Melting Curve Analysis

Melting Curve analysis, for short MCanalysis, is a program to detect the peak(s) in melting curve data generated after a run of the qPCR system. The program imports fluorescence data in a reaction per row, temperature per column format from Excel. Users of a LightCycler480 system can export the raw fluorescence data, with or without amplification data, to a delimited text file and import these data directly. The program performs smoothing and identifies peak(s) in the negative first derivative of the melting curve data. The Tm of the expected product should be determined from a positive control reaction. With this Tm, the program determines for each reaction the fraction of fluorescence in the correct melting peak. This factor can be used to correct the results of the amplification curve analysis see Reference).

FAQs

Why does the program want to change decimal commas into decimal points?
The program found that in the text file you got from your qPCR system the decimal separator was a comma, but that the Windows system on your computer uses decimal points. Because Excel will then interpret the comma as a thousand separator (converting 3,20 into 320, but 3,195 into 3195) the commas have to be replaced by points. However, when you have configured Excel to use decimal commas while Windows is using points you have to tell LC480Conversion NOT to convert commas to points. It is highly recommended to have Excel use the same decimal separator as Windows (in Excel: options menu - international Tab in the options dialog).

Q
A

Version History

MCanalysis, version 2019.0, June 2019:
This version of the program was released with the publication of the paper in FASEB Journal (Ruijter et al. FASEB Journal, In Press).

Reference

Ruijter et al. Removal of artefact bias from qPCR results using DNA melting curve analysis. FASEB J, In Press.