Bioinformatics Software Lab
Introduction to Analysis of Single Cell Sequencing

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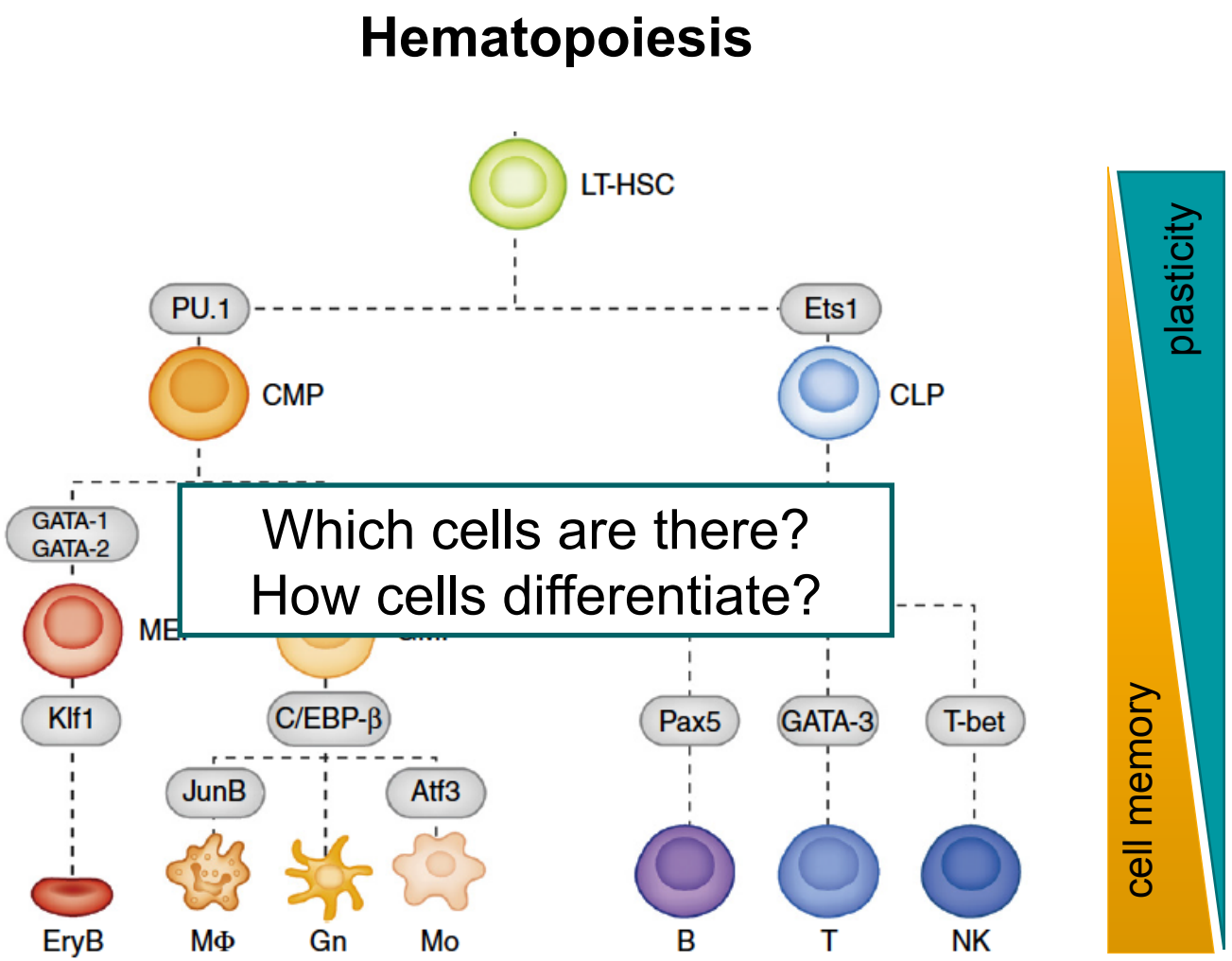
Objectives

1. basics of single cell sequencing
2. basic bioinformatics/computational problems
   - dimension reduction
   - clustering
   - data integration

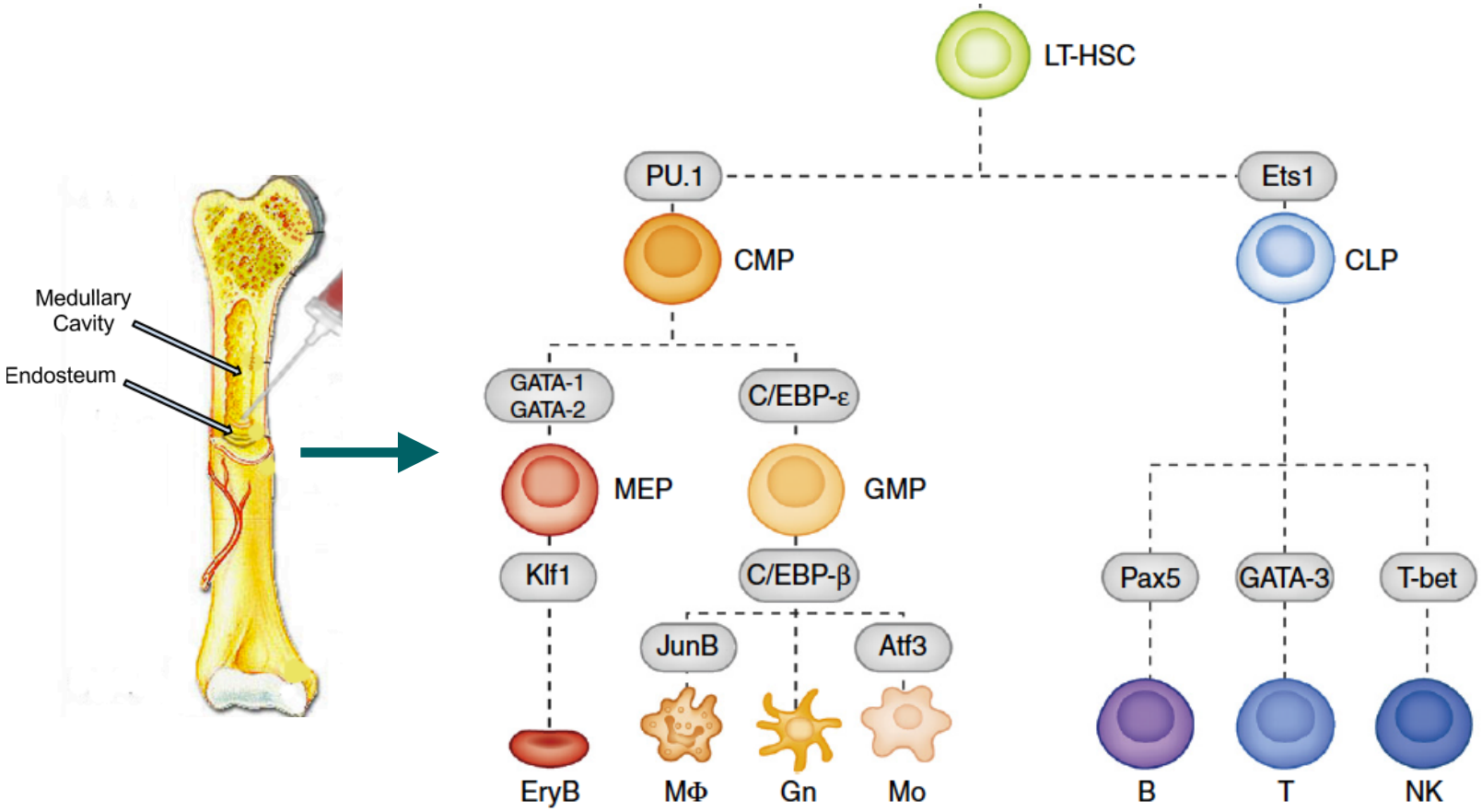
Expression at Single Cell Level

Cell Differentiation



Source: Amit (2016), *Nature Immunoloy.*

Cell Differentiation



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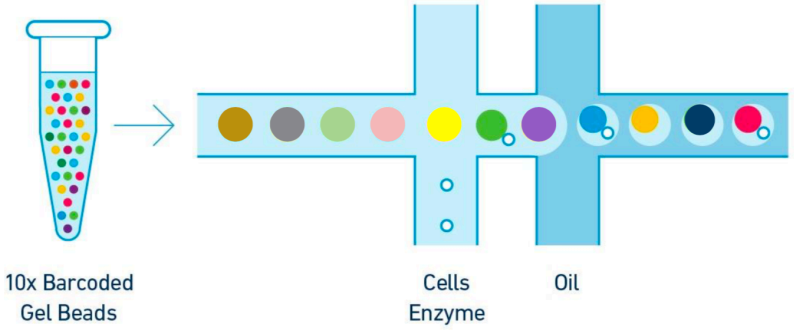
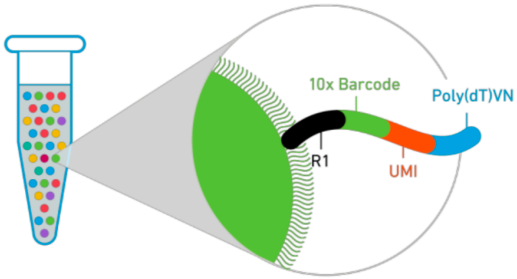
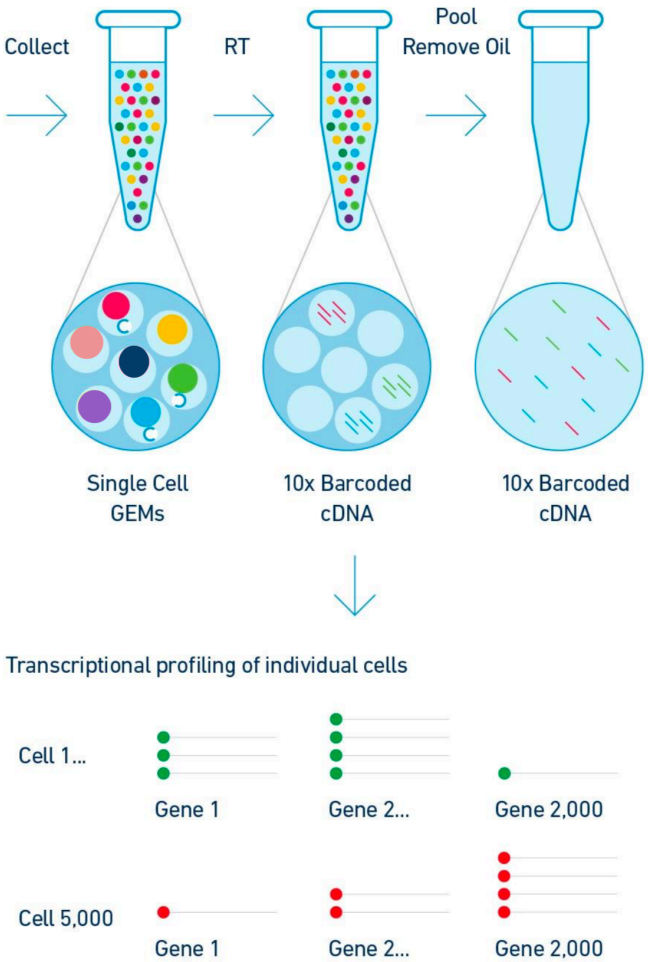
Droplet based RNA single cell sequencing



