

# 1<sup>st</sup> International qPCR Symposium & Application Workshop

26. Feb 2004

## Symposium Agenda

### Wednesday 3 March 2004

#### Welcome & Opening of the Symposium Lecture hall HS 14

- 08:00 – 10:00 Built-up for Industrial Exhibition
- 10:00 – 11:30 Arrival & Registration
- 11:30 – 12:30 **Get-together lunch in the student cafeteria**
- 12:30 "Welcome & Opening of the Symposium."  
Michael W. Pfaffl & Mikael Kubista  
Scientific coordination of the Symposium & Workshop
- 12:45 "Welcome at the Center of Food & Life Science in Freising Weihenstephan."  
Bertold Hock, Dean of the Center of Food & Life Science-Weihenstephan, Freising, Germany
- 13:00 "qPCR and Transcriptomics: Creation of a new tool to understand life."  
Heinrich H. D. Meyer, Physiology - Weihenstephan, Freising, Center of Food & Life Science-Weihenstephan, Germany
- 13:45 "Pitfalls in the quantification of RNA using real-time RT-PCR."  
Stephen Bustin, Reader in Molecular Medicine, School of Medicine, London
- 14:30 – 15:00 **Coffee break**

#### Session: Pre-Analytical Steps

Chairs: M. Kubista & D. Grove  
Lecture hall HS 14

- 15:00 Session introduction by M. Kubista & D. Grove
- 15:10 "Quantitative gene expression analysis by Real-time PCR - How to optimise the reverse transcription and real-time PCR reactions."  
Anders Stahlberg, Mikael Kubista, Chalmers University, Sweden
- 15:40 "Overcoming bias in quantitative RT-PCR: Different methods of reverse transcription influence sensitivity and accuracy of gene expression patterns."  
Ginzinger DG<sup>1</sup>, Yu M<sup>1</sup>, Schuster D<sup>2</sup> & A Rashtchian<sup>2</sup>;  
University of California San Francisco, Comprehensive Cancer Center, Genome analysis core facility, San Francisco, CA, USA; <sup>2</sup> Quanta BioSciences, Inc. Gaithersburg, MD 20877, USA
- 16:00 "RNA Integrity Number (RIN) –Standardization of RNA Integrity Measurements."  
Odilo Mueller<sup>1</sup>, Andreas Schroeder<sup>1</sup>, Samar Lightfoot<sup>2</sup>, Ruediger Salowsky<sup>1</sup>, Susanne Stocker<sup>1</sup>, Thomas Ragg<sup>3</sup>;  
1 Agilent Technologies, Waldbronn, Germany; 2 Agilent Technologies, Palo Alto, USA; 3 Quantom Bioinformatics, Weingarten, Germany
- 16:20 "Gene expression quantitated in PAXgene™ frozen stored blood as compared to freshly Immuno Magnetic Separated (IMS) blood cells."  
Ovstebo R, Haug KBF, Kierulf P.; The Research and Development Group, Department of Clinical Chemistry, Ullevål University Hospital, Oslo, Norway
- 16:40 "Modified silica-magnetite composite as a universal matrix for nucleic acids isolation."  
Zhao X, Huang Z, Luan G; Biovision Biotech, Inc, China

17:00 – 18:00 **Poster session**

18:00 – 22:00 **Get-together with the Companies  
Bavarian Dinner Buffet in the Aula**

### Thursday 4 March 2004

#### Session: qPCR Application in Clinical Diagnostics

Chairs: S. Bustin & M. Pazzagli  
Lecture hall HS 14

- 08:30 Session introduction by S. Bustin & M. Pazzagli
- 08:40 "Real-time PCR is the most sensitive technique for biomolecular detection. Possibilities and Limitations in Research and in Clinical Diagnostics."  
Mikael Kubista, Chalmers University of Technology and the TATAA Biocenter, Göteborg, Sweden
- 09:10 "Laser capture microdissection and real-time PCR in Human Cancer."  
Pamela Pinzani, Prof. Mario Pazzagli, Clinical Biochemistry Unit, Dep. of Clinical Physiopathology Florence, Italy
- 09:40 "Microarray data of pooled samples compared to real-time-PCR of individual samples in African Children with Malaria."  
Yvonne Kalmbach, Institute for Tropical Medicine, Dept. of Parasitology, Wilhelmstr. 27, D-72074, Tübingen, Germany.
- 10:00 "Quantitative analysis of gene expression – a valuable tool in clinical immunology."  
Giese T<sup>1</sup>, Stallmach A<sup>2</sup>, Zeier M<sup>3</sup> & Meuer SC<sup>1</sup>; <sup>1</sup>Institute of Immunology, University Hospital Heidelberg; <sup>2</sup>Dept. Gastroenterology, Catholic Hospital Essen-Nord; <sup>3</sup>Dept. Nephrology, University Hospital Heidelberg
- 10:20 – 10:45 **Coffee break**

