Analysis of single-cell RNA-seq data with R and *Bioconductor*.

Fanny Perraudeau, Kelly Street, Davide Risso

Tasks and packages

- Dimensionality reduction with zinbwave.
  - bioconductor.org/packages/zinbwave
- Cluster analysis with clusterExperiment.
  - bioconductor.org/packages/clusterExperiment
- Lineage inference and trajectory analysis with slingshot.
  - github.com/kstreet13/slingshot

Workshop material:
github.com/fperraudeau/bioc2017singlecell

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Experiments and analyses

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Core Facilities

QB3 Functional Genomics Laboratory
QB3 Genomics Sequencing Laboratory
Cancer Research Laboratory

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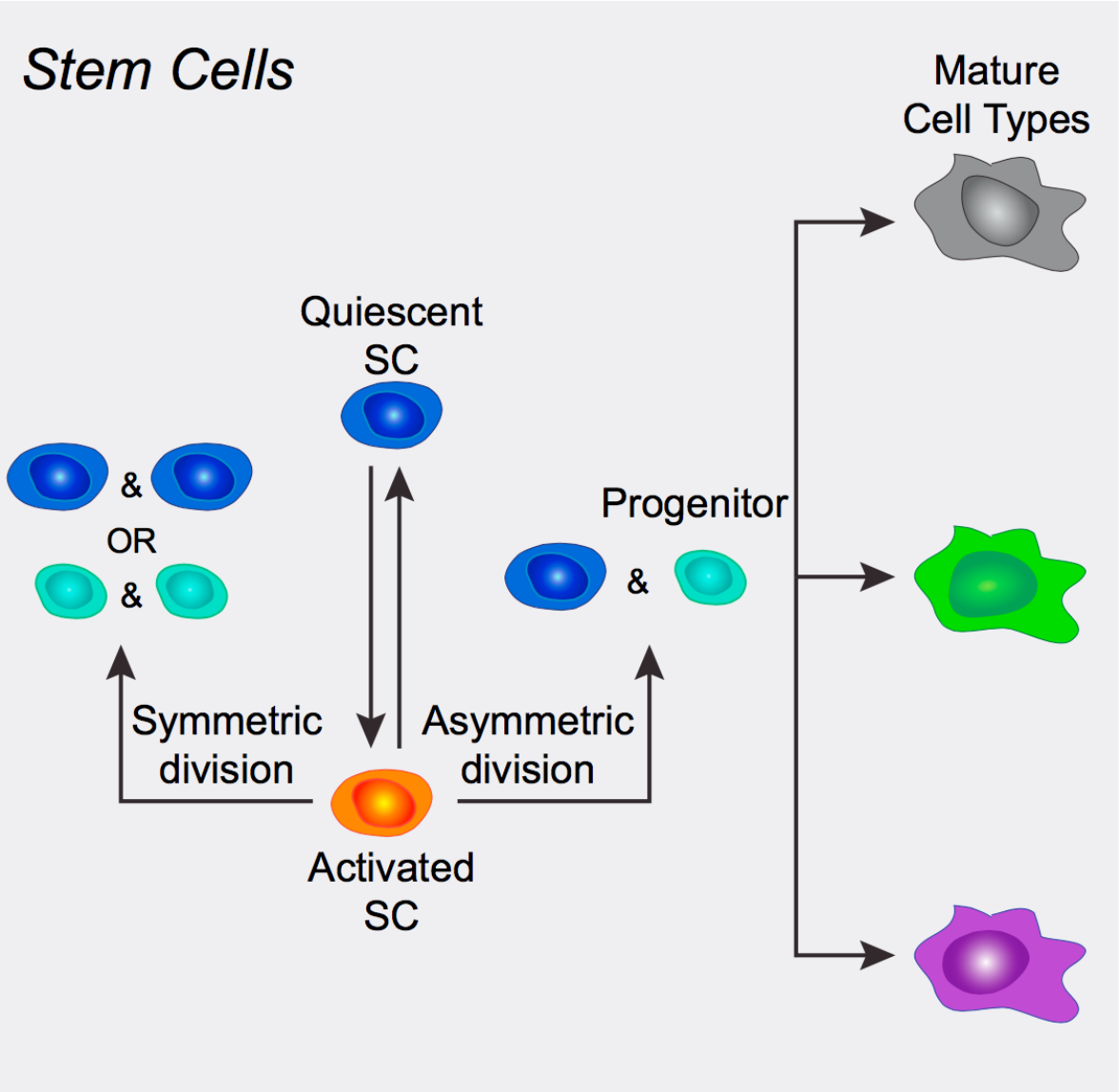
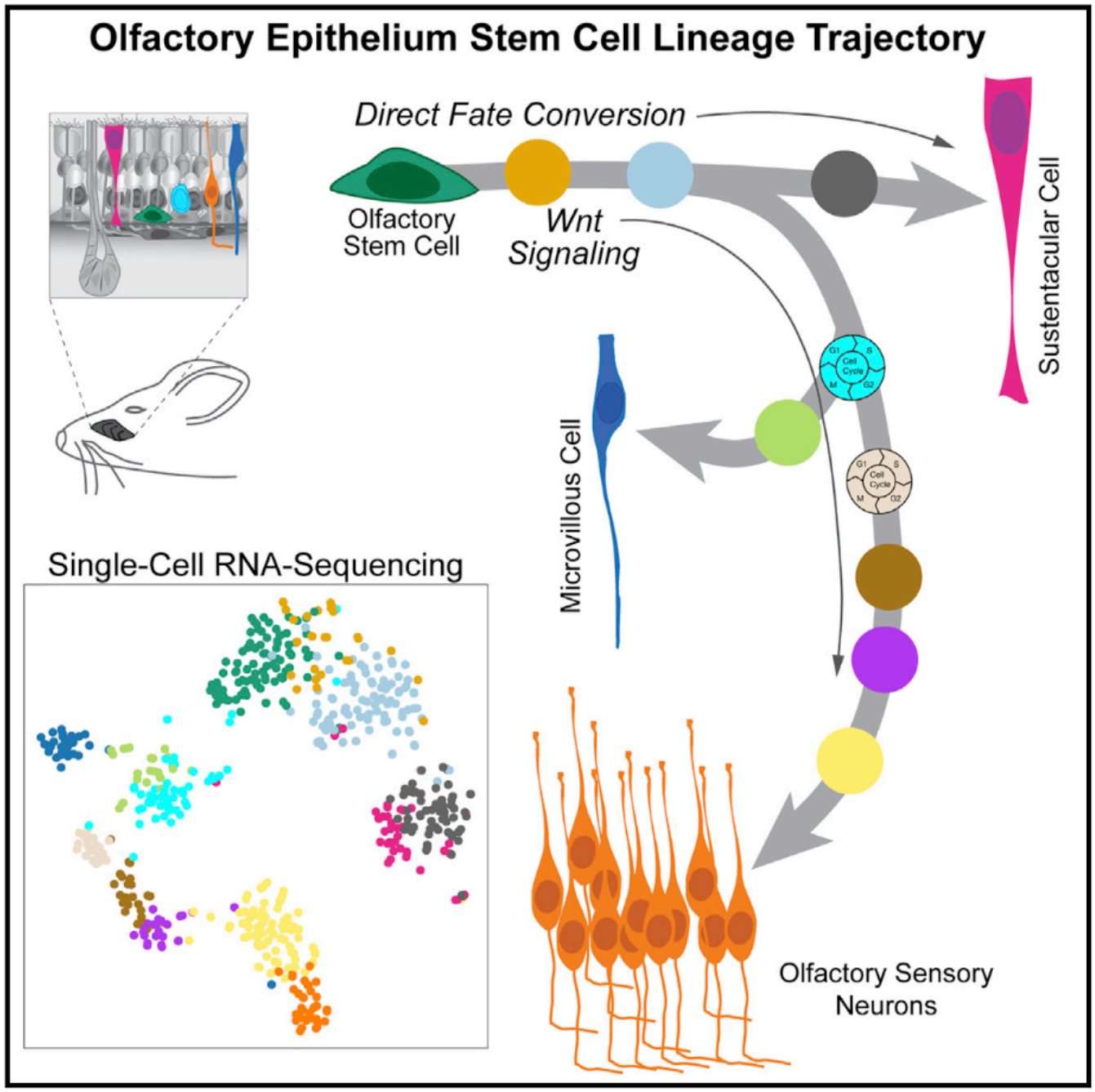
Adult stem cells maintain and regenerate tissues



