SMGR: Setup and Introduction

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

Vignette is updated on June 16, 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:

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

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

```r
# 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:

```r
# 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
}
```

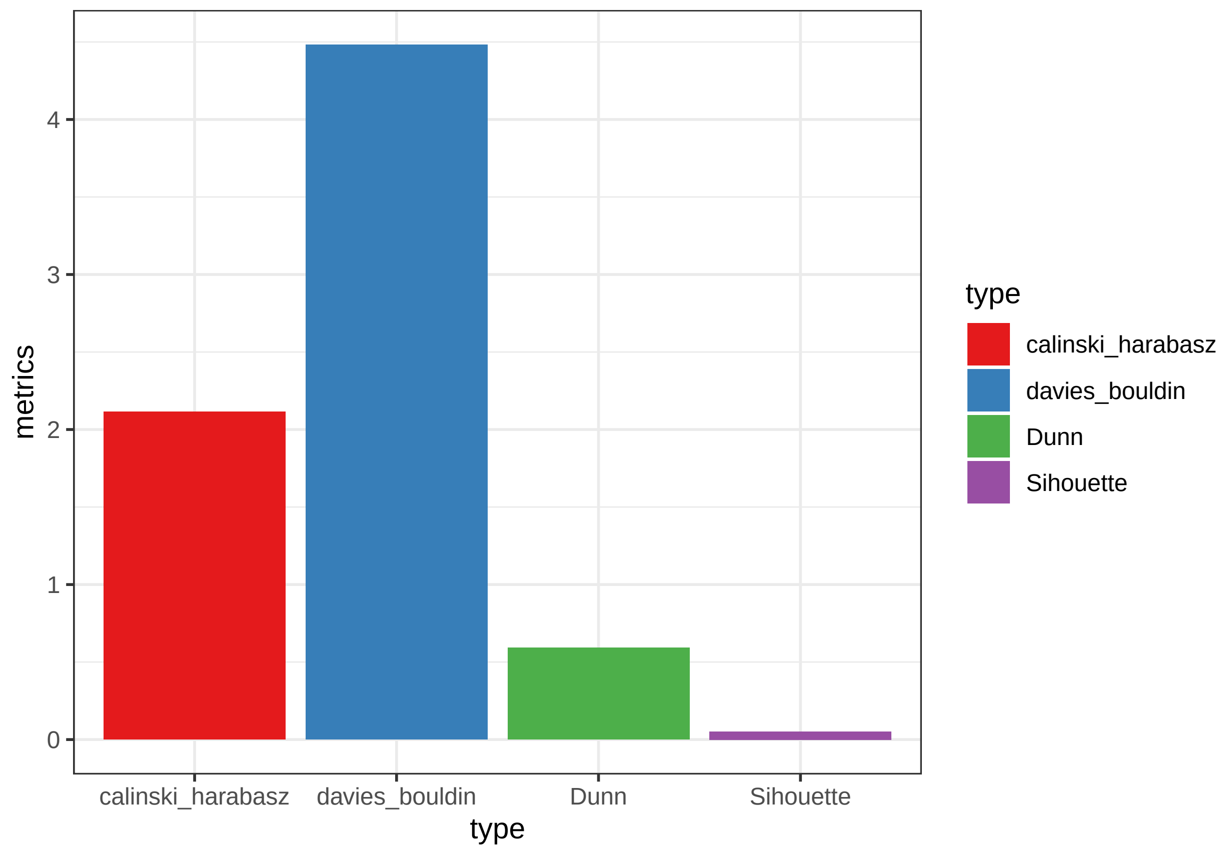
Evaluation index for scRNA-seq data

