

QPCR User Guide

Version 1.7

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1 Introduction

QPCR is a web application designed for storing, parsing, managing, and analyzing qPCR data. Including several different algorithms it can facilitate the analysis of qPCR results. The application is not intended to be installed on client machines. We suggest installing one instance for your group on a Linux server where users connect to by a standard Web browser. Additionally you can request an account on the server hosted by the TU Graz.

1.1 Purpose

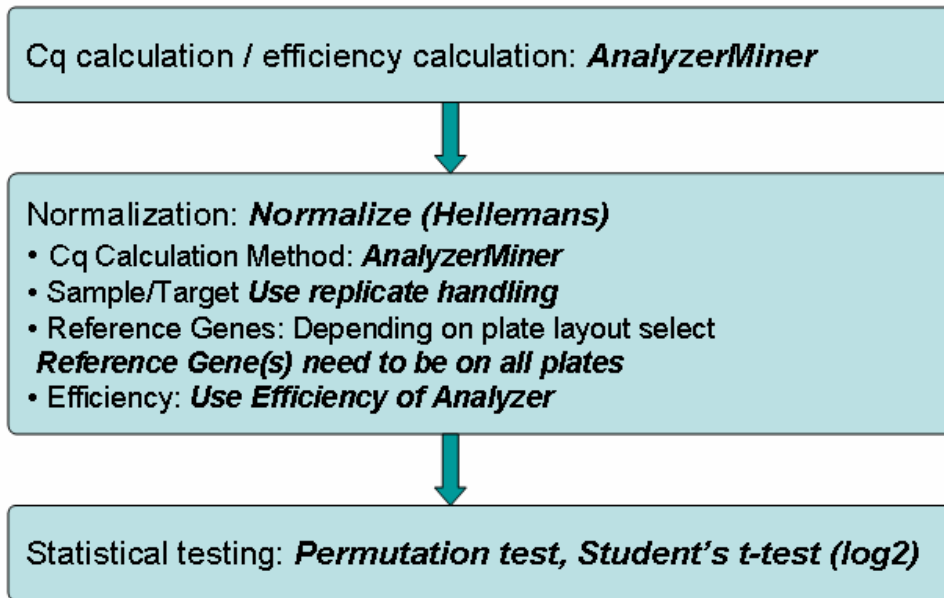
This document is not written to be read from the first to the last page. It is more a compendium that tries to tell what to do if one is puzzled. However for an introduction it makes sense to read chapters 3 to 8 to get general information about the application. These chapters are sorted according to a typical procedure of uploading, parsing, analyzing, and normalizing a qPCR experiment.

1.2 Hints for using the software

- Always use meaningful names, to be able to distinguish the different entries in the future.

1.3 Example of a standard workflow

- Upload files using the multiple file upload interface or upload them separately
- Parse and analyze the newly uploaded files using the multiple parse interface
- After parsing and analyzing is finished check the created runs:
 - Check hardware/software/instrument setting & SDS, Rn files
 - Check plate including wells
 - Display charts and check values (save them as picture)
 - Check Cq analyze result values
- Create experiment using the desired runs
- Analyze experiment (save analyze settings)
- Check normalized Cq values
- Export charts (picture) and Cq values (file)
- Perform statistical test
- Export fold change charts and test results

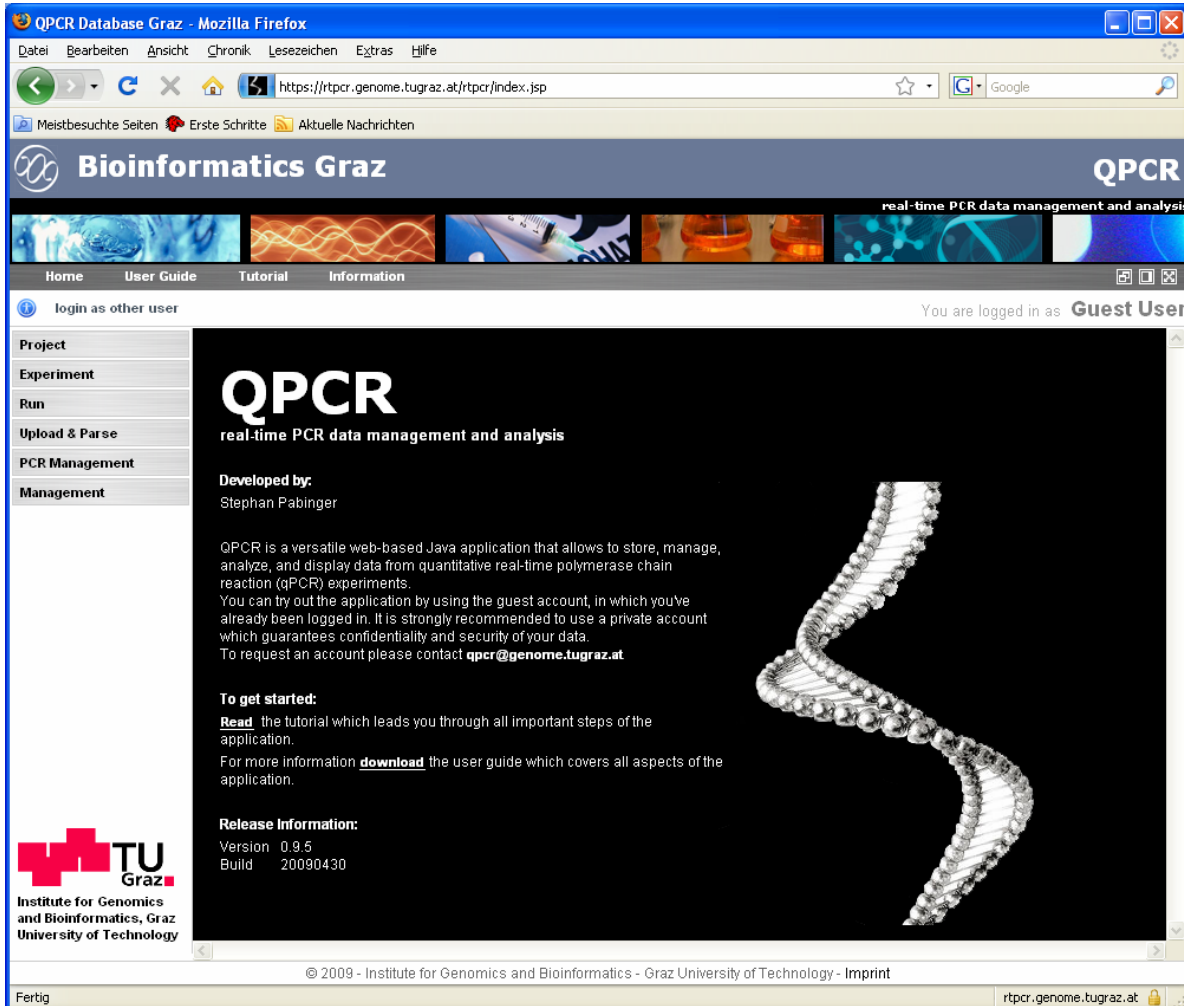


This flowchart displays the suggested methods (printed in bold) for each analysis step. Please keep in mind that these suggestions do not consider special instrumental setups or practices of your laboratory. The uniqueness of this application is the support of several different methods and a variety of setup parameters which are helpful to find the best setup for your data.

2 General information

This chapter contains information about the basic layout, the color and icon convention used throughout the whole application.

2.1 Welcome screen



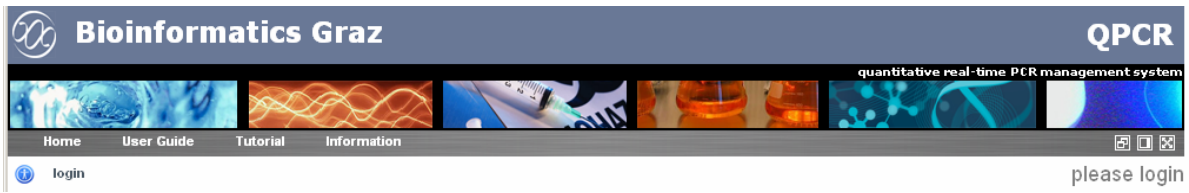
The picture above displays the welcome-screen of QPCR.

The main view is divided into 3 sections:

1. The header section consists of some images on the top, of one bar managing the display settings, and one bar displaying information about the AAS (Authentication and Authorization System)
2. The left bar contains the menu used for navigation
3. The center frame displays the selected information

2.2 Header section

2.2.1 Display bar



The Home- link sends the user back to the start page.


The User Guide- link opens this document.


The Tutorial link opens the tutorial

The Information link displays a page with useful information about the software

At the right side there are 3 icons where the user can change the spatial usage of the browser window:

 : resizes the window to the default size

 : stretches the window to the full width of the screen

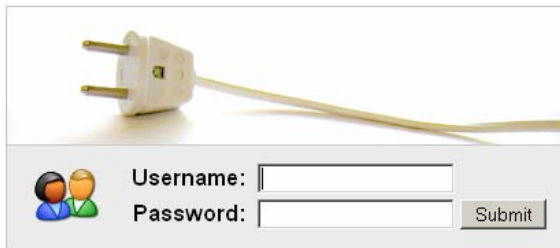
 : uses the full width of the window and the images at the header section disappear, only the display bar and the authentication bar will stay.

If the user is not logged in the following bar is shown:

 Log in |

please login

By clicking on the Log in link the user is directed to the login window.



A login window featuring a white power plug icon at the top. Below the icon are two input fields: 'Username:' and 'Password:'. A 'Submit' button is located to the right of the password field.

System Requirements

- JavaScript and cookies must be enabled in your browser
- Screen resolution of at least 1024x768 is strongly recommended

 **Mozilla Firefox:**
All Releases
recommended

 **Internet Explorer:**
Windows: Internet Explorer 6
Mac OS X: Internet Explorer 5.2

 **Netscape:**
Release 7.0

If the user is logged in the following bar is shown:

 [logout](#) | [User Settings](#) | [Progress Information](#) | [New Parser Log](#) | [New Analyzer Log](#) | [Run Deletion Log](#) | You are logged in as **Stephan Pabinger**

It provides the possibility to:

- Log out
- Change user settings
- Display new Analyzer Logs (only shown when new results are present)
- Display new Parser Logs (only shown when new results are present)
- Display new Run Deletion Logs (only shown when new results are present)

2.3 Navigation section

Project
Experiment
Run
Upload & Parse
Multiple File Upload
New File Upload
Find File Upload
Parse
PCR Management
Management

The navigation section is positioned at the right side of the screen. Grey fields with bold text are headers that reveal a submenu when the user clicks on them. A submenu can contain another submenu or links to a certain interface.

2.4 Information section

QPCR uses a list form to present overviews of data (shown in the figure below).

The header section contains 2 links:

- Customizable queries
- Customizable display

The table with the data is always enclosed by bars used for scrolling and most columns in the table are sortable. An arrow indicates the current sort direction of the corresponding column. Sort settings are saved in the database for each user and are loaded when the user logs in the next time.

