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Validation of Tuba1a as appropriate internal control for normalization of gene expression analysis during mouse lung development

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Abstract

© 2015 by the authors; licensee MDPI, Basel, Switzerland. The expression ratio between the analysed gene and an internal control gene is the most widely used normalization method for quantitative RT-PCR (qRT-PCR) expression analysis. The ideal reference gene for a specific experiment is the one whose expression is not affected by the different experimental conditions tested. In this study, we validate the applicability of five commonly used reference genes during different stages of mouse lung development. The stability of expression of five different reference genes (Tuba1a, Actb Gapdh, Rn18S and Hist4h4) was calculated within five experimental groups using the statistical algorithm of geNorm software. Overall, Tuba1a showed the least variability in expression among the different stages of lung development, while Hist4h4 and Rn18S showed the maximum variability in their expression. Expression analysis of two lung specific markers, surfactant protein C (SftpC) and Clara cell-specific 10 kDA protein (Scgb1a1), normalized to each of the five reference genes tested here, confirmed our results and showed that incorrect reference gene choice can lead to artefacts. Moreover, a combination of two internal controls for normalization of expression analysis during lung development will increase the accuracy and reliability of results.

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Keywords

Alpha 1A tubulin, geNorm, Mouse lung development, qRT-PCR, Reference gene, Tuba1a