B51 Optimal Small-Molecular Reference RNA for MicroRNA-Based Body Fluid Identification

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Learning Overview: After attending this presentation, attendees will better understand the importance of small-molecular reference RNA for forensically relevant Body Fluid Identification (BFID) based on quantitative PCR (qPCR) and the approach for determining the optimal reference RNA.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by identifying the optimal small-molecular reference RNA using the aggregate rank approach based on quantitative values and the expression consistency, and the development of miRNA-based BFID systems using the optimal small-molecular reference RNA identified.

Small-molecular RNAs such as microRNAs (miRNAs) are expressed in a cell-specific manner and resistant to environmental factors, and miRNA-based BFID systems have therefore attracted research attention worldwide. In qPCR-based BFID systems, the expression of the RNA is evaluated as ΔCq-value calculated by means of robust normalization performed using reference RNAs. The selected reference RNA must be expressed consistently among all samples and all individuals, and the copy number of the RNA should be high because the RNAs that are expressed at high levels tend to exhibit more stable expression in qPCR-based detections. In addition, normalization with multiple reference RNAs can be a reasonable solution in certain cases. The aim of this study is to identify the optimal reference RNA and the combination of reference RNAs in forensically relevant body fluids.

From five volunteers, each who provided their written informed consent, four forensically relevant body fluid samples were collected: venous blood, saliva, semen, and vaginal secretions. First, 11 candidate reference RNAs were quantified by qPCR using the miRCURY LNA™ Universal RT miRNA PCR System (Exiqon), which was a SYBR Green dye-based method to increase the specificity of binding to target sequences using locked nucleic acid technology in amplification primers. All qPCR results were obtained under compliance with the essential information provided in the MIQE guidelines.[1] Then, consistency of expression of candidate RNAs in body fluids was evaluated by NormFinder and BestKeeper, and last the optimal reference RNA and the combination of the multiple RNAs were determined based on each rank and index output from two tools using the RankAggreg package of R.[2-4] This study was approved by the ethics committee of the Graduate School of Medicine of Kyoto University.

The aggregate rank approach showed that 5S-rRNA was the best, it was followed by miR-103a-5p, miR-484, RNU1A1 and miR-92a-3p, that the best pair was the combination of 5S-rRNA and miR-92a-3p followed by 5S-rRNA and miR-484, and 5S-rRNA and miR-103a-5p, and that the best trio was the combination of 5S-rRNA, miR-484 and miR-92a-3p followed by 5S-rRNA, miR-103a-5p and miR-484, and 5S-rRNA, miR-103a-5p and miR-92a-3p. The present study concluded that 5S-rRNA was the optimal reference RNA for the normalization of miRNA expression in forensically relevant body fluids and 5S-rRNA combined with miR-92a-3p and/or miR-484 enhanced the normalization quality. Our findings enable us to perform stringent normalization of the expression of body fluid-specific RNAs, and thus can contribute to the development of small RNA-based BFID systems.

Reference(s):

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3. M.W. Pfaffl et. al. *Biotechnol. Lett.* 26 (2004) 509–515.
4. V. Pihur et. al. *BMC Bioinformatics* 10 (2009) 62.

MicroRNA, Body Fluid Identification, Quantitative PCR