Universitätsmedizin Mainz

# User Manual

## qPCR-VA a program to analyze qPCR-data

Murtagh, Justin

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# Content

# 1. About the program:

## 1.1 When to use this program:

If you ran a qPCR and now want to visualize your results in a plot and calculate the mean efficiency, standard deviation and significance of your data you should use this program. Multiple sheets (resulting from experiments ranging over multiple qPCRs) will be combined. With this program you can compare as many experiment conditions and groups as you like.

The quantification is realized using the $\Delta\Delta$Ct-method.

## 1.2 What you need to do before using this program:

This program is intended to be used as an analytical tool for qPCR-data. The required input is an Excel sheet. You will have to use LinReg on your data before using it with this program, to get the expression levels according to your individual probes. Please remark that you will have to use the group function of LinReg after calling the baselines in order for the program to work properly.

## 2. Requirements before Installation:

- OS: Windows 64-Bit
- R version 3.4.2 or higher
- (Get newest version at: https://www.r-project.org/)
- JRE version 1.8 or higher (Please make sure to only have one JRE installed, or the program will only start from command line!!!)
  - ➔ If you insist to have multiple JRE versions on your computer please refer to Chapter 8.3: Working with multiple JREs (Only recommended if you know what you are doing)

(Get newest version at:
https://www.oracle.com/technetwork/java/javase/downloads/index.html)

Optionally

- download R-Studio at:
  https://www.rstudio.com/products/rstudio/download/
  (Only if you intend to change the R-script working in the background)

# 3. Install:

## 3.1 Install with install file:

- Start R and type: source("C:\\Path\\to\\Inst.R")
    - Make sure that you have write permission by starting R as administrator. If this is not possible you need to copy all downloaded packages from your external library (should be in: "C:\Users\Documents\R\win-lib\R3.4\") into the main library of R (should be in: "C:\Program Files\R\R-3.4.2\library")
- Double click the file Install.bat
- Follow the instructions on the screen
- PlotAndStats should be ready to start

## 3.2 Manual Install

- Install JRI and rJava by opening R or R-Studio and typing "install.packages('rJava')"
- Install rmngb with "install.packages('rmngb')"
- Install reshape2 with "install.packages('reshape2')"
- Install ddCt with "source('https://bioconductor.org/biocLite.R')"
    - "biocLite('ddCt')"
- Go to the jri\bin directory ----------should be somewhere like: "C:\Program Files\R\R-3.4.2\library\rJava\jri\x64"
    - **!!! Make sure to use the jri.dll from the x64 directory!!!**
- Copy the jri.dll to the jre\bin ----------should be somewhere like: "C:\Program Files\Java\jre1.8.0_121\bin"
- Go to the R\bin\x64 directory ----------should be somewhere like: "C:\Program Files\R\R-3.4.2\bin\x64"
- Copy all .dll files to the jre\bin ----------should be somewhere like "C:\Program Files\Java\jre1.8.0_121\bin"

- Run PlotAndStats.jar
  - Due to known issues with multiple JREs on the computer the JAR-file might not start with a double click. If so, please start the program via the command line using: “java –jar PlotAndStats.jar “
    - ➔ If you are not familiar with the Windows command line see Chapter 8.2: Double clicking does not work

# 4. Usage:

This program is intended to work with data generated by LinReg. The Resulting Excel sheet should contain 4 Header rows with general Information and then the Data.

Please make sure the Columns are in the following order:

| name | indiv_PCR_eff | Amplicon | threshold | mean_PCR_eff | cq | N0 |
|---|---|---|---|---|---|---|
| | | | | | | |

The names must have the format:

Wellplate_Gene_Condition_Group (e.g. A1_TBP_0Gy_15min)

After controlling your Excel sheet start the program and fill in the mask according to the data of your Excel sheet

If you used spacebars or other separators (apart from "_") in the Excel sheet also use them while filling in the Mask

