YAPS

Daniel Huebschmann

26/08/2015

Contents

1 Introduction

The concept of mutational signatures was introduced in a series of papers by Ludmil Alexandrov et al. (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013) and (Alexandrov, Nik-Zainal, Wedge, Campbell, et al. 2013). A computational framework was published (Alexandrov 2012) with the purpose to detect a limited number of mutational processes which then describe the whole set of SNVs (single nucleotide variants) in a cohort of cancer samples. The general approach (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013) is as follows:

1. The SNVs are categorized by their nucleotide exchange. In total there are $4 \times 3 = 12$ different nucleotide exchanges, but if summing over reverse complements only $12/2 = 6$ different categories are left. For every SNV detected, the motif context around the position of the SNV is extracted. This may be a trinucleotide context if taking one base upstream and one base downstream of the position of the SNV, but larger motifs may be taken as well (e.g. pentamers). Taking into account the motif context increases combinatorial complexity: in the case of the trinucleotide context, there are $4 \times 6 \times 4 = 96$ different variant categories. These categories are called **features** in the following text. The number of features will be called n.
2. A cohort consists of different samples with the number of samples denoted by m. For each sample we can count the occurences of each feature, yielding an n-dimensional vector (n being the number of features) per sample. For a cohort, we thus get an $n \times m$ -dimensional matrix, called the **mutational catalogue** V. It can be understood as a summary indicating which sample has how many variants of which category, but omitting the information of the genomic coordinates of the variants.

3. The mutational catalogue V is quite big and still carries a lot of complexity. For many analyses a reduction of complexity is desirable. One way to achieve such a complexity reduction is a matrix decomposition: we would like to find two smaller matrices W and H which if multiplied would span a high fraction of the complexity of the big matrix V (the mutational catalogue). Remember that V is an $n \times m$ -dimensional matrix, n being the number of features and m being the number of samples. W in this setting is an $n \times l$ -dimensional matrix and H is an $l \times m$ -dimensional matrix. According to the nomeclature defined in (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013), the columns of W are called the **mutational signatures** and the columns of H are called **exposures**. l denotes the number of mutational signatures. Hence the signatures are n-dimensional vectors (with n being the number of features), while the exposures are l-dimensional vectors (l being the number of signatures). Note that as we are dealing with count data, we would like to have only positive entries in W and H. A mathematical method which is able to do such a decomposition is the **NMF** (**nonnegative matrix factorization**). It basically solves the problem as illustrated in the following figure:

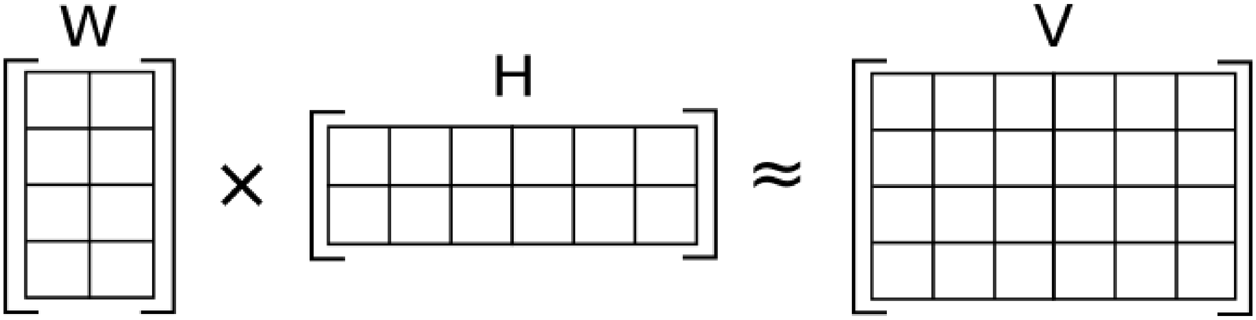


Figure 1: NMF, Image taken from https://en.wikipedia.org/wiki/Non-negative_matrix_factorization

Of course the whole concept only leads to a reduction in complexity if $l < n$, i.e. if the number of signatures is smaller than the number of features, as indicated in the above figure. Note that the NMF itself solves the above problem for a given number of signatures l. The framework of Ludmil Alexandrov et al. (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013) performs not only the NMF decomposition itself, but also identifies the appropriate number of signatures by an iterative procedure.

Another package to perform analyses of mutational signatures is available (Gehring et al. 2015) which also allows the matrix decomposition to be performed by PCA (principal component analysis). Both methods have in common that they can be used for **discovery**, i.e. for the **extraction of new signatures**. However, they only work well if the analyzed data set has a minimum size, i.e. a minimum number of samples and minimum numbers of counts per feature per sample.

2 The YAPS package

In a context where mutational signatures W are already known (because they were decribed and published as in (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013) or they are available in a database as under http://cancer.sanger.ac.uk/cosmic/signatures), we might want to just find the exposures H for these known signatures in the mutational catalogue V of a given cohort. Mathematically, this is a different and simpler task.

The **YAPS**-package (Yet Another Package for Signature analysis) presented here provides the function `LCD` (**l**inear **c**ombination **d**ecomposition) to perform this task. The advantage of this method is that there are **no constraints on the cohort size**, so `LCD` can be run for as little as one sample and thus be used e.g. for signature analysis in personalized oncology. In contrast to NMF, `LCD` is very fast and requires very little computational resources. The YAPS package provides additional functions for signature analysis, e.g. for stratifying the mutational catalogue to determine signature exposures in different strata, part of which will be discussed later in this vignette.

2.1 Linear Combination Decomposition (LCD)

In the following, we will denote the columns of V by $V_{(\cdot j)}$, which corresponds to the mutational catalogue of sample j. Analogously we denote the columns of H by $H_{(\cdot j)}$, which is the exposure vector of sample j. Then `LCD` is designed to solve the optimization problem:

$$\min_{H_{(\cdot j)} \in \mathbb{R}^l} ||W \cdot H_{(\cdot j)} - V_{(\cdot j)}|| \quad \forall j \in \{1...m\} \tag{1}$$

$$\text{under the constraint of non-negativity:} \quad H_{(ij)} >= 0 \quad \forall i \in \{1...l\} \quad \forall j \in \{1...m\}$$

Remember that j is the index over samples, m is the number of samples, i is the index over signatures and l is the number of signatures. `LCD` uses the function `lsei` from the package limsolve (Soetaert, Van den Meersche, and Van Oevelen 2009) and (Van den Meersche, Soetaert, and Van Oevelen 2009). Note that the optimization procedure is carried out for every $V_{(\cdot j)}$, i.e. for every column of V separately. Of course W is constant, i.e. the same for every $V_{(\cdot j)}$.

This procedure is highly sensitive: as soon as a signature has a contribution or an exposure in at least one sample of a cohort, it will be reported (within the floating point precision of the operating system). This might blur the picture and counteracts the initial purpose of complexity reduction. Therefore there is a function `LCD_cutoff`. This function takes as a second argument a cutoff (a value between zero and one). In the analysis, it will keep only those signatures which have a cumulative (over the cohort) normalized exposure greater than this cutoff. In fact it runs the LCD-procedure twice: once to find initial exposures, summing over the cohort and excluding the ones with too low a contribution as described just above, and a second time doing the analysis only with the signatures left over. Beside the exposures H corresponding to this reduced set of signatures, the function `LCD_cutoff` also returns the reduced set of signatures itself.