Run

Query
 Edit Display Settings

Runs per page: 15 [25] 50 100

5 Runs found | Page 1 of 1 | go to page go

Nr.	Name					Date			
1	20071108					2007-11-14			
2	Run					2007-11-14			
3	20071108_2					2007-11-14			
4	Run 1					2007-11-14			
5	Run 2					2007-11-14			

Runs per page: 15 [25] 50 100

5 Runs found | Page 1 of 1 | go to page go

2.4.1 Customizable queries

Queries are used to narrow the list of displayed entries according to the users needs. They

can be added, removed, and saved. When the operators LIKE and NOT LIKE are used a preceding or trailing asterisk needs to be entered.

The button **Submit Query** submits the entered query and updates the result table. **Reset Query** removes all entered queries and submits a query without any user-defined filters. **Restore Default** restores the default set of queries and submits them. **Save Queries** saves the current set of queries as default for this page. Unless the user changes the queries the data on that page will always be filtered with this default set of queries.

Query				✕	
Name	Like	*test*	AND	✕	
Description	Like	*SDS*	AND	✕	
Submit Query Reset Query Restore Default				Save Queries	

2.4.2 Customizable display

The information that will be displayed on the screen is customizable to the needs of the user. One can select the desired columns by clicking on the checkboxes and update the view on the data by pressing the button **Update**.

Save Settings allows the user to store his/her own display settings and whenever the user enters the same page his/her settings will be displayed by default.

Available fields		✕	
Required Information			
<input checked="" type="checkbox"/> Name	<input type="checkbox"/> User		
<input type="checkbox"/> Description	<input type="checkbox"/> Submitter		
<input checked="" type="checkbox"/> Date			
Update Display all Display default Save Settings			

2.4.3 Scrolling bar

The left side of the scrolling bar displays the number of found elements (depending on the query the user submitted). In the middle of the scrolling bar the actual page and the total number of pages are displayed. At the right side one can choose how many entries are shown per page. Moreover the user can directly jump to a page by entering its number.

		Runs per page: 15 [25] 50 100
4 Runs found	Page 1 of 1	go to page <input type="text"/> go

2.4.4 Table view

Nr.	ID	Upload Name	Category	Added Date				
1	4400	File1	plate	2007-01-29				
2	5200	20071108_2	plate	2007-11-14				
3	5201	20071108_2_Component	additionalplatefiles	2007-11-14				
4	5202	20071108_2_DeltaRn	additionalplatefiles	2007-11-14				
5	4600	File2	plate	2007-03-30				
6	5050	File3	plate	2007-09-26				
7	4450	File5	plate	2007-01-30				
8	4451	File5_clipped	additionalplatefiles	2007-01-30				
9	4500	test	plate	2007-01-30				

The table view consists by default of the following parts:

- The header: if one hovers the mouse over a column-name the colour changes to blue and one can sort the list by this column
- The number in the first column indicates the hit number of the entry corresponding to the order
- Detailed information about an entry is loaded by clicking on links of the corresponding entry
- Indicates that the data can be edited
- Indicates that there is information downloadable
- Indicates that the entry can be shared
- Indicates that the entry can be removed

By clicking on the share icon the user is redirected to the sharing page.

Sharing















You are about to share item: **384 S208 27062005**

	Name	E-Mail	
<input type="checkbox"/>	Institute for Genomics and Bioinformatics	zlatko.trajanoski@tugraz.at	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	Institute of Pathology, University of Graz	karin.wagner@klinikum-graz.at	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	Inserm U255	jerome@irgendwas.fr	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	Visitors	none	<input type="checkbox"/> <input type="checkbox"/>

When selecting a user or an institute the checkboxes for editing and deleting are enabled and the user can additionally specify if the shared entry can be edited or deleted.

2.5 Symbols

- : Indicates that one can edit the data
- : Indicates if there is some information downloadable
- : Indicates that one can delete this entry
- : Indicates that there is additional information available
- : Indicates that the user can share his/her data to other users of the system
- : Indicates that the entry is currently parsed
- : Indicates that the entry is currently analyzed
- : Indicates that the entry is currently deleted
- : Puts all entries from the left list into the right list
- : Puts all entries from the right list into the left list
- : Puts one or many (selected holding ctrl) entries from the left list into the right list
- : Puts one or many (selected holding ctrl) entries from the right list into the left list

3 Upload and Parse

3.1 File Upload

Files can be uploaded separately or in batches using the multiple file upload interface (described in 3.2).

When uploading a file the user has to specify the correct file type. Currently there are 2 file types available:

- File (.SDS – Applied Biosystems, .IXO – Roche, .CSV generic)
- Export File (Clipped Files, Component Files, DeltaRn Files, XML Files)

New File Upload

Name	<input type="text"/>
File	<input type="text"/> <input type="button" value="Durchsuchen..."/>
File Type	File <input type="button" value="v"/>
Comment	<input type="text"/>

File uploads are visible for all users of the same institute.

When deleting files the user can choose to delete associated runs, experiments, and additional files by ticking “Delete associated Runs and Experiment”.

<input type="button" value="Delete"/>	<input checked="" type="checkbox"/> Delete associated Runs and Experiments
<input type="button" value="Reset"/>	<input type="button" value="Cancel"/>

By pressing the **Delete** button a list of entries is shown that are going to be deleted.

You are going to delete:

File Type	Created Date
File(s):	2008-02-28
• test	
• test_component	uploads/21245
• test_deltaRn	746230
Include	FALSE
Run(s):	
• test	application/oc
Experiment	21245
Experiment(s):	

File Upload

 Query





































 Edit Display Settings

FileUpload per page: 15 [25] 50 100

19 FileUpload found

| Page 1 of 1 |

go to page go



Nr.	ID	Upload Name	Category	Added Date				
1	4400	File1	plate	2007-01-29				
2	5200	20071108_2	plate	2007-11-14				
3	5201	20071108_2_Component	additionalplatefiles	2007-11-14				
4	5202	20071108_2_DeltaRn	additionalplatefiles	2007-11-14				
5	4600	File2	plate	2007-03-30				
6	5050	File3	plate	2007-09-26				
7	4450	File5	plate	2007-01-30				
8	4451	File5_clipped	additionalplatefiles	2007-01-30				
9	4500	test	plate	2007-01-30				

3.2 Multiple Upload

The multiple file upload interface allows the user to upload several files at once. The type of the file (File, Export File) is determined automatically (if option is selected). Files are added using the Add Files button and after uploading them a status message is shown.

If this page does not show up please check if Java Version 1.5 is installed.

Multiple File Upload

 Add Files
 Remove

File name	Directory	Size (Kb)
File1.sds	C:\Documents and Settings\Administ...	1104
File1_Component.csv	C:\Documents and Settings\Administ...	273
File1_DeltaRn.csv	C:\Documents and Settings\Administ...	37
File2.sds	C:\Documents and Settings\Administ...	16925

4 file(s), 18340 Kb to upload left

Compress Files

automatic ▼

Upload

Cancel

You need a Java Plugin Version 1.5.x.

Using compression is only recommended for remote uploading data that can be well compressed (eg. plain text files, xml files and **not** images or already compressed files).

Cancel

3.3 Multiple Parse

The multiple parse section is put into the “Upload & Parse” menu in order to increase the usability, because typically files are parsed and analyzed immediately after uploading.

This interface shows by default only files (SDS, IXO, CSV) which are not linked to a run and therefore have not been parsed yet. Moreover the user can specify whether all files of the institute or only the files uploaded by the user are displayed. In addition all uploaded files can be shown in this interface.

Each file can be accompanied by a maximum number of three additional export files (Export file) which are automatically linked to the according file.

Remark: Export files need to contain the file name (e.g.: SDS: myFile.sds; rn: myFile_clipped.txt) in order to be correctly identified by the system.

For each File – Export file combination the user can specify whether the files should be (1) parsed and analyzed, (2) only parsed, or (3) skipped.

Pressing `submit` parses and analyzes the files in the background and creates a run for each selected file. The methods (one or multiple) used for analyzing can be set using the `user settings` interface (see 11).

The legend explains which files need to be exported and parsed (for each thermocycler) in order to guarantee a successful analysis.

More information about parsing is provided at 4.4 .

Multiple Parse

Display Files Owned By:	User
Display Files:	All
<input type="button" value="Update"/>	

Legend

Nr.	File	Export File 1	Export File 2	Export File 3	Parse	Analyze
1	test	test_component	test_deltaRn		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
2	templateCSV				<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
3	16samples	16samples_530Exp			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Top

Legend				
Thermocycler	File	Export File 1	Export File 2	Export File 3
ABI 7000	The saved SDS file	Exported component file	Exported deltaRn file	
ABI 7500	The saved EDS file	Exported file including Sample Setup and Amplification Data		
ABI 7900	The saved SDS file	Exported clipped file		
LightCycler 2.0	The saved IXO file	Exported Fluorescence history (over Cycles) as XML file		
Generic CSV file	The generated CSV file			

4 Run

4.1 Create a Run

New Run

Name:	test
Date:	14.11.2007
Category:	absolute
Hardware:	
Software:	
Instrument Setting:	
Plate:	
Protocol:	
Description:	
Experiments:	no experiment
SDS File:	test
Rn Files:	

November, 2007							
Today							
wk	Mon	Tue	Wed	Thu	Fri	Sat	Sun
44				1	2	3	4
45	5	6	7	8	9	10	11
46	12	13	14	15	16	17	18
47	19	20	21	22	23	24	25
48	26	27	28	29	30		
































Select date

Create

A Run in the application QPCR represents a performed qPCR run.

To create a run three properties need to be specified: a name, the date of creation, and a category (relative or absolute run). In order to attach existing plate information to a run, a file for parsing needs to be specified (SDS file -> the *.sds file of the thermocycler, Rn Files -> exported Rn files). Category, Hardware, Software, Instrument Setting, and Plate are created by the parser and are set during the parsing process. Nevertheless they can be set manually by the user.

4.2 Run List

Nr.	Name					Date				
1	20071108						2007-11-14			
2	Run						2007-11-14			
3	20071108_2						2007-11-14			
4	Run 1						2007-11-14			
5	Run 2						2007-11-14			

The general layout of the run list is equal to the layout described in 2.4.4 . In addition to the standard view the run list contains three more columns which indicate the state of a certain run. The first column describes whether the run is currently parsed (blue dot). The second column (green dot) indicates that the run is currently analyzed. The third column (red dot) informs the user that the run is currently getting removed. Moreover the plate icon sends the user directly to the plate of the run and the chart icon directs the user to the chart view.

4.3 Display Run

After a run has been parsed the combo boxes for run category, hardware, software, instrument settings, and plate are automatically set to the corresponding entry by the parser. The information section at the bottom of the page displays the current parse status and the latest successful parse job. Using the checkbox next to Options, the user can specify whether the names of wells (specified in the thermocycler software) are equal to sample names. Pressing the Parse button starts a parsing job using the files specified above. Whenever a parsing job is started the old plate including all plate information is deleted and a new plate is created.

Each run possesses a plate which can be displayed by clicking on the show button next to Plate.

If a run has not been added to an experiment the Create button next to Create Experiment including plate produces an experiment and adds the selected run to this experiment.

