

Extracting sparse mutational signatures via LASSO

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Overview. Point mutations occurring in a genome can be divided into 96 categories based on the base being mutated, the base it is mutated into and its two flanking bases. Therefore, for any patient, it is possible to represent all the point mutations occurring in that patient's tumor as a vector of length 96, where each element represents the count of mutations for a given category in the patient.

A mutational signature represents the pattern of mutations produced by a mutagen or mutagenic process inside the cell. Each signature can also be represented by a vector of length 96, where each element represents the probability that this particular mutagenic process generates a mutation of the 96 above mentioned categories. In this R package, we provide a set of functions to extract and visualize the mutational signatures that best explain the mutation counts of a large number of patients.

In this vignette, we give an overview of the package by presenting some of its main functions.

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1 Changelog

2.0.0 Migration from Travis-CI to Github Actions and Major refactoring.

1.0.4 Move NMF to Depends section.

1.0.3 Issue with the basis function solved.

1.0.0 package released on Bioconductor in May 2018.

2 Algorithms and useful links

Acronym	Extended name	Reference
SparseSignatures	De Novo Mutational Signature Discovery in Tumor Genomes using SparseSignatures	Publication

3 Using the SparseSignatures R package

We now present the main features of the package. To start, we show how to load data and transform them to a count matrix to perform the signatures discovery; first we load some example data provided in the package.

```
library("SparseSignatures")

## Loading required package: NMF
## Loading required package: pkgmaker
## Loading required package: registry
## Loading required package: rngtools
## Loading required package: cluster

## NMF - BioConductor layer [OK] | Shared memory capabilities [NO: windows] | Cores
79/80

data(ssm560_reduced)
head(ssm560_reduced)
```

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##	sample	chrom	start	end	ref	alt
## 1:	PD10014a	1	186484577	186484577	A	C
## 2:	PD10014a	7	141761948	141761948	G	A
## 3:	PD10014a	7	71266228	71266228	C	T
## 4:	PD10014a	8	82304475	82304475	A	T
## 5:	PD10014a	3	191275626	191275626	T	A
## 6:	PD10014a	4	135265376	135265376	C	T

These data are a reduced version with only 3 patients of the 560 breast tumors provided by Nik-Zainal, Serena, et al. (2016). We can transform such input data to a count matrix to perform the signatures discovery with the function `import.counts.data`. To do so, we also need to specify the reference genome as a `BSgenome` object and the format of the 96 nucleotides to be considered. This can be done as follows, where in the example we use `hs37d5` as our reference genome.

```
library("BSgenome.Hsapiens.1000genomes.hs37d5")

## Loading required package: BSgenome
## Loading required package: S4Vectors
## Loading required package: stats4
##
## Attaching package: 'S4Vectors'
## The following object is masked from 'package:NMF':
##
##     nrun
## The following object is masked from 'package:pkgmaker':
##
##     new2
## The following objects are masked from 'package:base':
##
##     I, expand.grid, unname
## Loading required package: IRanges
##
## Attaching package: 'IRanges'
## The following object is masked from 'package:grDevices':
##
##     windows
## Loading required package: GenomeInfoDb
## Loading required package: GenomicRanges
## Loading required package: Biostrings
## Loading required package: XVector
##
## Attaching package: 'Biostrings'
```

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```
## The following object is masked from 'package:base':
```

```
##
```

```
##      strsplit
```

```
## Loading required package: rtracklayer
```

```
bsg = BSgenome.Hsapiens.1000genomes.hs37d5
```

```
data(mutation_categories)
```

```
head(mutation_categories)
```

```
##      context alt      cat
```

```
## 1:      A:A C>A A[C>A]A
```

```
## 2:      C:A C>A C[C>A]A
```

```
## 3:      G:A C>A G[C>A]A
```

```
## 4:      T:A C>A T[C>A]A
```

```
## 5:      A:A C>G A[C>G]A
```

```
## 6:      C:A C>G C[C>G]A
```

```
imported_data = import.trinucleotides.counts(data=ssm560_reduced,reference=bsg,mutation_categories=mutation_categories)
```

