

A graph-based practice of evaluating collective identities of cell clusters

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Abstract

Random sentences

Introduction

It has been more than 10 years since the birth of single-cell RNA-sequencing (scRNA-seq), and the technology now is recognized as a prominent game changer of modern molecular biology. Likewise the pioneering technology, bulk RNA-seq, scRNA-seq can observe multidimensional gene expression profiles, while it also can provide such information in single-cell-level.

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In our previous research, we proposed a gene regulatory network (GRN)-based representation of cell clusters while edges of GRNs explain statistical dependencies between two genes[1]. We also demonstrated that similarity of two clusters can be defined as a quasi-pseudo-metric function[1]. Although we introduced a theory to form GRNs based on dependencies of gene expressions, we compromised to implement the algorithm relying on the statistical test of correlation to deal with the continuity of scRNA-seq data. Considering the fact that our method's primary application is the annotation of scRNA-seq data, an effective binarization method is needed to reduce computational costs and streamline the overall time required to initiate main analyses. Furthermore, we intended to make our framework dependent on researchers' expertises on the sample domains so that the metrics of cellular identities are tailor-made for the research scopes providing necessary and sufficient resolutions. Constrary, this design made our algorithm unfriendly to users. As the legitimacy our theory needs to be validated in various cases, A semi-automated system to help users select key marker genes is desired.

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Leveraging the backbone theory of GRN-based comparisons of cluster-wise cellular identities (i.e., cell classes), we implemented

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To simplify the contents of this study to highlight our foci, we would not discuss any practices of designing data spaces or clustering in depth.

Results

The framework of GRNet

In this reserch, we revisited the workflow of the GRN-based annotation

Automated marker-gene suggestion

Although we intended to require experimenters to curate marker genes to use in GRNs, Figure 1A-B

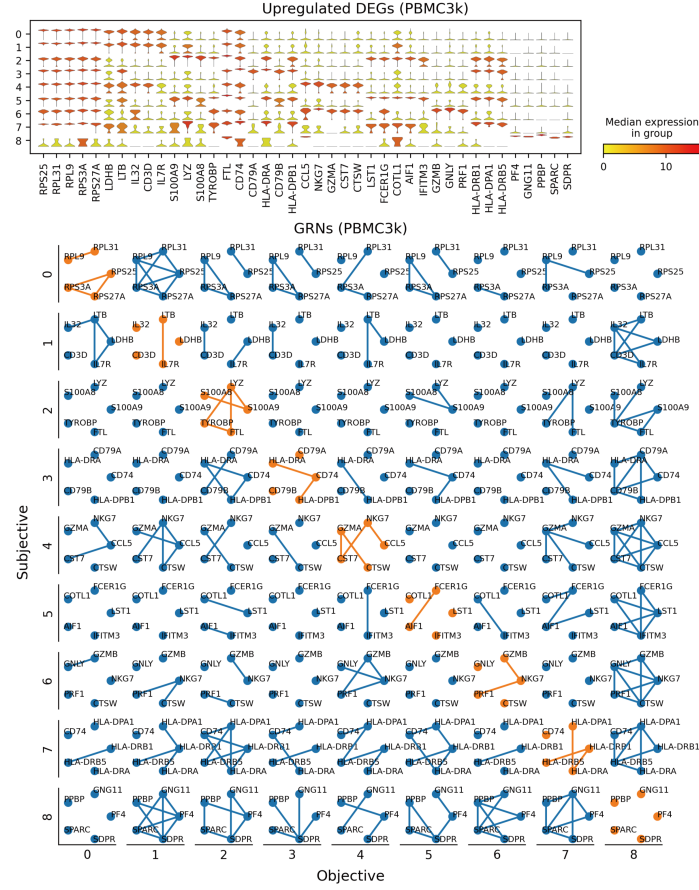


Figure 1: Gene expression patterns of clusters in PBMC3k

Dropout-based binarization

Benchmarking

Discussion

I have no idea.

Methods

GRNet Impletemntations

GO term-assisted gene selection referring Jaccard Index

$$J(A, B) := \frac{A \cap B}{A \cup B} \quad (1)$$

Jaccard Index of two sets A, B is defined as Eq. (1). We expanded this definition to pairwise comparisons of multiple elements by forming a matrix where each element is the corresponding Jaccard Index, and we named the matrix Jaccard index matrix (JIM). For example, the element in i -th row and j -th column (where $i, j, k \in \mathbb{N}$ and $i \leq k, j \leq k$), $JIM_{i,j}$, can be defined as follows when a JIM of sets X_1, X_2, \dots, X_k are considered:

$$JIM_{i,j} := J(X_i, X_j) \quad (2)$$

Especially for seed markers, sets of subscribed GO terms (let G_1, \dots, G_k) and their JIM are calculated in order to set $\min_{i,j}(J(G_i, G_j))$ as a threshold of biological correspondence.

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For detailed method of implementation, we calculated the JIM of the related GO terms of given seed markers. We used mygene.py[2] to query the GO database, and Numpy[3] to calculate JIM.

GRNs and the evaluation function

Following our previous report[1], we computed GRNs by calculating correlations of continuous gene expression values (e.g., $\log_2(RPM + 1)$) using Pgmpy[4]. In this study, we introduced

scRNA-seq data analysis

Dataset List

The scRNA-seq data we used in this research were publicly available as online resources as follows:

M1C10X: <https://portal.brain-map.org/atlas-and-data/rnaseq/human-m1-10x>

hFB: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165388>

PBMC3k: <https://support.10xgenomics.com/single-cell-gene-expression/datasets/1.1.0/pbmc3k>

aHSPC: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137864>

BCA: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE149938>

Preprocessing, dimensionality reduction, and visualization

We performed data preprocessing, dimensionality reduction, data visualization of the scRNA-seq datasets using Python packages (including Scanpy[5], Polars, Pandas[6], Numpy, Matplotlib[7], Seaborn[8]) and Julia packages.

Clustering and DE analysis

We performed leiden clustering, DE analysis using Scanpy.

Resource availability

Data availability

Not applicable

Code availability

GRNet and the analysis codes are available on GitHub (<https://github.com/yo-aka-gene/grnet>). Online documentation for GRNet is also provided (<https://grnet.readthedocs.io>).

Acknowledgements

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Abbreviations

GRN gene regulatory network

JIM Jaccard index matrix

scRNA-seq single-cell RNA-sequencing

References

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