

# evaluation of Quantitative PCR Master Mix sensitivity

One area of our group's current investigations is to elucidate the mechanism(s) by which immune tolerance is achieved in the female reproductive tract. As an immunocompetent structure exposed repeatedly to allogenic spermatozoa, the female reproductive tract must somehow be modified by tolerogenic factors so that sensitisation does not occur.

The development of a state of tolerance means that acute inflammatory reactions are avoided on repeated exposure and do not result in the destruction of the spermatozoa as they ascend the reproductive tract following coitus. To try to understand how tolerance is achieved we are studying the effects of a variety of extracts from human semen on dendritic cells (antigen-presenting cells), monocytes and neutrophils. We are using an ABI PRISM® 7700 Sequence Detector and ABI's TaqMan® methodology to measure the relative changes in cytokine expression following treatments.

RNA is extracted from the cells using standard methods and is then reverse transcribed using the TaqMan® Reverse Transcription Kit (ABI). Next, quantitative PCR is performed to measure changes in the expression of several cytokines, including IL-1 $\alpha$  and IL-8.

Some of the messages we are interested in are expressed at low levels and are detected only late in the PCR. This means that when looking for changes in expression (especially inhibition of expression) the methodology is stretched to the limits of sensitivity and may not be entirely reliable. We were therefore looking for a way of increasing amplification product without compromising the accuracy.

One alternative we chose was to evaluate the performance of our current chemistry against other, newly available chemistries. We compared ABgene's 2x Thermo-Start® PCR Master Mix\* with ROX to our current PCR mix (Supplier S). We did this by amplifying and quantifying expressions of IL-1 $\alpha$  and IL-8 using ABI's TaqMan® probes (final concentration 300 $\mu$ M, primers at 200 $\mu$ M) from a range of RNA samples (extracted from neutrophils differentiated from HL60 cells). We used 18-S ribosomal RNA as an internal standard using the TaqMan® Ribosomal RNA Control Reagents (VIC Probe Kit, ABI).

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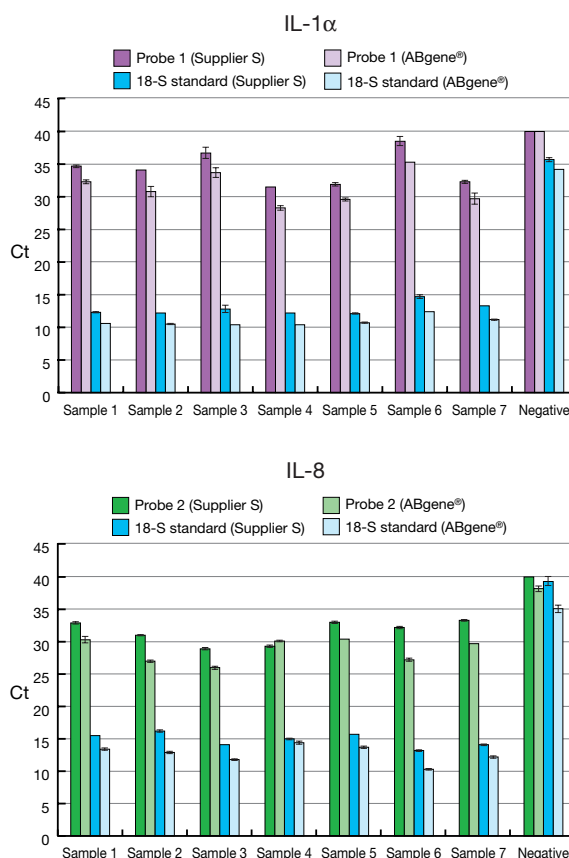
[e.faccenda@hrsu.mrc.ac.uk](mailto:e.faccenda@hrsu.mrc.ac.uk)

Specific probe and primer sequences were as follows:

- IL-1 $\alpha$ :** Probe: 5'-FAM-CAGGCATCTCCTTCAGCAGCACTGGTTG-TAMRA-3'  
Forward primer: 5'-TGTATGTGACTGCCCAAGATGAA-3'  
Reverse primer: 5'-CTACCTGTGATGGTTTTGGGATC-3'
- IL-8:** Probe: 5'-FAM-CCTTCCTGATTTCTGCAGCTCTGTGTGAA-TAMRA-3'  
Forward primer: 5'-CTGGCCGTGGCTCTCTTG-3'  
Reverse primer: 5'-TTAGCACTCCTTGGCAAACCTG-3'

We found that, for each sample of target RNA, the threshold cycle (Ct) was consistently lower using the ABgene® master mix\* (data shown below), indicating that the amplification was more powerful, resulting in target detection at an earlier PCR cycle. On average we found an approximate change in Ct of 10% for both

cytokines. Apart from the time/cost benefits, we expect this increase in quantitative sensitivity to be useful in studying gene regulation overall but especially in circumstances of low level or down regulation of mRNA transcription.



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