The Telomere as a Marker for Aging

James Qin, Applications Laboratory, QIAGEN Sciences, Inc., Germantown, MD
Rodrigo Calado, NHLBI, NIH, Bethesda, MD

The following presentation describes applications that are currently under development and are not commercially available. Therefore, they are presented here only for research purposes. They are not intended for diagnostic use.

Introduction

Telomeres are the ends of linear chromosomes and are composed of tandem hexameric nucleotide repeats of the six-nucleotide sequence, 5'-TTAGGG-3', coated by several proteins, which are collectively termed shelterin. The main function of telomeres is to protect the natural ends of chromosomes from being recognized as damaged DNA, hence contributing to chromosomal stability. Due to DNA polymerase's inability to fully duplicate the DNA strands at their extremities, telomeres shorten with each cell division and as a consequence telomeres shorten with aging. To maintain telomeres, cells with highly proliferative capacity express telomerase, a reverse transcriptase enzyme that uses RNA template to elongate the 3' end of the leading strand of telomeres, thus maintaining their length. Some genetic diseases are caused by deficient telomerase function in which mutations in the telomerase complex are etiologic. In these diseases, telomeres are extremely short and patients present the clinical manifestations of cell senescence and chromosomal instability. Patients with telomerase mutations usually present bone marrow failure and an increased propensity for leukemia development.

Thus, precise, reproducible, and simple methods aiming to measure telomere length are highly desired both in the laboratory and in clinical practice. Three major methods are available today in the laboratory: Southern blot, which is the gold standard method, but is labor intensive, time consuming, and requires large DNA quantities; flow-FISH, which combines flow cytometry and fluorescence in situ hybridization, but is labor intensive and requires intact cells for analysis; and quantitative PCR (qPCR), which requires low quantities of DNA, but the method until today requires several reagents to stabilize the reaction in a "laboratory-developed" mastermix. Here we describe a simplified and automated qPCR method to measure telomere length using standardized, commercially available chemistry. The method is demonstrated to be highly reproducible and accurate for human peripheral cells.

Method Part I – Telomere Assay

Method Design – Telomere Assay Standard Curve

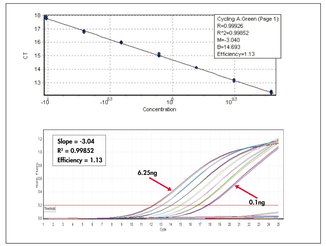

Figure 1: Telomere assay
25-cycle, 47 min qPCR was performed on the Rotor-Gene® Q real-time instrument with the QIAGEN Rotor-Gene SYBR Green Kit. gDNA from human peripheral cells was diluted at 1:2 in seven serial dilutions in the range of 0.1 ng – 6.25ng/ PCR in a 20µl reaction volume. The PCR condition used in the assay was : 95°C 5s; 98°C 7s, 60°C 10s (25 cycles). The assay was set up using a QIAgility® liquid handling instrument.

Human Samples Against a Standard Curve

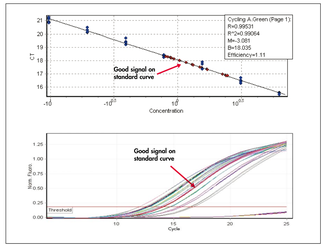

Figure 2: Telomere assay with diluted gDNA from human peripheral cells
The Telomere assay (25-cycle, 47min qPCR) was repeated using automated liquid-handling on the QIAgility in a Rotor-Disc™ 100 well format. Multiple human samples at 1 ng/ PCR were used along with a standard curve at seven point serial dilutions (in the range of 0.1 ng – 6.25 ng/ PCR). Human DNA diluted to 1 ng/ PCR is considered to be at an optimal sample input volume for this assay since the amplification signal falls within the middle of the standard curve.

Method Part II – Reference Assay

Method Design – Single Gene Standard Curve

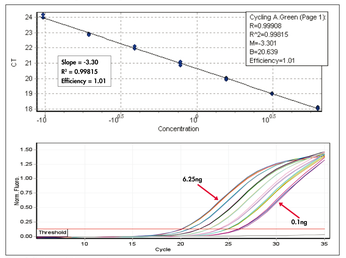

Figure 3: Single gene (reference gene) assay
35-cycle, 61 min qPCR was performed on the Rotor-Gene Q real-time instrument with the QIAGEN Rotor-Gene SYBR Green Kit. gDNA from human peripheral cells was diluted at 1:2 in seven serial dilutions in the range of 0.1 ng – 6.25 ng/ PCR in a 20µl reaction volume. The PCR condition used in the assay was: 95°C 5s; 98°C 7s, 58°C 10s (35 cycles). The assay was set up using a QIAgility liquid handling instrument.

