ELISA_BUNDLE

Finn Lobnow

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Contents

Prerequisites

This part should be interesting to you once you have performed an ELISA as described in . You should have data that ideally contains a positive and negative control, as well as a plate blank, and you should have the raw measurements from the plate reader.

Example Files

If you have access to data-tay, you can find everything here: ELISA_BUNDLE If you don't have access to data-tay, you can find everything on my Github: ELISA_BUNDLE

This folder contains the ELISA_BUNDLE with a complete example set. Transfer this folder to your computer (drag and drop or copy and paste to a location of your choice).

What you can find in the ELISA_BUNDLE folder

- archive: contains a static example output folder
- input: put your prepared excel files here
- example_data: I have prepared an example data set to ensure that you can run the script without any errors
- ELISA_CL_KEY.csv
  - a static copy of the cell line information
  - the most up-to-date version is stored on my GitHub
  - if you need to make changes, you can add your new cell lines to this file
  - column documentation:
    - CELL_LINE: what is the name you provided on the raw plate file?

        * CL_NUMBER: what is the number in our cell line database?
        * CL_NAME_ON_PLOT: what name should be displayed on the plot?
        * PLOT_ID: concatenated CL_NAME_ON_PLOT and EXPRESSION_LVL
        * PURPOSE: what was the purpose of the cell line? Was it a sample or a control?
        * PROMOTER: was a strong or weak promoter used? e.g. dSV40 or SFFV
        * ORIGIN: are the components from Mouse or something else?
        * PLOTTING_COLOR: what color should the cell line have in the plot?
        * EXPRESSION_LVL: were the cells sorted for endogenous expression or high expression?
        * ORDER_NO: how do you want the cell lines to be ordered in the plot?
        * INITIALS: who generated the cell line?
        * INFO: anything noteworthy?
        * COHORT: handy if you also analyzed this cell line with our Image Analysis Pipeline
- ELISA_BUNDLE.Rmd
    - a copy of what you are reading here
- ELISA_TEMPLATE.R
    - the script that will be run to analyze your data
    - keep this template and copy the folder for each analysis to keep a record of your analysis

Analysis Steps

How to prepare your excel sheets for Analysis

Take a look at the example data in the **example_data** folder. You can use this as a template for your own data. Please make sure that you name the files and sheets as described in the example data. (YYYYMMDD_Plate_X) The script will look for the following sheets in your excel files:

- MEASUREMENTS: contains the raw measured values per plate
    - each plate should contain at least two standard replicates, ideally also a positive + negative control
- CELL_LINES: contains the cell line names, standard curve concentrations, and where the BLANK is located
- STIM_DAYS: contains the stimulation days (numeric, e.g. 1,2,3,..)
- STIM_TIME: how long you stimulated the cells (e.g. 24 hours)
- STIM_CONCENTRATION: how high was the final stimulation concentration with your stimulant (IL-1ß, LPS)?
    - normal IL-1ß concentration is 5ng/mL
        * How is that calculated? We dilute 2µL IL-1ß in 10mL RPMI medium (essentially 20ng/mL), but since we add 50µL of this IL-1ß containing medium to 150µL medium with settled cells, the final concentration is 5ng/mL. For LPS stimulations, 100ng/mL were used.
        * Only the stimulated wells are marked, the unstimulated and the standards can be left blank or as NA
- CONDITIONS: which wells are stimulated, unstimulated, or used for calibration?
    - Stimulated wells –> STIM
    - Unstimulated wells –> UNSTIM
    - Wells that were used for calibration –> CALIBRATION
    - empty wells can be left blank or as NA
- DILUTIONS: did you dilute the samples? (e.g. 1:5 would mean that each cell contains a 5)
    - The standard protocol suggests a 1:5 dilution for all sample wells (control and other cell lines), but adjust for other dilutions as needed
    - Note for ELISA assay planning:
        * dilute the positive control 1:10 to allow all other samples more developing time
        * you need to stop the assay when the color reaches a high intensity
        * Measured values 1.3 are good, anything above should be taken with a grain of salt
- PATHWAY: what pathway are you interested in? (e.g. IL-1R, TLR4, etc.)
- STIMULANT: what stimulant did you use? (e.g. IL-1, LPS, etc.)

- Info: any additional information you want to add to the plot

How to run the example data

You don't need to change anything in the ELISA_TEMPLATE.R to run the example data. I woud advise to open the script in R and take a look at the script to understand what is happening. To run it, just open ELISA_TEMPLATE.R and click **Source** on the top right of the script window. This will run the script and create the output folder with the plots and tables. The output folder should now contain:

- ELISA_rltv.svg: a plot of the relative secretion values that was normalized based on the negative control (lowest value of the negative control was subtracted) and the positive control (~1)
- ELISA_real.svg: a plot of the real secretion values that were extrapolated based on the standard curve
- ELISA_fold_change.svg: a plot of the fold change values that were calculated based on the fold change between the stimulated and unstimulated wells per cell line. The statistice were performed based on the fold change value of the respective negative control.
- ELISA_ANALYSIS.xlsx: a table with the raw data, the normalized values, the real values, and the fold change values

How to prepare and run your own data

To run your own data, you should copy the ELISA_BUNDLE folder and rename it to your liking. Open the ELISA_TEMPLATE.R script and adjust file paths to your own data:

- line 60: instead of the example_data folder, you should point to the **input** folder that contains your data

Adjust the actual data:

- line 85 (part 1 ADD YOUR DATA & NORMALIZE IT): instead of the example data, you prepare your own data (e.g. you could filter for the plate dates of interest, stimulation days, whatever is necessary for you). You should also provide the respective positive and negative control names that you used in your experiment. This is important for the normalization of the data. Make sure you normalize the different data sets you provided.
- line 115 (part 2 BIND THE DATA OF INTEREST): bind the normalized data batches you wish to visualize below

Plot and save your data

- If you run the **RELATIVE_SECRETION** or the **REAL_SECRETION** chunks, you can plot the data as you wish
  - I have provided the simplest use case with a easy-plot function, but there is also a ggplot chunk that you can use to plot the data to your needs
- If you want to change the colors of the cell lines, you can do so in the **ELISA_CL_KEY.csv** file
- If you run the **SAVE** chunk, you can see that the plots and a new Excel file are created in the output folder
- If you run the **FOLD_CHANGE** chunk, you can see that the fold change plot is created in the output folder as well

Troubleshooting

If you run into problems, please make sure that you have the correct file paths and that the data is correctly formatted. If you still run into problems, please reach out to me (flobnow@gmail.com) and I will help you out.