Valid gene expression normalization by RT-qPCR in studies on hPDL fibroblasts with focus on orthodontic tooth movement and periodontitis

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Supplementary Information
## Contents

| Table/Figure/Supplementary Data | Page |
|---------------------------------|------|
| Supplementary Table 1 – MIQE checklist | 3 |
| Supplementary Table 2 – RNA quantity and quality | 10 |
| Supplementary Table 3 – Raw $C_q$ values of RT-qPCR | 11 |
| Supplementary Table 4 – Gene stability ranking for individual experimental groups | 12 |
| Supplementary Table 5 – Marker genes, primers and amplicons used for hPDL characterization | 13 |
| Supplementary Figure 1 – Characterisation of hPDL fibroblasts | 14 |
| Supplementary Figure 2 – Uncropped original gel of RT-qPCR products (amplification specifity) | 15 |
| Supplementary Data 1 – Splice variants and secondary structure analysis of amplicons and primers | 16 |
| Supplementary Data 2 – RNA integrity | 60 |
| Supplementary Data 3 – Amplification plot and Melting curve analysis (RT-qPCR) | 86 |
| Supplementary Data 4 – qPCR primer efficiency | 92 |
| Supplementary Data 5 – TUBB RT-qPCR, specifity and efficiency | 101 |
**Supplementary Table 1. MIQE checklist for authors, reviewers and editors. E = essential information; D = desirable information.**

| Item to check                                      | Importance | Description how item was addressed in study / article                                                                                                                                                                                                 |
|---------------------------------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Experimental design**                           |            |                                                                                                                |
| Definition of experimental and control groups     | E          | Control group: untreated hPDL fibroblasts (physiological conditions); Experimental groups: hPDL fibroblasts treated with compressive orthodontic force (model for orthodontic tooth movement) or bacterial lysate of Aggregatibacter actinomycetemcomitans (Agac, model for bacterial periodontitis) for 24h. For details see materials and methods and Figure 5. |
| Number within each group                          | E          | n = 6                                                                                                            |
| Assay carried out by the core or investigator’s   | D          | All assays were carried out in investigators’ laboratory.                                                          |
| laboratory?                                       |            |                                                                                                                |
| Acknowledgment of authors’ contributions          | D          | C.K. conceived the idea of the study/study design as well as designed/validated the used primer pairs. S.B., P.P. and A.S. contributed to discussion and study design. A.S. and C.K. conducted the experiments. A.S., C.K. and S.B. analysed the results. J.K. produced and contributed the Agac bacterial lysate. G.S. provided the primary hPDL fibroblasts. C.K. and A.S. wrote the manuscript and created the figures, tables and the supplementary material. All authors reviewed the manuscript. |
| **Sample**                                        |            |                                                                                                                |
| Description                                       | E          | Primary human periodontal ligament fibroblasts (hPDL) were cultivated from periodontal connective tissue isolated from the middle root section of human teeth free of decay, which had been freshly extracted for medical reasons. A pool of hPDL cell lines from four different patients was used (1 male, 3 female, age: 16-23 years). Cells were identified by means of hPDL-specific marker gene expression and their spindle-shaped morphology (Supplementary Table 5 and Supplementary Figure). Ethical consent was obtained from the local ethics committee (12-170-0150). |
| Volume/mass of sample processed                   | D          | Varying size of tissue sample / number of hPDL fibroblasts extracted. 70,000 cells were finally seeded per well / biological replicate for the experiments.                                                                                           |
| Microdissection or macrodissection                | E          | Microdissection                                                                                                  |
| Processing procedure                              | E          | Tissue samples were grown in 6-well cell culture plates until proliferation of adherently growing hPDL under normal cell culture conditions (37°C, 5% CO₂, water-saturated) in full media, then trypsinized and further cultivated and passaged until the 6th passage. |
| If frozen, how and how quickly?                   | E          | Until use hPDL fibroblasts were frozen in liquid nitrogen (90% FCS, 10% DMSO, freezing 1°C/minute in cryo-box with isopropanol).                                                                                                               |
| If fixed, with what and how quickly?              | E          | Not fixed.                                                                                                       |
Sample storage conditions and duration | E | Samples were directly isolated and cultivated under cell culture conditions in cell culture flasks and plates (37°C, 5% CO₂, water-saturated) in full media consisting of DMEM high glucose (D5796, Sigma–Aldrich®, S4438, St. Louis, MI, USA), 10% FCS (P30-3306, PAN-Biotech, Aidenbach, Germany), 1% L-glutamine (SH30034.01, GE Healthcare Europe, Munich, Germany), 100 µM ascorbic acid (A8960, Sigma–Aldrich, Munich, Germany) and 1% antibiotics/antimycotics (A5955, Sigma–Aldrich®, S4438).

Nucleic acid extraction

Procedure and/or instrumentation | E | After washing the cells twice with sterile phosphate-buffered saline, total RNA from hPDL cells was extracted by applying peqGOLD TriFast™ and further processing according to the manufacturer’s instructions. We eluted the resulting RNA pellet in nuclease-free water (25µl) with immediate ice-cooling.

Name of kit and details of any modifications | E | peqGOLD TriFast™ (1 ml / well, PEQLAB Biotechnology GmbH, Erlangen, Germany). We followed the manufacturer’s protocol exactly.

Source of additional reagents used | D | Chloroform (EMSURE®, 1.02445.1000; Merck KGaA, Darmstadt, Germany), 2-Propanol (20842.330, VWR International GmbH, Darmstadt, Germany), Ethanol (32205, Sigma–Aldrich, Munich, Germany); RNase-free water (T143, Bioscience-Grade, Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

Details of DNase or RNase treatment | E | 1 µg of RNA was treated with 40 U of RNase inhibitor (EO0381, Life Technologies) in a 22 µl final volume for cDNA synthesis. No DNase treatment was performed.

Contamination assessment (DNA or RNA) | E | For each primer pair and qPCR run we also tested a no-template-control (NTC) without cDNA and a -RT control (cDNA synthesis without enzyme reverse transcriptase added) on the same plate to exclude possible bias by primer dimers, contaminating or genomic DNA.

Nucleic acid quantification | E | RNA concentration was determined by measuring the absorbance at 260 nm UV light with 1 OD₂₆₀nm equalling 40 ng/µl total RNA. OD = optical density

Instrument and method | E | NanoDrop ND-2000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA)

Purity (A260/A280) | D | RNA purity was determined by measuring the absorbance ratio OD₂₆₀nm/280nm as well as OD₂₆₀nm/230nm. An OD₂₆₀nm/280nm ratio of >1.8 was considered protein-free RNA, and an OD₂₆₀nm/230nm ratio of >2.0 phenol-/ethanol-free RNA (Supplementary Table 2).

Yield | D | RNA yield was calculated as the amount of RNA obtained (µg) per well. Mean yield: 358.2 ng/µl x 2000 µl/well = 716.4 µg/well; Min./Max. yield: 218.6 / 495.4 ng/µl x 2000 µl/well = 437.2 / 990.8 µg/well (Supplementary Table 2).

RNA integrity: method/instrument | E | RNA integrity was determined with an Agilent 2100 Bioanalyzer (Agilent Technologies Inc. Santa Clara, CA, USA) according to the manufacturer’s protocol (Supplementary Data 2).

RIN/RQI or C₉ of 3´ and 5´ transcripts | E | RIN values ranged from 9.5 to 10 (mean 9.85, SD 0.15), indicating an absence of RNA degradation (Supplementary Data 2).

Electrophoresis traces | D | Electrophoresis traces were determined with an Agilent 2100 Bioanalyzer (Agilent Technologies Inc. Santa Clara, CA, USA) according to the manufacturer’s protocol (Supplementary Data 2).
Inhibition testing (C_q dilutions, spike, or other)  

For evaluation of qPCR and primer efficiency as well as absence of inhibitors a log_{10} serial dilution series of a random cDNA sample from the untreated group was amplified in triplet for each candidate reference gene and the limit of detection (LOD) as the highest dilution, at which 95% (all three) of the technical replicates are detectable (C_q values), was determined. A standard curve was created by linear regression of the resulting C_q values with the relative dilution within the linear dynamic range (LDR) and the coefficient of determination r^2 as well as qPCR reaction efficiencies (E) with 95% confidence intervals were determined from the slope of the standard curve: E = (10^{1/slope} -1) x 100%. Only primer pairs with a linear relation between C_q and log-transformed cDNA copy number (r^2>0.98) were considered as possible valid reference gene candidates. In addition, only efficiencies E within the range of 90-110% were deemed acceptable. (Table 2, Supplementary Data 4)

Reverse transcription

To synthesize cDNA, we transcribed a standardized quantity of 1µg RNA per sample using a random hexamer primer (0.1 nmol, 1 µl, SO142, Life Technologies), an oligo-dT18 primer (0.1 nmol, 1 µl, SO131, Life Technologies, Thermo Fisher Scientific Inc.), 5× M-MLV-buffer (4 µl, M1705, Promega, Fitchburg, WI, USA) and dNTP mix (40 nmol, 1 µl, 10 nmol/dNTP, Roti®-Mix PCR3, L785.2) ad 20 µl nuclease-free H_2O (Roth BioScience Grade T143, Carl Roth GmbH & Co. KG). After incubation for 3 min at 70°C the mixture was quickly cooled on ice (RNA denaturation). We then added reverse transcriptase (200 U, 1 µl, M1705, Promega) and an RNase inhibitor (40 U, 1 µl, EO0381, Life Technologies), continued incubation at 37°C for 60 min and inactivated the reverse transcriptase by heat (95°C, 2 min). To minimize experimental variations, synthesis of cDNA, which was stored at −20°C until use, was performed concurrently for all samples.

Amount of RNA and reaction volume  

Amount of RNA: 1 µg; Reaction volume: 22 µl

Priming oligonucleotide (if using GSP) and concentration  

0.1 nmol random hexamer primer; 0.1 nmol oligo-dT18 primer

Reverse transcriptase and concentration  

Reverse transcriptase (200 U, 1 µl, M1705, Promega) in a final concentration of 9.1 U/µl (200 U / 22 µl)

Temperature and time  

3 min at 70°C; 60 min at 37°C; 2 min at 95°C

Manufacturer of reagents and catalogue numbers  

Specified in “Complete reaction conditions”.

C_q with → without reverse transcription  

The signal of the amplification plot without reverse transcriptase was very late and there was a high C_q value difference between the -RT control and all cDNA samples.  
**GAPDH:** 15→29; **PPIB:** 16→34; **YWHAZ:** 22→32; **POLR2A:** 21→none; **TBP:** 23→35; **EEF1A1:** 14→27; **RPLP0:** 17→32; **RNA18S5:** 9→30; **RPL22:** 18→30

Storage conditions of cDNA  

-20°C

qPCR protocol

For qPCR amplification we used a Mastercycler® ep realplex-S thermocycler (Eppendorf AG, Hamburg, Germany) in conjunction with 96 well PCR plates (TW-MT, 712282, Biozym Scientific GmbH, Hessisch Oldendorf, Germany) and BZO Seal Filmcover sheeting (712350, Biozym Scientific GmbH). Into each well SYBR®Green JumpStart™ Taq ReadyMix™ (7.5 µl, Sigma–Aldrich®, S4438, St. Louis, MI, USA), consisting of Tris–HCl (20 mM, pH 8.3), KCl (100 mM), MgCl_2 (7 mM), dNTPs (0.4 mM per dATP, dCTP, dGTP, and dTTP),
dCTP, dGTP, dTTP), stabilizers, Taq-DNA-polymerase (0.05 U/µl), JumpStart Taq antibody and SYBR Green I, as well as the respective cDNA-solution (1.5 µl, dilution 1:10) and the respective primer pair (7.5 pmol, 0.75 µl - 3.75 pmol/primer) were pipetted ad 15 µl nuclease-free H2O (BioScience Grade T143, Carl Roth GmbH & Co. KG). A master-mix of all components except the cDNA solution was created to minimize technical errors during manual pipetting. We then amplified the cDNA in triplets (technical replicates) per candidate reference gene in 45 cycles (initial heat activation 95°C/5 min, per cycle 95°C/10 s denaturation, 60°C/8 s annealing, 72°C/8 s extension). At the end of each extension step SYBR Green I fluorescence was measured at 521 nm. For each biological replicate all genes were amplified in triplet on the same qPCR plate to minimize biasing effects of possible inter-run variations on relative reference gene stability assessment.

| Reaction volume and amount of cDNA/DNA | E | **Reaction volume**: 15 µl; **Amount of cDNA**: 1.5 µl of an 1:10 dilution of the cDNA stock solution |
|----------------------------------------|---|--------------------------------------------------|
| Primer, (probe), Mg2+, and dNTP concentrations | E | 3.75 pmol/primer; 3.5 mM MgCl2; 0.2 mM dNTP; 50 mM KCl |
| Polymerase identity and concentration | E | Taq-DNA polymerase in a final concentration of 0.025 U/µl (SYBR®Green JumpStart™ Taq ReadyMix™, Sigma–Aldrich®, S4438, St. Louis, MI, USA) |
| Buffer/kit identity and manufacturer | E | SYBR®Green JumpStart™ Taq ReadyMix™ (Sigma–Aldrich®, S4438, St. Louis, MI, USA) |
| Exact chemical composition of the buffer | D | 20 mM Tris–HCl, pH 8.3, final concentration 10 mM |
| Additives (SYBR Green I, DMSO, and so forth) | E | SYBR Green I, stabilizers, JumpStart Taq antibody, KCl , MgCl2 |
| Manufacturer of plates/tubes and catalogue number | D | 96 well PCR plates (TW-MT, 712282, Biozym Scientific GmbH, Hessisch Oldendorf, Germany) in combination with BZO Seal Filmcover sheeting (712350, Biozym Scientific GmbH) |
| Complete thermocycling parameters | E | Initial heat activation 95°C/5min; per cycle 95°C/10s denaturation, 60°C/8s annealing, 72°C/8s extension |
| Reaction setup (manual/robotic) | D | manual |
| Manufacturer of qPCR instrument | D | Mastercycler® ep realplex-S thermocycler (Eppendorf AG, Hamburg, Germany) |

**qPCR validation**

| Evidence of optimization | D | Primer optimization is evidenced by melting curve analysis and agarose gel electrophoresis (specificity), qPCR efficiency, technical reliability and in silico secondary structure analysis of primers and amplicons. Melting temperatures Tm of primers as validated by the manufacturer Eurofins MWG Operon LLC (Huntsville, AL, USA; High Purity Salt Free Purification HPSF®) are provided in Table 1. |
| Specificity (gel, sequence, melt or digest) | E | Specific amplification of target reference genes was assessed by agarose gel electrophoreses (single band, correct size) and a specific peak in melting curve analysis (95°C for 15s, 60°C for 15s, then continuous temperature increase to 95°C and fluorescence measurement for 20 min). For each primer pair and qPCR run we also tested a no-template-control (NTC) without cDNA and a -RT control (cDNA synthesis without enzyme reverse transcriptase added) on the same plate to exclude possible bias by unspecific amplification (primer dimers, contaminating or genomic DNA). (Figure 1, Supplementary Data 3). |
### Results for NTCs

| LOD for all genes (primer pairs) | E | The signal of the amplification plot during efficiency analysis for standard curve generation was very late and there was a high Cq value difference between the negative control and all cDNA dilutions. GAPDH: 40; PPIB: 36; YWHAZ: none; POLR2A: 37; TBP: none; EEF1A1: none; RPLP0: 40; RNA18S5: 35; RPL22: none. (Supplementary Data 4) |
|---------------------------------|--|--|

### Calibration curves with slope and y intercept

| Calibration curves with slope and y intercept | E | GAPDH: y=1E+9e^{-0.655x}, slope: -3.480; PPIB: y=5E+9e^{-0.651x}, slope: -3.508; YWHAZ: y=6E+9e^{-0.651x}, slope: -3.468; POLR2A: y=4E+10e^{-0.648x}, slope: -3.520; TBP: y=3E+12e^{-0.649x}, slope: -3.538; EEF1A1: y=7E+9e^{-0.665x}, slope: -3.315; RPLP0: y=2E+9e^{-0.646x}, slope: -3.509; RNA18S5: y=2E+6e^{-0.677x}, slope: -3.319; RPL22: y=1E+10e^{-0.674x}, slope: -3.403. (Supplementary Data 4) |
|--------------------------------------------|--|--|

### PCR efficiency calculated from slope

| PCR efficiency calculated from slope | E | GAPDH: 93.8%; PPIB: 92.8%; YWHAZ: 93.5%; POLR2A: 92.3%; TBP: 91.7%; EEF1A1: 100.3%; RPLP0: 92.7%; RNA18S5: 100.1%; RPL22: 96.7%. (Table 2, Supplementary Data 4) |
|-------------------------------------|--|--|

### CIs for PCR efficiency or SE

| CIs for PCR efficiency or SE | D | CIs of qPCR efficiencies E were calculated for all genes tested and are given in Supplementary Data 4. |
|-----------------------------|--|--|

### R² of calibration curve

| R² of calibration curve | E | GAPDH: 0.9998; PPIB: 0.9996; YWHAZ: 0.9993; POLR2A: 0.9984; TBP: 0.9974; EEF1A1: 0.9951; RPLP0: 0.9992; RNA18S5: 0.9974; RPL22: 0.9949. (Table 2, Supplementary Data 4) |
|---------------------------|--|--|

