PCR

Introduction

Materials

- PCR Machine
- Primers
- Sterile water
- NEB's Q5 2x Mastermix
- Template DNA

Procedure

1. 25µl reaction

   12,5µl 2x Mastermix
   1,25µl 10µM Forward Primer
   1,25µl 10µM Reverse Primer
   0,1pg-1,0ng template DNA
   Adjust final volume to 25µl with sterile H2O

2. Mix the tube gently by tapping or pipetting up and down (DO NOT VORTEX)

3. Quickly spin everything to the bottom of the tube

4. PCR program:

   1. 98°C - 30 sec
   2. 98°C - 10 sec
   3. X°C - 20 sec *
   4. 72°C - X sec **
   5. 72°C - 2 min
   6. 4°C - forever

   REPEAT STEPS 2.-4. 30 TIMES
   * Choose the temperature in step 3 according to your primers' annealing temperature. We used NEB's Tm calculator.
   **Chose the extension time according to your amplicon's length. We used 20-30sec/kb.

5. Run 5µl of the reaction on gel (add 1µl of 6x Loading dye) to check the results.

6. If the reaction was successful, clean with PCR purification kit

7. Store purified product in -20°C