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

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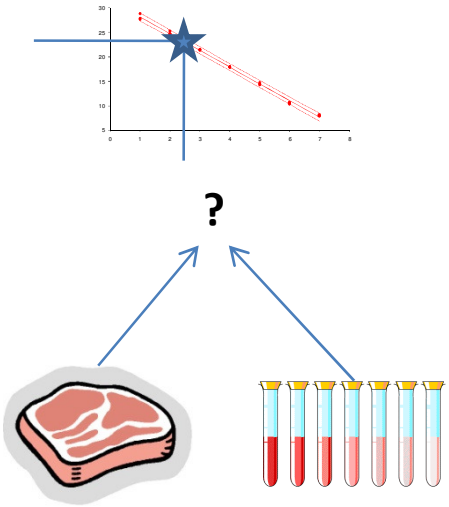
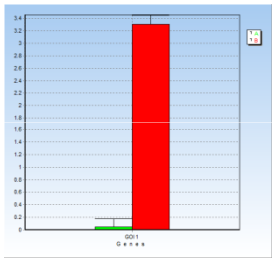
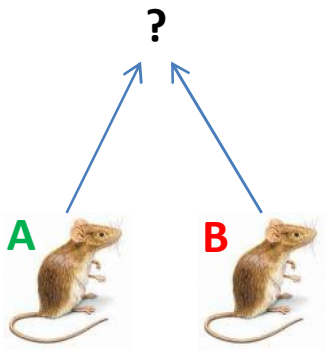
qPCR Data Analysis

Absolute Quantification

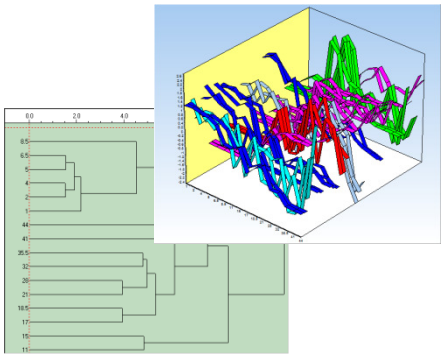


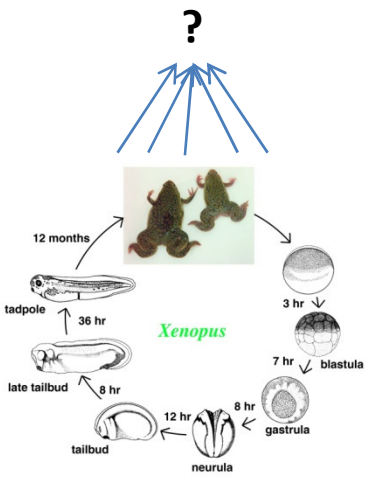
Relative Quantification





Expression Profiling





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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

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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

