BMDx Reference Manual with Sample Data Analysis

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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 μ g for Omeprazole and 10, 30 and 100 μ g 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 (<u>https://github.com/Greco-Lab/BMDx</u>).

Installation and execution

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

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).

alysis for expre	ession data \equiv			
Phenotype Data	Gene Expression Matrix	Filtering	BMD	Enrichment
	Phenotype Data	Phenotype Data Gene Expression Matrix	Phenotype Data Gene Expression Matrix Filtering	Phenotype Data Gene Expression Matrix Filtering BMD

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 (<u>https://github.com/Greco-Lab/BMDx</u>.

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**.

1		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	
•		omeoprazole	WY-14643	3 +		

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.

	nalvsis for expression data 🛛 🚍	
LOAD PHENOTYPE	Import Phenotype Data	×
Import Phenotype Data	Phenotype File Browse pheno_list_2_e	
LOAD EXPRESSION MATRIX	Upload complete Preview	
GENE FILTERING		
COMPUTE BMD	Clos	se
PATHWAY ENRICHMENT		

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	Data								×
Phenotype File Browse pheno_ Upload complete Preview	list_2_e	Samı Varia	bles: 48 bles: 4						
Variable	Tupo	Class	6.0	mplo1	Sample2	Sample?	Sample4	SampleF	5.2
	fastar	Class	5d		Samplez	Samples	Sample4	Samples	5d
BARCODE [1]	factor	character	1D_0030	017698023	ID_003017698024	ID_003017698025	ID_003017699005	ID_003017699006	ID_003
DOSE [2]	vector	numeric	0		0	0	0	0	0
DOSE_LEVEL [3]	factor	character	Control	l	Control	Control	Control	Control	Contro
SACRIFICE_PERIOD [4]	vector	numeric	4		4	4	8	8	8
Double-clic Sample ID Variable	to cl	hange ty	pe I	Dose Variab	le		Time Point Variab	le	
Variable 1		•		Variable 2		•	Variable 4		•
Select the	correc	et variabl	le ►	Variable 1 Variable 2 Variable 3 Variable 4					nport Close

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.

BMDx: dose response a	analysis for expre	ession data 🛛 😑									
LOAD PHENOTYPE DATA	Phenotype Data	Gene Expression Matrix	Filtering	BMD	Enrichment						
LOAD EXPRESSION MATRIX	Show 10 \$ entr	ies						Searc	h:		
		BARCODE			DOSE	DOSE_LEVEL			SA	CRIFICE_P	ERIOD
Import Expression Matrix	12800	ID_003017698023			0	Control					4
	12801	ID_003017698024			0	Control					4
GENE FILTERING	12802	ID_003017698025			0	Control					4
COMPUTE BMD	12803	ID_003017699005			0	Control					8
PATHWAY	12804	ID_003017699006			0	Control					8
	12805	ID_003017699007			0	Control					8
MORE INFO	12806	ID_003017667006			0	Control					15
GitHub	12807	ID_003017667007			0	Control					15
Manual Sample pheno data	12808	ID_003017667008			0	Control					15
Sample expression data	12809	ID_003017667018			0	Control					29
	Showing 1 to 10 of 4	8 entries					Previous	1 2	3	4 5	Next

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).

