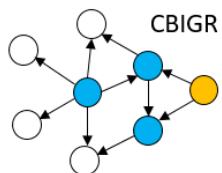


# HTSplotter

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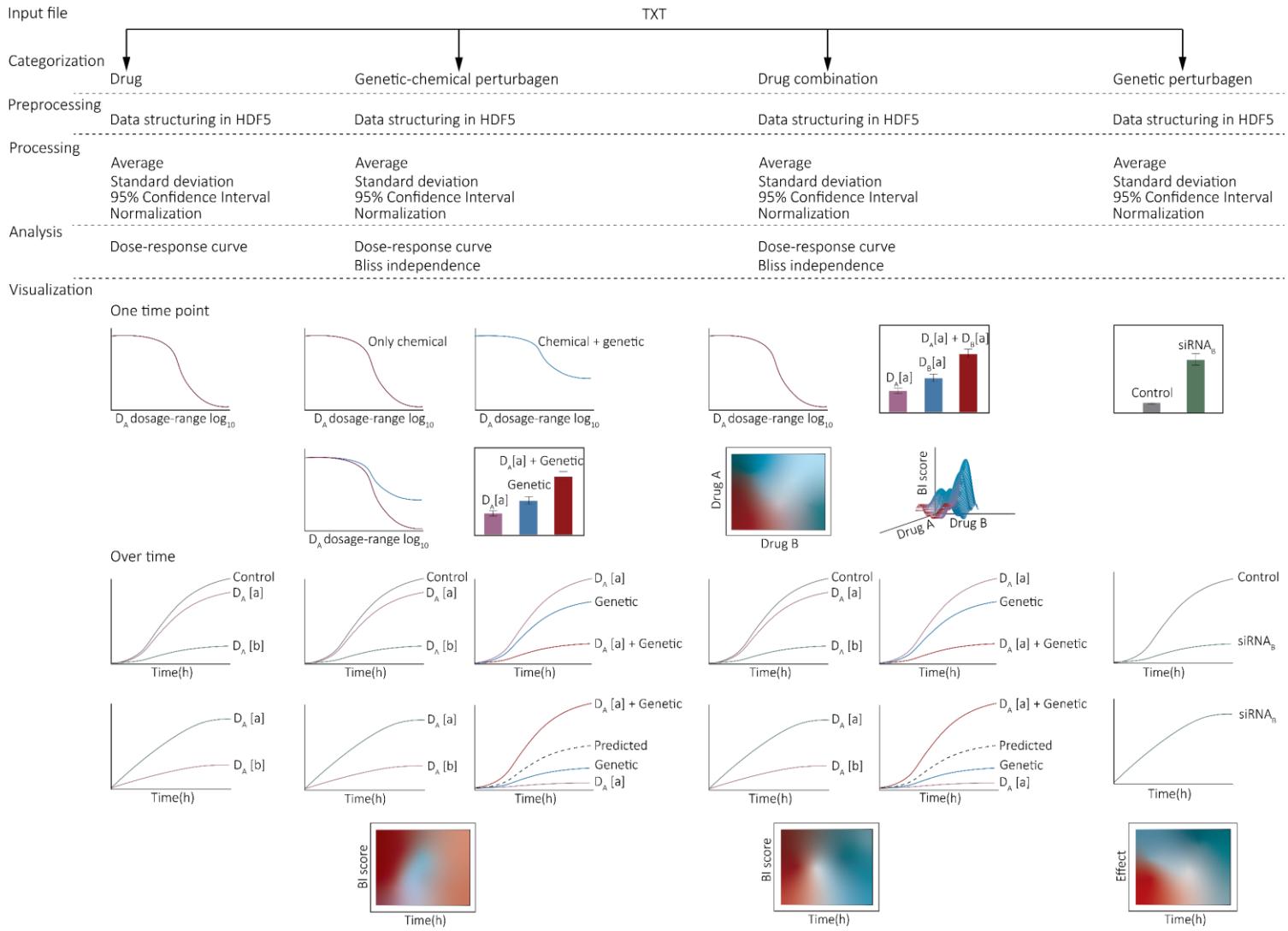
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## HTSplotter

HTSplotter allows an end-to-end data processing, analysis and visualization of chemical and genetic *in vitro* perturbation screens. It is freely available as a web tool at <https://htsplotter.cmgg.be/>, or as Python module, <https://github.ugent.be/vermeirssenlab/HTSplotter>.

HTSplotter is tailored to analyze drug, drug combination, genetic perturbagen and combinations of genetic-chemical perturbagen screens. These experiments can be conducted either in real-time or with endpoint readout. HTSplotter identifies the type of experimental setup through a conditional statement algorithm. It then performs a normalization and, in case of a drug screen, drug combination or genetic-chemical perturbagen experiment, identifies the dose-response relationship for each drug alone. Additionally, synergism or antagonism of drug or genetic-chemical combination screens is determined through the Bliss independence(BI) method. Finally, results are plotted and exported as PDF files, allowing a fast biological interpretation of the data.



*Figure 1: Overview of the HTSplotter steps for each type of HTS experiment. The input file, directly imported from high throughput screens (HTS) machines like Incucyte S3 as a TXT file, is automatically processed and analyzed by HTSplotter. As output, TXT and PDF files are generated. The PDF file contains different visualizations of each type of analysis.*

## Run analysis

On the “Run analysis” tab, one can submit one or more files from high throughput screening (HTS) machines, see Figure 2. Once all the fields are filled, click on the button “Run analysis”.

The screenshot shows the 'Run your own analysis' page of the HTSplitter interface. At the top right are buttons for 'Run analysis' (highlighted in blue), 'Manual', and 'Feedback'. The main area contains several input fields:

- 'Input file(s)': A 'Choose Files' button with the message 'No file chosen'. Below it is a note: 'Upload one or in case of biological replicates analyses more all TXT-files' and 'File names without space'.
- 'Biological replicate analysis': A dropdown menu showing 'no' (selected) and 'yes'.
- 'Biological replicate desired filename': An input field.
- 'Expected effect': A dropdown menu showing 'inhibition' (selected) and 'enhancement'.
- 'Information readout': An input field.
- 'Readout unit': An input field.

At the bottom are two buttons: 'Reset' and 'Run analysis'.

Figure 2: Main page of ‘Run analysis’. The analysis can be for one file or in case of biological replicates more than one file. The expected effect can be inhibition or enhancement.

Analysis of different biological replicates, please check Figure 3.

Analysis of one experiment, please check Figure 4.

## Run your own analysis

Input file(s)

Choose Files 3 files

Upload one or in case of biological replicates analyses more all TXT-files  
File names without space

Biological replicate analysis

yes

Biological replicate desired filename

drug\_combination\_biological\_replicates

Leave empty if no replicate analysis

Expected effect

inhibition

Information readout

confluency

Readout unit

%

Reset Run analysis

Figure 3: Example of the analysis of a biological replicate, in which each file has experimental data.

## Run your own analysis

Input file(s)

Choose Files drug\_combi...ditions.txt

Upload one or in case of biological replicates analyses more all TXT-files  
File names without space

Biological replicate analysis

no

Biological replicate desired filename

Leave empty if no replicate analysis

Expected effect

inhibition

Information readout

confluency

Readout unit

%

Reset Run analysis

Figure 4: Example of a file analysis.

## Run your own analysis

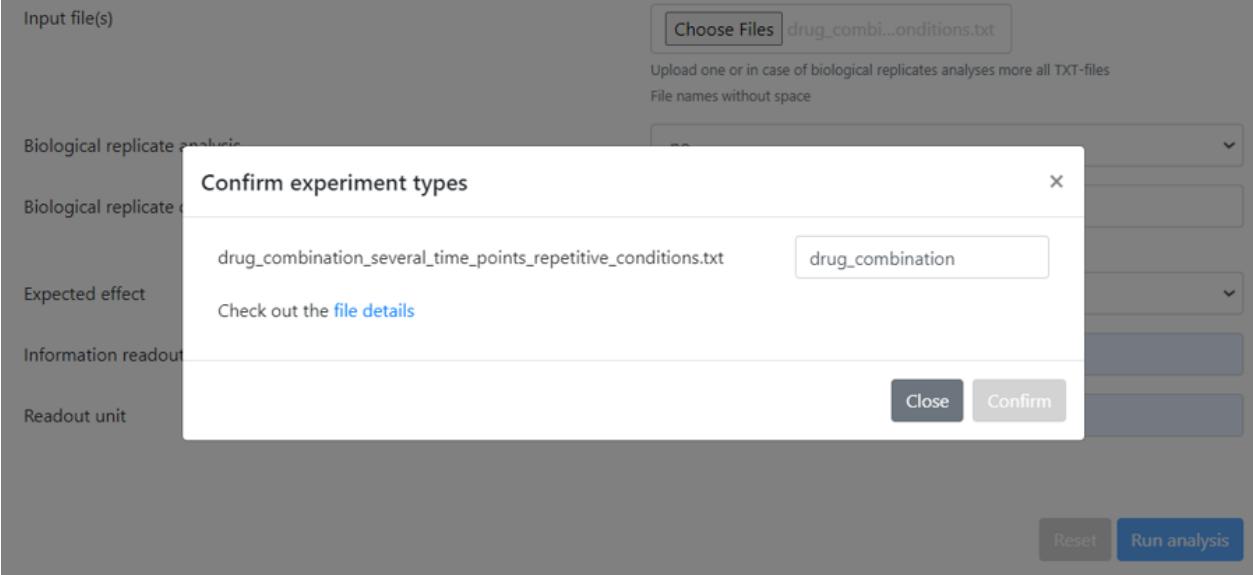


Figure 5: After analysis submission, a “Confirm experiment types” window pops-up. To check the details from the file, click on the “here” button. In case of a wrong experiment categorization, please correct in the box of the experiment type. Please check Figure 9 for more information regarding experiment types. In case of correct categorization click on the bottom “Confirm” and check Figure 7 for more details.

## Run your own analysis

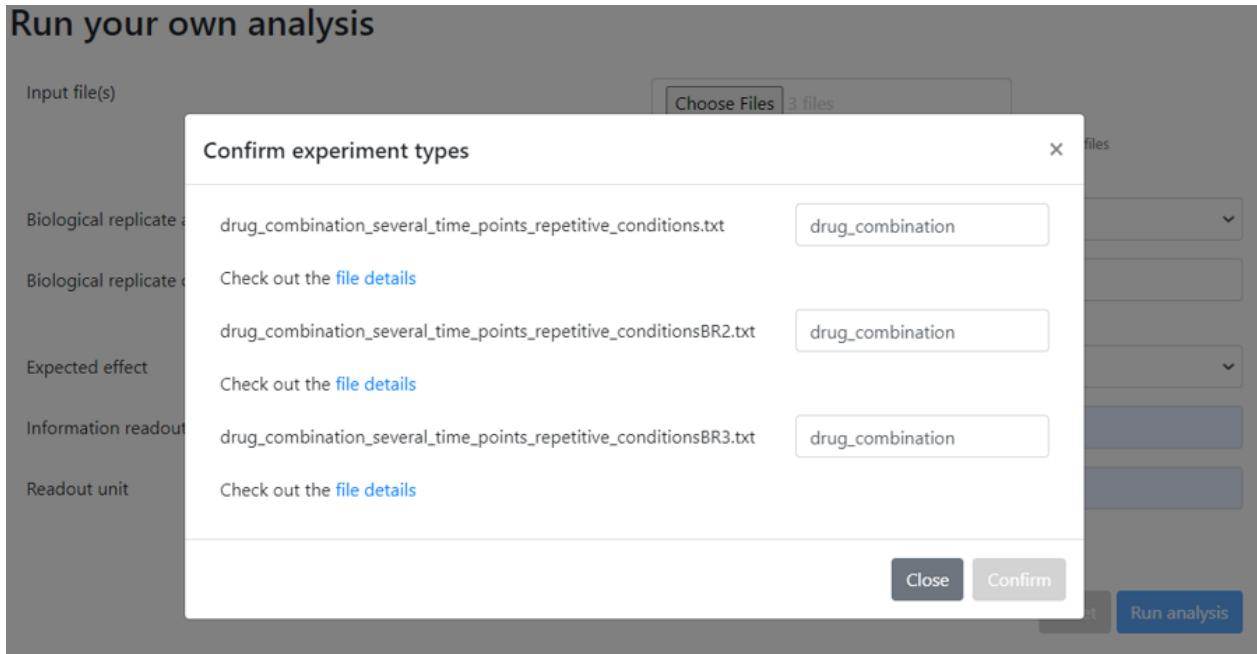


Figure 6: After analysis submission, a “Confirm experiment types” window pops-up. In case of more than one file, one can check details from each file, clicking on “here” button. In case of a wrong experiment categorization, please correct in the box of the experiment type. Please check Figure 9 for more information regarding experiment types. In case of correct categorization click on the bottom “Confirm” and check Figure 7 for more details.

## Run your own analysis

Input file(s)

Choose Files drug\_combi...onditions.txt  
Upload one or in case of biological replicates analyses more all TXT-files  
File names without space

Biological replicate analysis

no

Biological replicate desired filename

Leave empty if no replicate analysis

Expected effect

inhibition

Information readout

confluency

Readout unit

%

Analysis finished successfully. Download your results [here](#).

Reset Run analysis

Figure 7: Once the analysis is successfully run, one can access to the results by clicking on the button “Download all”.

## Run your own analysis

Input file(s)

Choose Files drug\_combi...\_on\_units.txt  
Upload one or in case of biological replicates analyses more all TXT-files  
File names without space

Biological replicate analysis

no

Biological replicate desired filename

Leave empty if no replicate analysis

Expected effect

inhibition

Information readout

confluency

Readout unit

%

Something went wrong. Please click [here](#) to see the error.

Reset Run analysis

Figure 8: If any main information is missing, such as concentrations, cell line name, seeding, drug name, concentration and units or control the following message pops-up. The button “click here” links to the “error” file generated by HTSplotter. For more details, go to the [Error file](#) section.

## Run your own analysis

Input file(s)

drug\_combi...timepoint.txt

Upload one or in case of biological replicates analyses more all TXT-files  
File names without space

Biological replicate analysis

Biological replicate desired filename

Leave empty if no replicate analysis

Expected effect

Information readout

Readout unit

Experiment type(s) did not match. [Click here](#) to see the error.  
Please fix this issue and resubmit.

Figure 9: If the experiment categorization by HTSplotter does not correspond to the expected one, the following message is shown. Click on the “click here” button to check the information file generated by HTSplotter.

## TXT file

Figure 10 is an example of a TXT file layout.

In grey is the information ignored by HTSplotter.

Headers should start with “Date Time” followed by the “Elapsed” word. From the third column onwards the experiment conditions should be present.

If each condition has a standard deviation, it should be included after the last condition and follow the same order. For all standard deviations, the labelling must be the same as the experiment condition but with “(Std)” at the end, as shown in Figure 10.

Repetitive experiment conditions are allowed in case of standard deviation being absent. The conditions can be randomly ordered in the file.

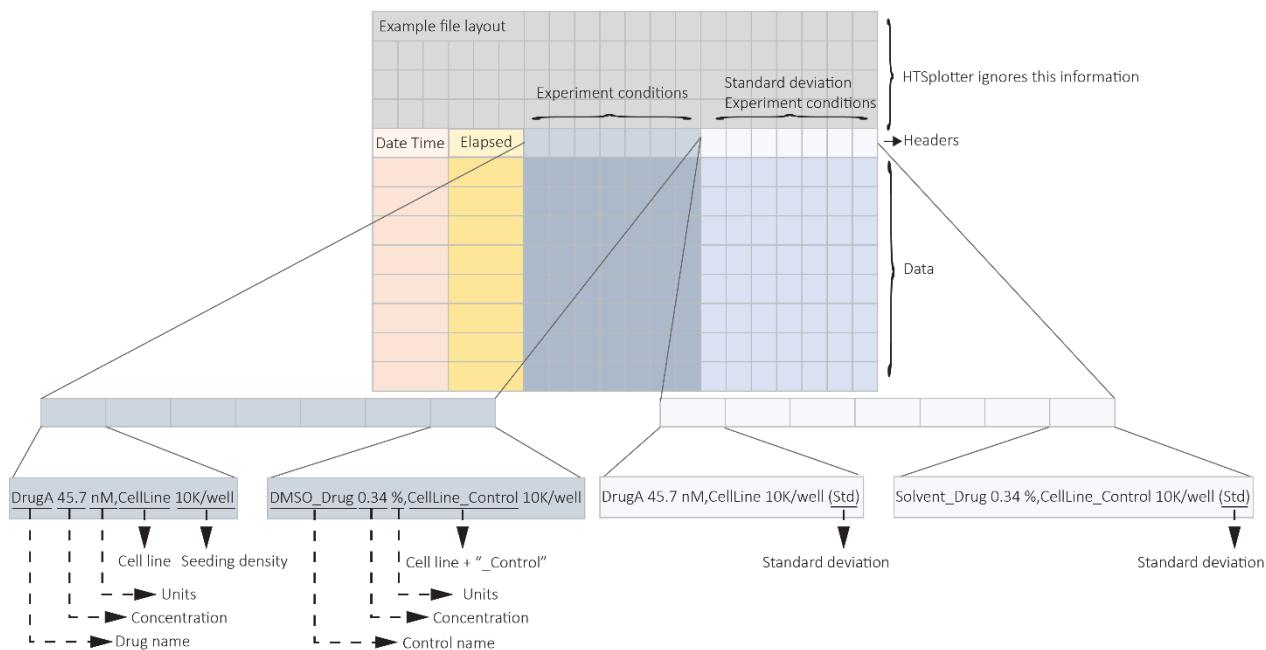


Figure 10: Example of a TXT file layout with a description of an example of an experiment condition and control. In the same order, is the standard deviation of the same experiment condition and of the same control.

### Date, Time and Elapsed

The “Date Time” column, should have date and time information about the read-out. This field cannot be empty.

- e.g. 09/07/2021 12:20 (Figure 11).

The “Elapsed” column is the experiments time point(s). Only integers numbers are allowed Figure 11.

The experiment may have more than one time point.

- e.g. read-out each 2h during a period of time of 16h Figure 2-A.

The experiment may also have a unique time point.

- e.g. read-out at 12h from the start of the experiment Figure 2-B.

A	B
Date Time	Elapsed
09/10/2020 12:20:00	0
09/10/2020 14:20:00	2
09/10/2020 16:20:00	4
09/10/2020 18:20:00	6
09/10/2020 20:20:00	10
10/10/2020 00:20:00	12
10/10/2020 02:20:00	14
10/10/2020 04:20:00	16

Figure 11: Example of “Data Time” and “Elapsed” information in case A when over time measurements were performed and B for one time point.

Experiment types and general information:

HTSplotter automatically identifies four experiment types per txt file:

Drug screen:

- If two or more dosages of a certain drug were tested in one or more cell lines.
- One control per compound is required, or one control for each cell line.

Drug combination screen:

- If at least two drugs were identified in one condition.
- One control per cell line is required.

Genetic perturbagen screen:

- In the case of a simple knockdown or overexpression of a gene, for example a CRISPR/Cas screen, CRISPRi or siRNA library.
- More than one control is allowed.

Genetic-chemical perturbagen screen:

- In the case of a genetic perturbagen in combination with a drug.
- Here a tag should be added to the drug name, for example: Drug-A\_GeneOff, where:
  - Drug-A is the name of the drug.
  - “\_GeneOff” is a tag indicating the genetic perturbagen. In this case it indicates that an expression of a certain gene is being repressed.

More details about each experiment type can be found in their respective section.

A correct labelling of experiment conditions is crucial, thus each one must contain drug and cell line information.

**Drug information** is the group of all drugs present in that condition.

- For each drug, a drug name following the respective concentration and units has to be present, see Figure 12.

- In case of a genetic perturbagen screen, the labelling of each condition should follow the same requirements as the drug, which is the genetic perturbagen name, concentration and units.
- In case of a genetic-chemical perturbagen screen, hence the combination of a drug with a genetic perturbagen, the drug name must have a tag indicating the genetic perturbagen, for example: Drug-A\_GeneOff, where the tag is “\_GeneOff”.

**Cell line information** is the cell line name and seeding description, see Figure 12.

The conditions may be noted in two different ways:

- Each information separated by “,”.
  - In this case the labelling should start with drug information and at the end the cell line information, see Figure 12-A.
- Each information separated by space.
  - In this case the cell line information must be in the first position followed by all drug information, see Figure 12-B.