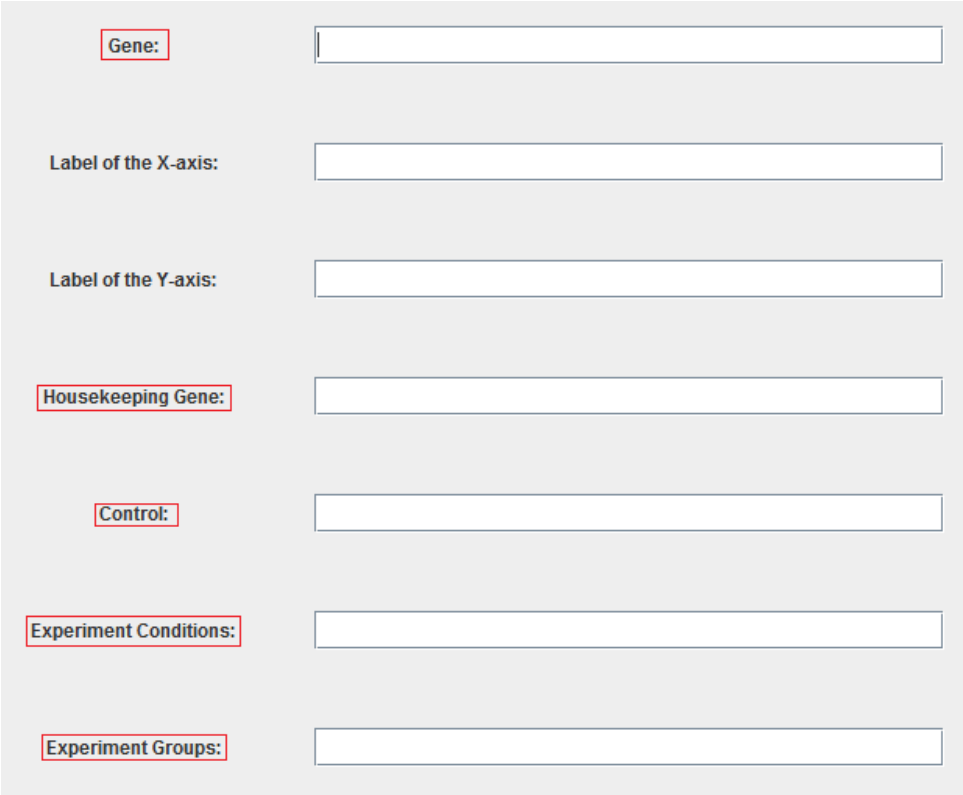
When you filled out the whole mask press 'Apply'. The program will now create a bar plot and show the corresponding data in a Table underneath.

If you would like to change the settings of the plot press ‘Change Plot’

➔ A dialog will pop up, asking you to change the settings of the plot. Press ‘OK’ to apply the new Settings.

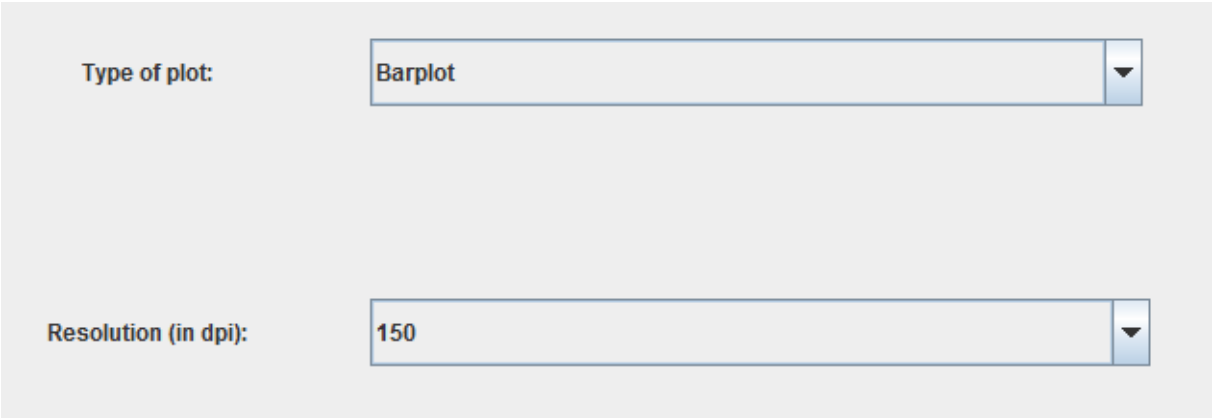
If you wish to save the Plot and Table press the save button and select the directory where the files should be saved. You will need to select another directory then the one where your excel-sheet is located or the files will be recognized as temporary files and will be deleted!

