



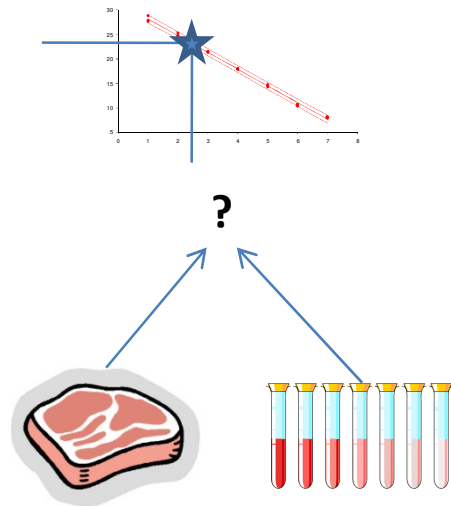
GenEx qPCR Data Analysis
2009-03-10

Presentation Outline

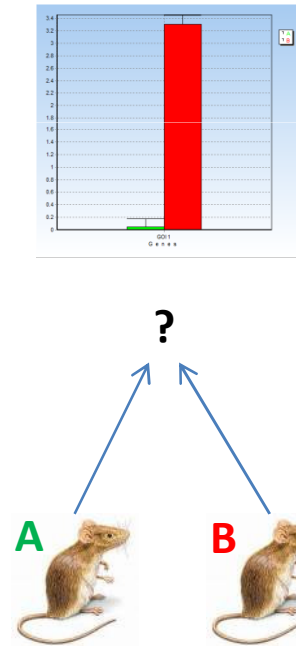
- **qPCR Data Analysis**
 - Absolute Quantification
 - Relative Quantification
 - Expression Profiling
- **GenEx Software Tool**
 - Modules
 - Pre-Processing
 - Statistical Tests
 - Visualizations

qPCR Data Analysis

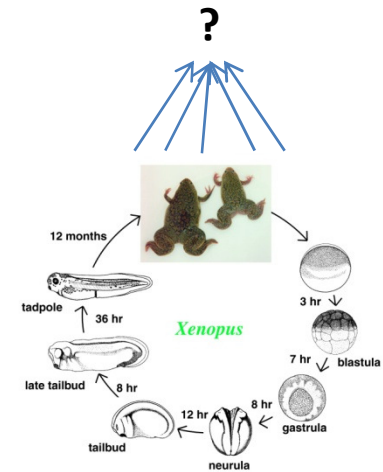
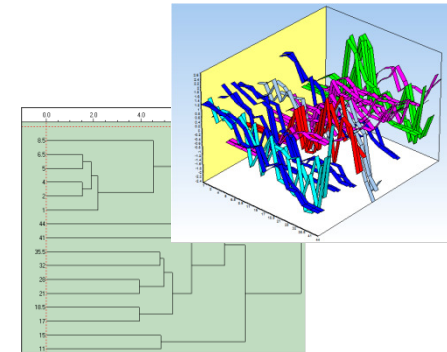
Absolute Quantification



Relative Quantification



Expression Profiling



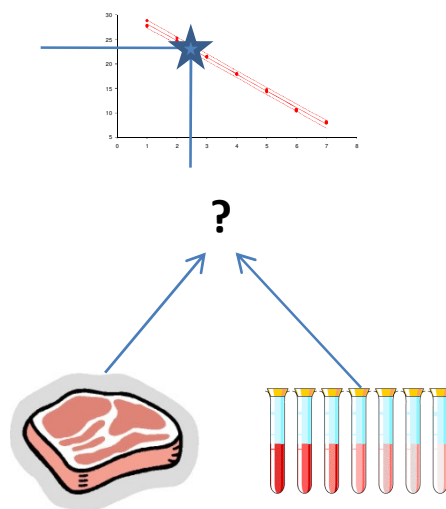
***GenEx Standard* includes modules for:**

- **Data editor**
 - Data corrections
 - Normalizations
 - Scaling
- **Data manager**
 - Definitions of groups and categories
 - Control of presentation parameters
 - Scaling
- **Plot functions**
 - 2D/3D functionalities
 - Line plots, Box plots, scatterplots and more
- **Reference gene identification**
 - geNorm
 - NormFinder
- **Statistics**
 - Descriptive statistics
 - Student's t-test
 - Non-parametric tests
- **Linear Regression**
 - Standard curve