2.2 Stratification of the Mutational Catalogue (SMC)

For some questions it is useful to assign the SNVs detected in the samples of a cohort to categories. In the following these categories have to be exclusive, i.e. one SNV must be in one and only one category. The categories will be called **strata** in the following and the procedure **stratification**. The number of strata will be denoted by s. For example one could evaluate whether an SNV falls into a region of high, intermediate or low mutation density by applying meaningful cutoffs on intermutation distance. Following the above convention, there are three strata: high, intermediate and low mutation density. If we have already performed an analysis of mutational signatures for the whole mutational catalogue of the cohort, we have identified a set of signatures of interest and the corresponding exposures. We now could ask the question if some of the signatures are enriched or depleted in one or the other stratum, yielding a strata-specific signature enrichment and depletion pattern. The function `SMC` (Stratification of the Mutational Catalogue) solves the stratified optimization problem:

$$\min_{H^k_{(\cdot j)} \in \mathbb{R}^l} ||W \cdot H^k_{(\cdot j)} - V^k_{(\cdot j)}|| \quad \forall j, k \tag{2}$$

$$\text{under the constraint of non-negativity:} \quad H^k_{(ij)} >= 0 \quad \forall i, j, k$$

$$\text{and the additional contraint:} \quad \sum_{k=1}^{s} H^k = H$$

$$\text{where H is defined by the optimization:} \quad \min_{H_{(\cdot j)} \in \mathbb{R}^l} ||W \cdot H_{(\cdot j)} - V_{(\cdot j)}|| \quad \forall j$$

$$\text{also under the constraint of non-negativity:} \quad H_{(ij)} >= 0 \quad \forall i, j \quad \text{and} \quad V = \sum_{k=1}^{s} V^k$$

Remember that j is the index over samples, m is the number of samples, i is the index over signatures, l is the number of signatures, k is the index over strata and s is the number of strata. Note that the last two lines of equation (2) correspond to equation (1). The very last part of equation (2) reflects the additivity of the stratified mutational catalogues V^k which is due to the fact that by definition the sets of SNVs they were constructed from (i.e. the strata) are exclusive.

The SMC-procedure can also be applied when an NMF analysis has been performed and the exposures $\widetilde{H}$ of this NMF analysis should be used as input and constraint for the SMC. It then solves the task:

$$(3) \qquad \begin{aligned} \min_{H^k_{(\cdot j)} \in \mathbb{R}^l} ||W \cdot H^k_{(\cdot j)} - V^k_{(\cdot j)}|| \quad \forall j,k \\ \text{under the constraint of non-negativity:} \quad H^k_{(ij)} >= 0 \quad \forall i,j,k \\ \text{and the additional contraint:} \quad \sum_{k=1}^{s} H^k = \widetilde{H} \end{aligned}$$

Applying `SMC` that way, the initial LCD decomposition of the unstratified mutational catalogue is omitted and it's result replaced by the exposures extracted by the NMF analysis.

3 Example: a cohort of B-cell lymphomas

We will now apply some functions of the YAPS package to Whole Genome Sequencing datasets published in Alexandrov et al. (2013) First we have to load this data and get an overview (first subsection). Then we will load data on published signatures (second subsection). Only in the third subsection we will actually start using the YAPS functions.

```
library(knitr)
opts_chunk$set(echo=TRUE)
opts_chunk$set(fig.show='asis')
```

3.1 Example data

In the following, we will load and get an overview of the data used in the analysis by Alexandrov et al. (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013)

3.1.1 Loading example data

```
lymphoma_Nature2013_ftp_path <- paste0("ftp://ftp.sanger.ac.uk/pub/cancer/AlexandrovEtAl/",
                                       "somatic_mutation_data/Lymphoma B-cell/",
                                       "Lymphoma B-cell_clean_somatic_mutations_",
                                       "for_signature_analysis.txt")
lymphoma_Nature2013_raw_df <- read.csv(file=lymphoma_Nature2013_ftp_path,
                                       header=FALSE,sep="\t")
```

This created a data frame with 128639 rows. The format is inspired by the vcf format with one line per called variant. Note that the files provided at that url have no header information, therefore we have to add some. We will also slightly adapt the data structure:

```r
names(lymphoma_Nature2013_raw_df) <- c("PID","TYPE","CHROM","START",
                                       "STOP","REF","ALT","FLAG")
lymphoma_Nature2013_df <- subset(lymphoma_Nature2013_raw_df,TYPE=="subs",
                                 select=c(CHROM,START,REF,ALT,PID))
names(lymphoma_Nature2013_df)[2] <- "POS"
head(lymphoma_Nature2013_df)
```

```
##   CHROM       POS REF ALT      PID
## 1     1 183502381   G   A 07-35482
## 2    18  60985506   T   A 07-35482
## 3    18  60985748   G   T 07-35482
## 4    18  60985799   T   C 07-35482
## 5     2 242077457   A   G 07-35482
## 6     6  13470412   C   T 07-35482
```

Here, we have selected only the variants characterized as `subs` (those are the single nucleotide variants we are interested in for the mutational signatures analysis, small indels are filtered out by this step), so we are left with 128212 variants or rows. Note that there are 48 different samples:

```r
unique(lymphoma_Nature2013_df$PID)
```

```
##  [1] 07-35482       1060           1061           1065
##  [5] 1093           1096           1102           4101316
##  [9] 4105105        4108101        4112512        4116738
## [13] 4119027        4121361        4125240        4133511
## [17] 4135350        4142267        4158726        4159170
## [21] 4163639        4175837        4177856        4182393
## [25] 4189200        4189998        4190495        4193278
## [29] 4194218        4194891        515            DLBCL-PatientA
## [33] DLBCL-PatientB DLBCL-PatientC DLBCL-PatientD DLBCL-PatientE
## [37] DLBCL-PatientF DLBCL-PatientG DLBCL-PatientH DLBCL-PatientI
## [41] DLBCL-PatientJ DLBCL-PatientK DLBCL-PatientL DLBCL-PatientM
## [45] EB2            FL009          FL-PatientA    G1
## 48 Levels: 07-35482 1060 1061 1065 1093 1096 1102 4101316 ... G1
```

For convenience later on, we annotate subgroup information to every variant (indirectly through the sample it occurs in). For reasons of simplicity, we also restrict the analysis to the Whole Genome Sequencing (WGS) datasets:

```r
library(YAPS)
lymphoma_Nature2013_df$SUBGROUP <- "unknown"
DLBCL_ind <- grep("^DLBCL.*",lymphoma_Nature2013_df$PID)
lymphoma_Nature2013_df$SUBGROUP[DLBCL_ind] <- "DLBCL_other"
MMML_ind <- grep("^41[0-9]+$",lymphoma_Nature2013_df$PID)
lymphoma_Nature2013_df <- lymphoma_Nature2013_df[MMML_ind,]
data(lymphoma_PID)
for(my_PID in rownames(lymphoma_PID_df)) {
  PID_ind <- which(as.character(lymphoma_Nature2013_df$PID)==my_PID)
  lymphoma_Nature2013_df$SUBGROUP[PID_ind] <-
    lymphoma_PID_df$subgroup[which(rownames(lymphoma_PID_df)==my_PID)]
}
lymphoma_Nature2013_df$SUBGROUP <- factor(lymphoma_Nature2013_df$SUBGROUP)
unique(lymphoma_Nature2013_df$SUBGROUP)
```

```
## [1] WGS_D WGS_F WGS_B WGS_I
## Levels: WGS_B WGS_D WGS_F WGS_I
```

3.1.2 Displaying example data

Rainfall plots provide a quick overview of the mutational load of a sample. To this end we have to compute the intermutational distances. But first we still do some reformatting…

```
lymphoma_Nature2013_df <- translate_to_hg19(lymphoma_Nature2013_df,"CHROM")
lymphoma_Nature2013_df$change <- attribute_nucleotide_exchanges(lymphoma_Nature2013_df)
lymphoma_Nature2013_df <- lymphoma_Nature2013_df[order(lymphoma_Nature2013_df$PID,
                                                       lymphoma_Nature2013_df$CHROM,
                                                       lymphoma_Nature2013_df$POS),]
lymphoma_Nature2013_df <- annotate_intermut_dist_cohort(lymphoma_Nature2013_df,
                                                        in_PID.field="PID")
colour_vector <- c("blue","black","red","purple","orange","green")
names(colour_vector) <- levels(lymphoma_Nature2013_df$change)
lymphoma_Nature2013_df$col <- colour_vector[lymphoma_Nature2013_df$change]
```

Now we can select one sample and make the rainfall plot. The plot function used here relies on the package gtrellis by Zuguang Gu (Zuguang Gu 2015).

```
#choice_PID <- "4163639"
choice_PID <- "4121361"
PID_df <- subset(lymphoma_Nature2013_df,PID==choice_PID)
trellis_rainfall_plot(PID_df,in_point_size=unit(0.5,"mm"))
```

This shows a rainfall plot typical for a lymphoma sample with clusters of increased mutation density e.g. at the immunoglobulin loci.