```
data(imported_data)
```

```
head(imported_data)
```

```
##      A[C>A]A A[C>A]C A[C>A]G A[C>A]T A[C>G]A A[C>G]C A[C>G]G A[C>G]T A[C>T]A
```

```
## PD10010a      37      25      8      24      35      5      16      25      49
```

```
## PD10011a     103      59      16      73     113      54      31     102     116
```

```
## PD10014a     235     241      37     234     158      71      26     180     229
```

```
##      A[C>T]C A[C>T]G A[C>T]T A[T>A]A A[T>A]C A[T>A]G A[T>A]T A[T>C]A A[T>C]C
```

```
## PD10010a      31     100      42      21      15      17      30      48      20
```

```
## PD10011a      73     228     109      61      70      56     165     184     116
```

```
## PD10014a      89     178     186     105      90     126     174     261     122
```

```
##      A[T>C]G A[T>C]T A[T>G]A A[T>G]C A[T>G]G A[T>G]T C[C>A]A C[C>A]C C[C>A]G
```

```
## PD10010a      29      44      8      6      10      23      34      28      8
```

```
## PD10011a     113     169      77      41      73     105     105      75     30
```

```
## PD10014a     167     211      76      27      84      59     244     238     35
```

```
##      C[C>A]T C[C>G]A C[C>G]C C[C>G]G C[C>G]T C[C>T]A C[C>T]C C[C>T]G C[C>T]T
```

```
## PD10010a      23      15      19      20      26      48      37      55      43
```

```
## PD10011a     102      60      37      22      65      71      52     108     103
```

```
## PD10014a     243     107     105      40     144     136     124     144     197
```

```
##      C[T>A]A C[T>A]C C[T>A]G C[T>A]T C[T>C]A C[T>C]C C[T>C]G C[T>C]T C[T>G]A
```

```
## PD10010a      12      7      18      16      14      17      20      30      6
```

```
## PD10011a     116      80      89     103     103      78     102     158     40
```

```
## PD10014a     116     139     145     217     103     144     112     129     47
```

```
##      C[T>G]C C[T>G]G C[T>G]T G[C>A]A G[C>A]C G[C>A]G G[C>A]T G[C>G]A G[C>G]C
```

```
## PD10010a      8      5      13      31      22      11      22      6      12
```

```
## PD10011a      65      55     188      78      50      14      55      55     66
```

```
## PD10014a      54      70     107     146     126      24     160      63     70
```

```
##      G[C>G]G G[C>G]T G[C>T]A G[C>T]C G[C>T]G G[C>T]T G[T>A]A G[T>A]C G[T>A]G
```

```
## PD10010a      9      14      40      32      82      25      6      6      6
```

```
## PD10011a      13      87      76      63     118      81      69      41     56
```

```
## PD10014a      25     120     141      99     180     163      62      66     83
```

```
##      G[T>A]T G[T>C]A G[T>C]C G[T>C]G G[T>C]T G[T>G]A G[T>G]C G[T>G]G G[T>G]T
```

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```
## PD10010a      13      22      9      16      24      7      1      8      10
## PD10011a      86      96     62     82     93     56     46     35     99
## PD10014a     126     110     81     102    135     32     18     61     78
##              T[C>A]A T[C>A]C T[C>A]G T[C>A]T T[C>G]A T[C>G]C T[C>G]G T[C>G]T T[C>T]A
## PD10010a      40      40      12     48     54     37     12     85     67
## PD10011a      78      80      12     83    116    104     29    194    119
## PD10014a     202     191      17    253    198    159     33    325    188
##              T[C>T]C T[C>T]G T[C>T]T T[T>A]A T[T>A]C T[T>A]G T[T>A]T T[T>C]A T[T>C]C
## PD10010a      55      53      71     39     13      3     35     19     13
## PD10011a      94      78     126    121     43     64     91    125     79
## PD10014a     153      93     184    124     89     73    221    143    118
##              T[T>C]G T[T>C]T T[T>G]A T[T>G]C T[T>G]G T[T>G]T
## PD10010a      11      25      18     11     11     35
## PD10011a      83     113     68     90    140    251
## PD10014a      75     148     71     54     76    160
```

