LinRegPCR update history

The most recent updates can be found on the LinRegPCR website.

LinRegPCR version 2021.1 released June 2021

In this version a problem with importing fluorescence data from the QuantStudio was fixed. LinRegPCR erroneously removed wells with negative data. Although negative fluorescence values should not be present, they can occur as result of the background correction by the machine (background defined as the fluorescence that is not dependent on the fluorochrome, e.g. autofluorescence of the plastics). Note that empty wells, and wells with only negative fluorescence values, will still be removed. In that case, a warning is shown and the user is urged to check the data.

When a lot of negative values are present in the raw data (Rn) it may be better to import the delta Rn values which are baseline-corrected by the machine (baseline defined as the fluorescence that is fluorochrome, but not amplification, dependent). This choice is made in the Read-from-Excel dialog. After reading delta Rn data, the baseline correction of LinRegPCR has to be performed by clicking the red button or skipped by setting the 'baseline corrected' menu option after data import.

LinRegPCR version 2020.2 released October 2020

In this version a bug in the handling of empty wells in Step-One-Plus input was corrected. Empty wells, those without fluorescence values in the Excel output of Step-One-Plus are removed during data import. NOTE: when you read amplicon and tissue annotation information from Excel you will have to remove these wells manually from the annotation sheets. You can export the imported data, without empty wells to Excel (choose tab Data - Input Data - grid to Excel) to help you find out which wells are removed. The first column in this grid is a concatenation of the well and the target name columns in the original input.

LinRegPCR version 2020.1 released March 2020

This version support RDML version 1.3. The main change are the pre-defined strings that distinguish the different monitoring chemistries in the RDML output. In the LinRegPCR interface some chemistry names have been extended and "saturating DNA binding dye" is added to list of choices in the Read-from-Excel dialog.

It was noted that in the compact output the column "Individual PCR Eff" was not present when no amplicon groups or tissue annotation were imported. This error has been corrected.

LinRegPCR version 2020.0.0.3 released January 2020

In this version two errors in the program’s behavior were corrected:

1. After setting amplicon groups, re-entering the Amplicon Groups tab triggered the program to run through all reactions and to set a common W-o-L. This unwanted action was undone

2. When no amplicon groups or tissues were defines, the program did not report individual PCR efficiencies. Although this was already the case since 2012, the quality check based on the individual PCR efficiency was deemed important enough to always report these values.

LinRegPCR version 2020.0.0.1 released January 2020

This version of the windows version of LinRegPCR was programmed in parallel with a web-version that will be released in 2020. The intention was to make sure that both versions give the same results. Most changes in the code are in the background and do not affect the reported results. However, some differences with results of earlier versions may occur:

- The assignment of the NoPlateau flag. The implementation of the rules was such that mainly NoAmplification reactions were reported as NoPlateau. Some NoPlateau reactions were not reported. The current version will not report a N0 value when NoPlateau or BaselineError are true.
- The default threshold and the Cq value reported. This change was implemented to accommodate the users who judge the results of their qPCR experiment from the reported Cq values. To make this a valid judgement the Cq values should be determined at a common threshold. Earlier versions of LinRegPCR determined an optimal threshold per assay. The Cq value for the common threshold is determined with the mean PCR efficiency per assay, whereas earlier versions used the PCR efficiency for the individual reactions. The common threshold does not change the reported target quantity (N0). The use of the mean efficiency to determine the Cq value is more in line with using the mean efficiency per assay to calculate N0. However, this change results in slightly different N0 values.
  NOTE 1: Users, who use Cq values from LinRegPCR in Pffafl-like efficiency corrected relative expression calculations should have set a common threshold for target/reference ratios (only 1 delta Cq); for fold difference calculations (two deltas) the threshold cancels out.
  NOTE 2: Users who use the individual efficiencies will not see any change. However, remember that this approach will lead to extra variability in your results (see our 2009 and 2013 papers).
  NOTE 3: The reported and applied common threshold is the geometric mean of the thresholds that the program determined for each assay. In earlier versions this was the threshold set when the user did not define groups of reactions per assay. Because of the new calculation of Cq values, this change does not affect the reported results.
- Some of the lesser used user choices were removed from the main interface and placed on the User Settings tab. Some only become visible when the are chosen in the Options Menu.
- An error in the import of LC96 data was corrected. Because in earlier versions these data were not imported, this correction does not affect the reported results.
- OK and cancel buttons on the ReadFromExcel dialog were moved to make sure they are always visible.
- In this version also a bug in the import of QuantStudio data was corrected. This import was impaired by the fact that the column headers of the QuantStudio Excel

