Protocol for PCR & Agarose Gel Electrophoresis - System A

·Material

Takara Prime STAR HS （Premix）
Template P16-1 plasmid
Template P16-2 plasmid
Template P18 plasmid
Primer
Double Distillation Water (ddH_2O)
Loading Buffer
DNA Marker

·Step

Group 1\2\3\4

① Adding 10μl of Takara Prime STAR HS, 1μl of tablet, 4μl of primer, 5μl of ddH_2O into a PCR tube. Different group use specific tube with distinctive sign. Those four group are arranged as table follow:

Group	Primer	Template
1	Seq-P16-F1 Seq-P16-R1	P16-1
2	Seq-P16-F1 Seq-P16-R1	P16-2
3	Seq-P16-F2 Seq-P16-R2	P16-1 or P16-2
4	Seq-P16-F2 Seq-P18-R2	P18

② Place those PCR tubes into Peltier thermal cycler.

③ Set the protocol as follow: begin at 98℃ for 5 mins, then keep 98℃ for 10 secs for denaturation, decrease to 54℃ for 15 secs, 72℃ for 1 min and repeat that cycle 30 times, finally maintain 4℃ infinity.

④ Adding 2μl different samples for each, 1μl loading buffer, 7μl ddH_2O into different wells(Adding DNA marker in the first well), then run the gel at 120V for about 30 minutes. Check the result under the UV rays.

Group 5

① Adding 10μl of Takara Prime STAR HS, 1μl of tablet, 4μl of primer, 5μl of ddH_2O into a PCR tube. Different group use specific tube with distinctive sign. Those four group are arranged as table follow:

Group	Primer	Template
5	Seq-P16-F1 Seq-P18-R1	P18

② Place the PCR tube into Peltier thermal cycler.

③ Set the protocol as follow: begin at 98℃ for 5 mins, then keep 98℃ for 10 secs for denaturation, decrease to 52℃ for 15 secs, 72℃ for 1 min and repeat that cycle 30 times, finally maintain 4℃ infinity.

④ Adding 2μl different samples for each, 1μl loading buffer, 7μl ddH2O into different wells(Adding DNA marker in the first well), then run the gel at 120V for about 30 minutes. Check the result under UV rays.