InterfaceqPCR package
a GUI to determine the concentration in the sample, obtained by qPCR after with or without PMA treatment

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Abstract

This article presents InterfaceqPCR a Graphical User Interface allowing to determine the concentration in the sample in CFU.mL^{-1} or in number of copies.mL^{-1} provided to qPCR results after with or without PMA treatment. This package is simply to use because no knowledge in R commands is necessary. A graphic represents the standard curve, and a table containing the result in CFU.mL^{-1} or copies.mL^{-1} for each sample is created. InterfaceqPCR package is available and running on several platforms: Windows, Unix and MacOS.

1 Introduction

The quantitative real-time ploymerase chain reaction (qPCR), is a molecular technique based on the quantification of DNA. This is a technique rapid, highly sensitive, accurate and generates a lot of datas quickly.

The live portion of the microbial communities is quantified by cultural methods. For some years, the molecular technique have developed new approaches for discriminating the viable and non-viable microorganisms (Nocker et al., 2007; Nocker and Camper, 2006; Nocker et al., 2006).

Treatment of microbial samples with propidium monoazide (PMA) before DNA extraction is a method that allows the selective detection solely of living cells. This technique coupled with qPCR has been called viability-qPCR or PMA-qPCR. The principle is based on the integrity of the bacterial cell membrane, PMA can penetrate only cells with a compromised membrane. Membrane integrity is one of the well-accepted criteria for distinguishing viable from dead cells (Pinto et al., 2013).

I present a new R package (R Core Team, 2016) called InterfaceqPCR with a graphical user interface (GUI) that helps the user to run the analysis method of qPCR datas. No programming knowledge is required to use it, thanks to its friendly GUI. All the options are realized with clickable or radio buttons.

The InterfaceqPCR package is a GUI based on the tcltk package developed and adapted to R by Dalgaard (Dalgaard, 2001, 2002; R Core Team, 2016).

It can i) create and import files containing the qPCR results treated or not with PMA, ii) select the analysis method, iii) establish the regression curve and export the graphic and iv) determine the concentration in CFU.mL^{-1} or in number of copies.mL^{-1} for each sample and export the results in text or Excel formats.

The aim of this paper is to introduce and describe the InterfaceqPCR package.

2 Launching InterfaceqPCR

Once R has started up, simply loading the InterfaceqPCR package by typing the command require(InterfaceqPCR) or library(InterfaceqPCR) into the R prompt.

Once loaded, you can write at the R prompt the following line command to run InterfaceqPCR: Start_qPCR(), InterfaceqPCR window should appear and the analyse can be run (Figure 1). InterfaceqPCR requires some packages in addition to several packages that are normally distributed with R, and loads these packages at startup. The packages require are: plyr, readxl, reshape2, tcltk, tkrplot and xlsx (Wickham, 2011, 2016, 2007; R Core Team, 2016; Tierney, 2011; Dragulescu, 2014).

Figure 1: The R Console and InterfaceqPCR window at start-up after write Start_qPCR()

3 Description of this package

This interface is composed of four steps.

1. The first step: select your language.
   The available languages are French and English, select the flag corresponding to continue (Figure 1).
2. The second step: Complete datas files.
   At this step, you click on the Standard icon and on the Sample icon to open a xlsx file and write datas (see model click on the model icon for the standard curve and for the samples) (Figure 2).

   There are several columns in the Standard file to complete and save this file in other folder.

   - **"Numero" column** represents the position in the range of each standard and the number of replicat.
   - **"Standard" column** represents the concentration of each standard written in scientific format.
   - **"log$_{10}$_Std" column** is the decimal logarithm of the concentration for each standard. It is calculated when a number is present in the "Standard" column.
   - **"Condition" column**, select for each row, if the standard is affiliated to PMA standard curve or Total standard curve.
   - **"Ct" column**, indicating the Ct (threshold concentration) value of each standard. These values provide to the qPCR results.

   For the Sample file, complete the file and save in other folder.

