

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)
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
```

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```
##          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")
```

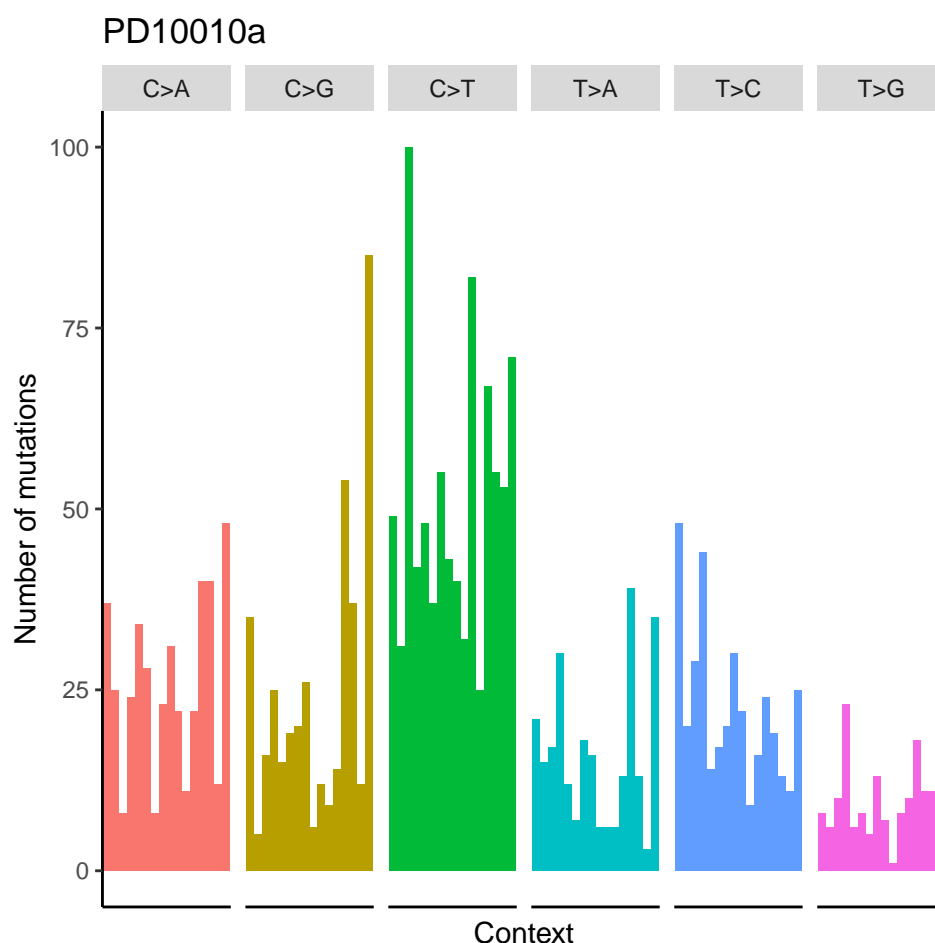


Figure 1: Visualization of the counts from patient PD10010a from the dataset published in Nik-Zainal, Serena, et al.

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

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##	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.

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```
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%...
```


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```
## 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)
```

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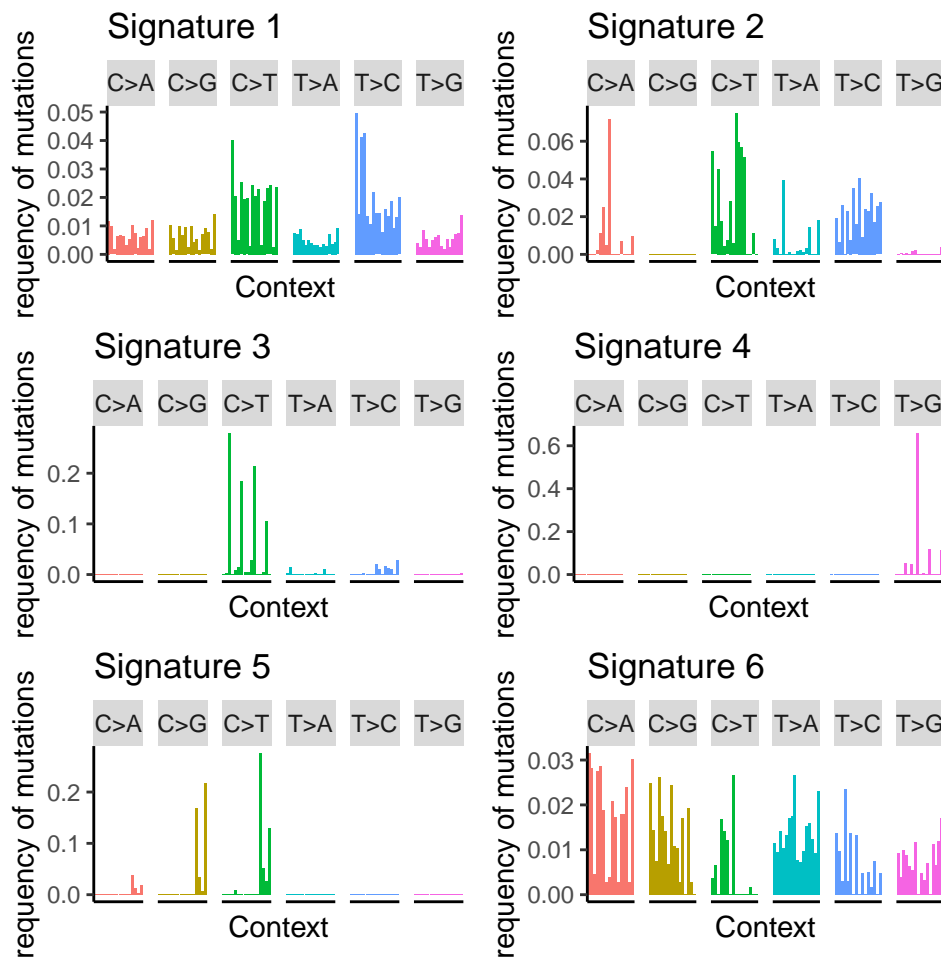


Figure 2: Visualization of the discovered signatures.

4 sessionInfo()

- R version 4.1.1 (2021-08-10), 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, stats, stats4, utils
- 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, cluster 2.1.2, knitr 1.36, pkgmaker 0.32.2, registry 0.5-1, rngtools 1.5.2, rtracklayer 1.54.0

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- 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, 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