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(PhireKit) Touchdown PCR Cycling Parameters

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Protocol status: Working
We use this protocol and it's working

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1

98 °C

5 min

ABSTRACT

This is the protocol for the PhireKit touchdown PCR assay

BEFORE START INSTRUCTIONS

The assay has been optimized and validated for the Bio-rad C1000 Touch Thermocycler.

- 2 98 °C 5 s
- 3 65 °C (-1 °C per cycle) 5 s
- 4 72 °C 1 min (20s per kb)
- 5 Go to step 2, 11 times
- 6 98 °C 5 s
- 7 55 °C 5 s
- 8 72 °C 1 min (20s per kb)
- 9 72 °C 2 min

10 18 °C FOREVER

11 End

Adapted from: Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. "Touchdown" PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 1991;19: 4008. doi:10.1093/nar/19.14.4008