quantGenius Manual

(last update 2-Feb-17)

1. Preface

Quantitative PCR (qPCR) is considered the golden standard molecular method for the quantification of nucleic acids. Due to several factors that can influence the final result, quantitative analysis and interpretation of qPCR data is not trivial. We have developed "quantGenius" (http://quantgenius.nib.si), a web-based tool for robust quantification of qPCR data using standard curve. It is designed as a workflow that guides the user through quality control (QC) and calculation steps. It deals with all the quantification-related issues of qPCR-based nucleic acid quantifications from data consistency, pipetting errors, standard curve parameters, individual sample efficiencies/inhibition, range of quantification and normalization to one or several reference genes. In this way it enables robust quality controlled quantification of nucleic acids (Figure 1).

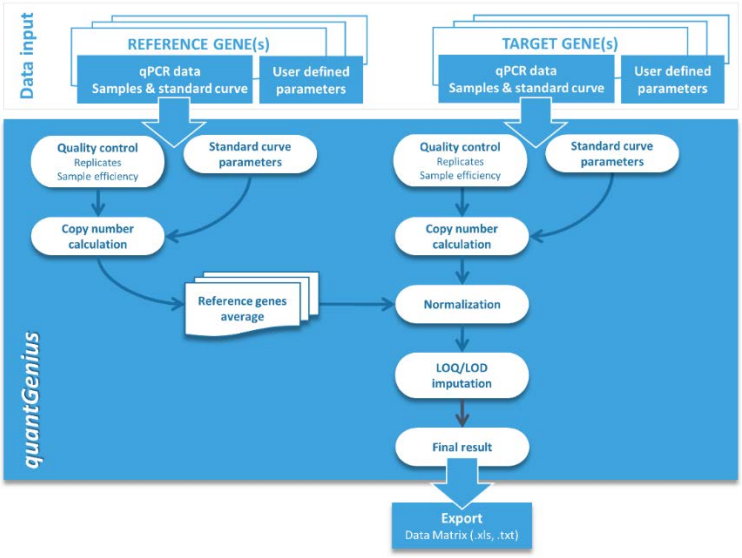


Figure 1: Simplified quantGenius workflow

The quantGenius was developed at the National Institute for Biology, based on the needs of the researchers. It is routinely used in our everyday research. The server version allows us to compare the data of different users, and to our internal primer and results databases. This manual will explain how to perform quantification analysis of qPCR data. It is important to note that the **qualitative QC steps** such as checking of fluorescence curves, qDNA contamination, non-template or other control**s should be performed by the users prior to analysis by the quantGenius**.

2. Registration and login

quantGenius is a web-based application, available at http://quantgenius.nib.si. It works in all the major web browsers (e.g. Internet Explorer, Firefox, Chrome...)

For simple, one time analysis, registration or login is not needed. To start using the application, click on "proceed without login" link below the login window. Please note that in this case your data will be stored on the server only for one session or 24 hours. Nevertheless, to store the experimental data on server for later modifications (adding data) or reanalysis (changing parameters), it is necessary to register. Click on

"Register" (top right on the screen) and enter your preferred username and your real name and surname, a valid email and your organization (see example figure below). Click "Register" (bottom right) to confirm (Figure 2). **User data is stored one year after last login to the application** and if you were not using the application for more than one year you must register again.

Figure 2: Registration window

3. Adding new experiments

After you login you are in the "Experiments page" where you can edit or delete already analysed experiments (listed in a table). To create new experiment analysis click on "Add experiment" button (bottom right; Figure 2). First you have to Name your experiment (use organism name/project name or similar) and click "Add". Your new experiment will now appear in the table. Click on the experiment name to open your experiment where you can add your reference and target genes results data.

All the experiment data is stored on the quantGenius server for one month after last modification (renaming, adding, deleting or changing genes), **and will be deleted after that.** Please export all the analysed data on time!

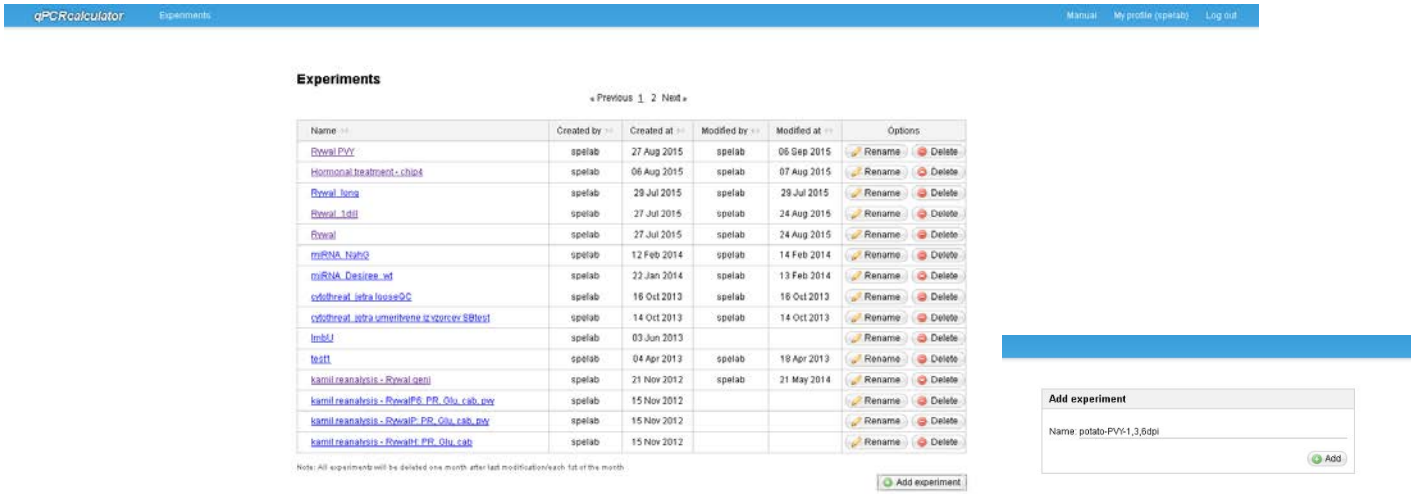


Figure 3: Experiment organization and addition

4. Adding a reference gene

For each experiment, at least one reference gene data must be added prior to any target gene data. Add a reference gene by clicking "Add reference gene" button (Figure 4).