The function `import.counts.data` can also take a text file as input with the same format as the one shown above. Now, we show an example of a visualization feature provided by the package, and we show the counts for the first patient PD10010a in the following plot.

```
patients.plot(trinucleotides_counts=imported_data,samples="PD10010a")
```

After the data are loaded, signatures can be discovered. To do so, we need to define a set of parameters on which to perform the estimation.

First of all, we need to specify the ranges for the number of signatures (variable K) and the LASSO penalty value (variable λ rate) to be considered. The latter is more complicated to estimate, as it requires that the values in the range not to be too small in order to avoid dense signatures, but also should not be too high in order to still perform a good fit of the observed counts.

Besides these parameters, we also need to estimate the initial values of β to be used during the estimation. We now show how to do this on the set of counts from 560 tumors provided in Nik-Zainal, Serena, et al. (2016).

```
data(patients)
head(patients)

##              A[C>A]A A[C>A]C A[C>A]G A[C>A]T A[C>G]A A[C>G]C A[C>G]G A[C>G]T A[C>T]A
## PD8623a      24      23       4     20     10     19      2     11     43
## PD8618a      29      19       2     15     11     12      2      8     31
## PD6418a      23      29       4     26     12      9      1     12     39
## PD7214a      19      20       5     18     11      5      4      7     30
## PD4968a      59      64       5     34     25     16      1     18     81
## PD4954a     102     87      19     82     80     48     13     88    117
##              A[C>T]C A[C>T]G A[C>T]T A[T>A]A A[T>A]C A[T>A]G A[T>A]T A[T>C]A A[T>C]C
## PD8623a      25      77      28     16     12     23     37     57      7
## PD8618a      17      91      24     10     10      8     18     50     23
## PD6418a      36     104      36     13     19     26     22     53     19
## PD7214a      22      65      21     12     18     17     18     41     12
## PD4968a      57     246      70     26     46     53     66     93     39
## PD4954a      53     125      79     64     48     37     52     97     41
##              A[T>C]G A[T>C]T A[T>G]A A[T>G]C A[T>G]G A[T>G]T C[C>A]A C[C>A]C C[C>A]G
```

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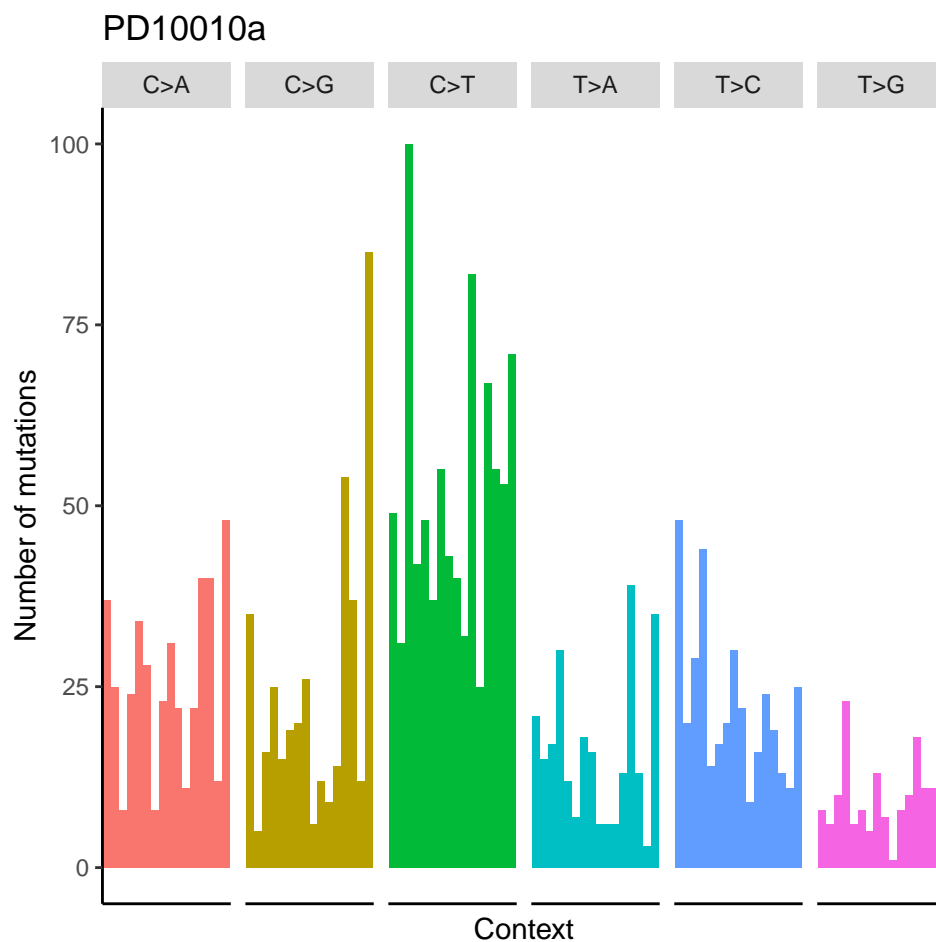