export have been changed. LinRegPCR now expects at least the words ‘Position’ in the header of the well description and ‘Target’ in the column that specifies the assay. Empty wells are removed from the dataset during import.
Note that QuantStudio, and other ‘chip’-based qPCR systems work with very small volumes and thus relatively low specific fluorescence. This leads to very low number of datapoints in the observed exponential phase and makes that the baseline correction of LinRegPCR can fail. To circumvent this issue, the user can decide to import the baseline-corrected data that the system has exported (rename the R column to X and the delta R to R and, in the Read-from-Excel dialog tell LinRegPCR that he data are baseline-corrected).

LinRegPCR version 2018.0 released September 2018

In this version the input format for the Excel export of Mic was added.

- The changes in this version have no effect on the analysis results.

LinRegPCR version 2017.1 released September 2017

In this version the input of raw data from the qTOWER3 system of Analytik Jena (both singleplex and multiplex) was implemented. LinRegPCR expects the range of data to include 1 header row with probe name and cycles numbers and 1 leading column with probe name and well codes. The well codes and probe name will be combined and imported as sample names.

An error in handling excluded samples was corrected. When the user read the excluded samples from Excel before determining the fluorescence baseline the excluded samples were reported in the quality checks but not excluded from the calculation of the mean efficiency. The current version handles the excluded samples correctly.

- This correction has effect on the analysis results when you excluded samples before you determined the baseline fluorescence.

LinRegPCR version 2017.0 released January 2017

In this version only a non-functional edit field was removed.

- The changes in this version have no effect on the analysis results.

LinRegPCR version 2016.2 released December 2016

This version includes an import option for the raw data exported from the Roche LC96 to a text file which is then read into Excel. Both exports, 'as rows' and 'as columns', are supported. The Excel file does contain one leading column and one header row in either format. There are no empty header rows or empty leading columns.

- The changes in this version have no effect on the analysis results.

LinRegPCR version 2016.1 released July 2016

An error that occurred in version 2016.0 while writing RDML 1.1 files was repaired. This makes that the results of the analysis of LinRegPCR can be further analysed with the current version of qbase-plus. When more than 1 tissue annotation column is used in LinRegPCR, these columns are concatenated to a single tissue identifier in RDML 1.1. Additionally an error that occurred when the RDML file contained reactions for which all fluorescence values were negative was solved. When negative values occur in a reaction, a constant is added to all cycles that brings the minimum fluorescence to 0.001 (with as many decimals as the system exports). Note that the applied correction assumes that the negative values are the result of the same constant value being subtracted from the fluorescence values in all cycles. Because LinRegPCR later on subtracts a constant baseline, this addition does hardly affect the results. The correction is applied to all RDML formats.

- The changes in this version may affect the analysis results when your RDML file contained negative values which should not be the case.

LinRegPCR version 2016.0 released January 2016

To allow the analysis of LinRegPCR output with the current version of qbase-plus, this version of LinRegPCR also enables data export in RDML1.1 format. Because LinRegPCR allows more than 1 column of tissue annotation, whereas RDML 1.1 only allows 1 column, the tissue annotation columns are concatenated to a single string per tissue sample.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2015.4 released December 2015

In this version of the program the link to the LinRegPCR website from the About box and the online Help was corrected. Moreover, the import of data from the Quantstudio system was added. Note that the number of reactions in this format make the processing slow. Therefore, create a shortcut to LinRegPCR and, in the command line of the shortcut add an S (outside the quotes when there are quotes). the program then runs in S (=speed) mode and does not wait for user input when problems are noted during baseline correction or window-of-linearity setting. Flags are set and reactions are excluded when needed. Check the flags (Options - Display flagged subsets) and the program's output for exlcuded reactions. Minimizing the program during baseline correction will further increase the processing speed.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2015.3 released April 2015

In this version the input from an Excel file exported by the Corbett 6000 system was implemented. In this format every well is represented by 3 columns: sample description, cycle and fluorescence. The header row has to be included in the range that has to be specified in the Read-from-Excel dialog.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2015.2 released March 2015

The handling of the #N/A entries in some data formats was automated and warning messages are avoided.