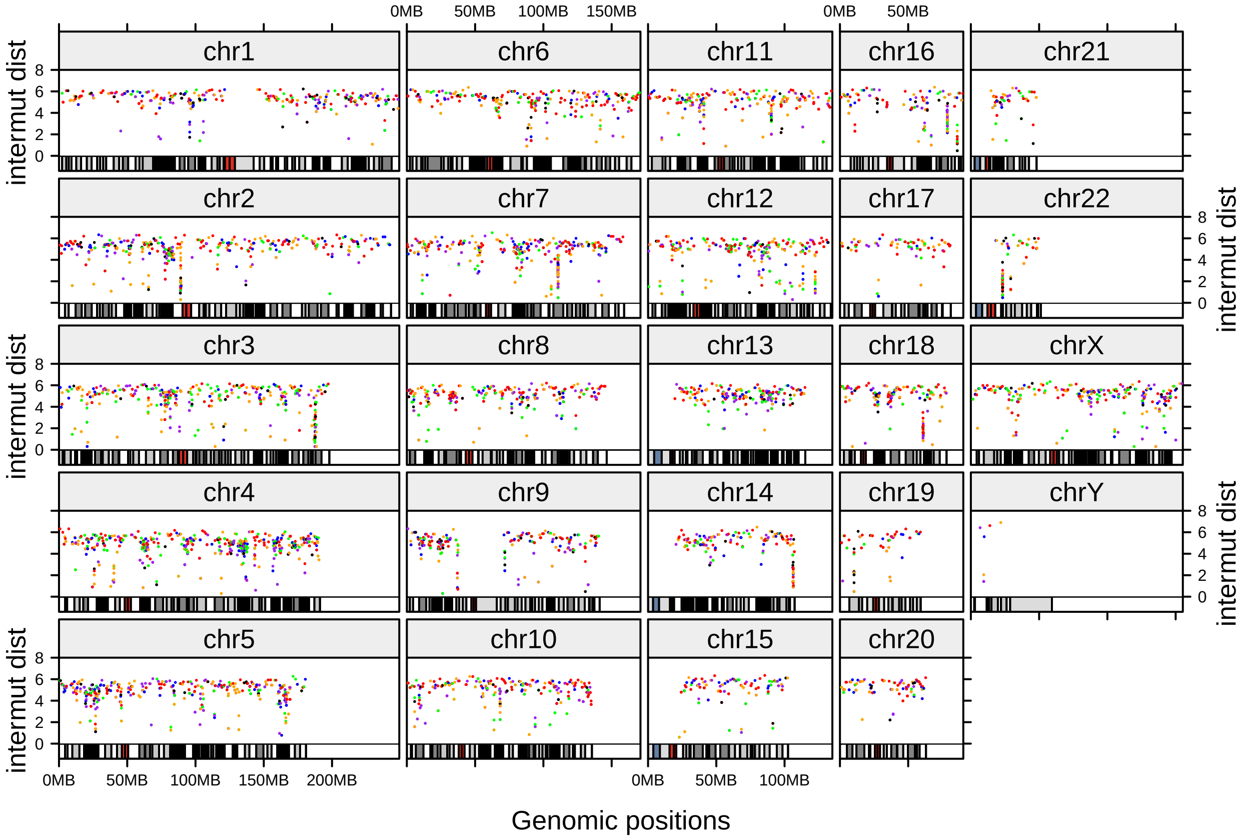


Figure 2: Example rainfall plot in a trellis structure.

3.2 Loading the signature information

As stated above, one of the functions in the YAPS package (LCD) is designed to do mutational signatures analysis with known signatures. There are (at least) two possible sources for signature data: i) the ones published initially by Alexandrov et al. (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013), and ii) an updated and curated current set of mutational signatures is maintained by Ludmil Alexandrov at http://cancer.sanger.ac.uk/cosmic/signatures. The following three subsections describe how you can load the data from these resources. Alternatively, you can bypass the three following subsections because the signature datasets are also included in this package:

```
data(sigs)
```

However, the curated set of signatures might change in the future, therefore it is recommended to rebuild it from the above mentioned website as described in the following subsections.

3.2.1 Loading the initial set of signatures

We first load the (older) set of signatures as published in Alexandrov et al. (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013):

```
Alex_signatures_path <- paste0("ftp://ftp.sanger.ac.uk/pub/cancer/",
                               "AlexandrovEtAl/signatures.txt")
Alex_signatures_df <- read.csv(Alex_signatures_path,header=TRUE,sep="\t")
Alex_signatures_df[c(1:9),c(1:4)]
```

```
##   Substitution.Type Trinucleotide Somatic.Mutation.Type Signature.1A
## 1               C>A           ACA               A[C>A]A       0.0112
## 2               C>A           ACC               A[C>A]C       0.0092
## 3               C>A           ACG               A[C>A]G       0.0015
## 4               C>A           ACT               A[C>A]T       0.0063
## 5               C>A           CCA               C[C>A]A       0.0067
## 6               C>A           CCC               C[C>A]C       0.0074
## 7               C>A           CCG               C[C>A]G       0.0009
## 8               C>A           CCT               C[C>A]T       0.0073
## 9               C>A           GCA               G[C>A]A       0.0083
```

We will now reformat the data frame:

```
Alex_rownames <- paste(Alex_signatures_df[,1],Alex_signatures_df[,2],sep=" ")
select_ind <- grep("Signature",names(Alex_signatures_df))
Alex_signatures_df <- Alex_signatures_df[,select_ind]
number_of_Alex_sigs <- dim(Alex_signatures_df)[2]
names(Alex_signatures_df) <- gsub("Signature\\.","A",names(Alex_signatures_df))
rownames(Alex_signatures_df) <- Alex_rownames
kable(Alex_signatures_df[c(1:9),c(1:6)],
      caption="Exemplary data from the initial Alexandrov signatures.")
```

	A1A	A1B	A2	A3	A4	A5
C>A ACA	0.0112	0.0104	0.0105	0.0240	0.0365	0.0149
C>A ACC	0.0092	0.0093	0.0061	0.0197	0.0309	0.0089

	A1A	A1B	A2	A3	A4	A5
C>A ACG	0.0015	0.0016	0.0013	0.0019	0.0183	0.0022
C>A ACT	0.0063	0.0067	0.0037	0.0172	0.0243	0.0092
C>A CCA	0.0067	0.0090	0.0061	0.0194	0.0461	0.0097
C>A CCC	0.0074	0.0047	0.0012	0.0161	0.0614	0.0050
C>A CCG	0.0009	0.0013	0.0006	0.0018	0.0088	0.0028
C>A CCT	0.0073	0.0098	0.0011	0.0157	0.0432	0.0111
C>A GCA	0.0083	0.0169	0.0093	0.0107	0.0376	0.0119

Table 1: Exemplary data from the initial Alexandrov signatures.

This results in a data frame for signatures, containing 27 signatures as column vectors. It is worth noting that in the initial publication, only a subset of these 27 signatures were validated by an orthogonal sequencing technology. So we can filter down:

```
Alex_validated_signatures_df <- Alex_signatures_df[,grep("^A[0-9]+",
                                          names(Alex_signatures_df))]
number_of_Alex_validated_sigs <- dim(Alex_validated_signatures_df)[2]
```

We are left with 22 signatures.