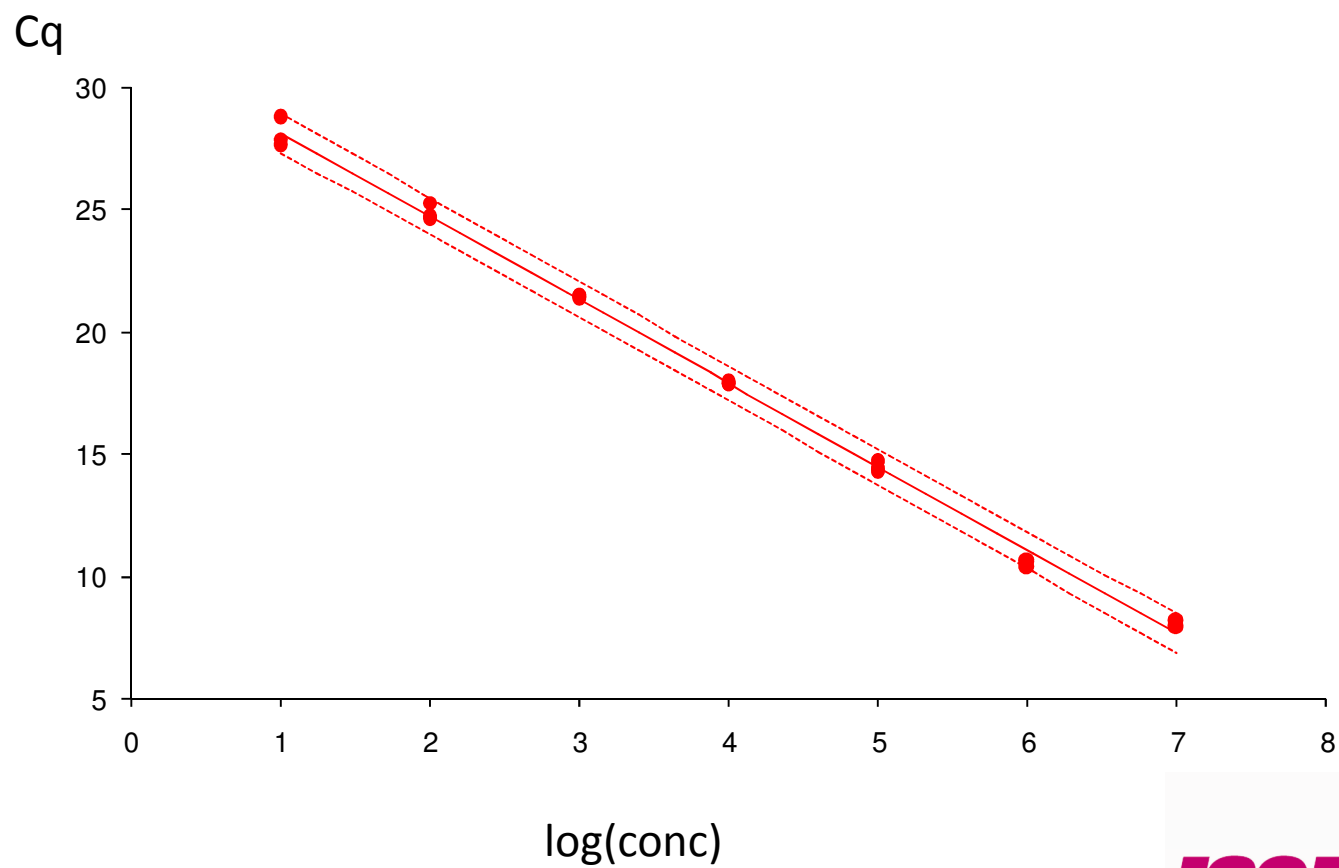
GenEx Pro also includes modules for:

- **Clustering**
 - Hierarchical/Phylogenetics
- **Principle component analyses**
 - Principle component analysis
 - Potential curve analysis
 - Non-parametric tests
- **Network analyses**
 - Artificial neural network analysis
 - Kohonen self organizing maps
- **Linear Regression**
 - Reverse calibration