### Linear dynamic range (LDR)

| Linear dynamic range (LDR) | E | The linear dynamic range (LDR) included the used 1:10 cDNA dilution in all cases and ranged from 3x log₁₀ (cDNA stock dilution 1:10 – 1:10⁶) to 6x log₁₀ (cDNA stock dilution 1:10 – 1:10⁶) for the individual genes (primer pairs), see Supplementary Data 4. Standard curves were calculated only considering dilutions within the LDR. (Supplementary Data 4) |
|-----------------------------|--|--|

### Cq variation at LOD

| Cq variation at LOD | E | GAPDH: SD=0.952; PPIB: SD=1.77; YWHAZ: SD=1.696; POLR2A: SD=1.004; TBP: SD=0.561; EEF1A1: SD=0.405; RPLP0: SD=0.176; RNA18S5: SD=0.000; RPL22: SD=0.202. (Supplementary Data 4) |
|---------------------|--|--|

### CIs throughout range

| CIs throughout range | D | CIs of Cq were calculated throughout the dilution range for all genes tested and are given in Supplementary Data 4. |
|----------------------|--|--|

### Evidence for LOD

| Evidence for LOD | E | Not detectable Cq value for ≥ 1 of the technical replicates (triplet) at the corresponding cDNA dilution level indicates LOD at the previous, more concentrated dilution level. LOD for all genes (primer pairs) detected at a cDNA quantity equivalent to ≤1 pg RNA, except for TBP with an LOD of 100 pg RNA equivalent (weak signal at 10 pg and 1 pg). (Supplementary Data 4) |
|------------------|--|--|

### If multiplex, efficiency and LOD of each assay

| If multiplex, efficiency and LOD of each assay | E | Not applicable. |
|-----------------------------------------------|--|--|

### Data analysis

| Data analysis | E | Mastercycler ep realplex software, version 2.2 (Eppendorf AG, Hamburg, Germany) |
|---------------|--|--|

| qPCR analysis program (source, version) | E | Second derivative maximum method (CalqPlex algorithm, Automatic Baseline, Drift Correction On) |
|----------------------------------------|--|--|

| Method of Cq determination | E | Second derivative maximum method (CalqPlex algorithm, Automatic Baseline, Drift Correction On) |
|---------------------------|--|--|

| Outlier identification and disposition | E | For analysis none of the Cq values was discarded. |
|---------------------------------------|--|--|

| Results for NTCs | E | The signal of the amplification plot was very late and there was a high Cq value difference between the negative control and all cDNA samples. GAPDH: 33; PPIB: 35; YWHAZ: 36; POLR2A: 36; TBP: none; EEF1A1: 35; RPLP0: none; RNA18S5: 35; RPL22: none. |
|------------------|--|--|
### Justification of number and choice of reference genes

Aim of this study - identification of optimal number and choice of reference genes for hPDL fibroblasts under physiological conditions, in a model for orthodontic tooth movement and a model for bacterial periodontitis.

### Description of normalization method

Samples were not normalized, since apart from the reference genes no target genes were quantified.

### Number and concordance of biological replicates

N = 1 (pool of hPDL fibroblasts from 4 different patients); n = 6 (pool cells seeded in 6 different wells per experimental group as biological replicates).

### Number and stage (RT or qPCR) of technical replicates

qPCR reactions were performed in triplets (technical replicates n = 3).

### Repeatability (intraassay variation)

The maximum SD (of the mean) across all biological replicates (n=18) of the means of C_q from the three technical replicates was ≤0.553 in all instances. 

\[ \text{GAPDH: 0.24; PPIB: 0.29; YWHAZ: 0.32; POLR2A: 0.35; TBP: 0.27; EEF1A1: 0.53; RPLP0: 0.36; RNA18S5: 0.20; RPL22: 0.33. (Table 2)} \]

### Reproducibility (interassay variation, CV)

High biological reproducibility was achieved as evidenced by the low SD of raw C_q values for all genes and experimental groups tested (see Figure 2, Supplementary Table 3).

### Power analysis

The number of biological replicates (n = 6) was based on previous studies and corresponds to the number of replicates generally used in cell culture RT-qPCR experiments.

### Statistical methods for results significance

All biological samples (n = 6) were measured in triplicate (n = 3) and an arithmetic mean of each C_q tripllett used for further analysis. The stability of each candidate was calculated with four different mathematical algorithms: geNorm, NormFinder, BestKeeper and the comparative ΔC_q method. Stability calculations were done with the official Microsoft-Excel-based software applets for geNorm, NormFinder and BestKeeper according to developers’ instructions. For the comparative ΔC_q method manual calculations were performed. The geNorm and NormFinder algorithms require the transformation of the raw C_q data to linear scale expression quantities Q corresponding to the qPCR efficiency (E) of each gene: 

\[ Q = E^{-(C_q_{\text{min}} - C_q_{\text{sample}})} \]

with the lowest C_q value corresponding to a quantity of 1 for each candidate reference gene. The genes were ranked according to their stability values (geNorm: M, NormFinder: \( \rho_{\text{ig}}/\sigma_{\text{i}} \), deltaCT: mean SD of \( \Delta C_q \); BestKeeper: Pearson’s r) for each algorithm and each experimental condition as well as combined experimental conditions (no treatment + compressive force, no treatment + Agac) and a rank sum of all algorithms calculated per gene for final stability assessment with the smallest rank sum indicating the most stable reference gene. Also a pooled overall ranking for all experimental conditions was calculated. The geNorm algorithm was used to calculate the ideal number of reference genes for reliable RT-qPCR normalization. If pairwise variation \( (\text{V}_n/\text{V}_{n+1}) \) between two sets of reference genes with one set including an additional reference gene was ≤0.15, this additional gene was deemed unnecessary for normalization. To assess ranking variations between the algorithms, we used IBM SPSS Statistics® 23 (IBM, Armonk, NY, USA) to create a correlation matrix of bivariate correlations (Pearson’s correlation coefficient r, normality confirmed by Shapiro-Wilk tests and histogram evaluation) of the overall pooled stability values as calculated by two respective algorithms. (see Figures 3 and 4, Table 3, Supplementary Table 4).

### Software (source, version)

Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA); IBM SPSS Statistics® 23 (IBM, Armonk, NY, USA)
| qPCR or raw data submission | D | Raw $C_{q}$ values are provided in Figure 2 and Supplementary Table 3. |
|----------------------------|---|---------------------------------------------------------------------|
| qPCR target information     |   | Provided in Table 1. We based our primer design on the officially registered target gene nucleotide sequences from the NCBI Nucleotide database (GeneBank, access: http://www.ncbi.nlm.nih.gov/nuccore). |
| Gene symbol                 | E | Provided in Table 1. We based our primer design on the officially registered target gene nucleotide sequences from the NCBI Nucleotide database (GeneBank, access: http://www.ncbi.nlm.nih.gov/nuccore). |
| Sequence accession number   | E | Provided in Table 1. We based our primer design on the officially registered target gene nucleotide sequences from the NCBI Nucleotide database (GeneBank, access: http://www.ncbi.nlm.nih.gov/nuccore). |
| Location of amplicon        | D | Provided in Table 1. Target amplicon sequences were chosen to range from 60 to 150 bp with a GC content of 35–65%. |
| Amplicon length             | E | Provided in Table 1. Target amplicon sequences were chosen to range from 60 to 150 bp with a GC content of 35–65%. |
| In silico specificity screen (BLAST, and so on) | E | Provided in Table 1. In-silico specify of constructed primers was ensured by PrimerBLAST (National Center for Biotechnology Information, Bethesda MD, USA, https://www.ncbi.nlm.nih.gov/tools/primer-blast; RefSeq mRNA, Splice variants allowed, Max. Product Size: 4000) and cross-checked using the UCSC in-silico-PCR Genome Browser (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr). Intron-flanking primer pairs were designed to prevent a co-amplification of genomic DNA and checked in silico for sufficient absence of hairpin structures and dimer formation at annealing temperature ($\Delta G \geq -3.5$ kcal/mol, BeaconDesigner™ Free Edition, Premier BioSoft International, Palo Alto, CA, USA, http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1). |
| Pseudogenes, retropseudogenes or other homologs | D | Sequence alignment, possible splicing and targeted transcript variants as well as absence of targeted pseudogenes, retropseudogenes or other homologs were assessed upon primer construction by NCBI PrimerBLAST (National Center for Biotechnology Information, Bethesda MD, USA, https://www.ncbi.nlm.nih.gov/tools/primer-blast) and PrimerCheck (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp). |
| Sequence alignment          | D | Provided in Supplementary Data 1. No secondary structures present at annealing temperature (60°C) were detected as determined in silico by UNAFold (http://eu.idtdna.com/UNAFold?, Suboptimality 50%; Integrated DNA Technologies Inc., Coralville, IA, USA). |
| Secondary structure analysis of amplicon | D | Provided in Supplementary Data 1. No secondary structures present at annealing temperature (60°C) were detected as determined in silico by UNAFold (http://eu.idtdna.com/UNAFold?, Suboptimality 50%; Integrated DNA Technologies Inc., Coralville, IA, USA). |
| Location of each primer by exon or intron | E | Provided in Table 1. Also see Supplementary Data 1. |
| What splice variants are targeted | E | Provided in Table 1. Also see Supplementary Data 1. |

### qPCR oligonucleotides

| Primer sequences | E | Provided in Table 1. |
| RTouchDB identification number | D | Not applicable, primers were constructed and validated by the authors. |
| Probe sequences   | D | Not applicable. |
| Location and identity of any modifications | E | Primers received no terminal or other modifications. |
| Manufacturer of oligonucleotides | D | Primers were synthesized by Eurofins MWG Operon LLC (Huntsville, AL, USA). |
| Purification method | D | Primers were purified by High Purity Salt Free Purification HPSF® (Eurofins MWG Operon LLC). |
Supplementary Table 2. Yield (quantity) and quality of extracted total RNA per biological replicate (well).

| Sample ID    | Nucleic Acid Concentration | Unit | A260 | A280 | A260/A280 | Sample Type | Factor |
|--------------|-----------------------------|------|------|------|-----------|-------------|--------|
| Control K7   | 450.8 ng/µl                 |      | 11.269 | 5.757 | 1.96      | RNA         | 40     |
| Control K8   | 495.4 ng/µl                 |      | 12.385 | 6.415 | 1.93      | RNA         | 40     |
| Control K9   | 488.2 ng/µl                 |      | 12.206 | 6.494 | 1.88      | RNA         | 40     |
| Control K10  | 486.2 ng/µl                 |      | 12.156 | 6.407 | 1.9       | RNA         | 40     |
| Control K11  | 444.4 ng/µl                 |      | 11.11  | 5.793 | 1.92      | RNA         | 40     |
| Control K12  | 472.8 ng/µl                 |      | 11.82  | 6.125 | 1.93      | RNA         | 40     |
| Compression D7| 295.1 ng/µl             |      | 7.377  | 3.922 | 1.88      | RNA         | 40     |
| Compression D8| 291.6 ng/µl              |      | 7.29   | 3.826 | 1.91      | RNA         | 40     |
| Compression D9| 225.2 ng/µl              |      | 5.631  | 3.002 | 1.88      | RNA         | 40     |
| Compression D10| 225.9 ng/µl            |      | 5.647  | 3.098 | 1.82      | RNA         | 40     |
| Compression D11| 218.6 ng/µl            |      | 5.464  | 2.934 | 1.86      | RNA         | 40     |
| Compression D12| 298.9 ng/µl           |      | 7.473  | 3.952 | 1.89      | RNA         | 40     |
| Agac7        | 441 ng/µl                  |      | 11.026 | 5.873 | 1.88      | RNA         | 40     |
| Agac8        | 245.5 ng/µl                |      | 6.138  | 3.262 | 1.88      | RNA         | 40     |
| Agac9        | 303 ng/µl                  |      | 7.575  | 3.935 | 1.93      | RNA         | 40     |
| Agac10       | 456.7 ng/µl                |      | 11.417 | 6.052 | 1.89      | RNA         | 40     |
| Agac11       | 295.2 ng/µl                |      | 7.38   | 3.902 | 1.89      | RNA         | 40     |
| Agac12       | 312.6 ng/µl                |      | 7.814  | 4.149 | 1.88      | RNA         | 40     |

A = absorbance = optical density (OD) at 260nm and 280nm; A260/A280 = absorbance ratio.
Factor = ng/µl total RNA per 1 unit OD_{260nm}. 
**Supplementary Table 3.** Raw $C_q$ values of RT-qPCR (triplet means) for 3 experimental groups and 9 candidate reference genes.

| RAW $C_q$ values | Gene | Gene | Gene | Gene | Gene | Gene | Gene | Gene | Gene |
|----------------|------|------|------|------|------|------|------|------|------|
| Sample         | Group | GAPDH | PPIB | YWHAZ | POLR2A | TBP | RPL22 | RPLP0 | EEF1A1 | RNA18S5 |
| Control K7     | 1     | 15.04 | 16.43 | 21.81 | 20.96 | 23.35 | 18.22 | 16.34 | 14.17 | 8.67   |
| Control K8     | 1     | 15.14 | 16.40 | 21.73 | 21.03 | 23.29 | 18.13 | 16.18 | 14.14 | 8.42   |
| Control K9     | 1     | 15.14 | 16.36 | 21.51 | 20.77 | 23.30 | 18.00 | 16.33 | 14.09 | 7.91   |
| Control K10    | 1     | 15.26 | 16.51 | 21.60 | 20.83 | 23.47 | 18.21 | 16.24 | 14.12 | 8.33   |
| Control K11    | 1     | 15.04 | 16.30 | 21.21 | 21.00 | 23.46 | 17.95 | 16.06 | 14.02 | 8.41   |
| Control K12    | 1     | 15.09 | 16.36 | 21.00 | 20.60 | 23.12 | 17.93 | 16.22 | 14.06 | 7.81   |
| Compression D7 | 2     | 15.13 | 16.98 | 22.57 | 21.78 | 24.08 | 18.39 | 16.37 | 14.26 | 8.97   |
| Compression D8 | 2     | 14.85 | 16.75 | 22.14 | 21.50 | 23.72 | 18.19 | 16.26 | 13.91 | 8.57   |
| Compression D9 | 2     | 14.70 | 16.80 | 21.74 | 21.66 | 23.75 | 18.26 | 16.42 | 13.97 | 8.52   |
| Compression D10| 2     | 15.05 | 16.74 | 21.11 | 21.45 | 23.50 | 18.20 | 16.46 | 13.97 | 8.08   |
| Compression D11| 2     | 15.11 | 16.52 | 21.01 | 21.51 | 23.64 | 17.95 | 16.26 | 13.75 | 8.27   |
| Compression D12| 2     | 14.85 | 16.71 | 21.28 | 21.57 | 23.67 | 18.21 | 16.06 | 13.85 | 8.05   |
| Agac7          | 3     | 15.58 | 16.43 | 21.84 | 21.07 | 23.32 | 17.82 | 16.19 | 14.15 | 8.76   |
| Agac8          | 3     | 15.41 | 16.63 | 21.46 | 21.45 | 23.67 | 18.36 | 16.55 | 14.44 | 8.46   |
| Agac9          | 3     | 15.27 | 16.48 | 20.97 | 21.18 | 23.46 | 18.21 | 16.42 | 14.41 | 7.99   |
| Agac10         | 3     | 15.37 | 16.48 | 21.03 | 21.15 | 23.43 | 18.36 | 16.43 | 14.37 | 8.02   |
| Agac11         | 3     | 15.58 | 16.71 | 21.04 | 21.36 | 23.69 | 18.41 | 16.83 | 14.57 | 8.32   |
| Agac12         | 3     | 15.40 | 16.66 | 20.89 | 21.09 | 23.50 | 18.35 | 16.65 | 14.46 | 7.91   |

| $C_q$ SD        |      |      |      |      |      |      |      |      |      |
|----------------|------|------|------|------|------|------|------|------|------|
| Control        | 1    | 0.08 | 0.07 | 0.31 | 0.16 | 0.13 | 0.13 | 0.10 | 0.05 | 0.33   |
| Compression    | 2    | 0.17 | 0.15 | 0.62 | 0.12 | 0.19 | 0.14 | 0.15 | 0.17 | 0.35   |
| Agac           | 3    | 0.12 | 0.12 | 0.37 | 0.15 | 0.14 | 0.22 | 0.22 | 0.14 | 0.33   |

$C_q$ = quantification cycle; SD = standard deviation of group mean. Gene symbols see Table 1. Agac = Aggregatibacter actinomycetemcomitans (periodontitis)
Supplementary Table 4. Gene stability ranking for individual experimental groups of the nine analysed candidate reference genes according to their expression stability as calculated by the algorithms geNorm, NormFinder, comparative $\Delta C_q$ and BestKeeper.