Name:	20071108
Date:	14.11.2007
Category:	absolute
Hardware:	SDS 7000
Software:	ABI Prism 7000 SDS v1.1
Instrument Setting:	new Instrument Setting - 2007
Plate:	20071108
Protocol:	
Description:	
Experiments:	
Create Experiment including plate:	Create
SDS File:	20071108
Rn Files:	20071108_Component
	20071108_DeltaRn
Plate:	Show
Parse Plate File:	
Currently parsing:	<input type="checkbox"/>
Latest successful parsing job:	2007-11-14 (20071108)
Options:	<input checked="" type="checkbox"/> Names specified for wells are equal to sample names
Parser:	Parse
Return	

4.4 Parse Run

Whenever a parsing job is started a notification message is displayed. The progress of the job can be monitored using the Progress Information list (described in 4.6).

To parse a thermocycler run at least the main file (.eds, .sds, .ixo, .csv) has to be specified. The following files need to be uploaded into the application:

Abi SDS 7000:

- SDS file
- Export and specify component file (e.g.: run1_Component.csv)
- Export and specify deltaRn file (e.g.: run1_DeltaRn.csv)

Abi SDS 7500

- SDS file
- Export file including Sample Setup and Amplification Data

Abi SDS 7900

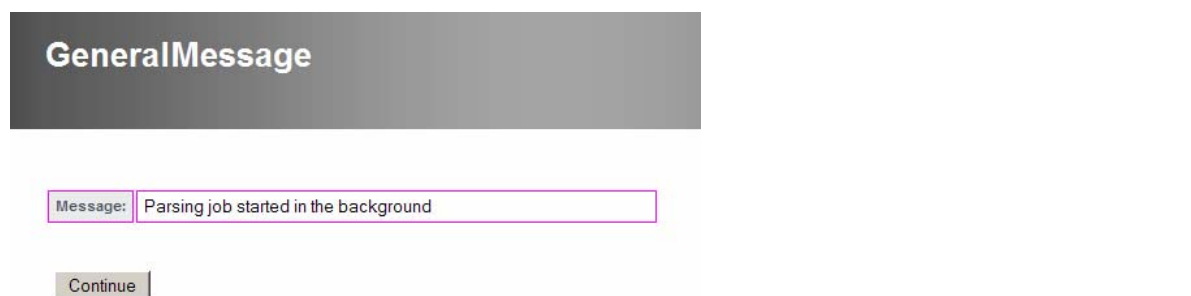
- SDS file
- Export and specify clipped file (e.g.: run1_Clipped.txt)

Roche Lightcycler 2.0 / 480

- IXO file
- Export the fluorescence history in the Run->Online Data Display window; Select all samples, then select “Fluorescence history” as the Chart and “Fluorescence over Cycles” as the Axis. Then right click on the chart, select export, pick the data tab and select XML.

CSV

- Generate a CSV file described in 15 .



After a parsing job is complete a notification pops up in the display bar (2.2.1) which links to the parser log page.

4.5 Parser Log

Displayed below is the parser log page.

Parser Result

Query

Legend

ParserResults per page: **15 [25] 50 100**

5 ParserResults found | Page 1 of 1 | go to page go

Nr.	Run Name	Successful	Date	Viewed	
1	20071108	☑	2008-02-06	☑	✗
2	templateCSV	☑	2008-09-11	☐	✗
3	16samples	☑	2008-09-11	☐	✗
4	templateCSV1	☑	2008-09-11	☑	✗
5	test	☑	2008-07-29	☐	✗

ParserResults per page: **15 [25] 50 100**

5 ParserResults found | Page 1 of 1 | go to page go

Top

Color Legend	
Color	Meaning
Red	Parsing was not successful
Orange	Warnings occurred - Parser result should be checked
Blue	Parsing was successful but result file has not been viewed
Black	Parsing was successful and result file has been viewed

The coloring of the parser logs is explained in the attached legend.

Each Parser log shows detailed information about the completed parsing job. It displays whether the job was successful, when it took place, and if a plate is attached to the run. Warnings produced during the parsing job are displayed in a box. Furthermore the result page shows the parsed run name and information about hardware, software, and instrument settings. Information about the created plate contain its id, name, size, input file, and plate status. Moreover the user can enter a description and update the plate using the `Update & goto plate` button. The three text fields for target, cDNA, and passive reference may contain entries that need to be added to the database.

Parser Result

successful:	successful
Date:	12.09.2008
Plate is:	existent

Warnings:	
-----------	--

Run:	File1
------	-------

Hardware:	found following hardware in system:
Hardware Name:	SDS 7000
Hardware Version:	18

Software:	found following software in system:
Software Name:	ABI Prism 7000 SDS v1.1
Software Version:	512

Instrument Setting:	found following instrument setting in system:
Instrument Setting Name:	new Instrument Setting - example

Plate:	3501
Plate Name:	File1
Plate Description:	
Plate Size:	96
Input File:	File1
Plate Status:	parsing - successful - run references parsed plate

Please add the following Detectors to the System:

Please add the following cDNAs to the System:

Please add the following Passive References to the System:

4.6 Progress Information

Progress Information is positioned in the run menu and shows the progress of parsing and analyzing jobs. The progress information list is automatically updated every five seconds. For each process the corresponding method is shown and by clicking on the run name the user is linked to the specified run.

Progress Information

The progress information page is reloaded every 5 seconds.

ProgressInformations per page: 15 [25] 50 100

10 ProgressInformations found

| Page 1 of 1 |

go to page go

Nr.	Type	Method(s)	Progress	Run	File	
1	analyzing	SoFARAnalyzer	<div style="width: 85%; background-color: red;"></div> 85%	20071108_2	20071108_2	✗
2	parsing	AbiSDS Parser: ABI V1d1	<div style="width: 100%; background-color: red;"></div> 100%	20071108_2	20071108_2	✗
3	analyzing	SoFARAnalyzer	<div style="width: 15%; background-color: red;"></div> 15%	20071108	20071108	✗
4	parsing	AbiSDS Parser: ABI V1d1	<div style="width: 100%; background-color: red;"></div> 100%	20071108	20071108	✗
5	parsing	AbiSDS Parser: V2d1	<div style="width: 100%; background-color: red;"></div> 100%	Run	File5	✗
6	analyzing	SoFARAnalyzer	<div style="width: 25%; background-color: red;"></div> 25%	Run 2	File2	✗
7	parsing	AbiSDS Parser: V2d1	<div style="width: 100%; background-color: red;"></div> 100%	Run 2	File2	✗
8	analyzing	AnalyzerMiner	<div style="width: 90%; background-color: red;"></div> 90%	Run 1	File4	✗
9	analyzing	AnalyzerMiner	<div style="width: 100%; background-color: red;"></div> 100%	Run 1	File4	✗
10	parsing	AbiSDS Parser: V2d1	<div style="width: 100%; background-color: red;"></div> 100%	Run 1	File4	✗

ProgressInformations per page: 15 [25] 50 100

10 ProgressInformations found

| Page 1 of 1 |

go to page go






5 Plate

The upper part of the plate view contains detailed information about a plate entry. It shows the plate name (which is equal to run name), barcode, description, SDS & Rn files, and plate size. The Show button next to Display Charts sends the user to the charts interface described in (5.2). As soon as there are analyze results available the user can display them by clicking on Show next to Display Ct Analyze Results.

Edit Plate

Name:	File1
Barcode:	
Description:	
SDS File:	File1
Rn Files:	File1_DeltaRn
	File1_Component
Size:	96 <input type="button" value="Edit"/>
Display Charts:	<input type="button" value="Show"/>
Display Ct Analyze Results:	<input type="button" value="Show"/>
Design:	

The lower part of the plate view shows the list of attached wells. Displayed are: well number, whether this well is set to omitted, passive reference, target(s), cDNA, and task. Each well can be shown in detail by clicking on the well number. Moreover the wells can be edited by clicking on the edit symbol of the according well.

Nr.	Well Number	Omitted	Passive Reference	Target(s)	cDNA	Task	
1	A1	<input type="checkbox"/>	ROX	detector1	sample1	Sample	
2	A2	<input type="checkbox"/>	ROX	detector1	sample1	Sample	
3	A3	<input type="checkbox"/>	ROX	detector1	sample2	Sample	
4	A4	<input type="checkbox"/>	ROX	detector1	sample2	Sample	
5	A5	<input type="checkbox"/>	ROX	detector1	sample3	Sample	
6	A6	<input type="checkbox"/>	ROX	detector1	sample3	Sample	
7	A7	<input type="checkbox"/>	ROX	detector1	sample4	Sample	
8	A8	<input type="checkbox"/>	ROX	detector1	sample4	Sample	
9	A9	<input type="checkbox"/>	ROX	detector1	sample5	Sample	
10	A10	<input type="checkbox"/>	ROX	detector1	sample5	Sample	
11	A11	<input type="checkbox"/>	ROX	detector1	sample6	Sample	
12	A12	<input type="checkbox"/>	ROX	detector1	sample6	Sample	
13	B1	<input type="checkbox"/>	ROX	detector2	sample1	Sample	
14	B2	<input type="checkbox"/>	ROX	detector2	sample1	Sample	
15	B3	<input type="checkbox"/>	ROX	detector2	sample2	Sample	
16	B4	<input type="checkbox"/>	ROX	detector2	sample2	Sample	
17	B5	<input type="checkbox"/>	ROX	detector2	sample3	Sample	
18	B6	<input type="checkbox"/>	ROX	detector2	sample3	Sample	
19	B7	<input type="checkbox"/>	ROX	detector2	sample4	Sample	
20	B8	<input type="checkbox"/>	ROX	detector2	sample4	Sample	
21	B9	<input type="checkbox"/>	ROX	detector2	sample5	Sample	
22	B10	<input type="checkbox"/>	ROX	detector2	sample5	Sample	
23	B11	<input type="checkbox"/>	ROX	detector2	sample6	Sample	

5.1 Well

A well represents the reaction room on a plate. Each well consists of the following attributes: well number, omitted, x and y position on the plate, reaction volume, task (e.g.: standard, target, ...), sample quantity, sample (cDNA), sample concentration, passive reference, realtime chemistries, targets, and description. Wells are a subunit of plates and can not be created without a plate reference.