Human Samples Against a Standard Curve

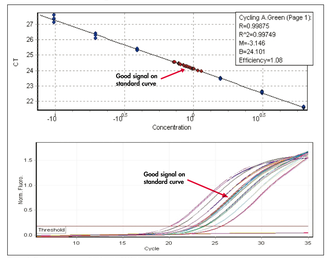

Figure 4: Single gene assay with diluted gDNA from human peripheral cells
The Single Gene assay (35-cycle, 61 min qPCR) was repeated using the QIAgility in a Rotor-Disc™ 100 well format. Multiple human samples at 1 ng/ PCR were used along with a seven point serial dilution curve (in the range of 0.1 ng – 6.25 ng/ PCR) in a 20µl reaction volume. Human DNA diluted to 1 ng/ PCR is considered to be at an optimal sample input volume for this assay since the amplification signal falls within the middle of the standard curve.

Method Validation

Telomere Length Correlation between Southern Blot and qPCR.

C_T values from the telomere assay were normalized to the single gene reference assay using the T/S ratio to determine telomere length. The telomere length (T/S ratio) from real-time PCR was then correlated against telomere length as determined by Southern Blot analysis. The telomere length (x) from each sample was based on the telomere to single copy gene ratio (T/S ratio) and was based on the calculation of the ΔC_T [$C_T^{(telomere)}/C_T^{(single\ gene)}$]. Telomere length was expressed as a relative T/S ratio, which was normalized to the average T/S ratio of the reference sample [$2^{-[\Delta C_T x - \Delta C_T]} = 2^{-\Delta\Delta C_T}$] for all standard curves, reference samples, and validation samples. In order to make results comparable from different assay runs, the results of each run were approved only if the relative T/S ratio of the validation reference sample fall within a 3% variation.

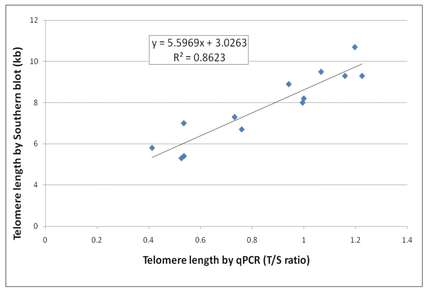

Figure 5: Correlation between Southern Blot and qPCR methods
Telomere length was measured in 13 samples using the gold standard Southern blot method and the new qPCR method described here. We have verified a high correlation between the two methods (R^2 = 0.8623), significantly higher than what was originally described (Cawthon NAS 2002).

Normal Population Data: Linear Regression between Age and Telomere Length

Telomere Length in Healthy Individuals

Data was obtained by quantitative PCR using a Rotor-Gene® Q real-time instrument. Signals from the telomere assay were normalized to a single gene reference (T/S ratio) before comparing to age information. Data shows good linear regression with R^2=0.4642 and p-value <0.0001 suggesting there is broad variation around the mean but telomere length is still highly age-dependent.

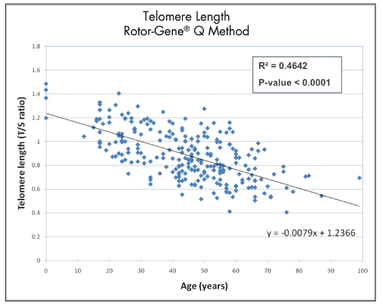

Figure 6: Telomere length of healthy individuals as a function of age
Using the T/S ratio qPCR method on the Rotor-Gene Q, the telomere length was measured for 299 healthy subjects varying in age from 0 (cord blood) to 99 years old. There was an inverse correlation between telomere length and age, as telomeres were shorter with aging.

Summary

This quantitative PCR method was demonstrated to be highly accurate, reproducible, and simple for human peripheral cells. The assay has enhanced sensitivity using a reaction volume of 20-µl with DNA at 1 ng/ assay, and has a good correlation of R^2 = 0.99064, slope = -3.081 and amplification efficiency at 100%. Furthermore, the real-time PCR assay takes only 47 min for up to 100 telomere targets.

We evaluated this assay with 299 healthy human subjects of varying ages ranging from 0 (cord blood) to 99 years old. The results are positive with an inverse correlation between telomere length and age. The telomere length was measured in 13 samples using the gold standard Southern Blot method and the new quantitative PCR method (correlation of R^2=0.8623), which is a significantly higher correlation than was previously described (Cawthon NAS 2002).

Description	q-PCR	Southern Blot
No. samples tested	299	13
Total time per process (Assay; Analysis)	90 min	2 days
Sensitivity: Sample (gDNA) required	1 ng	200 ng

This method has potential applications in a clinical setting for the diagnosis of age-related telomere diseases and conditions such as: macular degeneration (vision loss), atherosclerosis (hardening of arteries by plaque), impaired wound healing, heart disease, gray hair, and wrinkles.

It provides investigators with an easy-to-use technique for addressing the full extent and significance of telomere shortening in age-related telomere conditions, diseases and in the tumorigenic process. This fast, sensitive method is a powerful tool to study genomic instability, heart disease, cancer progression, and cancer therapy.