BMDx Reference Manual
with Sample Data Analysis

Contents

About BMDx

BMDx is an R-Shiny application created for easy benchmark dose (BMD) analysis on omics data across multiple time points and experiments. The tool guides the user through multiple steps starting from an analysis of variance, through BMD computing all the way to a functional enrichment of the dose-dependent genes. BMDx not only allows the user to compare the results between multiple time points, but also multiple experiments at once. Results along the way are visualised as several types of plots and the output can be downloaded as Excel files at multiple steps of the analysis.

The benchmark dose (BMD) is the dose or concentration of a substance that corresponds to a specified level of response above or below that observed in a control or background population. The specified level of response within this definition is referred to as the benchmark response (BMR), while the statistical lower confidence bound of the BMD (referred to as BMDL) and the statistical upper confidence bound on the BMD (BMDU) have been typically used by regulatory agencies to set safe levels of exposure.

BMD modelling involves fitting the experimental data, in this case, the gene expression values, to a selection of mathematical models, such as linear, second- or third- degree polynomial, an exponential model, hill model, asymptotic regression model, and Michaelis-Menten model. The best model is selected by using a goodness of fit criteria, such as the Akaike information and the goodness-of-fit p-value. A predefined response level of interest, the BMR, is identified and the optimal model is used to predict the corresponding dose (BMD) (Abraham et al. 2012). Moreover, the European Food Safety Authority (EFSA) suggest reporting both the lower and upper 95% confidence limit on the BMD called BMDL and BMDU respectively (EFSA Scientific Committee et al. 2017). The selection of models available in BMDx are presented on page 26 of this document with model descriptions included.

In this manual, we provide a detailed step-by-step guide to using BMDx and explanation of the analysis workflow (see Figure 1). As a result of the analysis, the user will retrieve the results of the analysis of variance, lists of dose-dependent genes with BMD, BMDL, BMDU and IC50/EC50 values, the model fitted for each gene and its corresponding lack-of-fit p-value. Moreover, the results of the functional enrichment can be downloaded providing a comprehensive view of the dose-dependent genes in the experiment.

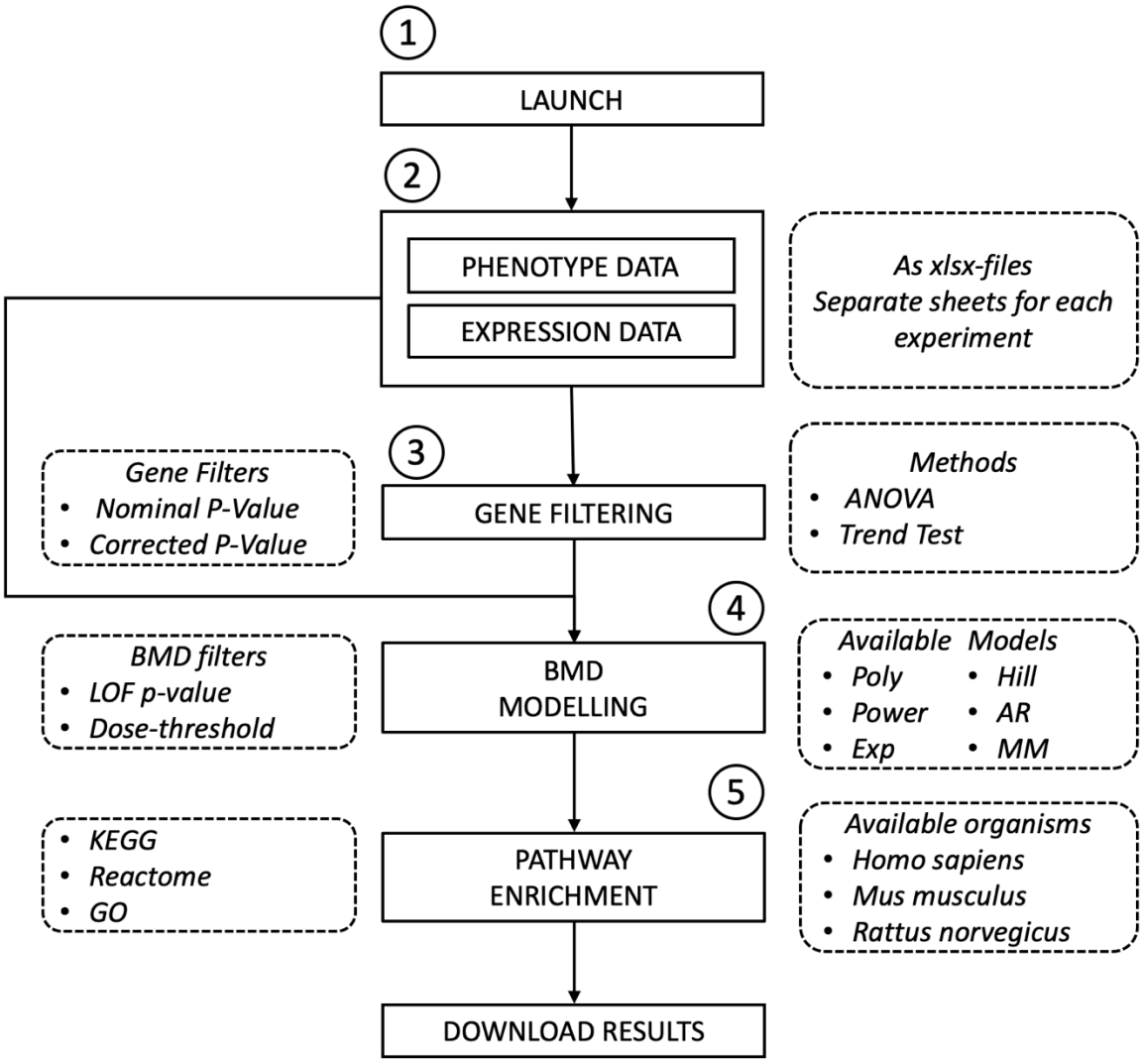


Figure 1: BMDx workflow. Schematic representation of the BMDx software workflow. The steps are represented by rectangular boxes with sharp edges, while the steps parameters are represented with rectangular boxes with rounded edges. Analytical steps are numerically coded with circular labels from 1 to 5.

Data Description

To demonstrate the use and effectiveness of our tool, we analysed gene expression data obtained from the Open TG-GATEs database (Igarashi et al. 2015). Out of the 170 compounds available in the database, we selected the expression data from the liver of rats exposed to either Omeprazole or Pirinixic acid (WY-14643). Omeprazole is a commonly used proton-pump inhibitor used to treat gastroesophageal reflux disease, while Pirinixic acid is a peroxisome proliferator linked to liver carcinogenesis (Woods et al. 2007). Both datasets include 48 samples as three doses (100, 300 and 1000 µg for Omeprazole and 10, 30 and 100 µg for Pirinixic acid) and their corresponding controls at four time points (4, 8, 15 and 29 days) were included in the experiment as triplicates.

Raw data were imported into R v. 3.4 by using the justRMA function from the Bioconductor utilities (Irizarry et al. 2003) to annotate the probes to Ensembl genes (rat2302rnensgcdf v. 22.0.0 annotation file obtained from http://brainarray.mbni.med.umich.edu/) and to quantile normalise the data. The experimental batch effect due to technical variables was estimated and removed using the ComBat algorithm implemented in the sva package (Leek et al. 2014). Linear models followed by eBayes pairwise comparisons (Ritchie et al. 2015) were performed to compute the log fold-change for each gene in all of the drug–control pairs. Genes with fold change > |1.5| and p-value < 0.05 were determined differentially expressed and used in this analysis. Finally, the Ensembl gene names were converted to official GeneSymbols.

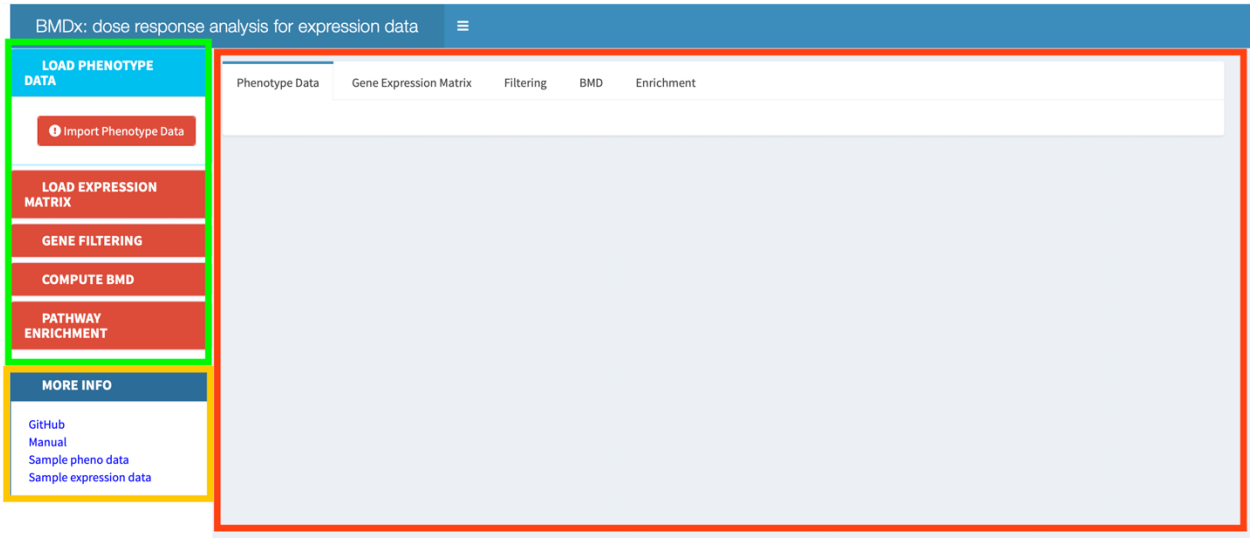
Data used as an example in this document are available on GitHub (https://github.com/Greco-Lab/BMDx).

Installation and execution

Instructions on how to install BMDx and its dependencies and how to launch the BMDx tool are available online at https://github.com/Greco-Lab/BMDx.

Workflow Interface

The workflow interface layout has a sidebar with input controls to configure and execute various steps (marked with green outline). Below this section, links to the GitHub page of the tool as well as the manual and sample data are provided (marked with orange outline). The output of the steps is visualized from the main display area (marked with red outline).



Input Description

BMDx takes as an input a phenotype file and an expression matrix, both provided as an Excel spreadsheet (xlsx). **If multiple experiments are included, both files must contain separate sheets for each experiment in corresponding orders.** Specific instructions for the file structures

are provided below and example files are available on GitHub (https://github.com/Greco-Lab/BMDx.

Phenotype Specification

The phenotype file is an Excel file containing separate sheets for each experiment. Each sheet contains information about the samples used in the specific experiment. In particular, the BMDx tool requires the spreadsheets to have at least three columns that specify the following characteristics: 1) Unique sample IDs (here BARCODE) corresponding to the column names in the expression matrix, 2) the dose and 3) the time points (here SACRIFICE_PERIOD) included in the experiment. **Each sheet must have the columns (sample ID, dose and time point) in the same positions**.

