MIXTURE

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A noise constrained Recursive Feature Extraction algorithm for robust deconvolution of cell-types mixture from molecular signatures

Since the significant impact of immunotherapy in cancer, the estimation of the immune cell-type proportions present in a tumor becomes crucial. Currently, the deconvolution of the cell mixture content of a tumor is carried out by different analytic tools, yet the accuracy of inferred cell type proportions has room for improvement. We improve tumor immune environment characterization developing MIXTURE, an analytical method based on a noise constrained recursive variable selection for a support vector regression

How to install MIXTURE

```
install.packages("devtools")
library(devtools)
install_github("elmerfer/MIXTURE")
```

Running MIXTURE

In this example we will use the LM22 signature matrix as a 22 subject expression matrix cohort

```
library(MIXTURE)
```

```
## Loading required package: BiocParallel
## Loading required package: e1071
## Loading required package: ComplexHeatmap
## Loading required package: grid
## ========================================
## ComplexHeatmap version 2.1.0
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
##
## If you use it in published research, please cite:
## Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
##   genomic data. Bioinformatics 2016.
## ========================================
## Loading required package: gridExtra
## Loading required package: ggExtra
## Loading required package: stringr
## Loading required package: plyr
```

```
## Loading required package: abind
## Loading required package: openxlsx
## Loading required package: ggplot2
## Loading required package: parallel
```

```
##Load signature matrix
data(LM22)
##  Run the self test on LM22 signature
mix.test <- MIXTURE(expressionMatrix = LM22,          #N x ncol(signatureMatrix) gene expression
                                                      ##matrix to evaluate
                                                      ##rownames(M) should be the GeneSymbols
              signatureMatrix = LM22,                 #the gene signature matrix (W) such that
                                                      ##M = W*betas'
                                                      #(i.e the LM22 from Newman et al)
              functionMixture = nu.svm.robust.RFE,    #cibersort, nu.svm.robust.rfe, ls.rfe.abbas,
              useCores = 10L,                         #cores for parallel processing
              verbose = TRUE,                         #TRUE or FALSE messages
              nullDist = "PopulationBased"
              )
```

```
##
## Running...
## Original Samples run
##
## Population based null distribution
##
## Building random population
##
## Building null distribution
##
## finish
```

```
# Showing the predicted proportions
head(GetMixture(mix.test)[,1:3])
```

```
##                          B cells naive B cells memory Plasma cells
## B cells naive                        1              0            0
## B cells memory                       0              1            0
## Plasma cells                         0              0            1
## T cells CD8                          0              0            0
## T cells CD4 naive                    0              0            0
## T cells CD4 memory resting           0              0            0
```

```
# Showing the predicted absolute coefficients
head(GetMixture(mix.test, type = "absolute")[,1:3])
```

```
##                          B cells naive B cells memory Plasma cells
## B cells naive               0.9596281       0.000000    0.0000000
## B cells memory              0.0000000       1.070502    0.0000000
## Plasma cells                0.0000000       0.000000    0.6977581
## T cells CD8                 0.0000000       0.000000    0.0000000
## T cells CD4 naive           0.0000000       0.000000    0.0000000
## T cells CD4 memory resting  0.0000000       0.000000    0.0000000
```

```r
# Showing the slots names of the MIXTURE object
names(mix.test)
```

```
## [1] "Subjects"       "PermutedMetrix" "method"         "usedGenes"
## [5] "p.values"
```

