

Touchdown PCR Protocol

Reagents	Volume
10x HotMaster Taq Buffer with Mg ²⁺	5μL
10mM dNTP mix	1μL
10μM Primer 1	0.5μL
10μM Primer 2	0.5μL
20ng/μL Template DNA	2μL
HotMaster Taq DNA polymerase	0.5μL
Sterile Water	37.5μL
DMSO	2.5μL
Total Volume	50μL

Run on PCR on HotStar PCR program:

95°C 4min

94°C 1min

75°C 1min; -1°C each cycle

68°C 2min

30X

94°C 30sec

55°C 30sec

72°C 1min

72°C 5min

4°C --end

30X