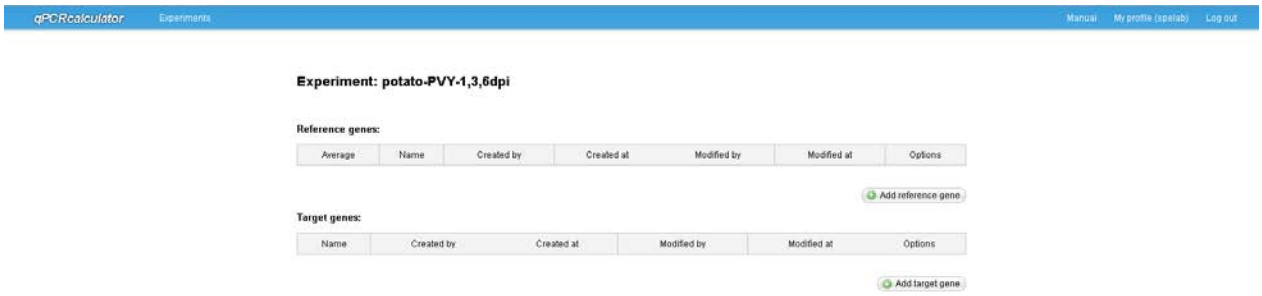


Figure 4: Adding new genes

The "Add reference gene" page is divided into four subpages: 1. Gene info & data, 2. Parsed data, 3. Check Sample names & dilutions, 4. Quality control & Calculations.

Gene info & data: Specify the gene name and then paste the sample data (Data) and standard curve data for the analysed reference gene. The data can be copied from the raw export file from the qPCR machine software.

The input data format for the samples is:

sample name [tab] Cq value [tab] sample dilution relative copy numbers (Figure 5).

The input data format for the standard curve is:

Cq value [tab] dilution relative copy numbers (Figure 5).

***Sample naming**: it is highly recommended to use Unique sample IDs to name your samples as this will enable easier further analysis workflow. If using two dilutions you should give the same sample names for both dilutions.*

***Dilutions**: organize your data in a way that less diluted reactions are preceding more diluted reactions (e.g. 10-fold dilution before 100-fold dilution).*

***Standard curve:** should include at least 4 –serial dilutions with at least 3 replicates each (see Svec at al., BDQ 2015, for details). If the same standard curve is used on different plates, the calculated copy numbers are comparable across plates, eliminating the need for any additional interpolate calibration.*

***Pipetting errors:** Do not correct or remove any wrongly pipetted reactions at this stage (or any previous manipulation of qPCR results data)!*

***Undetermined reactions:** the quantGenius recognizes "Undedermined", "Failed" or empty field as failed PCR amplification. If your qPCR platform gives different outputs (Eg. 40), it is best to delete the values, as only in this way they will be appropriately recognised and LOD imputation can be implemented (see below).*

***BioMark (Fluidigm) data**: For easier analysis of BioMark (Fluidigm) results, use the **Fluidigm data prep** tool that will convert the standard machine-output file into format, suitable for copy-pasting to the quantGenius. The tool is accessible from the quantGenius homepage window or at http://quantGenius.nib.si/fluidigm/.*

Click "Next" (bottom right) to proceed.

Figure 5: Data import

After importing the data, **Parsed data** page serves as a check-point to review if all data was correctly read by the application (Figure 6). The lines that will not be used for analysis are marked with in the "Accepted" field and the lines identified as appropriately formatted data are marked with and have a green background colour. If you are pleased with what you see, proceed by clicking "Next" at the bottom of the page (scroll down). If the parsing was incorrect click "Back" and correct the data format according to instructions above (hint: very common “mistake” is that you have different sample names for the two dilutions) (Figure 6).

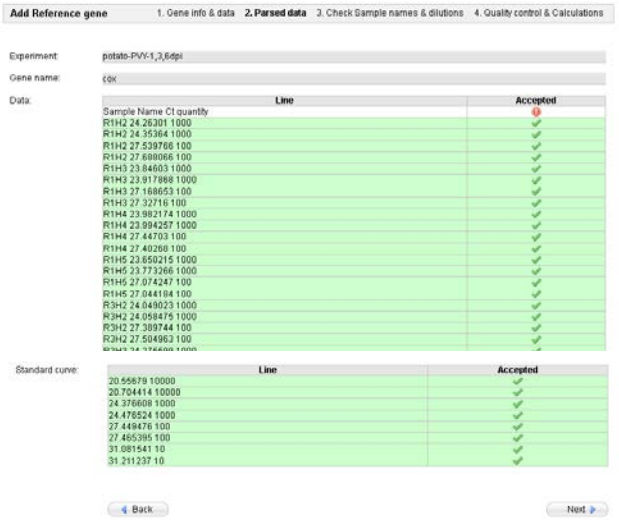


Figure 6: Format checking of the imported data

Reference Sample names & dilutions subpage lets you check if all reference genes added to the experiment have the same sample names and same sample number (Figure 7). Proceed by clicking "Next" (bottom right).

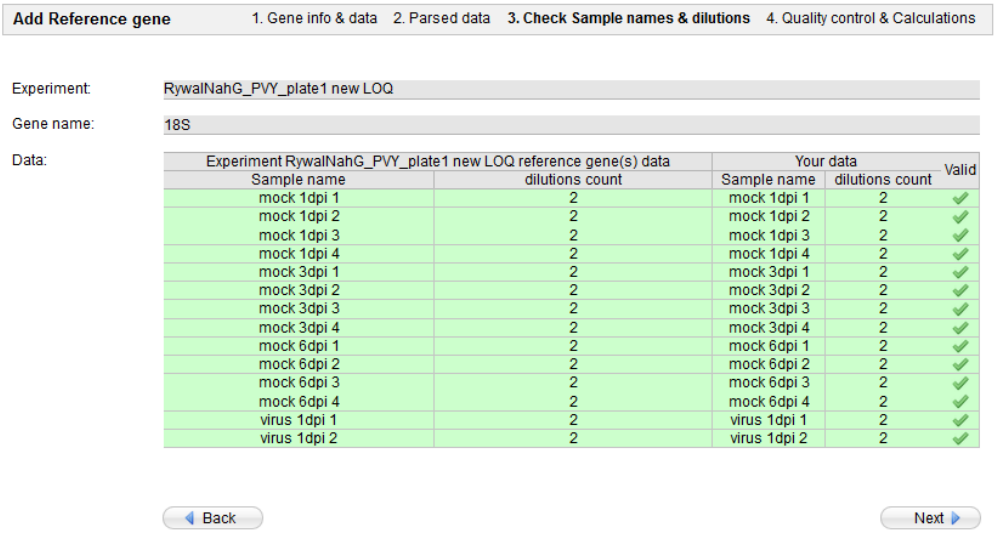


Figure 7: Consistency checking of the imported data: sample names and dilutions agreement between the imported reference genes

On **Quality control & Calculations** subpage you specify the "Extraction quality control (CqExtC)" which is the maximum Cq value that is considered OK for your reference gene (default 34, see note below). You can also define allowed sample slope ranges ('Slope between', default between -3.8 and -3) and slope difference between your sample and standard curve (default 0.5; Figure 8).