# 5.How to fill out the mask for one sheet/multiple sheets

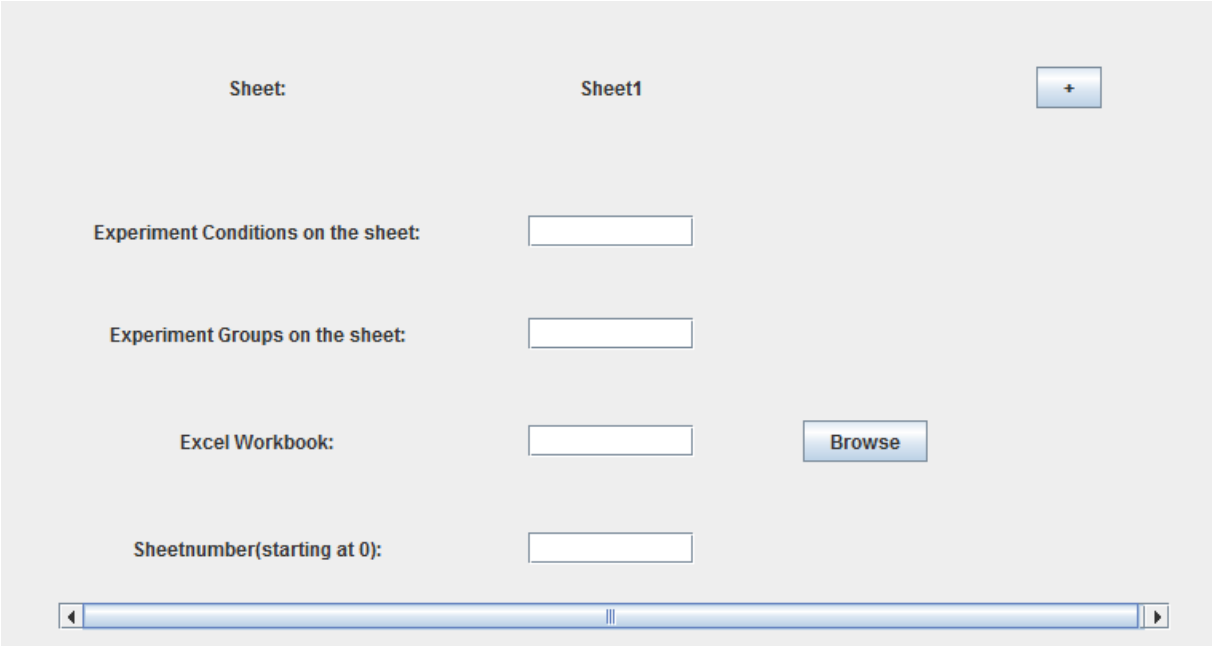



You need to fill in the mask with your data. Attention when filling in the marked sections, for these have to be filled exactly how the data is written in Excel. I.e. if you used separators for the gene names or conditions you will have to use the same ones to fill in the mask.

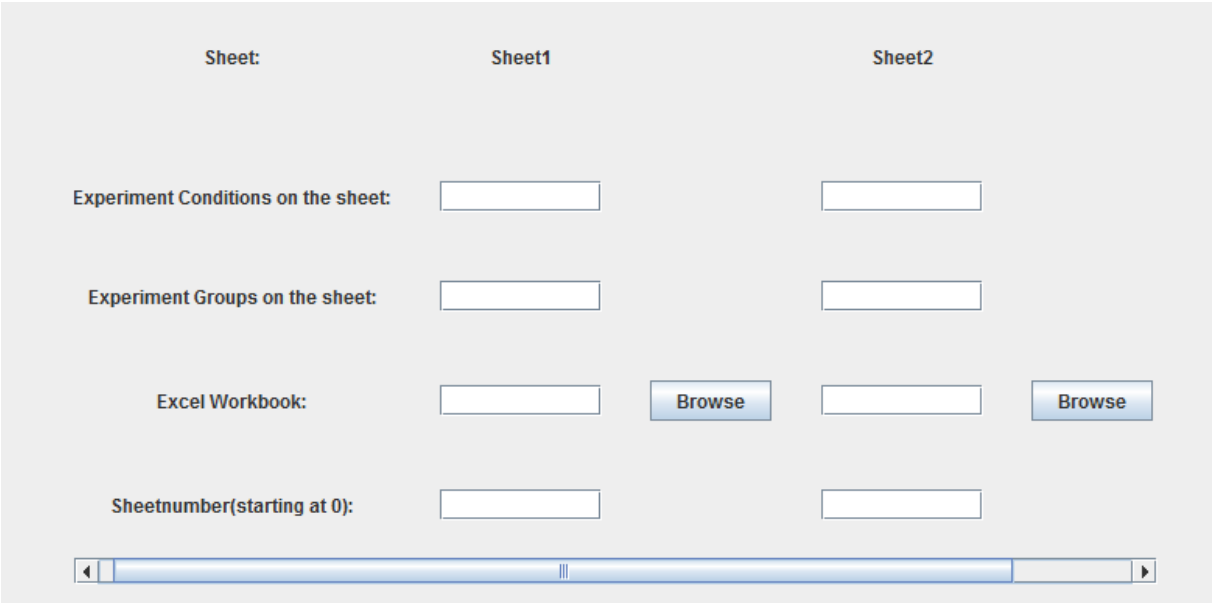
Now choose the type of plot and the resolution you would like to get.



Now it depends on how many Excel sheets your data is on. (e.g. If you had to run two qPCRs for your Samples the data should be split up on two sheets) Press the “+”-button until you have enough fields to input your data. (Do not add more sheets than you actually have or the program will not work)




Above is an example for one sheet.