3.2.2 Loading the updated set of mutational signatures

An updated and curated set of mutational signatures is maintained by Ludmil Alexandrov at http://cancer.sanger.ac.uk/cosmic/signatures. We will use this set for the following analysis:

```
Alex_COSMIC_signatures_path <- paste0("http://cancer.sanger.ac.uk/cancergenome/",
                                      "assets/signatures_probabilities.txt")
Alex_COSMIC_signatures_df <- read.csv(Alex_COSMIC_signatures_path,
                                      header=TRUE,sep="\t")
Alex_COSMIC_rownames <- paste(Alex_COSMIC_signatures_df[,1],
                              Alex_COSMIC_signatures_df[,2],sep=" ")
COSMIC_select_ind <- grep("Signature",names(Alex_COSMIC_signatures_df))
Alex_COSMIC_signatures_df <- Alex_COSMIC_signatures_df[,COSMIC_select_ind]
number_of_Alex_COSMIC_sigs <- dim(Alex_COSMIC_signatures_df)[2]
names(Alex_COSMIC_signatures_df) <- gsub("Signature\\.","AC",
                                         names(Alex_COSMIC_signatures_df))
rownames(Alex_COSMIC_signatures_df) <- Alex_COSMIC_rownames
kable(Alex_COSMIC_signatures_df[c(1:9),c(1:6)],
      caption="Exemplary data from the updated Alexandrov signatures.")
```

	AC1	AC2	AC3	AC4	AC5	AC6
C>A ACA	0.0110983	0.0006827	0.0221723	0.0365	0.0149415	0.0017
C>A ACC	0.0091493	0.0006191	0.0178717	0.0309	0.0089609	0.0028
C>A ACG	0.0014901	0.0000993	0.0021383	0.0183	0.0022078	0.0005

	AC1	AC2	AC3	AC4	AC5	AC6
C>A ACT	0.0062339	0.0003239	0.0162651	0.0243	0.0092069	0.0019
C>G ACA	0.0018011	0.0002635	0.0240026	0.0097	0.0116710	0.0013
C>G ACC	0.0025809	0.0002699	0.0121603	0.0054	0.0072921	0.0012
C>G ACG	0.0005925	0.0002192	0.0052754	0.0031	0.0023038	0.0000
C>G ACT	0.0029640	0.0006110	0.0232777	0.0054	0.0116962	0.0018
C>T ACA	0.0295145	0.0074416	0.0178722	0.0120	0.0218392	0.0312

Table 2: Exemplary data from the updated Alexandrov signatures.

This results in a data frame containing 30 signatures as column vectors. For reasons of convenience and comparability with the initial signatures, we reorder the features. To this end, we adhere to the convention chosen in the initial publication by Alexandrov et al. (Alexandrov, Nik-Zainal, Wedge, Aparicio, et al. 2013) for the initial signatures.

```
COSMIC_order_ind <- match(Alex_rownames,Alex_COSMIC_rownames)
Alex_COSMIC_signatures_df <- Alex_COSMIC_signatures_df[COSMIC_order_ind,]
kable(Alex_COSMIC_signatures_df[c(1:9),c(1:6)],
      caption="Exemplary data from the updated Alexandrov signatures, rows reordered.")
```

	AC1	AC2	AC3	AC4	AC5	AC6
C>A ACA	0.0110983	0.0006827	0.0221723	0.0365	0.0149415	0.0017
C>A ACC	0.0091493	0.0006191	0.0178717	0.0309	0.0089609	0.0028
C>A ACG	0.0014901	0.0000993	0.0021383	0.0183	0.0022078	0.0005
C>A ACT	0.0062339	0.0003239	0.0162651	0.0243	0.0092069	0.0019
C>A CCA	0.0065959	0.0006774	0.0187817	0.0461	0.0096749	0.0101
C>A CCC	0.0073424	0.0002137	0.0157605	0.0614	0.0049523	0.0241
C>A CCG	0.0008928	0.0000068	0.0019634	0.0088	0.0028006	0.0091
C>A CCT	0.0071866	0.0004163	0.0147229	0.0432	0.0110135	0.0571
C>A GCA	0.0082326	0.0003520	0.0096965	0.0376	0.0118922	0.0024

Table 3: Exemplary data from the updated Alexandrov signatures, rows reordered.

Note that the order of the features, i.e. nucleotide exchanges in their trinucleotide content, is changed from the fifth line on as indicated by the row names.

3.2.3 Preparation for later analysis

For every set of signatures, the functions in the YAPS package require an additional data frame containing meta information about the signatures. In that data frame you can specify the order in which the signatures are going to be plotted and the colours asserted to the different signatures. In the following subsection we will set up such a data frame. However, the respective data frames are also stored in the package. If loaded by `data(sigs)` the following code block can be bypassed.

```
signature_colour_vector <- c("darkgreen","green","pink","goldenrod",
                             "lightblue","blue","orangered","yellow",
                             "orange","brown","purple","red",
                             "darkblue","magenta","maroon","yellowgreen",
                             "violet","lightgreen","sienna4","deeppink",
                             "darkorchid","seagreen","grey10","grey30",
                             "grey50","grey70","grey90")
bio_process_vector <- c("spontaneous deamination","spontaneous deamination",
                        "APOBEC","BRCA1_2","Smoking","unknown","defect DNA MMR",
                        "UV light exposure","unknown","IG hypermutation",
                        "POL E mutations","temozolomide","unknown","APOBEC",
                        "unknown","unknown","unknown","unknown","unknown",
                        "unknown","unknown","unknown","nonvalidated",
                        "nonvalidated","nonvalidated","nonvalidated",
                        "nonvalidated")
signatures_indices_df <- data.frame(sig=colnames(Alex_signatures_df))
signatures_indices_df$index <- seq_len(dim(signatures_indices_df)[1])
signatures_indices_df$colour <- signature_colour_vector
signatures_indices_df$process <- bio_process_vector

COSMIC_signature_colour_vector <- c("green","pink","goldenrod",
                                    "lightblue","blue","orangered","yellow",
                                    "orange","brown","purple","red",
                                    "darkblue","magenta","maroon","yellowgreen",
                                    "violet","lightgreen","sienna4","deeppink",
                                    "darkorchid","seagreen","grey","darkgrey",
                                    "black","yellow4","coral2","chocolate2",
                                    "navyblue","plum","springgreen")
COSMIC_bio_process_vector <- c("spontaneous deamination","APOBEC",
                               "defect DNA DSB repair hom. recomb.",
                               "tobacco mutatgens, benzo(a)pyrene",
                               "unknown",
                               "defect DNA MMR, found in MSI tumors",
                               "UV light exposure","unknown","POL eta and SHM",
                               "altered POL E","alkylating agents, temozolomide",
                               "unknown","APOBEC","unknown",
                               "defect DNA MMR","unknown","unknown",
                               "unknown","unknown",
                               "associated w. small indels at repeats",
                               "unknown","aristocholic acid","unknown",
                               "aflatoxin","unknown","defect DNA MMR",
                               "unknown","unknown","tobacco chewing","unknown")
COSMIC_signatures_indices_df <- data.frame(sig=colnames(Alex_COSMIC_signatures_df))
COSMIC_signatures_indices_df$index <- seq_len(dim(COSMIC_signatures_indices_df)[1])
COSMIC_signatures_indices_df$colour <- COSMIC_signature_colour_vector
COSMIC_signatures_indices_df$process <- COSMIC_bio_process_vector
```

3.3 Performing an LCD analysis

Now we can start using the functions from the YAPS package. We will start with a mutational signatures analysis using known signatures (the ones we loaded in the above paragraph). For this, we will use the functions `LCD` and `LCD_cutoff`.

3.3.1 Building a mutational catalogue

This section uses functions which are to a large extent wrappers for functions in the package SomaticSignatures by Julian Gehring (Gehring et al. 2015).

```
library(BSgenome.Hsapiens.UCSC.hg19)
```

```
word_length <- 3

lymphoma_Nature2013_mutation_catalogue_list <- create_mutation_catalogue_from_df(
  lymphoma_Nature2013_df,
  this_seqnames.field = "CHROM", this_start.field = "POS",
  this_end.field = "POS", this_PID.field = "PID",
  this_subgroup.field = "SUBGROUP",
  this_refGenome = BSgenome.Hsapiens.UCSC.hg19,
  this_wordLength = word_length)
```

The function `create_mutation_catalogue_from_df` returns a list object with several entries. We will use the one called `matrix`.

```
names(lymphoma_Nature2013_mutation_catalogue_list)
```

```
## [1] "matrix" "frame"
```

```
lymphoma_Nature2013_mutation_catalogue_df <- as.data.frame(
  lymphoma_Nature2013_mutation_catalogue_list$matrix)
lymphoma_Nature2013_mutation_catalogue_df[c(1:9),c(5:10)]
```

```
##         4116738 4119027 4121361 4125240 4133511 4135350
## C>A ACA     127      31      72      34      49      75
## C>A ACC     104      36      39      19      36      80
## C>A ACG      13       2       2       1       6       8
## C>A ACT     102      33      48      22      47      56
## C>A CCA     139      43      47      29      51      70
## C>A CCC      66      34      35       7      25      42
## C>A CCG       9       7       6       3       7      11
## C>A CCT     167      47      50      32      58      84
## C>A GCA      90      47      66      29      45      66
```

