

The SDAMS package

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Abstract

This vignette introduces the use of the Bioconductor package SDAMS, which is designed for differential abundance analysis for metabolomics and proteomics data from mass spectrometry. These data may contain a large fraction of zero values and non-zero part may not be normally distributed. SDAMS considers a two-part semi-parametric model, a logistic regression for the zero proportion and a semi-parametric log-linear model for the non-zero values. A kernel-smoothed likelihood method is proposed to estimate regression coefficients in the two-part model and a likelihood ratio test is constructed for differential abundant analysis.

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1 Citation

The package SDAMS implements statistical methods from the following publication. If you use SDAMS in the published research, please cite:

Li, Y., Fan, T.W., Lane, A.N. et al. SDA: a semi-parametric differential abundance analysis method for metabolomics and proteomics data. BMC Bioinformatics 20, 501 (2019).

2 Quick Start

This section show the most basic SDAMS work flow for a differential abundance analysis for metabolomics and proteomics data from mass spectrometry:

1. Create a `SummarizedExperiment` object using function `createSEFromMatrix` or `createSEFromCSV`. In this section we use an example `SummarizedExperiment` object directly, which is an object of `SummarizedExperiment` class named `exampleSumExp` contained in this package.
2. Perform a differential abundance analysis using SDA.

```
> library("SDAMS")  
> data("exampleSumExp")  
> results <- SDA(exampleSumExp)
```

Here, the `SummarizedExperiment` class object `exampleSumExp` contained in the package is the proteomics dataset, which a matrix-like container for proteomic features with experimental subject grouping information. There are 560 features for 202 experimental subjects with 49 prostate cancer subjects and 153 healthy subjects (0 for healthy control and 1 for patient in this case). This is a 10% subsample of the original dataset. The features are stored as a matrix in the assay slot. Each row in this matrix represents a proteomic feature and each column represents a subject. See Reference [1] for detailed information regarding this dataset.

3 Data Input

3.1 Create SummarizedExperiment object from csv files

The proteomics or metabolomics data is stored as a matrix with each row being a feature and each column corresponding to a subject. All data in this matrix are non-negative. Another information required is the phenotype covariates. Here we focus on the binary grouping information, such as numeric 1 for control group and 0 for case group. But it can also be characters, such as "healthy" and

"disease". To utilize SDAMS package, we should have two separate csv files (for example 'feature.csv' and 'group.csv') as inputs for createSEfromCSV to create a SummarizedExperiment object.

Note:

1. The 1st column in 'feature.csv' represents feature names and the 1st row represents subject codes.
2. The 1st column in 'group.csv' represents subject codes, for example, Subject1, Subject2....

The format for "csv files" should look like as Figure 1 and Figure 2:

| | 9308 | 9324 | 9447 | 9449 | 9457 | 9459 | 9464 | 9466 | 9467 | 9471 |
|-----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 54 | 0 | 0 | 116.9700012 | 0 | 0 | 0 | 0 | 19.01000023 | 0 | 0 |
| 94 | 52.40000153 | 0 | 0 | 139.1199951 | 82.37000275 | 0 | 58.22999954 | 0 | 22.32999992 | 25.09000015 |
| 97 | 0 | 0 | 53.45000076 | 62.72999954 | 47.1599985 | 0 | 0 | 24.44000053 | 0 | 22.77000046 |
| 191 | 0 | 0 | 22.18000031 | 170.6499939 | 0 | 0 | 0 | 0 | 0 | 26.52000046 |
| 219 | 58.47000122 | 0 | 0 | 64.5 | 28.11000061 | 0 | 39.77999878 | 7.429999828 | 0 | 5.230000019 |
| 286 | 0 | 0 | 0 | 19.26000023 | 89.55999756 | 0 | 162.8200073 | 109.8600006 | 75.48999786 | 115.6999969 |
| 366 | 0 | 0 | 0 | 0 | 28.97999954 | 0 | 0 | 0 | 0 | 0 |
| 388 | 29.43000031 | 0 | 0 | 0 | 0 | 0 | 0 | 32.20000076 | 0 | 59.97000122 |
| 420 | 65.79000092 | 0 | 87.44000244 | 314.0100098 | 160.4400024 | 0 | 0 | 0 | 0 | 26.85000038 |
| 463 | 0 | 0 | 0 | 12.15999985 | 38.52999878 | 0 | 0 | 0 | 0 | 0 |
| 499 | 0 | 0 | 0 | 26.12999916 | 14.22000027 | 0 | 0 | 0 | 0 | 1.919999957 |
| 583 | 43.74000168 | 0 | 0 | 59.29000092 | 7.099999905 | 0 | 0 | 0 | 0 | 3.809999943 |
| 605 | 0 | 0 | 0 | 657.8400269 | 661.9500122 | 0 | 0 | 32.22999954 | 0 | 0 |
| 624 | 0 | 96.94999695 | 0 | 97.40000153 | 167.0599976 | 232.9499969 | 89.54000092 | 55.5 | 35.15999985 | 0 |
| 652 | 0 | 0 | 9.68999958 | 0 | 0 | 0 | 0 | 0 | 0 | 3.599999905 |
| 709 | 0 | 0 | 38.45000076 | 0 | 0 | 0 | 0 | 8.229999542 | 0 | 6.380000114 |
| 889 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 912 | 569.3200073 | 0 | 0 | 0 | 0 | 0 | 23.57999992 | 120.6800003 | 73.69000244 | 100.4100037 |

