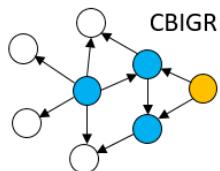


HTSplotter

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HTSplotter

HTSplotter allows an end-to-end data processing and analysis 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.com/CBIGR/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 as 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 BI method. Finally, results are plotted and exported as PDF files, allowing a fast biological interpretation of the data.

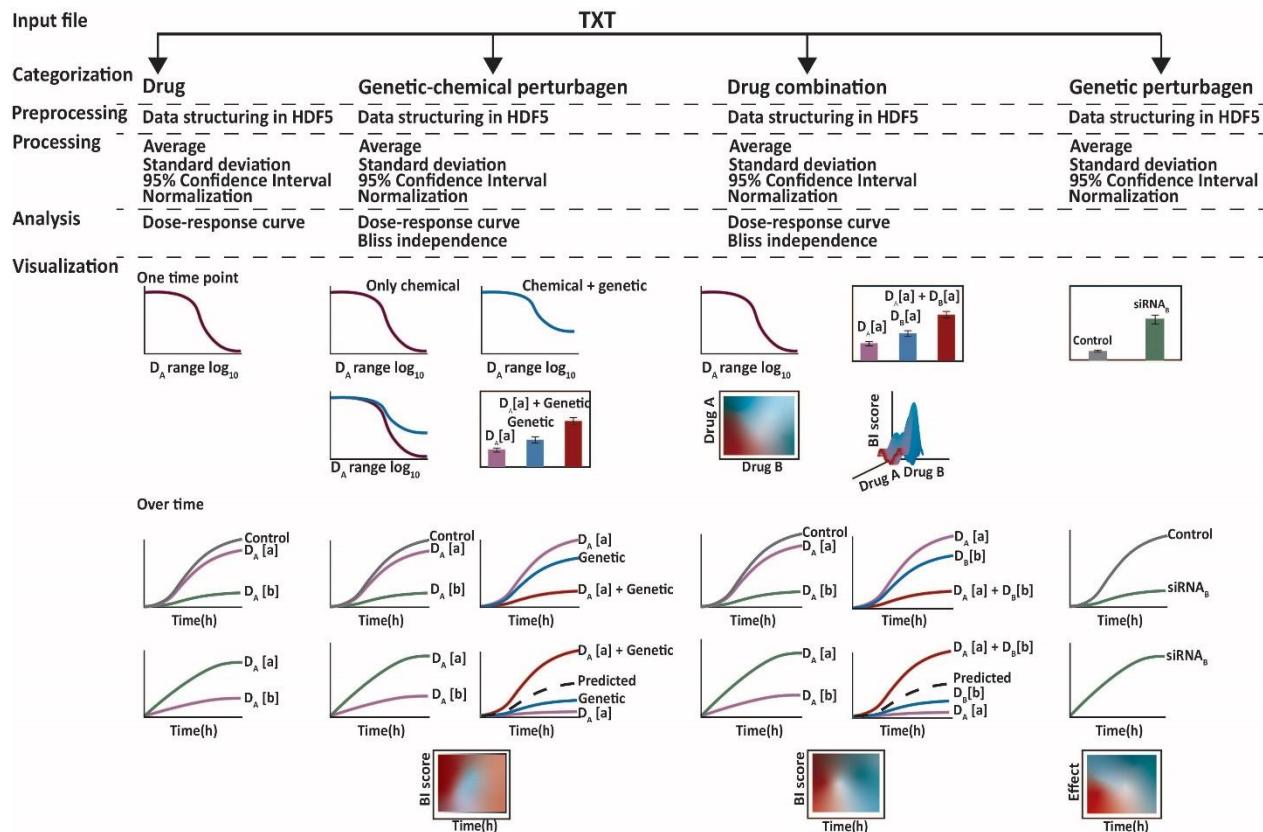


Figure 1: Overview of HTSplotter steps for each type of HTS experiment. The input file, directly imported from HTS machines like Incucyte S3 as a TXT file, is automatically processed and analyzed by HTSplotter. As output, TXT and PDF files are generated. The PDF file contains the plots from each type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

Run analysis

On the “Run analysis” tab, one can submit one or more files, Figure 2.

Analysis of different biological replicates, please check Figure 3.

Analysis of one experiment, please check Figure 4.

The screenshot shows the 'Run your own analysis' page of the HTSpotter interface. At the top, there is a navigation bar with tabs: 'HTSpotter' (selected), 'Run analysis' (highlighted in blue), 'Manual', 'Example files', and 'Feedback'. The main area is titled 'Run your own analysis'. It contains several input fields:

- 'Email address': A text input field with placeholder text 'We'll never share your email with anyone else'.
- 'Input file(s)': A 'Choose Files' button with 'No file chosen' displayed. 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 with options 'no' (selected) and 'yes'.
- 'Biological replicate desired filename': An empty text input field with placeholder text 'Leave empty if no replicate analysis'.
- 'Expected effect': A dropdown menu with options 'inhibition' (selected) and 'enhancement'.
- 'Information readout': An empty text input field.
- 'Readout unit': An empty text input field.

At the bottom right 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. Once all the fields are filled, click on the button “Run analysis”.

Run your own analysis

Email address

We'll never share your email with anyone else

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

biological_replicates_drug_combination_screen

Leave empty if no replicate analysis

Expected effect

inhibition

Information readout

confluency

Readout unit

%

Figure 3: Example of three files to be analyzed for biological replicate.

Run your own analysis

Email address

We'll never share your email with anyone else

Input file(s)

Choose Files drug_combi...,timepoint.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

enhancement

Information readout

confluency

Readout unit

%

Figure 4: Example of an analysis of one file.

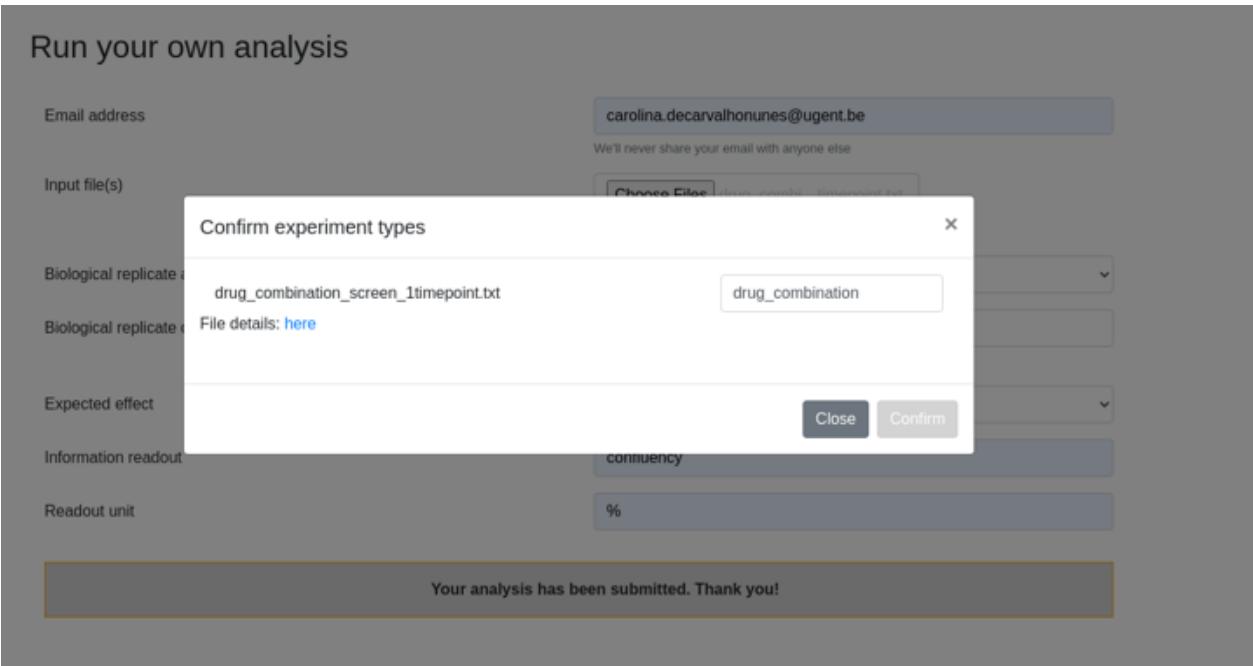


Figure 5: After submitted an analysis, a “confirm experiment types” window pops-up. To check details from the file, clicking on “here” button. In case of expecting a different experiment categorization, please re-write on the box with the experiment type. Please check Figure 9 for more information in case of mismatching experiment types. In case of correct categorization click on the bottom “confirm” and check Figure 7 for more details.

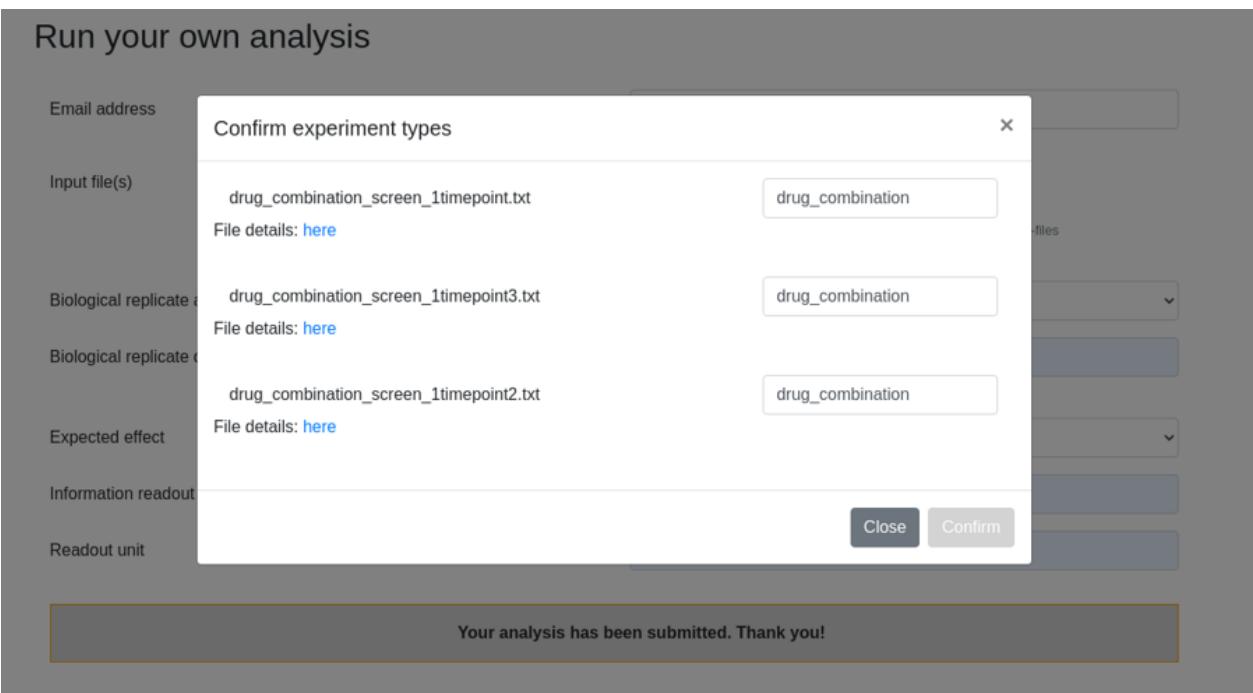


Figure 6: After submitted an analysis, a “confirm experiment types” window pops-up. In case of more than one file, one can check details from each file, clicking on “here” button. In case of expecting a different experiment categorization, please re-write on the box with the experiment type. Please check Figure 9 for more information in case of mismatching experiment types. In case of correct categorization click on the bottom “confirm” and check Figure 7 for more details.

Run your own analysis

Email address We'll never share your email with anyone else

Input file(s) 3 files
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

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

Figure 7: Once the analysis is successfully, one can check the PDF results by clicking on the button “here”. As to download all results files, including the input file, please click on “Download all”, in which a zip file should be downloaded to one’s computer.

Run your own analysis

Email address We'll never share your email with anyone else

Input file(s) 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

Biological replicate desired filename

Leave empty if no replicate analysis

Expected effect

Information readout

Readout unit

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

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](#) chapter.

Run your own analysis

Email address

We'll never share your email with anyone else

Input file(s)

Choose Files drugscreen...enhanced.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

%

The experiment type did not match. Please [click here](#) to see the error.

Figure 9: If the categorization by HTSplotter did not correspond to the expected one, the following message is shown. Please, 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 have standard deviation, it should be after the last condition and following the same order. For all standard deviation, the labelling must be the same as the experiment condition but with “(Std)” word at the end, as shown on Figure 10.

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

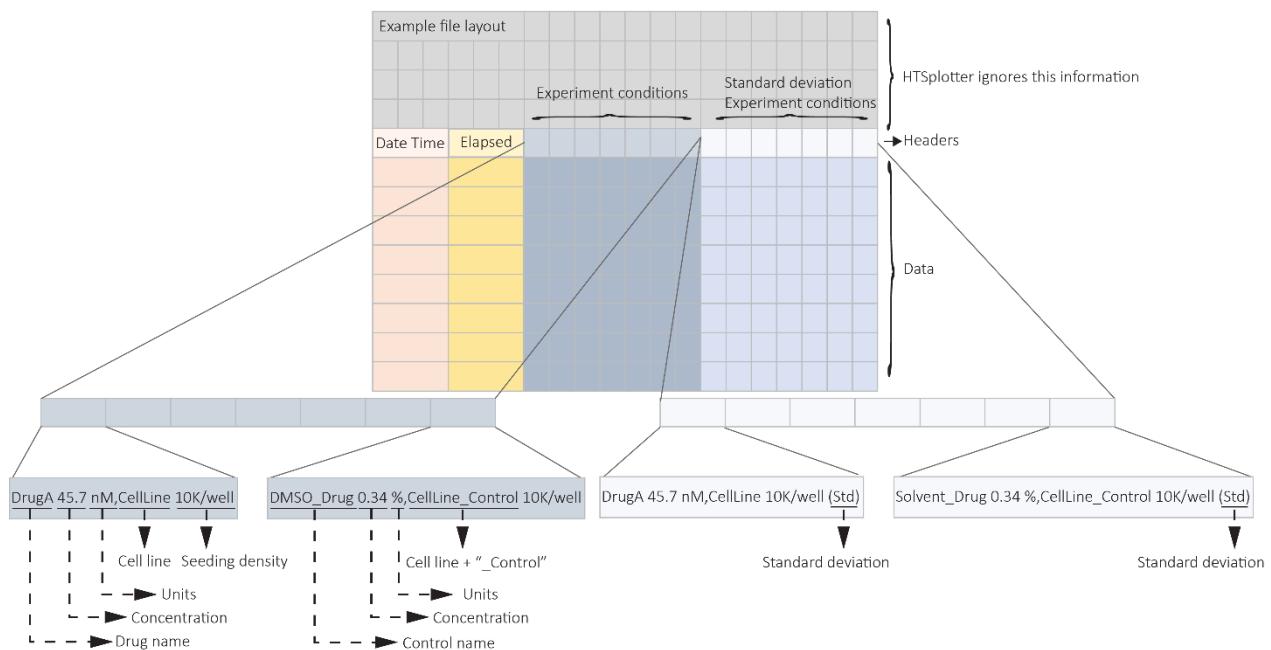


Figure 10: Example of TXT file layout with a description of an example of an experiment condition and control. Following the same order, is the standard deviation of the same experiment condition and of the same control.

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 has 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.

Experiments types and general information:

HTSplotter automatically identifies four experiment types per txt file:

Drug screen:

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

Drug combination screen:

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

Genetic perturbagen screen:

- A genetic perturbagen screen can be 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:

- Consist of a genetic perturbagen in combination with a drug.
- In this case a tag should be added to the drug name, for example: Drug-A_GeneOff, where:
- Drug-A is the name of the drug
- The tag, indicating the genetic perturbagen, is the “_GeneOff”.

More details about each experiment type can be found in their repetitive chapter.

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

Figure 12.

- In case of 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 genetic-chemical perturbagen, the condition where the drug is combined with the 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 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 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 Figure 12-B.

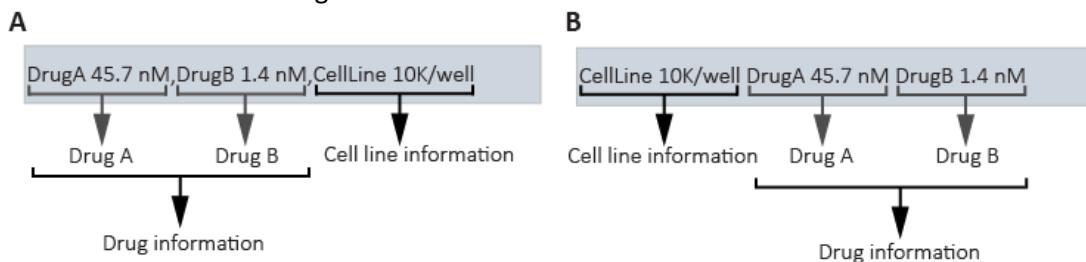


Figure 12: Example of different ways to annotate conditions. A) information separated by “,”. B) information separated by space.

Labelling conditions

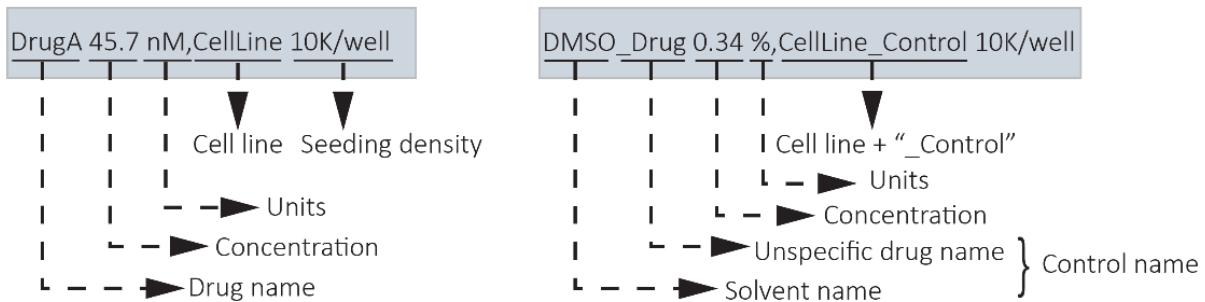
The labeling of the control differentiates from all the other conditions as shown on Figure 13.