3.3.2 LCD analysis without any cutoff

The LCD function performs the decomposition of a mutational catalogue into a priori known signatures and the respective exposures to these signatures as described in the second section of this vignette. We use the "new" signatures from the COSMIC website.

```
lymphoma_Nature2013_COSMIC_exposures_df <- LCD(lymphoma_Nature2013_mutation_catalogue_df,
                                               Alex_COSMIC_signatures_df)
```

Some adaptation (extracting and reformatting the information which sample belongs to which subgroup):

```
COSMIC_subgroups_df <- make_subgroups_df(lymphoma_Nature2013_COSMIC_exposures_df,
                                         lymphoma_Nature2013_df)
```

The resulting signature exposures can be plotted using custom plotting functions. First as absolute exposures:

```
abs_plot <- plot_exposures(lymphoma_Nature2013_COSMIC_exposures_df,
                           COSMIC_signatures_indices_df,
                           COSMIC_subgroups_df)
abs_plot
```

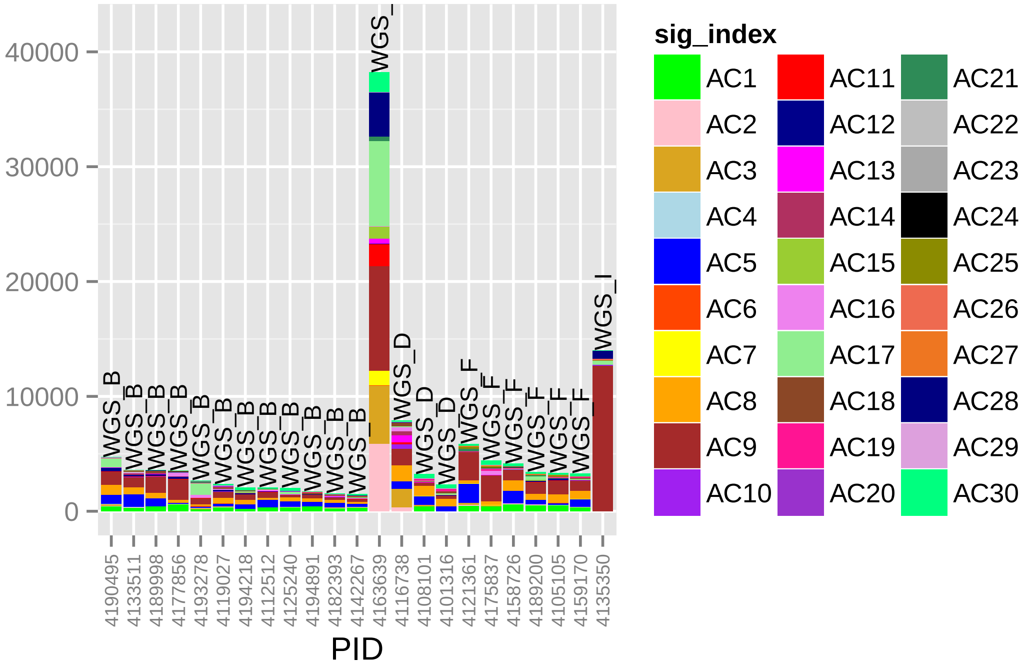


Figure 3: Absoute exposures of the COSMIC signatures in the lymphoma mutational catalogues, no cutoff for the LCD (Linear Combination Decomposition).

Second as relative exposures:

```
rel_plot <- plot_relative_exposures(lymphoma_Nature2013_COSMIC_exposures_df,
                                    COSMIC_signatures_indices_df,
                                    COSMIC_subgroups_df,
                                    in_show_subgroups=FALSE)
rel_plot
```

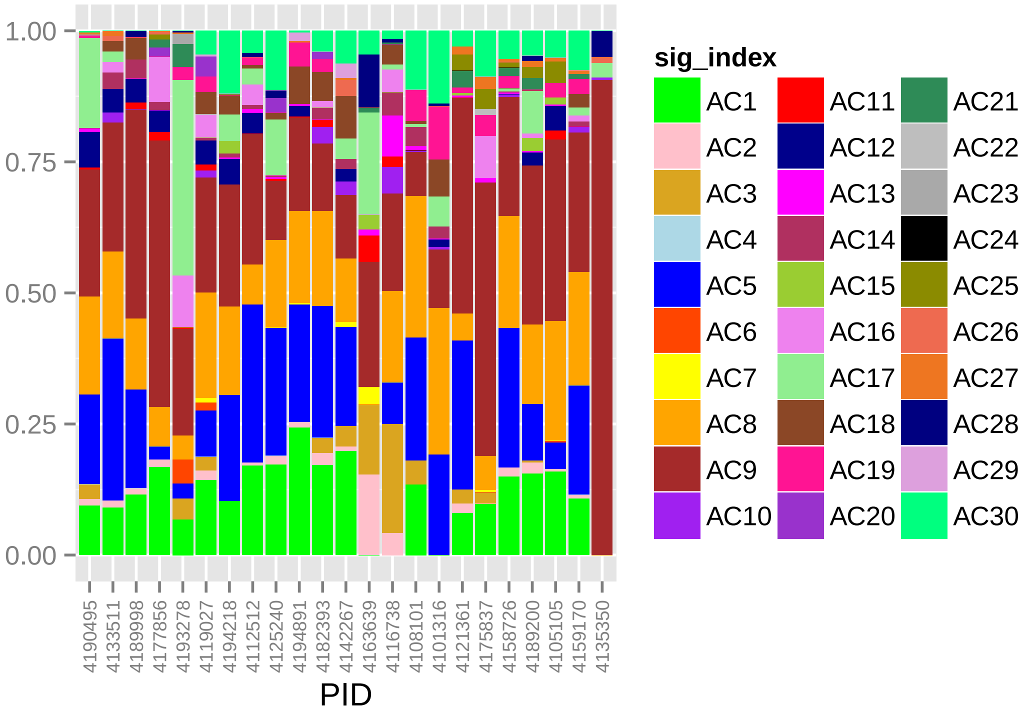


Figure 4: Relative exposures of the COSMIC signatures in the lymphoma mutational catalogues, no cutoff for the LCD (Linear Combination Decomposition).

We can see that all signatures are present in the exposures, even though some have a very small contribution.

3.3.3 LCD analysis with a cutoff

Now let's rerun the analysis with a cutoff to discard signatures with insufficient cohort-wide contribution.

```
my_cutoff <- 0.05
```

The cutoff of 0.05 means that a signature is kept if it's exposure represents at least 5% of all SNVs in the cohort. We will use the function `LCD_cutoff` instead of `LCD`.

```
COSMIC_LCD_cutoff_list <- LCD_cutoff(lymphoma_Nature2013_mutation_catalogue_df,
                                     Alex_COSMIC_signatures_df,in_cutoff=my_cutoff,
                                     in_filename=NULL)
lymphoma_Nature2013_COSMIC_cutoff_exposures_df <- COSMIC_LCD_cutoff_list$exposures
lymphoma_Nature2013_COSMIC_cutoff_signatures_df <- COSMIC_LCD_cutoff_list$signatures
lymphoma_Nature2013_COSMIC_cutoff_sig_ind <- COSMIC_LCD_cutoff_list$choice
lymphoma_Nature2013_COSMIC_cutoff_exposure_order <- COSMIC_LCD_cutoff_list$order
chosen_signatures_indices_df <- COSMIC_signatures_indices_df[lymphoma_Nature2013_COSMIC_cutoff_sig_ind,]
```

At the chosen cutoff of 0.05, we are left with 7 signatures. We can look at these signatures in detail and their attributed biological processes:

```
kable(chosen_signatures_indices_df, row.names=FALSE,
      caption=paste0("Signatures with cohort-wide exposures > ",my_cutoff))
```

sig	index	colour	process
AC1	1	green	spontaneous deamination
AC2	2	pink	APOBEC
AC3	3	goldenrod	defect DNA DSB repair hom. recomb.
AC5	5	blue	unknown
AC8	8	orange	unknown
AC9	9	brown	POL eta and SHM
AC17	17	lightgreen	unknown

