

Processing KO data

Step 1. Data normalization

Input files (required):

- | | | |
|---|---|--|
| 1 | map of the KO combinations | files/geneIndex.csv |
| 2 | map of gene indices to original gene name | files/geneNames.csv |
| 3 | list of lower matrix NaN gene indices | files/nan_lower_gene_index.csv |
| 4 | list of upper matrix NaN gene indices | files/nan_upper_gene_index.csv |
| 5 | raw data file for each condition | data/ 15C.csv and data/ No Treatment.csv |

Example:

Script: 1_normalize_data.R

Libraries required: amap, gplots, extrafont, optparse

Usage:

Rscript 1_normalize_data.R -d [data directory path] -f [files directory path] -o [output directory path] -r [number of replicates] -w [number of wildtype matrix]

Example:

Rscript 1_normalize_data.R -d /Users/violaholder/Desktop/GitHub_GI_Analysis/data -f /Users/violaholder/Desktop/GitHub_GI_Analysis/files -o /Users/violaholder/Desktop/GitHub_GI_Analysis/output -r 3 -w 15

Expected output files:

- | | | |
|---|--|--|
| 1 | normalized data for each condition and replicate | output/allData.csv |
| 2 | average normalized data | output/avgData.csv |
| 3 | heatmap of average normalized data | output/ 15C_ODfull.pdf & output/ No Treatment_ODfull.pdf |

Example:

Step 2. EPS (Genetic Interaction score) calculation

Script (jupyter notebook): 2_calculate_genetic_interaction.ipynb *Packages required:* numpy, pandas

User-defined variables: Definition:

- | | | |
|---|----------|--|
| 1 | cdn | which condition to process (15C or No Treatment) |
| 2 | num_reps | number of replicates |
| 3 | main_dir | main directory path |

Expected output file:

An Excel file per condition, with R+1 number of sheets, where R=number of replicates; last sheet is for average data.

Every sheet has the following columns: Gene_X, Gene_Y, Fitness_X, Fitness_Y, Fitness_XY, Fitness_YX, EPS_XY, EPS_YX

Average sheet has two additional columns - Average_Fitness_XY_Fitness_YX & Average_EPS_XY_EPS_YX

Example: 15C_fitness_and_eps.xlsx & No Treatment_fitness_and_eps.xlsx

Step 3. Run t-test to get p-values and check significant data for heatmaps

Script (jupyter notebook): 3_run_ttest.ipynb *Packages required:* numpy, pandas, scipy

<i>User-defined variables:</i>		<i>Definition:</i>
1	cdn	which condition to process (15°C or No Treatment)
2	num_reps	number of replicates
3	main_dir	main directory path
4	Pval_cutoff	Change between 0.05, 0.01, or 0.001 depending of level of significance wanted
5	Eps_cutoff_pos	positive interaction eps cutoff; update the value as needed
6	Eps_cutoff_neg	Negative interaction eps cutoff; update the value as needed

Expected output files:

- i. Excel files containing p-value data for the following comparisons:

a) experimental vs actual_XY
b) experimental vs actual_YX
c) actual_XY vs actual_YX
 $experimental = Fitness_X * Fitness_Y$
 $actual_XY = Fitness_XY$
 $actual_YX = Fitness_YX$

Examples:

output/cdn1_pvalue_exp_vs_actual_XY.csv
output/cdn1_pvalue_exp_vs_actual_YX.csv
output/cdn1_pvalue_actual_XY_vs_YX.csv

- ii. Excel file containing compiled data of above files

Example: output/15C_pvalue_compiled.csv & No_Treatment_pvalue_compiled.csv

Step 4. Generate heatmaps

Script (jupyter notebook): 4_generate_fitness_GI_significant_heatmap.ipynb

Packages required: numpy, pandas, scipy, seaborn

User-defined variables:		Definition:
1	cdn	which condition to process (15°C or No Treatment); can process both to generate hierarchical clustering
3	main_dir	main directory path

Expected output files:		Example:
1	Fitness heatmap per condition	15C_Fitness_Score_heatmap.png & No Treatment_Fitness_Score_heatmap.png
2	Genetic interaction heatmap per condition	15C_Genetic_Interaction_heatmap.png & No Treatment_Genetic_Interaction_heatmap.png
3	Significant data heatmap per condition	15C_Significant_data_heatmap.png & No Treatment_Significant_data_heatmap.png
4	All_condition fitness hierarchical clustering	All_cdn_Fitness_Score_clustermap.png
5	All_condition genetic interaction hierarchical clustering	All_cnd_Genetic_Interaction_clustermap.png
6	Single deletion Fitness score heatmap	Single_Deletion_Fitness_Score_heatmap.png