



**tata**biocenter

**Real-time PCR in Clinical  
Diagnostics**

**Mikael Kubista  
Institute of Biotechnology AS CR**



## NEWS

2008-05-29

Radek Sindelka from TATAA Biocenter and inventor of qPCR tomography defends his doctoral thesis "Gene expression in early development of *Xenopus laevis*" on May 28.

2008-05-15

TATAA Biocenter paper - Multiway real-time PCR...

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[Visit External Link](#)

2008-05-13

Seminar by Neven Zoric "Trends and applications of real-time PCR in biomedical research " May 29 in Leuven, Belgium.  
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## TESTIMONIALS

"State - of - the - art.



## TATAA BIOCENTER


TATAA Biocenter is a commercial research provider that offers commissioned research and training within molecular diagnostics and gene expression analysis using real-time PCR and other molecular techniques to quantify nucleic acids.

Our competence is based on knowledge and experience accumulated through years of research at leading European Universities. Our offer comprises the entire field of real-time PCR services including commissioned research, hands-on training, and custom design of real-time PCR assays.

### AMONG OUR ACTIVITIES ARE

- ▶ Open and tailor-made hands-on training courses
- ▶ Commissioned research and development
- ▶ Custom design of QPCR assays and assay validation
- ▶ Product development

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### UPCOMING COURSES

9:th-13:th of June 2008  
Göteborg - Sweden  
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16:th-20:th of June 2008  
Prague - Czech Republic  
[Register](#)

7:th-11:th of July 2008  
Freising - Germany  
[Register](#)

**View all courses.**

Tataa courses are supported  
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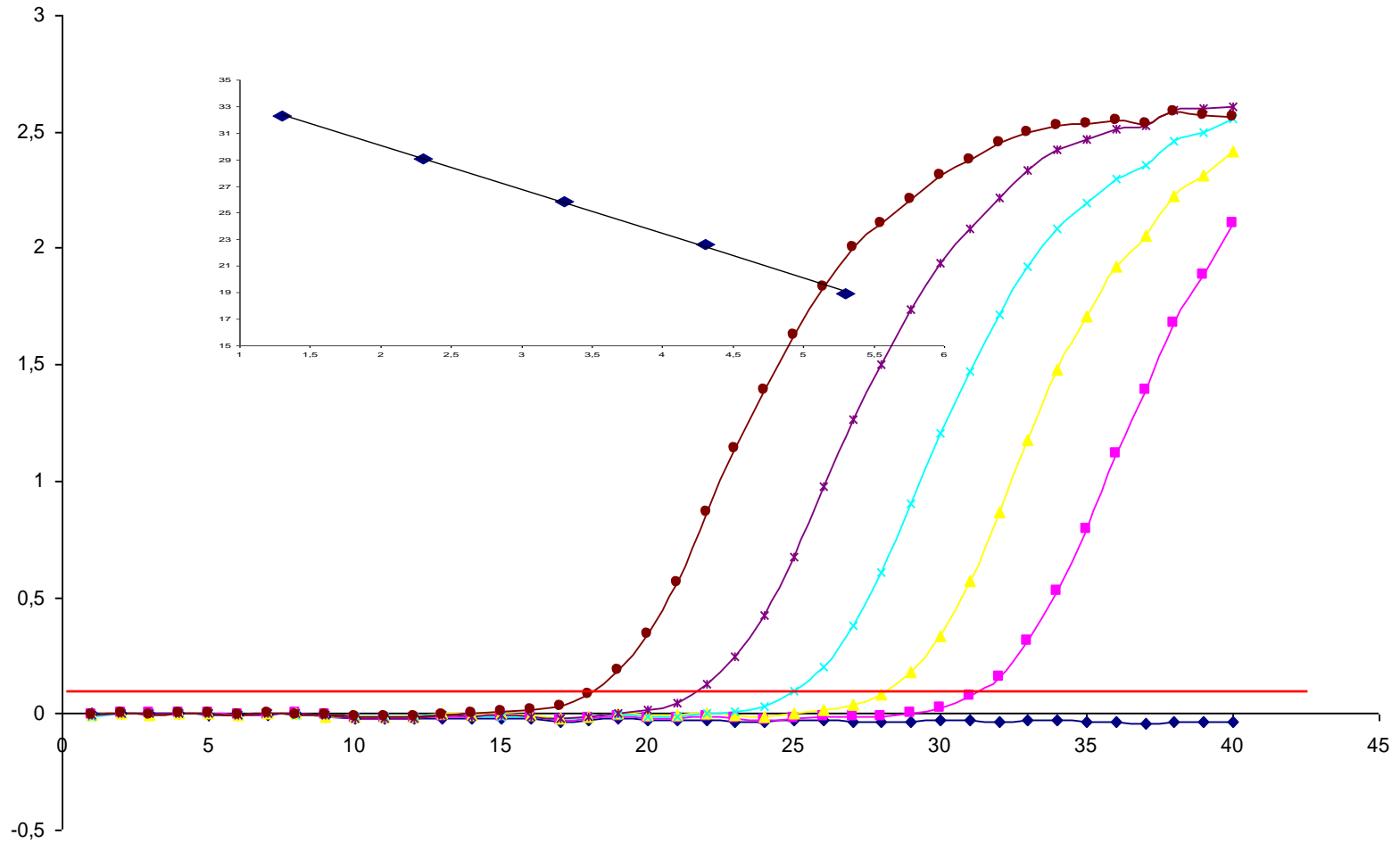
 **multiD**  
ANALYSES

# Real-time PCR experimental designs

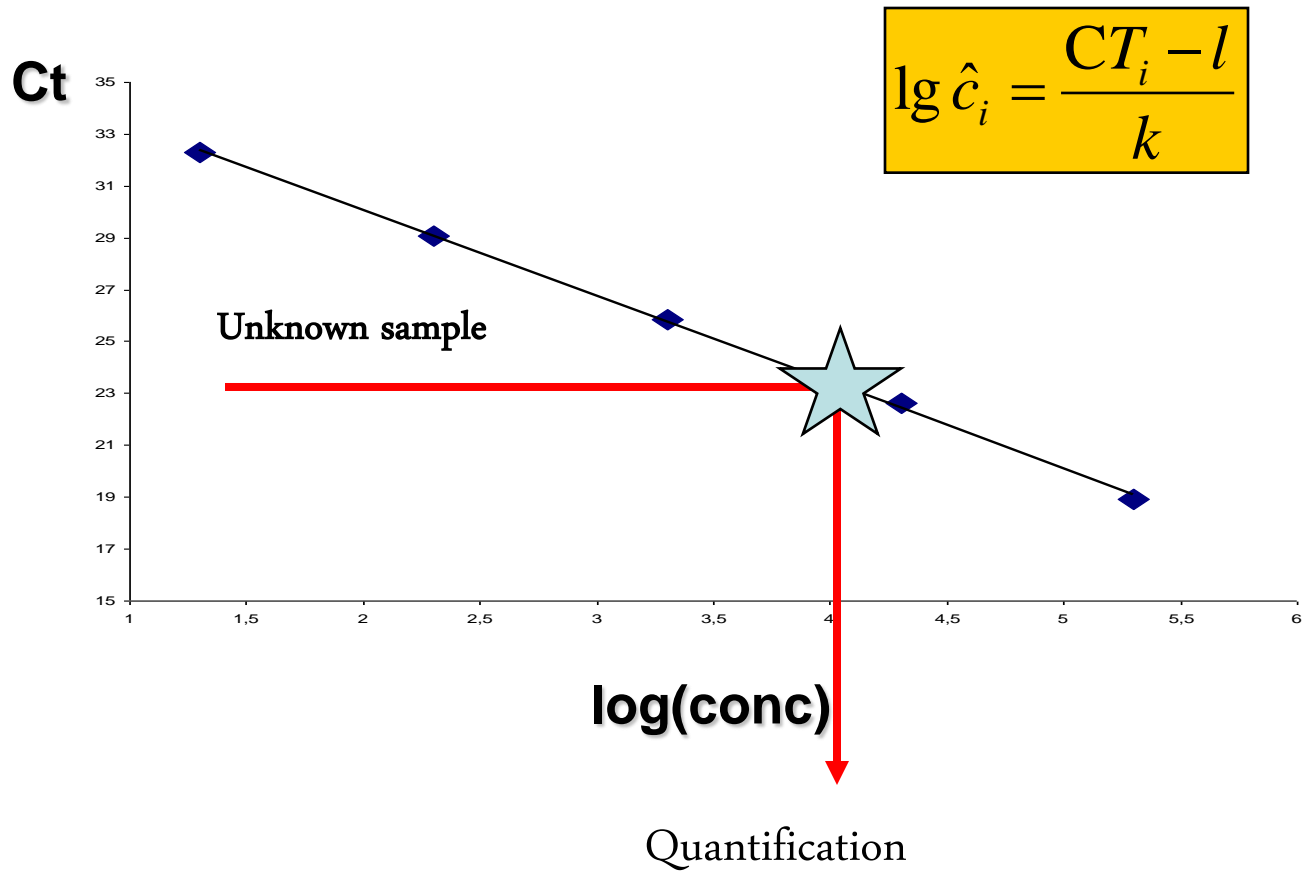
- Absolute quantification of a single marker by means of a standard curve
  - Viral load testing
- Relative quantification – the response of a single expression marker relative to an endogenous control
  - Diagnosis and monitoring of monogenic diseases
- Expression profiling
  - Staging, monitoring and prediction of complex diseases
- Protein detection
- Expression correlation