Bond, Ming, Song (2015) Cell Stem Cell



Fletcher et al. (2017)
Cell Stem Cell

Useful links

- ZINB-WaVE preprint:
  - https://doi.org/10.1101/125112
- Slingshot preprint:
  - https://doi.org/10.1101/128843

- Please submit bug reports and other issues at:
  - github.com/drisso/zinbwave/issues
  - github.com/epurdom/clusterExperiment/issues
  - github.com/kstreet13/slingshot/issues

Contact us!

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RSEC: Resampling-based Sequential Ensemble Clustering

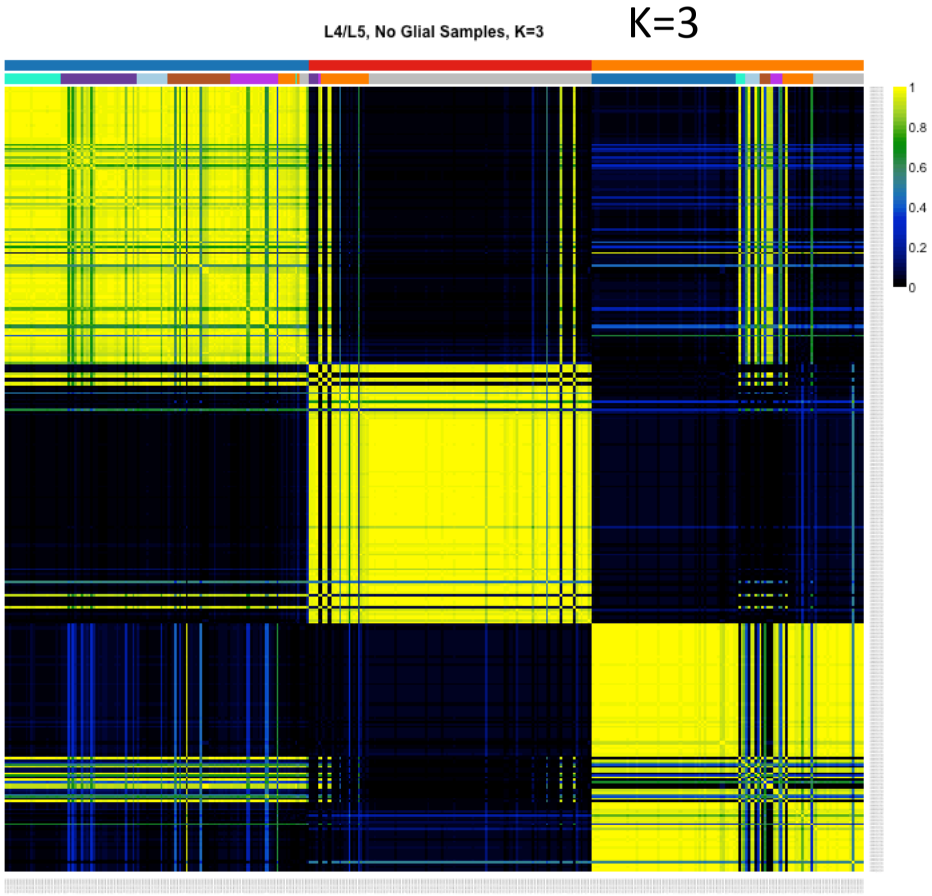
- Use a clustering routine that finds a large number of small, coherent clusters:
  - Subsampling of data to find robust clusters
  - Sequential clustering → find a group of coherent samples, remove them, start over
- Perform this routine over *many* different parameters
- Find a single consensus over the clusterings
- Merge together non-differential clusters
- Find biomarkers via differential expression with targeted comparisons
- Implemented with visualization tools in `clusterExperiment` package

What mean by subsample clustering?

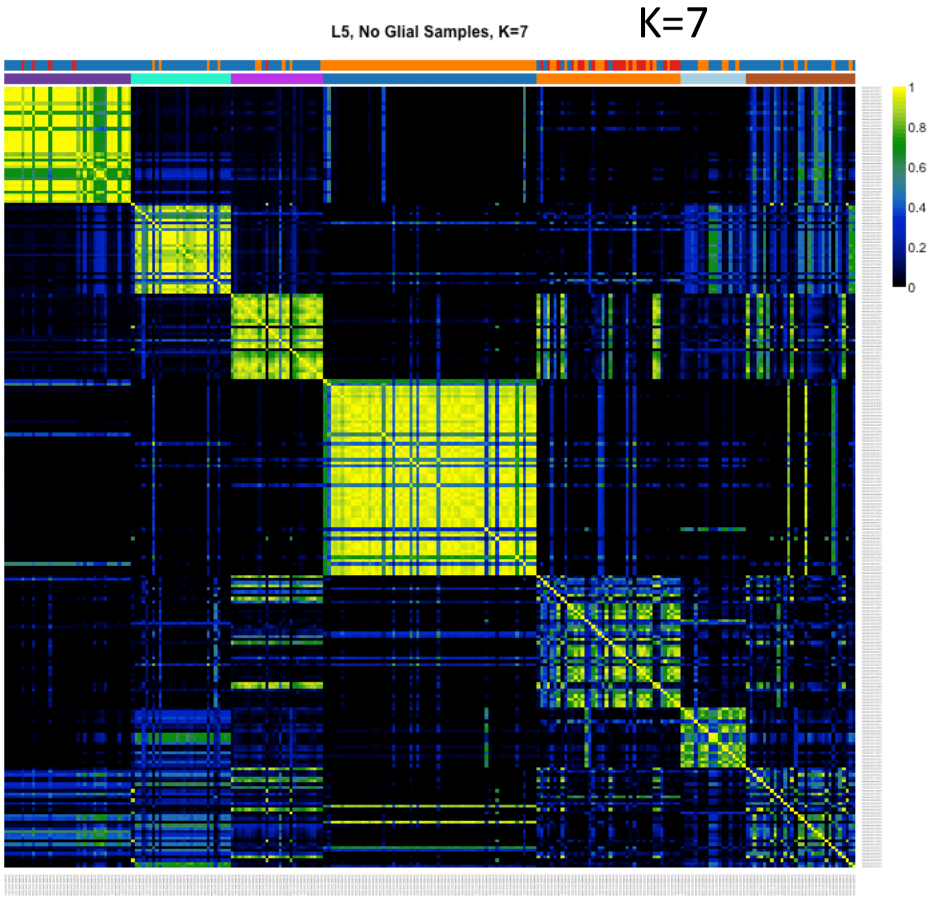
- Pick an underlying clustering strategy (e.g. kmeans or PAM with particular choice of K)
- Repeat the following:
  - Subsample the data, e.g. 70% of samples
  - Find clusters on the subsample
- Create coClustering matrix D: % of subsamples where samples were in same cluster

Examples of matrix D

K=3



K=7



Note, here forced the samples in order given by PAM
Also used kmeans in resampling, rather than PAM

What mean by subsample clustering?

