The Telomere as a Marker for Aging

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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 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. But in a DNA polymerase unable to fully replicate the DNA strands as they terminate, telomeres shorten with each cell division and as a consequence telomeres shorten with aging. To maintain telomeres, cells with highly proliferative

Method Part I – Telomere Assay
Method Design – Telomere Assay Standard Curve

Human Samples Against a Standard Curve

Method Validation
Telomere Length Correlation between Southern Blot and qPCR,

Ct values from the telomere assay were normalized to the single gene reference assay using the 1/2 ratio to determine telomere length. The telomere length (Ct value) from real-time PCR was then compared against telomere length as determined by Southern Blot analysis. The telomere length (Ct from each sample was based on the telomere to single copy gene ratio (T/S ratio) and was used as a relative T/S ratio. The reference sample (T/S ratio) was established on the average T/S ratio of the reference sample (T/S ratio = 1.00) for all standard curves, reference samples and validation samples. In order to keep results comparable from different assay runs, the ratio Ct values from the validation samples were averaged only if relative T/S ratio of the validation reference sample fell within a 5% variation.

Method Part II – Reference Assay
Method Design – Single Gene Standard Curve

Human Samples Against a Standard Curve

Method Part III – Reference Assay

Normal Population Data: Linear Regression between Age and Telomere Length

Telomere length in Healthy Individuals

Data was obtained by quantitative PCR using an Rotor-Gene® Q real-time instrument. Signals from the telomere assay were normalized to a single gene reference (T/S ratio) before linear regression analysis with R-squared and 95% confidence intervals (CI).

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 20µl with DNA at 1 ng/µl, and has a good correlation of R2 = 0.99064, slope = -3.081 and amplification efficiency at 100%. Furthermore, the real-time PCR assay takes only 47 min for one run.

We evaluated the assay with 299 healthy human subjects of varying ages ranging from 0 (cord blood) to 99 years old. The healthy data 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 R2 = 0.8623], which is a significantly higher correlation than was previously described [Cawthon NAS 2002].

This method has potential applications in a clinical setting for the diagnosis of age-related telomere diseases and conditions such as oncological degeneration (human leukemia, breast cancer) (formation of colony by plating), impaired wound healing, heart disease, grey hair, and wrinkles.

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