

Touchdown PCR Cycling Parameters

	Step	Temperature	Time
STAGE 1	Initial Denaturation	94 °C	00:03:00
STAGE 2 (10 cycles)	Denaturation	94 °C	00:00:40
	Annealing	65 °C (reduce 1°C each cycle)	00:00:40
	Extension	72 °C	00:01:00
STAGE 3 (30 cycles)	Denaturation	94 °C	00:00:40
	Annealing	55 °C	00:00:40
	Extension	72 °C	00:01:00
STAGE 4	Final Extension	72 °C	00:10:00
	Hold	4 °C	∞