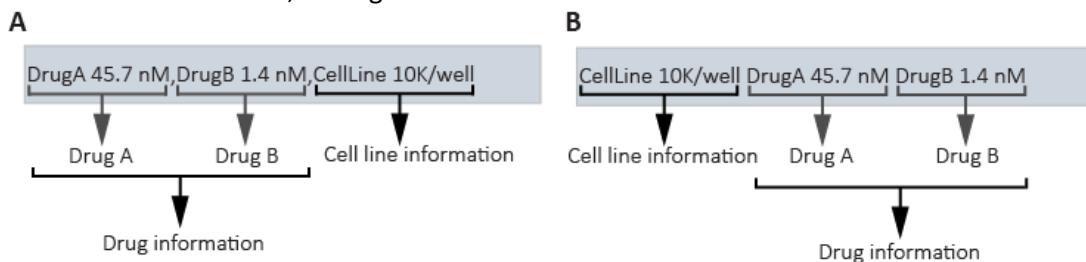
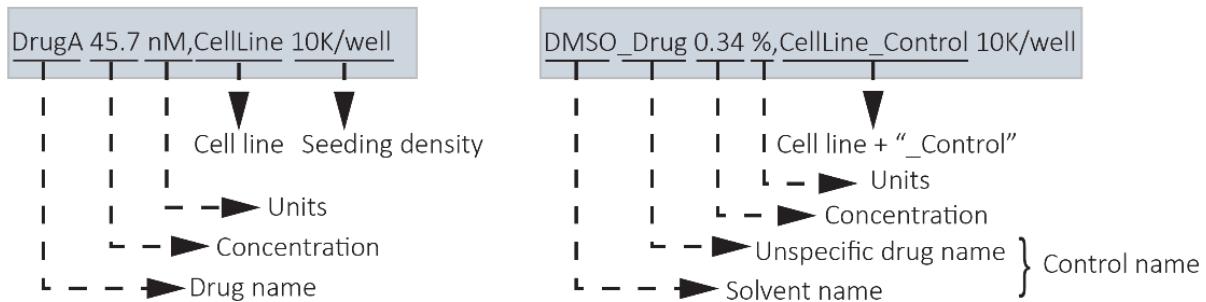


Figure 12: Example of different ways to annotate conditions. A) information separated by “,”. B) information separated by space.

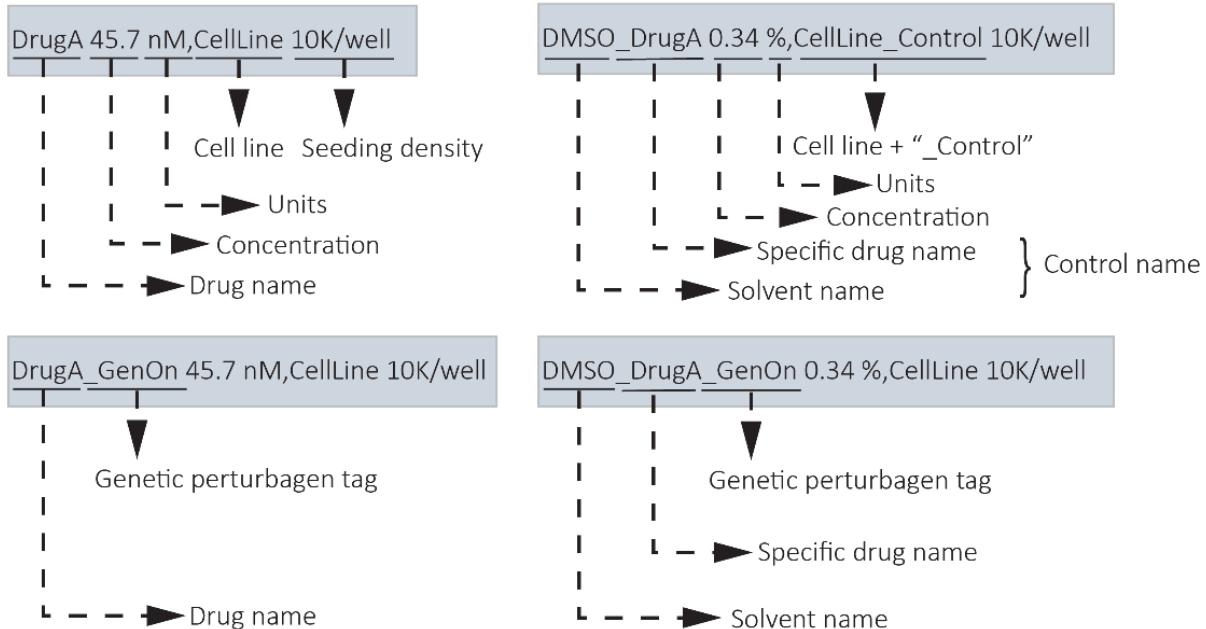
### Labelling conditions

The labeling of the control differentiates from all the other conditions as shown in Figure 13.

A



B



*Figure 13: Example of labeling the experiments conditions vs control. A) Example of a drug screen experiment, where the control name is not specific for the tested drug. The tag “\_Control” is added to the cell line name. B) Example of a genetic-chemical perturbagen experiment, where the control name must be specific for each tested drug. The tag “\_Control” is added to the cell line name. The control for this experiment type is the condition where the solvent is tested without the genetic perturbagen.*

Table 1 is the information required to label a perturbagen and a control condition.

*Table 1: Information required for labeling. \* in case of genetic perturbagen experiment the labelling follows the same rule as the drug screen, whereas in case of a genetic-chemical perturbagen screen, a tag is required in front of the drug name, e.g. “DrugA\_GenOn”. \*\*for Drug, genetic and genetic-chemical perturbagen, different controls are allowed. Therefore, in front of cell line name the tag “\_Control” needs to be put. Without this tag, HTSplotter cannot identify the experiment control.*

Perturbagen		Control	
Drug information	Cell line information	Solvent	Cell line
Drug name or gene name*	Cell line name	Control name**	Cell line name + “_Control”
Concentrations	Seeding	Concentration	Seeding
Units		Units	

## Error file

In case of any missing information, an **error file** is provided as shown in Figure 14 and Figure 15.

You have an error from your header, please check bellow

cellline, seeding, condition, compound, concentration, units, position from the input file

'unidentified', means that the information is missing.

check in front the column position in your file

Column number

Input file headers:

MCF7, 10Kperwell, Condition, MK-1775, 45.7, unidentified, [0]

MCF7, 10Kperwell, Condition, Prexasertib, 1.4, unidentified, [1]

MCF7, 10Kperwell, Condition, MK-1775\_Prexasertib, 45.7\_16, unidentified, [2]

MCF7, 10Kperwell, Condition, MK-1775\_Prexasertib, 45.7\_10.7, unidentified, [3]

Figure 14 Example of an error file when the units information is missing. HTSplotter writes "unidentified" at the expected position. At the end of each row, between straight brackets, is the referred position in the txt file.

You have an error from your header, please check bellow

Information order: cellline, seeding, condition, compound, concentration, units, position from the input file

'unidentified', means that the information is missing.

Between square brackets is the column position from your input file

**Control unidentified** : Please indicate the control adding "\_Control" to the cell line name

Figure 15 Example of an error file when the condition control was not identified. This information is at the end of the error file.

Notice that every "/" symbol will be replaced by "per" word.

## Numbers

HTSplotter only accepts "." as decimal markers.

## Information file

For each input file submitted successfully at the HTSplotter analysis, a txt file is generated with all the extracted information from the headers as shown in Figure 16. This file is called as the experiment file name plus "\_information.txt".

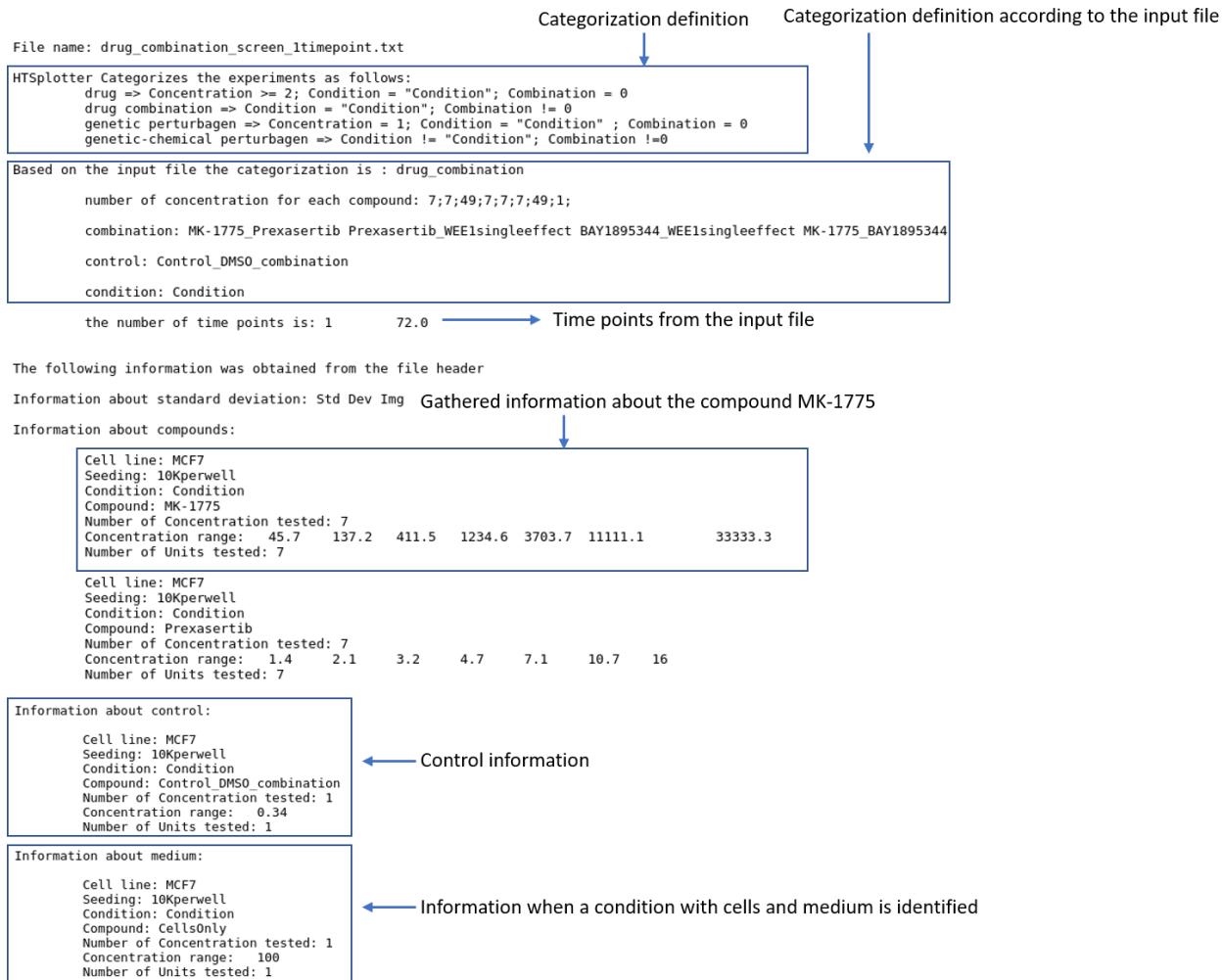


Figure 16: Example of an information file from a drug combination experiment with only one read-out. In case of drug combination, make sure that the number of combined conditions matches the product of the number of the dosage tested and non-combined compounds. For example for the combination MK-1775\_Prexasertib, the number of condition is 49, while for MK-1775 and Prexasertib alone is 7, so  $7 \times 7 = 49$ .

## Drug screen

HTSplotter categorizes an experiment as drug screen if for a certain drug, more than two dosages were tested in one or more cell lines.

For this experiment two different set-ups are accepted: for each cell line there is a unique control or for each tested drug there is a respective control.

There is no limit on the number of tested drugs and on the number of cell lines tested. Additionally, different drugs can be tested for each cell line.

### Example files

Example input files with their results are provided at :

<https://htsplotter.cmgg.be/>

#### Example of 1 time point

Input file:	drugscreen_1timepoint.txt
Experiment details:	Read-out: 1 time point Details: Dosage range of MK-1775, prexasertib and BAY1895344 tested on the cell line MCF-7 Only 1 control
Output file:	txt file      drugscreen_1timepoint_IC.txt (statistical parameters from the dose-response curve) pdf file      drugscreen_1timepoint_information.txt (Extracted information by HTSplotter) hdf5 file      drugscreen_1timepoint.pdf (plotted results) drugscreen_1timepoint.hdf5 (The hdf5 has the data structured, which can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

#### Example of more than 1 time point and only one control:

Input file:	drugscreen_severaltimepoint_1control.txt
Experiment details:	Read-out: each 2 hour during 72h Details: Dosage range of MK-1775, prexasertib and BAY1895344 tested on the cell line MCF-7 Only 1 control
Output file:	txt file      drugscreen_severaltimepoint_1control_IC.txt (statistical parameters from the dose-response curve) pdf file      drugscreen_severaltimepoint_1control_information.txt (Extracted information by HTSplotter) hdf5 file      drugscreen_severaltimepoint_1control.pdf (plotted results) drugscreen_severaltimepoint_1control.hdf5 (The hdf5 has the data structured, which can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

Example of more than 1 time point and one control for each drug:

Input file:	drugscreen_severaltimepoint_severalcontrols.txt
Experiment details:	Read-out: each 2 hour during 72h Details: Dosage range of MK-1775, prexasertib and BAY1895344 tested on the cell line MCF-7 1 control for each drug
Output file: txt file	drugscreen_severaltimepoint_severalcontrols_IC.txt (statistical parameters from the dose-response curve) drugscreen_severaltimepoint_severalcontrols_information.txt (Extracted information by HTSplotter)
pdf file	drugscreen_severaltimepoint_severalcontrols.pdf (plotted results)
hdf5 file	drugscreen_severaltimepoint_severalcontrols.hdf5 (The hdf5 has the data structured, which can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

## Experimental design

At least two concentrations must be tested for at least one drug in one or more cell lines.

In case of having one control per cell line the labelling is represented in Figure 17-A.

In case of having one control for each drug and cell line the labelling is represented in the Figure 17-B.

In this experiment type the drug name must not have the “\_” and “.” characters.

If the experiment has one control for each drug, HTSplotter uses the correspondent one to normalize all dosages of that drug.

If there is one condition without drug or solvent, the drug name should be named as “CellsOnly”. In this way, all controls are normalized to this condition as to observe the effect of the solvent.

**A**

Order: drug information and then cell line information

Drug-A 45.7 nM,CellLine1 10K/well	Solvent_Drugs 0.34 %,CellLine1_Control 10K/well
Drug-B 1.4 nM,CellLine1 10K/well	
Drug-A 45.7 nM,CellLine2 10K/well	Solvent_Drugs 0.34 %,CellLine2_Control 10K/well
Drug-C 16.4 nM,CellLine2 10K/well	

Order: cell line information and then drug information

CellLine1 10K/well Drug-A 45.7 nM	CellLine1_Control 10K/well Solvent_Drugs 0.34 %
CellLine1 10K/well Drug-B 1.4 nM	
CellLine2 10K/well Drug-A 45.7 nM	CellLine2_Control 10K/well Solvent_Drugs 0.34 %
CellLine2 10K/well Drug-C 16.4 nM	

**B**

Order: drug information and then cell line information

Drug-A 45.7 nM,CellLine1 10K/well	Solvent_Drug-A 0.34 %,CellLine1_Control 10K/well
Drug-B 1.4 nM,CellLine1 10K/well	Solvent_Drug-B 0.1 %,CellLine1_Control 10K/well
Drug-A 45.7 nM,CellLine1 10K/well	Solvent_Drug-A 0.34 %,CellLine2_Control 10K/well
Drug-C 16.4 nM,CellLine2 10K/well	Solvent_Drug-C 0.2 %,CellLine2_Control 10K/well

Order: cell line information and then drug information

CellLine1 10K/well Drug-A 45.7 nM	CellLine1_Control 10K/well Solvent_Drug-A 0.34 %
CellLine1 10K/well Drug-B 1.4 nM	CellLine1_Control 10K/well Solvent_Drug-B 0.1 %
CellLine2 10K/well Drug-A 45.7 nM	CellLine2_Control 10K/well Solvent_Drug-A 0.34 %
CellLine2 10K/well Drug-C 16.4 nM	CellLine2_Control 10K/well Solvent_Drug-C 0.2 %

Figure 17: Example of required fields to determine curve-response relationship of tested drugs. A) An example when one control is used for every tested drug. B) An example of a control per tested drug.

## Results plots

### Unique time point:

- Dose-response relationship for each drug, as shown on Figure 18.

### More than 1 time point:

- Raw data plotted as XY-plot shown on Figure 19.
- Dose-response relationship for each drug at main time points, e.g. 24h, 48h and 72h, see Figure 20.
- In case a perturbagen has an inhibition or enhancement effect, see Figure 21 and Figure 22, respectively.
  - XY-plot shows all dosage effect of a certain drug.

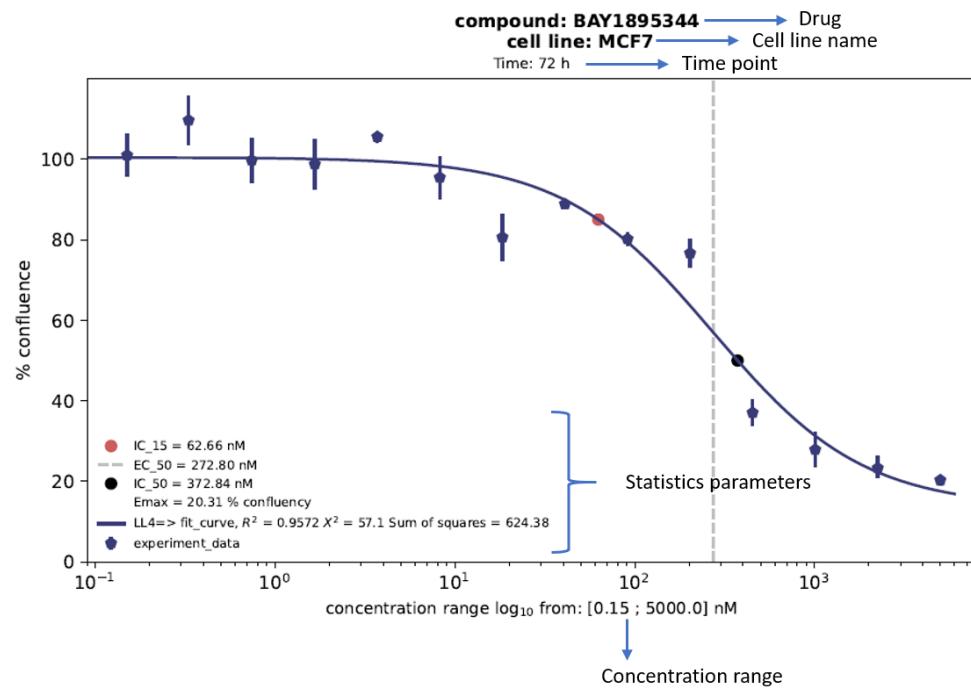


Figure 18: Dose response relationship for compound BAY1895344 tested on the cell line MCF-7. The Y-axis is the read-out provided by the user on the HTSplotter analysis. X-axis is the concentration range tested transformed into  $\log_{10}$ .

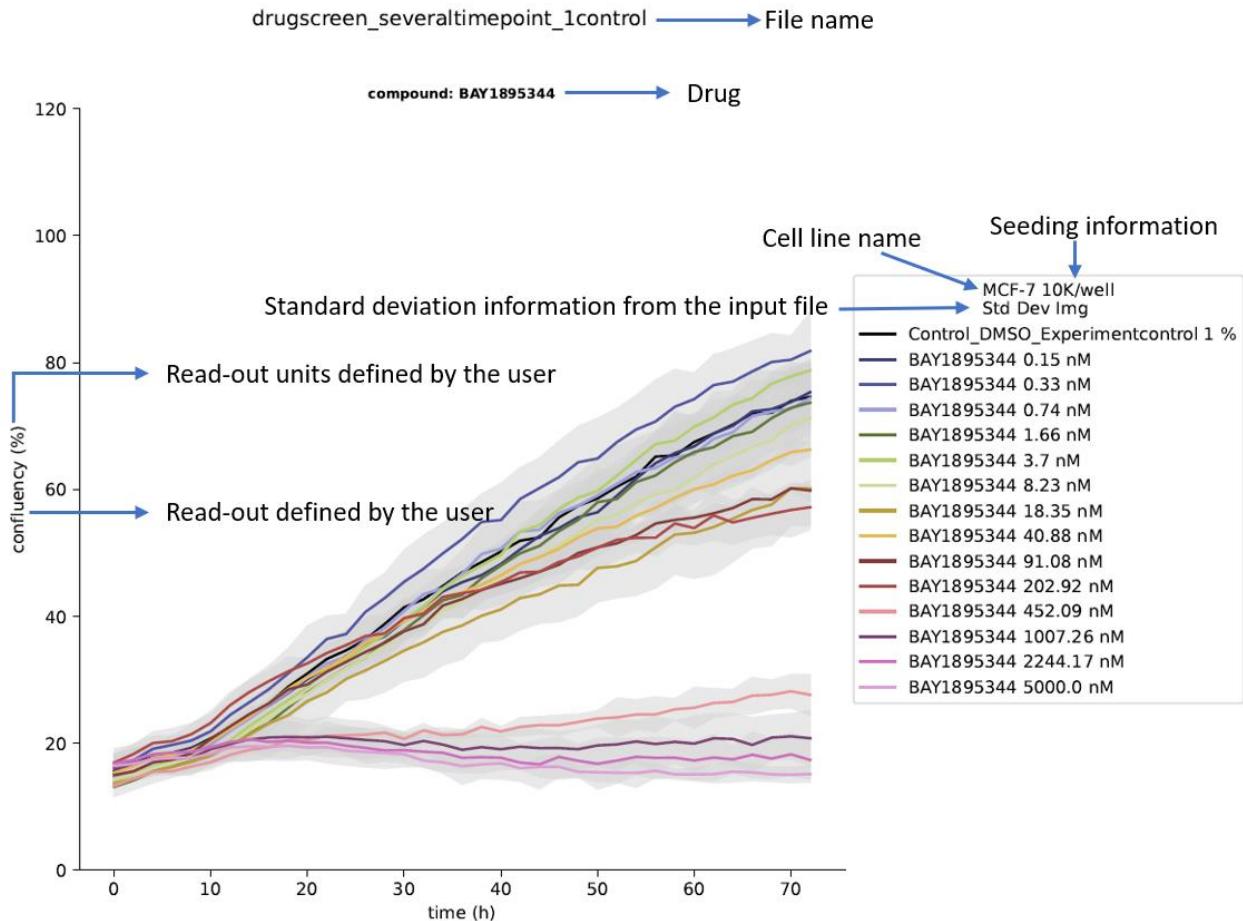


Figure 19: XY-plot example of raw data regarding to all dosages tested of BAY1895344. Y-axis is the read-out provided by the user and on the X-axis is time.