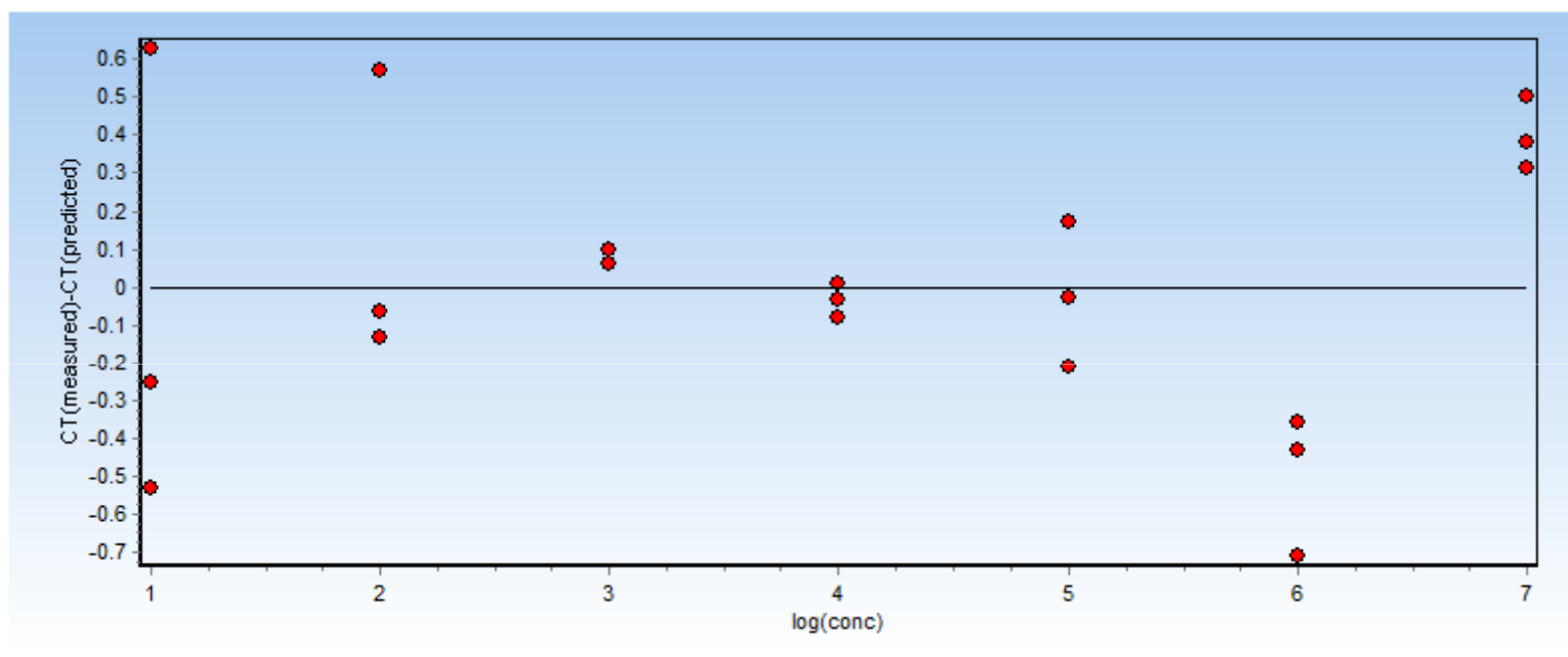
Absolute Quantification



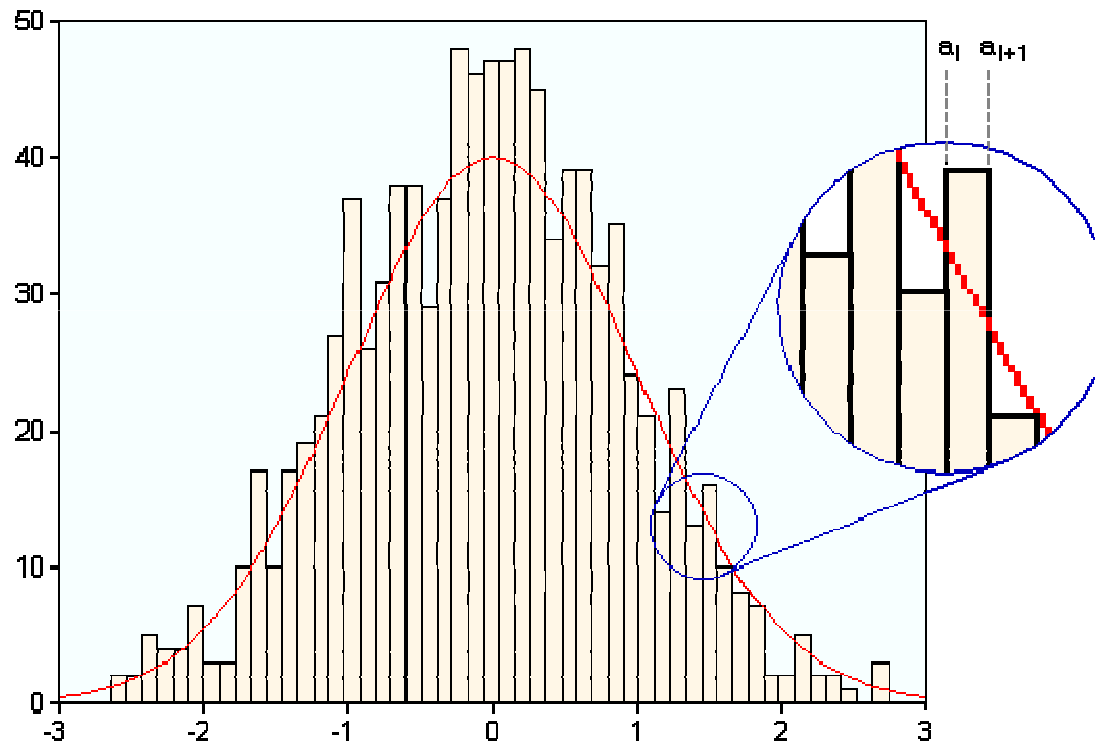
Standard Curve



Residual plot



Normal distribution = Gaussian distribution



Outlier detection of repeats

- If at least three repeats are available, the data can be tested for outliers with the Grubbs' test.