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

Table 1: * in case of genetic perturbagen experiment the labelling follows the same rule as the drug screen, whereas in case of genetic-chemical perturbagen, a tag is required in front of the drug name, e.g. “DrugA_GeneOn”. **for Drug, genetic and genetic-chemical perturbagen, different controls are allowed. Therefore, in front of cell line name add the tag “_Control”. Without this tag HTSplotter does not 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	
Units		Units	Seeding

A



B

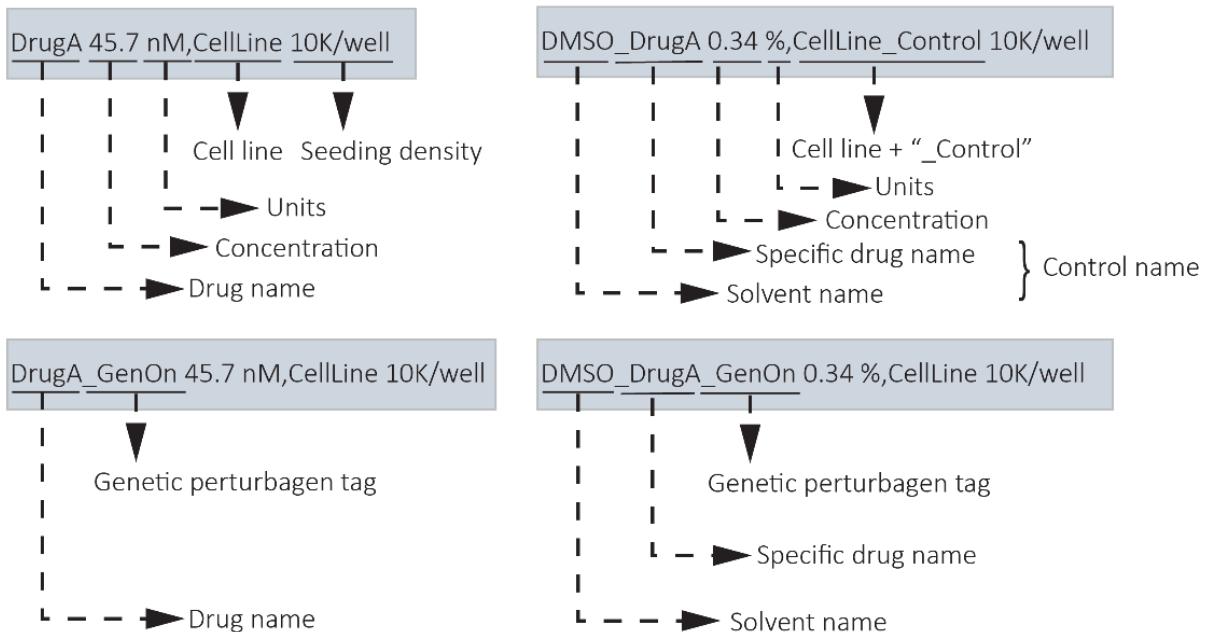


Figure 13: Example of labeling the experiments conditions vs control. A) Example of drug screen experiment, where the control name is not specific for the tested drug. The tag “_Control” is added to the cell line name. B) Example of genetic-chemical perturbagen, where the control name must be specific 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.

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 when the units information is missing. HTSplotter writes on the expected position as "unidentified". At the end of each row, between straight brackets, is the position on 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 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 on Figure 16. This file is called as the experiment file name plus "_information.txt".

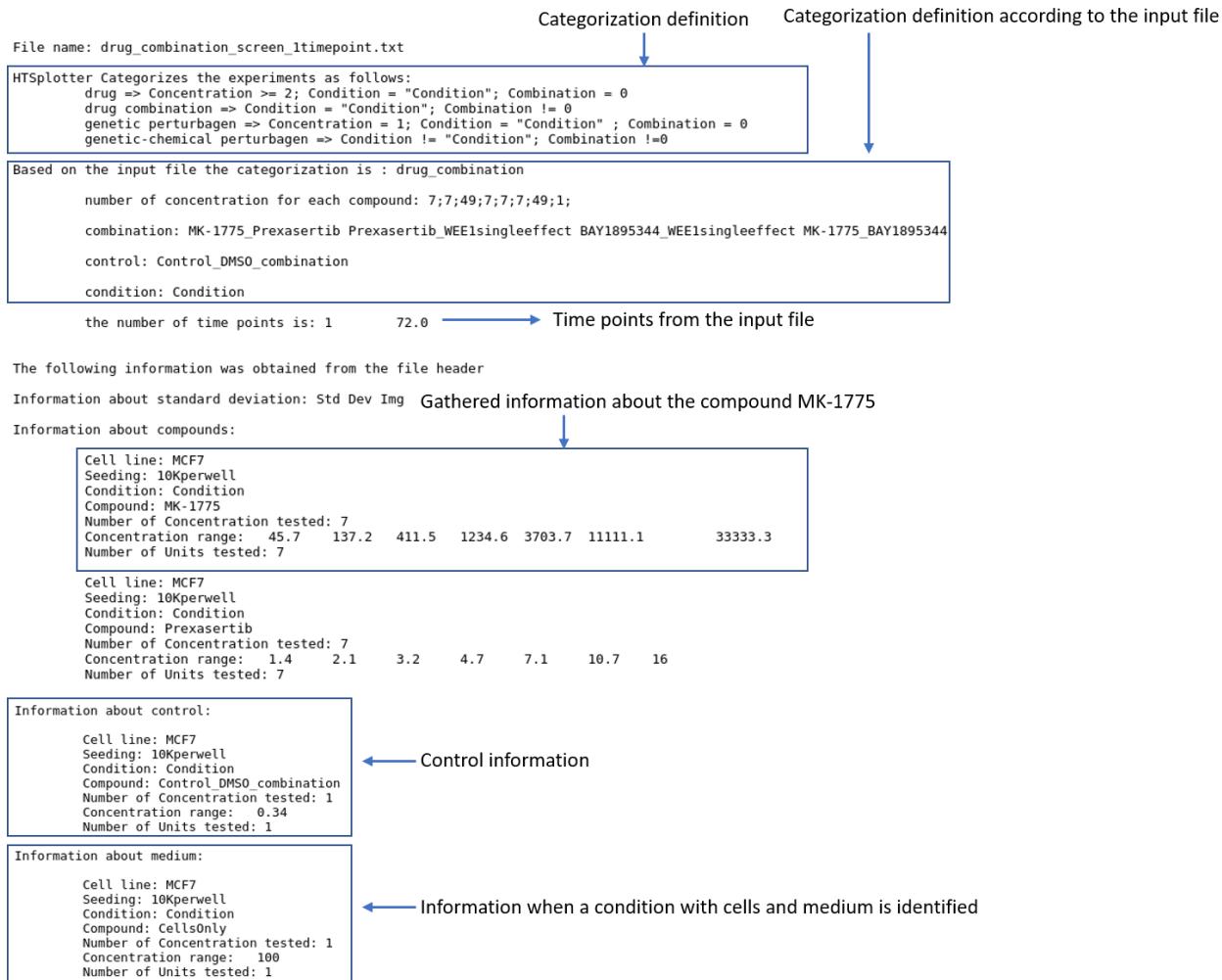


Figure 16: Example of an information file, from an drug combination experiment with only one read-out. In case of drug combination, please make sure that the number of dosage combined of each combination are the same as the ones from each compound alone.

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 (https://www.hdfgroup.org/downloads/hdfview/)).

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 (https://www.hdfgroup.org/downloads/hdfview/)).

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 (<https://www.hdfgroup.org/downloads/hdfview/>)).

Experiment 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 the 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 dosage from 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) It is an example when one control is used for every tested drug. B) It is 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 Figure 20.
- In case of inhibition or enhanced effect, Figure 21 and Figure 22, respectively.
 - XY-plot shows all dosage effect of a certain drug.

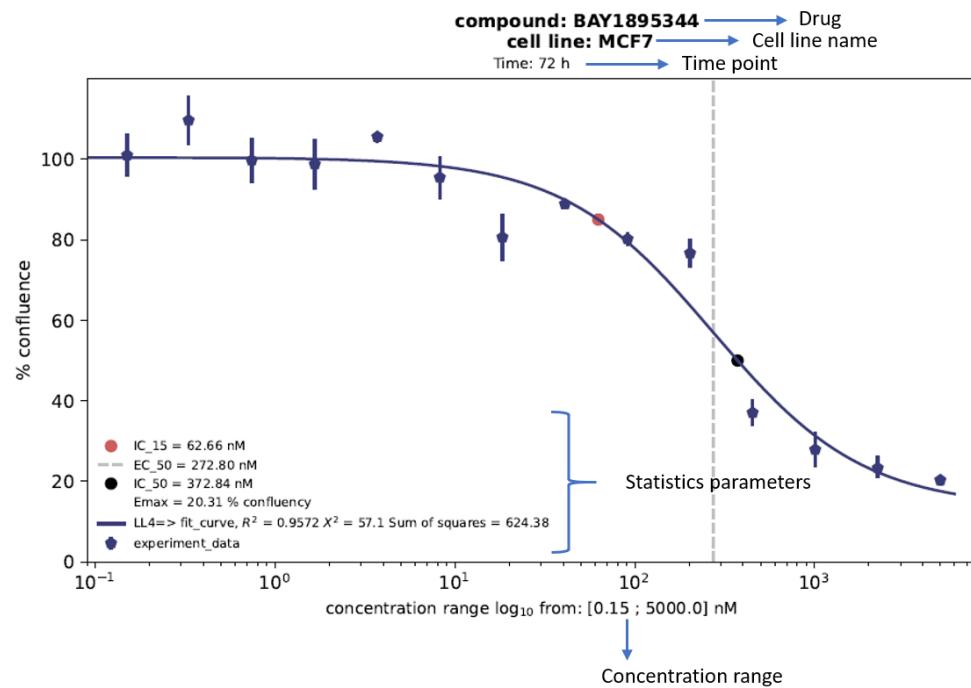


Figure 18: Dose response relationship for compound BAY1895344 tested on the cell line MCF-7. The Y-axis is the read-out provided by the user on the HTSplotter analysis. X-axis is the concentrations range tested transformed into \log_{10} , indicating the units.

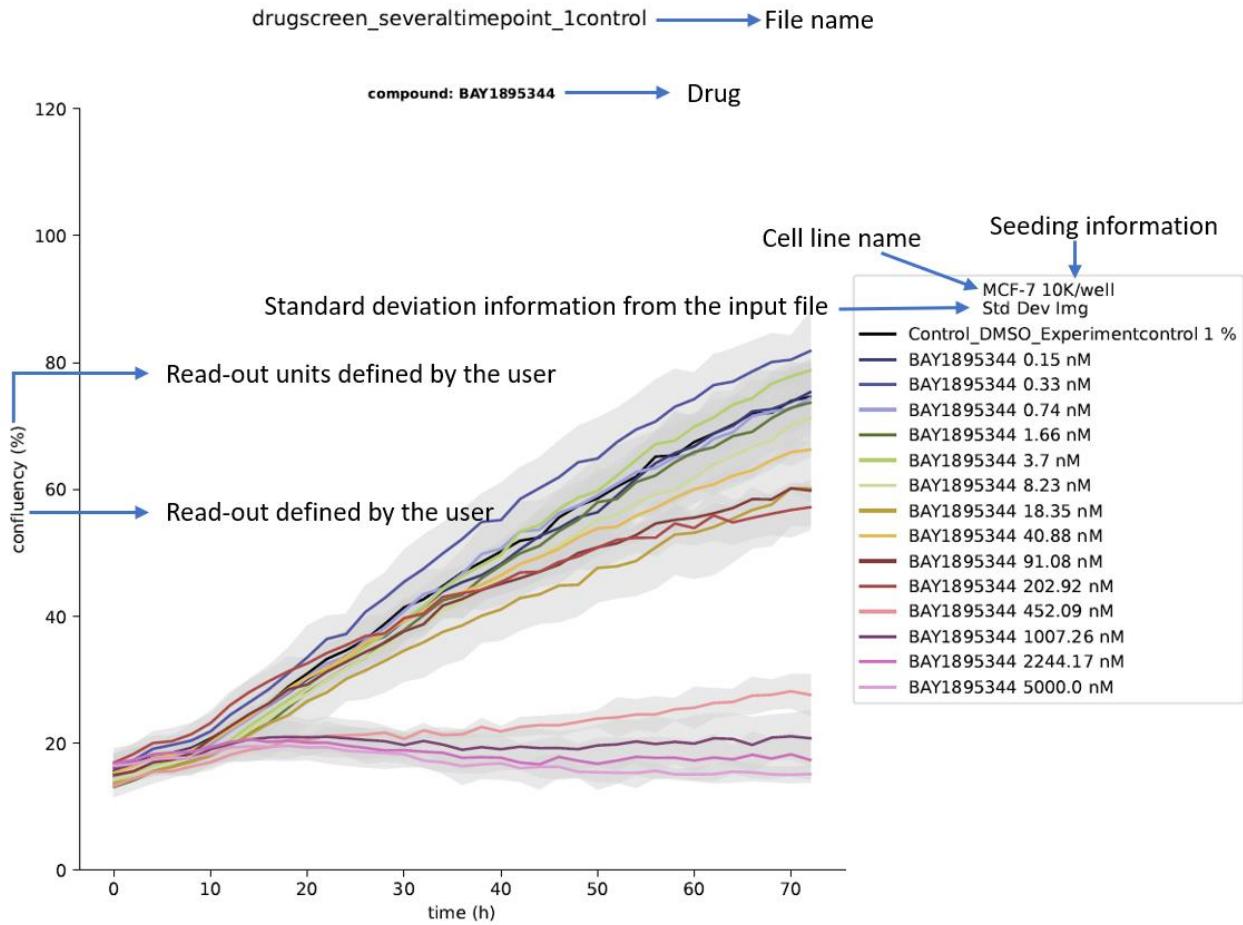


Figure 19: XY-plot example of raw data regarding to all dosage tested of BAY1895344. Y-axis is the read-out provided by the user and on the X-axis is time.

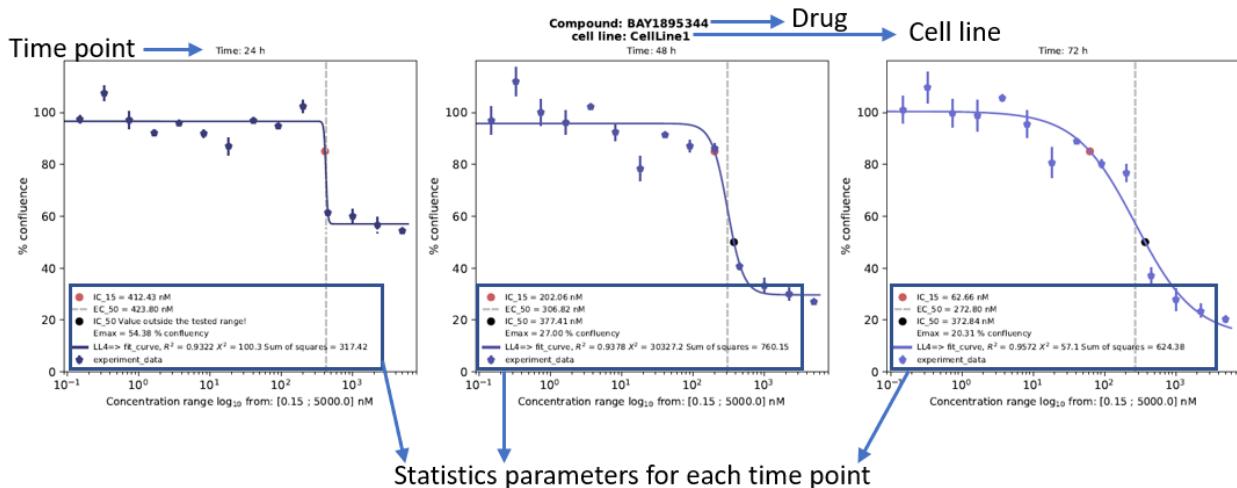


Figure 20: Dose-response relationship of BAY1895344 on the cell line MCF-7 for each main time point. The Y-axis is the read-out provided by the user on the HTSplotter analysis. X-axis is the concentrations range tested transformed into log₁₀, indicating the units.

