

Extracting sparse mutational signatures via LASSO

Daniele Ramazzotti^{1,2}, Avantika Lal¹, Keli Liu³, Luca De Sano⁴, Robert Tibshirani³, and Arend Sidow^{1,5}

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

²Department of Computer Science, Stanford University, Stanford, CA , USA.

³Department of Statistics, Stanford University, Stanford, CA , USA.

⁴Dipartimento di Informatica Sistemistica e Comunicazione, Università degli Studi Milano Bicocca Milano, Italy.

⁵Department of Genetics, Stanford University, Stanford, CA , USA.

April 28, 2020

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	<code>sessionInfo()</code>	11

1 Changelog

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
39/40

data(ssm560_reduced)
head(ssm560_reduced)

##      sample chrom      pos ref alt
## 1: PD10014a    1 186484577    A    C
## 2: PD10014a    7 141761948    G    A
## 3: PD10014a    7  71266228    C    T
```

Extracting sparse mutational signatures via LASSO

```
## 4: PD10014a      8  82304475  A   T
## 5: PD10014a      3 191275626  T   A
## 6: PD10014a      4 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 object is masked from 'package:base':
##
##   expand.grid
## 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'
## The following object is masked from 'package:base':
##
##   strsplit
## Loading required package: rtracklayer
```

Extracting sparse mutational signatures via LASSO

```

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.counts.data(input=ssm560_reduced,bsg=bsg,mutation_categories=mutation_categories)

## Warning in import.counts.data(input = ssm560_reduced, bsg = bsg, mutation_categories
= mutation_categories): Some samples have fewer than 100 mutations:
## PD10010a, PD10011a, PD10014a

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
##      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

```

Extracting sparse mutational signatures via LASSO

##	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.

```
patient.plot(countMatrix=imported_data,patientName="PD10010a")
```

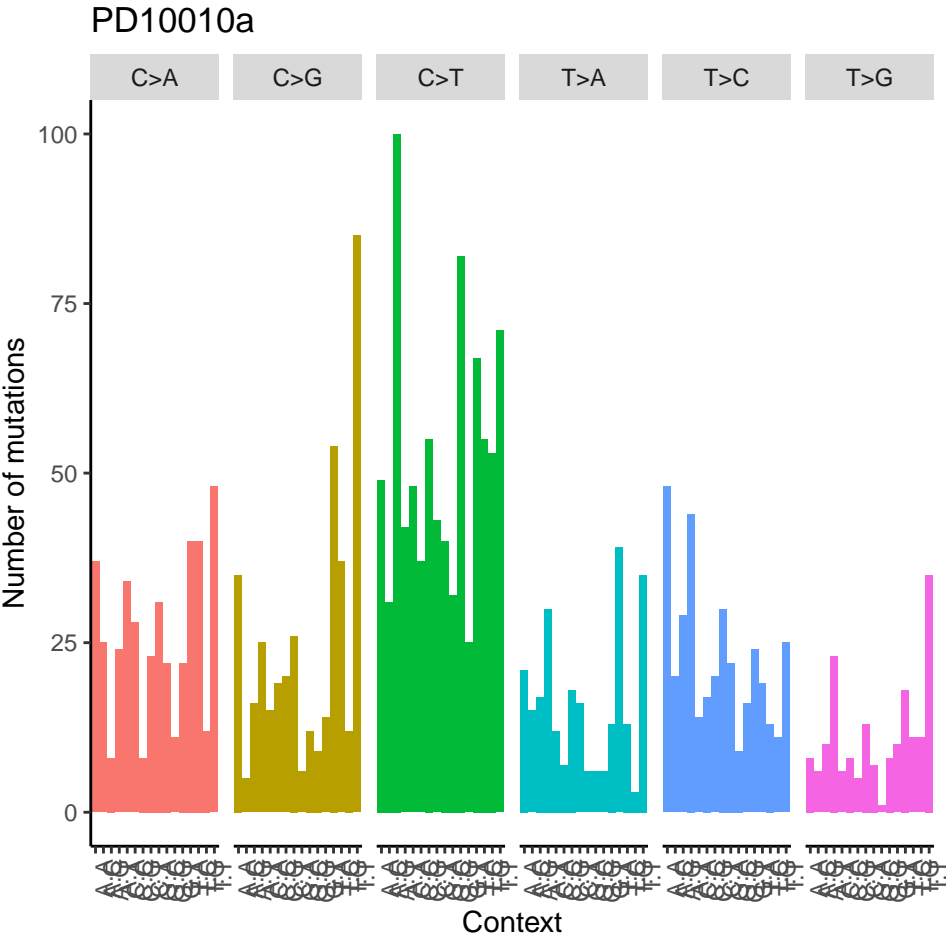


Figure 1: Visualization of the counts from patient PD10010a from the dataset published in Nik-Zainal, Serena, et al

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 = starting_betas.estimate(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 `evaluate.lambda.range` to test different values as follows.

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

Extracting sparse mutational signatures via LASSO

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 `starting.betas.estimation` and `evaluate.lambda.range` obtained with the commands above.

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

To evaluate the best lambda range, we need to carefully consider the log-likelihood of the solutions at each iteration of our method. This can be done by exploiting the `as.` functions that we provide. Here are some examples.