   - **"Nom_Echantillon" column** indicating the name of the sample.
   - **"Condition" column**, select for each row, if the sample is treated select PMA or not treated select Total.
   - **"Ct" column**, indicating the Ct value of each sample. These values provide to the qPCR results.

   When the two files are created, click on the arrow button to move to the next step.

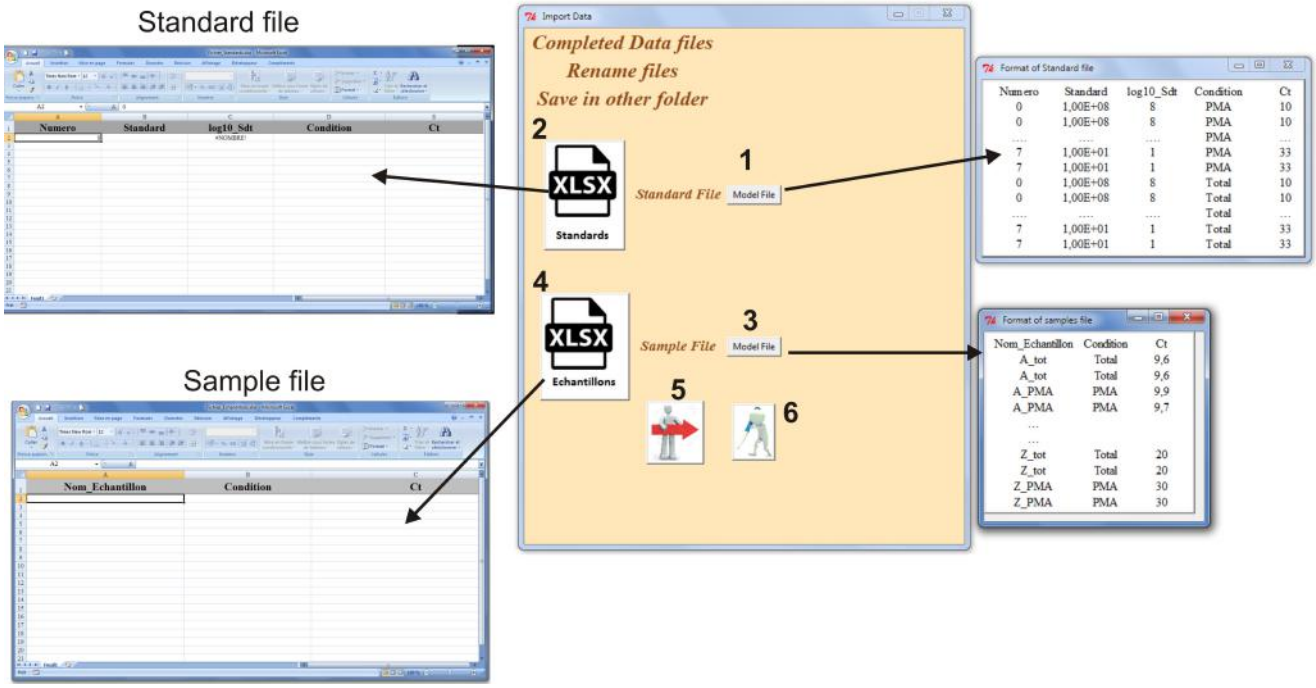

Figure 2: The second step: Input datas files
1: model file button helps to write the Standard file, 2: Standard icon opens the Standard.xlsx file, 3: model file button helps to write the Sample file, 4: Sample icon opens the Sample.xlsx file, 5: move to the next step, 6: About of this package.