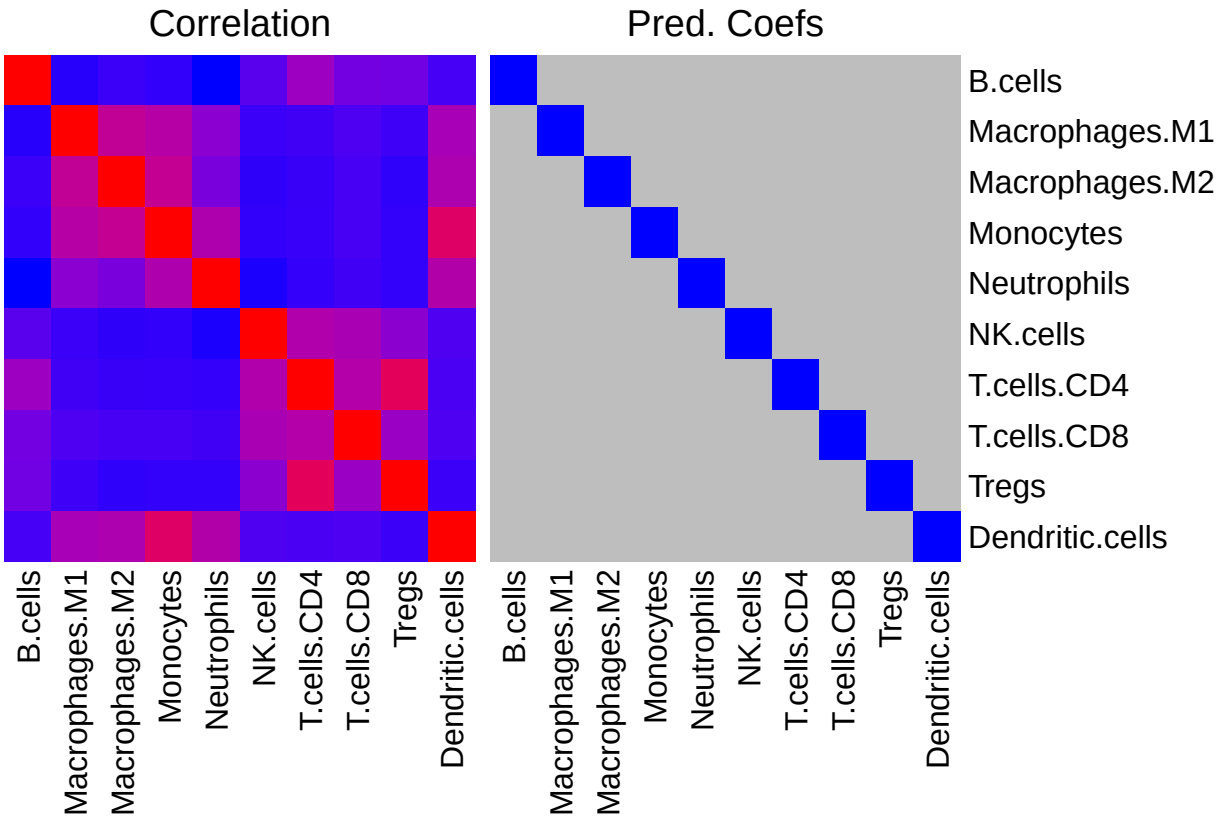
How to performe a molecular signature SelfTest analysis with MIXTURE ?

A self test analysis is intended to evaluate if the signature profiles can be accuratly predicted by using MIXTURE. This means that each signature profiles (Columns) will be assumed as a pure cell mixture profile, thus, the algorithm should only provide one coefficients as being 1 for each signature profile

```r
library(MIXTURE)
# Load the TIL10 signature from Finotello et al.
data(TIL10)
# Signature Format
head(TIL10)
```

