HeatmapGenerator3.1 Manual for Windows OS

I. Installation
   a. Prerequisites:
      i. HeatmapGenerator requires you to have R installed on your machine. Rscript must be accessible from the command line. To find out if you have this capability from Windows, create a command prompt by typing "cmd" in your Start search bar and clicking on "cmd.exe". Then type in: "where Rscript" (without the quotation marks, of course). If a directory path to the executable is successfully shown then the executable will run. If not, the user then needs to add the path to a global environment variable (see Appendix A) such that the program can find the Rscript executable.
      ii. *gplots* is a required R package for heatmap generation. A version of *gplots* is given with the downloaded executable bundle, so if it is not installed on your environment (for any reason) then the executable will install it. As of HeatmapGenerator3.1, *gplots* (and all internal package dependencies) should be automatically installed for you without any need for you to get involved in the process.
         1. *Note: as of HeatmapGenerator3.1, input files (i.e., text files) that are used to make a heatmap are no longer strictly required to be tab-delimited. They can be any mixture of spaces and tabs, as frequently happens when copy-pasting from an Excel spreadsheet environment into a plain text file.*
      iii. *stats* is also a required package for heatmap generation. *stats* is a general package that is downloaded in most R downloadable environments. As such, there is no need for you, as the user, to get involved in the process of installing it yourself.
   b. Installation
      i. To run HeatmapGenerator, simply click the .exe file to bring up the Graphical User Interface (GUI).

II. Create a new heatmap
   a. Change color
      i. To change the colors of the heatmap, click on the radio buttons for the low color value and for the high color value. Note that this must be done before the "Create New Heatmap" button is pressed, as this information is required by the R interpreter.
   b. Output file type
      i. The user can specify a .png output instead of a .tiff output and a .jpg output.
   c. Heatmap Type
      i. Simple heatmap uses the *heatmap* function
      ii. Advanced heatmap uses the *heatmap.2* function within *gplots*.
   d. Click on the "Create New Heatmap" button.
   e. Enter in the data matrix (i.e., select your .txt file of choice).

i. NOTE: Format of the input file must be exactly like the EXAMPLE.txt file. There can be no spaces in file names or gene names, and the file must be either tab-delimited or space-delimited or a mixture of spaces and/or tabs.
ii. Data can be continuous data or integers.

f. The resulting heatmap preview will show up in a new window.
i. Also created in the main directory of the program is a .jpg and a .tiff (by default). These images are high-resolution images that are publication-quality.

III. Get existing heatmap

a. To view existing heatmaps, the executable refers to the previously generated images in the main directory of the executable.
i. If the user clicks on a heatmap and clicks on "Choose from Existing Heatmaps" the same preview window will pop-up the heatmap that was previously generated.

Appendix A – Adding directory to global path in Windows

If the user does not have access to Rscript from the command line the user needs to do one of the following two options (depending on your computer setup):

1. Click on Start and open the Control Panel.
2. Click on User Accounts.
3. Click on the Change my environment variables in the left hand column.
4. Click on Edit for the PATH variable and add the path to the R executables. For example:
   a. Add ";C:\Program Files\R\R-3.2.2\bin" to the end of the "PATH variable"
      i. Notice here that the separation from what was written before is based on the semicolon; this could be different on your machine.
   b. Note: be sure to use the version of R that you have downloaded in the "PATH variable" name (e.g. if you have R version 3.1.2 on your computer but enter version 3.2.2 in the "PATH variable", you will get an error message. However, if you have R version 3.2.2 installed and add add ";C:\Program Files\R\R-3.2.2\bin" to the end of the "Path variable", it will work.
   c. Note: also make sure your command prompt window is closed when changing the "PATH variable." Otherwise, when you type "where Rscript" in the command prompt, the computer's response will not be updated.
5. Press OK on everything, close the Control Panel, and restart the executable.

OR:

1. Click on Start and **right-click** on Computer.
2. Click on Properties.
3. Click on Advanced system settings in the left hand column.
4. Click on Environmental Variables.
5. Find the variable "Path" and proceed to edit it: add the path to the R executables. For example:
   a. Add ";C:\Program Files\R\R-3.2.2\bin" to the end of the "PATH variable"

i. This is the directory that contains the R executable. It could be a different directory, depending on where you installed R on your computer. Simply make sure that you end your path with "\bin".

6. Press OK on everything and restart the executable.