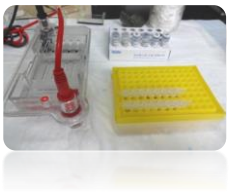
Colony PCR

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 one microcentrifuge tube.
3. Pipette up and down in the microcentrifuge tube, drain 25μL or 50.0μL solution to each PCR tube.
   a. **25.0μL reaction system**



18.3μL ddH2O
2.5μL 10x Buffer (mg^{2+})
2.0μL dNTPs
0.5μL forward primer
0.5μL reverse primer
0.2μL DNA polymerase
Colony stab (template DNA)
--------25.0μL Total

   b. **50.0μL reaction system**

37.75μL ddH2O
5.0μL 10x Buffer (mg^{2+})
4.0μL dNTPs
1.0μL forward primer
1.0μL reverse primer
0.25μL DNA polymerase
Colony stab (template DNA)
--------50.0μL Total

4. Pick colonies from plates, spot onto these PCR tubes.
5. Run the "Colony PCR" program, and adjust your extention time as described.

The "Colony PCR" program

Initial denaturation: 95°C for 5:00min
25 cycles of:
94°C for 0:30 min
55°C for 0:30 min (different primers different annealing temperature)
72°C for t min (“t” depends on the length of goal sequence, 1min per 1kb)
Final extension: 72°C for 10:00 min