Journal of Statistical Software

MMMMMM YYYY, Volume VV, Issue II. *doi: 10.18637/jss.v000.i00*

OmicSelector: Docker-based application and R package for biomarker signature selection from high-throughput omic experiments and deep learning model development.

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Abstract

The crucial phase of modern biomarker discovery studies is a selection of most promising features from the results of high-throughput screening assays. Here, we present the OmicSelector - Docker-based web application and R package that facilitates the analysis of such experiments. OmicSelector provides a consistent and overfitting-resilient pipeline that integrates 94 feature selection approaches based on 25 distinct variable selection methods. It identifies and ranks the best feature sets, basing on 12 modeling algorithms (including GPU-based deep learning) with hyperparameter optimization in hold-out or cross-validation. OmicSelector provides classification performance metrics for proposed feature sets, which allow researchers to choose the overfitting-resistant biomarker set with the most significant diagnostic potential. Lastly, it allows for development, validation and implementation of deep learning feedforward neural networks (up to 3 hidden layers) on selected signature. Application performs extensive grid search of hyperparameters including balancing and preprocessing with additional autoencoders. The pipeline is applicable for selecting candidate circulating or tissue miRNAs, RNAs, methylation data, metabolites, or proteins. The tool is open-source and available at `https://biostat.umed.pl/OmicSelector/`.

Keywords: feature selection, biomarker, data-mining, next-generation sequencing, omics, deep learning, artificial neural network, R, Docker.

1. Introduction

Broad-scale treatment personalization is one of the most significant modern medicine challenges, requiring accurate and cost-effective diagnostic tests. Such methods rely heavily on biomarkers, which are usually discovered using omic techniques. Although high-throughput experiments enable us to gather the biological measurements of an extensive amount of biomarker candidates, translating the results to the clinical bedside remains troublesome.

The typical biomarker study comprises of discovery and validation phases. (Goossens, Nakagawa, Sun, and Hoshida (2015)) In the former, high-throughput screening is usually performed to measure the values of multiple features. Those are further assessed to determine their diagnostic potential. In the validation phase, only selected variables are measured, typically in a new set of samples, with a cheaper and/or more accessible method. Our team has been working on microRNA (miRNA) biomarkers for radiation (Dinh, Fendler, Chałubińska-Fendler, Acharya, O'Leary, Deraska, D'Andrea, Chowdhury, and Kozono (2016)) and cancer (Elias, Fendler, Stawiski, Fiascone, Vitonis, Berkowitz, Frendl, Konstantinopoulos, Crum, Kedzierska, Cramer, and Chowdhury (2017)), but trouble with the reproducibility of selected biomarker performance (Acharya, Fendler, Watson, Hamilton, Pan, Gaudiano, Moskwa, Bhanja, Saha, Guha, Parmar, and Chowdhury (2015); Fendler, Malachowska, Meghani, Konstantinopoulos, Guha, Singh, and Chowdhury (2017); Małachowska, Tomasik, Stawiski, Kulkarni, Guha, Chowdhury, and Fendler (2020)) or reference identification (Pagacz, Kucharski, Smyczynska, Grabia, Chowdhury, and Fendler (2020)). Similar challenges, caused by bias and overfitting, hindered the attempts of other groups to develop validated, efficient omic-driven biomarkers. (Dobbin, Cesano, Alvarez, Hawtin, Janetzki, Kirsch, Masucci, Robbins, Selvan, Streicher, Zhang, Butterfield, and Thurin (2016))