- Pick an underlying clustering strategy (e.g. kmeans or PAM with particular choice of K)
- Repeat the following:
  - Subsample the data, e.g. 70% of samples
  - Find clusters on the subsample
- Create coClustering matrix D: % of subsamples where samples were in same cluster
- Cluster matrix D for final clustering
  - Could be with original clustering strategy, or different one
  - "Right" K may not be the K used in original clustering (e.g. kmeans)
  - We use a more flexible approach of hierarchical clustering and picking clusters so have at least 1-α similarity
  - Change from picking K to picking α, more intuitive choice
  - Not all samples get clustered

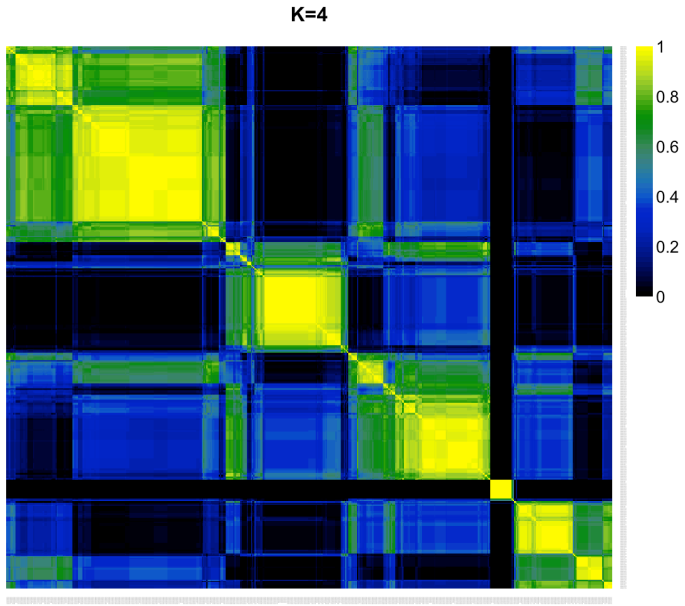
What mean by sequential clustering?

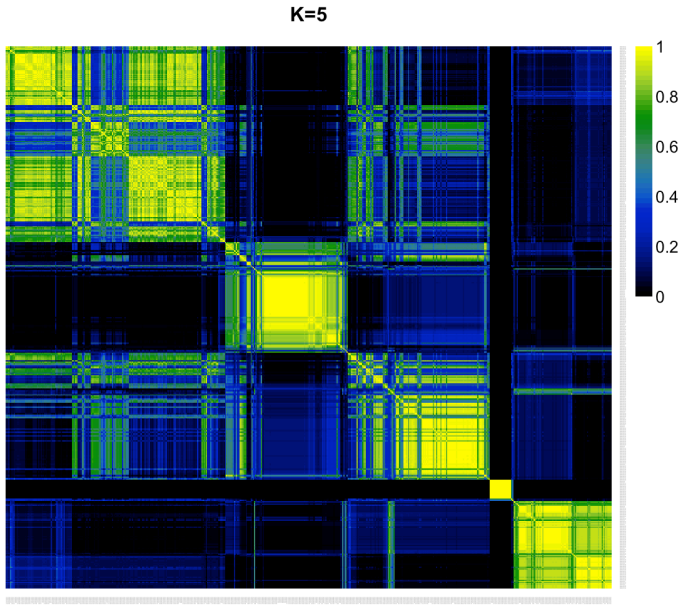
- Over range of starting parameters, do clustering
- The cluster that stays at least β similar, identify as cluster and remove
- Repeat until no more such clusters found, or not enough samples left
- Draws on ideas of "tight clustering" of genes of Tseng and Wong (2005)

Specifically,

- We range over K in underlying PAM in subsampling
- We find clusters based on results of subsample clustering (so may not be same K as input parameter)

