Class18

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Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas)

1. Exploring a cancer sequencing data portal

Discussion #1

Q. How many cancer samples are included in the dataset? 487

Q. Which is the most mutated gene? TP53

Q. Which is the most common treatment undergone by patients? Cisplatin

2. Downloading cancer sequencing data

We download the cancer sequencing data to use for the mutational signature analysis. The file that contains the mutation data is `data_mutations.txt`.

3. Generating mutational matrices and visualizing mutational profiles

First step, we need to install required packages:

```
# install.packages("BiocManager")
# BiocManager::install("BSgenome.Hsapiens.UCSC.hg19")
# BiocManager::install("maftools")
```

Reading the file:

```
# Read maf file
library(maftools)
coad = read.maf('data_mutations.txt')
```

```
-Reading
-Validating
--Removed 14038 duplicated variants
-Silent variants: 60539
-Summarizing
--Possible FLAGS among top ten genes:
  TTN
  MUC16
  USH2A
  SYNE1
-Processing clinical data
--Missing clinical data
-Finished in 19.0s elapsed (15.2s cpu)
```

Loading the matrix:

```
# Generate mutational matrix (SBS96 context)
mm_coad = trinucleotideMatrix(maf = coad, prefix = 'chr', add = TRUE,
                              ref_genome = "BSgenome.Hsapiens.UCSC.hg19")
```

```
Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
    table, tapply, union, unique, unsplit, which.max, which.min


Attaching package: 'S4Vectors'

The following objects are masked from 'package:base':

    expand.grid, I, unname
```

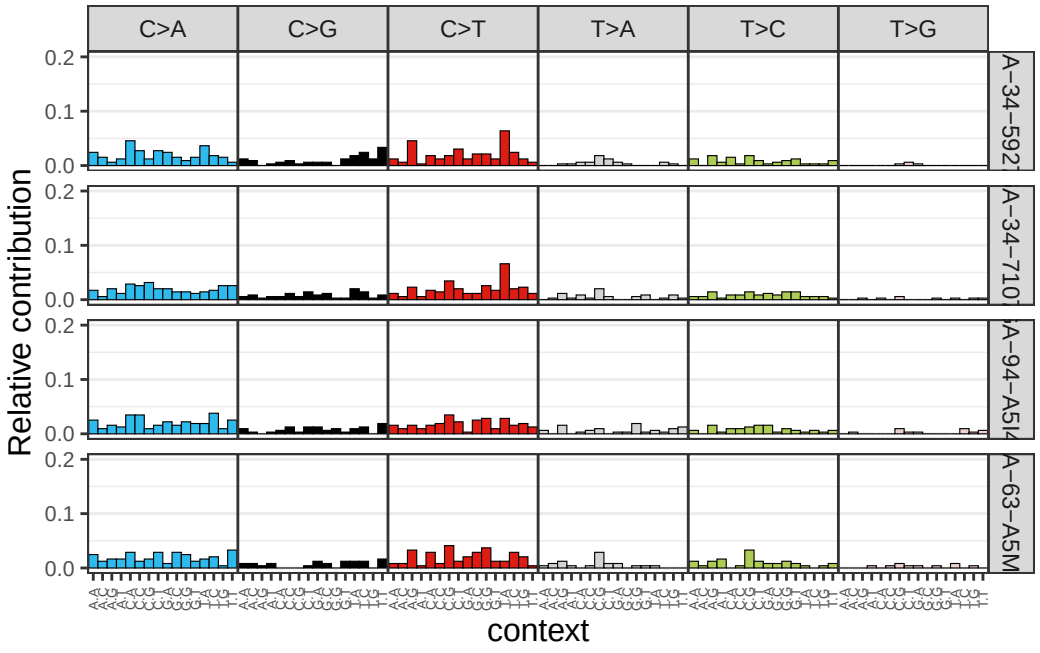
```
Attaching package: 'Biostrings'

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

    strsplit

-Extracting 5' and 3' adjacent bases
-Extracting +/- 20bp around mutated bases for background C>T estimation
-Estimating APOBEC enrichment scores
--Performing one-way Fisher's test for APOBEC enrichment
---APOBEC related mutations are enriched in  30.128 % of samples (APOBEC enrichment score > 2
-Creating mutation matrix
--matrix of dimension 469x96
```

```r
mm_coad = t(mm_coad$nmf_matrix)
```

