RELATIVE AND ABSOLUTE QUANTIFICATION OF THE GAPDH HOUSEKEEPING GENE

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The positive identification of human body fluid stains such as saliva, semen, and vaginal secretions can have important probative value in criminal cases. One confirmatory approach to stain identification is based on the differential expression of cellular genes. In this approach, stain-specific mRNA transcripts are detected and normalized against the expression of a housekeeping gene such as GAPDH, i.e. relative quantification. Several commercial QPCR assays are available to detect GAPDH expression; however, the described QPCR method offers certain advantages including enhanced detection of GAPDH expression in a sub-group of tested samples.

The described method amplifies a region of GAPDH cDNA which spans the second and third exons and thus virtually eliminates the possibility of amplifying the GAPDH gene itself or possible pseudogenes. The method may be utilized for either relative quantification as described above or for absolute quantification. In the latter approach, the determined level of GAPDH expression may be useful as an approximation of the total mRNA level in samples.

The standard for absolute quantification is a synthetic oligo which corresponds to a 165 nucleotide portion of the GAPDH cDNA. Using this standard, the method was found to be highly sensitive and capable of detecting fewer than 10 copies of GAPDH transcript. The standard was also suitable for a commercial GAPDH assay in which similar standard curve plots were produced. As an alternative, cDNA prepared from total human RNA may be used as the standard for absolute GAPDH quantification.

Using absolute quantification, certain samples which tested positive for the described method tested negative or exhibited reduced quantities when using a commercial method. This outcome may be explained by the existence of alternative GAPDH alleles in such samples. The presence of SNP(s) which could potentially affect primer and or probe binding, and subsequently amplification efficiency, is being investigated.

The GAPDH QPCR method presented is highly sensitive and can be used for normalizing gene expression, for determining absolute quantities of GAPDH cDNA, and for estimating the total mRNA levels within a sample. In addition, the method minimizes false negative results and underestimation of GAPDH expression in a subset of samples tested.