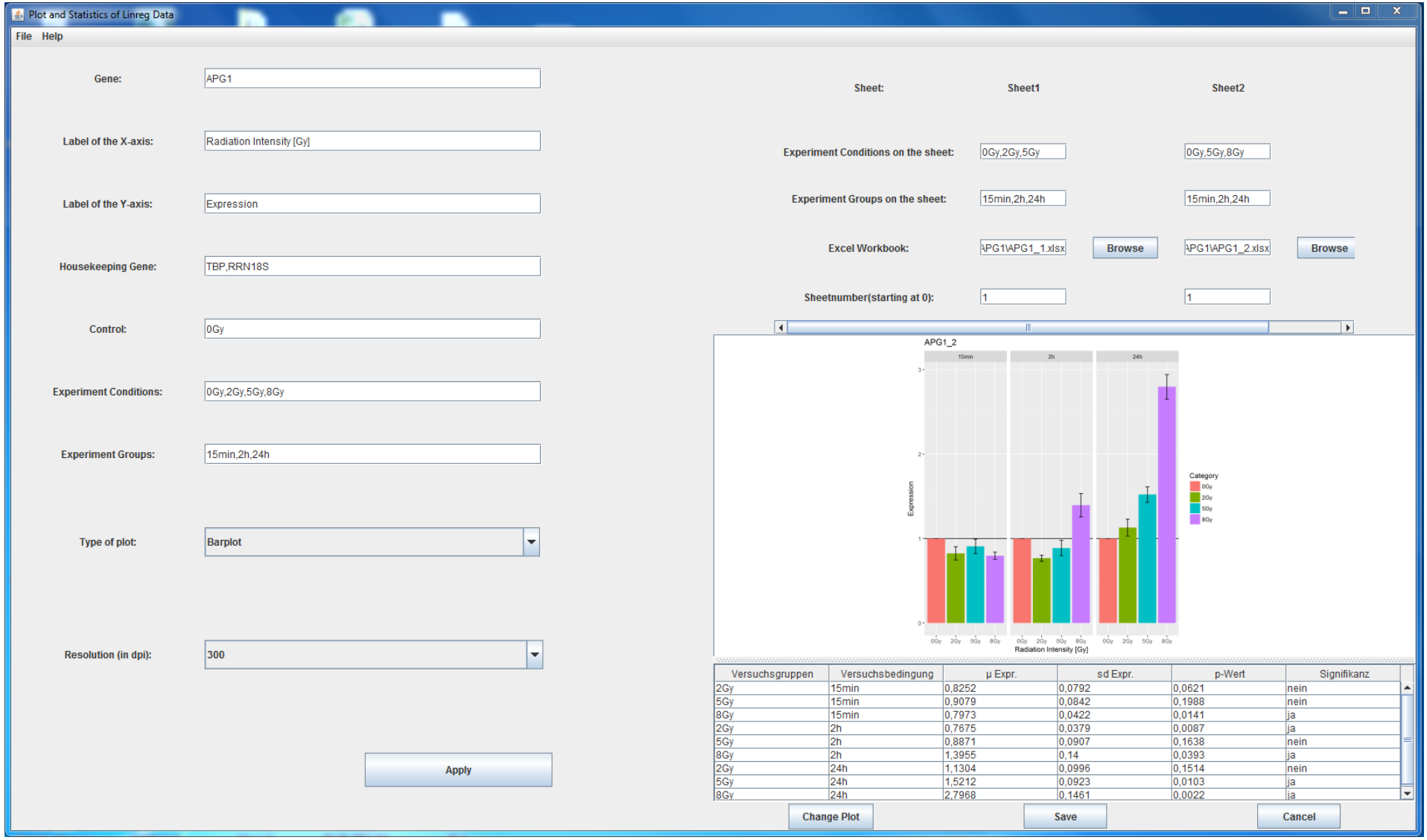



Here an example for two sheets.

Please fill in which data is on which sheet.

If you filled in the whole mask press ‘Apply’ to start computing.