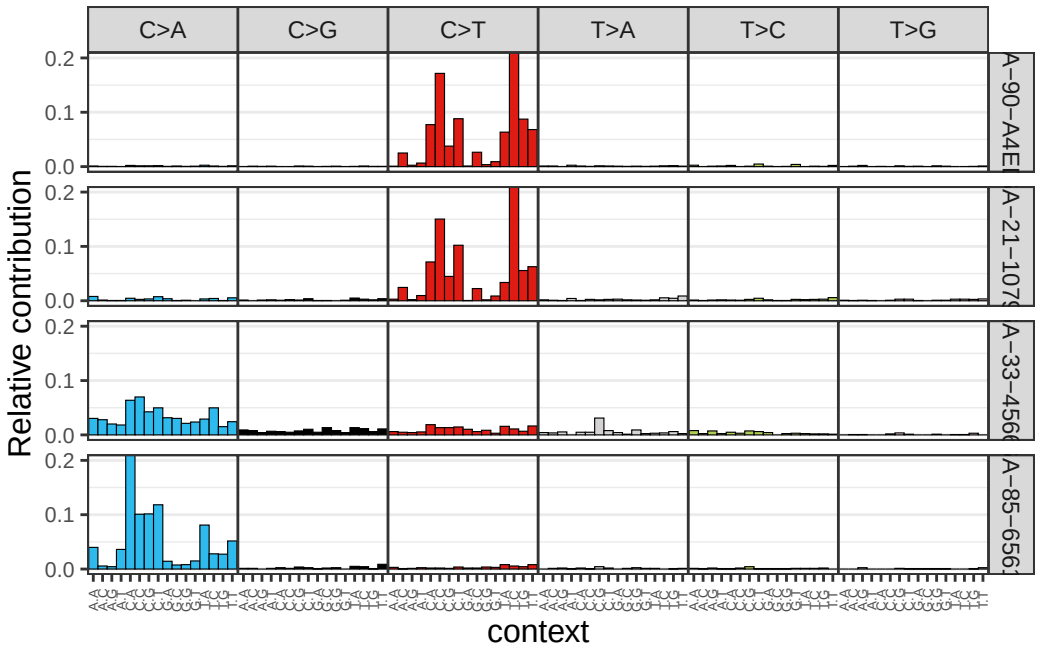
```r
# Generate mutational profiles (4 random samples)
library(MutationalPatterns)
```

```
Loading required package: NMF

Loading required package: registry

Loading required package: rngtools

Loading required package: cluster

NMF - BioConductor layer [OK] | Shared memory capabilities [NO: bigmemory] | Cores 2/2

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


Attaching package: 'NMF'

The following object is masked from 'package:S4Vectors':

    nrun
```

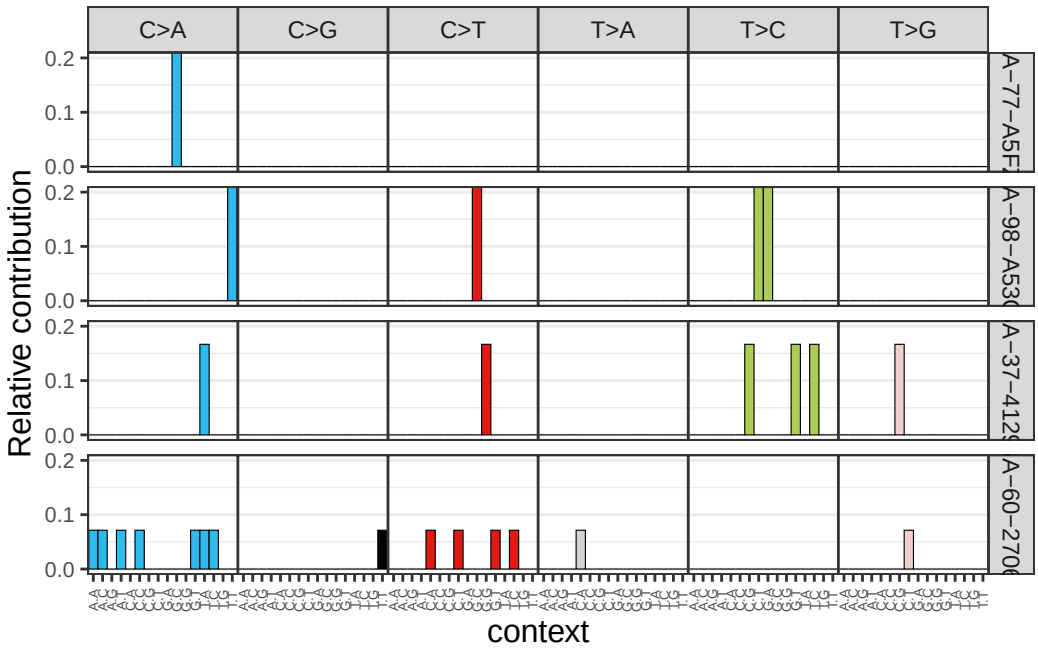
```r
set.seed(11111) # fixing the seed for random number generation

samples_to_plot = sample(1:ncol(mm_coad),4) # selecting 4 random samples
plot_96_profile(mm_coad[,samples_to_plot], condensed = T)
```