	Α	В	С	D	E	F	G	н	1	J
1		ID_003017698023	ID_003017698024	ID_003017698025	ID_003017699005	ID_003017699006	ID_003017699007	ID_003017667006	ID_003017667007	ID_0030176670
2	Gad1	6.41439102304723	6.46532837902453	6.15713257780585	6.38079246591397	6.48285490580646	6.56574785793586	6.68232330197958	6.80677729775191	6.82444037703
3	Cbln1	6.49548074605588	6.24746145003168	6.27445075342446	6.41063178671929	6.40066644578383	6.49783392284641	6.41888260148178	6.52749758647252	6.42275109523
4	Steap1	6.08725181707611	5.72392577452614	6.22159431386313	6.16469548011423	5.84907206426937	6.39590582054641	5.83780943848238	6.43277708130612	6.40249519808
5	Hebp1	8.16244677988902	7.98135071582213	7.30078550598878	7.37664557145522	7.56717620245261	7.87981448716478	6.95798232367227	6.76357210565463	6.54584649977
6	Tmcc2	6.30191849868739	6.08084669634948	6.12173437902059	6.22659240420387	6.15916599240876	6.44325544609901	6.06295206830075	6.22312347414791	6.48708339812
7	Nuak2	6.8608188928228	6.70585968433679	6.84342702783659	6.7861884544676	6.92896858163684	6.84461448501822	6.52149337211356	6.29071007301364	6.35225186865
8	Klhdc8a	6.70153179562915	6.49409288378762	6.6551999897453	6.64152966932325	6.63796441320896	6.65921658975372	6.26284294953698	6.59582961239123	6.37327199400
9	RGD1304622	6.15895311078878	5.76185279246279	6.11118369359443	6.43082784238366	6.13054145327246	6.40971712170263	6.52607578979618	6.4080867252917	6.80305592427
10	Slc26a1	7.78552753621991	7.05396358247276	7.30007101657089	6.98963036419745	7.31341541974814	7.08178203218198	6.72938943982973	6.54395271550508	6.47843053806
11	Cd82	6.56495105605652	6.44335816340407	6.33658735068232	6.50415179328371	6.50158016568822	6.56574785793586	6.11668555050335	5.96920847996047	5.88014545652
12	Gak	6.67361640520244	6.42020620132178	6.30647778993558	5.85568282526885	6.27717407300601	6.39823165499899	5.83401904109282	5.65078620662586	6.10430267868
13	Crp	7.88078857737421	7.66580053158294	7.70000806901788	7.36908120777646	7.80118607311789	8.10446795117725	7.37923434832878	6.90886740631087	7.25375987096
14	Abhd8	6.59852776436937	6.26406219642696	5.94937772417718	6.49174877782938	6.21556731267876	6.26890206072431	5.99958289056345	6.2287378268976	6.19215018296
15	Fcrl6	6.34498541593716	6.57499470445596	6.35004675732525	6.43657614607422	6.25536054004365	6.70757134232425	6.50190075428589	6.60951214467473	6.79793522534
16	Cplx1	6.57155895561969	6.22648987854162	6.15541626596436	6.19961431977232	6.33638370282558	6.24614056498139	6.4956951653433	6.24573332646841	6.37357962944
	omeoprazole	WY-14643 +								

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.

BMDx: dose response	analysis for ex	pression data	=							
LOAD PHENOTYPE DATA	Filter G	enes by Anova					×			
LOAD EXPRESSION MATRIX	Sho All	nts 🗸	Pvalue:	Anova	PValue Th:	Number of cores:	•	S	earch:	
GENE FILTERING			 Nominal 					007	ID_003017667006	ID_00
	Ga						Run Anova	93586	6.68232330197958	6.80
Anova Filtering	Ct						Close	84641	6.41888260148178	6.52
• Trend Filtering	St							54641	5.83780943848238	6.43:
O Skin Siltoring	Hebp1	8.16244677988902	7.98135071582213	7.30078550598878	7.37664557145522	7.56717620245261	7.87981448	716478	6.95798232367227	6.76:
O skip Pittering	Tmcc2	6.30191849868739	6.08084669634948	6.12173437902059	6.22659240420387	6.15916599240876	6.44325544	609901	6.06295206830075	6.22:

BMDx: dose response a	analysis for exr	pression data	=							
LOAD PHENOTYPE	Filter Ge	nes by Trend Test					×			
LOAD EXPRESSION MATRIX	Time Point	ts •	Pvalue:	Tren	I PValue Th:	Number of cores:	-	S	earch:	
GENE FILTERING	Ga		 Nominal 			L Ru	in Trend Test	93586	ID_003017667006	ID_00 6.80
• Anova Filtering	Ct						Close	84641 54641	6.41888260148178 5.83780943848238	6.52
Trend Filtering Skip Filtering	Hebpl	8.16244677988902	7.98135071582213	7.30078550598878	7.37664557145522	7.56717620245261	7.879814487	16478	6.95798232367227	6.76:
C sup ritering	Tmcc2	6.30191849868739	6.08084669634948	6.12173437902059	6.22659240420387	6.15916599240876	6.443255446	509901	6.06295206830075	6.22:

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*.