		BARCODE	DOSE	DOSE_LEVEL	SACRIFICE_PERIOD
2	12800	ID_003017698023	0	Control	4
3	12801	ID_003017698024	0	Control	4
4	12802	ID_003017698025	0	Control	4
5	12803	ID_003017699005	0	Control	8
6	12804	ID_003017699006	0	Control	8
7	12805	ID_003017699007	0	Control	8
8	12806	ID_003017667006	0	Control	15
9	12807	ID_003017667007	0	Control	15
10	12808	ID_003017667008	0	Control	15
11	12809	ID_003017667018	0	Control	29
12	12810	ID_003017667019	0	Control	29
13	12811	ID_003017667020	0	Control	29
14	12812	ID_003017698026	100	Low	4
15	12813	ID_003017698027	100	Low	4
16	12814	ID_003017698028	100	Low	4
17	12815	ID_003017699008	100	Low	8
18	12816	ID_003017699009	100	Low	8

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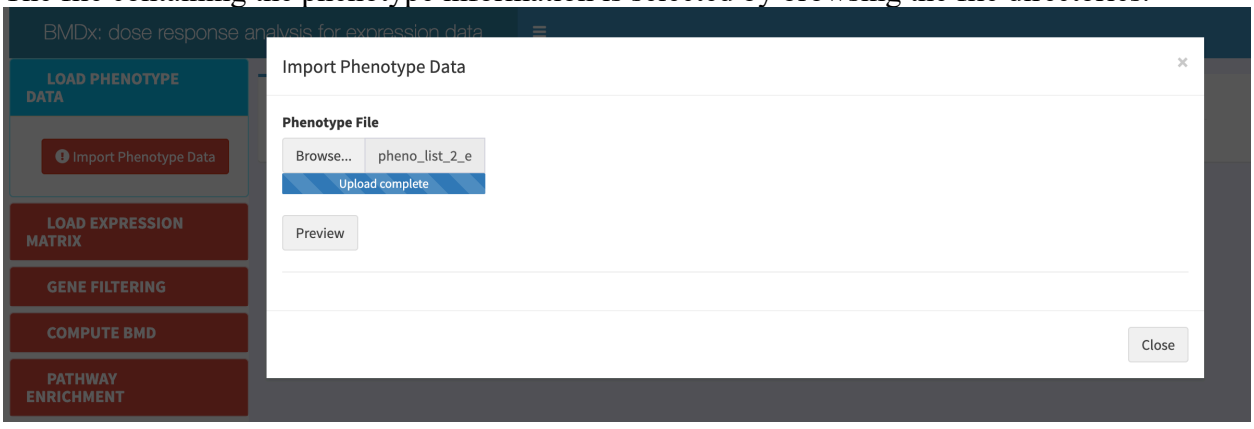
Load Phenotype

A popup window containing controls to configure the phenotype file import is launched by clicking *Import Phenotype Data* on the sidebar.

Select Phenotype File

The file containing the phenotype information is selected by browsing the file directories.



Phenotype Preview

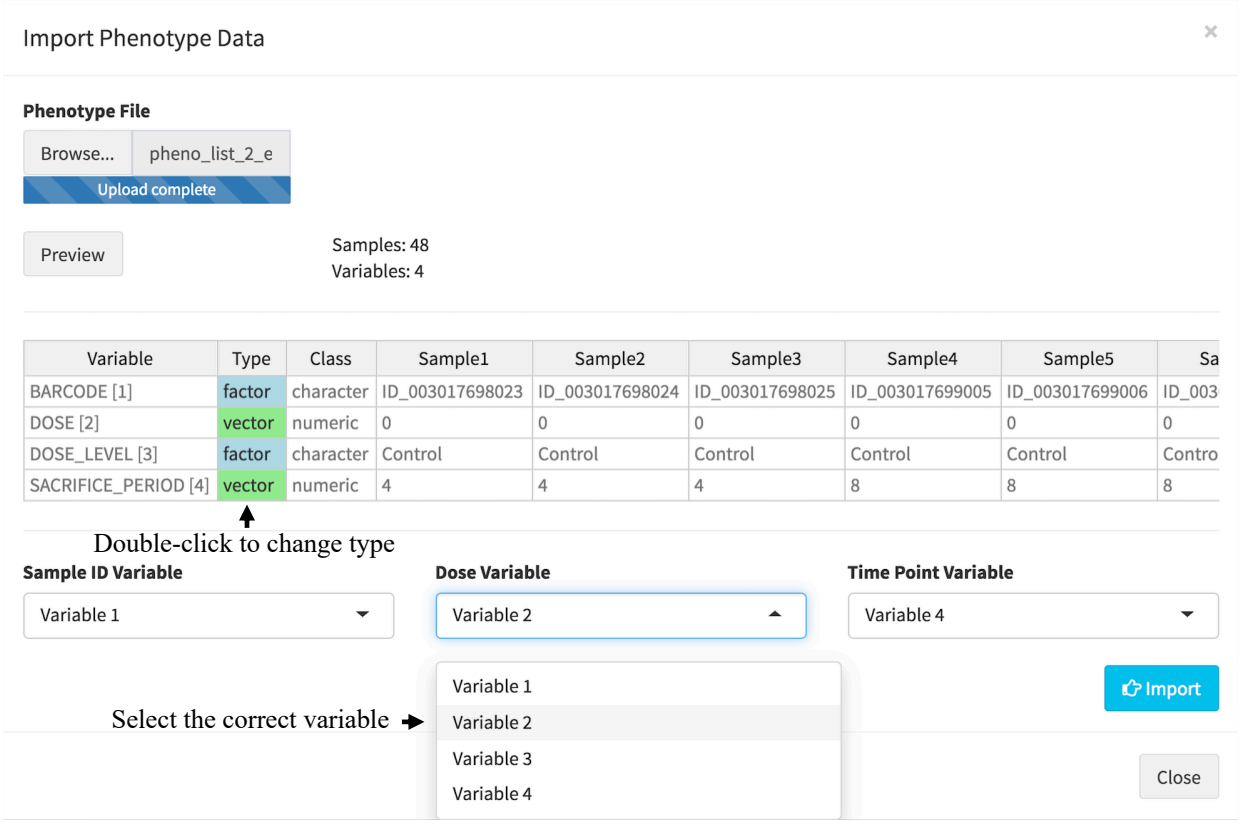
Preview of the phenotype file displays the columns from the first sheet in the phenotype file as variables. Each variable has an associated R class character, numeric, or integer and data representation type as factor or vector. Number of samples and variables are reported as text labels above the preview.

Configure Variable R Format

The user can change the default data representation type by double-clicking on the representative cell and selecting the alternative option (factor or vector).

Specify Sample ID, Dose and Time Point Variables

These variables are specified by the corresponding variable index from the phenotype preview.

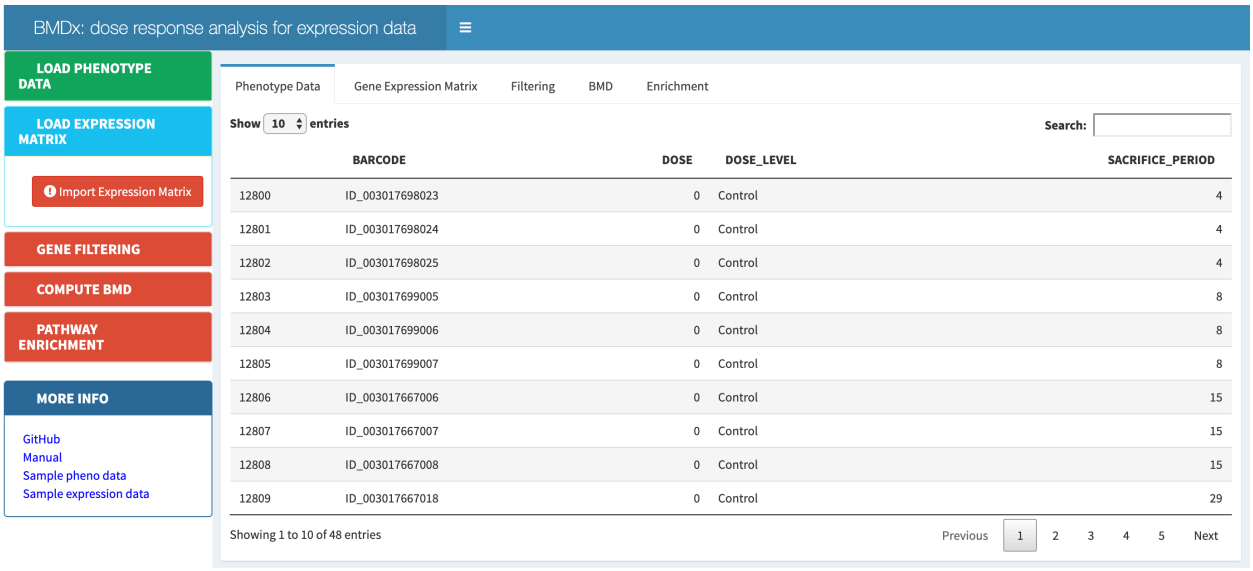


Import Phenotype

Finally, click on *Import* on the right bottom corner of the graphical window to import the configured phenotype file.

Phenotype View

The first sheet of the imported phenotype file is displayed in the main display area in the main *Phenotype Data* tab.



Expression matrix

The expression matrix is an Excel file with a separate sheet for each experiment. Sample columns are named with unique sample IDs (green outline) that match the sample IDs provided in the phenotype file. Gene names (orange outline) must be provided in the first column of each spreadsheet, and each following column specifies the expression values for those genes in each individual sample. The order of the sheets must match the order of the sheets in the phenotype file. Sample data can be found at https://github.com/Greco-Lab/BMDx (link also provided in the graphical interface).

	A	B	C	D	E	F	G	H	I	J
1		ID_003017698023	ID_003017698024	ID_003017698025	ID_003017699005	ID_003017699006	ID_003017699007	ID_003017667006	ID_003017667007	ID_0030176670
2	Gad1	6.414391023047236	6.465328379024536	6.157132577805856	6.380792465913976	6.482854905806466	6.565747857935866	6.682323301979586	6.806777297751916	6.824440377036
3	Cbln1	6.495480746055886	6.247461450031686	6.274450753424466	6.410631786719256	6.400666445783836	6.497833922846416	6.418882601481786	6.527497586472526	6.42275109523
4	Steap1	6.087251817076116	5.723925774526146	6.221594313863136	6.164695480114236	5.849072064269376	6.395905820546416	5.837809438423836	6.432777081306126	6.40249519808
5	Hebp1	8.162446779889026	7.981350715822136	7.300785505988786	7.376645571455226	7.567176202452616	7.879814487164786	6.957982323672276	6.763572105654636	6.545846499774
6	Tmcc2	6.301918498687356	6.080846696349486	6.121734379020556	6.226592404203876	6.159165992408766	6.443255446099016	6.062952068300756	6.223123474147916	6.48708339812
7	Nuak2	6.86081889282286	6.705859684336756	6.843427027836556	6.7861884544676	6.928968581636846	6.844614485018226	6.521493372113566	6.290710073013646	6.35225186865
8	Klhdc8a	6.701531795629156	6.494092883787626	6.6551999897453	6.641529669323256	6.637964413208966	6.659216589753726	6.262842949536986	6.595829612391236	6.37327199400
9	RGD1304622	6.158953110788786	5.761852792462756	6.111183693594436	6.430827842383666	6.130541453272466	6.409717121702636	6.526075789796186	6.4080867252917	6.80305592427
10	Slc26a1	7.785527536219916	7.053963582472766	7.300071016570856	6.989630364197456	7.313415419748146	7.081782032181986	6.729389439829736	6.543952715505086	6.47843053806
11	Cd82	6.564951056056526	6.443358163404076	6.336587350682326	6.504151793283716	6.501580165688226	6.565747857935866	6.116685550503356	5.969208479960476	5.88014545652
12	Gak	6.673616405202446	6.420206201321786	6.306477789935586	5.855682825268856	6.277174073006016	6.398231654998956	5.834019041092826	6.650786206625866	6.10430267868
13	Crp	7.880788577374216	7.665800531582946	7.700008069017886	7.369081207776466	7.801186073117856	8.104467951177256	7.379234348328786	6.908867406310876	7.25375987096
14	Abhd8	6.598527764369376	6.264062196426966	5.949377724177186	6.491748777829386	6.215567312678766	6.268902060724316	5.999582890563456	6.2287378268976	6.19215018296
15	Fcrl6	6.344985415937166	6.574994704455966	6.350046757325256	6.436576146074226	6.255360540043656	6.707571342324256	6.501900754285856	6.609512144674736	6.79793522534
16	Cplx1	6.571558955619666	6.226489878541626	6.155416265964366	6.199614319772326	6.336383702825586	6.246140564981356	6.495695165343316	6.245733326468416	6.37357962944

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Load Expression Matrix

The expression matrix file is imported similarly by clicking on *Import Expression Matrix* on the left side panel of the graphical interface. Expression matrix is viewed in the *Gene Expression Matrix* tab.