Calibration samples	log(dilution)	CT(1)	CT(2)	CT(3)
Calibration 1	1	27.63	28.79	27.91
Calibration 2	2	25.31	24.61	24.68
Calibration 3	3	21.42	21.38	21.42
Calibration 4	4	17.82	17.87	17.91
Calibration 5	5	14.65	14.27	14.45
Calibration 6	6	10.63	10.7	10.35
Calibration 7	7	7.95	8.02	8.14

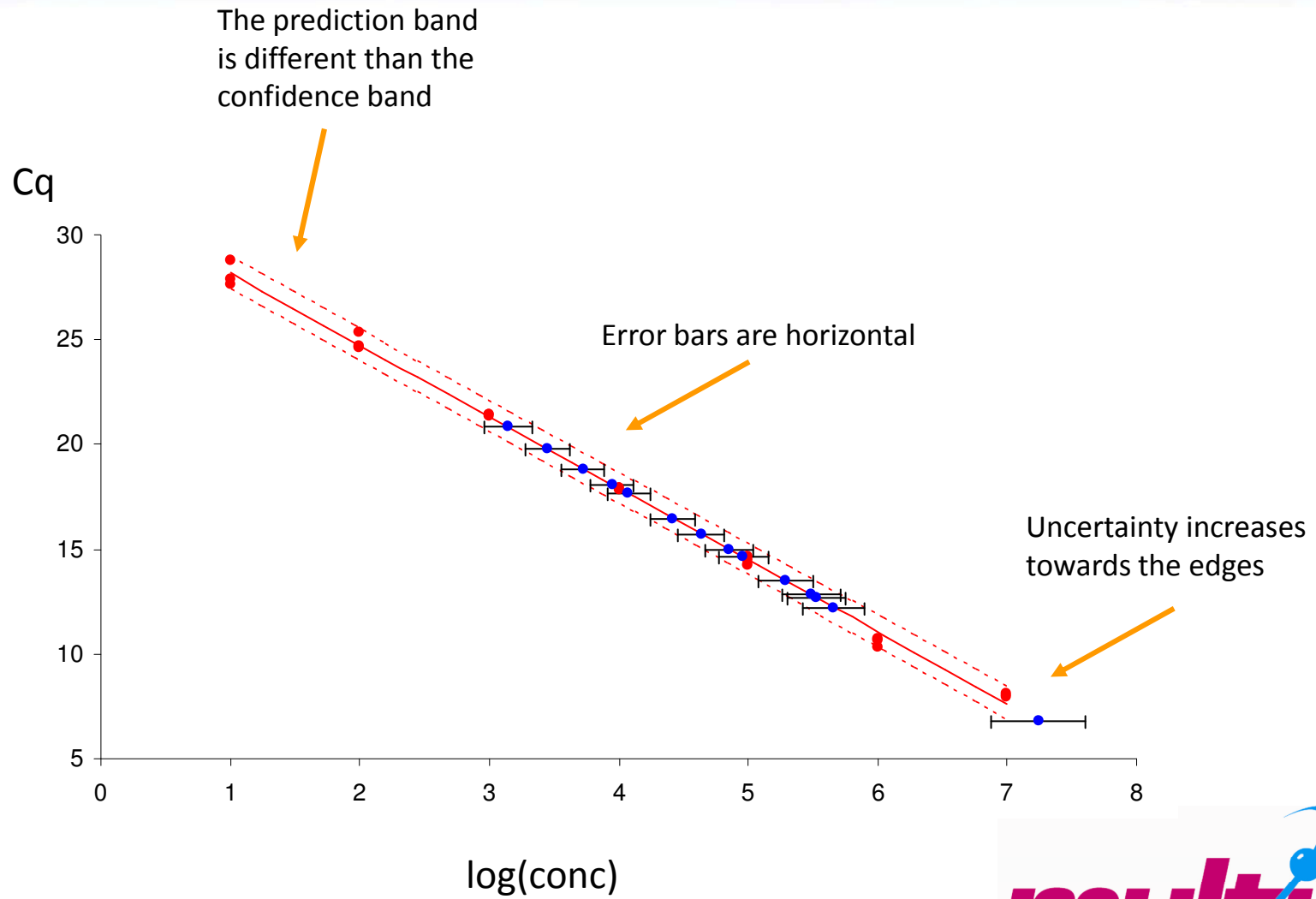
- Autoscale data:

$$Cq_{as} = (Cq - \overline{Cq}) / SD(Cq)$$

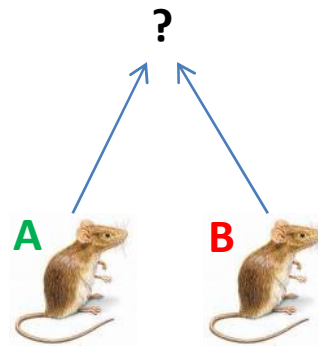
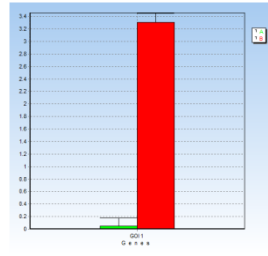
Autoscaled CT		
-0.79	1.12	-0.33
1.15	-0.67	-0.48
0.58	-1.15	0.58
-1.03	0.07	0.96
1.02	-0.98	-0.04
0.38	0.76	-1.13
-0.90	-0.17	1.08

N	90%	95%	99%
3	1.15	1.15	1.15
4	1.46	1.48	1.50
5	1.67	1.72	1.76
6	1.82	1.89	1.97
7	1.94	2.02	2.14
8	2.03	2.13	2.27
9	2.11	2.22	2.39
10	2.18	2.29	2.48

