Users' Guide
MORE

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1 Introduction

One of the most common questions to be addressed when performing a multi-omics experiment is how the levels of given biological entities are being regulated by other biological entities under certain conditions. An example of this type of study would be understanding the regulatory mechanisms behind the changes in gene expression.

Potential regulators of a given gene such as miRNAs, transcription factors (TF), methylation sites, etc., can be either retrieved or predicted from public databases or obtained by a combination of experimental and computational procedures. However, a methodology for selecting the specific regulators of a particular biological system studied under certain experimental conditions is required. This is the goal of the MORE (Multi-Omics REgulation) method: modeling gene expression as a function of experimental variables, such as diseases or treatments, and the potential regulators of a given gene. The idea is to obtain more specific candidate regulators for the biological system under study by applying regression models, specifically generalized linear models (GLM), or by applying Partial Least Squares (PLS). MORE facilitates the application of GLM or PLS to multi-omic data and although it was originally conceived to study gene expression regulation, its usage can be extended to protein or metabolite levels, for instance.

MORE requires several data inputs: gene expression data, regulators' omic data, experimental design, and potential associations between genes and regulators. With this input data, MORE generates the initial model equation, which is different for each gene because each one of them has different potential regulators. MORE admits numerical omic data (continuous or discrete) or binary data.

It is strongly recommended to fit MORE models only to genes that present significant changes in any of the experimental conditions studied, that is, to differentially expressed genes (DEGs). DEGs can be selected with the standard procedures depending on the experimental design, but DEGs selection is not included in the MORE algorithm and must be done by the user.

This idea can be extended to potential regulators since regulators that do not change across conditions are not good candidates to regulate gene expression. Removing non-DE regulators will also help to reduce the number of predictors in the model since an excess of them would prevent the estimation of regression coefficients. Even so, MORE has several functionalities to filter regulators with missing values or low variation, highly correlated regulators, and perform variable selection.

MORE package also includes a function to retrieve the significant regulations and the magnitude of the regulatory effect under each experimental condition considered and an additional function to graphically investigate the relationship between genes and regulators.

2 Getting started

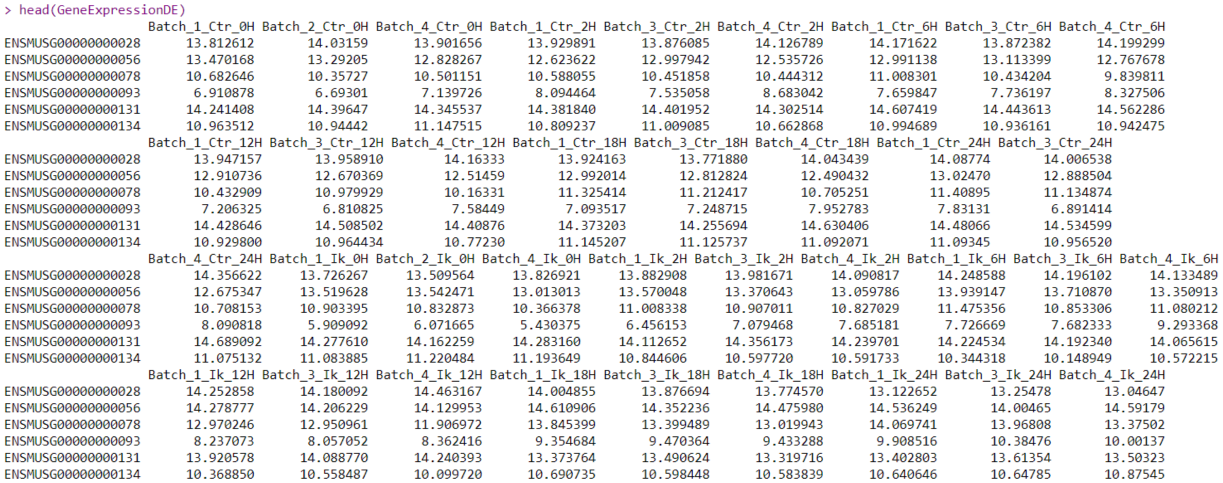
The MORE method is available as an R package from https://github.com/ConesaLab/MORE.git. As for other packages in GitHub, it can be installed from R with the following instructions:

```
> install.packages("devtools")
> devtools::install_github("ConesaLab/MORE")
```

3 Input data

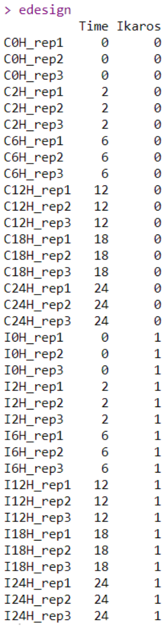
This section describes the main data files required by MORE to generate the regression models.

Gene expression data Expression values for each gene, in rows, under each experimental condition or replicate, in columns. MORE accepts either a **matrix** or a **data frame**. See an example below:



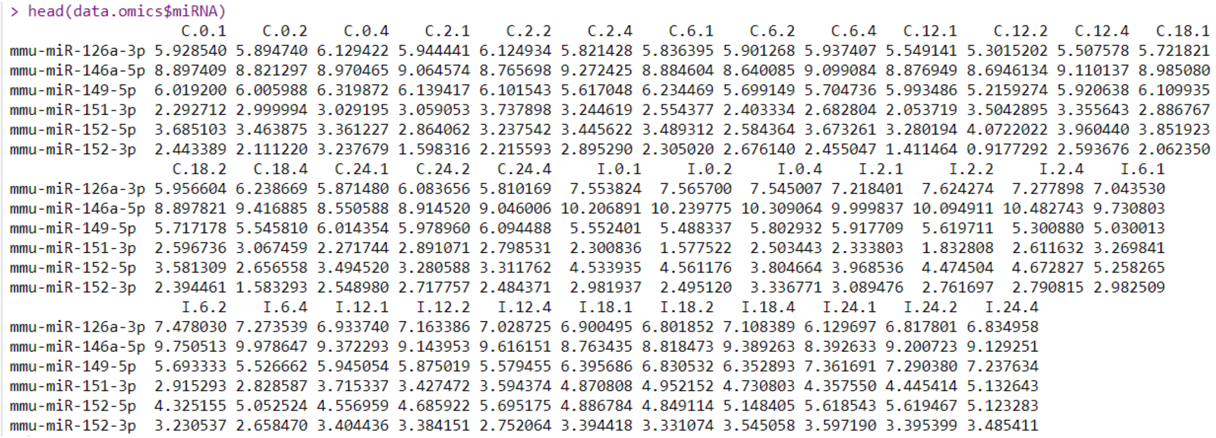
```
> head(GeneExpressionDE)
                   Batch_1_Ctr_0H Batch_2_Ctr_0H Batch_4_Ctr_0H Batch_1_Ctr_2H Batch_3_Ctr_2H Batch_4_Ctr_2H Batch_1_Ctr_6H Batch_3_Ctr_6H Batch_4_Ctr_6H
ENSMUSG00000000028      13.812612       14.03159      13.901656      13.929891      13.876085      14.126789      14.171622      13.872382      14.199299
ENSMUSG00000000056      13.470168       13.29205      12.828267      12.623622      12.997942      12.535726      12.991138      13.113399      12.767678
ENSMUSG00000000078      10.682646       10.35727      10.501151      10.588055      10.451858      10.444312      11.008301      10.434204       9.839811
ENSMUSG00000000093       6.910878        6.69301       7.139726       8.094464       7.535058       8.683042       7.659847       7.736197       8.327506
ENSMUSG00000000131      14.241408       14.39647      14.345537      14.381840      14.401952      14.302514      14.607419      14.443613      14.562286
ENSMUSG00000000134      10.963512       10.94442      11.147515      10.809237      11.009085      10.662868      10.994689      10.936161      10.942475
                   Batch_1_Ctr_12H Batch_3_Ctr_12H Batch_4_Ctr_12H Batch_1_Ctr_18H Batch_3_Ctr_18H Batch_4_Ctr_18H Batch_1_Ctr_24H Batch_3_Ctr_24H
ENSMUSG00000000028       13.947157       13.958910        14.16333       13.924163       13.771880       14.043439        14.08774       14.006538
ENSMUSG00000000056       12.910736       12.670369        12.51459       12.992014       12.812824       12.490432        13.02470       12.888504
ENSMUSG00000000078       10.432909       10.979929        10.16331       11.325414       11.212417       10.705251        11.40895       11.134874
ENSMUSG00000000093        7.206325        6.810825         7.58449        7.093517        7.248715        7.952783         7.83131        6.891414
ENSMUSG00000000131       14.428646       14.508502        14.40876       14.373203       14.255694       14.630406        14.48066       14.534599
ENSMUSG00000000134       10.929800       10.964434        10.77230       11.145207       11.125737       11.092071        11.09345       10.956520
                   Batch_4_Ctr_24H Batch_1_Ik_0H Batch_2_Ik_0H Batch_4_Ik_0H Batch_1_Ik_2H Batch_3_Ik_2H Batch_4_Ik_2H Batch_1_Ik_6H Batch_3_Ik_6H Batch_4_Ik_6H
ENSMUSG00000000028       14.356622     13.726267     13.509564     13.826921     13.882908     13.981671     14.090817     14.248588     14.196102     14.133489
ENSMUSG00000000056       12.675347     13.519628     13.542471     13.013013     13.570048     13.370643     13.059786     13.939147     13.710870     13.350913
ENSMUSG00000000078       10.708153     10.903395     10.832873     10.366378     11.008338     10.907011     10.827029     11.475356     10.853306     11.080212
ENSMUSG00000000093        8.090818      5.909092      6.071665      5.430375      6.456153      7.079468      7.685181      7.726669      7.682333      9.293368
ENSMUSG00000000131       14.689092     14.277610     14.162259     14.283160     14.112652     14.356173     14.239701     14.224534     14.192340     14.065615
ENSMUSG00000000134       11.075132     11.083885     11.220484     11.193649     10.844606     10.597720     10.591733     10.344318     10.148949     10.572215
                   Batch_1_Ik_12H Batch_3_Ik_12H Batch_4_Ik_12H Batch_1_Ik_18H Batch_3_Ik_18H Batch_4_Ik_18H Batch_1_Ik_24H Batch_3_Ik_24H Batch_4_Ik_24H
ENSMUSG00000000028      14.252858      14.180092      14.463167      14.004855      13.876694      13.774570      13.122652       13.25478       13.04647
ENSMUSG00000000056      14.278777      14.206229      14.129953      14.610906      14.352236      14.475980      14.536249       14.00465       14.59179
ENSMUSG00000000078      12.970246      12.950961      11.906972      13.845399      13.399489      13.019943      14.069741       13.96808       13.37502
ENSMUSG00000000093       8.237073       8.057052       8.362416       9.354684       9.470364       9.433288       9.908516       10.38476       10.00137
ENSMUSG00000000131      13.920578      14.088770      14.240393      13.373764      13.490624      13.319716      13.402803       13.61354       13.50323
ENSMUSG00000000134      10.368850      10.558487      10.099720      10.690735      10.598448      10.583839      10.640646       10.64785       10.87545
```