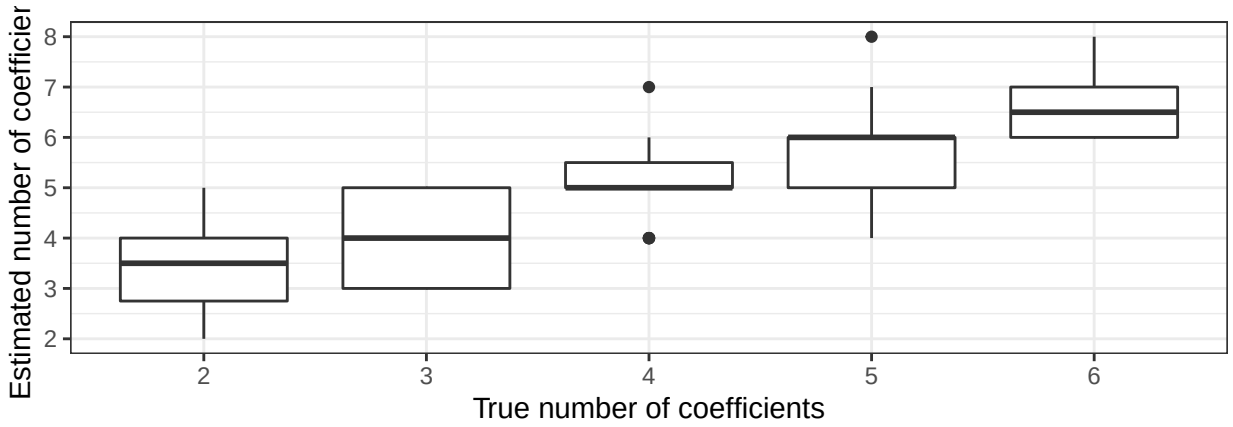
```
##            B.cells Macrophages.M1 Macrophages.M2   Monocytes Neutrophils
## ABCB4     22.71382      2.2317995      1.9715545  0.10599474  0.00000000
## ADAM28   138.12232     44.5491734      4.8615437  6.15419539  6.92361545
## ADAM6    311.52431      0.1855003      0.3871935  0.03094342  0.03136992
## AFF3      74.37987      1.4194759      1.7991814  2.90259698  1.79219822
## AKAP2     90.53809     17.5227275      3.3263218  0.22109067  0.00000000
## ARHGAP24  48.92311     10.4272520      2.5223667 17.63984171  3.12847521
##             NK.cells T.cells.CD4 T.cells.CD8       Tregs Dendritic.cells
## ABCB4     0.23659583  0.04195356  0.10700210  0.04641025      2.14790312
## ADAM28   16.03814946  0.08486099  0.07933068  0.66266673     18.91589470
## ADAM6     0.50123572  0.48061524  0.39069977  0.31171570      0.07778422
## AFF3      5.31305821  3.33874243  1.67199536  3.41073424      9.31677002
## AKAP2     6.84541045  5.89055945  1.27121635 18.36339914      3.30470201
## ARHGAP24  0.05250449  0.01065365  0.00000000  0.21506861      2.34976504
```

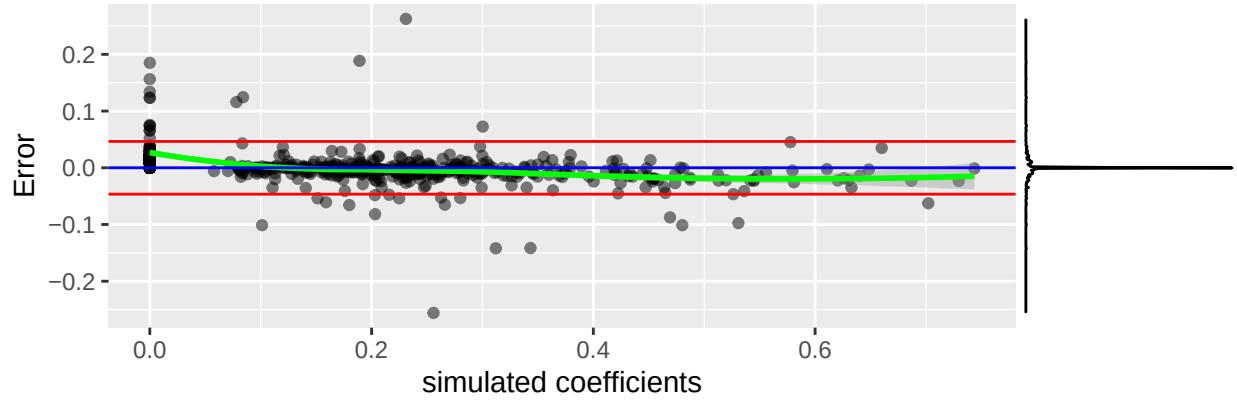
```r
SelfTest(TIL10)
```




```
# Run the MIXTURE on simulated samples built from the given signature
res <- SimulationTest(signatureMatrix = TIL10,
                      maxCoefs = 6, #maximum number of cell types to include in the mixture (from 2 to
                      maxSamples = 100,
                      noisy = TRUE, #Add a noisy profile to the simulated pure mix
                      useCores=3L)
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```