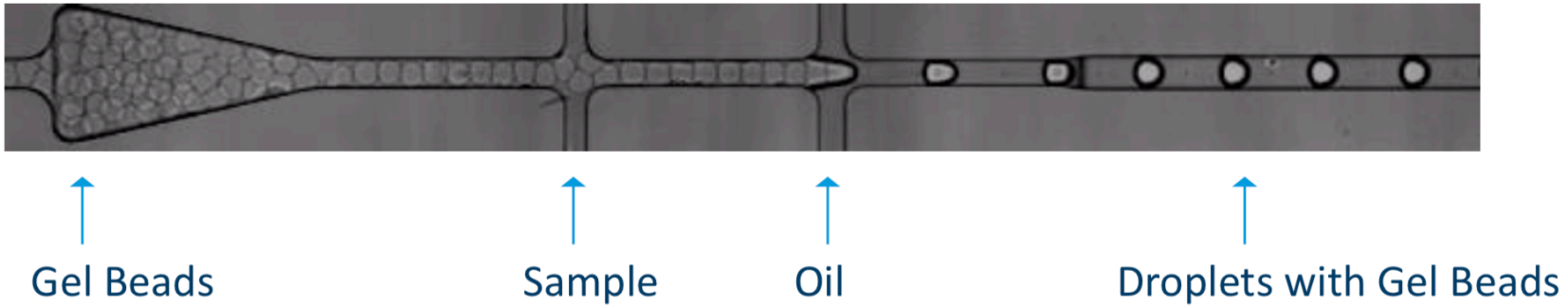


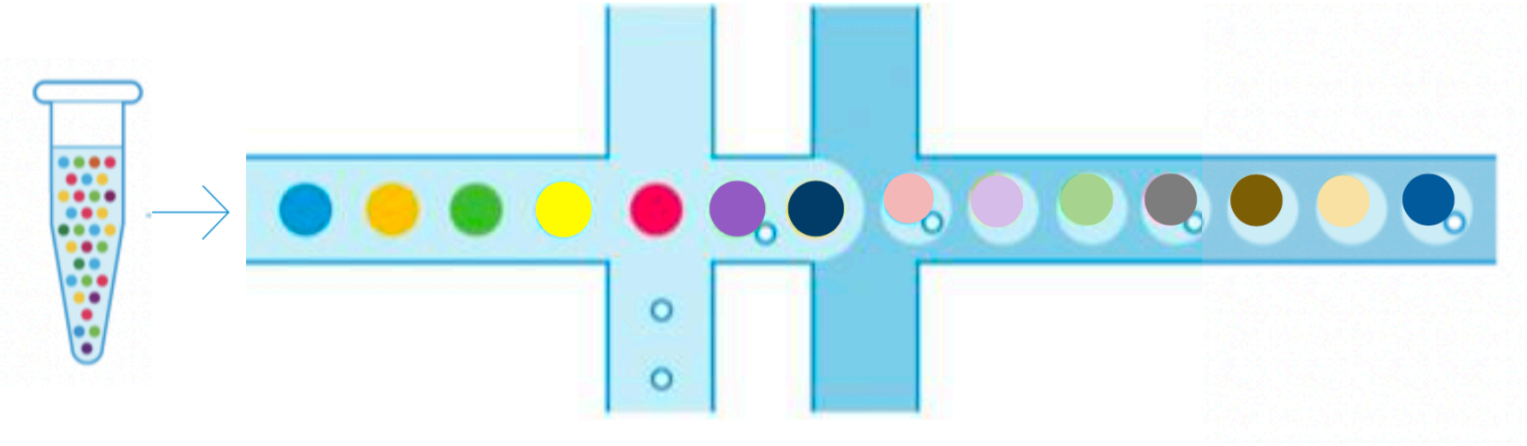


- Input: Single cells in suspension + 10x Gel Beads and Reagents
- Output: Digital gene expression profiles from every partitioned cell

Source: 10x genomics

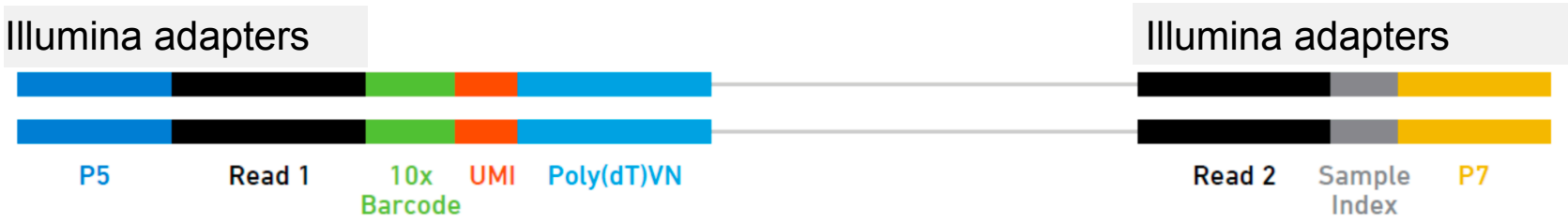
Droplet based RNA single cell sequencing





Source: 10x genomics

Basics Bioinformatics - Transcript Counts



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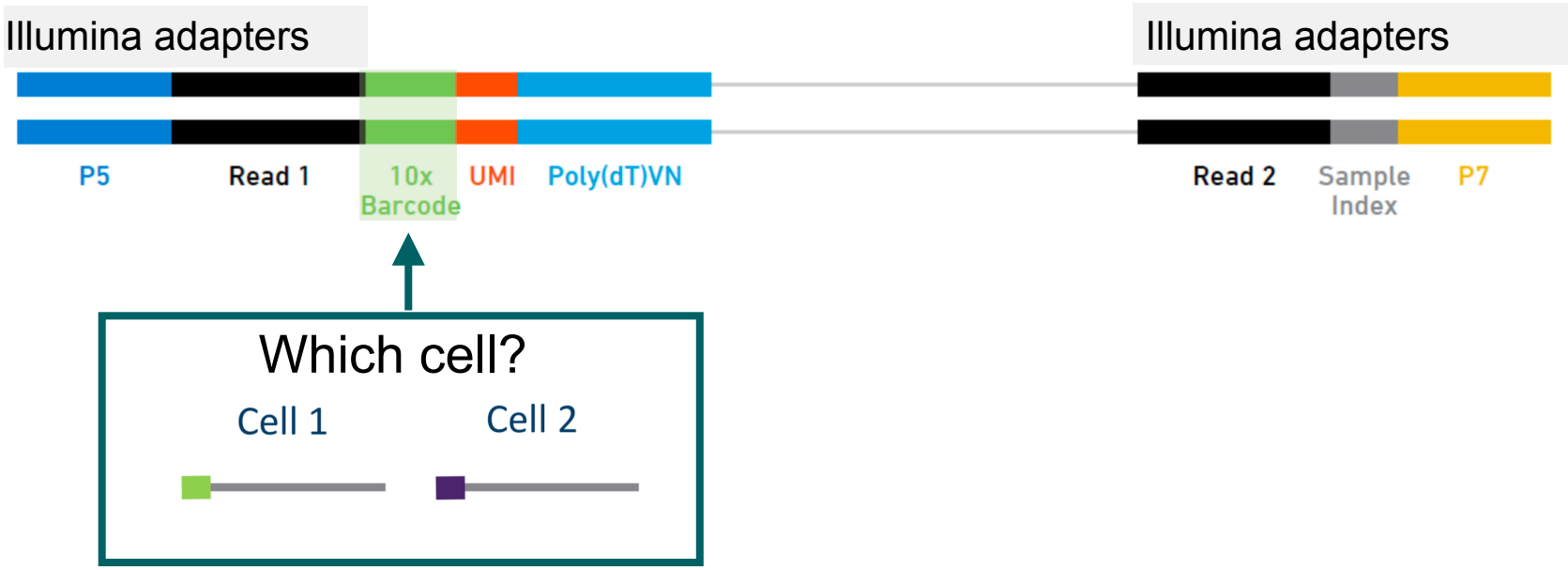

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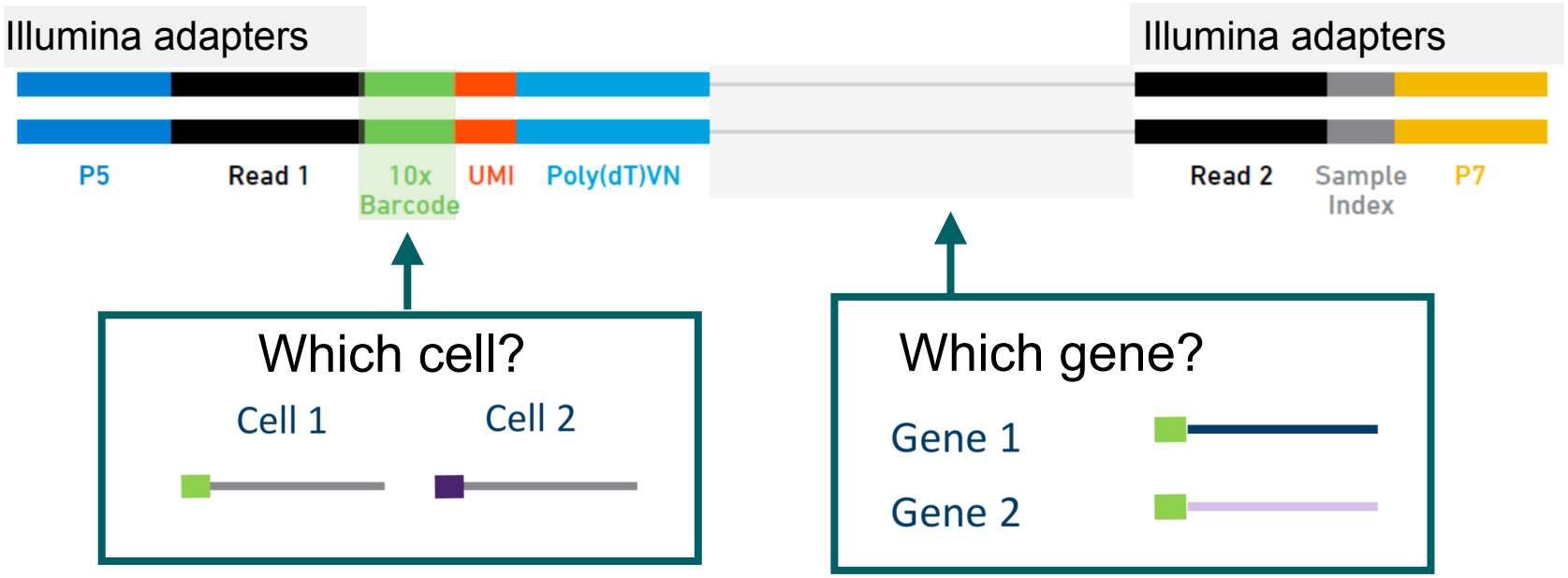

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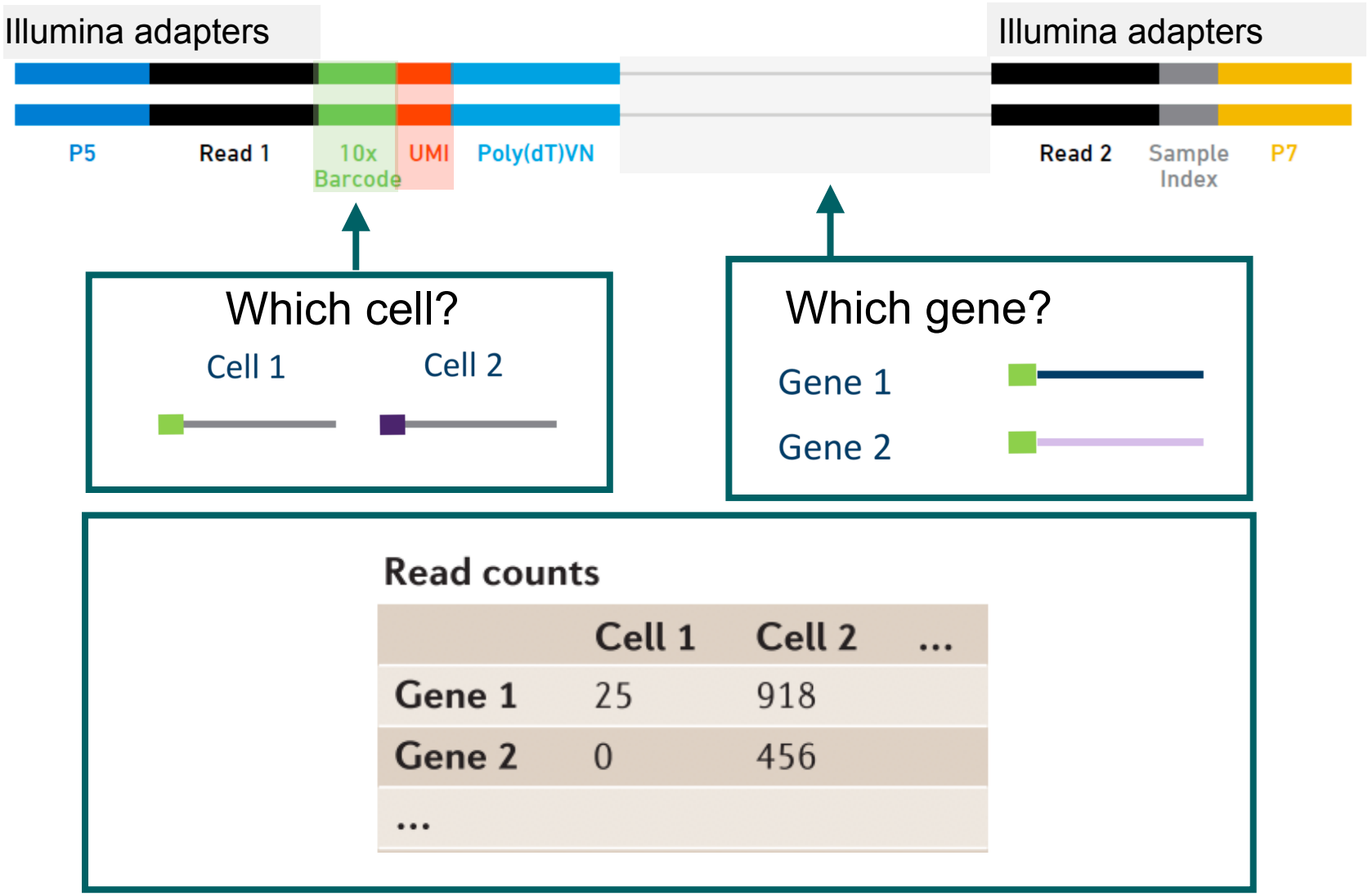
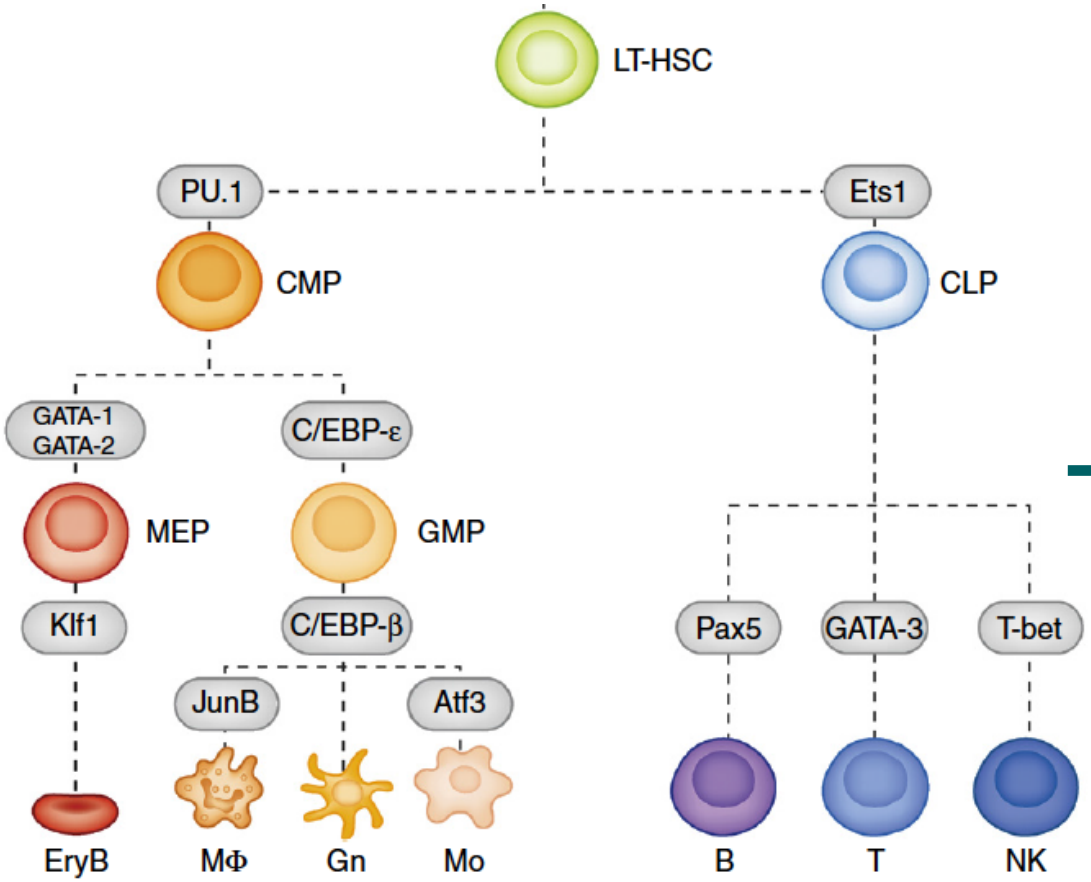


