

## The use of microRNAs as universal reference genes in quantitative PCR

Kulcheski, FR<sup>1</sup>; Marcelino, FC<sup>2</sup>; Nepomuceno, AL<sup>2</sup>; Abdelnoor, RV<sup>2</sup>; Margis, R<sup>1</sup>

<sup>1</sup> Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, UFRGS

<sup>2</sup> EMBRAPA Soja, Rodovia Carlos João Strass, Distrito de Warta, CEP 86001-970, Londrina, PR, Brazil

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Reverse transcription quantitative real-time PCR (RT-qPCR) is a robust and widely applied technique used to investigate gene expression. However, for correct analysis and interpretation of results, the choice of a suitable gene to use as an internal control is a crucial factor. These genes, such as housekeeping genes, should have a constant expression level in different tissues and across different conditions. The advances in genome sequencing have provided high-throughput gene expression analysis and have contributed to the identification of new genes, including microRNAs (miRNAs). The miRNAs are fundamental regulatory genes of eukaryotic genomes, acting on several biological functions. In this study, miRNA expression stability was investigated in different soybean tissues and genotypes as well as after abiotic or biotic stress treatments. The present study represents the first investigation into the suitability of miRNAs as housekeeping genes in plants. The transcript stability of ten miRNAs was compared to those of six previously reported housekeeping genes for the soybean. In this study, we provide evidence that miRNA expression stability can be greater than the expression stability of protein-coding genes. In addition, we conclude that miRNAs are optimal reference genes not only

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