The Real-Time Polymerase Chain Reaction, M. Kubista, J.M. Andrade, M. Bengtsson, A. Forootan, J. Jonak, K. Lind, R. Sindelka, R. Sjöback, B. Sjögren, L. Strömbom, A. Ståhlberg, N. Zoric, *Molecular Aspects of Medicine* (2006) 27, 95-125

# Viral load testing

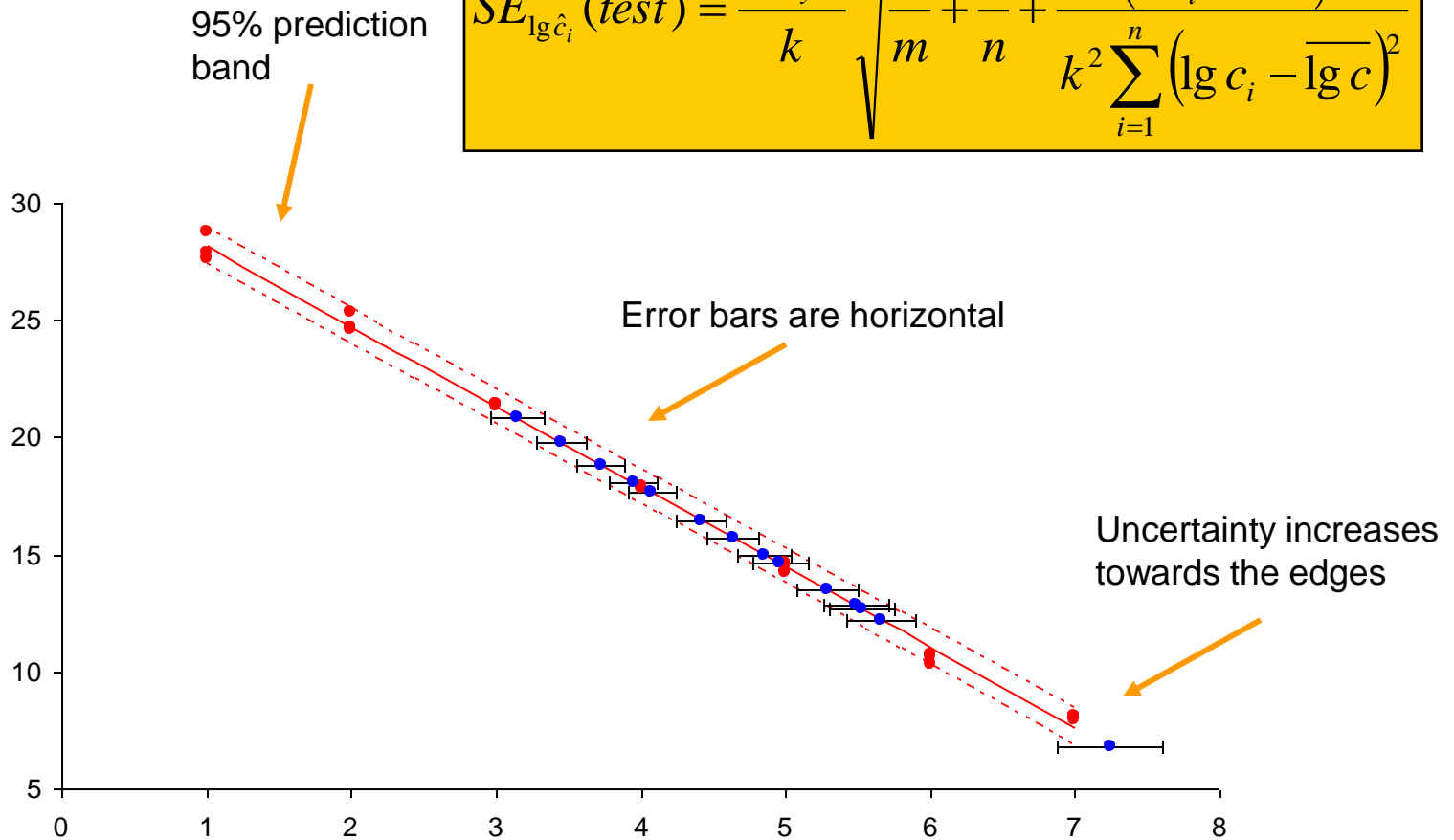


# Reverse calibration

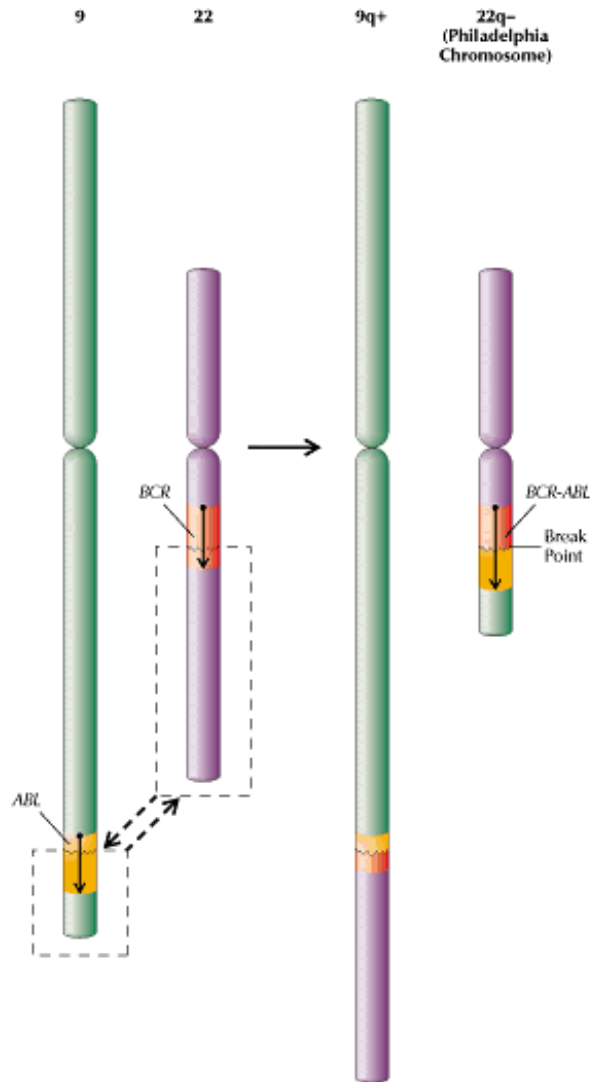


# Determination viral load

$$SE_{\lg \hat{c}_i} (test) = \frac{SE_{y.x}}{k} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(CT_i - \overline{CT})^2}{k^2 \sum_{i=1}^n (\lg c_i - \overline{\lg c})^2}}$$