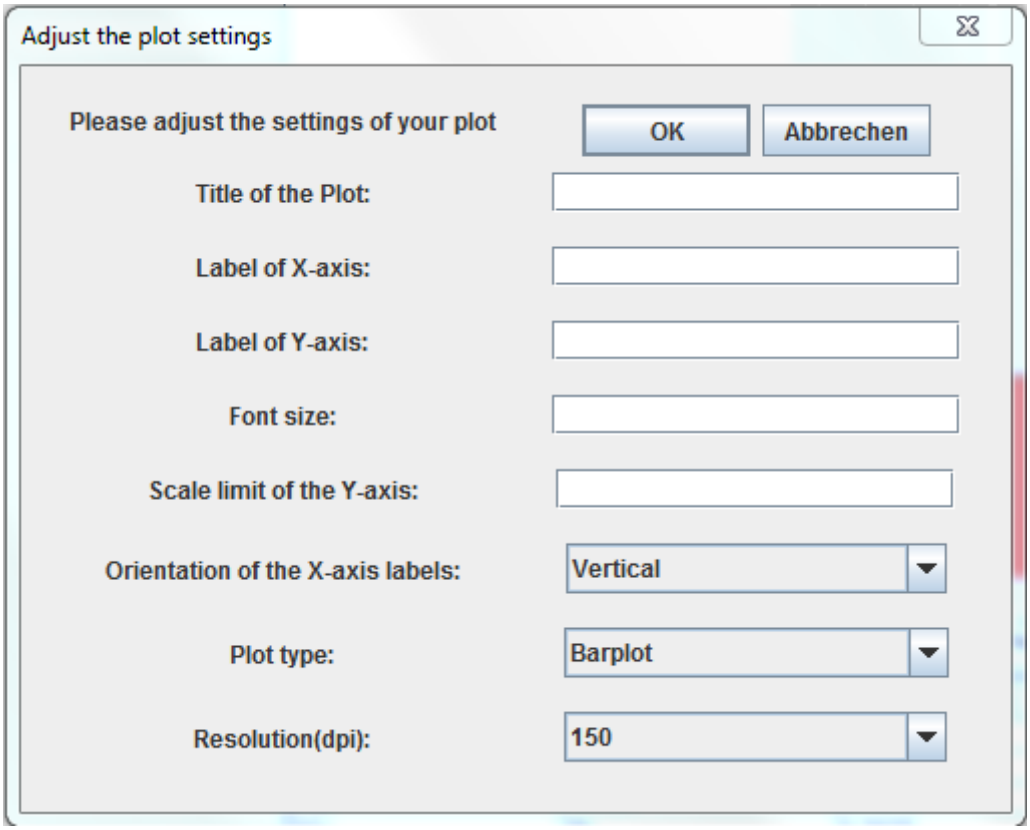
If you correctly filled out the mask you should get something like this:




| Versuchsgruppen | Versuchsbedingung | µ Expr. | sd Expr. | p-Wert | Signifikanz |
|---|---|---|---|---|---|
| 2Gy | 15min | 0,8252 | 0,0792 | 0,0621 | nein |
| 5Gy | 15min | 0,9079 | 0,0842 | 0,1988 | nein |
| 8Gy | 15min | 0,7973 | 0,0422 | 0,0141 | ja |
| 2Gy | 2h | 0,7675 | 0,0379 | 0,0087 | ja |
| 5Gy | 2h | 0,8871 | 0,0907 | 0,1638 | nein |
| 8Gy | 2h | 1,3955 | 0,14 | 0,0393 | ja |
| 2Gy | 24h | 1,1304 | 0,0996 | 0,1514 | nein |
| 5Gy | 24h | 1,5212 | 0,0923 | 0,0103 | ja |
| 8Gy | 24h | 2,7968 | 0,1461 | 0,0022 | ja |

You can now either directly save the generated plot and the table to your desired directory by pressing the save-button, or you can adjust the setting of your plot.

If you wish to adjust your plot press the ‘change plot’-button to get the following mask:




Here you can change all possible settings of your plot. Please remark that the scale limits of the Y-Axis needs a minimum and a maximum which has to be separated using a “,”. (e.g. 0,4)

If you do not enter a field the default settings will be used.

Click ok to apply the settings to your plot.

!! The plot sometimes does not show right away. If so move the dividing bar between plot and table to refresh the plot.