Figure 1: Visualization of the counts from patient PD10010a from the dataset published in Nik-Zainal, Serena, et al.

##	PD8623a	30	42	12	6	8	16	32	21	6
##	PD8618a	31	59	1	3	6	7	18	15	3
##	PD6418a	32	57	7	4	6	8	24	19	2
##	PD7214a	23	43	4	5	3	9	15	13	1
##	PD4968a	47	85	17	6	7	16	45	27	10
##	PD4954a	64	97	26	11	38	41	100	90	18
##		C[C>A]T	C[C>G]A	C[C>G]C	C[C>G]G	C[C>G]T	C[C>T]A	C[C>T]C	C[C>T]G	C[C>T]T
##	PD8623a	26	13	13	4	19	32	40	73	31
##	PD8618a	14	4	9	4	3	21	33	61	30
##	PD6418a	23	15	15	4	8	42	36	71	51
##	PD7214a	10	7	5	2	12	31	32	48	40
##	PD4968a	53	13	15	14	27	82	88	145	79
##	PD4954a	83	77	48	22	65	90	64	84	99
##		C[T>A]A	C[T>A]C	C[T>A]G	C[T>A]T	C[T>C]A	C[T>C]C	C[T>C]G	C[T>C]T	C[T>G]A
##	PD8623a	10	10	10	11	14	15	15	23	3
##	PD8618a	6	4	7	5	11	17	10	13	4
##	PD6418a	6	13	9	14	19	8	13	14	6

