

Last minute POSTER CALL

deadline until 31 January 2004

Please submit abstract as an attached **Word document** to:

gpcr2004@wzw.tum.de

The poster abstracts will be evaluated by the Scientific Committee, and acceptance notified to contributors until **6th of February 2004**.

The symposium proceedings including all **Abstract, Posters and Oral Presentations** will be published on the **Gene-Quantification** web page (<http://www.wzw.tum.de/gene-quantification/gpcr2004/>).

Until August 2004 the PDF download of the contributions is password protected and only accessible for Symposium or Application Workshop participants. The abstract should be a **one-page document** based on unpublished material and written in good standard English. The text should clearly state objectives, methodology, results, and conclusion. Simple tables and graphs are allowed.

Poster will be DIN A0 (portrait format :120 cm/48 inch high x 90 cm/35 inch wide)

Presentation of every communication at the Conference is subjected to **formal registration** of at least one co-author.

Abstract Layout:

Please type the abstract (**single line spacing**) using **Times New Roman font 12**.

Justify both right and left margins. Leave **1 inch (2.5 cm) margins all around** (top/bottom/left/right).

Title in bold (initial capital only)

Leave one line space

Author(s) family name followed by first name in capital letters. Please **underline** the presenting author.

e-mail of the corresponding author

Leave one line space

To indicate **affiliations** use superscripts 1, 2, 3, 4 etc using **Times New Roman font 10**

Leave one line spaces

Text

Advanced quantitative real-time PCR in clinical diagnostics and cDNA micro-array validation

STÅHLBERG A¹, HÅKANSSON J², XIAN X², SEMB H², PFAFFL MW³, ÅMAN P⁴ & M KUBISTA¹ (anders.stalberg@molbiotech.chalmers.se)

1 Department of Molecular Biotechnology, Chalmers University of Technology and TATAA Biocenter, Sweden

2 Department of Medical Biochemistry, Gothenburg University, Sweden

3 Institute of Physiology, FML-Weihenstephan, Technical University of Munich, Germany

4 Department of Pathology, Lundberg laboratory for cancer research, Gothenburg University, Sweden

Real-time PCR is the method of choice for quantitative studies of gene expression. The method has been an important tool in basic research for some years, and has now also started to replace more conventional methods in clinical diagnostics. Real-time PCR is characterized by a wide dynamic range of quantification, high sensitivity and high precision. One of the major problems in DNA quantification is to account for PCR inhibitions appropriately. We have developed an in situ calibration method based on either addition of known amount of target DNA or dilution of the test sample to determine sample specific PCR efficiencies. Relative gene expression in clinical samples and cDNA microarray validations are applications particular suitable for in situ calibration, resulting in high accuracy. In situ calibration is particular suitable for a few samples per gene investigations, for example: cDNA microarray validation and relative gene expression in clinical samples. Further, the efficiency and reproducibility of various reverse transcription assays has been carefully evaluated. Our results suggest that sample to sample variation in reverse transcription is significantly higher than in real-time PCR, except when quantifying very low copy numbers. The efficiency of reverse transcription differs significantly between genes and priming strategy. The reproducibility of reverse transcription and real-time PCR suggest that the least difference in mRNA that can be significantly measured is ~50 % when comparing two genes in one sample and ~100 % when comparing expression of genes in two samples.