

2X Multiplex PCR

9K-002-0037

store at -20°C



Protocol

2X MultiplexPCR is designed to amplify multiple target from single template in single PCR tube using h-Taq DNA polymerase. Chemically modified HotStart enzyme, h-Taq DNA polymerase is useful for multiplex PCR, genotyping, microsatellite due to its high specificity. Recommended to use of amplification for less than 1 kb PCR product.

| Reaction mixture (for 50µl reaction) | | Temperature cycle | |
|---|---------|-------------------|--------|
| 2X Multiplex PCR Premix | 25.0 µL | 95°C 15 min | x1 |
| primer 1 (10pmole/µl) | 2.0 µL | 95°C 20 sec | x30~50 |
| primer 2 (10pmole/µl) | 2.0 µL | AT* 40 sec | |
| template | X µL | 72°C 1 min/kb | |
| D.W. to | 50.0 µL | 72°C 5 min | x1 |

AT*: Annealing Temperature

Adjust to the lower Tm between two primers

AT = Tm(4-6°C)

Tm = 2°Cx(A+T)+4°Cx(G+C)

*It might be required to optimize PCR conditions depending on target size, Tm of primers, template nature, extension time, annealing temperature, enzyme quantity and cycles numbers.

Technical Information

A. Template (temperature cycle)

- . Animal genomic DNA
 - 50-200 ng (25-35 cycles)
 - 10-50 ng (30-40 cycles)
- . Bacterial genomic DNA
 - 10-50 ng (20-25 cycles)
 - 1-5 ng (30-35 cycles)
- . Plasmid and lambda DNA
 - 1-5 ng (20-30 cycles)

B. Primer design

- Primer can be designed using a primer design software or manually.
- Avoid repeated sequence at 3' end.
- In case 3'-end is G+C rich, the end has to be A or T.
- In case 3' end is A+T rich, the end have to be G or C.
- It is recommended that Tm of the designed primers is >64°C. and AT >58°C.

C. Extension time

- In general, extension should be performed at 0.5~1.0min/kb.
- If the amplification size is more than 3 kb, extension should be performed at 1.0~2.0 min/kb.
- If the amplification size is more than 5 kb, extension temperature should be assigned at 68°C.