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```
## PD7214a      9      4      3      6      8      9      9      8      0
## PD4968a     13     25     20     36     22     24     29     37     7
## PD4954a     41     48     55     57     46     53     40     74     17
##           C[T>G]C C[T>G]G C[T>G]T G[C>A]A G[C>A]C G[C>A]G G[C>A]T G[C>G]A G[C>G]C
## PD8623a      7     14     15     13     20      3     13      9      2
## PD8618a      4      6      5     17     13      9     14      2     10
## PD6418a      8      8     14     20     20      9     16      5      6
## PD7214a      7      8     12     24      7      2      8      6      6
## PD4968a     10      7     24     35     25     12     30      9     13
## PD4954a     19     37     42     53     67     13     42     40     28
##           G[C>G]G G[C>G]T G[C>T]A G[C>T]C G[C>T]G G[C>T]T G[T>A]A G[T>A]C G[T>A]G
## PD8623a      1      6     33     24     61     29      3     11      6
## PD8618a      0      5     23     33     67     29      3     12      4
## PD6418a      3      5     35     39     94     34      7     12      9
## PD7214a      3      4     31     47     50     24      1      8      6
## PD4968a      1     11     68     62    190     65      8     21     14
## PD4954a      1     63     72     69     85     67     19     29     22
##           G[T>A]T G[T>C]A G[T>C]C G[T>C]G G[T>C]T G[T>G]A G[T>G]C G[T>G]G G[T>G]T
## PD8623a      6     15     10      6     23      1      3      5      4
## PD8618a      5     17     10      8     23      0      1      1      0
## PD6418a      8     36     11     22     22      1      3      3      6
## PD7214a      8     26     12      8     18      1      3      2      2
## PD4968a     18     43     19     29     35      6      3      3     11
## PD4954a     49     61     37     34     54     12      7     32     36
##           T[C>A]A T[C>A]C T[C>A]G T[C>A]T T[C>G]A T[C>G]C T[C>G]G T[C>G]T T[C>T]A
## PD8623a     34     24      8     31     22     20      1     32    119
## PD8618a     22     17     10     25     15     14      1     30     47
## PD6418a     34     23      5     35      9     12      2     24     43
## PD7214a     14     22      6     24      9      7      2     24     52
## PD4968a     79     57      9     87     64     27      8    120    464
## PD4954a     92    109     11    106    158     89     17    279    166
##           T[C>T]C T[C>T]G T[C>T]T T[T>A]A T[T>A]C T[T>A]G T[T>A]T T[T>C]A T[T>C]C
## PD8623a     59     52     98     29     15      6     18     25     17
## PD8618a     26     37     37     20      4      3     13     21     12
## PD6418a     56     52     65     31      9      9     15     25     17
## PD7214a     38     41     62     14      8      7     16     19     14
## PD4968a    177    157    337    127     20     19     42     41     42
## PD4954a    114     48    150     62     44     27     71     58     38
##           T[T>C]G T[T>C]T T[T>G]A T[T>G]C T[T>G]G T[T>G]T
## PD8623a     11     26      9     11     10     27
## PD8618a     12     16      4      3      6     11
## PD6418a      9     36      9      6      9     20
## PD7214a     13     22      4     10      8     19
## PD4968a     23     44     15      8     15     38
## PD4954a     30     57     40     29     37     62
```

First, we can estimate the initial values of beta as follows.

```
starting_betas = startingBetaEstimation(x=patients,K=3:12,background_signature=background)
```

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Then, we also need to explore the search space of values for the LASSO penalty in order to make a good choice. To do so, we can use the function `lambdaRangeBetaEvaluation` to test different values to sparsify beta as follows. Notice that the package also provides the option to sparsify alpha and, in this case, we may use the function `lambdaRangeAlphaEvaluation` to explore the search space of values.

```
lambda_range = lambdaRangeBetaEvaluation(x=patients,K=10,beta=starting_betas[[8,1]],
                                         lambda_values=c(0.05,0.10))
```

As the executions of these functions can be very time-consuming, we also provide as examples together with the package a set of pre-computed results by the two functions `startingBetaEstimation` and `lambdaRangeBetaEvaluation` obtained with the commands above.

```
data(starting_betas_example)
data(lambda_range_example)
```

Now that we have evaluated all the required parameters, we need to decide which configuration of number of signatures and lambda value is the best. To do so, we rely on cross-validation.

```
cv = nmfLassoCV(x=patients,K=3:10)
```

We notice that the computations for this task can be very time consuming, especially when many iterations of cross validations are specified (see manual) and a large set of configurations of the parameters are tested. To speed up the execution, we suggest using the parallel execution options. Also, to reduce the memory requirements, we advise splitting the cross validation in different runs, e.g., if one wants to perform 100 iterations, we would suggest making 10 independent runs of 10 iterations each. Also in this case, we provide as examples together with the package a set of pre-computed results obtained with the above command and the following settings: $K = 3:10$, cross validation entries = 0.10, lambda values = `c(0.05,0.10,0.15)`, number of iterations of cross-validation = 2.

