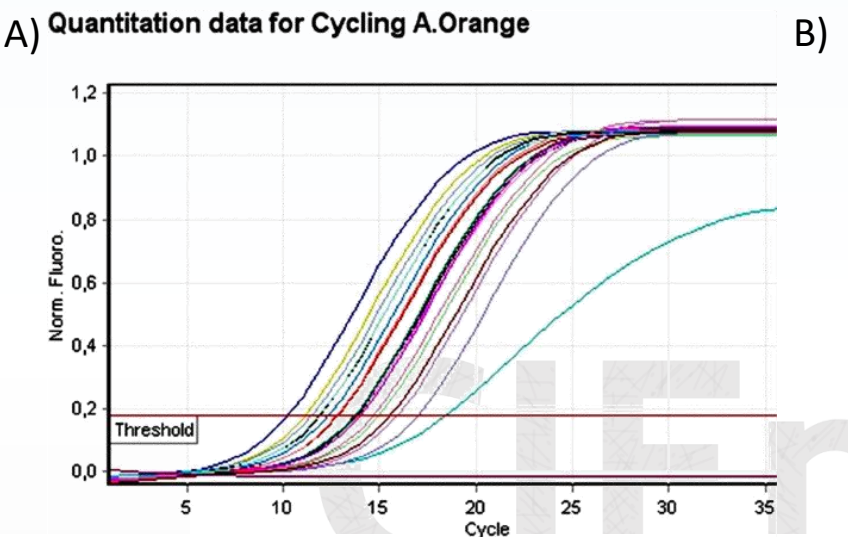
Efficacy

PCR (polymerase chain reaction) assay

Developed by Karry Mullis in 1983, the polymerase chain reaction (PCR) technique allows the amplification of small amounts of nucleic acids *in vitro*, which enabled the development of other techniques, such as quantification of gene expression, differential analysis of transcripts, among others (1- 3). Among the various techniques, the PCR-assay consists of detecting a range of genes simultaneously, making it possible to perform multi-gene expression screening quickly and efficiently (3-5). The detection is made by the intercalation of fluorescent substances during the amplification of specific segments. The difference in fluorescence is detected by the device in real time, making it possible to compare levels of gene expression in a given sample (4-5). Gene targets can be modified according to the pathway of interest analyzed.

Validation Data

A)



B)

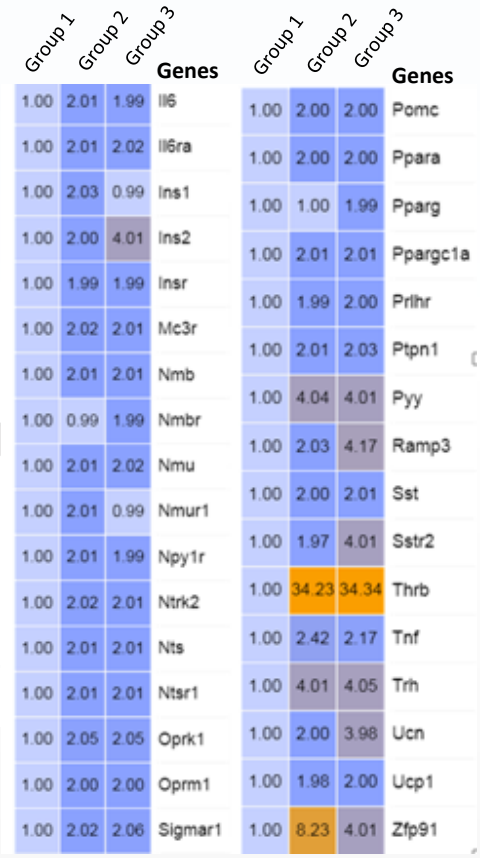


Figure: Graphical representation of the PCR-assay reaction. (A) Graphical representation of the difference in fluorescence intensity at each cycle of the PCR-assay reaction. We can observe the amplification difference from the Threshold established during the analysis of the reaction. (B) Results by mRNA expression levels were normalized to the expression level of housekeeping gene using the $2^{-\Delta\Delta Ct}$ formula. The results was expressed as the fold change. The heat map shows differences in expression between the indicated groups for every identified gene.

The PCR-assay technique has a high sensitivity, specificity, and speed in directing possible biological processes and mechanisms of action. With the PCR-assay technique it is possible to detect signaling pathways and gene families, thereby directing studies of pharmacological targets efficiently and at a lower cost.

References:

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[2] Mullis KB. **The Unusual Origin of the Polymerase Chain Reaction.** Sci Am. 1990 Apr;262(4):56-61, 64-5.
[3] Valasek MA, Repa JJ. **The Power of Real-Time PCR.** Adv Physiol Educ. 2005 Sep;29(3):151-9. doi: 10.1152/advan.00019.2005.
[4] Higuchi R, Fockler C, Dollinger G, Watson R. **Kinetic PCR Analysis: Real-Time Monitoring of DNA Amplification Reactions.** Biotechnology (N Y). 1993 Sep;11(9):1026-30.
[5] Bustin SA. **Absolute Quantification of mRNA Using Real-Time Reverse Transcription Polymerase Chain Reaction Assays.** J Mol Endocrinol. 2000 Oct;25(2):169-93.