AUTOR: Łukasz Fuławka

TYTUŁ PRACY: The assay for analysis of housekeeping genes expression – the reliable evaluation method of RNA quality with potential application in molecular pathology

AFILIACJA:

Introduction:
Reverse transcription quantitative (real-time) polymerase chain reaction (RT-qPCR) is an important molecular tool for the analysis of gene expression, intended for both research and diagnostic purposes. The first stage of RT-qPCR reaction is reverse transcription of RNA isolated from various types of biological material – blood, swabs, saliva and tissue sections. The product of this stage is cDNA, which serves as a template for subsequent qPCR reactions. The most available source of tissue samples for molecular analysis are paraffin-embedded tissue specimens (FFPEs). Unfortunately, the quality of the RNA, especially mRNA, in such material may be low, due to the fact that the RNA generally has a short half-life and, in addition, may be degraded during fixation, making it impossible to interpret the results correctly. To address this problem, we designed test to analyze the expression of housekeeping genes. The aim of this project is to prepare a simple, cost-effective assay for the analysis of RNA quality in biological material. Two housekeeping genes have been selected: beta actin (ACTB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Materials and Methods:
The reagent kits used were: Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific), QuantiFast SYBR Green PCR Kit (Qiagen), and two pairs of primers for ACTB and GAPDH. As a source of genetic material served remnant diagnostic samples from Cellgen Biobank (throat, buccal and cervical swabs). RNA was isolated using various kits (EliGene® Viral RNA/DNA FAST Isolation Kit, Invisorb® Spin Universal Kit, PowerPrep™ Viral DNA/RNA Extraction Kit, TANBead® Nucleic Acid Extraction Kit, ANDiS Viral RNA Auto Extraction & Purification Kit). The reference material was Universal Human Reference RNA (ThermoFisher Scientific).

Results:
Analysis of melting curves allowed to conclude that the reaction products were specific. Additionally, electorophoresis of products on agarose gel confirmed specific length of amplicons. A standard curve revealed very high correlation coefficient R2 = 0.9955-0.9956. The LOD (limit of detection) is less than 3* 10-3 ng/ µl. Moreover, the method has satisfactory linearity, repeatability and reproducibility. The expression of both genes appears to be correlated.

Conclusions:
A non-commercial simple but specific test for the analysis of two common housekeeping genes in human biological material has been developed. The direction of the research is very important, as it allows to evaluate RNA quality and therefore to minimize false negative results in molecular testing. The aim of the ongoing stage of this project is to validate the test on FFPEs. We believe that results of this step will improve the quality of research and diagnostic tests in molecular pathology.

Authors:

Łukasz Fuławka 1, Aleksandra Kuzan 1,2 , Łukasz Madej 1

1Molecular Pathology Centre Cellgen, Wrocław
2Wroclaw Medical University, Wrocław

 