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EXPERIMENTAL VALIDATION OF BIOINFORMATICALLY DESIGNED GAPDH PRIMERS FOR RT-QPCR NORMALIZATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND LEUKEMIA CELL LINES

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Annotation. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a highly sensitive and reproducible molecular method widely used for quantitative gene expression analysis. Reliable normalization of RT-qPCR data depends on the use of stable internal reference genes and well-validated primers. GAPDH is among the most commonly used housekeeping genes due to its constitutive expression in diverse human tissues and cell types. This study aimed to experimentally validate previously in silico-designed GAPDH primer pairs and assess their applicability as reference tools for RT-qPCR normalization in peripheral blood mononuclear cells (PBMCs) and leukemia cell lines (CCRF-CEM and Jurkat). Amplification analysis confirmed efficient and specific GAPDH detection in all tested samples. Among eight designed primer pairs, primer pair 5 demonstrated superior amplification efficiency, optimal GC content, balanced melting temperatures, absence of self-complementarity, and an appropriate amplicon length of 97 bp. Jurkat and CCRF-CEM cell lines exhibited earlier Ct values compared to PBMCs, reflecting differences in GAPDH expression levels. No amplification was observed in the negative control, confirming assay specificity. The findings indicate that the selected GAPDH primer pair is suitable for accurate RT-qPCR normalization in both normal blood-derived cells and leukemia models.

Keywords: RT-qPCR, GAPDH, housekeeping gene, primer validation, PBMC, leukemia cell lines, CCRF-CEM, Jurkat, gene expression normalization.

Introduction. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) remains one of the most widely used molecular techniques for quantitative gene expression studies because of its high sensitivity, specificity, and reproducibility. Despite these advantages, the reliability of RT-qPCR results strongly depends on proper normalization strategies, particularly the selection of stable internal reference genes whose expression remains constant across different cell types and experimental conditions. Inaccurate normalization may lead to misleading gene expression interpretations, especially in comparative studies involving healthy and diseased cells.

Housekeeping genes are commonly used as endogenous controls for normalization due to their essential cellular functions and presumed constitutive expression. Among them, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most frequently applied reference genes in molecular biology. GAPDH encodes a key glycolytic enzyme involved in cellular energy metabolism and is expressed ubiquitously across human tissues. Its stable

transcriptional activity and extensive historical use make it a practical candidate for RT-qPCR normalization in many biological systems, including hematological and oncological research.

However, successful use of GAPDH as a reference gene requires not only stable expression but also carefully designed primer pairs that ensure amplification specificity, efficiency, and reproducibility. Bioinformatic (in silico) primer design provides an effective preliminary strategy for selecting candidate primers based on GC content, melting temperature, amplicon size, and structural stability. Nevertheless, computational predictions must be experimentally validated to confirm practical performance under laboratory conditions.

This study focuses on validating GAPDH primers previously designed through in silico analysis and evaluating their suitability for RT-qPCR normalization in peripheral blood mononuclear cells (PBMCs) as representative normal blood cells, and in CCRF-CEM and Jurkat leukemia cell lines as malignant hematological models. The validation of reliable reference primers for these cellular systems is essential for improving the accuracy of gene expression studies in leukemia-related molecular research.

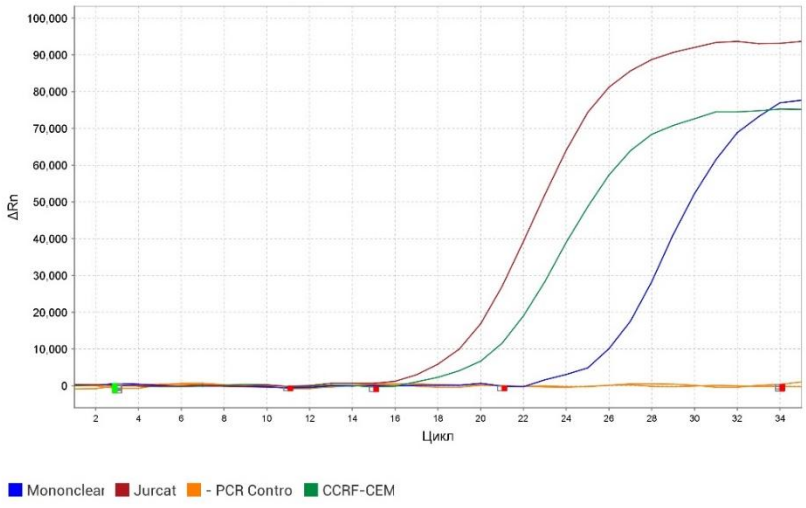
Reverse transcription quantitative PCR (RT-qPCR) is a widely applied method for quantitative analysis of gene expression, offering high sensitivity, specificity, and reproducibility [1]. Accurate data interpretation requires normalization using an internal reference gene with stable expression across different experimental conditions [1,2].

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most frequently used housekeeping genes for this purpose [3]. It encodes a key enzyme of the glycolytic pathway, is ubiquitously expressed in human cells, and is located on chromosome 12. Due to its constitutive expression and extensive application in molecular studies, GAPDH is widely considered a convenient reference gene for normalization [3,4]. Owing to its widespread application, GAPDH is considered a practical internal control in various experimental models, including blood-derived cells and leukemia cell lines [5].

The aim of this study was to validate GAPDH primers designed in a prior *in silico* analysis and to evaluate their suitability for qPCR-based normalization in peripheral blood mononuclear cells (PBMCs) and leukemia cell lines (CCRF-CEM and Jurkat).

The amplification analysis demonstrated specific and efficient detection of GAPDH across all examined samples, including peripheral blood mononuclear cells (PBMCs) and leukemia cell lines (CCRF-CEM and Jurkat) (Fig.1). All amplification curves demonstrated clearly defined exponential phases.

Figure 1.



Jurkat cells showed the earliest amplification (Ct ≈ 16), followed by CCRF-CEM (Ct ≈ 17), while PBMC samples demonstrated later amplification (Ct ≈ 23).

Among the eight designed primer pairs, primer pair 5 demonstrated the best performance; therefore, only its amplification data are presented in *Fig.1*, as it yielded superior results compared to the others. This primer pair was characterized by optimal GC content, closely matched melting temperatures, absence of self-complementarity, and an appropriate amplicon length (97 bp), collectively ensuring efficient and specific amplification across all samples.

No amplification was observed in the negative control.

Overall, the selected primer pair is suitable for RT-qPCR-based normalization in both PBMCs and leukemia cell lines.

References:

1. Bustin S. A., Benes V., Garson J. A., Hellemans J., Huggett J., Kubista M., Mueller R., Nolan T., Pfaffl M. W., Shipley G. L., Vandesompele J., Wittwer C. T. The MIQE guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments // Clinical Chemistry. – 2009. – Vol. 55, No. 4. – P. 611–622.
2. Vandesompele J., De Preter K., Pattyn F., Poppe B., Van Roy N., De Paepe A., Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes // Genome Biology. – 2002. – Vol. 3, No. 7. – P. research0034.
3. Barber R. D., Harmer D. W., Coleman R. A., Clark B. J. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues // Physiological Genomics. – 2005. – Vol. 21, No. 3. – P. 389–395.
4. Kozera B., Rapacz M. Reference genes in real-time PCR // Journal of Applied Genetics. – 2013. – Vol. 54, No. 4. – P. 391–406.
5. Chapman J. R., Waldenström J. With reference to reference genes: a systematic review of endogenous controls in gene expression studies // PLOS ONE. – 2015. – Vol. 10, No. 11. – e0141853.