#### Session: qPCR Application in Microbiology & Virology

Chairs: U. Reischl & H. Nitschko  
Lecture hall HS 14

- 10:45 Session introduction by U. Reischl & H. Nitschko
- 10:50 "LightCycler Applications in Diagnostic Bacteriology."  
Udo Reischl, Institute of Medical Microbiology & Hygiene, Regensburg, Germany
- 11:20 "Genotyping and quantification of hepatitis C virus using fluorescent probes."  
Hans Nitschko, Max-von-Pettenkofer Institut, LMU; Munich, Germany sponsored by Abgene
- 11:50 "Dissection of the retroviral life cycle using real-time PCR assays."  
Klein D, Nosek D, Leichsenring B & Knapp E; Institute of Virology, University of Veterinary Medicine Vienna, Austria
- 12:10 "Real-time quantitative PCR assays for the detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients."  
Suda Magdalena, Matthes-Martin Susanne and Lion Thomas; Div. Mol. Microbiology and Development of Genetic Diagnostics, Children's Cancer Research Institute, Vienna
- 12:30 – 13:30 **Lunch in the student cafeteria**

## Session: Normalization & Standardization

Chairs: T. Köhler & M.W. Pfaffl

### Lecture hall HS 14

- 13:30 Session introduction by T. Köhler & M.W. Pfaffl
- 13:40 "Accurate normalization of gene expression using multiple internal control genes."  
Jo Vandesompele, Center of Medical Genetics, Ghent University, Belgium
- 14:10 "Normalization genes for heart failure."  
Kristin Brevik Andersson, Institute of Experimental Medical Research, Oslo
- 14:40 "Validation of housekeeping genes for normalising RNA expression."  
Jim Huggett, Center for Infectious Diseases, London, UK
- 15:10 – 15:40 **Coffee break**
- 15:40 "Relative Gene Expression Studies using Multiplex Quantitative PCR on the Bio-Rad iCycler iQ Real Time PCR Detection System."  
Hilary Srere, R & D Bio-Rad Laboratories, Hercules, CA, USA
- 16:10 "Standardized gene expression profiling and tumor prognosis."  
Thomas Köhler, Roboscreen, Leipzig, Germany
- 16:50 "A Housekeeping-Gene Free Zone for Normalization."  
Tania Nolan, Stratagene Europe, Amsterdam, The Netherlands
- 17:10 "Housekeeping gene expression in human seminoma and normal testicular tissue."  
Neuvians TP, Sauer CG, Bleyl U & Grobholzer R; Pathologisches Institut, Universitätsklinikum Mannheim der Rupprecht-Karls-Universität Heidelberg, Deutschland
- 17:30 "Pitfalls in transfer of diagnostic duplex qPCR assays between technological platforms."  
Tobias Ruckes, Artus GmbH, Hamburg, Germany
- 17:50 – 19:00 **Poster session**
- 19:00 – 22:00 **Bavarian Gala Dinner in the „Oldest brewery of the world“ WEIHENSTEPHAN**
- or alternatively
- 19:00 – 22:00 **Mediterranean Gala Dinner in LINDENKELLER**

## Thursday 4 March 2004

### Session: New Approaches in qPCR & Automatisations

Chairs: C. Wittwer & L. Wangh

### Lecture hall HS 15

- 08:30 Session introduction by C. Wittwer & L. Wangh
- 08:40 "Real time PCR in a Core Facility: Helping others to help themselves."  
Deborah Grove, Pennsylvania State University, USA
- 09:10 "Determination of real-time PCR efficiency - An overview of different methods."  
Michael W. Pfaffl, Physiology - Weihenstephan, ZIEL, Center of Food & Life Science-Weihenstephan Germany
- 09:40 "LATE-PCR and Allied Technologies for Amplification and Utilization of Single-stranded DNA."  
Lawrence Wangh, Brandeis University, Boston, MA, USA
- 10:10 "Expression Profiling of Candidate Genes: Assays-on-Demand Gene Expression products based on TaqMan MGB chemistry."  
Manohar Furtado, Applied Biosystems R & D - Applera Deutschland GmbH, Germany
- 10:30 – 11:00 **Coffee break**

