

Extracting sparse mutational signatures via LASSO

Avantika Lal¹, Keli Liu², Luca De Sano³, Robert Tibshirani², Arend Sidow^{1,4}, and Daniele Ramazzotti^{1,3,5}

¹Department of Pathology, Stanford University, Stanford, CA , USA.

²Department of Statistics, Stanford University, Stanford, CA , USA.

³Department of Informatics, Systems and Communication, Università degli Studi Milano Bicocca Milano, Italy.

⁴Department of Genetics, Stanford University, Stanford, CA , USA.

⁵Department of Computer Science, Stanford University, Stanford, CA , USA.

⁶Department Medicine and Surgery, Università degli Studi Milano Bicocca Milano, Italy.

October 26, 2021

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.

Contents

- 1 Changelog. 2
- 2 Algorithms and useful links 2
- 3 Using the SparseSignatures R package. 2
- 4 `sessionInfo()`. 10

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: synchronicity]  
| Cores 71/72
```

Extracting sparse mutational signatures via LASSO

```
## To enable shared memory capabilities, try: install.extras('
## NMF
## ')

data(ssm560_reduced)
head(ssm560_reduced)

##      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
## Loading required package: GenomeInfoDb
## Loading required package: GenomicRanges
## Loading required package: Biostrings
## Loading required package: XVector
##
## Attaching package: 'Biostrings'
```

Extracting sparse mutational signatures via LASSO

```
## 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)
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
## 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
```

Extracting sparse mutational signatures via LASSO

```
##          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")
```

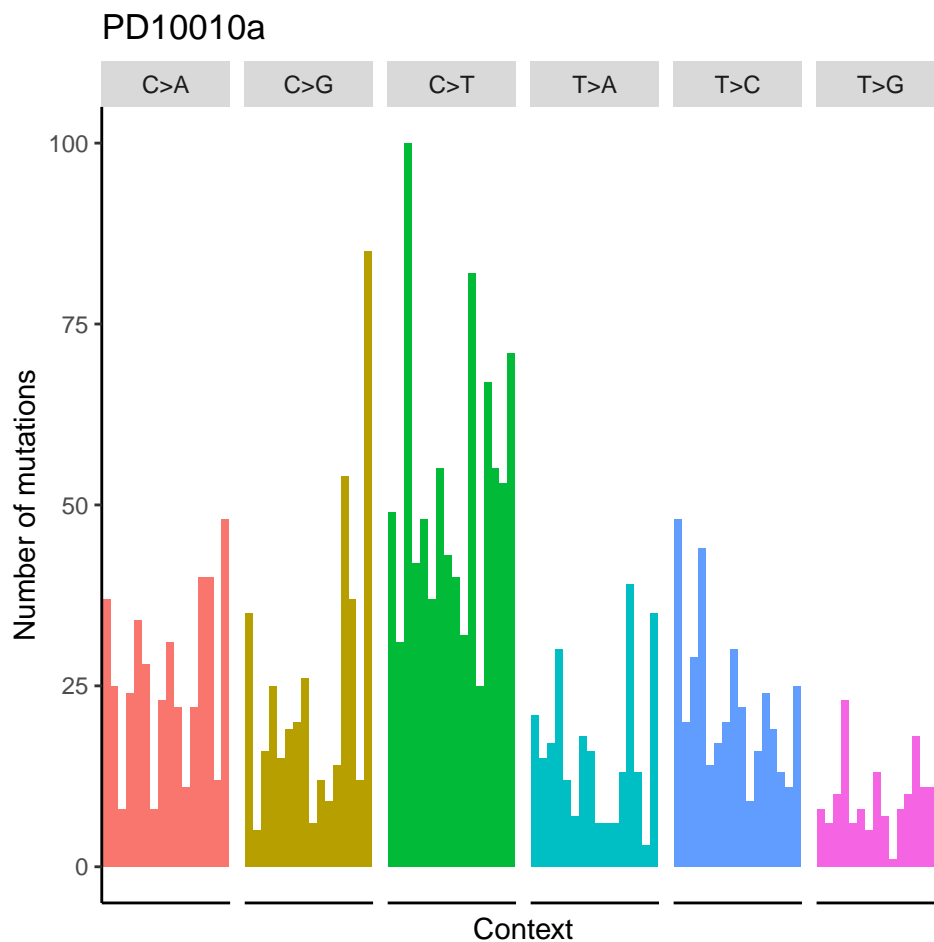


Figure 1: Visualization of the counts from patient PD10010a from the dataset published in Nik-Zainal, Serena, et al

Extracting sparse mutational signatures via LASSO

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 lambda 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 beta 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
## 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
## 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
```

Extracting sparse mutational signatures via LASSO

##	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)
```

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.

Extracting sparse mutational signatures via LASSO

```
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%...  
## Progress 46.666666666666667%...  
## Progress 50%...  
## Progress 53.333333333333333%...  
## Progress 56.666666666666667%...  
## Progress 60%...
```


Extracting sparse mutational signatures via LASSO