# Monogenic disease monitoring

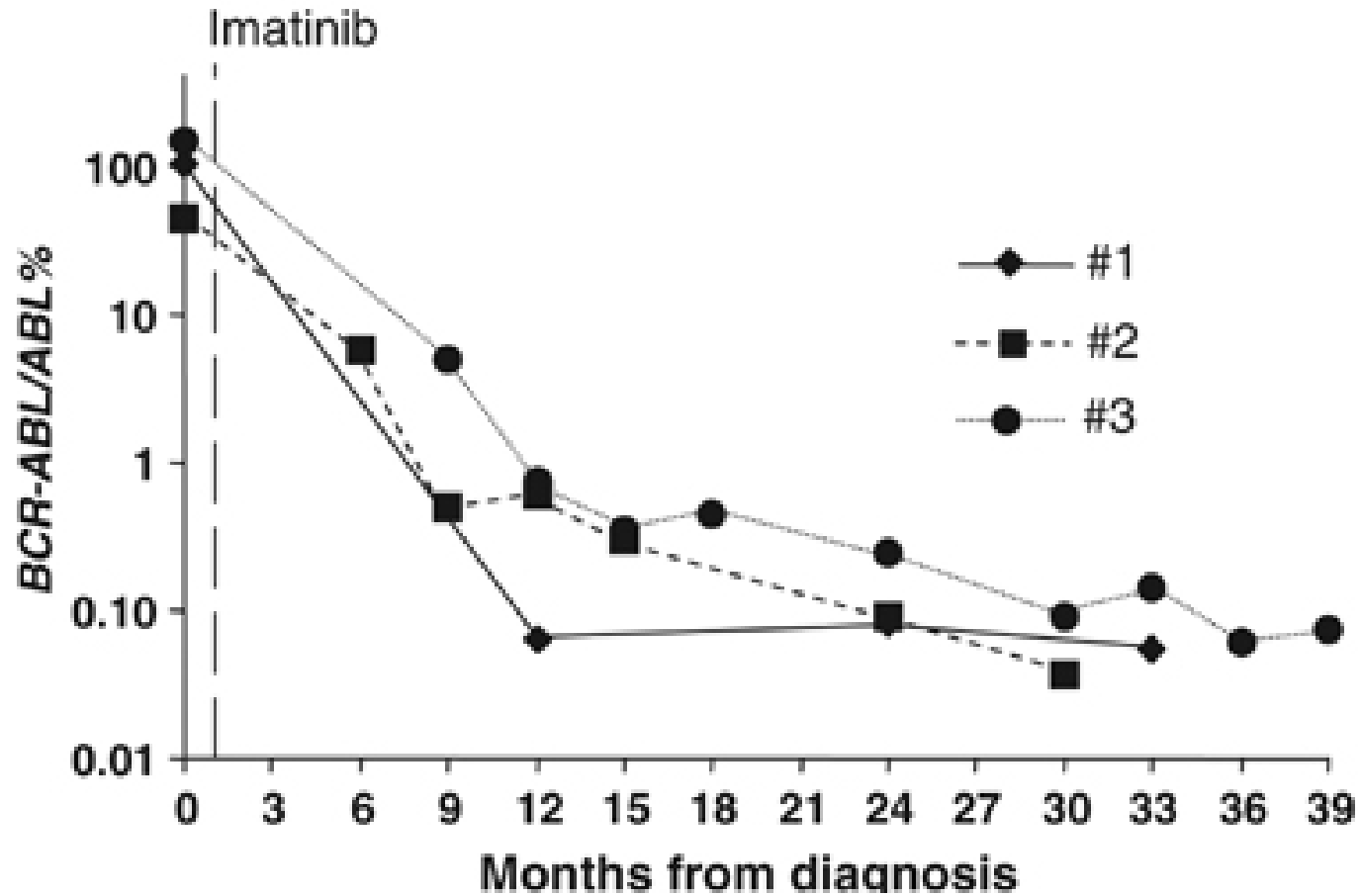


Biological samples

RT samples

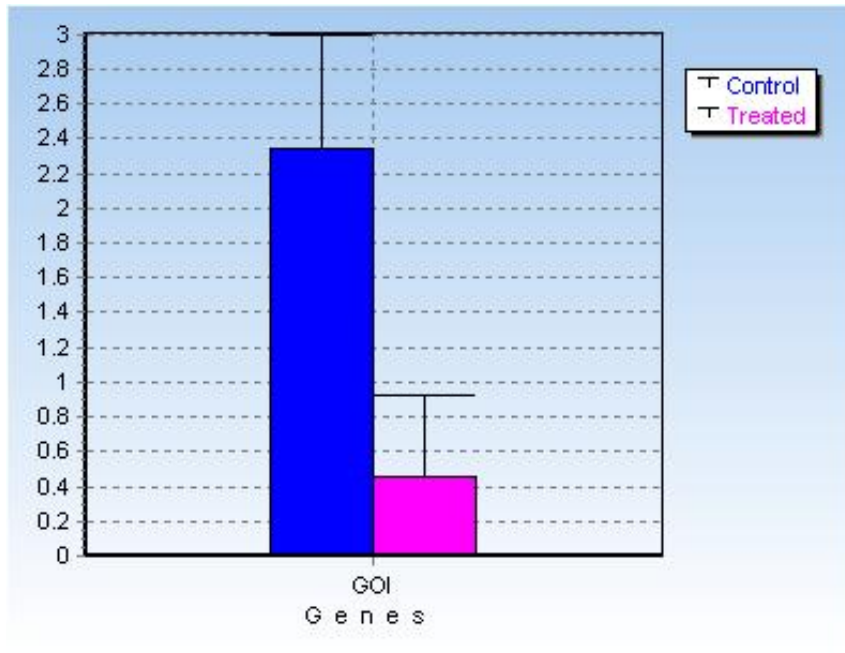
PCR samples

# Trend study

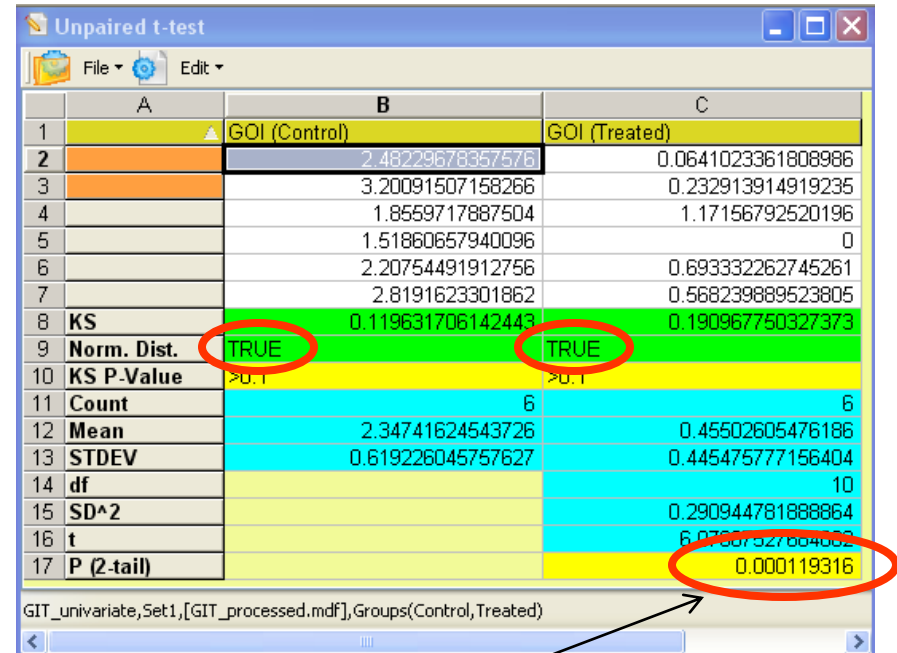