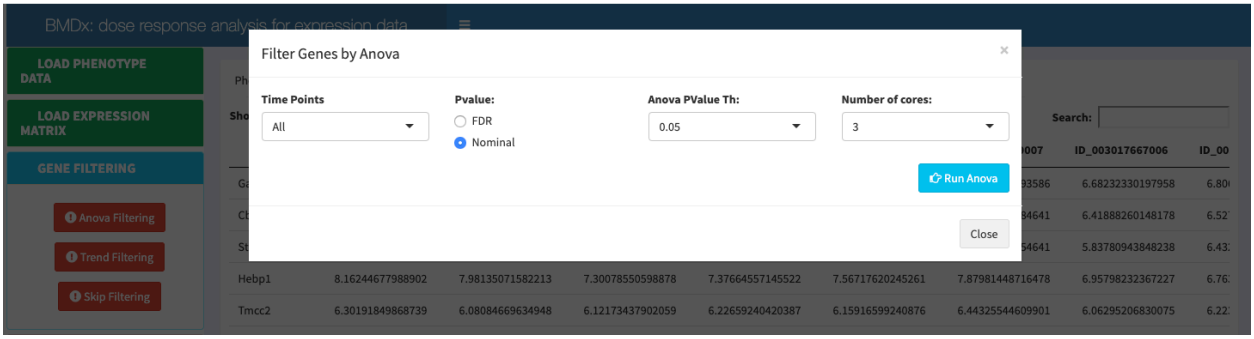
Gene Filtering

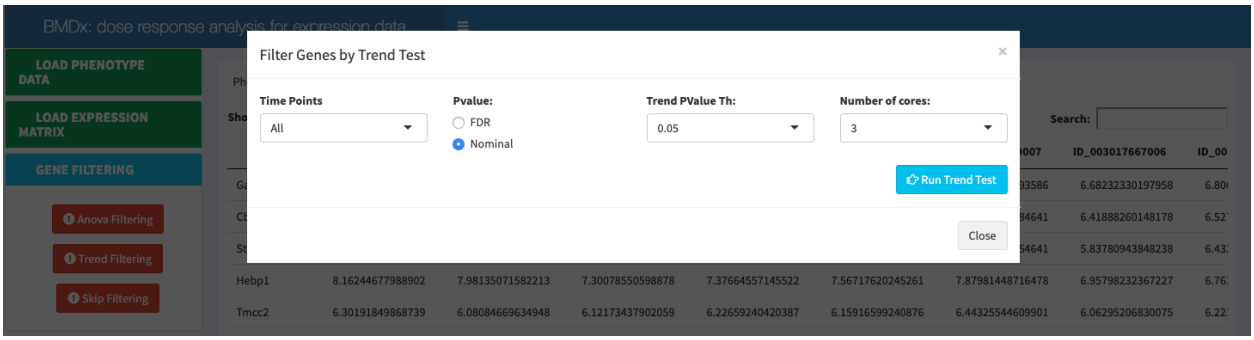
As the first step of the analysis, the genes can be filtered according to two different criteria that the user can select:

1. ANOVA: the genes that do not show variability across different doses are identified by performing an ANOVA test for each gene and removed from the analysis.

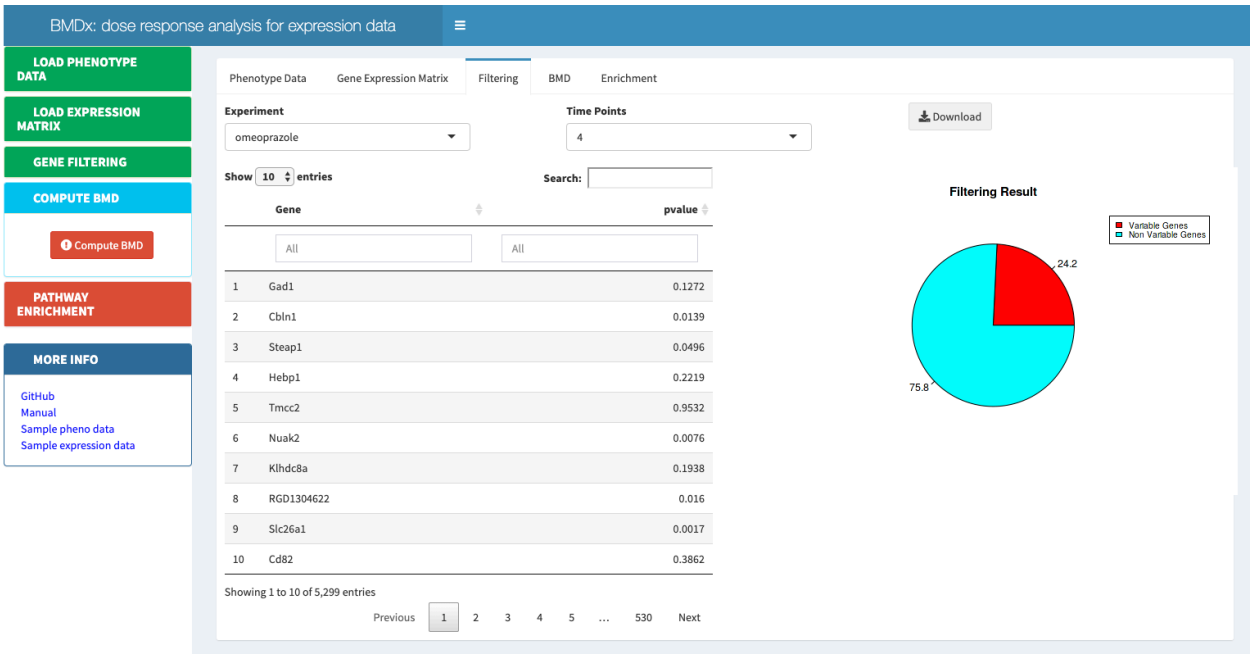
2. Trend Test: the genes that do not show a monotonical trend with respect to doses are identified by trend test for each gene and removed from the analysis.

Filtering is performed by click on the preferred method on the side bar. To run the filtering, time points included in the analysis are specified ("All" set as default). When multiple experiments are included in the analysis, all time points are included automatically and no less can be selected. P-value for the analysis can be specified between nominal and FDR corrected, and the p-value threshold can be set from the drop menu. Furthermore, the number of cores used for the analysis can be selected.





Once the gene filtering step has been run, the results are displayed in the *Filtering* tab. In particular, a table with the ANOVA/Trend Test p-value for every gene will be displayed for every time point in each experiment separately. The experiment under inspection can be changed from *Experiment* drop menu and the specific time point for which data is shown can be changed from the drop menu *Time Points*. Furthermore, a pie chart will show the percentage of genes surviving the filtering test for each time point. The results of the filtering test can be downloaded as one Excel file with multiple sheets, one for every time point at each experiment by clicking *Download*.



Model fitting and BMD computation

Parameter Selection

Clicking on *Compute BMD* on the side panel launches a graphical window for the selection of parameters and models for BMD analysis. The user selects the models to be fitted, the response level, the lack-of-fit p-value threshold and an upper and lower limit for the estimation of the minimum and maximum BMD allowed with respect to minimum and maximum doses. Moreover, an assumption of constant variance must be specified (see below).

BMD value filtering based on the doses

The two parameters *lowest/highest dose filter* are used to discard models where the predicted BMD value is x% lower than the lowest dose (lowest dose filter) or x% higher than the highest dose (highest dose filter). If 0 is selected, the exact minimum and maximum doses are used as limits. The value of x is selected from the drop menu for each parameter.

BMRF selection

As a default, the BMRF is set to 1.349. As described in Thomas et al. (2007), a BMR factor of 1.349 is the amount required to shift the mean transcriptional response of the control distribution such that the treated distribution contains 11% in a single tail, i.e., a 10% increase over the assume background rate of response. The 10% value for the shift in the tail area of the distribution is standard for BMD analysis.

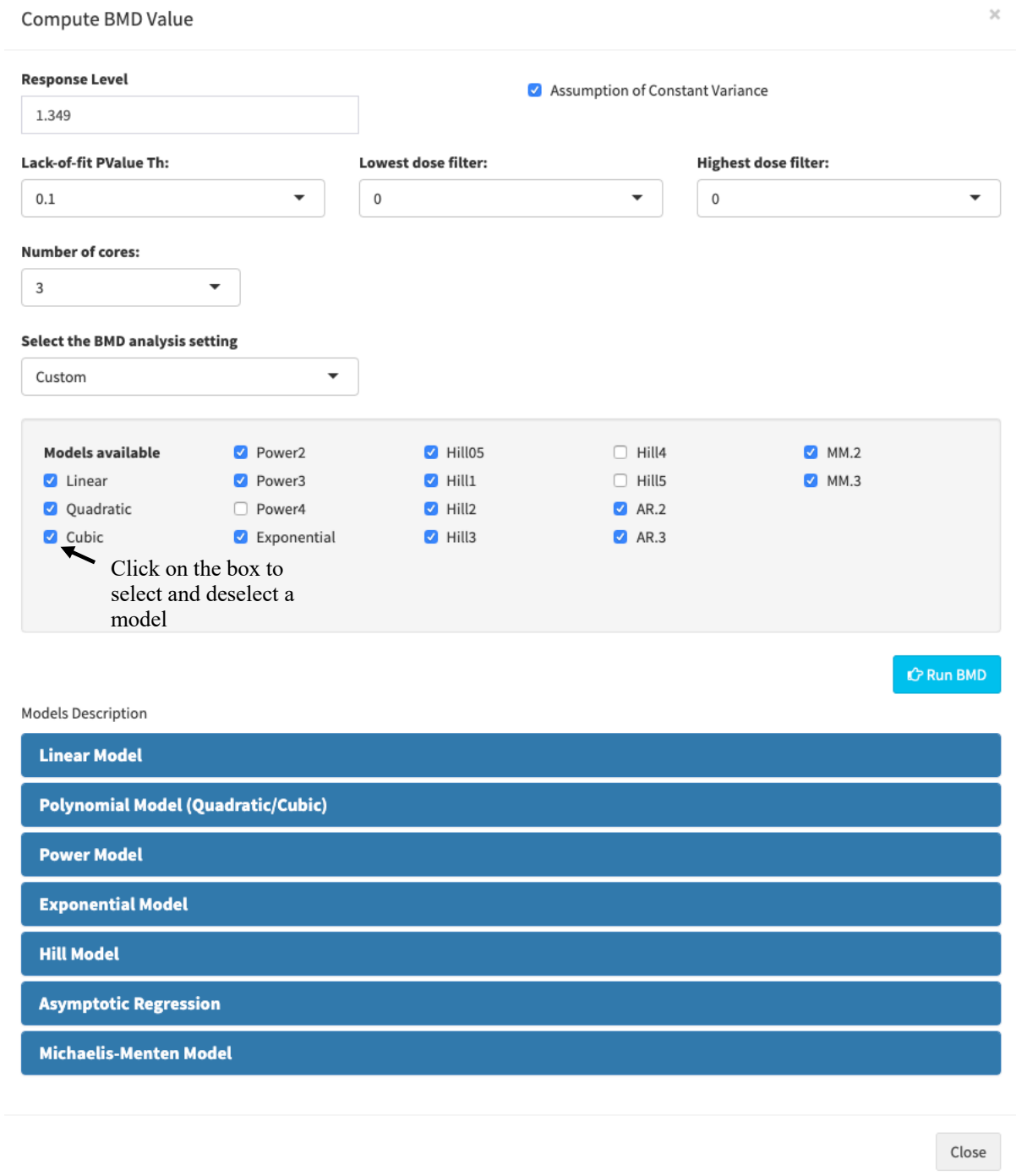
Assumption of constant variance model

If the tool is ran under the assumption of constant variance model, the BMRF is multiplied by the standard deviation of all the dose groups. Otherwise, the BMRF is multiplied by the standard deviation of the controls. (NTP, 2018)

