PCR Protocol

[PCR]

We used several PCR methods and reagents for our experiments.

1. Genotyping PCR

Genotyping PCR was used to clarify the recombinant plasmid sequence and test some gene sequence amplifying. We used 2X Taq Master Mix© from BioMed for genotyping PCR.

1.a. Genotyping PCR systems (10 μL/ reaction)

Template	Take colony sample by pipette
2X Taq Master Mix	5 μl
Primer Farward (10 μM)	0.5 μl
Primer Reverse (10 μM)	0.5 μl
Add ddH_2O to 10 μl	4 μl
Total	10 μl

1.b. Genotyping PCR program

Step1:94 °C pre-heating to start reactions for 3 min

Step2:94 °C unwinding DNA for 30 s

Step3:56 °C annealing DNA for 30 s (The annealing temperature depends on primer and template matching and we usually choose a range from 55 °C-58 °C)

Step4:72 °C elongation (The 2X Taq Master Mix can amplify DNA at about 2 kb/ min for segment short than 2 kb length, about 1 kb/ min for segment of 2-4 kb length, and about 800 bp/ min for segment longer than 5 kb length)

Step5:Go to Step2 for 30 cycles

Step6:72 °C elongation and complement for 5 min Step7:cool down to 16 °C

Step8:End

2. DNA amplification PCR

We used TranStart™ FastPfu Fly DNA polymerase by TransGen and Q5® High-Fidelity DNA polymerase by NEB for DNA amplification. The fidelity of these polymerase is about 100 times higher than Taq.

2.a. PCR systems of TransStart™ FastPfu Fly DNA polymerase (50 μL/ reaction)

5X FastPfu Fly Buffer	10 μl
2.5 mM dNTPs	4 μl
Primer Farward (10 uM)	1 μl
Primer Reverse (10 uM)	1 μl
Template	1 μl
Add ddH_2O to 50 μl	32 μl
FastPfu Fly DNA Ploymerase	1 μl
Total	50 μl

P.S. For target segment longer than 5 kb or high GC rate (more than 60%) template, 10 μL 5X TransStart PCR stimulant and 2 μL 50 mM $MgSO_4$ can help amplification.

2.b. PCR program of TransStart™ FastPfu Fly DNA polymerase

Step1:98 °C pre-heating to start reactions for 2 min

Step2:98 °C unwinding DNA for 20 s

Step3:56 °C annealing DNA for 20 s (The annealing temperature depends on
primer and template matching and we usually choose a range from 55 °C-58 °C)

Step4:72 °C elongation (The FastPfu Fly DNA polymerase can amplify DNA at about 4 kb/ min for segment short than 2 kb length, about 2 kb/ min for segment of 2-4 kb length, and about 1 kb/ min for segment longer than 5 kb length)

Step5:Go to Step2 for 35 cycles

Step6:72 °C elongation and complement 5 min

Step7:Cool down to16 °C

Step8:End

2.c. PCR systems of Q5® High-Fidelity DNA polymerase (50 μL/ reaction)

5X NEB Q5 Buffer	10 μl
10mM dNTPs	1 μl
Primer Forward (10 μM)	2.5 μl

Primer Reverse (10 μM)	2.5 μl
Template	1 μl
Add ddH_2O to 50 μl	32.5 μl
Q5 Ploymerase	0.5 μl
Total	50 μl

P.S. For high GC rate (more than 65%) template, 10 μL 5X Q5 High GC Enhancer can help amplification.

2.d. PCR program of Q5® High-Fidelity DNA polymerase

Step1:98 ℃ pre-heating to start reactions for 30 s

Step2:98 ℃ unwinding DNA for 10 s

Step3:56 ℃ annealing DNA for 20 s (The annealing temperature depends on primer and template matching and we usually choose a range from 55 ℃-58 ℃)

Step4:72 ℃ elongation (The Q5® High-Fidelity DNA polymerase can amplify DNA at about 4 kb/ min for segment short than 2 kb length, about 2 kb/ min for segment of 2-4 kb length, and about 1 kp/ min for segment longer than 5 kb length)

Step5:Go to Step2 for 35 cycles

Step6:72 ℃ elongation and complement 5 min

Step7:Cool down to16 ℃

Step8:End

3. quantitative PCR

We used SuperReal PreMix Plus (SYBR Green) from TIANGEN BIOTECH for qPCR.Our instrument was CFX96™ Touch Real-Time System from BIO-RAD.For data analyzing, we used program CFX Manager 3.1 from BIO-RAD.

3.a. qPCR systems

Template	less than 100 ng
2X SuperReal PreMix Plus	20 μl
Primer Farward (10 μM)	0.6 μl
Primer Reverse (10 μM)	0.6 μl
Add ddH_2O to 20 μl	
Total	20 μl

3.b. qPCR program of SuperReal PreMix Plus (SYBR Green)
Step1:95 °C pre-heating to start reactions for 15 min
Step2:95 °C unwinding DNA for 10 s
Step3:60 °C annealing DNA and elongation for 32 s, acquire the fluorescent signal.
Step4:Go to Step2 for 30 cycles.
Step5:Cool down to16 °C
Step6:End
3.c.Attention
In qPCR systems, the segment between the primers should not be too long. Segments about 80-200 bp are recommended.