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(PhireKit) Touchdown PCR Cycling Parameters



Forked from [TOUCHDOWN PCR](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.pxidpke>

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Protocol status: Working

We use this protocol and it's working

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Abstract

This is the protocol for the PhireKit touchdown PCR assay

Before start

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



- | | | |
|----|--------------------------|--------------------|
| 1 | 98 °C | 5 min |
| 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