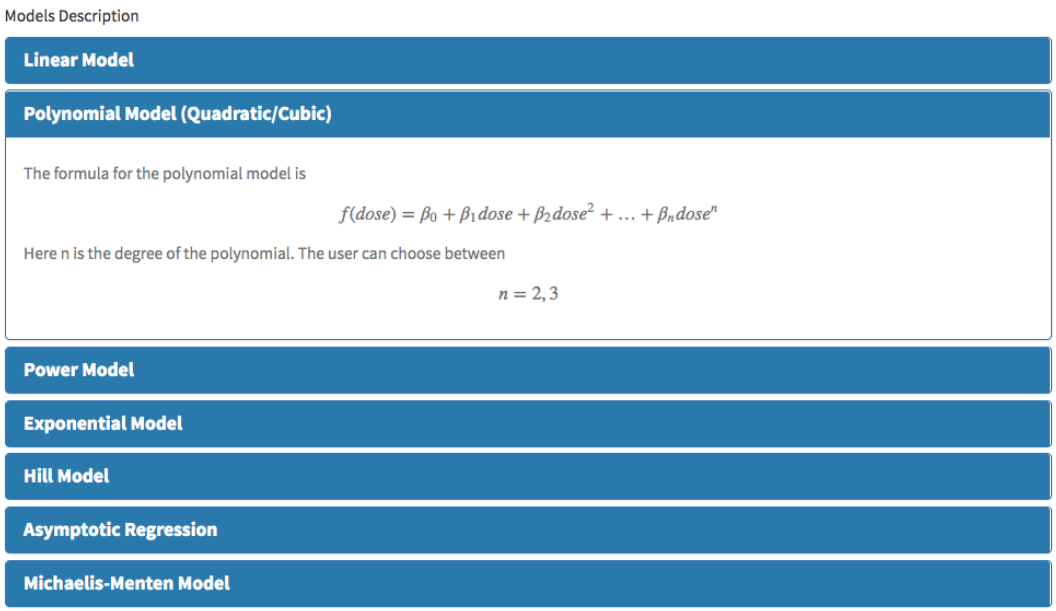
Model selection

For each gene, a list of models is computed and, for each fitting, a lack-of-fit p-value is provided. Models with non-statistically relevant fitting (lack-of-fit p-value < 0.1) and predicted BMD value outside the selected range are removed from the analyses. The optimal model is identified as the one with the minimum Akaike Information Criterion (AIC) value.

Select the BMD analysis setting section allows for the selection of models to be used for the analysis. The models can be selected from predefined sets (*All, Regulatory, Degree of Freedom, Custom*) or selected manually. *Regulatory* contains models used by the regulatory agencies, *Degree of Freedom* includes models with a degree of freedom smaller than $n_d - 1$, where n_d is the number of doses, while *Custom* allows for manual selection by clicking the models one at a time. Moreover, in the lower part of the compute BMD Value window, the user will find a description of the models available in the tool. Model descriptions can also be found on page 26 of this file.



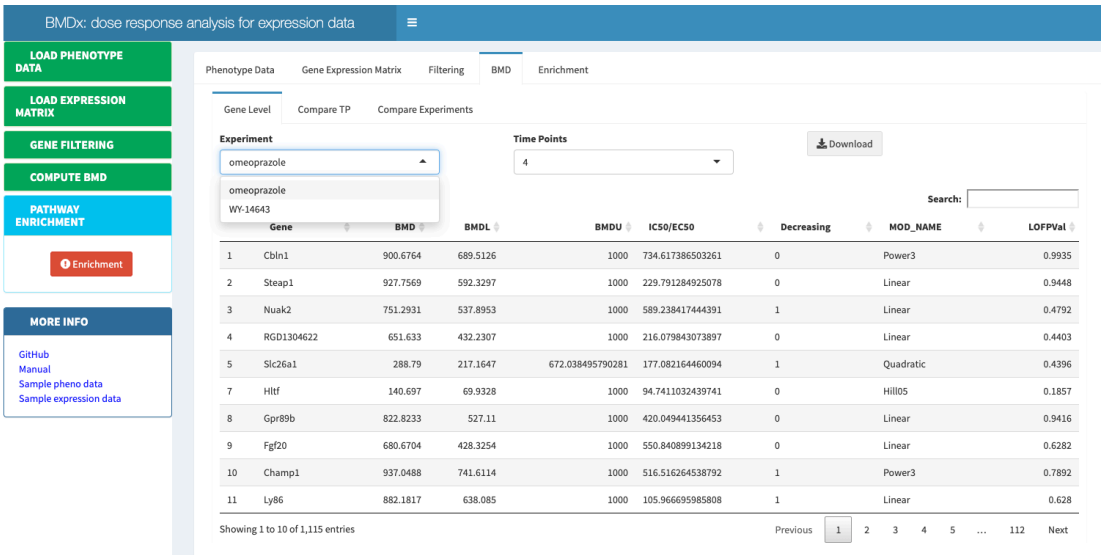
Descriptions of the models are viewed by clicking the name of the model.



Results investigation

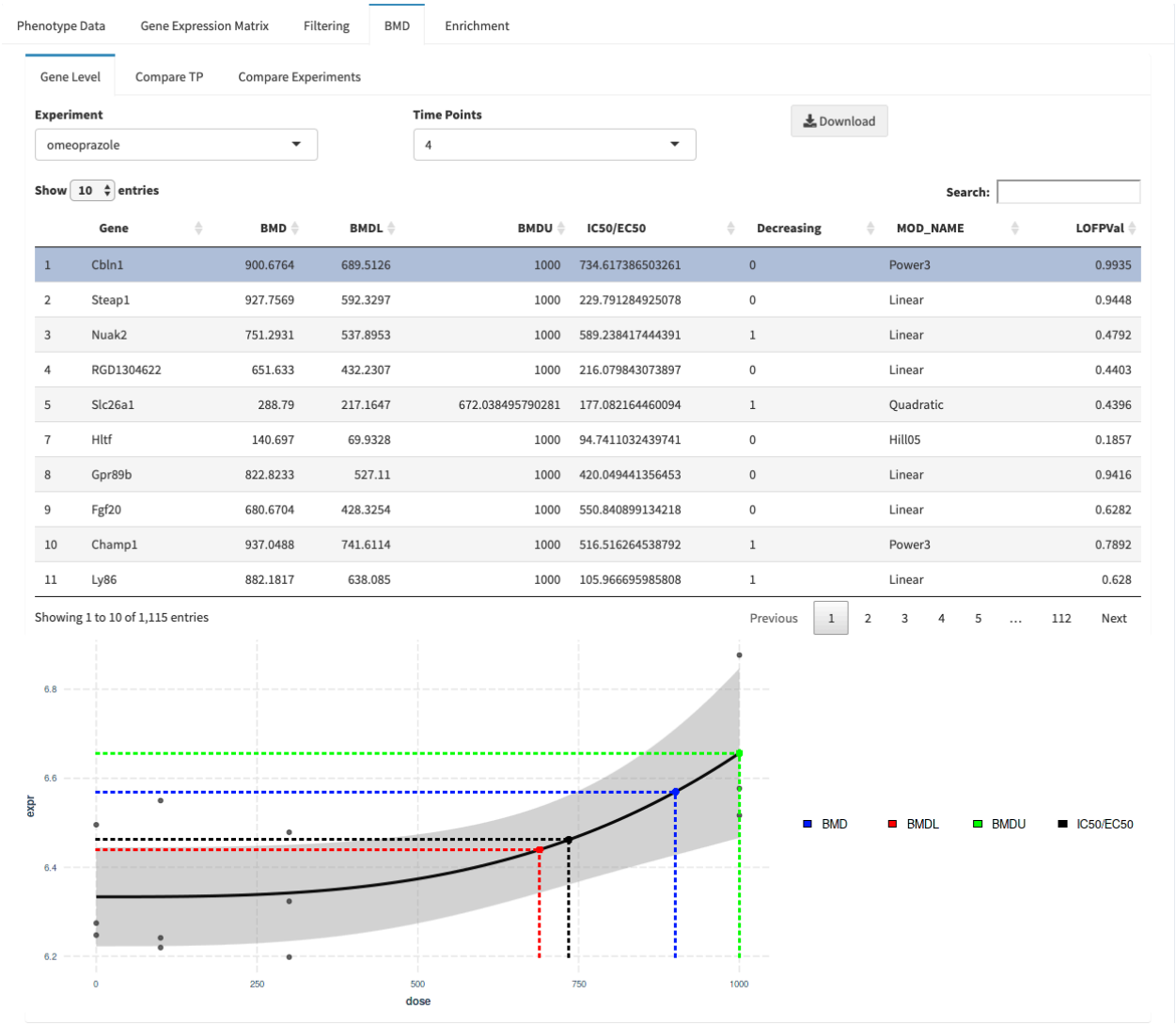
The results of the BMD analysis for each experiment can be explored on the *BMD* tab one time point at a time or different aspects of the results between time points can be visualised under Compare TP tab. Additionally, an UpSet plot representing the intersections between different doses and experiment can be visualised under *Compare Experiments* tab. The experiment under inspection can be changed from the *Experiment* drop menu.

On a gene level, BMD, BMDL and BMDU are calculated, as well as the IC50/EC50 value. The table also shows whether the expression of the gene is increasing or decreasing with dose, the optimal model and the lack-of-fit p-value of that model. Results can be downloaded as a single excel file with one sheet for each time point at each experiment by clicking *Download*.



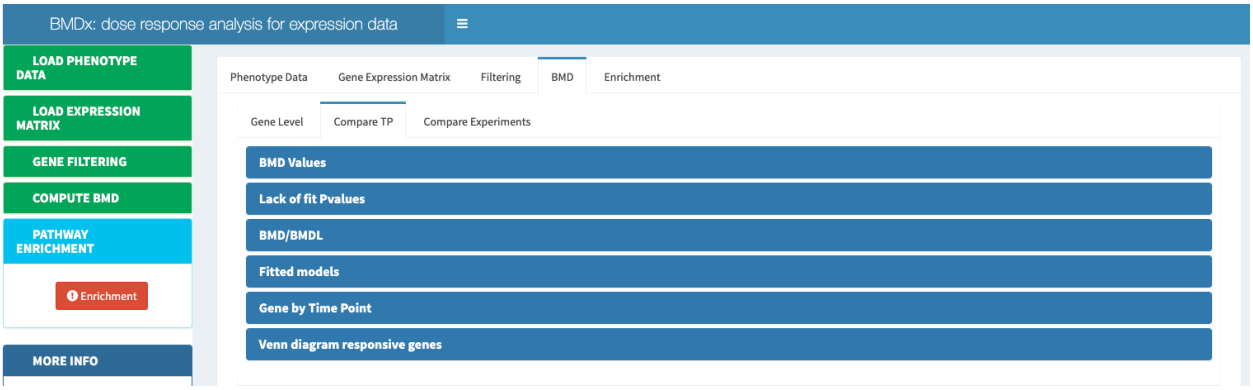
Fit of the model

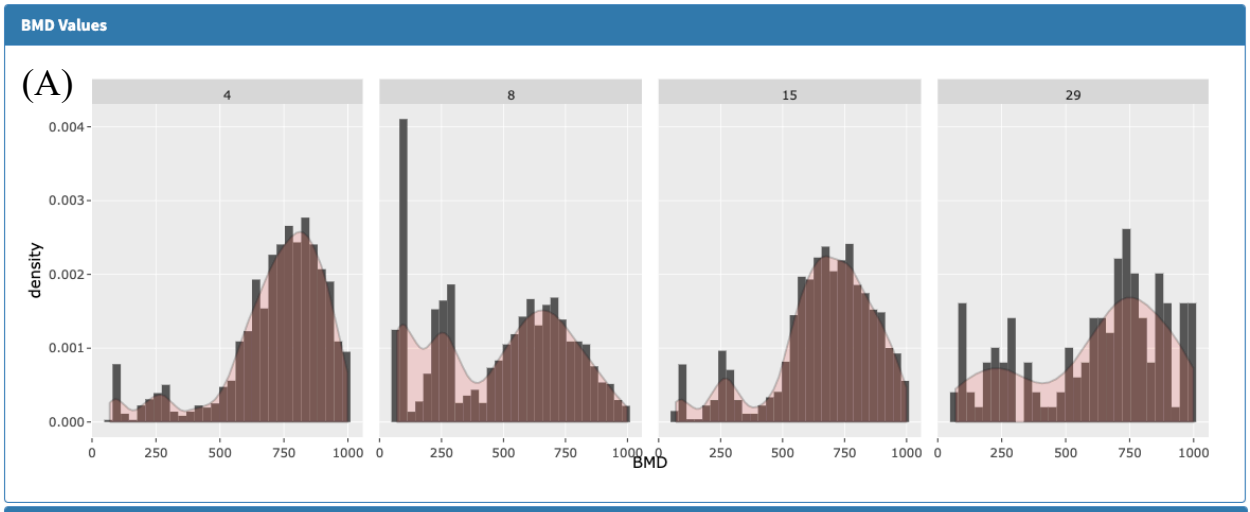
The fitting of the model can be visualised below the table by clicking on the row of the gene. Calculated values are shown in the figure with specific colours: red indicates the value for the BMDL, blue for BMD while black marks the BMDU value. IC50/EC50 is marked with black.