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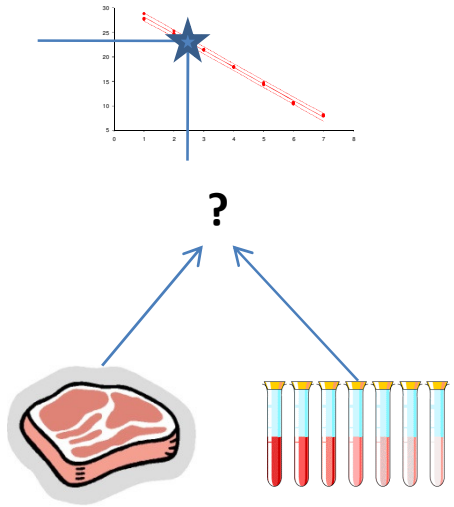
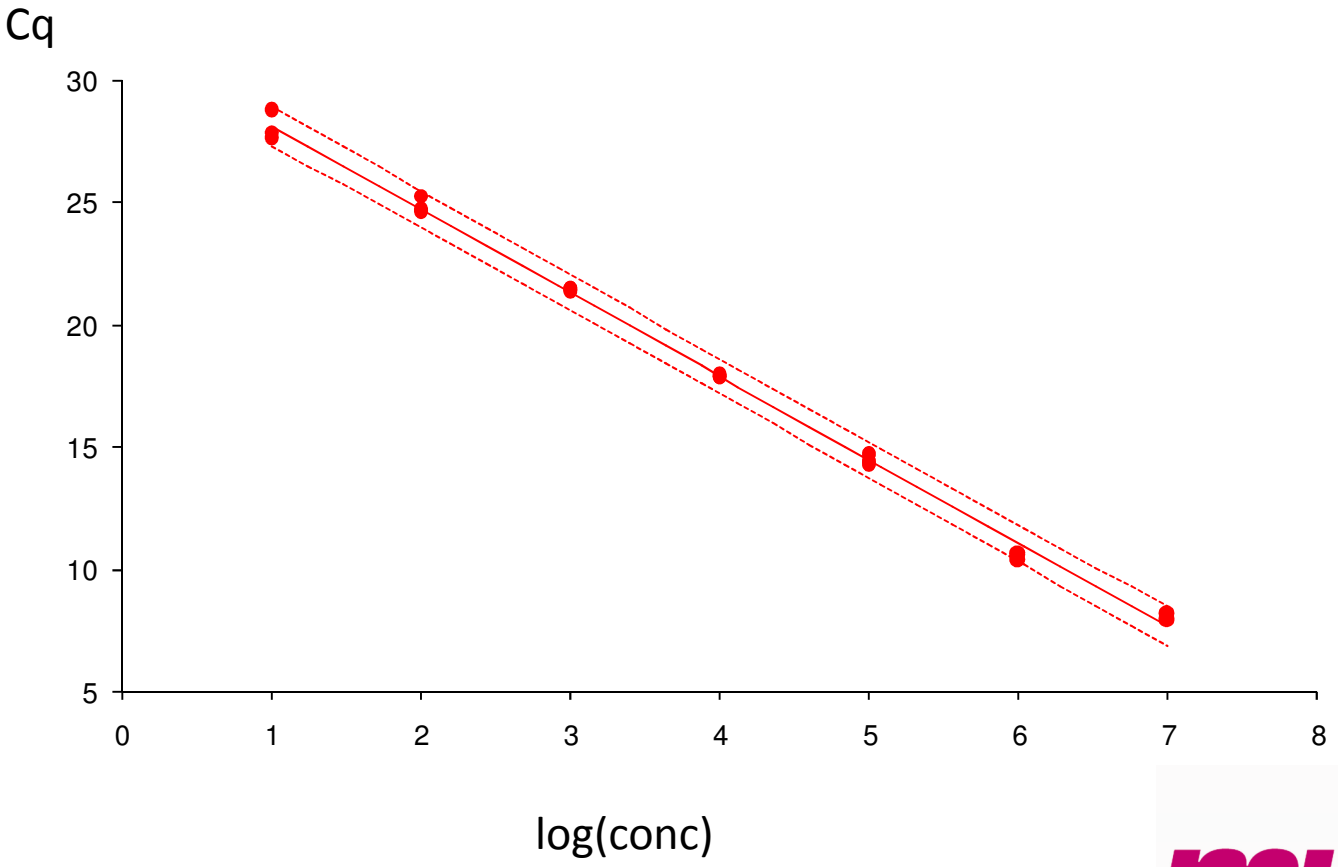
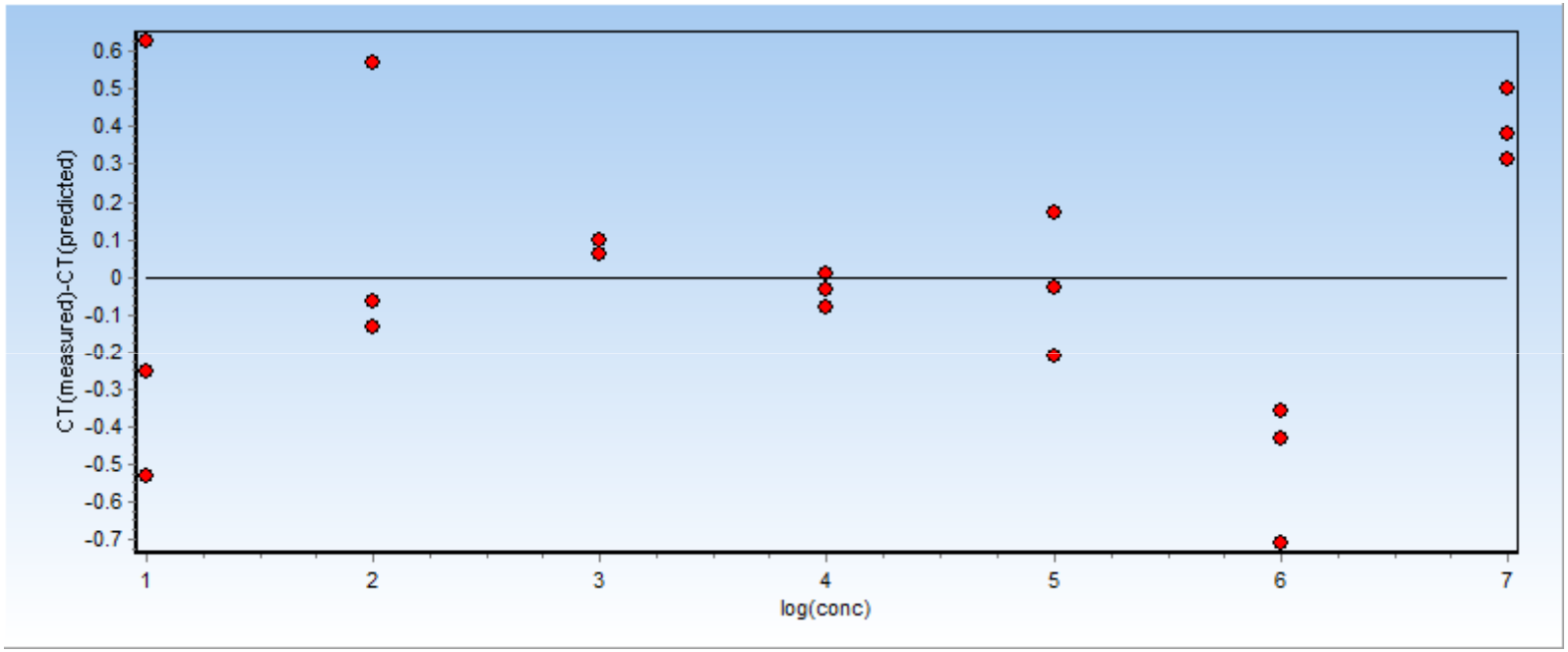
Absolute Quantification



