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Applications Note

Genome analysis

CellWalkR: an R package for integrating and visualizing single-cell and bulk data to resolve regulatory elements

Pawel F. Przytycki [1] **and Katherine S. Pollard**[1,2,3,*]

[1]Gladstone Institutes, San Francisco, CA, USA, [2]Chan Zuckerberg Biohub, San Francisco, CA, USA and [3]Department of Epidemiology and Biostatistics, Institute for Computational Health Sciences, University of California, San Francisco, CA, USA

*To whom correspondence should be addressed.

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Abstract

Summary: CellWalkR is an R package that integrates single-cell open chromatin data with cell type labels and bulk epigenetic data to identify cell type-specific regulatory regions. A Graphics Processing Unit (GPU) implementation and downsampling strategies enable thousands of cells to be processed in seconds. CellWalkR's user-friendly interface provides interactive analysis and visualization of cell labels and regulatory region mappings.

Availability and implementation: CellWalkR is freely available as an R package under a GNU GPL-2.0 License and can be accessed from https://github.com/PFPrzytycki/CellWalkR with an accompanying vignette.

Contact: katherine.pollard@gladstone.ucsf.edu

Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Gene regulatory elements active in specific cell types can be identified using single-cell open chromatin (scATAC-seq). However, analysis is difficult due to extremely low coverage per region per cell (Chen *et al.*, 2019). Bulk data have much higher resolution, enabling confident identification of regulatory regions, but it does not provide cell type-specific annotations. Single-cell RNA sequencing (scRNA-seq) is less sparse than scATAC-seq and hence can identify dozens of cell types in complex tissues, but it does not pinpoint regulatory elements (Chen *et al.*, 2019).

We developed CellWalkR, an R package that combines the strengths of these different technologies to resolve regulatory elements to cell types (Supplementary Fig. S1). We extended a previously introduced network diffusion model (Przytycki and Pollard, 2021) by adding filters based on bulk epigenetic data, flexible scATAC-seq formats, statistical thresholds for cell labels and multiple label sets. Key features of the package include a GPU implementation using TensorFlow (Falbel *et al.*, 2020) and an interactive R Shiny interface (Chang *et al.*, 2020). A suite of visualizations is provided for cell labeling, determining label similarity, embedding cells into low-dimensional space and mapping regulatory regions to cell types.

2 Results

CellWalkR builds a network consisting of two types of nodes representing cells and labels (i.e. cell types or states). Building this network requires two types of input: labeling data and scATAC-seq input as a cell-by-region matrix (e.g. cell-by-peak or cell-by-bin) or as processed by SnapATAC (Fang *et al.*, 2021), ArchR (Granja *et al.*, 2021) or Cicero (Pliner *et al.*, 2018). Cells are connected by edges weighted by similarity in the scATAC-seq experiment (Supplementary Fig. S2, edges between circle nodes). Labels are connected to cells by edges based on how open gene bodies, promoters or correlated distal peaks (Pliner *et al.*, 2018) of marker genes are in each cell (Supplementary Fig. S2, edges from square nodes to circle nodes). Marker gene names can be user-supplied or generated from scRNA-seq data using Seurat (Stuart *et al.*, 2019). This strategy can be used to build networks for an arbitrary number of label sets, representing cell types in different time points or disease states.

All label-cell and cell-cell edges are combined into a single network, with each set of labeling edges scaled by a parameter s. Once a complete network is built, a random walk with restarts model of diffusion is run to convergence (Przytycki and Pollard, 2021). CellWalkR can optionally run this step on a GPU using TensorFlow (Falbel *et al.*, 2020) for a greater than 15-fold speedup (Supplementary Fig. S3). The output of this process is an influence matrix where each column represents the amount of information that flows from each node to each other node.