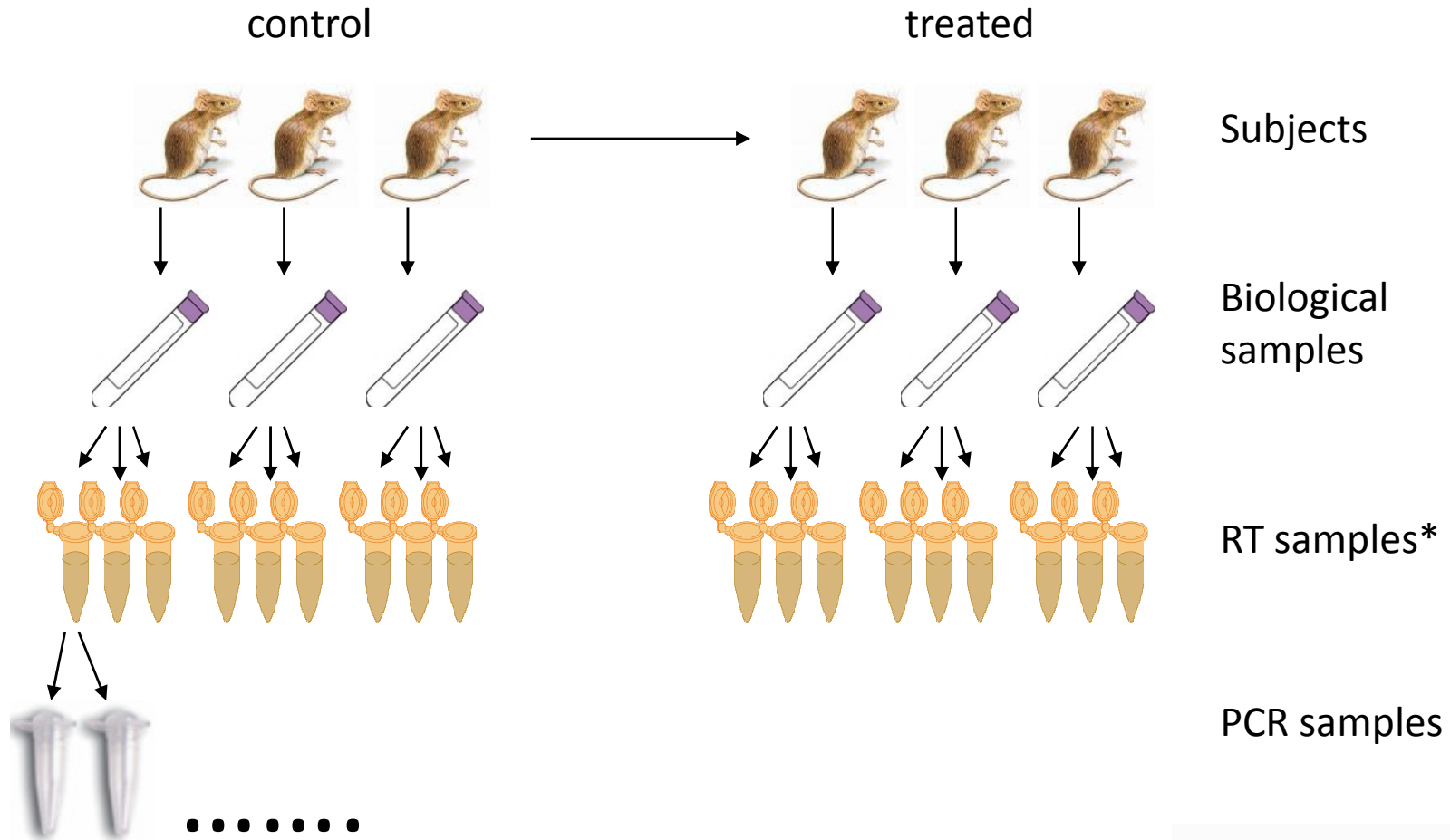
Prediction



Relative Quantification

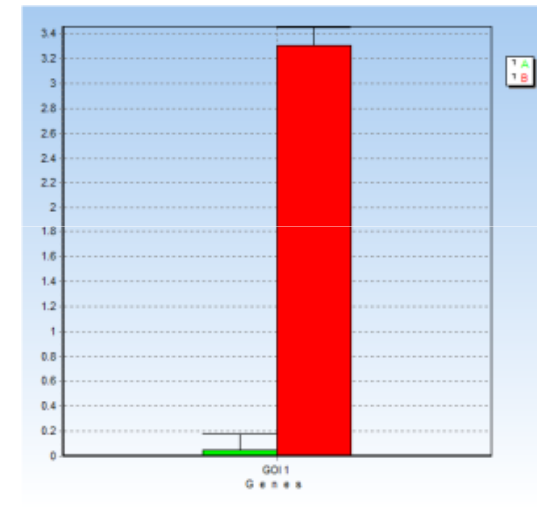
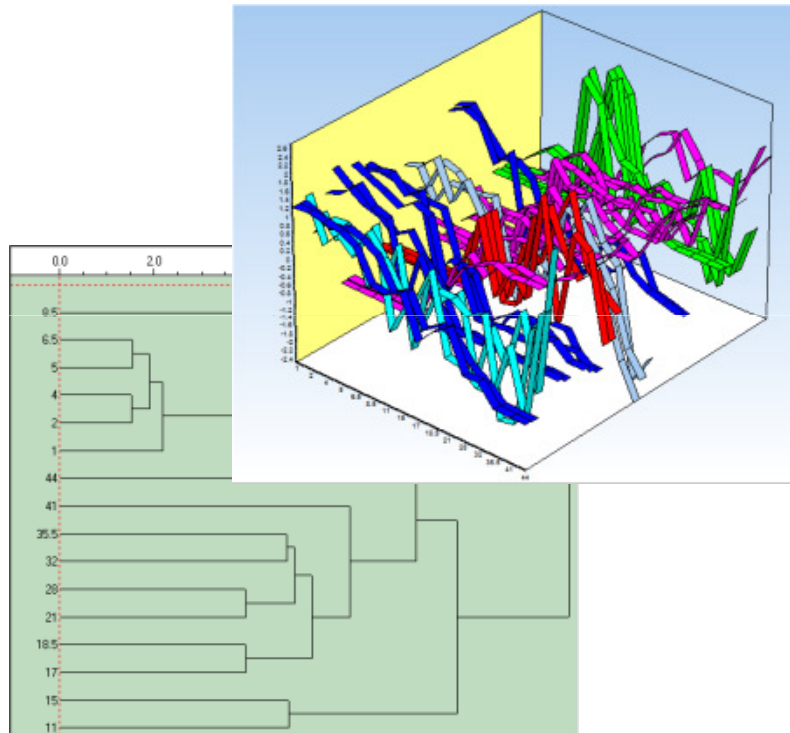


Experimental Design



*RT is the main source of variance. Therefore three replicates are built for each biological sample to assure more precise mean estimate.

