

# Polymerase chain reaction

1. The master mix is prepared by adding each component indicated in table 1.

**Table 1:** Recommended master mix

Reagent	Volume	Final concentration
CloneAmp HiFi PCR Premix	12.5 $\mu$ l	1X
Primer 1	0.5 $\mu$ l	10 $\mu$ M
Primer 2	0.5 $\mu$ l	10 $\mu$ M
Template (plasmid)	1 $\mu$ l (< 100 ng) (~1 $\mu$ g)	
Sterilized distilled water	Remaining volume (<25 $\mu$ l)	
Total volume per reaction	25.0 $\mu$ l	

2. The Master Mix is mixed by tapping the bottom of the tube, then centrifuging briefly.
3. 25  $\mu$ l of the Master Mix is transferred into individual PCR tubes or each well of a PCR plate, then centrifuged briefly.
4. The PCR machine is run with the following cycling conditions:

98°C                      20 sec

98°C                      10 sec

55°C                      15 sec

72°C                      5 sec / kb

**Repeat for 30 cycles**

72°C                      60 sec