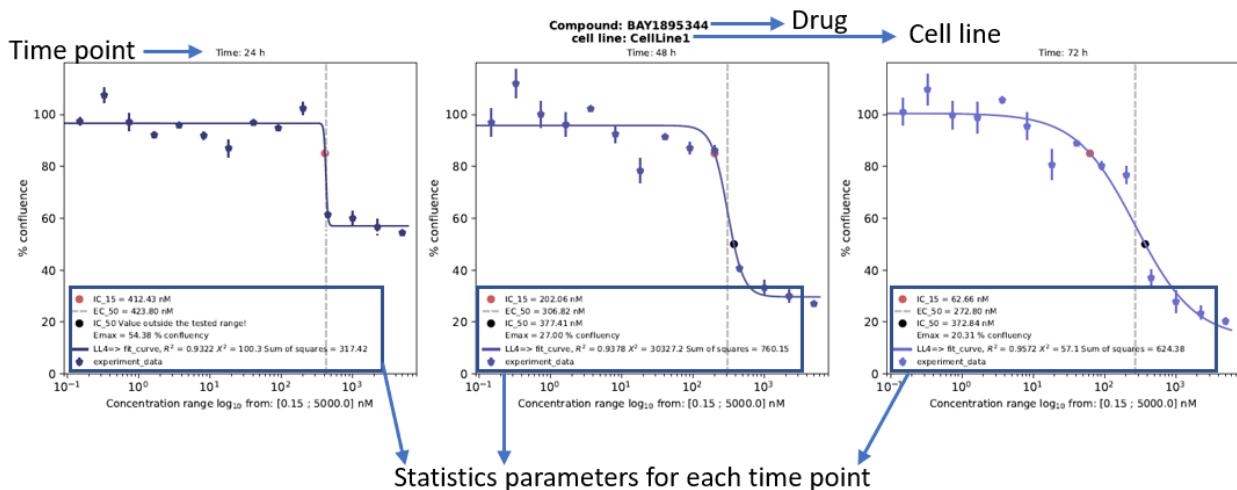


Figure 20: Dose-response relationship of BAY1895344 on the cell line MCF-7 for each main time point. The Y-axis is the read-out provided by the user for the HTSplotter analysis. X-axis is the concentrations range tested transformed into  $\log_{10}$ .

drugscreen\_severaltimepoint\_1control

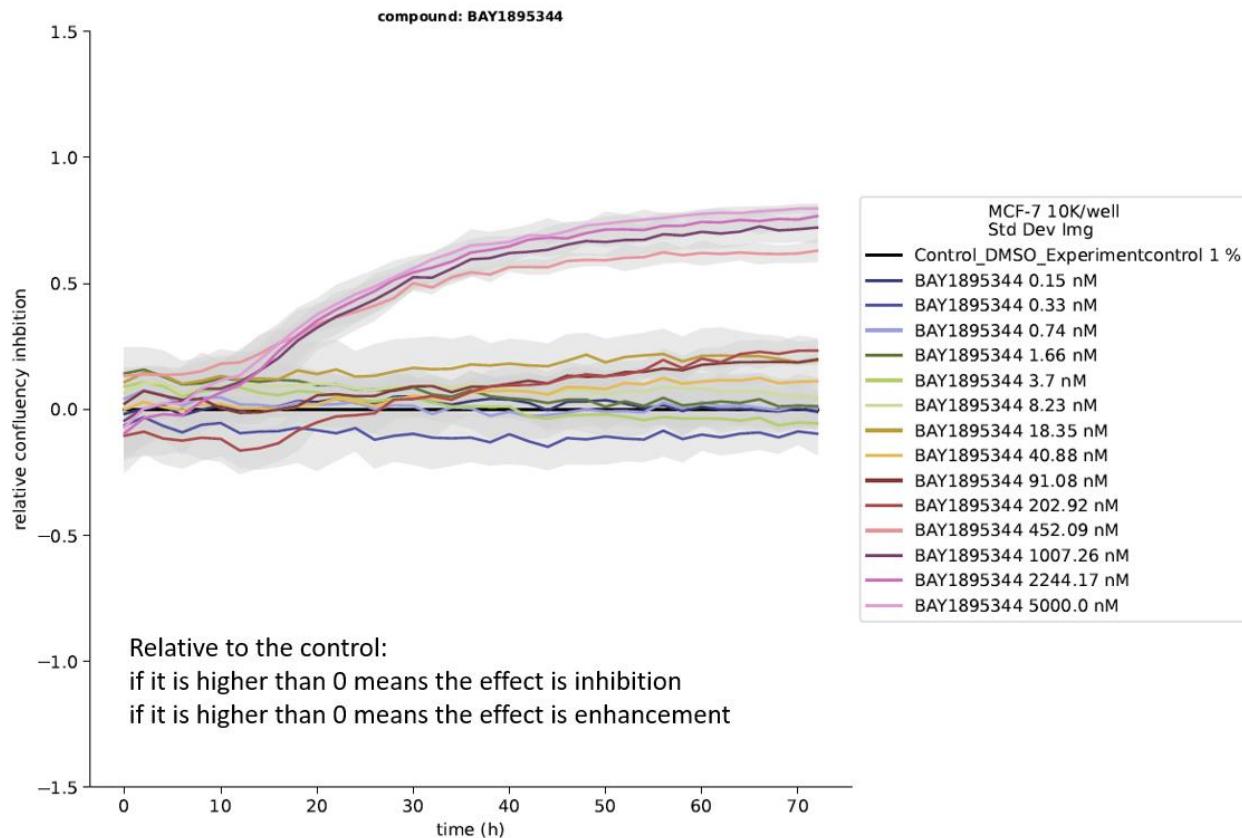


Figure 21: XY-plot example of inhibition effect of all dosages tested of BAY1895344 in relation to the Control, named as "Control\_DMSO\_Experimentcontrol". Y-axis is the read-out provided by the user with inhibition remark and on the X-axis is the time.

drugscreen\_severaltimepoint\_1control

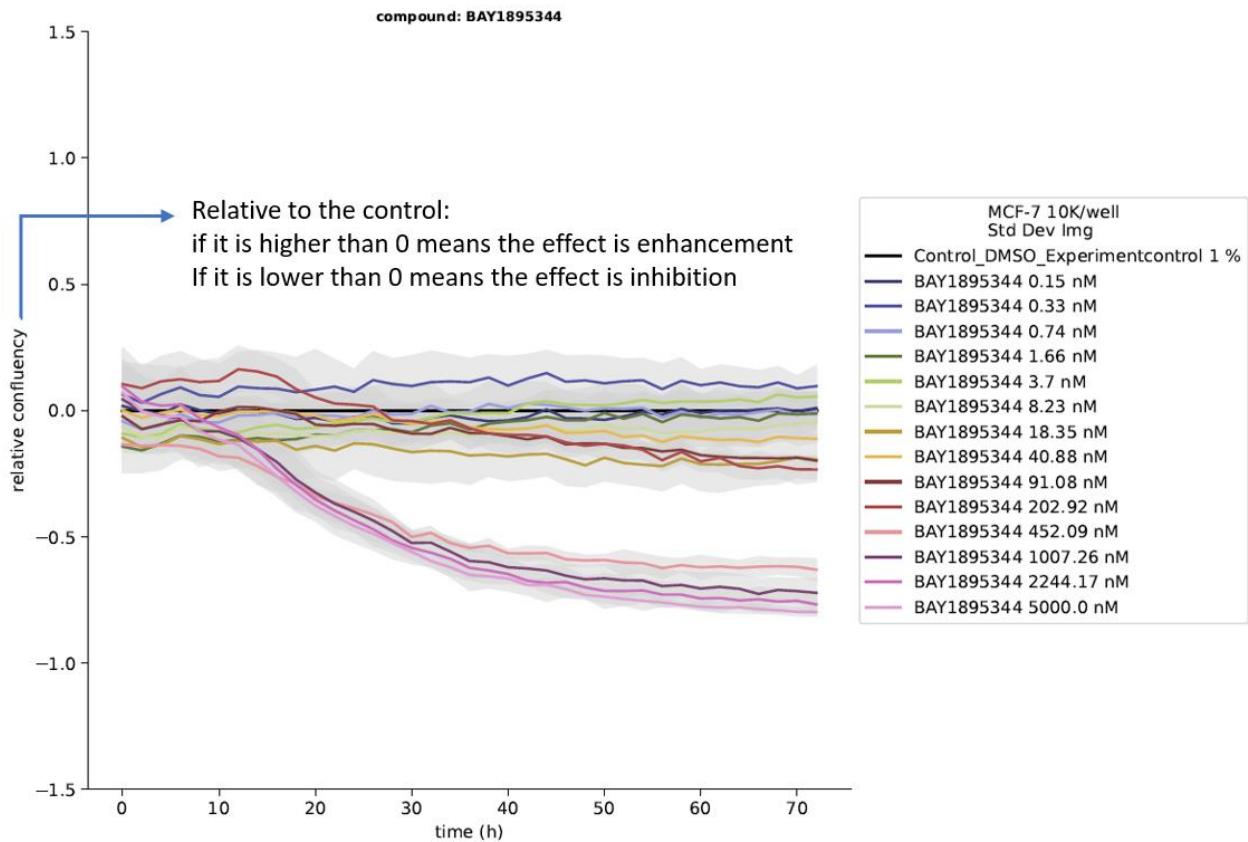


Figure 22: XY-plot example of the effect of all dosages tested of BAY1895344 in relation to the Control, named as "Control\_DMSO\_Experimentcontrol". Y-axis is the read-out provided by the user and on the X-axis is the time.

## Drug combination

HTSplotter categorizes the experiments as drug combination when more than one drug is identified in one experiment condition.

There is no limit of:

- Number of drugs in combinations
- Number of cell lines
- Matrix combination:
  - (m x n): e.g. 1 dosage of drug A combined with 7 different dosages of drug B
  - (n x n): e.g. 7 different dosages of drug A combined with 7 different dosages of drug B

## Example files

Example input files with their results are provided at :

<https://htsplotter.cmgg.be/>

Example of 1 time point:

Input file:	drug_combination_screen_1timepoint.txt
Experiment details:	Read-out: each 2 hour during 72h Details: Dosage range of MK-1775, preasertib and BAY1895344 tested on the cell line MCF-7 1 control for each drug
Output:	txt file      drug_combination_screen_1timepoint_IC.txt (statistical parameters from the dose-response curve) drug_combination_screen_1timepoint_information.txt (Extracted information by HTSplotter) drug_combination_screen_1timepoint_Blisscor.txt (Bl score for each combination) drug_combination_screen_1timepoint_Inhibitiondata.txt (Inhibition effect) drug_combination_screen_1timepoint_Predicted.txt (predicted effect for each combination) pdf file        drug_combination_screen_1timepoint.pdf (plotted results) hdf5 file        drug_combination_screen_1timepoint.hdf5 (data structured, can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

Example of more than 1 time point and repetitive conditions:

Input file:	drug_combination_several_time_points_repetitive_conditions.txt
Experiment details:	Read-out: each 2 hour during 72h Details: MK-1775 combined with prexasertib (7 x 7) and (1 X 7) and MK-1775 combined with BAY1895344 (7 x 7) and (1 X 7).
Output: txt file	drug_combination_several_time_points_repetitive_conditions_IC.txt (statistical parameters from the dose-response curve) drug_combination_several_time_points_repetitive_conditions.txt (Extracted information by HTSplotter) drug_combination_several_time_points_repetitive_conditions_Blisscor.txt (BI score for each combination) drug_combination_several_time_points_repetitive_conditions_Inhibitiondata.txt (Inhibition effect) drug_combination_several_time_points_repetitive_conditions_Predicted.txt (predicted effect for each combination)
pdf file	drug_combination_several_time_points_repetitive_conditions.pdf (plotted results)
hdf5 file	drug_combination_several_time_points_repetitive_conditions.hdf5 (data structured, can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

## Experimental design

In case of combination, please make sure to have a condition for each drug and dosage alone and for the combination, see Figure 23.

If the same drug is combined with another drug but with different ranges in combination, please give a different name to the drug. In this way HTSplotter will identify them as separated drugs in order to identify synergism.

The Bliss independence method is used to determine synergism or antagonism.

- Predicted effect ( $P_{A_\delta B_\delta}$ ) determined by the equation (1).
  - Observed effect  $O_{A_\delta B_\delta}$ , obtained from the experiment is compared to predicted effect in order to get the BI score, equation (2).
    - $B_\delta$  is the effect of drug B, at  $\delta$  dosage
    - $A_\delta$  is the effect of a drug A, at  $\delta$  dosage
    - $O_{A_\delta B_\delta}$  is the effect of  $B_\delta$  combined with  $A_\delta$
- $$P_{A_\delta B_\delta} = A_\delta + B_\delta - (A_\delta * B_\delta) \quad (1)$$
- $$BI \text{ score} = O_{A_\delta B_\delta} - P_{A_\delta B_\delta} \quad (2)$$

In this experiment type, one control is required and is used to normalize.

Notice that the drug name does not accept “\_” and “.” characters.

If there is one condition without drug or solvent, the drug name should be named as “CellsOnly”.

**A**

Order: drug information and then cell line information

Drug-A 45.7 nM,CellLine1 10K/well

Solvent\_Drugs 0.34 %,CellLine1\_Control 10K/well

Drug-A 137 nM,CellLine1 10K/well

Drug-B 1.4 nM,CellLine1 10K/well

Drug-A 45.7 nM,Drug-B 1.4 nM,CellLine1 10K/well

Drug-A 137 nM,Drug-B 1.4 nM,CellLine1 10K/well

Order: cell line information and then drug information

CellLine1 10K/well Drug-A 45.7 nM

CellLine1\_Control 10K/well Solvent\_Drug-A 0.34 %

CellLine1 10K/well Drug-A 137 nM

CellLine1 10K/well Drug-B 1.4 nM

CellLine1 10K/well Drug-A 45.7 nM Drug-B 1.4 nM

CellLine1 10K/well Drug-A 137 nM Drug-B 1.4 nM

*Figure 23: Example of the required fields to determine synergism or antagonism. Notice that for each combination there is a condition with each compound alone.*

## Results plots

### Unique time point:

- Dose-response relationship for each drug alone, as shown on Figure 18.
- In case of a perturbagen has a inhibition or enhancement effect, the bar plot is shown for each combination. The predicted effect according to the BI method is shown by a dash line in Figure 24 and Figure 25.
- 2D and 3D heatmap for the main time points, in cases of (n x n) and (m x n) matrices combinations, with m > 1, see Figure 26, for m = 1, see Figure 27.

### More than 1 time point:

- Raw data plotted as XY-plot
  - Grouped all dosages for a certain drug, see Figure 28.
  - Grouped by combination, control, each drug alone and the combination, see Figure 29.
- Dose-response relationship for each drug alone, see Figure 20.
- In case of a perturbagen has a inhibition and enhancement effect:
  - XY- plot grouped by dosage, see Figure 21 and Figure 22.
  - XY-plot grouped by combination. The predicted effect, according the BI method is plotted by a dash line, see Figure 31and Figure 32.
- Bar plot for each combination. A dash line shows the predicted effect according to the BI method, see Figure 24 and Figure 25.

- Heatmap over time for any type of matrix combination.
- BI score shown through a heatmap for each time point.
  - ( $n \times n$ ) matrix combination, see Figure 33.
  - ( $m \times n$ ) matrix combination, see Figure 34.
- 2D and 3D heatmap for the main time points, in cases of ( $n \times n$ ) and ( $m \times n$ ) matrices combinations, with  $m > 1$ , see Figure 26, for  $m = 1$ , see Figure 27.

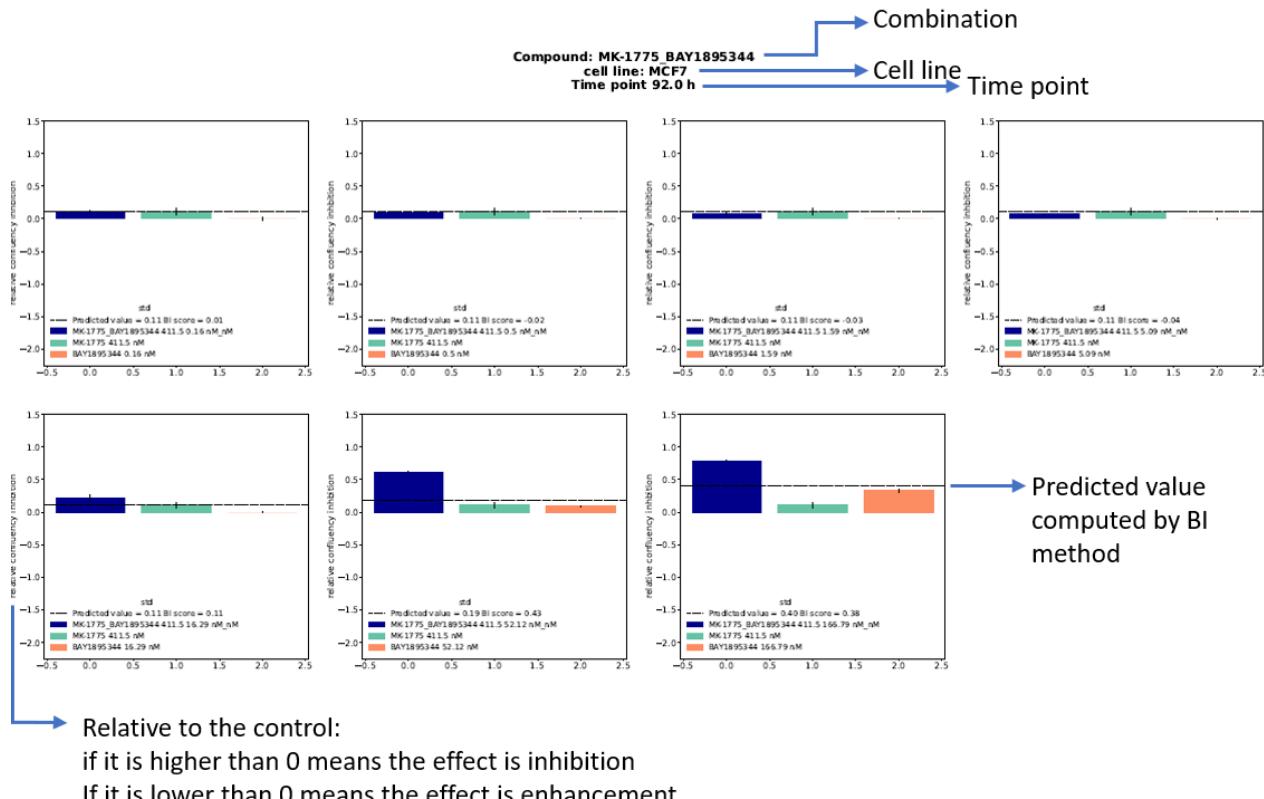


Figure 24: Bar plot with the inhibition effect of each combination and each compound alone for the main time point 72h. In each plot a dash line represents the predicted effect by the BI method.