| Rank | (of 4 methods) | geNorm | NormFinder | comparative $\Delta C_q$ | BestKeeper |
|------|----------------|--------|------------|--------------------------|------------|
|      | Total          | Order  | Stability  | Order                  | Stability  | Rank       | Stability  | SD | CV   |
|      | of 4 methods   |        | value (M)  |                        | value      |            |            |    |      |
|      |                |        |            |                          | mean $\Delta C_q$ |            |            |    |      |
|      |                |        |            |                          |            |            |            |    |      |
| Untreated control (physiological conditions) | | | | | | | |
| 1.) | RPL22 | 7 | EEF1A1 | 0.138 | RPL22 | 0.005 | 0.082 | EEF1A1 | 0.146 | RNA18S5 | 0.915 | 0.066 | 3.216 |
| 2.) | EEF1A1 | 9 | PPIB | 0.142 | EEF1A1 | 0.045 | 0.024 | RPL22 | 0.147 | RNA18S5 | 0.903 | 0.113 | 0.627 |
| 3.) | PPIB | 14 | RPL22 | 0.144 | PPIB | 0.055 | 0.025 | PPIB | 0.168 | YWHAZ | 0.892 | 0.248 | 1.154 |
| 4.) | TBP | 20 | GAPDH | 0.168 | TBP | 0.106 | 0.078 | GAPDH | 0.173 | POLR2A | 0.771 | 0.132 | 0.631 |
| 5.) | GAPDH | 23 | TBP | 0.165 | POLR2A | 0.173 | 0.029 | TBP | 0.177 | EEFC1A1 | 0.735 | 0.043 | 0.307 |
| 6.) | POLR2A | 23 | RPLP0 | 0.175 | GAPDH | 0.086 | 0.032 | RPLP0 | 0.182 | BNP | 0.621 | 0.095 | 0.407 |
| 7.) | RPLP0 | 27 | POLR2A | 0.178 | RPLP0 | 0.091 | 0.033 | POLR2A | 0.188 | PPIB | 0.579 | 0.053 | 0.325 |
| 8.) | YWHAZ | 27 | YWHAZ | 0.248 | YWHAZ | 0.148 | 0.049 | YWHAZ | 0.266 | RPLP0 | 0.186 | 0.075 | 0.462 |
| 9.) | RNA18S5 | 28 | RNA18S5 | 0.290 | RNA18S5 | 0.187 | 0.061 | RNA18S5 | 0.290 | GAPDH | 0.127 | 0.062 | 0.408 |
| Compressive orthodontic force (model for orthodontic tooth movement) | | | | | | | |
| 1.) | EEF1A1 | 9 | PPIB | 0.178 | EEF1A1 | 0.012 | 0.072 | PPIB | 0.185 | RNA18S5 | 0.958 | 0.277 | 3.290 |
| 2.) | PPIB | 10 | EEF1A1 | 0.161 | EEF1A1 | 0.021 | 0.045 | EEF1A1 | 0.187 | RPL22 | 0.938 | 0.508 | 2.349 |
| 3.) | TBP | 15 | POLR2A | 0.190 | PPIB | 0.036 | 0.034 | RPL22 | 0.200 | YWHAZ | 0.913 | 0.126 | 0.529 |
| 4.) | POLR2A | 17 | RPL22 | 0.190 | POLR2A | 0.065 | 0.033 | POLR2A | 0.201 | POLR2A | 0.905 | 0.115 | 0.824 |
| 5.) | RPL22 | 18 | TBP | 0.194 | RPL22 | 0.067 | 0.034 | TBP | 0.203 | EEFC1A1 | 0.672 | 0.093 | 0.557 |
| 6.) | RNA18S5 | 28 | RPLP0 | 0.246 | RPLP0 | 0.131 | 0.047 | RPLP0 | 0.258 | TBP | 0.804 | 0.094 | 0.438 |
| 7.) | RPLP0 | 26 | GAPDH | 0.276 | RNA18S5 | 0.140 | 0.050 | RNA18S5 | 0.286 | PPIB | 0.752 | 0.087 | 0.476 |
| 8.) | YWHAZ | 29 | RNA18S5 | 0.283 | GAPDH | 0.166 | 0.057 | RNA18S5 | 0.296 | RPLP0 | 0.390 | 0.112 | 0.685 |
| 9.) | GAPDH | 32 | YWHAZ | 0.474 | YWHAZ | 0.324 | 0.103 | YWHAZ | 0.515 | GAPDH | 0.177 | 0.148 | 0.992 |
| Bacterial lysate of Aggregatibacter actinomycetemcomitans (Agac, model for bacterial periodontitis) | | | | | | | |
| 1.) | TBP | 7 | TBP | 0.175 | TBP | 0.035 | 0.035 | TBP | 0.184 | POLR2A | 0.742 | 0.126 | 0.592 |
| 2.) | POLR2A | 10 | PPIB | 0.186 | POLR2A | 0.038 | 0.034 | PPIB | 0.187 | GAPDH | 0.715 | 0.097 | 0.626 |
| 3.) | PPIB | 11 | POLR2A | 0.192 | PPIB | 0.038 | 0.033 | EEF1A1 | 0.199 | RNA18S5 | 0.691 | 0.270 | 3.275 |
| 4.) | GAPDH | 16 | EEF1A1 | 0.194 | GAPDH | 0.055 | 0.032 | POLR2A | 0.203 | TBP | 0.650 | 0.112 | 0.477 |
| 5.) | EEF1A1 | 20 | GAPDH | 0.218 | EEF1A1 | 0.090 | 0.037 | GAPDH | 0.227 | PPIB | 0.537 | 0.102 | 0.614 |
| 6.) | RPLP0 | 25 | RPLP0 | 0.223 | RPLP0 | 0.124 | 0.045 | RPLP0 | 0.236 | YWHAZ | 0.483 | 0.297 | 1.399 |
| 7.) | RNA18S5 | 27 | RPL22 | 0.249 | RPL22 | 0.155 | 0.054 | RPL22 | 0.260 | RPLP0 | 0.379 | 0.165 | 0.999 |
| 8.) | RPL22 | 30 | RNA18S5 | 0.346 | RNA18S5 | 0.224 | 0.073 | RNA18S5 | 0.354 | EEF1A1 | 0.222 | 0.093 | 0.648 |
| 9.) | YWHAZ | 33 | YWHAZ | 0.386 | YWHAZ | 0.255 | 0.083 | YWHAZ | 0.410 | RPL22 | 0.078 | 0.158 | 0.864 |

$C_q$ = quantification cycle; SD = standard deviation; CV = coefficient of variation; r = Pearson’s correlation coefficient.
### Supplementary Table 5. Marker genes, primers and amplicons used for characterization of hPDL fibroblasts.

| Gene symbol | Gene name (Homo sapiens) | Accession number (NCBI GenBank) | Chromosomal location (length) | 5'-forward primer-3' (length / Tm / %GC / max. ΔG Hauppin &Self-Dimer / Self-Comp. / Self-3’-Comp.) | 5'-reverse primer-3' (length / Tm / %GC / max. ΔG Hauppin &Self-Dimer / Self-Comp. / Self-3’-Comp.) | Primer location (max. ΔG Cross-Dimer) | Amplicon (length, %GC, Tm, SSAT) | Amplicon location (bp of Start/Stop) | Intron flanking (length) | In silico qPCR specificity | Variants targeted (Transcript / Splice) |
|-------------|--------------------------|---------------------------------|-------------------------------|------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|----------------------------------------|-------------------------------|-----------------------------------|-----------------------------|-----------------------------------|----------------------------------------|
| VIM         | vimentin                 | NM_003380.3                     | 10p13 (215bp)                 | CTGAGGTCTACCTCCCTCTGGTG (22bp / 62.1°C / 54.6% / -1.3 / 5 / 0)                                 | CTTGATGTCCGAAATGGTCTCGTG (23bp / 60.6°C / 47.8% / -0.6 / 1 / 4 / 0)                                | exon 8/9 (-2.6)                         | 106bp 43.4% 82.3% no SSAT         | 1695/1800                        | Yes (850bp)                        | Yes                                  |
| P4HA1       | prolipoproteinase, alpha polyepitope 1 | NM_000917.3                  | 10q22.1 (2860bp)             | GCTTCTGCTGATGAAATCTCT (23bp / 60.6°C / 47.8% / 0.0 / 2 / 2)                                   | GTGAAGTCAATGGGGGTTCTG (22bp / 58.4°C / 45.3% / -3.4 / 4 / 0)                                    | exon 13/14 (-0.9)                       | 146bp 41.1% 82.2% no SSAT         | 1396/1541                        | Yes (1373bp)                       | Yes                                  |
| FN1         | fibronectin              | NM_212426.1                    | 2q34 (8813bp)                 | GCAAGTCATTCAACAGATTCTCTCTC (24bp / 62.7°C / 50.0% / -0.3 / 4 / 2)                             | GCTGTTCCTTCTGGTAAAGAG (23bp / 60.6°C / 47.8% / -2.5 / 4 / 1)                                    | exon 45/46 (-3.0)                      | 150bp 42.7% 83.1°C no SSAT         | 7579/7728                        | Yes (342bp)                        | Yes                                  |
| COL1A2      | collagen, type I, alpha 2 | NM_000089.3                    | 7q22.1 (5411bp)               | AGAACAACGTCTGTCTAGAG (21bp / 59.8°C / 52.4% / -3.3 / 4 / 2)                                   | GACTGAGGCCAGTTGGTTAG (21bp / 59.8°C / 52.4% / -2.3 / 4 / 5)                                    | exon 50/51 (-0.7)                      | 105bp 44.8% 83.3°C no SSAT         | 4139/4243                        | Yes (710bp)                        | Yes                                  |
| FMOD        | fibromodulin             | NM_002023.4                    | 1q32 (3271bp)                 | AGTCAACACCAACCTTGAAC (22bp / 60.3°C / 50.0% / -1.5 / 3 / 0)                                   | GAAGTTCAACGCTCACCAC (21bp / 61.8°C / 57.1% / -6.5 / 6 / 3)                                    | exon 2/3 (-2.8)                        | 97bp 51.6% 85.7°C no SSAT          | 1334/1430                        | Yes (4797bp)                       | Yes                                  |
| TNFRSF1B (OPG) | tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) | NM_002546.3             | 8q24 (2354bp)                 | TTTGTTTGTGCTTCGCTCACT (23bp / 60.6°C / 47.8% / 0.0 / 2 / 2)                                  | CCTGAAAGATCTGCTCTCACC (22bp / 62.1°C / 54.5% / -0.9 / 4 / 0)                                   | exon 3/4 (-1.8)                        | 124bp 42.7% 83.1°C no SSAT         | 824/947                          | Yes (2018bp)                       | Yes                                  |
| POSTN       | peristin                 | NM_006475.2                    | 13q13.3 (3390bp)             | AGACACACCGTGGGAAAG (19bp / 58.8°C / 35.9% / -1.3 / 4 / 0)                                    | GTTGACGGTTAGCTGAGGGT (24bp / 61.0°C / 45.8% / -2.6 / 4 / 2)                                      | exon 23/24 (-3.4)                      | 136bp 39.4% 81.9°C no SSAT         | 2548/2683                        | Yes (1148bp)                       | Yes                                  |
| RUNX2       | runt related transcription factor 2 | NM_001024630.3 | 6p21 (5533bp)                 | CAGTATAGGGACCTGGAAC (21bp / 61.8°C / 57.1% / 0.0 / 0 / 3 / 0)                                 | TGGGGGCTAGAGGACAAAC (21bp / 59.8°C / 52.4% / -0.9 / 1 / 3)                                      | exon 5/6 (-3.1)                        | 81bp 50.6% 83.7°C no SSAT          | 869/949                          | Yes (5388bp)                       | Yes                                  |
| SMAD1       | SMAD family member 1     | NM_005900.3                    | 4q31 (3056bp)                 | AGACAGACCTACCTCCACCTC (20bp / 61.4°C / 60.0% / 0.0 / 3 / 0)                                  | CTTGAGGAGCCGATCACCAG (21bp / 61.8°C / 57.1% / -0.5 / 1 / 3)                                     | exon 4/5 (-2.9)                        | 97bp 60.8% 90.4°C no SSAT          | 1014/1110                        | Yes (2520bp)                       | Yes                                  |
| ALPL        | alkaline phosphatase, liver/bone/kidney | NM_000478.4             | 1p36.12 (2060bp)             | ACAAGCACTCCTCCATCTCTG (22bp / 60.3°C / 50.0% / -0.5 / 3 / 2)                                 | GCTGCCATGCTGTTCTCGT (20bp / 61.4°C / 60.0% / -0.3 / 3 / 1)                                     | exon 7/8 (-2.1)                        | 132bp 56.1% 89.5°C no SSAT         | 1045/1176                        | Yes (3290bp)                       | Yes                                  |
| SCX         | scleraxis BHLL transcription factor | NM_001080514.2 | 8q24.3 (1027bp)               | CCAGGCCCCAACAGATGGTGCAC (21bp / 61.8°C / 57.1% / -7.9 / 8 / 2)                                | TGGCGATCTCTGCTTCTCAG (20bp / 63.0°C / 50.0% / -4.2 / 7 / 2)                                     | exon 1/2 (-3.8)                        | 83bp 54.2% 86.6°C no SSAT          | 575/657                          | Yes (923bp)                        | Yes                                  |
| S100A4      | S100 calcium binding protein A4 | NM_002961.2           | 1q21 (5129bp)                 | TCTCTACACCTCTCCTCGAG (23bp / 62.4°C / 52.2% / 0.0 / 3 / 3)                                   | GGAAGTGGAGACCTACATC (21bp / 62.1°C / 54.5% / -2.8 / 1 / 1)                                     | exon 1/3 (-1.5)                        | 103bp 54.1% 87.8°C no SSAT         | 11/118                           | Yes (943bp)                        | Yes                                  |
| NCAM1       | neural cell adhesion molecule 1 (NCAM1) | NM_000615.6         | 11q23.1 (5977bp)              | GCTCCACACCAACATACATG (21bp / 61.8°C / 57.1% / -1.5 / 3 / 2)                                 | CAGAGTTCTGCTCCACACG (20bp / 61.4°C / 60.0% / -1.3 / 6 / 2)                                     | exon 4/5 (-1.3)                        | 150bp 49.3% 86.6°C no SSAT         | 799/948                          | Yes (376bp)                        | Yes                                  |

\( T_m = \text{melting temperature of primer-specific qPCR product (amplicon)} \)

\( %\text{GC} = \text{guanine/cytosine content} \)

bp = base pairs

Comp. = Complementarity

SSAT = secondary structure at annealing temperature
Supplementary Figure 1. Characterization of human PDL fibroblasts. (a) Cell morphology of isolated hPDL cells. All cells show a spindle-shaped cell morphology. (b) Specific gene expression profile of hPDL markers (western blot of PCR products): untreated control samples of individual patients and of final hPDL cell pool (experimental groups). Abbreviations see Supplementary Table 5.

| Individual hPDL donors | VIM | P4HA1 | FN1 | COL1A2 | FMOD | OPG | POSTN | RUNX2 | SMAD1 | ALPL | SCX | S100A4 | NCAM1 |
|------------------------|-----|-------|-----|--------|------|-----|-------|-------|-------|------|-----|--------|-------|
| Patient 4 *1992 male   |     |       |     |        |      |     |       |       |       |      |     |        |       |
| Patient 7 *1995 female |     |       |     |        |      |     |       |       |       |      |     |        |       |
| Patient 8 *1991 female |     |       |     |        |      |     |       |       |       |      |     |        |       |
| Patient 16 *1989 female|     |       |     |        |      |     |       |       |       |      |     |        |       |
| Untreated              |     |       |     |        |      |     |       |       |       |      |     |        |       |
| hPDL pool (n = 4)      |     |       |     |        |      |     |       |       |       |      |     |        |       |

*Agac lysate (periodontitis)
Supplementary Figure 2. Uncropped original gel of RT-qPCR products (amplification specificity). For each candidate reference gene / primer pair we found a single fluorescent band at the expected amplicon size. bp = base pairs. Gene names see Table 1. All RT-qPCR products were run concurrently and adjacently on the same gel, which was recorded with the gel documentation system Genoplex 2 (VWR International GmbH, Darmstadt, Germany) and its software GenoCapture (version 7.01, Synoptics Ltd., Cambridge, UK - automatic exposure, exposure time 80 ms, no binning, transillumination) as secure gel data (*.sgd) and exported as TIF image, which was inverted and cropped to encompass the relevant gel area.
Supplementary Data 1. Splice variants and secondary structure analysis of amplicons and primers of the nine evaluated candidate reference genes.

