

The Screening of Housekeeping Genes for Quantitative PCR Studies in Rat Urinary Bladder with or without Obstruction

Introduction and Objectives: Rat bladder outlet obstruction (BOO) model is commonly used for the study of lower urinary tract obstruction and dysfunction. Both the mucosa and muscular layer of bladder play important roles. Quantitative real-time PCR is often used to investigate the regulation of transcription in the urinary bladder. A reliable normalization is crucial for the accurate quantification. In this study, we investigated the expression stability of seven housekeeping genes in the mucosa and muscular layer of rat bladder with or without obstruction.

Materials and Methods: The mucosa and muscular layer were harvested from rat urinary bladder with or without obstruction. The gene expression levels of 7 housekeepers, hypoxanthine guanine phosphoribosyltransferase (HPRT), tyrosine 3-monooxygenase (YWHAZ), succinate dehydrogenase complex (SDHA), TATA box binding protein (TBP), ubiquitin C (UBC), β -actin (ACTB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were investigated by using real-time PCR. The data was analyzed using different Visual Basic Applications: geNorm and NormFinder.

Results: In the muscular layer, YWHAZ and HPRT are the two most stably expressed housekeeping genes according to the analysis with geNorm. When using NormFinder, YWHAZ is still the most stable single one. However, because YWHAZ and HPRT show the same tendency in intergroup variation, NormFinder selected TBP and ACTB as the best combination for normalization. In the mucosa, geNorm also selected YWHAZ and HPRT as the best combination, while SDHA is the most stable single housekeeping gene when considering normalization factor value. According to the analysis with NormFinder, SDHA is the most stable single one, too. YWHAZ and HPRT also show the same tendency in intergroup variation. NormFinder selected SDHA and ACTB as the best combination for normalization.

Conclusions: To compare the expression level of target gene in the muscular layer of normal rat bladder with obstructed one, TBP and ACTB are proposed to the best combination for normalization. In the case of mucosa, SDHA and ACTB are recommended to calculate normalization factor. If a single reference gene is to be used, YWHAZ is recommended for muscular layer and SDHA for mucosa. Our results may facilitate the choice of reference genes for expression studies in rat BOO model.