# One disease marker for complex disease

## Mean and 95% CI

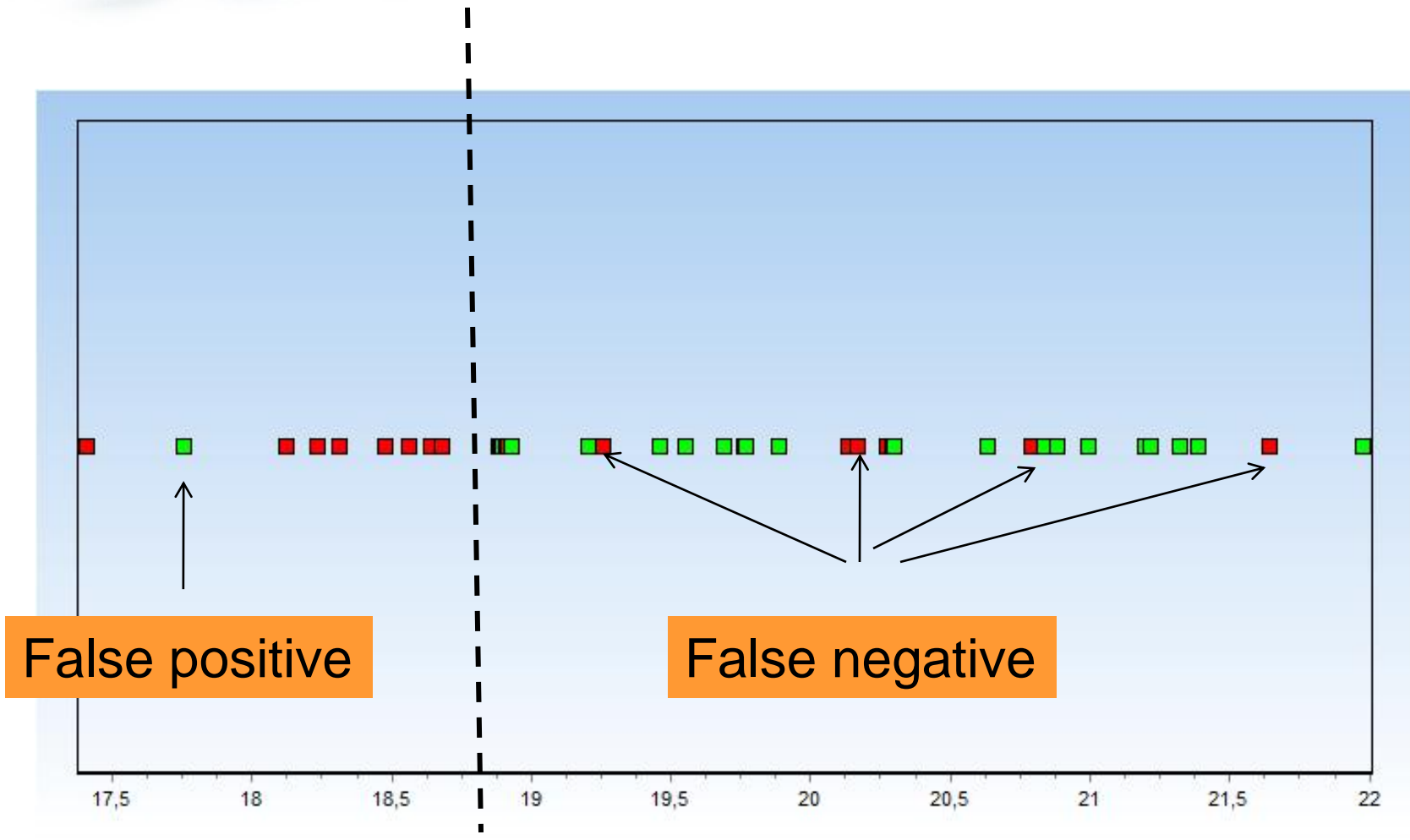


## Unpaired 2-sided t-test



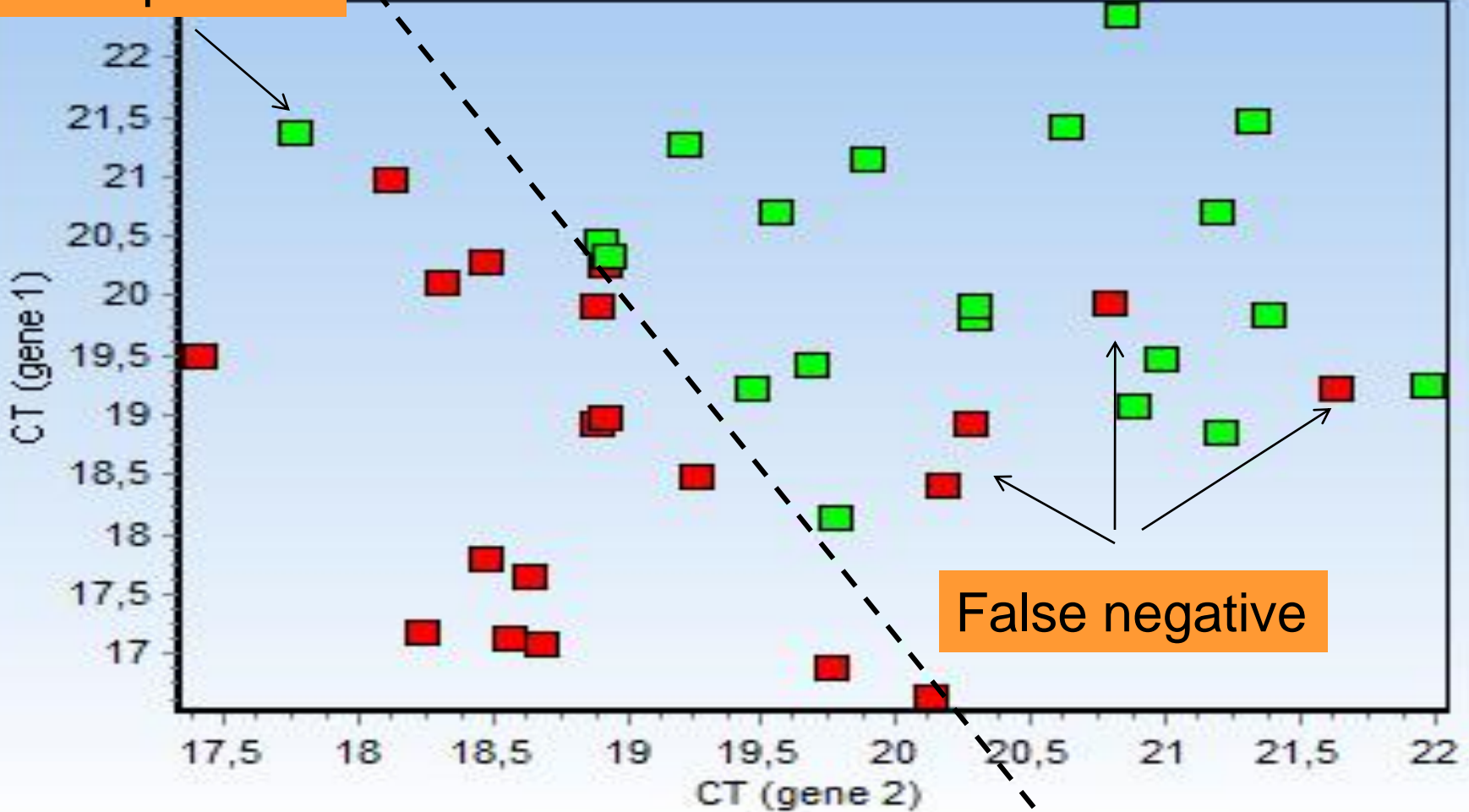
The probability that samples from two like populations shall produce the observed difference