Edit Well

Well Number:	A1		
Omitted:	<input type="checkbox"/>		
X Position:	1		
Y Position:	1		
Reaction Volume:			
Task:	Sample ▼		
Sample Quantity:			
Sample:	sample1 ▼		
Sample End Concentration:			
Passive Reference:	ROX ▼		
Realtime Chemistry:	Name	Concentration	Add
Target:	Name	Concentration	Add
	detector1 ▼	0.0	✖
Description:			

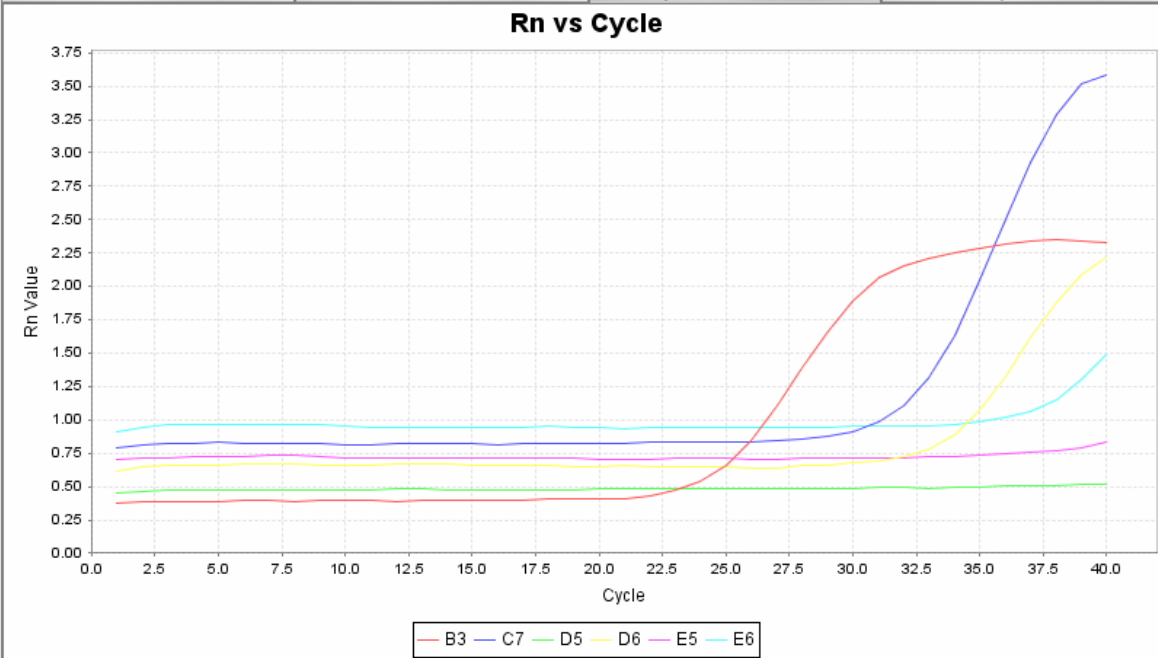
5.2 Charts

The chart interface can display four different datasets: raw dissociation data, derivative dissociation data, R_n vs. cycle, and ΔR_n vs. cycle. The Show button next to the plate name redirects the user back to the plate interface. Tabs are used to navigate between the different datasets. Charts includes a title, axis annotation, and a legend and can be saved as a picture by right clicking on the image or by using the Export As SVG button.

Below the chart, a grid or list is representing the plate layout, where each cell stands for a well of the plate. By clicking on a cell the well is added to the chart and the image is updated. Multiple cells can be selected by holding the ctrl key, clicking on the start well, holding the left mouse button, and dragging the mouse to the desired end point of the rectangle. When the mouse button is released the selected wells are added to the chart. Moreover entire rows and columns can be added to the chart by clicking on the respective header. Omitted wells are colored in light blue, whereas empty wells are colored in dark blue. To change the chart to a linear/logarithmic scale the user can click on the appropriate field. By clicking on the button Clear All the current cell selection is cleared.

Plate:

Dissociation Raw Dissociation Derivative **Rn vs. Cycle** DeltaRn vs. Cycle



	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

omitted empty linear log

5.3 Cq Analyze Results

Analyzer Results

Plate:	20071108	Show
--------	----------	------

Successful analyzed:

Analyzer	Date				
SoFARAnalyzer	2007-11-14 at 15:14:54	CSV	Export	View	✕

Not successful analyzed:

Analyzer	Date	Error Message	

The Analyzer Results page displays a short overview over completed analyzes of the selected plate. It is divided into two sections: successful analyzing jobs and not successful analyzing jobs.

Each entry in the successful analyzed list contains the name of the selected analyzer, the time of submission, and an export possibility. By clicking on the `View` button the user is guided to a detailed list of the selected analyzing result (described in 5.3.1). The same list is exported when the user presses the `Export` button, whereas the file format can be chosen using the list next to the `Export` button. Clicking on the delete symbol brings up a popup message which asks for confirmation to delete the analyzer result (including its Cq, efficiency, starting amount, and correlation value).

5.3.1 Detailed Analyzer Results

The detailed analyzer results page displays detailed information about a particular analyzer result. The header section contains the name of the plate including a link back to it, the name of the analyzer, and the submission date. The export feature is the same as described in 5.3 .

Each entry of the list contains information about a particular well of a plate. Displayed are: sample name, target(s), Cq value, and efficiency.

Detailed Analyzer Results

Plate:	example	Show
Analyzer:	AnalyzerMiner	
Date:	2008-07-08 at 9:37:27	
Export List:	CSV	Export
Back to Overview:	Show	

Plate	Well	Sample Name	Target(s)	Cq	Efficiency
example	A1	sample1	detector1	27.7037	1.7186
example	A2	sample1	detector1	27.4865	1.7415
example	A3	sample2	detector1	26.5125	1.6769
example	A4	sample2	detector1	26.4674	1.71
example	A5	sample3	detector1	27.2452	1.6978
example	A6	sample3	detector1	27.305	1.7513
example	A7	sample4	detector1	26.634	1.6809
example	A8	sample4	detector1	26.8422	1.6678
example	A9	sample5	detector1	28.0017	1.7338
example	A10	sample5	detector1	27.9115	1.7334
example	A11	sample6	detector1	27.0018	1.7098
example	A12	sample6	detector1	26.9511	1.6974
example	B1	sample1	detector2	26.9691	1.6687

6 Experiment

An experiment in the application QPCR maps a real world qPCR experiment. It consists of a name, a creation date, an optional description, and numerous runs.



Name:	Experiment 1		
Date:	14.11.2007		
Description:			
Runs:	<ul style="list-style-type: none"> 20071108 <li style="background-color: #003366; color: white;">Run 20071108_2 <li style="background-color: #003366; color: white;">Run 1 Run 2 		

The experiment list is somewhat different to normal table views (2.4.4) because it contains an additional symbol for each entry. By clicking on it () the user is guided to the analyze setting page (8.1). Experiments that cannot be analyzed have a grayed out symbol, experiments that can be analyzed have a colored symbol.



Experiments per page: 15 [25] 50 100

2 Experiments found | Page 1 of 1 | go to page go

Nr.	Name	Date ↑				
1	experiment - 20071108	2009-01-21				
2	experiment - 20070101	2008-02-12				

Experiments per page: 15 [25] 50 100

2 Experiments found | Page 1 of 1 | go to page go

The detailed view of an experiment contains its name, creation date, description, and added runs. The Go button next to analyze sends the user to the analyze setup page. Each added Run can be viewed by pressing the Show button next to the run and by clicking on Show Cq and Efficiency Results the user is sent to the Cq Analyze Results page (5.3).

Show Experiment


Name:	SDS experiment		
Date:	10.09.2008		
Description:	tutorial SDS experiment		
Runs:	File1	Show	Show Cq and Efficiency Results
Analyze:	Go		

[Return](#)

7 Project

Projects are used to group several different experiments. Each project consists of a name, date, and description. Experiments can be added to the project by assigning them to the right selection box.

New Project

Name:	Test Project	
Date:	09.05.2006	
Description:		
Experiments:	<ul style="list-style-type: none">IRC testLiver2Liver3Test Referencing ProblemtestLightCyclertestNewSort	<div style="border: 1px solid gray; padding: 2px; display: inline-block;">test</div>

Existing projects are displayed in a tree where the associated experiments and their runs are shown. The user can sort the list and its child nodes according to name or date. Moreover links to the detailed pages of projects, experiments, and runs are given and an icon is shown to quickly jump to the analyze setup page of an experiment.

Projects

Sort Project: [Name](#) ↓ [Date](#)

Sort Experiment: [Name](#) [Date](#) ↓

Sort Run: [Name](#) ↓ [Date](#)



8 Analyze

8.1 Analyze Setup

The Analyze Setup page is divided into two parts: the upper section contains information about the experiment, gives the user the possibility to save the current setting, and allows the user to load previously saved settings. The lower part contains a tabbed navigation which is used to define the different settings for analyzing an experiment.

In general one can differ between two classes of analyzes:

- Cq value / efficiency value calculation
- Normalization

Cq value / efficiency value calculation needs to be done before normalization can be started, because normalization is dependent on Cq values (and if chosen also on efficiency values). Therefore whenever a Cq calculation method is selected, the application checks if these values are existent. If they are not present a message is shown at the bottom and the button changes from `Analyze` to `Calculate values`. By pressing this button the calculation of Cq/Efficiency values is started in the background. Because of the fact that this process is very time consuming the calculation is performed in the background.

The normalization of Cq values is a very quick task and is therefore not performed in the background. It is only started if Cq values and efficiency values (if selected) exist.

Normalization of the Cq values contains the following steps:

- Technical replicate handling – samples having the exact same name (cDNA) are averaged
- Efficiency correction
- Normalization using the selected reference genes – multiple reference genes are geometrically averaged
- Inter run calibration (on a gene specific base) – samples that are present on all used runs are used as calibrators

For more information please consult the paper “qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data” by Hellemans *et al.* (2007).

8.1.1 Cq Calculation Methods

The first tab displays the available Cq calculation methods. Shown is the name of the method and a short description. Whenever a calculation method is chosen the application checks if Cq values are existent and displays the corresponding message at the bottom of the page.

Analyze Setup

Experiment:	example	Show
Save Setting:		Save
Setting:		

Cq Calculation Methods	Sample/Target	Reference Genes	Normalization										
<table border="1"> <thead> <tr> <th>Use Name</th> <th>Description</th> </tr> </thead> <tbody> <tr> <td><input checked="" type="radio"/> AnalyzerMiner</td> <td>AnalyzerMiner implements the model described by Zhao and Fernald in [Zhao and Fernald, 2005] (PMID: 16241897). It operates on the raw fluorescence data and calculates Cq value, efficiency, and starting</td> </tr> <tr> <td><input type="radio"/> AnalyzerCy0</td> <td>Efficiency: Use the efficiency calculated by another method or determined by primer validation! AnalyzerCy0 implements the model described by Michele Guescini and Davide Sisti et al. in [A new</td> </tr> <tr> <td><input type="radio"/> SoFARAnalyze</td> <td>AnalyzerSoFar implements the algorithm described by Wilhelm in [Wilhelm, 2003] and Wilhelm et al. in [Wilhelm et al., 2003] (PMID: 12613255). SoFar stands for <Software For the Analysis of Real-time</td> </tr> <tr> <td><input type="radio"/> SDSAnalyzer</td> <td>AnalyzerSDS implements an algorithm similar to the one used by the SDS 2.2.2 software from Applied Biosystems. It uses a dynamic baseline created by a line fitted into the area prior to the exponential</td> </tr> </tbody> </table>	Use Name	Description	<input checked="" type="radio"/> AnalyzerMiner	AnalyzerMiner implements the model described by Zhao and Fernald in [Zhao and Fernald, 2005] (PMID: 16241897). It operates on the raw fluorescence data and calculates Cq value, efficiency, and starting	<input type="radio"/> AnalyzerCy0	Efficiency: Use the efficiency calculated by another method or determined by primer validation! AnalyzerCy0 implements the model described by Michele Guescini and Davide Sisti et al. in [A new	<input type="radio"/> SoFARAnalyze	AnalyzerSoFar implements the algorithm described by Wilhelm in [Wilhelm, 2003] and Wilhelm et al. in [Wilhelm et al., 2003] (PMID: 12613255). SoFar stands for <Software For the Analysis of Real-time	<input type="radio"/> SDSAnalyzer	AnalyzerSDS implements an algorithm similar to the one used by the SDS 2.2.2 software from Applied Biosystems. It uses a dynamic baseline created by a line fitted into the area prior to the exponential			
Use Name	Description												
<input checked="" type="radio"/> AnalyzerMiner	AnalyzerMiner implements the model described by Zhao and Fernald in [Zhao and Fernald, 2005] (PMID: 16241897). It operates on the raw fluorescence data and calculates Cq value, efficiency, and starting												
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<input type="radio"/> SDSAnalyzer	AnalyzerSDS implements an algorithm similar to the one used by the SDS 2.2.2 software from Applied Biosystems. It uses a dynamic baseline created by a line fitted into the area prior to the exponential												
Cq Values exist	Efficiency Values exist		Analyze										

8.1.2 Sample/Target selection

The second tab allows the user to select samples and targets that will be used for normalization. On the left side, available samples and targets are displayed (omitted wells are not considered for normalization) in list form and on the right side the samples and targets used for normalization are shown. Using the arrow buttons the user can add or remove one or many entries from each list.