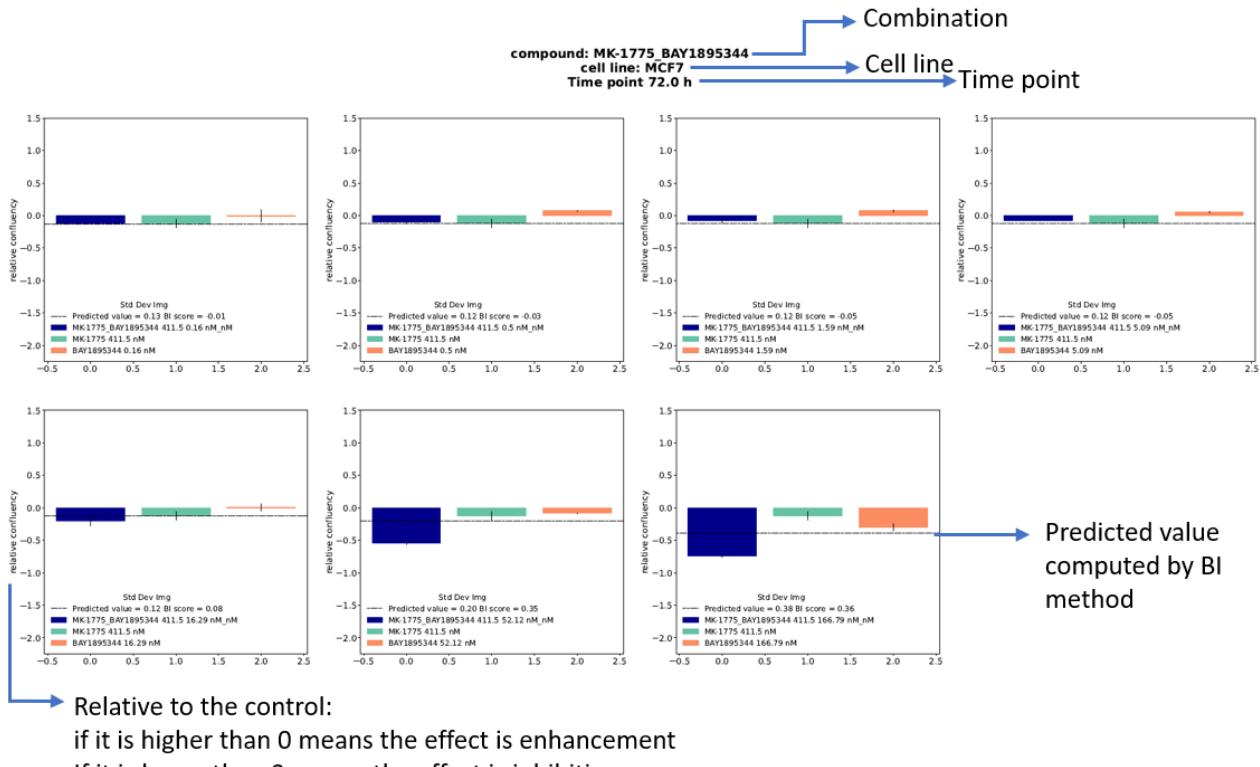


Figure 25: Bar plot with the enhancement effect of each combination and each compound alone for the main time point 72h. In each plot a dash line represents the predicted effect by the PI method.

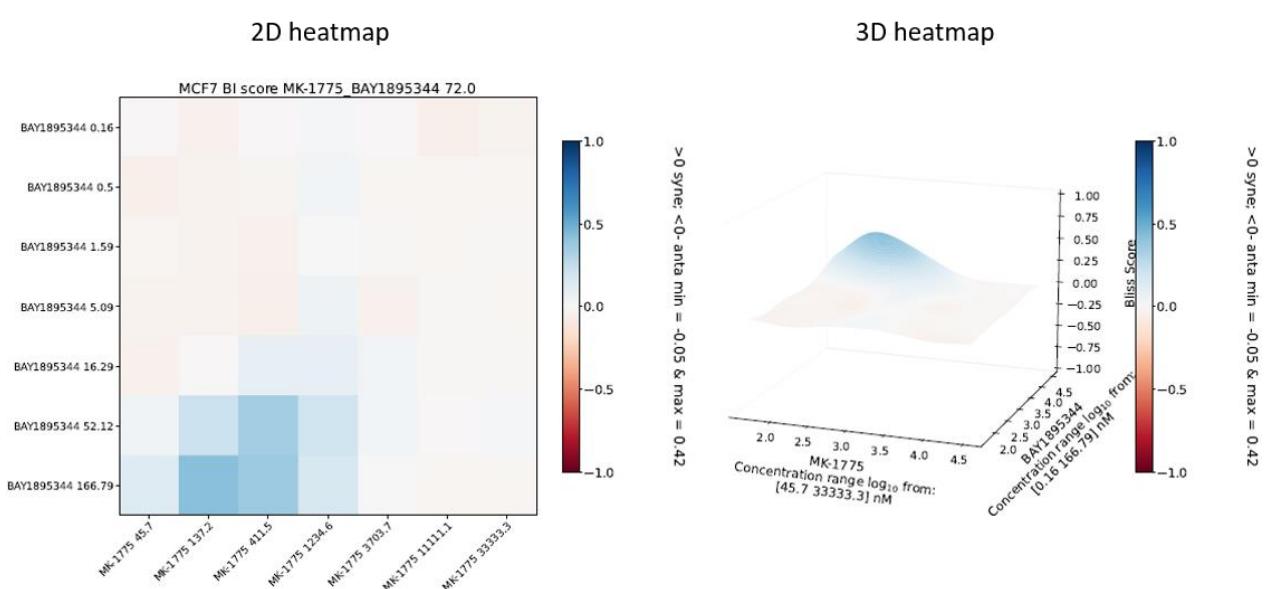


Figure 26: A 2D and 3D heatmap shown for the time point 72h, in cases of a ( $n \times n$ ) or ( $m \times n$ ) matrices combinations, with  $m > 1$ .

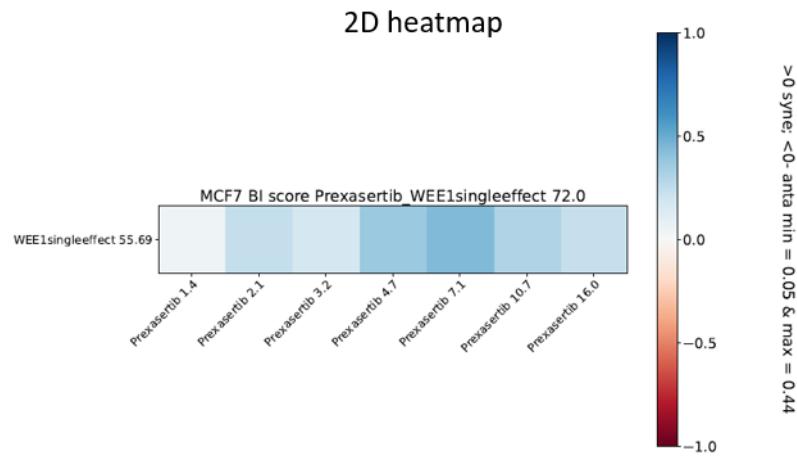


Figure 27: A 2D heatmap shown for the time point 72h, in case of a ( $m \times n$ ) matrix combination, with  $m = 1$ .

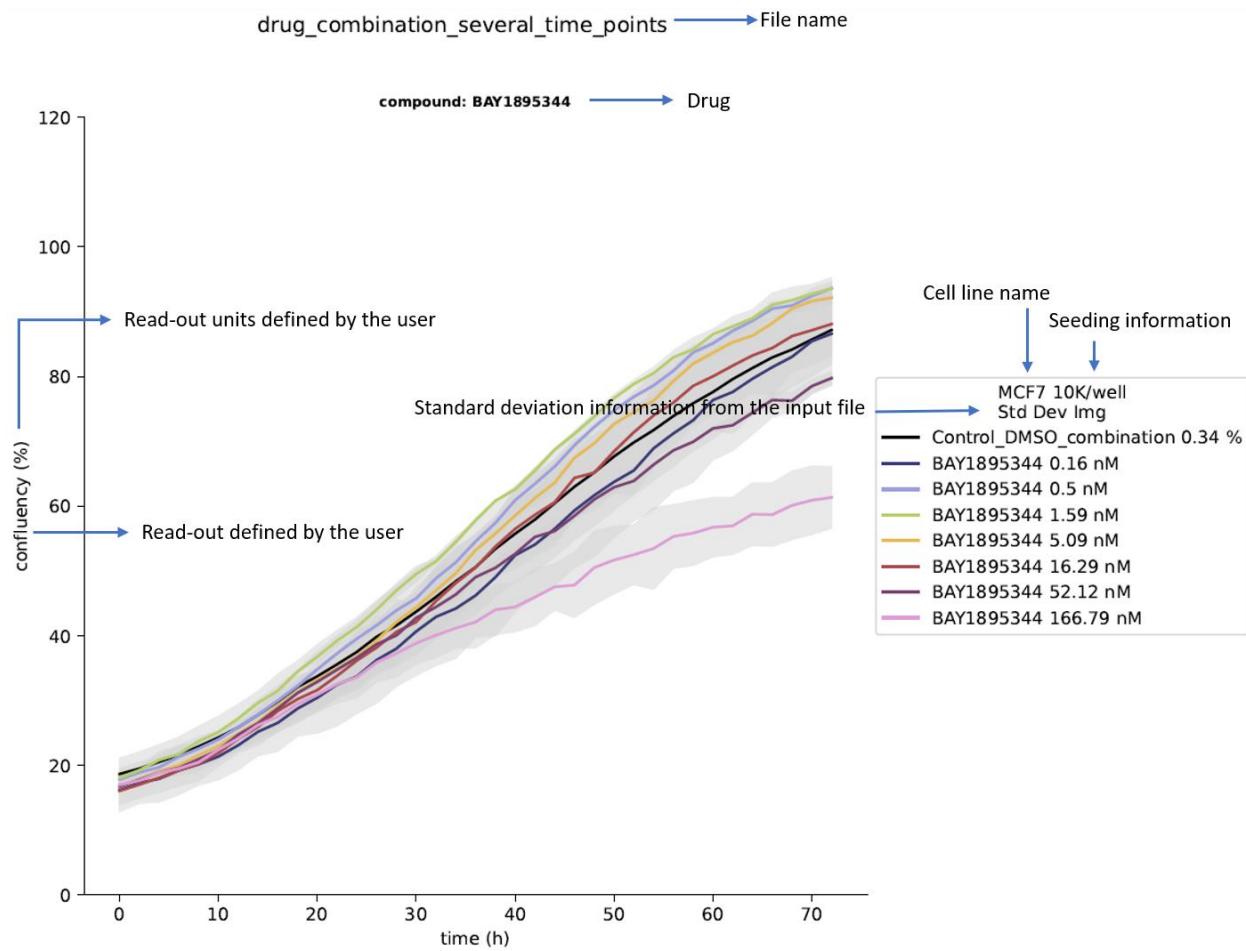


Figure 28: XY-plot example of raw data regarding all dosages tested of BAY1895344. Y-axis is the read-out provided by the user and on the X-axis is the time.

### drug\_combination\_several\_time\_points

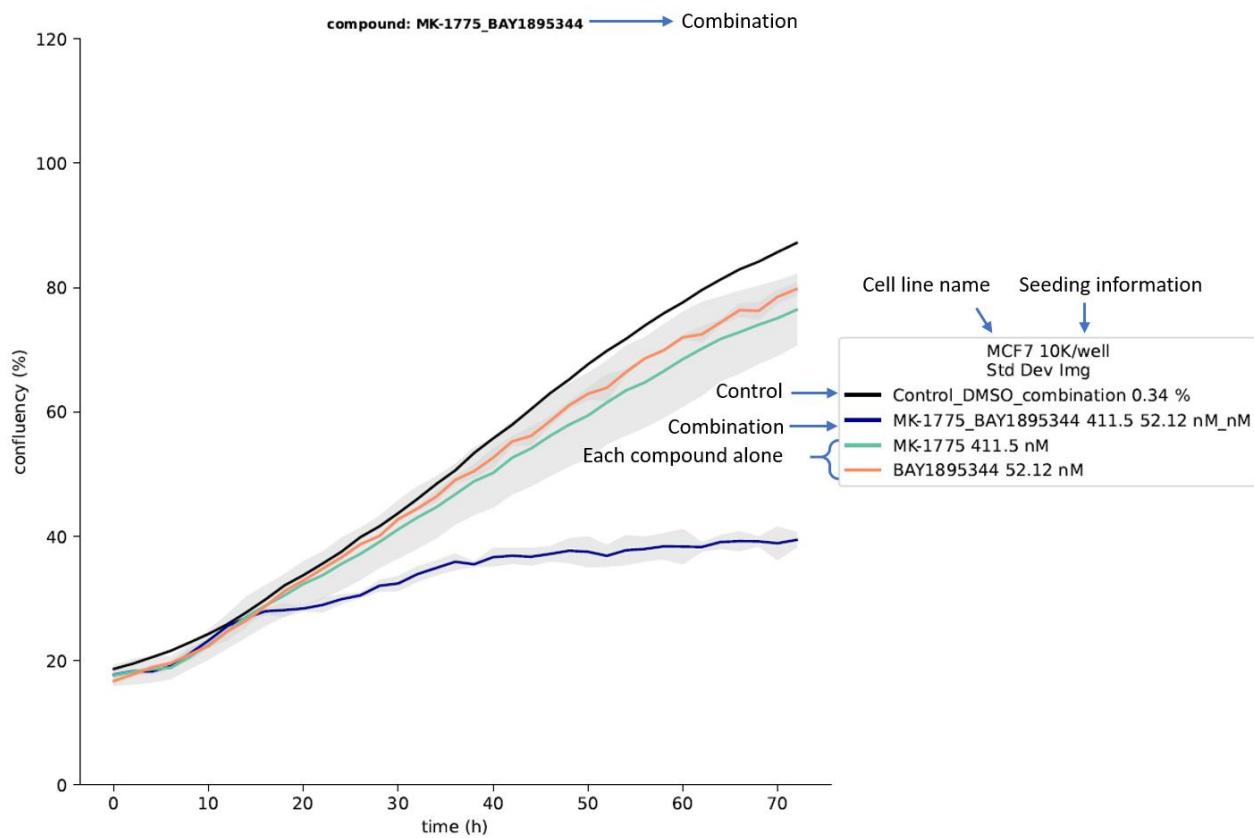


Figure 29: XY-plot showing the raw data over time for the combination condition (MK-1775 at 411.5 nM and BAY1895344 52.12 nM), each compound alone and the experiment control. Y-axis is the confluence (%) read-out provided by the user. On the X-axis is the time course of the experiment.

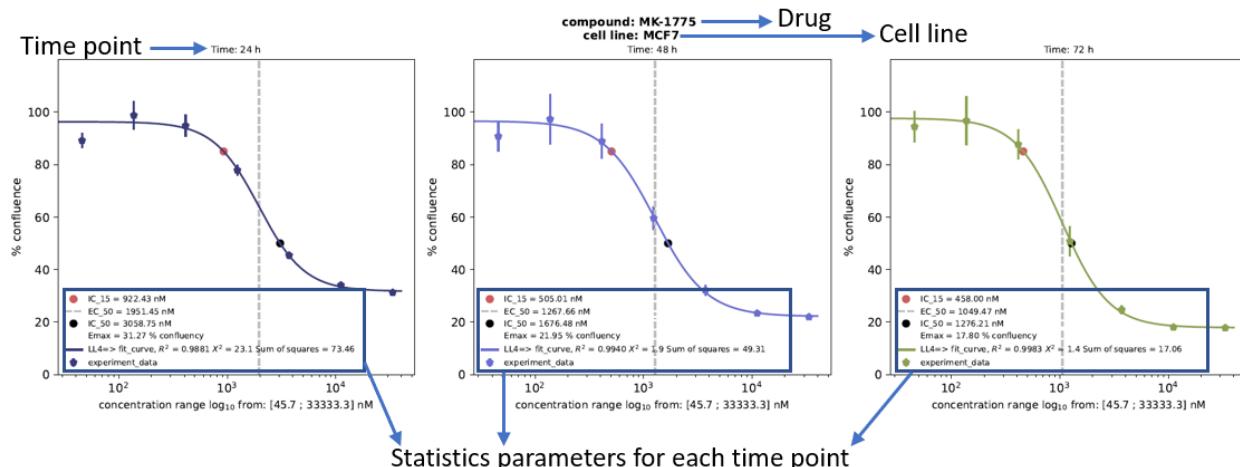


Figure 30: Dose-response relationship of MK-1775 on the cell line MCF-7 for each main time point. The Y-axis is the read-out provided by the user on the HTSplotter analysis. The X-axis plots the concentration range tested transformed into  $\log_{10}$ .

drug\_combination\_several\_time\_points

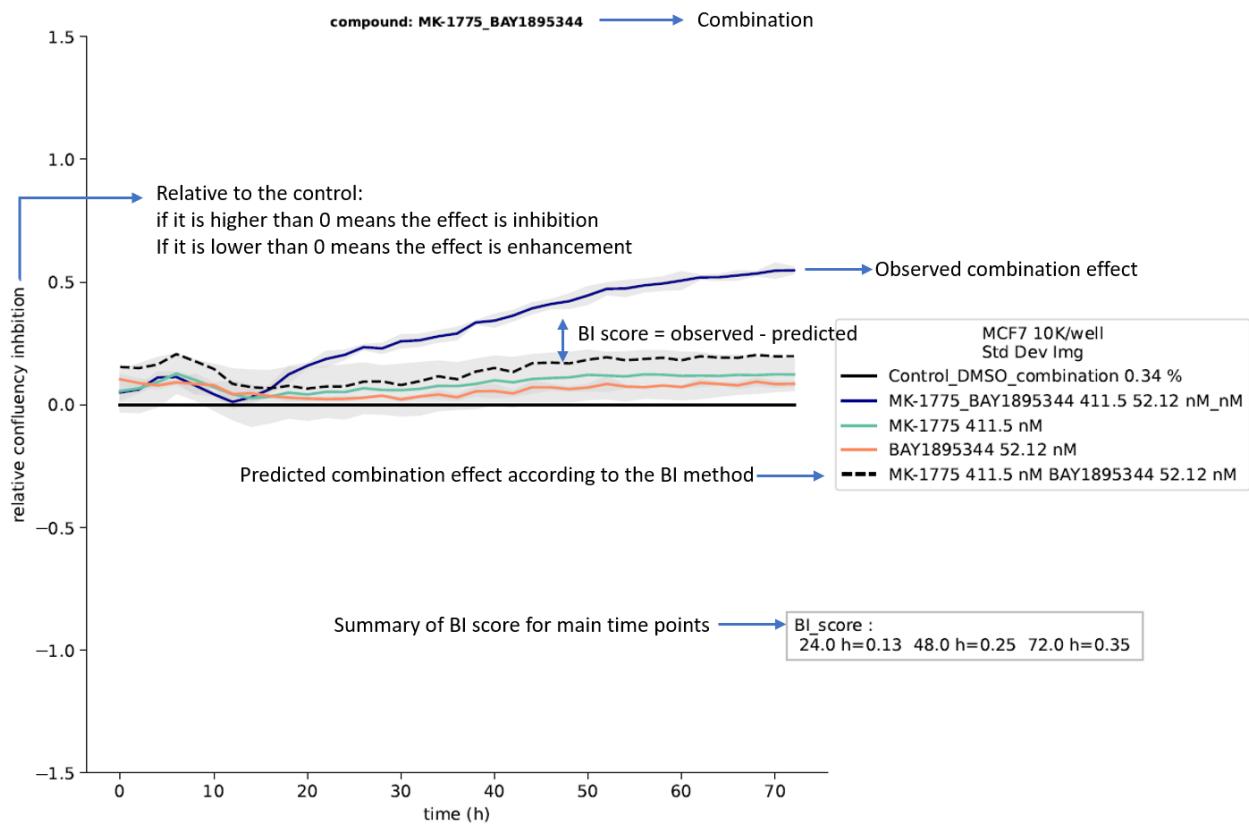


Figure 31: XY-plot showing the inhibition effect over time for the combination condition (MK-1775 at 411.5 nM and BAY1895344 52.12 nM), each compound alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

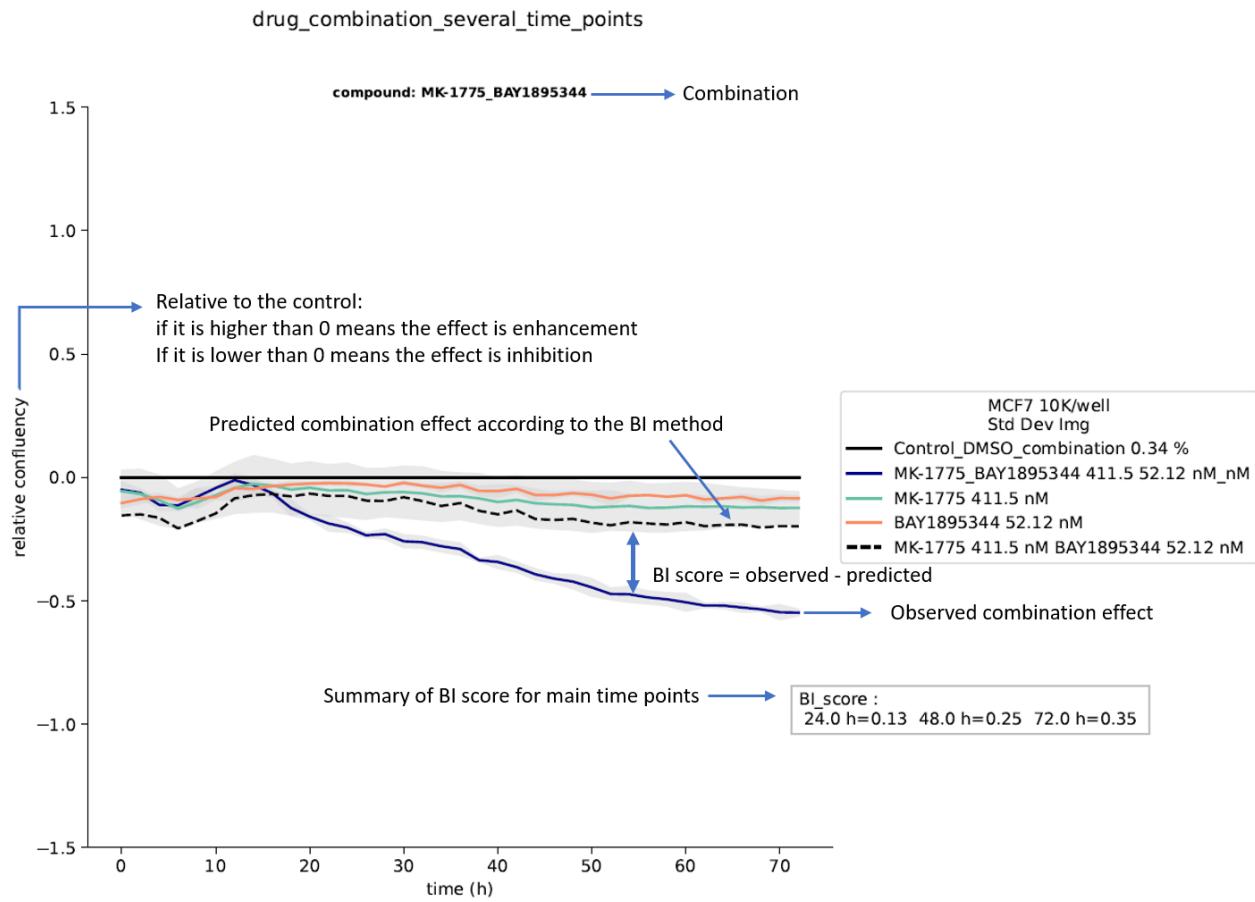


Figure 32: XY-plot showing the effect over time for the combination condition (MK-1775 at 411.5 nM and BAY1895344 52.12 nM), each compound alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