Experimental design Matrix or data frame containing the experimental covariates, such as treatments, diseases, strains, dose of a drug, etc. The rows of the object must be the same as the columns in **Gene expression data** and in the same order, as shown below. There is no restriction for the number of columns, but it must be taken into account that MORE will combine all the experimental covariates into a single variable. For instance, in the example below, the new single covariate would combine Time and Group2 to obtain the values: 1_0, 2_0, ...,7_1, 8_1. Therefore, in this case, it makes more sense to exclude Time from the experimental design and just include the covariate Group2.



```
> edesign
          Time Ikaros
C0H_rep1     0      0
C0H_rep2     0      0
C0H_rep3     0      0
C2H_rep1     2      0
C2H_rep2     2      0
C2H_rep3     2      0
C6H_rep1     6      0
C6H_rep2     6      0
C6H_rep3     6      0
C12H_rep1   12      0
C12H_rep2   12      0
C12H_rep3   12      0
C18H_rep1   18      0
C18H_rep2   18      0
C18H_rep3   18      0
C24H_rep1   24      0
C24H_rep2   24      0
C24H_rep3   24      0
I0H_rep1     0      1
I0H_rep2     0      1
I0H_rep3     0      1
I2H_rep1     2      1
I2H_rep2     2      1
I2H_rep3     2      1
I6H_rep1     6      1
I6H_rep2     6      1
I6H_rep3     6      1
I12H_rep1   12      1
I12H_rep2   12      1
I12H_rep3   12      1
I18H_rep1   18      1
I18H_rep2   18      1
I18H_rep3   18      1
I24H_rep1   24      1
I24H_rep2   24      1
I24H_rep3   24      1
```

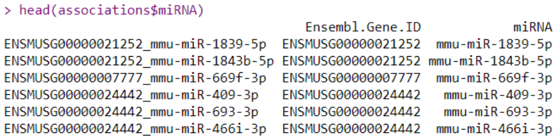
Regulatory omic data This object must be a list where each element is a matrix or data frame containing the data for each "regulatory" omic (miRNA expression, transcription factor expression, etc.), with a structure similar to gene expression data: regulators in rows and experimental conditions in columns (the columns must be the same as in gene expression and in the same order). See the example below (TestData$data.omics$'miRNA-seq').



```
> head(data.omics$miRNA)
                   C.0.1    C.0.2    C.0.4    C.2.1    C.2.2    C.2.4    C.6.1    C.6.2    C.6.4   C.12.1    C.12.2   C.12.4   C.18.1
mmu-miR-126a-3p 5.928540 5.894740 6.129422 5.944441 6.124934 5.821428 5.836395 5.901268 5.937407 5.549141 5.3015202 5.507578 5.721821
mmu-miR-146a-5p 8.897409 8.821297 8.970465 9.064574 8.765698 9.272425 8.884604 8.640085 9.099084 8.876949 8.6946134 9.110137 8.985080
mmu-miR-149-5p  6.019200 6.005988 6.319872 6.139417 6.101543 5.617048 6.234469 5.699149 5.704736 5.993486 5.2159274 5.920638 6.109935
mmu-miR-151-3p  2.292712 2.999994 3.029195 3.059053 3.737898 3.244619 2.554377 2.403334 2.682804 2.053719 3.5042895 3.355643 2.886767
mmu-miR-152-5p  3.685103 3.463875 3.361227 2.864062 3.237542 3.445622 3.489312 2.584364 3.673261 3.280194 4.0722022 3.960440 3.851923
mmu-miR-152-3p  2.443389 2.111220 3.237679 1.598316 2.215593 2.895290 2.305020 2.676140 2.455047 1.411464 0.9177292 2.593676 2.062350
                  C.18.2   C.18.4   C.24.1   C.24.2   C.24.4     I.0.1     I.0.2     I.0.4    I.2.1     I.2.2     I.2.4    I.6.1
mmu-miR-126a-3p 5.956604 6.238669 5.871480 6.083656 5.810169  7.553824  7.565700  7.545007 7.218401  7.624274  7.277898 7.043530
mmu-miR-146a-5p 8.897821 9.416885 8.550588 8.914520 9.046006 10.206891 10.239775 10.309064 9.999837 10.094911 10.482743 9.730803
mmu-miR-149-5p  5.717178 5.545810 6.014354 5.978960 6.094488  5.552401  5.488337  5.802932 5.917709  5.619711  5.300880 5.030013
mmu-miR-151-3p  2.596736 3.067459 2.271744 2.891071 2.798531  2.300836  1.577522  2.503443 2.333803  1.832808  2.611632 3.269841
mmu-miR-152-5p  3.581309 2.656558 3.494520 3.280588 3.311762  4.533935  4.561176  3.804664 3.968536  4.474504  4.672827 5.258265
mmu-miR-152-3p  2.394461 1.583293 2.548980 2.717757 2.484371  2.981937  2.495120  3.336771 3.089476  2.761697  2.790815 2.982509
                   I.6.2    I.6.4   I.12.1   I.12.2   I.12.4   I.18.1   I.18.2   I.18.4   I.24.1   I.24.2   I.24.4
mmu-miR-126a-3p 7.478030 7.273539 6.933740 7.163386 7.028725 6.900495 6.801852 7.108389 6.129697 6.817801 6.834958
mmu-miR-146a-5p 9.750513 9.978647 9.372293 9.143953 9.616151 8.763435 8.818473 9.389263 8.392633 9.200723 9.129251
mmu-miR-149-5p  5.693333 5.526662 5.945054 5.875019 5.579455 6.395686 6.830532 6.352893 7.361691 7.290380 7.237634
mmu-miR-151-3p  2.915293 2.828587 3.715337 3.427472 3.594374 4.870808 4.952152 4.730803 4.357550 4.445414 5.132643
mmu-miR-152-5p  4.325155 5.052524 4.556959 4.685922 5.695175 4.886784 4.849114 5.148405 5.618543 5.619467 5.123283
mmu-miR-152-3p  3.230537 2.658470 3.404436 3.384151 2.752064 3.394418 3.331074 3.545058 3.597190 3.395399 3.485411
```

Associations For each regulatory omic, associations between regulators and genes which indicate which are the potential regulators of each gene that will be consequently incorporated into the initial equation of the regression model. The association objects must be data frames and stored in a single **list** (attached below the example of miRNA-seq, TestData$associations$'miRNA-seq'). The names of the elements of this list must be the names of the list collecting regulatory omic data and must be in the same order.

If the user wants to consider all regulators of an omic as potential regulators they must set to NULL the object of this omic in the **associations** list. Moreover, if the user does not provide the list of **associations**, all regulators of all omics in **data.omics** will be considered potential regulators for all genes. However, this option is very time-consuming. By default, NULL.



```
> head(associations$miRNA)
                                       Ensembl.Gene.ID            miRNA
ENSMUSG00000021252_mmu-miR-1839-5p  ENSMUSG00000021252  mmu-miR-1839-5p
ENSMUSG00000021252_mmu-miR-1843b-5p ENSMUSG00000021252 mmu-miR-1843b-5p
ENSMUSG00000007777_mmu-miR-669f-3p  ENSMUSG00000007777  mmu-miR-669f-3p
ENSMUSG00000024442_mmu-miR-409-3p   ENSMUSG00000024442   mmu-miR-409-3p
ENSMUSG00000024442_mmu-miR-693-3p   ENSMUSG00000024442   mmu-miR-693-3p
ENSMUSG00000024442_mmu-miR-466i-3p  ENSMUSG00000024442  mmu-miR-466i-3p
```

4 Generating the regression models with MORE

The **more** function in MORE adjusts a generalized linear model (GLM) with elastic net regularization regression method for each gene (protein, metabolite, etc.) in the *GeneExpresion* object to determine which regulators and experimental covariates have a significant effect on the response variable (gene expression, protein levels, etc.) if the selected method by the user is 'glm'. If the selected method is 'pls', it adjusts a partial least squares (PLS) model instead of the GLM. These are the arguments the function accepts, described in detail in Section 4.1.

```
more(GeneExpression, data.omics, associations, omic.type = NULL,
     edesign = NULL, clinic = NULL, clinic.type = NULL,
     center = TRUE, scale = TRUE, epsilon = 0.00001, alfa = 0.05,
     family = gaussian(), elasticnet = NULL, interactions.reg = TRUE,
     min.variation = 0,  min.obs = 10, col.filter = 'cor',
     correlation = 0.7, scaletype = 'auto', p.method = 'jack',
     vip = 0.8, method  ='glm')
```