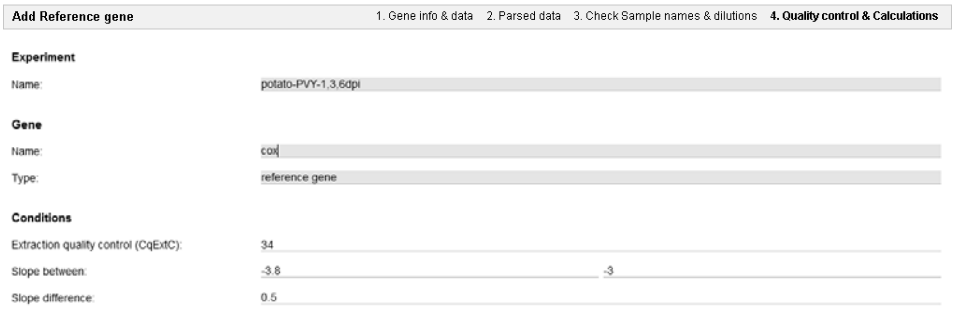


Figure 8: Definition of quality control parameters

***CqExtC**: CqExtC lets you specify the limit of the RNA in the reaction to achieve optimal results and is not to be mixed with CqLOQ (explained below). The Cq values of your samples can be higher than CqExtC because RNA isolation yield of the sample was too low, reverse transcription has gone wrong or the sample is too much diluted. The wells with Cq values higher than CqExtC will not be used for calculations for any of the target genes. By default CqExtC is set to 34, which rarely affects quantification. For well-expressed reference genes it is recommended to set this control to 28.*

***Slope between** and **Slope difference**: Ideally, if PCR reaction is 100% efficient, the slope between dilutions is -3.3, which in real life is not always the case. That is why we are allowing the slopes of our samples to be between -3.8 and -3 (83-115% efficiency). The thresholds can be changed but keep in mind that by widening the range you are increase measurement uncertainty/precision. The same goes for slope. To assure accurate quantification, the slope of the sample should not be more than 0.5 units different from the slope of the standard curve.*

5. Reviewing reference gene data

After the conditions are entered the application automatically calculates all parameters of the standard curve and the sample reference gene copy numbers. In the standard curve table, you can check the calculated partial slopes. On the right you should check table with dilution curve slope, correlation coefficient and intercept that are used for calculation of gene copy numbers. For each calculated number you can see its formula by clicking on the header column (Figure 9).

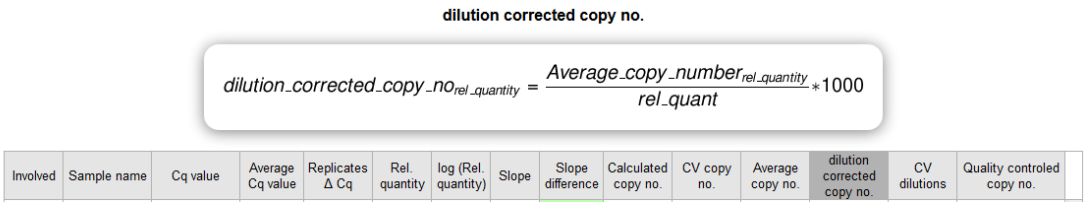


Figure 9: Calculated data table header with the formula of one of the columns shown

All the **data that are out of the preset boundaries is highlighted t**o assess the data quality and help with manual corrections (Figure 10):

Standard curve:

- *Replicates ΔCq > 0.5,*
- *Slope out in the predefined range ("Slope between")*
- *Correlation coefficient < 0.98*

Data

- *Average Cq Value > CqExtcC*
- *Replicates ΔCq > 0.5,*
- *Slope out in the predefined range ("Slope between")*
- *Slope difference > predefined Slope difference*
- *CV copy number > 30*

You can use the checkmarks in the "Involve" column of the tables to remove values (individual wells) where errors occurred during pipetting both in the Standard curve results as well as in Data results (Figure 10). You can also remove the dilutions of the standard curve where Cq values are out of the linear amplification range.

Standard curve:

Involved	Cq value	Rel. quantity
☑	20.55679	10000
☑	20.704414	10000
☑	24.376608	1000
☑	24.476524	1000
☑	27.449476	100
☑	27.465395	100
☑	31.081541	10
☑	31.211237	10

Cq	Replicates Δ Cq	Rel. quantity	log (Rel. quantity)	Partial slope
20.55679	0.15	10000	4	-3.796
20.704414		10000	4	
24.376608	0.1	1000	3	-3.0309
24.476524		1000	3	
27.449476	0.02	100	2	-3.689
27.465395		100	2	
31.081541	0.13	10	1	/
31.211237		10	1	

Slope:	-3.457823
Inverse slope:	-0.288638
Correlation cofficient (rsq):	0.998058
Intercept:	9.980114

Data:

Involved	Sample name	Cq value	Average Cq value	Replicates Δ Cq	Rel. quantity	log (Rel. quantity)	Slope	Slope difference	Calculated copy no.	CV copy no.	Average copy no.	dilution corrected copy no.	CV dilutions	Quality controled copy no.
☑	R3H5	23.882208			1000	3			1221.27					
☑	R3H5	27.190796	27.2124	0.04	100	2			135.47	2.03	133.55	13.35		13.35
☑	R3H5	27.23404			100	2			131.63					
☑	R6H2	24.636354	24.6857	0.1	1000	3	-3.3112	0.15	739.84	4.64	716.34	7.16	7.17	7.16
☑	R6H2	24.73512			1000	3			692.83					
☑	R6H2	27.97427	27.9969	0.05	100	2			80.48	2.13	79.29	7.93		7.93
☑	R6H2	28.019604			100	2			78.09					
☑	R6H3	23.974873	24.0139	0.08	1000	3	-3.3544	0.1	1148.33	3.67	1119.3	11.19	5.19	11.19
☑	R6H3	24.05292			1000	3			1090.28					
☑	R6H3	27.316357	27.3683	0.1	100	2			124.62	4.88	120.47	12.05		12.05
☑	R6H3	27.420155			100	2			116.31					
☑	R6H4	23.986359	27.2413	6.51	1000	3	0.0709	3.53	1139.59	137.73	577.33	5.77	58.32	Explain
☑	R6H4	30.496176			1000	3			15.06					
☑	R6H4	26.952732	27.1703	0.44	100	2			158.69	20.31	138.76	13.88		Explain
☑	R6H4	27.387918			100	2			118.83					
☑	R6H4	23.986359	23.9864	/	1000	3	-3.184	0.27	1139.59	/	1139.59	11.4	13.88	11.4
☐	R6H4	30.496176			1000	3			/					
☑	R6H4	26.952732	27.1703	0.44	100	2			158.69	20.31	138.76	13.88		13.88
☑	R6H4	27.387918			100	2			118.83					

Figure 10: Reviewing reference gene data and removal of a pipetting error (encircled in red).

CAUTIONS

- **Do not remove sample reactions that are out of linear range. These will be handled properly by the application itself!**
- **If you remove all replicates of one dilution, the sample efficiency quality control will not be implemented**

*For the reference gene, the following **quality control** checks/modifications are performed (see Figure 11 for details):*

- *If you have only one dilution, only CqExtC limit is checked. If the reference gene Cq is above CqExtC limit, it indicates that the extraction, reverse transcription or PCR reaction were not efficient enough and no quantification can be performed for this sample.*
- *In the case of two dilutions, after CqExtC, relative copy numbers are not calculated if the sample slope is outside the limits or the slope difference is bigger than set.*

If no "Quality controlled copy no." is calculated for the sample clicking the "Explain" button shows you why this is so (the rule appears above the data table). The parameters for which the reaction failed are highlighted.

When all the checking/editing is done, scroll down and click "Save" button (bottom right) to proceed. This reference gene is now saved in the quantGenius database. Click "Back to Experiment" to return to the Experiment page and add other data.

A) Simple calculation

Conditions - reference gene	Decision/Result
Average Cq	
<= CqExtC	dilution corrected copy no.

B) two dilution calculation

	Conditions - reference gene				Decision/Result
1. block	Dilutions involved/enabled	Cq average		CV Copy number (1 or 2)	
	One of two dilutions is enabled/involved in calculation	Cq_average < CqLOQ	AND	CV copy number (1. or 2. dilution) < 30	if(all_block_conditions) quality_controled_copy_number[dilution] = dilution_corrected_copy_no.[dilution] else match_block_2
2. block	Cq average	slope		slope difference	
	<CqExtC	slope > Slope between first and slope < Slope between second	AND	< Slope difference	quality_controled_copy_number[dilution] = dilution corrected copy no.[dilution]

Figure 11: Decision tree for the reference gene in the case of simple (one dilution; A) or two dilution calculation (B)

6. Adding more reference genes

If you use more than one reference gene in your experiment (highly recommended) follow the steps above to add additional reference genes. When adding additional reference genes the "**Check Sample names & dilutions**" subpage shows you if the reference data you are adding has the same sample names and dilution counts as the previously added reference genes (Figure 12).

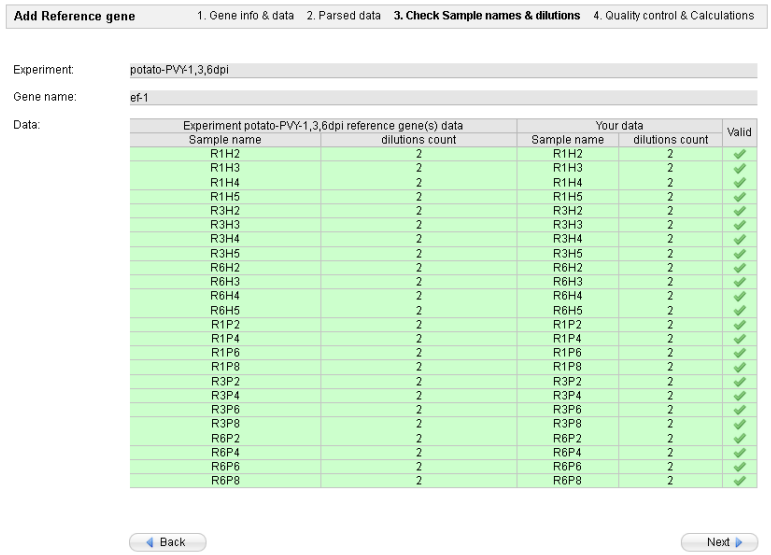


Figure 12: Format checking of the imported data for the subsequent genes

The following procedure is the same as described for the first reference gene in the experiment.
When you have several reference genes an average will be calculated from all of them. The calculation can be viewed by clicking on the "Average" link in the reference gene table (Figure 13):

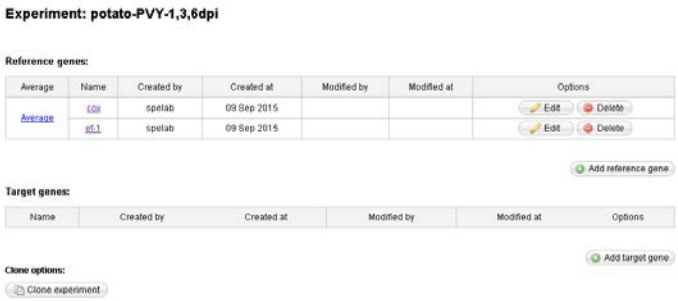


Figure 13: Reference gene data organization

In the Reference gene average page, you can see which reference gene's copy number values have been used for averaging and how the reference copy number was calculated (Figure 14).

