Factor Correction qPCR can be used to remove multiplicative between-run variation that occurs when a quantitative PCR experiment has to include multiple plates to accomodate all samples, targets and replications to collect all data. These replicate runs show similar proportional differences between experimental conditions, but different absolute values, even though the measurements were presumably carried out under identical circumstances.
In most cases, between-session variation is multiplicative and can, therefore, be removed by division of the data in each session with a session-specific correction factor. Assuming one level of multiplicative between-session error, unbiased session factors can be calculated from all available data through the generation of a between-session ratio matrix. Alternatively, these factors can be estimated with a maximum likelihood approach. The effectiveness of this correction method, dubbed "factor correction", is demonstrated with examples from the field of molecular biology and retrovirology. Especially when not all conditions are included in every measurement session, factor correction results in smaller residual error than normalisation and standardisation and therefore allows the detection of smaller treatment differences. See: Ruijter et al. Retrovirology 3:2, 2006.

FAQs

- **How do I distinguish between 'session' and 'condition'?**
  The session is the variable that causes the variation that you want to remove. In most cases it is the measurement identified by 'date' or 'run'. The condition is the variable or combination of variables of which you want to preserve the effects.
- **Can I correct a data set with positive and negative values?**
  The Ratio method only accepts positive values.
- **Can the data set contain zero's?**
  Yes, but in the calculation of the session factors the value zero is ignored. However, the session factors are applied to zeros, which results of course in the corrected value of zero.
- **Can I correct a data set without overlap between sessions?**
  No, there has to be at least one condition that is shared by each pair of sessions. When this is not the case, calculations wil not be performed because the resulting factors and condition means are completely useless. Note that the shared condition can be different for each pair of sessions.
- **Q**
  A

References

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- Ruijter JM, Thygesen HH, Schoneveld JLM, Das A, Berkhout B, and Lamers WH. Factor correction as a tool to eliminate between-session variation in replicate experiments: application to molecular biology and retrovirology. Retrovirology 3:2, 2006.
- Lefever S, Hellemans J, Pattyn F, Przybylski DR, Taylor C, Geurts R, Untergasser A, Vandesompele J; RDML consortium.. RDML: structured language and reporting guidelines for real-time quantitative PCR data. Nucleic Acids Res. 37: 2065-9. 2009.
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Version History

Factor_qPCR version 2016.0. Released January 2016.

When a column Run was already preent in the Excel input of Factor-qPCR the sheet format of the third input was not recognized as similar. To avoid this confusion, the column added by the program is now labeled RunFC and the user is warned when he/she already included a column with that name. In that case, the user has to rename or remove the column RunFC. Note that a column indicating the run number is not required because the program adds it automatically. This change has no effect on the results.

Factor_qPCR version 2015.2. Released July 2015.

- The original 2015.2 version did not enable the Create RDML file button when the data were read from Excel and thus prevented saving the data to RDML. This bug is repaired in this new 2015.2 version. This fix has no effect on the results.

Factor_qPCR version 2015.2. Released June 2015.

- In this version saving to RDML includes the choice "version 1.1 or version 1.2 ?". This is because downstream analysis programs are not yet all able to read RDML 1.2 files. The different tissue annotation elements that can be used in RDML 1.2 are concatenated into one tissue description in RDML 1.1 and the AmplificationEfficiencySE element is not yet present. The result of the removal of between-plate variation is the same.

Factor_qPCR version 2015.1. Released June 2015.

- The ZIP file that you can download includes the program file, Factor_qPCR.exe and a Demo_dataset_Factor_qPCR.xls that contains the analysis results of a two-plate experiment. The read-me sheet in this file explains the experiemnt and shows you how to read the data, perform the between-run correction and to save the results to either Excel or to RDML. There are also 2 RDML files (QPCRCourseApril2015_plate_n.rdml, with n = 1 or 2) that contain the raw fluorescence data and the analysis results after analysis in LinRegPCR (see the download on this website for details). The correction of the run difference in these RDML files is similar to the correction after reading Excel. Saving the corrected results to RDML is much easier because the program 'remembers' the structure of the original RDML files.

Factor_qPCR version 2015.0. Released January 2015.

- This version of Factor qPCR allows the removal of run variation in a multi-plate qPCR experiment. It is based on the Excel or RDML output of the amplification curve analysis program LinRegPCR (see the LinRegPCR download on this site). However, Factor qPCR can handle Excel data from other sources that, at least, contain the columns Run (or plate), tissue annotations, PCR efficiency, quantification threshold, Cq value and target quantity (N0).
- RDML output of Factor qPCR can be inported into qbase+ and is then handled as one, combined, Run.