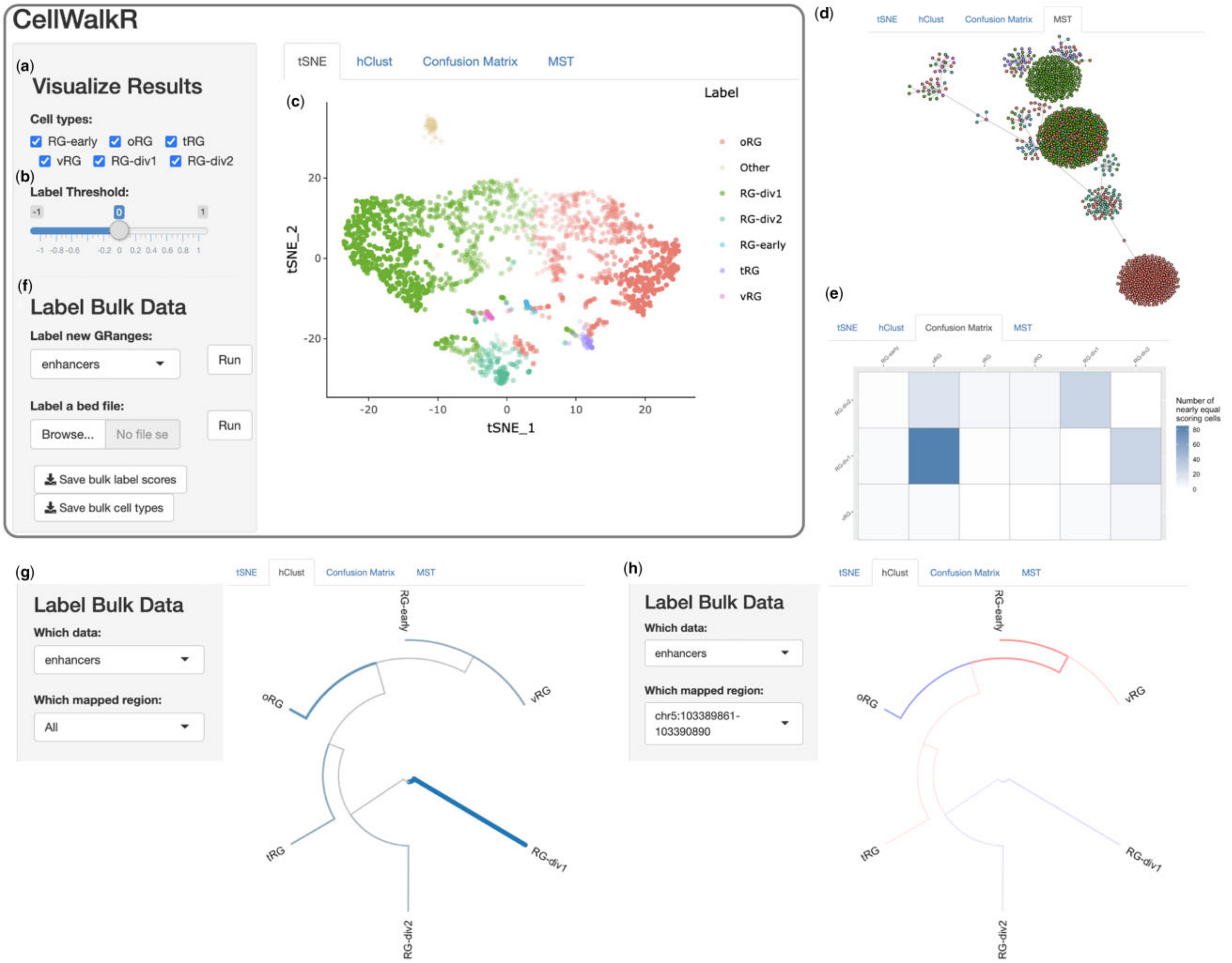
In addition to labeling data, CellWalkR can also take bulk epigenetic data as input. This data serves as a filter through which scATAC-seq peaks are passed before being considered for calculating cell-label edges. Each filter has a scaling parameter f for each label set that determines the strength of the filter. Filters are restrictive by default (remove scATAC-seq peaks overlapping filter regions) but can also be set to permissive (allow only overlapping peaks).

They can apply to the entire associated gene or just overlapping peaks.