```
data(cv_example)
```

Finally, we can compute the signatures for the best configuration, i.e., $K = 5$.

```
beta = starting_betas_example[["5_signatures","Value"]]
res = nmfLasso(x = patients, K = 5, beta = beta, background_signature = background, seed = 12345)

## Performing the discovery of the signatures by NMF with Lasso...
## Performing a total of 30 iterations...
## Progress 3.333333333333333%...
## Progress 6.666666666666667%...
## Progress 10%...
## Progress 13.333333333333333%...
## Progress 16.666666666666667%...
## Progress 20%...
## Progress 23.333333333333333%...
## Progress 26.666666666666667%...
## Progress 30%...
## Progress 33.333333333333333%...
## Progress 36.666666666666667%...
## Progress 40%...
## Progress 43.333333333333333%...
```


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```
## Progress 46.6666666666667%...
## Progress 50%...
## Progress 53.3333333333333%...
## Progress 56.6666666666667%...
## Progress 60%...
## Progress 63.3333333333333%...
## Progress 66.6666666666667%...
## Progress 70%...
## Progress 73.3333333333333%...
## Progress 76.6666666666667%...
## Progress 80%...
## Progress 83.3333333333333%...
## Progress 86.6666666666667%...
## Progress 90%...
## Progress 93.3333333333333%...
## Progress 96.6666666666667%...
## Progress 100%...

## Warning in nmfLassoDecomposition(x, beta, lambda_rate_alpha, lambda_rate_beta,
: The likelihood is not increasing, you should try a lower value of lambda! Current
settings: K = 6, lambda_rate_alpha = 0.05, lambda_rate_beta = 0.05...
```

We conclude this vignette by plotting the discovered signatures.

```
data(nmf_LassoK_example)
signatures = nmf_LassoK_example$beta
signatures.plot(beta=signatures, xlabels=FALSE)
```

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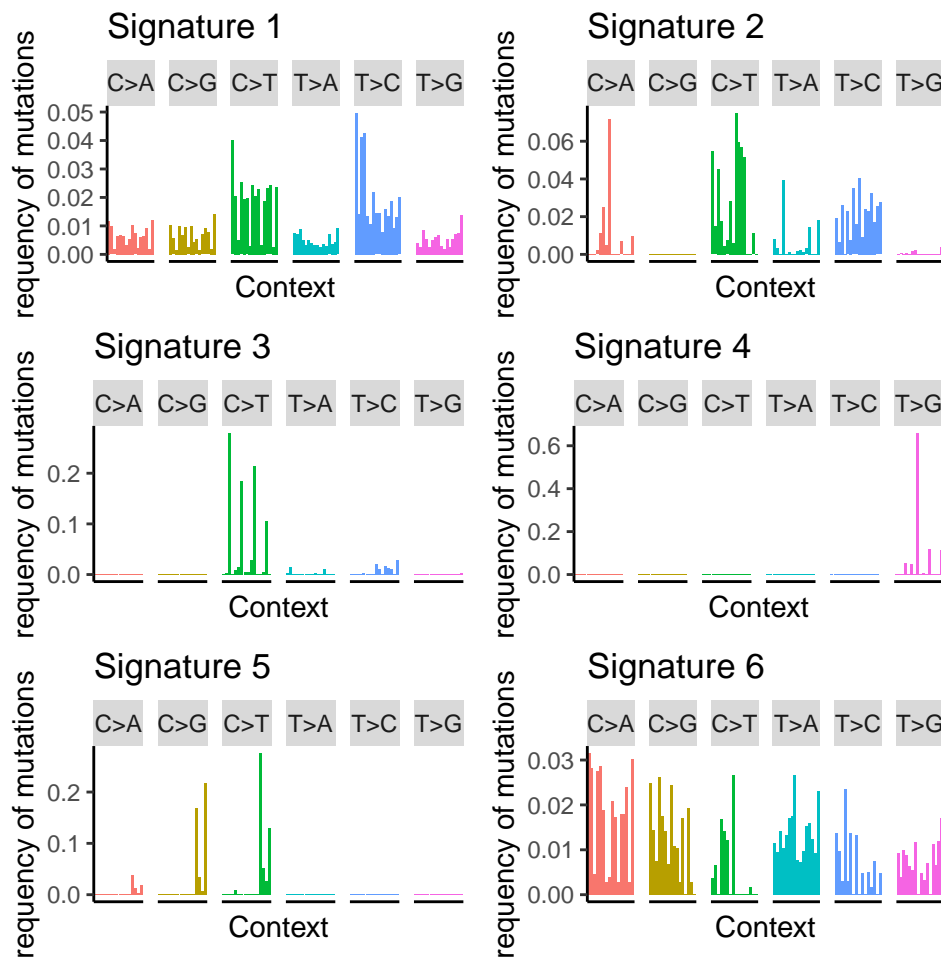