3. The third step: Select the method to analyse the samples. Two icons are present in the window to select the method of analysis (Figure 3).
   - **First icon:** Select to analyse samples treated with PMA (corresponding in the sample file at the PMA condition)
   - **Second icon:** Select to analyse samples not treated with PMA (corresponding in the sample file at the Total condition)

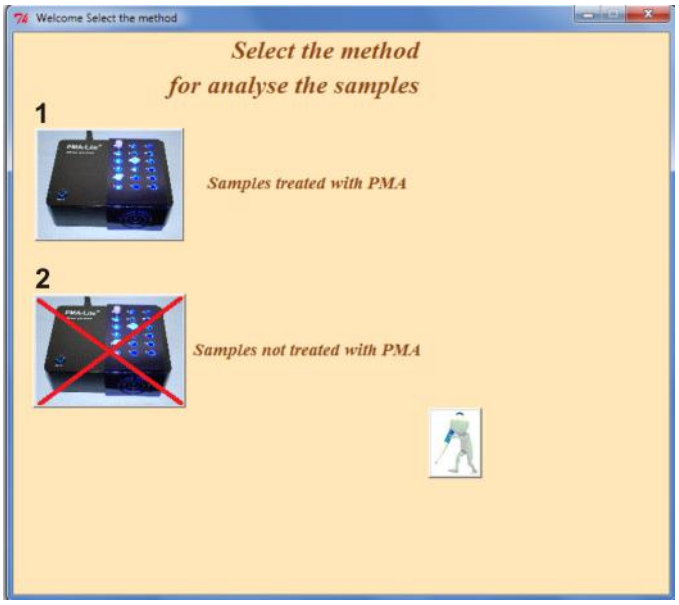

Figure 3: The third step: Select the method to analyse the samples
1: Analyse samples treated with PMA (corresponding in the sample file at the PMA condition), 2: Analyse samples not treated with PMA (corresponding in the sample file at the Total condition)

4. The fourth step: Analyse the samples and determine the concentration.
When the InterfaceqPCR starts up, there is no active data set (indicating by the 'No' in the cases present in the block names 'Import files'. You can import datas from xlsx files created during the second step (Figure 4).

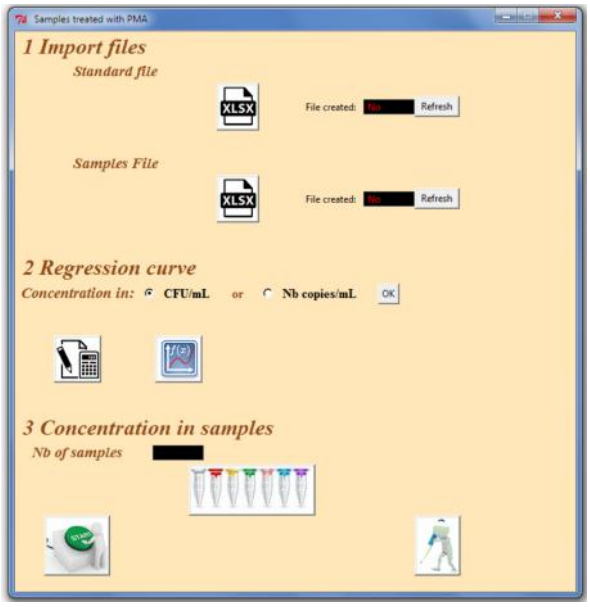

Figure 4: The fourth step: Analyse the samples
No datas are imported

The interface is divided into three parts:

- **1 Import files:** You click on xlsx icon to open the File Browser and select the standard file then click on refresh button. This action verifies if the file loaded is correct or not, if no problem, in the case the 'No' changes in 'Yes'. The same action is repeated to import the sample file. When you click on refresh, the 'No' is replaced by 'Yes' and in the Concentration in the sample section, the case

next to 'number of samples' indicates the number present in the file (Figure 5). If two 'yes' are obtained, you can move to the next block.