GAPDH PrimerBLAST (National Center for Biotechnology Information, Bethesda MD, USA, https://www.ncbi.nlm.nih.gov/tools/primer-blast )

| Primer pair 1 | Sequence 5'→3' | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3' complementarity |
|---------------|----------------|--------|-------|------|----|-----|----------------------|------------------------|
| Forward primer | TGCCCTAACGACACITTG | 29     | 1091  | 1110 | 63.28 | 50.00 | 3.00 | 2.00 |
| Reverse primer | CCACACACCTGGTGGCTTGA | Minus  | 20    | 1154 | 1145 | 63.08 | 60.00 | 4.00 |
| Product length | 74             |        |       |      |     |      |                     |                        |
| Total intron size | 164  (between pos. 657096 and 6628101 on NT_009759.17) |        |       |      |     |      |                     |                        |

Products on intended target

| Product length | Forward primer | Reverse primer | Template |
|----------------|---------------|----------------|----------|
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1091         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1077         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1150         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1123         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1123         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1144         |

Products on allowed transcript variants

| Product length | Forward primer | Reverse primer | Template |
|----------------|---------------|----------------|----------|
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1096         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1106         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1131         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1202         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1237         |
| 74             | TGCCCTAACGACACCTTGG | CCACACACCTGGTGGCTTGA | 1144         |
GAPDH PrimerCheck (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp)
GAPDH UNAFold (Integrated DNA Technologies Inc., Coralville, IA, USA, http://eu.idtdna.com/UNAFold? , Suboptimality 50%)

**GAPDH Amplicon Sequence**

5' TGCCCTCAACGACCACTTTGTCAGCTCATTCTTCTGGTTATGACAACGAATTGGCTACAGCAGCAACAGGGTGTTGGG 3'

| Structures | Image | ΔG (kcal.mole⁻¹) | Tₘ (°C) | ΔH (kcal.mole⁻¹) | ΔS (cal.K⁻¹.mole⁻¹) | Output |
|------------|-------|------------------|--------|------------------|----------------------|--------|
| 1          |       | 1.04             | 47     | -25.6            | -79.96               | Ct Det |
| 2          |       | 1.33             | 49.9   | -42.7            | -132.17              | Ct Det |
| 3          |       | 1.49             | 41.3   | -25              | -79.51               | Ct Det |
| 4          |       | 1.58             | 47.1   | -39.2            | -122.4               | Ct Det |
| 5          |       | 1.66             | 41.2   | -27.8            | -68.44               | Ct Det |
| 6          |       | 1.82             | 30.4   | -4.9             | -20.19               | Ct Det |
| 7          |       | 1.92             | -26.6  | -5.3             | -21.67               | Ct Det |
| 8          |       | 1.98             | 34.3   | -23.6            | -76.77               | Ct Det |
**UCSC In-silico PCR**

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.
GAPDH BeaconDesigner™ Free Edition (Premier BioSoft International, Palo Alto, CA, USA, http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1)

**Secondary Structures for Sense Primer**

**Dimer:**

5’ GCCCTGAGACACGACCTTTG 3’

| | | | | |
|---|---|---|---|---|
3’ GTCCTGACACGACCTTTG 5’

-0.7

**Hairpin:**

/CAGACCTTTG 3’

| | | | | |
\CAGACCTTTG 3’

-0.7

**Secondary Structures for Anti-sense Primer**

**Dimer:**

Not Found

**Hairpin:**

Not Found

**Cross Dimer between Sense Primer and Anti-sense Primer:**

5’ GCCCTGAGACACGACCTTTG 3’

| | | | | |
3’ GATCTGAGACACGACCTTTG 5’

-2.4
**PPIB PrimerBLAST** (National Center for Biotechnology Information, Bethesda MD, USA, [https://www.ncbi.nlm.nih.gov/tools/primer-blast](https://www.ncbi.nlm.nih.gov/tools/primer-blast))

**PPIB PrimerCheck** (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, [http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp](http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp))
**PPIB UCSC In-silico-PCR Genome Browser** (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr)

**UCSC In-Silico PCR**
The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

>pp002and_3  PPIB:446-533 55bp TTCCATCGTGTAATCAAGGACTTCATGATCCAGGGCGGAGACTTCACCAGGGGAGATGGCACAGGAGGAAAGAGCATCTACGGTGAGC

**PPIB UNAFold** (Integrated DNA Technologies Inc., Coralville, IA, USA, http://eu.idtdna.com/UNAFold?, Suboptimality 50%)

**PPIB Amplicon Sequence** 5' TTCCATCGTGTAATCAAGGACTTCATGATCCAGGGCGGAGACTTCACCAGGGGAGATGGCACAGGAGGAAAGAGCATCTACGGTGAGC 3'

**Structures**

| Structure Name | Image | ΔG (kcal.mole⁻¹) | T_M (°C) | ΔH (kcal.mole⁻¹) | ΔS (cal.K⁻¹.mole⁻¹) | Output |
|----------------|-------|-----------------|----------|-----------------|---------------------|--------|
| 1              | ![Image](image1.png) | 0.45           | 49       | -13.1           | -40.66              | Ct, Det |
| 2              | ![Image](image2.png) | 0.64           | 47.3     | -16.1           | -50.24              | Ct, Det |
| 3              | ![Image](image3.png) | 0.66           | 41.5     | -11.2           | -35.6               | Ct, Det |
| 4              | ![Image](image4.png) | 1.25           | 49       | -36.6           | -113.6              | Ct, Det |
| 5              | ![Image](image5.png) | 1.32           | 35.1     | -16.4           | -53.2               | Ct, Det |
PPIB BeaconDesigner™ Free Edition (Premier BioSoft International, Palo Alto, CA, USA, [http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1](http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1))

Secondary Structures for Sense Primer

Dimer:-

5' TTCCATCGTGCATAGGCTCTC 3'
   |||| ||| ||
3' CTTCTCTGAGAGCCACTCG 5'
   1.3

Hairpin:-

/CTTCTCTGAGAGCCACTCG 5'
7 : : ||||
\CTTCTCTGAGAGCCACTCG 3'
   -1.3

Secondary Structures for Anti-sense Primer

Dimer:-

5' CTCTCTGAGAGCCACTCG 3'
   |||| ||| ||
3' CTTCTGATGAGCAGGCTCTC 5'
   0.7

Hairpin:-

/CTTCTGATGAGCAGGCTCTC 5'
0 : : ||||
\CTTCTCTGAGAGCCACTCG 3'
   -0.7

Cross Dimer between Sense Primer and Anti-sense Primer:-

5' TTCCATCGTGCATAGGCTCTC 3'
   |||| ||| ||
3' CTTCTCTGAGAGCCACTCG 5'
   2.1

5' CTCTCTGAGAGCCACTCG 3'
   |||| ||| ||
3' CTTCTGATGAGCAGGCTCTC 5'
   1.3

5' TTCCATCGTGCATAGGCTCTC 3'
   |||| ||| ||
3' CTTCTGATGAGCAGGCTCTC 5'
   -0.5

5' TTCCATCGTGCATAGGCTCTC 3'
   |||| ||| ||
3' CTTCTGATGAGCAGGCTCTC 5'
   -0.4
**YWHAZ PrimerBLAST** (National Center for Biotechnology Information, Bethesda MD, USA, [https://www.ncbi.nlm.nih.gov/tools/primer-blast](https://www.ncbi.nlm.nih.gov/tools/primer-blast))

| Primer pair 1 | Sequence (5’→3’) | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3’ complementarity |
|---------------|------------------|-----------------|--------|-------|------|----|-----|----------------------|------------------------|
| **Forward primer** | AGGAGGATCTACGGTACTTGGC | Plus | 24 | 504 | 527 | 62.43 | 45.83 | 4.00 | 2.00 |
| **Reverse primer** | AGCTTCTGTAGCTGTGGTGTG | Minus | 23 | 594 | 572 | 62.24 | 43.48 | 4.00 | 0.00 |
| **Product length** | 91 | | | | | | | | |
| **Total intron size** | 617 (between pos. 15210694 and 15210706 on NT_000046.17) | | | | | | | | |

**Products on intended target**

>**NM_003430.3** Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ), transcript variant 1, mRNA

- **product length = 91**
  - **Forward primer 1** AGGAGGATCTACGGTACTTGGC 24
  - **Template** 504 527
  - **Reverse primer 1** AGCTTCTGTAGCTGTGGTGTG 23
  - **Template** 594 572

**Products on allowed transcript variants**

>**XM_01157289.1** PREDICTED: Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ), transcript variant X4, mRNA

- **product length = 91**
  - **Forward primer 1** AGGAGGATCTACGGTACTTGGC 24
  - **Template** 809 832
  - **Reverse primer 1** AGCTTCTGTAGCTGTGGTGTG 23
  - **Template** 899 877

>**XM_005251063.2** PREDICTED: Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ), transcript variant X3, mRNA

- **product length = 91**
  - **Forward primer 1** AGGAGGATCTACGGTACTTGGC 24
  - **Template** 663 686
  - **Reverse primer 1** AGCTTCTGTAGCTGTGGTGTG 23
  - **Template** 753 731

>**XM_005251062.2** PREDICTED: Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ), transcript variant X2, mRNA

- **product length = 91**
  - **Forward primer 1** AGGAGGATCTACGGTACTTGGC 24
  - **Template** 676 699
  - **Reverse primer 1** AGCTTCTGTAGCTGTGGTGTG 23
  - **Template** 766 744
| Accession | Description                                                                 | Forward Primer | Template | Reverse Primer | Template |
|-----------|------------------------------------------------------------------------------|----------------|----------|----------------|----------|
| XM_005251061.2 | Homo sapiens tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta (YWHAZ), transcript variant X1, mRNA | AGGAGATTACTACCTGAGACCTTG 24 | 901        | AGCTTCCTGGCTAGCTGTGGTG 23 | 991      |
| NM_001135702.1 | Homo sapiens tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta (YWHAZ), transcript variant 6, mRNA | AGGAGATTACTACCTGAGACCTTG 24 | 543        | AGCTTCCTGGCTAGCTGTGGTG 23 | 611      |
| NM_001135701.1 | Homo sapiens tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta (YWHAZ), transcript variant 5, mRNA | AGGAGATTACTACCTGAGACCTTG 24 | 524        | AGCTTCCTGGCTAGCTGTGGTG 23 | 592      |
| NM_001135700.1 | Homo sapiens tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta (YWHAZ), transcript variant 4, mRNA | AGGAGATTACTACCTGAGACCTTG 24 | 475        | AGCTTCCTGGCTAGCTGTGGTG 23 | 543      |
| NM_001135699.1 | Homo sapiens tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta (YWHAZ), transcript variant 3, mRNA | AGGAGATTACTACCTGAGACCTTG 24 | 521        | AGCTTCCTGGCTAGCTGTGGTG 23 | 589      |
| NM_145690.2 | Homo sapiens tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta (YWHAZ), transcript variant 2, mRNA | AGGAGATTACTACCTGAGACCTTG 24 | 578        | AGCTTCCTGGCTAGCTGTGGTG 23 | 646      |
YWHAZ PrimerCheck (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp)
**YWHAZ UCSC In-silico-PCR Genome Browser** (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, [http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr](http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr))

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### UCSC In-Silico PCR

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

| Gene | Chrm | Start | End | Sequence 1 | Sequence 2 |
|------|------|-------|-----|------------|------------|
| uc0111p1.1_YWHAZ:543+633 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc0111h1.1_YWHAZ:524+614 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc0111h1.1_YWHAZ:273+363 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc003iy2.1_YWHAZ:475+565 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc010mbo.1_YWHAZ:410+500 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc003iy2.1_YWHAZ:578+668 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc010mbo.2_YWHAZ:521+611 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
| uc003iy2.2_YWHAZ:504+594 | 91bp | AGGAGATIATACGGTTACTTGGC AGCTTCCTGATGCTGTTG | AGGAGATIATACGGTTACTTGGC Tgagtgtccgtgtgatcgaaga |
YWHAZ UNAFold (Integrated DNA Technologies Inc., Coralville, IA, USA, [http://eu.idtdna.com/UNAFold](http://eu.idtdna.com/UNAFold), Suboptimality 50%)

YWHAZ Amplicon Sequence

5' AGGAGATTACTACCGTGACGGGACCCGCTGGATGACAAGAAAGGGATTGTCGATCAGTCA
CAACAAGCATACCAAGAAGCT
3'

| Structures | | | | | | |
|---|---|---|---|---|---|
| Structure Name | Image | $\Delta G$ (kcal.mole$^{-1}$) | $T_M$ (°C) | $\Delta H$ (kcal.mole$^{-1}$) | $\Delta S$ (cal.K$^{-1}$mole$^{-1}$) | Output |
| 1 |   | 0.98 | 32.5 | -10.9 | -35.66 | Ct Det |
| 2 |   | 1.03 | 41.9 | -18 | -57.14 | Ct Det |
| 3 |   | 1.06 | 46.6 | -25.3 | -79.13 | Ct Det |
| 4 |   | 1.3 | 41.4 | -22 | -69.94 | Ct Det |
| 5 |   | 1.47 | 35.7 | -18.6 | -60.23 | Ct Det |
| 6 |   | 1.52 | 26 | -13.4 | -44.79 | Ct Det |
| 7 |   | 1.57 | 39 | -23.3 | -74.64 | Ct Det |
| 8 |   | 1.74 | 41.1 | -28.9 | -91.96 | Ct Det |
| 9 |   | 1.88 | 18.8 | -13.3 | -45.56 | Ct Det |
**YWHAZ BeaconDesigner™ Free Edition** (Premier BioSoft International, Palo Alto, CA, USA, [http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1](http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1))

### Secondary Structures for Sense Primer

**Dimer:**

Not Found

**Hairpin:**

Not Found

### Secondary Structures for Antisense Primer

**Dimer:**

| Primer | Secondary Structure | Stability |
|--------|---------------------|-----------|
| 5' ACCTCCCTGGATATCCCTGG 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | |
| 5' ACCTCCCTGGATATCCCTGG 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | |

**Hairpin:**

| Primer | Secondary Structure | Stability |
|--------|---------------------|-----------|
| 5' GCTTGGCTGATATCCCTGG 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | |

**Cross Dimer:**

Cross Dimer between Sense Primer and Antisense Primer:

| Primer | Secondary Structure | Stability |
|--------|---------------------|-----------|
| 5' AGGAGTTACCTACCTAGGCC 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | -3.0 |
| 5' AGGAGTTACCTACCTAGGCC 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | -2.2 |
| 5' AGGAGTTACCTACCTAGGCC 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | -0.7 |
| 5' AGGAGTTACCTACCTAGGCC 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | -5.5 |

| Primer | Secondary Structure | Stability |
|--------|---------------------|-----------|
| 5' AGGAGTTACCTACCTAGGCC 3' | | |
| 3' GCTTGGCTGATATCCCTGG 5' | | -0.3 |
**POLR2A PrimerBLAST** (National Center for Biotechnology Information, Bethesda MD, USA, [https://www.ncbi.nlm.nih.gov/tools/primer-blast](https://www.ncbi.nlm.nih.gov/tools/primer-blast))

| Primer pair 1 | Sequence (5'->3') | Template strand | Length | Start | Stop | Tm  | GC% | Self complementarity | Self 3' complementarity |
|---------------|------------------|-----------------|--------|-------|------|-----|-----|----------------------|------------------------|
| Forward primer | TGGCTTACTGTCTTCCGTGTTG | Plus | 22    | 3798  | 3819 | 62.77 | 50.00 | 3.00 | 0.00                 |
| Reverse primer | TGTGTGGGCACTCACAATTC | Minus | 20    | 3065  | 3086 | 62.95 | 55.00 | 3.00 | 3.00                 |

**Product length**: 108

**Total intron size**: 668 (between pos. 7019488 and 7019557 on [NT_010718.17](https://www.ncbi.nlm.nih.gov/nuccore/NT_010718.17))

**Products on intended target**

>NM_000537.4 Homo sapiens polymerase (RNA) II (DNA directed) polypeptide A, 220kDa (POLR2A), mRNA

**product length** = 108

| Forward primer | TGGCTTACTGTCTTCCGTGTTG | 22 |
| Template       | 3798  .......................... | 3819 |
| Reverse primer | TGTGTGGGCACTCACAATTC | 20 |
| Template       | 3905  .......................... | 3886 |

**POLR2A PrimerCheck** (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, [http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp](http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp))
**POLR2A UCSC In-silico-PCR Genome Browser** (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, [http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr](http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr))

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

```
>yeO32esq.1    POLR2A:379643008  108bp TCAGCTACTGTCTCTCTCTGCCC  TGGTGTGGAGAGCACACCTCC
TCAGCTACTGTCTCTCTCTGCCC  TGGTGTGGAGAGCACACCTCC
5'caagatatctgtgctctggtgcatataaagttggtggtaggagtGACTGCTTCATAGTGGCAACACTGCGCCAGCTAGGCTGGT
3'                                           CCAACACA
```

**POLR2A UNAFold** (Integrated DNA Technologies Inc., Coralville, IA, USA, [http://eu.idtdna.com/UNAFold](http://eu.idtdna.com/UNAFold), Suboptimality 50%)

**POLR2A Amplicon Sequence**

5' TCGCTTACTGTCTTCATTGGGCCAGTCCGCTCGAGATGCTGAGAGAGCCAAGGATATTCTGTGCCGCTTGAGCATACAAACGTGAGGAAGTTGAGTTGACTGCTTCATAGTGGCAACACTGCGCCAGCTAGGCTGGT
3'

### Structures

| Structure Name | Image | ΔG (kcal.mole⁻¹) | Tm (°C) | ΔH (kcal.mole⁻¹) | ΔS (cal.K⁻¹.mole⁻¹) | Output |
|----------------|-------|-----------------|---------|-----------------|---------------------|--------|
| 1              | ![Structure Image] | 0.11            | 58.7    | -28             | -84.38              | Ct Det       |
| 2              | ![Structure Image] | 0.63            | 48.7    | -18.1           | -56.23              | Ct Det       |
| 3              | ![Structure Image] | 0.64            | 50.7    | -22.2           | -68.55              | Ct Det       |
| 4              | ![Structure Image] | 0.83            | 49.9    | -26.6           | -82.35              | Ct Det       |
**POLR2A BeaconDesigner™ Free Edition** (Premier BioSoft International, Palo Alto, CA, USA, [http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1](http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1))