drugscreen_severaltimepoint_1control

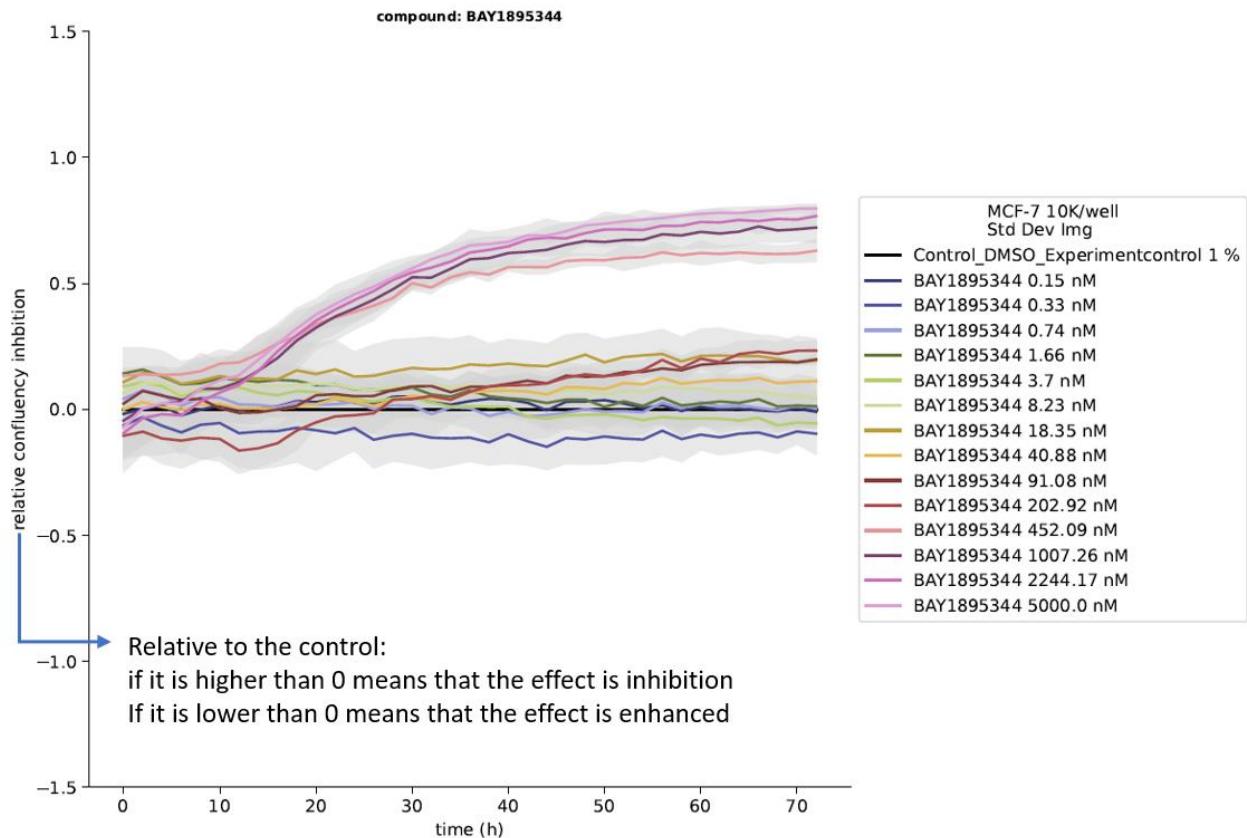


Figure 21: XY-plot example of inhibited effect of all dosage tested of BAY1895344 in relation to the Control, named as "Control_DMSO_Experimentcontrol". Y-axis is the read-out provided by the user with inhibition remark and on the X-axis is the time.

drugscreen_severaltimepoint_1control

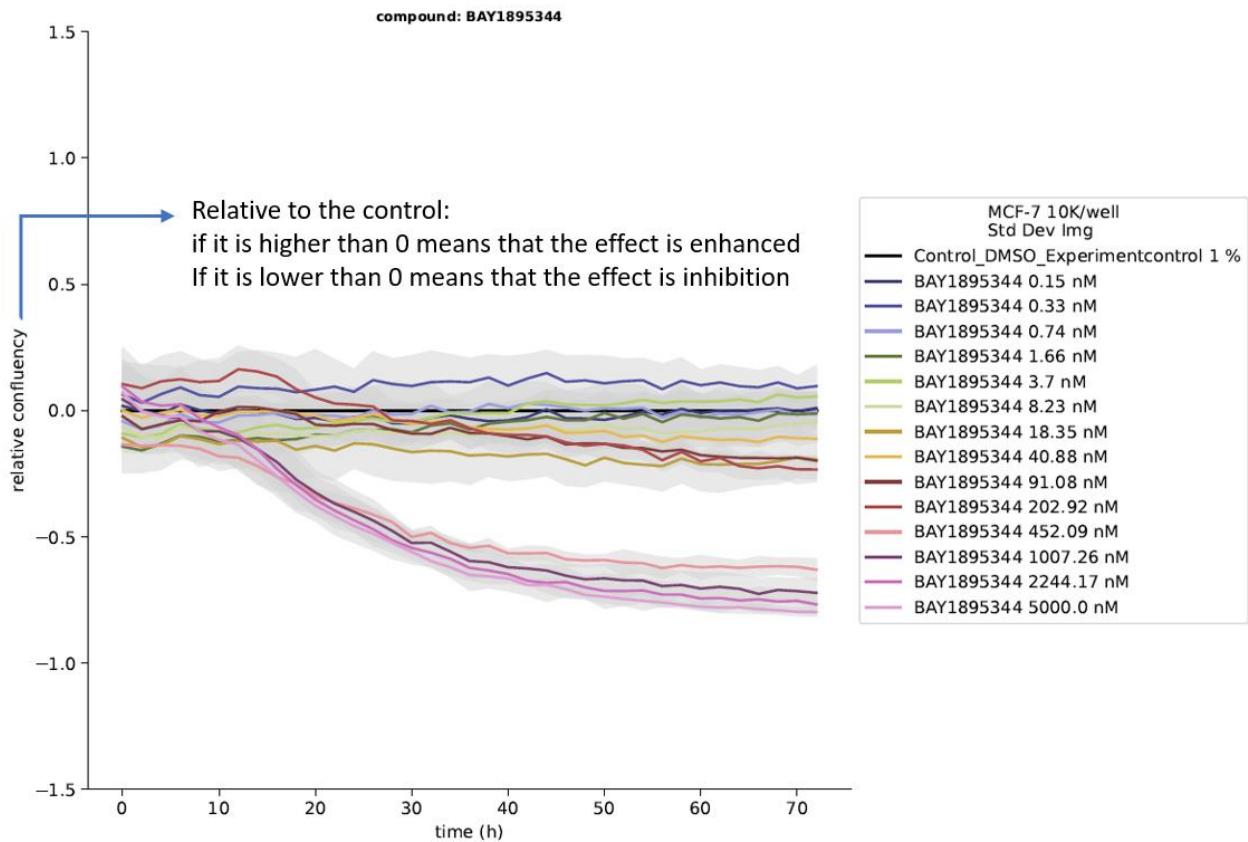


Figure 22: XY-plot example of the effect of all dosage tested of BAY1895344 in relation to the Control, named as "Control_DMSO_Experimentcontrol". Y-axis is the read-out provided by the user and on the X-axis is the time.

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 drug in combinations

- Number of cell lines
- Matrix combination:
- (m x n): e.g. 1 dosage of drug A combined with 7 different dosage of drug B
- (n x n): e.g. 7 different dosage of drug A combined with 7 different dosage 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 (https://www.hdfgroup.org/downloads/hdfview/)).

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 (<https://www.hdfgroup.org/downloads/hdfview/>)).

Experiment design

In case of combination, please make sure to have a condition for each drug and dosage alone and for the combination 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 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_δ is the effect of drug B, at δ dosage
 - A_δ is the effect of a drug A, at δ dosage
 - $O_{A_\delta B_\delta}$ is the effect of B_δ combined with A_δ
- $$P_{A_\delta B_\delta} = A_\delta + B_\delta - (A_\delta * B_\delta) \quad (1)$$
- $$BI \text{ score} = O_{A_\delta B_\delta} - P_{A_\delta B_\delta} \quad (2)$$

In this experiment requires one control, which 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 andd 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 required fields to determine synergism or antagonism. Notice that it is required conditions with each drug at certain dosage alone.

Results plots

Unique time point:

- Dose-response relationship for each drug alone, as shown on Figure 18.
- In case of inhibition or enhanced effect, the bar plot is shown for each combination. The predicted effect according to the BI method is shown by a dash line Figure 24 and Figure 25.
- 2D and 3D heat map in case of (n x n) matrix combination, Figure 26.

More than 1 time point:

- Raw data plotted as XY-plot
 - Grouped all dosage for a certain drug Figure 27.
 - Grouped by combination, control, each drug alone and the combination Figure 28.
- Dose-response relationship for each drug alone Figure 20.
- In case of inhibition or enhanced effect:
 - XY- plot grouped by dosage Figure 21 and Figure 22.
 - XY-plot grouped by combination. The predicted effect, according the BI method is plotted by a dash line Figure 30and Figure 31.
- Bar plot for each combination. Represented with a dash line, it is shown the predicted effect according to the BI method Figure 24 and Figure 25.
- Heatmap over time for any type of matrix combination
- BI score shown through a heat map for each time point.
 - (n x n) matrix combination Figure 32.

- (m x n) matrix combination Figure 33.
- Heatmap 2D and 3D for the main time points, only in case of (n x n) matrix combination, Figure 26.

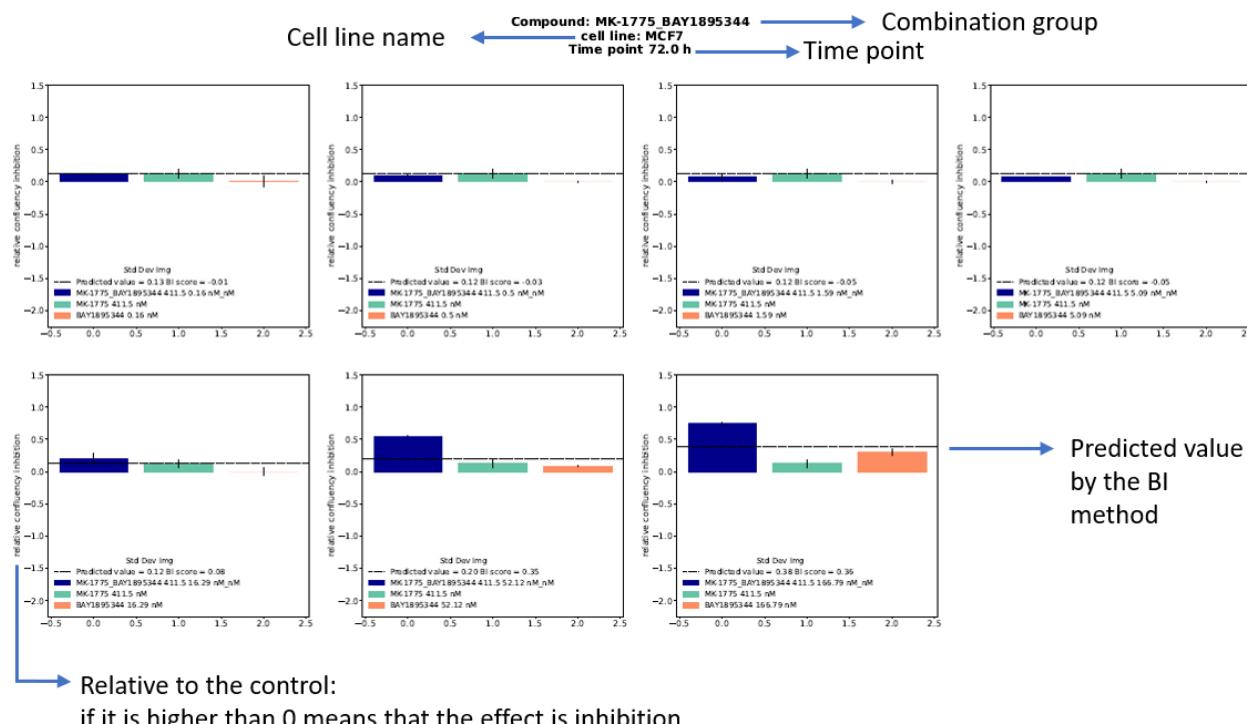


Figure 24: Bar plot with the inhibition effect of each combination and each compound alone for the main time point 72h. In each plot a dash line represents the predicted effect by the BI method. On the legend of each bar plot.

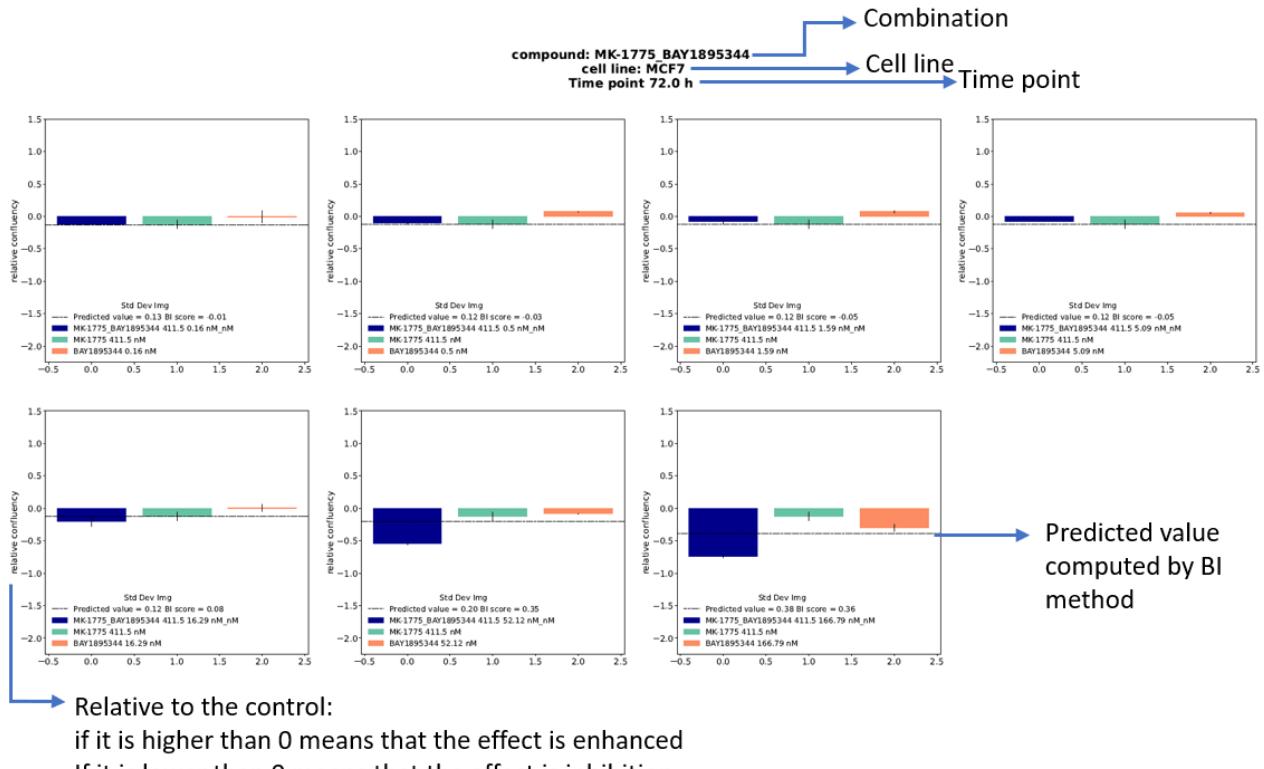


Figure 25: Bar plot with the enhanced effect of each combination and each compound alone for the main time point 72h. In each plot a dash line represents the predicted effect by the BI method.

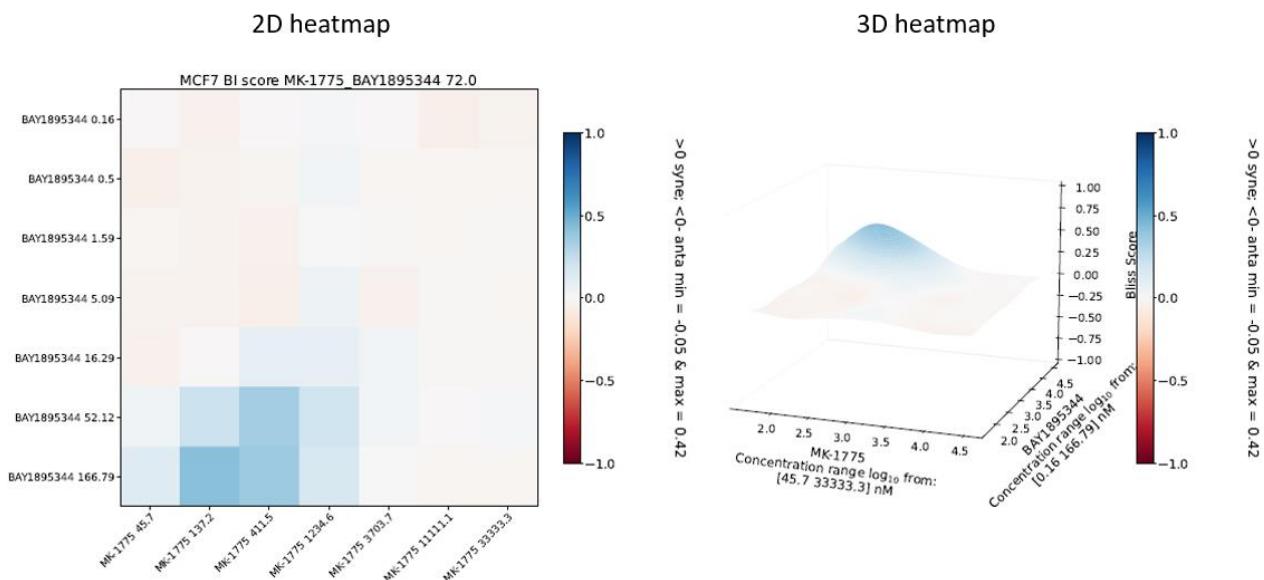


Figure 26: A heatmap 2D and 3D shown for the time point 72h, in case of ($n \times n$) matrix combination.

