

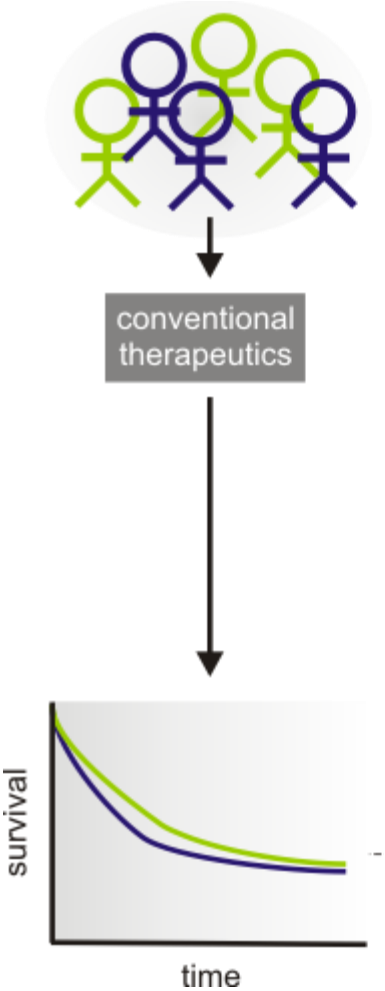


## **External oligonucleotide standards enable cross laboratory and exchange of real-time PCR data**

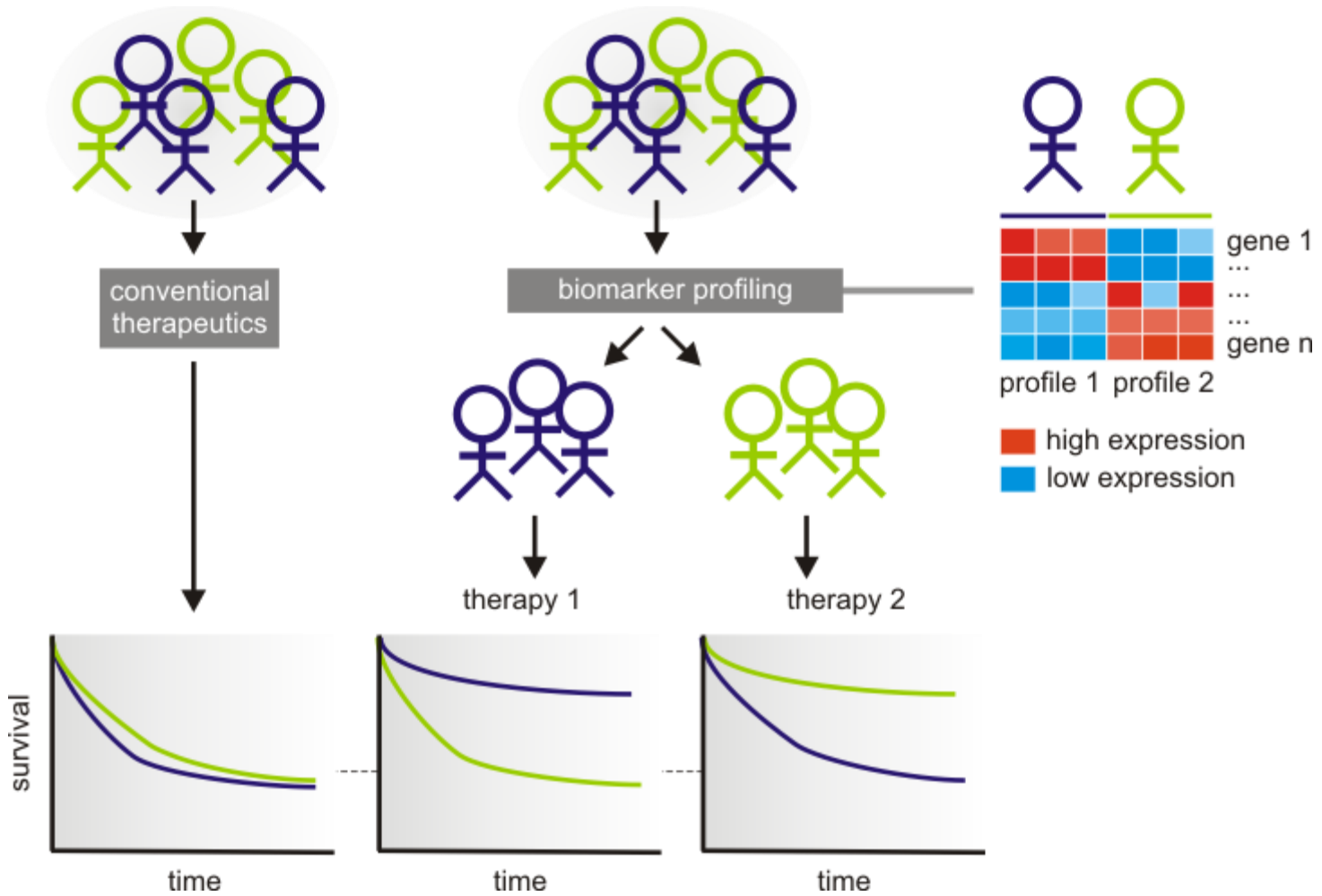
Jo Vandesompele  
professor, Ghent University  
co-founder and CEO, Biogazelle