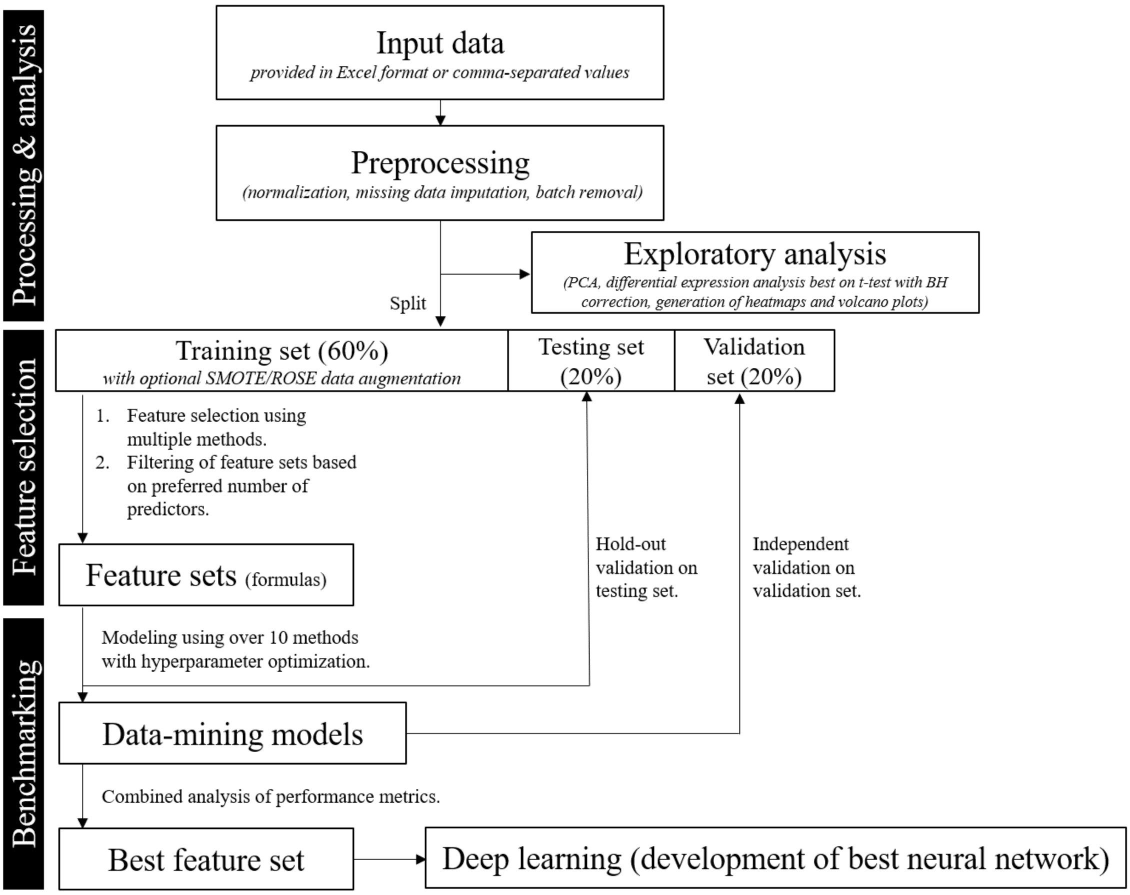
Cohorts used in the discovery phase are usually small due to the high cost of high-throughput assays, which makes the experiments vulnerable to overfitting and results in false-positive biomarker candidates that fail in external validation. (Smialowski, Frishman, and Kramer (2009)) For example, a recent review of serum miRNA biomarkers for pancreatic cancer (Xue, Jia, Ren, Lindsay, and Yu (2019)) highlights how various miRNA sets are chosen in different studies, with each study reporting unrealistically optimistic results. Thus, correct and overfitting-resistant feature selection is critical in biomarker studies.

In this paper, we try to tackle this problem by designing software for systematic, overfitting-resistant, and informative feature selection. The analytical steps of our package entail (1Figure 1): splitting of the dataset into training, testing and validation sets, differential expression analysis and performing up to 94 different feature selection procedures on the training set. Feature sets (formulas) are further validated by training 12 models of various architectures with hyperparameter optimization based on hold-out- or cross-validation. Our toolset enables the users to make an informed decision about the most appropriate feature selection method and informs them about their predictive abilities using different modeling approaches. Finally, as the most flexible method, users are able to train and implement final deep feedforward neural network (up to 3 hidden layers, with or without autoencoders; grid search of hyperparameters) for classification (diagnostic) problem.

2. Models and software

The basic Poisson regression model for count data is a special case of the GLM framework

Figure 1: ***The pipeline of OmicSelector analysis.*** *Abbreviations: PCA - principal component analysis, BH - Benjamini-Hochberg procedure, SMOTE/ROSE - data balancing methods explained in the main text.*


Processing & analysis
Input data
provided in Excel format or comma-separated values
Preprocessing
(normalization, missing data imputation, batch removal)
Exploratory analysis
(PCA, differential expression analysis best on t-test with BH correction, generation of heatmaps and volcano plots)
Split
Feature selection
Training set (60%)
with optional SMOTE/ROSE data augmentation
Testing set (20%)
Validation set (20%)
1. Feature selection using multiple methods.
2. Filtering of feature sets based on preferred number of predictors.
Feature sets (formulas)
Hold-out validation on testing set.
Independent validation on validation set.
Benchmarking
Modeling using over 10 methods with hyperparameter optimization.
Data-mining models
Combined analysis of performance metrics.
Best feature set
Deep learning (development of best neural network)

?. It describes the dependence of a count response variable y_i $(i = 1, \dots, n)$ by assuming a Poisson distribution $y_i \sim \text{Pois}(\mu_i)$. The dependence of the conditional mean $\mathsf{E}[y_i \,|\, x_i] = \mu_i$ on the regressors x_i is then specified via a log link and a linear predictor

$$\log(\mu_i) \quad = \quad x_i^\top \beta, \tag{1}$$

where the regression coefficients β are estimated by maximum likelihood (ML) using the iterative weighted least squares (IWLS) algorithm.