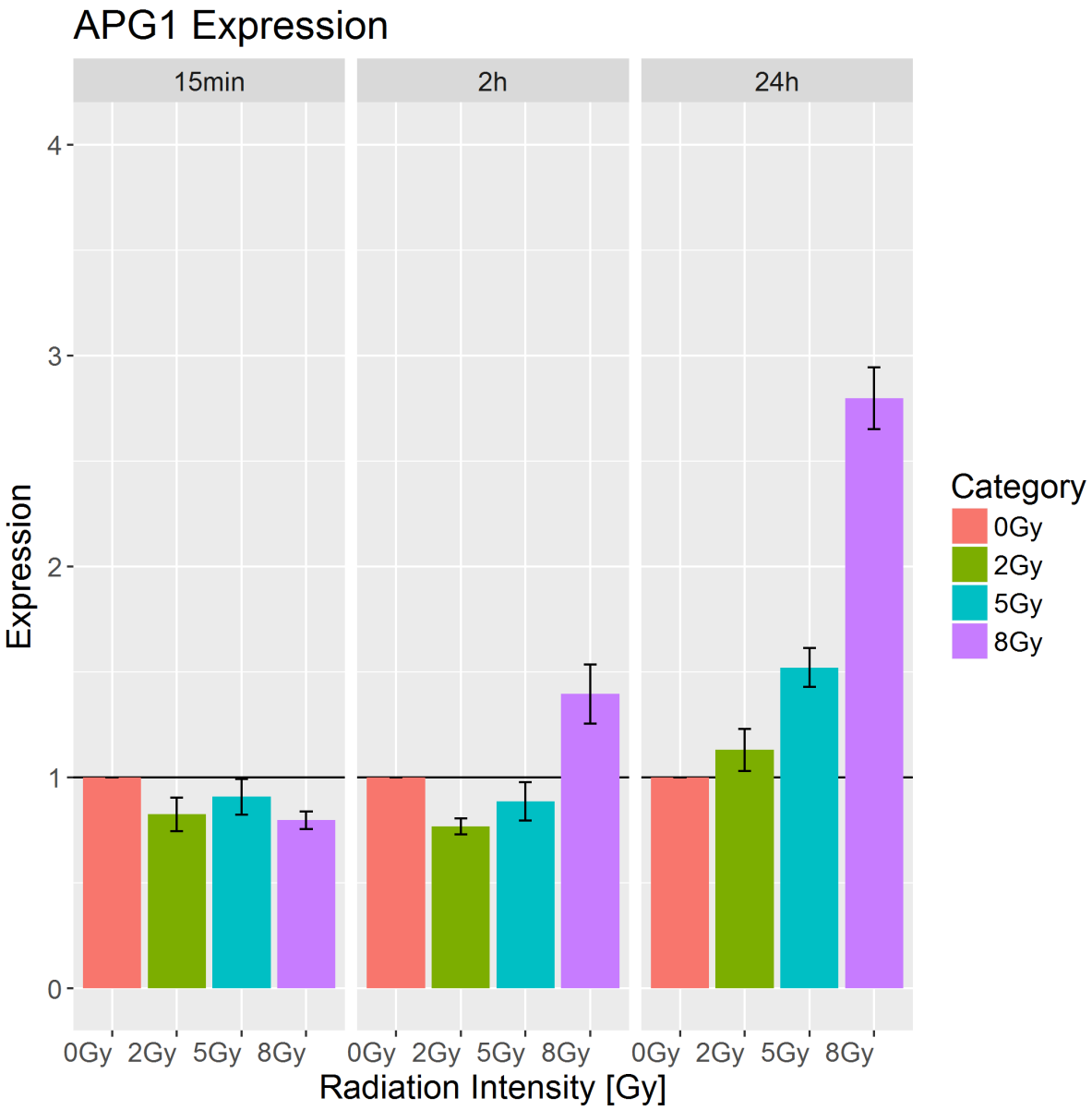
## 6. Resulting Data

If everything went correctly you should have two files in your chosen save directory.

1. A '.png' file containing the plot
2. A '.csv' file containing the results of the statistical evaluation of the data

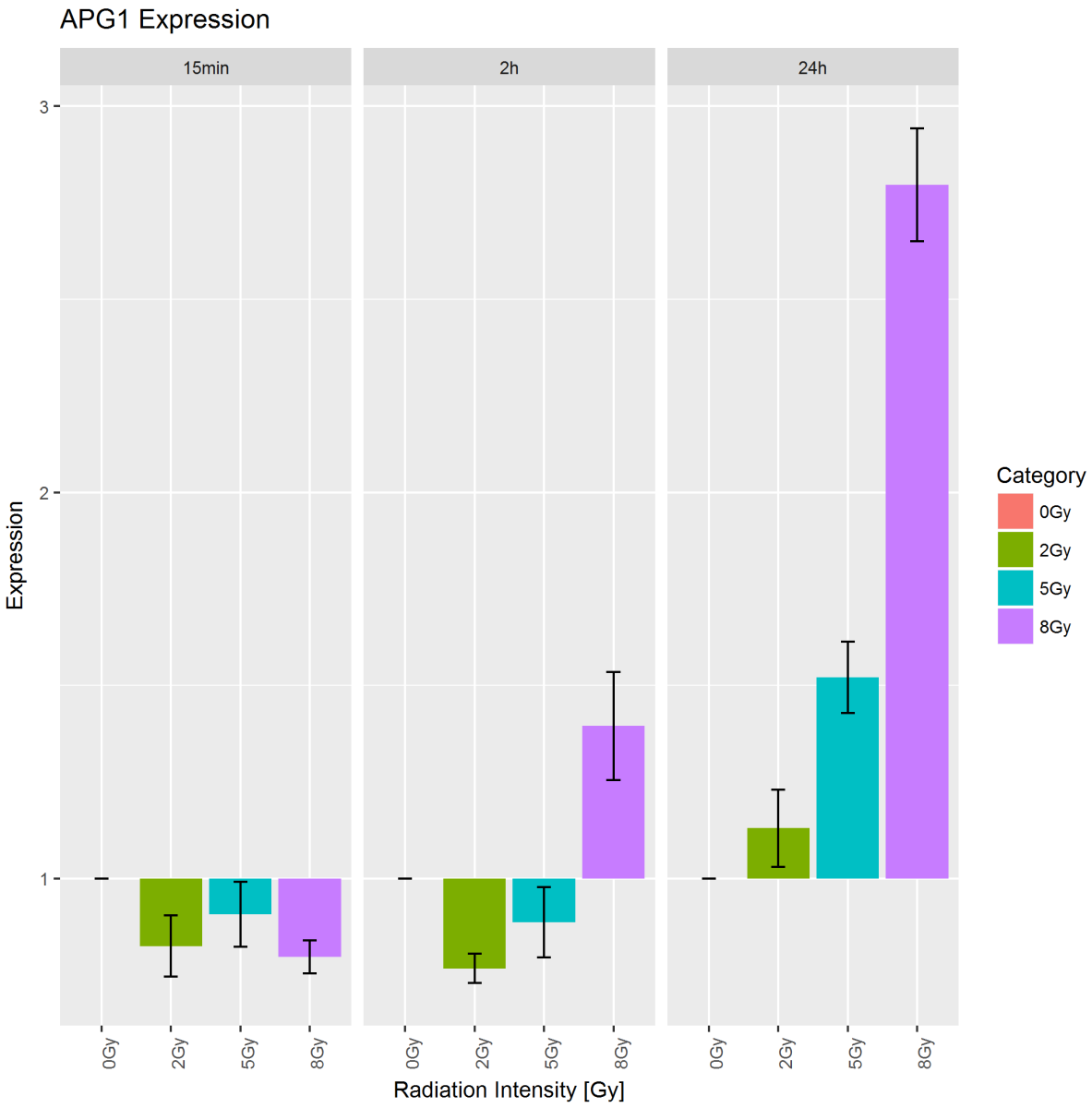
The main reason to use this program should be to find out if the difference in expression levels during the PCR is significant or not, and to gain a visualization of the results.