```
# Getting simulated data
# Simulated Samples
dim(res$SimulatedData$M)
```

```
## [1] 170 100
```

```
head(res$SimulatedData$M[, 1:4])
```

```
##               Sim1      Sim2      Sim3       Sim4
## ABCB4     13.29021  11.26067 163.45946  4.5800447
## ADAM28    76.79587 127.90301  39.40495 25.5264146
## ADAM6    177.38637  39.54803  88.54633  0.1701104
## AFF3      46.30495 315.18722  24.60387  4.4425339
## AKAP2     54.71688  22.70742  28.19767  7.3201416
## ARHGAP24  32.86084  12.70722  15.00088 11.2859041
```

```
#Simulated betas (coefficients)
head(res$SimulatedData$B)
```

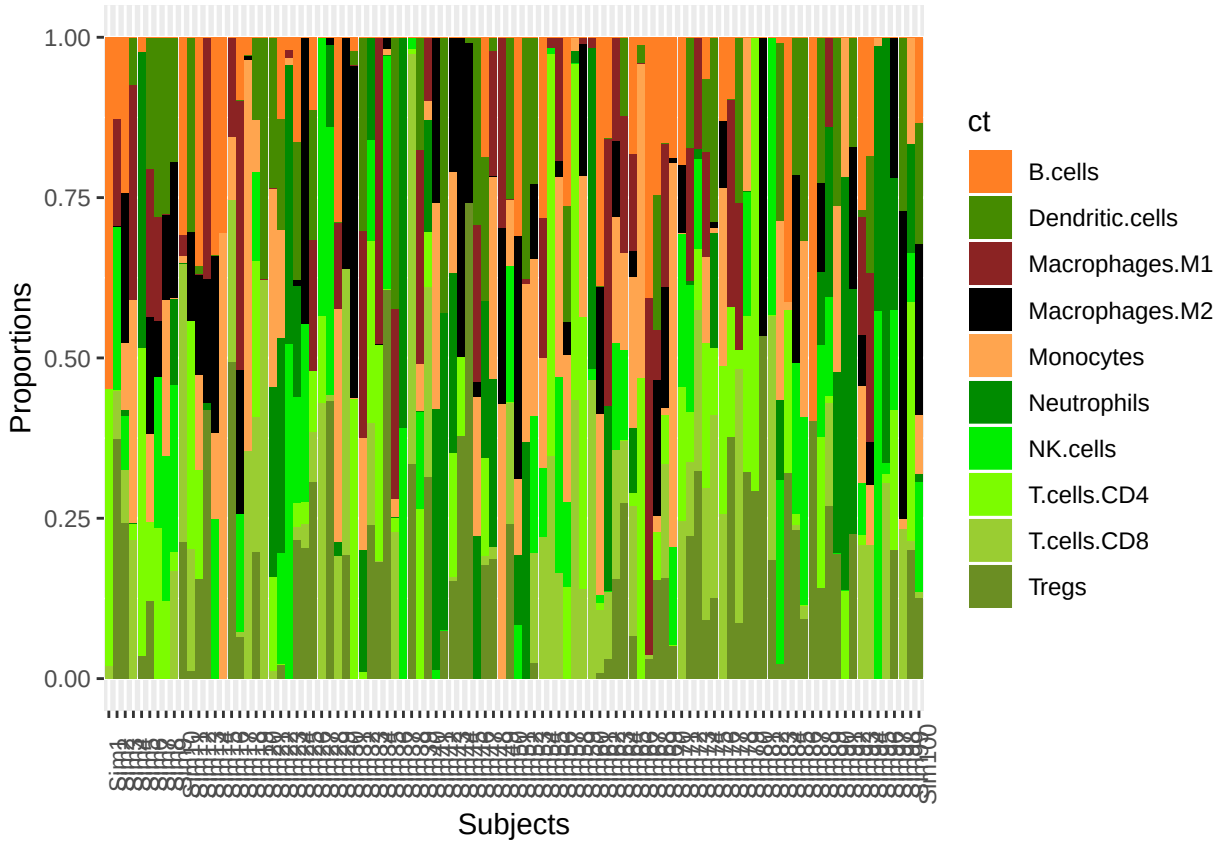
```
##        B.cells Macrophages.M1 Macrophages.M2 Monocytes Neutrophils   NK.cells
## [1,] 0.5557261      0.0000000      0.0000000 0.0000000   0.0000000 0.00000000
## [2,] 0.1258823      0.1655962      0.0000000 0.0000000   0.0000000 0.24247739
## [3,] 0.2444789      0.0000000      0.2370051 0.1132436   0.0000000 0.08577184
## [4,] 0.0000000      0.3449028      0.0000000 0.3439071   0.0000000 0.00000000
## [5,] 0.0000000      0.0000000      0.0000000 0.0000000   0.4737942 0.00000000
## [6,] 0.0000000      0.2401874      0.1864803 0.1449138   0.0000000 0.00000000
##      T.cells.CD4 T.cells.CD8     Tregs Dendritic.cells
## [1,]   0.4442739  0.00000000 0.0000000       0.0000000
## [2,]   0.0000000  0.11075218 0.3552919       0.0000000
```