When “Use Replicate Handling” is checked Cq values of replicates (all sample/target combinations on one plate) are averaged. When ticking “Average technical replicates over plates” then technical replicates are not only averaged within one plate but averaged over all plates that are in this experiment.

Cq Calculation Methods	Sample/Target	Reference Genes	Normalization														
Use Replicate Handling <input checked="" type="checkbox"/> Average technical replicates over plates <input type="checkbox"/>																	
<table border="1"> <thead> <tr> <th>Samples</th> <th>Used Samples</th> </tr> </thead> <tbody> <tr> <td></td> <td>sample1</td> </tr> <tr> <td></td> <td>sample2</td> </tr> <tr> <td></td> <td>sample3</td> </tr> <tr> <td></td> <td>sample4</td> </tr> <tr> <td></td> <td>sample5</td> </tr> <tr> <td></td> <td>sample6</td> </tr> </tbody> </table>	Samples	Used Samples		sample1		sample2		sample3		sample4		sample5		sample6			
Samples	Used Samples																
	sample1																
	sample2																
	sample3																
	sample4																
	sample5																
	sample6																
<table border="1"> <thead> <tr> <th>Targets</th> <th>Used Targets</th> </tr> </thead> <tbody> <tr> <td></td> <td>detector1</td> </tr> <tr> <td></td> <td>detector2</td> </tr> <tr> <td></td> <td>detector3</td> </tr> <tr> <td></td> <td>detector4</td> </tr> <tr> <td></td> <td>detector5</td> </tr> <tr> <td></td> <td>detector6</td> </tr> </tbody> </table>	Targets	Used Targets		detector1		detector2		detector3		detector4		detector5		detector6			
Targets	Used Targets																
	detector1																
	detector2																
	detector3																
	detector4																
	detector5																
	detector6																
Cq Values exist	Efficiency Values exist		Analyze														

8.1.3 Housekeeping Genes selection

The selection mechanism for reference genes is the same as described in 8.1.2. If a reference gene is put into the used list the corresponding target is put into its corresponding used list, because technically a reference gene is a target. When analyzing multiple runs in one experiment the reference gene(s) can be:

- on only one plate and Cq values of the other plates are normalized to the values of the reference genes present on only one plate
- on every plate and Cq values are normalized to the values of the reference gene on the current plate

If the checkbox next to “Reference Gene(s) need to be on all plates” is checked reference gene(s) need to be present on every plate.

The screenshot shows the 'Reference Genes' tab of a software interface. At the top, there are four tabs: 'Cq Calculation Methods', 'Sample/Target', 'Reference Genes', and 'Normalization'. The 'Reference Genes' tab is active. Below the tabs, there is a checkbox labeled 'Reference Gene(s) need to be on all plates' which is checked. Underneath, there are two lists: 'Reference Gene list' and 'Used Reference Genes'. The 'Reference Gene list' contains 'detector2', 'detector3', 'detector4', 'detector5', 'detector6', and 'detector7'. The 'Used Reference Genes' list contains 'detector1'. Between the two lists are four buttons: '>>', '<<', '<', and '>'. At the bottom of the window, there are two status indicators: 'Cq Values exist' and 'Efficiency Values exist'. An 'Analyze' button is located in the bottom right corner.

8.1.4 Normalization

The last tab is used for setting the normalization parameters. It is divided into two parts: definition of efficiency, specification of normalization algorithm.

The efficiency of each well can be specified in three ways:

- Global efficiency: each well has the same user defined efficiency
- Efficiency determined by an analyzer: each well uses the calculated efficiency of a certain analyzer
- Target dependent efficiency: the user can specify the efficiency for each used target. If the target has a stored efficiency (see 13.6.2), it is automatically loaded into the corresponding field. Moreover the efficiencies calculated by Primer Validation (see 13.6) can be loaded for a particular primer validation run by selecting the corresponding entry in the combo box.

To normalize the Cq values one of the three methods needs to be picked. In addition to these methods efficiency can be calculated using dilution series. Such a series is determined by the task attribute of a well which needs to be set to `standard`.



The selection of normalization algorithm is similar to the selection of Cq calculation method described in 8.1.1.

Cq Calculation Methods	Sample/Target	Reference Genes	Normalization																				
<p>Define Efficiency</p> <p><input type="checkbox"/> Calculate Efficiency (if possible) from dilution series</p> <p><input type="radio"/> Global Efficiency</p> <p style="margin-left: 20px;">Efficiency: <input style="width: 100px;" type="text" value="2"/></p> <p style="margin-left: 20px;">SE Efficiency: <input style="width: 100px;" type="text" value="0.05"/></p> <p><input checked="" type="radio"/> Use Efficiency of Analyzer AnalyzerMiner ▼</p> <p style="margin-left: 20px; font-size: x-small;">AnalyzerMiner implements the model described by Zhao and Fernald in [Zhao and Fernald, 2005] (PMID: 16241897). It operates on the raw fluorescence data ▼</p> <p><input type="radio"/> Specify Efficiency for each Detector</p> <p style="margin-left: 20px;">Use Primer Validation Plate: ▼</p> <table border="1" style="width: 100%; border-collapse: collapse; font-size: x-small;"> <thead> <tr> <th style="width: 10%;">Detector</th> <th style="width: 15%;">Efficiency</th> <th style="width: 15%;">SE Eff</th> <th style="width: 15%;">Plate</th> </tr> </thead> <tbody> <tr> <td>detector1</td> <td><input style="width: 50px;" type="text" value="2"/></td> <td><input style="width: 50px;" type="text" value="0.05"/></td> <td><input style="width: 100px;" type="text"/></td> </tr> <tr> <td>detector2</td> <td><input style="width: 50px;" type="text" value="2"/></td> <td><input style="width: 50px;" type="text" value="0.05"/></td> <td><input style="width: 100px;" type="text"/></td> </tr> <tr> <td>detector3</td> <td><input style="width: 50px;" type="text" value="2"/></td> <td><input style="width: 50px;" type="text" value="0.05"/></td> <td><input style="width: 100px;" type="text"/></td> </tr> <tr> <td>detector4</td> <td><input style="width: 50px;" type="text" value="2"/></td> <td><input style="width: 50px;" type="text" value="0.05"/></td> <td><input style="width: 100px;" type="text"/></td> </tr> </tbody> </table>				Detector	Efficiency	SE Eff	Plate	detector1	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>	detector2	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>	detector3	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>	detector4	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>
Detector	Efficiency	SE Eff	Plate																				
detector1	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>																				
detector2	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>																				
detector3	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>																				
detector4	<input style="width: 50px;" type="text" value="2"/>	<input style="width: 50px;" type="text" value="0.05"/>	<input style="width: 100px;" type="text"/>																				
Cq Values exist		Efficiency Values exist																					
			<input type="button" value="Analyze"/>																				

8.2 Cq Analyze Log

When a Cq calculation is finished a message is displayed in the header section (2.2). By clicking on this link the user gets redirected to the Cq Analyzer Log page which lists the completed analyzing jobs. Entries that have not been viewed are colored blue and are put at the beginning of the list.

Cq/Efficiency Analyzer Log

 Query
 Edit Display Settings

Legend

Analyzer Logs per page: **15** [25] 50 100

2 Analyzer Logs found | Page 1 of 1 | go to page go

Nr.	User	Viewed ↓	Plate Name	Successful	Date	
1	Stephan Pabinger	<input type="checkbox"/>	example	true	2008-07-08	✘
2	Stephan Pabinger	<input checked="" type="checkbox"/>	example1	true	2008-07-29	✘

Analyzer Logs per page: **15** [25] 50 100

2 Analyzer Logs found | Page 1 of 1 | go to page go

Top

Color Legend	
Color	Meaning
Blue	Analyzer Log has not been viewed

The detailed Cq Analyzer Log contains information about the plate name, plate id, whether the analyzing job was successful, submission date, and in case of an error the error message.

Show Cq/Efficiency Analyzer Log

Plate Name:	Isopn_20090430_2
Plate Id:	46704
Successful:	yes
Date:	14.05.2009
Viewed:	<input checked="" type="checkbox"/>

Analyzers	Successful	Error Message
AnalyzerMiner <input checked="" type="checkbox"/>		no error

[Return](#) [go to plate](#)

In case it was not successful:

Analyzers	Successful	Error Message
LinRegAnalyzer <input type="checkbox"/>		plate has no values to analyze attached

8.3 Normalization Result

The normalization result view contains information about the normalization process of Cq values. The header section displays the experiment and provides a link back to it. Pressing the button called `Display Bars & Quality Control` sends the user to the `Bars` section of normalize results (8.4). The button `Back to Analyze Setup` sends the user back to the setup page. `Perform Statistical Test` sends the users to the statistical test setup page.

By selecting on or many samples in the `Reference Samples` box the calculated values of each sample are referenced to (divided by) the values of the selected samples. If more than one reference sample is picked the average of the selected samples is used.

`Save Normalize Results` saves the current results to the database. These entries are used if external applications request for normalized QPCR results (MARS database).

Using the export feature the user can export the result list in various file formats.

By pressing on the Show/Hide log2 button the user can choose to display the calculated log2 results.

Each entry in the list contains information about:

- cDNA
- target
- task
- average Cq value (averaged value of replicates)
- CV value
- relative Cq value (rel Cq – relative quantity of Cq value using gene specific efficiency ($E^{-\Delta Cq}$))
- normalized relative Cq value (NRCq – normalized using geometric mean of reference genes)
- calibrated normalized Cq value (CNRCq – normalized NRCq values using calibrators)
- their respective standard errors.

The legend at the bottom of the page explains each calculated result.