Standard Curve



Residual plot



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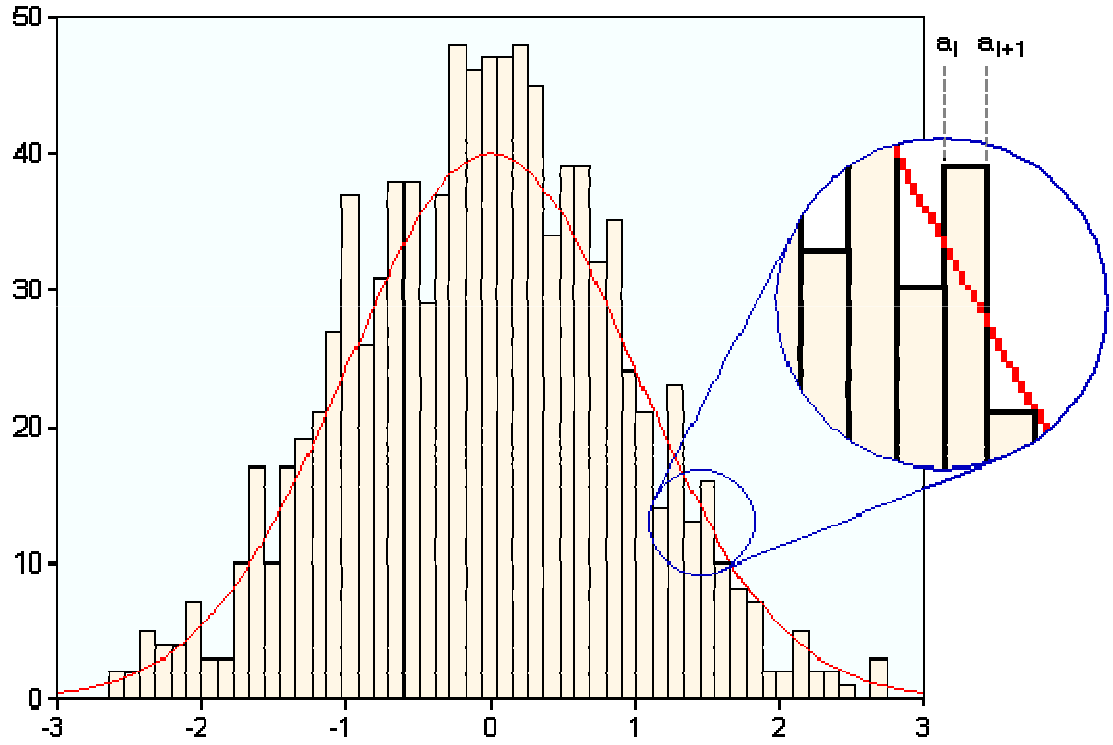
Normal distribution = Gaussian distribution