Roche Molecular Biology Days  
Vilvoorde, 7 oktober, 2009

# biomarker signature based stratification



# biomarker signature based stratification



# study workflow

selection of a top ranking list of 59 prognostic markers

RNA quality control 700 samples

sample pre-amplification (WT-Ovation)

qPCR assay design and validation

real-time PCR  
384-well plates (LC480)

qbase<sup>PLUS</sup> data-analysis

- meta-analysis of 7 published microarray gene expression studies
- literature screening of almost 800 abstracts from single-gene studies

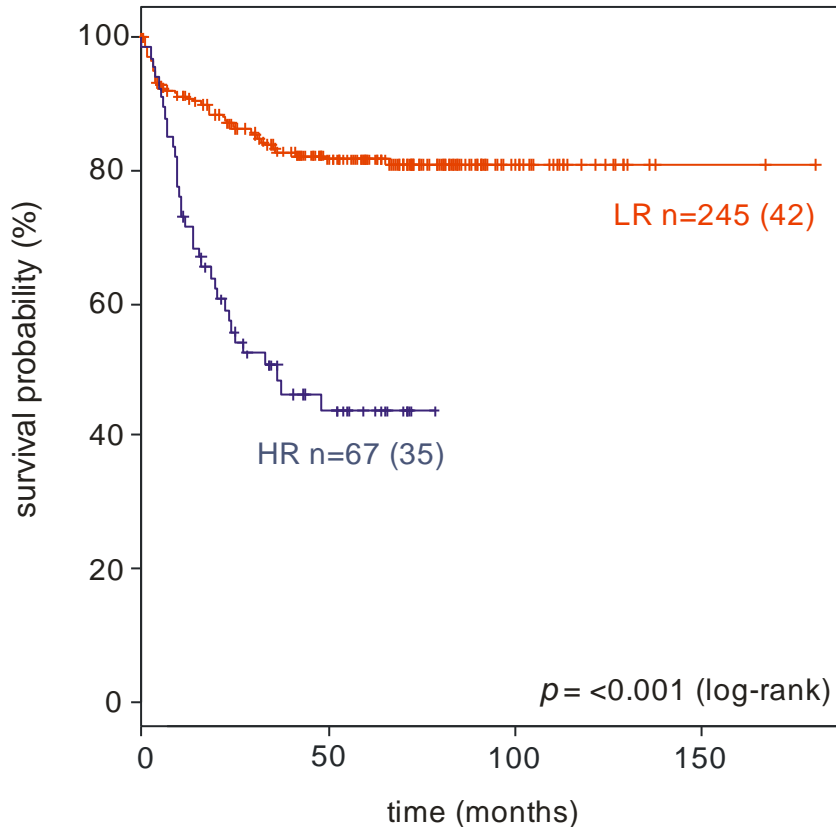
- two PCR-based assays
- capillary gel electrophoresis (Experion)

- [www.rtprimerdb.org](http://www.rtprimerdb.org)