# 7. Example Output




Example with:

- Title = APG1 Expression
- X-label = Radiation Intensity
- Y-label = Expression
- Font size = 15
- Y-limits = 0, 4
- Orientation = Horizontal
- Plot type = Bar plot
- Resolution = 300 dpi




Example with:

- Title = APG1 Expression
- X-label = Radiation Intensity
- Y-label = Expression
- Font size = 15
- Y-limits = 0, 3
- Orientation = Vertical
- Plot type = Bar plot – deviation from mean
- Resolution = 300 dpi

| Versuchsgruppen | Versuchsbedingung | µ Expr. | sd Expr. | p-Wert | Signifikanz |
|---|---|---|---|---|---|
| **2Gy** | 15min | 0,8252 | 0,0792 | 0,0621 | nein |
| **5Gy** | 15min | 0,9079 | 0,0842 | 0,1988 | nein |
| **8Gy** | 15min | 0,7973 | 0,0422 | 0,0141 | ja |
| **2Gy** | 2h | 0,7675 | 0,0379 | 0,0087 | ja |
| **5Gy** | 2h | 0,8871 | 0,0907 | 0,1638 | nein |
| **8Gy** | 2h | 1,3955 | 0,14 | 0,0393 | ja |
| **2Gy** | 24h | 1,1304 | 0,0996 | 0,1514 | nein |
| **5Gy** | 24h | 1,5212 | 0,0923 | 0,0103 | ja |
| **8Gy** | 24h | 2,7968 | 0,1461 | 0,0022 | ja |

Example with:

- Two sheets
- Three groups (15min, 2h, 24h)
- Four Conditions (0Gy, 2Gy, 5Gy, 8Gy)
- Statistical test = t-test

# 8. ERROR HELP

## 8.1 Installation Errors:

- If you get an Error saying something like:
  "Could not move temporary file from 'C:\\Path' to 'C:\\Path'"

  Try typing: debug(utils:::unpackPkgZip) and then installing the package again

  ➔ You will have to press Enter a lot until it should say:
  "Package downloaded successfully ...something... check sum MD5"

  Sometimes it can be necessary to repeat the step mentioned above to get rid of any issues

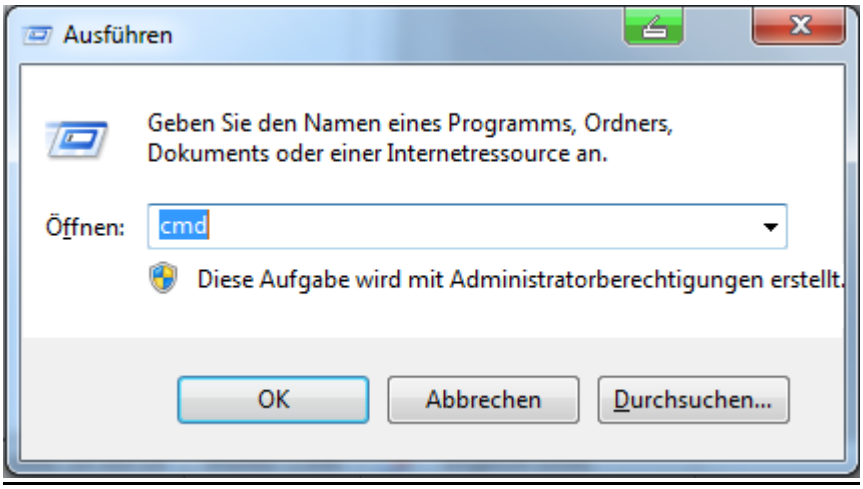
- Check if all necessary packages are downloaded correctly, by typing:

  ```
  library("rmngb")
  library("reshape2")
  library("ddCt")
  library("ggplot2")
  ```