```
# example of using too small a value of lambda
# the log-likelihood is very unstable across the iterations
res = as.loglik.progression.in.range(lambda.range.result=lambda_range_example, lambda_value=0.01)
```

```
plot(res)
```

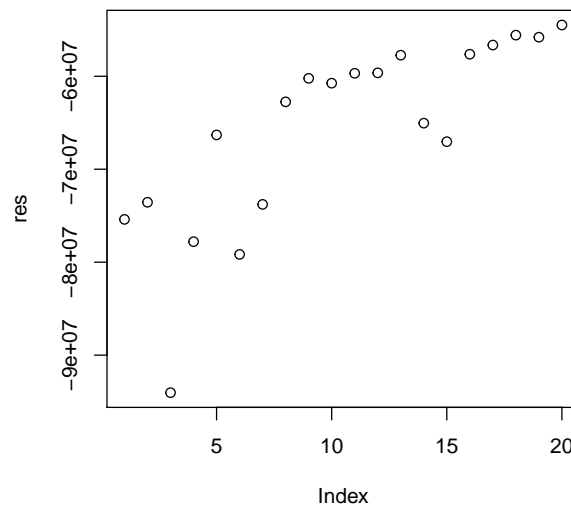


Figure 2: Example of using too small a value of lambda: the log-likelihood is very unstable across the iterations

```
# example of using too high a value of lambda
# the log-likelihood drops after the first iteration
res = as.loglik.progression.in.range(lambda.range.result=lambda_range_example, lambda_value=0.30)
```

```
plot(res)
```

```
# example of using a good value of lambda
# the log-likelihood is increasing across the iterations
res = as.loglik.progression.in.range(lambda.range.result=lambda_range_example, lambda_value=0.15)
```

```
plot(res)
```

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.

Extracting sparse mutational signatures via LASSO

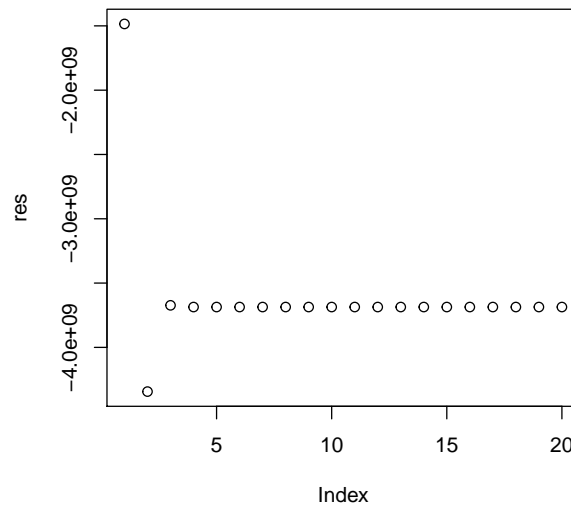


Figure 3: Example of using too high a value of lambda: the log-likelihood drops after the first iteration

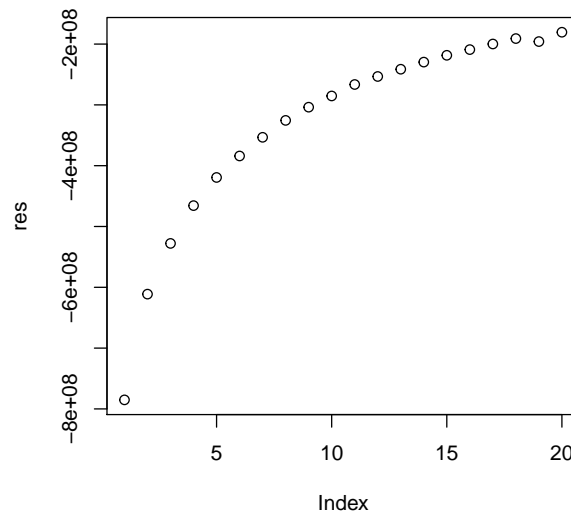


Figure 4: Example of using a good value of lambda: the log-likelihood is increasing across the iterations