```
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)
```

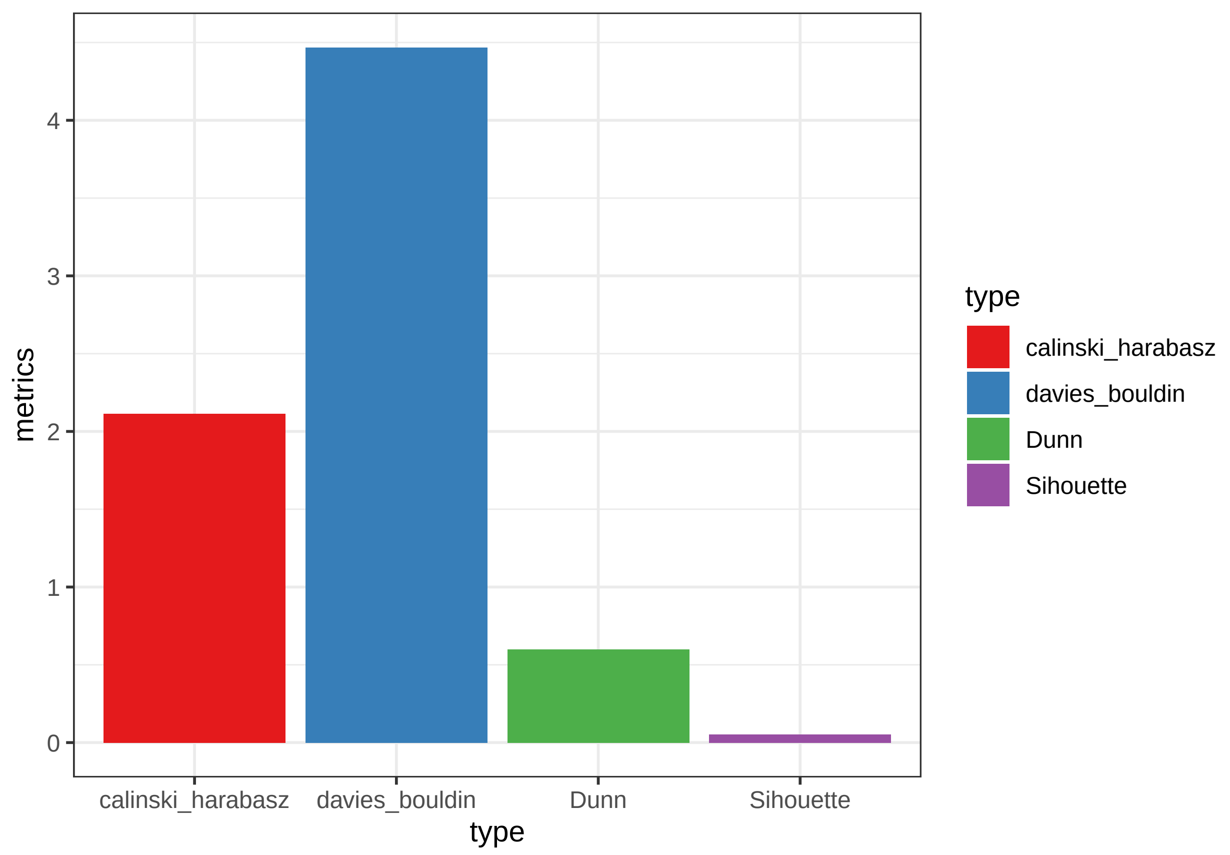


Evaluation index for scATAC-seq data

```
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

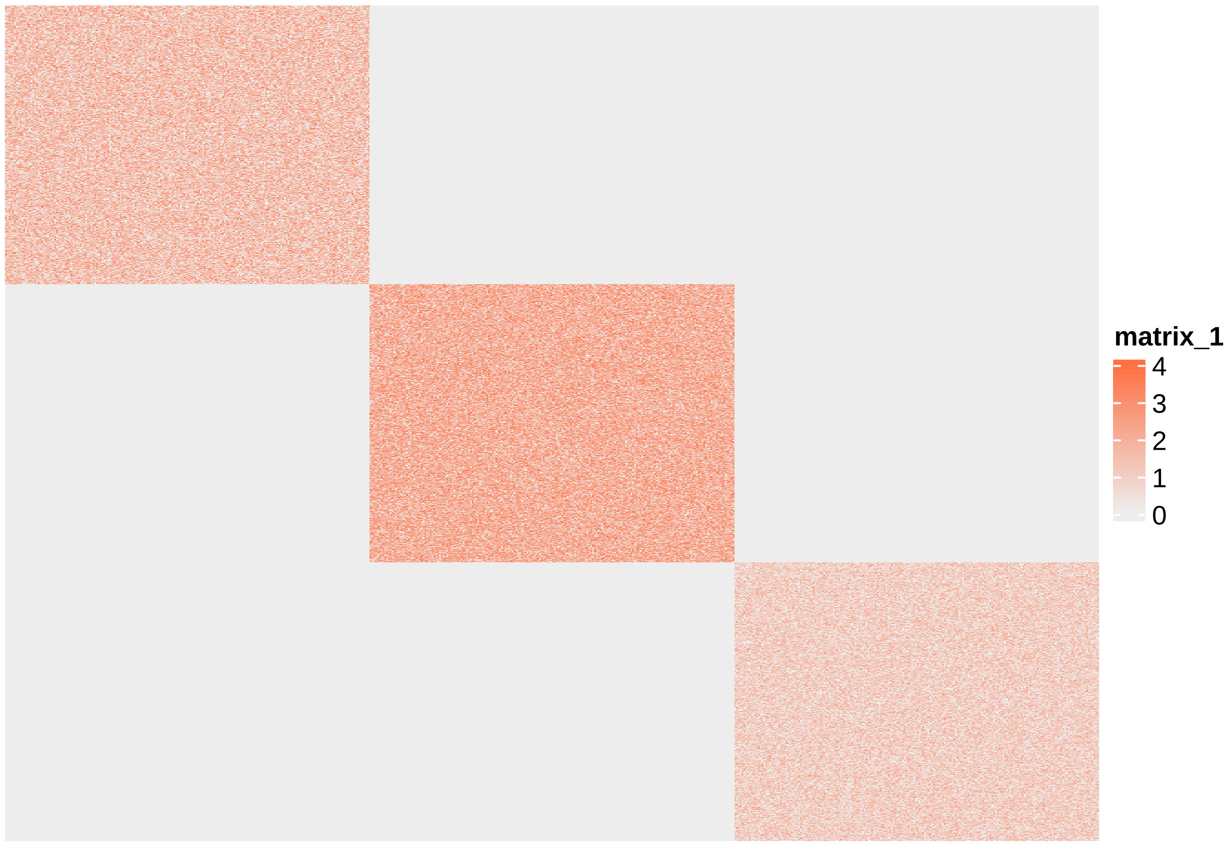
```
m1 <- d1[programs==1,]; m2 <- d1[programs==2,]; m3 <- d1[programs==3,]
program.d <- rbind(cbind(m1,matrix(0,nrow=nrow(m1),ncol=400)),
                   cbind(matrix(0,nrow=nrow(m2),ncol=200),m2,matrix(0,nrow=nrow(m2),ncol=200)),
                   cbind(matrix(0,nrow=nrow(m3),ncol=400),m3))
```