4.1 Arguments for more() function

GeneExpression Matrix or data frame containing gene expression data with genes in rows and experimental samples in columns. The row names must be the gene IDs.

data.omics List where each element corresponds to a different omic data type (miRNAs, transcription factors, methylation, etc.). The names of this list will be the omics, and each element of the list is a matrix or data frame with omic regulators in rows and samples in columns.

associations List where each element corresponds to a different omic data type (miRNAs, transcription factors, methylation, etc.). The names of the elements of the list will be the omics (in the same order as in **data.omics**). Each element is a data frame with two columns (optionally three) describing the potential interactions between genes and regulators for that omic. The first column must contain the regulators, the second the gene IDs, and an additional column can be added to describe the type of interaction (for example, in methylation data, if a CpG site is located in the promoter region of the gene, in the first exon, etc.). Optionally, the user can set the **associations** data frame of an omic equal to NULL if they want to consider all the regulators of that omic as potential regulators for all the genes. They can even set **associations** to NULL if they want to consider all regulators of all omics in **data.omics** as potential regulators to all genes. Even if it can be done, we do not recommend the user to do it as it can be very time-consuming.

omic.type Vector with as many elements as the number of omics, indicating whether the omic values are numeric (0) or binary (1). When NULL is indicated, **MORE** will estimate which type of omics are provided and display them on the screen. If a single value is provided, the type for all the omics is set to that value. By default, NULL. If the estimated type of omics are incorrect, the user must halt the process and manually specify the omic.type.

edesign Data frame or matrix describing the experimental design. Rows must be the samples, that is, the columns in the **GeneExpression**, and columns must be the experimental covariates to be included in the model, such as disease, treatment, etc.

clinic Data frame or matrix containing clinical variables values where rows must represent samples and columns variables.

clinic.type Vector with as many elements as the number of clinical variables, indicating whether the variables values are numeric (0) or categorical/binary (1). When NULL is indicated, **MORE** will estimate which type of variables are provided and display them on the screen. If a single value is provided, the type for all the variables is set to that value. By default, NULL. If the estimated type of variables are incorrect, the user must halt the process and manually specify the clinic.type.

center If TRUE (default), the omic data are centered.

scale If TRUE (default), the omic data are scaled.

epsilon A threshold for the positive convergence tolerance in the GLM model. By default, 0.00001.

alfa Significance level. By default, 0.05.

family Error distribution and link function to be used in the GLM model (see `glm` for more information). By default, `gaussian()`.

elasticnet ElasticNet mixing parameter (α). By default, NULL. These are the values that can be passed to this argument:

NULL α parameter will be automatically optimized for *cvup*, which is the mean cross-validated error plus the estimate of the standard error. For computational efficiency, it will only be tested with values ranging from 0 to 1 in increments of 0.1.

Value between 0 and 1 ElasticNet is applied with this α being the combination between ridge and lasso penalization.

Vector of α's ElasticNet will be applied for each of the α values provided in the vector, and the one that optimizes the *cvup* will be selected.

Value 0 The ridge penalty.

Value 1 The lasso penalty.

We make clear that the shrinkage parameter (λ) will in all cases be optimized by cross-validation.

interactions.reg If TRUE (default), **MORE** allows for interactions between each regulator and the experimental covariate.

min.variation Vector with as many elements as the number of omics (names of this vector will be the omics), indicating the minimum change in the standard deviation that a regulator must show across conditions in order not to be considered as having low variation and be removed from the regression models, for numerical regulators. Or the minimum change in the proportion a regulator must show across conditions for binary regulators. When a single value is given, the minimum change will be considered the same for all omics. The user has the option to set this value to NA if they do not want to provide a value but are sure that they want to filter more than constant regulators across conditions. In this case, the value will be calculated as the 10% of the maximum observed variability across conditions for continuous regulators and as the 10% of the maximum observed proportion difference across conditions for binary regulators. Additionally, the user can combine both functionalities; indeed, the user has the option to provide a vector containing the minimum change in the standard deviation for some omics and NA for those omics for which they do not want to provide a value. By default, its value is 0. If the user has been very restrictive an error message will be provided.

min.obs Minimum number of observations a gene must have to compute the GLM model. By default, 10.

col.filter Type of filtering to be applied when adjusting a GLM model. This filter looks for highly correlated groups of regulators and considers the selected filter and the considered correlation threshold to select a representative. It can be 'cor' or 'pcor' if the partial correlation wants to be considered.

correlation Correlation threshold (in absolute value) to decide which regulators are correlated, in which case, a representative of the group of correlated regulators is chosen to enter the model. By default, 0.7.

scaletype Type of scaling to be applied when adjusting a model if scaling is requested. It can be: 'auto', 'pareto', or 'block'. The first applies the autoscaling method; so that scales each variable independently. The second applies the Pareto scaling to the omics. The third applies the block scaling considering as block each of the omics in **data.omics** and the interactions of experimental design variables with them if they were.

p.method This parameter is only required when adjusting a PLS model and is used for computing the p-values of the variables within the model. There are two available options: 'jack,' which refers to the Jack-Knife resampling method, and 'perm,' which corresponds to the response variable permutation method for obtaining the distribution of the coefficients and compute then their associated p-value.

method This parameter indicates whether a GLM model will be applied, 'glm', or if a PLS model will be applied instead. In this case, the user can ask for a PLS1 model using 'pls1' in which for each of the genes in **GeneExpression** a different PLS model will be applied or for a PLS2 model using 'pls2' in which a single PLS model will be computed which creates a single model for all genes. The user must be aware that in the 'pls2' model, the **association** list will not be considered and must be set to NULL.

4.2 more output

The object returned by the **more** function varies depending on the selected method.

4.2.1 more output for GLM

The object returned by the **more** when fitting a GLM model is a list that contains the following elements:

ResultsPerGene is a list with as many elements as genes in the GeneExpression object. For each gene, there is a list containing the following information:

Y Data frame with the response variable values for that gene (y), the values fitted by the model (fitted.y), and the residuals of the model (residuals).

X Data frame with all the predictors included in the final model.

coefficients Matrix with the estimated coefficients for the regulators selected as relevant by the elastic net regularization method.

allRegulators Data frame with all the initial potential regulators in rows and the following information in columns: gene, regulator, omic, area (the third optional column in associations), filter (if the regulator has been filtered out of the model, this column indicates the reason), and Rel (1 if the regulator is considered relevant and 0 if not). Regarding the filter column, several values are possible:

- *MissingValue*: If the regulator has been filtered out of the study because it has missing values.
- *LowVariation*: If the regulator has been filtered out of the study because it has lower variability than the threshold set by the user in min.variation parameter.
- *Model*: When the regulator is included in the initial equation model.
- *omic_mcX_X_X*: For example, *TF_mc1_1_R*. This notation is related to highly correlated regulators and how they are treated to avoid the multicollinearity problem. Following the *TF_mc1_1_R* example, two or more regulators, which potentially regulate the gene, are highly correlated (in absolute value). In such cases, one is chosen as the representative and indicated with _R. The rest of the regulators considered if they are directly highly correlated to it are labeled with _P, which means that they are positively correlated with the representative and with _N if negatively correlated. Once a representative is taken for them, if there are still highly correlated regulators, the process is repeated and indicated in the _1 of the example. An additional row is then added to this table, with the regulator *TF_mc1_1_R* and the filter label being *Model*, since only this representative is considered in the model. When there are several groups of correlated regulators for the same omic, it is indicated with _mc1_, _mc2_, etc.

relevantRegulators A character vector containing the relevant regulators.

GlobalSummary List that contains the following elements:

GoodnessOfFit Matrix that collects the R-squared value (which for GLMs is defined as the percentage of deviance explained by the model), the Root Mean Square Error (RMSE), the Coefficient of Variation of the Root Mean Square Error (CV(RMSE)) and the number of relevant regulators for all the genes that had at least a relevant regulator.

ReguPerGene Matrix containing, for each omic and gene, the number of initial regulators, the number of regulators included in the initial model, and the number of relevant regulators.

GenesNOmodel List of genes for which the final GLM model with the elastic net regularization could not be obtained. There are three possible reasons for that, and they are indicated: “Too many missing values”, “-Inf/Inf values”, and ”No regulators left after NA/LowVar filtering".

GenesNoregulators List of genes for which there were no initial regulators, only generated in case any gene was under this condition.

MasterRegulators Vector of the top 10 regulators that relevantly regulate more genes.

HubGenes Vector of the top 10 genes that are relevantly regulated by more regulators.

Arguments List with the arguments used to generate the model: experimental design matrix, minimum degrees of freedom in the residuals, significance level, family distribution, etc.

4.2.2 more output for PLS

The object returned by the **more** when fitting a PLS (PLS1 or PLS2 indifferently) model is a list that contains the following elements:

ResultsPerGene is a list with as many elements as genes in the GeneExpression object. For each gene, there is a list containing the following information:

Y Data frame with the response variable values for that gene (y), the values fitted by the model (fitted.y), and the residuals of the model (residuals).

X Data frame with all the predictors included in the final model.

coefficients Matrix with the estimated coefficients for the regulators selected as significant by the selected p-value computation method (p.method) and whose Variable Importance in Projection (VIP) has been higher than 0.8.

allRegulators Data frame with all the initial potential regulators in rows and the following information in columns: gene, regulator, omic, area (the third optional column in associations), filter (if the regulator has been filtered out of the model, this column indicates the reason), and Sig (1 if the regulator is considered significant and 0 if not). Regarding the filter column, several values are possible:

- *MissingValue*: If the regulator has been filtered out of the study because it has missing values.
- *LowVariation*: If the regulator has been filtered out of the study because it has lower variability than the threshold set by the user in min.variation parameter.
- *Model*: When the regulator is included in the initial equation model.

significantRegulators A character vector containing the significant regulators.