# Classification based on 1 marker



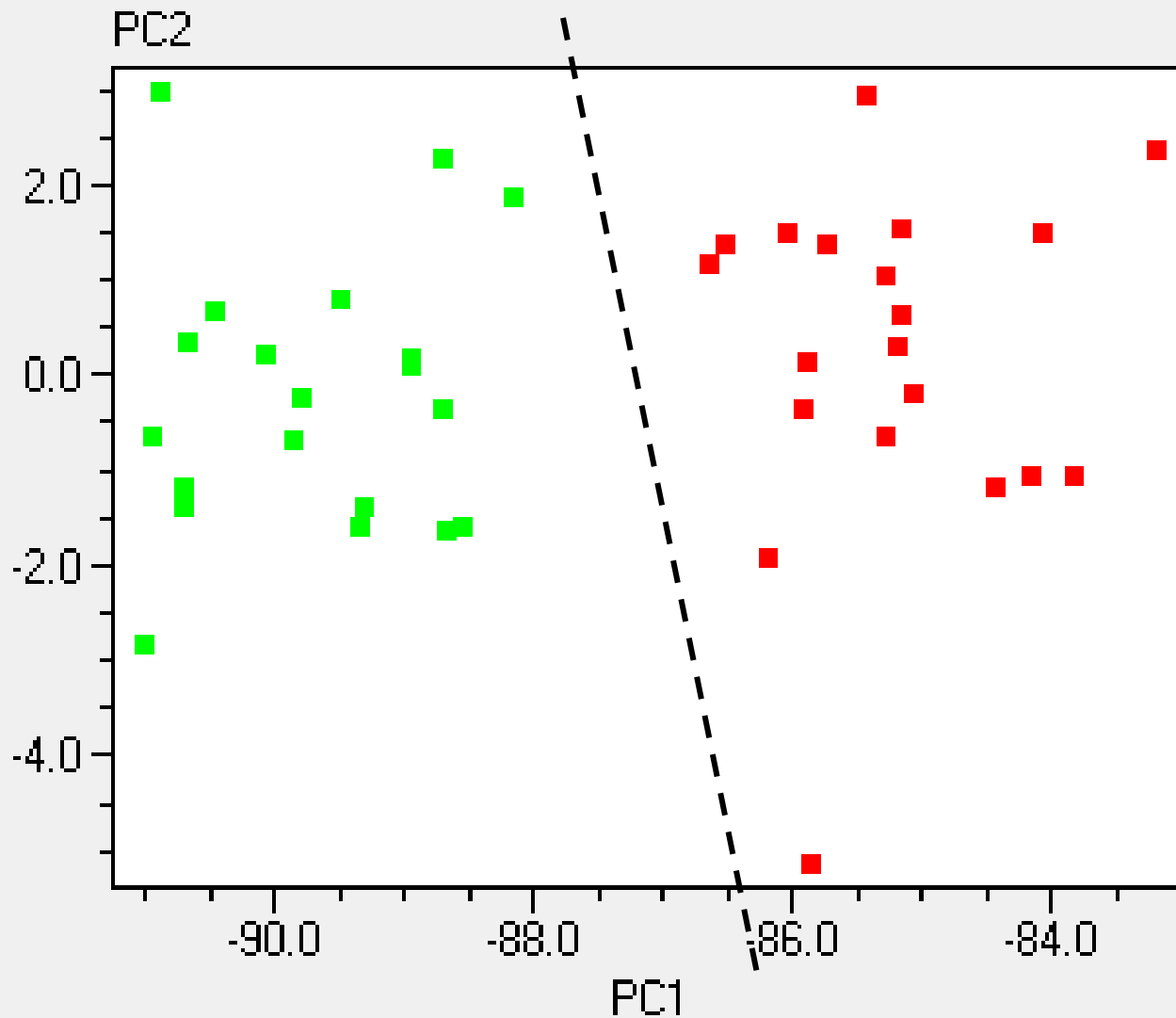
# Classification based on 2 genes

False positive

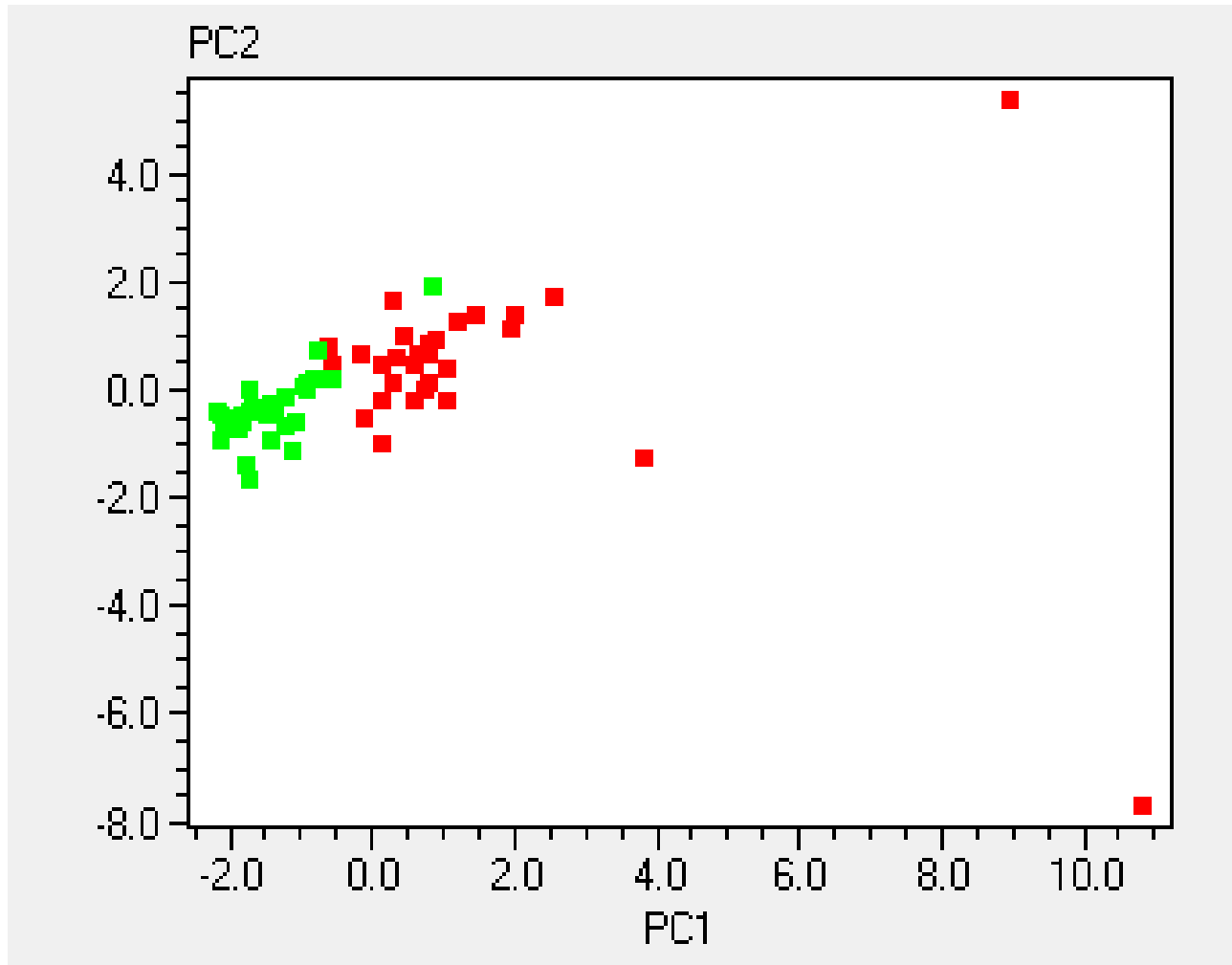


False negative

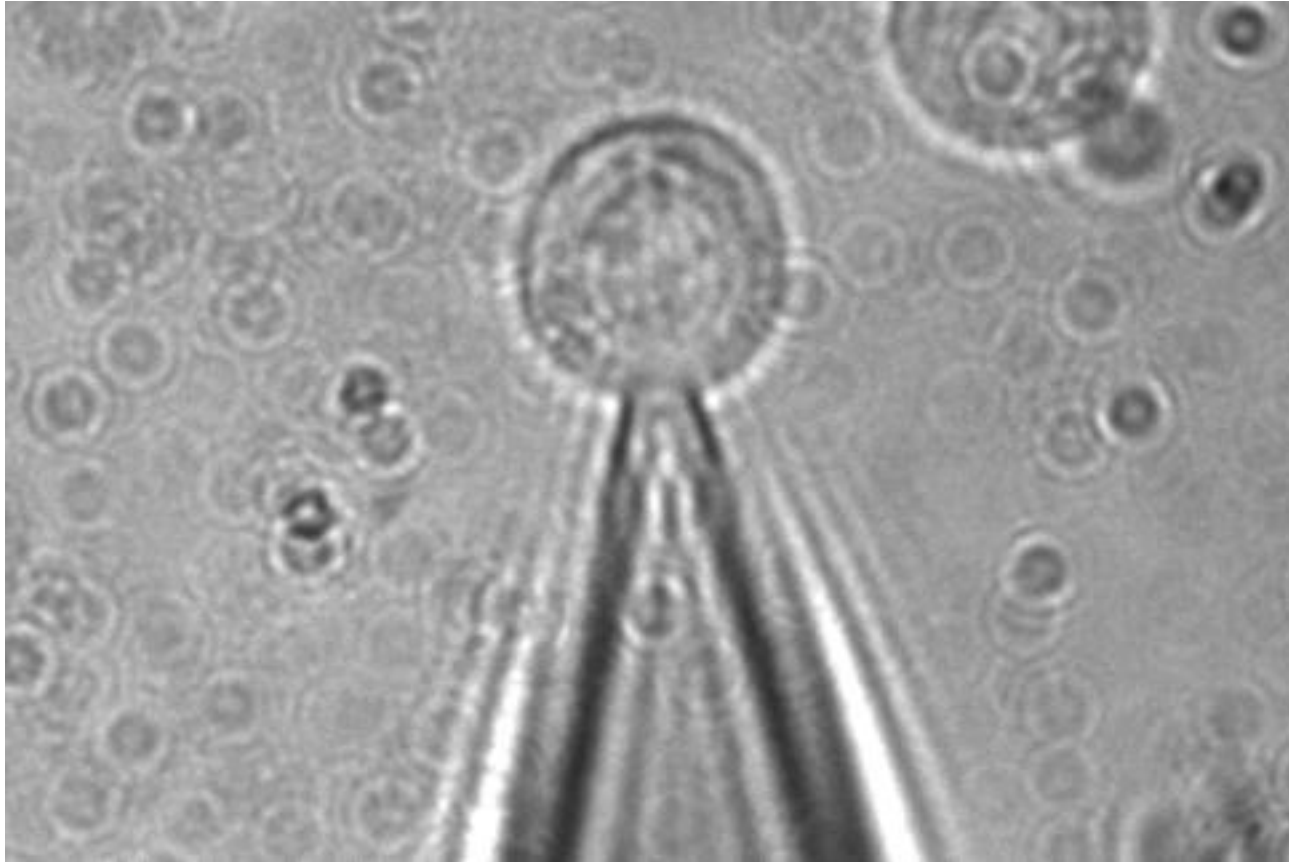
# Principal component analysis based on 14 markers