Wells for which all data are missing, or all values are negative, are no longer removed before analysis, but set to a low positive constant. This makes that they are assigned the 'no amplification' flag. Their presence in the data makes that the annotation columns remain synchronized.

The elements AmplificationEfficiencyMethod, BackgroundCorrectionMethod and CqDetectionMethod were added to the RDML, version 1.2, output.

Some cosmetic changes were made.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2015.1 released February 2015

In this version, the option to read the exclusion of samples, e.g. because of a deviating melting curve, from an Excel file was extended with information or warning messages to assure the correct handling of these exclusions.

Secondly, the import format of the Step-One-Plus ViiA system was extended. The presence of #N/A as entry in the Target Name column has to be checked and replaced because LinRegPCR cannot, yet, handle this error code. Moreover, fully empty wells are no longer removed to avoid loss of synchronisation within the system export.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2015.0 released February 2015

In this version, the extended annotation of tissue samples was fully implemented. Export of these annotation columns to RDML now uses the new Annotation element of RDML 1.2. Similarly, data stored in the sample annotation element can now be read from a version 1.2 RDML file.

- Grid-to-Excel buttons were added to some pages of LinRegPCR.
- The changes in this version do not affect the analysis results.

LinRegPCR version 2014.8 released December 2014

In this version, the annotation of tissue samples has been extended to include more than just the one column; a number of columns can be read from Excel and thus added to the output of LinRegPCR to facilitate downstream statistical analysis. Export of these annotation columns to RDML is still restricted to the first column. Full implementation of the extended annotation option of RDML 1.2 is in preparation.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2014.7 released November 2014

In this version, a piece of code that tried to calculate a SEM for a group with only 1 observation was corrected. When a group of 1 occurred, this bug prevented the results from being saved and a 'division by zero' error was displayed.

- The changes in this version do not affect the analysis results; results that could be saved were correct.

LinRegPCR version 2014.6 released October 2014

This version in compliant with RDML version 1.2. RDML files of version 1.0 and 1.1 will be read. However,when results are saved to RDML, these will be saved as version 1.2, which includes the SE of the mean PCR efficiency per amplicon. RDML files with decimal commas can be read, but the results will always be saved with decimal points, as is required by the XML format.

- The changes in this version do not affect the analysis results.

LinRegPCR version 2014.5 released September 2014

In this version the assignment of the 'no_plateau' flag is repeated afer setting the W-o-L per group because this assignment is dependent on the W-o-L.

Similarly, the quality flags are reset after reading of baseline values that were manually set in an earlier analysis of the same data and then saved to Excel.

- The first change may affect the analysis results when plateau heights are very different between groups. When plateaus were similar, there is no effect. The second change only affects the results when the baseline is not determined automatically before baseline values are read. This is because not all flags can be set when fluorescence data are baseline-corrected.

LinRegPCR version 2014.4 released August 2014

In this version an input option for the Excel export of the Bio-Rad CFX software was added. The 'Quantification Amplification Results' that have been exported to Excel can be read into LinRegPCR. NOTE: the empty column A in the Excel sheet has to be included in the range to be read. Otherwise the values of the first sample are used as cycle numbers.

In this version an error was corrected that made that after import of an RDML file the results could not be saved for a second time. Changes in target and sample assignment are now also saved to an existing RDML file.

- The last change has effect on the analysis results because the change of target assignment will affect the mean PCR efficiency per group.

LinRegPCR version 2014.3 released July 2014

In this version for the Step-One-Plus format, a replacement of negative values in the raw Rn data was implemented. See version 2014.2 for an explanation.

- This change has effect on the analysis results of Step-One-Plus files that contained such negative raw data and are re-analysed.

A bug that prevented LinRegPCR_help.pdf to be opened from the Help menu was corrected.