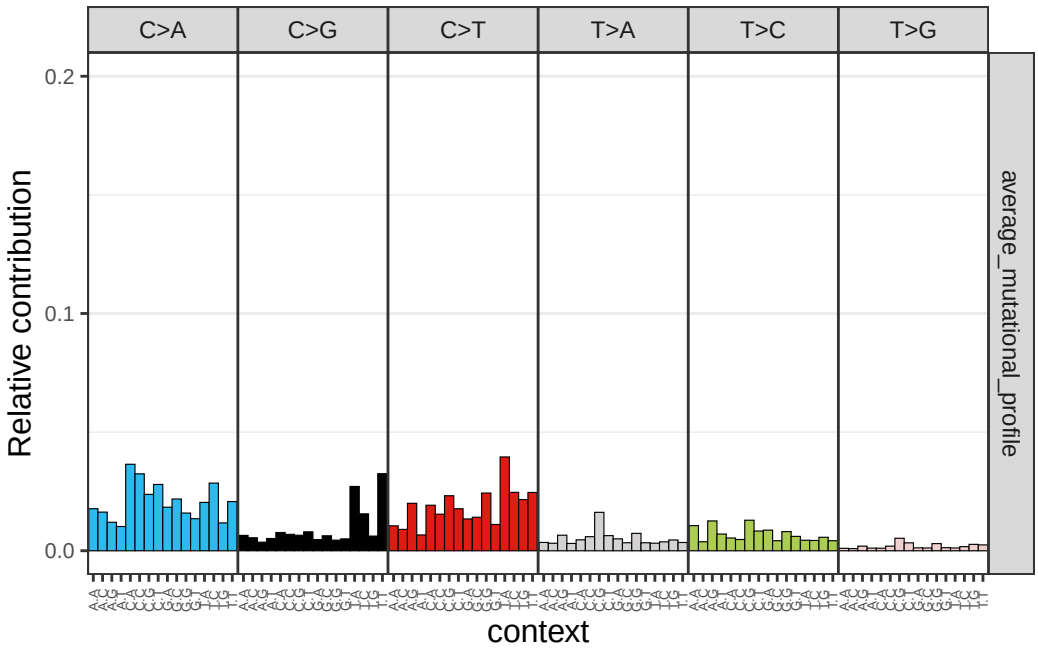

```r
# Generate mutational profiles (top 4 mutated samples and top 4 less mutated)
mutations_in_samples = colSums(mm_coad)
mutations_in_samples = sort(mutations_in_samples, decreasing = T)
samples_to_plot = names(mutations_in_samples)[1:4]
plot_96_profile(mm_coad[,samples_to_plot], condensed = T)
```




```
mutations_in_samples = sort(mutations_in_samples, decreasing = F)
samples_to_plot = names(mutations_in_samples)[1:4]
plot_96_profile(mm_coad[,samples_to_plot], condensed = T)
```




```
# Generate average mutational profiles
relative_mutational_profile = apply(mm_coad, 2, prop.table) # obtained relative
                                                            # mutational matrix
average_mutational_profile = rowMeans(relative_mutational_profile)
average_mutational_profile = data.frame(average_mutational_profile)
plot_96_profile(average_mutational_profile, condensed = T)
```



4. COSMIC reference mutational signatures

We use the COSMIC Mutational Signature website to gather more information about the most active signatures in the cancer.