Source: 10x genomics

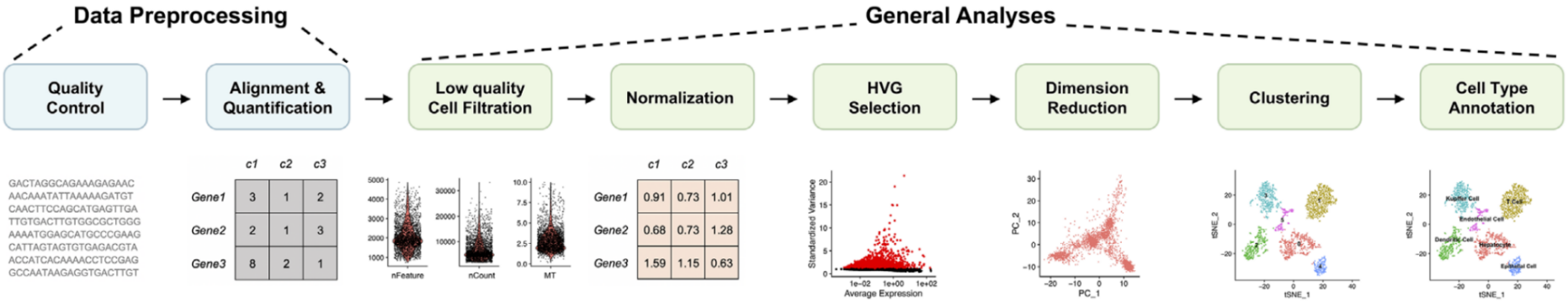
Cell Differentiation & Gene Expression



	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

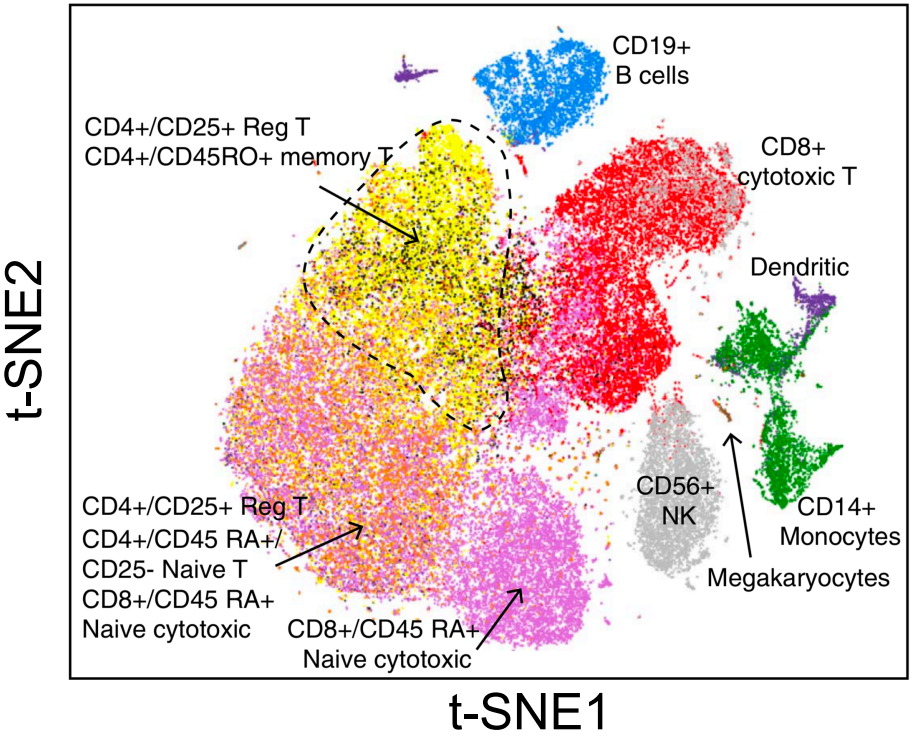
Source: Amit (2016), *Nature Immunoloy.*

Basics Bioinformatics - single cell RNA-seq



Gene Expression of Lymphoid Cells

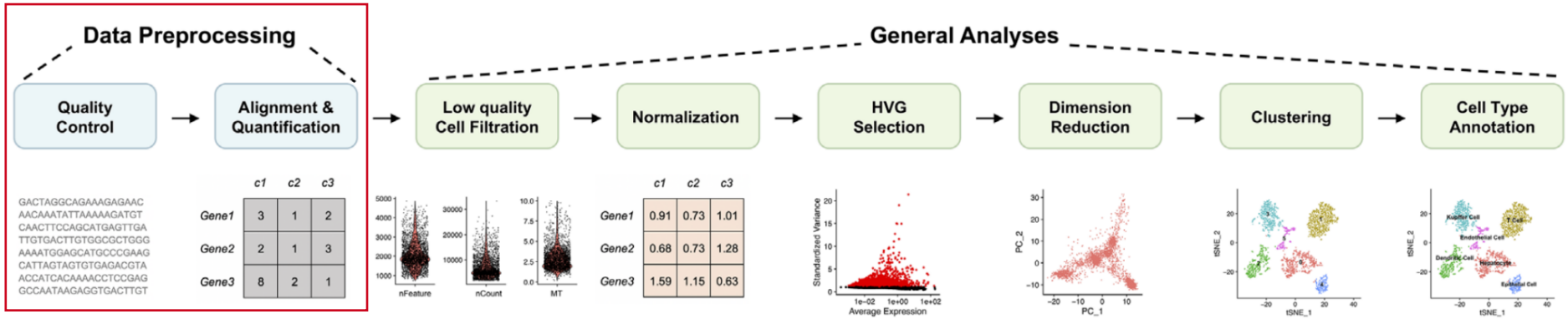
PBMCs from Humans



Single cell RNA-seq from 68k cells

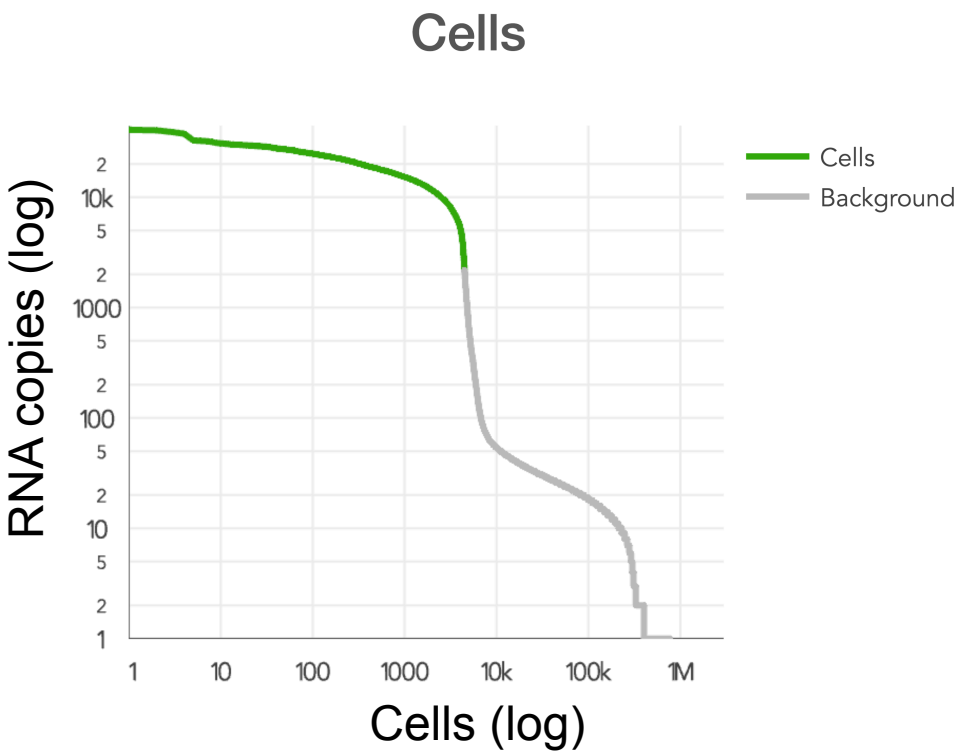
Source: Zheng et al. 2017 & Buenrostro et al. 2018

Basics Bioinformatics - single cell RNA-seq



Basics Bioinformatics - Cell Filtering

1. sum UMIs (copy of transcripts) per cell
2. consider cells with total UMI count > 99th of expected recovered cells



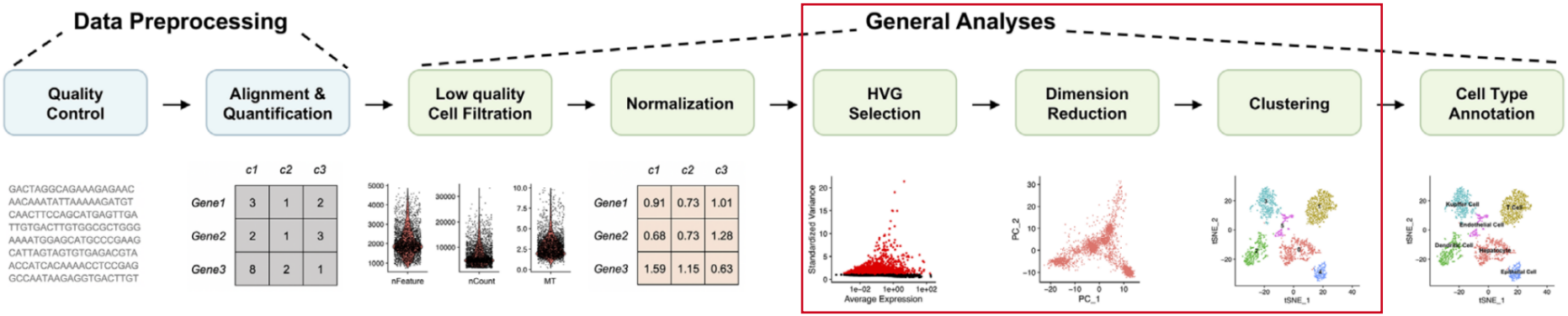
Estimated Number of Cells
4,495

Post-Normalization Mean Reads per Cell
89,289

Median Genes per Cell
2,504

cell ranger - 10x genomics

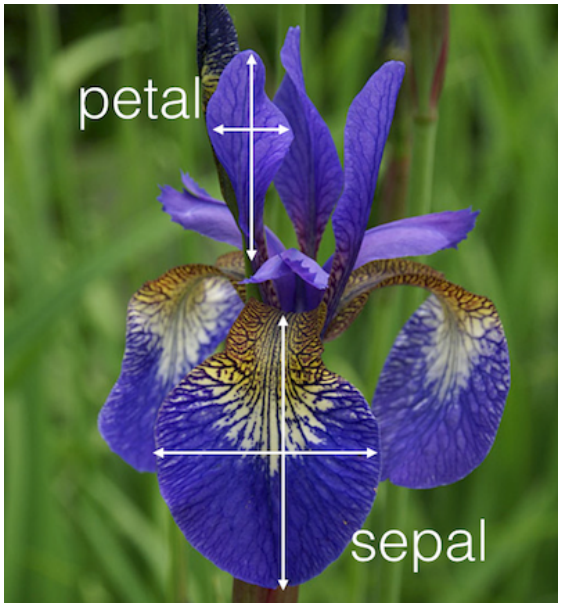
Basics Bioinformatics - single cell RNA-seq