**Secondary Structures for Sense Primer**

**Dimer:**

Not Found

**Hairpin:**

Not Found

**Secondary Structures for Anti-sense Primer**

**Dimer:**

5' TGTGGCTGCACTACCGCTGCTGCTTG 3'  
||| ||| |||  
3' CCTCCCAGCTGACCGTGTGTG 5'  
-1.3

**Cross Dimer**

5' TCGCTACTGCTCTCTCTTGG 3'  
||| ||| |||  
3' CCTCCCAGCTGACCGTGTGTG 5'  
-2.5

**Cross Dimer between Sense Primer and Anti-sense Primer:**

5' TCGCTACTGCTCTCTCTTGG 3'  
||| |||  
3' CCTCCCAGCTGACCGTGTGTG 5'  
-1.1

5' TGTGGCTGCACTACCGCTGCTGCTTG 3'  
||| ||| |||  
3' CCTCCCAGCTGACCGTGTGTG 5'  
-1.3
TBP PrimerBLAST (National Center for Biotechnology Information, Bethesda MD, USA, https://www.ncbi.nlm.nih.gov/tools/primer-blast)

| Primer pair 1 | Sequence (5’->3’) | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3’ complementarity |
|---------------|------------------|-----------------|--------|-------|------|----|-----|----------------------|------------------------|
| Forward primer | CGGCTGTAAACTGCGTTCC | Plus | 21 | 79 | 99 | 62.54 | 52.38 | 5.00 | 0.00 |
| Reverse primer | TGGTTTATCTTCACACGCAAG | Minus | 22 | 164 | 143 | 63.37 | 50.00 | 3.00 | 2.00 |
| Product length | 86 | | | | | | |
| Total intron size | 2418 (between pos. 110324529 and 110326948 on NT_025741.16) | | | | | | |

Products on intended target
>NM_003194.4 Homo sapiens TATA box binding protein (TBP), transcript variant 1, mRNA
product length = 86
Forward primer 1 GGGCTGTAAACTGCGTTCC 21
Template 79 ....................... 99
Reverse primer 1 TGGTTTATCTTCACACGCAAG 22
Template 164 ....................... 143

TBP UCSC In-silico-PCR Genome Browser (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr )

UCSC In-Silico PCR

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

>uc003qwu.3_TBP:79+164 86bp CGGCTGTAAACTGCGTTCC TGGTTTATCTTCACACGCAAG
CGGCTGTAAACTGCGTTCCGcggcccatagcttttgcagtgacc
cacgcatcactgtttCTTGCCGTGTGAAGATAACCCA

>uc003qxt.3_TBP:79+167 89bp CGGCTGTAAACTGCGTTCC TGGTTTATCTTCACACGCAAG
CGGCTGTAAACTGCGTTCCGtggcccatagcttttgcagtgacc
cacgcatcactgtttCTTGCCGTGTGAAGATAACCCA
TBP PrimerCheck (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp)
**TBP UNAFold** (Integrated DNA Technologies Inc., Coralville, IA, USA, [http://eu.idtdna.com/UNAFold](http://eu.idtdna.com/UNAFold), Suboptimality 50%)

**TBP Amplicon Sequence**

5’ CGGCTGTTTAACCTTGCTTCCGGCCATAGTGATCTTTGCGATGACCAGCATCAGTGTGTCTTTGGCCTGGTGAAGATAACCCA 3’

### Structures

| Structure Name | Image | ΔG (kcal.mole⁻¹) | Tm (°C) | ΔH (kcal.mole⁻¹) | ΔS (cal.K⁻¹.mole⁻¹) | Output |
|---------------|-------|------------------|---------|------------------|---------------------|--------|
| 1             | ![Image](image1.png) | 0.26             | 58.1    | -46.1            | -139.15             | Ct Det |
| 2             | ![Image](image2.png) | 0.69             | 53.2    | -42.5            | -130.23             | Ct Det |
| 3             | ![Image](image3.png) | 1.15             | 44.4    | -23.5            | -74                 | Ct Det |

![Graphs](image4.png)
**TBP BeaconDesigner™ Free Edition** (Premier BioSoft International, Palo Alto, CA, USA, [http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1](http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1))

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**Secondary Structures for Sense Primer**

**Dimer:**

3' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

5' CGCGCTTTTAACCTCGCTCC 3'

5' CGACCGCAACTCTTATTTGGGT 5'

**Hairpin:**

Not Found

---

**Secondary Structures for Anti-sense Primer**

**Dimer:**

5' CGCGCTTTTAACCTCGCTCC 3'

5' CGACCGCAACTCTTATTTGGGT 5'

3' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

**Hairpin:**

/TATTTTATTTT/ 5'

/TATTTTATTTT/ 3'

/AATTATTATTATT/ 5'

/AATTATTATTATT/ 3'

---

**Cross Dimer**

Cross Dimer between Sense Primer and Anti-sense Primer:

5' CGCGCTTTTAACCTCGCTCC 3'

5' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

3' CGACCGCAACTCTTATTTGGGT 5'

---

**Secondary Structures for Cross Dimer**

5' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

5' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

5' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

5' CGCGCTTTTAACCTCGCTCC 3'

3' CGACCGCAACTCTTATTTGGGT 5'

---

**Hairpin:**

Not Found

---

**Secondary Structures for Hairpin**

/TATTTTATTTT/ 5'

/TATTTTATTTT/ 3'

/AATTATTATTATT/ 5'

/AATTATTATTATT/ 3'

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RPL22 PrimerBLAST (National Center for Biotechnology Information, Bethesda MD, USA, [https://www.ncbi.nlm.nih.gov/tools/primer-blast](https://www.ncbi.nlm.nih.gov/tools/primer-blast))

| Primer pair 1 | Sequence (5'→3') | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3' complementarity |
|---------------|------------------|----------------|--------|-------|------|----|-----|-----------------------|------------------------|
| Forward primer | TGATGCCACCCACCTGTAG | Plus | 20 | 115 | 134 | 62.18 | 55.00 | 4.00 | 2.00 |
| Reverse primer | GGTCCCCAGCTTTCCGTTT | Minus | 20 | 212 | 193 | 61.84 | 55.00 | 4.00 | 0.00 |
| Product length | 98 |
| Total intron size | 4597 (between pos. 5611664 and 5607066 on NT_032977.10) |

Products on intended target

>NM_000983.3 Homo sapiens ribosomal protein L22 (RPL22), mRNA

| Product length | 98 |
| Forward primer | TGATGCCACCCACCTGTAG | 20 |
| Template | 115 | 134 |
| Reverse primer | GGTCCCCAGCTTTCCGTTT | 20 |
| Template | 212 | 193 |

RPL22 UCSC In-silico-PCR Genome Browser (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, [http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr](http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr)).

UCSC In-Silico PCR

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

> prod1b4_a3_chr22:115:212 99bp TGGATGCCAAGCTTGTAG GGTCCCCAGCTTTCCGTTT
TGGATGCCAAGCTTGTAG actgacttatgtagactgactattttttt
acgtttttttacagtgaaggttcctcaaggtgacactgacccgaacaagtgggagacc
> prod1b4_a3_chr22:115:212 99bp TGGATGCCAAGCTTGTAG GGTCCCCAGCTTTCCGTTT
TGGATGCCAAGCTTGTAG actgacttatgtagactgactattttttt
acgtttttttacagtgaaggttcctcaaggtgacactgacccgaacaagtgggagacc
**RPL22 PrimerCheck** (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, [http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp](http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp))

**Exons**

| Exon | Primer 1 | Primer 2 |
|------|----------|----------|
| BC035566 |          |          |
| BC058687 |          |          |
| CR458573 |          |          |
| D17652   |          |          |
| NM_000963 | Primer 1 | Primer 2 |

**RPL22 UNAFold** (Integrated DNA Technologies Inc., Coralville, IA, USA, [http://eu.idtdna.com/UNAFold?](http://eu.idtdna.com/UNAFold?), Suboptimality 50%)

**RPL22 Amplicon Sequence**

5' TGATTGCACCACCCTGTAGAAGATGGATGTGCTGCAATTTTGTAGCAGTTTTTGCAAGAAAGGATCAAAGTGAACGGAAAAGCTGGGAACC 3'

**Structures**

| Structure Name | Image | $\Delta G$ (kcal mol$^{-1}$) | $T_M$ (°C) | $\Delta H$ (kcal mol$^{-1}$) | $\Delta S$ (cal K$^{-1}$ mol$^{-1}$) | Output |
|---------------|-------|-----------------|---------|----------------------------|---------------------------------|--------|
| 1             | ![Image](image1.png) | 0.34            | 56.4    | -30.8                      | -90.47                          | Ct Det |
| 2             | ![Image](image2.png) | 0.34            | 52.5    | -14.9                      | -45.75                          | Ct Det |
| 3             | ![Image](image3.png) | 0.68            | 52.9    | -31                        | -95.09                          | Ct Det |
| 4             | ![Image](image4.png) | 1.17            | 44.3    | -23.8                      | -74.97                          | Ct Det |
Secondary Structures for Sense Primer

Dimer: -

5' TGAATTGCACCCACCGCTTAG 3'
   || ||| |||
3' GATGCTCCCACCCACCGTTAG 5'

Hairpin: -
Not Found

Secondary Structures for Anti-sense Primer

Dimer: -

5' CTTGCCCTTCGGACCCCTGG 3'
   ||||
3' CTGGCCCTTTCGGACCCCTGG 5'

Cross Dimer

Cross Dimer between Sense Primer and Anti-sense Primer: -

5' TGAATTGCACCCACCGCTTAG 3'
   || ||| |||
3' CTGGCCCTTTCGGACCCCTGG 5'

5' TGAATTGCACCCACCGCTTAG 3'
   ||||
3' CTGGCCCTTTCGGACCCCTGG 5'

Hairpin: -
Not Found

---

RPL22 BeaconDesigner™ Free Edition (Premier BioSoft International, Palo Alto, CA, USA, http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1)
**EEF1A1 PrimerBLAST** (National Center for Biotechnology Information, Bethesda MD, USA, [https://www.ncbi.nlm.nih.gov/tools/primer-blast](https://www.ncbi.nlm.nih.gov/tools/primer-blast))

| Primer pair 1 | Sequence (5'-3') | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3' complementarity |
|---------------|------------------|-----------------|--------|-------|------|----|-----|----------------------|------------------------|
| **Forward**   | CTGGGTCTCAGGATGTTCTAC | Plus            | 22     | 604   | 825  | 64.13 | 59.09 | 5.00                | 2.00                   |
| **Reverse**   | GGAGAACGGATGCTCAGCATC | Minus           | 22     | 908   | 887  | 63.70 | 54.55 | 6.00                | 2.00                   |
| **Product length** | 105               |                 |        |       |      |      |       |                      |                        |
| **Total intron size** | 87 (between pos. 12388604 and 12388670 on NT_025741.16) |                 |        |       |      |      |       |                      |                        |

**Products on intended target**

*NM_001492.5 Homo sapiens eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), mRNA*

**product length = 105**

**Forward primer** 1: CTGGGTCTCAGGATGTTCTAC 22

**Template**

| Product | Sequence (5'-3') | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3' complementarity |
|---------|------------------|-----------------|--------|-------|------|----|-----|----------------------|------------------------|
| 1       | GGAGAACGGATGCTCAGCATC | Minus           | 22     | 908   | 887  | 63.70 | 54.55 | 6.00                | 2.00                   |

**Products on allowed transcript variants**

*XM_011325514.1 PREDICTED: Homo sapiens eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), transcript variant X1, mRNA*

**product length = 105**

**Forward primer** 1: CTGGGTCTCAGGATGTTCTAC 22

**Template**

| Product | Sequence (5'-3') | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3' complementarity |
|---------|------------------|-----------------|--------|-------|------|----|-----|----------------------|------------------------|
| 1       | GGAGAACGGATGCTCAGCATC | Minus           | 22     | 908   | 887  | 63.70 | 54.55 | 6.00                | 2.00                   |

**UCSF In-silico-PCR Genome Browser** (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, [http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr](http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr))

**UCSF In-Silico PCR**

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

[Sequence links]

---

**46**
EEF1A1 PrimerCheck (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, [http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp](http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp))
**EEF1A1 UNAFold** (Integrated DNA Technologies Inc., Coralville, IA, USA, [http://eu.idtdna.com/UNAFold](http://eu.idtdna.com/UNAFold), Suboptimality 50%)

**EEF1A1 Amplicon Sequence**

5' CCTGCCTCTCCAGGATGTCTACAAAAATTTGGTGATTTGTACTGTCTCTGTTGGCCGAGTGAGACTGTGTTCCTCAAACCCGTTATGTTGTCACCTTTGCTCC 3'

### Structures

| Structure Name | Image | \(\Delta G\) (kcal mole\(^{-1}\)) | \(T_m\) (°C) | \(\Delta H\) (kcal mole\(^{-1}\)) | \(\Delta S\) (cal. K\(^{-1}\) mole\(^{-1}\)) | Output |
|----------------|-------|----------------------------------|--------------|---------------------------------|---------------------------------|--------|
| 1              | ![Image](image1.png) | 0.5                             | 54.3         | -28.9                           | -88.25                          | Ct Det |
| 2              | ![Image](image2.png) | 0.59                            | 49.7         | -18.5                           | -57.3                           | Ct Det |
| 3              | ![Image](image3.png) | 0.63                            | 48.7         | -18.1                           | -56.23                          | Ct Det |
| 4              | ![Image](image4.png) | 0.84                            | 45.1         | -18                             | -56.56                          | Ct Det |
| 5              | ![Image](image5.png) | 0.67                            | 51.3         | -32.5                           | -100.19                         | Ct Det |
| 6              | ![Image](image6.png) | 1.12                            | 40.6         | -18.2                           | -58.01                          | Ct Det |
| 7              | ![Image](image7.png) | 1.33                            | 43.5         | -25.4                           | -80.22                          | Ct Det |
| 8              | ![Image](image8.png) | 1.39                            | 18.4         | -9.7                            | -33.29                          | Ct Det |
| 9              | ![Image](image9.png) | 1.44                            | 15.8         | -9.4                            | -32.53                          | Ct Det |
Cross Dimer between Sense Primer and Anti-sense Primer:

5' CCTGCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-2.9

5' CCTGCTCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-2.0

5' CCTGCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-1.3

5' CCTGCTCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-1.3

5' CCTGCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-1.3

5' CCTGCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-1.1

5' CCTGCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-1.0

5' CCTGCTCTCTCCAGGATGCTCTAC 3'
    ||||| 3' CATACCACASTGGGAAACGAGG 5'
-0.5
RPLP0 PrimerBLAST (National Center for Biotechnology Information, Bethesda MD, USA, https://www.ncbi.nlm.nih.gov/tools/primer-blast)

| Primer pair 1 | Sequence (5’→3’) | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3’ complementarity |
|--------------|------------------|----------------|--------|-------|------|----|-----|---------------------|------------------------|
| Forward primer | GAAACTCCTGATCTGCTGCC | Plus | 22 | 802 | 823 | 62 | 34 | 50.00 | 4.00 | 0.00 |
| Reverse primer | GACCTGTTGATCCCCGTGAAG | Minus | 22 | 921 | 900 | 62 | 01 | 50.00 | 4.00 | 0.00 |
| Product length | 120 | | | | | | | | |
| Total intron size | 1091 (between pos. 82903362 and 82902921 on NT_029416.13) | | | | | | | | |

Products on intended target

>NM_001602.3 Homo sapiens ribosomal protein, large P0 (RPLP0), transcript variant 1, mRNA
product length = 120
Forward primer 1 | GAAACTCCTGATCTGCTGCC | 22
Template | 802 | 823
Reverse primer 1 | GACCTGTTGATCCCCGTGAAG | 22
Template | 921 | 900

Products on allowed transcript variants

>NM_003757.3 Homo sapiens ribosomal protein, large P0 (RPLP0), transcript variant 2, mRNA
product length = 120
Forward primer 1 | GAAACTCCTGATCTGCTGCC | 22
Template | 862 | 883
Reverse primer 1 | GACCTGTTGATCCCCGTGAAG | 22
Template | 981 | 960

RPLP0 UCSC In-silico-PCR Genome Browser (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, http://genome-mirror.genomediak.au.dk/cgi-bin/hgPcr)

UCSC In-Silico PCR

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

http://genome-mirror.genomediak.au.dk/cgi-bin/hgPcr

52
RPLP0 PrimerCheck (SpliceCenter der Genomics and Bioinformatics Group, LMP, CCR, NCI, http://projects.insilico.us/SpliceCenter/PrimerCheck.jsp )

RPLP0
### RPLP0 UNAFold

(Integrated DNA Technologies Inc., Coralville, IA, USA, [http://eu.idtdna.com/UNAFold](http://eu.idtdna.com/UNAFold), Suboptimality 50%)

### RPLP0 Amplicon Sequence

5' GAAACTCTGATTCTCCTGCTGAGGGGTGTCGCCAGTGTCTGCAGATTGGGTACCCACTGTTGCATCGATCCCCATTCTATCATCAACGGGTACAAACGAGTC 3'

### Structures

| Structure Name | Image | ΔG (kcal.mole⁻¹) | T_M (°C) | ΔH (kcal.mole⁻¹) | ΔS (cal.K⁻¹.mole⁻¹) | Output |
|----------------|-------|-----------------|----------|-----------------|---------------------|--------|
| 1              | ![Image](image1.png) | 0.15            | 59       | -51             | -153.54             | Ct Det |
| 2              | ![Image](image2.png) | 0.67            | 52.1     | -27.6           | -84.86              | Ct Det |
| 3              | ![Image](image3.png) | 0.69            | 52.3     | -29.1           | -99.41              | Ct Det |
| 4              | ![Image](image4.png) | 0.76            | 50.8     | -26.9           | -83.03              | Ct Det |
| 5              | ![Image](image5.png) | 0.85            | 49.4     | -25.9           | -80.29              | Ct Det |
| 6              | ![Image](image6.png) | 0.95            | 55.1     | -63.5           | -193.46             | Ct Det |
| 7              | ![Image](image7.png) | 1.12            | 42.1     | -19.7           | -62.49              | Ct Det |
**Secondary Structures for Sense Primer**