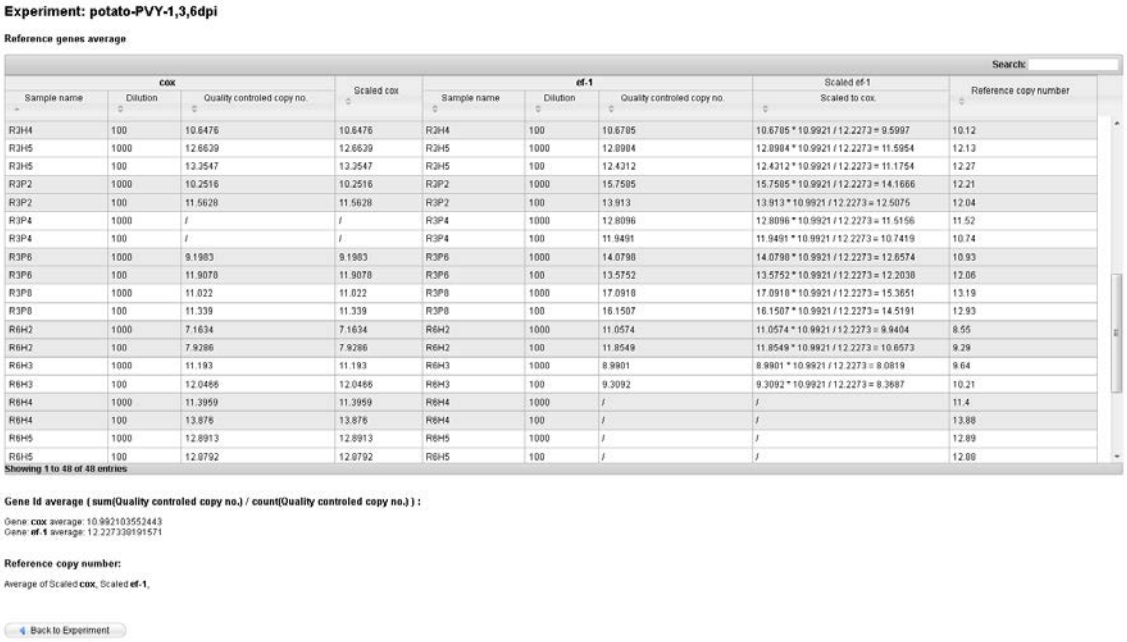


Figure 14: Reference gene average page

> ***Average reference copy no****.: To avoid unequal contribution of each gene's relative copy number value effect to the average (due to different expression rates), the second reference gene is scaled to the average of the first reference gene. The order of the reference gene input might influence the absolute but not the relative expression values. In the cases when the copy numbers are not calculated for one gene due to QC issues, the reference copy number of other gene(s) is used for normalization.*

Click on "Back to Experiment" to go back.

7. Adding target genes

To add target genes, go to your experiment's page and click "Add target gene" button (Figure 15).

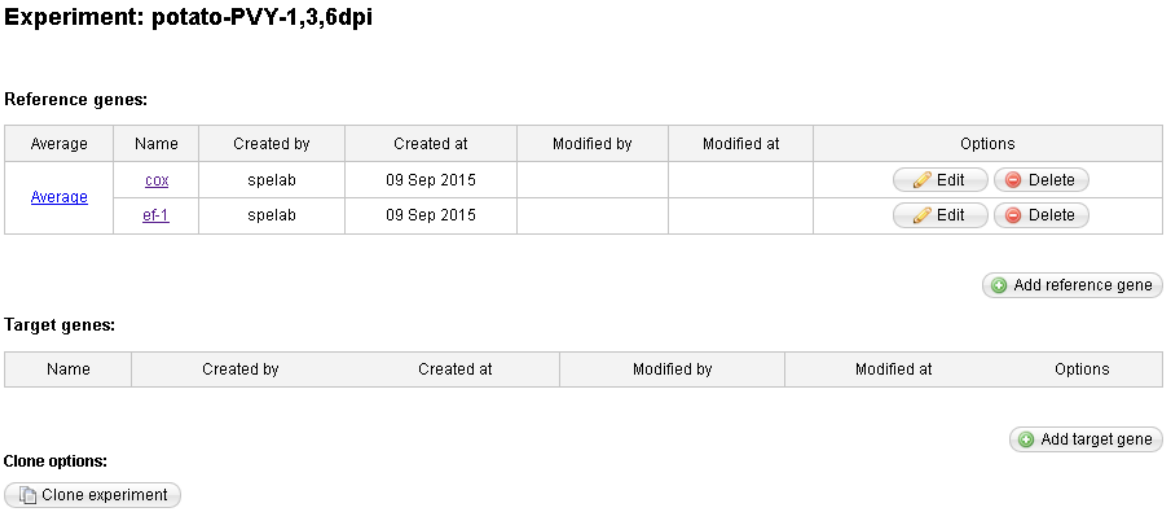


Figure 15: Adding a target gene

Specify the gene name and input the sample data and standard curve data in the same way as described for the reference gene (see above) for the analysed target gene.
The subpage "**Check Sample names & dilutions**" shows you if the target gene data you are adding have the same sample names and dilution counts as the reference genes. Click "Next" to proceed.
The subpage "**Quality control & Calculations**" is used to specify the "Cq at Limit of quantification (CqLOQ)" which is the minimum Cq value that is considered below the limit of quantification for specific target gene.

> ***CqLOQ**: The Cq values indicate that the target gene copy numbers are below limit of quantification when the reproducibility of analysis is low (e.g. coefficient of variation between copy numbers calculated from the analysis of the same sample is above 30%) or the formation of primer-dimers was detected already when inspecting results in the original real-time PCR software. Set the CqLOQ for your target gene according to the results of amplification of standard curve and samples included in the analysis.*

Moreover, you have to set limits for sample slopes (described above in the section Add reference gene) and specify if the analysis is to be done on both/all (if more than 1 dilutions are analysed per sample) or only one (first) dilution.

> ***Simple or two dilution calculation**: If your qPCR was performed on only one dilution the software will recognize that from the input data. It is only necessary to select simple – one (first) dilution if qPCR was done for two dilutions, but the second one is out of the quantification range.*

8. Reviewing target gene data

After all the conditions are entered the application automatically calculates all parameters of the standard curve, determines quality control parameters for analysis of samples and calculates the target gene copy numbers in all samples. In the standard curve table, you can check the calculated partial slopes.
As for the Reference gene the **data that are out of the preset boundaries is highlighted** to assess the data quality and help with manual corrections (Figure 16).