Clustering & Dimension reduction

Clustering

- **Given a data description**
  - i.e. measurement of size of iris flowers
- **Find groups of similar observations**
  - i.e. iris flower sub-types



	Sepal Length	Sepal Width	Petal Length	Petal Width
Flower 1	5.1	3.5	1.4	0.2
Flower 2	4.9	3.0	1.4	0.2
Flower 3	4.7	3.2	1.3	0.2
Flower 4	4.6	3.1	1.5	0.2
…	…	…	…	…

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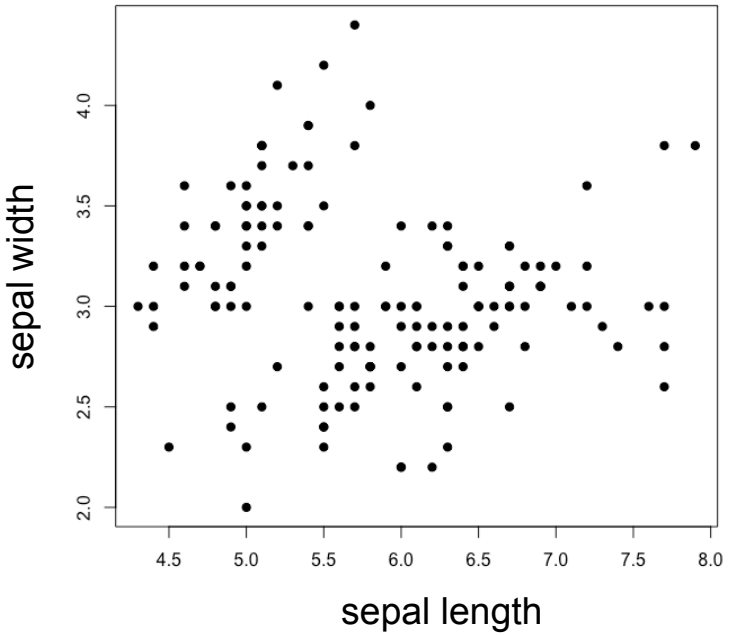

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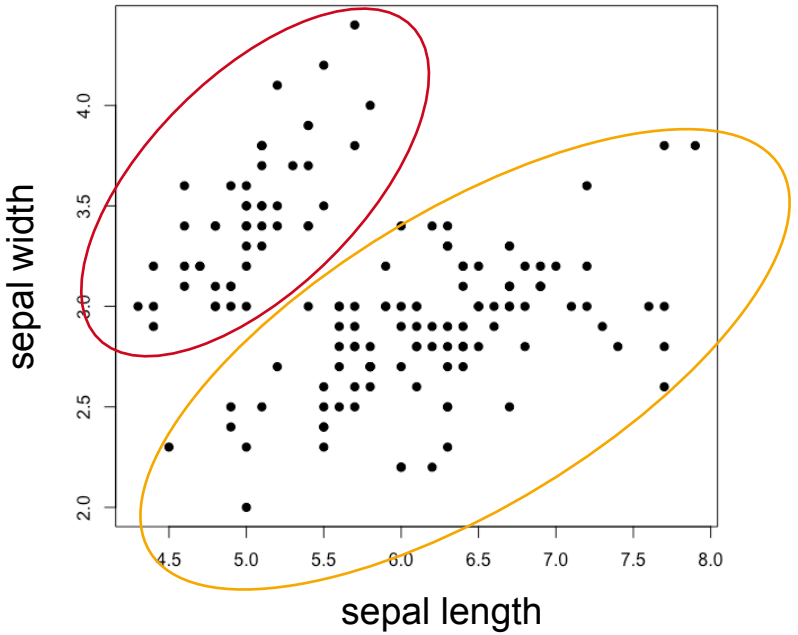

Clustering

- **Given a data description**
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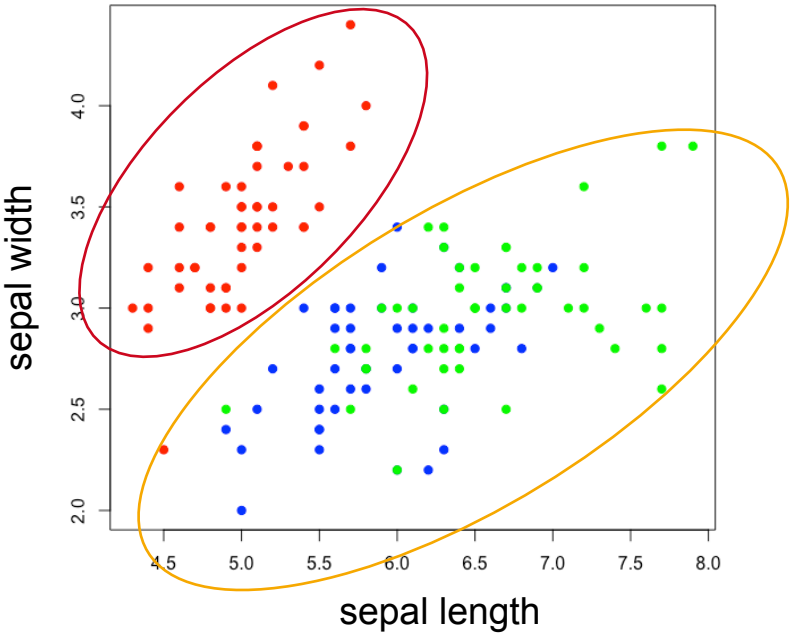
Iris Setosa



Iris Virginia

Iris Versicolor

Clustering Formalism

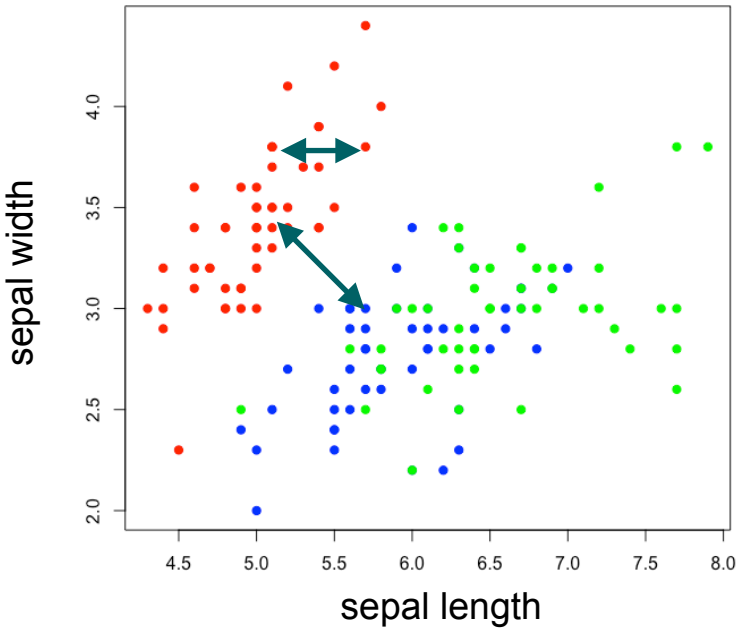
- **For a given data:**
  - Matrix X with N observations and L dimensions
    where x_i is a vector representing observation i

x_{11}	x_{12}	…	x_{1L}
x_{21}	x_{22}	…	x_{2L}
x_{31}	x_{32}	…	x_{3L}
…	…	…	…
x_{N1}	x_{N2}	…	x_{NL}

- **find groups of similar observations**
  - *vector* $Y = (y_1, \ldots, y_N)$
    where $y_i \in \{1, \ldots, K\}$ indicates the cluster of observation i

Distance

- **A important concept in clustering is a distance (similarity) between a pair of objects x_i and x_j**
  - Observations of a same group should be close in space



Euclidean distance
(sensitive to scale)

$$d(x_i, x_j) = \sqrt{\sum_{l=1}^{L} (x_{il} - x_{jl})^2}$$

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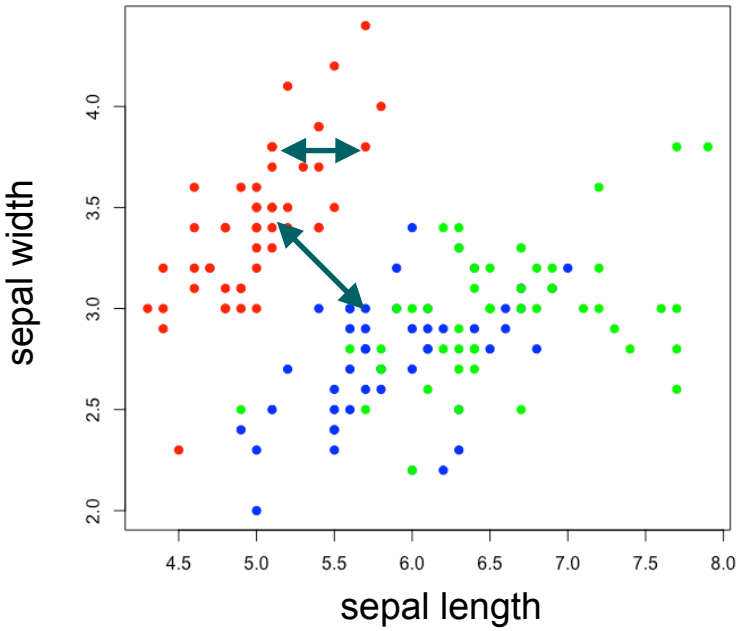

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Pearson Correlation

(scale insensitive/ similarity)

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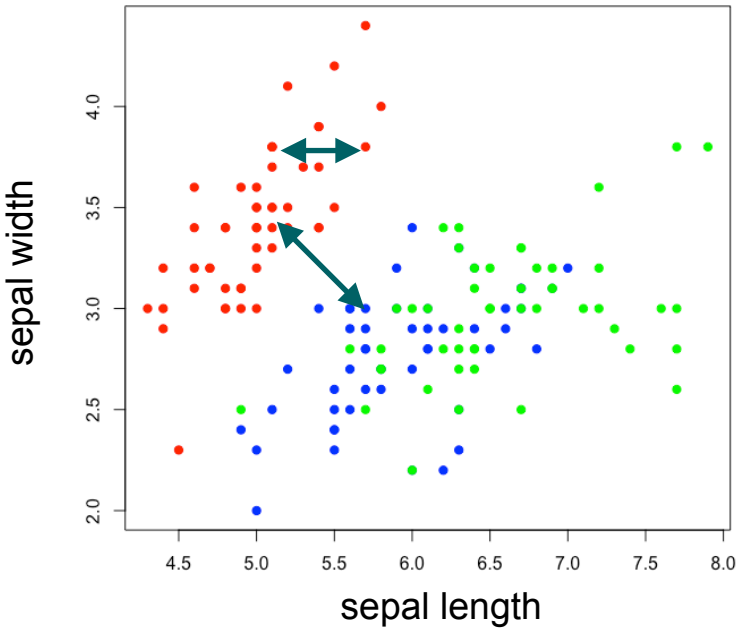

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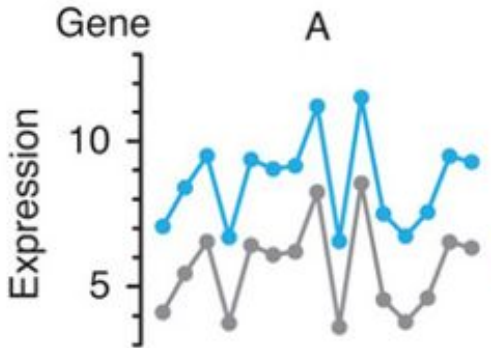
Distance and Scale

- **In some problems scale can be important!**
  - Similarly in changes are more important / not absolute values.

