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Destin: toolkit for single-cell analysis of chromatin accessibility

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Abstract

Summary: Single-cell assay of transposase-accessible chromatin followed by sequencing (scATAC-seq) is an emerging new technology for the study of gene regulation with single-cell resolution. The data from scATAC-seq are unique - sparse, binary, and highly variable even within the same cell type. As such, neither methods developed for bulk ATAC-seq nor single-cell RNA-seq data are appropriate. Here, we present Destin, a bioinformatic and statistical framework for comprehensive scATAC-seq data analysis. We evaluated performance of Destin using downsampled bulk ATAC-seq data of purified samples and scATAC-seq data from seven diverse experiments. Compared to existing methods, Destin was shown to outperform across all data sets and platforms. For demonstration, we further applied Destin to 2,088 adult mouse forebrain cells and identified cell type-specific association of previously reported schizophrenia GWAS loci.
Availability: Destin toolkit is freely available as an R package at https://github.com/urrutiag/destin.
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1 Introduction

Single-cell assay of transposase-accessible chromatin followed by sequencing (scATAC-seq) is an emerging new technology for the study of gene regulation with single-cell resolution. Unlike conventional regulomics technologies, scATAC-seq measures chromatin accessibility within each individual cell, which circumvents the averaging artifacts associated with traditional bulk population data, yielding new insights into epigenetic regulation at the cellular level. Technologically, Buenrostro *et al.* (2015) adapted the bulk ATAC-seq technology to single cells, utilizing microfluidic device to physically isolate single cells. Cusanovich *et al.* (2015) adopted a two-step "split-and-pool" strategy, where cells undergo several rounds of barcoding procedures to be uniquely labeled. Recently, Preissl *et al.* (2018) developed single nucleus ATAC-seq (snATAC-seq), adapting the two-step combinatorial indexing strategy to frozen tissues. The Chromium Single Cell ATAC Solution by the 10X Genomics can further profile chromatin accessibility across 500 to 10,000 nuclei in parallel. Refer to Supplementary Table 1 for a summary of existing platforms and technologies.

The data from scATAC-seq are unique - sparse, binary, noisy with biases and artifacts, and highly variable even within cell types. Supplementary Fig. 1 shows a snapshot of both single-cell and bulk-tissue chromatin accessibility within a 800kb region from chromosome 1, using human monocyte cells and purified bulk samples (Corces *et al.*, 2016), respectively. ScATAC-seq data is sparse and noisy, and the signals share low similarity across cells. On the contrary, bulk signals are highly conserved across the five purified samples. Total depth of coverage in single cells is also greatly reduced, several orders of magnitude lower than bulk. Additionally, since most of the genome has only two copies in a cell and the transposase can cleave and add adaptors only once per copy, for each open chromatin region, at most two sequenceable fragments can be generated and equivalently at most two reads per locus can be obtained after removing PCR duplicates. As such, scATAC-seq data is also highly binary, indicating an open/closed status. Because of the aforementioned uniqueness of scATAC-seq data, neither methods developed for bulk ATAC-seq nor single-cell RNA-seq (scRNA-seq) can be directly applied.

Several cell type clustering methods for scATAC-seq have been proposed. ScAsAT (Baker *et al.*, 2017) first performs dimension reduction on cell-by-cell Jaccard distance matrix, followed by t-SNE (Maaten and Hinton, 2008) and k-mediods for clustering. scABC (Zamanighomi *et al.*, 2018) begins by clustering cell types using weighted k-mediods, up-weighting cells with higher sequencing depth. To address the sparsity and noise observed in scATAC-seq and to reduce dimension, SCRAT (Ji *et al.*, 2017) and chromVar (Schep *et al.*, 2017) aggregate scATAC-seq read counts across biological features such as transcription factor binding motifs, DNase I hypersensitivity sites (DHSs), genes, or gene sets of interest. This is followed by dimension reduction and clustering.

Here, we propose Destin (*De*tection of cell-type *s*pecific difference in chroma*tin* accessibility), a bioinformatic and statistical framework for comprehensive scATAC-seq data analysis. For cell type clustering, instead of aggregating peaks based on genomic annotations, Destin adopts weighted principal component analysis (PCA), with peak-specific weights calculated based on the distances to transcription start sites (TSSs) as well as the relative frequency of chromatin accessibility peaks based on a broad range of reference experiments. The weights, together with the number of clusters, and the number of principle components, are cast as tuning parameters and are determined based on the likelihood calculated from a post-classification multinomial model. Destin is evaluated on scATAC-seq data of 5,800 cells from seven experiments and is benchmarked against existing methods. We show that Destin outperforms the other methods across different data sets and platforms. As a proof of concept, we demonstrate Destin on a scATAC-seq data set of 2,088 adult mouse forebrain cells and identify cell-type specific association of previously reported GWAS loci for schizophrenia.

