

qPCR workshop Freising 5th-6th March 2004

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TUMTECH

TUM
TECHNISCHE
UNIVERSITÄT
MÜNCHEN

Workshop Supporting Companies

CR CORBETT
RESEARCH

BIO-RAD

ABgene

PYROSEQUENCING

MJ Research

Roche

Cepheid

BIOSEARCH
TECHNOLOGIES
Chemistry for Genomics

BD
BD Biosciences
Complete Library
Immunity Library System
Immune Monitoring System

STRATAGENE
EUROPE

Applied
Biosystems

eppendorf

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Schedule Friday 5th March

- 12:00 – 14:00 Registration
- 14:00 – 14:30 Welcome & Opening of the Application Workshop by Prof. M. Kubista
- 14:30 – 18:00 Lectures by the Workshop participating Companies
- 14:30 "The new LightCycler 2.0: An advanced multi-channel system for rapid real-time PCR."
Oliver Geulen
Roche Diagnostics, LightCycler Development Group
- 14:50 "Complete Solutions for Real-Time PCR approaches - Bio-Rad Real-Time PCR Systems."
Marcus Neusser & Luis Ugozzoli
R & D Bio-Rad Laboratories
- 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.
- 15:30 – 16:00 Coffee break
- 16:00 "New tools for genetic research: Whole genome microarrays and customized low density solutions"
Thomas Ryuge & Thomas Schild
Applied Biosystems (Germany)

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Schedule Friday 5th March

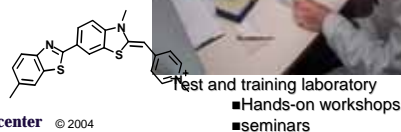
- 16:20 "Multiplexing your assay, from simplex to fourplex."
Fabrice Magnino
Stratagene Europe
- 16:40 "Optimizing Assays in real time amplification."
Thomas Kaiser
Corbett Research R&D, Australia sponsored by Biotage (former Pyrosequencing)
- 17:00 "Normalization using the F3 Channel of the Lightcycler- a New Reporter Enables Multiplexing with 5'Nuclease Probes."
Mary Katherine Johansson
- "A Two-Color TaqMan Assay on the LightCycler 1.2."
Brian E. Caplin
Biosearch Technologies
- 17:20 "The effect of consumable type on the sensitivity and reproducibility of qPCR."
Sarah Freshwater
ABgene
- 17:40 "Test Systems for Fast and Automated Molecular Diagnostics."
William A. McMillan
Cepheid
- 18:00 "QZyme Assays"
Robert Larsen
BD Biosciences

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TATAA training courses

- Education and support
 - » Several nordic and global authorities and institutes
 - » On location or at TATAA facility
 - » Open courses in Göteborg, Sweden

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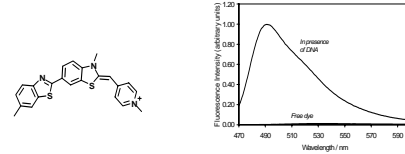
Services

- » Validation of RNAi experiments
- » Replacement of tedious Northern/Southern Blot
- » Microarray validation
- » Setup of disease monitoring tools for hospitals
- » Setup of custom assays

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Improving probe technology

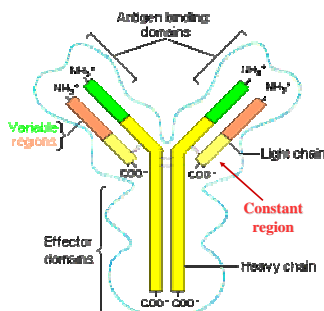
- BEBO – a novel asymmetric cyanine dye that binds in the minor groove of DNA
- 250-fold increase in fluorescence upon binding dsDNA



Collaboration with Dr Gunnar Westman, Dept of Organic Chemistry, Chalmers

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B cell lymphoma

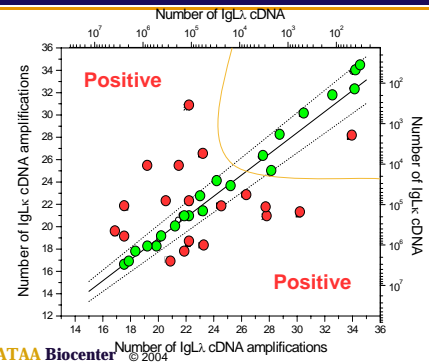


Two versions of the light chain constant region, κ and λ , are expressed in 60 and 40 % of B cells in healthy individuals.

In Non Hodgkin lymphoma the 60:40 expression ratio is altered due to clonality.