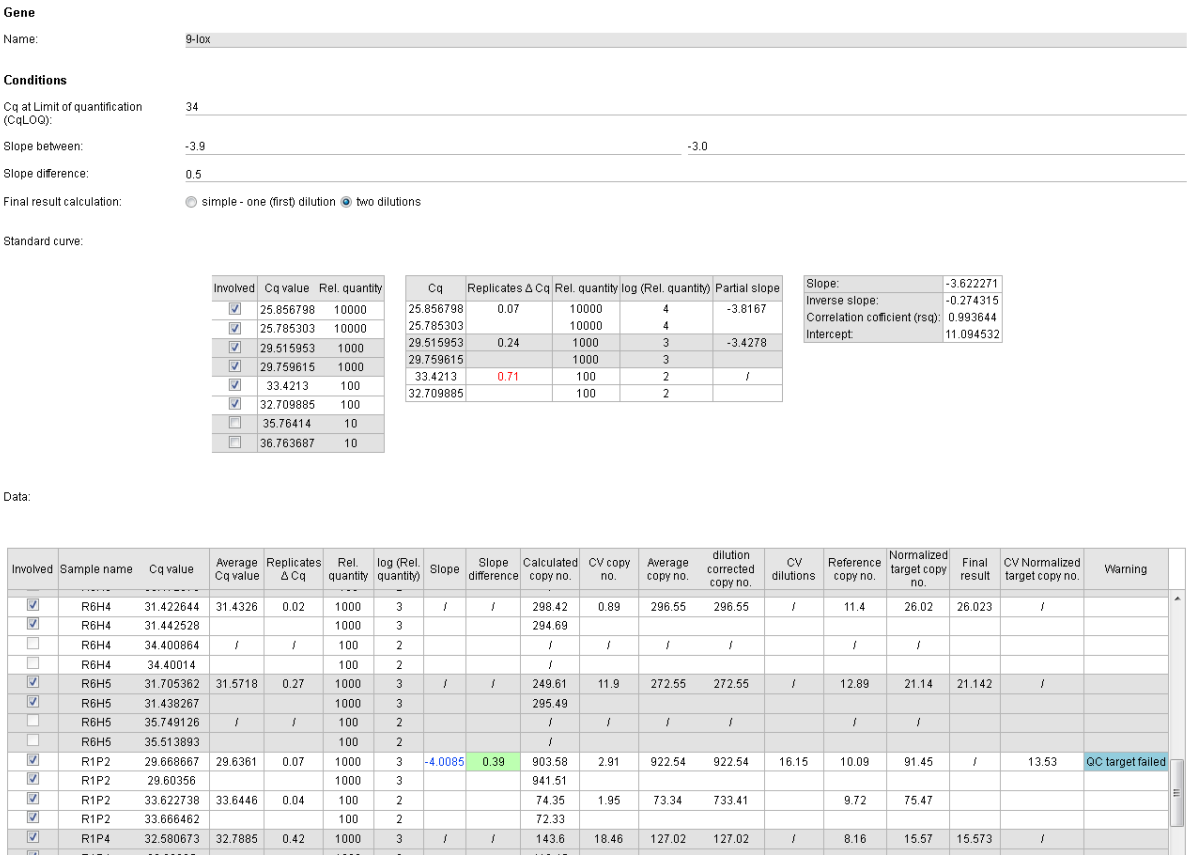
Standard curve:

- *Replicates ΔCq > 0.5,*
- *Slope out in the predefined range ("Slope between")*
- *Correlation coefficient < 0.98*

Data

- *Average Cq Value > CqLOQ*
- *Replicates ΔCq > 0.5,*
- *Slope out in the predefined range ("Slope between")*
- *Slope difference > predefined Slope difference*
- *CV copy number > 30*

You can check the results; and at this stage omit the individual wells (pipetting errors and standard curve dilutions outside the quantification range) by unchecking the box of the reaction row (Figure 16).



Gene

Name: 9-lox

Conditions

Cq at Limit of quantification (CqLOQ): 34

Slope between: -3.9 -3.0

Slope difference: 0.5

Final result calculation: simple - one (first) dilution two dilutions

Standard curve:

Involved	Cq value	Rel. quantity
☑	25.856798	10000
☑	25.785303	10000
☑	29.515953	1000
☑	29.759615	1000
☑	33.4213	100
☑	32.709885	100
☐	35.76414	10
☐	36.763687	10

Cq	Replicates Δ Cq	Rel. quantity	log (Rel. quantity)	Partial slope
25.856798	0.07	10000	4	-3.8167
25.785303		10000	4	
29.515953	0.24	1000	3	-3.4278
29.759615		1000	3	
33.4213	0.71	100	2	/
32.709885		100	2	

Slope:	-3.622271
Inverse slope:	-0.274315
Correlation coefficient (rsq):	0.993644
Intercept:	11.094532

Data:

Involved	Sample name	Cq value	Average Cq value	Replicates Δ Cq	Rel. quantity	log (Rel. quantity)	Slope	Slope difference	Calculated copy no.	CV copy no.	Average copy no.	dilution corrected copy no.	CV dilutions	Reference copy no.	Normalized target copy no.	Final result	CV Normalized target copy no.	Warning
☑	R6H4	31.422644	31.4326	0.02	1000	3	/	/	298.42	0.89	296.55	296.55	/	11.4	26.02	26.023	/	
☑	R6H4	31.442528			1000	3			294.69									
☐	R6H4	34.400864	/	/	100	2			/	/	/	/		/	/			
☐	R6H4	34.40014			100	2			/									
☑	R6H5	31.705362	31.5718	0.27	1000	3	/	/	249.61	11.9	272.55	272.55	/	12.89	21.14	21.142	/	
☑	R6H5	31.438267			1000	3			295.49									
☐	R6H5	35.749126	/	/	100	2			/	/	/	/		/	/			
☐	R6H5	35.513893			100	2			/									
☑	R1P2	29.668667	29.6361	0.07	1000	3	-4.0085	0.39	903.58	2.91	922.54	922.54	16.15	10.09	91.45	/	13.53	QC target failed
☑	R1P2	29.60356			1000	3			941.51									
☑	R1P2	33.622738	33.6446	0.04	100	2			74.35	1.95	73.34	733.41		9.72	75.47			
☑	R1P2	33.666462			100	2			72.33									
☑	R1P4	32.580673	32.7885	0.42	1000	3	/	/	143.6	18.46	127.02	127.02	/	8.16	15.57	15.573	/	

Figure 16: Definition of QC parameters and implementation of QC modifications for a taget gene: For this assay the last dilution of the standard curve was excluded. The limit of quantification was set to 34 and allowed efficiency limit was set to -3.9. For the samples that had even worse efficiencies, the final result was not calculated. For the samples with Cq values above CqLOQ in the second dilution, the Final result was calculated only from the first dilution.