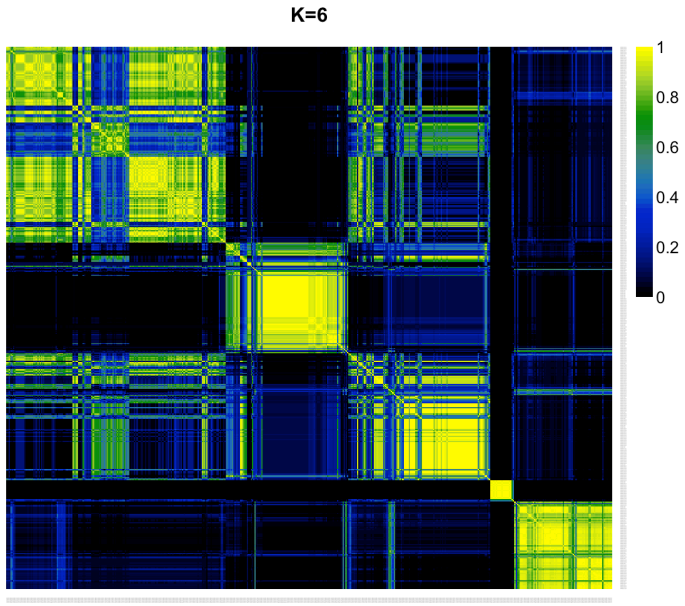
K=4



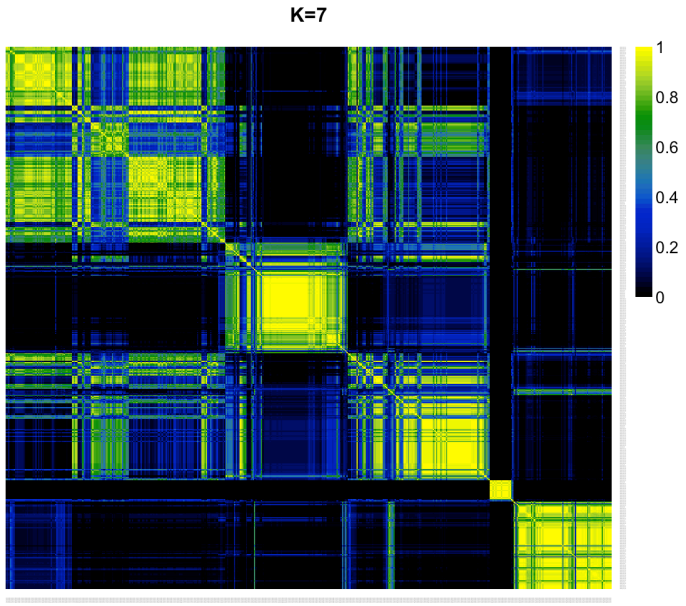


K=5

Because of subsampling, changing K is more a perturbation



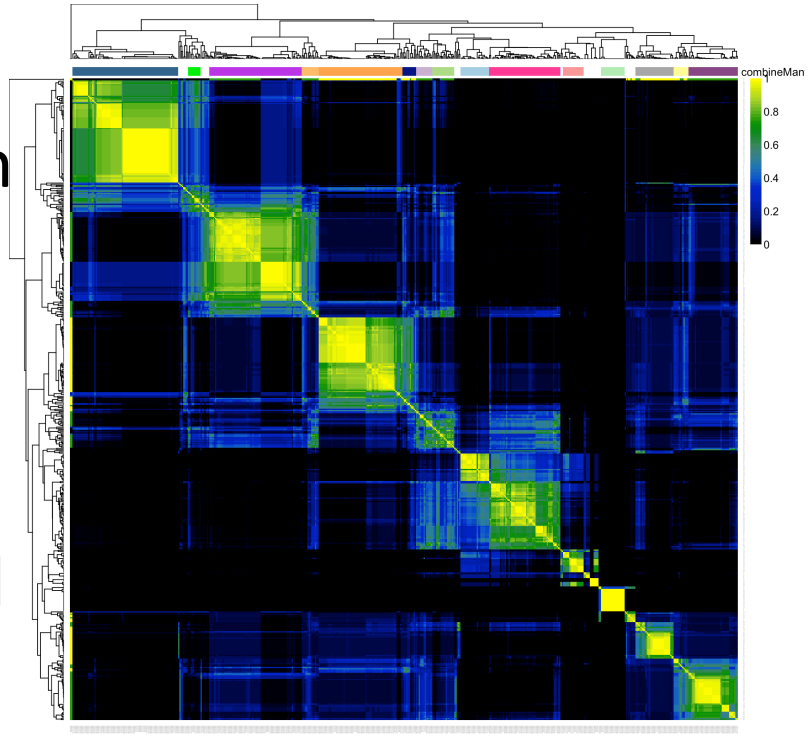
K=6



K=7

Find consensus cluster

- Create a co-Clustering matrix D of how many times co-cluster together and cluster D
  - Like with subsampling, on now across different parameters
  - Again, important that clusters are largely perturbations, not radical different clusters



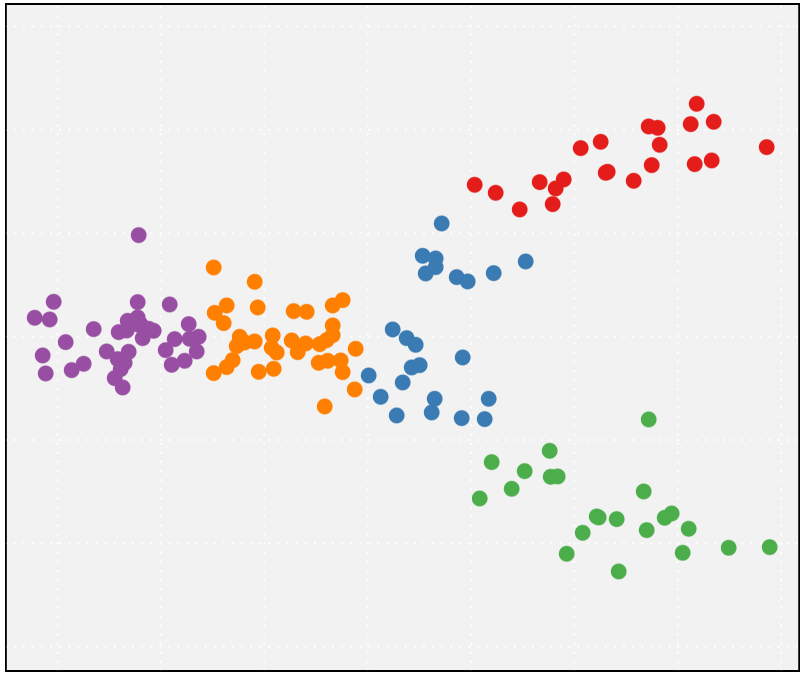
Bioconductor workflow for single-cell RNA-seq data analysis: dimensionality reduction, clustering, and pseudotime ordering.