Setting the *s* parameter and the *f* parameters correctly for each label set affects how information is propagated through the network. CellWalkR has built-in functions that tune these parameters to optimize cell homogeneity, a measure of influence between cells of the same label versus between cells of different labels (Przytycki and Pollard, 2021). To accelerate optimization, CellWalkR can downsample cells, run these calculations in parallel or compute an approximate solution for the random walk (Supplementary Fig. S4).

Once an influence matrix has been computed, the user has many options for downstream analysis. In addition to writing outputs to files or manipulating them with R code, one can launch CellWalkR's Shiny interface (Fig. 1) to visualize and perform interactive analysis with different portions of the influence matrix. The label-to-cell influence portion is used to assign labels to cells, a subset of which can be excluded (Fig. 1a). These label estimates are fuzzy, meaning each cell has a distribution of scores from each label. Cells scoring below a chosen threshold can be determined to belong to no known type (Fig. 1b). Visualizing cells in a 2D space is a helpful strategy for understanding how cells relate to each other and to their labels. CellWalkR uses t-SNE (Krijthe and Van der Maaten, 2015) or Uniform Manifold Approximation and Projection (UMAP) (Melville *et al.*, 2021) to embed cell-to-cell influence (Fig. 1c). By default, cells are colored by their highest scoring label. When only one cell type is selected, CellWalkR generates a t-SNE or UMAP plot showing the amount of influence a single label has on each cell (Supplementary Fig. S3a). If two labels are selected, it shows the difference in influence between two labels (Supplementary Fig. S5b), allowing the user to identify transition regions. Overall, embedding cell-to-cell influence rather than sparse, noisy cell accessibility profiles has clear advantages for separating cells of different types, especially rare cell types (Supplementary Fig. S6). To visualize relationships between cell types, cells can be drawn as a minimum spanning tree (MST) built by including edges of maximal influence (Fig. 1d). CellWalkR can determine how many cells receive nearly the same score for two different labels, which it summarizes in an uncertainty matrix (Fig. 1e).

To resolve cell types/states for bulk-identified genomic regions (e.g. genetic variants, enhancers, protein binding sites and epigenetic segmentations) CellWalkR uses cell-to-label influence (Fig. 1f). Cell types for which labels are enriched across the set of regions are


CellWalkR

(a) Visualize Results

Cell types: ☑ RG-early ☑ oRG ☑ tRG ☑ vRG ☑ RG-div1 ☑ RG-div2

(b) Label Threshold: 0

(f) Label Bulk Data

Label new GRanges: enhancers

Label a bed file: No file se

(g) Label Bulk Data

Which data: enhancers

Which mapped region: All

(h) Label Bulk Data

Which data: enhancers

Which mapped region: chr5:103389861-103390890

Fig. 1. Overview of CellWalkR interface. CellWalkR provides an interactive interface for downstream analysis and visualization of influence matrices. The user selects (**a**) their cell types of interest and (**b**) the label threshold, and CellWalkR dynamically generates (**c**) a t-SNE embedding and (**d**) an MST of cell-to-cell influence, and (**e**) a confusion matrix counting how many cells have very similar label scores. The user can then calculate cell type-specific labels for bulk data (**f**) either from their active session or from a BED format file. After bulk data are selected, new menu options are displayed for analyzing labels, allowing the user to (**g**) view enrichment for cell types across all bulk data in a hierarchical clustering of cell types, and (**h**) examine how a specific range is enriched or depleted in each cell type. Shown data are for cell type labels (Nowakowski *et al.*, 2017) and scATAC-seq from a population of radial glia in the developing brain (Ziffra *et al.*, 2021). RG, Radial Glia; oRG, outer RG; tRG, truncated RG and vRG, ventral RG

shown in a hierarchical clustering built using label-to-label influence (Fig. 1g). The user can then select a single region to see how strongly it maps to each cell type (Fig. 1h).

3 Conclusions

Many regulatory elements are only mapped in bulk data, and scATAC-seq data can be difficult to use due to its sparsity. CellWalkR is a flexible R package for scATAC-seq data that incorporates bulk epigenetic and expression data to multilabel cells. The package can resolve rare cell types or states and associate regulatory elements with those labels.

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Conflict of Interest: none declared.

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