GlobalSummary List that contains the following elements:

GoodnessOfFit Matrix that collects the R-squaredY value (the R squared of the response variable), the Q-squared (the goodness of prediction), the square root of the mean error between the actual and the predicted responses (RMSEE), and the number of significant regulators for all the genes that had at least a significant regulator.

ReguPerGene Matrix containing, for each omic and gene, the number of initial regulators, the number of regulators included in the initial model, and the number of significant regulators.

GenesNOmodel List of genes for which the final GLM model with the elastic net regularization could not be obtained. There are three possible reasons for that, and they are indicated: “Too many missing values”, “-Inf/Inf values”, and ”No regulators left after NA/LowVar filtering”.

GenesNoregulators List of genes for which there were no initial regulators, only generated in case any gene was under this condition.

MasterRegulators Vector of the top 10 regulators that significantly regulate more genes.

HubGenes Vector of the top 10 genes that are significantly regulated by more regulators.

Arguments List with the arguments used to generate the model: experimental design matrix, minimum degrees of freedom in the residuals, significance level, family distribution, etc.

4.2.3 more summary

Making use of the output object returned by **more**, in both cases in GLM and PLS models, the user can ask for a summary of the results obtained by:

```
summary(object, plot.more=FALSE)
```

This summary takes two arguments as input:

object MORE object obtained from applying **more** function, indifferent to the method that has been used (’glm’, ’pls1’ or ’pls2’).

plot.more If TRUE, the top 10 master regulators will be plotted against the genes they regulate. By default, FALSE. It could be very time-consuming if the master regulators regulate a huge number of genes, so it is not recommended unless the user knows that there are only a few of them. Instead, it is recommended to use the **plotmore** function to plot the specific regulations.

Once the function is used the following information will be printed on the screen:

1. Number of genes for which a model was computed.
2. Number of genes that did not have initial regulators.
3. Number of genes for which the final model could not be obtained.
4. The mean of relevant/significant regulators of the genes.
5. Top 10 hub genes and the number of relevant regulators for each.
6. Top 10 hub genes per omic and the number of relevant regulators for each.
7. Top 10 master regulators and the number of genes they regulate.
8. If required with plot.more = TRUE the plots of the master regulators against the genes they regulate.

4.3 Running an example

An example of the execution of **more** function for the 'glm' option is shown next by using simulated data. Even if the data file `TestData.RData` is available in the package; the results shown below are related to the STATegra database available here.

In this file, the gene expression matrix corresponds to the omic RNA-seq (**GeneExpressionDE**), and there is a list with four matrices of regulators in the **data.omics** object:

miRNA-seq miRNA expression data.

DNase-seq measures the chromatin accessibility expression.

Methyl-seq Methylation per CpG site (M values).

TF TF expression data.

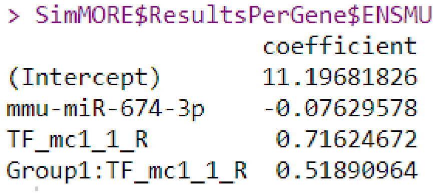
All values are normalized

The experimental design matrix (**edesign**) consists of 6 time points in two conditions, which results in a total of 12 experimental samples, but time is not to be considered as an experimental covariate since we are interested in comparing temporal profiles for the two experimental groups.

We can run the following **more** code to obtain the regression models for our genes:

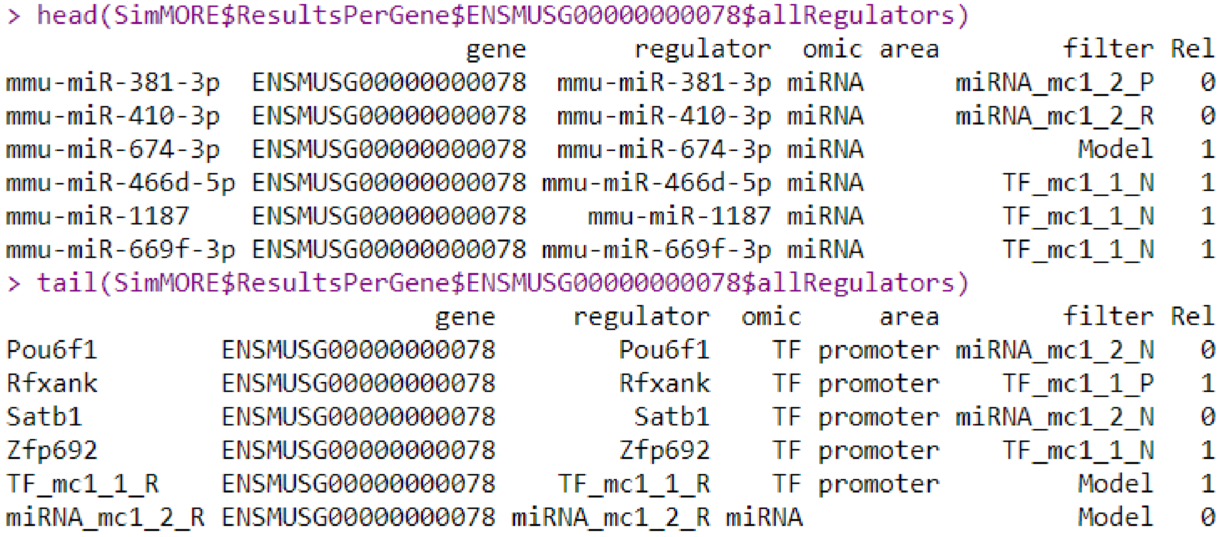
```
> set.seed(123)
> SimGLM = more(GeneExpression = GeneExpressionDE,
associations = associations, data.omics = data.omics,
edesign = edesign[,-1, drop = FALSE], center = TRUE, scale = TRUE,
family = gaussian(), elasticnet = 0.5, interactions.reg = TRUE,
min.variation = NULL, min.obs = 10, omic.type = 0,
col.filter = 'cor', correlation = 0.7,  method ='glm')
```

The estimated coefficients of the relevant regulators in the final GLM model computed by **more** for the gene ENSMUSG00000000078 are



```
> SimMORE$ResultsPerGene$ENSMUSG00000000078$coefficients
                   coefficient
(Intercept)        11.19681826
mmu-miR-674-3p     -0.07629578
TF_mc1_1_R          0.71624672
Group1:TF_mc1_1_R   0.51890964
```

The **allRegulators** table shows, for each gene, their regulators, omic, area, the kind of filter applied, and if the regulator is considered relevant or not. In this case (see **filter** column), in miRNA-seq, the regulators mmu-miR-381-3p and mmu-miR-410-3p are correlated, and the last has been chosen as representative regulator (R). In addition, it is indicated that the correlation with the representative is positive (P). In the same column, `Model` means that the regulator was included in the model by itself. On the other hand, the **Rel** column returns 1 if the regulator was considered relevant in the final model and 0 if not.



```
> head(SimMORE$ResultsPerGene$ENSMUSG00000000078$allRegulators)
                             gene       regulator  omic area        filter Rel
mmu-miR-381-3p  ENSMUSG00000000078  mmu-miR-381-3p miRNA      miRNA_mc1_2_P   0
mmu-miR-410-3p  ENSMUSG00000000078  mmu-miR-410-3p miRNA      miRNA_mc1_2_R   0
mmu-miR-674-3p  ENSMUSG00000000078  mmu-miR-674-3p miRNA              Model   1
mmu-miR-466d-5p ENSMUSG00000000078 mmu-miR-466d-5p miRNA         TF_mc1_1_N   1
mmu-miR-1187    ENSMUSG00000000078    mmu-miR-1187 miRNA         TF_mc1_1_N   1
mmu-miR-669f-3p ENSMUSG00000000078 mmu-miR-669f-3p miRNA         TF_mc1_1_N   1
> tail(SimMORE$ResultsPerGene$ENSMUSG00000000078$allRegulators)
                           gene     regulator  omic     area        filter Rel
Pou6f1       ENSMUSG00000000078        Pou6f1    TF promoter miRNA_mc1_2_N   0
Rfxank       ENSMUSG00000000078        Rfxank    TF promoter    TF_mc1_1_P   1
Satb1        ENSMUSG00000000078         Satb1    TF promoter miRNA_mc1_2_N   0
Zfp692       ENSMUSG00000000078        Zfp692    TF promoter    TF_mc1_1_N   1
TF_mc1_1_R   ENSMUSG00000000078    TF_mc1_1_R    TF promoter         Model   1
miRNA_mc1_2_R ENSMUSG00000000078 miRNA_mc1_2_R miRNA                 Model   0
```

5 Retrieving significant regulations from MORE results

The function **RegulationPerCondition** is applied to the **more** output. It returns a summary table containing all the relevant/significant regulations, that is, all the pairs gene-regulator considered relevant/significant in MORE models (depending if a GLM or a PLS model was applied). Moreover, it provides the regression coefficient that relates the gene and the regulator for each experimental condition after testing if this coefficient is relevant/significant or not.

```
RegulationPerCondition(output)
```