LinRegPCR version 2014.2 released March 2014

In this version for the Illumina-Eco and the Rotor-Gene (6 leading columns) formats, a replacement of negative values was implemented. It turns out that the background that these systems subtract to correct for technical fluorescence can lead to negative values in the raw data. This is solved by adding, to all cycles, the smallest constant that leads to all positive values. Additonally, an error in the functonality of the February release was repaired.

- These changes have effect on the analysis results of Illumna-Eco and Rotor-Gene files that contained such negative raw data. You are advised to re-analyse those raw data.

- Note that raw data (so-called component data) of the Illumna-Eco can only be exported after closing and re-opening the data file in the system software.

LinRegPCR version 2014.1 released February 2014

In this version we included the option to read the 'User excluded sample' assignment from a column in Excel. When you have, based on your melting curve analysis or gel electroforesis decided that some samples should be excluded from further analysis, you can easily add a column to the Excel file and thus keep the structure of the raw fluorescence data untouched.

- These changes have no effect on the analysis results, provided you had already assigned these exclusions by hand.

LinRegPCR version 2014.0 released January 2014

In this version we implemented a coupling between the display of amplicon groups and the display of flagged subsets of samples: only flagged samples in the chosen amplicon group are shown, and vice versa, when you browse through subsets or groups. When amplicon groups are defined, the Amplicon Groups Tab now shows a table with statistics (mean efficiency, standard deviation, SEM and RSE) per group.

- These changes have no effect on the analysis results.

LinRegPCR version 2013.1 released December 2013

In this version the processing of data files originating from monitoring with different chemistries has been extended. The user can choose the applied chemistry in the Read dialog. For each chemistry, the shift that occurs in Cq values because of accumulation of fluorescence or lag in occurence of fluorescence will be corrected. Based on Ruijter et al., Microchimica Acta, accepted for publication.

- This extension has no effect on the analysis results; the described Cq corrections were implemented since version 12.5 (August 2010).
- Note that the choice of targetted sequence, when a probe-based chemistry is used, has been removed. The latest paper shows that the probe target does not noticibly affect the Cq value.

Very rarely 'no amplification' samples escape the criterion that amplification should at least result in a 7-times increase of fluorescence (after subtraction of the minimum observed fluorescence as temporary baseline) between the first and last cycle. Therefor, an extra criterion was implemented: the two cycles directly before the SDM should both show increasing fluorescence. Samples that do not fullfil this criterion are also assigned the 'no amplification' flag.

- This change may have effect on the analysis of samples that were borderline between 'no amplification' and 'noisy' but has no effect on samples with more than 4 cycles in the exponential phase.

LinRegPCR version 2013.0 released March 2013

In this version the input format for the PikoReal system of Thermo Scientific was added in the Read-from-Excel dialog. Export the raw data, resulting in an Aquired_dat_1 sheet in Excel. Ommit the 10 lines header and read only the range of data, including the header row with well identifiers and the 3 leading columns, labelled Channel, Cycle and Temp.

- These changes have no effect on the analysis results.

LinRegPCR version 2012.3 released December 2012

In this version the input from RDML was updated to enable import of RDML files that use comma as decimal separator. The definition of RDML states that floating point values have to be written with a point as decimal separator. Therefore, the analysis results will always be written with decimal points to maintain compatibility with downstream analysis programs that use RDML. RDML files containing decimal commas can be read by LinRegPCR, but results will be saved to a new RDML file with decimal points. To avoid having to save to a new file: set your computer system to decimal points when you use RDML files.

- These changes have no effect on the analysis results.

LinRegPCR version 2012.2 released October 2012

In this version the input from RDML was updated to enable import of RDML version 1.1 and version 1.0. The analysis results will always be saved in RDML version 1.1 to maintain compatibility with downstream analysis programs.

Other corrections made in this version of the program are:

- Note that the XML file in an RDML file is essentially a text file. Therefore it is very important that the decimal separator of the qPCR apparatus is the same as the decimal separator of the computer on which the data analysis is performed.
- Zip files that cannot be handled will be captured. The online Help and the Manual tell you how to re-zip those files.
- Formatting of results in Excel is now conditional upon checking a box in the Save-to-Excel dialog. In some computer configurations formatting should be turned off.
- These changes have no effect on the analysis results.

