PCR and Colony PCR

Polymerase Chain Reaction

Phanta Max Master Mix

Materials

· 2 × Phanta Max Master Mix（Vazyme, catalog no. P515-02)
· Forward primer
· Reverse primer
· template DNA
· dd water

Procedure

1. To a PCR tube add the following as table 1.
2.

Table 1

Compon	Volume（ul）
Forward primer	2
Reverse primer	2
Template DNA	1
2 × Phanta Max Master Mix	25
ddH2O	Add to 50

2. The PCR program was designed according to the table 2.

Table 2

Step	Temperature	Time
Pre denaturation	95℃	3min
Denaturation	95℃	15sec
Annealing	56-72℃	15sec
Extension	72℃	60sec/kb
Final Extension	72℃	5min

Notes

1. All operations shall be carried out on ice, and each group shall be fully mixed after decomposition and freezing.
2. Prolonging the extension time is helpful to improve the amplification yield.

KOD-Plus-Neo

Materials

· KOD-401
· Forward primer
· Reverse primer
· template DNA
· dd water

Procedure

1. Dilute the template DNA and the final concentration was 50ng/50ul.
2. To a PCR tube add the following as table 3.

Table 3

Components	Volume（ul）
Forward primer	1.5
Reverse primer	1.5
Template DNA	1
2 mM dNTPs	5
25 mM MgSO4	3
10x PCR Buffer for KOD-Plus-Neo	5
KOD-Plus-Neo	1
ddH2O	Add to 50

3. The PCR program was designed according to table 4.

Table 4

Step	Temperature	Time
Pre denaturation	94℃	2min
Denaturation	98℃	10sec
Extension	68℃	30sec/kb

Notes

1. All operations shall be carried out on ice, and each group shall be fully mixed after decomposition and freezing.

2. Prolonging the extension time is helpful to improve the amplification yield.

PrimeSTAR Max DNA Polymerase

Materials

· PrimeSTAR Max Premix(2x)
· Forward primer
· Reverse primer
· template DNA
· dd water

Procedure

1. Dilute the template DNA and the initial concentration below 200ng/50ul.
2. To a PCR tube add the following as table 5.
3.

Table 5

Components	Volume（ul）
Forward primer	1
Reverse primer	1
Template DNA	1
PrimeSTAR Max Premix	25
ddH2O	Add to 50

3. The PCR program was designed according to table 6.

Table 6

Step	Temperature	Time
Pre denaturation	98℃	10sec
Annealing	55℃	15sec
Extension	72℃	10sec/kb

Notes

1. All operations shall be carried out on ice, and each group shall be fully mixed after decomposition and freezing.
2. Prolonging the extension time is helpful to improve the amplification yield.

2×EasyTaq PCR SuperMix

Materials

· 2×EasyTaq PCR SuperMix (+dye ,AS112-12)
· Forward primer
· Reverse primer
· template DNA
· dd water

Procedure

1. To a PCR tube add the following as table 7.

Table 7

Components	Volume（ul）
Forward primer	0.5
Reverse primer	0.5
PrimeSTAR Max Premix	5
ddH2O	Add to 10

2. Single colony was picked from the plate and placed in the PCR tube.
3. The PCR program was designed according to table 8.

Table 8

Step	Temperature	Time
Pre denaturation	94℃	5min
Denaturation	94℃	30sec
Annealing	50-60℃	30sec
Extension	72℃	1min/kb
Final extension	72℃	5min

PS: 35 cycles are required from pre denaturation to extension

Notes

1. All operations shall be carried out on ice, and each group shall be fully mixed after decomposition and freezing.
2. Prolonging the extension time is helpful to improve the amplification yield.