5.1 RegulationPerCondition input parameters

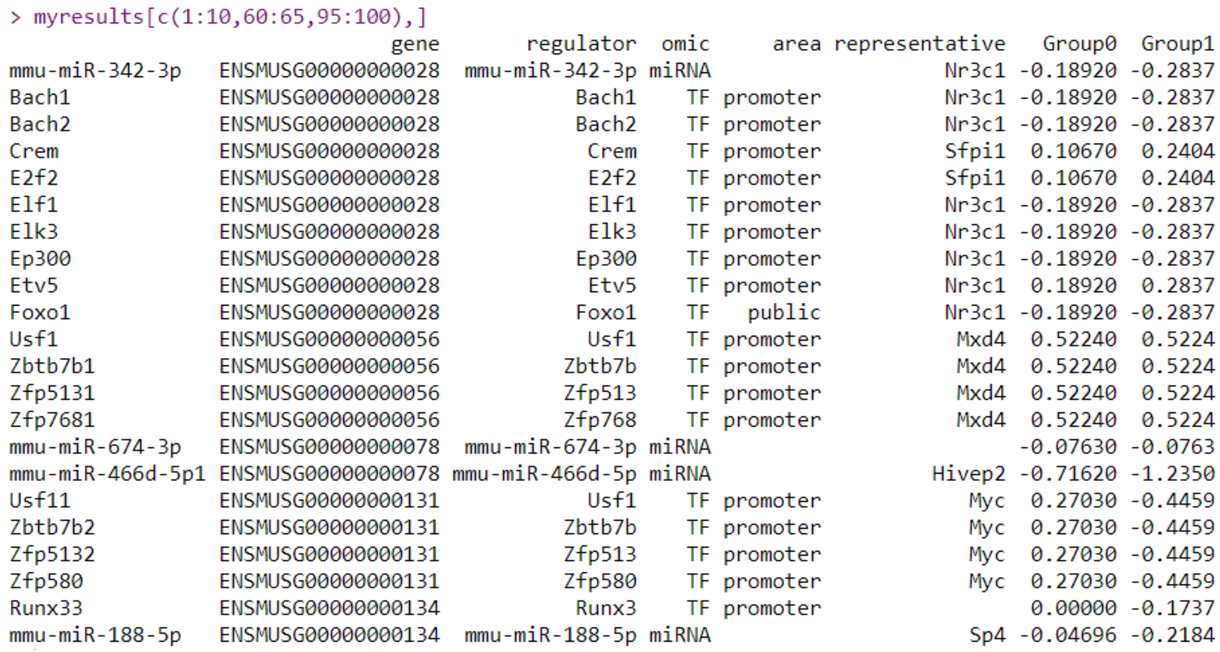
output Object containing the output of **more** function.

5.2 Interpreting RegulationPerCondition output with an example

Following the previous example, we can run the **RegulationPerCondition** function.

```
> myresults = RegulationPerCondition(SimMORE)
```

The output is the following table, where some pairs gene-regulator are selected to have a complete vision of the output of this function:



```
> myresults[c(1:10,60:65,95:100),]
                                   gene       regulator  omic     area representative   Group0  Group1
mmu-miR-342-3p   ENSMUSG00000000028  mmu-miR-342-3p miRNA                   Nr3c1 -0.18920 -0.2837
Bach1            ENSMUSG00000000028           Bach1    TF promoter          Nr3c1 -0.18920 -0.2837
Bach2            ENSMUSG00000000028           Bach2    TF promoter          Nr3c1 -0.18920 -0.2837
Crem             ENSMUSG00000000028            Crem    TF promoter          Sfpi1  0.10670  0.2404
E2f2             ENSMUSG00000000028            E2f2    TF promoter          Sfpi1  0.10670  0.2404
Elf1             ENSMUSG00000000028            Elf1    TF promoter          Nr3c1 -0.18920 -0.2837
Elk3             ENSMUSG00000000028            Elk3    TF promoter          Nr3c1 -0.18920 -0.2837
Ep300            ENSMUSG00000000028           Ep300    TF promoter          Nr3c1 -0.18920 -0.2837
Etv5             ENSMUSG00000000028            Etv5    TF promoter          Nr3c1  0.18920  0.2837
Foxo1            ENSMUSG00000000028           Foxo1    TF   public          Nr3c1 -0.18920 -0.2837
Usf1             ENSMUSG00000000056            Usf1    TF promoter           Mxd4  0.52240  0.5224
Zbtb7b1          ENSMUSG00000000056          Zbtb7b    TF promoter           Mxd4  0.52240  0.5224
Zfp5131          ENSMUSG00000000056          Zfp513    TF promoter           Mxd4  0.52240  0.5224
Zfp7681          ENSMUSG00000000056          Zfp768    TF promoter           Mxd4  0.52240  0.5224
mmu-miR-674-3p   ENSMUSG00000000078  mmu-miR-674-3p miRNA                         -0.07630 -0.0763
mmu-miR-466d-5p1 ENSMUSG00000000078 mmu-miR-466d-5p miRNA                  Hivep2 -0.71620 -1.2350
Usf11            ENSMUSG00000000131            Usf1    TF promoter            Myc  0.27030 -0.4459
Zbtb7b2          ENSMUSG00000000131          Zbtb7b    TF promoter            Myc  0.27030 -0.4459
Zfp5132          ENSMUSG00000000131          Zfp513    TF promoter            Myc  0.27030 -0.4459
Zfp580           ENSMUSG00000000131          Zfp580    TF promoter            Myc  0.27030 -0.4459
Runx33           ENSMUSG00000000134           Runx3    TF promoter                 0.00000 -0.1737
mmu-miR-188-5p   ENSMUSG00000000134  mmu-miR-188-5p miRNA                     Sp4 -0.04696 -0.2184
```

This table shows the relevant regulators for each gene. The **representative** column indicates if the regulator was chosen as the random representative of a correlated group of regulators or, otherwise, which regulator was taken as the representative of the group. When no information is provided in this column, it means that the regulator was not part of a correlated group of regulators. Regulators correlated positively with the representative will have the same coefficients (same sign) as the representative, while negatively correlated regulators will have the same coefficients as the representative but with the opposite sign.

The final columns correspond to the regression coefficients of each regulator for each experimental group. In this case, the experimental design matrix (**edesign**) contained two conditions, so the column `Group0` corresponds to the first condition, and `Group1` corresponds to the second one. These are the conclusions we can draw from the coefficients:

- If two experimental groups have the same coefficients, it means that the regulator has the same effect on the gene in both groups.
- If one of the coefficients is 0, it means that the regulator has no effect on the gene under this experimental condition.
- Experimental groups with different non-zero coefficients indicate that the regulator affects the gene in all these experimental groups but the magnitude of the effect is not the same for all these groups.

6 Plotting MORE results

MORE package includes several plots for the interpretation of the results.

6.1 summary_plot function

MORE package includes the function **summary_plot** to graphically represent the summary of the relationship between genes and regulators found when creating the models. It creates two types of summary plots depending on user specifications.

```
summary_plot(output, output_regpcond, by_genes =TRUE)
```

6.1.1 summary_plot input parameters

output Object generated by the function **more**.

output_regpcond Object generated by the function **RegulationPerCondition** when applied to a **more** object.

by_genes If TRUE (default), the function plots the percentage of genes with significant regulators globally and per omic. If FALSE, it plots the percentage of significant regulations per omic.

6.2 plotmore function

MORE package includes the function **plotmore** to graphically represent the relationship between genes and regulators: for a given pair gene-regulator, to explore the regulators of a given gene, or to analyze which genes are regulated by a specific regulator.

```
plotmore(output, gene, regulator = NULL, reguValues = NULL,
plotPerOmic = FALSE, gene.col = 1, regu.col = NULL, order = TRUE,
xlab = "", cont.var = NULL, cond2plot = NULL)
```

6.2.1 plotmore input parameters

output Object generated by the function **more**.

gene ID of the gene to be plotted.

regulator ID of the regulator to be plotted. If NULL (default value), all the regulators of the gene are plotted.

reguValues Vector containing the values of a regulator that the user can optionally provide. If NULL (default value), these values are taken from **GLMoutput** as long as they are available.

plotPerOmic If TRUE, all the relevant regulators of the given gene and the same omic are plotted in the same graph. If FALSE (default value), each regulator is plotted in a separate plot.

gene.col Color to plot the gene. By default, 1 (black).

regu.col Color to plot the regulator. If NULL (default), a color will be assigned by the function, that will be different for each regulatory omic.

order If TRUE (default), the values in X-axis are ordered.

xlab Label for the X-axis.

cont.var Vector with length equal to the number of observations in data, which optionally may contain the values of the numerical variable (e.g. time) to be plotted on the X-axis. By default, NULL. It plots a range for each observation in which the observation could take values taking into account the numerical variable introduced.

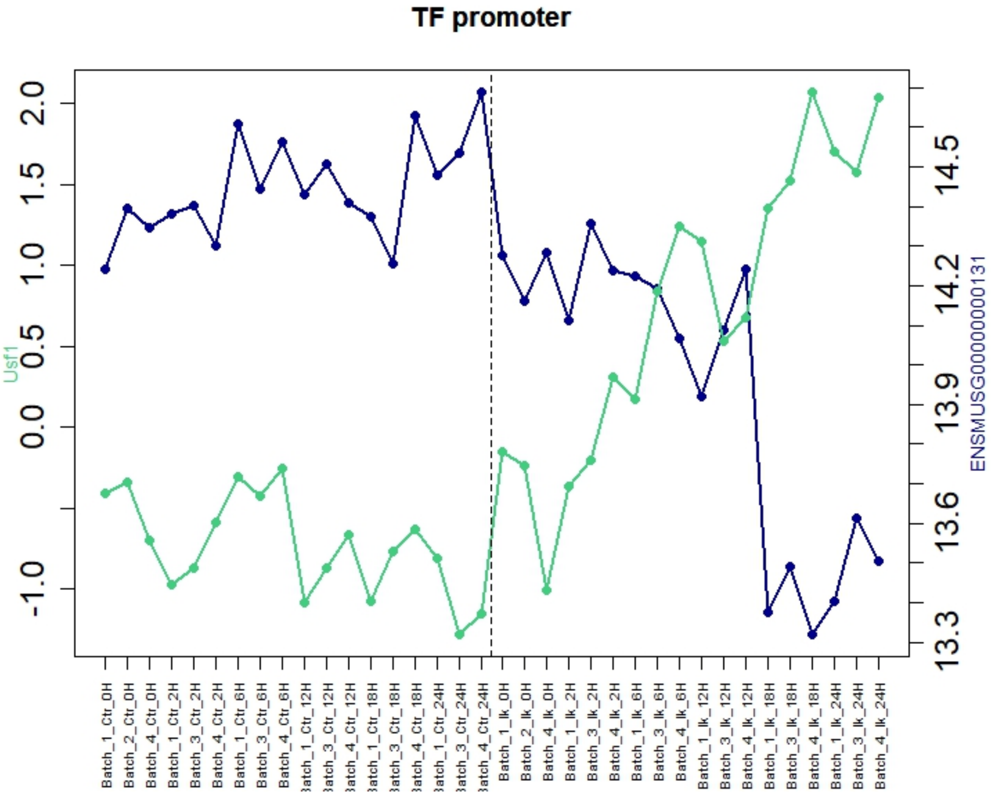
cond2plot Vector or factor indicating the experimental group of each value to represent. If NULL (default), the labels are taken from the experimental design matrix.