BMDx: dose response	analysis for expression data	
LOAD PHENOTYPE DATA	Phenotype Data Gene Expression Matrix Filtering BMD Enrichment	
LOAD EXPRESSION MATRIX	Experiment Time Points	🛓 Download
GENE FILTERING	Show 10 ¢ entries Search:	
COMPUTE BMD	Gene 🔶 pvalue 🗄	Filtering Result
Compute BMD	All	Non Variable Genes
PATHWAY	1 Gad1 0.1272	
ENRICHMENT	2 Cbln1 0.0139	
MORE INFO	3 Steap1 0.0496	
	4 Hebp1 0.2219	75.8
GitHub Manual	5 Tmcc2 0.9532	
Sample pheno data Sample expression data	6 Nuak2 0.0076	
	7 Klhdc8a 0.1938	
	8 RGD1304622 0.016	
	9 Sic26a1 0.0017	
	10 Cd82 0.3862	
	Showing 1 to 10 of 5,299 entries	
	Previous 1 2 3 4 5 530 Next	

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.

Compute BMD Value

Response Level		Assumption of Constant Variance							
1.349									
Lack-of-fit PValue Th:		Lowest dose filter:		Highest dose filter:					
0.1	•	0	•	0	•				
Number of cores:									
3	•								
Select the BMD analysis se	etting								
Custom	•								
Models available	Power2	✓ Hill05	🗆 Hill4	✓ MM.2					
🗹 Linear	Power3	🗹 Hill1	🗆 Hill5	MM.3					
🗹 Quadratic	Power4	🗹 Hill2	AR.2						
Cubic	🗹 Exponential	🗹 Hill3	🗹 AR.3						
Click on t select and model	he box to deselect a								

Iodels Description					
Linear Model					
Polynomial Model (Quadratic/Cubic)					
Power Model					
Exponential Model					
Hill Model					
Asymptotic Regression					
Michaelis-Menten Model					

Close

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

Models Description
Linear Model
Polynomial Model (Quadratic/Cubic)
The formula for the polynomial model is
$f(dose) = \beta_0 + \beta_1 dose + \beta_2 dose^2 + \dots + \beta_n dose^n$
Here n is the degree of the polynomial. The user can choose between
n = 2, 3
Power Model
Exponential Model
Hill Model
Asymptotic Regression
Michaelis-Menten 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*.

BMDx: dose response	analysis for expression dat	ta ≡						
DAD PHENOTYPE	Phenotype Data Gene Expr	ession Matrix Filteri	ng BMD	Enrichment				
DAD EXPRESSION RIX	Gene Level Compare T	P Compare Experim	ients					
NE FILTERING	Experiment		1	lime Points		🛓 Down	beol	
OMPUTE BMD	omeoprazole	•		4	•			
	omeoprazole						Search:	
ICHMENT	Gene	¢ BMD ¢	BMDL 🔶	BMDU 🔶	IC50/EC50	Decreasing	♦ MOD_NAME ♦	LOFPVa
Enrichment	1 Cbln1	900.6764	689.5126	1000	734.617386503261	0	Power3	0.99
Emicimient	2 Steap1	927.7569	592.3297	1000	229.791284925078	0	Linear	0.94
DE INEO	3 Nuak2	751.2931	537.8953	1000	589.238417444391	1	Linear	0.47
	4 RGD1304622	651.633	432.2307	1000	216.079843073897	0	Linear	0.44
b al	5 Slc26a1	288.79	217.1647	672.038495790281	177.082164460094	1	Quadratic	0.43
e pheno data e expression data	7 Hltf	140.697	69.9328	1000	94.7411032439741	0	Hill05	0.18
·	8 Gpr89b	822.8233	527.11	1000	420.049441356453	0	Linear	0.94
	9 Fgf20	680.6704	428.3254	1000	550.840899134218	0	Linear	0.62
	10 Champ1	937.0488	741.6114	1000	516.516264538792	1	Power3	0.78
	11 Ly86	882.1817	638.085	1000	105.966695985808	1	Linear	0.6
	Showing 1 to 10 of 1,115 entri	es				Previous 1	2 3 4 5	112 Ne:

