

Oct 01, 2024

Relative Telomere Length Measurement

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvw1rn7lmk/v1>

Sinan Gültekin¹

¹Vetmeduni Vienna



Karyna Tarasova

Veterinary Medicine University

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Protocol Citation: Sinan Gültekin 2024. Relative Telomere Length Measurement. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.e6nvw1rn7lmk/v1>

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Protocol status: Working

We use this protocol and it's working

Created: October 01, 2024



Last Modified: October 01, 2024

Protocol Integer ID: 108714

Keywords: telomere length measurement relative telomere length measurement, length, measurement

Abstract

Relative Telomere Length Measurement by qPCR



Relative Telomere Length Measurement

1 DNA isolation



1.1 Genomic

DNA was extracted using the **DNeasy Blood and Tissue Kit** (Qiagen, Hilden, Germany)



qPCR

2 10 ng/ μ L DNA was amplified in a 5x EvaGreen[®] mix (No ROX) (Bio&Sell, Nurnberg, Germany). The thermal cycling protocol of Telomere A: 5'-CGGTTTGGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3', Telomere B: 5'-GGCTTGCCCTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3' and **B2M** was: 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 56°C for 20 s, and 72°C for 20 s. The thermal cycling protocol of **Telomere Primer Pair 1** was: 95°C for 15 min, followed by 2 cycles of 15 s at 94°C, 1 cycle of 15 s at 49°C, 40 cycles of 15 s at 94°C, 1 cycle 10 s at 62°C, 1 cycle 15 s at 74°C with signal acquisition, 10 s at 84°C, and 15 s at 88°C with signal acquisition. A no-template control (NTC) and the all templates were run in triplicate for each reaction



2.1 Primer sequences

Relative telomere length (RTL) was determined using two different primer pairs for telomere amplification and a primer pair for the single-copy reference gene **beta-2-microglobulin (B2M)**. The following primers were employed: **Telomere Primer Pair 1 (Hermann C, 2023)**: Telg: 5'-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3', Telc: 5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3'. **Telomere Primer Pair 2 (Mugdha, 2020)**: Telomere A: 5'-CGGTTTGGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3', Telomere B: 5'-GGCTTGCCCTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'. **Single Copy Gene: Beta-2-microglobulin (B2M)**: Forward (F): 5'-CAAGACACCCGCCAGAAGAT-3', Reverse (R): 5'-CAGCGTGGGACAGAAGGTAG-3' (Designed by our lab).



Data analysis

3 Data were analyzed using **ViiA7 software** (ThermoFisher, MA, USA), and RTL was calculated using the $\Delta\Delta C_t$ method: $\Delta\Delta C_t = (\text{Sample B2M } C_t - \text{Sample Telomere } C_t) - (\text{Control B2M } C_t - \text{Control Telomere } C_t)$.