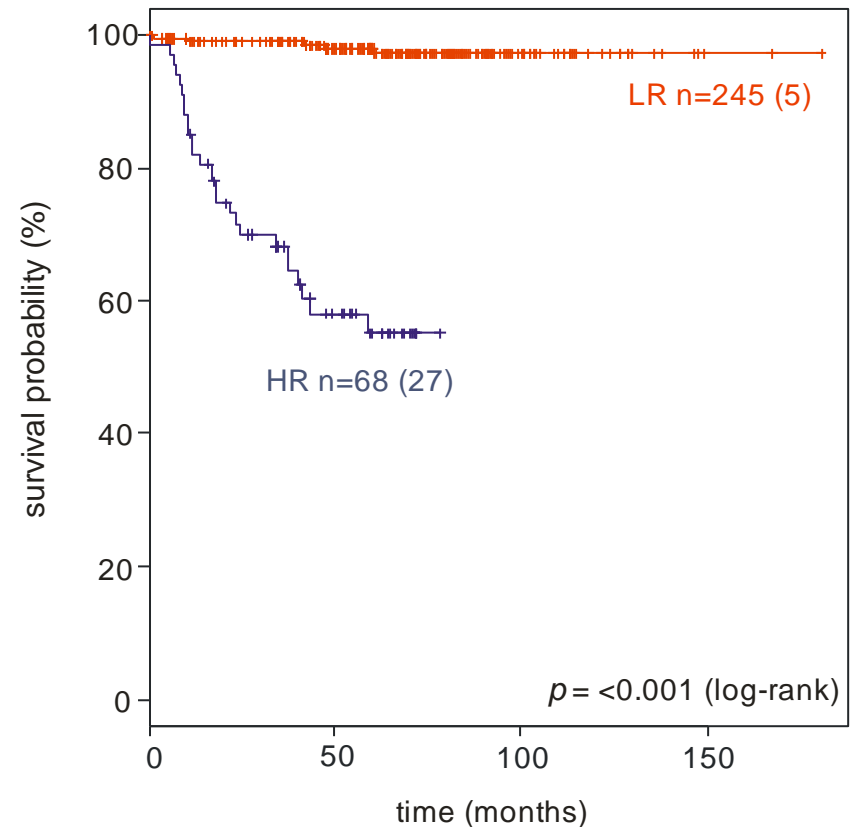
- Prediction Analysis of Microarrays
- Kaplan-Meier
- Cox proportional hazards