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.

perin	nent		Tin	ne Points		+ Downlo	ad	
meo	prazole	-	4	1	•	2 Downlo	au	
	10 1							
w	10 y entries						Search:	
	Gene	BMD ≑	BMDL ≑	BMDU ≑	IC50/EC50	Decreasing	♥ MOD_NAME	LOFPVal
	Cbln1	900.6764	689.5126	1000	734.617386503261	0	Power3	0.993
	Steap1	927.7569	592.3297	1000	229.791284925078	0	Linear	0.944
	Nuak2	751.2931	537.8953	1000	589.238417444391	1	Linear	0.479
	RGD1304622	651.633	432.2307	1000	216.079843073897	0	Linear	0.440
	Slc26a1	288.79	217.1647	672.038495790281	177.082164460094	1	Quadratic	0.439
	Hltf	140.697	69.9328	1000	94.7411032439741	0	Hill05	0.185
	Gpr89b	822.8233	527.11	1000	420.049441356453	0	Linear	0.941
	Fgf20	680.6704	428.3254	1000	550.840899134218	0	Linear	0.628
D	Champ1	937.0488	741.6114	1000	516.516264538792	1	Power3	0.789
1	Ly86	882.1817	638.085	1000	105.966695985808	1	Linear	0.62
owin: B — -	g 1 to 10 of 1,115 entrie	S				Previous	2 3 4 5	112 Nex
5	•	•				■ BMD	BMDL BMDU	IC50/EC

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	Phenotype Data Gene Expression Matrix Filtering BMD Enrichment						
LOAD EXPRESSION MATRIX	Gene Level Compare TP Compare Experiments						
GENE FILTERING	BMD Values						
COMPUTE BMD	Lack of fit Pvalues						
PATHWAY	BMD/BMDL						
	Fitted models						
Enrichment	Gene by Time Point						
	Venn diagram responsive genes						
MOREINPO							









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).

	rsis for expression data		
LOAD PHENOTYPE DATA PH	Enrichment	x	
LOAD EXPRESSION MATRIX	1. Input gene lists		
GENE FILTERING	1) Organisms human	2) GeneID symbols	hway Pathways Table
COMPUTE BMD	o mouse	ensemble entrez	
PATHWAY			
ENRICHMENT	2. Functional annotation parameters		
C Enrichment	Select Functional Annotation KEGG	Select GO BP	_
MORE INFO	GO GO	○ CC ○ MF	
GitHub Manual Samela abasa data	Correction Method	P-value threshold:	Metabolism
Sample expression data	 tdr bonferroni Nominal 		
	Annotated genes only	Minumum number of genes in the intersection:	enetic Information Processing
			onmental Information Processing
	3. Display parameters		Cellular Processes value
	Aggregation Function min max	Choose Values Type O Pvalue GenesModifications	
	 meán median Plot modification value 	GenesModifications_Pvalue	Organismat Systems
	⊖ sign		
	4. Filter BMD Values		Human Diseases
	5. Download gene lists as excel file		
	L Download		
		Close	

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*).

1. Input gene lists

1) Organisms	2) GenelD
human	symbols
o mouse	ensemble
In at a start of the start o	entrez

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). ^{2. Functional annotation parameters}

Select Functional Annotation	Select GO
• KEGG	O BP
○ REACTOME	\bigcirc cc
⊖ GO	○ MF
Correction Method	P-value threshold:
⊖ gSCS	0.01
⊙ fdr	
🔿 bonferroni	
🔿 Nominal	
	Minumum number of genes in the intersection:
Annotated genes only	0
	O <u></u>

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. 4. Filter BMD Values

C Run Enrichment

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.