```
## [3,]   0.0000000  0.07259511 0.2469054       0.0000000
## [4,]   0.0000000  0.22780415 0.0000000       0.0833859
## [5,]   0.5262058  0.00000000 0.0000000       0.0000000
## [6,]   0.0000000  0.00000000 0.2031668       0.2252518
```

```
#Retrieving MIXTURE results
dim(GetMixture(res$MIXTURE))
```

```
## [1] 100  10
```

```
# Displaying the cell type proportions
ProportionPlot(res$MIXTURE)
```



How to Download CDC1000 pure cell lines data to test the MIXTURE algorithm ?

```
library(data.table)
library(openxlsx)
library(org.Hs.eg.db)
library(limma)

#download and unzip expression from
url <- "https://www.cancerrxgene.org/gdsc1000/GDSC1000_WebResources/
/Data/preprocessed/Cell_line_RMA_proc_basalExp.txt.zip"
fname <- basename(url)
# this will save the downloaded file in your working directory
download.file(url  =  url, destfile = fname, method = "auto")
unzip(fname)

#load expression matrix
a.data<- fread("Cell_line_RMA_proc_basalExp.txt")
```

```
#update annotation
b.annot<- a.data[,1:2]
colnames(b.annot)<- c("symbol", "name")
columns(org.Hs.eg.db)
b.entrezids <- mapIds(org.Hs.eg.db, keys=b.annot$symbol, column="ENTREZID",
                      keytype="SYMBOL", multiVals="first")
b.entrezids[sapply(b.entrezids, is.null)]<- NA
b.annot$entrezid<- unlist(b.entrezids)

#fix colnames
colnames(a.data)<- gsub("DATA.", "", colnames(a.data))

#make elist
b.elist<- new("EList", list(E=a.data[,-c(1,2)], genes= b.annot))
dim(b.elist)

#remove missing entrezid
b.elist<- b.elist[which(!is.na(b.elist$genes$entrezid)),]

#combine repeated entrezid expression
b.elist<- avereps(x = b.elist, ID = b.elist$genes$entrezid)
# this will save the cell lines gene expression matrix in your working directory
saveRDS(b.elist, file = "celllines.rds")
```

Once the file has been downloaded and stored in your hard disk, the you can proceed to analyze

