

4. OE PCR & Agarose Gel Electrophoresis

Material

2 x Phanta Max Master Mix
Primer
Double Distillation Water (ddH₂O)
Loading Buffer
DNA Marker

Step

- ① Adding 25 μ l of 2 x Phanta Max Master Mix(5 μ M), 1 μ l per fragment, 4 μ l of primer mix, then add ddH₂O up to 50 μ l into a PCR tube. Different group use specific tube with distinctive sign.
- ② Place those PCR tubes into Peltier thermal cycler.
- ③ Set the protocol as follow: begin at 95 $^{\circ}$ C for 30 secs, then keep 95 $^{\circ}$ C for 15 secs for denaturation, decrease to 60 $^{\circ}$ C for 15 secs, 72 $^{\circ}$ C for 1 min/kb and repeat that cycle 30 times, finally maintain 16 $^{\circ}$ C infinity.
- ④ 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.

Note

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