# classification of patients with respect to PFS and OS

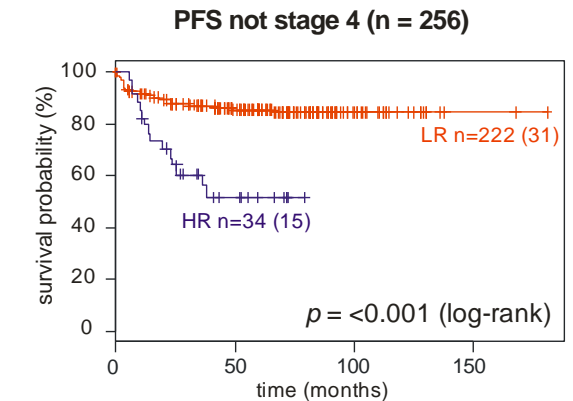
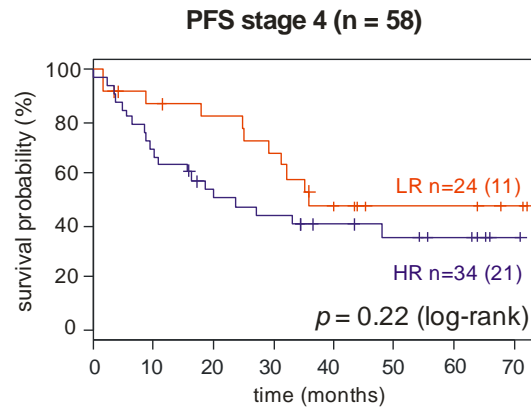
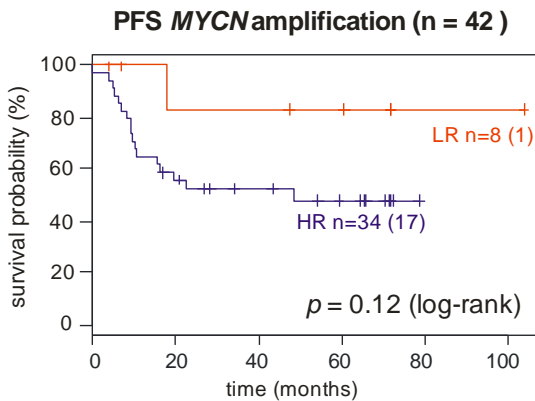
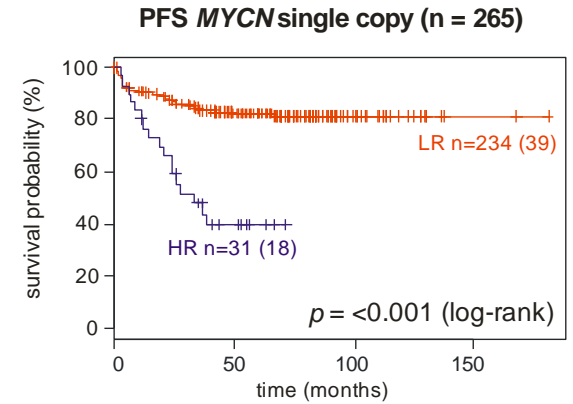
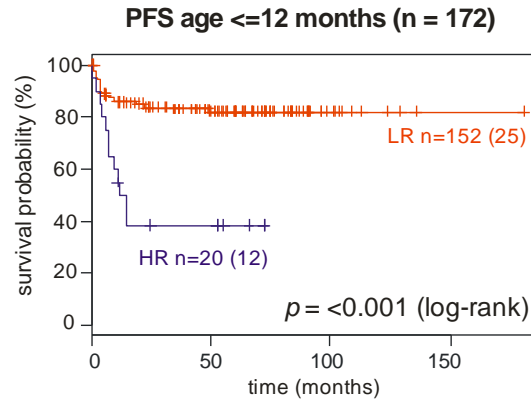
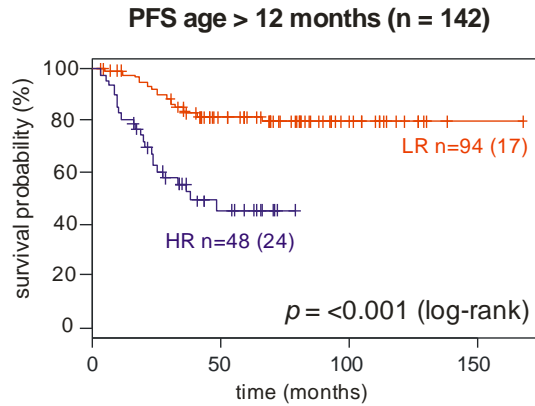
PFS total SIOPEN cohort (n = 312)



OS total SIOPEN cohort (n = 313)



# value of the classifier in relation to currently used risk factors: PFS



PAM classifier

multivariate  
cox analysis

independent  
predictor  
(age, stage,  
MYCN)

strong independent predictor:

patients with high molecular risk have

a **19-fold higher risk** to die from disease

a **4-fold higher risk** for relapse/progression

compared to patients with low molecular risk

- Vermeulen et al., The Lancet Oncology, 2009

---

## Predicting outcomes for children with neuroblastoma using a multigene-expression signature: a retrospective SIOPEN/COG/GPOH study

*Joëlle Vermeulen, Katleen De Preter, Arlene Naranjo, Liesbeth Vercruyssen, Nadine Van Roy, Jan Hellemans, Katrien Swerts, Sophie Bravo, Paola Scaruffi, Gian Paolo Tonini, Bruno De Bernardi, Rosa Noguera, Marta Piqueras, Adela Cañete, Victoria Castel, Isabelle Janoueix-Lerosey, Olivier Delattre, Gudrun Schleiermacher, Jean Michon, Valérie Combaret, Matthias Fischer, André Oberthuer, Peter F Ambros, Klaus Beiske, Jean Bénard, Bárbara Marques, Hervé Rubie, Janice Kohler, Ulrike Pötschger, Ruth Ladenstein, Michael D Hogarty, Patrick McGrady, Wendy B London, Geneviève Laureys, Frank Speleman, Jo Vandesompele*