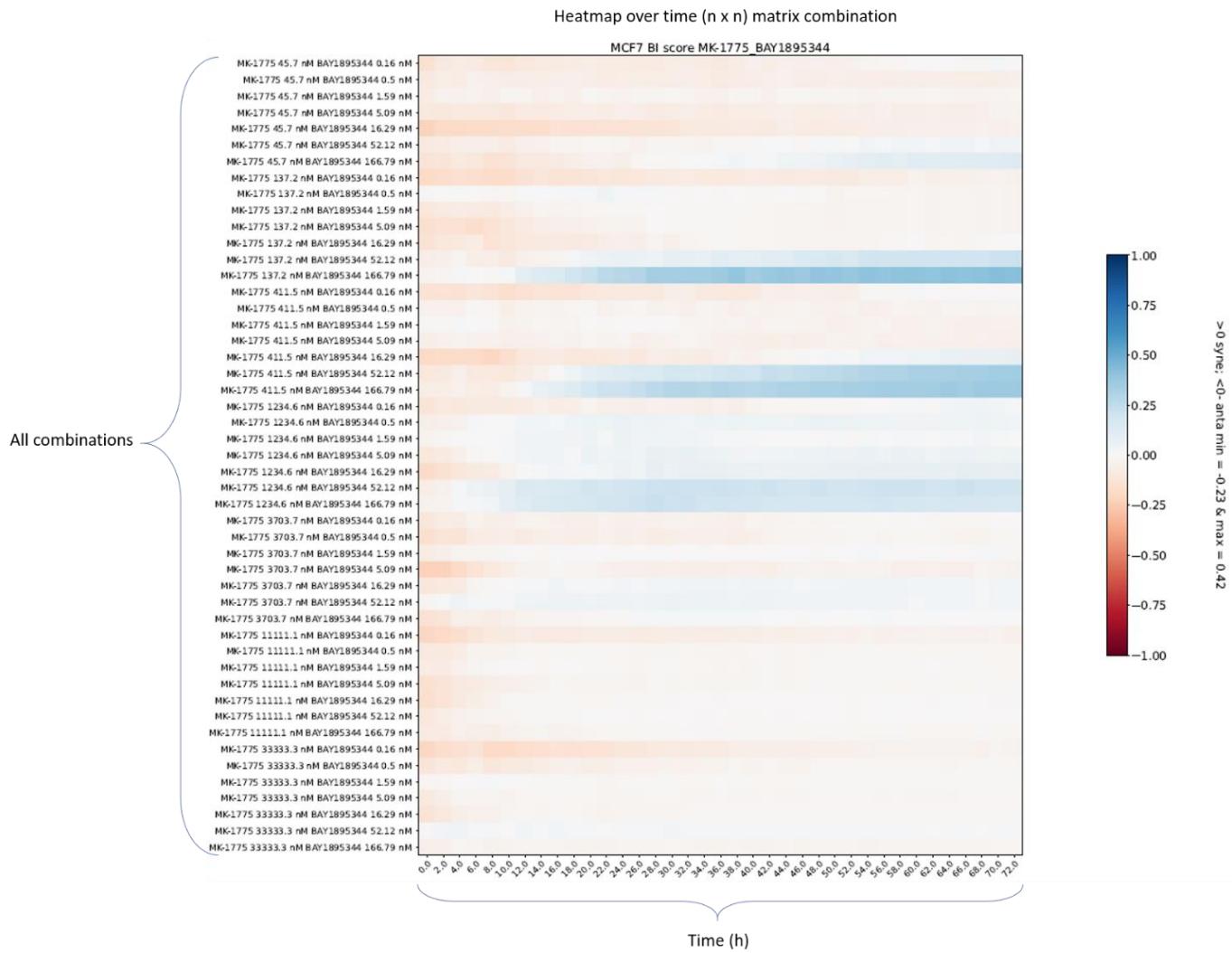


Figure 33: Heatmap over time for (n x n) matrix combination.

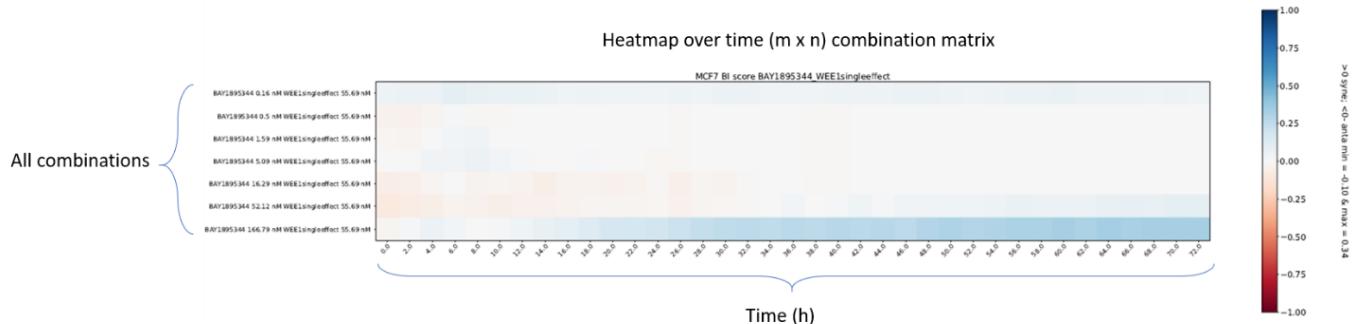


Figure 34: Heatmap over time for (m x n) matrix combination.

## Genetic perturbagen

HTSplotter categorizes an experiment as a genetic-perturbagen screen when for each drug only one dosage is tested. Additionally, only one drug information is allowed.

If more than one control is identified, HTSplotter computes first the average, standard deviation and 95 % confidence interval of all controls. Then it normalizes all conditions to the average control.

There is no limit of:

- Number of genetics perturbagens.
- Number of cell lines.
- Number of controls per cell line.

### Example files

Example input files with their results are provided at:

<https://htsplotter.cmgg.be/>

Example of 1 time point:

Input file:	gene_perturbagen_1timepoint_1control.txt
Experiment details:	Read-out: each 2 hour during 72h Details: several transcriptomic perturbagens in 1 cell line. 1 control
Output:	txt file gene_perturbagen_1timepoint_1control_information.txt (Extracted information by HTSplotter) pdf file gene_perturbagen_1timepoint_1control.pdf (plotted results) hdf5 file gene_perturbagen_1timepoint_1control.hdf5 (data structured, can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

Example of more than 1 time point and more than one control:

Input file:	Gene_perturbagen_severaltimepoints.txt
Experiment details:	Read-out: each 2 hour during 72h Details: several transcriptomic perturbagens in 1 cell line. More than one control
Output:	txt file Gene_perturbagen_severaltimepoints_information.txt (Extracted information by HTSplotter) pdf file Gene_perturbagen_severaltimepoints.pdf (plotted results) hdf5 file Gene_perturbagen_severaltimepoints.hdf5 (data structured, can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

### Experimental design

For each genetic perturbation condition, a name, dosage and units are required. It is crucial a unique concentration for each gene name is provided. In case of for example of siRNA targeting the same transcript, please differentiate the name, as shown on Figure 35-A.

If HTSplotter identifies more than one dosage, the experiment will not be categorized as genetic-perturbagen.

In case of more than one control, HTSplotter first computes its average, standard deviation and 95% CI, and then normalize all conditions.

Figure 35-B is an example of a genetic perturbagen screen performed, where more than 1 control might be required.

If there is one condition without drug or solvent, the drug name should be named as “CellsOnly”.

## A

Order: drug information and then cell line information

gene-A-1 40 ng/well,CellLine1 10K/well

siRNA-NTC 40 ng/well,CellLine1\_Control 10K/well

gene-A-2 40 ng/well,CellLine1 10K/well

gene-A-3 40 ng/well,CellLine1 10K/well

Order: cell line information andd then drug information

CellLine1 10K/well gene-A-1 40 ng/well

CellLine1\_Control 10K/well siRNA-NTC 40 ng/well

CellLine1 10K/well gene-A-2 40 ng/well

CellLine1 10K/well gene-A-3 40 ng/well

## B

Order: drug information and then cell line information

gene-1 40 ng/well,CellLine1 10K/well

gene-5 40 ng/well,CellLine1\_Control 10K/well

gene-2 40 ng/well,CellLine1 10K/well

gene-6 40 ng/well,CellLine1\_Control 10K/well

gene-3 40 ng/well,CellLine1 10K/well

Order: cell line information andd then drug information

CellLine1 10K/well gene-1 40 ng/well

CellLine1\_Control 10K/well gene-5 40 ng/well

CellLine1 10K/well gene-2 40 ng/well

CellLine1\_Control 10K/well gene-6 40 ng/well

CellLine1 10K/well gene-3 40 ng/well

Figure 35: Example of labeling each condition in case of a genetic-perturbagen screen. A) Refers to an experiment where the genetic perturbagen targets the same gene, but different perturbations were tested. B) Refers to an experiment targeting different genes and where more than one control was required.

## Results plots

Unique time point:

- Bar plot is with all perturbagens, shown as inhibition (Figure 36) or as enhancement effect (Figure 37).

More than 1 time point:

- Raw data plotted as XY-plot, for each perturbagen, see Figure 38.
- XY-plot for each perturbagen, inhibition (Figure 39) or enhancement effect (Figure 40).
- Heatmap over time with all perturbagens (Figure 41).

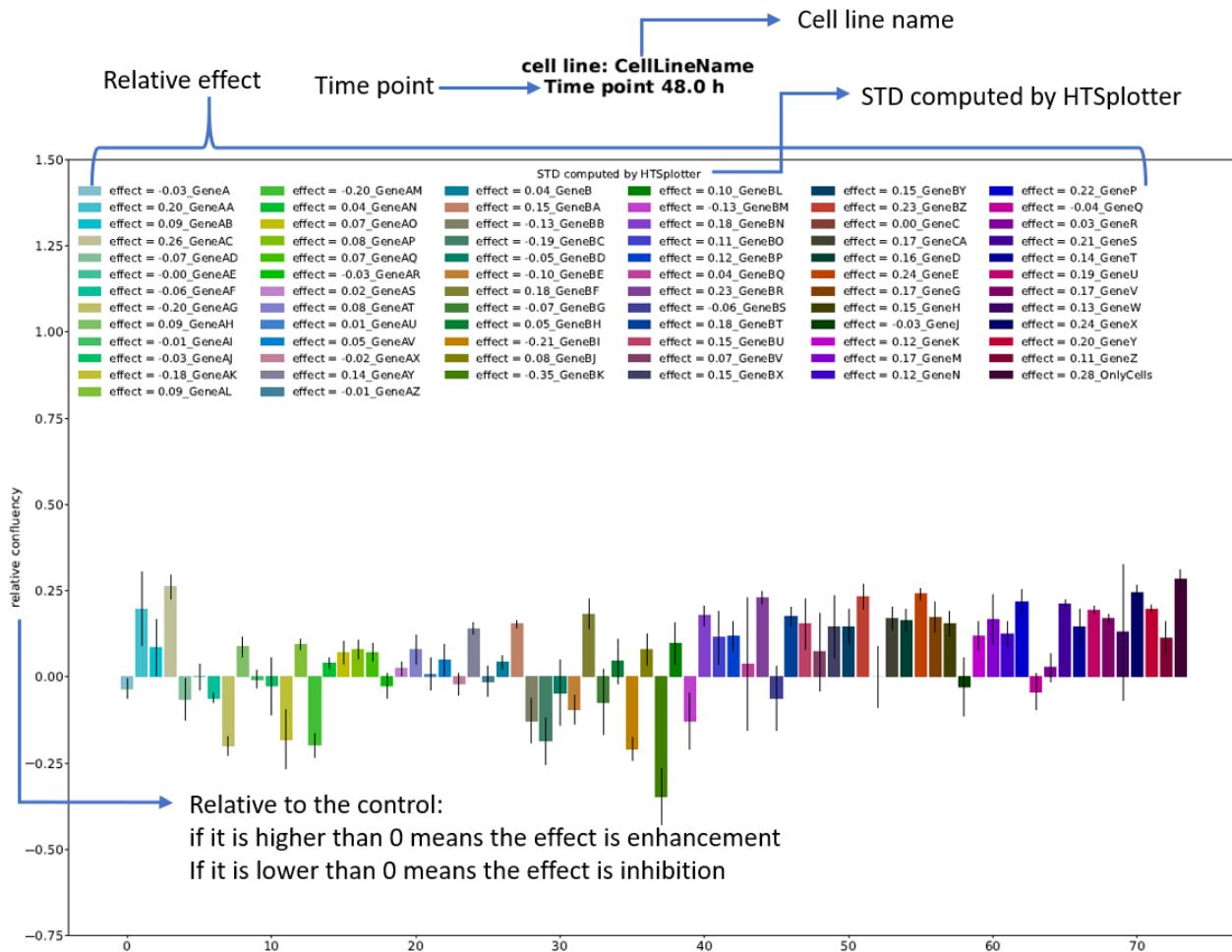


Figure 36: Inhibition effect of all genetic perturbagens plotted. On the legend, the inhibition effect relative to the control is shown.

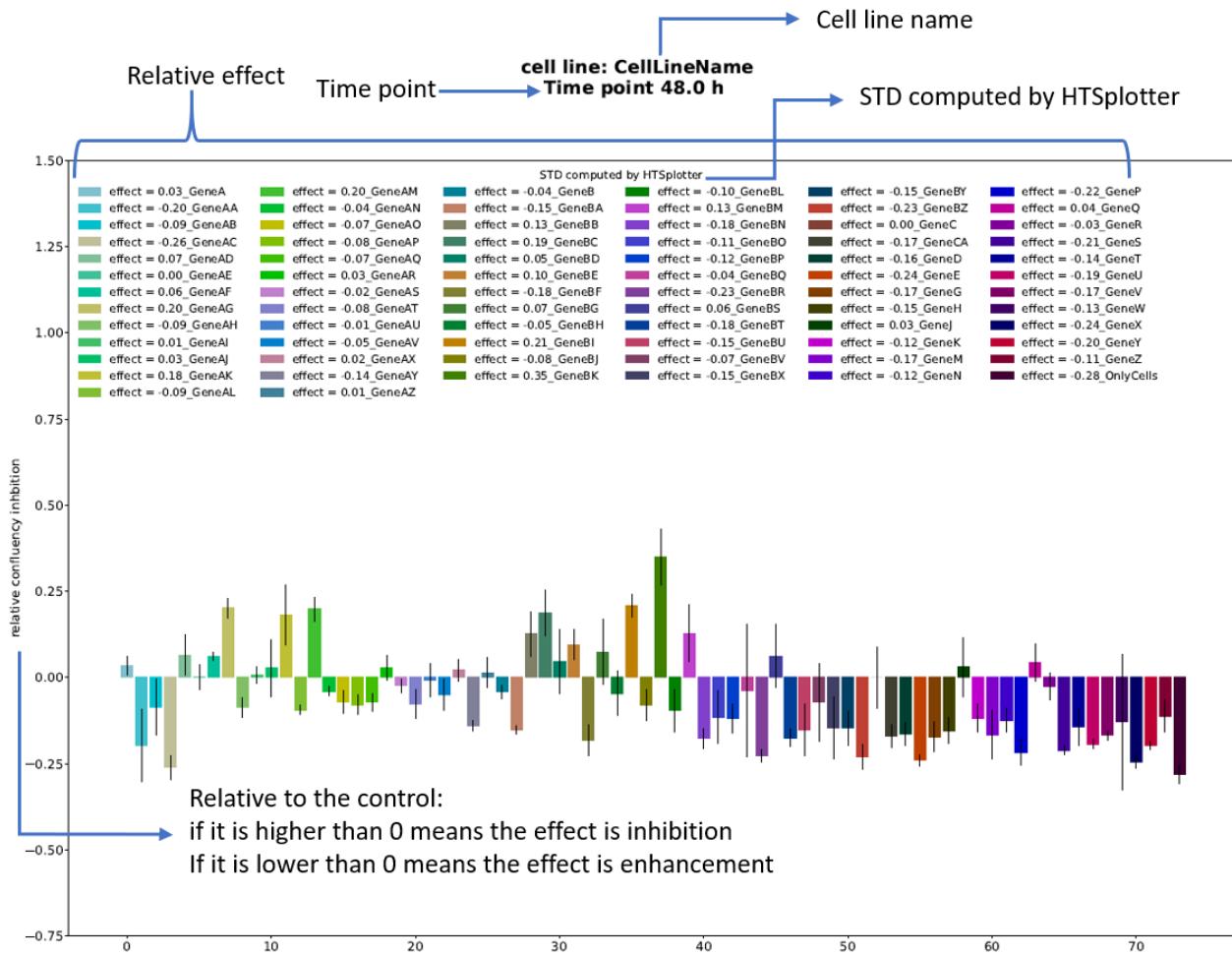


Figure 37: Effect of all genetics perturbagens plotted. On the legend, the effect relative to the control is shown.

Gene\_perturbagen\_severaltimepoints → File name

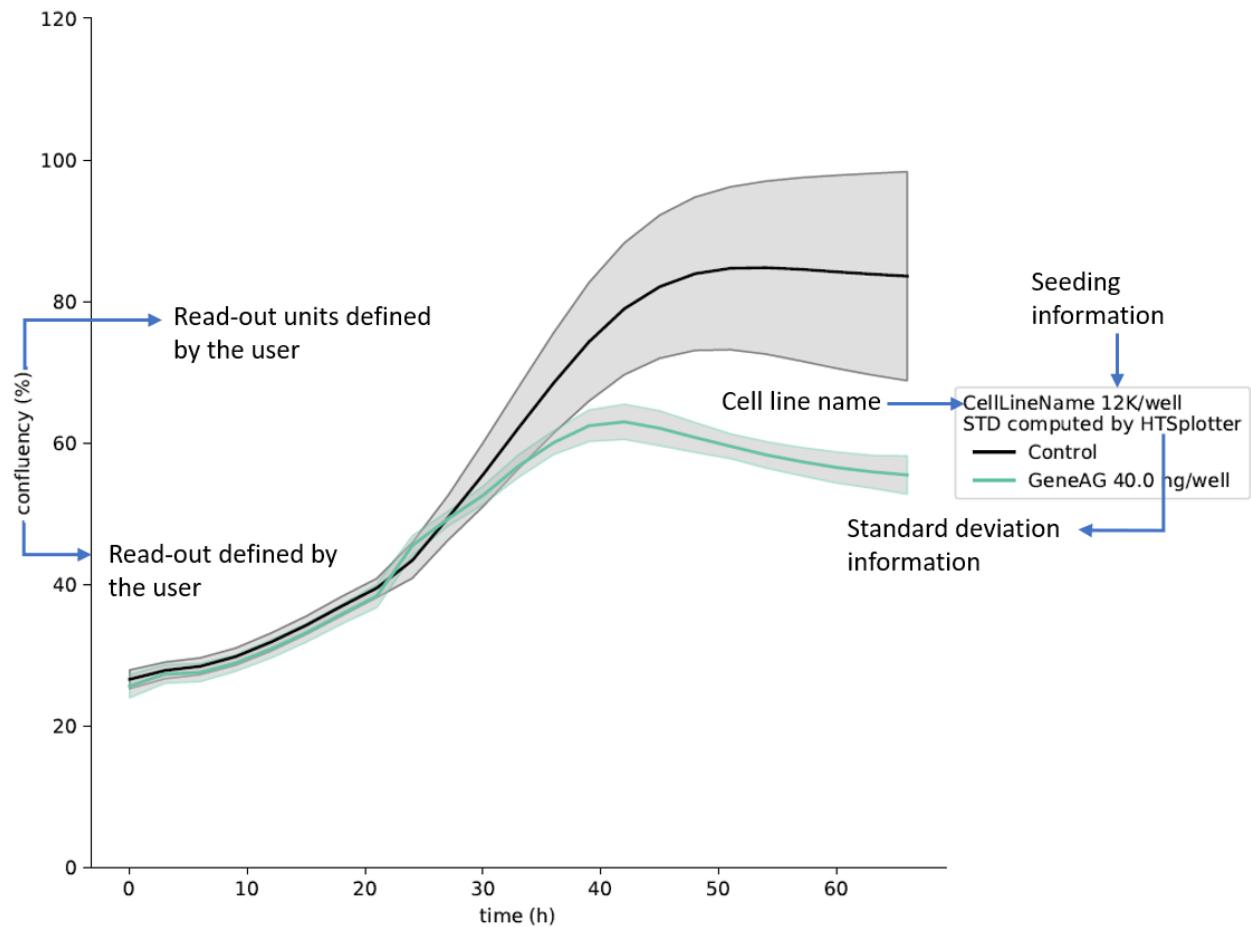


Figure 38: XY-plot showing the raw data for control and GeneAG conditions.

