

3<sup>rd</sup> – 6<sup>th</sup> March 2004 in Freising-Weihenstephan, Germany

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

## preliminary Symposium Agenda

22. December 2003

### Welcome & Opening of the Symposium

Michael W. Pfaffl & Mikael Kubista  
Scientific coordination of the Symposium & Workshop

Bertold Hock  
Dean of the Center of Food & Life Science-Weihenstephan

#### "qPCR and Transcriptomics: Creation of a new tool to understand life."

Heinrich H. D. Meyer, Physiology - Weihenstephan, Freising, Germany, Center of Food & Life Science-Weihenstephan, Germany

#### "Pitfalls in the quantification of RNA using real-time RT-PCR."

Stephen Bustin, Reader in Molecular Medicine, School of Medicine, London

### Session 1: Pre-Analytical Steps

Chairs: M. Kubista & S. Adams

#### "Quantitative gene expression analysis by Real-time PCR - How to optimize the reverse transcription and real-time PCR reactions:"

Anders Stahlberg, Chalmers University, Sweden

#### "Overcoming bias in quantitative RT-PCR: Different methods of reverse transcription influence sensitivity and accuracy of gene expression patterns."

Ginzinger A<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 94143, USA; <sup>2</sup> Quanta BioSciences, Inc. Gaithersburg, MD 20877, USA

#### "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>; <sup>1</sup> Agilent Technologies, Waldbronn, Germany; <sup>2</sup> Agilent Technologies, Palo Alto, USA; <sup>3</sup> Quantom Bioinformatics, Weingarten, Germany

#### "Gene expression quantitated in PAXgene™ frozen stored blood as compared to freshly Immuno Magnetic Separated (IMS) blood cells."

Øvstebo R, Haug KBF, Kierulf P.; The Research and Development Group, Department of Clinical Chemistry, Ullevål University Hospital, Oslo, Norway

#### "Modified silica-magnetite composite as a universal matrix for nucleic acids isolation."

Zhao X, Huang Z, Luan G; Biovision Biotech, Inc, China

### Session 2: qPCR Application in Clinical Diagnostics

Chairs: S. Bustin & M. Pazzagli

#### "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

#### "Laser capture microdissection and real-time PCR in Human Cancer."

Pamela Pinzani & Prof. Mario Pazzagli, Clinical Biochemistry Unit, Dep. of Clinical Physiopathology Florence, Italy

#### "Real Time PCR for the identification of genes associated with complex diseases."

Brooks, P, Marccailou, C, Stockholm, D, & Hager, J; IntegraGen, Evry, France; Genethon, Association Francais Myopathy, Evry, France

#### "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

### Session 3: qPCR Application in Microbiology & Virology

Chairs: U. Reischl & H. Nitschko

#### "LightCycler Applications in Diagnostic Bacteriology."

Udo Reischl, Institute of Medical Microbiology & Hygiene, Regensburg, Germany

#### "Genotyping and quantification of hepatitis C virus using fluorescent probes."

Hans Nitschko, Max-von-Pettenkofer Institut, LMU sponsored by Abgene

#### "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

#### "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, A-1090 Vienna

### Session 4: Normalization & Standardization

Chairs: T. Köhler & M.W. Pfaffl

#### "Accurate normalization of gene expression using multiple internal control genes."

Jo Vandesompele, Center of Medical Genetics, Ghent University, Belgium

#### "Normalization genes for heart failure."

Kristin Brevik Andersson, Institute of Experimental Medical Research, Oslo

#### "Validation of housekeeping genes for normalising RNA expression."

Jim Huggett, Center for Infectious Diseases, London, UK

#### "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

#### "Standardized gene expression profiling and tumor prognosis."

Thomas Köhler, Roboscreen, Leipzig

#### "A Housekeeping-Gene Free Zone for Normalization."

Tania Nolan, Stratagene Europe, Amsterdam, The Netherlands

#### "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

**Session 5:  
Nutrigenomics**  
Chair: H. Daniel

"Nutrigenomics: the road that leads to new insights into nutritional processes."

Hannelore Daniel, Molecular Nutrition Unit, ZIEL, Center of Food & Life Science Weihenstephan, Germany

"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 & Kayademir T; Institute for Anthropology and Human Genetics, Division of Molecular Genetics, University of Tübingen, Germany

"Effect of chromium on gene expression in yeast."

Jenko-Brincovec Š.<sup>1</sup>, Plaper A.<sup>2</sup> and Raspor P.<sup>1</sup>; <sup>1</sup>University of Ljubljana, Biotechnical Faculty, Chair of Biotechnology, Ljubljana, Slovenia. <sup>2</sup>KRKA d.d., Research Dept. of New Entities, Novo mesto, Slovenia

"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, Concepción, Chile; <sup>2</sup>Department of Physiopathology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile; <sup>3</sup>Department of Cell Biology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile

**Session 6:  
Transcriptomics & Expression profiling**  
Chairs: M. Kubista & M.W. Pfaffl

"Different approaches of data analysis in real-time amplification."

Thomas Kaiser, Corbett Research R&D, Australia sponsored by Pyrosequencing, Sweden

"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

"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

"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

"Human Transcriptome Probe Library - detecting 37,000 genes with 90 probes."

