5. PCR & Agarose Gel Electrophoresis – Colony PCR

·*Material*

Green Taq Mix
Primer
Double Distillation Water (ddH_2O)
Eight tube centrifuge
EP Tube
Tweezers
plastic wrap
Shaker
PCR Eight-Tube
DNA Marker

·*Step(Take 5 tubes of eight tube centrifuge for example)*

1) Pick bacteria
   ① Add 1mL Amp LB to 1.5mL EP Tube.
   ② Use a small tip with tweezers to pick out a single clone and put them into the EP Tube.
   ③ Put the EP tube on the Rank and wrap it with plastic wrap.
   ④ Insert it into the shaker obliquely, shake for 2h.

2) Colony PCR （after shaking for 2h）

   ① Adding 6.6 Primer Mix(5μl), 41.3μl 2 x Green Taq, 29.1μl of ddH_2O to the first tube of eight-tube, then draw 14ul from the first tube to the next 4 tubes.
   ② Centrifuge the eight-tube in a centrifuge for several seconds.
   ③ Take the eight-tube into Clean bench.
   ④ Add 1μl Bacteria Liquid.
   ⑤ Set the protocol as follow: begin at 95℃ for 3 mins, then keep 95℃ for 15 secs for denaturation, decrease to 55℃ for 15 secs, 72℃ for 1 min and repeat that cycle 30 times, 72℃ for 1 min, finally maintain 4℃ infinity.
   ⑥ Adding all the samples to the hole. Run the gel at 120V for about 25 minutes. Check the result under the Blue Light Gel Imager.
   ⑦ Select the right System for Shaking bacteria and Plasmid Extraction.

·Note

① The lid of the PCR Eight-tube is placed in the ultra-clean workbench to make sure we add Bacteria Liquid at the ultra-clean workbench.

② As we use Green Taq Mix, we needn’t add Loading when Agarose Gel Electrophoresis.