```
# we will run over the first 10 cell lines
library(limma)
library(MIXTURE)
cells <- readRDS("celllines.rds")
# gene expression cell line data is in log2 scale
# so we anti log the data
M <- 2^cells$E

cn <- colnames(cells$E)
cn[1:10]
```

```
##  [1] "906826"  "687983"  "910927"  "1240138" "1240139" "906792"  "910688"
##  [8] "1240135" "1290812" "907045"
```

```
rownames(M) <- cells$genes$symbol
# we will only process the first ten cell lines
cells.mix <- MIXTURE(expressionMatrix = M[, 1:10], signatureMatrix =  LM22, useCores = 3L)
head(GetMixture(cells.mix))
```

```
##         B cells naive B cells memory Plasma cells T cells CD8 T cells CD4 naive
## 906826              0              0   0.31962758           0         0.0000000
## 687983              0              0   0.41648729           0         0.0000000
## 910927             NA             NA           NA          NA                NA
## 1240138             0              0   0.00000000           0         0.2482379
## 1240139             0              0   0.09858075           0         0.2474324
## 906792              0              0   0.26761851           0         0.0000000
##         T cells CD4 memory resting T cells CD4 memory activated
## 906826                   0.4185829                            0
## 687983                   0.0000000                            0
```

```
## 910927                          NA                            NA
## 1240138                  0.0000000                             0
## 1240139                  0.0000000                             0
## 906792                   0.0000000                             0
##         T cells follicular helper T cells regulatory (Tregs)
## 906826                   0.0000000                           0
## 687983                   0.2297501                           0
## 910927                          NA                          NA
## 1240138                  0.0000000                           0
## 1240139                  0.0000000                           0
## 906792                   0.2209942                           0
##         T cells gamma delta NK cells resting NK cells activated Monocytes
## 906826                    0                0                  0         0
## 687983                    0                0                  0         0
## 910927                   NA               NA                 NA        NA
## 1240138                   0                0                  0         0
## 1240139                   0                0                  0         0
## 906792                    0                0                  0         0
##         Macrophages M0 Macrophages M1 Macrophages M2 Dendritic cells resting
## 906826      0.00000000              0              0                       0
## 687983      0.00000000              0              0                       0
## 910927              NA             NA             NA                      NA
## 1240138     0.05308345              0              0                       0
## 1240139     0.00000000              0              0                       0
## 906792      0.07261115              0              0                       0
##         Dendritic cells activated Mast cells resting Mast cells activated
## 906826                 0.05252302          0.2092665                    0
## 687983                 0.35376259          0.0000000                    0
## 910927                         NA                 NA                   NA
## 1240138                0.26332460          0.4353540                    0
## 1240139                0.25975621          0.0000000                    0
## 906792                 0.43877617          0.0000000                    0
##         Eosinophils Neutrophils
## 906826    0.0000000           0
## 687983    0.0000000           0
## 910927           NA          NA
## 1240138   0.0000000           0
## 1240139   0.3942307           0
## 906792    0.0000000           0
```

How to process TCGA Data ?

```r
#Download FPKM data with TCGAbioLinks
library(TCGAbiolinks)
library(SummarizedExperiment)

query_all_brca_fpkm <- GDCquery(project = "TCGA-BRCA",
                            data.category = "Transcriptome Profiling",
                            data.type = "Gene Expression Quantification",
                            experimental.strategy = "RNA-Seq",
                            workflow.type = "HTSeq - FPKM")

GDCdownload(query_all_brca_fpkm)
```

```r
data_all_brca_fpkm <- GDCprepare(query_all_brca_fpkm, save = TRUE, save.filename = "BRCA.All.FPKM.rda")
##Chage the directory according to wahre you save your downloaded data
```

```
## Loading required package: GenomicRanges
## Loading required package: stats4
## Loading required package: BiocGenerics
##
## Attaching package: 'BiocGenerics'
## The following object is masked from 'package:limma':
##
##     plotMA
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB
## The following object is masked from 'package:gridExtra':
##
##     combine
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##     anyDuplicated, 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, which.max, which.min
## Loading required package: S4Vectors
##
## Attaching package: 'S4Vectors'
## The following object is masked from 'package:plyr':
##
##     rename
## The following object is masked from 'package:base':
##
##     expand.grid
## Loading required package: IRanges
##
## Attaching package: 'IRanges'
## The following object is masked from 'package:plyr':
##
##     desc
## Loading required package: GenomeInfoDb
```

