20µL reactions

5µL sample

2.4 µL pH2O

4µL 5x buffer > final 1x

1µL PCR1 10µM >final 0.5 µM

1µL PCR2 10µM >final 0.5 µM

0.4µL dNTPs 10mM > final 0.2mM

0.2µL Phusion polymerase > final 1U per 50µL reaction

Run Phusion program in thermocycler:

98˚ for :30 (98˚ :10, 68.4 ˚ :30, 72˚ :30) 12x, 72˚ 10:00 followed by an infinite hold at 4˚