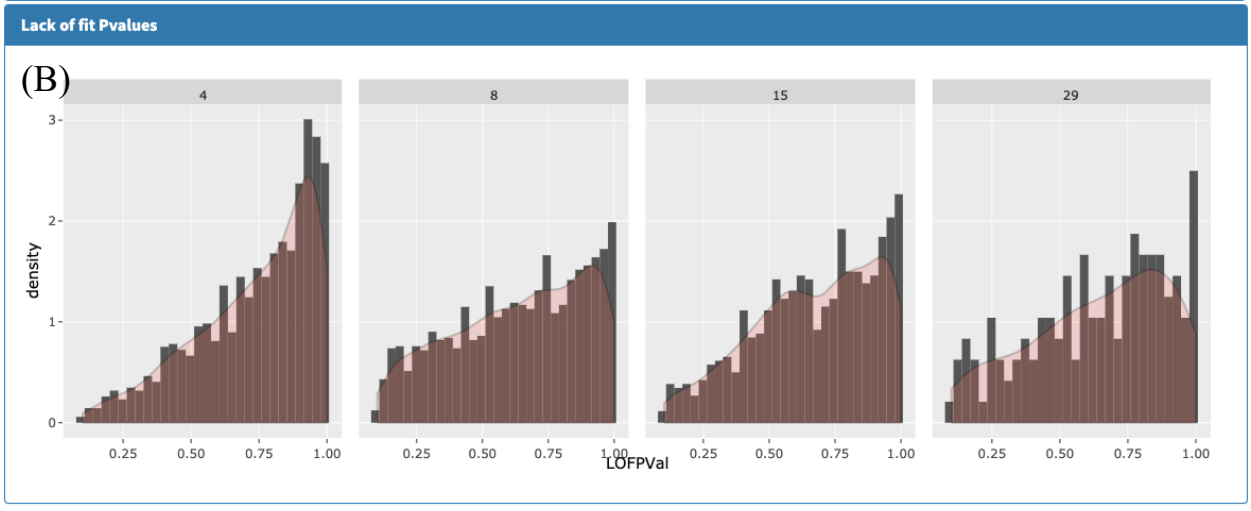


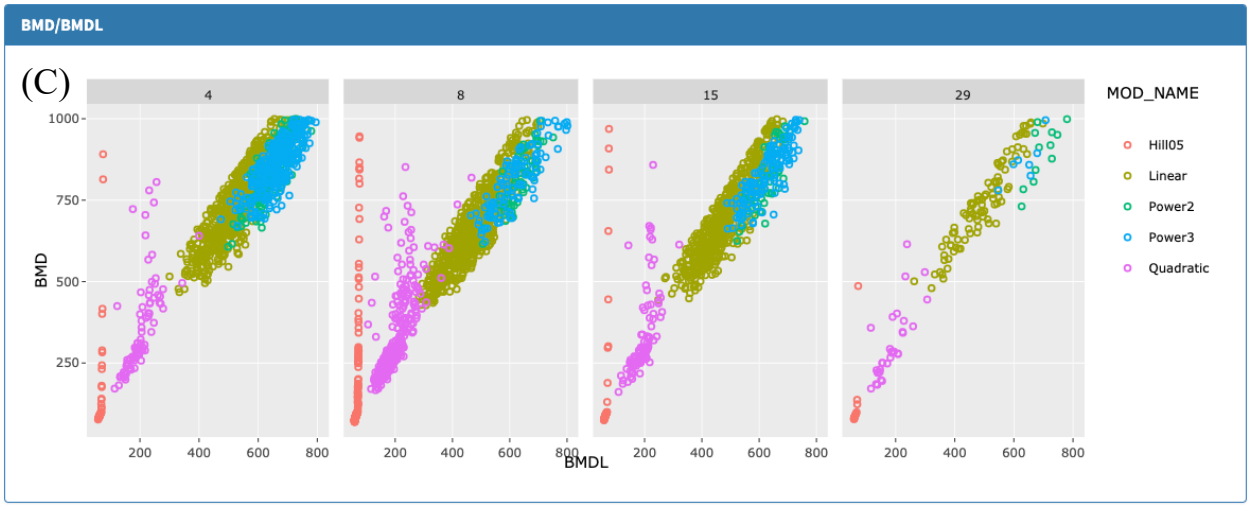
Comparing results between time points

When multiple time points are included in the analysis, the results between them can be visualised on the *Compare TP* tab. BMDx allows for the visualisation of the density of the BMD values (A) as well the lack-of-fit p-values (B). The BMD values obtained at each time point with each of the optimal models are plotted (C), the proportion of the models at each time point are visualised (D). The number of dose-dependent genes at each time point are shown as bar plots for easy comparison (E), and finally, a Venn diagram of the responsive genes is shown and the gene lists at all of the intersections can be explored (F). Plots C, D, and F are interactive, and the plotted features can be deselected and selected by clicking the feature (e.g. the gene names in the lower part of the plot F).


BMDx: dose response analysis for expression data
LOAD PHENOTYPE DATA
LOAD EXPRESSION MATRIX
GENE FILTERING
COMPUTE BMD
PATHWAY ENRICHMENT
Enrichment
MORE INFO
Phenotype Data
Gene Expression Matrix
Filtering
BMD
Enrichment
Gene Level
Compare TP
Compare Experiments
BMD Values
Lack of fit Pvalues
BMD/BMDL
Fitted models
Gene by Time Point
Venn diagram responsive genes


BMD Values
(A)
4
8
15
29
density
BMD

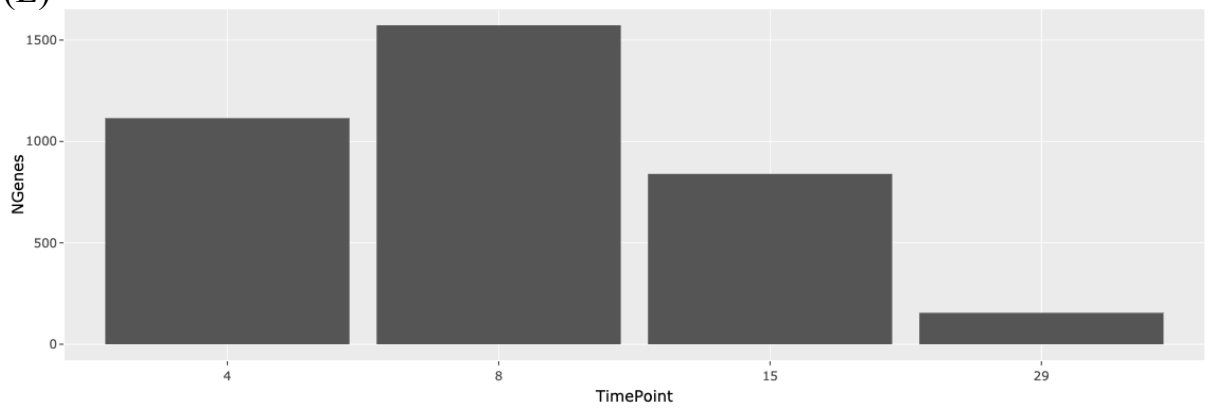

Lack of fit Pvalues
(B)
4
8
15
29
density
LOFPVal


BMD/BMDL
(C)
4
8
15
29
BMD
BMDL
MOD_NAME
Hill05
Linear
Power2
Power3
Quadratic


Fitted models
(D)
Freq
Model
Value
ValueType
BMDL
BMD
BMDU

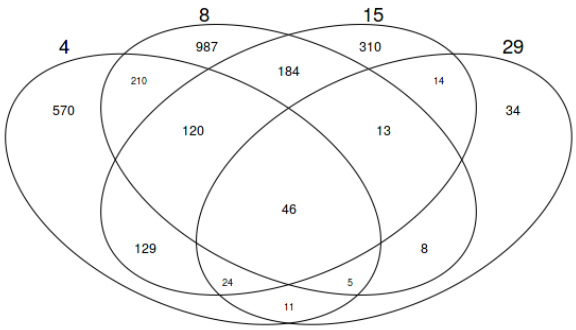
Gene by Time Point

(E)



Venn diagram responsive genes

(F)



Show 5 entries Search:

4	8	15	29	4:8	4:15
Steap1	Gad1	Hebp1	Tspyl4	Cbln1	Nuak2
Hltf	Klhdc8a	Crp	Clic2	RGD1304622	Slc26a1
Gpr89b	Pde6b	Lamp2	Smpdl3a	Fgf20	Ano10
Champ1	Thoc3	Mpst	Bace2	Notch4	Cdkn1a
Ly86	Rnf19b	Csf2rb	Tmem100	Pi16	Marcks

