A single-cell clusters similarity measure for different batches, datasets, and samples

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Abstract

Summary: Since the inception of single-cell level measuring techniques, identification of distinct cell stages, phenotypes and populations has been a challenge. Clustering is the most popular approach to overcome the difficulty and heterogeneity of single-cell data, but as public repositories continue to grow in number, integrative analyses, and the merging of large pools of samples from very different and heterogeneous datasets become a reality, with new added difficulties, which showcases the impossibility of scalability of some of the existing methods. Here we present *ClusterFoldSimilarity*, an R package that calculates a measure of similarity between clusters from different datasets/batches, without the need of correcting for batch effect or normalizing and merging the data, thus avoiding artifacts and the loss of information derived from these kinds of techniques. The similarity metric is based on the average vector module and sign of the product of logarithmic fold-changes. *ClusterFoldSimilarity* compares every single pair of clusters from any number of different samples/datasets, including different number of clusters for each sample. Additionally, the algorithm is able to select the top genes that contributed the most to the similarity of two specific clusters, serving also as a feature selection tool.

Availability and implementation: The algorithm is freely available as an R package at: https://github.com/OscarGVelasco/ClusterFoldSimilarity

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Introduction

Single-cell sequencing is a new technology that is able to capture the biomolecular cellular status and measure multiple cellular markers for hundreds to millions of cells, and thus researchers are able to elucidate genomic, epigenomic, and transcriptomic heterogeneity in cellular populations and subpopulations, and the variability at these levels (Kashima et al., 2020).

The number of large-scale single-cell transcriptomic datasets generated using different technologies is continuously growing with the advent of public databases. But wide data integration remains difficult because the inherent batch-specific systematic variations that present a challenge to batch-effect removal. A number of specific tools for batch-effect removal exists, but each has its advantages and limitations, with no clearly superior method (Tran et al., 2020). Here we present a new methodology that is able to analyze the similarity between clusters from different and heterogeneous datasets, without the need of batch-effect removal or merging: it is based on the fold change of the sequenced signal between clusters, the idea is that overall signal expression changes across different cell populations, subpopulations or phenotypes in a dataset should be conserved in external datasets with similar populations.

Materials and methods

Pairwise fold changes

We can think of fold-changes (FC) as an Euclidean vector with origin at 0 and direction equal to the expression change, the scalar product of two vectors in the same space gives as a result a perpendicular vector to the plane. In the case of the dot product of two fold-changes the direction of the perpendicular vector will act as a concordance value of the direction of the FCs (see Figure 1C). The FCs are computed using the normalized count matrix. They are calculated pairwise between each cluster i and the other clusters belonging to the same sample. FC for each gene is calculated as the log2 of the ratio of the mean expression from cells in cluster i and the mean expression from cells in cluster j $\forall j \neq i$. In such manner, we obtain k-1 FCs (k: number of clusters in a given sample) for each of the clusters in that sample.

Similarity measurement

Once all the possible combinations of FCs between all clusters from each sample/batch are computed, we start by comparing the cluster 1 from sample/batch 1 with the cluster 1 in sample/batch 2 and continue iteratively. We achieve this by computing the scalar product of every possible combination of FCs from the two clusters: $combinations = (k_1 - 1) \times (k_2 - 1)$ being k_1: number of clusters in sample 1; k_2: number of clusters in sample 2. Then we calculate for each gene the mean of the scalar products. This value will serve as a measure of gene importance, being the gene with the highest positive value the one that contributes the most to the similarity between the two clusters (negative values indicate opposite FC values). Finally, the similarity value for a pair of clusters is calculated as the square root of the sum of the mean of genes (calculated in the previous step):

$$Similarity = \sqrt{\left|\sum \overline{FC}\right|} * SIGN\left(\sum \overline{FC}\right) * \omega$$

The weight ω is based on the concordance of positive vs negative FC's and is calculated as:

$$\omega = \sqrt{\frac{|NEG_{FC} - POS_{FC}|}{NEG_{FC} + POS_{FC}}}$$

The maximum similarity positive value calculated this way will correspond with the most similar cluster: it is expected that, for a specific population of cells, overall changes in gene expression across different clusters (other cell populations) are conserved on different samples, and thus the sign of the product of FCs should be positive and its module higher than less concordant expression changes.

