**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.

Table 1

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

1. 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 |

1. 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.

Table 5

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

1. 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 |

1. Single colony was picked from the plate and placed in the PCR tube.
2. 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.