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**

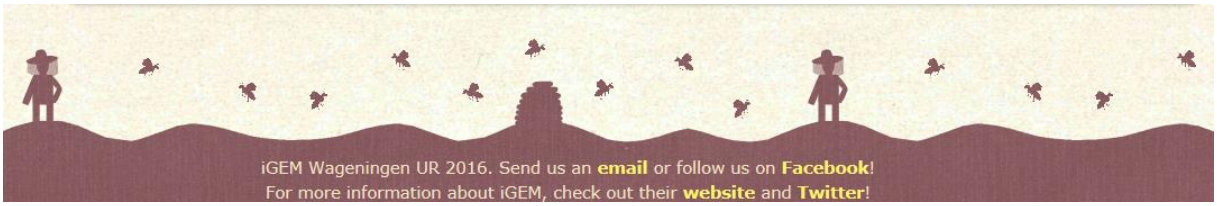
      18.375μL ddH2O
      5μL 5X OneTaq® Standard Reaction Buffer
      0.5μL dNTPs
      0.5μL forward primer
      0.5μL reverse primer
      0.125μL OneTaq DNA polymerase
      Colony stab (template DNA)
      --------**25.0μL** Total

   b. **50.0μL reaction system**

      36.75μL ddH2O
      10μL 5X OneTaq® Standard Reaction Buffer
      1.0μL 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, dilute in 10 μL ddH2O and add 1 μL into PCR tubes.
5. Run the "Colony PCR" program, and adjust your extention time as described below.

   The "Colony PCR" program

   Initial denaturation: 95°C for 5:00min
   30 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 1000bp)



Final extension: 72°C for 10:00 min