```
## Loading required package: Biobase
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
## Loading required package: DelayedArray
## Loading required package: matrixStats
##
## Attaching package: 'matrixStats'
## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians
## The following object is masked from 'package:plyr':
##
##     count
##
## Attaching package: 'DelayedArray'
## The following objects are masked from 'package:matrixStats':
##
##     colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges
## The following objects are masked from 'package:base':
##
##     aperm, apply, rowsum
#set path where your BRCA.All.FPKM.rda is located
load(file.path(path,"BRCA.All.FPKM.rda"))

 # Prepare your gene expression data matrix
 gene_annot_all <- rowData(data)
 exp.all <- assay(data)
 target.data.all <- as.data.frame(colData(data))
 tumor.type <- do.call(rbind,str_split(as.character(target.data.all$barcode),"-"))
 # Tissue type distribution
 table(tumor.type[,4])
##
##  01A  01B  01C  06A  11A  11B
## 1077   24    1    7   99   14
 rownames(exp.all)[1:10]
##  [1] "ENSG00000000003" "ENSG00000000005" "ENSG00000000419" "ENSG00000000457"
##  [5] "ENSG00000000460" "ENSG00000000938" "ENSG00000000971" "ENSG00000001036"
##  [9] "ENSG00000001084" "ENSG00000001167"
 # only ensembl gene ids
 exp.all <- exp.all[gene_annot_all$ensembl_gene_id,]
 #MIXTURE use rownames to identify genes and macth with the molecular signature
 rownames(exp.all) <- gene_annot_all$external_gene_name
 rownames(exp.all)[1:10]
```

```
##  [1] "TSPAN6"   "TNMD"     "DPM1"     "SCYL3"    "C1orf112" "FGR"
##  [7] "CFH"      "FUCA2"    "GCLC"     "NFYA"
```

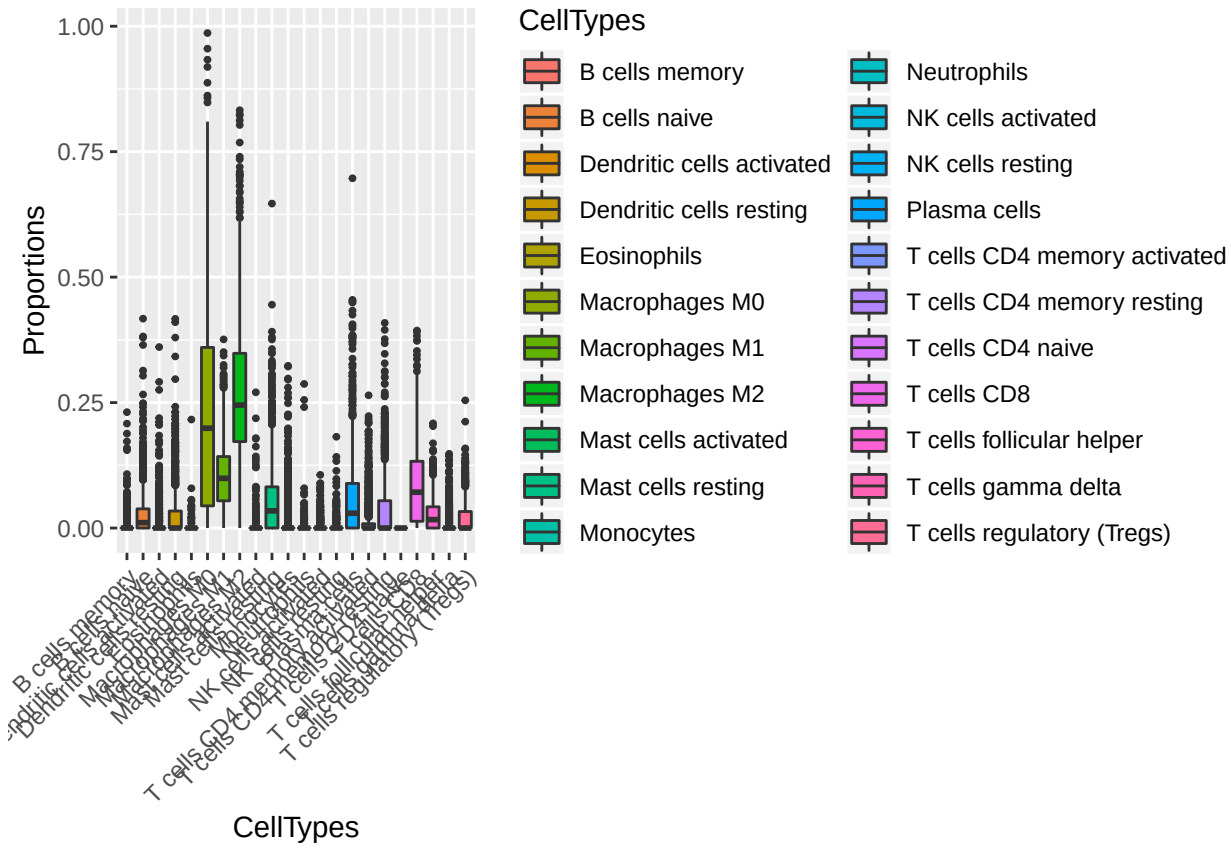
```
#an E list from limma package
a.data <- new("EList",list(E = exp.all, genes = gene_annot_all, targets = target.data.all))
a.data$targets$TissueType <- do.call(rbind,str_split(as.character(target.data.all$barcode),"-"))[,4]
# processing with MIXTURE
brca.mix <- MIXTURE(expressionMatrix = a.data$E, signatureMatrix =  LM22, useCores = 6L)