- **2 Regression curve:** In first time select the concentration, The choice is CFU.mL^{-1} or number of copies.mL^{-1} and click on the Ok button to valide, a new window appears and indicates your choice. If you are agreed click on the Ok button (Figure 6).
  Then the concentration selected, click on the icon representing a sheet with a calculator to determine the standard curve. After click on the graphic icon, a new window appears, showing the regression curve between the Ct results and the concentration in $\log_{10}$ of qPCR standards. The standard deviation are indicated for each dilution. On this graphic two x-scales are represented. The first x-axis is on decimal logarithm of the concentration and the second x-axis is the value written in scientific format of the the concentration.
  The regression equation, the coefficient of determination, the qPCR efficiency and the limits of detection and quantitation are mentionned.
  The coefficient of determination (R^2) is a number that indicates the proportion of the variance in the dependent variable that is predictable from the independent variable. The R^2 ranges from 0 to 1, indicating perfect negative correlation at -1, absence of correlation at zero, and perfect positive correlation at $+1$. The qPCR assay with a R^2 higher than 0.985 are acceptable.
  The qPCR efficiency is determined in percentage by the formula: $E = (-1 + 10^{(-1/slope)}) * 100$, where the slope was obtained by the linear regression analysis between the threshold (Ct) and the number of cell/or copies enumerated in log10. E comprised between 90% and 110% is acceptable.
  The limit of detection (LOD) is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test (Shrivastava et al., 2011).
  The limit of quantitation (LOQ) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test (Shrivastava et al., 2011).
  For a linear calibration curve, it is assumed that the instrument response y is linearly related to the standard concentration x for a limited range of concentration. It can be expressed in a model such as $y = ax + b$. The LOD and LOQ can be computed as : $LOD = 3 * SD/a$ and $LOQ = 10 * SD/a$
  Where a is the slope of the calibration curve, b is the y-intercept of the calibration curve and SD is the standard error of y-intercepts.
  You can output the graphic into the directory of your choice in vector or bitmap formats, for this click on the tiff button or/and the jpeg button or/and the pdf button. When the graphic is saved, you can close this window (Figure 7).