2 Materials and methods

Destin begins with a bioinformatic pipeline to preprocess raw sequencing files and and follows with statistical analysis for cell type clustering and cell type enrichment of previously reported GWAS loci. Specifically, the bioinformatic pipeline includes

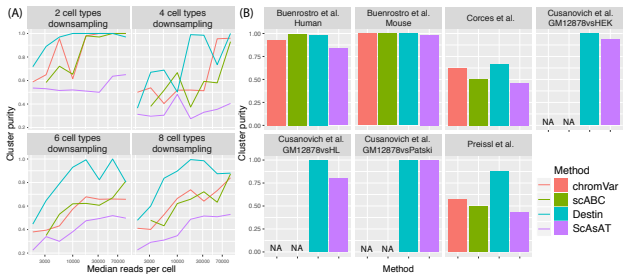

Fig. 1. Benchmark results against existing methods via downsampling and empirical data analysis. (A) Purified bulk ATAC-seq data from (Corces et al. (2016)) were downsampled with different numbers of cell types and different median reads per cell. (B) Cluster results across seven scATAC-seq data sets. chromVAR and scABC cannot be applied to the three data sets from Cusanovich et al. (2015) due to unavailability of required input as bam files.

demultiplexing (for platforms with cellular barcodes), trimming adaptors, mapping reads, filtering blacklist regions, calling peaks in pseudo-bulk samples as aggregates of single cells. After a further quality control procedure, this results in a peak-by-cell chromatin accessibility matrix. Refer to Supplementary Materials for details on bioinformatic analysis.

Destin utilizes biological annotation in order to prioritize chromatin accessible regions that are more informative to distinguish cell types. Specifically, Corces *et al.* (2016) showed that distal regulatory elements (e.g., enhancers) provide sharper ability for clustering than do proximal elements (e.g., promoters), and thus, for analysis, focused on chromatin accessible regions 1kb upstream from the TSS. Similarly, Preissl *et al.* (2018) focused on chromatin accessible regions outside a 2kb window from the TSS. However, Corces *et al.* (2016) also showed that there is predictive value in the promoter region, while (Zamanighomi *et al.*, 2018) further identified cluster-specific open promoters that distinguish expression. As such, Destin retains all peaks in analysis and opts to give higher weights to distal regulatory elements compared to promoter regions. The second set of peak-specific weights is defined by the reference frequency of chromatin accessibility peaks across different cell types. We hypothesize that chromatin accessible regions shared among fewer cells types have higher predictive value for classification compared to chromatin accessible regions shared across many cell types. Therefore, we created a reference frequency map for chromatin accessibility peaks using DHS data of 50 to 100 cell lines/types from the ENCODE Project (Consortium *et al.*, 2012), depending on species. The reference DHS frequency was created by calculating the proportion of reference cell types or tissue types containing a DHS peak in each 500bp genomic region. Higher weight is given to chromatin accessible region with lower reference DHS frequency.

Weighted PCA is then performed with the above peak-specific weights to reduce dimension, followed by k-means for clustering. Destin resorts to weigthed PCA for incorporation of existing regulomic data and, meanwhile, for its high computation efficiency in such an ultra-high dimensional setting, where binary matrix factorization cannot be run. The values of weights, as well as the number of PCs and the number of clusters, are optimized via grid search to maximize the post-classification likelihood based on a multinomial model, which is also used to re-assign cluster memberships for cells. Refer to Supplementary Materials for details of the proposed algorithm.

	MAGMA *p*-value			ECWC *p*-value
Cell Type	SCZ	MDD	ADHD	SCZ
Excitatory neuron	0.466	0.164	1	0.255
Inhibitory neuron 1	0.009*	0.151	0.774	0.035*
Inhibitory neuron 2	0.434	0.204	1	1
Neuroglia	1	1	1	1
Oligodendrocyte	1	1	1	1

Table 1. Cell type enrichment for previously reported GWAS loci. Bonferroni corrected p-values are calculated by MAGMA and ECWC for association of mouse forebrain cell types with previously reported GWAS loci for schizophrenia (SCZ), major depressive disorder (MDD), and attention deficit hyperactivity disorder (ADHD).

3 Results

We benchmarked Destin against three existing scATAC-seq methods: scABC, ScAsAT and chromVar. The online GUI by SCRAT takes bam files as input and cannot handle datasets with large number of cells. We began our benchmarks by downsampling bulk ATAC-seq data of purified bulk samples from Corces *et al.* (2016). We varied the number of cell types (2, 4, 6, or 8) and downsampled 50 cells per cell type. Median read depth per cell was also varied, ranging from 3,000 (combinatorial barcode indexing) to 70,000 (Fluidigm C1). with mean cluster purity shown in Fig. 1A. Our results show that performance for all methods increased with number of reads and that Destin outperformed all other methods in almost every scenario. Next, we benchmarked Destin against the other methods using the seven full single cell ATAC-seq data sets (Supplementary Table 2). Destin performed as well as or better than all other methods in terms of cluster purity across all data sets (Fig. 1B), including cluster purity at nearly 100% in five out of seven data sets.

We further applied Destin to snATAC-seq data of 2,088 cells from adult mouse forebrain Preissl *et al.* (2018). Destin's cell type clustering results, by its default, differ from the results by the original publication – Destin clustered together both the three excitatory neuron subtypes and the related microglia and astrocytes, the latter of which we refer to as neuroglia (Supplementary Fig. 7A). Notably, the subclusters can be resolved by increasing the number of clusters (Supplementary Fig. 7B). A key application of single cell omics is to identify specific cell types that are associated with disease. Compared to scRNA-seq, the scope is broadened to outside the gene bodies to include, e.g., promoters and enhancers. In a similar fashion to Skene *et al.* (2018), we determined the "cell type specificity" for each gene, based on gene annotations of chromatin accessible regions and clustering results by Destin. The cell type specificity was then tested for association with GWAS p-values mapped to each gene from three psychiatric studies of schizophrenia (Ripke *et al.*, 2014), major depressive disorder (Wray *et al.*, 2018), and attention deficit hyperactivity disorder (Demontis *et al.*, 2017). We discovered significant association between schizophrenia GWAS loci and inhibitory neuron 1 by two independent methods MAGMA (de Leeuw *et al.*, 2015) and ECWC (Skene and Grant, 2016).

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