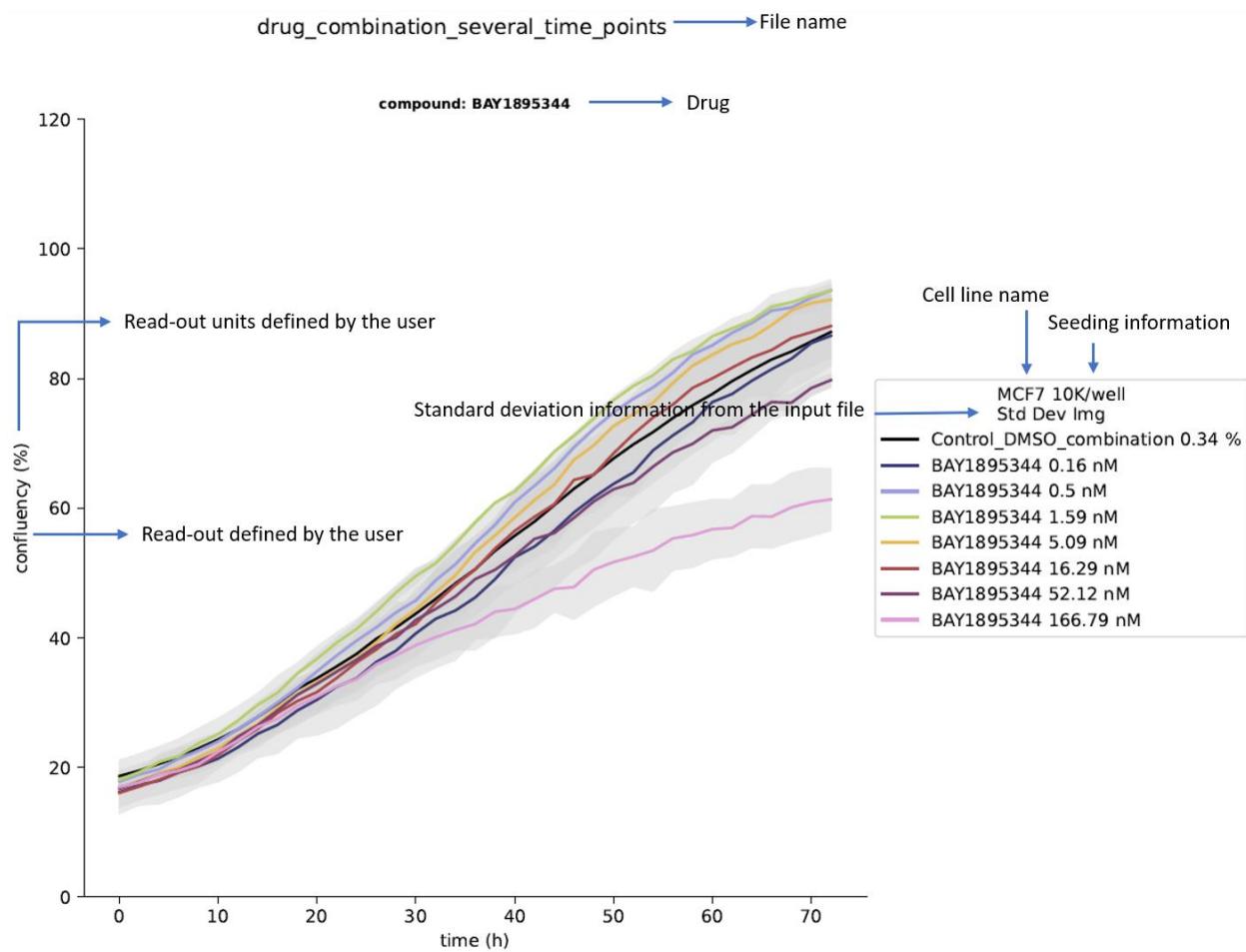


Figure 27: XY-plot example of raw data regarding to all dosage tested of BAY1895344. Y-axis is the read-out provided by the user and on the X-axis is time.

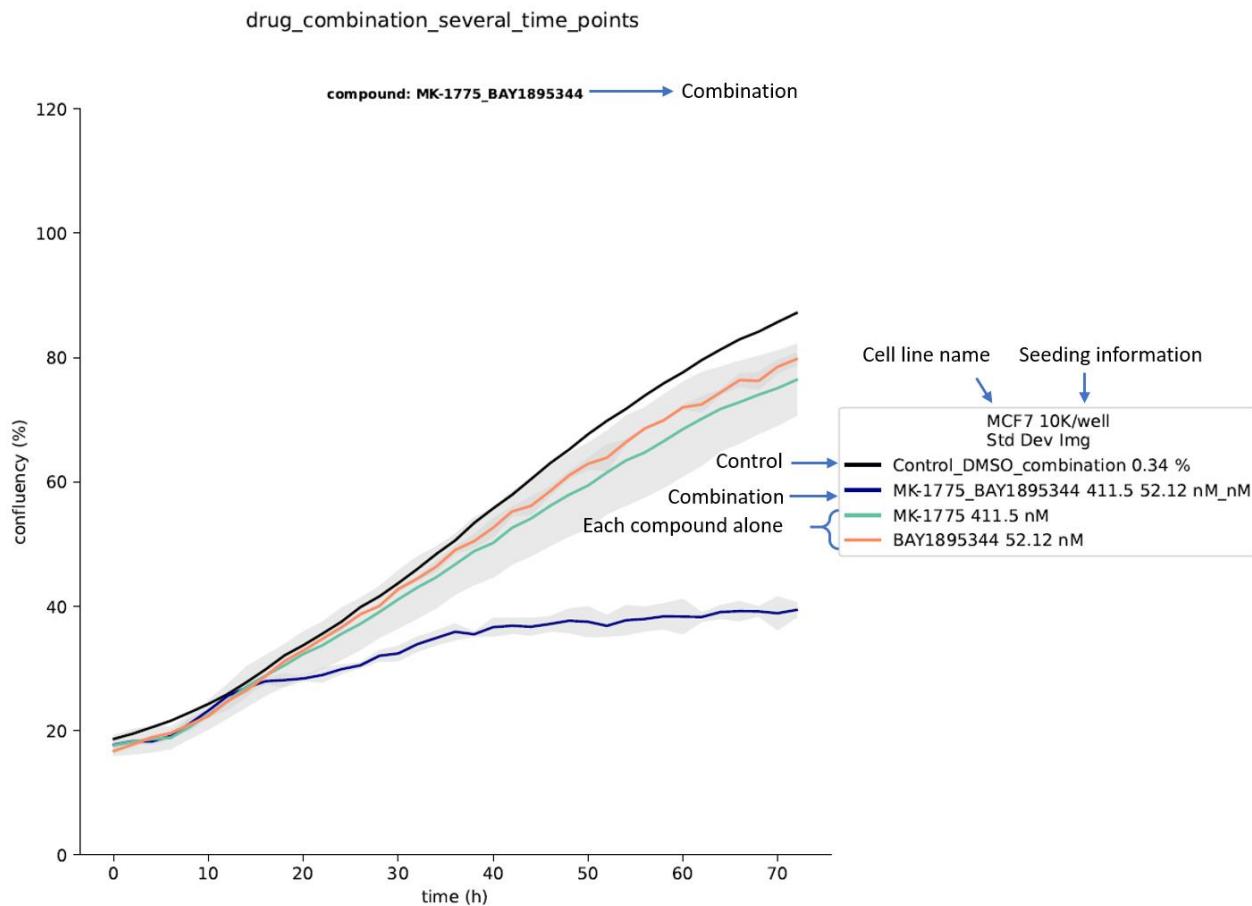


Figure 28: XY-plot showing the raw data over time for the combination condition (MK-1775 at 411.5 nM and BAY1895344 52.12 nM), each compound alone and the experiment control. Y-axis is the confluency (%) read-out provided by the user. On the X-axis is the time course of the experiment.

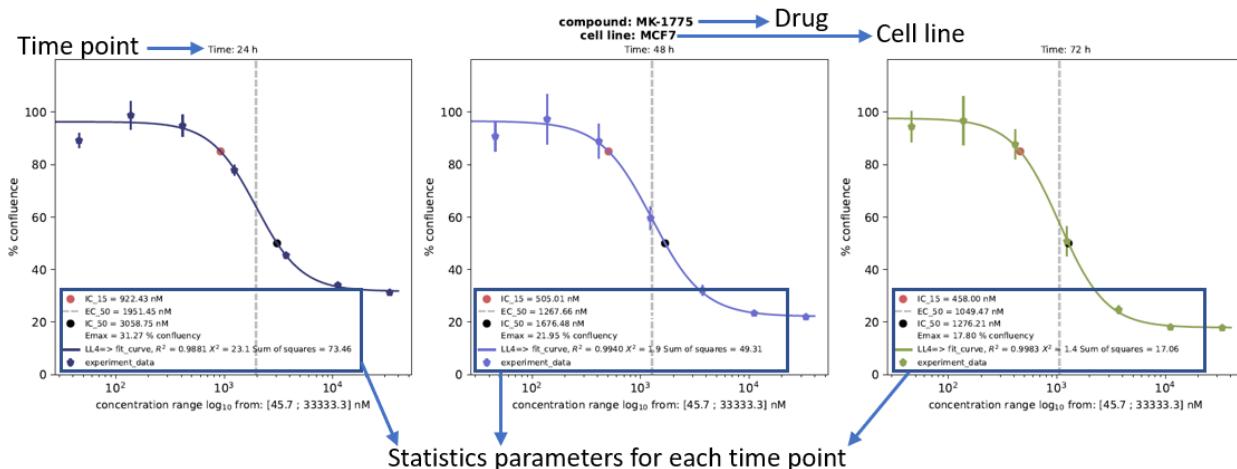


Figure 29: Dose-response relationship of MK-1775 on the cell line MCF-7 for each main time point. The Y-axis is the read-out provided by the user on the HTSplotter analysis. X-axis is the concentrations range tested transformed into log₁₀, indicating the units.

drug_combination_several_time_points

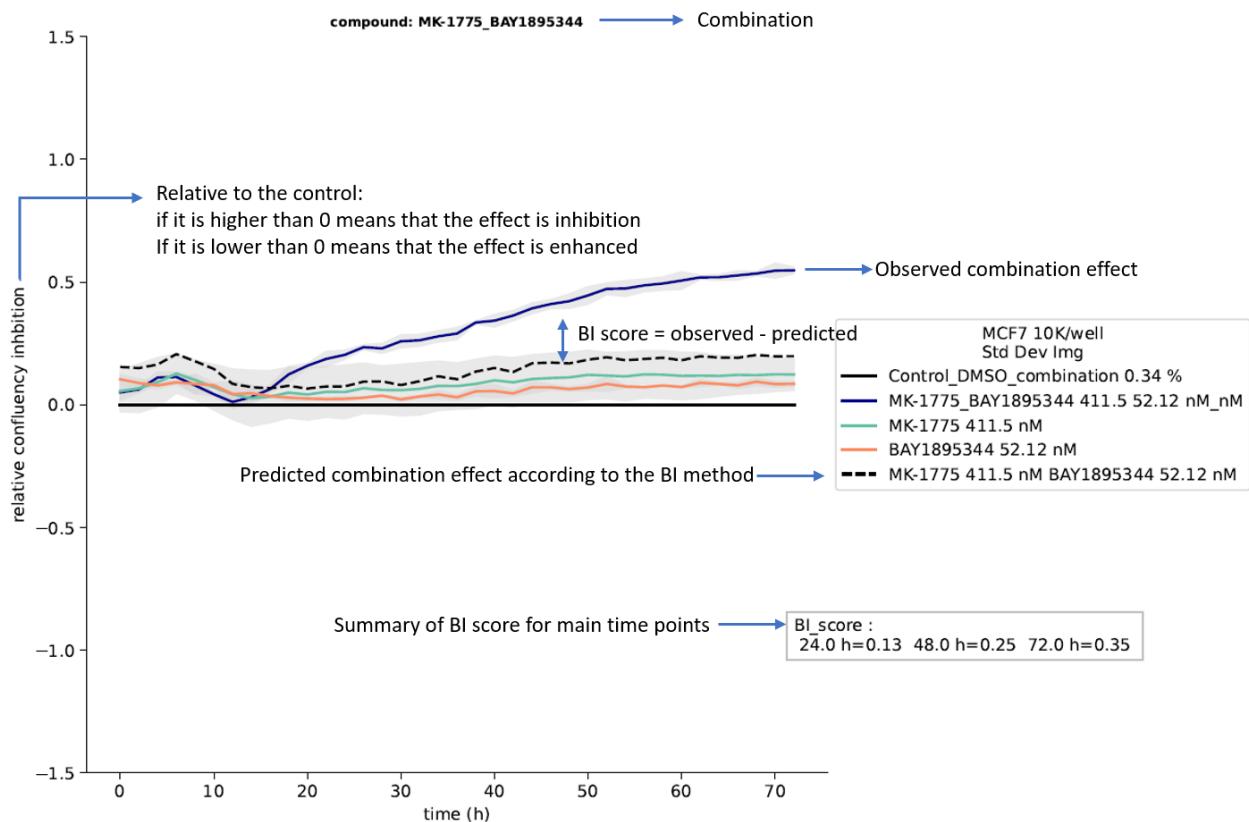


Figure 30: XY-plot showing the inhibition effect over time for the combination condition (MK-1775 at 411.5 nM and BAY1895344 52.12 nM), each compound alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

drug_combination_several_time_points

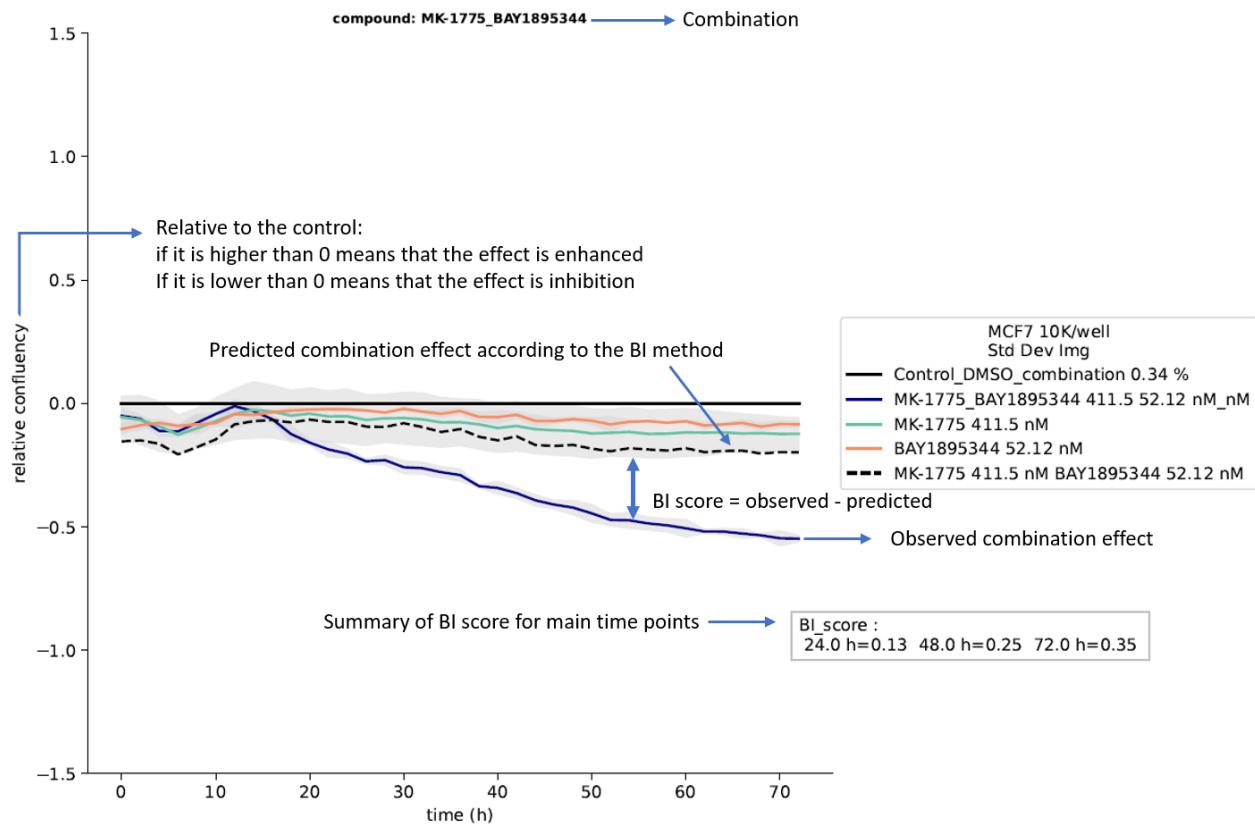


Figure 31: XY-plot showing the effect over time for the combination condition (MK-1775 at 411.5 nM and BAY1895344 52.12 nM), each compound alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the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

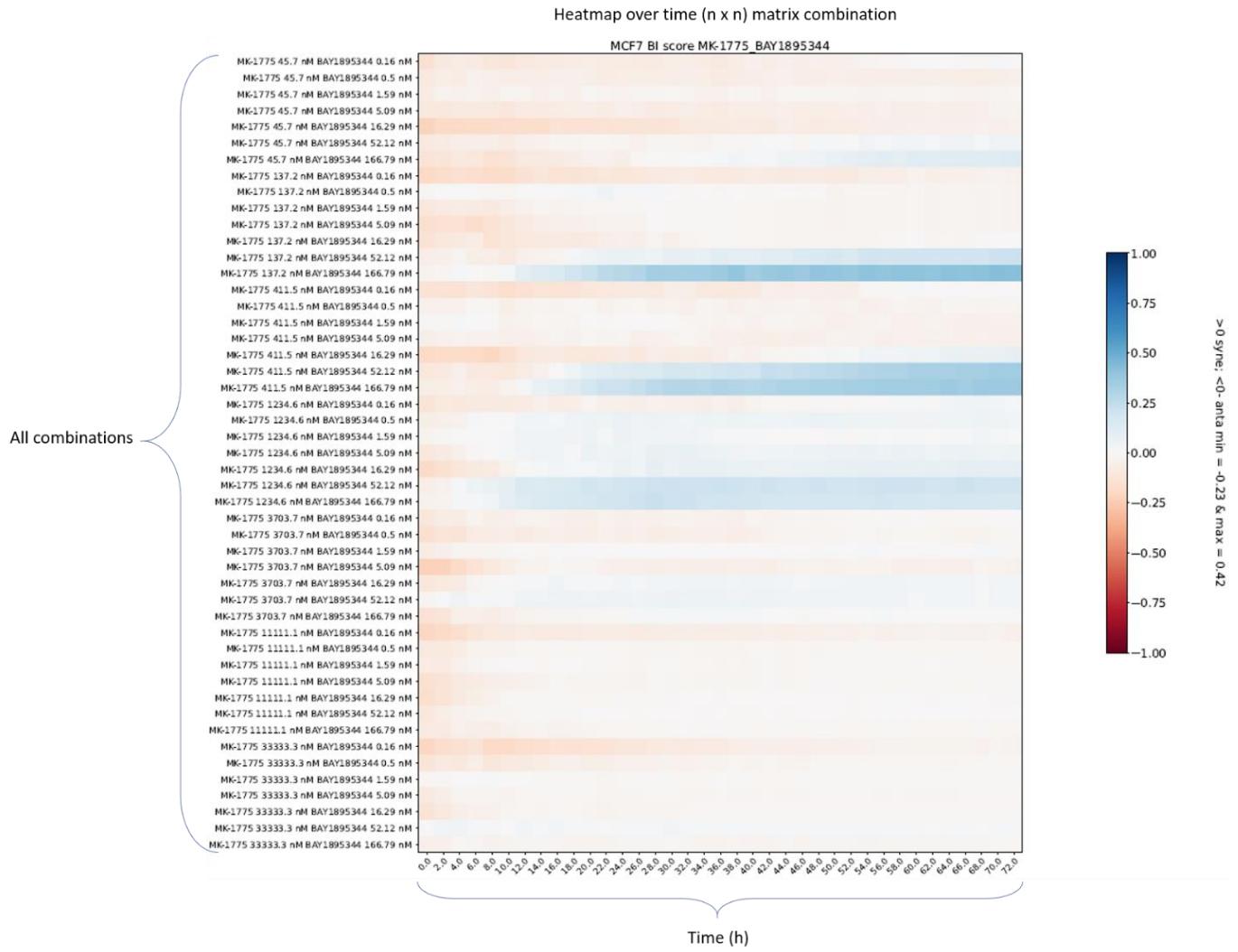


Figure 32: Heatmap over time for (n x n) matrix combination.

