

# 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 µl                          | 1X                  |
| Primer 1                   | 0.5 µl                           | 10 µM               |
| Primer 2                   | 0.5 µl                           | 10 µM               |
| Template (plasmid)         | 1 µl (< 100 ng) ( <b>~1 µg</b> ) |                     |
| Sterilized distilled water | Remaining volume (<25 µl)        |                     |
| Total volume per reaction  | 25.0 µl                          |                     |

2. The Master Mix is mixed by tapping the bottom of the tube, then centrifuging briefly.
3. 25 µl 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