Display Normalization Results

Experiment:

Back To Analyze Setup:

Display Bars & Quality Control:

Perform Statistical Test:

Reference Samples:

Save Normalized Results:

CSV

cDNA	target	task	avg Cq	SE avg Cq	SD avg Cq	CV	rel Cq	SE rel Cq	SD rel Cq	NRCq	SE NRCq	SD NRCq	CNRCq	SE CNRCq	SD CNRCq	Log2 NRCq	Log2 Upper SE NRCq	Log2 Lower SE NRCq	Log2 Upper	Log2 Lower
sample1	detector1	Sample	27.5951	0.1006	0.1536	0.3936	0.793	0.0473	0.0660	1.0	0.0844	0.1192	1.0	0.0844	0.1192	0.0	0.1169	0.1272	0.1625	0.1625
sample2	detector1	Sample	26.4899	0.0226	0.0319	0.0852	1.4323	0.0217	0.0276	1.0	0.0215	0.0272	1.0	0.0215	0.0272	0.0	0.0306	0.0313	0.0388	0.0388
sample3	detector1	Sample	27.2751	0.0299	0.0423	0.1097	0.9453	0.0156	0.0219	1.0	0.0233	0.0328	1.0	0.0233	0.0328	0.0	0.0332	0.034	0.0465	0.0465
sample4	detector1	Sample	26.7381	0.1041	0.1472	0.3892	1.2506	0.0672	0.0849	1.0	0.0759	0.1073	1.0	0.0759	0.1073	0.0	0.1056	0.1139	0.1471	0.1471
sample5	detector1	Sample	27.9566	0.0451	0.0630	0.1614	0.6494	0.0161	0.0220	1.0	0.0351	0.0497	1.0	0.0351	0.0497	0.0	0.0480	0.0516	0.0699	0.0699
sample6	detector1	Sample	26.9764	0.0253	0.0359	0.0939	1.1097	0.015	0.0212	1.0	0.0191	0.027	1.0	0.0191	0.027	0.0	0.0273	0.0279	0.0395	0.0395
sample1	detector2	Sample	26.8743	0.0848	0.1341	0.3527	0.8157	0.0398	0.0583	1.0287	0.0793	0.1121	1.0287	0.0793	0.1121	0.0468	0.1072	0.1158	0.1492	0.1492
sample2	detector2	Sample	25.5159	0.0303	0.0429	0.1188	1.7201	0.0747	0.0893	1.201	0.0562	0.0606	1.201	0.0562	0.0606	0.2642	0.0649	0.0679	0.071	0.071
sample3	detector2	Sample	26.2716	0.2141	0.3028	0.8149	1.118	0.1291	0.1825	1.1827	0.138	0.1949	1.1827	0.138	0.1949	0.2421	0.1592	0.179	0.2201	0.2201
sample4	detector2	Sample	25.0416	0.1476	0.2088	0.5717	1.4016	0.1897	0.1551	1.1907	0.1064	0.1504	1.1907	0.1064	0.1504	0.1645	0.1388	0.1439	0.1816	0.1816

8.4 Normalization Result – Bars

The normalization result bars page is divided into two sections. The upper part contains information about the normalized experiment and provides links to the normalization result page, statistical test setup page and normalization setup page.

The lower part consists of a tabbed navigation interface with three tabs: Multiple Targets, Single Target, and Quality Control.

8.4.1 Multiple Targets

The “multiple targets” tab allows the user to choose one or many targets. The chosen targets are shown in the legend and colored in different colors. The x-axis contains the selected samples and the y-axis shows the normalized Cq value whereas each bar displays the calculated error.

The user can customize the chart in the following ways:

- Select the displayed error (standard error, standard deviation, confidence interval)
- Select the samples used for referencing
- Set a title for the chart
- Group the bars by sample or target
- Select which sample should be displayed in the chart

The order of samples is equal to the order set in the single target chart (see 8.4.2).

The chart can be saved either by right clicking on it and selecting the corresponding option or by using the **Export As SVG** button.



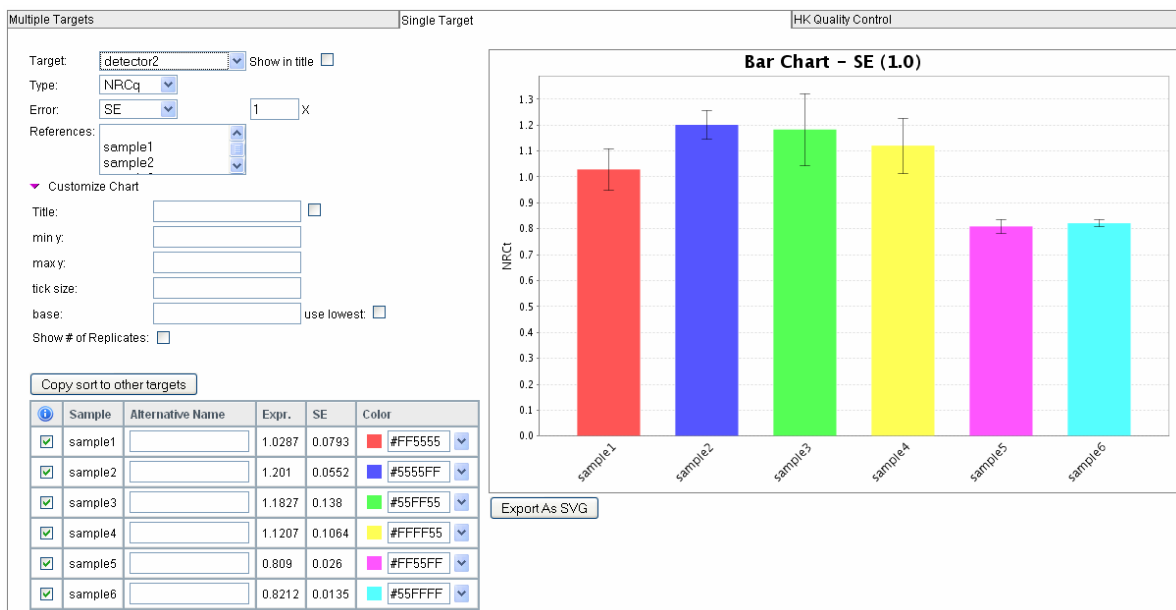
8.4.2 Single Target

The single target view shows samples of one specific target.

The user can customize the chart in the following ways:

- Switch between Cq value, normalized Cq value, and calibrated normalized Cq value and their log₂ values
- Select the displayed error (standard error, standard deviation, confidence interval)
- Select the samples used for referencing
- Set a title for the chart
- Specify the displayed minimum and maximum value
- Set the tick size
- Set the base to an arbitrary number or the lowest number displayed in the chart
- Include the number of replicates of each sample in the chart
- Customize and view the samples displayed in the chart
 - Include/Exclude the sample from the chart
 - Set an alternative name
 - Set a specific color for each sample
 - Rearrange the list using drag and drop

The selected color, alternative name, and position of each sample is stored in the database and loaded when this experiment is analyzed again.



8.4.3 Quality Control

Quality Control is only useful when multiple reference genes have been selected.

The CV value represents the coefficient of variation of the normalized reference gene expression levels. A value lower than 50% for heterogeneous panels (lower than 25 % for homogeneous) is typically observed for stably expressed reference genes.

The M value (geNorm) represents the mean stability measure of the used reference genes. A value lower than 1 for heterogeneous panels (lower than 0.5 % for homogeneous) is typically observed for stably expressed reference genes.

The lower these quality values are, the more stable the reference genes are expressed in the tested samples.

The next section checks if a NTC has been used for each selected target.

In the next table the differences between the technical replicates is calculated and the user can choose to color the ones that are above a certain user defined threshold.

Multiple Targets		Single Target		HK Quality Control	
	CV	M (geNorm)			
detector1	9.04 %	0.2565			
detector2	8.72 %	0.2565			
Mean	8.88 %	0.2565			
target	has NTC				
detector1	false				
detector2	false				
detector3	false				
detector4	false				
detector5	false				
detector6	false				
detector7	false				
detector8	false				
ExperimentReplicates threshold:		0.3	<input type="button" value="Show"/>		
target	cDNA	difference	#		
detector8	sample6	0.1799	2		
detector8	sample5	0.3121	2		
detector8	sample4	0.1712	2		
detector8	sample3	0.2569	2		
detector8	sample2	0.0497	2		
detector8	sample1	0.0477	2		
detector7	sample6	0.2121	2		
detector7	sample5	0.1909	2		
detector7	sample4	0.2016	2		
detector7	sample3	0.1536	2		
detector7	sample2	0.2071	2		
detector7	sample1	0.0564	2		
detector6	sample6	0.0	1		
detector6	sample5	0.3483	2		
detector6	sample4	1.3124	2		
detector6	sample3	0.3483	2		

9 Statistical Test

Statistical tests are used to test several groups (in the software named as class) of samples for significant difference between them. It can be used, for example, to test whether several biological replicates of certain samples are differentially expressed to several biological replicates acting as a control.

9.1 Test Setup

The header section of the setup page provides links back to the experiment, to the Analyze Setup (see 8.1) and to the Normalization Result page.

The following parameters can be set for the statistical test:

- Define which samples are included in the test
- Select the reference sample(s) used for referencing the calculated values. This has nothing to do with the actual statistical test. It is used to calculate the fold change ratios. By selecting a class all samples in this class will be used as reference samples.
- Select which test should be used
- Select the p-Value type
- Select multiple testing correction: Choose between four established methods to correct the calculated p-value; Additionally calculate your p-values without multiple testing correction
- Select the data type: choose between CNRCq and log2 CNRCq
- Select if samples should be averaged in each class. This causes that the output chart displays only the averaged value (only on bar) for each class.

The next part of the setup page is used to define the classes (groups) and their attributes. The user can define as many classes as needed which are then used in the statistical test. One class acts as the statistical reference (reference class) and all other classes are tested for their statistical significant difference to this reference class. Do not confuse this with the sample references which are used to reference the samples to a given set of samples (no statistical test).

Each class has a color or pattern associated, is given a specific name, and needs to consist of at least one sample. In one class the property `Set As Statistical Reference` is set which specifies to which class all other classes are compared (previously described).

Classes can be added or removed from the list and the complete statistical setup is stored in the database and loaded whenever the test is repeated.

Perform delta delta Cq calculation

Experiment:	<input type="text" value="example"/>	<input type="button" value="Show"/>
Back To Analyze Setup:	<input type="button" value="Show"/>	
Display Normalization Result:	<input type="button" value="Show"/>	

Reference Calculation:

Samples:	<input type="text" value="sample3"/> <input type="text" value="sample4"/> <input type="text" value="sample5"/> <input type="text" value="sample6"/>
Reference:	<input checked="" type="radio"/> Samples: <input type="text" value="sample1"/> <input type="text" value="sample2"/> <input type="radio"/> Class: <input type="text" value="class 1"/>

Statistical Test:

Choose Test:	<input type="text" value="Permutation Mean Test"/>
Choose p-Value Type:	<input type="text" value="TWO SIDED"/>
Choose Testing Correction:	<input type="text"/>
Choose Datatype:	<input type="text" value="CNRCq"/>
Average samples in class:	<input type="checkbox"/>
Choose Classes:	<input type="button" value="Add Class"/> <input type="button" value="Remove Last Class"/>

Set As Statistical Reference

<input type="text" value="sample1"/> <input type="text" value="sample2"/> <input type="text" value="sample3"/> <input type="text" value="sample4"/> <input type="text" value="sample5"/> <input type="text" value="sample6"/>
--

Set As Statistical Reference

<input type="text" value="sample1"/> <input type="text" value="sample2"/> <input type="text" value="sample3"/> <input type="text" value="sample4"/> <input type="text" value="sample5"/> <input type="text" value="sample6"/>
--

9.2 Graphical Result

The upper section of the statistical result page displays links back to the various analysis pages and provides the functionality to export the generated results.