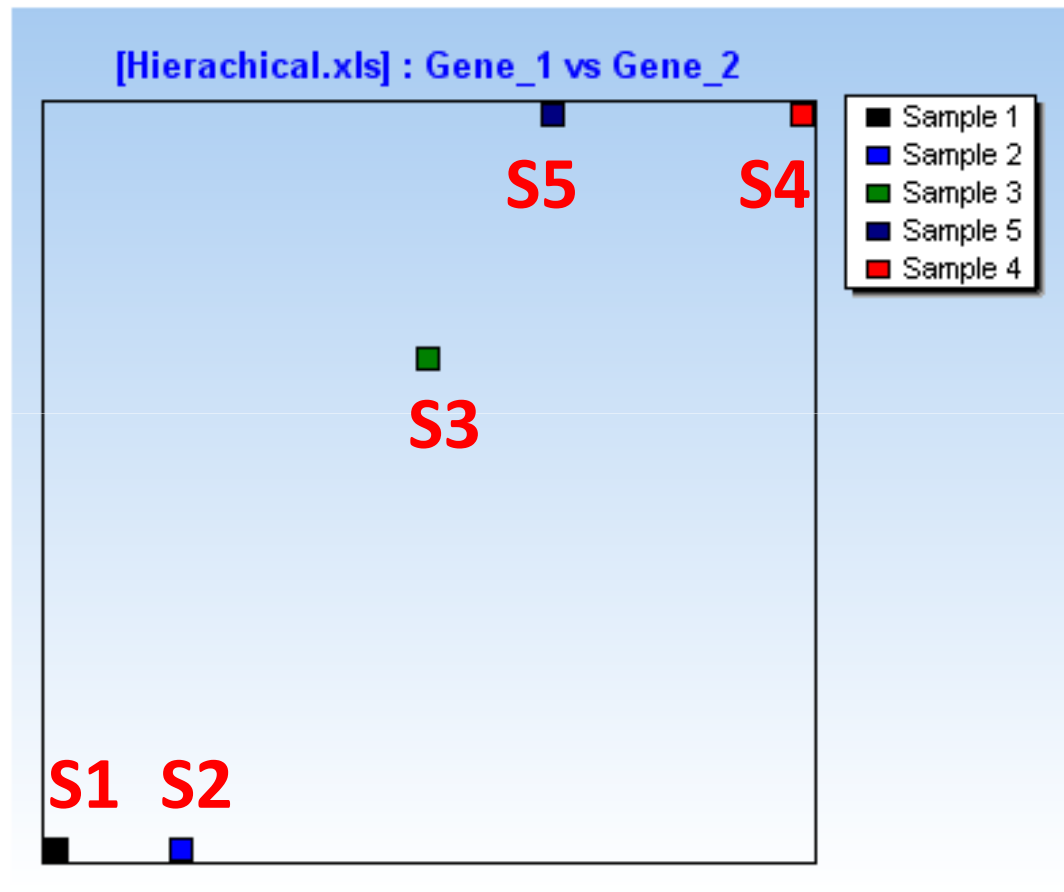
Statistical Analysis & Visualization Tools