round(apply(GetMixture(brca.mix),2,function(x) sum(x>0))/nrow(GetMixture(brca.mix)),2)
```

```
##                B cells naive                B cells memory
##                         0.55                          0.06
##                Plasma cells                   T cells CD8
##                         0.57                          0.77
##           T cells CD4 naive   T cells CD4 memory resting
##                         0.00                          0.44
## T cells CD4 memory activated    T cells follicular helper
##                         0.25                          0.58
##   T cells regulatory (Tregs)          T cells gamma delta
##                         0.48                          0.18
##             NK cells resting           NK cells activated
##                         0.05                          0.09
##                   Monocytes               Macrophages M0
##                         0.18                          0.80
##               Macrophages M1               Macrophages M2
##                         0.95                          0.99
##      Dendritic cells resting    Dendritic cells activated
##                         0.44                          0.14
##           Mast cells resting         Mast cells activated
##                         0.67                          0.04
##                 Eosinophils                 Neutrophils
##                         0.01                          0.05
```

```
brca.prop <- GetMixture(brca.mix)
df <- data.frame(Proportions = c(brca.prop), CellTypes = rep(colnames(brca.prop), each = nrow(brca.prop

ggplot(df,aes(x=CellTypes, y=Proportions, fill = CellTypes)) +  geom_boxplot(outlier.size = 0.7) +
  theme(axis.text.x = element_text(angle=45, vjust = 1,hjust = 1))
```


Proportions
1.00
0.75
0.50
0.25
0.00
CellTypes
CellTypes
B cells memory
B cells naive
Dendritic cells activated
Dendritic cells resting
Eosinophils
Macrophages M0
Macrophages M1
Macrophages M2
Mast cells activated
Mast cells resting
Monocytes
Neutrophils
NK cells activated
NK cells resting
Plasma cells
T cells CD4 memory activated
T cells CD4 memory resting
T cells CD4 naive
T cells CD8
T cells follicular helper
T cells gamma delta
T cells regulatory (Tregs)