5. Assigning reference mutational signatures

```
library(MutationalPatterns)
# Mutational signature assignment
cosmic_signatures = get_known_signatures(source = 'COSMIC_v3.2')
fit_res = fit_to_signatures(mm_coad, cosmic_signatures)

# Top contributing signatures
contributions = fit_res$contribution

top_contributing_signatures_abs = rowMeans(contributions)
top_contributing_signatures_abs = sort(top_contributing_signatures_abs,
                                       decreasing = T)[1:4]

## Top 4 contributing signatures (absolute values)
```

```
  top_contributing_signatures_abs
```

```
    SBS4    SBS24    SBS39    SBS13
104.9223  30.2159  29.8143  26.5996
```

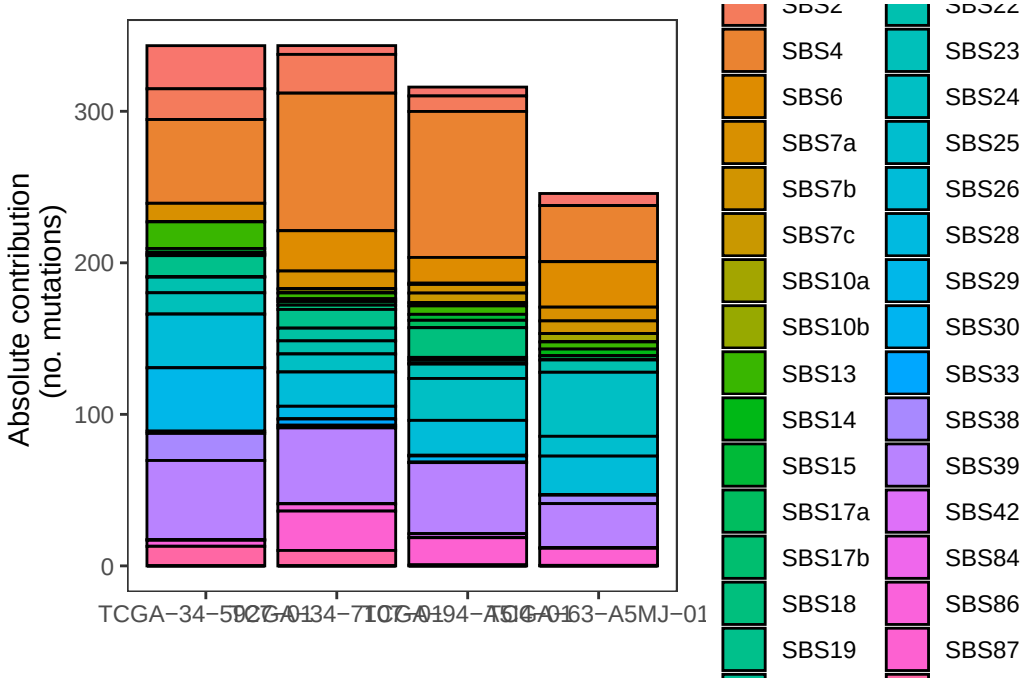
```
  relative_contributions = apply(contributions,2,prop.table)
  top_contributing_signatures_rel = rowMeans(relative_contributions)
  top_contributing_signatures_rel = sort(top_contributing_signatures_rel,
                                         decreasing = T)[1:4]

  ## Top 4 contributing signatures (relative values)
  top_contributing_signatures_rel
```

```
     SBS4      SBS24      SBS39      SBS13
0.23615327 0.08534249 0.08064435 0.06469107
```

