Quantitative RT-PCR: Principles, applications, and possible translational approaches"

Polymerase Chain Reaction (PCR) has become the cornerstone of modern molecular biology. Quantitative RT-PCR PCR is an advanced form of the Polymerase Chain Reaction that maximizes the potential of the technique: The progress of a PCR reaction is monitored simultaneously to the reaction ("real-time"), and quantitative statements about gene expression can be made. Generally, the technique is used to compare the expression of genes of interest in different samples of RNA. The PCR reaction generates fluorescence signals, and as the number of gene copies increases during the reaction, so the fluorescence increases. Taking into account certain experimental prerequisites, it is possible to calculate the relative expression rates of the targets of interest. The technique enables to evaluate differences between different cells, between different treatments, with or without genetic manipulation, between health and disease, etc. and thus is of outstanding scientific and medical relevance.

In lecture and course we will introduce basic principles of real-time PCR, and we will give an overview about different techniques and applications. The course will provide a hands-on qPCR training, including sample extraction and preparation, experimental design, reagents and methods, primer design, housekeeper evaluation, data analysis and statistics, and practical advice. Also, we will discuss the value of the technique as a tool in translational approaches.

In experimental setup we will use a human macrophage/monocyte cell line, we will incubate with or without inflammatory stimuli, and the expression of IL1b will be measured as an experimental read-out.

Literatur:

Stephen A Bustin et al.
The need for transparency and good practices in the qPCR literature
Nature Methods 11:1063-1067 (November 2013)

Jim F. Huggett, et al.
Guidelines for Minimum Information for Publication of Quantitative Digital PCR Experiments
Clin Chem 59:6 (2013)