Table 4: Signatures with cohort-wide exposures > 0.05

Again we can plot absolute exposures:

```
abs_plot <- plot_exposures(lymphoma_Nature2013_COSMIC_cutoff_exposures_df,
              chosen_signatures_indices_df,
              COSMIC_subgroups_df)
abs_plot
```

And relative exposures:

```
rel_plot <- plot_relative_exposures(lymphoma_Nature2013_COSMIC_cutoff_exposures_df,
              chosen_signatures_indices_df,
```

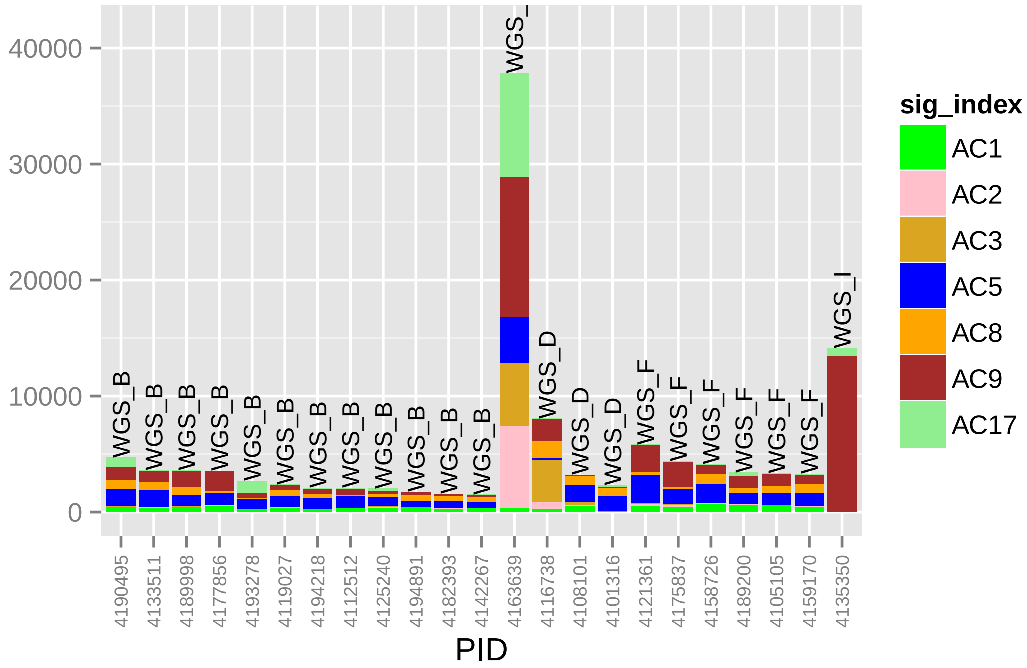
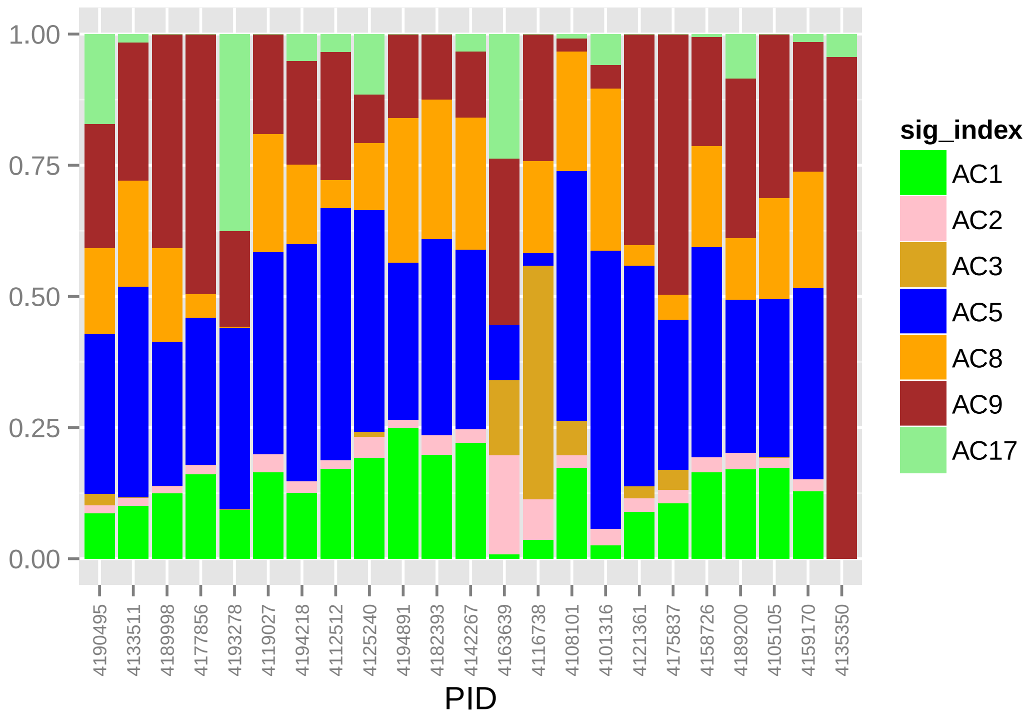


Figure 5: Absoute exposures of the COSMIC signatures in the lymphoma mutational catalogues, cutoff of 5% for the LCD (Linear Combination Decomposition).

```
                COSMIC_subgroups_df,
                in_show_subgroups=FALSE)
rel_plot
```



3.4 Cluster samples based on their signature exposures

To identify groups of samples which were exposed to similar mutational processes, the exposure vectors of the samples can be compared. The YAPS package provides a custom function for this task: `complex_heatmap_exposures`, which uses the package ComplexHeatmap by Zuguang Gu (Z Gu 2015). It produces output as follows:

```
rel_lymphoma_Nature2013_COSMIC_cutoff_exposures_df <-
  normalize_df_per_dim(lymphoma_Nature2013_COSMIC_cutoff_exposures_df,2)
library(circlize)
complex_heatmap_exposures(rel_lymphoma_Nature2013_COSMIC_cutoff_exposures_df,
                          COSMIC_subgroups_df,
                          chosen_signatures_indices_df,
                          in_data_type="norm exposures",
                          in_subgroup_colour_column="col",
                          in_method="manhattan",
                          in_subgroup_column="subgroup")
```