## external oligonucleotide standards

### ■ synthetic control

FP

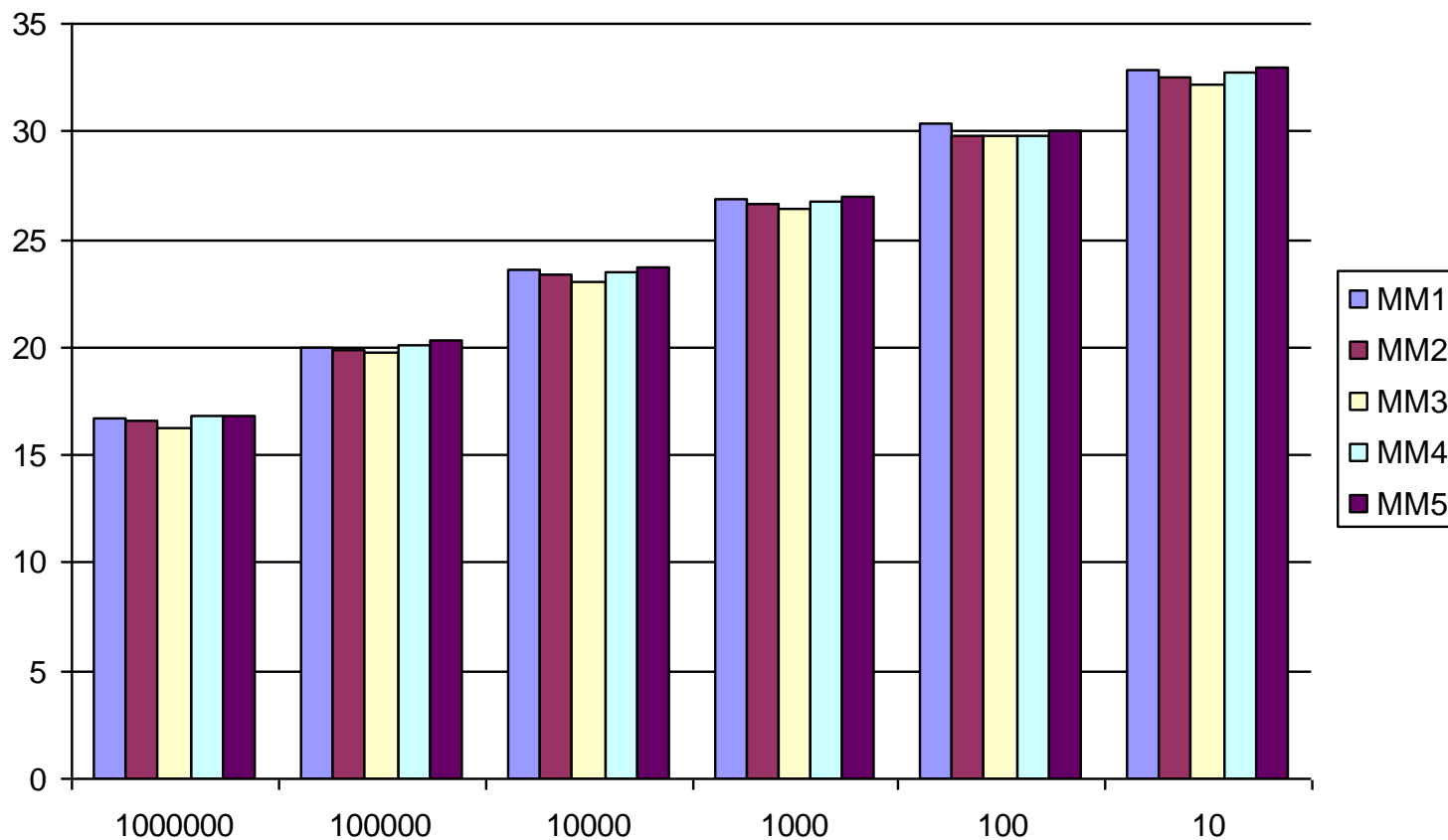
stuffer

RCRP

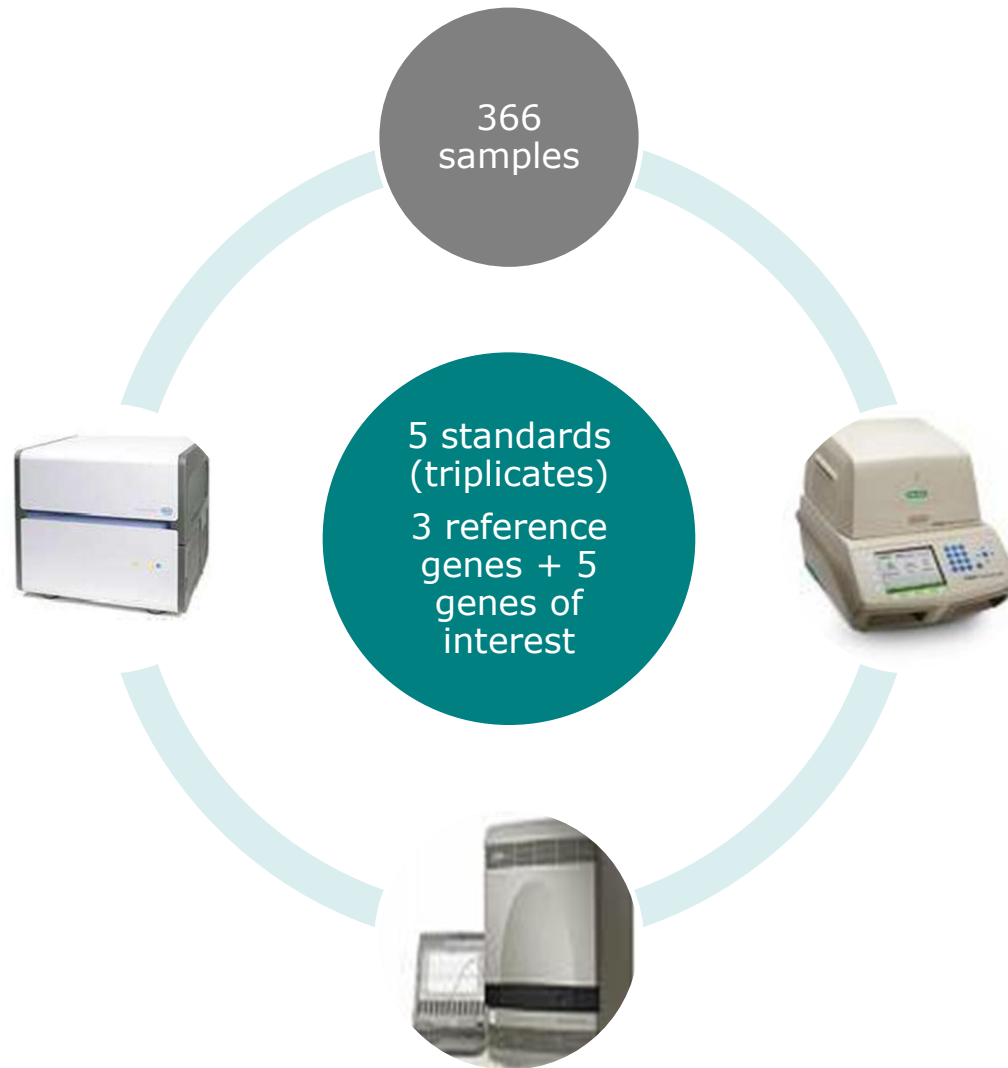
- 55 nucleotides
- PAGE purification
- blocking group
- 5 points dilution series: 150 000 molecules > 15 molecules

# external oligonucleotide standards

■ reproducibility across master mixes (5) and instruments (2)

