

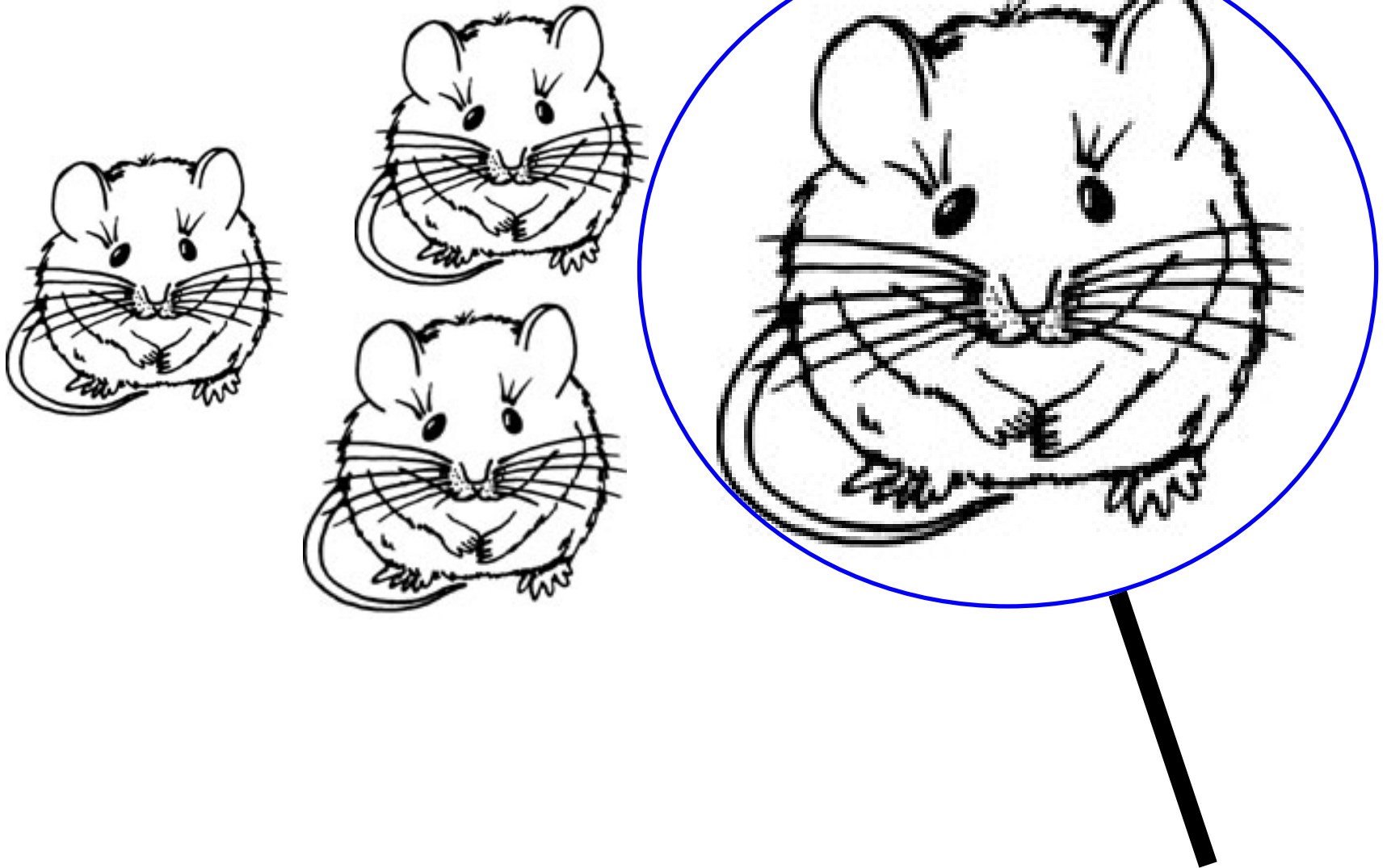
Session: Quality Assessment in qPCR

Lecture hall HS 15

16:50 Session introduction by T. Bar



Can we trust the results?



