Göteborg, 2005-04-19

qPCR workshop Freising 7-9th September 2005

This workshop is aimed at giving participants a deep and objective understanding of real-time quantitative PCR and its applications. The courses are intended for persons considering working with qPCR or scientists currently working with qPCR seeking a deeper understanding.

The course covers all aspects in qPCR, from sample preparation to data analysis and is held during 3 days. The course is approximately 50% hands-on as is limited to 20 participants, resulting in very interactive teaching and everybody given the opportunity to try the instrumentation.

Examples of topics covered in the workshop:

- Basic Principles of PCR and qPCR
- Comparison of different detection technologies
- Applications of qPCR
- Probe and Primer design
- Data Analysis
- Relative Quantification-considerations and limitations
- Experimental Design
- Reverse Transcription
- Extraction methods
- Multiplex considerations

After the course participants will be able to plan and perform qPCR experiments themselves, as well as interpret and analyze data.

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Preliminary Schedule for TATAA Biocenter qPCR workshop Freising 7-9th September 2005

Day 1-Basic qPCR

12.30-14.00 **Basic PCR and qPCR theory and applications**
-Amplification and detection
-Detection chemistries
-Selected applications

14.00-15.00 **qPCR experiment by participants**
-Display of various instrument platforms
-Demonstration of qPCR software
-practical considerations when preparing PCR reactions
-programming qPCR machines

15.00-15.15 **Coffee Break**

15.15-16.00 **Primer and probe design and considerations**
-What does primer design affect?
-What are primer dimers?
-How do we minimize formation of primer dimers?
-Design of Molecular Beacons and TaqMan probes

16.00-17.00 **Data analysis**
-How does qPCR software process the data?
-How are standard curves used and created?
-How are melt curves used?
-Principle of quantification using standard curves
-Principle of relative quantification

17.00-17.30 Analysis of performed qPCR experiments

17.30-17.45 **Discussion and Q&A**
17.45 End of qPCR workshop day 1.

Lunch, coffee and snacks are included in the course fee. The course is focused on practical issues for qPCR and are partly hands-on, performed by the course participants in the lab (marked in blue).

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Day 2-Advanced qPCR - Quantification, Normalization and experimental design

09.00-09.50 **qPCR quantification strategies**
-standard curves
-relative quantification
-how to compensate for inhibition in biological samples

09.50-10.15 **Normalization of qPCR data**
-What levels of normalization can be used?
-How to choose a good reference gene?

10.15-10.30 **Break**

10.30-11.45 **Experiment comparing different quantification strategies**
-relative and standard curve quantification
-different efficiency calculations/assumptions

11.45-12.45 **Lunch**

12.45-13.30 **Optimization of qPCR protocols**
-What parameters can/should be optimized?
-A optimization strategy

13.30-15.00 **Quantification calculation examples**
-what effect will efficiency have on quantification
-quantification methods, and equations

15.00-15.15 **Coffee Break**

15.15-16.45 **Analysis of experimental data**
-differences in quantifications strategies
-effect of efficiency estimations on results
-calculations of relative abundance of genes
-pros and cons of different methods

16.45-17.00 **Discussion and Q&A**

17.00 End of qPCR workshop day 2

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Day 3-Advanced qPCR - Sample Preparation and reverse transcription

09.00-10.00 **Principle of RT and different RT priming strategies**
-Pros and cons of different methods

10.00-10.45 **Principle of RNA and DNA extraction**
-How it works
-Available methods and products suitable for qPCR
-Practical considerations

10.45-11.00 **Break**

11.00-11.45 **Reverse transcription experiment using different priming methods**
-Oligo(dt)
-Random Hexamers
-Gene specific primers

11.45-12.45 **Lunch**

12.45-13.30 **qPCR experiment evaluating RT using the generated cDNA**
-Is there a best RT priming method?

13.40-14.30 **Quality Control in qPCR using Kinetic Outlier Detection**
-How to detect samples with significant inhibition

14.30-14.45 **Coffee Break**

14.45-15.30 **SNP detection. Multiplexing possibilities and problems**.
-qPCR for SNP/mutation detection. What alternatives are there?
-Multiplex optimization

15.30-16.15 **Analysis of experimental data**
-Which priming method for RT is best?
-How should experiments be planned to take RT priming into consideration?

16.15-16.30 **Probes and Dyes**
-What dyes/quenchers are typically used in qPCR
-How to measure the maximum fluorescence available in a dual-labelled probe.

16.30-16.45 **Discussion and Q&A**

16.45 End of qPCR workshop day 3

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