6.2.2 Interpretation of MORE plots

Following the previous example, the MORE graphic below represents the expression profile of a given gene (ENSMUSG00000000131) and the values for a relevant regulator of this gene (TF promoter regulator Usf1) and can be generated with the following code:

```
> plotmore(output = SimMORE,
          gene = "ENSMUSG00000000131",
          regulator = "Usf1",
          plotPerOmic = FALSE,
          gene.col = "blue4",
          order = FALSE,
          regu.col = "seagreen3")
```

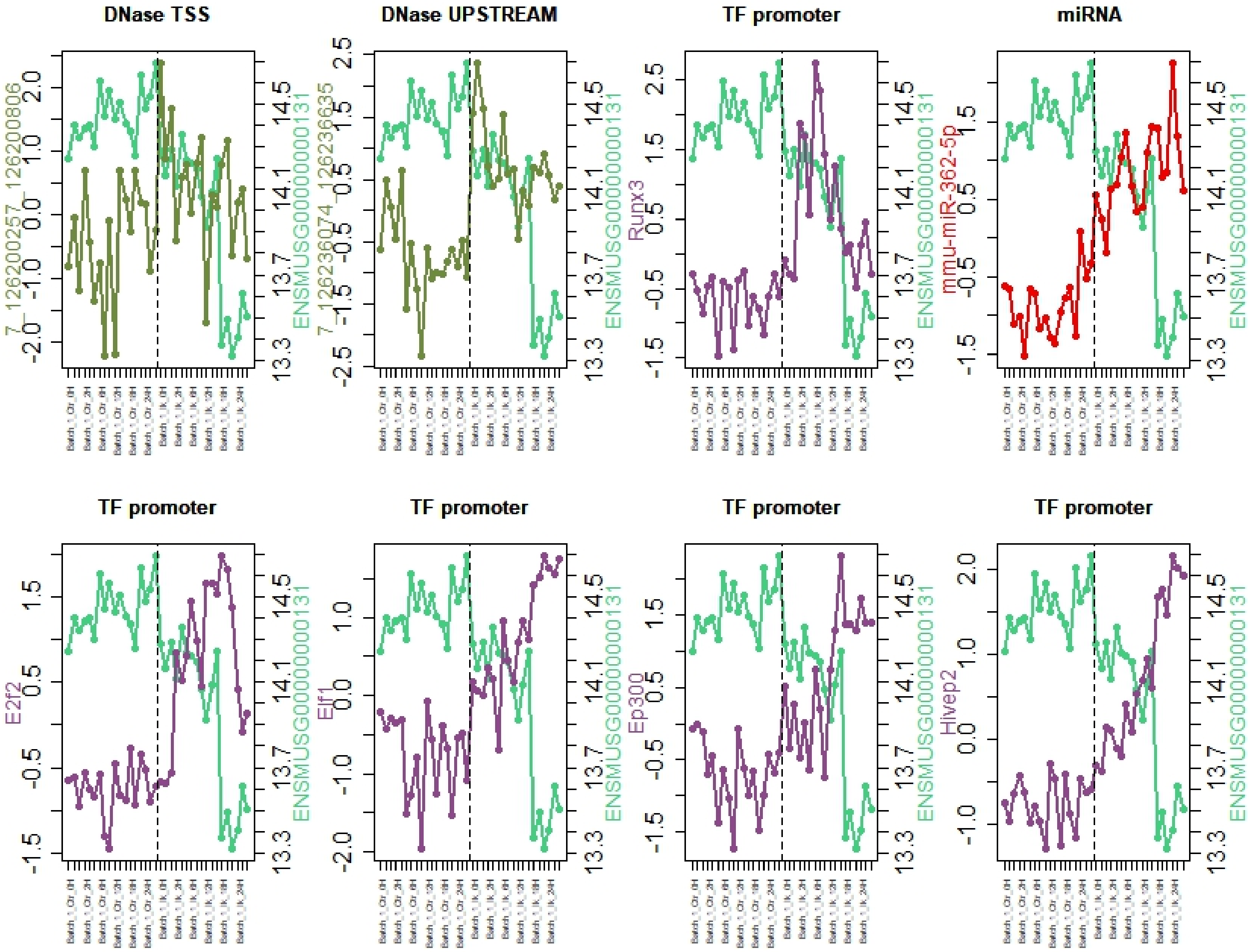
The X-axis is divided into two conditions (1 or 2), and within each condition, the observations are displayed, which correspond to different time points in this case. The right Y-axis shows the expression values for the gene (plotted in blue), while the left Y-axis indicates the values for the regulator (plotted in green).



If we set the regulator argument to NULL, all the relevant regulators of gene ENSMUSG00000000131 will be plotted (18 regulators from three different omics: miRNA-seq, DNase-seq, and TF).
Only 8 will be presented:

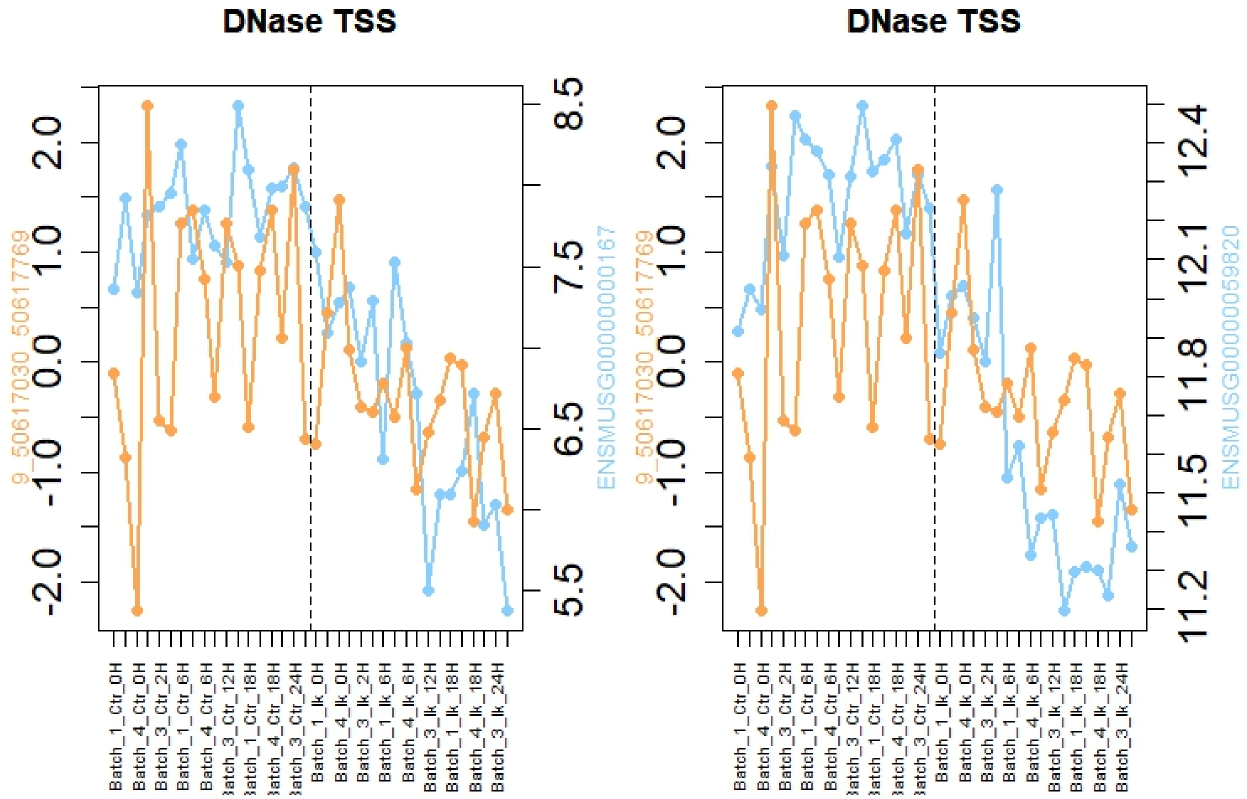
```
> par(mfrow = c(2,4))
> plotmore(output = SimMORE,
           gene = "ENSMUSG00000000131",
           regulator = NULL,
           order =FALSE,
           plotPerOmic = FALSE,
           gene.col = "seagreen3")
```

The title of each plot indicates the omic represented in that plot and the area. The values for relevant regulators are plotted in different colors according to the omic. The values for the gene are plotted in sea green, as indicated in the previous code.