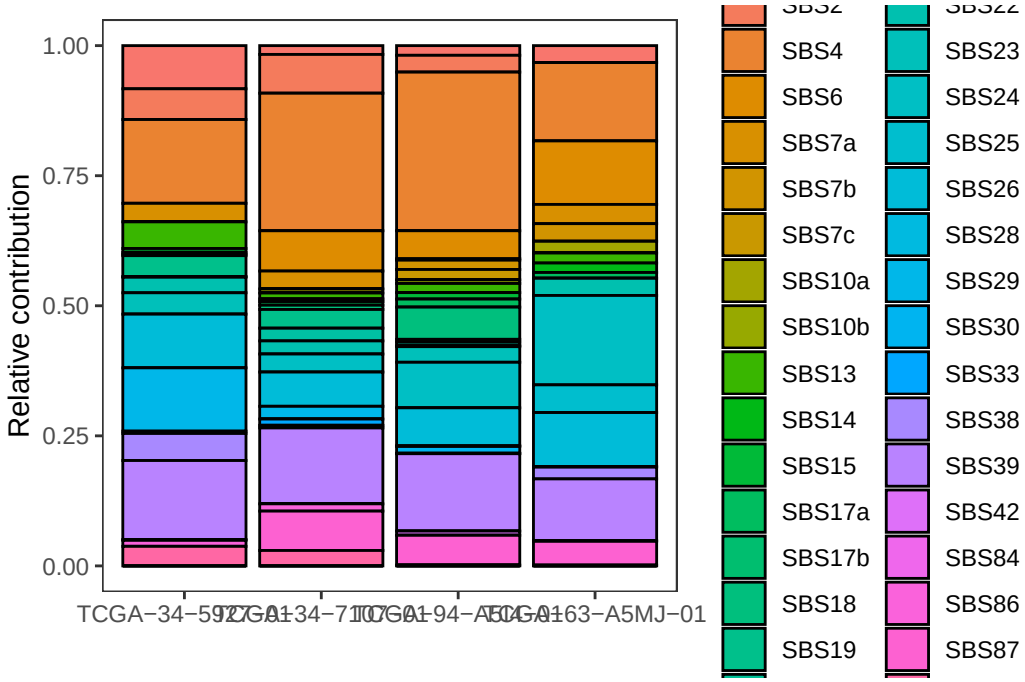
```
  # Mutational signature assignment strict
  fit_res_strict = fit_to_signatures_strict(mm_coad, cosmic_signatures)
  fit_res_strict = fit_res_strict$fit_res
  contributions_strict = fit_res_strict$contribution
```

6. Visualizing mutational signature assignment results

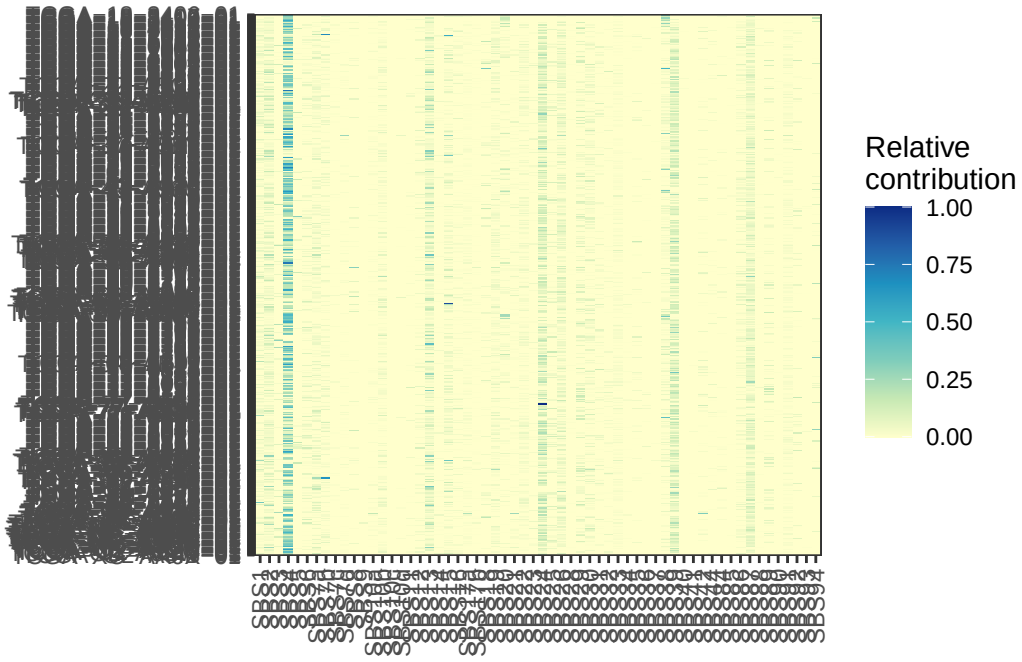
```
  # Visualization of signature assignment results (fit_to_signatures)
  set.seed(11111)
  samples_to_plot = sample(1:ncol(mm_coad),4)

  plot_contribution(contributions[,samples_to_plot], mode = "absolute")
```