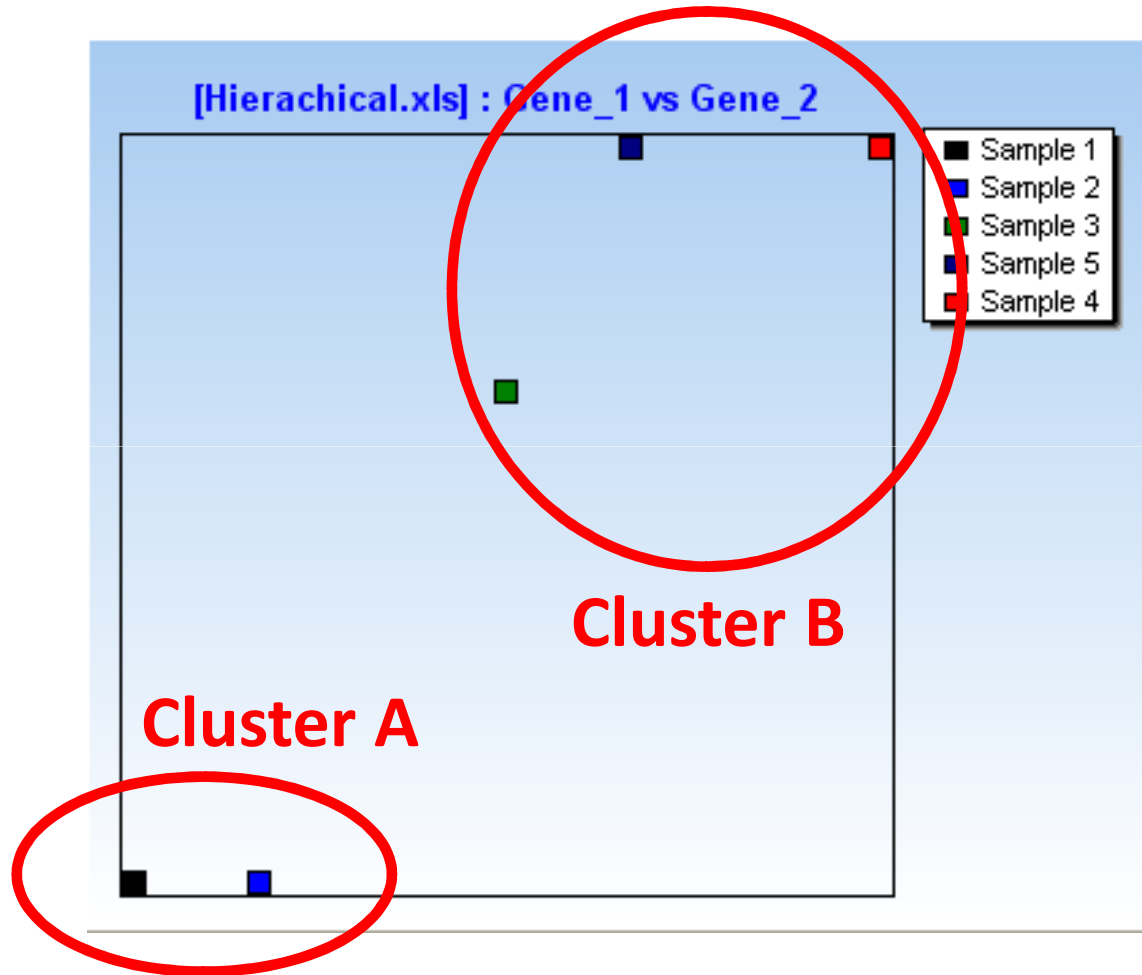
Multivariate Analysis

	Gene_1	Gene_2
Sample 1	1	1
Sample 2	2	1
Sample 3	4	5
Sample 4	7	7
Sample 5	5	7

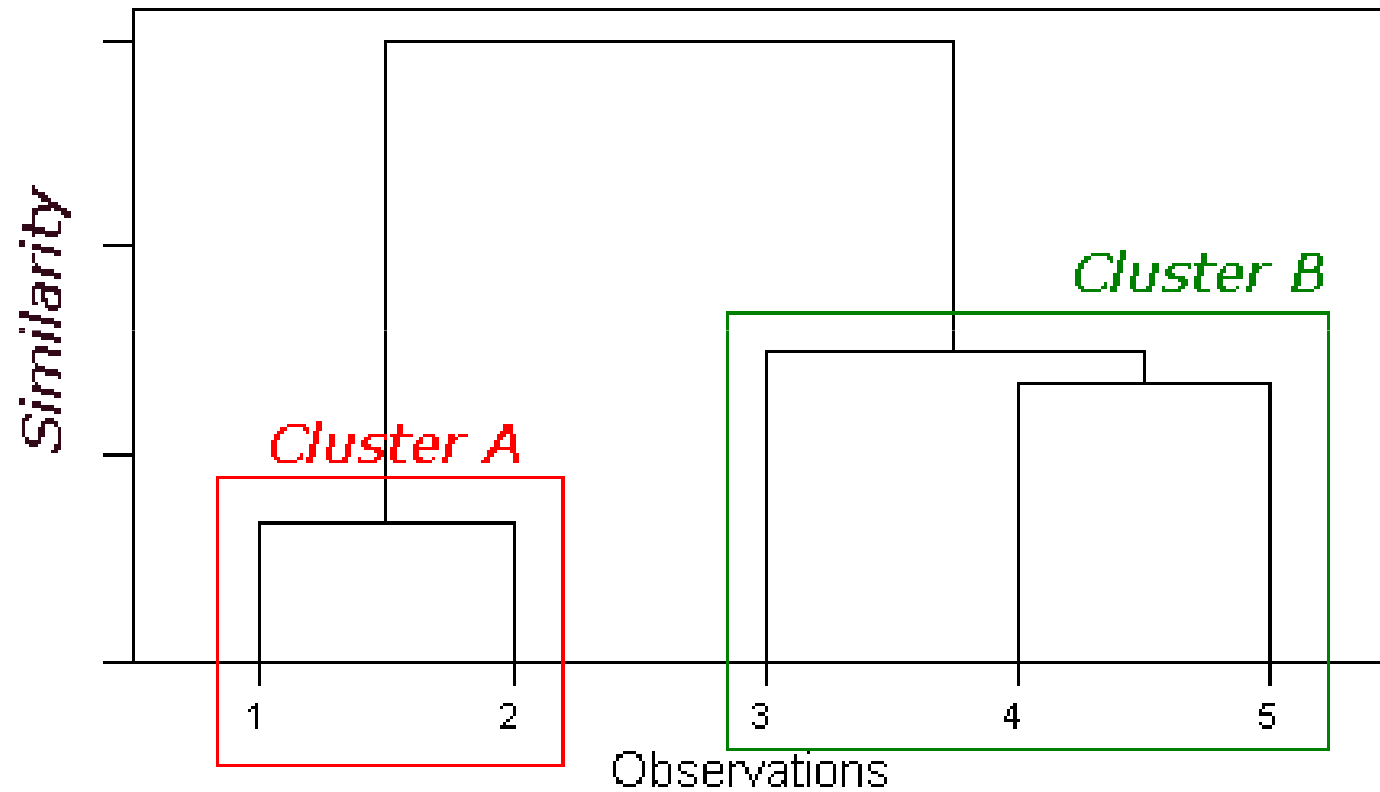
Scatter plot



Scatter plot



Dendrogram



Summary

- **qPCR Data Analysis**
 - Plan your experiments ahead of time
 - Use replicates to evaluate variation
- **GenEx Software Tool**
 - Fully functional one-month free trial
 - www.multid.se
 - www.qpcrforum.com
- **Come to MultiD's booth in room S2 and ask us about:**
 - Software support
 - Biostatistics training
 - Data analysis consulting
 - Research collaborations (SmartHEALTH, CD-Medics, ...)

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