> Note that around the `{equation}` above there should be no spaces (avoided in the LaTeX code by `%` lines) so that "normal" spacing is used and not a new paragraph started.

R provides a very flexible implementation of the general GLM framework in the function `glm()` (?) in the **stats** package. Its most important arguments are

```
glm(formula, data, subset, na.action, weights, offset,
  family = gaussian, start = NULL, control = glm.control(...),
  model = TRUE, y = TRUE, x = FALSE, ...)
```

Type	Distribution	Method	Description
GLM	Poisson	ML	Poisson regression: classical GLM, estimated by maximum likelihood (ML)
		Quasi	"Quasi-Poisson regression": same mean function, estimated by quasi-ML (QML) or equivalently generalized estimating equations (GEE), inference adjustment via estimated dispersion parameter
		Adjusted	"Adjusted Poisson regression": same mean function, estimated by QML/GEE, inference adjustment via sandwich covariances
	NB	ML	NB regression: extended GLM, estimated by ML including additional shape parameter
Zero-augmented	Poisson	ML	Zero-inflated Poisson (ZIP), hurdle Poisson
	NB	ML	Zero-inflated NB (ZINB), hurdle NB

Table 1: Overview of various count regression models. The table is usually placed at the top of the page (`[t!]`), centered (`centering`), has a caption below the table, column headers and captions are in sentence style, and if possible vertical lines should be avoided.

where `formula` plus `data` is the now standard way of specifying regression relationships in R/S introduced in ?. The remaining arguments in the first line (`subset`, `na.action`, `weights`, and `offset`) are also standard for setting up formula-based regression models in R/S. The arguments in the second line control aspects specific to GLMs while the arguments in the last line specify which components are returned in the fitted model object (of class '`glm`' which inherits from '`lm`'). For further arguments to `glm()` (including alternative specifications of starting values) see `?glm`. For estimating a Poisson model `family = poisson` has to be specified.

> As the synopsis above is a code listing that is not meant to be executed, one can use either the dedicated `{Code}` environment or a simple `{verbatim}` environment for this. Again, spaces before and after should be avoided.
>
> Finally, there might be a reference to a `{table}` such as Table 1. Usually, these are placed at the top of the page (`[t!]`), centered (`\centering`), with a caption below the table, column headers and captions in sentence style, and if possible avoiding vertical lines.

3. Illustrations

For a simple illustration of basic Poisson and NB count regression the `quine` data from the **MASS** package is used. This provides the number of `Days` that children were absent from school in Australia in a particular year, along with several covariates that can be employed as regressors. The data can be loaded by

```
R> data("quine", package = "MASS")
```

and a basic frequency distribution of the response variable is displayed in Figure 2.

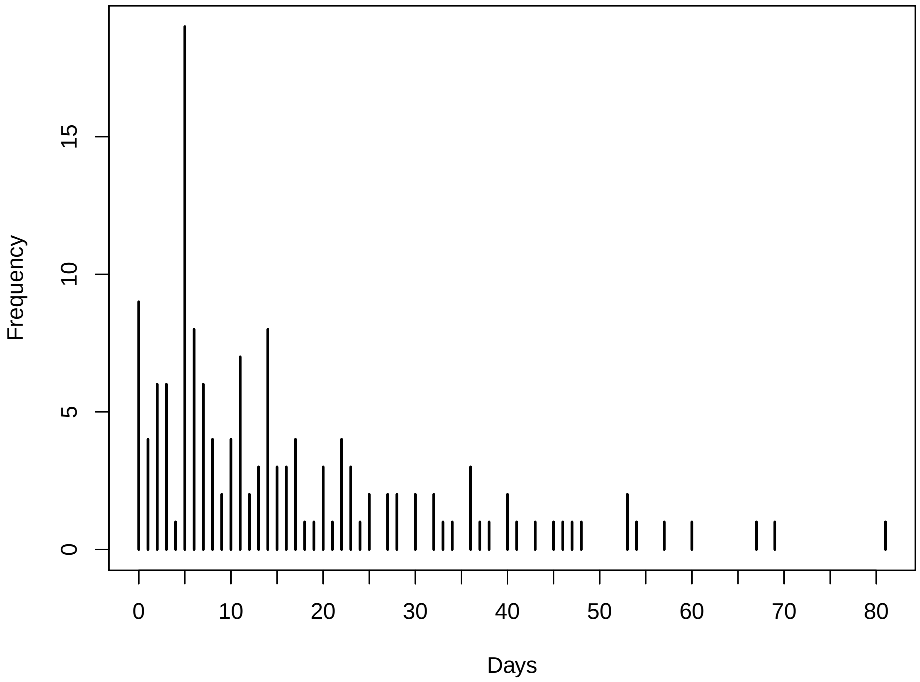


Figure 2: Frequency distribution for number of days absent from school.

