New England BioLabd- Phusion PCR:

1) Set up the following reaction on ice:

COMPONENT	AMOUNT
5X Phusion HF Buffer	10 µl
10mM dNTPs	1 µl
10mM Forward Primer	2,5 µl
10mM Reverse Primer	2,5 µl
Phusion DNA Polymerase	0,5 µl
Template DNA	0,5 µl
ddH_2O	33 µl
Total amount	50 µl

2) Split 50 µl into two tubes with 25 µl.
3) Set up following PCR program on thermocycler:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25-30 cycles	98°C 68°C 72°C	10 seconds 10 seconds 15-30 seconds/kb
Final Extension	72°C	10 seconds
Hold	12°C	