Showing 1 to 5 of 987 entries

Previous 1 2 3 4 5 … 198 Next

Select Gene Set

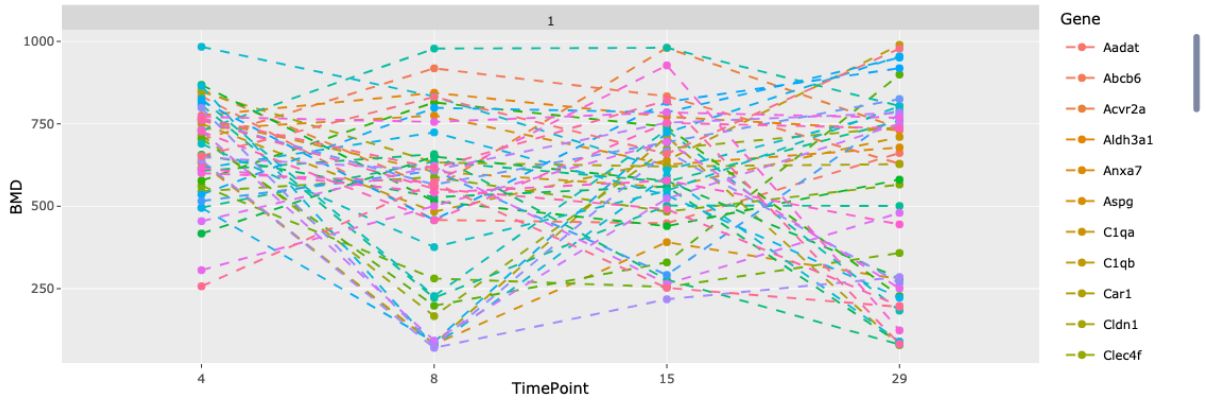
4:8:15:29

Number of clusters

1

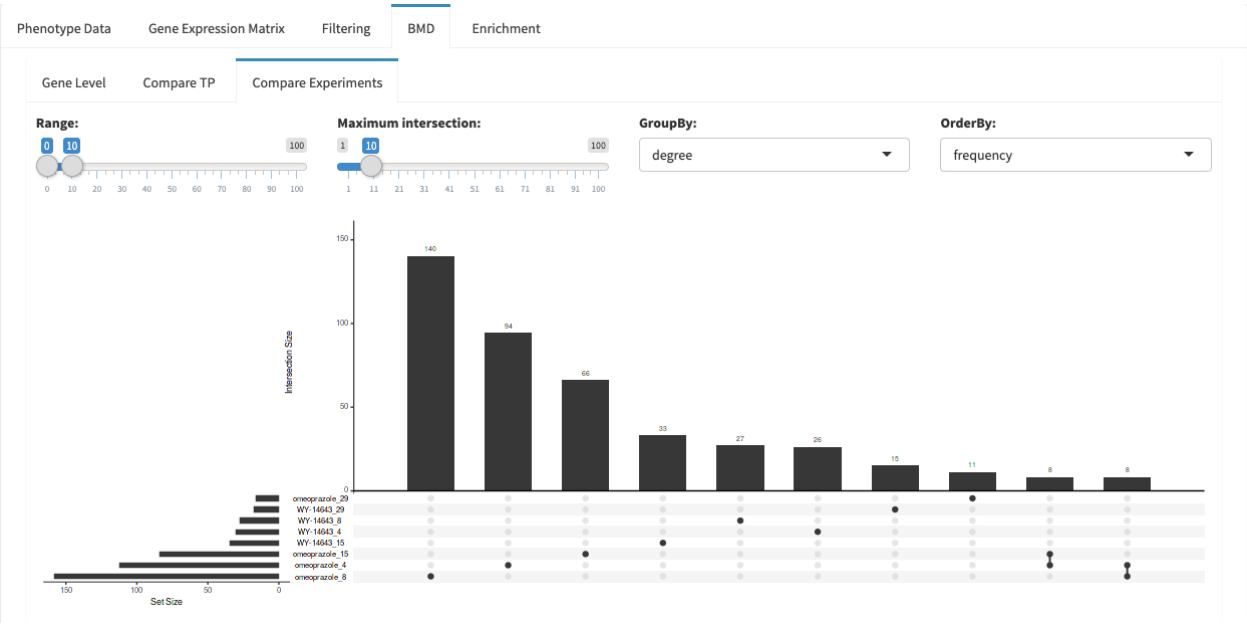
Agglomerative Method

ward



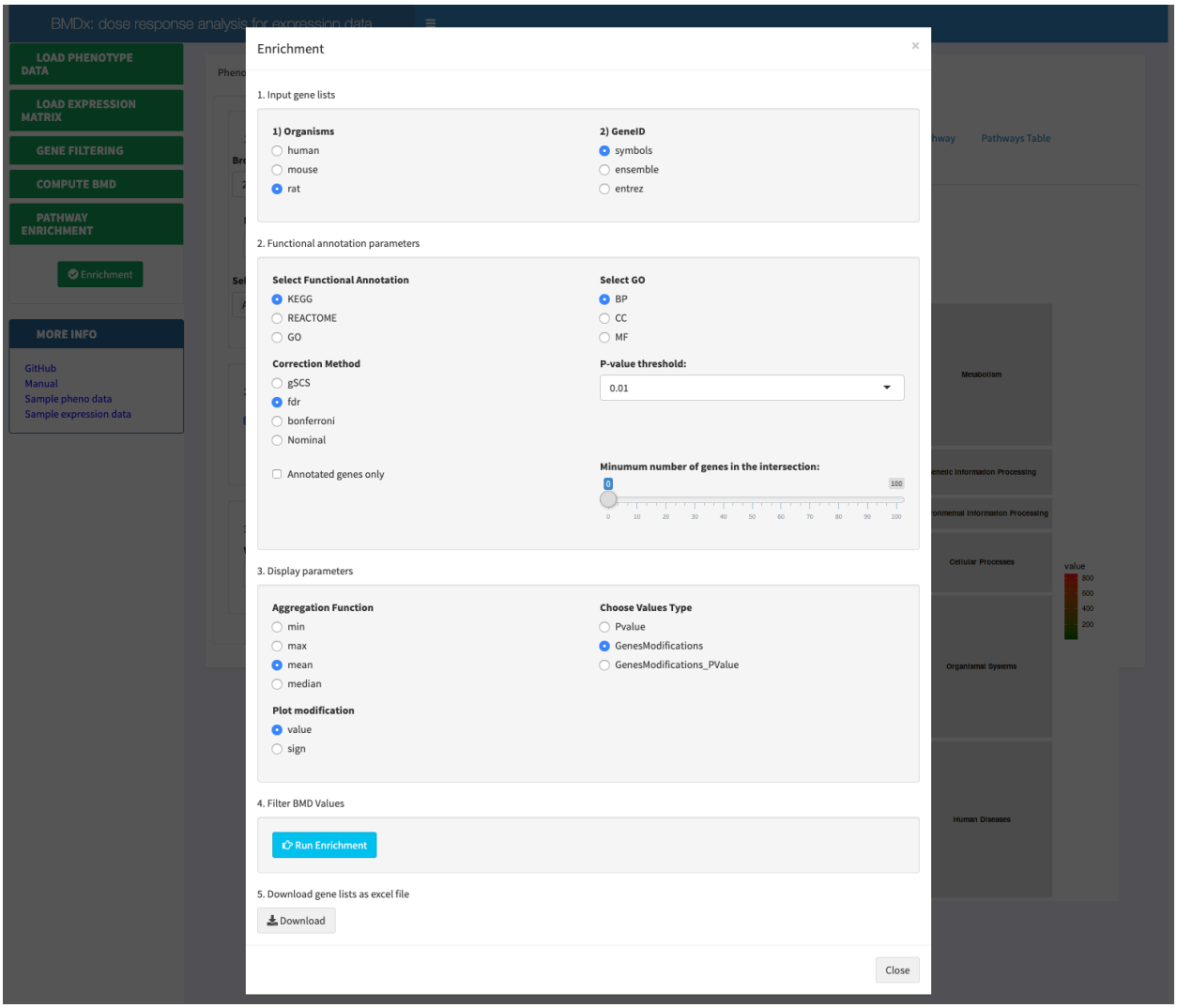
Compare Experiments

An UpSet plot can be viewed to represent the intersecting genes between time points and experiments.



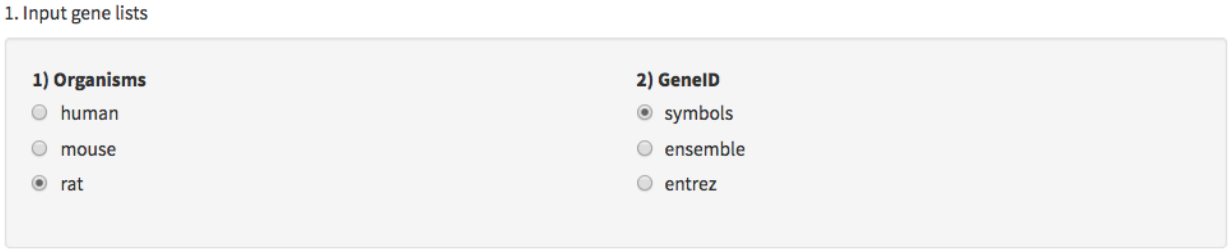
Functional Enrichment

Finally, the results of the BMD analysis can be explored in the form of a pathway enrichment analysis by launching a graphical window by clicking on *Enrichment* on the side panel. For detailed information on the enrichment tool, please refer to Scala et al. (2019).

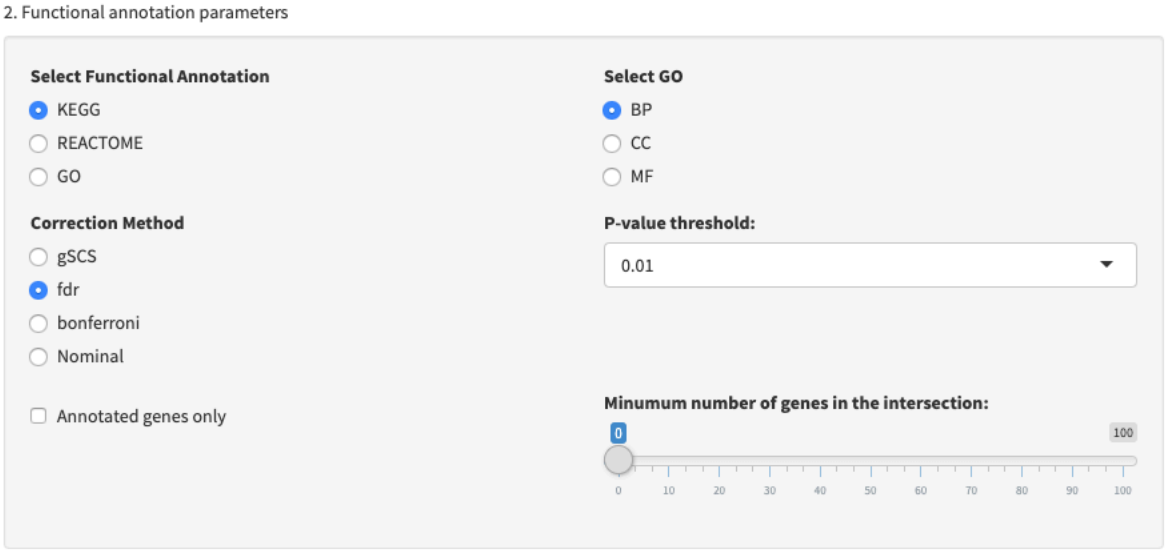


Enrichment parameters

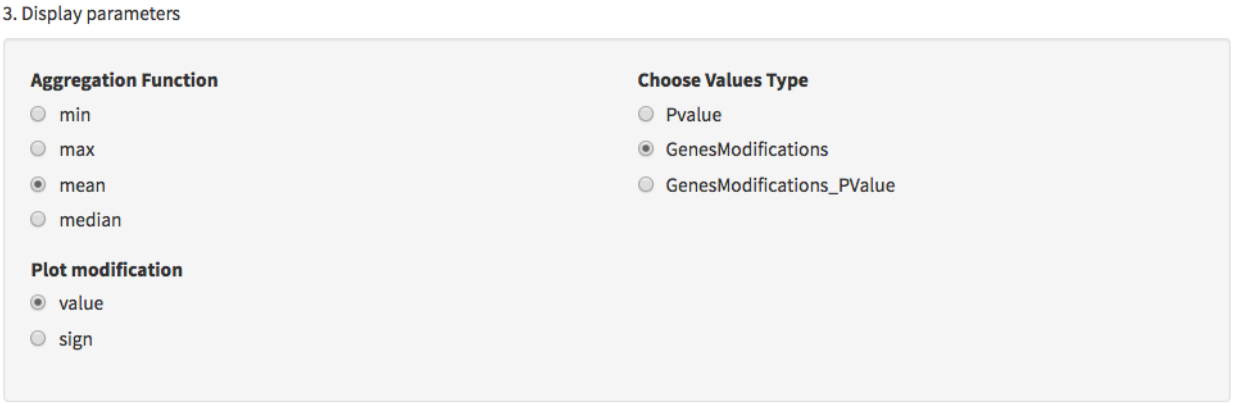
The enrichment analysis supports human, mouse and rat genes expressed in official gene symbols or Ensemble or Entrez gene IDs. The right parameters are selected from the options provided in the first part of the graphical window (*1. Input gene lists*).