> For code input and output, the style files provide dedicated environments. Either the "agnostic" `{CodeInput}` and `{CodeOutput}` can be used or, equivalently, the environments `{Sinput}` and `{Soutput}` as produced by `Sweave()` or **knitr** when using the `render_sweave()` hook. Please make sure that all code is properly spaced, e.g., using `y = a + b * x` and *not* `y=a+b*x`. Moreover, code input should use "the usual" command prompt in the respective software system. For R code, the prompt `"R> "` should be used with `"+ "` as the continuation prompt. Generally, comments within the code chunks should be avoided – and made in the regular LaTeX text instead. Finally, empty lines before and after code input/output should be avoided (see above).

As a first model for the `quine` data, we fit the basic Poisson regression model. (Note that JSS prefers when the second line of code is indented by two spaces.)

```
R> m_pois <- glm(Days ~ (Eth + Sex + Age + Lrn)^2, data = quine,
+    family = poisson)
```

To account for potential overdispersion we also consider a negative binomial GLM.

```
R> library("MASS")
R> m_nbin <- glm.nb(Days ~ (Eth + Sex + Age + Lrn)^2, data = quine)
```

In a comparison with the BIC the latter model is clearly preferred.

```
R> BIC(m_pois, m_nbin)
```

```
       df      BIC
m_pois 18 2046.851
m_nbin 19 1157.235
```

Hence, the full summary of that model is shown below.

```
R> summary(m_nbin)

Call:
glm.nb(formula = Days ~ (Eth + Sex + Age + Lrn)^2, data = quine,
    init.theta = 1.60364105, link = log)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.0857  -0.8306  -0.2620   0.4282   2.0898

Coefficients: (1 not defined because of singularities)
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  3.00155    0.33709   8.904  < 2e-16 ***
EthN        -0.24591    0.39135  -0.628  0.52977
SexM        -0.77181    0.38021  -2.030  0.04236 *
AgeF1       -0.02546    0.41615  -0.061  0.95121
AgeF2       -0.54884    0.54393  -1.009  0.31296
AgeF3       -0.25735    0.40558  -0.635  0.52574
LrnSL        0.38919    0.48421   0.804  0.42153
EthN:SexM    0.36240    0.29430   1.231  0.21818
EthN:AgeF1  -0.70000    0.43646  -1.604  0.10876
EthN:AgeF2  -1.23283    0.42962  -2.870  0.00411 **
EthN:AgeF3   0.04721    0.44883   0.105  0.91622
EthN:LrnSL   0.06847    0.34040   0.201  0.84059
SexM:AgeF1   0.02257    0.47360   0.048  0.96198
SexM:AgeF2   1.55330    0.51325   3.026  0.00247 **
SexM:AgeF3   1.25227    0.45539   2.750  0.00596 **
SexM:LrnSL   0.07187    0.40805   0.176  0.86019
AgeF1:LrnSL -0.43101    0.47948  -0.899  0.36870
AgeF2:LrnSL  0.52074    0.48567   1.072  0.28363
AgeF3:LrnSL       NA         NA      NA       NA
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(1.6036) family taken to be 1)

    Null deviance: 235.23  on 145  degrees of freedom
Residual deviance: 167.53  on 128  degrees of freedom
AIC: 1100.5

Number of Fisher Scoring iterations: 1
```

```
              Theta:  1.604
          Std. Err.:  0.214

 2 x log-likelihood:  -1062.546
```

4. Summary and discussion

As usual . . .

Computational details

If necessary or useful, information about certain computational details such as version numbers, operating systems, or compilers could be included in an unnumbered section. Also, auxiliary packages (say, for visualizations, maps, tables, . . .) that are not cited in the main text can be credited here.

The results in this paper were obtained using R 4.0.3 with the **MASS** 7.3.53 package. R itself and all packages used are available from the Comprehensive R Archive Network (CRAN) at `https://CRAN.R-project.org/`.

Acknowledgments

All acknowledgments (note the AE spelling) should be collected in this unnumbered section before the references. It may contain the usual information about funding and feedback from colleagues/reviewers/etc. Furthermore, information such as relative contributions of the authors may be added here (if any).

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Cleaning up BIBTEX files is a somewhat tedious task – especially when acquiring the entries automatically from mixed online sources. However, it is important that informations are complete and presented in a consistent style to avoid confusions. JSS requires the following format.

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- Journal titles should not be abbreviated and in title case.
- DOIs should be included where available.
- Software should be properly cited as well. For R packages `citation("pkgname")` typically provides a good starting point.

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Journal of Statistical Software http://www.jstatsoft.org/
published by the Foundation for Open Access Statistics http://www.foastat.org/
MMMMMM YYYY, Volume VV, Issue II *Submitted:* yyyy-mm-dd
doi:10.18637/jss.v000.i00 *Accepted:* yyyy-mm-dd