The rules for calculating the Final Result (which is quality controlled and normalized relative copy number of the target gene of the sample), can be observed by clicking on the "Final result" column header (see

also explanation and Figure 17 below). Any possible warnings regarding performed modifications are written in the Warning column.

*The following **QC check and modifications** are performed for the target gene copy number calculation (see figure 17 for details):*

In simple calculation, the only thing that can be checked is whether the sample is under the LOD (missing values) or LOQ (determined by set LOQ or high replicates coefficient of variation), the final result is modified (LOQ copy number divided by 2 and normalized to the average reference gene copy no. overall samples included in the experiment)
In two (or many) dilution calculation it is first determined whether the sample Cq is

- *Under LOD (missing or undetermined): LOQ copy number divided by 10 and normalized to the average reference gene copy no. (Note: here we could assign value 0 to our sample results. Due to potential further analytical steps it is preferably to instead assign (impute) a very small number as a measured relative copy number. Be careful in qualitative qPCR experiments (+/-) eg. virus, transgene etc.)*
- *Both dilutions under LOQ: LOQ copy number divided by 2 and normalized to the average reference gene copy no.)*
- *Second dilution Under LOQ: calculated from the first dilution only*

If both dilutions above LOQ

- *if replicate CV is higher than 30 in 2nd dilution): calculate the final result from the 1st dilution (Note: correct pipetting errors only above LOQ)*
- *Final results is only calculated if the slope and slope range are within the pre-set limits.*

Possible warnings:

- ***QC ref failed**: bad results for reference gene, final result is not calculated*
- ***Under LOQ**: Cq in both dilutions above set LOQ (final result calculated as LOQ copy number divided by 2 and normalized to the average reference gene copy no.)*
- ***Under LOD:** no result in all of the analysed wells (final result calculated as LOQ copy number divided by 2 and normalized to the average reference gene copy no.)*
- ***QC target failed:** sample efficiency estimate out of set range, final result is not calculated*

When all the checking/editing is done scroll down and click "Save" button (bottom right) to proceed.

A) simple calculation, one dilution

Block no.	Conditions - target gene			Decision/Result
1. block	if(count(Cq = 0 or empty or Undetermined or Failed) == count(Cq))			if(all_block_conditions) final_result = LOQ_copies / 10 / average_reference_copy_no., warning "Under LOD" else match_block_2
	(first) Cq average		(first) CV copy number	
2. block	> CqLOQ	OR	> 30	if(all_block_conditions) final_result = LOQ copies / 2 / average_reference_copy_no., warning "Under LOQ" else final_result = Normalized target copy no.

B) two-dilution calculation

Block no.	Conditions - target gene	Decision
1. block	if(second_Cq_average > CqLOQ) OR count(second_dilution_Cqs = 0 or empty or undetermined or failed) == count(second_dilution_Cqs)	if(all_block_conditions) match simple calculation (remove second dilution from calculation)
2. block	if(count(Cq = 0 or empty or Undetermined or Failed) == count(Cq))	if(all_block_conditions) final_result = LOQ_copies / 10 / average_reference_copy_no., warning "Under LOD"

	Dilutions enabled	Cq average (dil. 1 or 2)		CV copy number (dil. 1 or 2)	Decision
3. block	One dilution is enabled	< CqLOQ	AND	< 30	if(all_block_conditions) final_result = Normalized_target_copy_number else , match_block_4

Block no.	1st dil CV copy No.		slope		Slope difference		2nd dil CV copy No.	Decision
4. block	> 30 OR first _Cq_average > CqLOQ	AND	any	AND	any	AND	any	if(all_block_conditions) final_result = LOQ copies / 2 / average_reference_copy_no. , warning "Under LOQ" else match_block_5
5. block	any	AND	any	AND	any	AND	> 30 OR not calculated OR second_Cq_average > CqLOQ	if(all_block_conditions) final_result = Normalized target copy no. 1 else match_block_6
6. block	<= 30 OR not calculated	AND	slope between first < x < slope between second	AND	< slope difference	AND	< 30 OR not calculated	if(all_block_conditions) final_result = average(Normalized target copy no. 1, 2) else final_result = /, warning "QC target failed"

Figure 17: Decision tree for the target gene in the case of simple (one dilution; A) or two dilution calculation (B)

9. Editing and deleting experiments/genes

In the Experiments view, experiments can be sorted by name, creation and modification dates. Individual experiments can be renamed and deleted by clicking on "Rename" and "Delete" buttons (Figure 18). You can edit the experiment by clicking on its name. Please note that **all the experiment data is stored on the quantGenius server for one month after last modification** (renaming, adding, deleting or changing genes), **and will be deleted after that.** Please export all the analysed data on time!

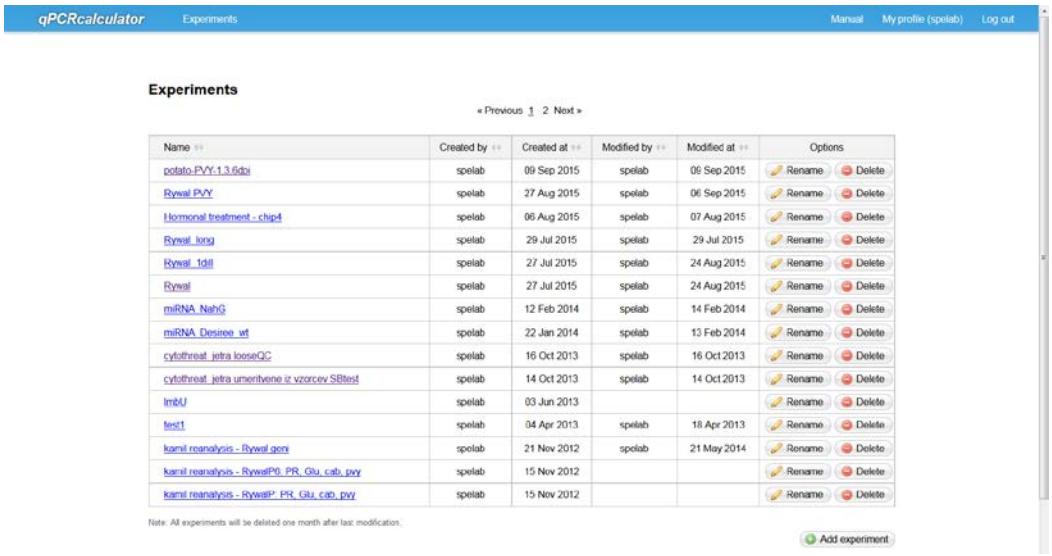


Figure 18: Experiment management

The whole experiment can be cloned (useful when you want to analyse the same data using different conditions) by clicking on the “Clone experiment” button. Moreover, you can delete individual genes or add new ones and you can modify the analysis parameters by clicking on “Edit” button”(Figure 19).