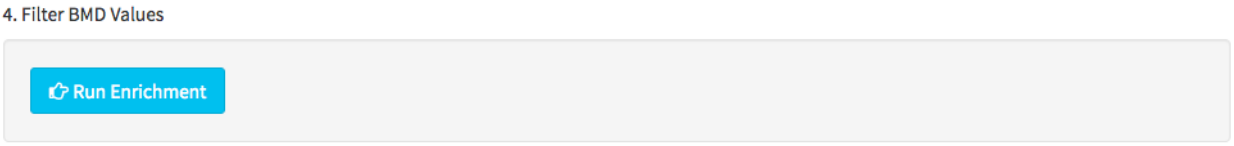
Functional annotation can be selected between KEGG and Reactome Pathways or GO terms. For GO terms, specify BP for Biological Pathways, CC for Cellular Components or MF Molecular Functions. P-value threshold for the enrichment is selected from the drop menu and the correction method for the p-value can be selected from several methods (gSCS, FDR, Bonferroni).



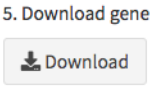
In the bottom of the window, the user can specify the display parameters used for the plotting of the enrichment map. *Aggregation function* (min, max, mean, median) specifies the function to be used when all the genes annotated to the same pathway are aggregated, while *Plot modification* specifies whether the enrichment map is plotted in chromatic scale or in one colour. *Choose values type* determines if the values plotted in the map are the p-values of the enrichment, the genes modifications (i.e. the BMD value) or a combination of the two.



To run the enrichment with selected parameters, *Run Enrichment* is clicked. If the user wants to change the parameters or enrichment type later, the window is launched again from the side panel.

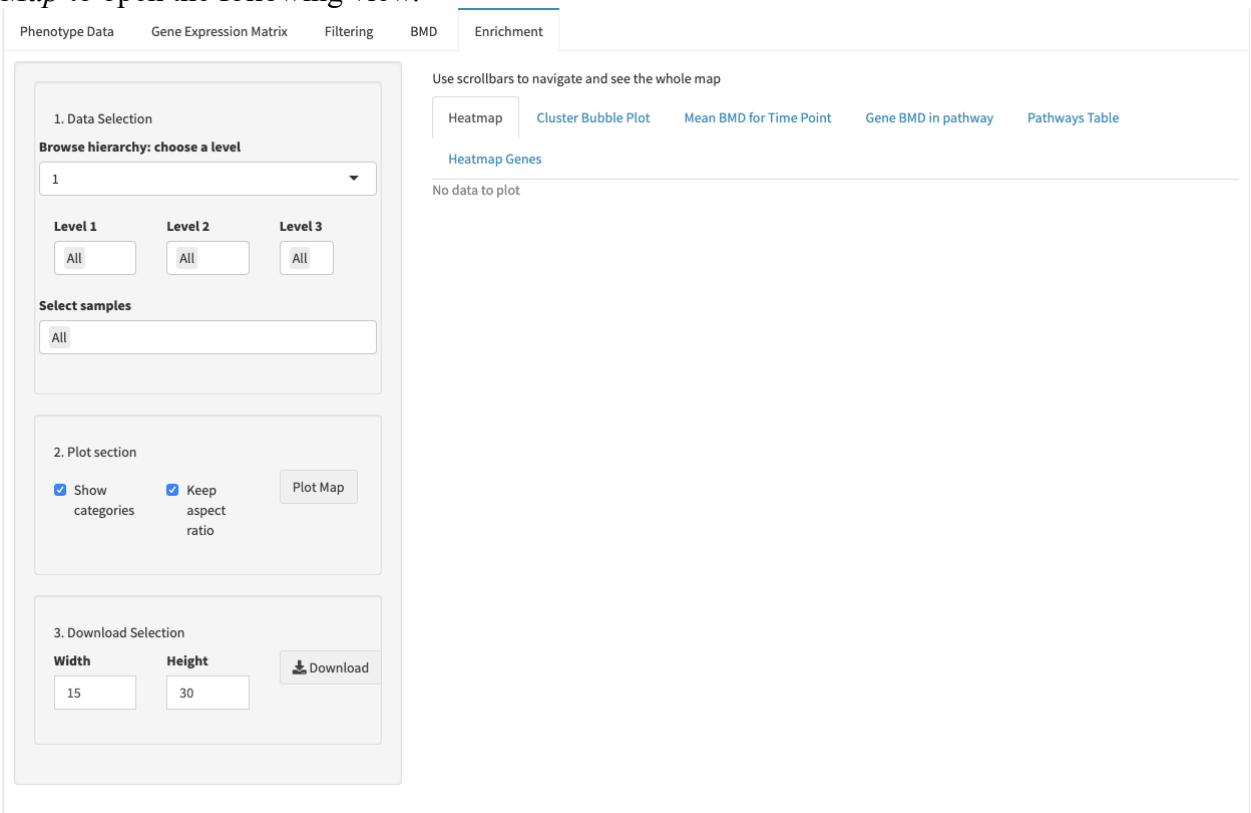


The input for the enrichment tool can be downloaded by clicking the *download* button on the bottom of the graphical window. The file contains multiple sheets, each representing the genes and their BMD values at each time point of the included experiments.



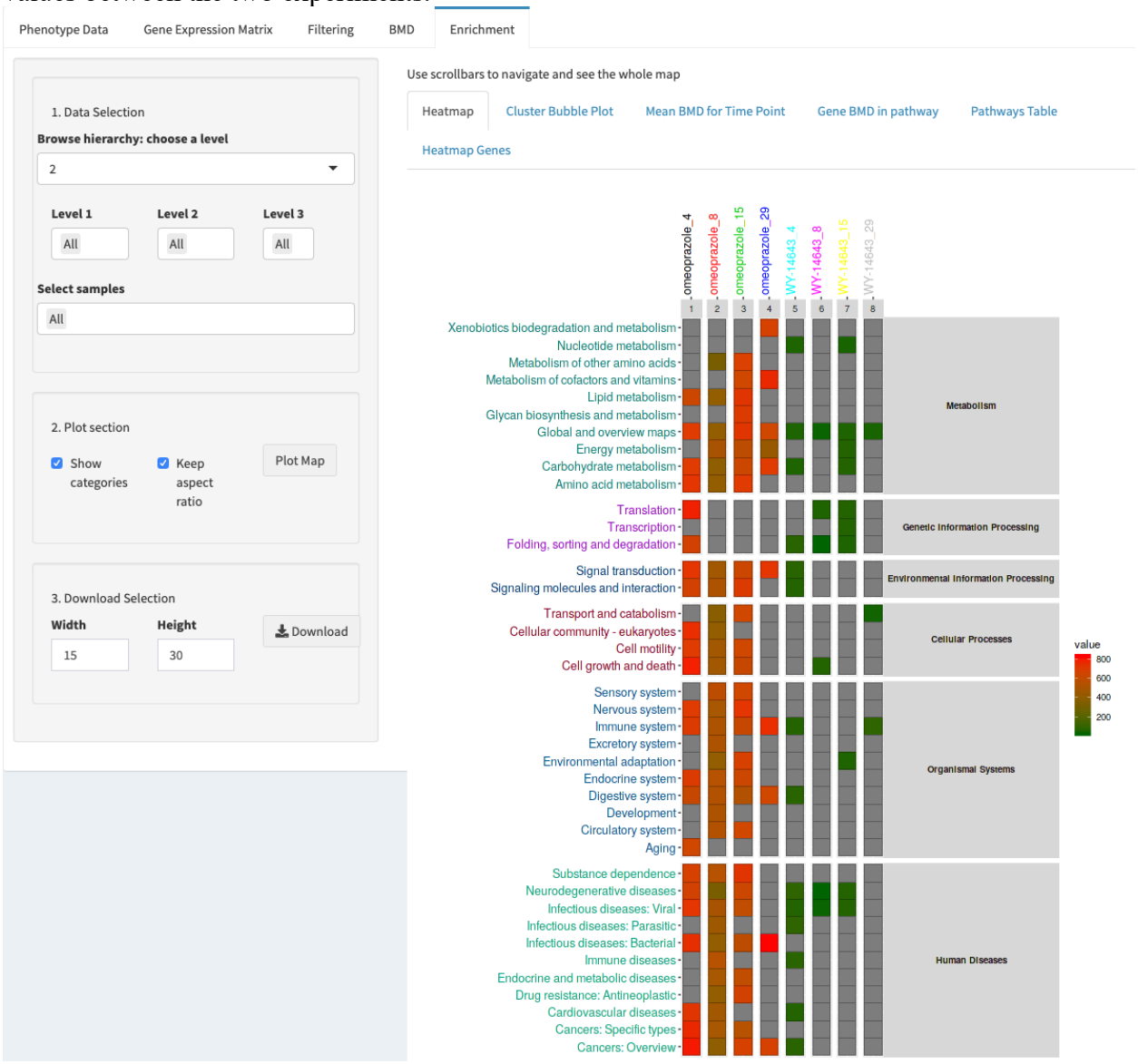
Enrichment results

After running the enrichment, the results can be explored on the *Enrichment* tab. Before visualizing the map, the user must specify the hierarchy level on which the results are shown and click on *Plot Map* to open the following view.



Heatmap

The enrichment heatmap shows each time point in each experiment as a separate column. Pathways are shown in the rows, and coloured boxes indicate enrichment of the pathway at the specific condition. When plotted values are shown in chromatic scale, the colour of the box changes according to the value. For example, in the figure below, the colour indicates the mean BMD value of the genes contributing to the enrichment of the pathway, showing the difference in the BMD values between the two experiments.



Cluster Bubble Plot

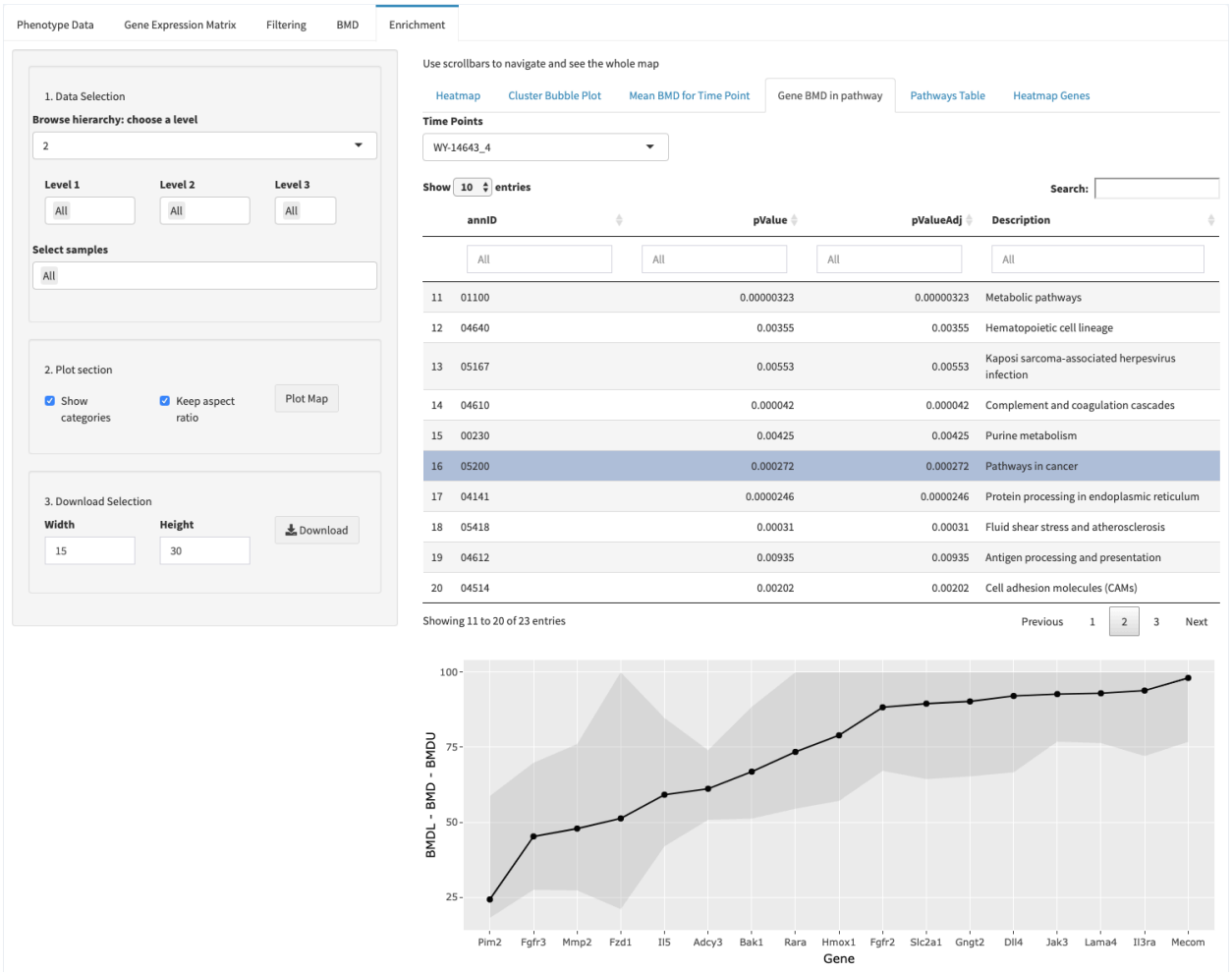
The functional enrichment can be visualised in the form of a bubble plot. Slots consisting of each time point in each experiment show bubbles characterising the size of the enrichment. The bigger the bubble, the more terms are included in that category.

