SMGR: Setup and Introduction

SMGR (Joint statistical method for integrative analysis of single-cell multi-omics data)

Vignette built on April 20, 2022.

SMGR workflow

This tutorial goes through the steps in the SMGR workflow:

- Identify conistent signals of genes and peaks based on scRNA-seq & scATAC-seq data.
- Reveal the latent representation of co-expressed genes and peaks, i.e. co-regulatory program
- Identify co-expressed TF and target genes, based on single-cell multi-omics analysis

The setup and running steps are shown as follows in this tutorial:

Requirements

- Input: expression matrix The input to SMGR is a list of scRNA-seq expression matrix and scATAC-seq matrix.

For scRNA-seq matrix: Each column corresponds to a sample (cell) and each row corresponds to a gene. Expression units: The preferred expression values are gene-summarized raw counts. Other measurements (such as FPKM) are also accepted.

For scATAC-seq matrix: Each column corresponds to a sample (cell) and each row corresponds to a peak.

Installation

The R implementation of SMGR is based on R packages as below.

Therefore, you will need to install these packages, and some extra dependencies, to run SMGR:

```
if (!requireNamespace("BiocManager", quietly = TRUE)) install.packages("BiocManager")
BiocManager::version()
```

```
## [1] '3.12'
```

```
# If your bioconductor version is previous to 4.0, see the section bellow
# Required
packages = c('glmnet','MASS','purrr','mpath','zic','pscl','parallel')
# BiocManager::install(packages)
```

Now you are ready to install SMGR:

```
# devtools::install_github("QSong-github/SMGR")
library('SMGR')
```

Note: please invoke these packages to run the following codes:

```
pkg = c('glmnet','MASS','purrr','mpath','zic','pscl','parallel')
sapply(pkg, require, character.only = TRUE)
```

```
##   glmnet     MASS    purrr    mpath      zic     pscl parallel
##     TRUE     TRUE     TRUE     TRUE     TRUE     TRUE     TRUE
```

Run SMGR with example data

Example data is deposited in the data folder.

```
# @param rna.cts simulated scRNA-seq data
rna.cts <- readRDS('simuation_scRNA-seq.RDS')

# @param atac.cts simulated scATAC-seq data
atac.cts <- readRDS(file='simuation_scATAC-seq.RDS')

input_data <- list(rna.cts, atac.cts)
```

SMGR process

Input data is a list of scRNA-seq and scATAC-seq data

```
input_data <- list(as.matrix(rna.cts),as.matrix(atac.cts))

result1 <- smgr_main(sm.data = input_data, K=nrow(input_data[[1]]))
```

```
## ## ============================================================================
## ## |                              running SMGR                                |
## ## ----------------------------------------------------------------------------
## ## |            scRNA-seq data and scATAC-seq data identified ....              |
## ## |                            Set initial values ....                         |
## ## |                                iter= 1.....                                |
## ## |                                iter= 2.....                                |
## ## |                                iter= 3.....                                |
## ## |                                iter= 4.....                                |
## ## |                                iter= 5.....                                |
## ## |                                iter= 6.....                                |
## ## |                                iter= 7.....                                |
## ## |                                iter= 8.....                                |
## ## |                                iter= 9.....                                |
## ## |                                iter=10.....                                |
## ## |                                iter=11.....                                |
## ## |                                iter=12.....                                |
## ## |                                iter=13.....                                |
## ## |                                iter=14.....                                |
## ## |                                iter=15.....                                |
## ## |                                iter=16.....                                |
## ## |                                iter=17.....                                |
## ## |                                iter=18.....                                |
## ## |                                iter=19.....                                |
## ## |                                iter=20.....                                |
```

if output is set to 'all', you will obtain the coefficients and BIC values

```
# member1: is the ground truth
member1 <- c(rep(1,600),rep(2,600),rep(3,600))

# programs: co-expressed gene & peaks from latent representation
programs <- result1$clusters
```

report ARI score

```
library(clues)
print (adjustedRand(programs, member1))
```

```
##    Rand      HA      MA      FM Jaccard
##       1       1       1       1       1
```