Dimer:

```
5' GAAACTCTGTACATCTGCTCC 3' 
| | | |  |
3' GTAGTGGCCATGTGCTGTCG 5' 
```

Hairpin:

```
/CTCTCAAGS 5' 
|  
\TCTCTAGG 3' 
```

**Cross Dimer**

**Cross Dimer between Sense Primer and Anti-sense Primer:**

```
5' GAAACTCTGTACATCTGCTCC 3' 
| | | |  |
3' GTAGTGGCCATGTGCTGTCG 5' 
```

**Secondary Structures for Anti-sense Primer**

Dimer:

```
5' GTAGTGGCCATGTGCTGTCG 3' 
| | | |  |
3' GAAACTCTGTACATCTGCTCC 5' 
```

Hairpin:

```
Not Found 
```
**RNA18S5 PrimerBLAST** (National Center for Biotechnology Information, Bethesda MD, USA, [https://www.ncbi.nlm.nih.gov/tools/primer-blast](https://www.ncbi.nlm.nih.gov/tools/primer-blast))

| Primer pair 1 | Sequence (5’→3’) | Template strand | Length | Start | Stop | Tm | GC% | Self complementarity | Self 3’ complementarity |
|---------------|------------------|-----------------|--------|-------|------|----|-----|----------------------|------------------------|
| Forward primer | AACTGGGAATGGCTCATAAATC | Plus | 23 | 84 | 106 | 60.55 | 39.13 | 6.00 | 3.00 |
| Reverse primer | GCCCGTCGCCATGTATTAG | Minus | 10 | 186 | 186 | 60.86 | 57.89 | 5.00 | 1.00 |
| Product length | 103 | |

**RNA18S5 UCSC In-silico-PCR Genome Browser** (Dec. 2013 GRCh38/hg38; UCSC Genes; Max. Product Size: 4000; Min. Perfect Match: 15; Min. Good Match: 15; Jim Kent, [http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr](http://genome-mirror.genomedk.au.dk/cgi-bin/hgPcr)).
RNA18S5-UNAFold (Integrated DNA Technologies Inc., Coralville, IA, USA, http://eu.idtdna.com/UNAFold?, Suboptimality 50%)

RNA18S5 Amplicon Sequence

5' AACTGCGAATGGCTCATTAATCAGTTATGGTTTCTTTGCTGCTGCTCCTCTCCTACTTTGATATCGTGAATTCTAGAGCTAATACATGCACAGGACGGCGG 3'

| Structures |  |  |  |  | Output |
|---|---|---|---|---|---|
| Structure Name | Image | ΔG (kcal.mole⁻¹) | Tm (°C) | ΔH (kcal.mole⁻¹) | ΔS (cal.K⁻¹.mole⁻¹) |
| 1 |  | 0.06 | 59.9 | -25.6 | -77.09 | Ct | Det |
| 2 |  | 0.77 | 47.2 | -19.4 | -60.55 | Ct | Det |
| 3 |  | 0.99 | 46.1 | -22.8 | -71.42 | Ct | Det |
RNA18S5 BeaconDesigner™ Free Edition (Premier BioSoft International, Palo Alto, USA, http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1)

Cross Dimer

Cross Dimer between Sense Primer and Anti-sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 1.7   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | -2.4 |

Cross Dimer between Anti-sense Primer and Anti-sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 1.7   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | -2.4 |

Cross Dimer between Sense Primer and Sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 2.4   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | -0.5 |

Cross Dimer between Anti-sense Primer and Sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 2.4   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | -2.4 |

Cross Dimer between Anti-sense Primer and Sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 0.0   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | 0.0 |

Cross Dimer between Anti-sense Primer and Anti-sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 0.0   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | 0.0 |

Cross Dimer between Sense Primer and Sense Primer:

| Dimer | Sequence 1 | Sequence 2 | Energy |
|-------|------------|------------|--------|
| 0.3   | 5' AACTGGGCTTCATTAAAATC 3'  |
|       | 3' GATTATGACGCGCTGCCCG 5'   | 0.3 |
Supplementary Data 2. RNA integrity analysis. Experimental groups: K7-K12 = control; D7-D12 = compressive orthodontic force; Agac7-Agac12 = bacterial lysate (periodontitis).
### Electrophoresis File Run Summary (Chip Summary)

| Sample Name | Sample Comment | Status | Result Label | Result Color |
|-------------|----------------|--------|--------------|--------------|
| 58 Z2 K     |                | ✔️     | RIN:10       |              |
| 58 Z2 D     |                | ✔️     | RIN:10       |              |
| 60 Z2 K     |                | ✔️     | RIN: 9.70    |              |
| 60 Z2 D     |                | ✔️     | RIN: 9.60    |              |
| 62 Z2 K     |                | ✔️     | RIN:10       |              |
| 62 Z2 D     |                | ✔️     | RIN: 9.80    |              |
| K7          |                | ✔️     | RIN:10       |              |
| K8          |                | ✔️     | RIN: 9.90    |              |
| K9          |                | ✔️     | RIN:10       |              |
| K10         |                | ✔️     | RIN:10       |              |
| K11         |                | ✔️     | RIN: 9.90    |              |
| K12         |                | ✔️     | RIN: 9.90    |              |
| Ladder      |                | ✔️     | All Other Samples |              |

### Chip Comments:

- 

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**Assay Class:** Eukaryote Total RNA Nano  
**Data Path:** E:\...Eukaryote Total RNA Nano_DE72901710_2017-03-08_10-01-40.xad  
**Created:** 08.03.2017 10:01:40  
**Modified:** 08.03.2017 10:25:31

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Printed: 08.03.2017 10:13:43
Electrophoresis Assay Details

General Analysis Settings
Number of Available Sample and Ladder Wells (Max.) : 13
Minimum Visible Range [s] : 17
Maximum Visible Range [s] : 70
Start Analysis Time Range [s] : 19
End Analysis Time Range [s] : 69
Ladder Concentration (ng/µl) : 150
Lower Marker Concentration (ng/µl) : 0
Upper Marker Concentration (ng/µl) : 0
Used Lower Marker for Quantitation
Standard Curve Fit is Logarithmic
Show Data Aligned to Lower Marker

Integrator Settings
Integration Start Time [s] : 19
Integration End Time [s] : 69
Slope Threshold : 0.6
Height Threshold [FU] : 0.5
Area Threshold : 0.2
Width Threshold [s] : 0.5
Baseline Plateau [s] : 6

Filter Settings
Filter Width [s] : 0.5
Polynomial Order : 4

Ladder

| Ladder Peak | Size |
|-------------|------|
| 1           | 25   |
| 2           | 200  |
| 3           | 500  |
| 4           | 1000 |
| 5           | 2000 |
| 6           | 4000 |
Electropherogram Summary Continued ...

Overall Results for sample 7: **K7**

- RNA Area: 1.347.6
- RNA Integrity Number (RIN): 10 (B.02.08)
- RNA Concentration: 471 ng/μl
- Result Flagging Color: **RIN:10**
- Result Flagging Label: **RIN:10**
- rRNA Ratio (28s / 18s): 1.8

Fragment table for sample 7: **K7**

| Name | Start Time [s] | End Time [s] | Area   | % of total Area |
|------|----------------|--------------|--------|-----------------|
| 18S  | 41.44          | 42.97        | 298.3  | 22.1            |
| 28S  | 47.40          | 50.59        | 547.4  | 40.6            |
### Overall Results for sample 8: K8

- **RNA Area:** 1,892,6
- **RNA Integrity Number (RIN):** 9.9 (B.02.08)
- **RNA Concentration:** 661 ng/µl
- **rRNA Ratio [28s / 18s]:** 1.8
- **Result Flagging Color:**
- **Result Flagging Label:** RIN: 9.90

### Fragment table for sample 8: K8

| Name | Start Time [s] | End Time [s] | Area   | % of total Area |
|------|----------------|--------------|--------|-----------------|
| 18S  | 41,39          | 42,92        | 424,1  | 22.4            |
| 28S  | 47,31          | 50,51        | 757,6  | 40.0            |
Overall Results for sample 9: **K9**

- RNA Area: 1.413.9
- RNA Integrity Number (RIN): 10 (B.02.08)
- RNA Concentration: 494 ng/μl
- rRNA Ratio [28s / 18s]: 1.8

Result Flagging Color: [Color representation]
Result Flagging Label: RIN:10

Fragment table for sample 9: **K9**

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 41,34          | 42,87        | 312,2| 22,1            |
| 28S  | 47,23          | 50,47        | 568,9| 40,2            |
**Electropherogram Summary Continued ...**

**Overall Results for sample 10 : K10**

- RNA Area: 1,661,9
- RNA Concentration: 581 ng/ul
- rRNA Ratio [28s / 18s]: 1,8
- RNA Integrity Number (RIN): 10 (B.02.08)
- Result Flagging Color: [Gray]
- Result Flagging Label: RIN:10

**Fragment table for sample 10 : K10**

| Name | Start Time [s] | End Time [s] | Area  | % of total Area |
|------|----------------|--------------|-------|-----------------|
| 18S  | 41,27          | 42,76        | 358,9 | 21,6            |
| 28S  | 47,14          | 50,36        | 652,4 | 39,3            |
Overall Results for sample 11: **K11**

| Parameter                      | Value    |
|-------------------------------|----------|
| RNA Area                      | 1.426.2  |
| RNA Integrity Number (RIN)    | 9.9 (B.02.08) |
| RNA Concentration             | 499 ng/μl|
| rRNA Ratio [28s / 18s]        | 1.8      |

Result Flagging Label: RIN: 9.90

Fragment table for sample 11: **K11**

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 41,26          | 42,75        | 306,4| 21,5            |
| 28S  | 47,15          | 50,19        | 555,1| 38,9            |
Overall Results for sample 12 : **K12**

| RNA Area:     | 1,415,8          | RNA Integrity Number (RIN): | 9.9 (B.02.08) |
|--------------|------------------|----------------------------|----------------|
| RNA Concentration: | 495 ng/μl       | Result Flagging Color:     |                |
| rRNA Ratio [28s / 18s]: | 1,7             | Result Flagging Label:    | RIN: 9.90      |

Fragment table for sample 12 : **K12**

| Name | Start Time [s] | End Time [s] | Area  | % of total Area |
|------|---------------|--------------|-------|-----------------|
| 18S  | 40,54         | 42,93        | 318,9 | 22,5            |
| 28S  | 47,21         | 50,50        | 528,1 | 37,3            |
### Electrophoresis File Run Summary (Chip Summary)

| Sample Name | Sample Comment | Status | Result Label | Result Color |
|-------------|----------------|--------|--------------|--------------|
| D7          |                | ✔      | RIN: 9.50    |              |
| D8          |                | ✔      | RIN: 9.80    |              |
| D9          |                | ✔      | RIN:10       |              |
| D10         |                | ✔      | RIN: 9.80    |              |
| D11         |                | ✔      | RIN: 9.80    |              |
| D12         |                | ✔      | RIN: 9.80    |              |
| Agac7       |                | ✔      | RIN: 9.90    |              |
| Agac8       |                | ✔      | RIN:10       |              |
| Agac9       |                | ✔      | RIN: 9.80    |              |
| Agac10      |                | ✔      | RIN: 9.80    |              |
| Agac11      |                | ✔      | RIN: 9.50    |              |
| Agac12      |                | ✔      | RIN: 9.90    |              |
| Ladder      |                | ✔      | All Other Samples |          |

**Chip Lot #**

**Reagent Kit Lot #**

**Chip Comments:**
Assay Class: Eukaryote Total RNA Nano
Data Path: E:\..\Eukaryote Total RNA Nano_DE72901710_2017-03-08_10-30-01.xad

Electrophoresis Assay Details

General Analysis Settings
Number of Available Sample and Ladder Wells (Max.): 13
Minimum Visible Range [s]: 17
Maximum Visible Range [s]: 70
Start Analysis Time Range [s]: 19
End Analysis Time Range [s]: 69
Ladder Concentration [ng/μl]: 150
Lower Marker Concentration [ng/μl]: 0
Upper Marker Concentration [ng/μl]: 0
Used Lower Marker for Quantitation
Standard Curve Fit is Logarithmic
Show Data Aligned to Lower Marker

Integrator Settings
Integration Start Time [s]: 19
Integration End Time [s]: 69
Slope Threshold: 0.6
Height Threshold [FU]: 0.5
Area Threshold: 0.2
Width Threshold [s]: 0.5
Baseline Plateau [s]: 6

Filter Settings
Filter Width [s]: 0.5
Polynomial Order: 4

Ladder

| Ladder Peak | Size |
|-------------|------|
| 1           | 25   |
| 2           | 200  |
| 3           | 500  |
| 4           | 1000 |
| 5           | 2000 |
| 6           | 4000 |
Electropherogram Summary

Overall Results for sample 1:

RNA Area: 479.7
RNA Concentration: 201 ng/μl
rRNA Ratio [28s / 18s]: 1.6

RNA Integrity Number (RIN): 9.5 (B.02.08)
Result Flagging Color:
Result Flagging Label: RIN: 9.50

Fragment table for sample 1:

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 40,71          | 42,91        | 97,1 | 20,2            |
| 28S  | 47,39          | 50,41        | 156,1| 32,5            |
Overall Results for sample 2:

RNA Area: 718.9
RNA Concentration: 302 ng/μl
rRNA Ratio [28s / 18s]: 1.7

RNA Integrity Number (RIN): 9.8 (B.02.08)
Result Flagging Color: 
Result Flagging Label: RIN: 9.80

Fragment table for sample 2:

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 41.32          | 42.92        | 153.6| 21.4            |
| 28S  | 47.13          | 50.61        | 267.8| 37.2            |
Electropherogram Summary Continued...

Overall Results for sample 3: **D9**

| Parameter                      | Value   |
|--------------------------------|---------|
| RNA Area                       | 520.9   |
| RNA Integrity Number (RIN)     | 10      |
| RNA Concentration              | 219 ng/µl|
| rRNA Ratio 28s / 18s           | 1.6     |

Fragment table for sample 3: **D9**

| Name | Start Time [s] | End Time [s] | Area  | % of total Area |
|------|----------------|--------------|-------|-----------------|
| 18S  | 40.72          | 42.93        | 120.5 | 23.1            |
| 28S  | 47.60          | 50.58        | 196.5 | 37.7            |
Electropherogram Summary Continued ...

Overall Results for sample 4 : **D10**

| Parameter                      | Value   |
|--------------------------------|---------|
| RNA Area                       | 534.3   |
| RNA Integrity Number (RIN)     | 9.8 (B.02.08) |
| RNA Concentration              | 224 ng/μl |
| rRNA Ratio [28s / 18s]         | 1.6     |

Result Flagging Color: [Blank]
Result Flagging Label: RIN: 9.80

Fragment table for sample 4 : **D10**

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 40.84          | 43.04        | 122.9| 23.0            |
| 28S  | 47.35          | 50.60        | 197.5| 37.0            |
Electropherogram Summary Continued ...

Overall Results for sample 5 :

- D11

RNA Area: 687.2
RNA Concentration: 289 ng/µl
rRNA Ratio [28s / 18s]: 1.6

RNA Integrity Number (RIN): 9.8
Result Flagging Color: [Red]
Result Flagging Label: RIN 9.80

Fragment table for sample 5 :

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 40,69          | 43,09        | 156,2| 22,7            |
| 28S  | 47,39          | 50,65        | 247,1| 36,0            |
Overall Results for sample 6 :  

**D12**

- RNA Area: 942.6
- RNA Concentration: 396 ng/µl
- RNA Integrity Number (RIN): 9.8 (B.02.08)
- Result Flagging Color:
- Result Flagging Label: RIN: 9.80

### Fragment table for sample 6 :  

**D12**

| Name | Start Time [s] | End Time [s] | Area  | % of total Area |
|------|----------------|--------------|-------|-----------------|
| 18S  | 41.31          | 42.89        | 207.1 | 22.0            |
| 28S  | 47.15          | 50.55        | 357.4 | 37.9            |
**Electropherogram Summary Continued ...**

**Overall Results for sample 7:** *Agac7*
- RNA Area: 1,665,2
- RNA Integrity Number (RIN): 9.9 (B.02.08)
- RNA Concentration: 657 ng/µl
- rRNA Ratio [28s / 18s]: 1.7
- Result Flagging Color: [Color Swatch]
- Result Flagging Label: RIN: 9.90

**Fragment table for sample 7:** *Agac7*

| Name | Start Time [s] | End Time [s] | Area   | % of total Area |
|------|----------------|--------------|--------|-----------------|
| 18S  | 41.26          | 42.79        | 341.8  | 21.8            |
| 28S  | 47.01          | 50.37        | 595.8  | 38.1            |
Electropherogram Summary Continued...