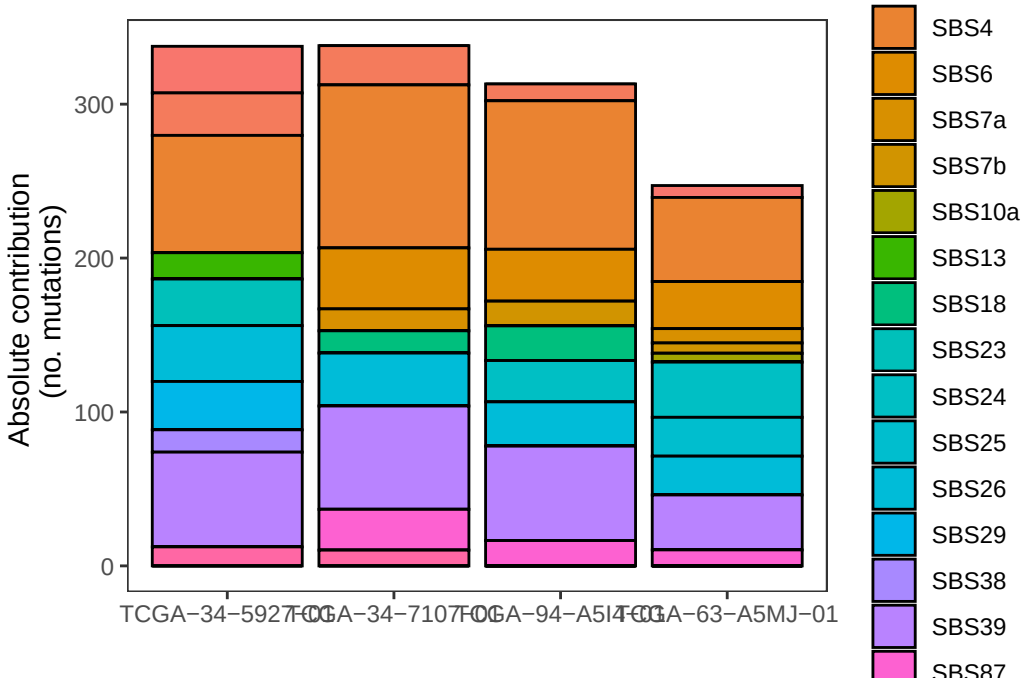

```
plot_contribution(contributions[,samples_to_plot], mode = "relative")
```



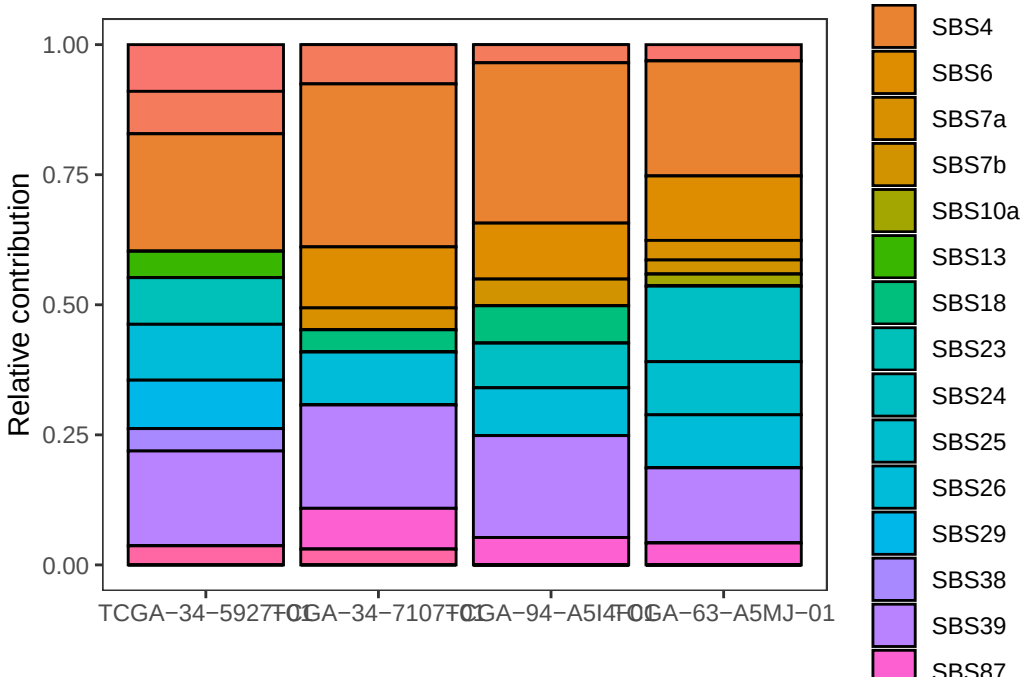
```
plot_contribution_heatmap(contributions, cluster_samples = F)
```