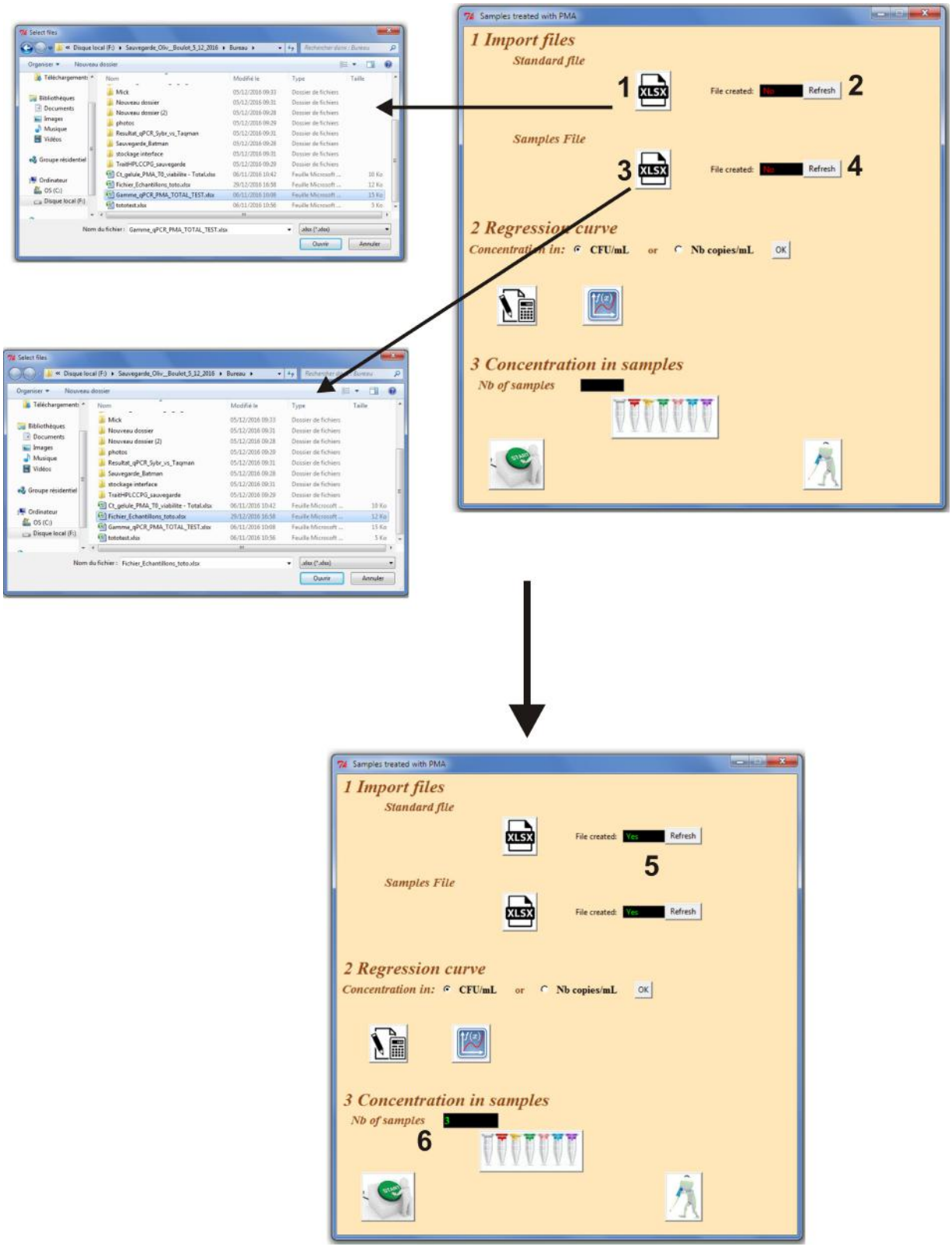

Figure 5: The fourth step: Import datas
1: Standard file click on xlsx file button to open the File Browser and select the the standard file, 2: Refresh button to verify if the input file is correct ('Yes' replace 'No'), 3: Samples file click on xlsx file button to open the File Browser and select the the samples file, 4: Refresh button to verify if the input file is correct ('Yes' replace 'No'), 5: Two 'yes' are indicated, you can move to the next block, 6: Number of samples presents in the file

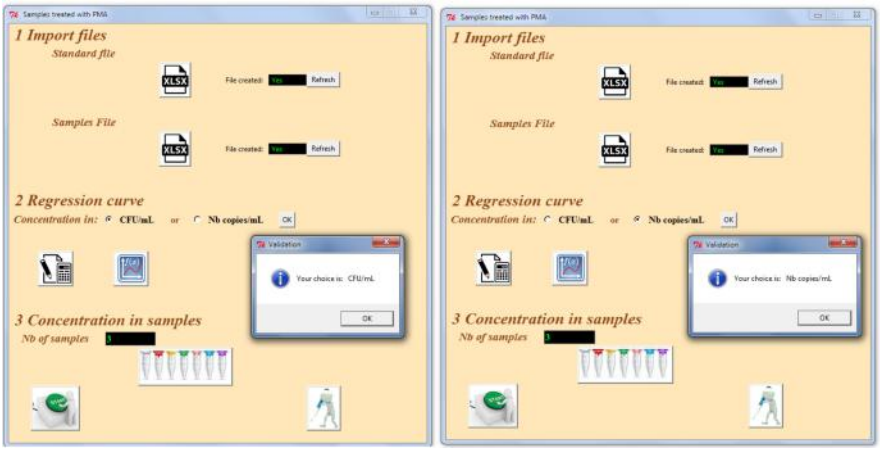

Figure 6: The fourth step: Selected units : CFU.mL^{-1} or number of copies.mL^{-1}

