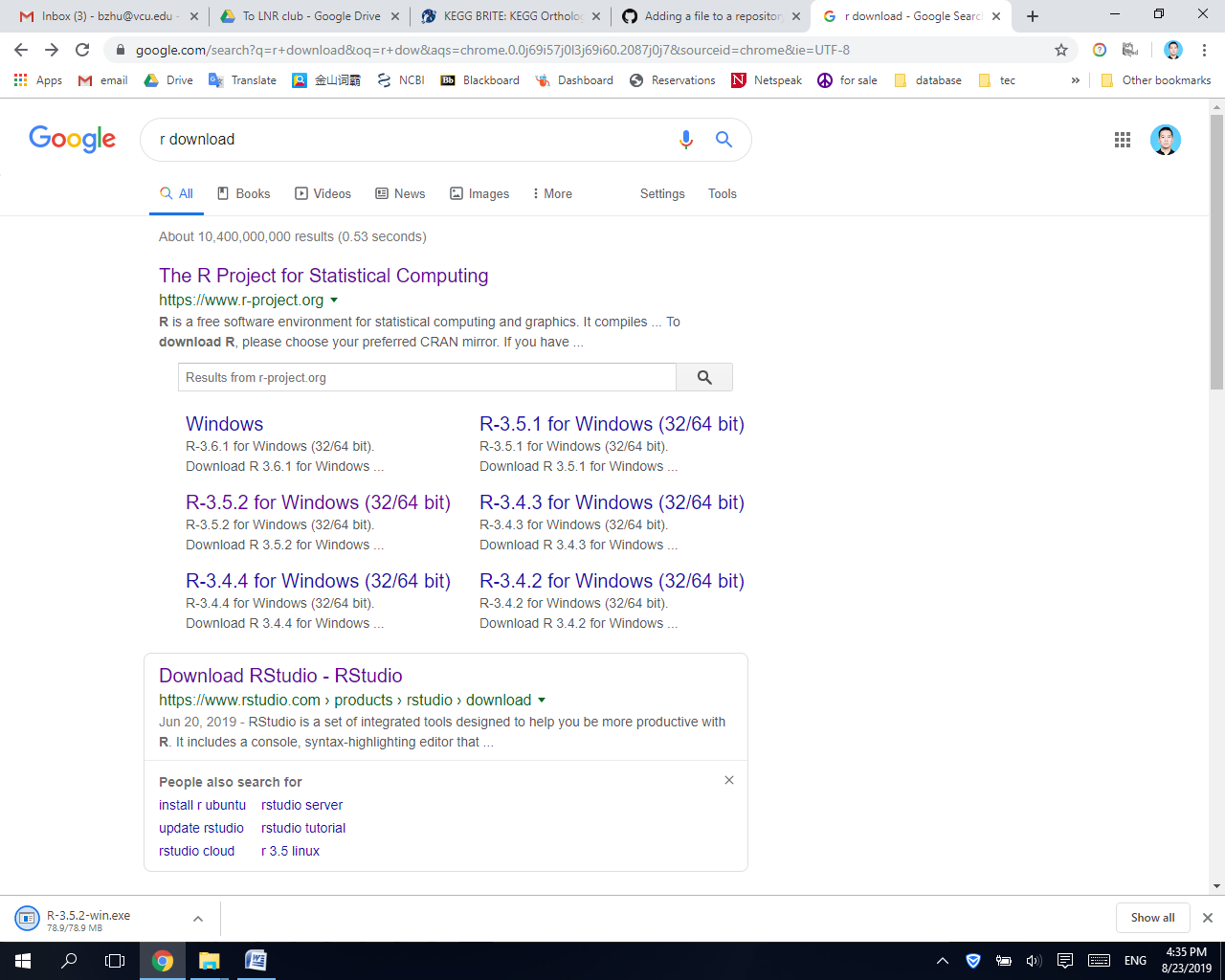
Before using the script, please install R and Rstudio in your computer.



If this is the first time to use the script, you should open ‘Installation.R’, choose all of the commands and run the commands to install necessary packages for the analysis. From the second time on, you don’t need to run it again.