Display Statistical Test Results - Bars

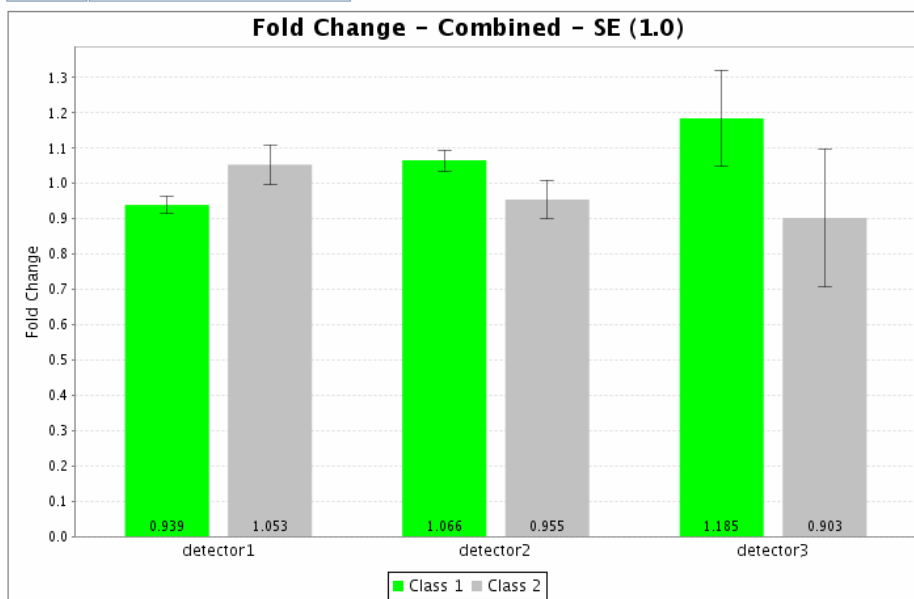
Experiment:	example	Show
Back To Analyze Setup:	Show	
Display Normalization Result:	Show	
Perform Statistical Test:	Show	
Display Test Result:	Show	
Statistical Test:	Permutation Mean Test	

CSV	Export
-----	--------

The combined target view displays the averaged results of each class (in this case the classes replicates1 and replicates2) for the selected targets. It is possible to select multiple targets and to set a title for the generated chart.

Combined Targets:

Select Targets:	detector1 detector2 detector3
Title:	<input type="checkbox"/>



Export As SVG

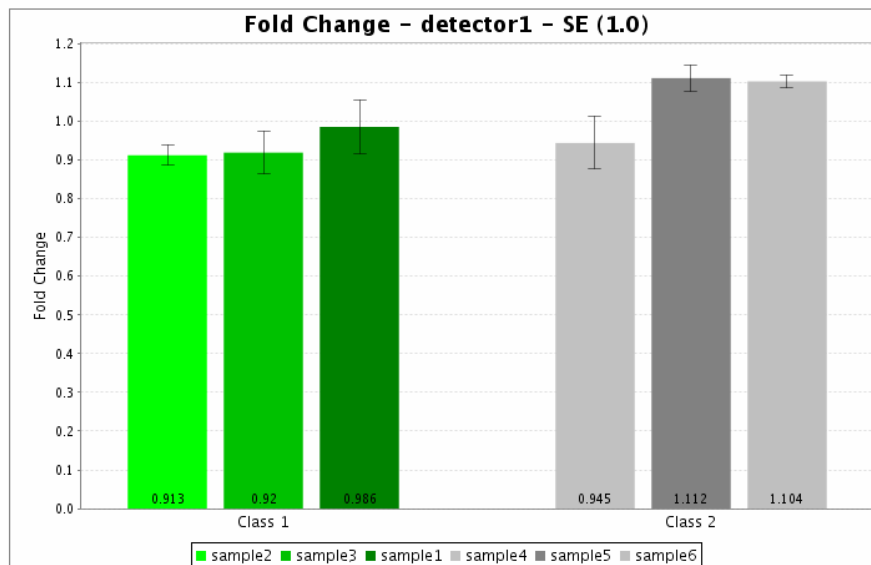
Next the results for each target are shown. Both the calculated values and the created chart are displayed.

Displayed are the following values for each class:

- The calculated p-Value
- The average ddCq value of the class divided by the average value of the statistical reference class (NOT reference samples)
- The average SD of the class divided by the average SD of the statistical reference class (propagated standard deviation)
- Whether this class was set as statistical reference

Target: detector1

Class	p-Value	Ø ddCq / reference	Ø SE ddCq / reference	Ø SD ddCq / reference	Is Statistical Reference
Class 1	-	-	-	-	true
Class 2	0.1975	1.1214	[+0.0655; -0.0655]	[+0.1134; -0.1134]	false



Export As SVG

In addition to the graphical view results of the statistical test can be displayed in text format. This view is accessed by clicking on show next to Display Test Result.

For each class the p-Value, average ddCq/reference and the corresponding error are shown. In addition, the fold change values and the corresponding errors of the samples used for the statistical test are displayed in a list.

detector1

Class	p-Value	$\bar{\Delta} \Delta C_t$ / reference	$\bar{\Delta} SE \Delta C_t$ / reference	$\bar{\Delta} SD \Delta C_t$ / reference	Is Statistical Reference																								
replicates 1	-	-	-	-	true																								
<table border="1"> <thead> <tr> <th>Sample</th> <th>ΔC_t</th> <th>SE ΔC_t</th> <th>SD ΔC_t</th> </tr> </thead> <tbody> <tr> <td>sample1</td> <td>1.0</td> <td>0.023332</td> <td>0.032996</td> </tr> <tr> <td>sample2</td> <td>1.0</td> <td>0.028336</td> <td>0.040074</td> </tr> <tr> <td>sample3</td> <td>1.0</td> <td>0.05177</td> <td>0.073213</td> </tr> <tr> <td colspan="4"> </td> </tr> <tr> <td>Avg replicates 1</td> <td>1.0</td> <td>0.017272</td> <td>0.029916</td> </tr> </tbody> </table>						Sample	ΔC_t	SE ΔC_t	SD ΔC_t	sample1	1.0	0.023332	0.032996	sample2	1.0	0.028336	0.040074	sample3	1.0	0.05177	0.073213					Avg replicates 1	1.0	0.017272	0.029916
Sample	ΔC_t	SE ΔC_t	SD ΔC_t																										
sample1	1.0	0.023332	0.032996																										
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Avg replicates 1	1.0	0.017272	0.029916																										
replicates 2	1.0	1.0	0.0306	0.053	false																								
<table border="1"> <thead> <tr> <th>Sample</th> <th>ΔC_t</th> <th>SE ΔC_t</th> <th>SD ΔC_t</th> </tr> </thead> <tbody> <tr> <td>sample4</td> <td>1.0</td> <td>0.067074</td> <td>0.094856</td> </tr> <tr> <td>sample5</td> <td>1.0</td> <td>0.06139</td> <td>0.086818</td> </tr> <tr> <td>sample6</td> <td>1.0</td> <td>0.019126</td> <td>0.027049</td> </tr> <tr> <td colspan="4"> </td> </tr> <tr> <td>Avg replicates 2</td> <td>1.0</td> <td>0.025289</td> <td>0.043801</td> </tr> </tbody> </table>						Sample	ΔC_t	SE ΔC_t	SD ΔC_t	sample4	1.0	0.067074	0.094856	sample5	1.0	0.06139	0.086818	sample6	1.0	0.019126	0.027049					Avg replicates 2	1.0	0.025289	0.043801
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sample6	1.0	0.019126	0.027049																										
Avg replicates 2	1.0	0.025289	0.043801																										

10 Error propagation

Throughout the whole analysis pipeline error propagation is performed to ensure statistical relevant results. It is based on truncated Taylor series expansion and uses general valid error propagation laws.


Included in the pipeline is:

- Technical replicate handling
- Efficiency correction
- Normalization using reference genes
- Inter-run calibration
- Referencing the normalized Cq values to sample(s)
- Averaging of biological replicates

11 User settings

The user settings interface can be reached by clicking on the corresponding link described in chapter 2.2.1 . Preferred Cq Analyzer(s) and Preferred Efficiency Analyzer(s) defines the analyzer(s) that is/are used when the user starts a multiple parse (described in 3.3). Use NTC's in Cq analysis / Normalization defines whether the non template controls are used in the corresponding analysis. Chart Background defines the background color that is used in every chart (default is white).



User Settings

Information:	
Preferred Cq Analyzer(s):	<ul style="list-style-type: none">AnalyzerMinerAnalyzerCy0SoFARAnalyzer
Preferred Efficiency Analyzer(s):	<ul style="list-style-type: none">AnalyzerMinerAnalyzerRutledGeneLinRegAnalyzer
Use NTCs in Cq analysis:	yes
Use NTCs in Normalization:	yes
Chart Background:	<input type="checkbox"/> #FFFFFF

12 Run Deletion Log

Deleting a run is a very time consuming operation and is therefore performed in the background. Whenever such an operation is completed the user gets informed that a new Run Deletion Log is available. Results that have not been viewed are colored in blue and are put at the top of the list. After a Run Deletion Log run deletion result has been viewed by a user it is automatically deleted.

Run Deletion Log

 Query
 Edit Display Settings

Legend

Run Deletion Logs per page: 15 [25] 50 100

1 Run Deletion Logs found | Page 1 of 1 | go to page go

Nr.	Run	Date ↑	Viewed	
1	test	2008-07-29	<input type="checkbox"/>	✘

Top

Color Legend	
Color	Meaning
Blue	Run Deletion Log has not been viewed

Run Deletion Logs per page: 15 [25] 50 100

1 Run Deletion Logs found | Page 1 of 1 | go to page go

Each Run Deletion Result entry presents information about the deleted run, whether the job was successful, the submission date, an error message if the deletion job was not successful, and the view status.

Show Run Deletion Log

Run:	test
Successful:	yes
Date:	13.05.2009
Error Message:	
Viewed:	<input checked="" type="checkbox"/>

[Return](#)

13 PCR Management

The menu PCR Management groups properties that are necessary to specify a qPCR run.

13.1 CDNA

A cDNA entry represents a physical cDNA (sometimes called sample) in qPCR experiments. Each entry consists of a name, amount, concentration, an optional protocol, an option to specify whether DNase treatment was performed, and a description. RNA Extract defines the extract where the cDNA was created from and is linked to the MARS database.