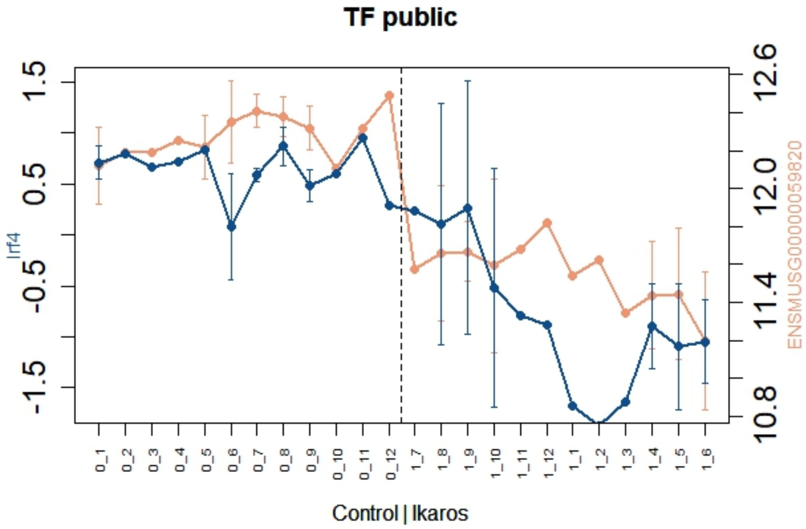
If we want to plot all the genes that are considered to be relevantly regulated by a given regulator (e.g. 9_50617030_50617769), we must set the gene argument to NULL as follows. In this case, the TF regulates two genes: ENSMUSG00000000167 and ENSMUSG00000059820.

```
> par(mfrow = c(1,2))
> plotmore(output = SimMORE,
          gene = NULL,
          regulator = "9_50617030_50617769",
          plotPerOmic = FALSE,
          order =FALSE,
          gene.col = "skyblue1",
          regu.col = "tan1")
```



Users can also define their own values for time points with the vector **cont.var**. In addition, they can assign a label for axis X to differentiate between two conditions, Control or Ikaros, which is the **xlab** parameter. The code and the resulting graph, where the gene is plotted in light orange, and the regulator is plotted in blue, can be found below.

```
> plotmore(output = SimGLM,
           gene = "ENSMUSG00000059820",
           regulator = "Irf4",
           plotPerOmic = FALSE,
           gene.col = "lightsalmon2",
           regu.col = "dodgerblue4",
           cont.var = c(1,2,3,4,5,6,7,8,9,10,11,12),
           xlab = "Control␣|␣Ikaros")
```



7 How to use MORE with R Shiny

Shiny is an R package that allows for building web applications from R packages or scripts so users that are not familiar with R language can still easily use R packages. We have generated a MORE web application with R Shiny for this purpose. To use the MORE shiny tool, users must first install the Shiny and the Shiny themes packages from CRAN repository with `install.packages()` function.

```
> install.packages("shiny")
> install.packages("shinythemes")
```

Moreover, users must also have previously installed the MORE package described in section 2.

The MORE shiny scripts needed to run the tool are available in the Downloads bitbucket folder for MORE package (`https://bitbucket.org/ConesaLab/more/downloads/`). There is a file called `app.R`, an example dataset stored in the file `TestDataShiny.RData` and a folder called `www`.

app.R Script to run the web application for MORE method. Users must open this file from RStudio to start using the application.

TestDataShiny.RData Example data file to test the application. We used it as an example of an execution of MORE Shiny.

www Folder containing the style options. Please, do not delete anything in this folder.

Therefore, in order to run the application, please open the `app.R` file, where the MORE method application is located, and execute it using the button `Run App` (the red box in the following picture).

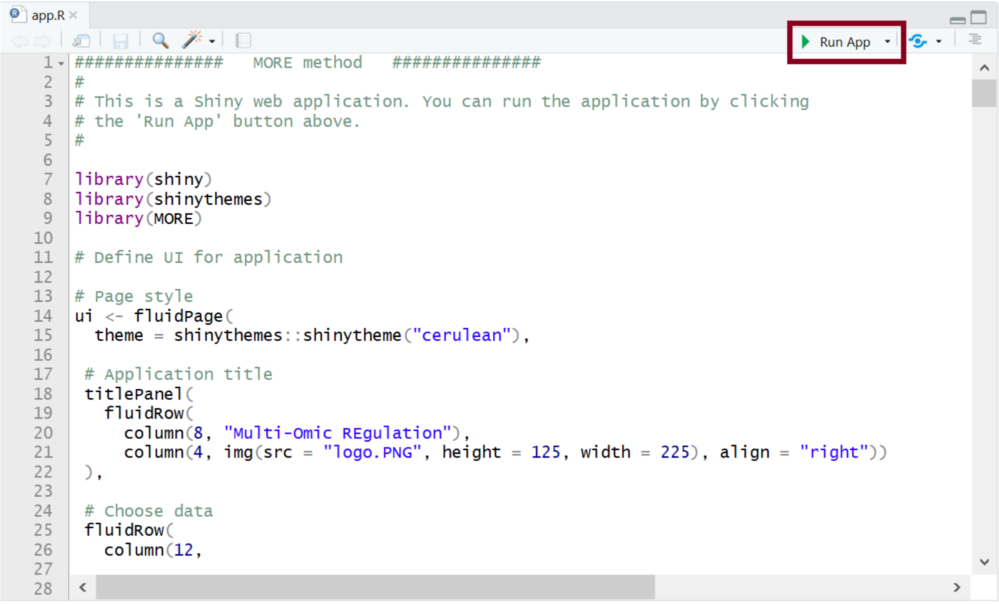
A window will open with the MORE application, as shown in the following figure.

The different options have been explained in the previous sections, but next, we run an example to clarify how to use them. This example can also be visualized in this video: `https://youtu.be/SSIaeFRNsXg`.

7.1 MORE Shiny application example

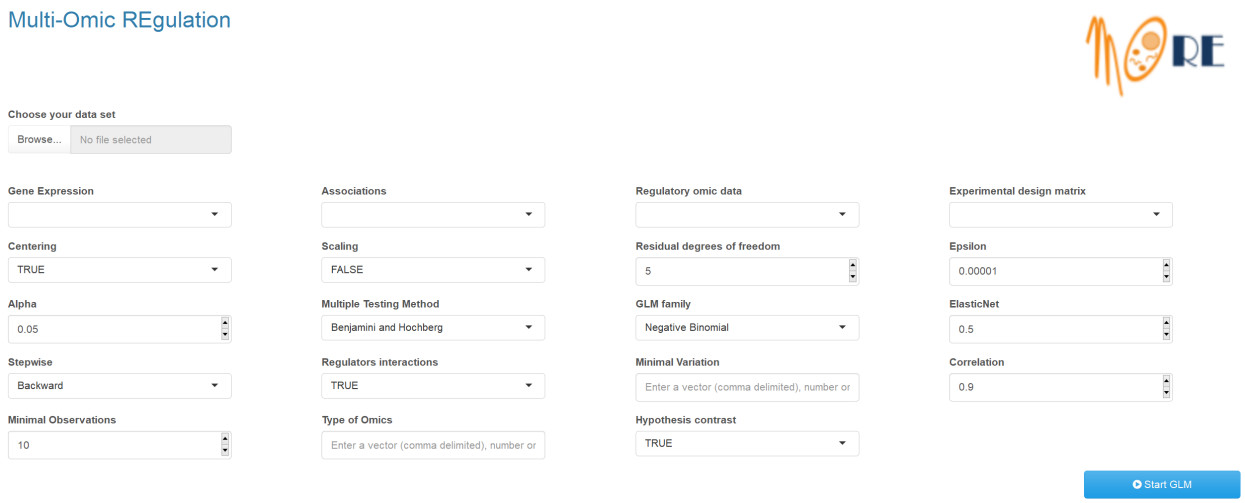
Please take into account that MORE Shiny only supports `.RData` files at this time. Once the RData is loaded, the user must indicate the names of the data files in the RData that corresponds to (see the input data defined in the **section 3**): gene expression matrix, experimental design matrix, regulators matrix and association matrix. In summary (for more details see **section 3**):

Gene expression matrix Matrix or data frame that contains expression values for each gene, in rows, under each experimental condition or replicates, in columns.



```r
###############    MORE method    ###############
#
# This is a Shiny web application. You can run the application by clicking
# the 'Run App' button above.
#

library(shiny)
library(shinythemes)
library(MORE)

# Define UI for application

# Page style
ui <- fluidPage(
  theme = shinythemes::shinytheme("cerulean"),

 # Application title
 titlePanel(
   fluidRow(
     column(8, "Multi-Omic REgulation"),
     column(4, img(src = "logo.PNG", height = 125, width = 225), align = "right"))
 ),

 # Choose data
 fluidRow(
   column(12,

```

Figure 1: Run MORE application, click `Run App` button



Multi-Omic REgulation

Choose your data set: No file selected

Gene Expression:

Associations:

Regulatory omic data:

Experimental design matrix:

Centering: TRUE

Scaling: FALSE

Residual degrees of freedom: 5

Epsilon: 0.00001

Alpha: 0.05

Multiple Testing Method: Benjamini and Hochberg

GLM family: Negative Binomial

ElasticNet: 0.5

Stepwise: Backward

Regulators interactions: TRUE

Minimal Variation: Enter a vector (comma delimited), number or

Correlation: 0.9

Minimal Observations: 10

Type of Omics: Enter a vector (comma delimited), number or

Hypothesis contrast: TRUE

Figure 2: MORE Shiny

Experimental design matrix Matrix or data frame that contains the experimental covariates, such as treatments, points of time...

