First steps

Katarzyna Sikora

Overview

- 10x scRNAseq output data
- Seurat Object
- PCA
- Shared nearest neighbourgh graph
- Louvain clustering

Standard 10x scRNAseq output

- matrix.mtx -> feature(gene) x cell counts
- genes.tsv -> gene annotation (ensembl ID > gene symbol)
- barcodes.tsv -> barcode list

Seurat Object Seurat Object RNA assay NormalizeData SCTransform - . - . ----- ScaleData Seurat Object Seurat Object RNA assav **Seurat Object** RNA/SCT assay DotPlot counts VlnPlot CellScatter FeaturePlot FeatureScatter VariableFeaturePlot scale.data RidgePlot RunPCA DoHeatmap JackStrawPlot ElbowPlot FindClusters FindAllMarkers

markers

DimPlot

FindVariableFeatures

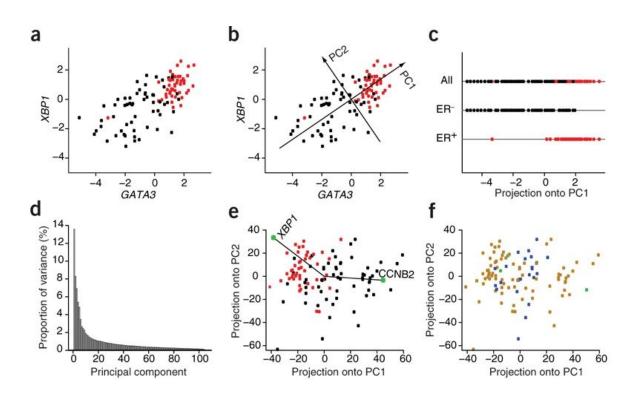
Seurat Object

Accessors & accessor functions:

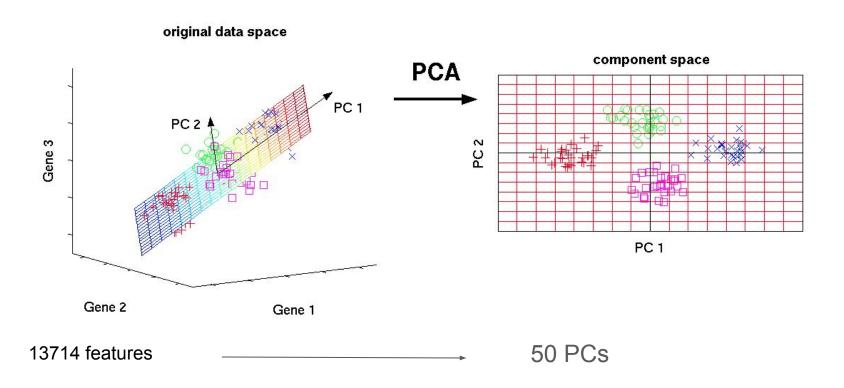
- obj@meta.data
- obj[[assay]]@layers\$slot
- LayerData()
- FetchData()
- Layers()
- Assays()
- Features()
- Cells()
- Embeddings()

https://satijalab.org/seurat/articles/essential_commands#seurat-object-data-access

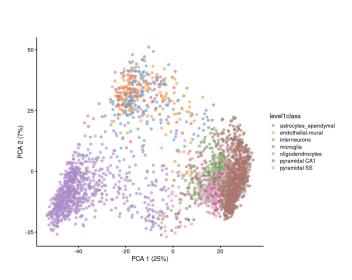
PCA: a simple example

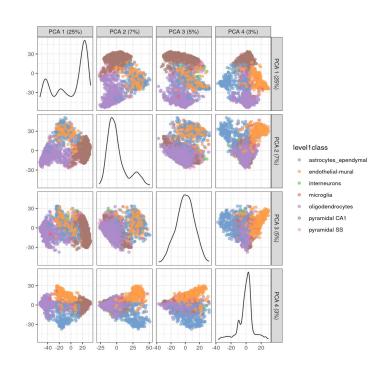


PCA: multiple dimensions

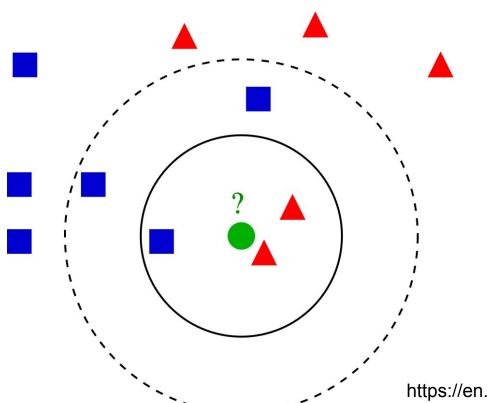


PCA on a scRNAseq dataset





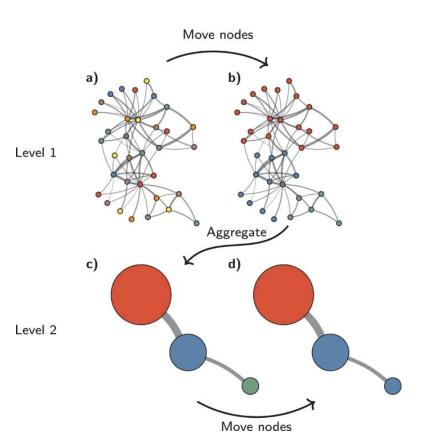
Shared nearest neighbour graph construction



- 1. find k nearest neighbours for each cell
- 2. calculate neighbourhood overlap (Jaccard index)
- 3. construct SNN graph

https://en.wikipedia.org/wiki/K-nearest_neighbors_algorithm

Louvain/Leiden clustering on the SNN graph



Resolution parameter:
Higher resolutions lead to more communities, while lower resolutions lead to fewer communities.

https://www.nature.com/articles/s41598-019-41695-z