Fanny Perraudeau, Davide Risso, Kelly Street

BioC2017

July 28th, 2017

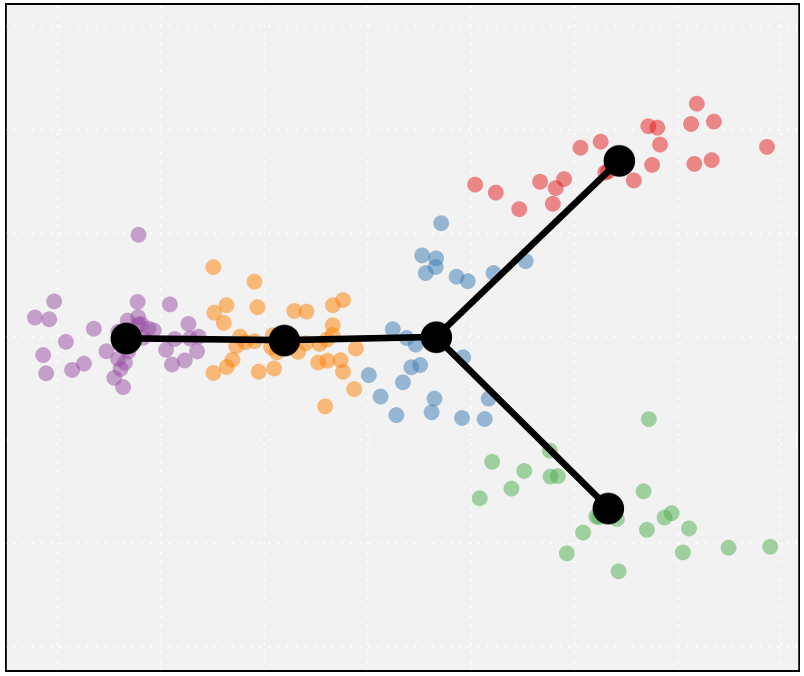
Step 0: Input



Clustered data in low-dimensional space.

We consider dimensionality reduction and clustering to be separate problems, but generally prefer PCA and RSEC.

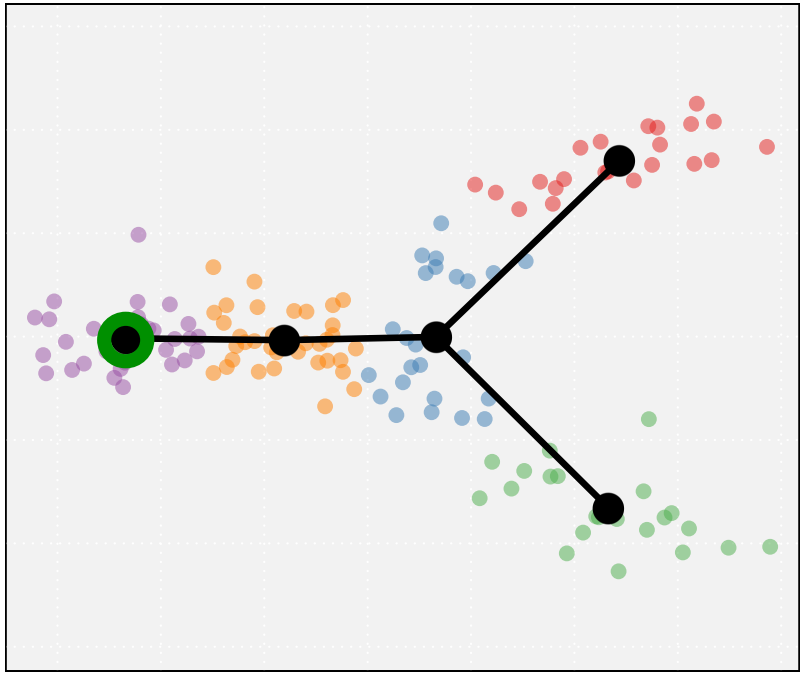
Step 1: Cluster MST



Construct minimum spanning tree (MST) on clusters.

The nodes are clusters, not cluster centers. Requires a distance metric.

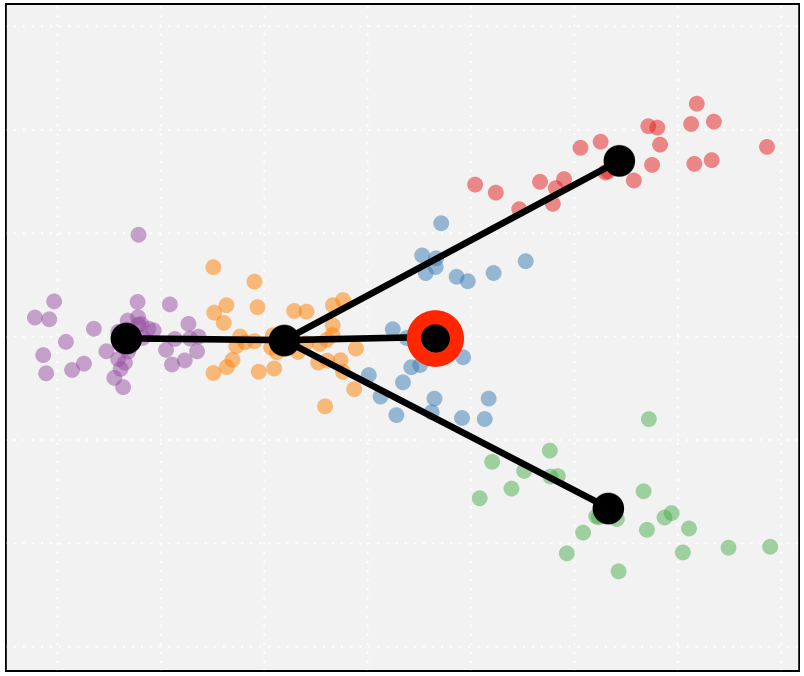
Step 1: Cluster MST



Construct minimum spanning tree (MST) on clusters.