Mouritzen P, Tolstrup N, Ramsing NB; Exiqon A/S, Byggestubben 9, DK2950 Vedbaek, Denmark

"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>), Prella 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, Technical University of Munich, Freising, Germany; <sup>2</sup>Institute of Molecular Animal Breeding, Ludwig-Maximilians University, Munich, Germany; <sup>3</sup>Gene Center of the Ludwig-Maximilians University, Munich, Germany; <sup>4</sup>Bavarian Research Center for Biology of Reproduction, Oberschleissheim, Germany; <sup>5</sup>present address: Institute of Veterinary Biochemistry, Free University of Berlin, Berlin, Germany

"Multiplex BD QZyme Assays – a reliable real-time qPCR chemistry for analyzing the effects of gene silencing models."

Larsen Robert, BD Biosciences, Belgium

**Session 7:  
Detection methods**  
Chairs: D. Whitcomb

"Scorpions- Application in Genotyping and Real time PCR."

David Whitcombe, DxS Genotyping, Manchester, UK

"SNP Genotyping."

Thomas Fröhlich, Roche Diagnostics R & D LightCycler Development Group, Penzberg, Germany

"Real-time PCR genotyping using strand displacement probes."

Li Q, Cheng J, Zhang Y  
Molecular Diagnostics Laboratory, Xiamen University, China

"LUX™ Fluorogenic Detection System and other new approaches in qPCR."

Debra Nicksen, Invitrogen Europe

"LNA probes, a new tool to enhance your real Time QPCR applications."

Khalil Arar, Director of Research & Development Proligo SAS, Paris, France

"Novel amplification and detection chemistries for real-time PCR."

Dirk Löffert, Qiagen, Hilden, Germany

"Highly sensitive analysis of allele-specific gene expression by MALDI-TOF MS."

Christian Jurinke, Sequenom, Hamburg, Germany

**Session 8:  
New Approaches in qPCR & Automatisisation**  
Chairs: C. Wittwer & L. Wangh

"Real time PCR in a Core Facility: Helping others to help themselves."

Pamela Scottie Adams, Trudeau Institute, Saranac Lake, NY, USA

"Determination of real-time PCR efficiency - An overview of different methods."

Michael W. Pfaffl, Physiology - Weihenstephan, ZIEL, Center of Food & Life Science-Weihenstephan Germany

"LATE-PCR and Allied Technologies for Amplification and Utilization of Single-stranded DNA."

Lawrence Wangh, Brandeis University, Boston, MA, USA

"Expression Profiling of Candidate Genes: Assays-on-Demand Gene Expression products based on TaqMan MGB chemistry."

Falko Kräusche & Dr. Roland Wicki, Applied Biosystems R & D - Applera Deutschland GmbH, Germany

"Efficient non-linear analysis of kinetic amplification for quantification and automated results calling."

Martin Lee, BioGene Limited, Kimbolton, UK

"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

"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 Laboratoriumsmedizin und Pathobiochemie, Westend, Haus 31, Spandauer Damm 130, 14050 Berlin; <sup>2</sup> Charité - Universitätsmedizin für Berlin, Institut für Laboratoriumsmedizin und Pathobiochemie, Augustenburger Platz 1, 13353 Berlin; <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

"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

**Session 9:  
Quality Assessment in qPCR**

Chair: T. Bar

**"High Resolution Melting Curve Analysis."**

Carl T. Wittwer, School of Medicine, University of Utah, USA

**"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 7B 405 30, <sup>2</sup>TATAA Biocenter, Medicinargatan 7B 405 30, <sup>3</sup>Department of Mathematical statistics, Eklandagatan 86, 412 96, Gothenburg, Sweden

**"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

**"Data processing in real time PCR."**

Larionov A.A., Miller W.R.; Breast Unit Research Group, Western General Hospital, Edinburgh, UK

**preliminary Workshop Agenda**

Lectures by the participating Companies:

**"LightCycler 2.0"**

N. N., Roche Diagnostics, LightCycler Development Group, Germany

**"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

**"Haplotype Analysis using a Novel Real-Time Amplification Strategy on the MJ Research Opticon Continuous Fluorescence Detection System."**

Andree Chas, MJ Research Inc., Waltham, MA, USA

**"New tools for genetic research: Whole genome microarrays and customized low density solutions"**

Dr. Thomas Rygus & Dr. Thomas Schild, Applied Biosystems, Germany

**"No title submitted"**

N. N., Stratagene Europe, Amsterdam, The Netherlands

**"Optimizing Assays in real time amplification."**

Thomas Kaiser, Corbett Research R&D, Australia sponsored by Pyrosequencing, Sweden

**"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

**Session 10:  
Food Hygiene & GMO**

Chair: H.H.D. Meyer

**"Real-time Detection and Quantitation of Genetically Modified Soy."**

Babette Fahey, MJ Research Inc., Waltham, MA, USA

**"Detection and quantitation of GMO in official food and feed control."**

S. Pecoraro, Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim, Germany

**"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

**"Finding the traces - real-time PCR assays for quantitative and qualitative detection of animal DNA in food and feed."**

Bruns U, Müller M, Steinbohn R & Müller S; Institute of Animal Breeding and Genetics, Veterinary University Vienna, Austria

**"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>; <sup>1</sup> Istituto Sperimentale per la Cerealicoltura, Via San Protaso 302, 29017-Fiorenzuola d'Arda (PC), Italy; <sup>2</sup> IACR-Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK

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