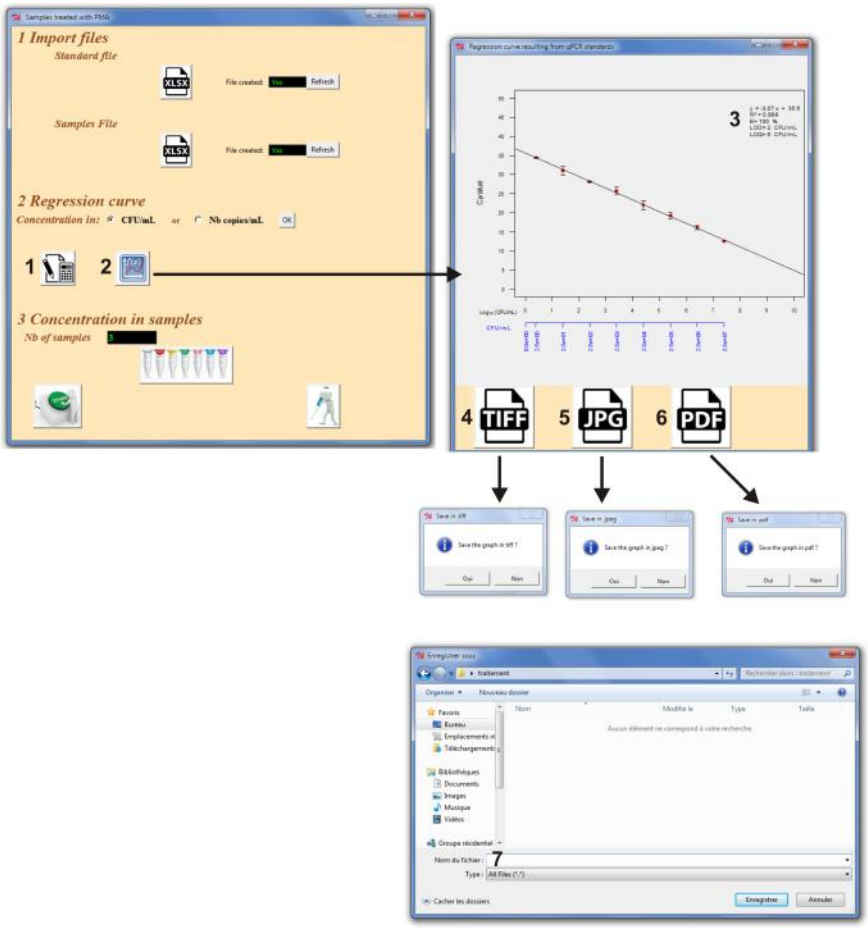

Figure 7: The fourth step: Regression curve
1: Calculator button to calculate the standard curve, 2: Graphic button to show the regression curve in a new window, 3: The regression curve and these parameters in topright (regression equation, coefficient of determination (R^2), qPCR efficiency (E), limit of detection (LOD) and limit of quantitation (LOQ), 4: Export graphic in tiff format , 5: Export graphic in jpeg format, 6: Export graphic in pdf format, 7: In the File Browser, choice your directory, name the file without the extension and save.

- **3 Concentration in samples:**
  Click on the tubes icon and a new window appears representing a table. This table contains the datas coming from sample file added of a new column mentionning the concentration in CFU.mL^{-1} or in number of copies.mL^{-1}.

The case filter is upper the table and filters the table in according to a word or a number.
You can save the table into the directory of your choice in Excel (xlsx) or/and text (csv) formats. For that, click on the csv button or/and the xlsx button (Figure 8).
If you have finished, you close the interface else click on the Start icon to return to the third step: select the analysis method (Figure 3).