- 11:00 "Establishment and the use of multiplex applications."  
Böll Inga, Roche Applied Science, Penzberg, Germany
- 11:20 "Efficient non-linear analysis of kinetic amplification for quantification and automated results calling."  
Martin Lee, BioGene Limited, Kimbolton, UK
- 11:40 "Quantitative PCR – a novel tool for protein quantification."  
Andreas Kage<sup>1</sup>, Wolfgang Henke<sup>2</sup>, Heiko Witt<sup>3</sup>, Claudia Dahmen<sup>4</sup>; <sup>1</sup> Charité - Universitätsmedizin für Berlin, Institut für Laboratoriums-mezizin und Pathobiochemie, <sup>2</sup> Charité - Universitätsmedizin für Berlin, Institut für Laboratoriumsmedizin und Pathobiochemie, <sup>3</sup> Charité - Universitätsmedizin für Berlin, Abt. für Pädiatrie, Augustenburger Platz 1, 13353 Berlin; <sup>4</sup> AptaRes AG, Im Biotechnologiepark TGZ 1, 14943 Luckenwalde, Germany
- 12:00 "PurAmp - a New Quantitative Method for Preparation, Synthesis, and Amplification of Both cDNA and Genomic DNA in a Single Tube."  
Lawrence Wangh, Brandeis University, Boston, MA, USA
- 12:20 "A sensitive method for the quantitation of residual DNA using Alu based sequences and real-time PCR amplification."  
Nussbaum O, Oppenheimer-Shaanan Y, Eren R, Dagan S, Zaubermann, A; XTL Biopharmaceuticals Ltd., Rehovot, Israel
- 12:40 - 13:40 **Lunch in the student cafeteria**

### Session: Transcriptomics & Expression profiling

Chair: M. Kubista

### Lecture hall HS 15

- 13:40 Session introduction by M. Kubista
- 13:50 "Different approaches of data analysis in real-time amplification."  
Thomas Kaiser, Corbett Research R&D, Australia sponsored by Pyrosequencing, Sweden
- 14:20 "Real-Time RT-PCR profiling of over 1,400 Arabidopsis transcription factors: Unprecedented sensitivity reveals novel root- and shoot-specific genes."  
Czechowski T, Bari R, Stitt M, Scheibele WR & Udvardi MK; Max-Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Golm, Germany
- 14:40 "PCR amplification efficiency, relative quantification and the analysis of alterations in cellular gene expression patterns."  
Petrauskene OV, MacLean J, Wong a, & Furtado MR; Applied Biosystems, 850 Lincoln Center Dr. Foster City, CA, USA
- 15:00 "A rapid method to measure reactivity of brain regions upon stress exposure employing real-time qPCR analysis of activity regulated gene transcripts and calculation of recommended sample size."  
Koya E<sup>1</sup>, Spijkers S<sup>1</sup>, Homberg J<sup>2</sup>, Schoeffelmeer ANM<sup>2</sup>, De Vries TJ<sup>2</sup>, Smit AB<sup>1</sup>; <sup>1</sup> Department of Molecular and Cellular Neurobiology, Vrije Universiteit Amsterdam, The Netherlands; <sup>2</sup> Department of Medical Pharmacology, VU-Medical Center, The Netherlands
- 15:20 – 15:50 **Coffee break**
- 15:50 "Using real-time quantitative RT-PCR to validate a transcriptomics analysis advancing embryo-maternal communication."  
Ulbrich S<sup>1</sup>, Bauernsachs S<sup>2</sup>, Rehfeld S<sup>2</sup>, Mallok S<sup>3</sup>, Prell K<sup>1</sup>, Wenigerkind H<sup>4</sup>, Blum H<sup>3</sup>, Wolf E<sup>2</sup> & Einspanier R<sup>1,5</sup>; <sup>1</sup>Institute of Physiology, TUM, Freising, Germany; <sup>2</sup>Institute of Molecular Animal Breeding, LMU, Munich, Germany; <sup>3</sup>Gene Center of the LMU Munich, <sup>4</sup>Bavarian Research Center for Biology of Reproduction, Ober-schleissheim, Germany; <sup>5</sup> present address: Institute of Veterinary Biochemistry, Free University of Berlin, Berlin, Germany