Figure 1: Example of 'feature.csv' pattern

After creating the two csv files, we need the paths for the two csv files:

```
> path1 <- "/path/to/your/feature.csv/"
> path2 <- "/path/to/your/group.csv/"
```

Here for demonstration purpose, we use the data stored in inst/extdata directory. This is the csv format of the data in exampleSumExp which is a SummarizedExperiment object we described before.

```
> directory1 <- system.file("extdata", package = "SDAMS", mustWork = TRUE)
> path1 <- file.path(directory1, "ProstateFeature.csv")
> directory2 <- system.file("extdata", package = "SDAMS", mustWork = TRUE)
> path2 <- file.path(directory2, "ProstateGroup.csv")
```

then use the function createSEFromCSV after loading the SDAMS package.

| | grouping |
|------|----------|
| 9308 | 0 |
| 9324 | 0 |
| 9447 | 0 |
| 9449 | 0 |
| 9457 | 0 |
| 9459 | 0 |
| 9464 | 0 |
| 9466 | 0 |
| 9467 | 0 |
| 9471 | 0 |
| 9495 | 0 |
| 9497 | 0 |
| 9507 | 0 |
| 9512 | 0 |
| 9959 | 1 |
| 9960 | 1 |

Figure 2: Example of 'group.csv' pattern

```
> library("SDAMS")
> exampleSE1 <- createSEFromCSV(path1, path2)
> exampleSE1
```

```
class: SummarizedExperiment
dim: 560 202
metadata(0):
assays(1): counts
rownames(560): 93922 87209 ... 180624 130855
rowData names(0):
colnames(202): 9512 9963 ... 49341 49586
colData names(1): grouping
```

The feature data and grouping information can be accessed using SummarizedExperiment commands:

```
> head(assay(exampleSE1)[,1:10])
```

| | 9512 | 9963 | 9965 | 9975 | 9979 | 9997 | 10015 | 10034 | 10044 | 10047 |
|--------|------|--------|------|-------|------|-------|-------|-------|--------|-------|
| 93922 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 68.97 | 0.00 | 0.00 | 0.00 |
| 87209 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 43.87 |
| 29633 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 57.21 | 0.00 | 0.00 |
| 40225 | 0.00 | 226.40 | 0.00 | 19.65 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 126342 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 20.43 | 0.00 | 0.00 | 109.93 | 0.00 |

```
42832  52.32 137.76 70.25  0.00 453.23 92.20  0.00 352.55 496.71  0.00
```

```
> head(colData(exampleSE1)$grouping)
```

```
[1] 0 1 1 1 1 1
```