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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

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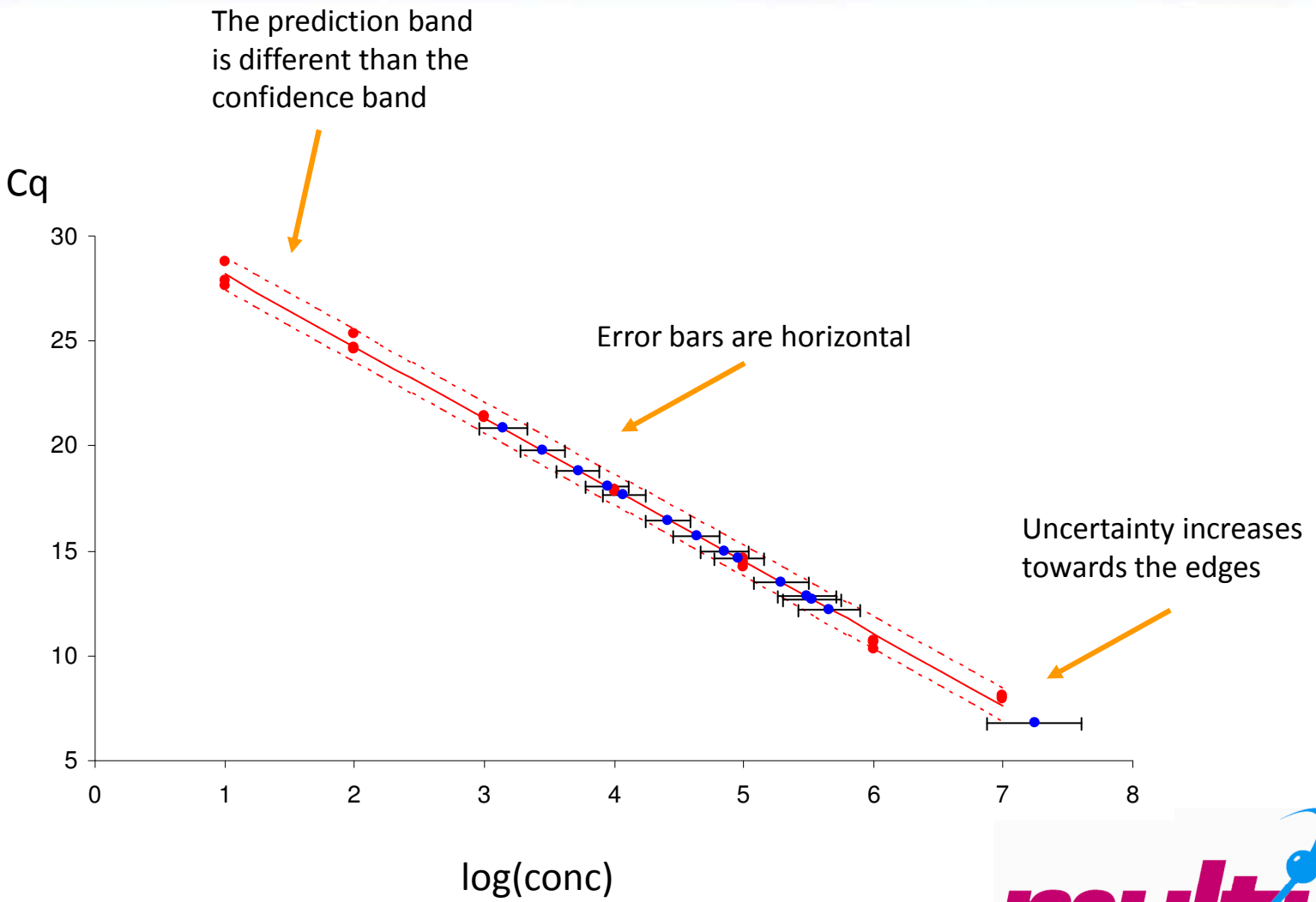
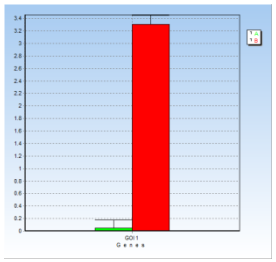
Prediction