Nucleic acid extraction



Purification



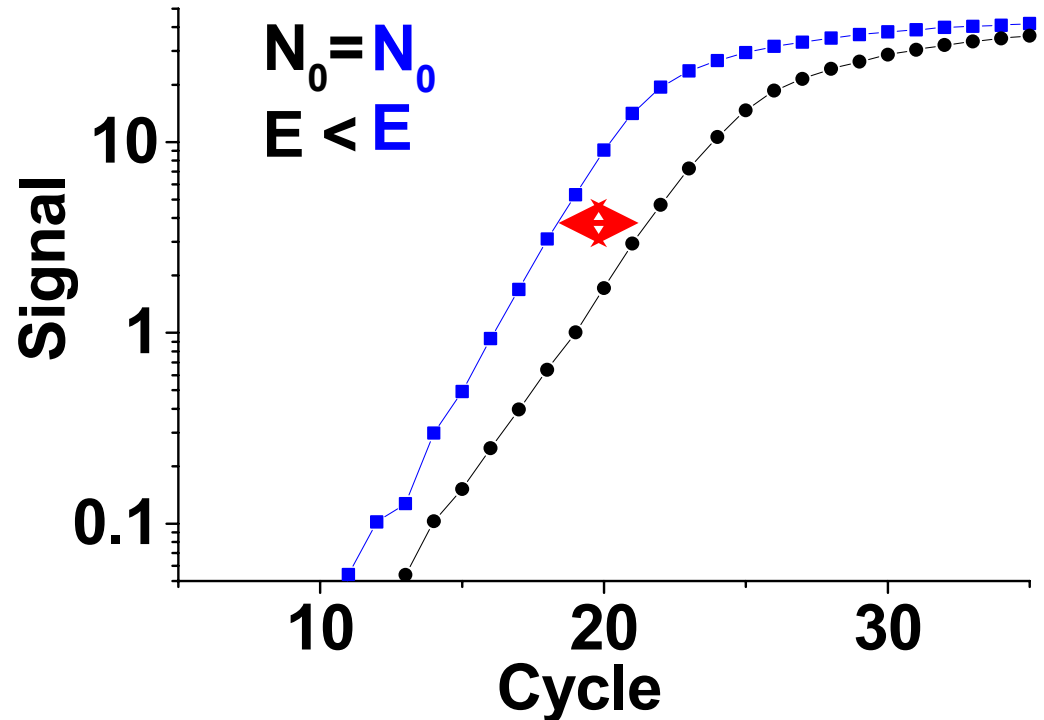
(Reverse transcription)



Real-time PCR

Controlled by
reference
gene(s) or
spiking

Where is the main problem?



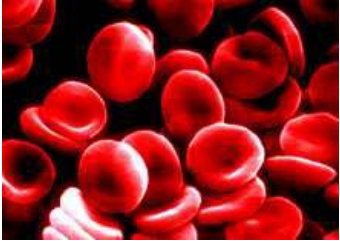
Most methods
require similar
PCR efficiencies
for reliable
quantification

$$\text{ratio} = \frac{\left(E_{\text{target}}\right)^{\Delta\text{CP}_{\text{target}}(\text{control-sample})}}{\left(E_{\text{ref}}\right)^{\Delta\text{CP}_{\text{ref}}(\text{control-sample})}}$$

Pfaffl, M. W. (2001). "A new mathematical model for relative quantification in real-time RT-PCR." Nucleic Acids Res **29**(9): E45-E45.

Stahlberg, A., P. Aman, et al. (2003). "Quantitative Real-Time PCR Method for Detection of B-Lymphocyte Monoclonality by Comparison of kappa and lambda Immunoglobulin Light Chain Expression." Clin Chem **49**(1): 51-59.