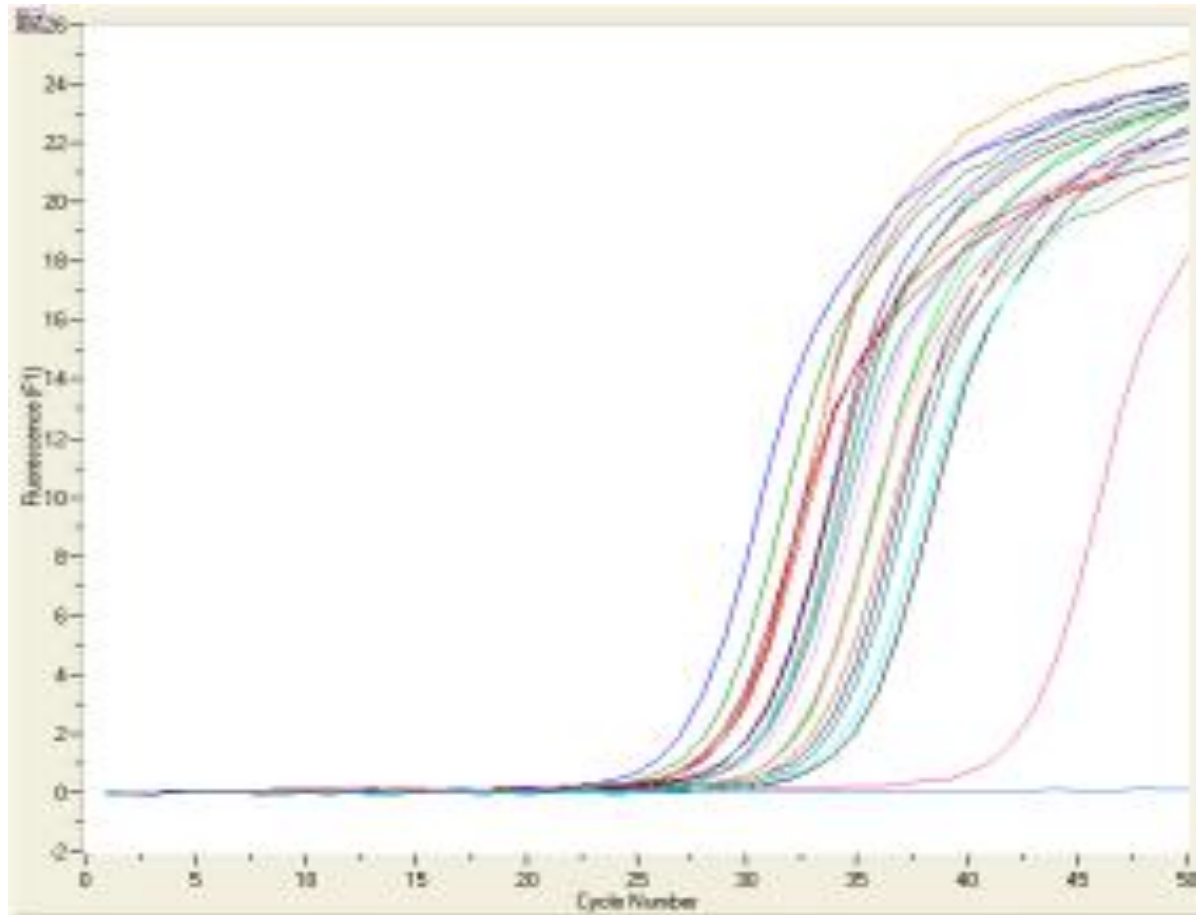
## Breast cancer vs. Healthy individuals (14 markers)



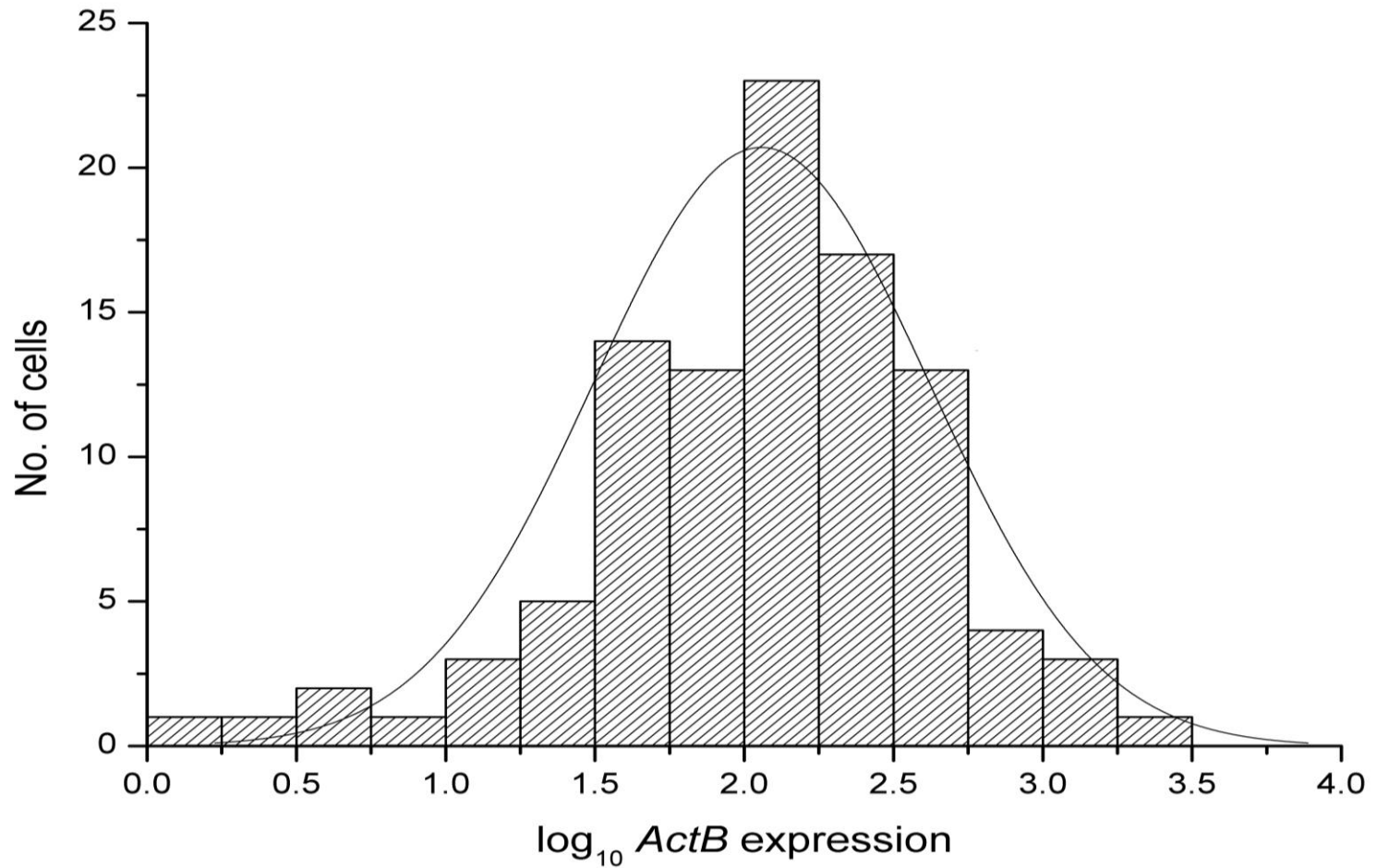
# Single-cell quantitative RT-PCR



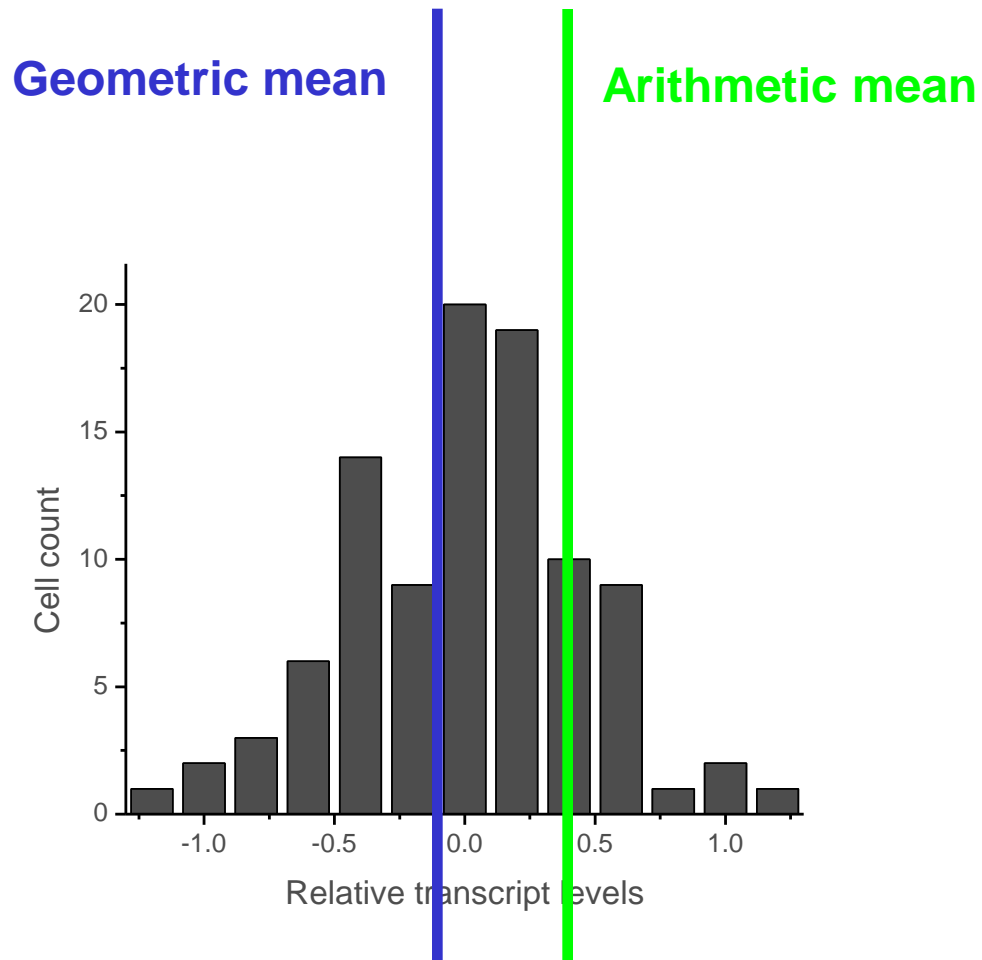
## Expression of Ins2 in individual $\beta$ cells



and in logarithmic scale....



