scLAPA: Learning association for single-cell transcriptomics by integrating single-cell profiling of gene expression and alternative polyadenylation

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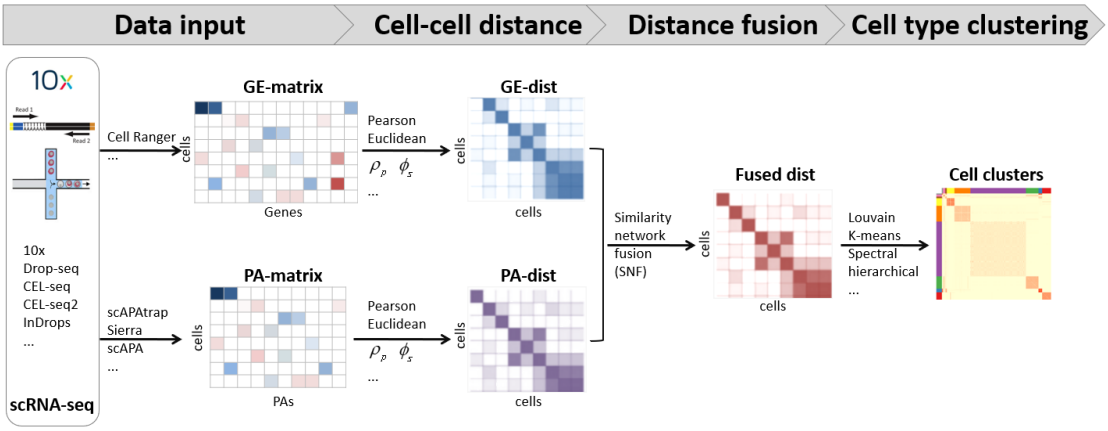
1 Introduction

High variability and dropout rate inherent in scRNA-seq confounds the reliable quantification of cell-cell associations based on the gene expression profile alone. Lately bioinformatics studies have emerged to capture key transcriptome information on alternative polyadenylation (APA) from standard scRNA-seq and revealed APA dynamics among cell types, suggesting the possibility of discerning cell identities with the APA profile. We proposed a toolkit called scLAPA for learning association for single-cell transcriptomics by combing single-cell profiling of gene expression and alternative polyadenylation. As a comprehensive toolkit, scLAPA provides a unique strategy to learn cell-cell associations, improve cell type clustering and discover novel cell types by the augmentation of gene expression profiles with polyadenylation information, which can be incorporated in most existing scRNA-seq pipelines.

scLAPA mainly consists of four modules: (i) the input module, (ii) cell-cell distance, (iii) distance fusion, (iv) cell type clustering. The input module prepares the input for scLAPA, including a poly(A) site expression matrix (hereinafter referred as PA-matrix) and a gene expression matrix (hereinafter referred as GE-matrix). In the module of cell-cell distance, a cell-cell distance matrix is learned for PA-matrix (called PA-dist) and GE-matrix (called GE-dist), respectively. The module of distance fusion employs similarity network fusion (SNF) to integrate the two distance matrices (PA-dist and GE-dist) into one cell-cell distance matrix. The cell type clustering module clusters cells based on the fused distance matrix with various clustering methods.



2 Installation

You can install scLAPA from github with:

```
install.packages("devtools")
library(devtools)
install_github("BMILAB/scLAPA")
library(scLAPA)
```

3 Preparations

The input module prepares the input for scLAPA, including a poly(A) site expression matrix (hereinafter referred as PA-matrix) and a gene expression matrix (hereinafter referred as GE-matrix). The PA-matrix is generated from raw scRNA-seq with scAPAtrap, which stores expression levels of poly(A) sites, with each row denoting a poly(A) site and each column denoting a cell. The GE-matrix can be obtained from websites like NCBI GEO and 10x Genomics, or generated by various routine scRNA-seq analysis tools like Cell Ranger. . In this study, we will use the Mouse Hypothalamus as example.

```
>####loading DE-matrix
> data("gene_Hypothalamus")
> gene.data<-gene_Hypothalamus
> dim(gene.data)
[1] 14374   727
> gene.data[1:3,1:3]
                AAAACAGCGTGA AAAACGCCGTCG AAAAGGTGACTC
0610007C21RIK            0     1.956876     2.293011
0610007L01RIK            0     1.395896     0.000000
0610007N19RIK            0     0.000000     0.000000
>####loading PA-matrix
> data("PA_Hypothalamus")
> PA.data<-PA_Hypothalamus$counts
> dim(PA.data)
[1] 7114  727
> PA.data[5:7,1:3]
        AAAACAGCGTGA AAAACGCCGTCG AAAAGGTGACTC
PA8389      0.000000            0     0.000000
PA8396      1.584963            0     3.584963
PA8640      0.000000            0     0.000000
```