### Gene\_perturbagen\_severaltimepoints

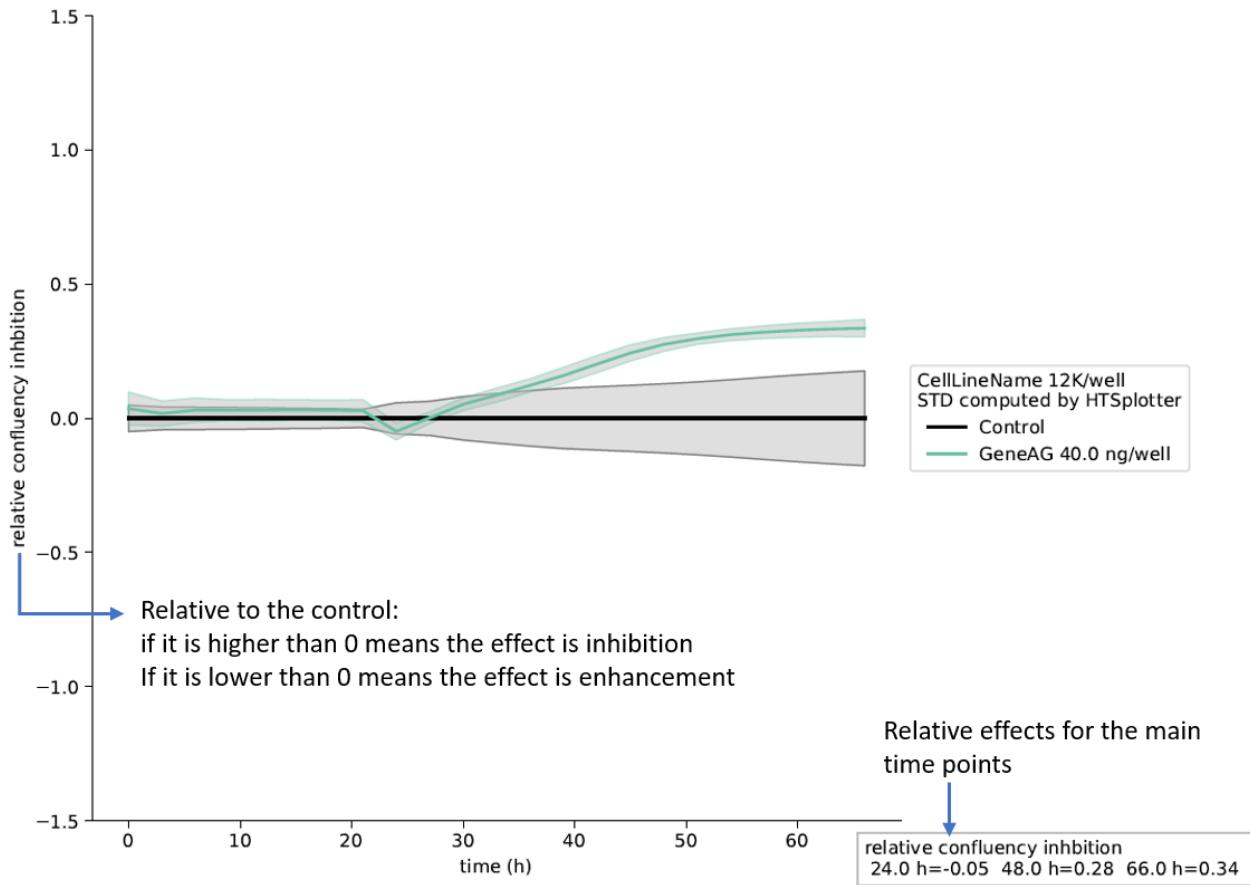


Figure 39: XY-plot with the inhibition effect of GeneAG. A summary with the relative inhibition effect is shown for the main time points.

### Gene\_perturbagen\_severaltimepoints

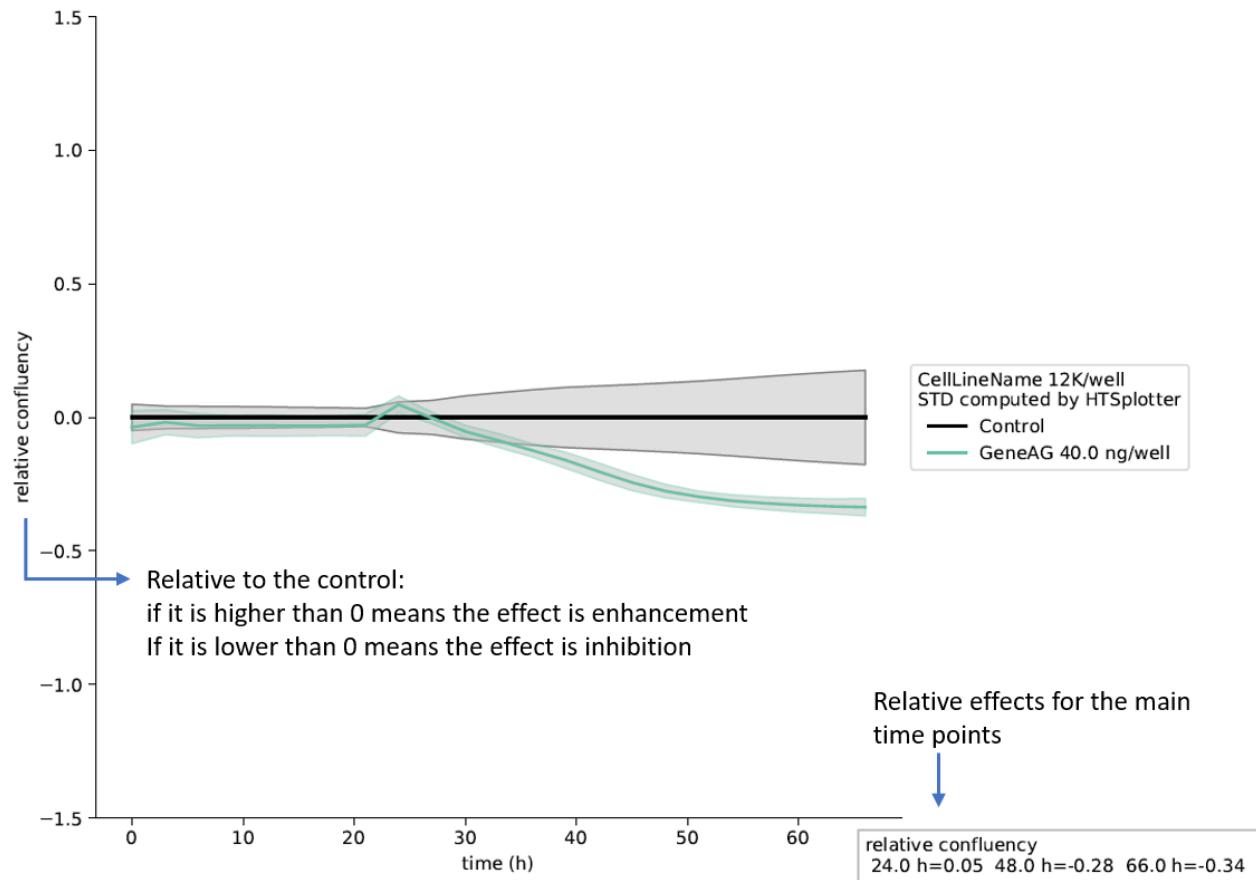


Figure 40: XY-plot with the effect of GeneAG. A summary with the relative condition effect is shown for the main time points.

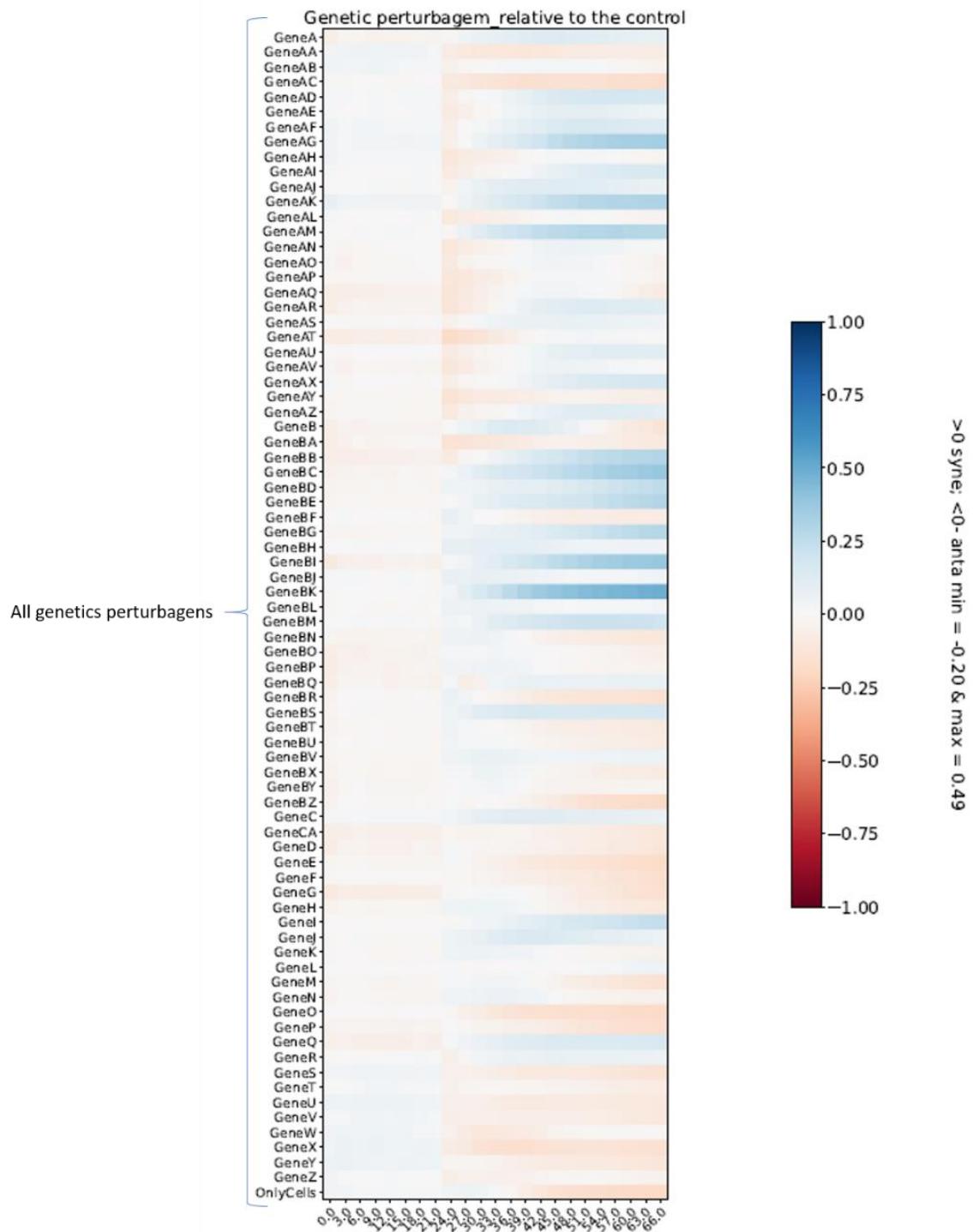


Figure 41: Heatmap over time with the effect of all genetics perturbagens relative to the control. Noticed that the condition with only cells is also shown, as to compare with the experiment control.

## Genetic-chemical perturbagen

A genetic-chemical perturbagen screen is identified by HTSplotter when the drug name has a tag, which is identified by the presence of the “\_” character followed by an extra word, such as “GeneOff”. For example a condition where a drug-A was tested in combination with an overexpression of a certain gene should be referred as “drug-A\_GeneOff”.

There is no limit on:

- Number of drugs.
- Number of cell lines.
- ( $m \times n$ ) matrix combination:
  - The dosage range of a certain drug must be tested without the genetic perturbagen, being this the drug effect for each dosage alone.
  - The genetic perturbagen with drug solvent is considered the genetic perturbagen effect alone.
  - The combination of genetic and chemical perturbagen is identified on the condition where the drug name has a tag, e.g. “\_GeneOff”.
  - Thus the Predicted BI method ( $P_{A_\theta B_\delta}$ ) is computed according to equation (1), where:
    - $B_\delta$  is the solvent with the genetic perturbagen.
    - $A_\theta$  is the dosage effect of a certain drug.
    - The BI score (*BI score*) is computed according to equation (2), where:
      - $O_{A_\theta B_\delta}$  is the drug at certain dosage combined with a genetic perturbagen

$$P_{A_\theta B_\delta} = A_\theta + B_\delta - (A_\theta * B_\delta) \quad (3)$$

$$BI \text{ score} = O_{A_\theta B_\delta} - P_{A_\theta B_\delta} \quad (4)$$

## Example files

Example input files with their results are provided at :

<https://htsplotter.cmgg.be/>

Example of 1 time point:

Input file:	genetic-chemical_perturbagen_1time_point.txt
Experiment details:	Read-out: each 2 hours during 72h Details: several transcriptomic perturbagens in 1 cell line. 1 control
Output: txt file	genetic-chemical_perturbagen_1time_point.txt (Extracted information by HTSplotter) genetic-chemical_perturbagen_1time_point_BIscor.txt (BI score for each combination) genetic-chemical_perturbagen_1time_point_Inhibitiondata.txt (Inhibition effect) genetic-chemical_perturbagen_1time_point_Predicted.txt (predicted effect for each combination) genetic-chemical_perturbagen_1time_point_IC.txt (statistical parameters from the dose-response curve)
pdf file	genetic-chemical_perturbagen_1time_point.pdf (plotted results)
hdf5 file	genetic-chemical_perturbagen_1time_point.hdf5 (data structured, can be open by hdf5view software ( <a href="https://www.hdfgroup.org/downloads/hdfview/">https://www.hdfgroup.org/downloads/hdfview/</a> )).

Example of more than 1 time point and more than one control:

Input file: genetic-chemical\_perturbagen\_several\_time\_points.txt  
Experiment details: Read-out: each 2 hours during 72h  
Details: several transcriptomic perturbagens in 1 cell line.  
1 control

Output: txt file      genetic-chemical\_perturbagen\_several\_time\_points.txt      (Extracted information by HTSplotter)  
genetic-chemical\_perturbagen\_several\_time\_points\_Blisscor.txt      (Bl score for each combination)  
genetic-chemical\_perturbagen\_several\_time\_points\_Inhibitiondata.txt (Inhibition effect)  
genetic-chemical\_perturbagen\_several\_time\_points\_Predicted.txt (predicted effect for each combination)  
genetic-chemical\_perturbagen\_several\_time\_points\_IC.txt (statistical parameters from the dose-response curve)

pdf file      genetic-chemical\_perturbagen\_several\_time\_points.pdf (plotted results)  
hdf5 file      genetic-chemical\_perturbagen\_several\_time\_points.hdf5 (data structured, can be open by hdf5view software (<https://www.hdfgroup.org/downloads/hdfview/>)).

## Experimental design

A combination of genetic and chemical perturbagens requires a control, in which the drug solvent was tested without genetic perturbagen.

The control condition is used to normalize all conditions, where the drug was tested with and without the genetic perturbagen, including the solvent with genetic perturbagen. This last condition determines the effect of the genetic perturbagen alone. Therefore, a solvent condition in combination with genetic perturbagen is required for each tested drug.

The Figure 42 shows an example of conditions labeling for each situation. Noticed that all conditions where the drug was tested in combination with genetic perturbagen are indicated with the tag “\_GeneOff”, including the solvent condition.

**A**

Order: drug information and then cell line information

Solvent_Drug-A_GeneOff 0.3 %,CellLine1 10K/well	Solvent_Drug-A 0.3 %,CellLine1_Control 10K/well
Drug-A_GeneOff 100 nM,CellLine1 10K/well	
Drug-A_GeneOff 5 nM,CellLine1 10K/well	
Drug-A 100 nM,CellLine1 10K/well	
Drug-A 5 nM,CellLine1 10K/well	
Solvent_Drug-B_GeneOff 0.3 %,CellLine1 10K/well	Solvent_Drug-B 0.3 %,CellLine1_Control 10K/well
Drug-B_GeneOff 20 nM,CellLine1 10K/well	
Drug-B_GeneOff 15 nM,CellLine1 10K/well	
Drug-B 20 nM,CellLine1 10K/well	
Drug-B 15 nM,CellLine1 10K/well	

Order: cell line information and then drug information

CellLine1 10K/well Solvent_Drug-A_GeneOff 0.3 %	CellLine1_Control 10K/well Solvent_Drug-A 0.3 %
CellLine1 10K/well Drug-A_GeneOff 100 nM	
CellLine1 10K/well Drug-A_GeneOff 5 nM	
CellLine1 10K/well Drug-A 100 nM	
CellLine1 10K/well Drug-A 5 nM	
CellLine1 10K/well Solvent_Drug-B_GeneOff 0.3 %	CellLine1_Control 10K/well Solvent_Drug-B 0.3 %
CellLine1 10K/well Drug-B_GeneOff 20 nM	
CellLine1 10K/well Drug-B_GeneOff 15 nM	
CellLine1 10K/well Drug-B 20 nM	
CellLine1 10K/well Drug-B 15 nM	

Figure 42: Example of labeling each condition in case of a genetic-chemical perturbagen screen. Drug-A tested on dosage of 100 and 5 nM and the same dosages of drug-A in combination with genetic perturbagen ("Drug-A\_GeneOff"). Drug-B tested on a dosage range of 20 and 15 nM and the same dosages of drug-B in combination with genetic perturbagen ("Drug-B\_GeneOff"). Notice that for each tested drug there is a condition where the solvent was combined with the genetic perturbagen ("Solvent\_Drug-A\_GeneOff" and "Solvent\_Drug-B\_GeneOff").

## Results plots

Unique time point:

- Dose-response relationship: without genetic perturbagen (Figure 43-A), with genetic perturbagen (Figure 43-B), and both curves (Figure 43-C).
- In case of a perturbagen has a inhibition and enhancement effect, the bar plot is shown for each combination, and the predicted value according the BI method is shown by a dash line, see Figure 44 and Figure 45, respectively.
- 2D heatmap with the BI score, see Figure 46.

More than 1 time point:

- Raw data plotted as XY-plot
  - Grouped all dosages for a certain drug, such as all dosage tested for drug A, see Figure 47-A.
  - Grouped all dosages for a certain drug combined with the genetic perturbagen, see Figure 47-B.
  - Grouped by combination: control, drug alone, genetic perturbagen (condition with drug solvent) and the combination of both, see Figure 48.
- Dose-response relationship for the main time points: without genetic perturbagen (Figure 43-A), with genetic perturbagen (Figure 43-B), and both curves (Figure 43-C).
- In case of inhibition or enhancement effect, XY-plot:
  - Grouped by each type of perturbagen alone, see Figure 50 and Figure 51.
  - Grouped by combination, in which the predicted effect, according the BI method is plotted by a dash line, see Figure 52 and Figure 53.
- Heatmap of BI score over time, see Figure 54.