```
cv = nmf.LassoCV(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.

Extracting sparse mutational signatures via LASSO

```
data(cv_example)
```

We can now estimate the best configuration of the parameters in terms of median mean squared error by cross validation, where the best configuration is the one with lowest error.

```
res = as.mean.squared.error(cv_example)$median
res_best = which(res==res[which.min(res)],arr.ind=TRUE)
best_K = rownames(res)[res_best[1]]
best_lambda = colnames(res)[res_best[2]]
best_K

## [1] "5_signatures"

best_lambda

## [1] "0.1_lambda"
```

Finally, we can compute the signatures for the best configuration, i.e., $K = 5$ and $\lambda = 0.10$.

```
beta = starting_betas_example[["5_signatures", "Value"]]
res = nmf.LassoK(x=patients, K=5, beta=beta, background=background, lambda_rate=0.10,
                iterations=5, num_processes=NA)

## Performing the discovery of the signatures by NMF with Lasso...
## Performing a total of 5 iterations...
## Progress 20%...
## Progress 40%...
## Progress 60%...
## Progress 80%...
## Progress 100%...
```

We conclude this vignette by plotting the discovered signatures.

```
signatures = as.beta(res)
signatures.plot(beta=signatures, xlabels=FALSE)

## Warning in melt(beta, varnames = c("signature", "cat")): The melt generic in
data.table has been passed a matrix and will attempt to redirect to the relevant
reshape2 method; please note that reshape2 is deprecated, and this redirection is
now deprecated as well. To continue using melt methods from reshape2 while both
libraries are attached, e.g. melt.list, you can prepend the namespace like reshape2::melt(beta).
In the next version, this warning will become an error.
```

