Selection of reference genes for quantitative RT-PCR (RT-qPCR) analysis of rat tissues under physiological and toxicological conditions

In biological research, the analysis of gene expression levels in cells and tissues can be a powerful tool to gain insights into biological processes. For this, quantitative RT-PCR (RT-qPCR) is a popular method that often involve the use of constitutively expressed endogenous reference (or ‘housekeeping’) gene for normalization of data. Thus, it is essential to use reference genes that have been verified to be stably expressed within the specific experimental setting. Here, we have analysed the expression stability of 12 commonly used reference genes (Actb, B2m, Gapdh, Hprt, Pgk1, Rn18s, Rpl13a, Rps18, Rps29, Sdha, Tbp and Ubc) across several juvenile and adult rat tissues (liver, adrenal, prostate, fat pad, testis and ovaries), both under normal conditions and following exposure to various chemicals during development. Employing NormFinder and BestKeeper softwares, we found Hprt and Sdha to be amongst the most stable genes across normal and manipulated tissues, with several others also being suitable for most tissues. Tbp and B2m displayed highest variability in transcript levels between tissues and developmental stages. It was also observed that the reference genes were most unstable in liver and testis following toxicological exposure. For future studies, we propose the use of more than one verified reference gene and the continuous monitoring of their suitability under various experimental conditions, including toxicological studies, based on changes in threshold (Ct) values from cDNA samples having been reverse-transcribed from a constant input concentration of RNA.

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