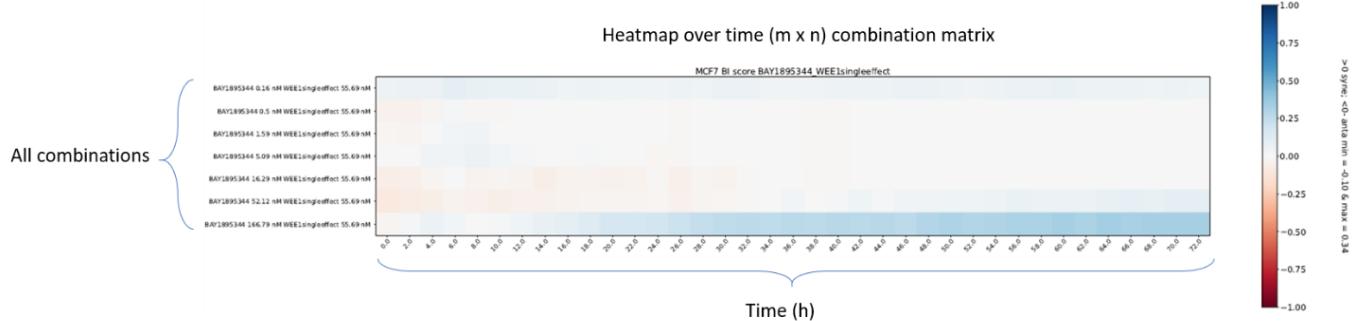


Figure 33: Heatmap over time for (m x n) matrix combination.

Genetic perturbagen

HTSplotter categorizes an experiment as genetic-perturbagen 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 an average, standard deviation and 95 % of confidence interval of all controls. Then normalize 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 (https://www.hdfgroup.org/downloads/hdfview/)).

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 (https://www.hdfgroup.org/downloads/hdfview/)).

Experiment design

For each genetic perturbation condition, a name, dosage and units are required. It is crucial a unique concentration for each gene name. In case of for example of siRNA targeting the same transcript, please differentiate the name, as shown on Figure 34-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% of CI, and then normalize all conditions.

Figure 34-B is an example of a genetic perturbagen 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 34: Example of labeling each condition in case of genetic-perturbagen. 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 more than one control was required.

Results plots

Unique time point:

- Bar plot is with all perturbagens, shown as inhibition (Figure 35) and as enhanced (Figure 36)

More than 1 time point:

- Raw data plotted as XY-plot, for each perturbagen Figure 37.
- XY-plot for each perturbagen, inhibition Figure 38 or enhanced Figure 39.
- Heatmap over time with all perturbagens Figure 40.

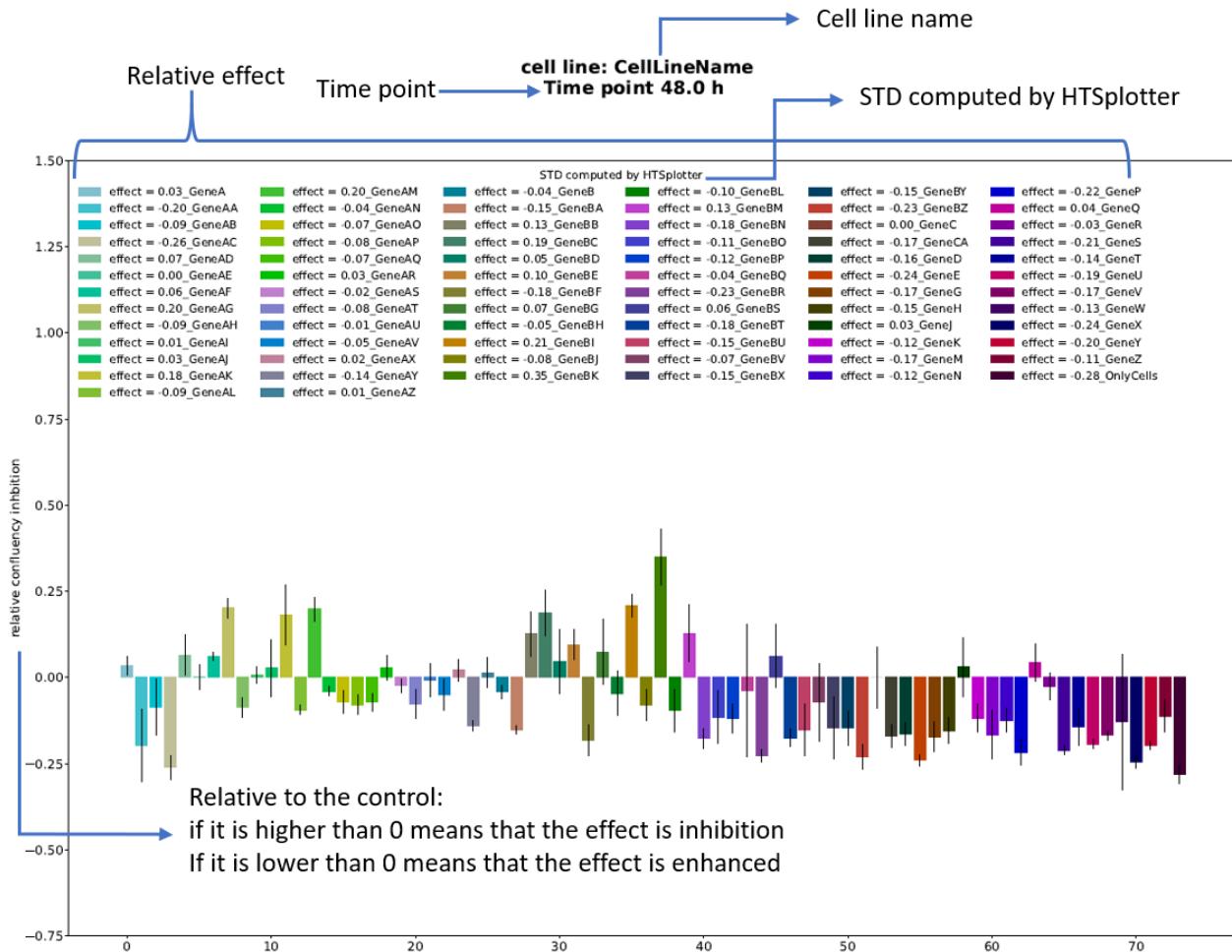


Figure 35: Inhibition effect of all genetics perturbagens plotted. On the legend, the inhibition effect relative to the control is shown.

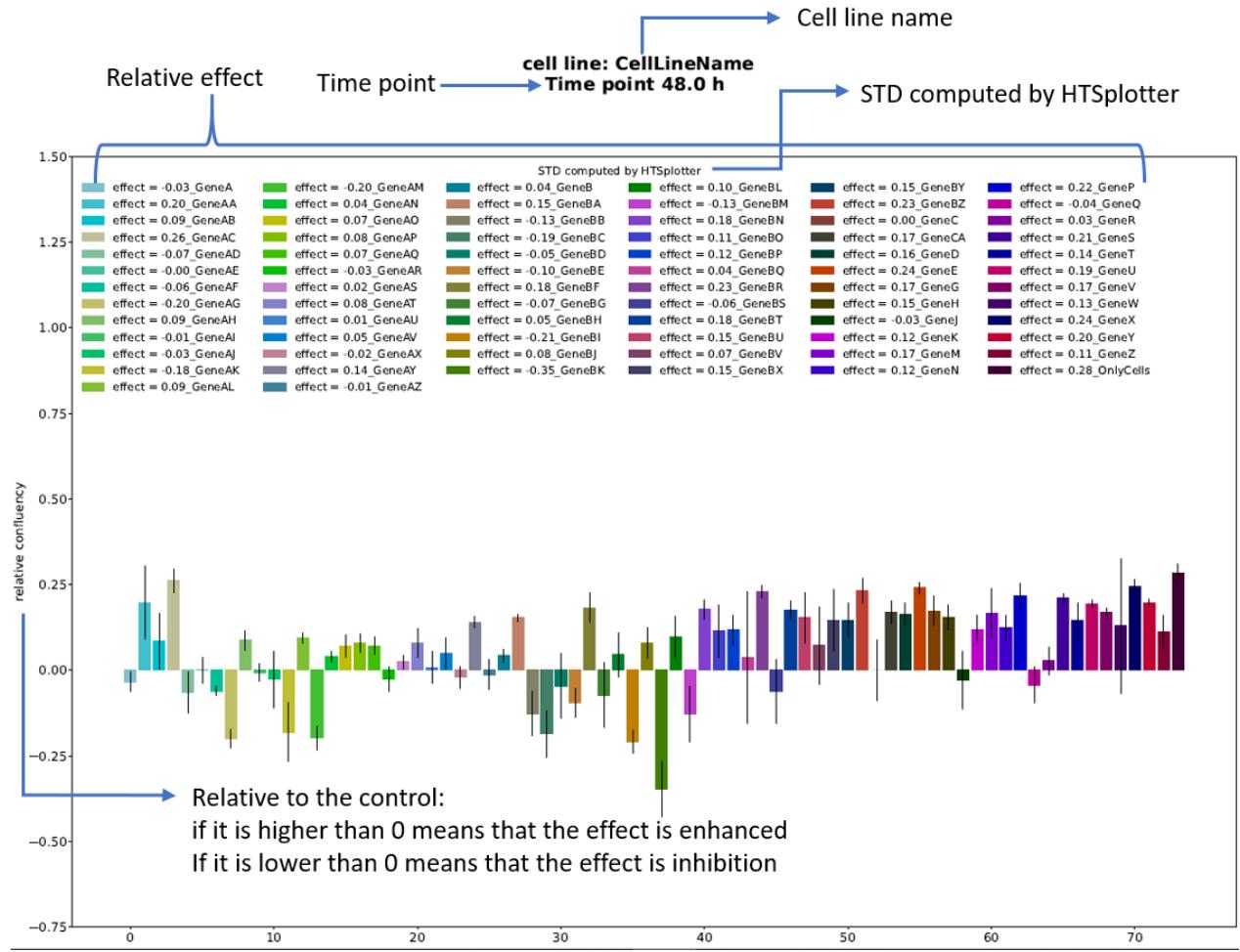


Figure 36: Effect of all genetics perturbagens plotted. On the legend, the effect relative to the control is shown.

Gene_perturbagen_severaltimepoints

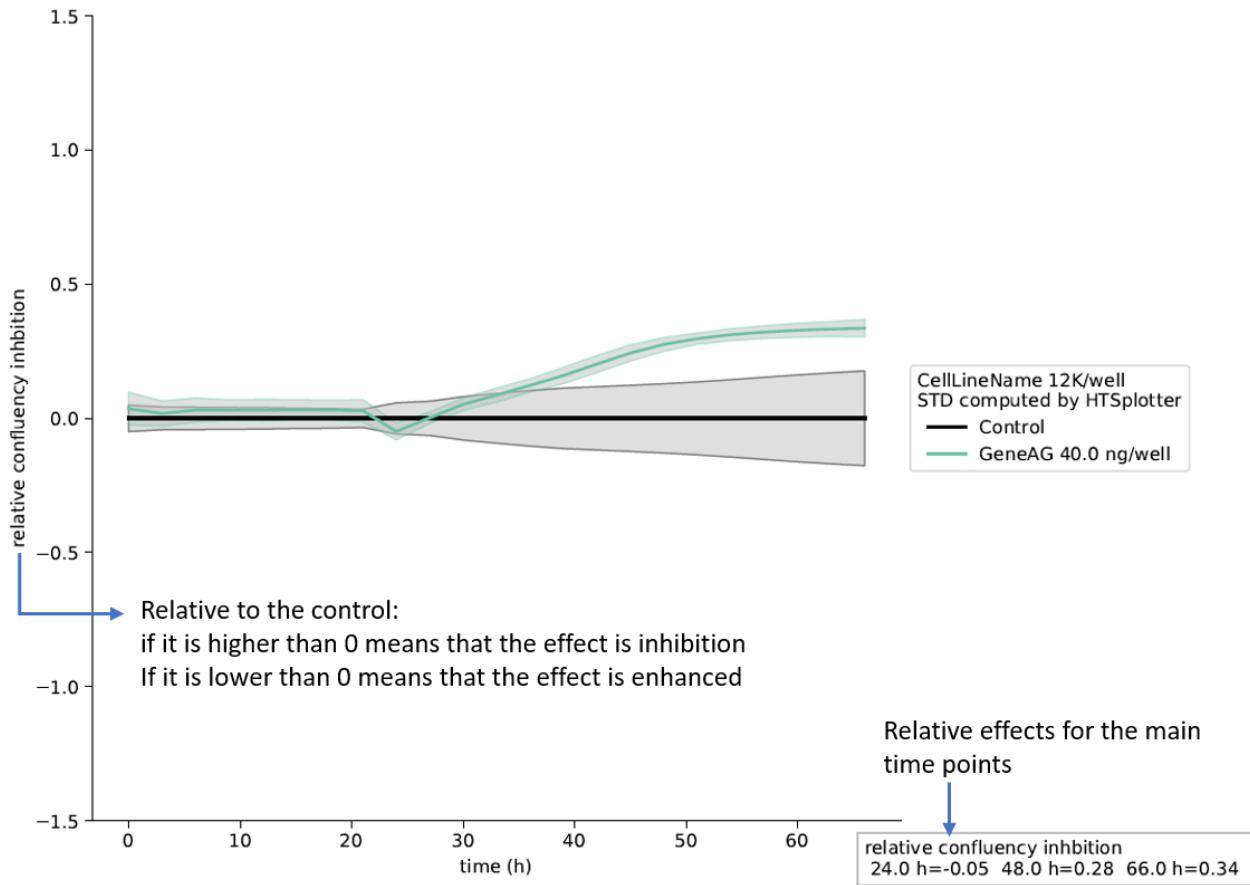


Figure 37: XY-plot showing the raw data for control and GeneAG conditions.