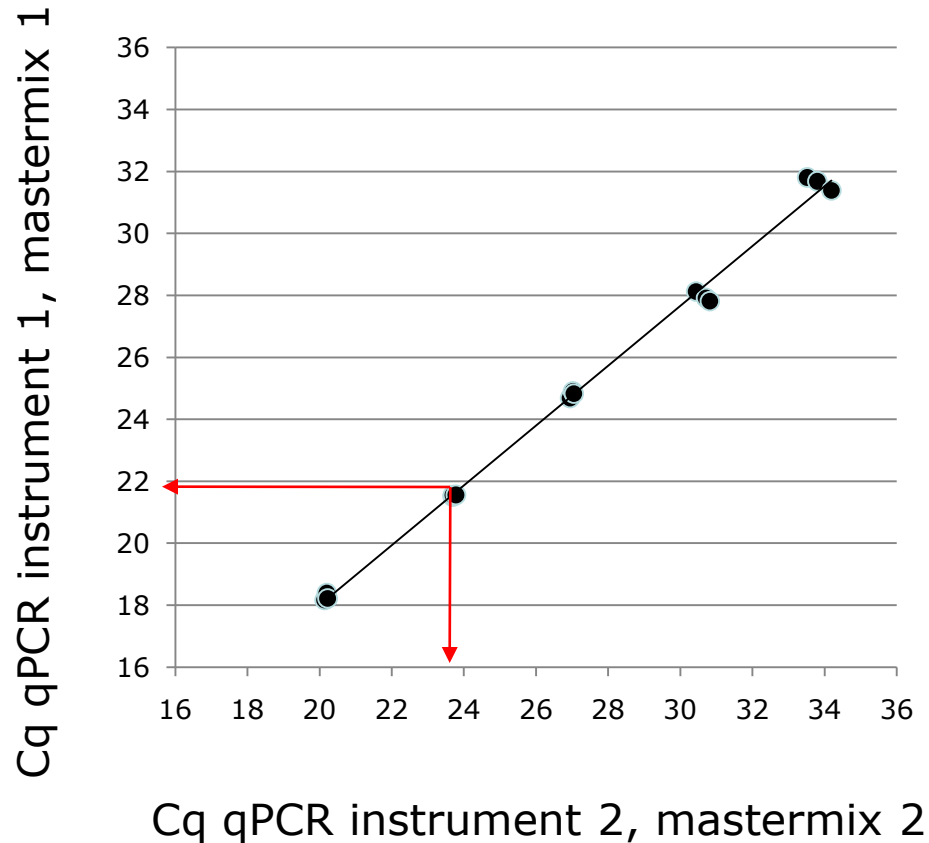


# external oligonucleotide standards cross lab comparison



# external oligonucleotide standards cross lab comparison

- 5 standards (triplicates)



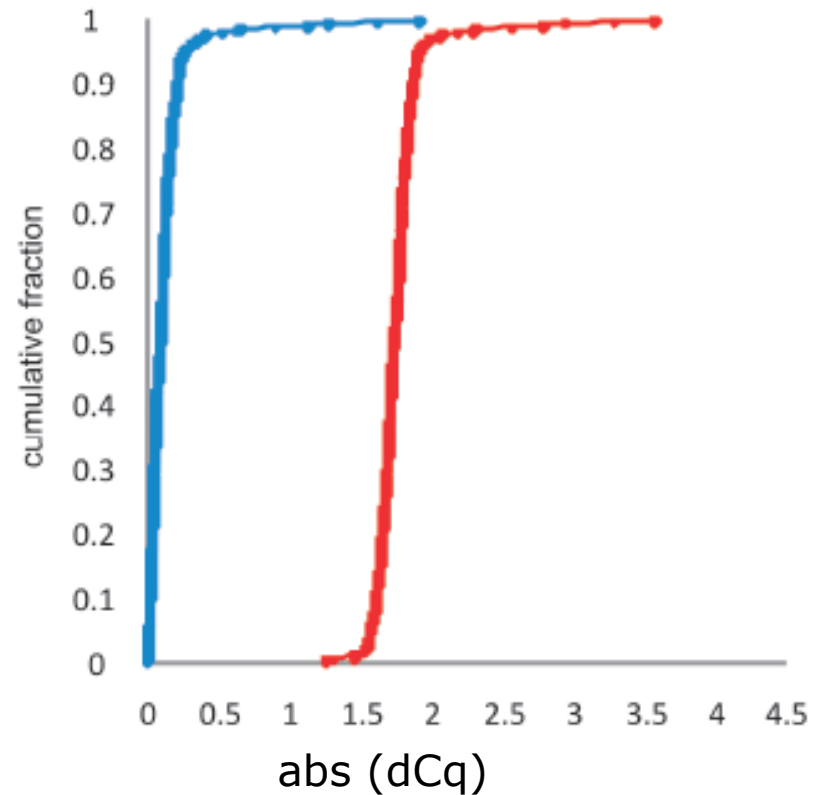
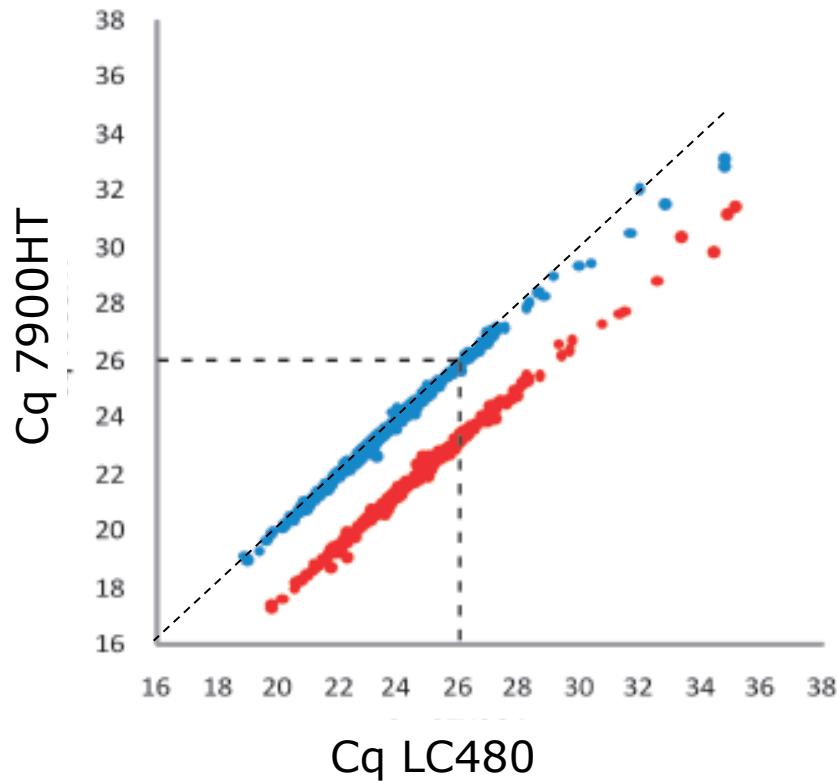
average  $\Delta Cq$  standards