latent.var : latent representation of genes & peaks

```
latent.var <- result1$latent_f
```

evaluation metrics

eval : evaluation metrics

```
library(clValid); library(clusterCrit)

eval <- vector('list',length=2)
for ( i in 1:2 ){
    data <- log2(input_data[[i]]+1)
    ind1 <- Evaluate(data,programs)
    ind2 <- intCriteria(data,as.integer(programs),
                        c("Calinski_Harabasz",'Davies_Bouldin'))
    inds <- as.data.frame(rbind(do.call(rbind,ind2),ind1))
    if (i==1){ inds$data <- 'scRNA-seq' } else { inds$data <- 'scATAC-seq' }
    colnames(inds) <- c('metrics','data')
    eval[[i]] <- inds
}
```

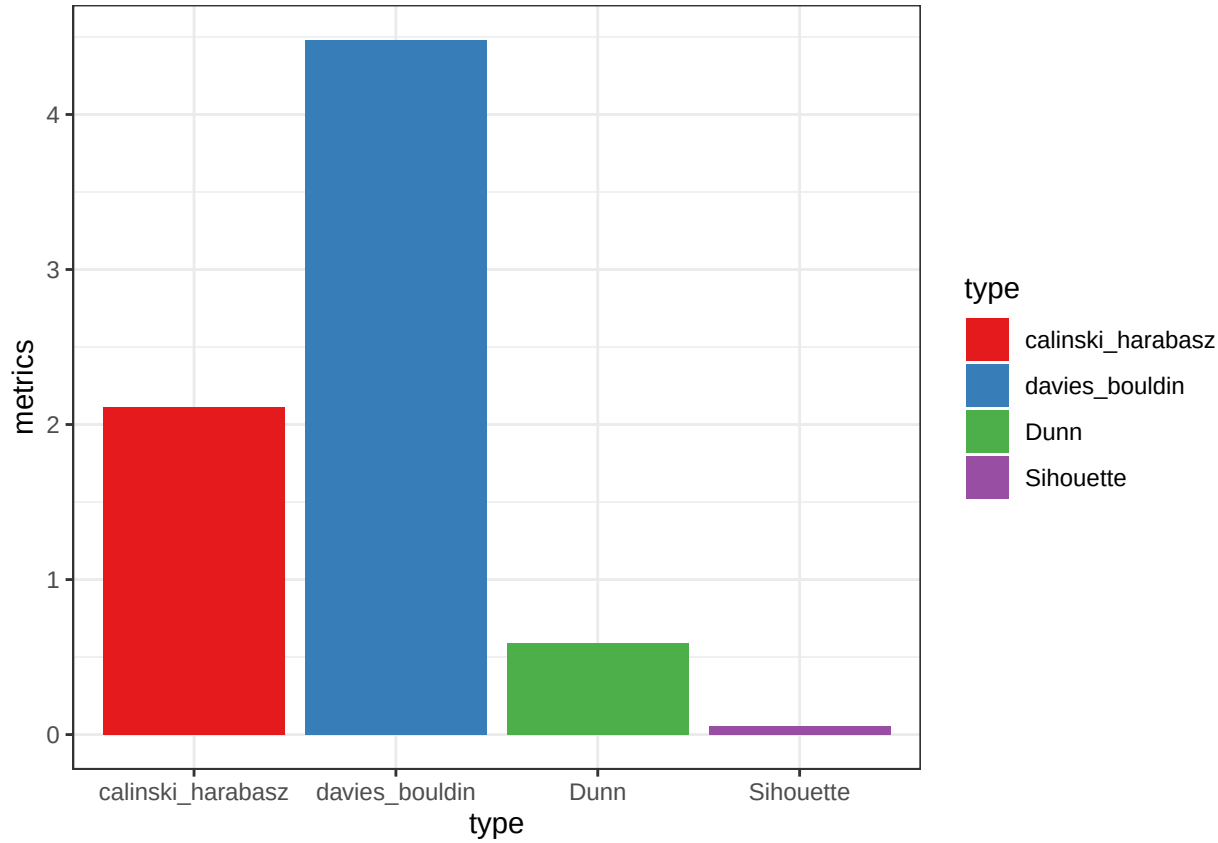
Evaluation index for scRNA-seq data

```
print (eval[[1]])
```

```
##                        metrics      data
## calinski_harabasz 130.00004149 scRNA-seq
## davies_bouldin      4.48195970 scRNA-seq
## Dunn                0.59328525 scRNA-seq
## Sihouette           0.05238342 scRNA-seq
```

```
eval[[1]][1,1] = log10(eval[[1]][1,1])
eval[[1]]$type = rownames(eval[[1]])
library(ggplot2)
```

```
p <- ggplot(eval[[1]], aes(x=type, y=metrics, fill=type)) +
    geom_bar(stat='identity')+
    scale_fill_brewer(palette='Set1')+ theme_bw()
print (p)
```