The prediction band is different than the confidence band
Cq
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
Error bars are horizontal
Uncertainty increases towards the edges
log(conc)

Relative Quantification



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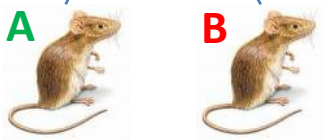

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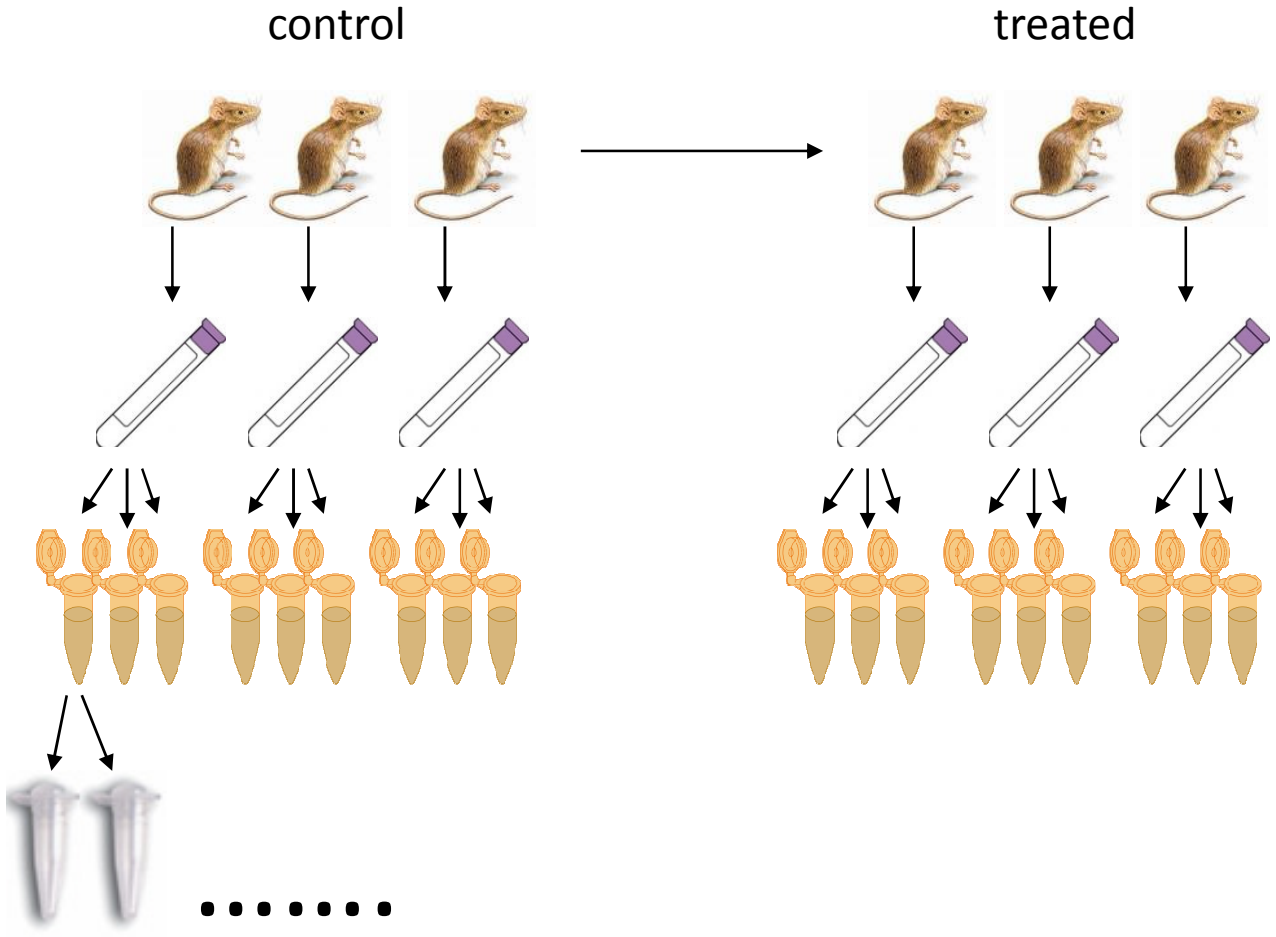
Experimental Design



