

(PhireKit) Touchdown PCR Cycling Parameters

Chen Wang

Abstract

This is the protocol for the PhireKit touchdown PCR assay

Citation: Chen Wang (PhireKit) Touchdown PCR Cycling Parameters . **protocols.io**

dx.doi.org/10.17504/protocols.io.pxidpke

Published: 05 May 2018

Before start

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

Protocol

Step 1.

98 °C 5 min

Step 2.

98 °C 5 s

Step 3.

65 °C (-1 °C per cycle) 5 s

Step 4.

72 °C 1 min (20s per kb)

Step 5.

Go to step 2, 11 times

Step 6.

98 °C 5 s

Step 7.

55 °C 5 s

Step 8.

72 °C 1 min (20s per kb)

Step 9.

72 °C 2 min

Step 10.

18 °C FOREVER

Step 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