Gene synthesis using PCR amplification of primers

Reagents

- 28uL ddH2O
- 10uL S_{15} buffer (5x): HuTao
- 1uL dNTP mix (10mM mix) (Synbio Tech Co., Ltd.)
- Primers (20pM stock)
- 1uL S_{15}^{TM} DNA polymerase (2.5U/µl) (Synbio Tech Co., Ltd.)

A. PCR I.

1. Take 3uL of each primer into a 1.5mL centrifuge tube
2. Add ddH_2O into the tube (to make up the volume to 200uL)
3. Finger flick to mix, then centrifuge briefly.
4. Prepare the PCR system as the following order:
   28uL ddH2O
   10uL S_{15} buffer (5x)
   1uL dNTP mix (10mM mix)
   10uL primer mix (20pM stock)
   1uL S_{15}^{TM} DNA polymerase (2.5U/µl)
5. Finger flick to mix, then centrifuge briefly.
6. Place the reaction tubes in the Thermal Cycler.
7. PCR will be run with the following programme:
   1 cycle of 98 °C, 2 min
   18cycles of 98 °C, 10 sec
   58 °C, 20 sec
   72 °C, 30 sec
   Final extension 72 °C, 1 min
   Hold at 4 °C

B. PCR II

1. Prepare the PCR system as the following order:
   35uL ddH2O
   10uL S_{15} buffer (5x)
   1uL dNTP mix (10mM mix)
   1uL forward primer the first (20pM stock)
   1uL reverse primer the last (20pM stock)
   1uL S_{15}^{TM} DNA polymerase (2.5U/µl)
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 98 °C, 2 min
   25cycles of 98 °C, 10 sec
   58 °C, 20 sec
   72 °C, 30 sec
   Final extension 72 °C, 1 min

Hold at 4 °C

5. Run the detection gel electrophoresis to test the results.