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

```r
knitr::opts_chunk$set(eval = FALSE)
#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:

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

# rna.cts: toy scRNA-seq data
```

```r
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

```r
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

```r
knitr::opts_chunk$set(eval = FALSE)
library(SMGR)
result1 <- smgr_main(sm.data = input_data, K=nrow(input_data[[1]]))
```

Generate simulation data for SMGR

Here we use the negative bionomila distribution to simulate data for SMGR testing. We use different seeds and different parameters to generate the ground truth of the co-regulatory programs across scRNA-seq data and scATAC-seq data.

```r
knitr::opts_chunk$set(eval = FALSE)
library(MASS)
mu1 = 0.2; theta1= 0.3; mu2 = 2; theta2 = 0.4; mu3= 4; theta3=0.3; M = 600; N = 200
# these parameters can be changed for different simulation data

set.seed(123); rna1 <- matrix(rnegbin(n=M*N,mu=mu1,theta=theta1),ncol=N)
set.seed(123); atac1 <- matrix(rnegbin(n=M*N,mu=mu1,theta=theta1),ncol=N)

set.seed(123); rna2 <- matrix(rnegbin(n=M*N,mu=mu2,theta=theta2),ncol=N)
set.seed(123); atac2 <- matrix(rnegbin(n=M*N,mu=mu2,theta=theta2),ncol=N)

set.seed(123); rna3 <- matrix(rnegbin(n=M*N,mu=mu3,theta=theta3),ncol=N)
set.seed(123); atac3 <- matrix(rnegbin(n=M*N,mu=mu3,theta=theta3),ncol=N)

# *rna.sim* simulated scRNA-seq data
rna.sim <- rbind(rna1,rna2,rna3)
# *atac.sim* simulated scATAC-seq data
atac.sim <- rbind(atac1,atac2,atac3)

sim_data = list(rna.sim, atac.sim)
sim.result <- smgr_main(sm.data = sim_data, K=nrow(sim_data[[1]]))
```

Notes of applying SMGR to real data

Cell-level filtering: For real data, we will have the metadata with columns include: - orig.ident: the identity of cells - nCount_RNA: number of UMIs per cell - nFeature_RNA: number of genes detected per cell

```r
knitr::opts_chunk$set(eval = FALSE)
# Assume "metadata" is a dataframe
# Add number of genes per UMI for each cell to metadata
metadata$log10GenesPerUMI <- log10(metadata$nFeature_RNA) / log10(metadata$nCount_RNA)
# Compute percent mito ratio
metadata$mitoRatio <- PercentageFeatureSet(object = metadata, pattern = "^MT-")
metadata$mitoRatio <- meta.data$mitoRatio / 100
# Add cell IDs to metadata
metadata$cells <- rownames(metadata)
# Rename columns
metadata <- metadata %>%
        dplyr::rename(seq_folder = orig.ident,
                      nUMI = nCount_RNA,
                      nGene = nFeature_RNA)
# We recommend to filter out low quality cells before using SMGR.
filtered_cells <- subset(x = metadata,
                         subset= (nUMI >= 200) &
                           (nGene >= 200) &
                           (log10GenesPerUMI > 0.80) &
                           (mitoRatio < 0.20))
```

Gene-level filtering: Real data will have many genes with zero counts. We will remove genes that have zero expression in all cells. Additionally, if a gene is expressed in only very small portion of cells, it is not particularly meaningful as well. Thus we recommend to keep only genes which are expressed in 10 or more cells.

```r
knitr::opts_chunk$set(eval = FALSE)
# Sums all TRUE values and returns TRUE if more than 10 TRUE values per gene
keep_genes <- Matrix::rowSums(counts > 0) >= 10
# Filtered data by cells and genes
filtered_counts <- counts[keep_genes, filtered_cells$cells]
```