PCR amplification of DNA fragment from plasmid

Reagents

- 73uL ddH2O
- 20uL S_{15} buffer (5x): HuTao
- 2uL dNTP mix (10mM mix) (Synbio Tech Co., Ltd.)
- 1uL forward primer (20pM stock)
- 1uL reverse primer (20pM stock)
- 1uL S_{15}^{TM} DNA polymerase (2.5U/µl) (Synbio Tech Co., Ltd.)
- 2uL plasmid

Procedure

1. For one PCR reaction, the above reagents should be added into a PCR tube in order.
2. Finger flick to mix, then centrifuge briefly.
3. Place the reaction tubes in the Thermal Cycler.
4. PCR will be run with the following programme:
   1 cycle of 96 °C, 5 min
   25cycles of 96 °C, 30 sec
   58 °C, 30 sec
   72 °C, 30-50 sec (depend on the size of DNA fragment)
   Final extension 72 °C, 2 min
   Hold at 4 °C
5. Check PCR results on an Agarose gel
   (Refer to Protocol: Detection gel electrophoresis)