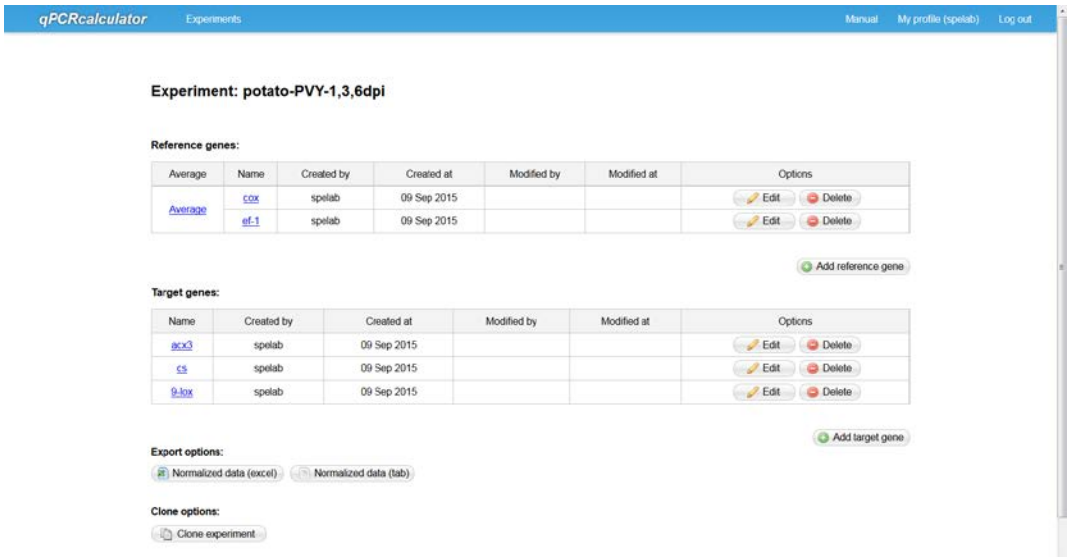


Figure 19: Experiment management: individual genes

10. Export analysis

You can export the calculation table of the individual gene as Excel file by clicking on the gene name and then clicking “Export to Excel” button on the bottom right of the page Figure 20).

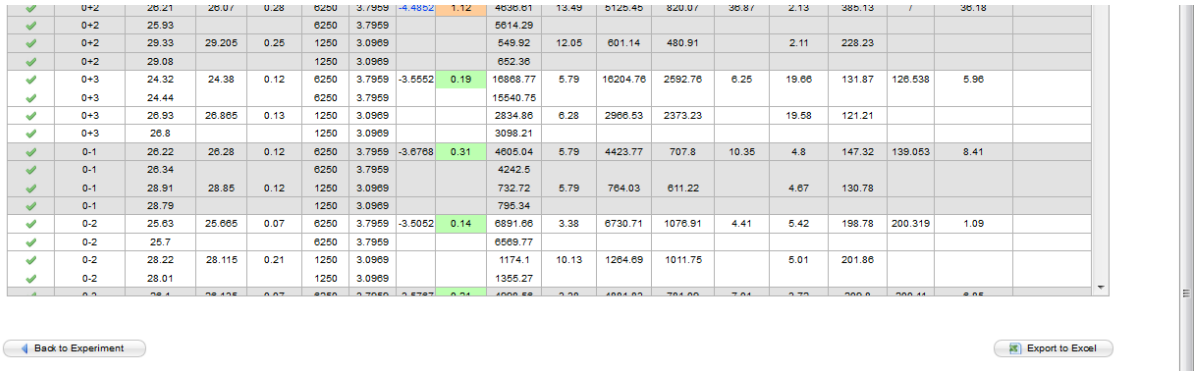


Figure 20: Individual gene export

Final result data for all the target genes in the experiment (taking into account QC parameters and normalized to average reference gene) can be exported from the Experiment view (Figure 19) in .txt or .xls formats. In the .txt file only data matrix is exported. In the .xls file two sheets are created, one containing the same data as in .txt file and the other containing any possible warnings regarding the values (see chapter 8)

	Experiment name:	zebrafish_liver_treatment	
		Target gene name	
	Sample name	vtg1	hsd17b3
	1	64.35	817.31
	2	94.58	5.22
	3	494087.83	5.89
	4	62.35	2173.49
	5	19.43	/
	6	56.21	6.95
	7	709534.9	2.71
	8	944223.4	0.38
	9	410784.12	6.07
	10	172.06	747.65
	11	346.17	1305.46
	12	/	0.08
	13	/	13.71
	14	/	3.55
	15	693201.17	17.75
	16	41.62	882.65
	17	/	0.08
	18	42.48	246.12
	19	369796.96	5.55
	20	247320.68	5.12
	21	/	65.14
	22	26.93	4.5
	23	/	96.47
	24	174.99	2117.96
	25	/	19.37
	26	888934.6	11.36
	27	607206.83	10.45

zebrafish_liver_treatment | Warning zebrafish_liver_tre...

READY

	Experiment name:	zebrafish_liver_treatment	
		Target gene name	
	Sample name	vtg1	hsd17b3
	1	64.35	817.31
	2	94.58	5.22
	3	494087.83	5.89
	4	62.35	2173.49
	5	19.43	QC target failed
	6	56.21	6.95
	7	709534.9	2.71
	8	944223.4	Under LOQ
	9	410784.12	6.07
	10	172.06	747.65
	11	346.17	1305.46
	12	QC ref. 1 failed	Under LOD
	13	QC target failed	13.71
	14	QC target failed	3.55
	15	693201.17	17.75
	16	41.62	882.65
	17	QC ref. 1 failed	Under LOD
	18	42.48	246.12
	19	369796.96	5.55
	20	247320.68	5.12
	21	QC target failed	65.14
	22	26.93	4.5
	23	QC target failed	96.47
	24	174.99	2117.96
	25	QC target failed	19.37
	26	888934.6	11.36
	27	607206.83	10.45

zebrafish_liver_treatment | Warning zebrafish_liver_tre...

READY

Figure 21: Comparison of two versions of the exported nomalized data tables: left – with imputed values and without Warnings, right: without imputed values, including QC warnings