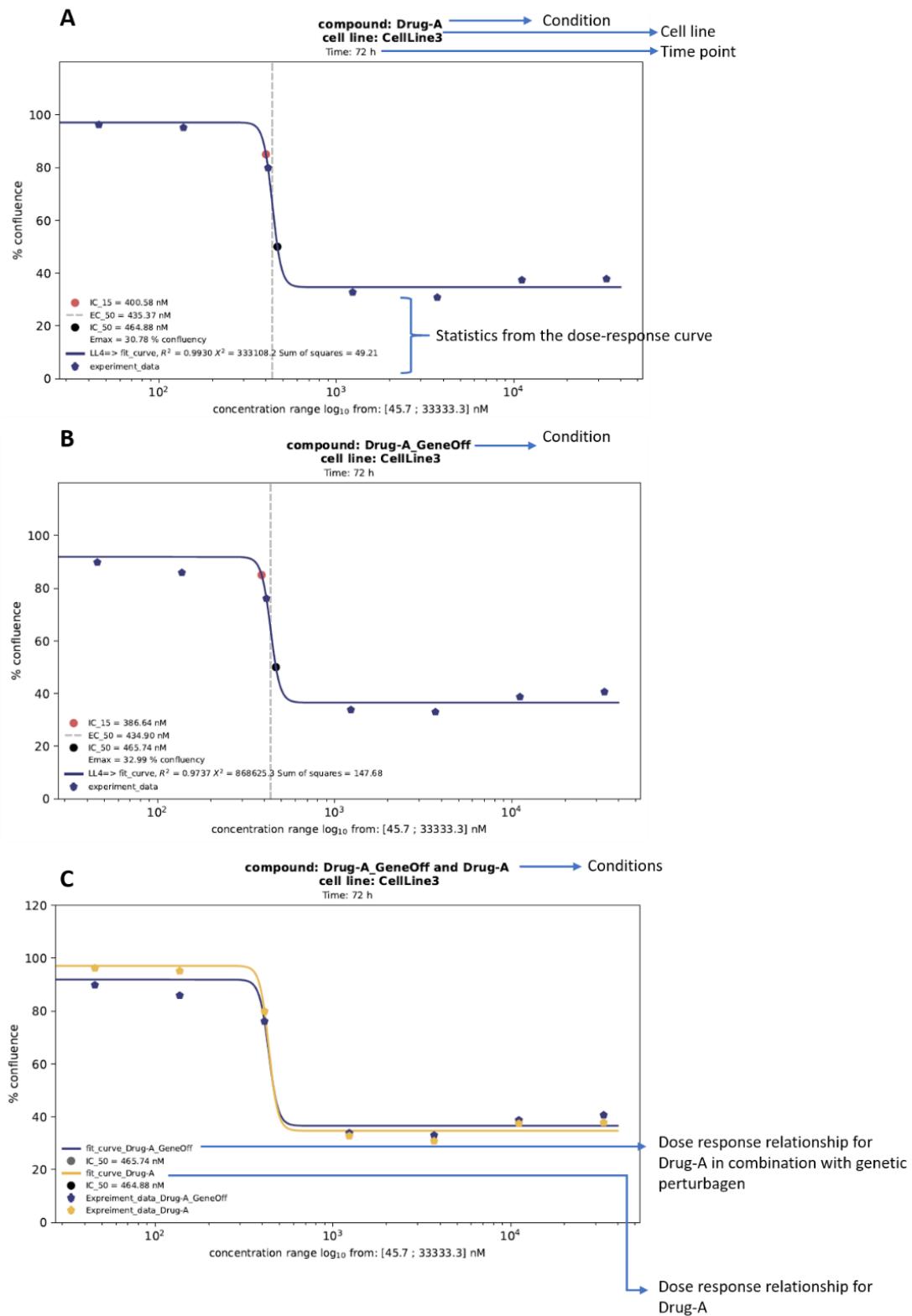


Figure 43: Drug-A dose response relationship. The Y-axis is the read-out provided by the user on the HTSplotter analysis and the X-axis is the concentration range tested transformed into  $\log_{10}$ . A) Dose-response curve of drug-A alone. B) Dose-response curve of drug-A in combination with a genetic perturbagen. C) Dose-response curve of both situations.

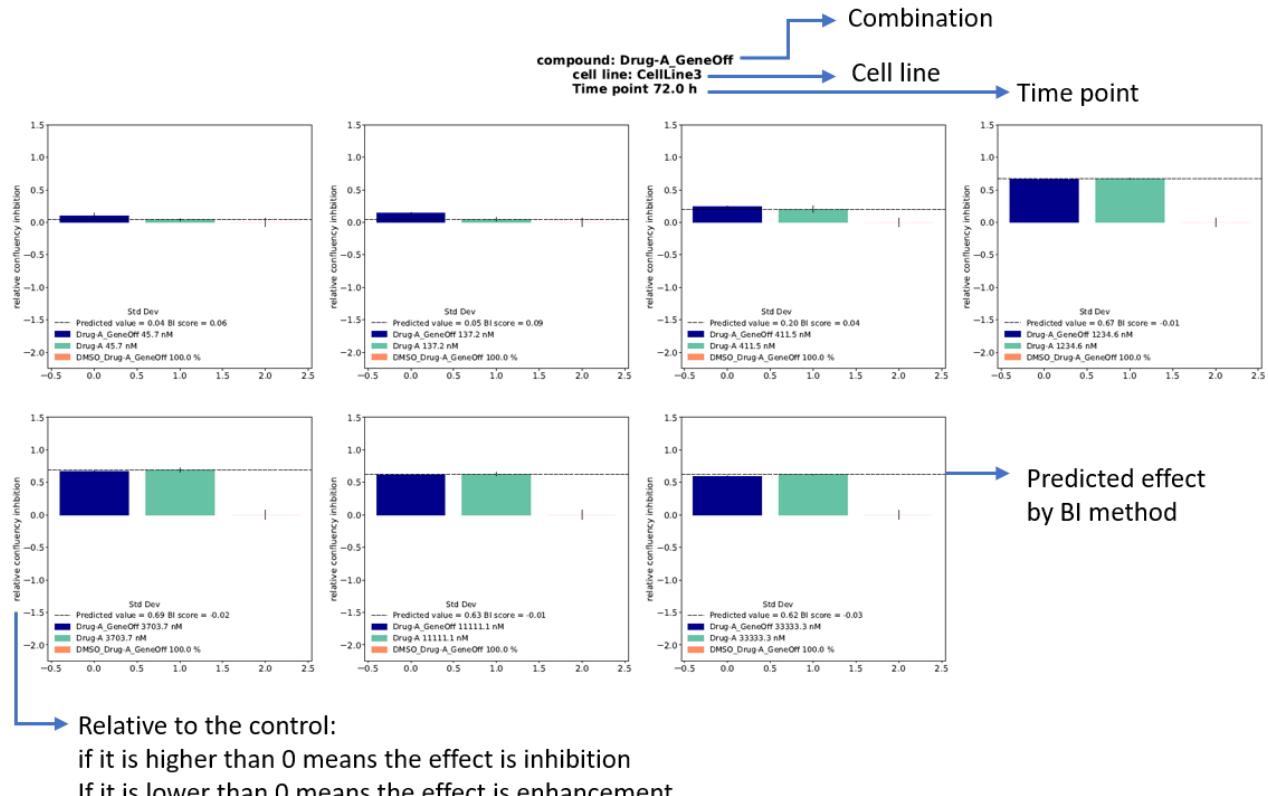


Figure 44: Bar plot of inhibition effect of each combination, drug-A at certain dose, genetic perturbagen with the drug-A solvent and the combination of both. The dash line, is the predicted effect by the BI method.

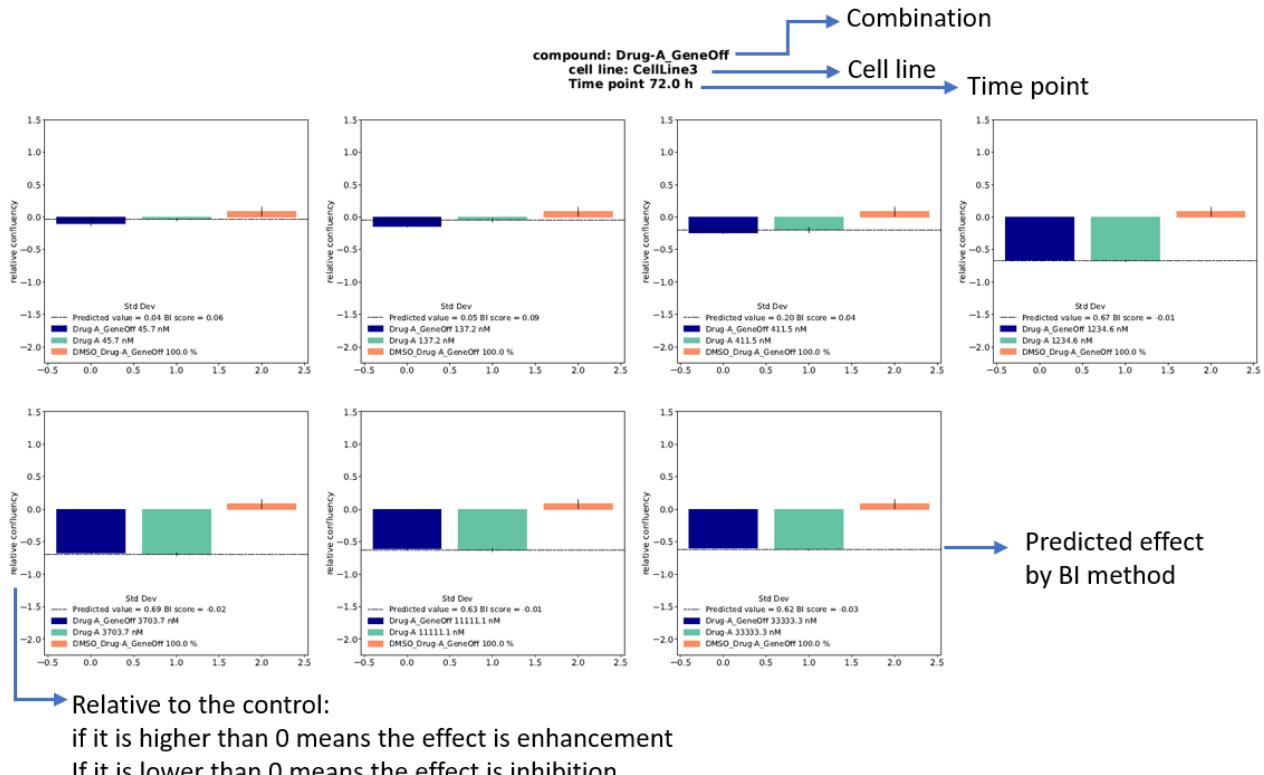


Figure 45: Bar plot of enhancement effect of each combination, drug-A at certain dose, genetic perturbagen with the drug-A solvent and the combination of both. The dash line, is the predicted effect by the BI method.

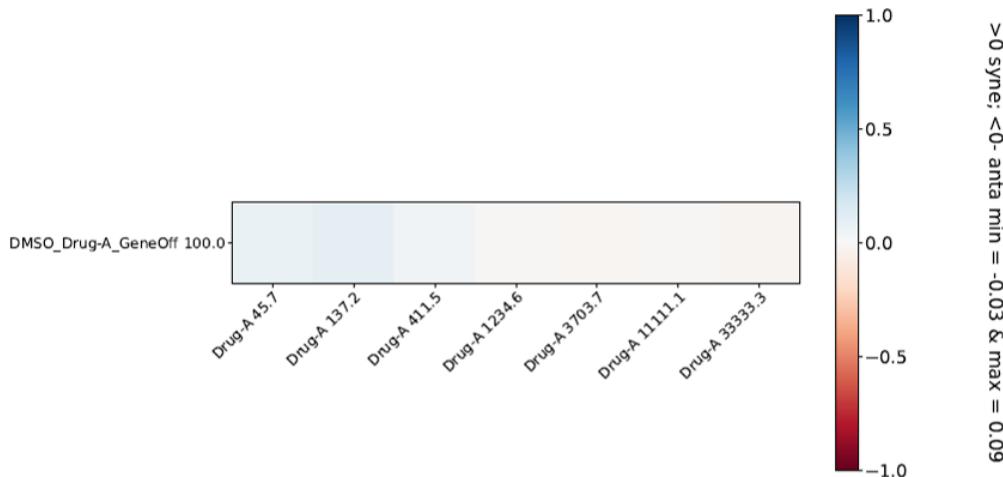


Figure 46: 2D heatmap for the time point 72h, in case of a ( $m \times n$ ) matrix combination.

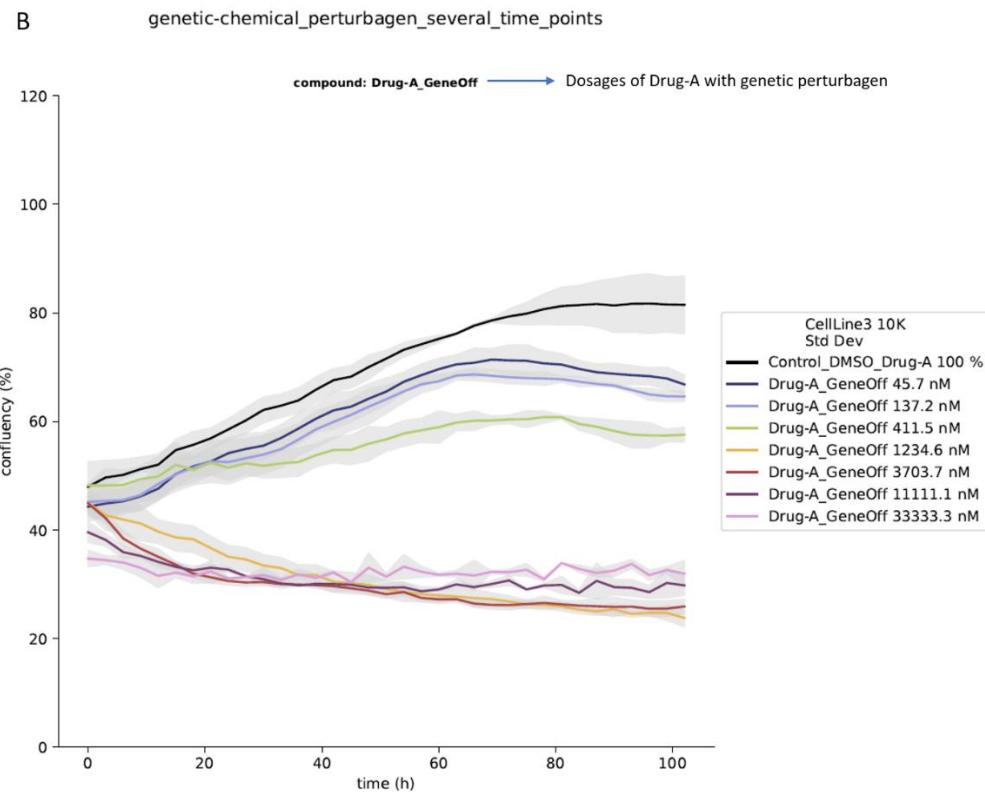
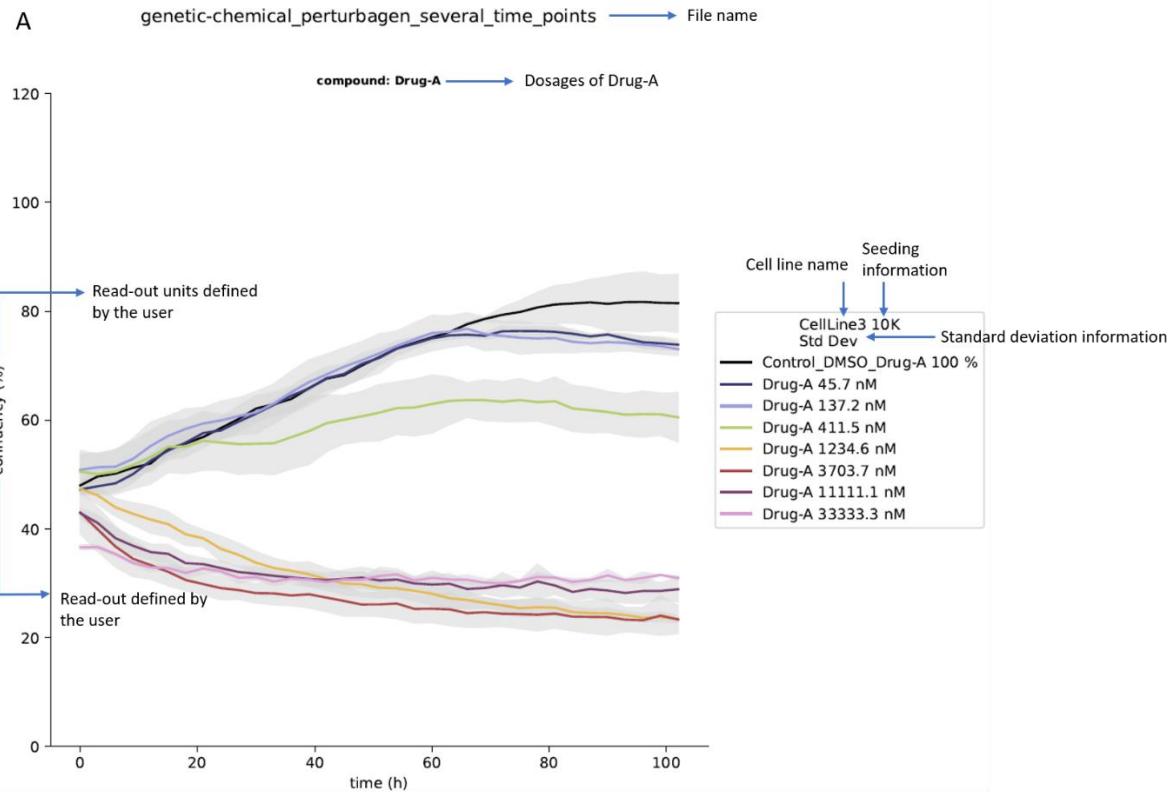


Figure 47: XY-plot example of raw data regarding to all dosages tested of drug-A. Y-axis is the read-out provided by the user and on the X-axis is the time. A) All dosages tested of drug-A alone. B) All dosages tested of drug-A in combination with genetic perturbagen.

genetic-chemical\_perturbagen\_several\_time\_points

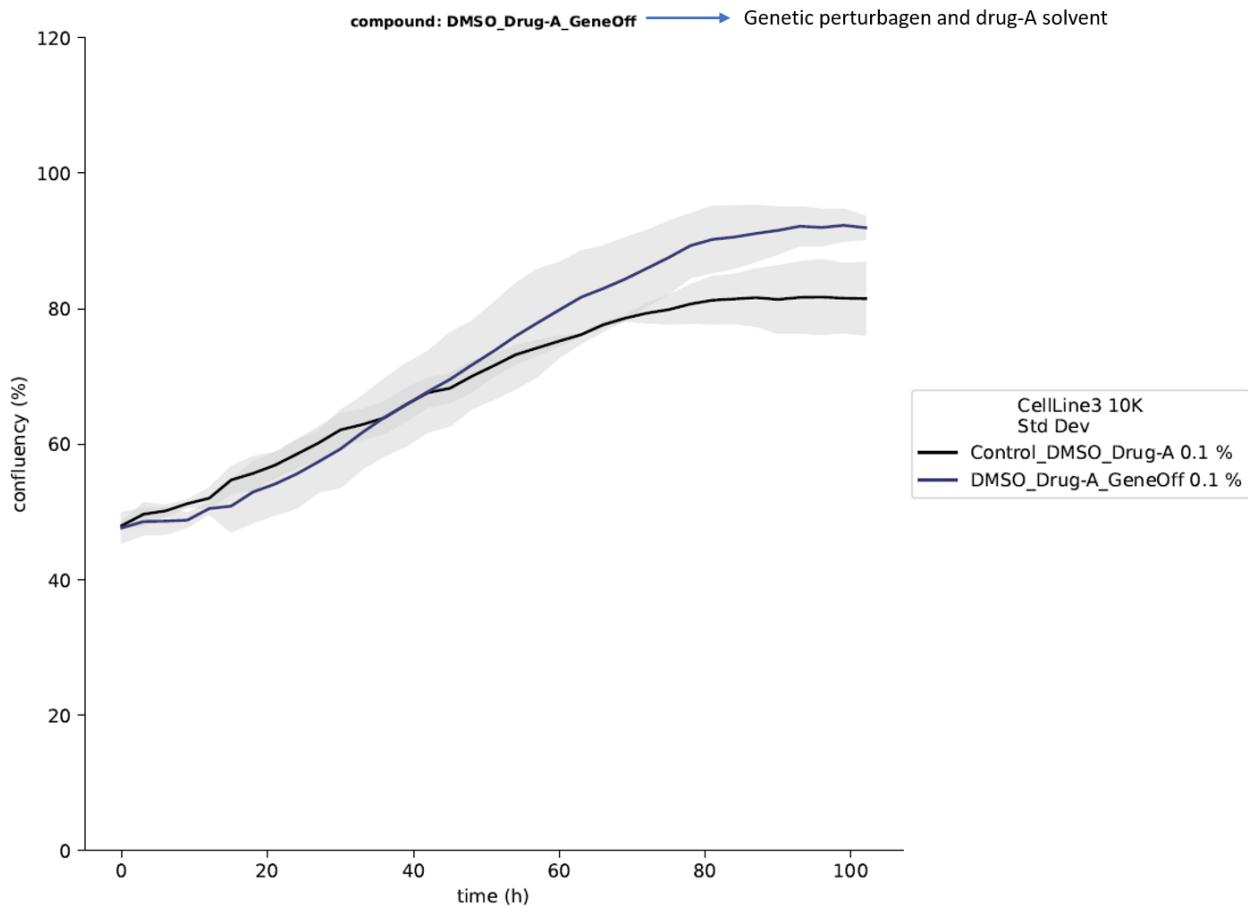


Figure 48: XY-plot example of raw data regarding the genetic perturbagen of a certain gene in combination with the solvent and the solvent alone. Y-axis is the read-out, confluency, and on the X-axis is the time course of the experiment.