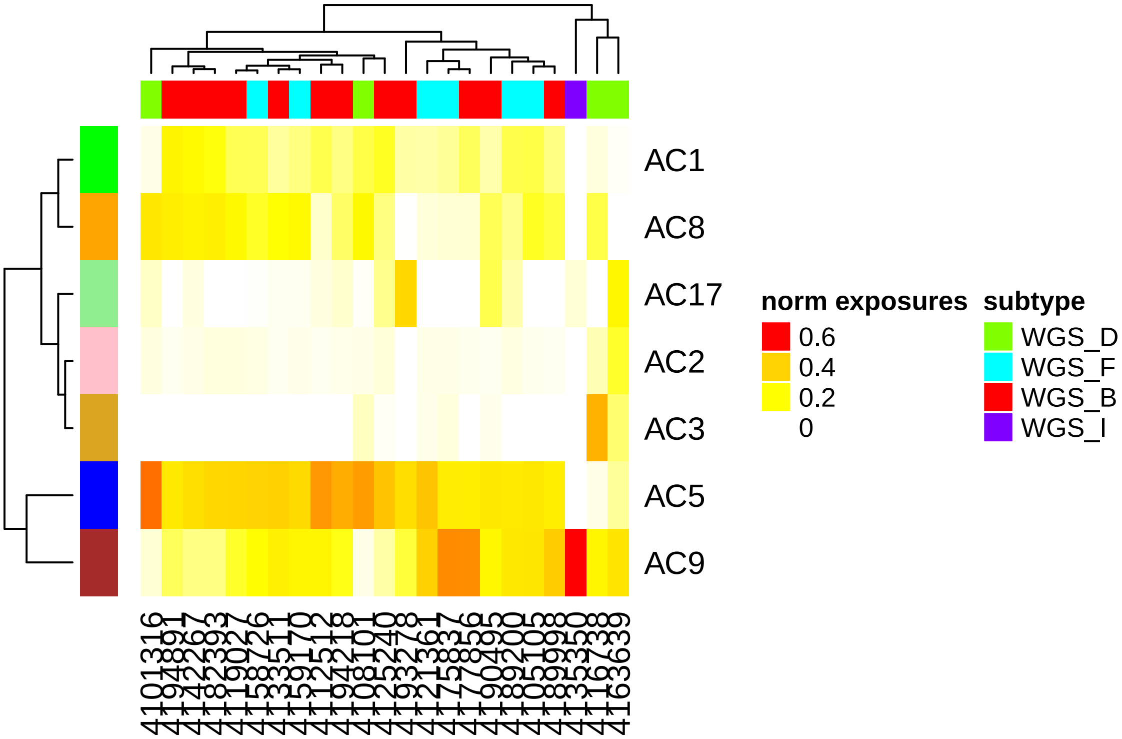


Figure 6: Clustering of Samples and Signatures based on the relative exposures of the COSMIC signatures in the lymphoma mutational catalogues.

If you are interested only in the clustering and not in the heatmap information, you could also use `hclust_exposures`:

```
hclust_list <- hclust_exposures(rel_lymphoma_Nature2013_COSMIC_cutoff_exposures_df,
                                COSMIC_subgroups_df,
                                in_method="manhattan",
                                in_subgroup_column="subgroup")
```

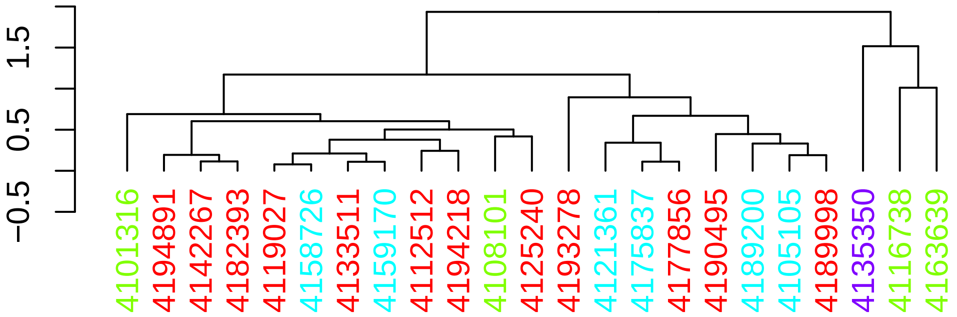
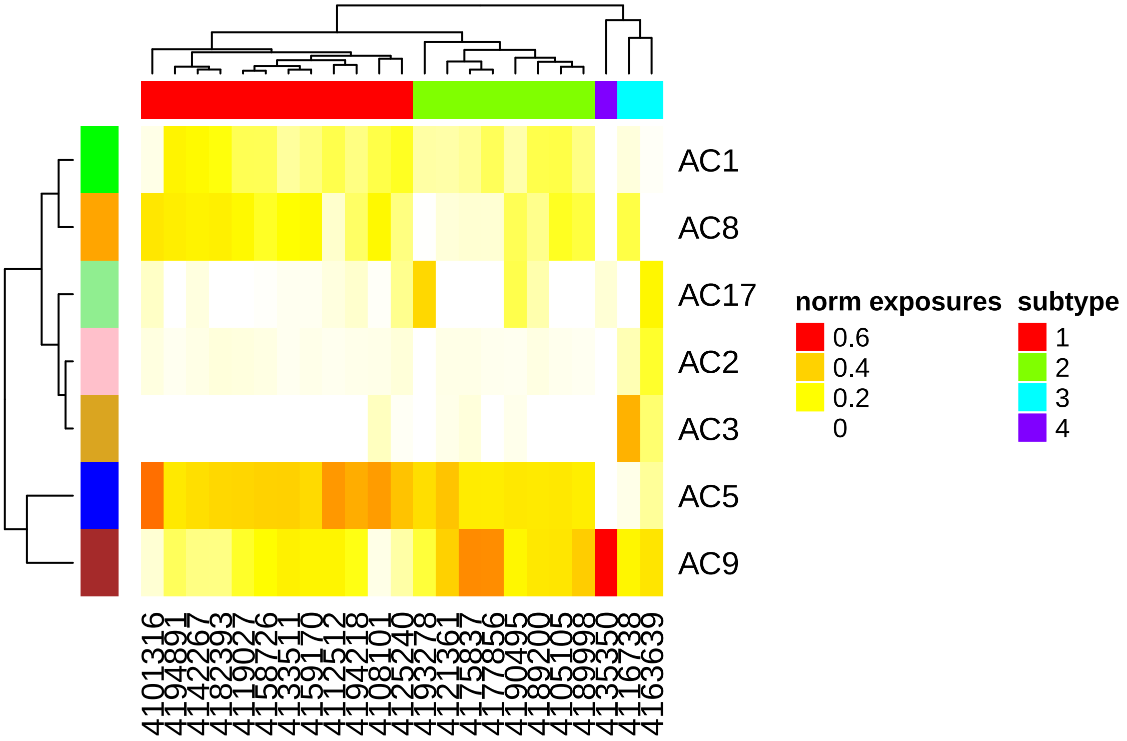


Figure 7: Clustering of the Samples based on the relative exposures of the COSMIC signatures in the lymphoma mutational catalogues.

The dendrogram produced by either the function `complex_heatmap_exposures` or the function `hclust_exposures` can be cut to yield signature exposure specific subgroups of the PIDs.

```
cluster_vector <- cutree(hclust_list$hclust,k=4)
COSMIC_subgroups_df$cluster <- cluster_vector
subgroup_colour_vector <- rainbow(length(unique(COSMIC_subgroups_df$cluster)))
COSMIC_subgroups_df$cluster_col <- subgroup_colour_vector[factor(COSMIC_subgroups_df$cluster)]
complex_heatmap_exposures(rel_lymphoma_Nature2013_COSMIC_cutoff_exposures_df,
                          COSMIC_subgroups_df,
                          chosen_signatures_indices_df,
                          in_data_type="norm exposures",
                          in_subgroup_colour_column="cluster_col",
                          in_method="manhattan",
                          in_subgroup_column="cluster")
```



3.5 Performing a stratification based on mutation density

We will now use the intermutational distances computed above. We set cutoffs for the intermutational distance at 1000 and 100000 bp, leading to three strata. We annotate to every variant in which stratum it falls.

```
lymphoma_Nature2013_df$density_cat <- cut(lymphoma_Nature2013_df$dist,
                                          c(0,1001,100001,Inf),
                                          right=FALSE,
                                          labels=c("high","intermediate","background"))
```

The following table shows the distribution of variants over strata:

```
temp_df <- data.frame(table(lymphoma_Nature2013_df$density_cat))
names(temp_df) <- c("Stratum","Cohort-wide counts")
kable(temp_df, caption=paste0("Strata for the SMC of mutation density"))
```

Stratum	Cohort-wide counts
high	6818
intermediate	62131
background	50675

Table 5: Strata for the SMC of mutation density

We now have everything at hand to carry out a stratified signature analysis:

```
strata_order_ind <- c(1,3,2)
mut_density_list <- run_SMC(lymphoma_Nature2013_df,
                            lymphoma_Nature2013_COSMIC_cutoff_signatures_df,
                            chosen_signatures_indices_df,
                            COSMIC_subgroups_df,
                            column_name="density_cat",
                            refGenome=BSgenome.Hsapiens.UCSC.hg19,
                            cohort_method_flag="norm_PIDs",
                            in_strata_order_ind=strata_order_ind)
```

This produces a multi-panel figure with 4 rows of plots. The first row visualizes the signature distribution over the whole cohort without stratification, followed by one row of plots per stratum. Hence in our example we have four rows of graphs with three (exclusive) strata as input. Each row consists of three plots. The left plots show absolute exposures in the respective stratum as stacked barplots on a per sample basis. The middle plots show relative exposures in the respective stratum on a per sample basis as stacked barplots. The right plots shows cohort-wide averages of the relative exposures in the respective stratum. The error bars indicate the standard error of the mean (SEM).