5. Download gene lists as excel file					
🛓 Download					

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.

enotype Data	Gene Expression	Matrix Filtering	BMD Enrichr	nent			
			Use scrollbars	to navigate and see the w	hole map		
1. Data Selection	1		Heatmap	Cluster Bubble Plot	Mean BMD for Time Point	Gene BMD in pathway	Pathways Table
Browse hierarchy:	choose a level		Heatmap G	enes			
1		-	No data to plo	t			
Level 1	Level 2	Level 3					
All	All	All					
Select samples							
All							
2. Plot section							
Show	🗹 Keep	Plot Map					
categories	aspect ratio						
3. Download Sele	ection						
Width	Height	🕹 Download					
15	30						

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*.

Phenotype Data Gene Expression Matrix Filtering BMD	ichment	
1. Data Selection	Use scrollbars to navigate and see the whole map Heatmap Cluster Bubble Plot Mean BMD for Time Point Gene BMD in pathway Pathways Table Heatmap Genes	
Browse hierarchy: choose a level	Time Points	
2		
	oneoprazore_4	
Level 1 Level 2 Level 3	Lownload	
All All	Show 10 \$ entries Search	:
Select samples	Description 🗍 annID 🝦 gID	
All	All	
	1 KEGG 00000 STEAP1,FGF20,CD01,ALAS2,GATM,PLPP2,CCL7,PRODH1,NOTCH4 PSMB9,CDKN1A,PRF1,MARCKS,AMD N3,MRPS18B,GJA1,FABP7,RBMX,SLC44A4,RAN,MCOLN1,NCOR2,EIF2B1,EIF2AK1,VPS37B,KL,PRKAB1,G	1,HDAC2,IPMK,JMJD1C,PTP LP1R,MSI1,ABCG1,COQ5,TRI
2. Plot section	2 Pertussis 05133 ITGB2,C4BPA,C4BPB,SERPING1,CASP3,C1S,C1QB,C1QA,IRF8	
Show Keep aspect Plot Map categories	Insulin 3 signaling 04910 ACACB,PRKAB1,PPP1CC,SOCS3,SOS2,PRKACA,PYGL,PRKCI,FLOT2,TSC2,TSC1,EIF4EBP1,FBP2 pathway	
	4 RNA transport 03013 RPP21,RAN,EIF2B1,NUP54,GEMIN5,ELAC2,PNN,EIF2B4,NUP88,EIF2B2,NUP107,STRAP,CASC3,NUP205,	PRMT5,EIF4EBP1,EIF3F,SUN
3. Download Selection Width Height 🛓 Download	Ascorbate and 5 aldarate 00053 ALDH2,UGT2A1,UGT2B1,UGDH,RGN,ALDH1B1 metabolism	
15 30	6 Focal 04510 COL6A2,PPP1CC,PDGFRA,ITGA3,IGF1,PAK3,SOS2,BIRC3,HGF,FLNC,RAC2,VTN,BIRC2,COL9A2,FN1,PDGF	RB
	7 Amphetamine 05031 PPP1CC,ATF2,MA0A,GRIN2C,DDC,PRKACA,SLC18A2,CREB3L2,TH	
	8 Prion diseases 05020 PRKACA,C8B,C8A,CCL5,C1QB,C1QA	
	Cysteine and 9 methionine 00270 CD01,AMD1,SDS,GCLC,APIP,MAT1A,GOT2 metabolism	
	Synaptic 04721 NAPA,ATP6V1A,CACNA1B,SYT1,ATP6V1G1,SLC18A2,ATP6V1D,SLC17A6,TCIRG1,AP2A2 vesicle cycle	
	Showing 1 to 10 of 53 entries Previous 1 2 3	4 5 6 Next

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:

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

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