genetic-chemical\_perturbagen\_several\_time\_points

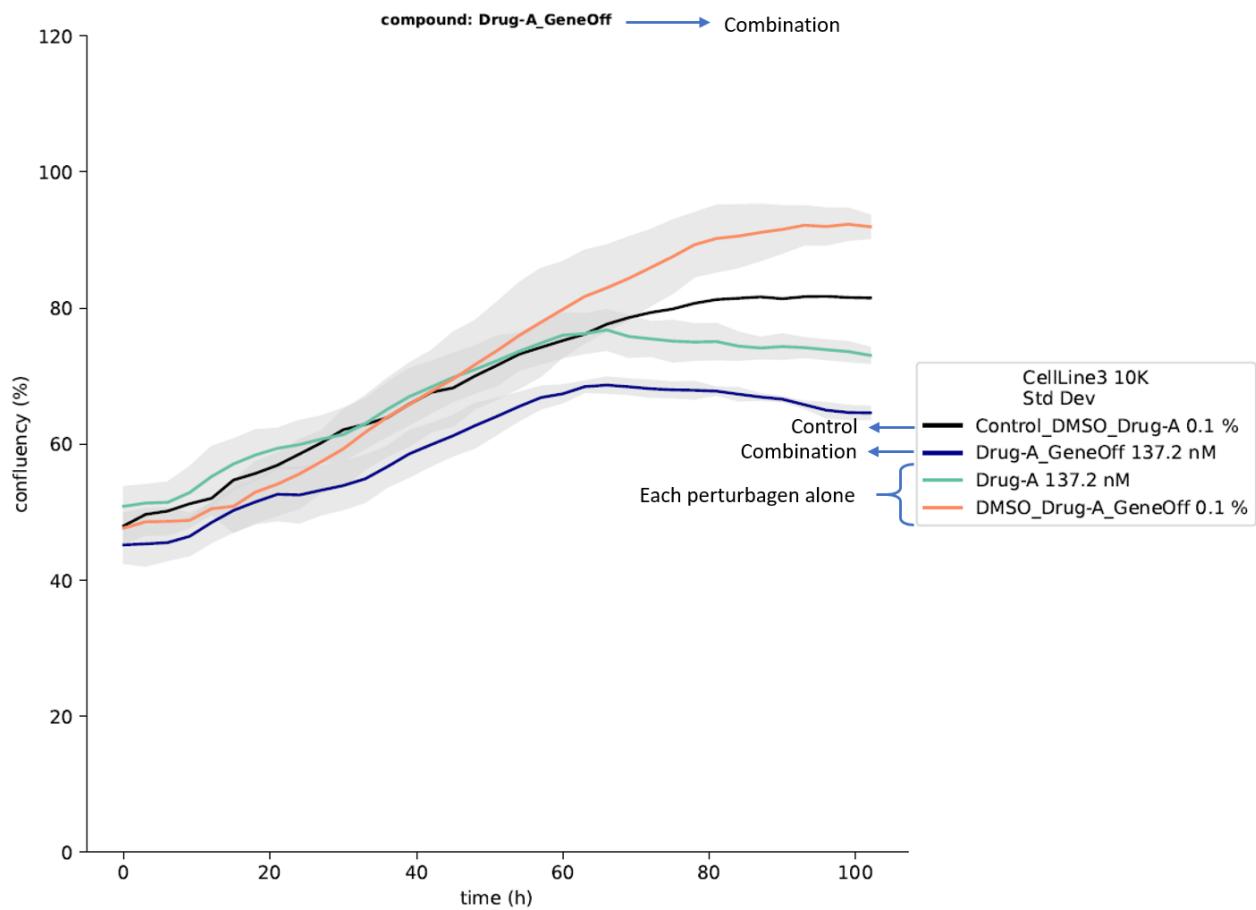
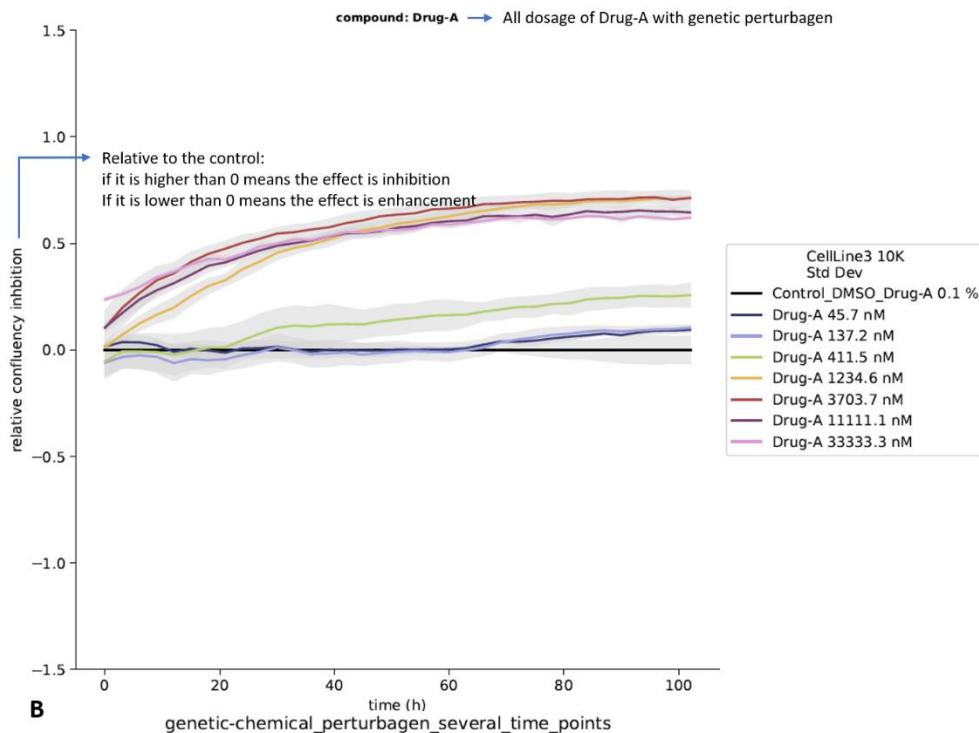


Figure 49: XY-plot of raw data over time for the combination condition (drug-A at 137.2 nM in combination with a genetic perturbagen in which a certain gene is silenced), each perturbagen alone and the experiment control. Y-axis is the read-out, confluency, and on the X-axis is the time course of the experiment.

**A**

genetic-chemical\_perturbagen\_several\_time\_points

**B**

genetic-chemical\_perturbagen\_several\_time\_points

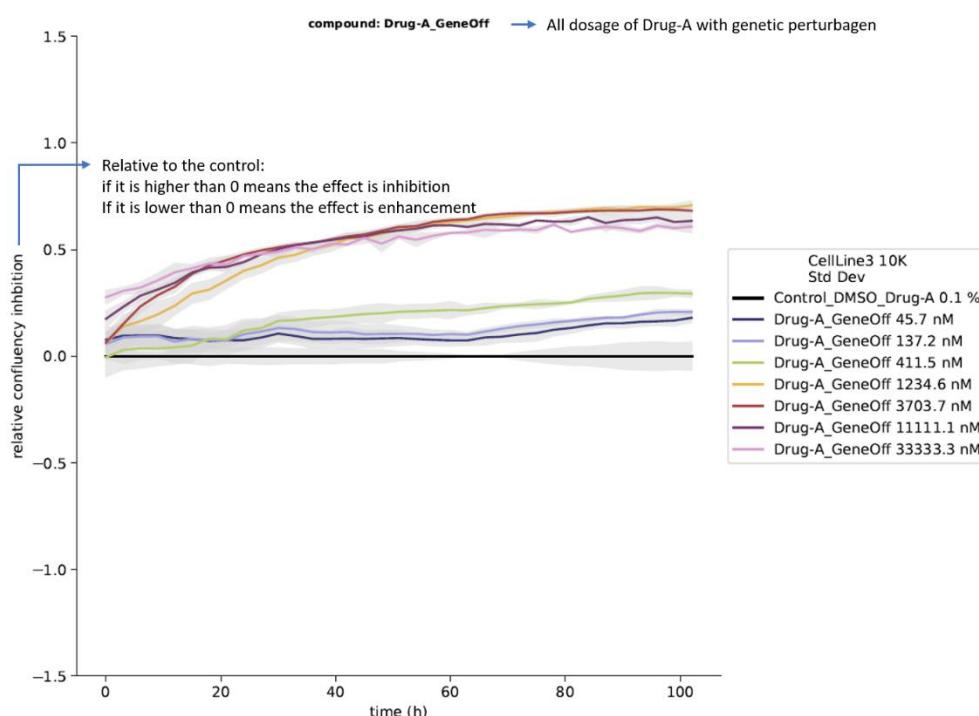


Figure 50: XY-plot example of inhibition effect data regarding to all dosage tested of drug-A. Y-axis is the read-out provided by the user and on the X-axis is the time. A) All dosages tested of drug-A alone. B) All dosages tested of drug-A in combination with genetic perturbagen.

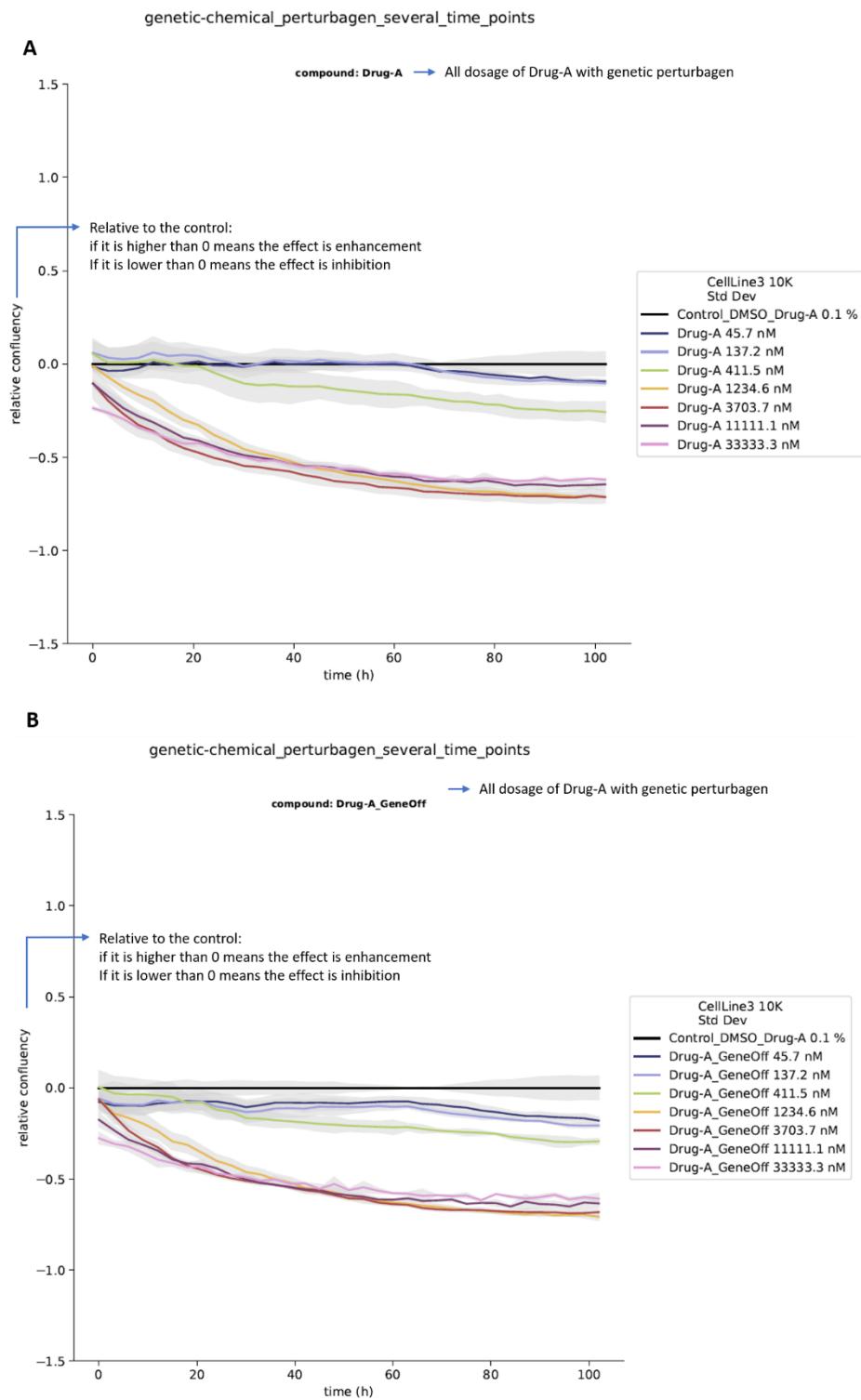


Figure 51: XY-plot example of enhancement effect data regarding to all dosage tested of drug-A. Y-axis is the read-out provided by the user and on the X-axis is the time. A) All dosages tested of drug-A alone. B) All dosages tested of drug-A in combination with genetic perturbagen.

genetic-chemical\_perturbagen\_several\_time\_points

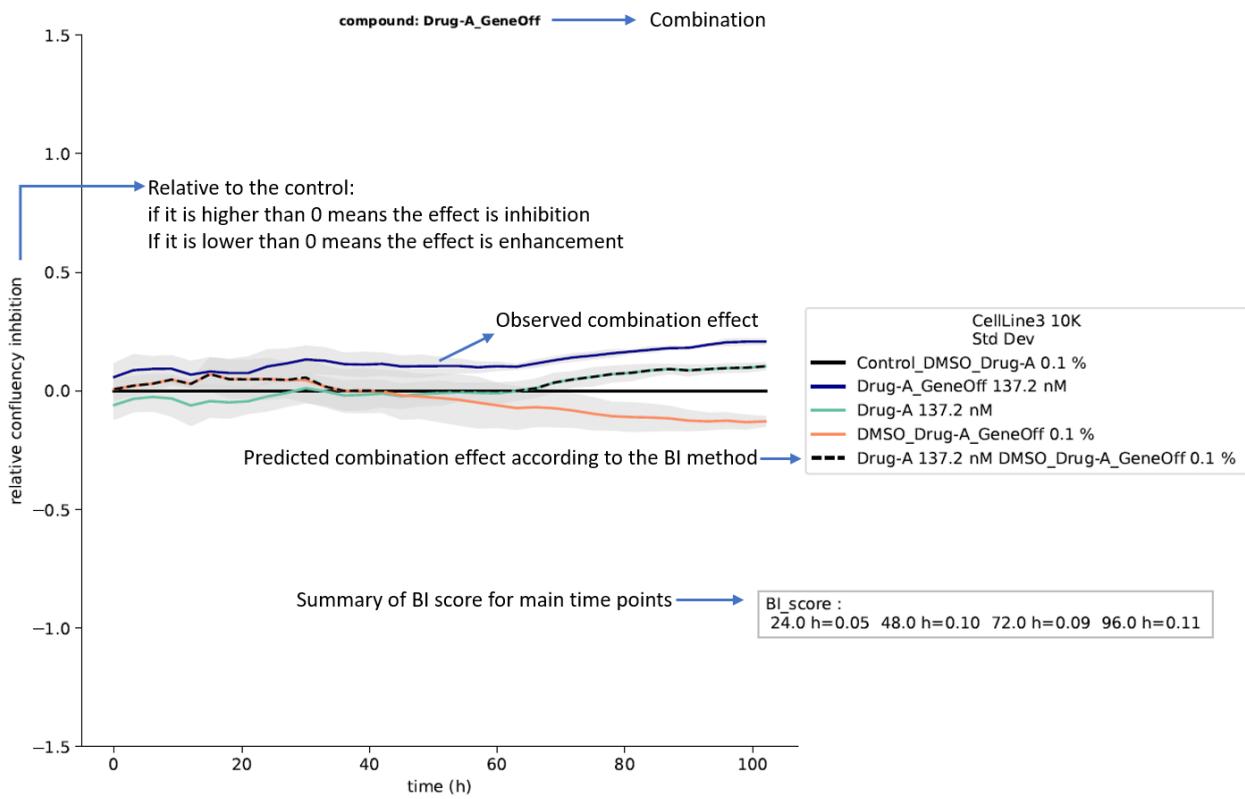


Figure 52: XY-plot showing the inhibition effect over time for the combination condition (drug-A at 137.2 nM in combination with a genetic perturbagen in which a certain gene is silenced), each perturbagen alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

genetic-chemical\_perturbagen\_several\_time\_points

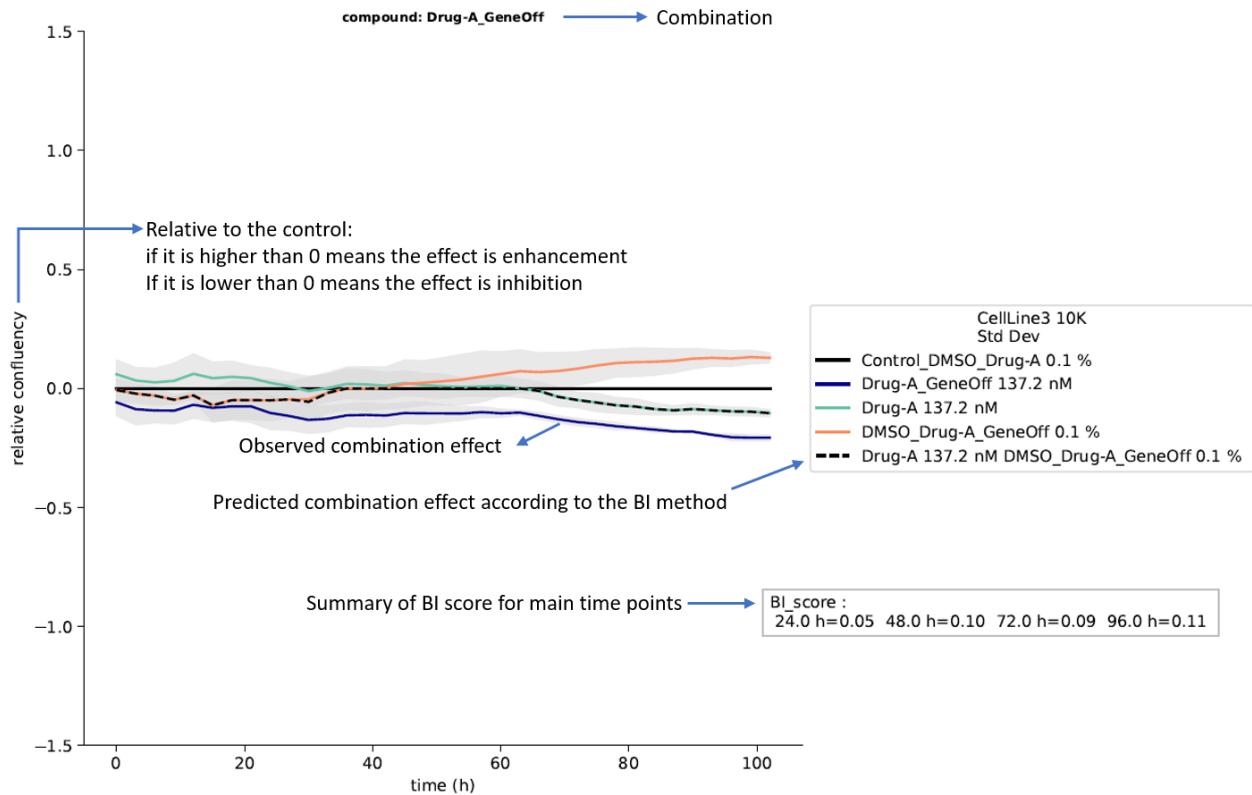


Figure 53: XY-plot showing the enhancement effect over time for the combination condition (drug-A at 137.2 nM in combination with a genetic perturbagen in which a certain gene is silenced), each perturbagen alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

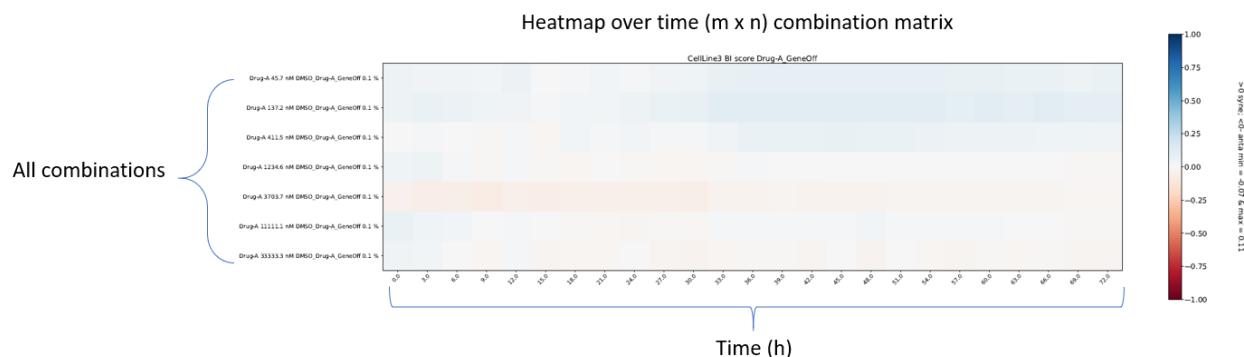


Figure 54: Heatmap over time for (m x n) matrix combination.