LinRegPCR version 2012.1 uploaded October 2012

In this upload only a few lay-out errors were corrected

LinRegPCR version 2012.1 released August 2012

In this version the input format for the Illumina Eco system was added

- Export 'Component data' from the Eco system
- Start the input range with the 'Well' cell
- The sample name will be composed of the entries in the Well and the Dye columns
- Excluded and Empty wells will be ignored

The ViiA7 format was added to the Step-One Plus format option

- Note that ViiA7 has a different number of header rows

LinRegPCR version 2012.0 released April 2012

In this version the following features have been implemented:

- This version is fully RDML compatible
- Data can be read from an RDML file
- Results can be saved to an RDML (version 1.1) file
- Saved results can be imported in qbasePLUS for normalisation and statistical analysis

NOTE:

- For RDML input into LinRegPCR it is very important that the qPCR machine is using the decimal separator that is set with the Regional Settings of Windows
- Keep in mind that also the RDML export of the qPCR machine should contain raw, not baseline-corrected, data
- The RDML output of the BioRad CFX software is based on RDML, version 1.0, and can therefore not be handled (yet)

LinRegPCR version 12.18 released March 2012

- Release in preparation of the full RDML compatible release
- Added tab page for assignment of Tissue Samples per reaction
- Added option to read amplicon group, tissue sample and baseline from Excel
- Note that spaces will be removed from the given identifiers
- Moved user choices to separate tab page 'User Settings'
- Note that the LinRegPCR_help.pdf is not yet updated
- When you want to test the RDML input and output: please contact us at info@linregpcr.nl

LinRegPCR version 2012.0 released April 2012

In this version the following features have been implemented:

This version is fully RDML compatible

Data can be read from an RDML file

Results can be saved to an RDML file

Saved results can be imported in qbasePLUS for normalisation and statistical analysis

LinRegPCR version 12.18 released March 2012

Release in preparation of the full RDML compatible release

Added tab page for assignment of Tissue Samples per reaction

Added option to read amplicon group, tissue sample and baseline from Excel

Note that spaces will be removed from the given identifiers

Moved user choices to separate tab page 'User Settings'

Note that the LinRegPCR_help.pdf is not yet updated

When you want to test the RDML input and output: please contact us at info@linregpcr.nl

LinRegPCR version 12.17 released November 2011

Enabled the import of data from Excel files with column codes AAA and further. Previous versions were restricted to ZZ.

LinRegPCR version 12.16 released September 2011

Corrected visibility of Amplicon Group Tab when baseline-corrected data are read (this error was introduced in version 12.13).

Added option to read baseline values that were saved in an earlier analysis of the data set.

Corrected a bug that occurred when the plateau phase data were not fully continuously increasing. This then resulted in a two plateau phase points being selected and the sample being excluded. Note that this correction can lead to changed results when data are re-analysed.

LinRegPCR version 12.15 released August 2011

Repaired an error in placing of the Legends column that was introduced when adding the Tissue sample column in version 12.13.

This change has no effect on the results.

LinRegPCR version 12.14 released August 2011

Repaired an error that sometimes occurred in setting of Window-of-Linearity: when the variation between curves decreased close to the baseline, the W-o-L was set too low (see Manual for details). NOTE: this change can lead to different results when data are re-analysed.

LinRegPCR version 12.13 released August 2011

Release of second (test) version for RDML output.

Lay-out of Read data dialog was changed to accomodate future RDML input.

Added Tab-page for annotation of tissue samples.

Added functionality to read Amplicon groups and Tissue sample annotation from Excel.

Changes have no effect on calculations.

LinRegPCR version 12.12 released June 2011

Release of first (test) version for RDML output. No other changes were implemented

LinRegPCR version 12.11 released April 2011

Improved the handling of different decimal separators used by different Regional Settings of Windows

LinRegPCR version 12.10 released March 2011

Added handling of fully negative samples to Step-One-Plus format. Was already implemented for Eppendorf Realplex. When fully negative samples occur processing stops, the user is notified, given the well numbers and the program waits for new input.

LinRegPCR version 12.9 released March 2011

Added input format for the new Applied Biosystems export format with 5 leading columns. Ignore the first lines: given range should start at cell with content 'Well'. The empty 'Rn' column has to be included. See the manual for further details.

