Polymerase Chain Reaction

1. Keep everything on ice. The PCR reaction mixture in the ice preparation, and then placed in a PCR reaction in the PCR reaction apparatus. This cold start (Cool Start Method) can enhance the specificity of PCR amplification to reduce non-specific reactions in the PCR process, can get good PCR results.
2. Make up a master mix of everything into PCR tubes.
   a. **25.0µL reaction system**

15.75µL ddH2O
5µL 5X Q5 Reaction Buffer
0.5µL dNTPs
1.25µL forward primer
1.25µL reverse primer
0.25µL Q5 DNA polymerase
5µL 5X Q5 High GC Enhancer (optional)
1.0µL template DNA

--------25.0µL Total

   b. **50.0µL reaction system**

32.5µL ddH2O
10µL 5X Q5 Reaction Buffer
1.0µL dNTPs
2.5µL forward primer
2.5µL reverse primer
0.5µL Q5 DNA polymerase
10µL 5X Q5 High GC Enhancer (optional)
1.0µL template DNA

--------50.0µL Total

3. Chose a suitable program, and adjust your annealing temperature and extention time as described below:

Annealing: 55°C for 0:30 min (different primers different annealing temperature)

Extention: 72°C for t min ("t" depends on the length of goal sequence, 1min per 1000bp)

Final extension: 72°C for 10:00 min