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🌐 Relative Telomere Length Measurement

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

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Abstract

Relative Telomere Length Measurement by qPCR

Relative Telomere Length Measurement
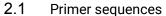
1 DNA isolation

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

qPCR

2 10 ng/µL DNA was amplified in a 5x EvaGreen® mix (No ROX) (Bio&Sell, Nurnberg, Germany). The thermal cycling protocol of Telomere A: 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3', Telomere B: 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-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'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3', Telomere B: 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-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 ΔΔCt method: ΔΔCt = (Sample B2M Ct - Sample Telomere Ct) - (Control B2M Ct - Control Telomere Ct).