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Optimization of qPCR condition for some specific genes of equine mesenchymal stem cell

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Abstract

Equine mesenchymal stem cells (MSCs) serve as a valuable resource for equine regenerative medicine in orthopedics. The first step on the way of their application is identification of characteristics and differentiation capabilities of these cells by different methods. Quantitative - polymerase chain reaction (qPCR) is one the most powerful technique for assessment of gene expression profile at mRNA level. The exact quantification (absolute or relative) depends on an accurate qPCR conditions. Because of limited data on gene expression analysis of equine stem cells, we aimed to optimize qPCR condition for some specific genes of equine MSCs.Total RNA was extracted from equine adipose-derived MSCs (AD-MSCs) using TRIzol reagent and subsequently cDNAs was synthesized through standard procedure. Primers were designed using the software Beacon and Oligo7 for common genes in equine MSC studies including GAPDH, RUNX2, SSP1, PPARG, SCX, MKX, COL1 A1, TNMD, SOX9, TGFBRIII, BMPR2, and CTNNB1. Seven points serial dilutions of cDNA template were prepared. All samples were run in triplicate samples using fluorescent SYBR Green. We achieved high efficiency for equine AMSCs reference gene (GAPDH) as well as other target amplicons including osteogenic markers (RUNX2, SSP1), adipogenic markers (PPARG), tenogenic markers (SCX,MKX,COL1 A1, TNMD), chondrogenic markers (SOX9) and some other ones associated with specific cell signaling pathways (TGFBRIII,BMPR2, CTNNB1). The optimization of reaction condition especially quantification of designed primers is critical for accurate interpretation of qPCR data. Our study achieved the appropriate efficiency for given reference and target genes of equine AMSCs which can be used in quantitative gene expression studied.
Keywords: Equine, Mesenchymal Stem Cell, Optimization, qPCR

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