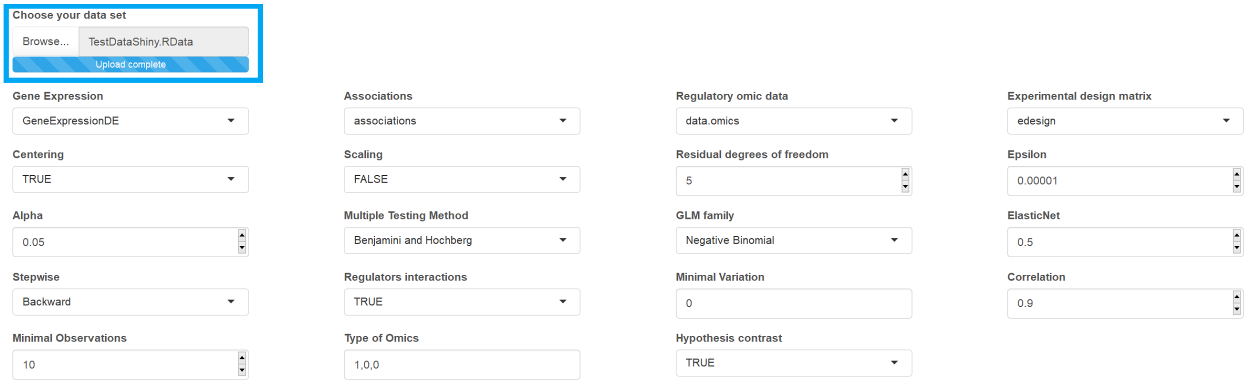
Regulatory omic data List that contains matrices or data frames containing the data for each regulatory omic, e.g. miRNA expression. The data frame structure is similar to gene expression data.

Association matrix List that contains data frames with the potential regulators for each regulatory omic considered. The association objects must be data frames and stored in a single list.

In this case, the file `TestDataShiny.RData` contains the objects described in **section 4.3**, unlike the experimental design matrix, which contains only one column with two conditions. By clicking on the button `Browse...`, users can choose their own data file (see Figure 3 blue box). Once the data is loaded, the user must enter the same input parameters of the GetGLM and RegulationPerConditon functions as defined in **section 4.1** and **section 5.1**.

It should be taken into account that if users want to enter a NULL value for a given parameter, they must leave the box empty.

In the example of the application, we will consider the input parameters shown in the following figure, leaving blank those we want to be NULL.



Choose your data set
Browse... TestDataShiny.RData
Upload complete

Gene Expression
GeneExpressionDE

Associations
associations

Regulatory omic data
data.omics

Experimental design matrix
edesign

Centering
TRUE

Scaling
FALSE

Residual degrees of freedom
5

Epsilon
0.00001

Alpha
0.05

Multiple Testing Method
Benjamini and Hochberg

GLM family
Negative Binomial

ElasticNet
0.5

Stepwise
Backward

Regulators interactions
TRUE

Minimal Variation
0

Correlation
0.9

Minimal Observations
10

Type of Omics
1,0,0

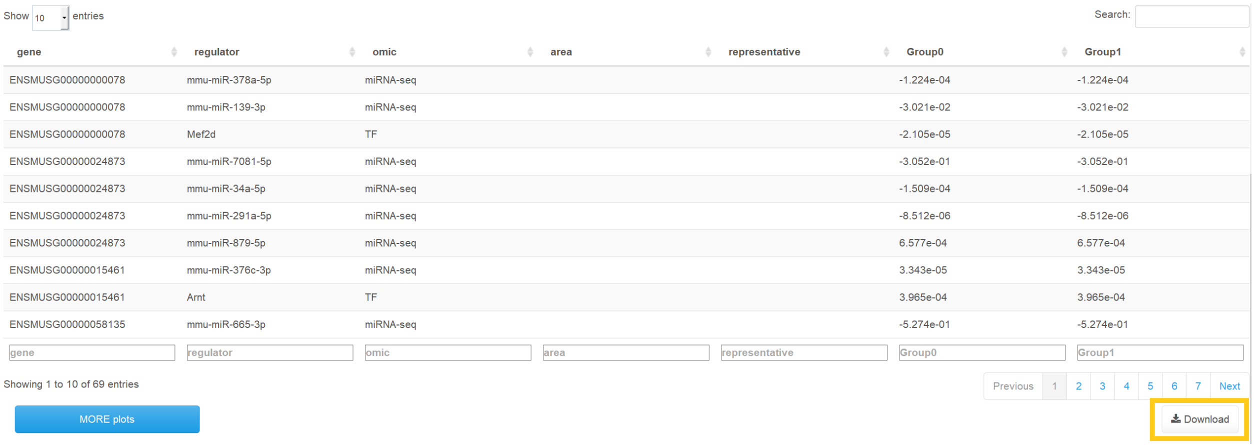
Hypothesis contrast
TRUE

Figure 3: MORE Shiny: inputs for running example

Now, clicking the button `Start GLM`, we will obtain a summary table (see Figure 4). Specifically, this is the table defined in the **section 5.2**, the output of the **RegulationPerCondition()** function. The user can download the table in CSV format by pressing the button `Download` (see Figure 4 orange box). It is necessary to save the file that will contain the table with name and extensión **.csv**.

The button `MORE plots` (see Figure 4) will generate plots to visualize the relationship between genes and regulators. The user can change the different parameters without re-executing the application to tune the plots or plot new elements.

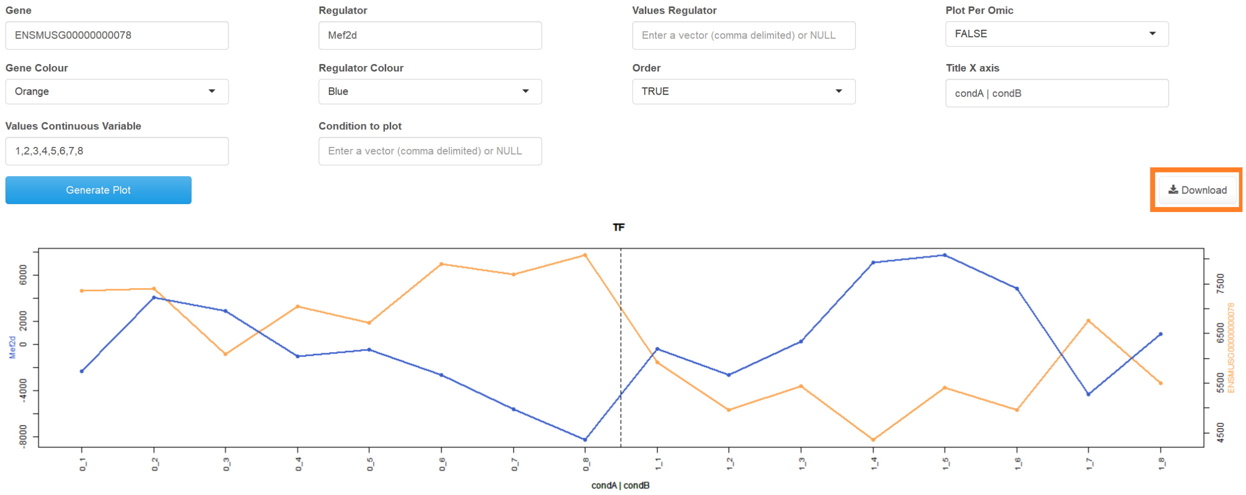
Here we show the example for the gene ENSMUSG00000000078 (orange) and TF Mef2d (blue). Pressing the button `Generate Plot`, Shiny generates the first graph. However, if it is expected to obtain more than one graphic, the user can see all of them in a pdf file. This pdf file is generated by pressing the button `Download` (box orange in Figure 5) and saving the document, for example, as plotsGLM.pdf. It is essential to save the file with name and extension **.pdf**.



gene	regulator	omic	area	representative	Group0	Group1
ENSMUSG00000000078	mmu-miR-378a-5p	miRNA-seq			-1.224e-04	-1.224e-04
ENSMUSG00000000078	mmu-miR-139-3p	miRNA-seq			-3.021e-02	-3.021e-02
ENSMUSG00000000078	Mef2d	TF			-2.105e-05	-2.105e-05
ENSMUSG00000024873	mmu-miR-7081-5p	miRNA-seq			-3.052e-01	-3.052e-01
ENSMUSG00000024873	mmu-miR-34a-5p	miRNA-seq			-1.509e-04	-1.509e-04
ENSMUSG00000024873	mmu-miR-291a-5p	miRNA-seq			-8.512e-06	-8.512e-06
ENSMUSG00000024873	mmu-miR-879-5p	miRNA-seq			6.577e-04	6.577e-04
ENSMUSG00000015461	mmu-miR-376c-3p	miRNA-seq			3.343e-05	3.343e-05
ENSMUSG00000015461	Arnt	TF			3.965e-04	3.965e-04
ENSMUSG00000058135	mmu-miR-665-3p	miRNA-seq			-5.274e-01	-5.274e-01
gene	regulator	omic	area	representative	Group0	Group1

Showing 1 to 10 of 69 entries

Figure 4: Summary table and `MORE plots` button. The user can download the whole table by pressing the button `Download` (orange box)



Gene: ENSMUSG00000000078

Regulator: Mef2d

Values Regulator: Enter a vector (comma delimited) or NULL

Plot Per Omic: FALSE

Gene Colour: Orange

Regulator Colour: Blue

Order: TRUE

Title X axis: condA | condB

Values Continuous Variable: 1,2,3,4,5,6,7,8

Condition to plot: Enter a vector (comma delimited) or NULL

Figure 5: Inputs and plot for the pair gen–regulator (ENSMUSG00000000078–Mef2d). The user can download all plots by pressing the button `Download` (orange box)

8 How to cite MORE package

Tarazona, S., Tomás-Riquelme, B., Martínez-Mira, C., Clemente-Císcar, M., Conesa, A. (2018). MORE: Multi-Omics REgulation by regression models. R package version 0.1.0.