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

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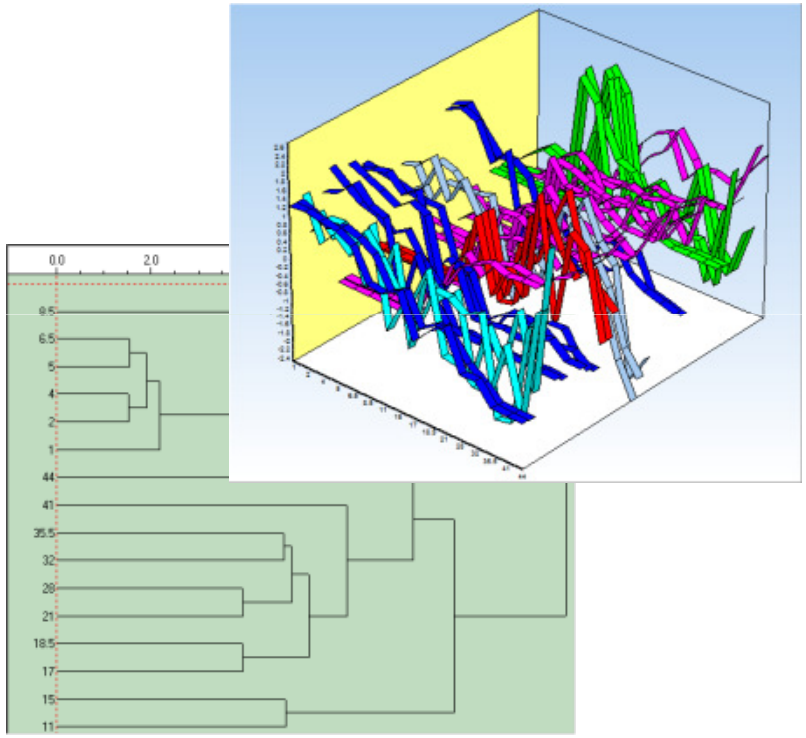
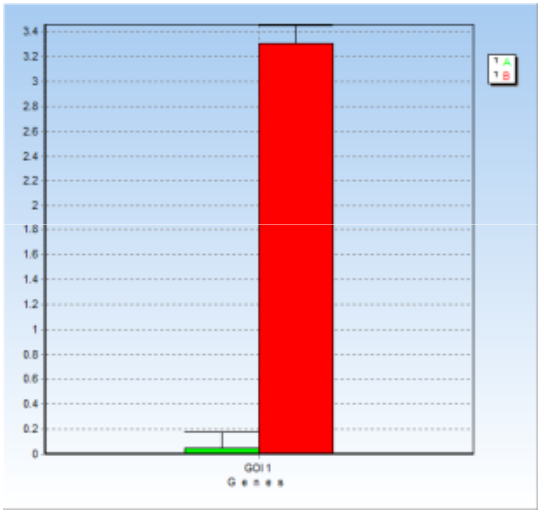
Statistical Analysis & Visualization Tools





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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