16:10 "Multiplex BD QZyme Assays – a reliable real-time qPCR chemistry for analyzing the effects of gene silencing models." Larsen Robert, BD Biosciences, Belgium

16:30 "Human Transcriptome Probe Library - detecting 37,000 genes with 90 probes." Mouritzen P, Tolstrup N, Ramsing NB; Exiqon A/S, Bygstubben 9, DK2950 Vedbaek, Denmark

### Session: Quality Assessment in qPCR

Chair: T. Bar

Lecture hall HS 15

16:50 Session introduction by T. Bar

17:00 "High Resolution Melting Curve Analysis." Carl T. Wittwer, School of Medicine, University of Utah, USA

17:30 "Comparative Quality Assessment (CoQA) for real-time PCR." Tzachi Bar<sup>1</sup> Neven Zoric<sup>2</sup>, Anders Muszta<sup>3</sup> and Mikael Kubista<sup>1,2</sup>; <sup>1</sup>Department of Chemistry and Biosciences, Chalmers University of Technology, Medicinargatan, <sup>2</sup>TATAA Biocenter, Medicinargatan, <sup>3</sup>Department of Mathematical statistics, Eklandagatan 86, 412 96, Gothenburg, Sweden

17:50 "An Italian external quality control program for quantitative PCR assay based on the use of TaqMan probes: results of a 42 laboratory survey." Orlando C<sup>1</sup>, Casini Raggi C<sup>1</sup>, Pinzani P<sup>1</sup>, Simi L<sup>1</sup>, Verderio P<sup>2</sup>, Marubini E<sup>2</sup>, Pazzagli M<sup>1</sup>. <sup>1</sup>Clinical Biochemistry Unit, Department of Clinical Physiopathology, University of Florence, Italy; <sup>2</sup>Operative Unit of Medical Statistics and Biometry, European Institute of Oncology, Milan, Italy

18:10 "Data processing in real time PCR." Larionov A.A., Miller W.R.; Breast Unit Research Group, Western General Hospital, Edinburgh, UK

18:30 – 19:00 **Poster session**

19:00 – 22:00 **Bavarian Gala Dinner in the „Oldest brewery of the world“ WEIHENSTEPHAN**

or alternatively

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## Friday 5 March 2004

### Session: Nutrigenomics

Chair: H. Daniel

Lecture hall HS 14

08:30 Session introduction by H. Daniel

08:40 "Nutrigenomics: the road that leads to new insights into nutritional processes." Hannelore Daniel, Molecular Nutrition Unit, ZIEL, Center of Food & Life Science Weihenstephan, Germany

09:10 "Nuclear Factors and Cytokines control TFFs down regulation in tumor cell lines of the digestive tract." Baus-Loncar M, Dossinger V, Blin N, Gött P & Kayademi T; Institute for Anthropology and Human Genetics, Division of Molecular Genetics, University of Tübingen, Germany

09:40 "Differential regulation of the sodium-ascorbate co-transporters SVCT1 and SVCT2 expression in glutathione depleted CaCo-2 cells as assessed by functional analysis and quantitative real-time PCR." Maulen NP<sup>1</sup>, Kempe S<sup>2</sup>, Nualart F<sup>3</sup>, Bustamante ME<sup>1</sup> & Vera JC<sup>2</sup>; <sup>1</sup>Laboratory of Molecular Biology, Faculty of Medicine, Universidad Católica de la Santísima Concepción; <sup>2</sup>Department of Physiopathology, <sup>3</sup>Department of Cell Biology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile

10:00 – 10:30 **Coffee break**

### Session: Food Hygiene & GMO

Chair: H.H.D. Meyer

Lecture hall HS 14

10:30 Session introduction by Heinrich H.D. Meyer

10:40 "Real-time Detection and Quantitation of Genetically Modified Soy." Babette Fahey, MJ Research Inc., Waltham, MA, USA

11:10 "Detection and quantitation of GMO in official food and feed control." S. Pecoraro, Bavarian Health and Food Safety Authority Oberschleißheim, Germany

11:30 "CaMV virus detection with Real-time PCR – identification of false positive results in 35S screening for genetically modified organisms (GMOs)." Cankar K, Gruden K, Tusek M, Toplak N, Ravnikar M, Žel J; National Institute of Biology, Department of Plant Physiology and Biotechnology, Večna pot 111, 1000 Ljubljana, Slovenia