4 Standard analysis work-flow

4.1 cell-cell distance

A cell-cell distance matrix is learned for *PA-matrix* (called *PA-dist*) and *GE-matrix* (called *GE-dist*), respectively.In this module, Various distance metrics can be chosen, including Euclidean distance, Pearson correlation, two metrics of proportionality ($\boldsymbol{\rho_p}$ and $\boldsymbol{\emptyset_s}$)[1], RAFSIL (Random Forest based SImilarity Learning)[2] and SIMLR (Single-cell Interpretation via Multikernel Learning)[3].

```
># PA-matrix convert to Pearson similarity matrix(PA-dist)
> PA.pearson<-calculate_distance(PA.data,method = "pearson")
> PA.pearson[1:3,1:3]
                AAAACAGCGTGA AAAACGCCGTCG AAAAGGTGACTC
AAAACAGCGTGA       0.0000000       0.9076725       0.4434940
AAAACGCCGTCG       0.9076725       0.0000000       0.9019272
AAAAGGTGACTC       0.4434940       0.9019272       0.0000000
># GE-matrix convert to Pearson similarity matrix(DE-dist)
> gene.pearson<-calculate_distance(gene.data,method = "pearson")
```

4.2 cell-cell distance

After learning PA-dist and GE-dist, similarity network fusion (SNF)[4] is utilized to flexibly integrate the two layers of cell-cell similarities into one distance matrix. First, PA-dist and GE-dist are iteratively and gradually fused to a consensus network, utilizing the non-linear method of message passing theory. Then weak similarities representing potential noise are discarded, and strong similarities are retained. By generating coherent cell-cell similarities from both APA isoform and gene layers, SNF profiles a more comprehensive biological relationship among cells, beyond the scope of methods solely based on GE-matrix.

```
> fusion<-network_fusion(PA.pearson,gene.pearson,K=20,alpha = 0.5,T=10)
> fusion[1:3,1:3]
                AAAACAGCGTGA AAAACGCCGTCG AAAAGGTGACTC
AAAACAGCGTGA 0.0008185671 0.0004694652 0.0333463433
AAAACGCCGTCG 0.0004694652 0.0008264428 0.0005650480
AAAAGGTGACTC 0.0333463433 0.0005650480 0.0008335804
```

4.3 Clustering

Four widely-used clustering methods were provided in scLAPA to cluster cells on the

basis of the fused cell-cell similarity matrix, including Louvain clustering[5] , hierarchical clustering (HC)[6], spectral clustering (SC) [7], and k-means.

```
>#louvain clustering
> d.pr<-1/(fusion+1)
> fusion_clu<-Clustering(d.pr,method = "louvain")
> label<-as.character(PA_Hypothalamus$colData$celltype)
>#get clustering index
> fusion_index<-index_index(fusion_clu,label = label)
> fusion_index
     purity          Rand            HA            MA            FM      Jaccard          nmi
0.9807428 0.9948049 0.9845614 0.9846390 0.9878699 0.9760239 0.9756830
```

5 Session information

```
sessionInfo()
R version 3.5.3 (2019-03-11)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 10 x64 (build 19041)
Matrix products: default
locale:
[1] LC_COLLATE=Chinese (Simplified)_China.936
[2] LC_CTYPE=Chinese (Simplified)_China.936
[3] LC_MONETARY=Chinese (Simplified)_China.936
[4] LC_NUMERIC=C
[5] LC_TIME=Chinese (Simplified)_China.936
attached base packages:
[1] stats4    parallel  stats     graphics  grDevices utils     datasets
[8] methods   base
other attached packages:
[1]  SIMLR_1.8.1       SNFtool_2.3.0      flashClust_1.01    igraph_1.2.5    clues_0.5.9
[6]  psych_2.0.9       RAFSIL_0.2.5       WGCNA_1.69         scLAPA_0.1.0
loaded via a namespace (and not attached):
[1]rappdirs_0.3.1    ClusterR_1.2.1    prabclus_2.3-2        GGally_2.0.0    coda_0.19-3
[6]tidyr_1.1.0    bit64_0.9-7    knitr_1.2    irlba_2.3.3           dclone_2.3-0    rpart_4.1-15
[12]inline_0.3.15 hwriter_1.3.2 RCurl_1.98-1.2 AnnotationFilter_1.6.0    generics_0.0.2
[17]callr_3.4.3    cowplot_1.0.0    RSQLite_2.2.0    future_1.18.0    bit_1.1-15.2    readxl_1.3.1
[22]caTools_1.16    DBI_1.1.0    geneplotter_1.6  htmlwidgets_1.5.1          reshape_0.8.8
[27]purrr_0.3.4    ellipsis_0.3.1 backports_1.1.8 annotate_1.60.1
```

6 References

1. Skinnider, M.A., J.W. Squair, and L.J.J.N.M. Foster, *Evaluating measures of association for single-cell transcriptomics.* 2019. **16**(5).
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3. Wang, B., et al., *Visualization and analysis of single-cell RNA-seq data by kernel-based similarity learning.* 2017. **14**(4): p. 414.
4. Wang, B., et al., *Similarity network fusion for aggregating data types on a genomic scale.* 2014. **11**(3): p. 333-337.
5. Blondel, V.D., et al., *Fast unfolding of communities in large networks.* 2008.
6. Eisen, M.B., et al., *Eisen, M.B., Spellman, P.T., Brown, P.O. & Botstein, D. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA 95, 14863–14868.* 1999. **95**(25): p. 14863-14868.
7. Ng, A.Y., M.I. Jordan, and Y. Weiss. *On Spectral Clustering: Analysis and an Algorithm*. in *Proceedings of the 14th International Conference on Neural Information Processing Systems: Natural and Synthetic*. 2001.