

Pipetting scheme and Thermocycler protocol:

Protocol using SuperHOTTAQ

Components	Volume / 50µl PCR-Reaction	Final concentration
10 x PCR-Buffer	5µl	1x
dNTP-Mix (40mM)	1µl	800 µM (200µM each)
Upstream Primer	variable	0,1-0,5 µM
Downstream Primer	variabel	0,1-0,5 µM
SuperHOTTAQ 5u/µl	0,25µl-1,0µl	1,25-5,0 units
Template DNA	variable	10 to 500ng /reaction
Steril dest. water	Adjust to 50 µl final volume	
Reaction volume	50 µl	

Separate MgCl₂ solution can be used, if incomplete buffer is used, or if you have to titrate MgCl₂ for optimal PCR results:

Final MgCl ₂ conc. mM	1,5	2,0	2,5	3,0	3,5	4,0	4,5	5,0	5,5	6,0
Volume in µl of 100 mM MgCl ₂ per 50 µl reaction	0,75	1,0	1,25	1,5	1,75	2,0	2,25	2,5	2,75	3,0

Thermocycler protocol:

2 min 94°C

10 sec 94°C

20 sec 55°C depending on the primers (start with 0,5°C below the recommended temperature for your primer)

1 min 30 sec 72°C
(30 cycles)

5 min 72°C

Notes:

Program the cycler according manufacturers instructions. Each program should start with an initial denaturation step at 94°C for 2 to max. 5 min.

Recommended elongation time is 1 min per 1kb of target.

For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.