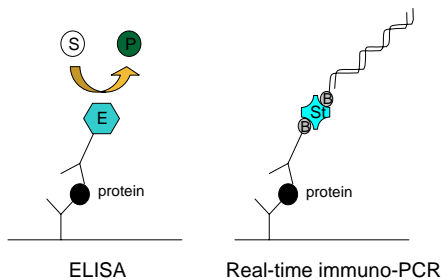
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Diagnosis of B-cell Lymphomas



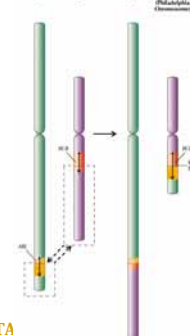
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Real-time Immuno PCR



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Disease monitoring



Treatment of Chronic Myelogenous Leukemia (CML) Novartis drug Glivec effect is monitored by measuring expression of bcr-abl fusion transcript relative to reference transcript.

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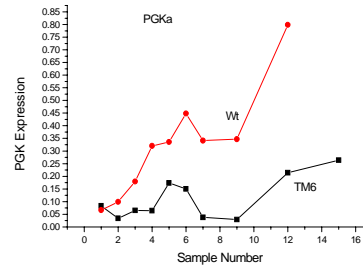
Single cell realtime PCR



Gene expression is measured in individual β cells of the islet of langerhans and correlated to ion flux as measured by patch clamp.

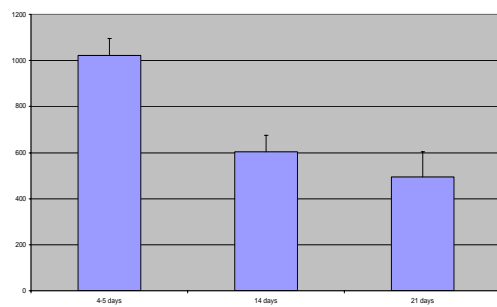
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Time-studies using qPCR



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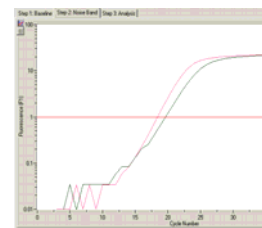
Studying embryonic stem cell differentiation using qPCR



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Kinetic Outlier Detection

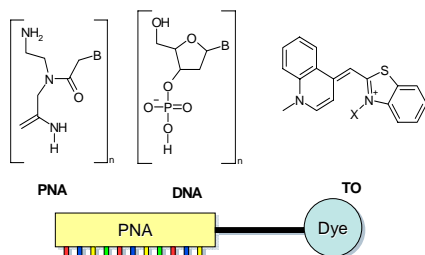
- Even slight differences in the efficiency in samples with equal start amount results in app. 1 cycle difference in CT.
- PCR inhibition is common.
- The Kinetic Outlier Detection method confidently detects outliers that should be reinvestigated.



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LightUp probe

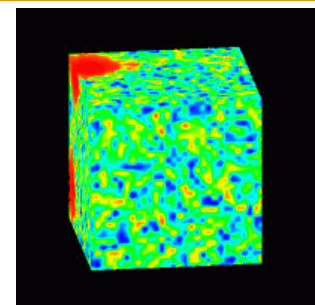
- PNA tethered to fluorescent dye



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Gene expression profiling

- Determining a multidimensional expression profile using a number of interesting genes will give valuable information for diagnostics of complex diseases



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Schedule Saturday 6th March

Group 1

- 08:30-10:00 Introduction to qPCR applications, gene quantification and normalization
- 10:00-11:00 Primer and Probe Design, Assay Optimization
- 11:00-12:00 Reverse Transcription
- 12:00-12:30 Nucleic acids isolation and purification
- 12:30-13:30 Lunch
- 13:30-14:15 qPCR experiment on 1 instrument in groups of 4
- 14:15-17:30 20 minutes per additional instrument

Group 2

- 08:30-09:15 qPCR experiment on 1 instrument in groups of 4
- 09:15-12:30 20 minutes per additional instrument
- 12:30-13:30 Lunch
- 13:30-15:00 Introduction to qPCR applications, gene quantification and normalization
- 15:00-16:00 Primer and Probe Design, Assay Optimization
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- 17:00-17:30 Nucleic acids isolation and purification

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Instruments Platforms

- ABI Prism 7500
- Rotorgene 3000
- Bio-Rad iQ
- Bio-Rad myQ
- Roche LightCycler 2.0
- Stratagene mx3000p
- MJ Research Opticon 2
- Cepheid SmartCycler II

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Experiments

- LightUp probes for CMV viral load diagnostics
- Molecular Beacon for quantification of human telomerase
- SNP detection using hybridization probes
- Hybridization probes for GMO quantification
- siRNA knockdown quantification using SYBR Green
- In situ calibration for determining sample specific efficiencies
- New unspecific dyes for qPCR
- Universal TaqMan assay from Assay-on-Demand
- New QZyme probe technology from BD Bioscience
- Smartcycler pelleted TaqMan assays
- Molecular Beacon Duplex assay

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