New cDNA

Name:	<input type="text"/>
RNA Extract:	<input type="text"/>
Amount:	<input type="text"/>
Concentration:	<input type="text"/>
Protocol:	no protocol
DNase Treatment:	<input type="checkbox"/>
Description:	<input type="text"/>

13.2 Target

A target contains information about the target's name, type, barcode, description, and used primers.

New Target

Name:	<input type="text"/>
Type:	<input type="text"/> <input type="button" value="Edit"/>
Barcode:	<input type="text"/>
Description:	<input type="text"/>
Primers:	<input type="text" value="no primer"/> <input type="text" value="testPrimer"/> <input type="text" value="eqrw"/>
Efficiency:	<input type="text"/>
SE Efficiency:	<input type="text"/>

13.3 Instrument Setting

An Instrument Setting represents a thermocycler setup for a qPCR run. The general information contains a name and a description.

Each setting consists of stages which are divided into steps.

Each stage has a stage-number and a number of repetitions. Steps are also numbered and each step consists of a duration and a temperature.

During the parsing process these values are extracted from the SDS file and stored in the database.

Users can manually insert an instrument setting with at least one stage and one step in each stage. The number of stages and steps is not limited.

New Instrument Setting

Name :	my Instrument Setting				
Description :					
Stage :	1	Reps :	5	✘	
	Step:	1	Duration:	15:00 min	Temperature: 67 °C ✘ Add Step
Stage:	2	Reps:	14	✘	Add Stage
	Step:	1	Duration:	3:00 min	Temperature: 73 °C ✘
	Step:	2	Duration:	5:00 min	Temperature: 93 °C ✘ Add Step
Create					

Show Instrument Setting

Name:	new Instrument Setting - test				
Description:	2007-01-30 - EDIT PLEASE				
Stage:	1	Reps:	1		
	Step:	1	Duration:	2:00 min	Temperature: 50.0 °C
Stage:	2	Reps:	1		
	Step:	1	Duration:	10:00 min	Temperature: 95.0 °C
Stage:	3	Reps:	40		
	Step:	1	Duration:	0:15 min	Temperature: 95.0 °C
	Step:	2	Duration:	1:00 min	Temperature: 60.0 °C
Stage:	4	Reps:	1		
	Step:	1	Duration:	0:15 min	Temperature: 95.0 °C
	Step:	2	Duration:	0:20 min	Temperature: 50.0 °C
	Step:	3	Duration:	19:25 min	Temperature: 85.0 °C
Return					

13.4 Passive Reference

A passive reference entry consists of name and description and can be linked to a well.

Edit Passive Reference

Name:	ROX
Description:	Standard Passive Reference

[Return](#)[Update](#)

13.5 Primer

To create a primer six mandatory properties need to be set: name, sequence (the actual DNA sequence), sequence position, primer length, primer concentration, and type (forward, reverse, or probe). Optionally temperature, logNr, provider, author, and description can be specified.

New Primer

Name:	<input type="text"/>
Sequence:	<input type="text"/>
Sequence Position:	<input type="text"/>
Length:	<input type="text"/>
Temperature:	<input type="text"/>
Concentration:	<input type="text"/>
LotNr:	<input type="text"/>
Provider:	<input type="text"/>
Type:	forward <input type="button" value="Edit"/>
Author:	<input type="text"/>
Description:	<input type="text"/>

13.6 Primer Validation

Primer Validation is used to determine the efficiency of targets by using serial dilution series. After calculating the Cq values linear regression is used to calculate the efficiency of the target. The efficiency is then stored in the database and can be loaded into the analysis of an experiment (see 8.1.4). In order to calculate the efficiency, wells on the plate need to be marked by setting the task as standard!

13.6.1 Perform Validation

First a Run, that has been used for primer validation (standard curve), needs to be parsed and analyzed using the standard procedure. Dependent on the selected Cq analyzer the systems checks if Cq values exist for this analyzer. If they are not present, the Cq value calculation can be started directly from this interface.

If the system has found Cq values for this analyzer the calculation of efficiency values can be started by pressing the Analyze button. If this plate has been analyzed before the system displays a warning that the results will be overridden.

When the calculation is finished the results for each target on this plate having a serial dilution series are shown in a list. Displayed are the efficiency, the standard error, R2, and the slope of the calculated line. The efficiency of each target can be stored by ticking the checkbox and pressing the save button.

Primer Validation

Plate:	test	▼	Analyze
Cq Analyzer:	AnalyzerMiner	▼	Cq values exist
Validation Method:	Linear Regression	▼	

Target	E	SE	R2	Slope	Save
18S rRNA (MM)	1.84409	0.07152	0.97647	-3.76249	✓
GAPDH (MM)	1.81782	0.04924	0.98583	-3.85282	✓
HBMS (MM)	2.07412	0.15234	0.95177	-3.15623	✓
TBP (MM)	1.61007	0.03878	0.98241	-4.83452	✓
TF II B (MM)	1.85769	0.11479	0.971	-3.71783	✓

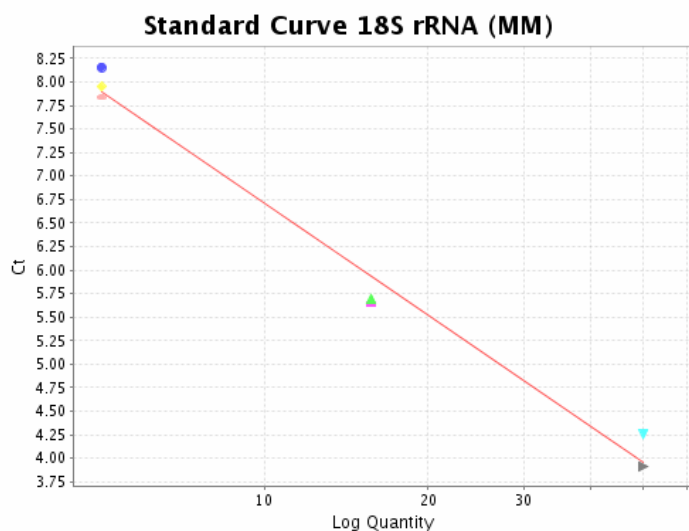
Displayed below is the message shown when Cq values don't exist.

Plate:	templateCSV1	▼	
Cq Analyzer:	AnalyzerMiner	▼	Cq values do NOT exist
Validation Method:	Linear Regression	▼	<input type="button" value="Calculate Values"/>

The lower part of the page shows a graphical representation of the fitted line for the selected target. Targets can be selected by choosing the corresponding entry in the nearby combo box.

Below the chart, a representation of the plate layout is displayed that marks the wells currently used to calculate the efficiency. The system allows the user to de/select wells used for calculating the curve (either by pressing on the point in the graph or by pressing on the colored well in the grid). Results are updated on the fly after the particular well has been in/excluded from the calculation. The color code used in the plate layout representation is explained in the legend.

After the user is satisfied with the result of the calculated efficiency (using the slope of the curve) it can be stored in the database. The choice which wells are used for the calculation is stored in the database and loaded when the primer validation is performed again.



Target	18S rRNA (MM)
E	1.794019
SE	0.060335
Slope	-3.939691
R2	0.983714
Intercept	10.646703

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
G	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
H	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY

 omitted
 empty
 used
 available for calculation

13.6.2 Target Efficiencies

By pressing on Target Efficiencies a list of the currently stored efficiencies is shown.

For each target the following information is displayed:


- Efficiency
- Standard error of this efficiency
- Slope
- R2
- If this efficiency is used for this target
- The run used to calculate the efficiency (run used to perform the primer validation)
- If available a button to set this efficiency as the current on (activate)
- A link to delete this efficiency

It is possible to perform several primer validations for one target and store each calculated efficiency. Therefore the system provides the opportunity to set one efficiency as the active one which is loaded into the analysis settings when an experiment is analyzed (see 8.1.4). To switch the currently active efficiency the button in the Activate column has to be pressed.

Target Efficiencies

 Query

 Edit Display Settings

There are currently **670** targets with no efficiency in the database. 

DetectorEfficiencys per page: **15 [25] 50 100**

5 DetectorEfficiencys found

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go to page go

Nr.	Target	Efficiency	Se Efficiency	Slope	R2	Used	Run	Activate
1	18S rRNA (MM)	1.79402	0.06033	-3.93969	0.98371		test	
2	GAPDH (MM)	1.81782	0.04924	-3.85282	0.98583		test	
3	HBMS (MM)	2.07412	0.15234	-3.15623	0.95177		test	
4	TBP (MM)	1.61007	0.03878	-4.83452	0.98241		test	
5	TF II B (MM)	1.85769	0.11479	-3.71783	0.971		test	

DetectorEfficiencys per page: **15 [25] 50 100**

5 DetectorEfficiencys found

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13.7 Realtime Chemistry

A realtime chemistry (e.g. TaqMan, Scorpions, SYBR, ...) entry consists of name, catalog number, concentration, provider, and description. Realtime chemistries are linked to wells.

New Realtime Chemistry

Name:	<input type="text"/>
Catalog Number:	<input type="text"/>
Concentration:	<input type="text"/>
Provider:	<input type="text"/>
Description:	<input type="text"/>


14 Resource Management

The management section groups properties which are of general information in the QPCR application.

14.1 Hardware

A hardware entry in the application QPCR describes a physical device. It stores information about the name, type, creation date, version, shown attribute, and description of a specific hardware. Hardware entries are linked to runs.

Edit Hardware

Name:	SDS 7900
Type:	Thermocycler 
Date:	30.01.2007
Version:	12
Shown:	<input checked="" type="checkbox"/>
Description:	2007-01-30 - EDIT PLEASE

[Return](#)[Update](#)

14.2 Protocol

A protocol entry is used to store protocols of analyzes, runs, and cDNAs. It consists of a name, a type, its files, and a description. The specified files are uploaded to the system when a new protocol is created.

New Protocol

Name:	Analyze 44_57 sk
Type:	Analyze Protocol <input type="button" value="Edit"/>
Txt File:	abinger\Desktop\prot.txt <input data-bbox="699 824 831 869" type="button" value="Browse..."/>
Other File:	binger\Desktop\prot.pdf <input data-bbox="699 880 831 925" type="button" value="Browse..."/>
Description:	<input type="text"/>

14.3 Provider

A provider stores information about name, abbreviation, street, city, province, country, phone, fax, email, web address, and description. Providers can be linked to realtime chemistries and primes.

New Provider


Name:	Test Provider
Abbreviation:	TP
Street:	Nowherestr 3
City:	Gotham
Province:	
Country:	USA
Phone:	
Fax:	
Email:	me@gotham.com
Url:	www.gotham.com
Description:	

Create

14.4 Software

Software entries describe the software of a particular hardware (see 14.1). It stores information about the name, type, creation date, version, shown attribute, and description. A software entry can be linked to a run.

New Software

Name:	<input type="text"/>
Type:	<input type="text"/>  <input type="button" value="Edit"/>
Date:	<input type="text"/>
Version:	<input type="text"/>
Shown:	<input type="checkbox"/>
Description:	<input type="text"/>

15 CSV file format

If no parser for the used thermocycler is available the generic CSV file format can be used. The information section provides a sample file and explains the specification.

[Information about the CSV file format](#)