Function: Normalization\_ionomycin()

Filename: Normalization\_ionomycin.R

Description: This function takes drug response file and ionomycin response file from a Calcium Imaging dataset as input and gives out a file with F/Fmax values for all the cells that meet threshold (mean+5st.dev) for both ionomycin and drug as shown below:

To compute the steps in the flow chart this function calls two other functions: Threshold() [all the blue boxes in the flow chart] and Threshold\_iono() [all the red boxes] and only does the green box work on its own. Threshold() and Threshold\_iono() can also be used as standalone functions – their functionality is fully explained below.

Variable List:

Numeric

* i,j - row and column identifiers within loops
* percent - % of cells that responded to the drug and had an ionomycin response
* flag- used to flag presence of columns with NA

Matrices

* Analyzed- ionomycin normalized matrix
* new.analyzed – analyzed matrix of responder cells only with column names intact from original input file
* A\_new (from Threshold()) – see below

Array

* orig.cols – stores original column names
* new.cols – after deleting all the cells that did not respond to either drug or ionomycin

In-built R function list:

* readline()- to prompt users what to enter
* paste() -concatenate words
* array() – make array
* isTRUE() – tests if a statement is true
* write.csv() – make a .csv file as output
* identical(, FALSE) – see if a statement is false
* is.na() – tests if there is NA in the file
* rbind() – appends row in a matrix
* apply() – recursive function

Function: Threshold()

Filename: Threshold.R

Description:

This function computes Ca Imaging response of a drug application by subtracting the peak response from baseline as shown in flowchart below:

A .csv file (obtained from ImageJ), frame numbers that denote baseline and frame numbers that denote peak response are taken as input from the user

Only cells that meet the threshold of (baseline mean+2St.dev) and their column names are returned

The output from this function are:

* Matrix of the red box in flowchart (Data after subtraction baseline mean from all points in responder cells) – There is an option to print this matrix in a file in the function.
* Percentage of responders

Variable List:

Numeric variables

* i,j – row and column identifiers within loops
* r1,r2,r3,r4- inputing range of row numbers for baseline mean and peak mean calculation
* count –counting number of cells that cross the threshold (responders)
* percent - % of responders

Matrices

* A – The input file is converted into this matrix
* nob- matrix after background intensity is subtracted
* nor- matrix after mean of baseline is subtracted from each intensity data point
* M- subset matrix of nob to calculate baseline mean for all columns
* M1, Mp – subset matrices of nor to find threshold
* A\_new- final matrix with processed numbers of responder cells

Vectors

* Back –background intensity
* Mm– mean of baseline in nob
* St1- standard deviation of mean of baseline in columns

Arrays

* Mma- array of M
* orig.cols- original column names of the input file is preserved here
* Mm1, threshold, Mmp- arrays of mean of baseline, threshold and peak respectively

Strings

* File, filename- makes input filename into readable format
* outputfile – accepts the output file name from user

List

* Matandcolnames – list of A\_new and orig.cols

In-built R function list:

* readline()- to prompt users what to enter
* paste() -concatenate words
* read.csv() – input a .csv file
* array() – make array
* ncol(), nrow() – total number of columns, rows in a matrix/array
* as.numeric() – convert input row numbers into numbers. R treats all input as strings
* colMeans() – compute mean of columns
* matrix() – create a matrix
* isTRUE() – tests if a statement is true
* write.csv() – make a .csv file as output
* list() – since R does not allow return of multiple objects from a function, this is a way to get more than one thing from a function – in this case both the final matrix and the column names are returned
* print() – to print
* assign() – in this case it is used to make a list available to the global environment so that all functions that call this function can use it

Function: Threshold\_iono()

Filename: Threshold\_iono.R

Description: THIS FUNCTION IS FOR IONOMYCIN ONLY – but very similar to Threshold(). Inputs Ionomycin response file to return a matrix with ionomycin response subtracted from baseline mean for the cells that respond to ionomycin (Threshold=mean+5st.dev). There is an option to save this file. Also returns mean peak response of those cells and percentage of cells that responded to ionomycin.

Variable list: Very similar to Threshold() above. Some differences are listed below:

Deletions: orig.cols, matandcolnames

Replacements: A.new – does same work as A\_new in Threshold()

Additions:

Matrix:

* A.iono – A matrix to preserve peak responses after background removal
* Peak =subset matrix of A.iono to hold peak responses

Array

* Mean\_peak – column mean of Peak matrix described above

In-built function list: Same as in Threshold()