Gene_perturbagen_severaltimepoints → File name

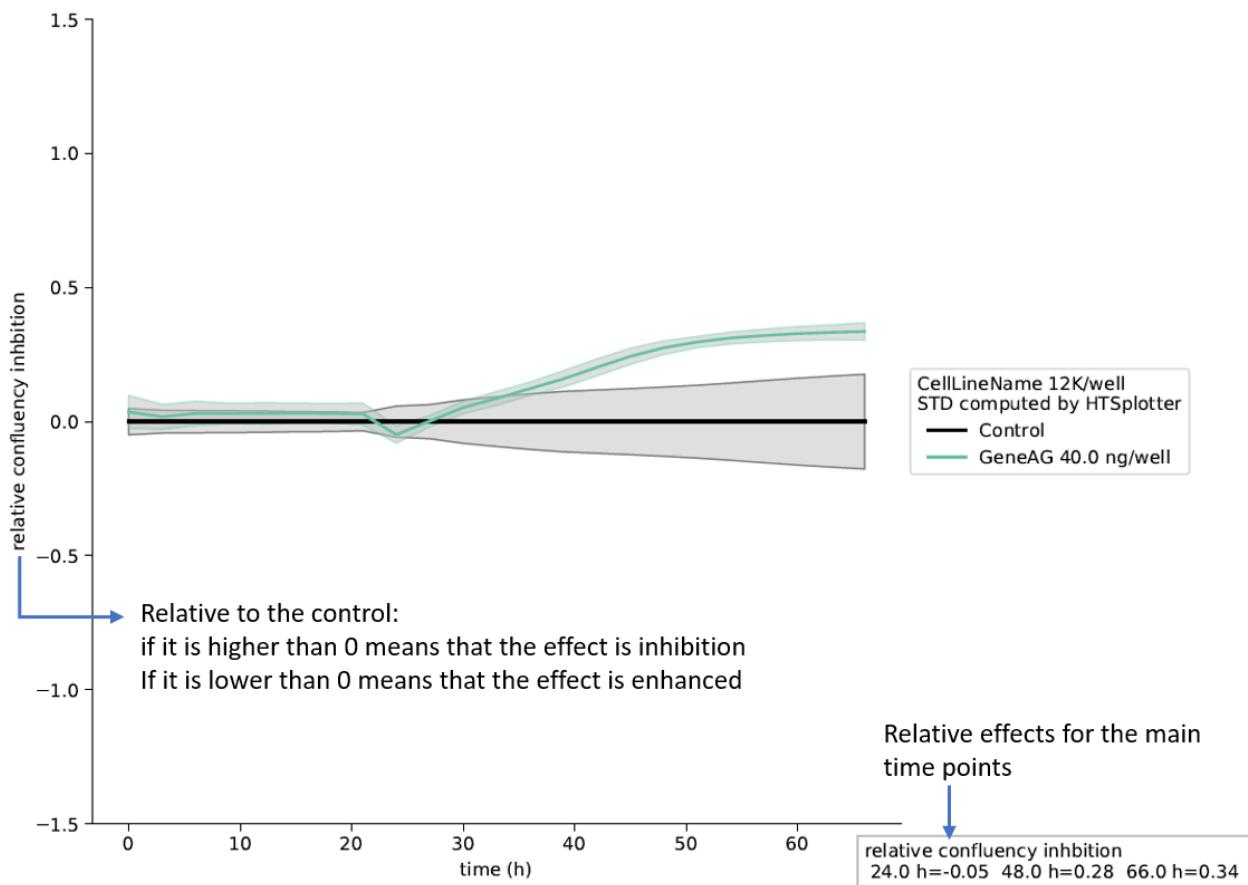


Figure 38: XY-plot with the inhibition effect of GeneAG. A summary with the relative inhibition effect is shown for the main time points.

Gene_perturbagen_severaltimepoints

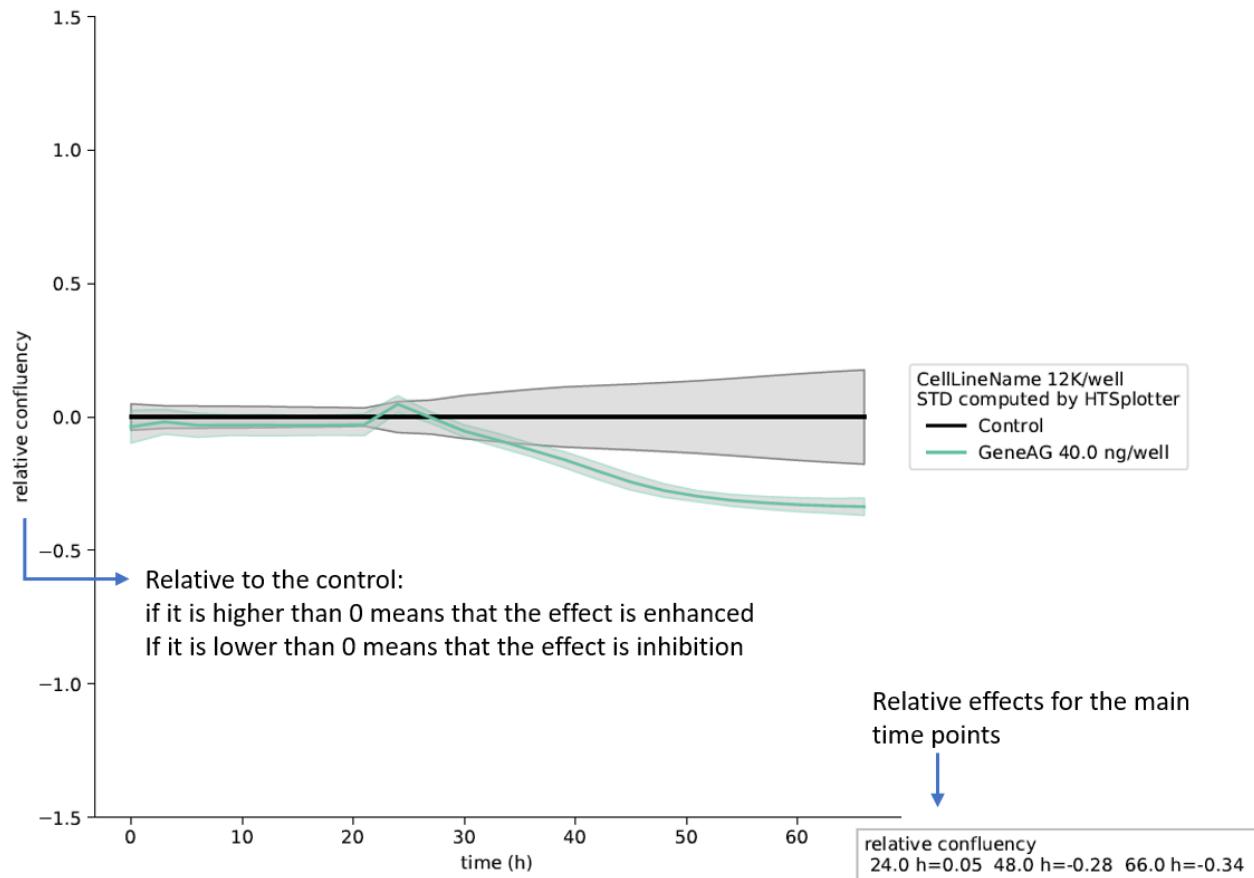


Figure 39: XY-plot with the effect of GeneAG. A summary with the relative condition effect is shown for the main time points.

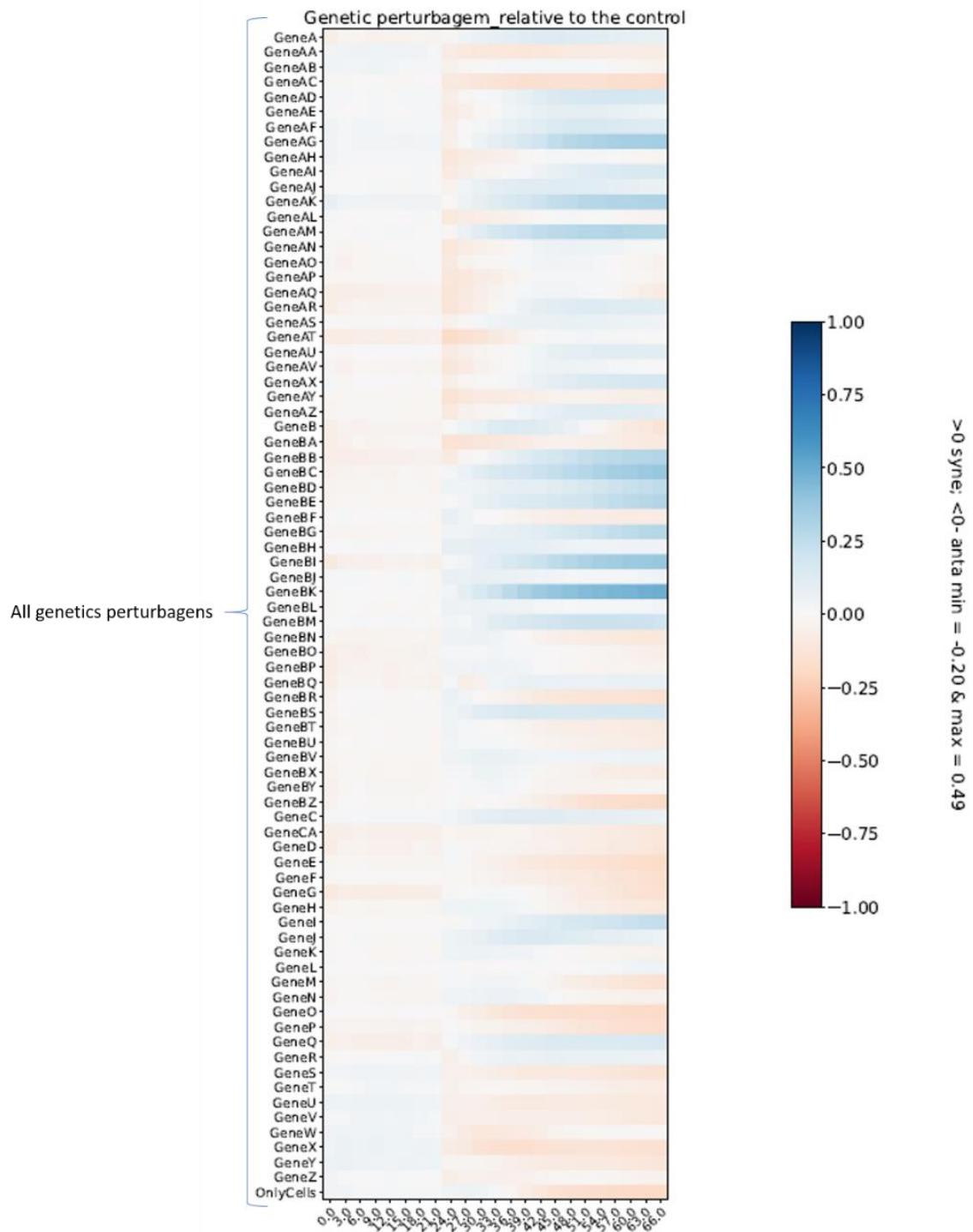


Figure 40: Heatmap over time with the effect of all genetics perturbagens relative to the control. Noticed that the condition with only cells is also shown, as to compare with the experiment control.

Genetic-chemical perturbagen

Genetic-chemical perturbagen is categorized by HTSplotter when the drug name has a tag, which is identified by the presence of the “_” character followed by 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 x n) matrix combination:
 - 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 alone 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 ($P_{A_\delta B_\delta}$) is computed according equation (1), where:
 - B_δ is the solvent with the genetic perturbagen
 - A_δ is the dosage effect of a certain drug
 - The BI score (*BI score*) is computed according equation (2), where:
 - $O_{A_\delta B_\delta}$ is the drug at certain dosage combined with a genetic perturbagen

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

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

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_Blisscor.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 (https://www.hdfgroup.org/downloads/hdfview/)).

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 (BI 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/>)).

Experiment design

Genetic combined with chemical perturbagen requires one control, which is the condition where the drug solvent was tested without the 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 41 shows an example of conditions labeling for each situation. Noticed that all condition 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 41: Example of labeling each condition in case of genetic-chemical perturbagen. Drug-A tested on dosage of 100 and 5 nM and the same dosages of drug-A in combination with genetic perturbagen ("Drug-A_GeneOn"). 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_GeneOn"). Notice that for each tested drug there is a condition where the solvent was combined with the genetic perturbagen ("Solvent_Drug-A_GeneOn" and "Solvent_Drug-B_GeneOn").

Results plots

Unique time point:

- Dose-response relationship: without genetic perturbagen Figure 42-A, with genetic perturbagen Figure 42-B and both curves Figure 42-C.
- In case of inhibition or enhanced effect, the bar plot is shown for each combination, and the predicted value according the BI method is shown by a dash line Figure 43 and Figure 44.

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, Figure 45-A.
 - Grouped all dosages for a certain drug combined with the genetic perturbagen Figure 45-B.
 - Grouped by combination: control, drug alone, genetic perturbagen (condition with drug solvent) and the combination of both Figure 46.
- Dose-response relationship for the main time points: without genetic perturbagen Figure 42-A, with genetic perturbagen Figure 42-B and both curves Figure 42-C.
- In case of inhibition or enhanced effect, XY-plot:
 - Grouped by each type of perturbagen alone Figure 48 and Figure 49.
 - Grouped by combination, in which the predicted effect, according the BI method is plotted by a dash line Figure 50 and Figure 51.
- Heatmap of BI score over time Figure 52.

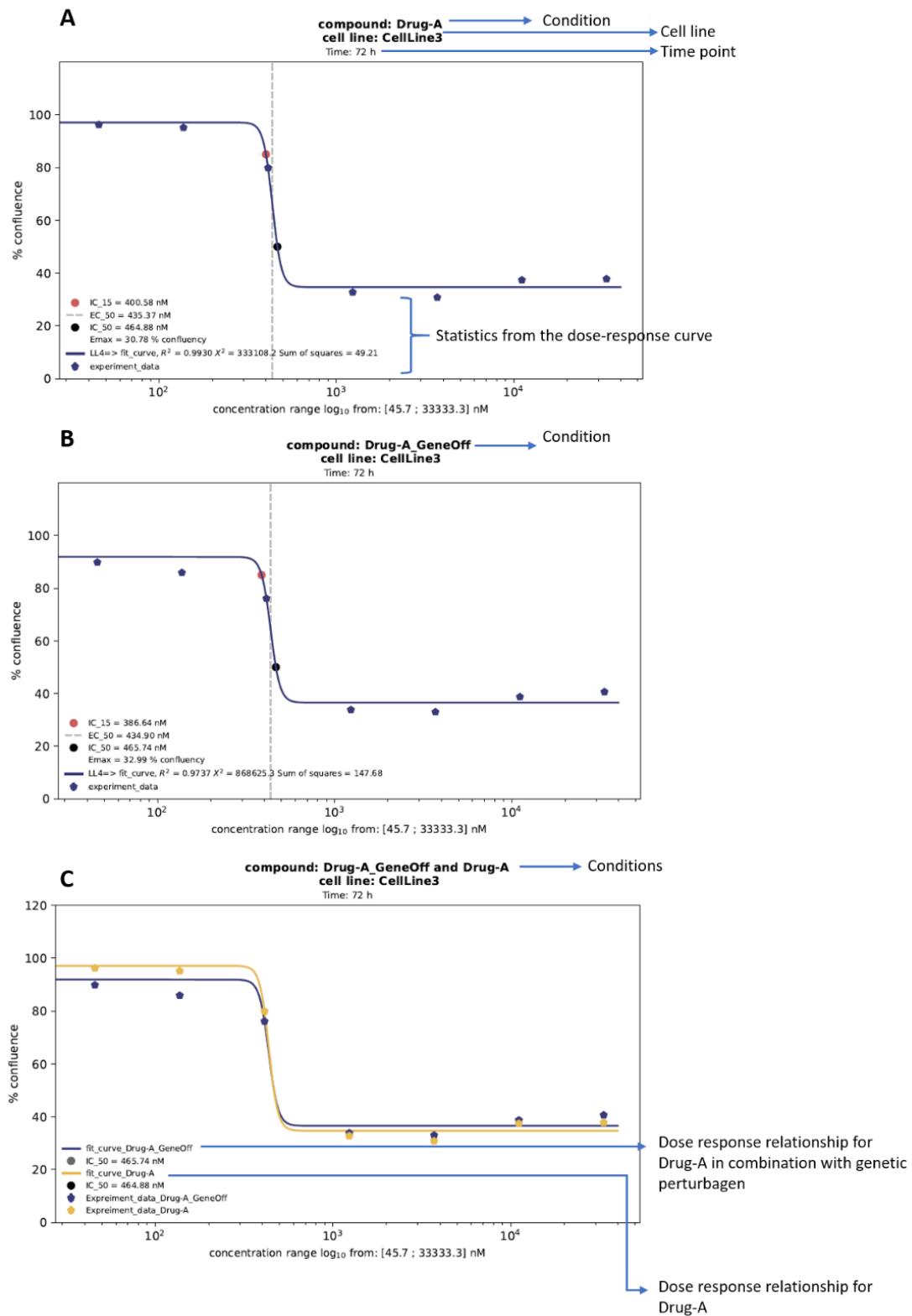


Figure 42: Drug-A dose response relationship. The Y-axis is the read-out provided by the user on the HTSplotter analysis and the X-axis is the concentrations range tested transformed into \log_{10} , indicating the units. A) Dose-response curve of drug-A alone. B) Dose-response curve of drug-A in combination with a genetic perturbagen. C) Dose-response curve of both situation.