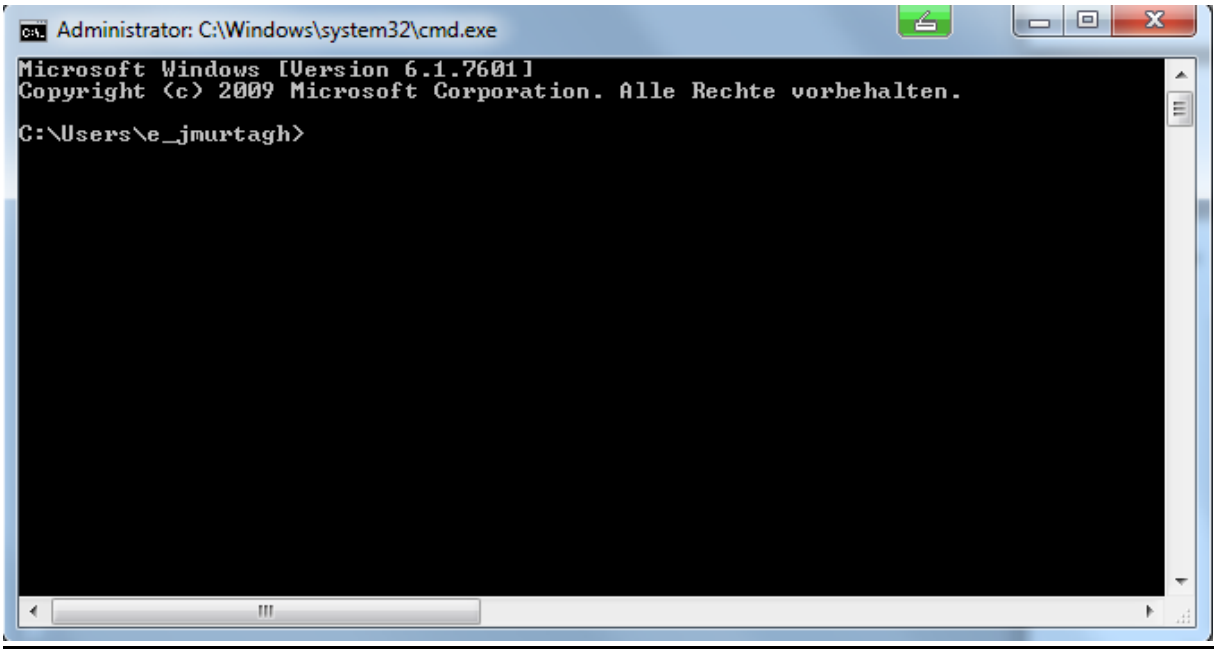
  ➔ If there is a Warning like:
  Could not find package "NameOfPackage",
  try installing it manually with "install.packages('NameOfPackage')"

## 8.2 Double clicking does not work:

- Open the command line by pressing the windows button + R at the same time. You should see this:




- Type in “cmd” and press Enter
- The command line should come up an show you something like this:




- Now you need to change to the directory of the program
  - E.g. if you installed it in a directory on the desktop type in:
    - cd C:\Users\YourUser\Desktop\PlotAndStats
    - (instead of “YourUser” type in the name of the User your working on and instead of “PlotAndStats” the name of the directory the program is in)
- You should now be in the right directory and be able to start the program by typing: “java –jar PlotAndStats.jar”

## 8.3 Working with multiple JREs:

Change the Registry:

First open the Windows command line by [Windows-button] + [R] and type regedit. Confirm by pressing [Enter].

You now may have to enter the administrator password or you have to confirm the security check of the User Access Control (UAC).

Next the Registry-Editor should open. On the left hand side you will find the code key.

You now need to click until you reach:

HKEY_CLASSES_ROOT\jarfile\shell\open\command

or (depending on the Windows-Version)

HKEY_CLASSES_ROOT\jar_auto_file\shell\open\command

Now open the key editor by double clicking the name standard on the right side of the window.

The value of the key consists of the path to the interpreter and the Wildcards, who act as placeholders for the given data-name:

"C:\Program Files (x86)\Java\jre8\bin\javaw.exe" -jar "%1" %*

The path to the JRE has to be read or copied from the system and then, respective to the template in the line with grey background, pasted to the key-editor. Pressing OK confirms the edit. The Registry-Editor can now be closed.

The Jar-Files should now start by double clicking.