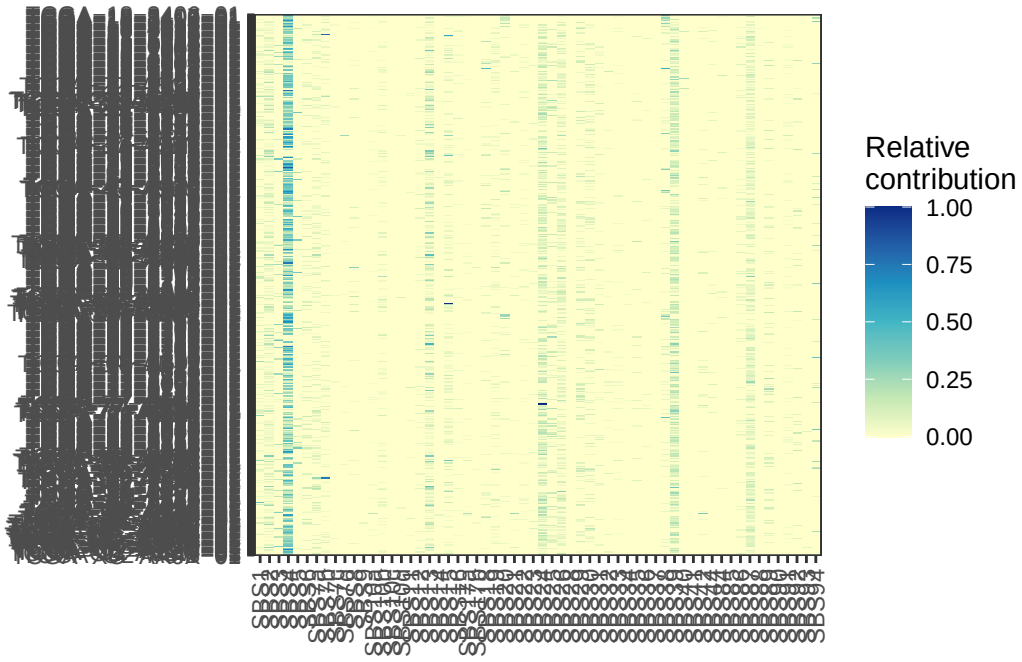

```
# Visualization of signature assignment results (strict)
plot_contribution(contributions_strict[,samples_to_plot], mode = "absolute")
```



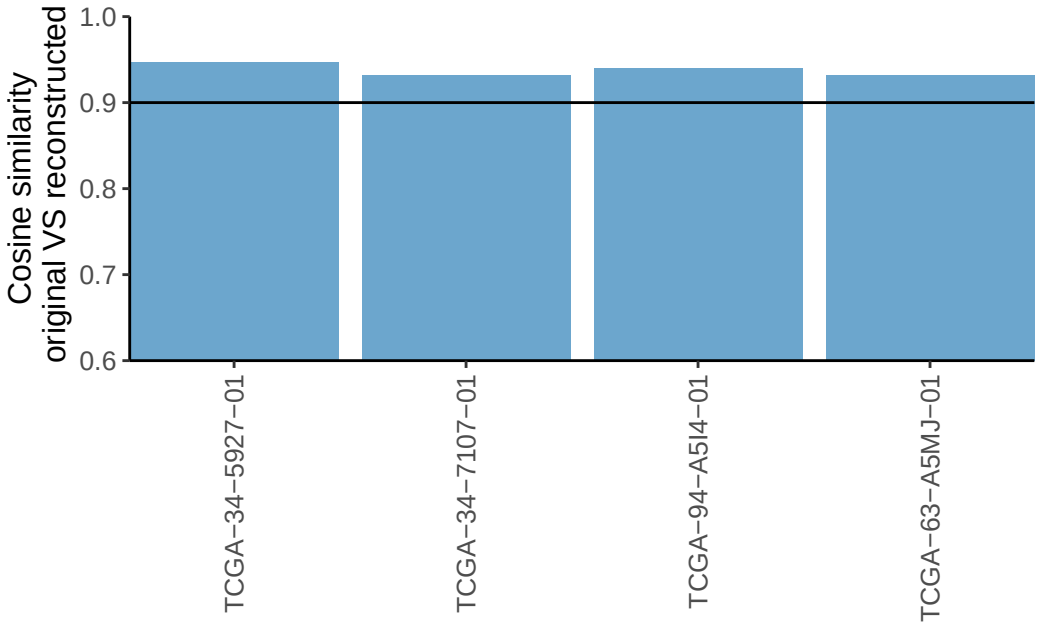

```
plot_contribution(contributions_strict[,samples_to_plot], mode = "relative")
```



