

Instability of standard PCR reference genes in adipose-derived stem cells during propagation, differentiation and hypoxic exposure.

Fink T, Lund P, Pilgaard L, Rasmussen JG, Duroux M, Zachar V.

Laboratory for Stem Cell Research, Aalborg University, Denmark. trinef@hst.aau.dk

BACKGROUND: For the accurate determination of gene expression changes during growth and differentiation studies on adipose-derived stem cells (ASCs), quantitative real-time RT-PCR has become a method of choice. The technology is very sensitive, however, without a proper selection of reference genes, to which the genes of interest are normalized, erroneous results may be obtained. **RESULTS:** In this study, we have compared the gene expression levels of a panel of twelve widely used reference genes during hypoxic culture, osteogenic and chondrogenic differentiation, and passaging of primary human ASCs. We found that several of the commonly used reference genes including 18S rRNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin were unsuitable for normalization in the conditions we tested, whereas tyrosine 3/tryptophan 5-monoxygenase activation protein (YMHAZ), TATAA-box binding protein (TBP), beta-glucuronidase (GUSB) were the most stable across all conditions. **CONCLUSION:** When determining gene expression levels in adipose-derived stem cells, we recommend normalizing transcription levels to the geometric mean of YMHAZ, TBP and GUSB.