Extracting sparse mutational signatures via LASSO

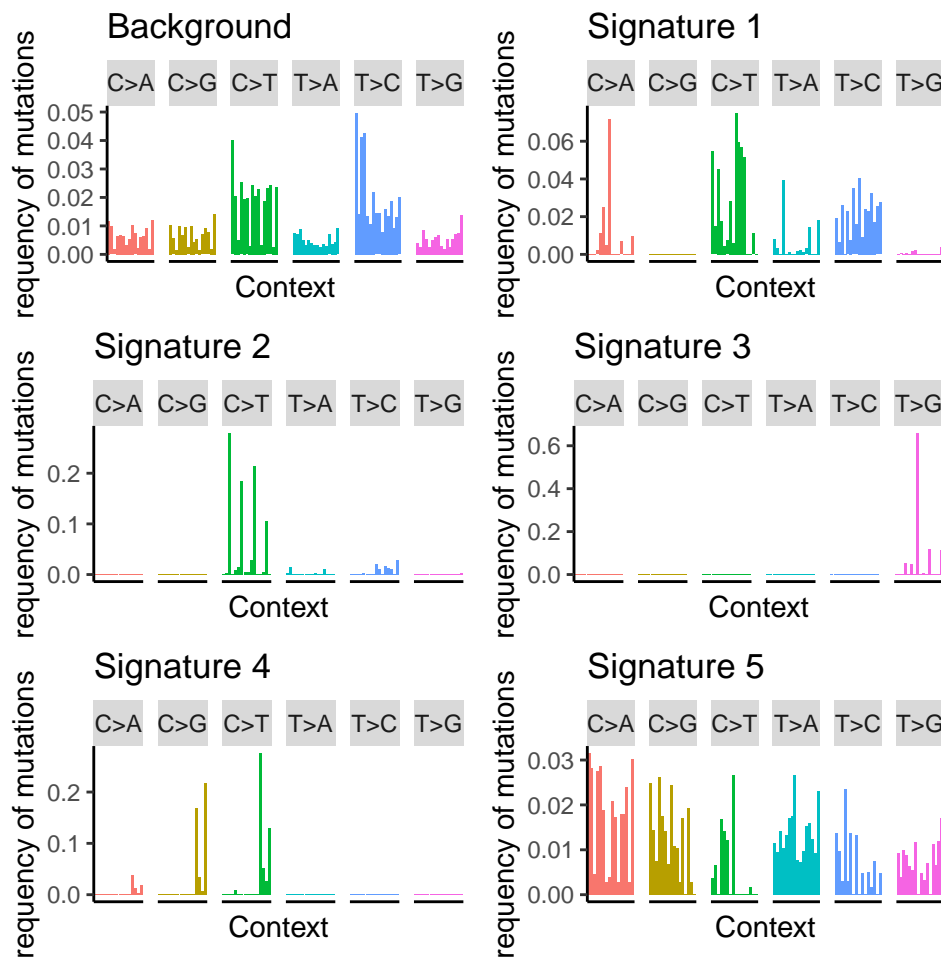


Figure 5: Visualization of the discovered signatures

4 sessionInfo()

- R version 4.0.0 (2020-04-24), 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 2012 R2 x64 (build 9600)
- Matrix products: default
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: BSgenome 1.56.0, BSgenome.Hsapiens.1000genomes.hs37d5 0.99.1, Biobase 2.48.0, BiocGenerics 0.34.0, Biostrings 2.56.0, GenomeInfoDb 1.24.0, GenomicRanges 1.40.0, IRanges 2.22.0, NMF 0.22.0, S4Vectors 0.26.0, SparseSignatures 1.8.0, XVector 0.28.0, cluster 2.1.0, knitr 1.28, pkgmaker 0.31.1, registry 0.5-1, rngtools 1.5, rtracklayer 1.48.0

Extracting sparse mutational signatures via LASSO

- Loaded via a namespace (and not attached): BiocManager 1.30.10, BiocParallel 1.22.0, BiocStyle 2.16.0, DelayedArray 0.14.0, GenomeInfoDbData 1.2.3, GenomicAlignments 1.24.0, Matrix 1.2-18, R6 2.4.1, RColorBrewer 1.1-2, RCurl 1.98-1.2, Rcpp 1.0.4.6, Rsamtools 2.4.0, SummarizedExperiment 1.18.0, XML 3.99-0.3, assertthat 0.2.1, bibtex 0.4.2.2, bitops 1.0-6, codetools 0.2-16, colorspace 1.4-1, compiler 4.0.0, crayon 1.3.4, data.table 1.12.8, digest 0.6.25, doParallel 1.0.15, dplyr 0.8.5, ellipsis 0.3.0, evaluate 0.14, farver 2.0.3, foreach 1.5.0, ggplot2 3.3.0, glue 1.4.0, grid 4.0.0, gridBase 0.4-7, gridExtra 2.3, gtable 0.3.0, highr 0.8, htmltools 0.4.0, iterators 1.0.12, labeling 0.3, lattice 0.20-41, lifecycle 0.2.0, magrittr 1.5, matrixStats 0.56.0, munsell 0.5.0, nnlasso 0.3, nnls 1.4, pillar 1.4.3, pkgconfig 2.0.3, plyr 1.8.6, purrr 0.3.4, reshape2 1.4.4, rlang 0.4.5, rmarkdown 2.1, scales 1.1.0, stringi 1.4.6, stringr 1.4.0, tibble 3.0.1, tidyselect 1.0.0, tools 4.0.0, vctrs 0.2.4, withr 2.2.0, xfun 0.13, xtable 1.8-4, yaml 2.2.1, zlibbioc 1.34.0