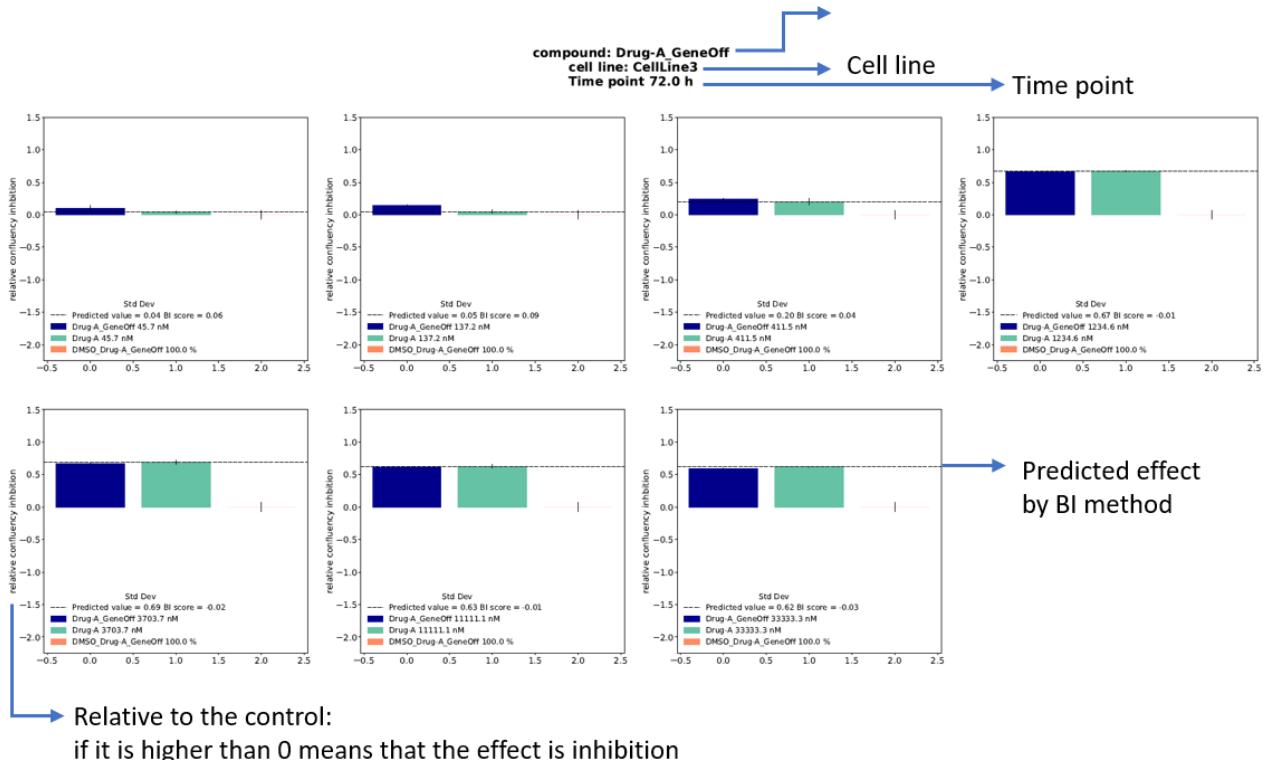


Figure 43: Bar plot of inhibition effect of each combination, drug-A at certain dose, genetic perturbagen with the drug-A solvent and the combination of both. The dash line, is the predicted effect by the BI method.

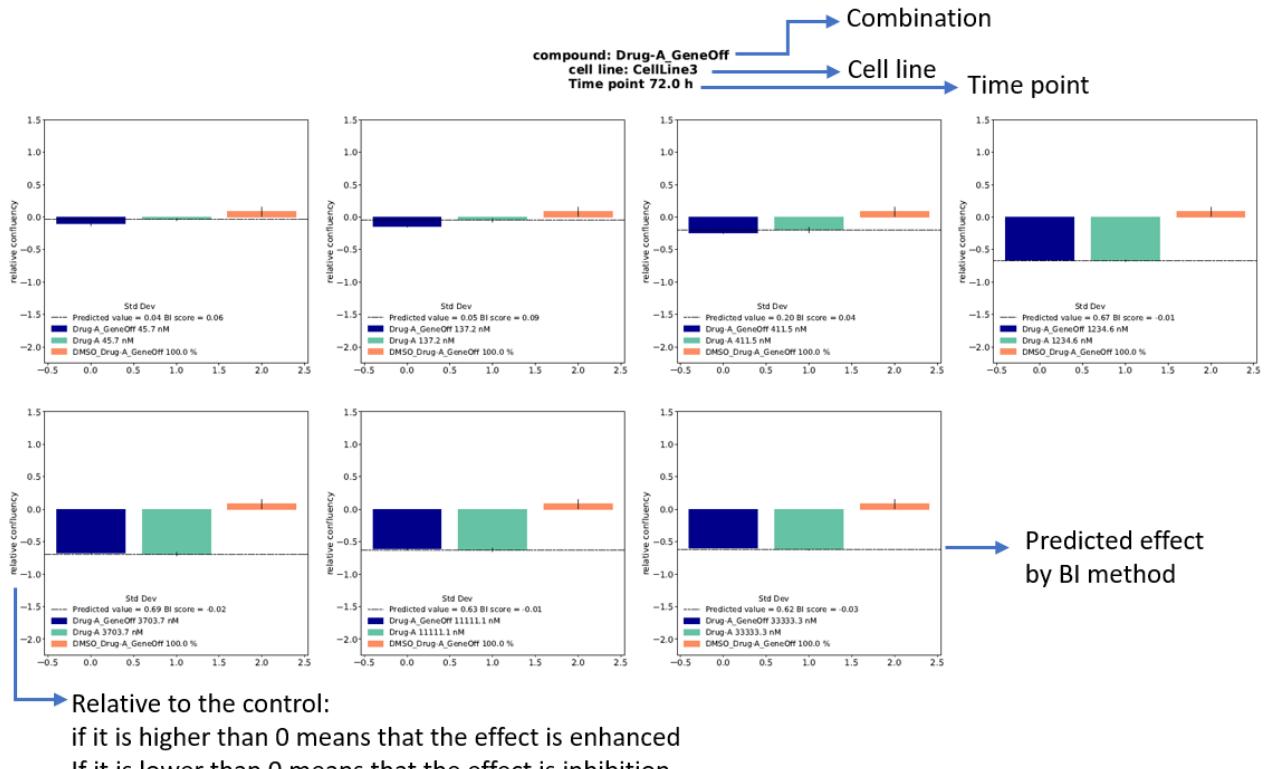


Figure 44: Bar plot of enhanced effect of each combination, drug-A at certain dose, genetic perturbagen with the drug-A solvent and the combination of both. The dash line, is the predicted effect by the BI method.

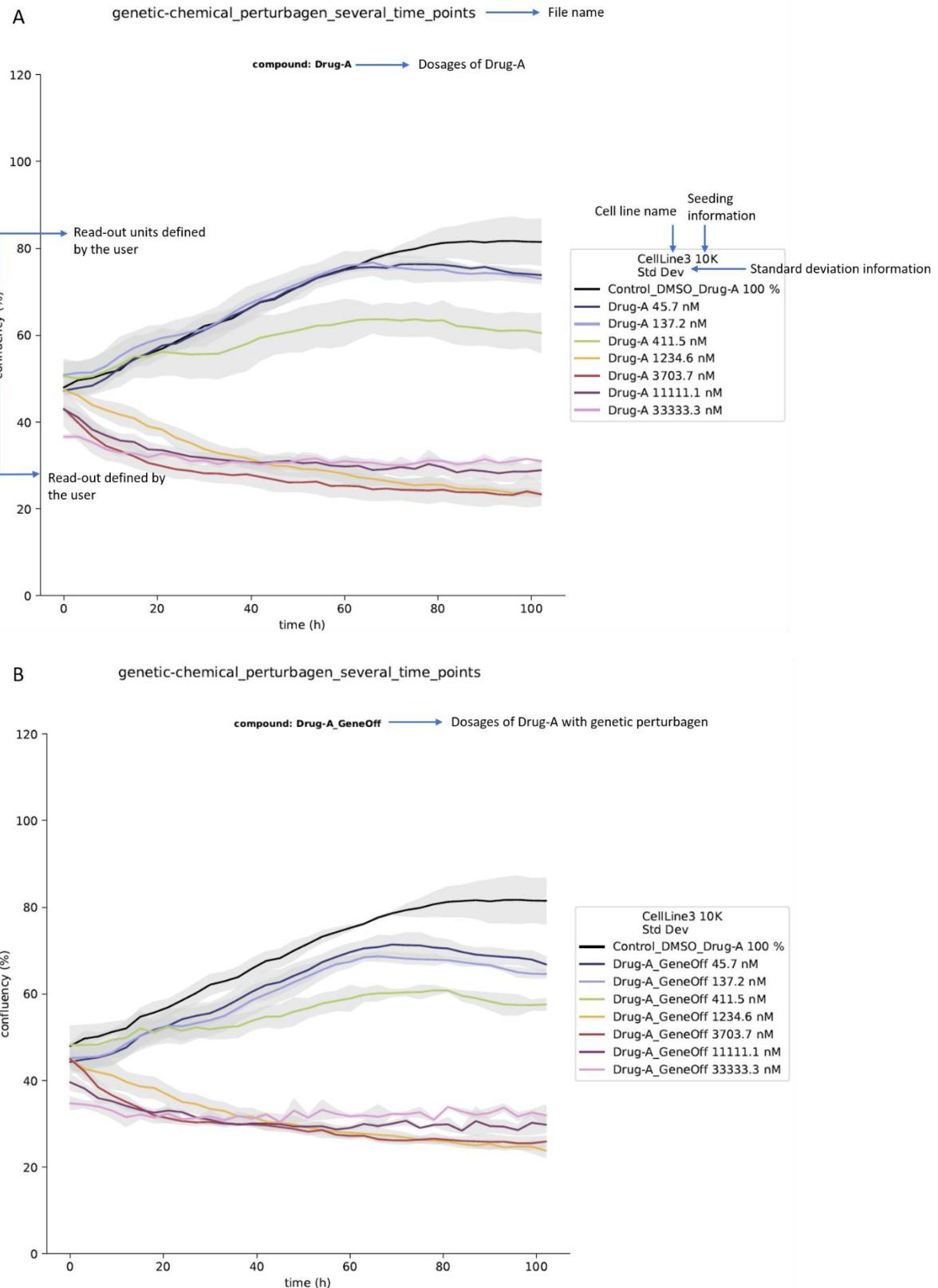


Figure 45: XY-plot example of raw data regarding to all dosage tested of drug-A. Y-axis is the read-out provided by the user and on the X-axis is the time. A) All dosages tested of drug-A alone. B) All dosages tested of drug-A in combination with genetic perturbagen.

genetic-chemical_perturbagen_several_time_points

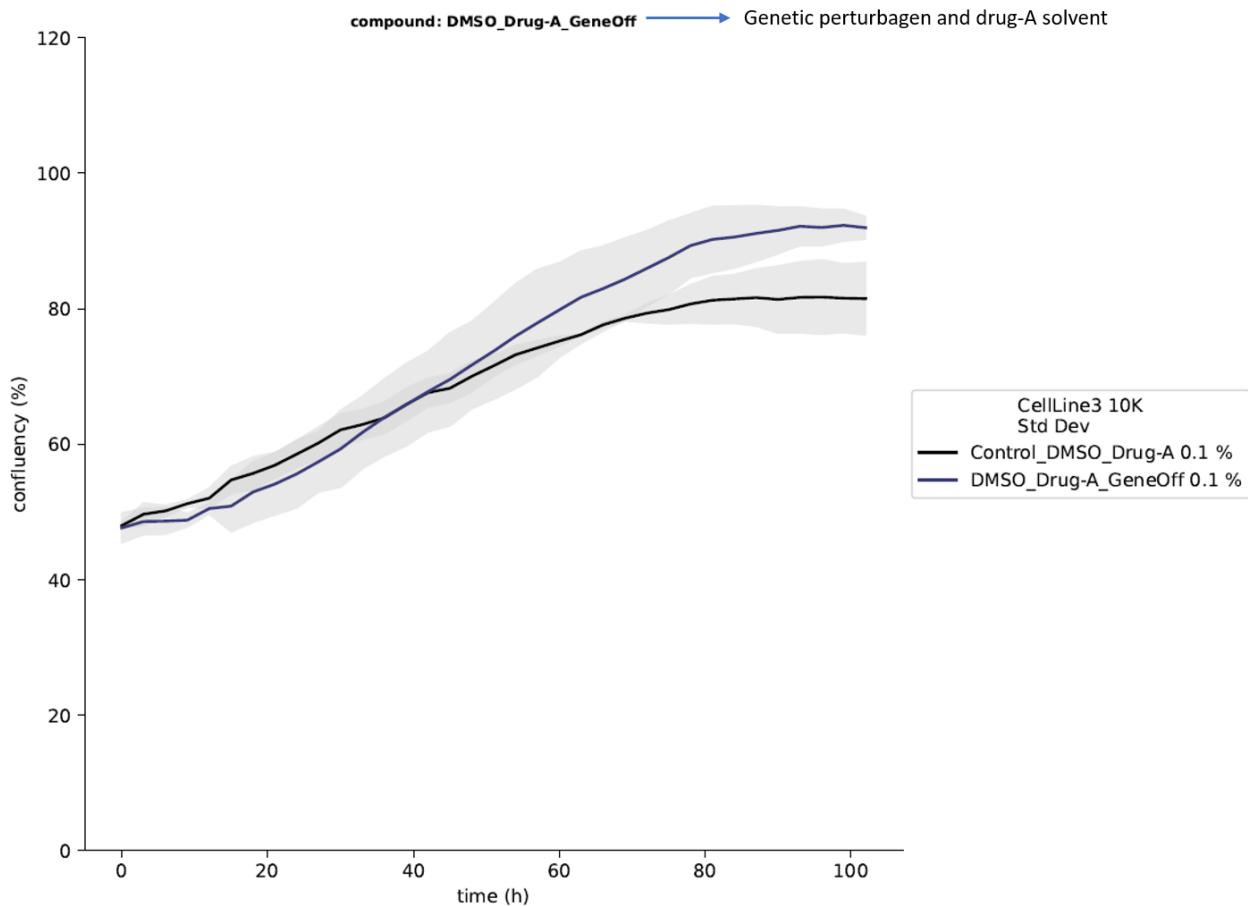


Figure 46: XY-plot example of raw data regarding the genetic perturbagen of a certain gene in combination with the solvent and the solvent alone. Y-axis is the read-out, confluency, and on the X-axis is the time course of the experiment.

genetic-chemical_perturbagen_several_time_points

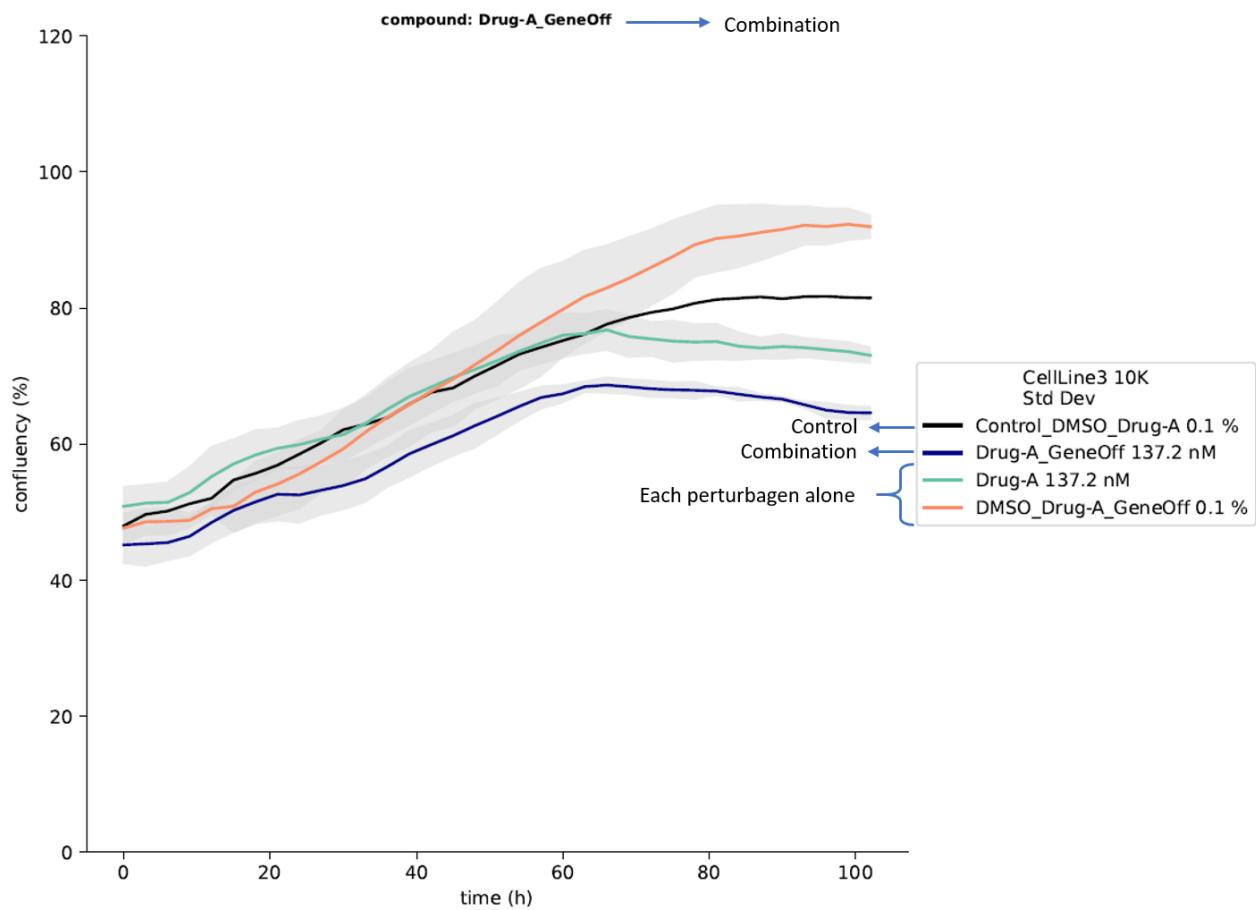
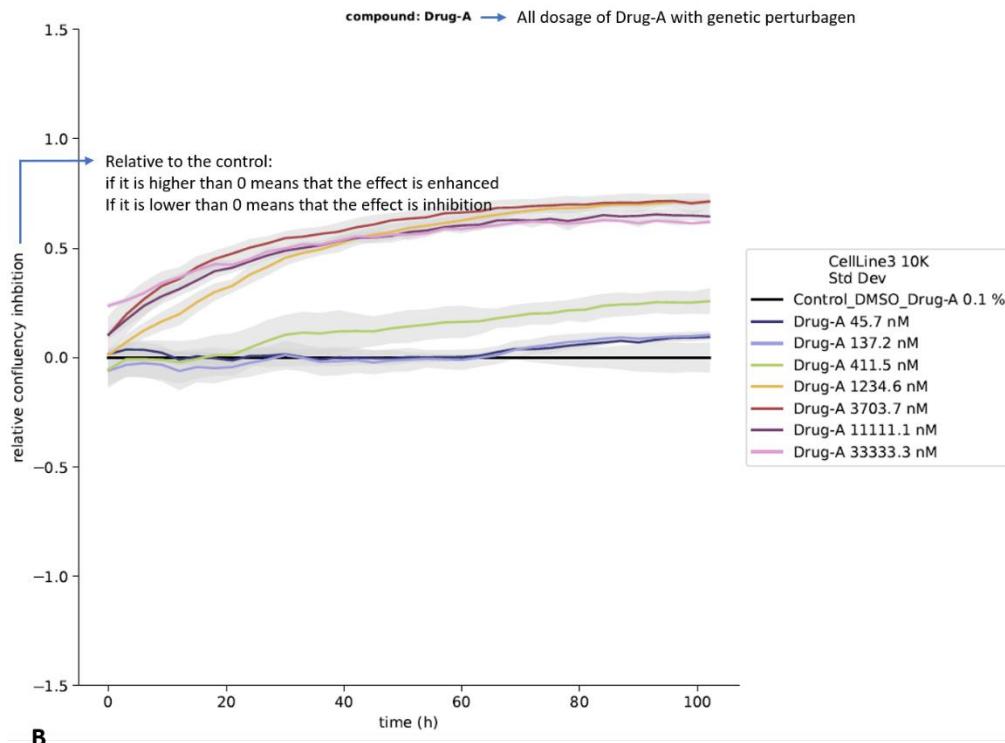


Figure 47: XY-plot of raw data over time for the combination condition (drug-A at 137.2 nM in combination with a genetic perturbagen in which a certain gene is silenced), each perturbagen alone and the experiment control. Y-axis is the read-out, confluency, and on the X-axis is the time course of the experiment.