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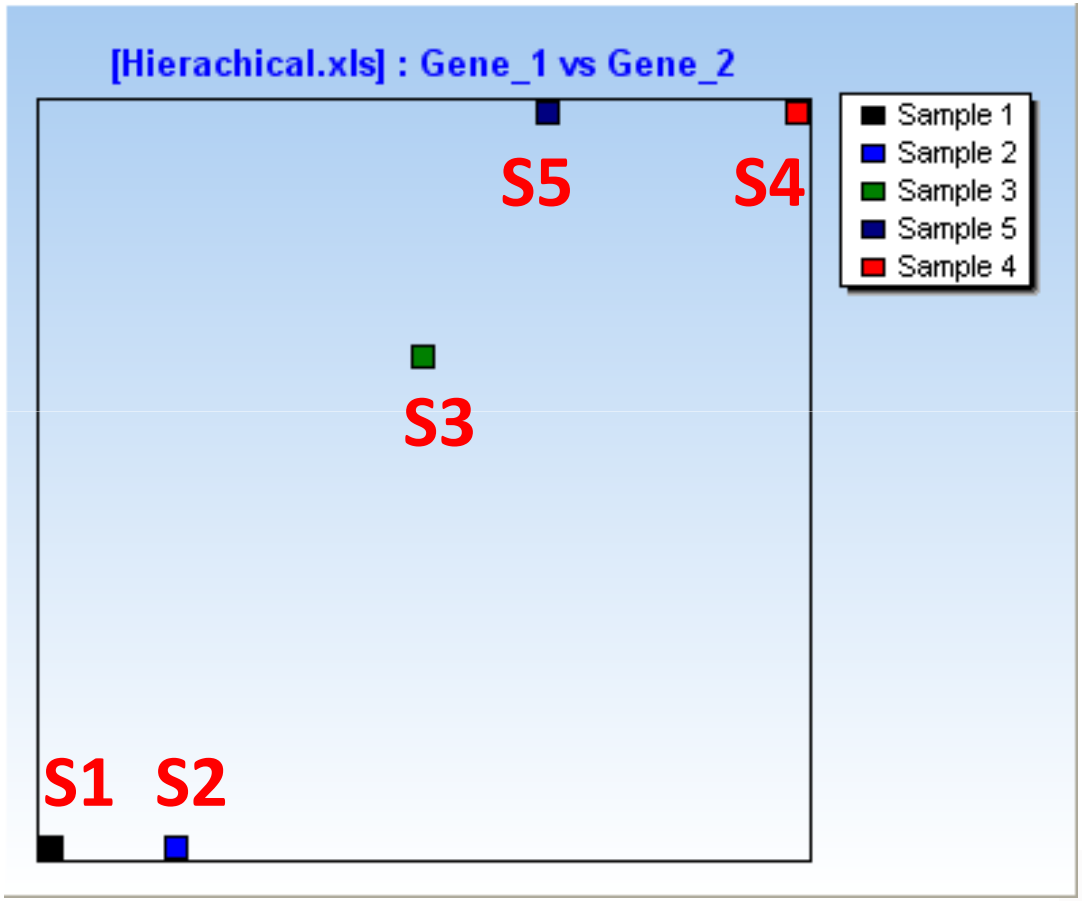
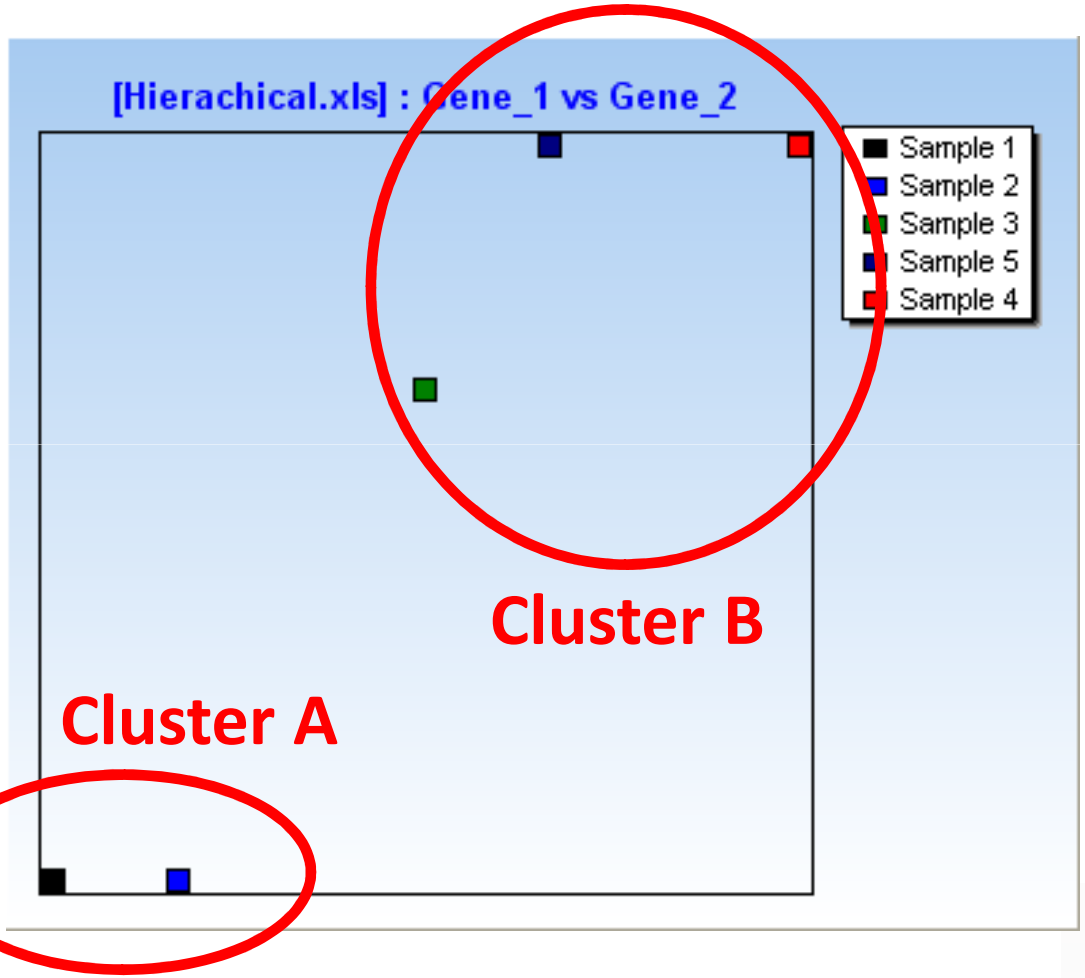
Scatter plot