Select starting cluster based on marker genes or parsimony.

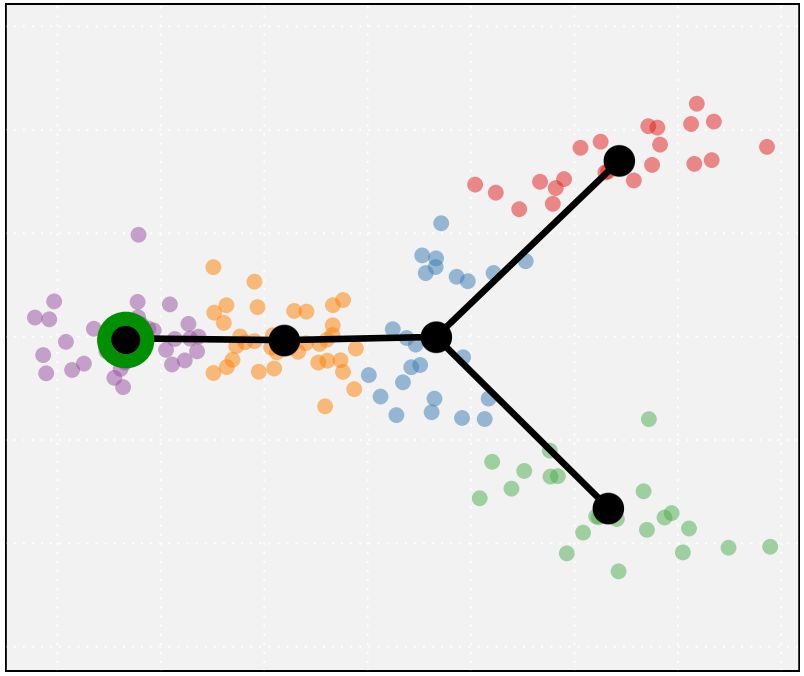
Step 1: Cluster MST



Specify known terminal clusters for additional supervision.

This results in the construction of a constrained MST.

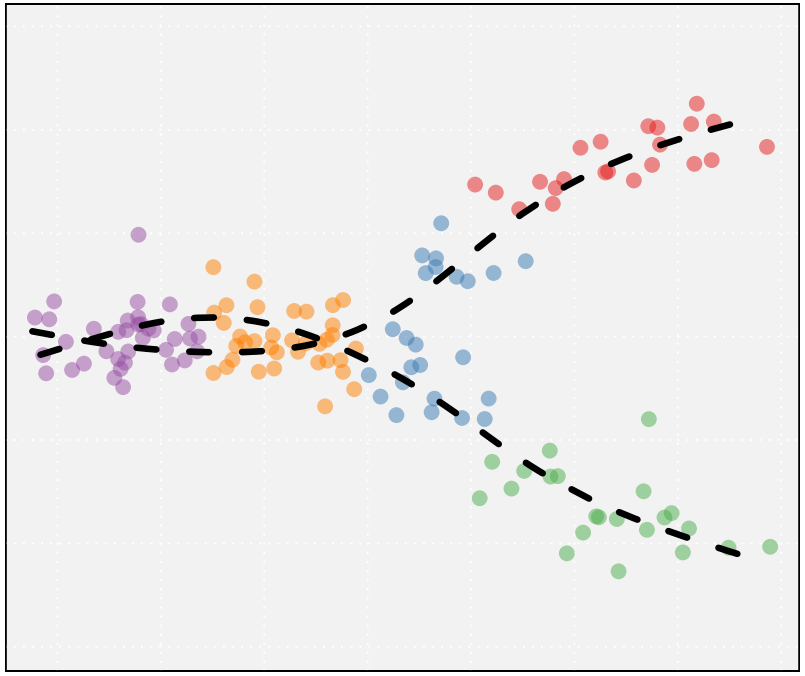
Step 1: Cluster MST



Construct minimum spanning tree (MST) on clusters.

Select starting cluster based on marker genes or parsimony.