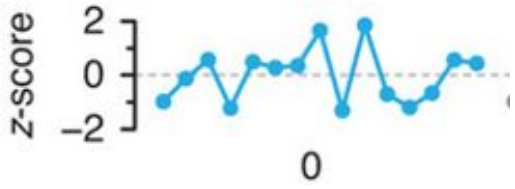
unscaled data



Euclidean - not similar

Correlation - similar

z-score normalised data



$$z = \frac{x_{ij} - \mu_i}{\sigma_i}$$

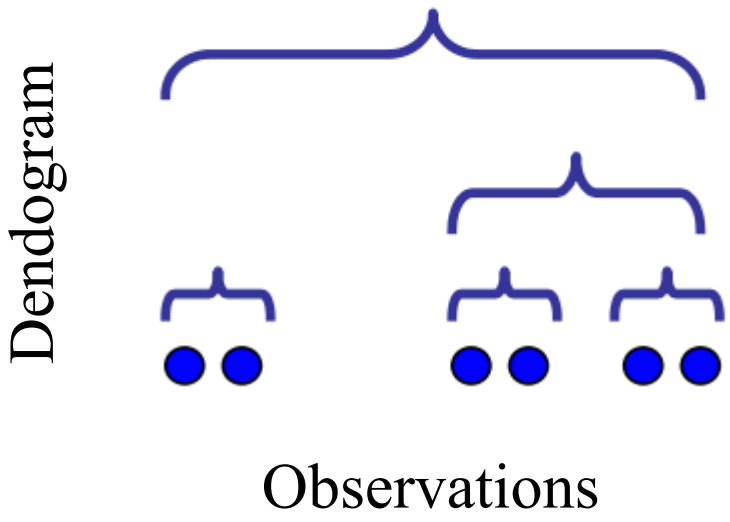
Euclidean - similar

Correlation - similar

Clustering Methods

- **Hierarchical methods**
  - Mostly bottom up
  - based on distance / simple to interpret
- **Partitional methods (k-means or mixture models)**
  - Mostly top down
  - Use models of groups, centroids
- **Graph based methods**
  - Use graph formalisms to represent data:
    - nodes are objects
    - edges weights represent similarities
    - find well connected graphs

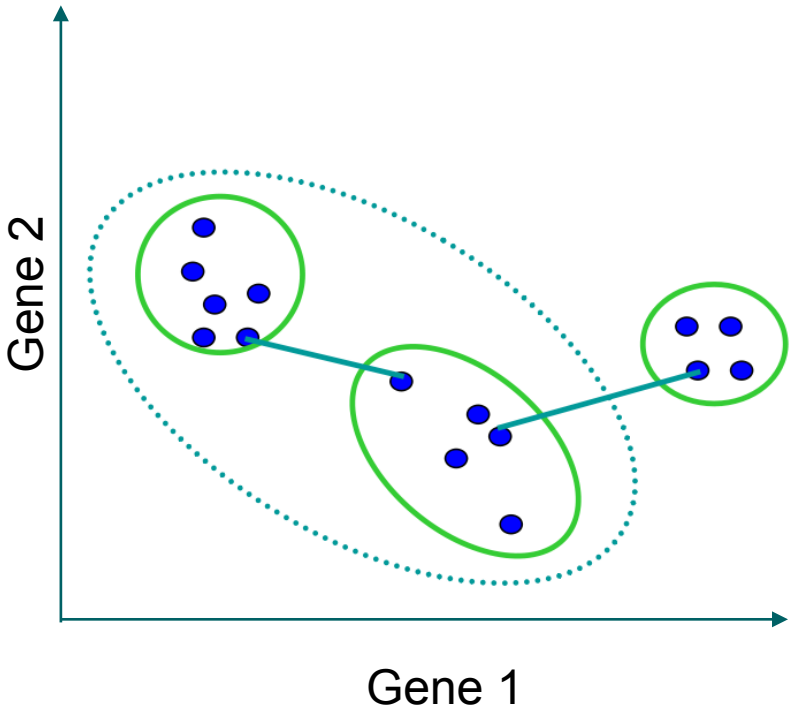
Hierarchical Clustering



- Botton up method
- Starting with a distance (similarity) matrix and each object as a group
- Repeat:
  - Joint two most similar groups
- Until the dendrogram has only one group

Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering



Single-Linkage

- Join two groups where two examples are close
- Find groups with linear shapes

Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering

Distance Matrix

	1	2	3	4	5
1	0				
2	2	0			
3	6	3	0		
4	10	9	7	0	
5	9	8	5	4	0

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Hierarchical Clustering

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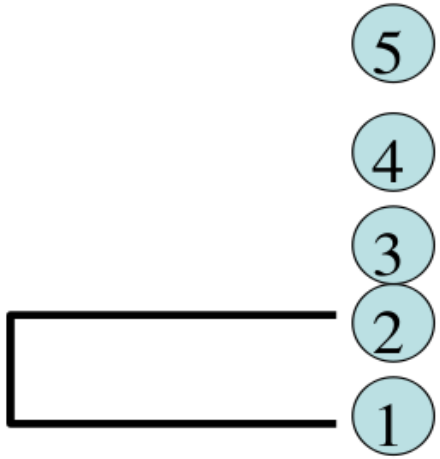

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Hierarchical Clustering

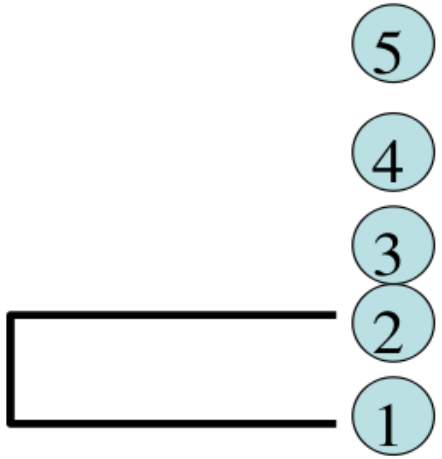
Distance Matrix

$$\begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array} \begin{array}{c} \begin{array}{ccccc} 1 & 2 & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & & & \\ 2 & 0 & & & \\ 6 & 3 & 0 & & \\ 10 & 9 & 7 & 0 & \\ 9 & 8 & 5 & 4 & 0 \end{bmatrix} \end{array} \Rightarrow \begin{array}{c} \\ (1,2) \\ 3 \\ 4 \\ 5 \end{array} \begin{array}{c} \begin{array}{cccc} (1,2) & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & & \\ 3 & 0 & & \\ 9 & 7 & 0 & \\ 8 & 5 & 4 & 0 \end{bmatrix} \end{array}$$

$$d_{(1,2),3} = \min\{d_{1,3}, d_{2,3}\} = \min\{6,3\} = 3$$

$$d_{(1,2),4} = \min\{d_{1,4}, d_{2,4}\} = \min\{10,9\} = 9$$

$$d_{(1,2),5} = \min\{d_{1,5}, d_{2,5}\} = \min\{9,8\} = 8$$



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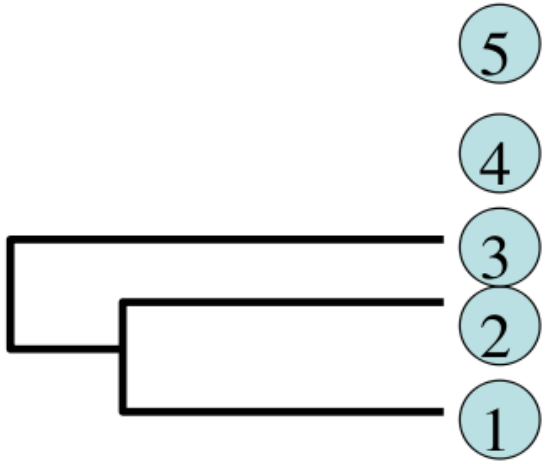
$$d_{(1,2,3),4} = \min\{d_{(1,2),4}, d_{3,4}\} = \min\{9,7\} = 7$$

$$d_{(1,2,3),5} = \min\{d_{(1,2),5}, d_{3,5}\} = \min\{8,5\} = 5$$



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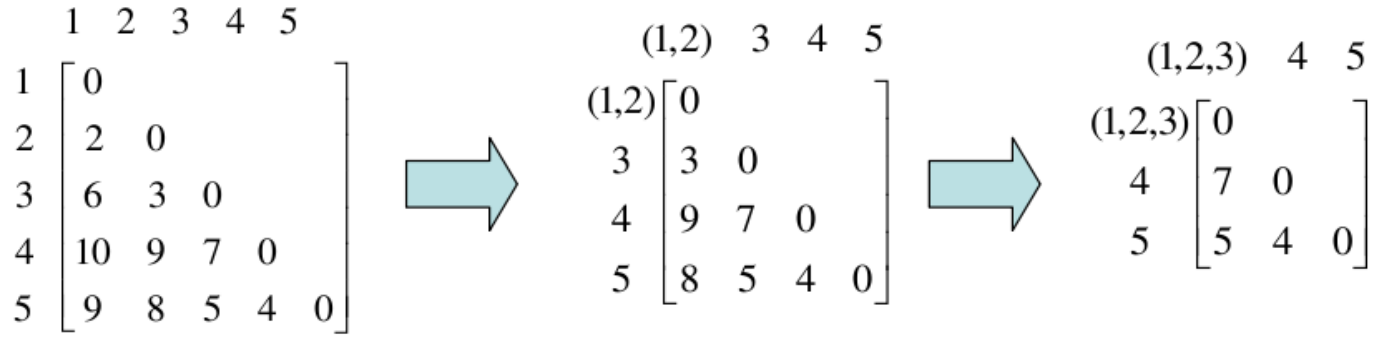
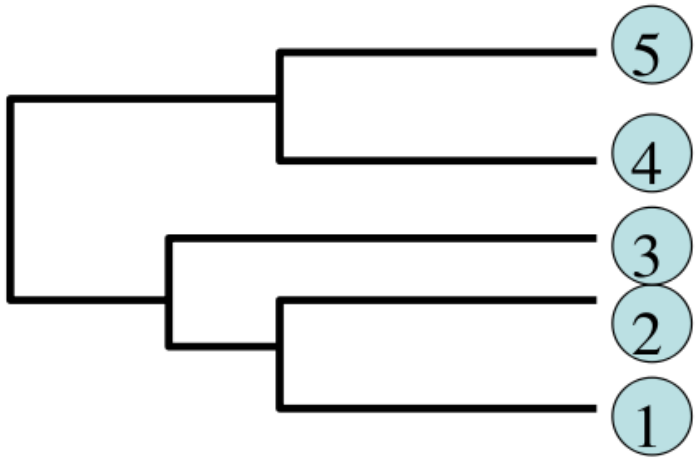
Hierarchical Clustering



$$d_{(1,2,3),(4,5)} = \min\{d_{(1,2,3),4}, d_{(1,2,3),5}\} = 5$$



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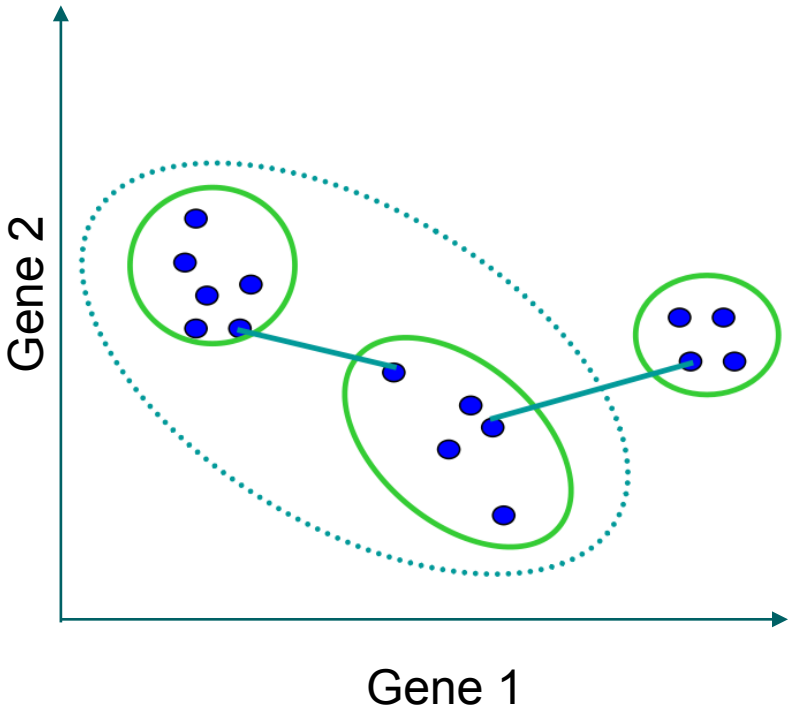
Hierarchical Clustering



Single-Linkage

- Groups with closest genes
- linear shapes

Complete-Linkage

- Closest groups with more far genes
- Compact clusters

Average Linkage

- Groups with closest centroids (middle)
- Outlier robust

Source: Slides adapted from Ziv-Bar Joseph

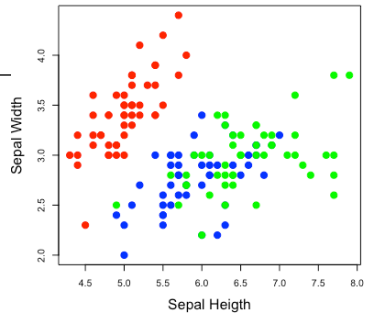
Hierarchical Clustering

Which linkage?

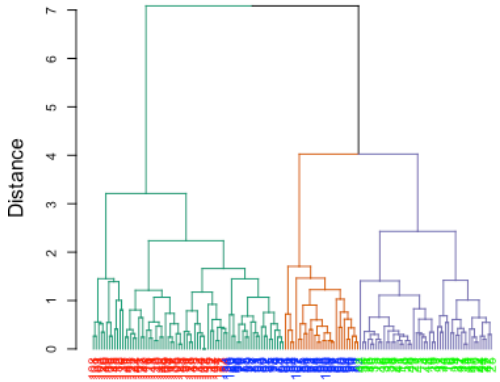
Which distance?