3.2 Create SummarizedExperiment object from separate matrix

If the two datasets have been already cleaned and loaded into R as matrices, then we can use `createSEFromMatrix` to create a `SummarizedExperiment` object.

```
> set.seed(100)
> featureInfo <- matrix(runif(800, -2, 5), ncol = 40)
> featureInfo[featureInfo<0] <- 0
> rownames(featureInfo) <- paste("feature", 1:20, sep = '')
> colnames(featureInfo) <- paste('subject', 1:40, sep = '')
> groupInfo <- data.frame(grouping=matrix(sample(0:1, 40, replace = TRUE),
+                                       ncol = 1))
> rownames(groupInfo) <- colnames(featureInfo)
> exampleSE2 <- createSEFromMatrix(feature = featureInfo, colData = groupInfo)
> exampleSE2
```

```
class: SummarizedExperiment
```

```
dim: 20 40
```

```
metadata(0):
```

```
assays(1): counts
```

```
rownames(20): feature1 feature2 ... feature19 feature20
```

```
rowData names(0):
```

```
colnames(40): subject1 subject2 ... subject39 subject40
```

```
colData names(1): grouping
```

```
> head(assay(exampleSE2)[,1:10])
```

```
      subject1 subject2 subject3 subject4 subject5 subject6 subject7
feature1 0.1543628 1.7506781 0.3146237 1.2459082 1.216678 0.2919056 0.7766364
feature2 0.0000000 2.9756269 4.0558438 2.5297084 2.195787 0.7263509 0.7489158
feature3 1.8662570 1.7684409 3.4430911 4.7240116 4.438053 0.0000000 1.3078982
feature4 0.0000000 3.2428056 3.7911241 2.7347872 4.879769 0.5297764 2.0855984
feature5 1.2798450 0.9407102 2.2232705 1.1160362 0.000000 1.9968466 0.4667102
```

```

feature6 1.3863951 0.0000000 1.4386228 0.5044165 2.045562 2.7941617 0.0000000
      subject8 subject9  subject10
feature1 2.712745 3.705652 0.02105356
feature2 0.000000 1.978800 3.02481591
feature3 0.000000 1.360440 4.46378210
feature4 4.359349 0.000000 2.72457641
feature5 0.000000 0.000000 0.00000000
feature6 3.102040 0.000000 0.43647014

> head(colData(exampleSE2)$grouping)

[1] 1 0 1 1 1 1

>

```

4 Data Analysis

Finally, we perform differential abundance analysis using SummarizedExperiment object created in the last section. This can be done by using function SDA. The theory behind SDA can be reached at section 5. A list with point estimates, p-values, q-values and corresponding feature names is returned. Below is the results generated by using the SummarizedExperiment object exampleSE1.

```

> results <- SDA(exampleSE1)
> head(results$gamma)

[1] 0.1100009 0.8629447 -0.7151261 0.2876821 -0.1251631 0.6292037

> head(results$pv_gamma)

[1] 0.8290701 0.1703578 0.2356975 0.5571851 0.7227301 0.1248023

> head(results$qv_gamma)

[1] 0.4092097 0.1914385 0.2191259 0.3505466 0.3887992 0.1651784

```

In this example, there is only one group covariate applied to each subject. Here \mathbf{X}_i is one dimension. The covariate effect on the fraction of zero values for feature g is γ_g , which is estimated to be 0.11 for the first feature, and 0.86 for the second feature, etc. The corresponding hypothesis is $H_0: \gamma_g = 0$ vs. $H_1: \gamma_g \neq 0$. The p-values calculated from likelihood ratio test are returned in `pv_gamma`. Users can determine their own significance level to make inference, such as 0.05 nominal level. We also provide a

FDR adjustment method [2] used in SDA for multiple comparison issues. Those results for γ_g are stored in `qv_gamma`.

```
> head(results$beta)

[1] -0.04912170 -1.11354659 -1.30566809  0.02484749  0.53967121 -0.22075205

> head(results$pv_beta)

[1] 0.88988924 0.11334266 0.06081223 0.95500620 0.06770261 0.13832035

> head(results$qv_beta)

[1] 0.7466379 0.2979437 0.2525717 0.7599717 0.2673415 0.3235222
```

The model parameter β_g is the log fold change in the non-zero abundance comparing different values of the single group covariate for feature g . The corresponding two-sided hypothesis is $H_0: \beta_g = 0$ vs. $H_1: \beta_g \neq 0$. Again, SDA will return p-values and adjusted p-values(q-values) for parameter β_g , and they are stored in `pv_beta` and `qv_beta` respectively.

```
> head(results$pv_2part)

      [,1]
[1,] 0.96764635
[2,] 0.11153821
[3,] 0.08538208
[4,] 0.84038731
[5,] 0.17695215
[6,] 0.10266642

> head(results$qv_2part)

      [,1]
[1,] 0.4622205
[2,] 0.1380966
[3,] 0.1192246
[4,] 0.4323376
[5,] 0.1806273
[6,] 0.1345612
```

Hypothesis testing on overall effect of group covariate on the g th feature is performed by assessing γ_g and β_g . The null hypothesis $H_0: \gamma_g = 0$ and $\beta_g = 0$ against alternative hypothesis $H_1: \text{at least one}$

of the two parameters is non-zero. The p-values are calculated based on chi-square distribution with 2 degrees of freedom. And the corresponding q-values are calculated using the same procedure as in one-part model.

```
> head(results$feat.names)

[1] "93922" "29633" "40225" "126342" "42832" "127351"
```

A vector of feature names is returned for convenience which corresponds to the results in the other components.