Compatibility

ClusterFoldSimilarity is compatible with the most popular single-cell pipelines for an easy downstream integration: Seurat (Hao et al., 2021) and SingleCellExperiment (Amezquita et al., 2020).

Results

ClusterFoldSimilarity has been tested in several single-cell datasets in which cell-type information was available: four human pancreatic single-cell RNA-Seq datasets from the scRNAseq package (Risso D, 2021) (GSE84133 (Baron et al., 2016), GSE86469 (Lawlor et al., 2017), GSE81608 (Xin et al., 2016), ArrayExpress: E-MTAB-5061 (Segerstolpe et al., 2016)). In four peripheral blood mononuclear cell (PBMC) single-cell RNA-Seq datasets (GSE149938 (Xie et al., 2021), 10x Genomics 10k Peripheral blood mononuclear cells (PBMCs) from a healthy donor, Single Indexed, GSE120221 (Oetjen et al., 2018), PRJEB40448 (Lawlor et al., 2021))

Results show that our similarity metric is able to pair clusters by cell-type and sub-type with high accuracy (all clusters corresponding with shared cell-types across datasets are paired together), including subpopulations in the case of PBMC, and the top gene markers selected by similarity correspond to markers reported on scientific literature (see Sup. Material).

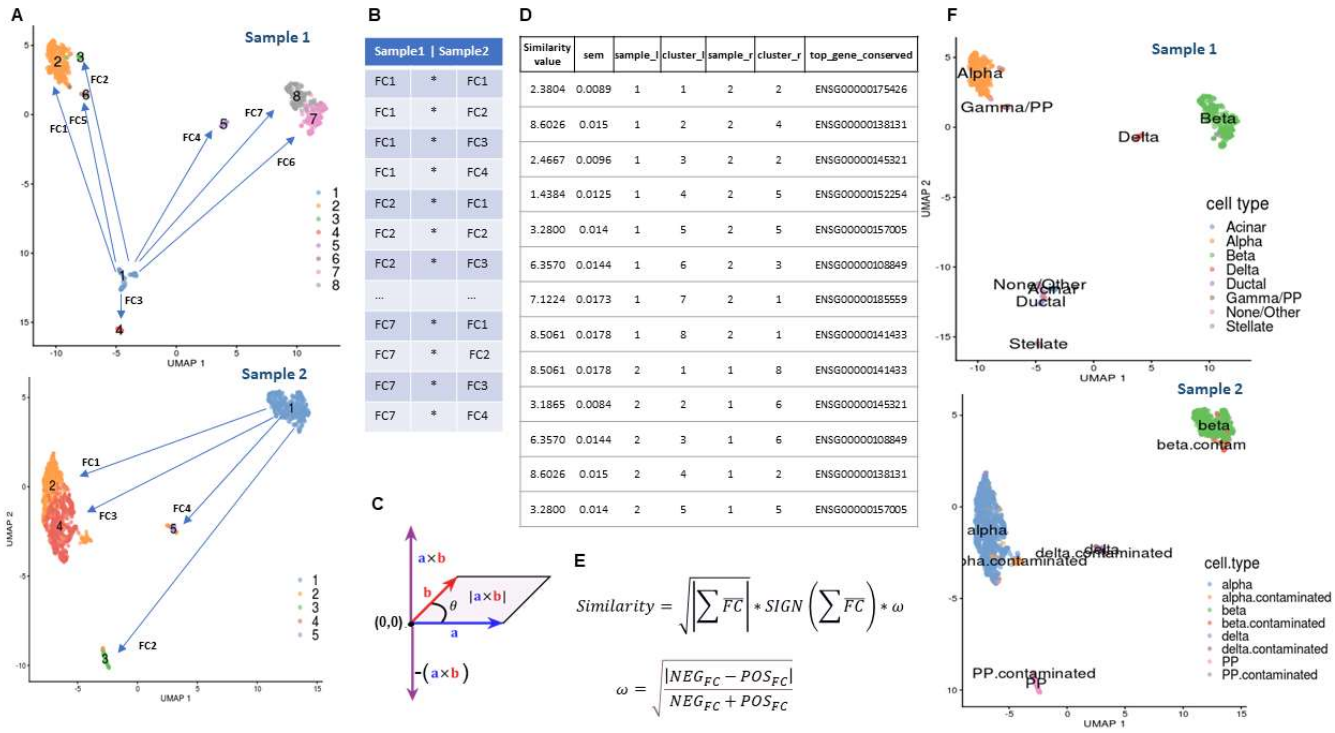

Figure 1: Pipeline representation of the similarity measurement done by ClusterFoldSimilarity. A: Fold changes (FC) are calculated between all possible combination of clusters for each dataset, figure shows the FC calculation for cluster 1 in both sample 1 and 2 in a UMAP corresponding with pancreatic cells. B: The scalar product of the computed FCs between all pairs is calculated. C: Representation of the vectorial space of FCs and their product, FC that points in the same direction will have positive product sign, meanwhile opposite FC will have negative product sign. D: Table with the similarity values and output information given by ClusterFoldSimilarity. E: Equation used for calculating the similarity. F: UMAP representation of two pancreatic datasets with annotated cell-types. Note that exact cell-type clusters are matched together by the table given at D.

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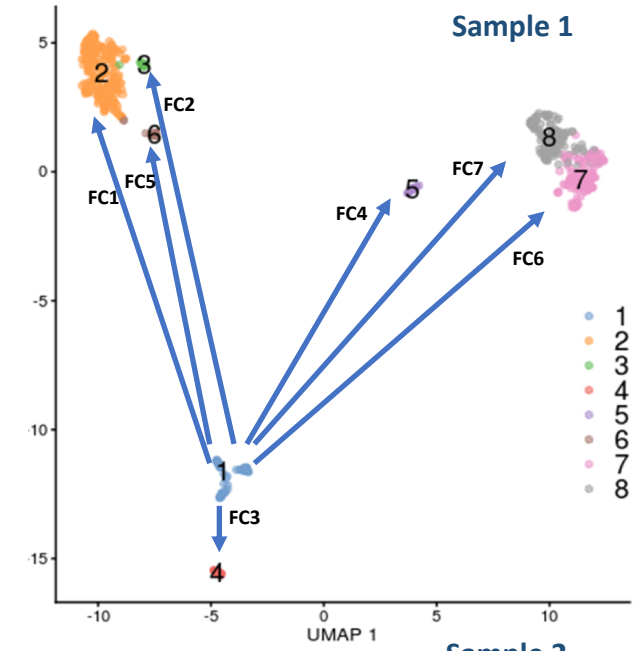
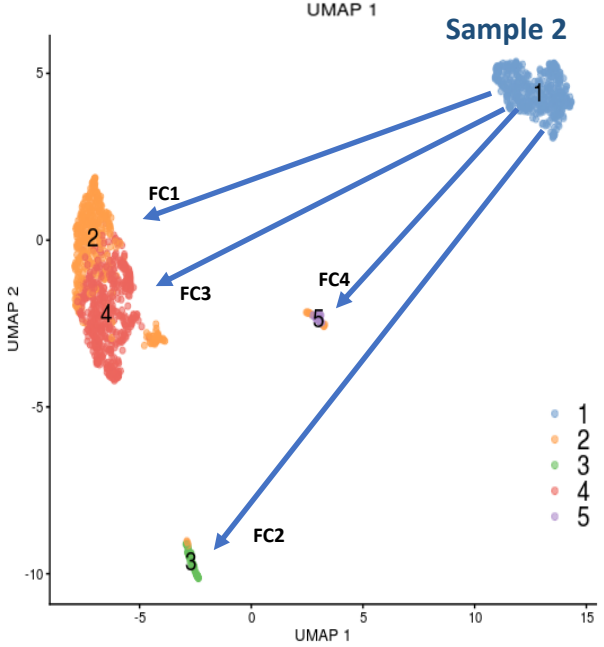
A

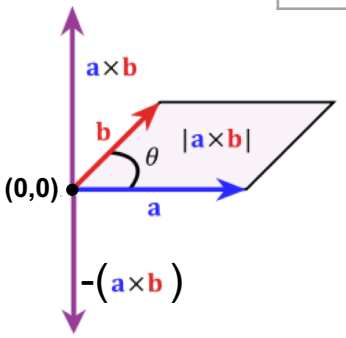




B

Sample1	Sample2	
FC1	*	FC1
FC1	*	FC2
FC1	*	FC3
FC1	*	FC4
FC2	*	FC1
FC2	*	FC2
FC2	*	FC3
...		...
FC7	*	FC1
FC7	*	FC2
FC7	*	FC3
FC7	*	FC4

