction Buffer	10.00 µL	1X
10 mM dNTPs	1.00 µL	200 µM
10 µM Forward Primer	2.50 µL	0.5 µM
10 µM Reverse Primer	2.50 µL	0.5 µM
Template DNA	X	10 ng
Q5 High-Fidelity DNA Polymerase	0.50 µL	0.02 U/µl
Nuclease-free water	Add to 50 µL	

After quick mixing, an individually adjusted heat-cycler program was used for the PCR. The basic heat cycler program (see table 2) was adjusted regarding the denaturation time (depending on the GC content of the template), the annealing temperature and time (using the NEB tool provided at http://tmcalculator.neb.com/#!/main) and the elongation time (depending on the template length).

Table 2 – General heat-cycler program used for PCR experiments. The cycle steps were repeated 25-30 times.

STEP	TEMP	TIME
Preheat	98°C	
Initial Denaturation	98°C	30 s
Cycle: Denaturation	98°C	5–10 s
Cycle: Annealing	50-65°C	10–30 s
Cycle: Elongation	72°C	20-30 s/kb
Final Extension	72°C	2 min
Hold	4–10°C	

After the PCR was finished, the amplified DNA template was purified using the DNA Clean & Concentrator kit from Zymo Research Inc. The concentration of the template was determined using a Nanodrop 2000 (Thermo Fisher Inc.) and the quality was controlled using agarose gel electrophoresis.