Overall Results for sample 8:  **Agac8**

| Parameter                | Value       |
|--------------------------|-------------|
| RNA Area:                | 713.4       |
| RNA Integrity Number (RIN): | 10 (B.02.08) |
| RNA Concentration:       | 300 ng/μl   |
| rRNA Ratio [28s / 18s]:  | 1.6         |
| Result Flagging Color:   | RIN:10      |
| Result Flagging Label:   |             |

Fragment table for sample 8:  **Agac8**

| Name  | Start Time [s] | End Time [s] | Area  | % of total Area |
|-------|----------------|--------------|-------|-----------------|
| 18S   | 41.25          | 42.84        | 147.8 | 20.7            |
| 28S   | 47.36          | 50.34        | 267.2 | 37.7            |
**Overall Results for sample 9:**  
**Agac9**

- **RNA Area:** 944.4
- **RNA Concentration:** 397 ng/µl
- **rRNA Ratio [28s / 18s]:** 1.8
- **RNA Integrity Number (RIN):** 9.8 (B.02.08)
- **Result Flagging Color:**
  - **Result Flagging Label:** RIN: 9.80

**Fragment table for sample 9:**  
**Agac9**

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|------|----------------|--------------|------|-----------------|
| 18S  | 41,23          | 42,73        | 186.9| 19.8            |
| 28S  | 46,98          | 50,21        | 333.0| 35.3            |
Overall Results for sample 10:  **Agac10**

| RNA Area:       | 1.401.0 | RNA Integrity Number (RIN): | 9.8 (B.02.08) |
|-----------------|---------|----------------------------|----------------|
| RNA Concentration: | 588 ng/µl | Result Flagging Color: | - |
| rRNA Ratio [28s / 18s]: | 1.8 | Result Flagging Label: | RIN: 9.80 |

Fragment table for sample 10:  **Agac10**

| Name | Start Time [s] | End Time [s] | Area   | % of total Area |
|------|----------------|--------------|--------|-----------------|
| 18S  | 41,12          | 42,62        | 294,2  | 21,0            |
| 28S  | 46,21          | 50,14        | 527,0  | 37,6            |
Electropherogram Summary Continued ...

Overall Results for sample 11 : **Agac11**
- RNA Area: 705,5
- RNA Concentration: 296 ng/μl
- rRNA Ratio [28s / 18s]: 1,6
- RNA Integrity Number (RIN): 9.5 (B.02.08)
- Result Flagging Color: 
- Result Flagging Label: RIN: 9.50

Fragment table for sample 11 : **Agac11**

| Name | Start Time [s] | End Time [s] | Area   | % of total Area |
|------|----------------|--------------|--------|-----------------|
| 18S  | 40,32          | 42,76        | 133,9  | 19,0            |
| 28S  | 47,14          | 50,36        | 218,9  | 31,0            |
Overall Results for sample 12: **Agac12**

- RNA Area: 985.1
- RNA Concentration: 414 ng/ul
- rRNA Ratio [28s / 18s]: 1.6

**RNA Integrity Number (RIN):** 9.9 (B.02.08)

**Result Flagging Color:**
- **Result Flagging Label:** RIN: 9.90

Fragment table for sample 12: **Agac12**

| Name | Start Time [s] | End Time [s] | Area   | % of total Area |
|------|---------------|-------------|--------|-----------------|
| 18S  | 40.52         | 42.71       | 216.0  | 21.9            |
| 28S  | 47.09         | 50.26       | 354.0  | 35.9            |
Supplementary Data 3. Amplification plot and Melting curve analysis (RT-qPCR).

**qPCR program** (used in all qPCR runs)

![qPCR program diagram](image)

**GAPDH (main qPCR)**

Amplification plot

Melting curve

**PPIB (main qPCR)**

Amplification plot

Melting curve

**YWHAZ (main qPCR)**

Amplification plot

Melting curve
POLR2A (main qPCR)

Amplification plot

Melting curve

TBP (main qPCR)

Amplification plot

Melting curve

RPL22 (main qPCR)

Amplification plot

Melting curve
**EEF1A1** (main qPCR)

Amplification plot

Melting curve

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**RPLP0** (main qPCR)

Amplification plot

Melting curve

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**RNA18S5** (main qPCR)

Amplification plot

Melting curve
GAPDH (efficiency qPCR – standard curve)

Amplification plot

Melting curve

PPIB (efficiency qPCR – standard curve)

Amplification plot

Melting curve

YWHAZ (efficiency qPCR – standard curve)

Amplification plot

Melting curve
**POLR2A** (efficiency qPCR – standard curve)

Amplification plot

Melting curve

**TBP** (efficiency qPCR – standard curve)

Amplification plot

Melting curve

**RPL22** (efficiency qPCR – standard curve)

Amplification plot

Melting curve
**EEF1A1** (efficiency qPCR – standard curve)

Amplification plot

Melting curve

**RPLP0** (efficiency qPCR – standard curve)

Amplification plot

Melting curve

**RNA18S5** (efficiency qPCR – standard curve)

Amplification plot

Melting curve
**Supplementary Data 4.** Evaluation of qPCR primer efficiency (factor-specific). Log_{10} serial dilution of cDNA stock solution (1,000,000 pg RNA equivalent) was performed in triplets. From the resulting C_q values a standard curve was created by linear regression.

**GAPDH (factor-specific primer efficiency)**

| Gene   | RNA equivalent [pg] | C_q Triplet | C_q 95%CI | C_q Mean | C_q SD | cDNA dilution |
|--------|---------------------|-------------|-----------|----------|--------|---------------|
| GAPDH  | 100000              | 14.60       | 14.56/14.69 | 14.62    | 0.025  | 1:10          |
| GAPDH  | 100000              | 14.65       | 14.63      | 14.67    | 0.025  | 1:10          |
| GAPDH  | 100000              | 17.91       | 17.74/18.15 | 17.94    | 0.081  | 1:10^2        |
| GAPDH  | 100000              | 17.89       | 18.04      | 18.05    | 0.081  | 1:10^2        |
| GAPDH  | 100000              | 21.56       | 21.11/21.76 | 21.43    | 0.130  | 1:10^3        |
| GAPDH  | 100000              | 21.30       | 21.44      | 21.47    | 0.130  | 1:10^3        |
| GAPDH  | 100000              | 24.90       | 24.74/25.29 | 25.01    | 0.111  | 1:10^4        |
| GAPDH  | 100000              | 25.03       | 25.12      | 25.18    | 0.111  | 1:10^4        |
| GAPDH  | 100000              | 28.27       | 27.95/29.14 | 28.55    | 0.240  | 1:10^5        |
| GAPDH  | 100000              | 28.69       | 28.68      | 28.74    | 0.240  | 1:10^5        |
| GAPDH  | 100000              | 33.26       | 29.80/34.53 | 32.16    | 0.952  | 1:10^6        |
| GAPDH  | 100000              | 31.55       | 31.68      | 31.68    | 0.952  | 1:10^6        |
| GAPDH  | 100000              | 31.68       | 31.68      | 31.68    | 0.952  | 1:10^6        |
| GAPDH  | NTC                 | 39.62       |           |          |        |               |

- **y = 1E+09e^{0.659x}**
- **R² = 0.9998**

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval
PPIB (factor-specific primer efficiency)

| Gene | RNA equivalent [pg] | C_q | C_q 95%CI | C_q Mean | C_q SD | cDNA dilution |
|------|---------------------|-----|-----------|----------|--------|---------------|
| PPIB | 100000              | 16.91 | 16.88/16.92 | 16.900   | 0.010  | 1:10          |
| PPIB | 100000              | 16.90 | 16.89     | 16.900   | 0.010  | 1:10          |
| PPIB | 100000              | 16.89 |           | 16.900   | 0.010  | 1:10          |
| PPIB | 10000               | 20.21 | 20.00/20.29 | 20.143   | 0.058  | 1:10²         |
| PPIB | 10000               | 20.11 | 20.00     | 20.143   | 0.058  | 1:10²         |
| PPIB | 10000               | 20.11 |           | 20.143   | 0.058  | 1:10²         |
| PPIB | 1000                | 23.72 | 23.60/23.90 | 23.750   | 0.061  | 1:10⁵         |
| PPIB | 1000                | 23.71 | 23.60     | 23.750   | 0.061  | 1:10⁵         |
| PPIB | 1000                | 23.71 |           | 23.750   | 0.061  | 1:10⁵         |
| PPIB | 100                 | 27.19 | 26.90/27.94 | 27.420   | 0.210  | 1:10⁴         |
| PPIB | 100                 | 27.47 | 26.90     | 27.420   | 0.210  | 1:10⁴         |
| PPIB | 100                 | 27.61 |           | 27.420   | 0.210  | 1:10⁴         |
| PPIB | 10                  | 31.14 | 30.21/31.66 | 30.933   | 0.291  | 1:10⁵         |
| PPIB | 10                  | 30.60 | 30.21     | 30.933   | 0.291  | 1:10⁵         |
| PPIB | 10                  | 31.06 |           | 30.933   | 0.291  | 1:10⁵         |
| PPIB | 1                   | 33.62 | 31.18/39.97 | 35.573   | 1.770  | 1:10⁶         |
| PPIB | 1                   | 36.03 | 31.18     | 35.573   | 1.770  | 1:10⁶         |
| PPIB | 1                   | 37.07 |           | 35.573   | 1.770  | 1:10⁶         |
| PPIB | NTC                 | 35.92 |           |         |        |               |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval

y = 5E+09e\(^{-0.651x}\)
R² = 0.9996

**Slope** [95% CI] = -3.509 [-3.808/-3.209]
**% Efficiency** [95% CI] = 92.7 [83.1/104.9]
**LDR (dilution range)** = 1:10 – 1:10⁵
**LOD (dilution)** ≤ 1:10⁶
**YWHAZ** (factor-specific primer efficiency)

| Gene | RNA equivalent [pg] | cDNA dilution | C_q Triplet | C_q 95%CI | C_q Mean | C_q SD | Slope [95% CI] | % Efficiency [95% CI] | LDR (dilution range) | LOD (dilution) |
|------|---------------------|---------------|-------------|-----------|----------|--------|---------------|------------------|-----------------|---------------|
| YWHAZ | 1000000             | 1:10          | 17.20       | 16.85/17.35 | 17.100   | 0.100  | -3.488/-3.062 | 93.5             | 1:10 – 1:10^5   | ≤1:10^6        |
| YWHAZ | 100000              | 1:10          | 17.10       | 16.26/16.39 | 17.000   | 0.036  | -3.07/1.18   | 99.5             | 1:10 – 1:10^3   | ≤1:10^3        |
| YWHAZ | 10000               | 1:10          | 17.00       | 16.21/16.39 | 17.000   | 0.036  | -3.07/1.18   | 99.5             | 1:10 – 1:10^3   | ≤1:10^3        |
| YWHAZ | 1000                | 1:10          | 20.33       | 20.21/20.39 | 20.000   | 0.036  | -3.07/1.18   | 99.5             | 1:10 – 1:10^3   | ≤1:10^3        |
| YWHAZ | 100                 | 1:10          | 20.26       | 20.00/20.39 | 20.000   | 0.036  | -3.07/1.18   | 99.5             | 1:10 – 1:10^3   | ≤1:10^3        |
| YWHAZ | 100                 | 1:10          | 20.31       | 20.00/20.39 | 20.000   | 0.036  | -3.07/1.18   | 99.5             | 1:10 – 1:10^3   | ≤1:10^3        |
| YWHAZ | NTCP                | -              | -           | -          | -        | -      | -             | -               | -              | -             |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval
**POLR2A (factor-specific primer efficiency)**

| Gene    | RNA equivalent [pg] | C<sub>q</sub> Triplet | C<sub>q</sub> 95%CI | C<sub>q</sub> Mean | C<sub>q</sub> SD | cDNA dilution |
|---------|---------------------|------------------------|---------------------|------------------|---------------|---------------|
| POLR2A  | 100000              | 20.01                  | 19.93/20.13         | 20.033           | 0.040         | 1:10          |
|         | 100000              | 20.08                  |                     |                  |               |               |
|         | 100000              | 20.01                  |                     |                  |               |               |
| POLR2A  | 10000               | 23.11                  | 23.01/23.37         | 23.193           | 0.072         | 1:10<sup>2</sup> |
|         | 10000               | 23.23                  |                     |                  |               |               |
|         | 10000               | 23.24                  |                     |                  |               |               |
| POLR2A  | 1000                | 26.72                  | 26.31/26.71         | 26.810           | 0.201         | 1:10<sup>3</sup> |
|         | 1000                | 27.04                  |                     |                  |               |               |
|         | 1000                | 26.67                  |                     |                  |               |               |
| POLR2A  | 100                 | 30.51                  | 29.72/30.98         | 30.593           | 0.352         | 1:10<sup>4</sup> |
|         | 100                 | 30.29                  |                     |                  |               |               |
|         | 100                 | 30.98                  |                     |                  |               |               |
| POLR2A  | 10                  | 36.85                  | 34.35/34.98         | 36.993           | 1.004         | 1:10<sup>6</sup> |
|         | 10                  | 32.99                  |                     |                  |               |               |
|         | 10                  | 34.64                  |                     |                  |               |               |
| POLR2A  | 1                   | 37.83                  | 34.50/34.99         | 36.993           | 1.004         | 1:10<sup>6</sup> |
|         | 1                   | 35.88                  |                     |                  |               |               |
|         | 1                   | 37.27                  |                     |                  |               |               |
| POLR2A  | NTC                 | 37.11                  |                     |                  |               |               |

**SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval**
### TBP (factor-specific primer efficiency)

| Gene | RNA equivalent [pg] | \(C_q\) Triplet | \(95\%\) CI | \(C_q\) Mean | \(C_q\) SD | cDNA dilution |
|------|--------------------|-----------------|--------------|-------------|----------|---------------|
| TBP  | 100000             | 26.53           | 26.46/26.64  | 26.550      | 0.035    | 1:10          |
| TBP  | 100000             | 26.53           | 26.46/26.64  | 26.550      | 0.035    | 1:10          |
| TBP  | 100000             | 26.59           | 26.46/26.64  | 26.550      | 0.035    | 1:10          |
| TBP  | 100000             | 26.59           | 26.46/26.64  | 26.550      | 0.035    | 1:10          |
| TBP  | 100000             | 29.80           | 29.72/29.83  | 29.773      | 0.023    | 1:10^2        |
| TBP  | 100000             | 29.76           | 29.72/29.83  | 29.773      | 0.023    | 1:10^2        |
| TBP  | 100000             | 29.76           | 29.72/29.83  | 29.773      | 0.023    | 1:10^2        |
| TBP  | 100000             | 29.76           | 29.72/29.83  | 29.773      | 0.023    | 1:10^2        |
| TBP  | 100000             | 33.35           | 32.96/34.30  | 33.627      | 0.270    | 1:10^3        |
| TBP  | 100000             | 33.64           | 32.96/34.30  | 33.627      | 0.270    | 1:10^3        |
| TBP  | 100000             | 33.64           | 32.96/34.30  | 33.627      | 0.270    | 1:10^3        |
| TBP  | 100000             | 33.64           | 32.96/34.30  | 33.627      | 0.270    | 1:10^3        |
| TBP  | 100000             | 35.19           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |
| TBP  | 100000             | 35.55           | 34.28/37.07  | 35.677      | 0.561    | 1:10^4        |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; \(R^2\) = coefficient of determination; CI = confidence interval

![Graph showing the relationship between TBP RNA equivalent and qC](chart.png)

\[
y = 3E+12e^{-0.649x} \\
R^2 = 0.9974
\]

**Slope**

-3.538 [95% CI]

**Efficiency**

91.7 [95% CI]

**LDR (dilution range)**

1:10 - 1:10^3

**LOD (dilution)**

1:10^4

[-7.540/0.465]
**RPL22** (factor-specific primer efficiency)

| Gene   | RNA equivalent [pg] | C_q Triplet | C_q 95%CI | C_q Mean | C_q SD | cDNA dilution |
|--------|---------------------|-------------|-----------|----------|--------|---------------|
| RPL22  | 100000              | 18.09       | 17.88/18.19 | 18.037   | 0.061 | 1:10          |
| RPL22  | 100000              | 20.86       | 20.77/20.91 | 20.840   | 0.026 | 1:10²         |
| RPL22  | 10000               | 24.37       | 24.33/24.40 | 24.367   | 0.015 | 1:10³         |
| RPL22  | 1000                | 28.18       | 28.04/28.45 | 28.247   | 0.083 | 1:10⁴         |
| RPL22  | 100                 | 32.68       | 31.59/33.24 | 32.413   | 0.333 | 1:10⁵         |
| RPL22  | 10                  | 34.16       | -          | 34.160   | 0.000 | 1:10⁶         |
| RPL22  | NTC                 | -           | -          | -        | -      | -             |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval

![Graph showing the relationship between RNA equivalent and C_q for RPL22, with a linear equation and R² value provided.]
**EEF1A1** (factor-specific primer efficiency)

| Gene   | RNA equivalent [pg] | C_q Triplet | C_q 95%CI | C_q Mean | C_q SD | cDNA dilution |
|--------|---------------------|-------------|-----------|----------|--------|---------------|
| EEF1A1 | 100000              | 16.72       | 16.25/17.20 | 16.727   | 0.190  | 1:10          |
| EEF1A1 | 100000              | 16.92       | 16.54/17.20 | 16.727   | 0.190  | 1:10          |
| EEF1A1 | 100000              | 16.54       | 16.25/17.20 | 16.727   | 0.190  | 1:10          |
| EEF1A1 | 100000              | 19.16       | 18.76/19.61 | 19.187   | 0.172  | 1:10^2        |
| EEF1A1 | 100000              | 19.37       | 19.03/19.61 | 19.187   | 0.172  | 1:10^2        |
| EEF1A1 | 100000              | 19.03       | 18.76/19.61 | 19.187   | 0.172  | 1:10^2        |
| EEF1A1 | 100000              | 22.54       | 22.21/23.44 | 22.820   | 0.248  | 1:10^3        |
| EEF1A1 | 100000              | 23.01       | 22.21/23.44 | 22.820   | 0.248  | 1:10^3        |
| EEF1A1 | 100000              | 22.91       | 22.21/23.44 | 22.820   | 0.248  | 1:10^3        |
| EEF1A1 | 100000              | 25.53       | 24.81/27.44 | 26.127   | 0.529  | 1:10^4        |
| EEF1A1 | 100000              | 26.31       | 24.81/27.44 | 26.127   | 0.529  | 1:10^4        |
| EEF1A1 | 100000              | 26.54       | 24.81/27.44 | 26.127   | 0.529  | 1:10^4        |
| EEF1A1 | 100000              | 30.23       | 29.38/30.60 | 29.987   | 0.245  | 1:10^5        |
| EEF1A1 | 100000              | 29.74       | 29.38/30.60 | 29.987   | 0.245  | 1:10^5        |
| EEF1A1 | 100000              | 29.99       | 29.38/30.60 | 29.987   | 0.245  | 1:10^5        |
| EEF1A1 | 100000              | 33.03       | 31.70/33.71 | 32.703   | 0.405  | 1:10^6        |
| EEF1A1 | 100000              | 32.83       | 31.70/33.71 | 32.703   | 0.405  | 1:10^6        |
| EEF1A1 | 100000              | 32.25       | 31.70/33.71 | 32.703   | 0.405  | 1:10^6        |
| EEF1A1 | 100000              | -           | -          | -        | -      | -             |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval

\[
y = 7E+09e^{0.685x}
\]

\[
R^2 = 0.9951
\]

**slope**

-3.315 [95% CI -4.291/-2.339]

**efficiency**

100.3 [95% CI 71.0/167.6]

**LDR (dilution range)**

1:10 – 1:10^5

**LOD (dilution)**

\(\leq 1:10^6\)
**RPLP0 (factor-specific primer efficiency)**

| Gene | RNA equivalent [pg] | C_q Triplet | C_q 95%CI | C_q Mean | C_q SD | cDNA dilution |
|------|---------------------|-------------|-----------|----------|--------|---------------|
| RPLP0 | 100000              | 15.15       | 15.35     | 15.45    |        |               |
|       | 100000              | 14.94       | /15.70    | 15.317   | 0.153  | 1:10          |
| RPLP0 | 100000              | 18.52       | 18.40     | 18.60    |        |               |
|       | 100000              | 18.26       | /18.76    | 18.507   | 0.101  | 1:10²         |
| RPLP0 | 100000              | 22.30       | 22.34     | 22.25    |        |               |
|       | 100000              | 22.18       | /22.41    | 22.297   | 0.045  | 1:10³         |
| RPLP0 | 100000              | 25.61       | 26.20     | 26.27    |        |               |
|       | 100000              | 25.13       | /26.93    | 26.027   | 0.363  | 1:10⁴         |
| RPLP0 | 100000              | 29.30       | 29.22     | 29.54    |        |               |
|       | 100000              | 28.94       | /29.77    | 29.353   | 0.167  | 1:10⁵         |
| RPLP0 | 100000              | 32.49       | 32.16     | 32.22    |        |               |
|       | 100000              | 31.85       | /32.73    | 32.290   | 0.176  | 1:10⁶         |
| RPLP0 | 100000              | 39.62       |           |          |        |               |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; \( R^2 \) = coefficient of determination; CI = confidence interval
RNA18S5 (factor-specific primer efficiency)

| Gene   | RNA equivalent [pg] | C_q Triplet | C_q 95%CI | C_q Mean | C_q SD | cDNA dilution |
|--------|---------------------|-------------|-----------|----------|--------|---------------|
| RNA18S | 100000              | 4.65        | 4.66/4.71 | 4.57     | 0.026  | 1:10          |
| RNA18S | 100000              | 4.66        | 4.61      | 4.60     | 0.026  | 1:10          |
| RNA18S | 100000              | 4.66        | 4.61      | 4.60     | 0.026  | 1:10          |
| RNA18S | 7.06                | 7.09/7.13   | 7.063     | 0.025    | 1:10^2 |
| RNA18S | 7.04                | 7.09/7.13   | 7.063     | 0.025    | 1:10^2 |
| RNA18S | 10.68               | 10.54/10.95 | 10.747    | 0.083    | 1:10^3 |
| RNA18S | 10.72               | 10.54/10.95 | 10.747    | 0.083    | 1:10^3 |
| RNA18S | 14.23               | 13.98/14.88 | 14.433    | 0.182    | 1:10^4 |
| RNA18S | 14.49               | 13.98/14.88 | 14.433    | 0.182    | 1:10^4 |
| RNA18S | 14.58               | 13.98/14.88 | 14.433    | 0.182    | 1:10^4 |
| RNA18S | 17.66               | 17.50/18.05 | 17.777    | 0.111    | 1:10^5 |
| RNA18S | 17.88               | 17.50/18.05 | 17.777    | 0.111    | 1:10^5 |
| RNA18S | 17.79               | 17.50/18.05 | 17.777    | 0.111    | 1:10^5 |
| RNA18S | 21.00               | 20.73/21.74 | 21.233    | 0.202    | 1:10^6 |
| RNA18S | 21.35               | 20.73/21.74 | 21.233    | 0.202    | 1:10^6 |
| RNA18S | 21.35               | 20.73/21.74 | 21.233    | 0.202    | 1:10^6 |
| RNA18S | NTC                 | 35.26       |           |          |        |               |

SD = standard deviation; NTC = no-template control; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval

y = 2E+06e^(-0.677x)
R² = 0.9974

Slope [95% CI] | % Efficiency [95% CI] | LDR (dilution range) | LOD (dilution) |
---------------|------------------------|-----------------------|----------------|
-3.319 [-3.967/-2.670] | 100.1 [78.7/136.9] | 1:10 – 1:10^6 | ≤1:10^6 |
Supplementary Data 5. Evaluation of a commercially available primer pair for TUBB (Qiagen, PPH17836A). Primer specificity was evaluated and confirmed by melting curve analysis (a,b) and agarose gel electrophoresis (c). d To calculate primer efficiency $E_p$, which was within the pre-specified acceptable range, a serial log$_{10}$ dilution of cDNA stock solution (1,000,000 pg RNA equivalent) was performed in triplets. From the resulting C$_q$ values a standard curve was created by linear regression. The 1:10 dilution used for qPCR for all genes/primers, however, was beyond the linear dynamic range LDR. e Amplification efficiency $E_A$ was calculated with LinRegPCR and within the pre-specified acceptable range. f Raw qPCR C$_q$ values for TUBB (triplet means). g Reference gene stability rankings including TUBB as 10th candidate reference gene indicate low intergroup expression stability in hPDL experiments on orthodontic tooth movement and periodontitis.
(d) TUBB Primer efficiency (factor-specific)

| Gene  | RNA equivalent [pg] | C_q Triplet   | C_q Mean | C_q SD | cDNA dilution |
|-------|---------------------|---------------|----------|--------|---------------|
| TUBB  | 1000000             | 19.65/19.79   | 19.583   | 0.083  | 1:10          |
| TUBB  | 1000000             | 19.49/19.61   | 19.38/19.79 | 0.631  | 1:10²         |
| TUBB  | 1000000             | 19.45/19.20   | 18.777   | 0.175  | 1:10³         |
| TUBB  | 1000000             | 21.67/21.51   | 21.500   | 0.046  | 1:10⁴         |
| TUBB  | 1000000             | 24.87/24.96   | 24.920   | 0.270  | 1:10⁵         |
| TUBB  | 1000000             | 28.16/28.67   | 28.467   | 0.379  | 1:10⁶         |
| TUBB  | 1000000             | 30.40/30.87   | 30.807   | 0.379  |               |
| TUBB  | NTC                 | 42.23/32.31   |          |        |               |

SD = standard deviation; NTC = no-template control; -RT = control without reverse transcriptase; LDR = linear dynamic range; LOD = limit of detection; R² = coefficient of determination; CI = confidence interval
(e) Primer efficiency (factor-specific) and coefficients of determination derived from a standard curve for TUBB (6x log_{10} dilution of cDNA stock solution, random untreated sample) as well as technical repeatability (intraassay reliability, n = 18) and amplification efficiency (sample-specific), calculated using LinRegPCR software (http://LinRegPCR.HFRC.nl; n = 18 in triplets).

* of three technical replicates (triplet) among all biological replicates (n = 18). CI = confidence interval

| Gene symbol | Slope  | Primer efficiency E_P [%] (2^{E_P/100%}) | Coefficient of determination R^2 | Intraassay reliability SD of mean of C_q* (mean, min./max.) | Amplification Efficiency E_A [%] (2^{E_A/100%}) |
|-------------|--------|----------------------------------------|---------------------------------|-------------------------------------------------------------|-----------------------------------------------------|
| TUBB        | -3.230 | 104.0 (2.056)                          | 0.9965                          | 0.26                                                        | 91.3                                                 |

* of three technical replicates (triplet) among all biological replicates (n = 18). CI = confidence interval

(f) Raw C_q values (triplet means) of TUBB RT-qPCR for the three experimental groups.

| Sample       | Group | Gene |
|--------------|-------|------|
| Control K7   | 1     | 20.10|
| Control K8   | 1     | 20.71|
| Control K9   | 1     | 19.58|
| Control K10  | 1     | 19.53|
| Control K11  | 1     | 19.54|
| Control K12  | 1     | 19.30|
| Compression D7 | 2     | 18.84|
| Compression D8 | 2     | 19.06|
| Compression D9 | 2     | 19.88|
| Compression D10 | 2    | 19.10|
| Compression D11 | 2    | 18.55|
| Compression D12 | 2    | 19.50|
| Agac7        | 3     | 19.30|
| Agac8        | 3     | 18.49|
| Agac9        | 3     | 18.29|
| Agac10       | 3     | 18.53|
| Agac11       | 3     | 19.19|
| Agac12       | 3     | 18.38|

C_q = quantification cycle; SD = standard deviation of group mean.
Agac = Aggregatibacter actinomycetemcomitans (periodontitis)
(g) Reference gene stability ranking including TUBB for hPDL experiments on orthodontic tooth movement (compressive orthodontic force vs. untreated control), experiments on periodontitis (Agc, toxins/bacterial lystate vs. untreated control) and pooled/overall experimental conditions as calculated by the algorithms geNorm, NormFinder, comparative ΔCq and BestKeeper. A higher rank denotes lower expression stability.

| Rank | Total (of 4 methods) | geNorm | NormFinder | comparative deltaCq | BestKeeper |
|------|----------------------|--------|------------|---------------------|-----------|
|      | Rank | Rank | Stability value (M) | Stability value (pM) | Standard error | Ranking | Stability value (mean SD of mean ΔCq) | Ranking | Stability value (r) | SD (+/- Cq) | CV (Cq) |
| hPDL untreated + compressive orthodontic force (experiments on orthodontic tooth movement, n = 12) |
| 1. | RPL22 | 6 | RPL22 | 0.263 | RPL22 | 0.043 | 0.033 | RPL22 | 0.271 | RNA18S5 | 0.910 | 0.259 | 3.110 |
| 2. | PPIB | 13 | PPIB | 0.286 | EEFF1A1 | 0.092 | 0.031 | PPIB | 0.296 | YWHAZ | 0.905 | 0.373 | 1.728 |
| 3. | RPLP0 | 17 | RPLP0 | 0.296 | RPLP0 | 0.097 | 0.031 | RPLP0 | 0.307 | RPL22 | 0.856 | 0.121 | 0.665 |
| 4. | TBP | 18 | TBP | 0.299 | PPIB | 0.099 | 0.031 | EEFF1A1 | 0.311 | TBP | 0.657 | 0.202 | 0.860 |
| 5. | EEFF1A1 | 18 | EEFF1A1 | 0.302 | TBP | 0.121 | 0.034 | TBP | 0.314 | | | |
| 6. | RNA18S5 | 19 | RNA18S5 | 0.347 | RNA18S5 | 0.152 | 0.039 | RNA18S5 | 0.365 | POLR2A | 0.533 | 0.357 | 1.681 |
| 7. | YWHAZ | 20 | YWHAZ | 0.350 | GAPDH | 0.166 | 0.041 | POLR2A | 0.475 | YWHAZ | 0.805 | 0.315 | 0.601 |
| 8. | POLR2A | 30 | POLR2A | 0.399 | POLR2A | 0.230 | 0.053 | POLR2A | 0.423 | RPLP0 | 0.364 | 0.098 | 0.601 |
| 9. | GAPDH | 31 | GAPDH | 0.424 | YWHAZ | 0.234 | 0.054 | YWHAZ | 0.449 | TUBB | 0.187 | 0.420 | 2.157 |
| 10. | TUBB | 39 | TUBB | 0.678 | TUBB | 0.449 | 0.097 | TUBB | 0.664 | GAPDH | -0.154 | 0.117 | 0.776 |
| hPDL untreated + Agc toxins/bacterial lystate (experiments on periodontis, n = 12) |
| 1. | PPIB | 8 | PPIB | 0.250 | PPIB | 0.066 | 0.031 | PPIB | 0.255 | RNA18S5 | 0.815 | 0.269 | 3.261 |
| 2. | TBP | 10 | TBP | 0.259 | TBP | 0.077 | 0.031 | PPIB | 0.265 | POLR2A | 0.599 | 0.176 | 0.836 |
| 3. | POLR2A | 18 | EEFF1A1 | 0.269 | GAPDH | 0.116 | 0.034 | EEFF1A1 | 0.270 | YWHAZ | 0.567 | 0.318 | 1.488 |
| 4. | RPL22 | 20 | RPL22 | 0.284 | RPL22 | 0.122 | 0.035 | RPL22 | 0.290 | TBP | 0.514 | 0.121 | 0.518 |
| 5. | EEFF1A1 | 21 | POLR2A | 0.286 | POLR2A | 0.128 | 0.036 | POLR2A | 0.296 | GAPDH | 0.400 | 0.159 | 1.044 |
| 6. | GAPDH | 21 | GAPDH | 0.286 | RPLP0 | 0.092 | 0.031 | RPLP0 | 0.307 | YWHAZ | 0.427 | 0.187 | 1.271 |
| 7. | RNA18S5 | 25 | GAPDH | 0.287 | RPLP0 | 0.140 | 0.038 | RPLP0 | 0.307 | TUBB | 0.324 | 0.558 | 2.897 |
| 8. | RPLP0 | 29 | RNA18S5 | 0.380 | RNA18S5 | 0.166 | 0.042 | RNA18S5 | 0.381 | TUBB | 0.138 | 0.678 | 1.717 |
| 9. | YWHAZ | 30 | YWHAZ | 0.420 | YWHAZ | 0.203 | 0.049 | YWHAZ | 0.493 | EEFF1A1 | 0.313 | 0.167 | 1.170 |
| 10. | TUBB | 38 | TUBB | 0.815 | TUBB | 0.554 | 0.119 | TUBB | 0.792 | RPLP0 | 0.291 | 0.172 | 1.049 |
| hPDL pooled/overall (experiments on orthodontic tooth movement and periodontitis n = 18) |
| 1. | PPIB | 7 | PPIB | 0.296 | PPIB | 0.076 | 0.026 | PPIB | 0.306 | RNA18S5 | 0.859 | 0.266 | 3.199 |
| 2. | TBP | 12 | TBP | 0.304 | TBP | 0.093 | 0.026 | RPL22 | 0.313 | YWHAZ | 0.759 | 0.381 | 1.777 |
| 3. | RPL22 | 12 | RPL22 | 0.304 | TBP | 0.100 | 0.026 | TBP | 0.316 | TBP | 0.625 | 0.173 | 0.735 |
| 4. | RPLP0 | 19 | RPLP0 | 0.326 | RPLP0 | 0.135 | 0.030 | RPLP0 | 0.338 | PPIB | 0.587 | 0.158 | 0.935 |
| 5. | RNA18S5 | 19 | RNA18S5 | 0.357 | RNA18S5 | 0.159 | 0.033 | RNA18S5 | 0.362 | POLR2A | 0.526 | 0.230 | 1.320 |
| 6. | EEFF1A1 | 24 | POLR2A | 0.373 | EEFF1A1 | 0.171 | 0.035 | RNA18S5 | 0.383 | RPL22 | 0.485 | 0.141 | 0.776 |
| 7. | POLR2A | 25 | POLR2A | 0.379 | POLR2A | 0.184 | 0.037 | POLR2A | 0.391 | RPLP0 | 0.262 | 0.149 | 0.913 |
| 8. | YWHAZ | 29 | YWHAZ | 0.465 | YWHAZ | 0.253 | 0.047 | YWHAZ | 0.491 | TUBB | 0.236 | 0.501 | 2.805 |
| 9. | GAPDH | 34 | YWHAZ | 0.465 | YWHAZ | 0.253 | 0.047 | YWHAZ | 0.491 | TUBB | 0.236 | 0.501 | 2.805 |
| 10. | TUBB | 39 | TUBB | 0.744 | TUBB | 0.491 | 0.085 | TUBB | 0.726 | GAPDH | 0.057 | 0.189 | 1.245 |

Cq = quantification cycle; SD = standard deviation; CV = coefficient of variation; r = Pearson’s correlation coefficient