C

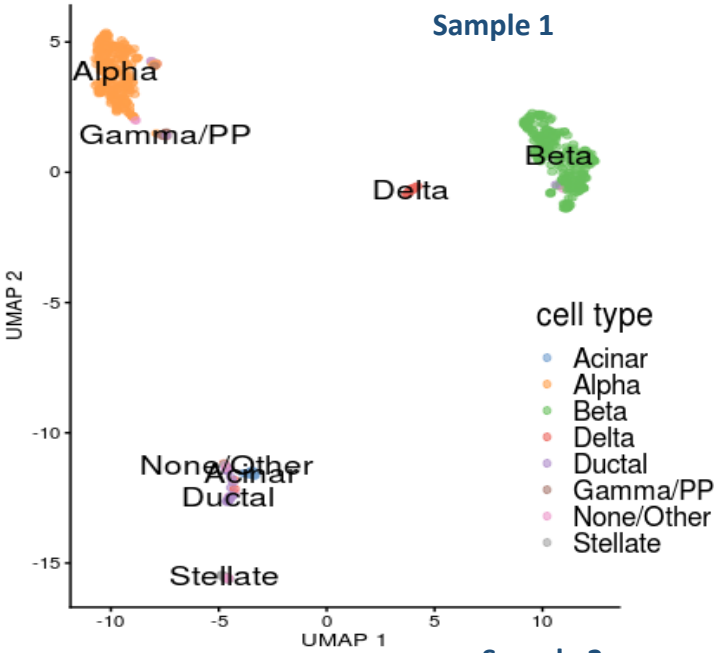
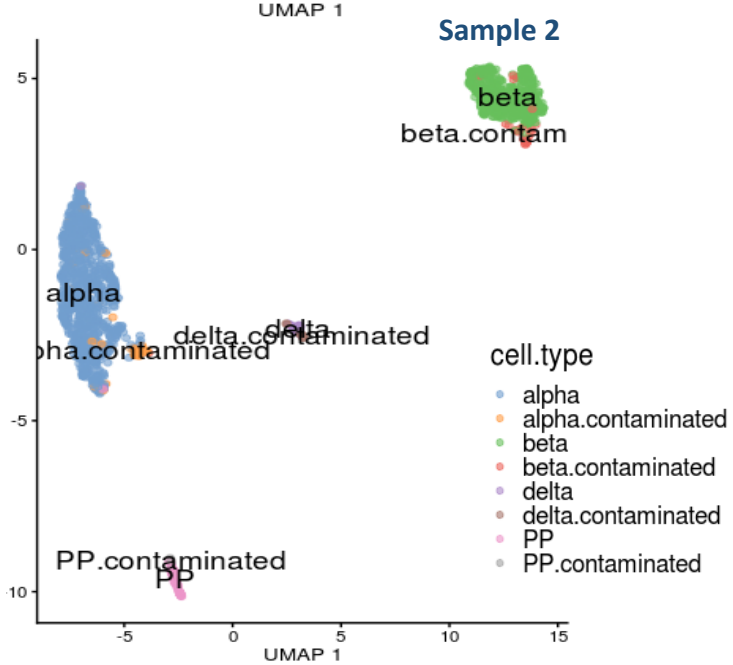


D

Similarity value	sem	sample_l	cluster_l	sample_r	cluster_r	top_gene_conserved
2.3804	0.0089	1	1	2	2	ENSG00000175426
8.6026	0.015	1	2	2	4	ENSG00000138131
2.4667	0.0096	1	3	2	2	ENSG00000145321
1.4384	0.0125	1	4	2	5	ENSG00000152254
3.2800	0.014	1	5	2	5	ENSG00000157005
6.3570	0.0144	1	6	2	3	ENSG00000108849
7.1224	0.0173	1	7	2	1	ENSG00000185559
8.5061	0.0178	1	8	2	1	ENSG00000141433
8.5061	0.0178	2	1	1	8	ENSG00000141433
3.1865	0.0084	2	2	1	6	ENSG00000145321
6.3570	0.0144	2	3	1	6	ENSG00000108849
8.6026	0.015	2	4	1	2	ENSG00000138131
3.2800	0.014	2	5	1	5	ENSG00000157005

E

$$Similarity = \sqrt{\left|\sum \overline{FC}\right|} * SIGN\left(\sum \overline{FC}\right) * \omega$$

$$\omega = \sqrt{\frac{|NEG_{FC} - POS_{FC}|}{NEG_{FC} + POS_{FC}}}$$

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