5 Theory for SDAMS

As mentioned in the abstract, MS data is a mixture of zero intensity values and possibly non-normally distributed non-zero intensity values. Therefore, the differential abundance analysis needs to be performed to compare both the zero proportion and the mean of non-zero values between groups and also allows adjustment of covariates. SDA is a two-part model which addresses these issues that uses a logistic regression model to characterize the zero proportion and a semiparametric model to characterize non-zero values.

5.1 A two-part semi-parametric model

The differential abundance analysis in SDAMS has the following forms. For binary part:

$$\log\left(\frac{\pi_{ig}}{1 - \pi_{ig}}\right) = \gamma_{0g} + \gamma_g \mathbf{X}_i,$$

For continuous non-zero part:

$$\log(Y_{ig}) = \beta_g \mathbf{X}_i + \varepsilon_{ig},$$

where Y_{ig} is the random variable representing the abundance of feature g in subject i , $\pi_{ig} = Pr(Y_{ig} = 0)$ is the probability of point mass. $\mathbf{X}_i = (X_{i1}, X_{i2}, \dots, X_{iQ})^T$ is a Q -vector covariates that specifies the treatment conditions applied to subject i . The corresponding Q -vector of model parameters $\gamma_g = (\gamma_{1g}, \gamma_{2g}, \dots, \gamma_{Qg})^T$ quantify the covariates effects on the fraction of zero values for feature g and γ_{0g} is the intercept. $\beta_g = (\beta_{1g}, \beta_{2g}, \dots, \beta_{Qg})$ is a Q -vector of model parameters quantifying the covariates effects on the non-zero values for the feature, and ε_{ig} 's ($i = 1, 2, \dots, n$) are independent error terms with a common but completely unspecified density function f_g . Importantly, we do not impose any distributional assumption on f_g . Without assuming a specific parametric distribution for ε_{ig} , this model is much more flexible to characterize data with unknown and possibly non-normal distribution.

5.2 Identification of differentially abundant features

We replace f_g by its kernel density estimator in the likelihood function. The maximum likelihood estimator is obtained through a trust region maximization algorithm. The likelihood ratio test is performed on the null hypothesis $H_0 : \gamma_{qg} = 0$ and $\beta_{qg} = 0$ against alternative hypothesis H_1 : at least one of the two parameters is non-zero. We also consider the hypotheses for testing $\gamma_{qg} = 0$ and $\beta_{qg} = 0$ separately. To adjust for multiple comparisons across features, the false discovery rate (FDR) q-value is calculated based on the `qvalue` function in `qvalue` package in R/Bioconductor.

6 Session Info

```
> toLatex(sessionInfo())
```

- R version 4.0.0 alpha (2020-04-05 r78150), x86_64-apple-darwin17.0
- Locale: C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
- Running under: macOS Mojave 10.14.6
- Matrix products: default
- BLAS:


```
/Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
```
- LAPACK:


```
/Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
```
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: Biobase 2.47.3, BiocGenerics 0.33.3, DelayedArray 0.13.10, GenomInfoDb 1.23.16, GenomicRanges 1.39.3, IRanges 2.21.8, S4Vectors 0.25.15, SDAMS 1.7.1, SummarizedExperiment 1.17.5, matrixStats 0.56.0
- Loaded via a namespace (and not attached): GenomInfoDbData 1.2.2, Matrix 1.2-18, R6 2.4.1, RCurl 1.98-1.1, Rcpp 1.0.4.6, XVector 0.27.2, assertthat 0.2.1, bitops 1.0-6, cli 2.0.2, colorspace 1.4-1, compiler 4.0.0, crayon 1.3.4, dplyr 0.8.5, ellipsis 0.3.0, fansi 0.4.1, ggplot2 3.3.0, glue 1.4.0, grid 4.0.0, gtable 0.3.0, lattice 0.20-41, lifecycle 0.2.0, magrittr 1.5, munsell 0.5.0, pillar 1.4.3, pkgconfig 2.0.3, plyr 1.8.6, purrr 0.3.3, qvalue 2.19.0, reshape2 1.4.3, rlang 0.4.5, scales 1.1.0, splines 4.0.0, stringi 1.4.6, stringr 1.4.0, tibble 3.0.0, tidyr 1.0.0, tools 4.0.0, trust 0.1-8, vctrs 0.2.4, zlibbioc 1.33.1

References

- [1] Justyna Siwy, William Mullen, Igor Golovko, Julia Franke, and Petra Zürgb. Human urinary peptide database for multiple disease biomarker discovery. *PROTEOMICS-Clinical Applications*, 5(5-6):367–374, 2011.
- [2] John D Storey and Robert Tibshirani. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences*, 100(16):9440–9445, 2003.