Dissimilarities in PCR efficiency due to inhibition are common



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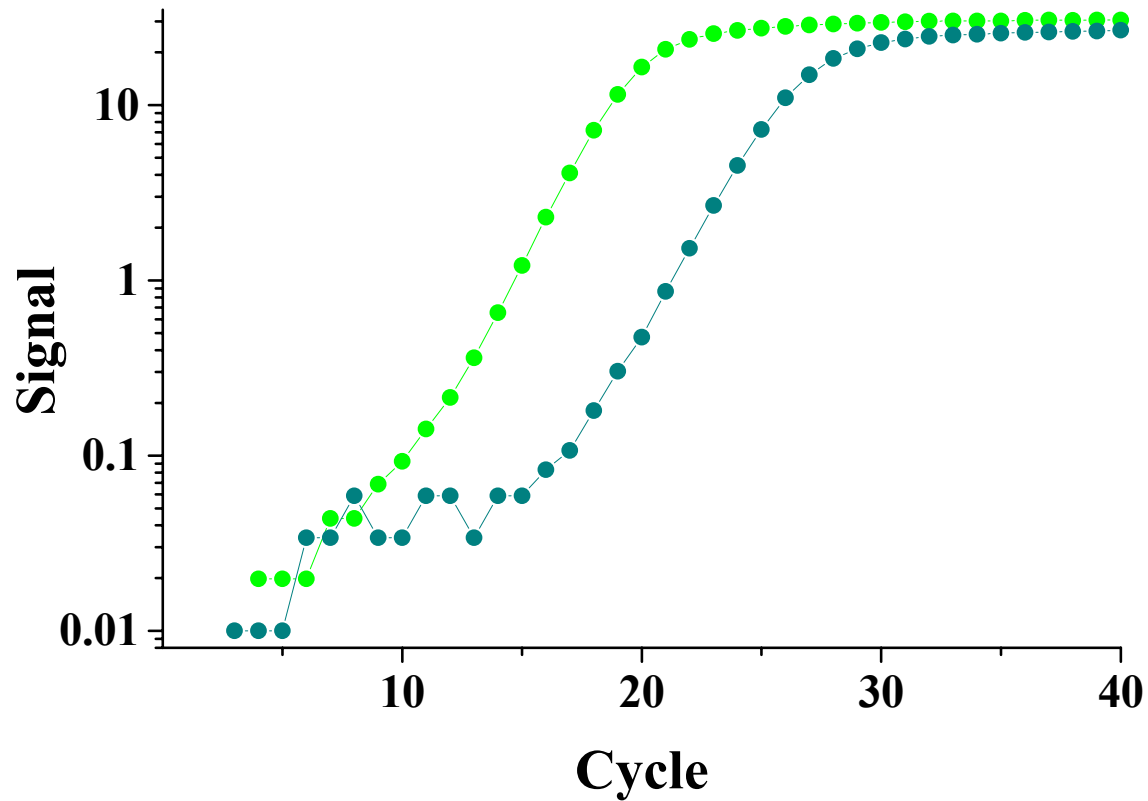


Watson, R. J. and B. Blackwell (2000). "Purification and characterization of a common **soil** component which inhibits the polymerase chain reaction." Can J Microbiol 46(7): 633-42.



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Can we trust the results?



“In God we trust,
all the others need to supply
evidence.”

Jacob, J. P. a. C. (1998). Statistical Estimations of PCR Amplification Rates. Gene Quantification. F. Ferre.

Schlereth, W., I. D. Bassukas, et al. (1998). "Use of the recursion formula of the Gompertz function for the quantitation of PCR-amplified templates." Int J Mol Med **1**(2): 463-7.

Meijerink J, M. C., van de Locht L, Tönnissen E, Goodsaid F, Raemaekers J (2001). "A novel method to compensate for different amplification efficiencies between patient DNA samples in quantitative real-time PCR." Journal of Molecular Diagnostics **3**(2): 55-61.

Liu, W. and D. A. Saint (2002). "Validation of a quantitative method for real time PCR kinetics." Biochem Biophys Res Commun **294**(2): 347-53.

Liu W, S. D. (2002). "A New Quantitative Method of Real Time Reverse Transcription Polymerase Chain Reaction Assay Based on Simulation of Polymerase Chain Reaction Kinetics." Analytical Biochemistry **302**(1): 52-59.

Tichopad, A., A. Dzidic, et al. (2002). "Improving

Session: Quality Assessment in qPCR

Chair: T. Bar

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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 Bar¹ Neven Zoric², Anders Muszta³ and Mikael Kubista^{1,2}; ¹Department of Chemistry and Biosciences, Chalmers University of Technology, Medicinargatan, ²TATAA Biocenter, Medicinargatan, ³Department 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 C¹, Casini Raggi C¹, Pinzani P¹, Simi L¹, Verderio P², Marubini E², Pazzagli M¹. ¹Clinical Biochemistry Unit, Department of Clinical Physiopathology, University of Florence, Italy; ²Operative 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**