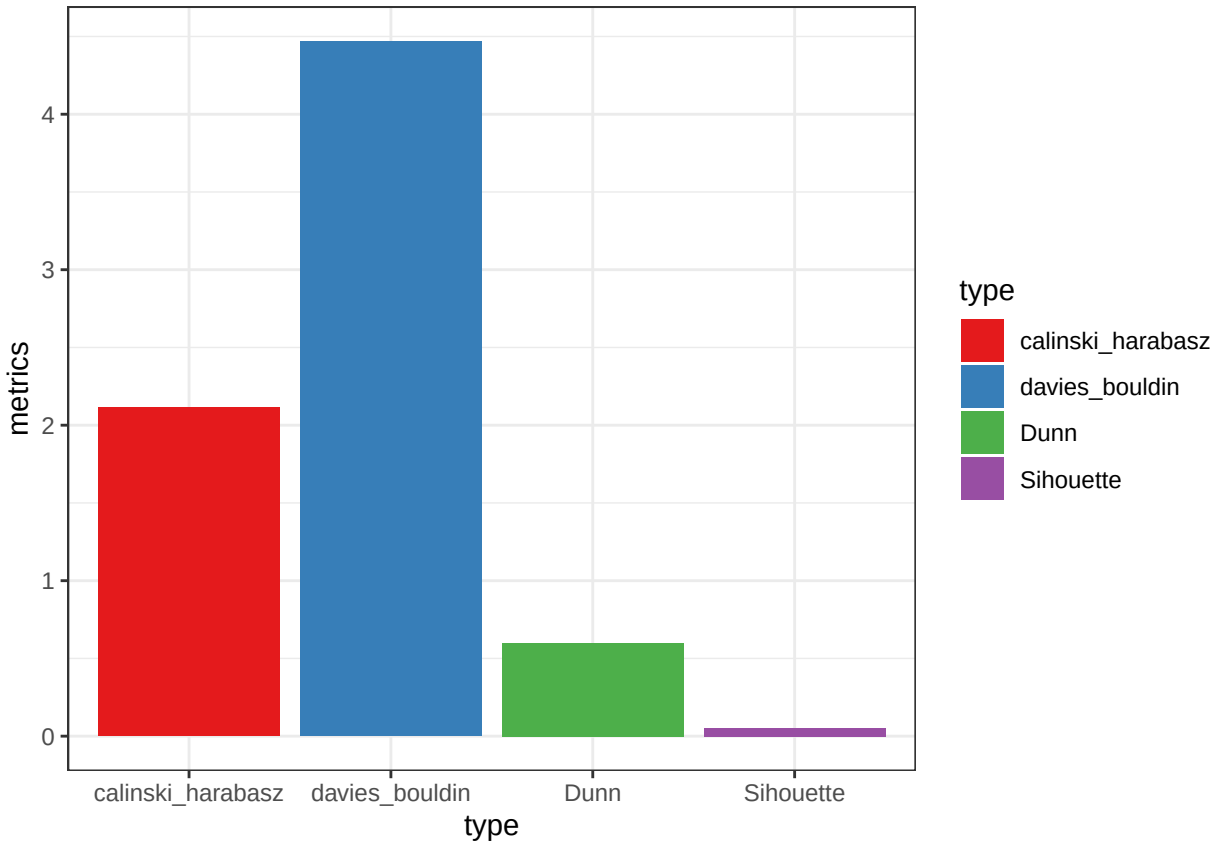


Evaluation index for scATAC-seq data

```
print (eval[[2]])
```

```
##                       metrics       data
## calinski_harabasz 130.09797714 scATAC-seq
## davies_bouldin      4.46870408 scATAC-seq
## Dunn                0.59913972 scATAC-seq
## Sihouette           0.05267272 scATAC-seq
```

```
eval[[2]][1,1] = log10(eval[[2]][1,1])
eval[[2]]$type = rownames(eval[[2]])
p <- ggplot(eval[[2]], aes(x=type, y=metrics, fill=type)) +
    geom_bar(stat='identity')+
    scale_fill_brewer(palette='Set1')+ theme_bw()