# The typical (median) cell



# Which mean do you mean?

There is considerable variation in gene-expression levels between individual cells. Bengtsson *et al.* show that these levels are distributed log-normally rather than normally, which implies that the arithmetic mean does not represent the situation in a typical cell. They also show that the levels of expression of different genes in the same cell do not generally correlate, and suggest that mechanistic conclusions can be drawn when they do.

Using reverse transcriptase quantitative real-time PCR, they measured the transcript levels of 5 genes in 169 mouse pancreatic cells. For each gene the results were distributed log-normally across the sample cells, making the geometric mean a more appropriate representation of the data than the more commonly quoted arithmetic mean. For the insulin genes, *Ins1* and *Ins2*, up to 9-fold differences were found between the arithmetic and geometric means.

Of the five genes studied, only *Ins1* and *Ins2* expression levels correlated at the level of the individual cell. Levels of *ActB*, the  $\beta$ -actin gene, correlated with these two only at the overall population level, whereas levels of the final two genes did not correlate with any of the others. This indicates that expression-level differences in individual genes are not due to cells having different levels of overall transcription. The authors suggest that genes that correlate at the individual cell level are coordinately regulated, whereas those

that correlate at the population level merely respond to the same environmental stimuli.

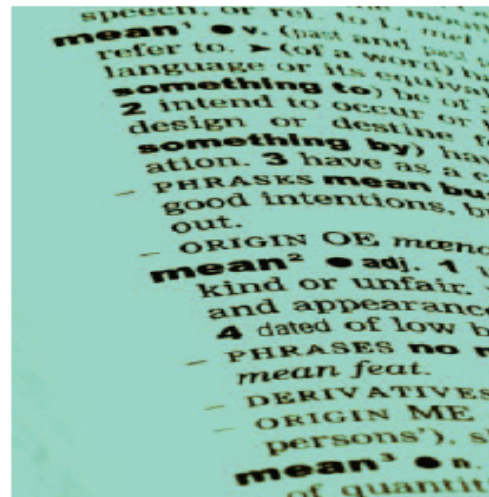
The importance of these findings is demonstrated by the fact that we might have underestimated the effect of glucose on insulin expression by almost 4-fold, which could be important in the administration of therapeutic insulin.

Patrick Goymer

## References and links

### ORIGINAL RESEARCH PAPER

Bengtsson, M. *et al.* Gene-expression profiling in single cells from the pancreatic islets of Langerhans reveals lognormal distribution of mRNA levels. *Genome Res.* **15**, 1388–1392 (2005)



# Correlation in genes' expression identifies the cell

## Pearson's coefficient

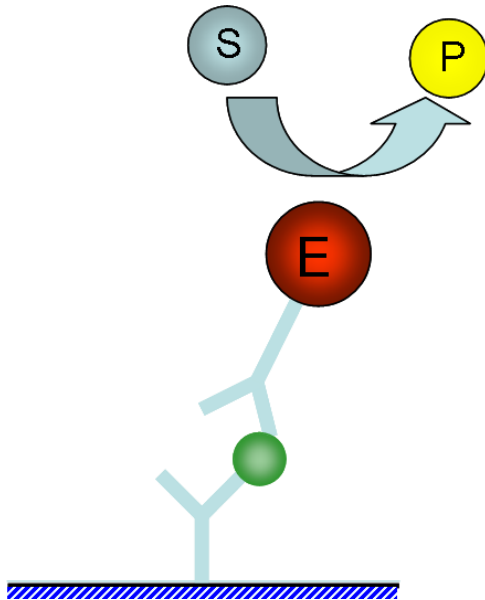
	<b>ActB</b>	<b>Ins1</b>	<b>ins2</b>	<b>Sur1</b>	<b>Kcnj11</b>
<b>ActB</b>	1				
<b>Ins1</b>	0.15	1			
<b>ins2</b>	0.12	0.90	1		
<b>Abcc8</b>	-0.02	-0.01	0.06	1	
<b>Kcnj11</b>	0.11	-0.02	0.24	-0.15	1

M. Bengtsson, A. Stålberg, P. Rorsman, M. Kubista, Genome Research (2005) 1388-1392

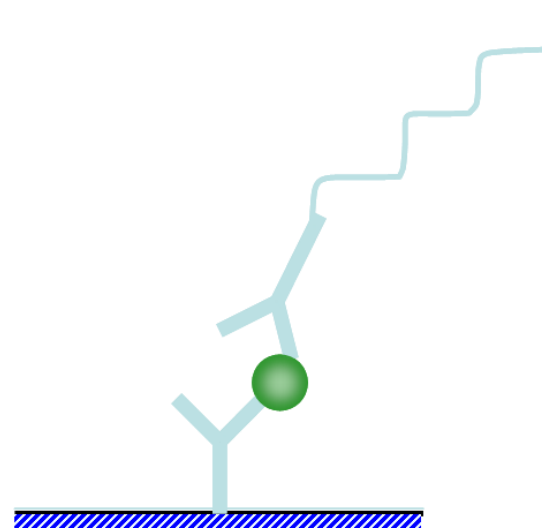
With pre-amp 50 genes per cell

# Protein detection

ELISA

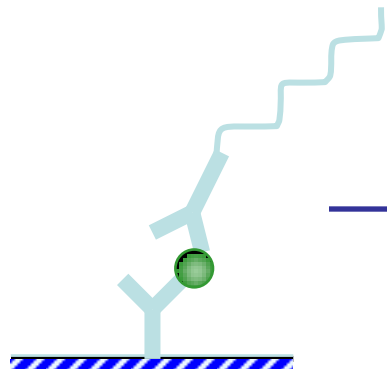


Immuno PCR

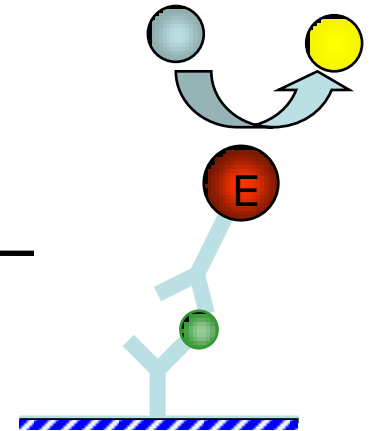
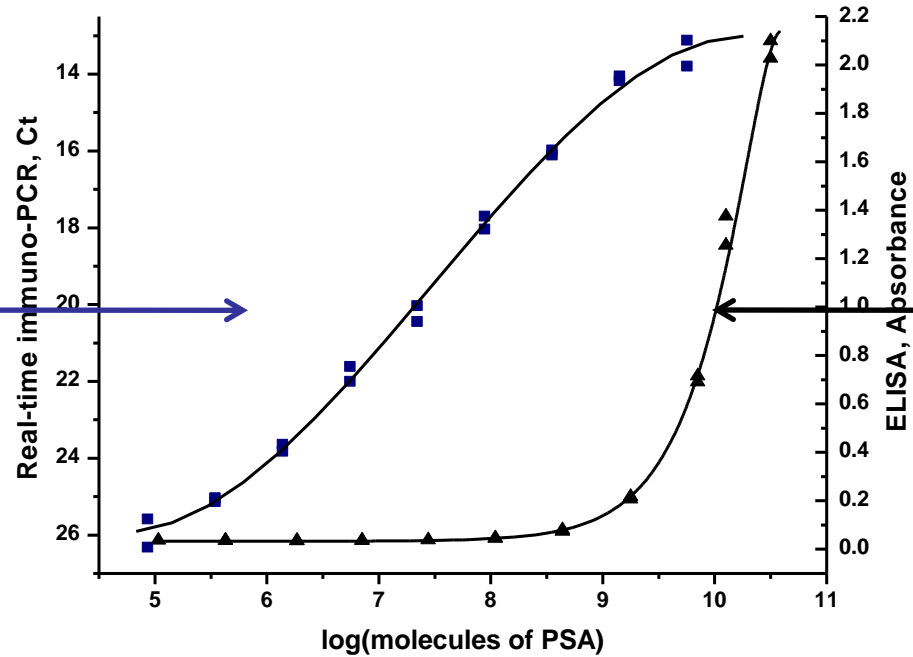