Hierarchical Clustering of Iris

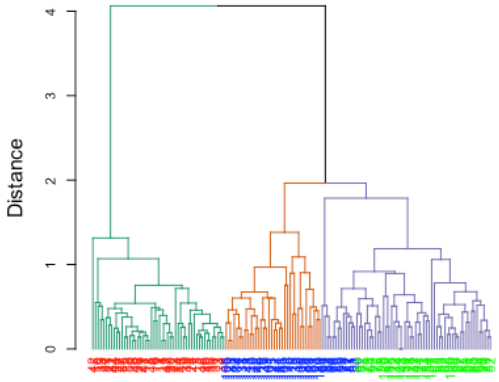
True labels



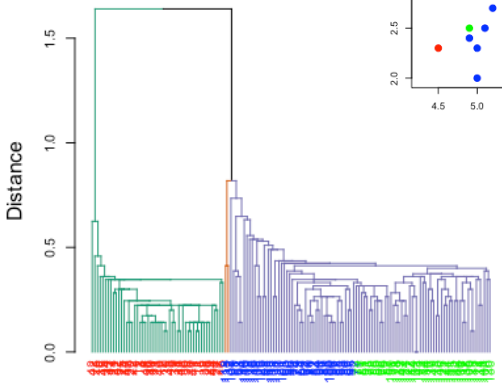
Euclidean distance



Complete



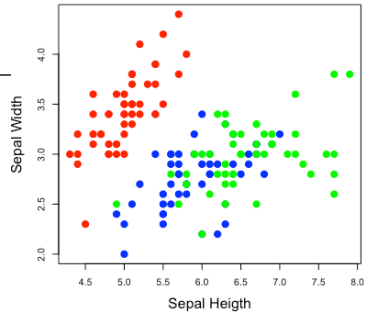
Average



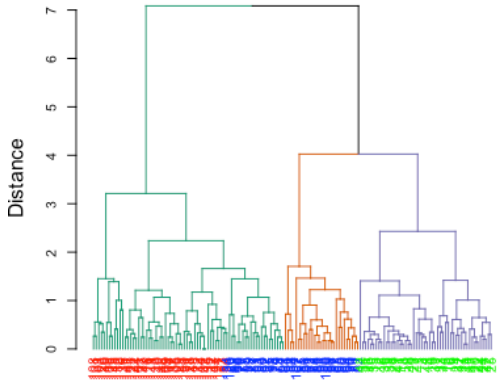
Single

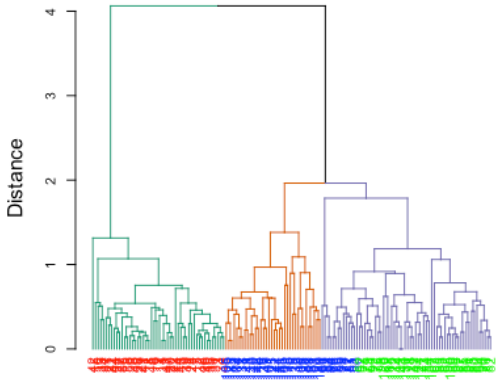
Hierarchical Clustering of Iris

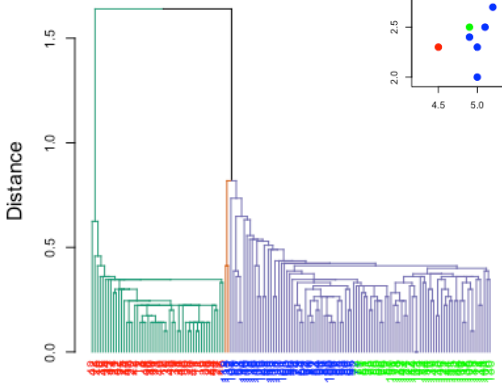
True labels



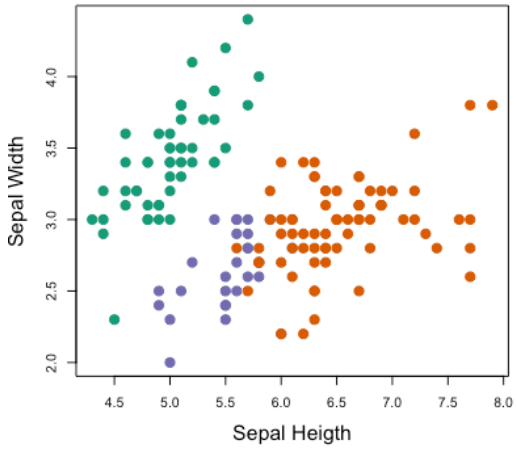
Euclidean distance



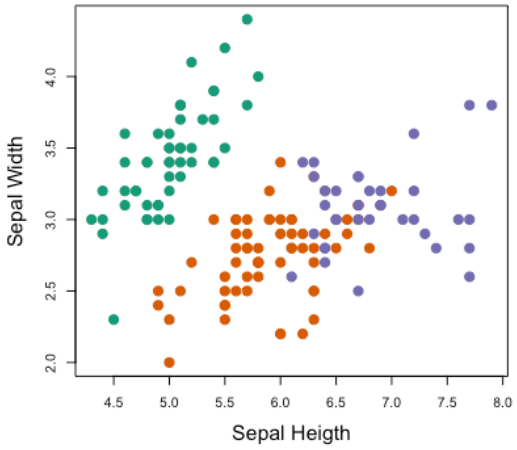




Complete



Average



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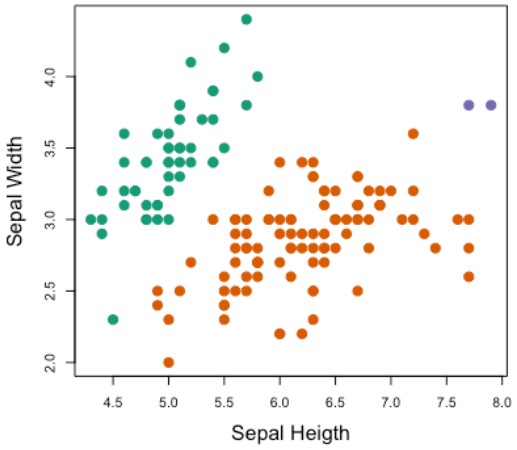

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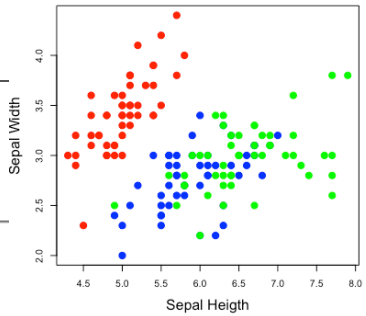
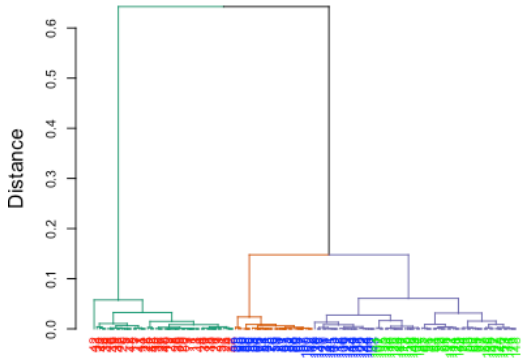
Hierarchical Clustering of Iris

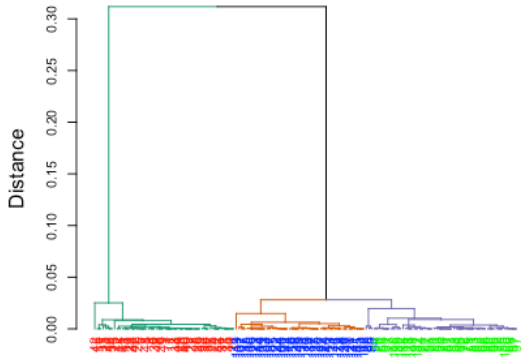
True labels



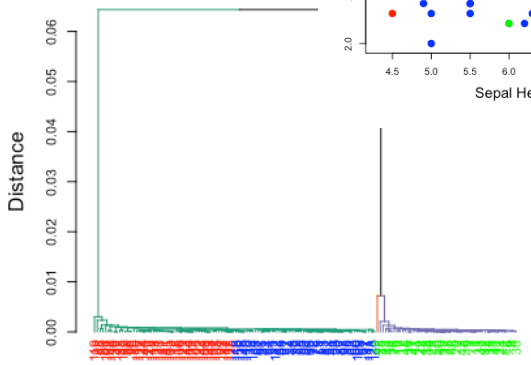
Pearson distance



Complete



Average



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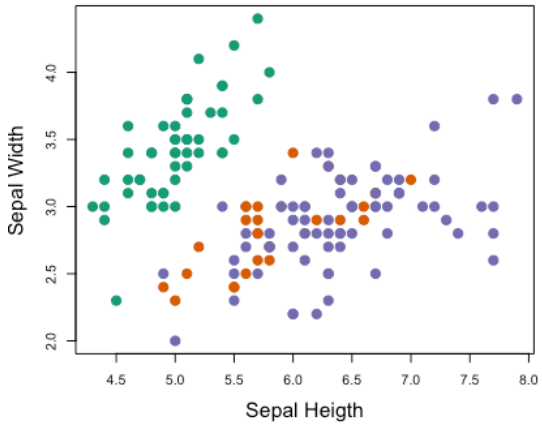

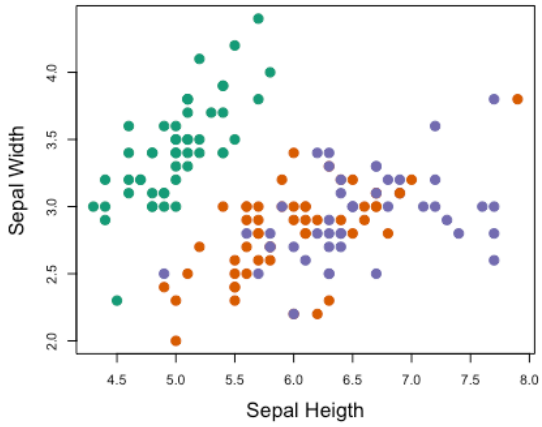

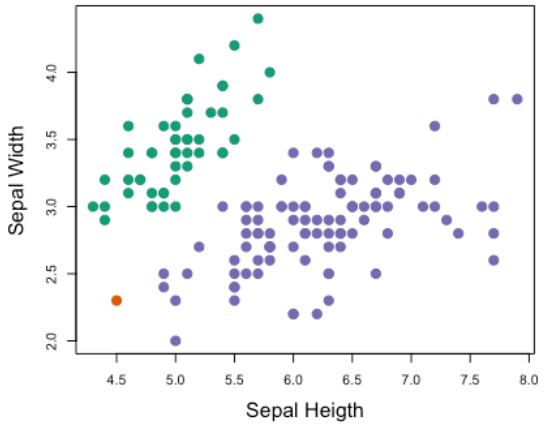

- Hierarchical cluster is sensitive to noise/outliers
- High computational cost O(n^2)

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K-means

Iterative algorithm using **centroids** as cluster representations

Requires specification of number of clusters (***K***)

Algorithm:

Start cluster (Y) randomly

Repeat for a number of iterations

- estimate centroid (m_k) for each cluster

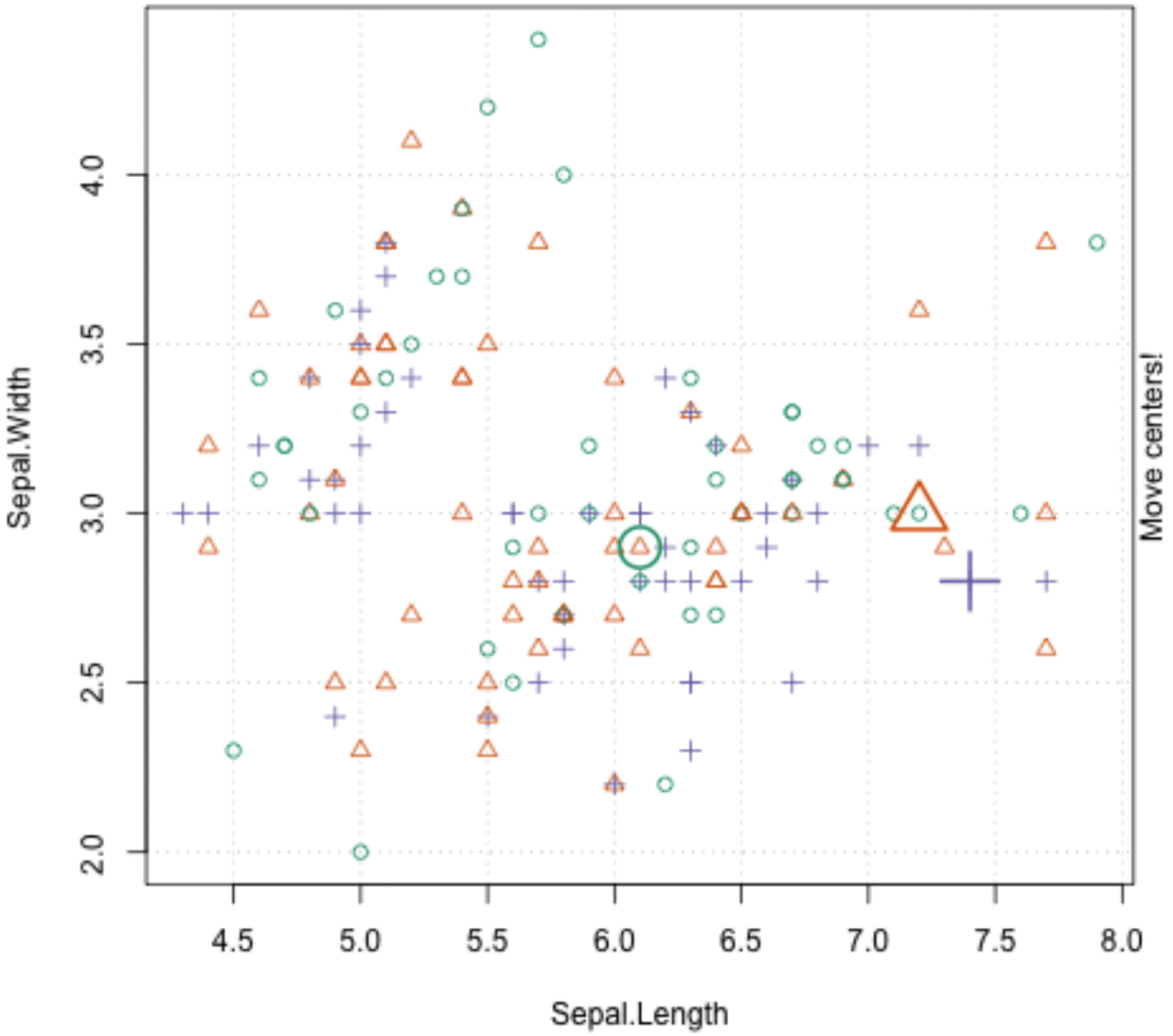
$$m_k = \frac{\sum_{i=1}^{N} 1(y_i = k) x_i}{\sum_{i=1}^{N} 1(y_i = k)}$$