print (p)
```



Visualization of results data

for scRNA-seq data

```
library(ComplexHeatmap)
d1 <- log2(input_data[[1]]+1)
```

Suppose these programs are identified in differnt cell types

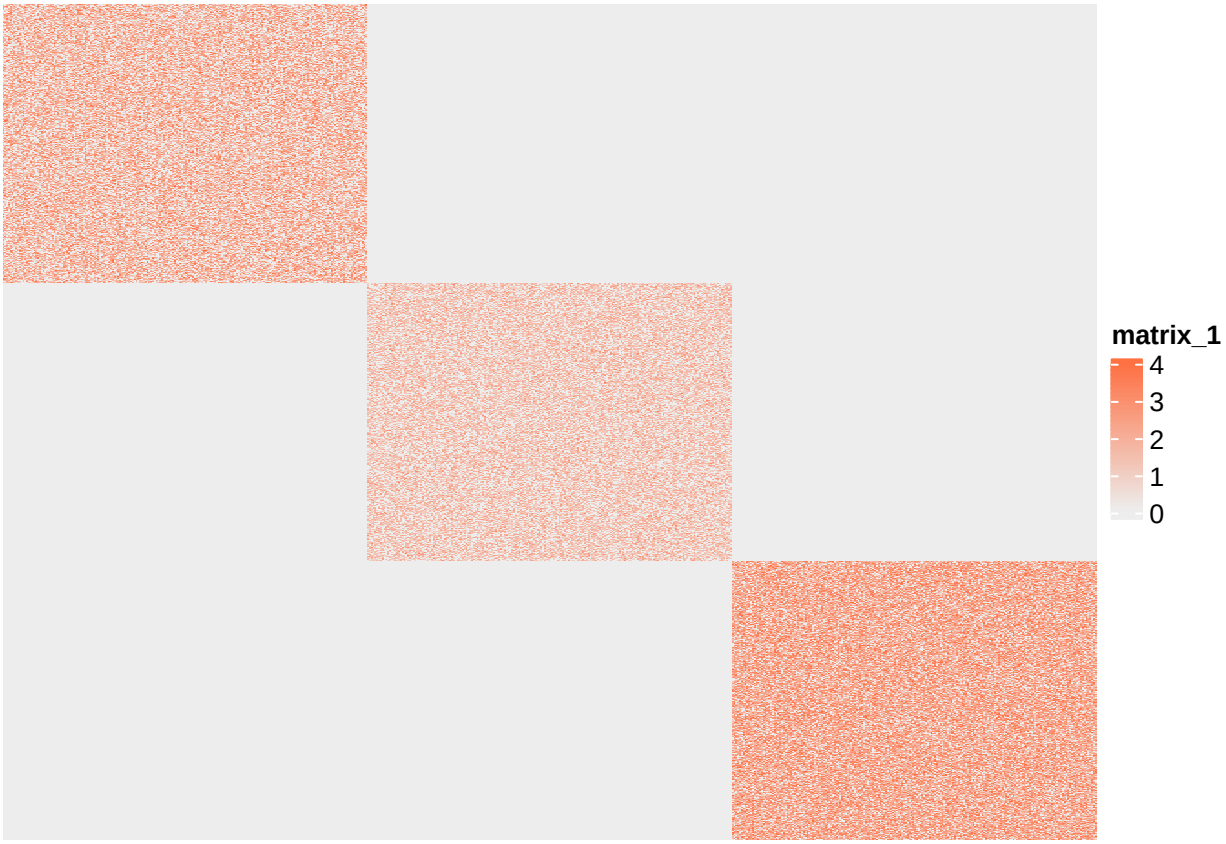
```
m1 <- d1[programs==1,]; m2 <- d1[programs==2,]; m3 <- d1[programs==3,]
program.d <- rbind(cbind(m1,matrix(0,nrow=nrow(m1),ncol=400)),
                   cbind(matrix(0,nrow=nrow(m2),ncol=200),m2,matrix(0,nrow=nrow(m2),ncol=200)),
                   cbind(matrix(0,nrow=nrow(m3),ncol=400),m3))
```