A menu item 'Open Manual (pdf)' was added to the 'Help' menu. LinRegPCR.pdf should be stored in the program directory

LinRegPCR version 12.8 released February 2011

Added input format for Eppendorf Realplex. Ignore the first lines: given range should start at cell with content 'Position'.

From this version onwards LinRegPCR writes its version number to the Windows Registry. When, sometime in the future, your system manager has installed a newer version you will be notified

LinRegPCR version 12.7 released November 2010

Corrected error in browsing though groups that was introduced in version 12.6. This error had no effect on the output

LinRegPCR version 12.6 released November 2010

Solved 'floating point division by zero' error that occurred when all samples in an amplicon group were excluded from the calculation of the mean efficiency

LinRegPCR version 12.5 released August 2010

Updated Read-from-Excel dialog to deal with different targets for hydrolysis probes

Adjusted Cq correction to handle different targetting as well as different input (see 12.3)

LinRegPCR version 12.4 released July 2010

Bug fix in baseline estmation. Bug caused a crash but had no effect on estimated baselines.

Bug fix in import of Step-One Plus output: Rn column is used, delta-Rn column is ignored.

LinRegPCR version 12.3 released May 2010

Restricted application of Cq-shift correction to hydrolysis probe monitered qPCR with input of ds-DNA.

LinRegPCR version 12.2 released March 2010

Updated manual released.

Tuomi et al reference completed.

LinRegPCR version 12.1 released February 2010

Correction of an error in the display of flagged samples.

Exclusion of deviating PCR efficiency values was adapted to handle skewed distiributions.

LinRegPCR version 12.0 released February 2010

Update because of acceptance of the paper in Methods (Tuomi et al.)

This paper describes how to deal with cumulative fluorescence data like those obtained with TaqMan hydrolysis probes.

LinRegPCR version 11.5

Updated Rotor-Gene input format to include raw and baseline-corrected data

Raw data are exported with the "Excel data sheet" option of Rotor-Gene

The LinReg export format exports baseline-corrected data (constant baseline per sample)

LinRegPCR version 11.4

not released

LinRegPCR version 11.3

Updated the code to read the Step-One Plus format to allow for different output versions

Enabled a 'relaxed' baseline estimation for datasets with a short continuous log-linear phase due to measurement noise

Extended 'Legends' column in output with list of choices made by the User

LinRegPCR version 11.2

Added import formats to Read-from-Excel dialog:

- Step-One Plus system (ABI)

- LightCycler 480, 2 columns per sample

- Rotor-Gene (Corbett Reseach; LinRegPCR export)

Corrected program flow after detection of noisy samples

Enabled easier handling of baseline error samples

LinRegPCR version 11.1

Removed 'overflow' error that occurred sometimes when empty wells were included in the data

LinRegPCR version 11.0 released January 2009.

Version number was increased because of the acceptance of the paper in Nucleic Acids Research (Ruijter et al.).

Added input format for Stratagene systems (Format 1, Vertically Grouped data)

Added export of input data in ‘1 row per sample, 1 column per cycle’ format

LinRegPCR version 10.3

Detection of noisy datasets

Extended quality check with ‘noisy sample’

Removed error that lead to crash in the setting of the window-of-linearity in noisy datasets

Help on the handling of noisy data

LinRegPCR version 10.2

Corrected error in the setting of the Ct value

Corrected assignment of upper W-o-L limit for first amplicon group

Implemented full reset of the values when ‘determine baseline’ is pressed twice

LinRegPCR version 10.1

‘division by zero’ error that occurred when not enough data were present at low W-o-L settings was captured and handled

Freezing of the tab pages when a grouping error occurred was removed

LinRegPCR version 10.0 fully updated version. Released March 2008.

In this version the following features have been implemented:

import of raw data files

estimation of baseline fluorescence

new approaches to determine the window-of-linearity and fluorescence threshold

definition of sample groups per amplicon

calculation of the mean PCR efficiency per amplicon group

calculation of starting concentrations based on mean efficiency and individual Ct values

reports on data quality for each sample

LinRegPCR versions 8 and 9

Non-released. these versions were intermediate versions while implementing baseline subtraction and amplicon grouping.

LinRegPCR version 7.4

Version released with Ramakers et al Neurosci Lett 2003.

The use of this version is no longer recommended and its distribution was discontinued in March 2008