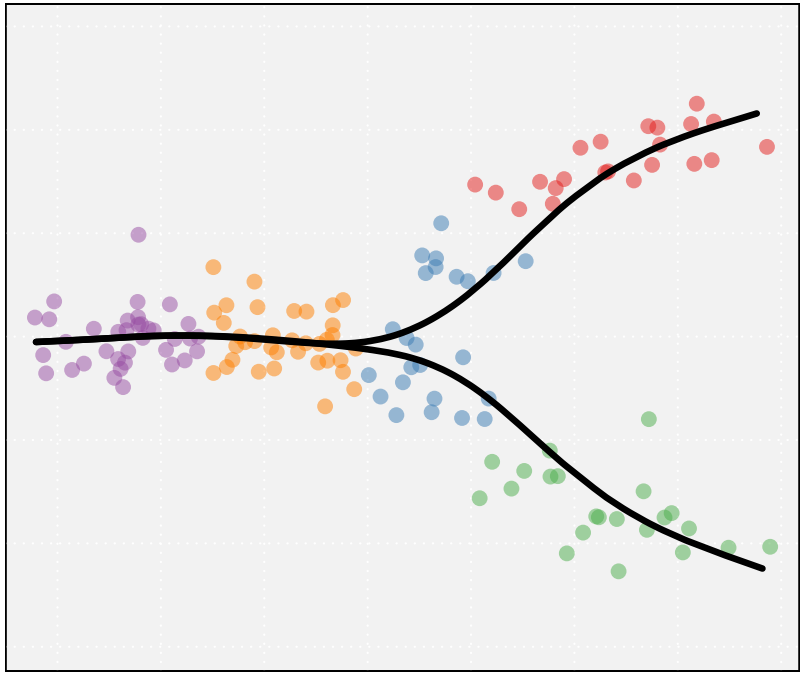
Step 1.5: Principal Curves



Highly stable, nonlinear generalization of principal components. Fits a curve to the “middle” of the data.

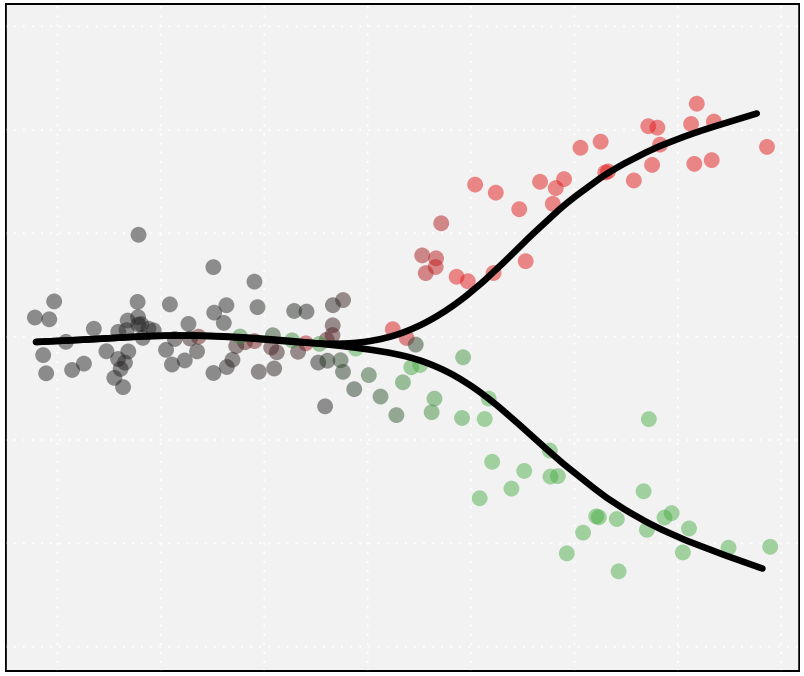
Inconsistent, fails to reflect underlying biology (ie. branching).

Step 2: Simultaneous Principal Curves



Still highly stable and nonlinear. Extends the concept of principal curves to multiple, branching curves with common origin (smooth tree structures).

Step 2: Simultaneous Principal Curves



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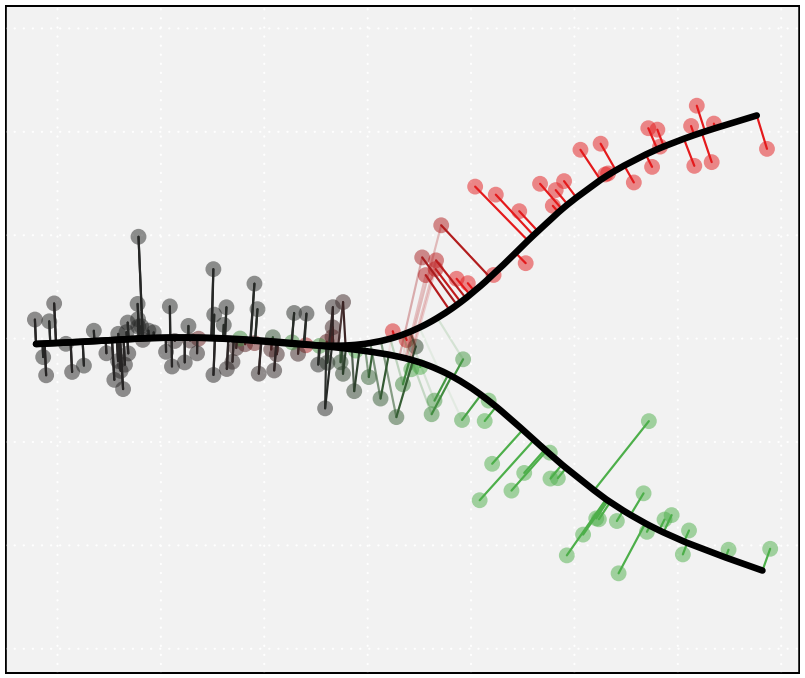
Step 2: Simultaneous Principal Curves



Project cells onto curves to obtain pseudotime values.

Like linear principal components, the curves seek to minimize squared projection distance (subject to some constraints).