

# Recent Advances in Relative Quantitation of Gene Expression

## with ABI PRISM™ 7700

PE Biosystems has developed guidelines enabling streamlined design and implementation of real-time quantitative PCR assays. The use of these guidelines makes it easy to apply either the fluorogenic 5' nuclease assay, or SYBR® Green 1 double stranded DNA binding dye chemistry, to any real-time quantitative PCR system. Specific assay design and optimization guidelines minimize the time and cost of assay implementation, whilst providing reliable and robust assay performance. Furthermore the guidelines enable all assays to be run using the same thermal cycling parameters with a single reagent master mix.

· TaqMan® Universal PCR Master Mix has been developed for this purpose and contains enhanced buffer components to provide robust performance even when encountering the most difficult G/C-rich sequences. TaqMan® Universal PCR Master Mix is suitable for all real-time assays using DNA or cDNA as a substrate.

Multiplex TaqMan® assays can be performed on the ABI PRISM® 7700 Sequence Detection System due to its capability to detect multiple dyes with distinct emission wavelengths. The most common application where this is used is for relative quantitation of gene expression, where one probe labeled with FAM dye is used to detect the target species, and another probe labeled with VIC™ dye is used to detect an endogenous control gene (the utilization of the VIC™ dye provides both improved spectral resolution and signal strength over previously available dyes). Running both assays in a single tube reduces both the running costs, and the dependence on accurate pipetting when splitting a sample into two separate tubes.

PE Biosystems has utilized these features in developing the TaqMan® Cytokine Gene Expression Plate 1, a research tool for in vitro quantitative evaluation of cytokine gene expression. The TaqMan® Cytokine Plate I detects the expression of twelve human cytokine target sequences and an endogenous control in cDNA samples, and is ideally suited for relative quantitation studies using the comparative CT method. This method eliminates the need for standard curves because all quantities are expressed relative to a calibrator sample. The targets incorporated in the TaqMan® Cytokine Gene Expression Plate 1 are IL-1, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFNγ, and TNFα. The primers and probe for each target are dried down into eight wells, with all ninety-six wells containing dried down primers and probe for the detection of ribosomal RNA as the endogenous control. All primer and probe concentrations are optimized for use with TaqMan® Universal Master Mix, making the addition of a specific cDNA sample the only step required before loading and running the plate.

The next generation of this product will be the TaqMan® Cytokine Card. This future product will enable reaction volumes to be reduced from 50 µl to 1 µl on the present instrument platform, resulting in a significant reduction in reagent consumption. The TaqMan® Cytokine Card will detect 24 human cytokine targets (in replicates of four). In addition to the pre-deposited primers and probe for the cytokine targets, each well will contain primers and probe for detection of ribosomal RNA endogenous control. This product will be accompanied by application specific software, which will perform all calculations required when utilizing the comparative CT method in relative quantitation of gene expression studies.

By developing application specific products such as the TaqMan® Cytokine Plate and Card in addition to providing rapid and robust assay optimization guidelines, PE Biosystems is facilitating rapid and easy to use assay solutions for researchers whether they wish to develop their own assay, or purchase a pre-developed assay.