Tutorial rShiny App Jelle Bonthuis, last updated 26-02-2023

**The program will run if all the correct settings have been selected, please note that it *will* generate errors if not everything is filled in. Additionally please use the different parts of the program separately, that is, once you have created an AGAR file, please rerun the program, fill in the necessary information and run the creation of the graphs. Or run the whole group analysis if you have the correct wholematrix files.**

**In case of error, rerun the R script. When performing a new section, rerun R script (should not be necessary but saver to do so).**

* **To always do (Tab1):**
  + - **The following options need to be done for *every* analysis.**
    - **Open app.R in R studio, run (ctrl+alt+r)**
    - **Data input: choose proteinGroup.txt files (provided numbered. If you want to analyse file 50, you have to input file 1 till 50). Whatever you input is still filtered by the excel file, so if you only want to analyse file 50, you will specify so in the excel file.**
    - **Choose excel file with experimental setup. Columns in file are grouped, so if you have: Agar1, agar2, agar3, GFP1, GFP2, GFP3, column 1 is : Agar1-3 and column 2 is GFP1-3. The order inside the files can be read with “create matrix with names of samples”.**
    - **Name of run**
    - **Select directory (will create a new folder with the name of the run in the selected directory)**
  + **In order to check which names are inside the proteinGroup.txt files you uploaded, you can click: “create matrix with names of samples”. You can use this to fill in the excel file.**
  + **Creating AGAR pool files (Tab1)** 
    - **select any AGAR pool options you want**
    - **Set the amount to reduce SD width of pooled agar**
    - **Click: “click here to create agar files”**
    - **You will find the created agar files inside the R saved data frame folder inside your current run folder (selected directory/[name of run]**
  + **Creating graphs (tab 2)**
    - **Upload a complexes excel file with each column being a complex and gene names of members in the rows below.**
    - **Check options that you would like (Mendoza imputation changes the imputation rules so that very large or very small values do not move towards the mean as much during imputation).**
    - **[optional] If you want to use AGAR pool files, click the checkbox then uploads the AGAR LFQ file, then select it in the drop down window**
    - **Set options you would like for the graphs.**
    - **Create all graphs, or cycle through them (will give error if PDF’s of files are open when they are remade. Therefore either change the run name/folder when changing options, or close all graphs before rerunning).**
  + **Performing whole group analysis (tab 3)**
    - **Upload a complexes excel file in tab 2**
    - **Upload the 4 different RDS files found in R saved dataframes (created after running the previous steps) (tab 3).**
    - **Uploaded RDS files can be accessed/changed (deselecting particular experiments is useful as they can obscifucate some results).**
    - **Check/set options**
    - **Click (IN ORDER!!!): create whole matrix graphs, create UMAP samples, create heatmap.**
    - **Open graphs (UMAP heatmap etc) when rerunning with different settings will lead to errors, please close all before running.**