- Assign objects to closest centroid:

$$y_i = \text{argmin}_k \, d(x_i, m_k)$$

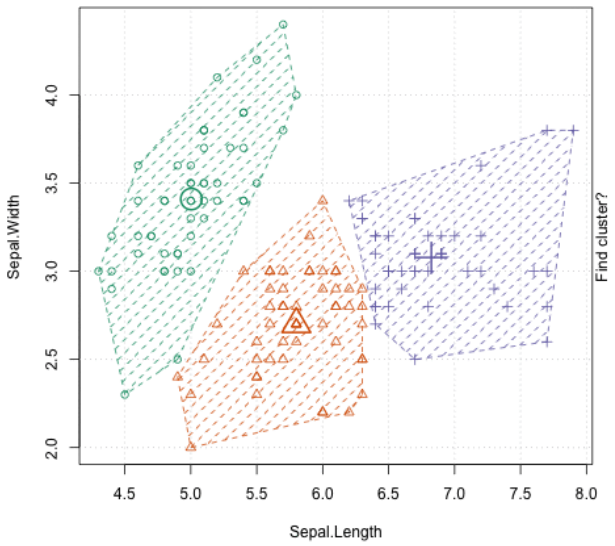
* convergence is only guaranteed for Euclidean distance

K-means on Iris

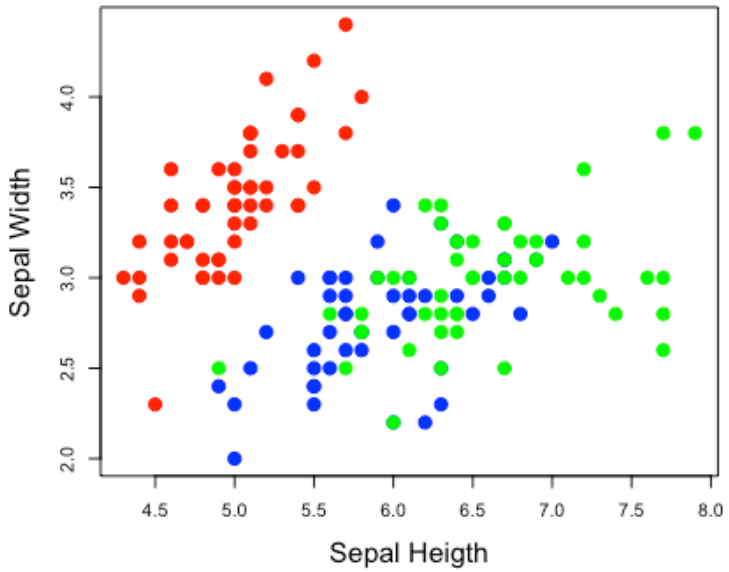


K-means on Iris

K-means solutions

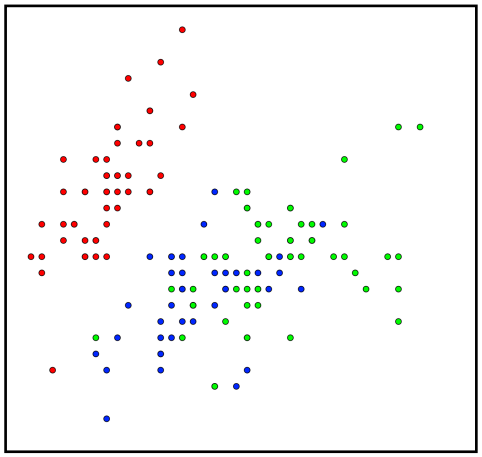


True labels



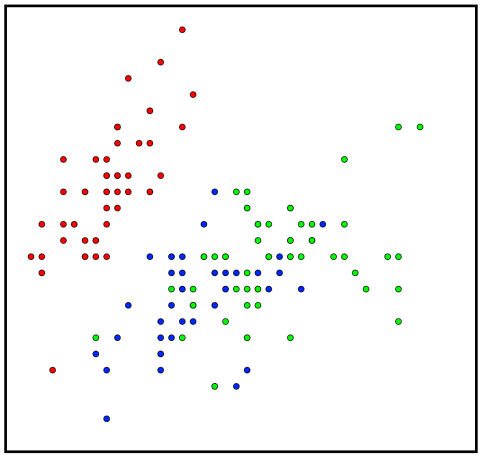
- K-means tends to find spherical clusters
- Sensitive to initialisation

Graph based clustering



- data points are nodes

Graph based clustering



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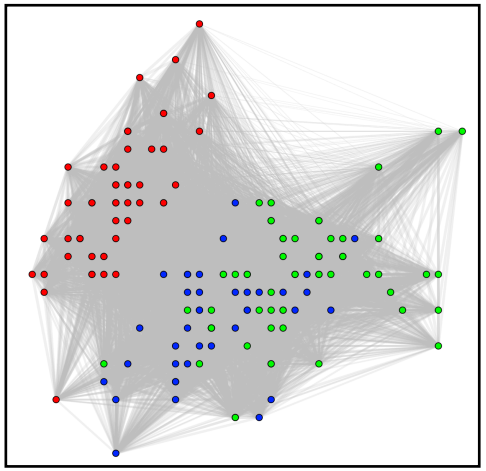
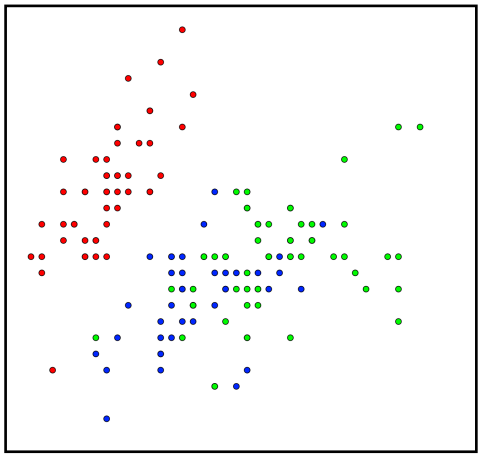


- edges represent similarities

Graph based clustering



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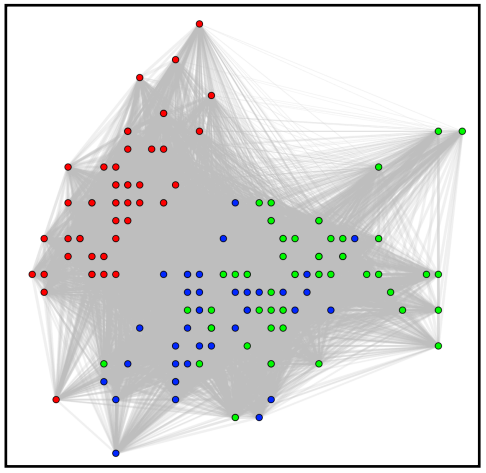

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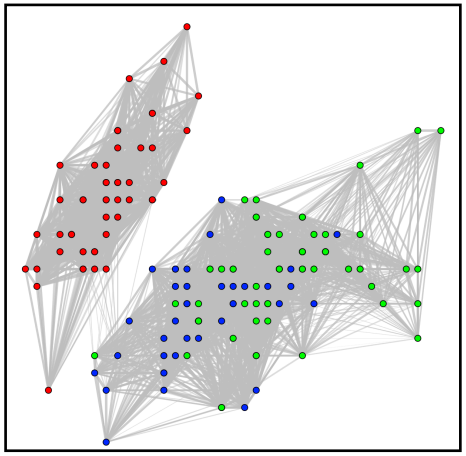
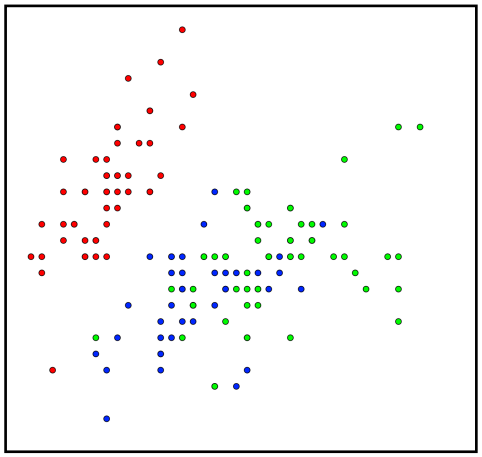


- k-nearest neighbours (KNN) -> sparse graphs

Graph based clustering



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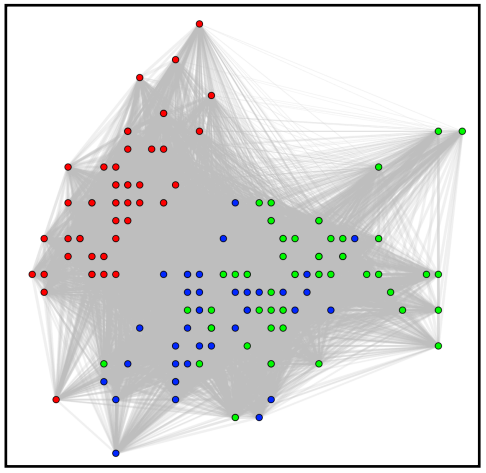

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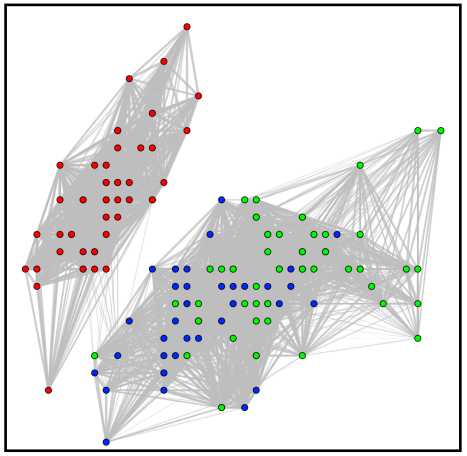
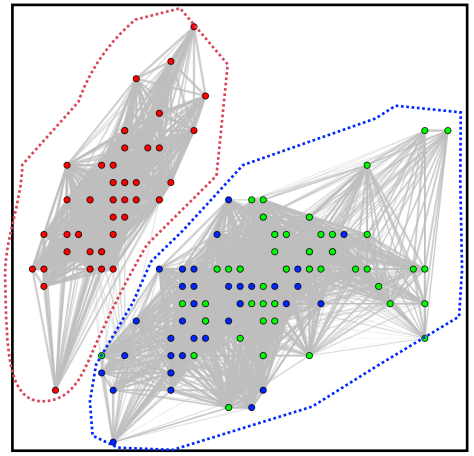
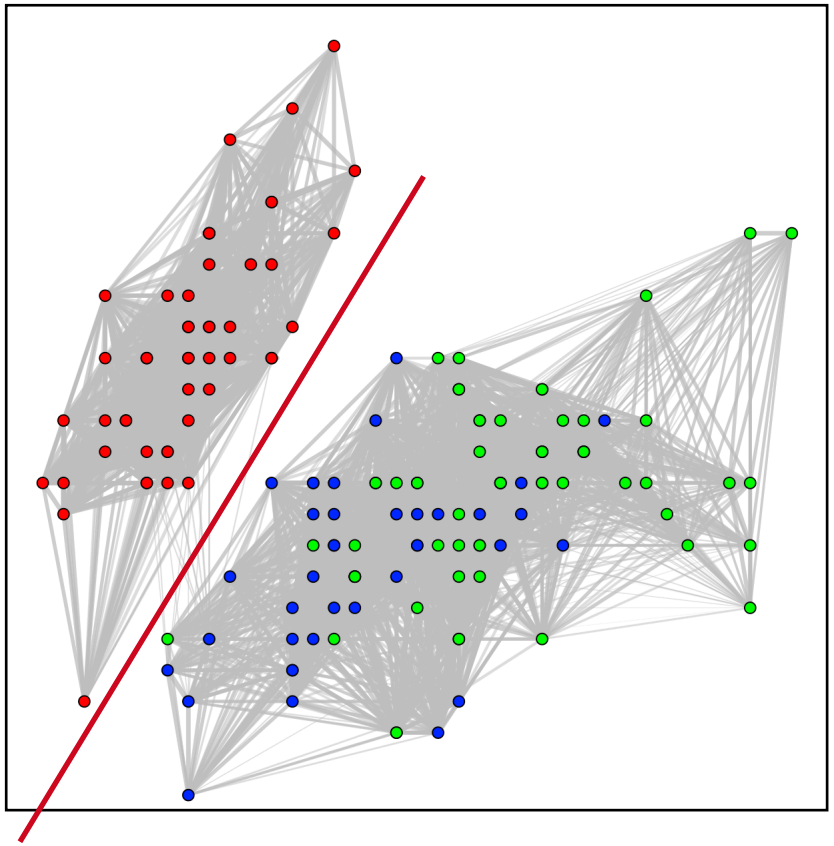