color setting

```
library(circlize)
c1 <- c("#EDEDEDFF","dodgerblue"); c1 <- c("#EDEDEDFF","#FF7043FF")
myCol1 <- colorRampPalette(c1)(100); myBreaks1 <- seq(0, 4, length.out = 100)
```

Heatmap

```
print (Heatmap(program.d,
               cluster_columns = FALSE,cluster_rows =F,
               show_column_names = FALSE,show_row_names=FALSE,
               col = colorRamp2(myBreaks1, myCol1)))
```



Run SMGR with your own data

For your own real data: here we use the simulated toy data to demonstrate the process

```
#devtools::install_github("bowang-lab/simATAC")
library(simATAC);
count <- getCountFromh5("GSE99172.snap")  # GSE99172.snap is accessbile from simATAC package: https://d
obj1 <- simATACEstimate(t(count))
obj1 <- setParameters(obj1, noise.mean = -0.3, noise.sd = 0.1)
obj1 <- simATACSimulate(obj1, nCells = 200)
```

the .gtf file can be downloaded from https://grch37.ensembl.org/info/data/ftp/index.html

map the link between peak regions and genes:

```
# atac.cts: toy scATAC data
atac.cts = linkActivityMatrix(peak.matrix = counts(obj1)[1:6000,1:200],
                              annotation.file = 'Homo_sapiens.GRCh37.82.gtf',
                              seq.levels = c(1:22,'X','Y'),
                              upstream = 2000)$activity_matrix[1:600,1:200]

# rna.cts: toy scRNA-seq data
library(splatter); library(scater)
sim.groups <- splatSimulate(group.prob =1, method = "groups",
```

```
                            verbose = FALSE, nGenes=600, batchCells=200)
rna.cts <- counts(sim.groups)
rownames(rna.cts) <- rownames(atac.cts);
colnames(rna.cts) <- paste0('cell_',1:ncol(rna.cts))
```

input_data: with a list of scRNA-seq and scATAC-seq toy data

```
input_data <- list(rna.cts, atac.cts)
```

from here, you can start with the smgr function and use the evaluation index to check if you achieve good performance of idnetifying co-regulation program

```
library(SMGR)
result1 <- smgr_main(sm.data = input_data, K=nrow(input_data[[1]]))
```

```
## ## ================================================================================
## ## |                              running SMGR                                    |
## ## --------------------------------------------------------------------------------
## ## |            scRNA-seq data and scATAC-seq data identified ....                |
## ## |                            Set initial values ....                           |
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## ## |                                iter= 3.....                                  |
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## ## |                                iter= 8.....                                  |
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## ## |                                iter=15.....                                  |
## ## |                                iter=16.....                                  |
## ## |                                iter=17.....                                  |
## ## |                                iter=18.....                                  |
## ## |                                iter=19.....                                  |
## ## |                                iter=20.....                                  |
```