```
## Progress 63.333333333333%...
## Progress 66.666666666667%...
## Progress 70%...
## Progress 73.333333333333%...
## Progress 76.666666666667%...
## Progress 80%...
## Progress 83.333333333333%...
## Progress 86.666666666667%...
## Progress 90%...
## Progress 93.333333333333%...
## Progress 96.666666666667%...
## 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)
```

Extracting sparse mutational signatures via LASSO

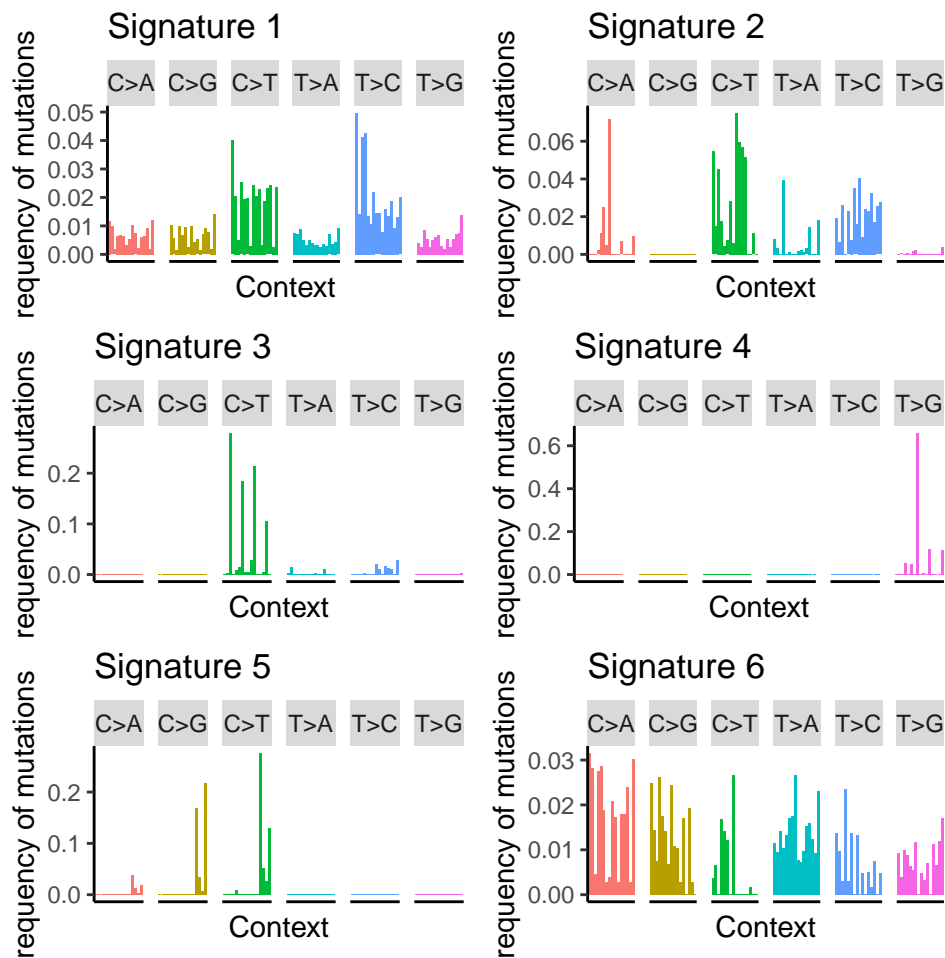


Figure 2: Visualization of the discovered signatures

4 sessionInfo()

- R version 4.1.1 (2021-08-10), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_GB, LC_COLLATE=C, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Running under: Ubuntu 20.04.3 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.14-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.14-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, stats, stats4, utils

Extracting sparse mutational signatures via LASSO

- Other packages: BSgenome 1.62.0, BSgenome.Hsapiens.1000genomes.hs37d5 0.99.1, Biobase 2.54.0, BiocGenerics 0.40.0, Biostrings 2.62.0, GenomInfoDb 1.30.0, GenomicRanges 1.46.0, IRanges 2.28.0, NMF 0.23.0, S4Vectors 0.32.0, SparseSignatures 2.4.0, XVector 0.34.0, bigmemory 4.5.36, cluster 2.1.2, knitr 1.36, pkgmaker 0.32.2, registry 0.5-1, rngtools 1.5.2, rtracklayer 1.54.0
- Loaded via a namespace (and not attached): BiocIO 1.4.0, BiocManager 1.30.16, BiocParallel 1.28.0, BiocStyle 2.22.0, DBI 1.1.1, DelayedArray 0.20.0, GenomInfoDbData 1.2.7, GenomicAlignments 1.30.0, Matrix 1.3-4, MatrixGenerics 1.6.0, R6 2.5.1, RColorBrewer 1.1-2, RCurl 1.98-1.5, Rcpp 1.0.7, Rsamtools 2.10.0, SummarizedExperiment 1.24.0, XML 3.99-0.8, assertthat 0.2.1, bigmemory.sri 0.1.3, bitops 1.0-7, codetools 0.2-18, colorspace 2.0-2, compiler 4.1.1, crayon 1.4.1, data.table 1.14.2, digest 0.6.28, doParallel 1.0.16, dplyr 1.0.7, ellipsis 0.3.2, evaluate 0.14, fansi 0.5.0, farver 2.1.0, fastmap 1.1.0, foreach 1.5.1, generics 0.1.1, ggplot2 3.3.5, glue 1.4.2, grid 4.1.1, gridBase 0.4-7, gridExtra 2.3, gtable 0.3.0, highr 0.9, htmltools 0.5.2, iterators 1.0.13, labeling 0.4.2, lattice 0.20-45, lifecycle 1.0.1, magrittr 2.0.1, matrixStats 0.61.0, munsell 0.5.0, nnlasso 0.3, nnls 1.4, parallel 4.1.1, pillar 1.6.4, 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 0.4.12, rmarkdown 2.11, scales 1.1.1, stringi 1.7.5, stringr 1.4.0, tibble 3.1.5, tidyselect 1.1.1, tools 4.1.1, utf8 1.2.2, vctrs 0.3.8, withr 2.4.2, xfun 0.27, xtable 1.8-4, yaml 2.2.1, zlibbioc 1.40.0