Lind & Kubista, Bio-Rad amplification tech note 2805

# ELISA vs immuno-qPCR

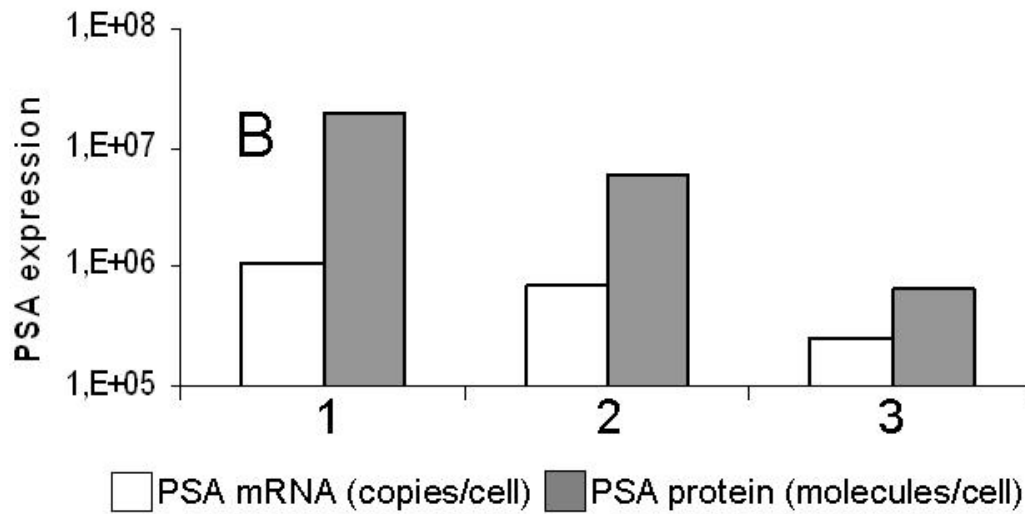
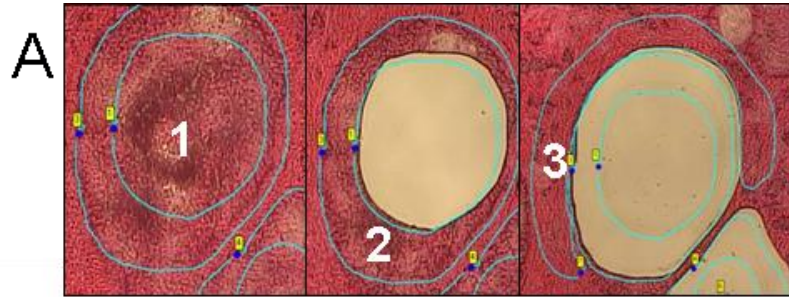


Immuno-qPCR



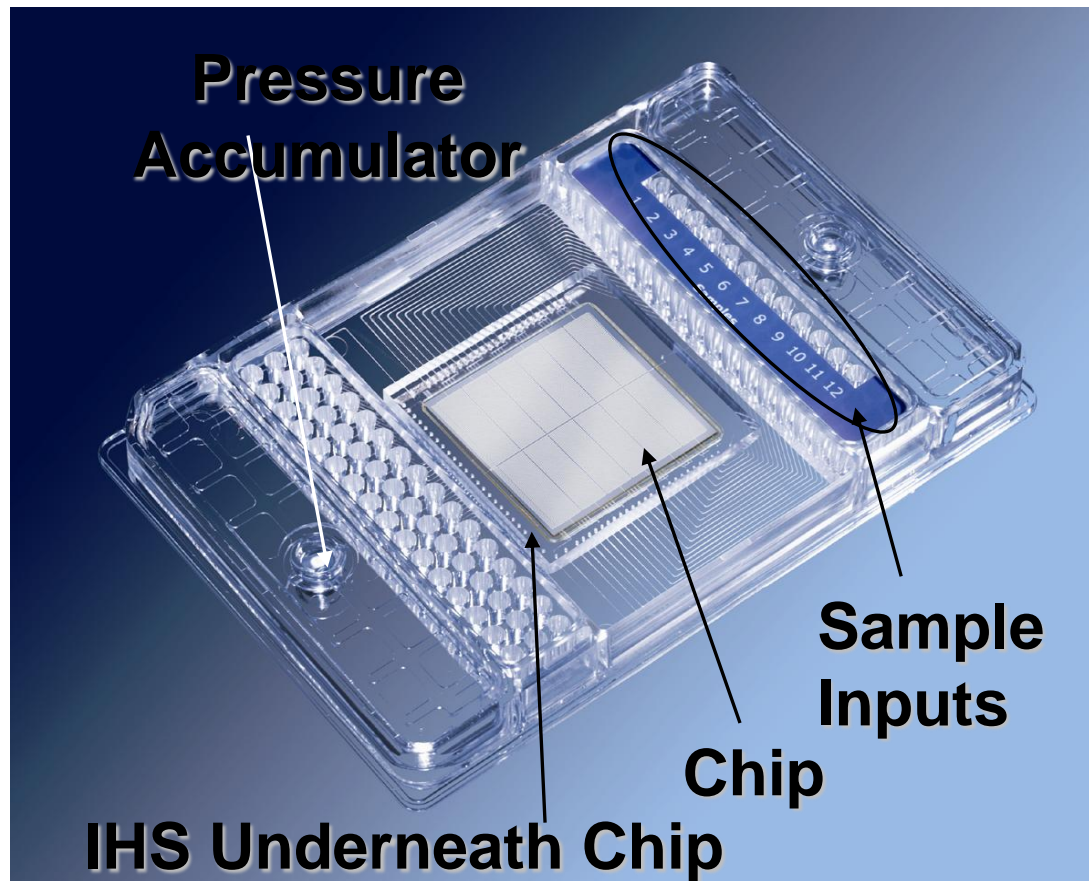
ELISA

# Measurements of PSA mRNA and protein



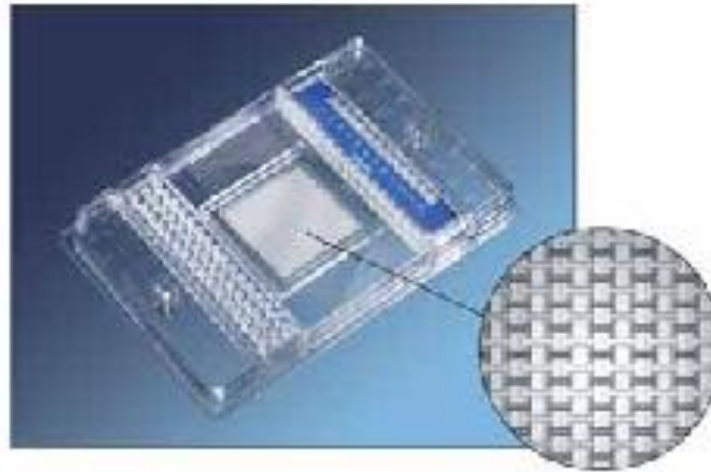
# High throughput expression profiling

48 (genes) × 48 (samples) = 2304 reactions!



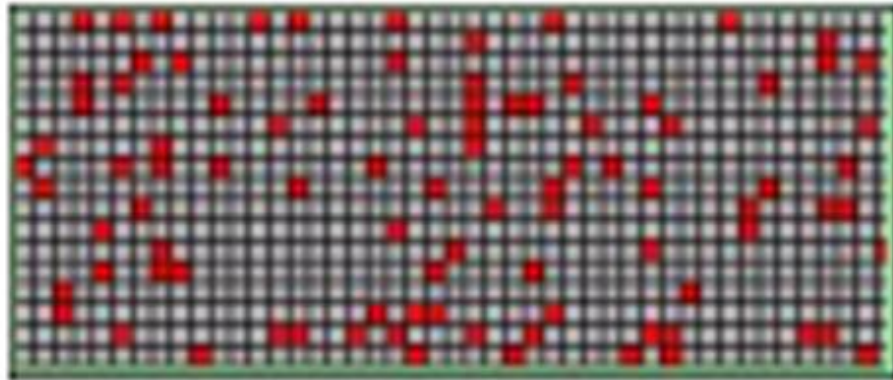
# Digital PCR

Sample is diluted into chambers that each contains in average less than 1 molecule



## Digital PCR

Sample is diluted into chambers that each contains in average less than 1 molecule



Rare mutations are easily detected because the background in a chamber containing the target has been reduced by the dilution

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## **Tooth development**

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Renata Peterková  
Jan Prochazka

## **Molecular Diagnostics**

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Patrik Rorsman  
Vlasta Ctrnacta  
Vendula Rusnaková  
Katarina Kolostova  
Daniela Pintérová

## **Neurospheres**

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Milos Pekny  
Maryam Faiz

## **Multivariate/multiway expression profiling**

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Amin Forootan  
Daniel Lindh  
Anders Bergkvist  
José Manuel Andrade  
Ales Tichopad

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