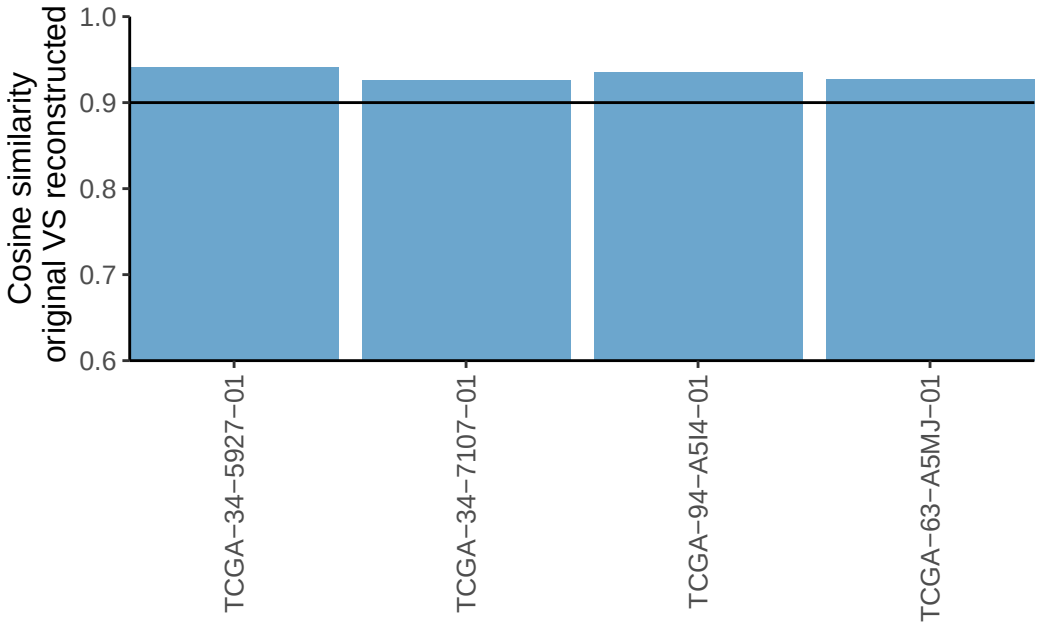
```
plot_contribution_heatmap(contributions_strict, cluster_samples = F)
```




```
# Cosine similarity reconstruction vs. original mutational profile (fit_to_signatures)
set.seed(11111)
samples_to_plot = sample(1:ncol(mm_coad),4)

plot_original_vs_reconstructed(mm_coad[,samples_to_plot],
                               fit_res$reconstructed[,samples_to_plot],
                               y_intercept = 0.90)
```




```
# Cosine similarity reconstruction vs. original mutational profile (strict)
plot_original_vs_reconstructed(mm_coad[,samples_to_plot],
                               fit_res_strict$reconstructed[,samples_to_plot],
                               y_intercept = 0.90)
```



Discussion #2

Q. Which is the etiology of the top absolute contributing signature for liver cancer? Aristolochic acid exposure

Q. Which is the most prominent mutational context for the top contributing signature in skin cancer? C>T

Q. The etiology of the top contributing signature for lung cancer corresponds to an endogenous cellular mechanism. FALSE

Q. SBS4 is one of the most common signatures found in lung cancer and is associated with tobacco smoking. TRUE

Q. SBS7d is one of the most common signatures in skin cancer and is associated with UV light exposure and high numbers of C>T mutations. FALSE