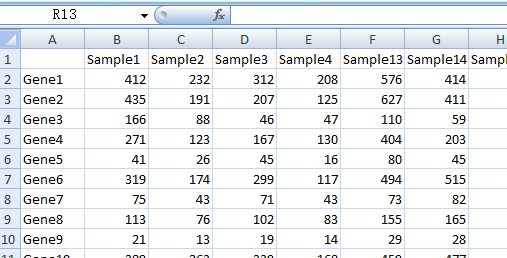
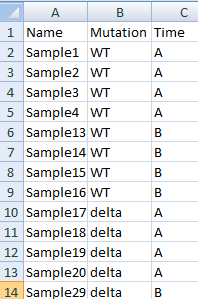
To run commands, you can choose the commands using your mouse, or you could move cursor to command windows, press ‘Control+A’ in Windows or ‘command+A’ in Mac to choose all of the commands and click ‘run’.

A screenshot of a cell phone

Description automatically generated

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1. Prepare your reads data in ‘data.csv’ file and design information in ‘design.csv’ file. If you have only one factor in your experiment design, you can delete the column ‘C’ in ‘design.csv’. You can use Google Sheets or EXCEL to edit .csv files. If you want to annotate gene names in your volcano plot, you need to put the related gene names in ‘lable.csv’

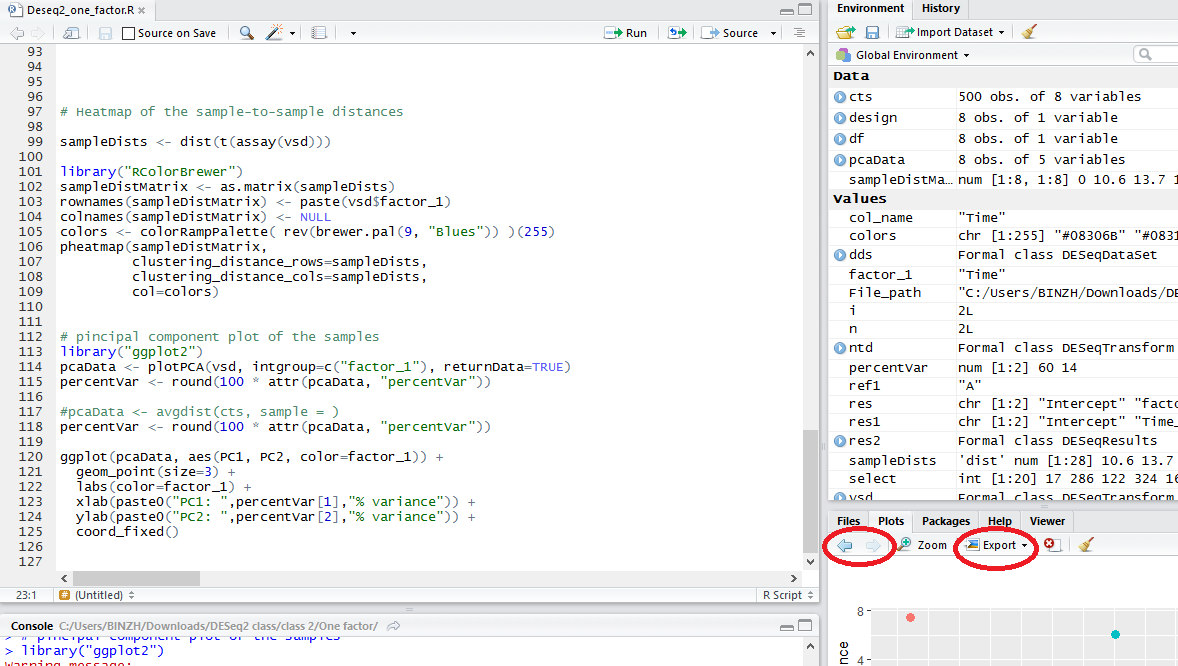
 

2. Open ‘EZ2RNAseq.R’ in Rstudio. Move cursor to command windows, press ‘Control+A’ in Windows or ‘command+A’ in Mac to choose all of the commands and click ‘run’.

A screenshot of a computer

Description automatically generated

3. Click arrows to choose figures and click ‘Export’ to export figures. The results of differentially expressed genes are exported to .csv files in the same folder.



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Notes:

1. Make sure there is no space in any cell of your input files.

2. The order of sample names in the first row of ‘data.csv’ should be the same as that in the first column of ‘design.csv’.

3. There will be two volcano plots for one differential expressed genes result. The second plot is restricted on the maximum value of x and y axis.