11:50 "Finding the traces - real-time PCR assays for quantitative and qualitative detection of animal DNA in food and feed." Bruns U, Müller M, Steinbohrn R & Müller S; Institute of Animal Breeding and Genetics, Veterinary University Vienna, Austria

12:10 "qPCR and Small Grain Cereals: Species and Transgene Detection." Terzi V<sup>1</sup>, Shewry PR<sup>2</sup>, Stanca AM<sup>1</sup>, Faccioli P<sup>1</sup>; 1 Istituto Sperimentale per la Cerealcoltura, Via San Protaso 302, 29017-Fiorenzuola d'Arda (PC), Italy; 2 IACR-Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK

### Session: Detection methods

Chair: D. Whitcomb

Lecture hall HS 15

08:30 Session introduction by D. Whitcombe

08:40 "Scorpions- Application in Genotyping and Real time PCR." David Whitcombe, DxS Genotyping, Manchester, UK

09:10 "Genotyping of SNPs via Fluorescent Melting Curve Analysis." Thomas Fröhlich, Roche Diagnostics R & D LightCycler Development Group, Penzberg, Germany

09:40 "Real-time PCR genotyping using strand displacement probes." Li Q, Cheng J, Zhang Y, Molecular Diagnostics Laboratory, Xiamen University, China

10:00 – 10:30 **Coffee break**

10:30 "LUX Fluorogenic Detection System and other new approaches in qPCR." Debra Nickson, Invitrogen Europe

11:00 "LNA probes, a new tool to enhance your real Time QPCR applications." Khalil Arar, Proligo SAS, Paris, France

11:30 "Novel amplification and detection chemistries for real-time PCR." Dirk Löffert, Qiagen, Hilden, Germany

12:00 "Highly sensitive analysis of allele-specific gene expression by MALDI-TOF MS." Stephen Sharp, Sequenom, Inc., San Diego, Germany

### Closing of the Symposium

Lecture hall HS 14

12:30 – 13:00 "Closing of the Symposium." Michael W. Pfaffl & H. H. D. Meyer

13:00 – 14:00 **Lunch in the student cafeteria**

**Friday 5 March 2004**

**Workshop Agenda**

- 12:00 – 14:00 Registration
- 14:00 – 14:30 Welcome & Opening of the Application Workshop  
by Prof. M. Kubista

**Lectures by the Workshop participating Companies:  
Lecture hall HS 15**

- 14:30 "The new LightCycler 2.0: an advanced multi-channel system for rapid real-time PCR."  
Oliver Geulen, Roche Diagnostics, LightCycler Development Group, Germany
- 14:50 "Complete Solutions for Real-Time PCR approaches – Bio-Rad Real-Time PCR Systems."  
M. Neusser & Luis Ugozzoli, Bio-Rad Laboratories GmbH, 80939 München, Germany
- 15:10 "Haplotype Analysis using a Novel Real-Time Amplification Strategy on the MJ Research Opticon Continuous Fluorescence Detection System."  
Chas Andre, MJ Research Inc., Waltham, MA, USA
- 15:30 – 16:00 **Coffee break**
- 16:00 "New tools for genetic research: Whole genome microarrays and customized low density solutions"  
Thomas Rygus & Thomas Schild, Applied Biosystems, Germany
- 16:20 "Multiplexing your assay, from simplex to fourplex."  
Fabrice Magnino, Stratagene Europe, Amsterdam, The Netherlands
- 16:40 "Optimizing Assays in real time amplification."  
Thomas Kaiser, Corbett Research R&D, Australia sponsored by Pyrosequencing, Sweden
- 17:00 "Normalization using the F3 Channel of the Lightcycler- a New Reporter Enables Multiplexing with 5'Nuclease Probes."  
Mary Katherine Johansson, Biosearch Technologies, Novato, CA, USA
- 17:20 "The effect of consumable type on the sensitivity and reproducibility of QPCR."  
Sarah Freshwater, ABgene, Blenheim Road, Epsom, UK
- 17:40 "Test Systems for Fast and Automated Molecular Diagnostics."  
William A. McMillan, Cepheid
- 18:00 **Open evening & Visit the Nightlife of Freising**  
[www.freising.de](http://www.freising.de)

**Saturday 6 March 2004**

**qPCR Application Workshop**

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