A23 Determination of an Effective Housekeeping Gene for the Quantification of mRNA for Forensic Applications

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After attending this presentation, attendees will understand the underlying concepts of current serological methods, the need to establish techniques that confidently and precisely identify biological fluids other than those commonly used in blood and semen identification, the importance of mRNA quantification, and the impact that this procedure might have on serological applications.

This presentation will impact the forensic science community by providing insight into a method that might improve the already existing technologies for the identification of body fluids.

The potential application of mRNA for the identification of biological fluids using molecular techniques is a recent development in

forensic serology. However, the rate of degradation and instability of mRNA is relatively high compared to DNA. Since the amount of biological material is often limited in forensic cases, it is critical to determine the appropriate quantification method to gather the most amount of information from a given sample. Constitutively expressed housekeeping genes can be used to assess the amount of mRNA recovered from a sample and establishes its suitability for downstream applications using real-time polymerase chain reaction (RT-PCR).

After an in-silico query to determine commercial availability, amplicon size, level and consistency of expression among a variety of tissues, six housekeeping genes were selected for this study. Relative quantification was utilized to compare the degree of expression of the selected genes from forensic-like body fluid stains obtained from semen, saliva, blood, menstrual blood, and vaginal secretions, in order to establish which would be the most appropriate housekeeping gene for the assessment of human mRNA quantity prior to profiling. Five consenting donors provided the samples of the various fluids. The housekeeping genes glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta actin (ACTB), beta-2-microglobulin (B2M), cyclophilin A (PPIA), phosphoglycerate kinase 1 (PGK1), and ribosomal protein large P0 (RPLP0), were compared to determine which was the most consistently expressed between the different donors for any one of the examined body fluids. The number of cycles necessary for the sample fluorescence to exceed that of the background fluorescence, called the cycle threshold (Ct), were compared using GAPDH as a reference for normalization across samples.

The results indicated that overall, β-2-microglobulin and β-actin exhibited the highest expression level across all body fluids examined. The same results were observed when an across donor comparison was made. In addition, both genes were highly expressed after exposure to environmental conditions (June, no precipitation) for up to 24 hours, indicating that mRNA is suitable for analysis if recovered during this period of time.

Consequently, ACTB or B2M appear to be the best candidates from the set of housekeeping genes analyzed for human-specific mRNA quantification prior to mRNA profiling for forensic applications. **Serology, Messenger RNA, Real-Time PCR**

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