correction Cq samples

# external oligonucleotide standards cross lab comparison

- ARHGEF7 gene
  - 366 samples
  - use of 5 standards (triplicates) for correction



- Vermeulen et al., Nucleic Acids Research, 2009

*Nucleic Acids Research*, 2009, 1–9  
doi:10.1093/nar/gkp721

## **External oligonucleotide standards enable cross laboratory comparison and exchange of real-time quantitative PCR data**

Joëlle Vermeulen<sup>1</sup>, Filip Pattyn<sup>1</sup>, Katleen De Preter<sup>1</sup>, Liesbeth Vercruysse<sup>1</sup>, Stefaan Derveaux<sup>1</sup>, Pieter Mestdagh<sup>1</sup>, Steve Lefever<sup>1</sup>, Jan Hellemans<sup>1,2</sup>, Frank Speleman<sup>1</sup> and Jo Vandesompele<sup>1,2,\*</sup>

## inter-run calibration requires specialized software

- data analysis using qbase<sup>PLUS</sup>
  - based on Ghent University's **geNorm** and **qBase** technology
  - up to fifty 384-well plates
  - multiple reference genes for accurate normalization
  - detection and correction of **inter-run variation**
    - multiple IRC > *more accurate*
    - normalized relative quantities > *greater flexibility*
  - dedicated error propagation
  - automated analysis; no manual interaction required

qbase<sup>PLUS</sup>

<http://www.qbaseplus.com>

- standardization is hot in real-time PCR
  - MIQE and RDML contribute to higher quality and transparency
  - external oligonucleotides enable cross-laboratory studies

# acknowledgements

- Jan Hellemans (UGent, Biogazelle)
- Filip Pattyn (UGent)
- Steve Lefever (UGent)
- Joëlle Vermeulen (UGent)
  
- MIQE & RDML consortium



# ADVANCES

## IN GENOMICS

### SYMPOSIUM



**REAL-TIME  
QUANTITATIVE  
PCR**

28.01.2010

**NEXT  
GENERATION  
SEQUENCING**

29.01.2010

**WWW.ADVANCES-IN-GENOMICS.ORG**

January 28-29, 2010  
Ghent, Belgium

[www.advances-in-genomics.org](http://www.advances-in-genomics.org)

early bird registration  
October 31, 2009