Scatter plot



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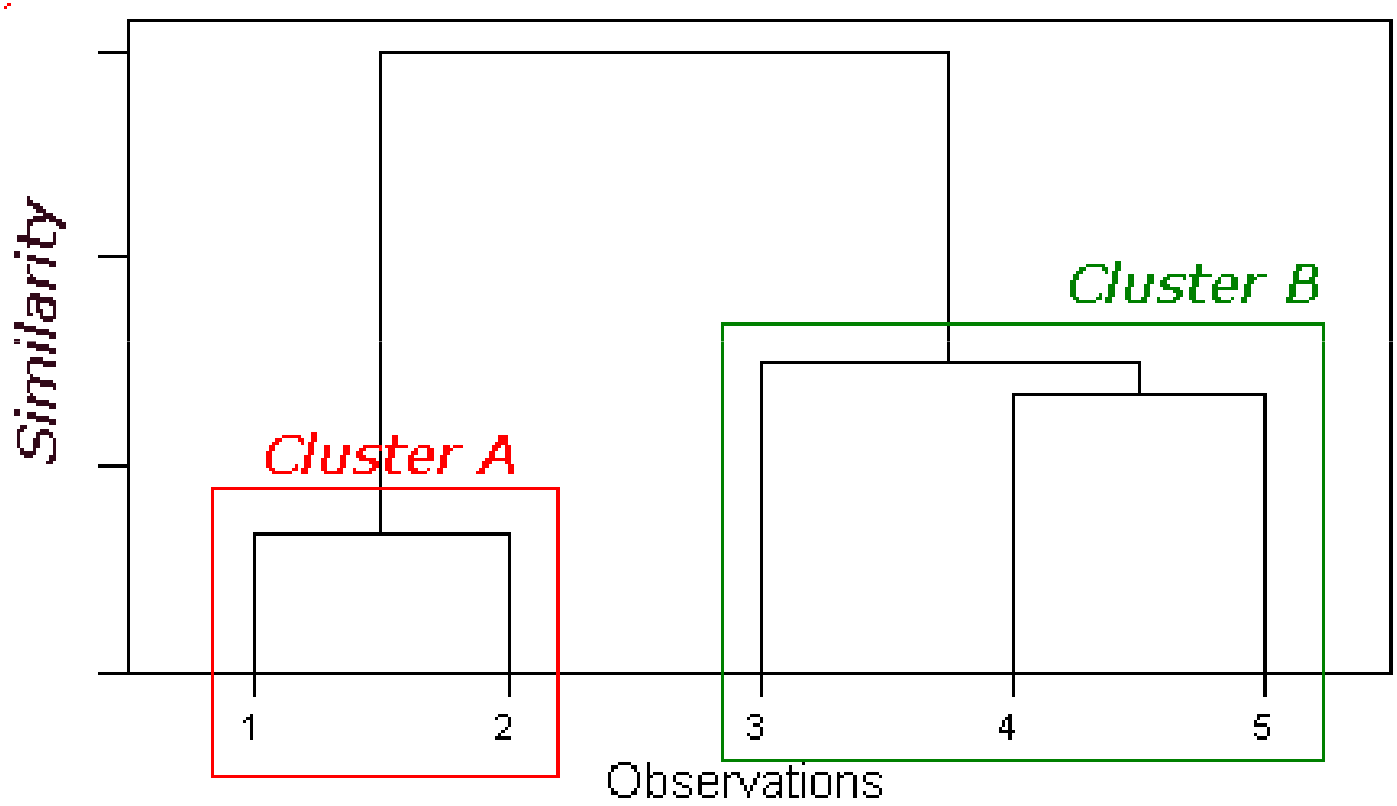
Dendrogram



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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