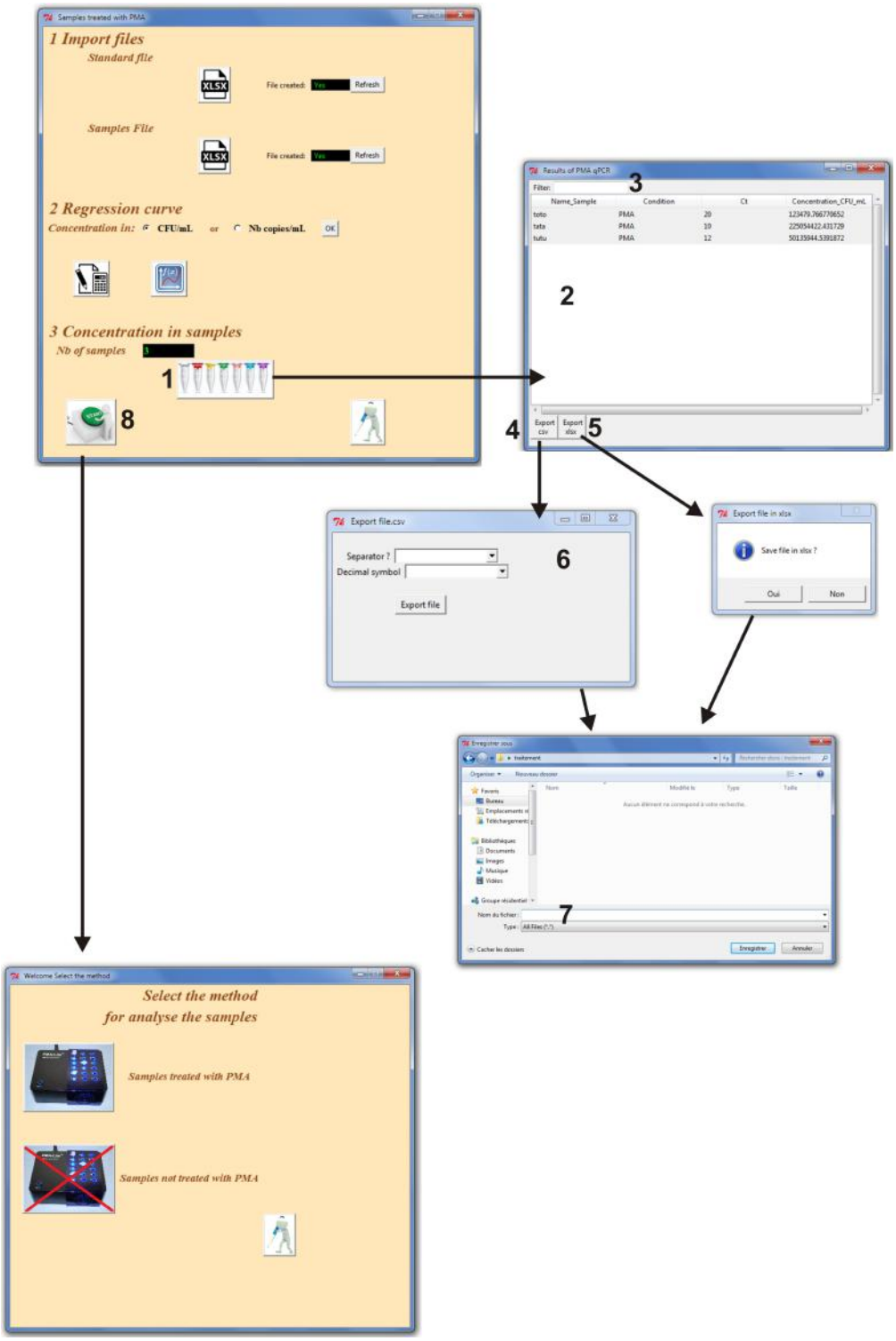

Figure 8: The fourth step: Concentration in samples
1: Tubes button to calculate the concentration in each sample, 2: Table, in a new window, shows the concentration for all samples, 3: Filter case can to filter the table in according to a word or a number, 4: Export table in csv format, 5: Export table in xlsx format, 6: Select the field separator (tabulation, comma, semicolon, dot and space) then the decimal separator (dot or comma) and click on Export csv button, 7: In the File Browser, choice your directory, name the file without the extension and save. 8: Start button to return to the third step: Select the method.

4 Conclusion

The novel InterfaceqPCR package is helpful for microbial researchers to analyse qPCR results treated or not with PMA. Furthermore it is a free program, easy to use, with a graphical user interface and running on numerous platforms (Windows, Unix and MacOS).

References

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