To test for statistical significance of potential differences in the signature exposures (i.e. signature enrichment and depletion patterns) between the different strata, we can use the Kruskal-Wallis test, as the data is grouped into (potentially more than two) categories and might not follow a normal distribution. As we are testing the different signatures on the same stratification, we have to correct for multiple testing. In order to control the false discovery rate (FDR), the Benjamini-Hochberg correction is appropriate.

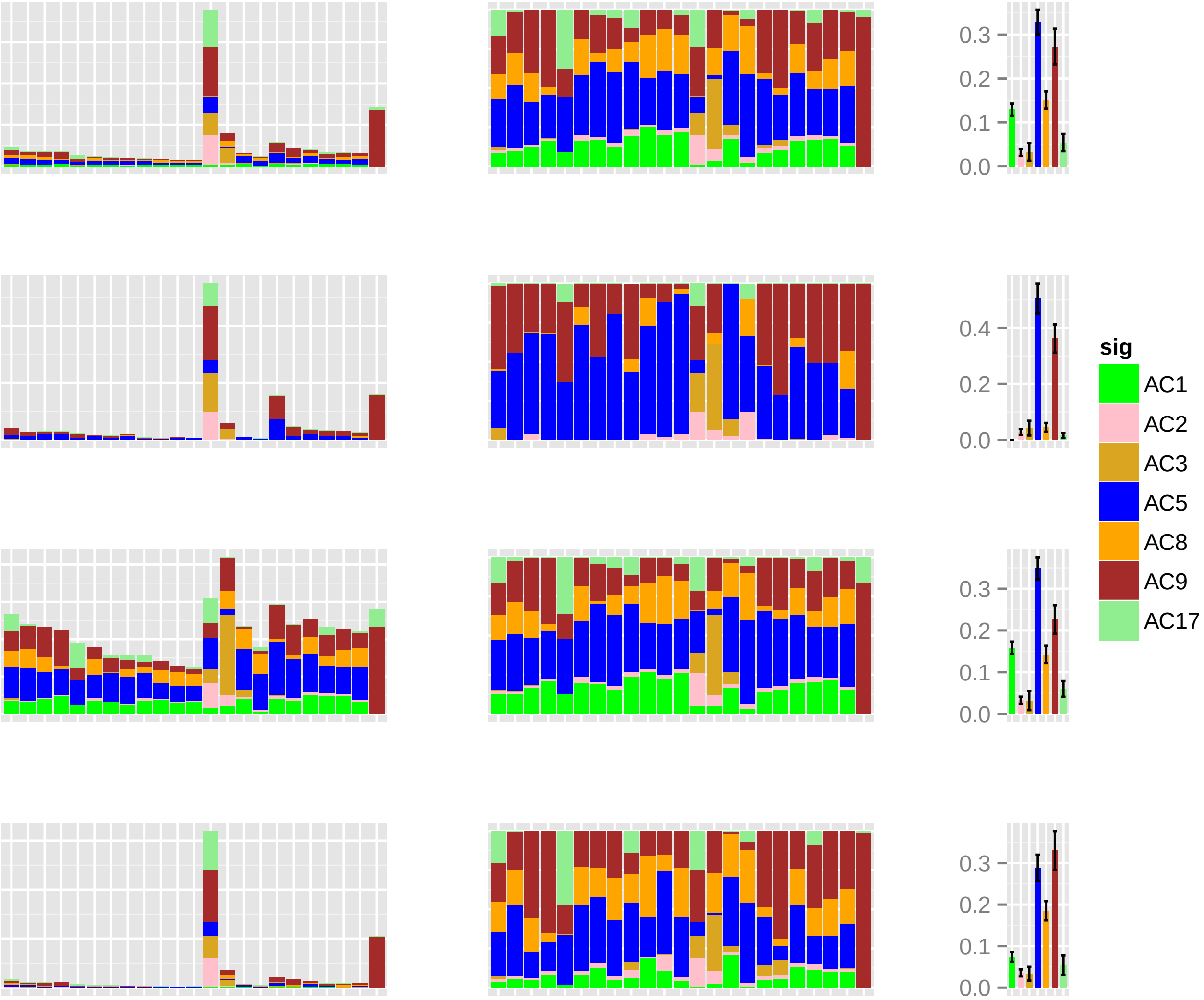


Figure 8: SMC (Stratification of the Mutational Catalogue) based on mutation density.

```r
stat_mut_density_list <- stat_test_SMC(mut_density_list,in_flag="norm")
kable(stat_mut_density_list$kruskal_df,
      caption=paste0("Results of Kruskal tests for cohort-wide exposures over strata ",
                     "per signature without and with correction for multiple testing."))
```

	Kruskal_statistic	df	Kruskal_p_val	Kruskal_p_val_BH
AC1	50.109358	2	0.0000000	0.0000000
AC2	3.968018	2	0.1375168	0.1604363
AC3	1.098077	2	0.5775047	0.5775047
AC5	13.013503	2	0.0014933	0.0034844
AC8	19.651074	2	0.0000541	0.0001892
AC9	5.917688	2	0.0518788	0.0726304
AC17	6.914406	2	0.0315178	0.0551561

Table 6: Results of Kruskal tests for cohort-wide exposures over strata per signature without and with correction for multiple testing.

In the following paragraph we perform post-hoc tests for those signatures where the Kruskal-Wallis test, as evaluated above, has given a significant result.

```
significance_level <- 0.05
for(i in seq_len(dim(stat_mut_density_list$kruskal_df)[1])){
  if(stat_mut_density_list$kruskal_df$Kruskal_p_val_BH[i]<significance_level){
    print(paste0("Signature: ",rownames(stat_mut_density_list$kruskal_df)[i]))
    print(stat_mut_density_list$kruskal_posthoc_list[[i]])
  }
}
```

```
## [1] "Signature: AC1"
##
##  Pairwise comparisons using Tukey and Kramer (Nemenyi) test
##                    with Tukey-Dist approximation for independent samples
##
## data:  sig_exposures_vector and sig_strata_vector
##
##              background high
## high         2.6e-11    -
## intermediate 0.033      4.5e-05
##
## P value adjustment method: none
## [1] "Signature: AC5"
##
##  Pairwise comparisons using Tukey and Kramer (Nemenyi) test
##                    with Tukey-Dist approximation for independent samples
##
## data:  sig_exposures_vector and sig_strata_vector
##
##              background high
## high         0.0402     -
## intermediate 0.5171     0.0012
##
## P value adjustment method: none
## [1] "Signature: AC8"
##
##  Pairwise comparisons using Tukey and Kramer (Nemenyi) test
##                    with Tukey-Dist approximation for independent samples
##
## data:  sig_exposures_vector and sig_strata_vector
##
##              background high
## high         0.0066     -
## intermediate 0.4391     5.9e-05
##
## P value adjustment method: none
```

From this analysis, we can see that a distinct signature enrichment and depletion pattern emerges:

1. Stratum of high mutation density: Enrichment of signatures AC5 (significant) and AC9, depletion of signatures AC1 (significant), AC2, AC8 (significant) and AC17
2. Background: signature distribution very similar to the one of the complete mutational catalogue (first row)
3. Stratum of intermediate mutation density: intermediate signature enrichment and depletion pattern between the strata of high mutation density and background.

4 Features of the package not covered in this vignette

- Normalization of a mutational catalogue if the sequencing was not performed on the whole genome level, but instead a target capture approach like Whole Exome Sequencing (WES) or panel sequencing has been used. If the target capture regions are supplied as a .bed file, the trinucleotide content (or more general the motif content) of the target capture regions is extracted and the mutational catalogue normalized to this different background motif distribution to make the signature analysis comparable. For this purpose, a perl script called `kmer_frenquencies.pl` and a wrapper function `run_kmer_frequency_pl` in R are available.
- An external perl script `annotate_vcf` and a corresponding wrapper function in R are available to annotate whatever feature is available to the vcf-like file from which the mutational catalogue is then created. This additionally annotated information may then be used for stratification.
- Automatic report generation for either whole cohorts or individual samples.

References

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