Mean BMD for Time Point

The mean BMD values for each time point are shown as stacked bar plots for each experiment separately.

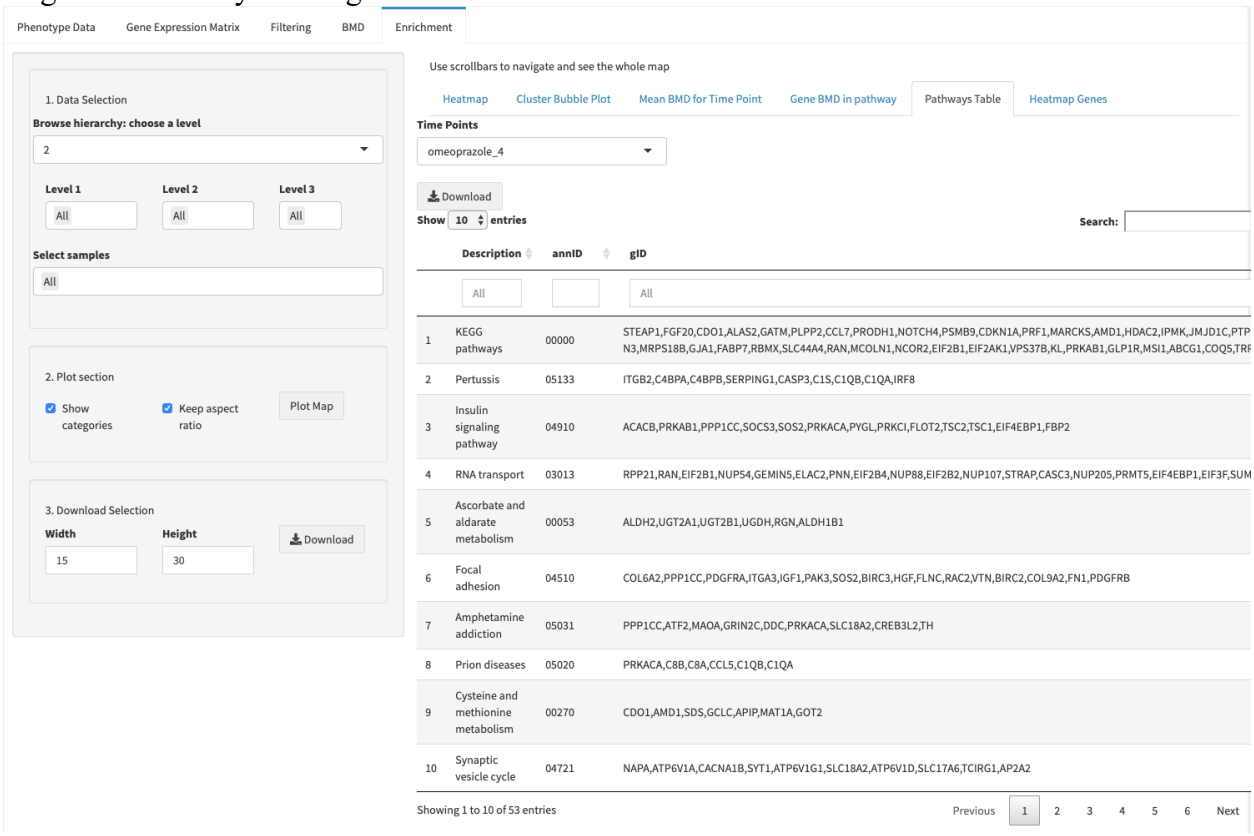
Gene BMD in Pathway

Gene BMD in a Pathway tab shows all pathways with their enrichment p-values. Selecting a row of the table plots a graph under the table with all the genes in the pathway, their BMD values as well as the lower and upper confidence bound BMD. *Note! Deselect the row before selecting the next row to only include the genes in the desired pathway.*



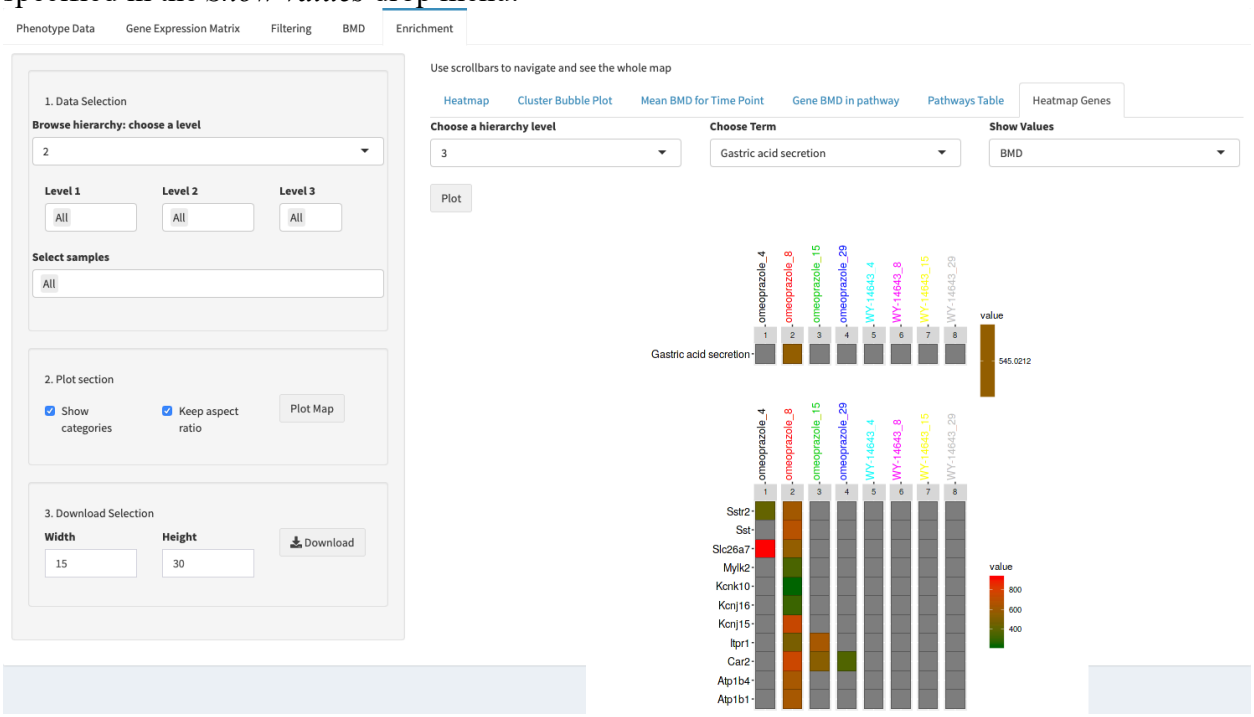
Pathways Table

The genes mapped to each term are shown as a table in *Pathways table* tab. Time point for which data is shown can be changed from the *time point* drop menu. The tables can be downloaded as a single Excel file by clicking *download*.



Heatmap Genes

Finally, the genes in different pathways can be explored in the form of a heatmap. The drop menus allow for the specification of the hierarchy level, the terms belonging to that level and specification of values shown on the heatmap. The heatmap then shows the genes mapped to the selected term on rows with the experiments and time points as columns, and coloured boxes indicating the value specified in the *Show values* drop menu.



Model descriptions

The models available for the evaluation of the BMD are:

Linear Model:

$$f(dose) = \beta_0 + \beta_1\, dose$$

Polynomial model:

$$f(dose) = \beta_0 + \beta_1 dose + \beta_2 dose^2 + \ldots + \beta_n dose^n$$

Here n is the degree of the polynomial. The user can choose between $n = 2, 3$

Power model:

$f(dose) = \beta_0 + (dose)^{\delta}$

The user can choose between $\delta = 2,3,4$

Exponential model:

$f(dose) = \beta_0 + expr(dose)$

Hill model:

$f(dose) = \beta_0 + \frac{dose^n}{Kd + dose^n}$

The user can choose between $n = 0.5, 1,2,3,4,5$, while $Kd = 10$

Asymptotic regression model:

$f(dose) = c + (d - c) \times (1 - expr(-dose/e))$

The parameter c is the lower limit (at x=0), the parameter d is the upper limit and the parameter e>0 is determining the steepness of the increase of dose.

The AR.3 model is the one depending from c, d and e parameters. The AR.2 model depends only on d and e parameters, while c is set to zero

Michaelis-Menten Model:

The model is defined by the three-parameter model (MM.3) function

$f(dose, (c, d, e)) = c + \frac{d-c}{1+(e/dose)}$

It is increasing as a function of the dose, attaining the lower limit at dose 0 (x=0) and the upper limit d for infinitely large doses. The parameter e corresponds to the dose yielding a response halfway between c and d.

The common two-parameter Michaelis-Menten model (MM.2) is obtained by setting c equal to 0.

References

Abraham, K., Mielke, H. and Lampen, A. 2012. Hazard characterization of 3-MCPD using benchmark dose modeling: Factors influencing the outcome. *European Journal of Lipid Science and Technology* 114(10), pp. 1225–1226.

EFSA Scientific Committee, Hardy, A., Benford, D., et al. 2017. Guidance on the use of the weight of evidence approach in scientific assessments. *EFSA Journal* 15(8).

Igarashi, Y., Nakatsu, N., Yamashita, T., Ono, A., Ohno, Y., Urushidani, T., & Yamada, H. 2015. Open TG-GATEs: a large-scale toxicogenomics database. *Nucleic acids research*, *43*(Database issue), D921–D927.

Leek, J.T., Johnson, W.E., Parker, H.S., Jaffe, A.E. and Storey, J.D. 2012. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 28(6), pp. 882–883.

National Toxicology Program. 2018. NTP Research Report on National Toxicology Program Approach To Genomic Dose-Response Modeling.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W. and Smyth, G.K. 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43(7), p. e47.

Scala, G., Serra, A., Marwah, V.S., Saarimäki, L.A. and Greco, D. 2019. FunMappOne: a tool to hierarchically organize and visually navigate functional gene annotations in multiple experiments. *BMC Bioinformatics* 20(1), p. 79.

Thomas, R.S., Allen, B.C., Nong, A., et al. 2007. A method to integrate benchmark dose estimates with genomic data to assess the functional effects of chemical exposure. *Toxicological Sciences* 98(1), pp. 240–248.

Woods, CG., Burns, A.M., Bradford, B.U., Ross, P.K., Kosyk., O., Swenberg, J.A., Cunningham, M.L. and Rusyn, I. 2007. WY-14,643 Induced Cell Proliferation and Oxidative Stress in Mouse Liver are Independent of NADPH Oxidase. *Toxicological Sciences, 98*(2), 366.