

## Abstract

Housekeeping genes (HKGs) are metabolic (also known as constitutive genes) which remain potentially active throughout the life span of an organism. Selection of appropriate housekeeping genes (HKGs) is the prime step for the validity of data normalization of real time PCR (RT-PCR). In present study, it is focused to compare the stability of HKGs systematically to identify stable endogenous internal control for all control Covid-19 experimental groups (healthy candidates), and Covid-19 effected groups. Expression levels of 10 housekeeping genes (HKGs) from cohort of 40 blood samples (10 from healthy candidates, 10 from mild, 10 from moderate, and 10 from ICU admitted patients) is exploited for the identification of most stable housekeeping genes i.e., potentially expression level remained active in all molecular conditions (normal verses viral infected cells). Gene expression stability has determined by using three different biostatistical based applets, geNorm, NormFinder, and Bestkeeper, which are specially designed software to determine stability character of each housekeeping gene by automated generation of stability values. The results suggested that HPRT1, RPL13a and UBE2D2 showed significant variation in their expression levels, and graded as the least stable housekeeping genes (HKGs). However, GAPDH,  $\beta$ -actin, and B2M housekeeping genes were identified as the most stable housekeeping genes (HKGs) to carry out genetic based expression analysis of all samples associated with Covid-19. This study has applied the knowledge of bioinformatics, molecular biology, immunological, pathological, and biotechnological aspects in an integrated manner to pave the ways for SARS-CoV-2 transcriptome analysis for the development of effective vaccines against Covid-19 in future.