B102 Optimization and Validation of an Assay for the Determination of Telomere Length Using the ABI Prism 7500 Real-Time PCR Instrument

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The goal of this presentation is to discuss a research project to optimize and validate an assay for the determination of relative telomere length using the Applied Biosystems Prism 7500 Real-Time PCR instrument, an instrument already in use in many forensic laboratories. The assay utilizes the ABI Power SybrGreen Master Mix, thus minimizing reagent preparation. This assay validation is a necessary first step in a series of experiments to determine if age range can truly be determined for forensic samples using telomere length. Telomeres are hexamer repeats located at the ends of chromosomes that protect terminal genes; studies report that telomere length shortens throughout an individual's lifetime as their cells replicate.

This presentation will impact the forensic community by demonstrating an age range determination technique. Detection of relative telomere length using the ABI Prism 7500 allows for rapid and easy analysis of large numbers of samples in a forensic laboratory. This technique proved to be highly precise and reproducible from run to run, both between and within lot numbers of reaction mix. A successful age range determination technique would potentially have a significant

impact on the forensic community by providing investigative leads for cases with no active suspect.

The real-time PCR telomere length measurement technique employs the use of unique primer sets that hybridize to and amplify the hexamer repeats found at the telomeric regions of chromosomes, while at the same time minimizing the amplification artifacts commonly seen when targeting repeating sequences. The relative telomere length assay using quantitative real-time PCR measures two targets for each sample: the telomere repeat regions and a single copy gene (36B4, a gene that encodes acidic phosphoprotein PO). Relative telomere length is determined by comparing the C_t ratio of the telomere and 36B4 amplifications (T/S ratio) against the ratio of a known standard or pooled sample.

While the development of the relative telomere length assay has been reported in other fields, successful modification and validation of the assay for use with instrumentation commonly used in forensic casework was necessary. The ABI Prism 7500 Real-Time instrument has been validated for use in the forensic community, and the ease of use as well as programmability makes it amenable to other forensic uses in addition to human DNA quantitation. In addition, the ABI Power SYBR Green Master Mix simplifies sample preparation by integrating most of the reaction components into a single master mix, and makes for simple quality control records. The Power SYBRGreen master mix includes all necessary components for amplification.

A DNA sample, extracted from whole blood using the Qiagen DNA Mini kit, was quantified using the Applied Biosystems Quantifiler kit. A single set of standard curve samples was made from this sample and used for the entire validation series. Once the method was optimized for use with the ABI Prism 7500, several replicates of both telomere and 36B4 standard curves were analyzed for both between- and within-run precision analysis.

Standard curves were generated for both telomere and 36B4 reactions using the ABI Prism 7500 SDS software over multiple runs. Between run slope values for telomere and 36B4 reactions averaged - 4.20 ± 0.48 and -3.15 ± 0.3, respectively (n=9 for each assay). R^2 values averaged 0.987 ± 0.02 and 0.996 ± .01, respectively (n=9). Both telomere and 36B4 amplifications were shown to be reliable and reproducible over a large range of template quantities tested. When samples were measured multiple times, average standard deviations were 0.182 and 0.262 (telomere) and 0.086 and 0.228 (36B4), for within and between run, respectively. Lower limits of detection were also investigated: 36B4 was detectable from 100 ng to .2 ng, and telomere amplification product was detected from 100 ng down to 3.125 ng of sample. Sample quantities were not tested above 100 ng.

Genomic DNA extracts from several individuals were tested in duplicate for final validation of the technique. Relative telomere length for each sample was measured by determining the ratio of the real-time amplification of the telomere repeats (T) versus the single copy 36B4 (S), as compared to a reference sample, also known as the T/S ratio. While most replicates had only small variations in the T/S ratios as calculated, others had larger variations, but were still well within the expected range. Actual telomere length was also calculated, and found to be concordant in most cases.

Detection of relative telomere length using the ABI Prism 7500 allows for rapid and easy analysis of large numbers of samples in a forensic laboratory. This technique proved to be highly precise and reproducible from run to run, both between and within lot numbers of reaction mix. A successful age range determination technique would potentially have a significant impact on the forensic community by providing investigative leads for cases with no active suspect. **Telomeres, Real-Time PCR, Age Determination**

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