A

genetic-chemical_perturbagen_several_time_points

**B**

genetic-chemical_perturbagen_several_time_points

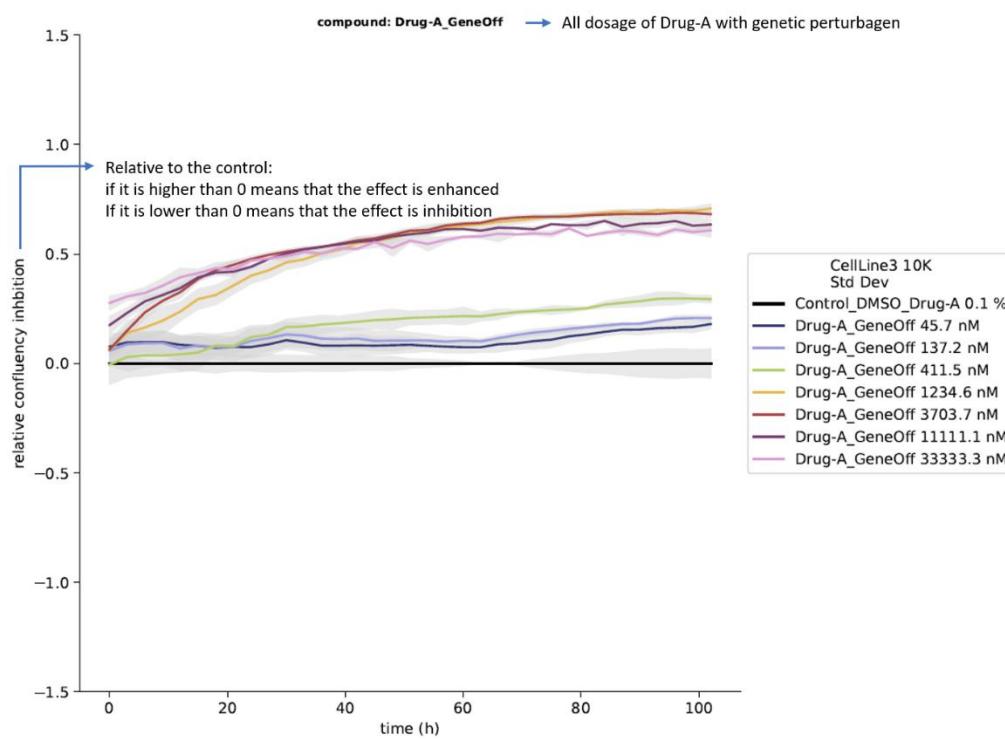
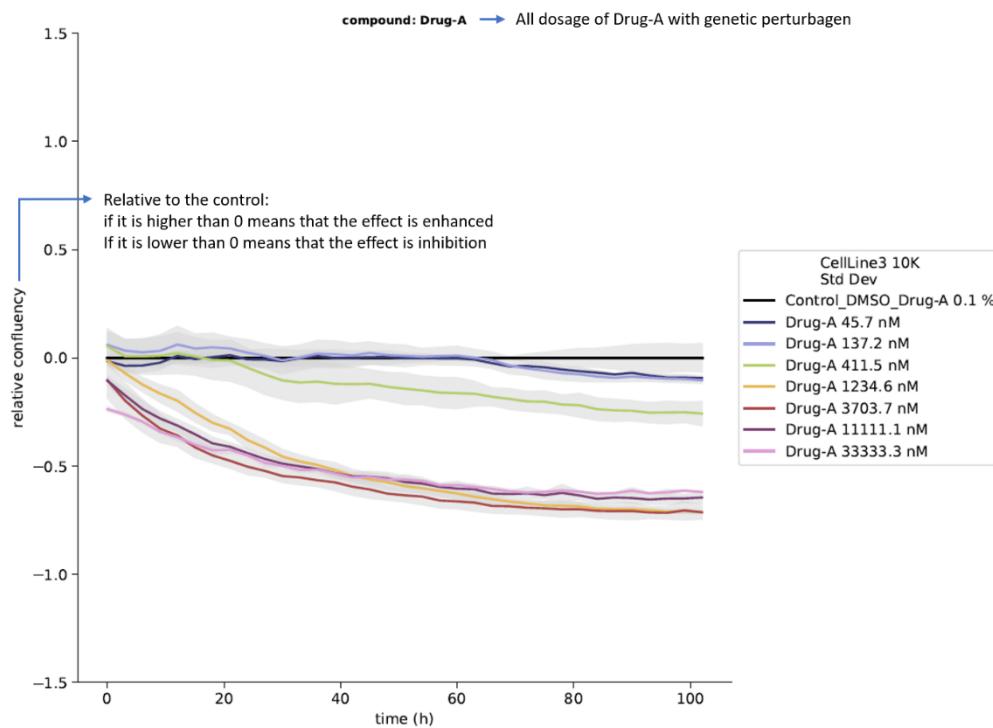


Figure 48: XY-plot example of inhibition effect data regarding to all dosage tested of drug-A. Y-axis is the read-out provided by the user and on the X-axis is the time. A) All dosages tested of drug-A alone. B) All dosages tested of drug-A in combination with genetic perturbagen.

genetic-chemical_perturbagen_several_time_points

A



B

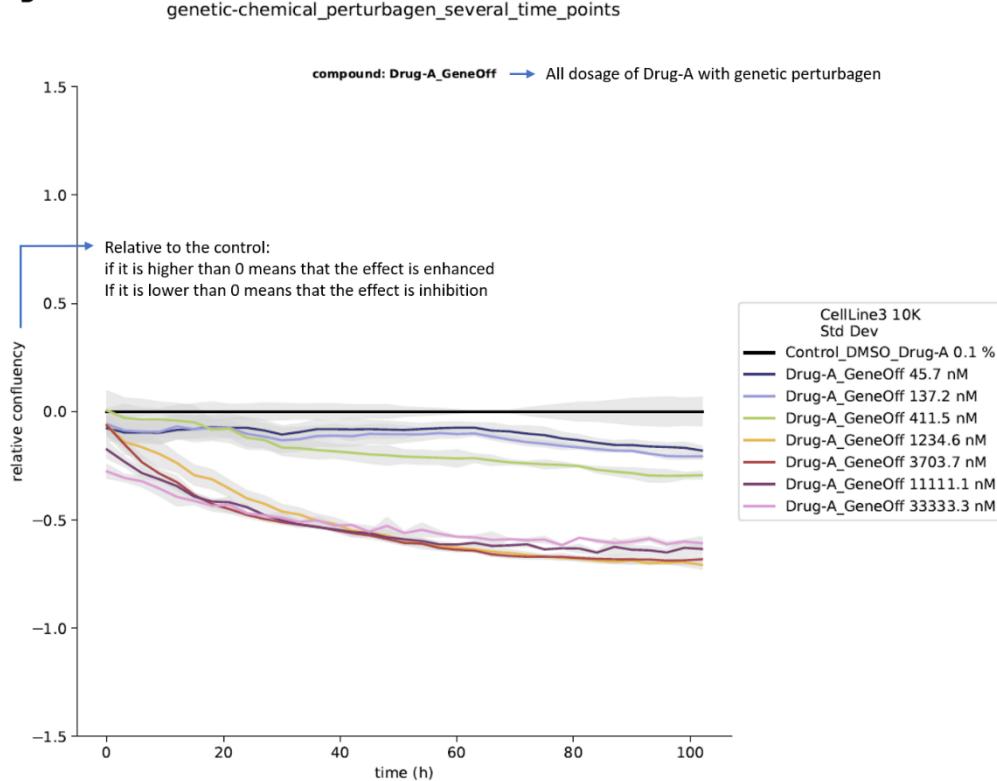


Figure 49: XY-plot example of enhanced effect data regarding to all dosage tested of drug-A. Y-axis is the read-out provided by the user and on the X-axis is the time. A) All dosages tested of drug-A alone. B) All dosages tested of drug-A in combination with genetic perturbagen.

genetic-chemical_perturbagen_several_time_points

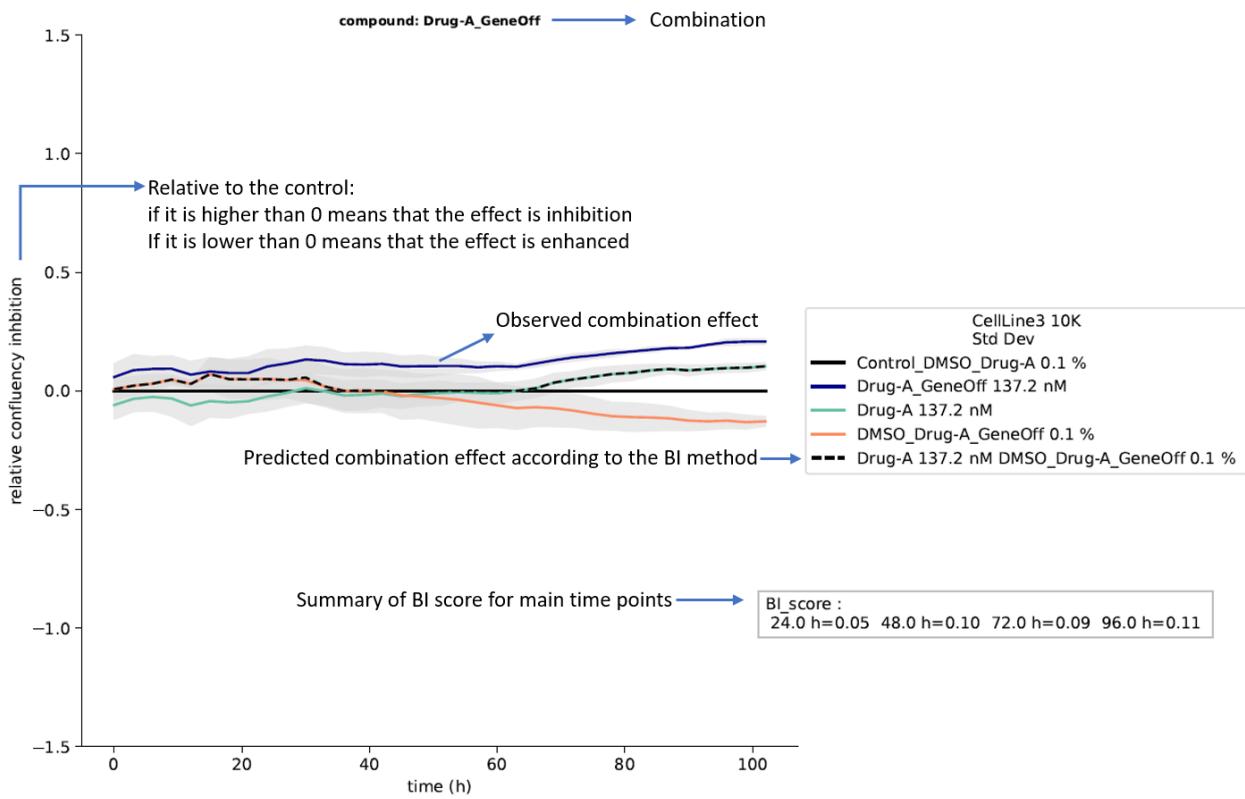


Figure 50: XY-plot showing the inhibition effect over time for the combination condition (drug-A at 137.2 nM in combination with a genetic perturbagen in which a certain gene is silenced), each perturbagen alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

genetic-chemical_perturbagen_several_time_points

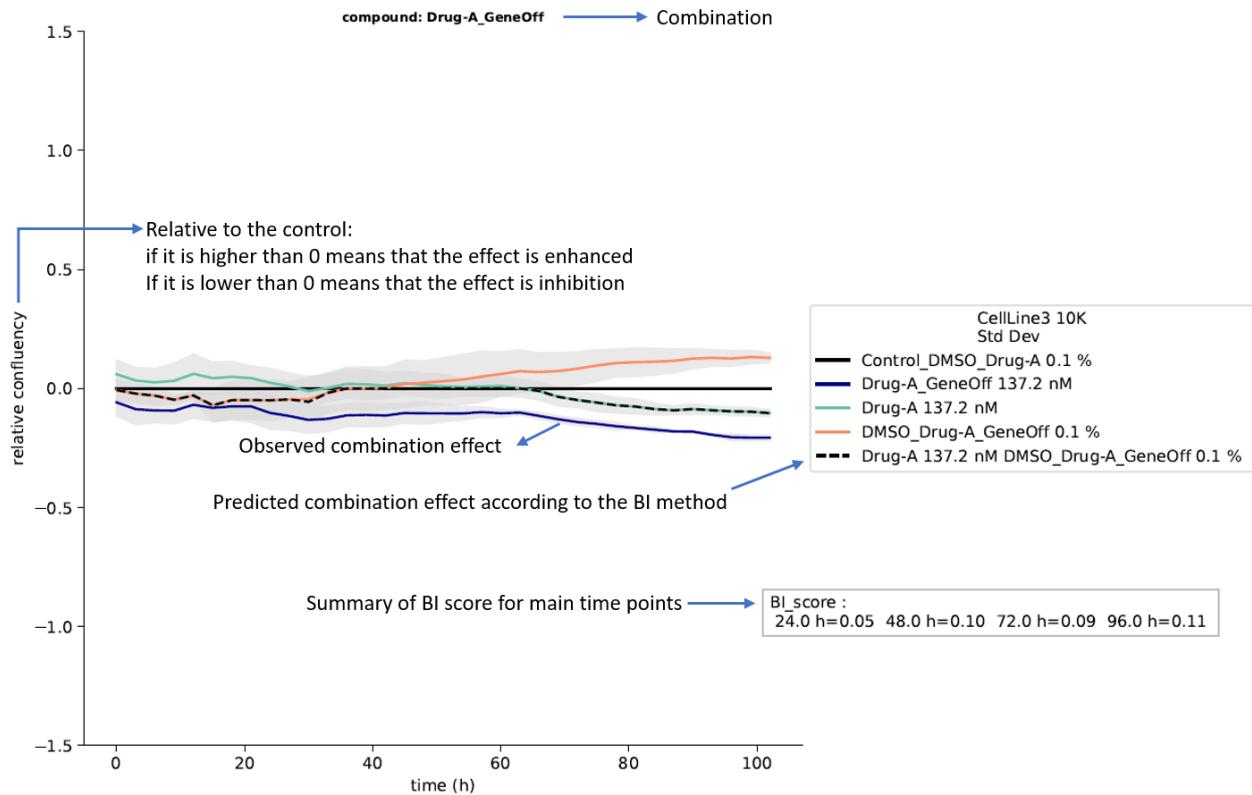


Figure 51: XY-plot showing the enhanced effect over time for the combination condition (drug-A at 137.2 nM in combination with a genetic perturbagen in which a certain gene is silenced), each perturbagen alone and the experiment control. Y-axis is the relative confluency. On the X-axis is the time course of the experiment. A summary of the BI scores is shown for the main time points.

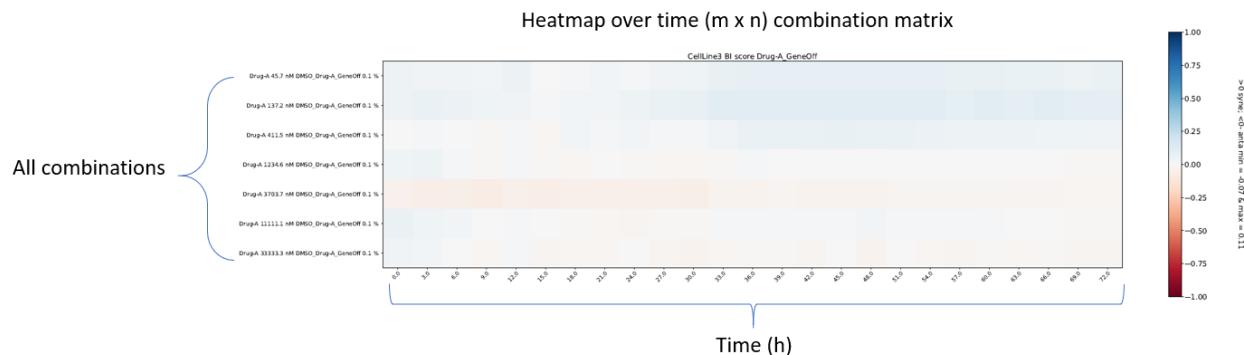


Figure 52: Heatmap over time for (m x n) matrix combination.