Figure 2: Visualization of the discovered signatures.

4 sessionInfo()

- R version 4.1.0 RC (2021-05-10 r80283), x86_64-w64-mingw32
- Locale: LC_COLLATE=C, LC_CTYPE=English_United States.1252, LC_MONETARY=English_United States.1252, LC_NUMERIC=C, LC_TIME=English_United States.1252
- Running under: Windows Server x64 (build 17763)
- Matrix products: default
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: BSgenome 1.60.0, BSgenome.Hsapiens.1000genomes.hs37d5 0.99.1, Biobase 2.52.0, BiocGenerics 0.38.0, Biostrings 2.60.0, GenomInfoDb 1.28.0, GenomicRanges 1.44.0, IRanges 2.26.0, NMF 0.23.0, S4Vectors 0.30.0, SparseSignatures 2.2.0, XVector 0.32.0, cluster 2.1.2, knitr 1.33, pkgmaker 0.32.2, registry 0.5-1, rngtools 1.5, rtracklayer 1.52.0

Extracting sparse mutational signatures via LASSO

- Loaded via a namespace (and not attached): BiocIO 1.2.0, BiocManager 1.30.15, BiocParallel 1.26.0, BiocStyle 2.20.0, DBI 1.1.1, DelayedArray 0.18.0, GenomeInfoDbData 1.2.6, GenomicAlignments 1.28.0, Matrix 1.3-3, MatrixGenerics 1.4.0, R6 2.5.0, RColorBrewer 1.1-2, RCurl 1.98-1.3, Rcpp 1.0.6, Rsamtools 2.8.0, SummarizedExperiment 1.22.0, XML 3.99-0.6, assertthat 0.2.1, bitops 1.0-7, codetools 0.2-18, colorspace 2.0-1, compiler 4.1.0, crayon 1.4.1, data.table 1.14.0, digest 0.6.27, doParallel 1.0.16, dplyr 1.0.6, ellipsis 0.3.2, evaluate 0.14, fansi 0.4.2, farver 2.1.0, foreach 1.5.1, generics 0.1.0, ggplot2 3.3.3, glue 1.4.2, grid 4.1.0, gridBase 0.4-7, gridExtra 2.3, gtable 0.3.0, highr 0.9, htmltools 0.5.1.1, iterators 1.0.13, labeling 0.4.2, lattice 0.20-44, lifecycle 1.0.0, magrittr 2.0.1, matrixStats 0.58.0, munsell 0.5.0, nnlasso 0.3, nnls 1.4, pillar 1.6.1, pkgconfig 2.0.3, plyr 1.8.6, purrr 0.3.4, reshape2 1.4.4, restfulr 0.0.13, rjson 0.2.20, rlang 0.4.11, rmarkdown 2.8, scales 1.1.1, stringi 1.6.2, stringr 1.4.0, tibble 3.1.2, tidyrselect 1.1.1, tools 4.1.0, utf8 1.2.1, vctrs 0.3.8, withr 2.4.2, xfun 0.23, xtable 1.8-4, yaml 2.2.1, zlibbioc 1.38.0