- k-nearest neighbours (KNN) -> sparse graphs



- find well connected sub-graphs

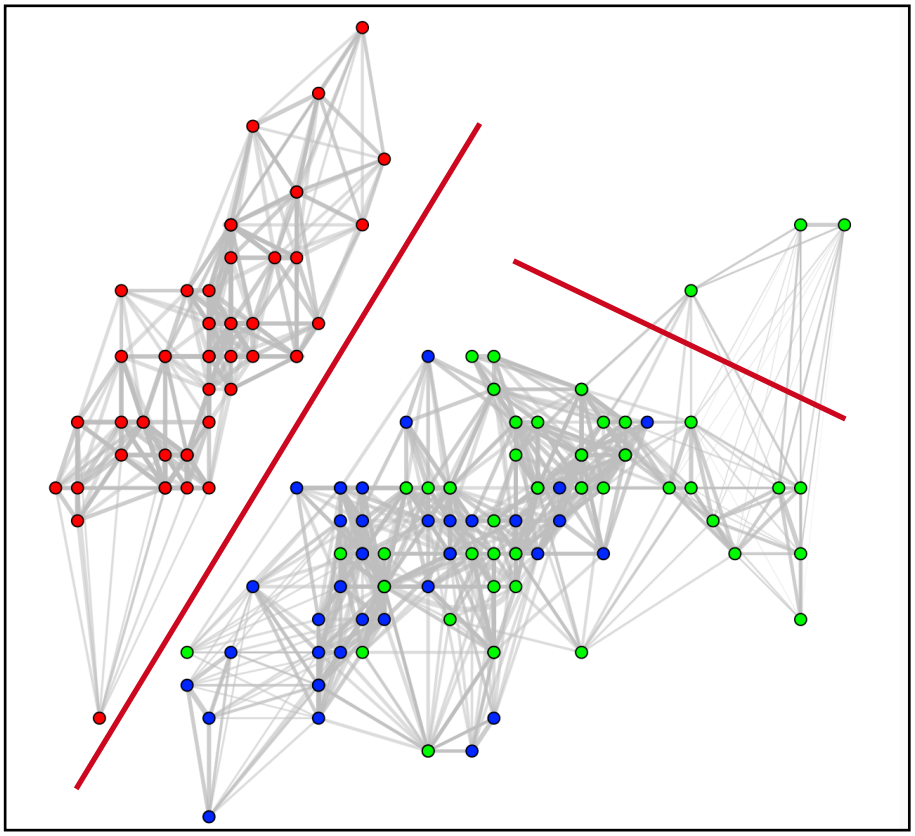
Graph cut



KNN = 50

- Cluster by finding cuts in the graph
- Cut cost **C(*A*,*B*)** = sum of edge weights in cut

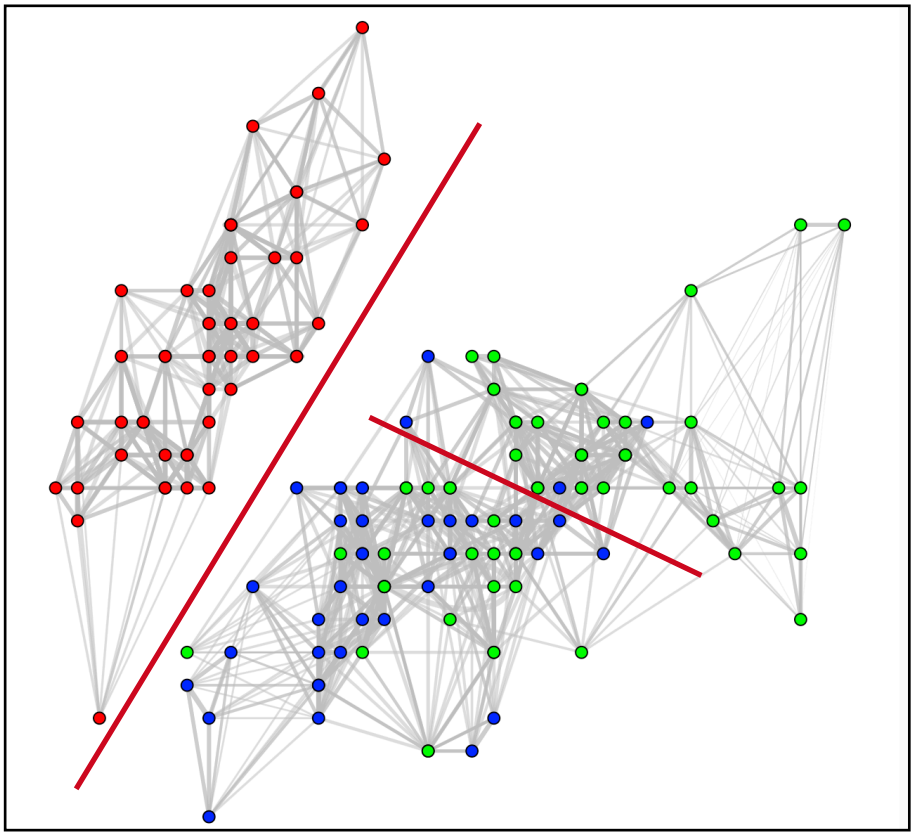
Graph cut



KNN = 10

- Cluster by finding cuts in the graph
- Cut cost **C(*A*,*B*)** = sum of edge weights in cut
  - smallest cuts might not be the best

Normalized graph cut



KNN = 10

- Normalized graph cut avoids small graphs

$$normCUT(A,B) = \frac{CUT(A,B)}{VOL(A)} + \frac{CUT(A,B)}{VOL(B)}$$

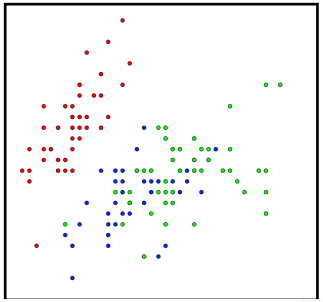
where $VOL(A)$ is the weight sums of cluster A.

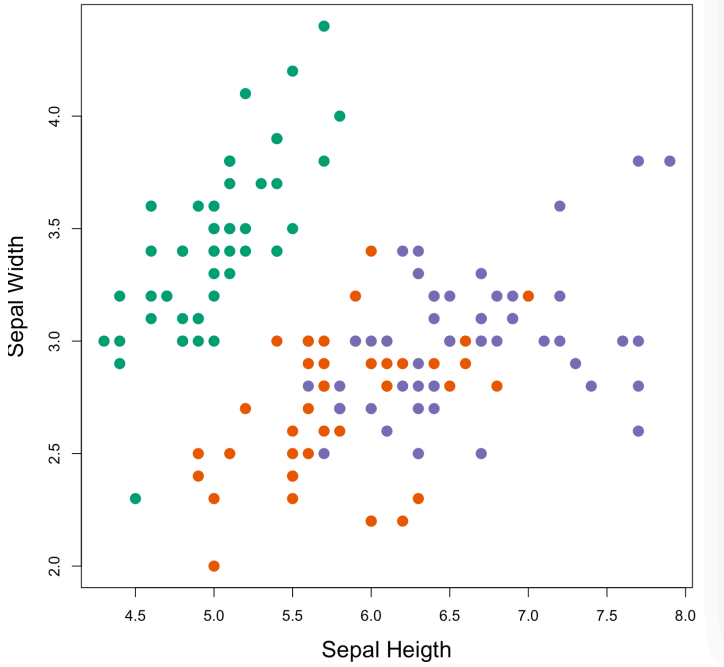
Spectral Clustering

From a graph weight matrix *W* derived from a (i.e. KNN or kernel function)

1. Estimate the laplacian *L=D-W*
   where *D* is a diag. matrix $d_{ii} = \sum w_{ij}$
2. Estimate eigenvalues of *L*
3. Perform *k*-means on lowest K eigenvalues

- spectral clustering is equivalent to find normCUT in graphs
- eigenvalues equal to 0 represent connected graphs
- graph sparsity (KNN) makes spectral clustering efficient for large *n*





Von Luxburg, arXiv, 0711.0189, 2007.

Single cell Clustering Methods / Leiden algorithm

Optimize cluster modularity

$$\mathcal{H} = \sum_c [e_c - \gamma(\binom{n_c}{2})],$$

where n_c is the size of cluster and

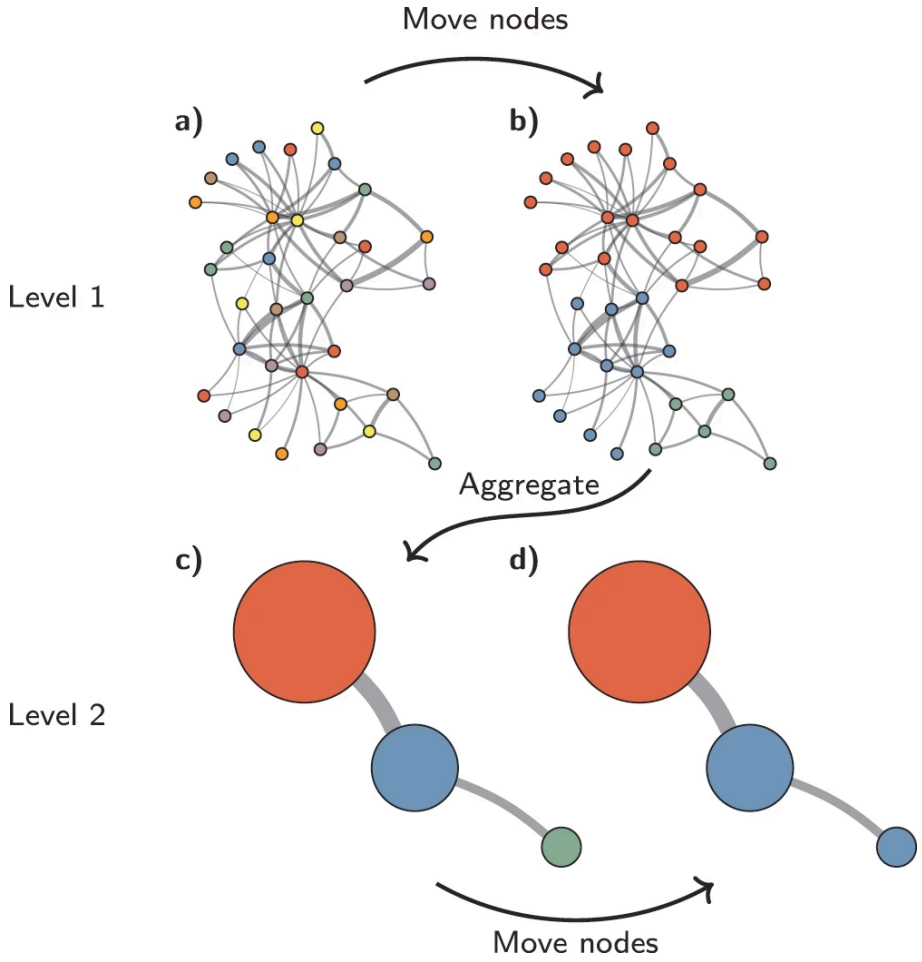
e_c is the number of expected edges

A) Start with singleton partition

B) Move nodes improving H

C) Create a meta-graph level

D) Move nodes improving H



Van Traag, Scientific Reports, 2019.
Blondel, Journal of Statistical Mechanics, 2008

Resume / Clustering Methods

- K-means, hierarchical clustering, spectral clustering

  - standard algorithms with standard performance on simple clustering problems

- Clustering of single cell algorithms
  - Leiden and louvain clustering
  - Robust and scale well to large data sets
- Further issues:
  - Data dimensionality:
    - distances do not work well on high dimension
    - visualisation is easier in low level space
  - Validation:
    - How many clusters is present in the data?
    - Which is the best method?

More details on clustering

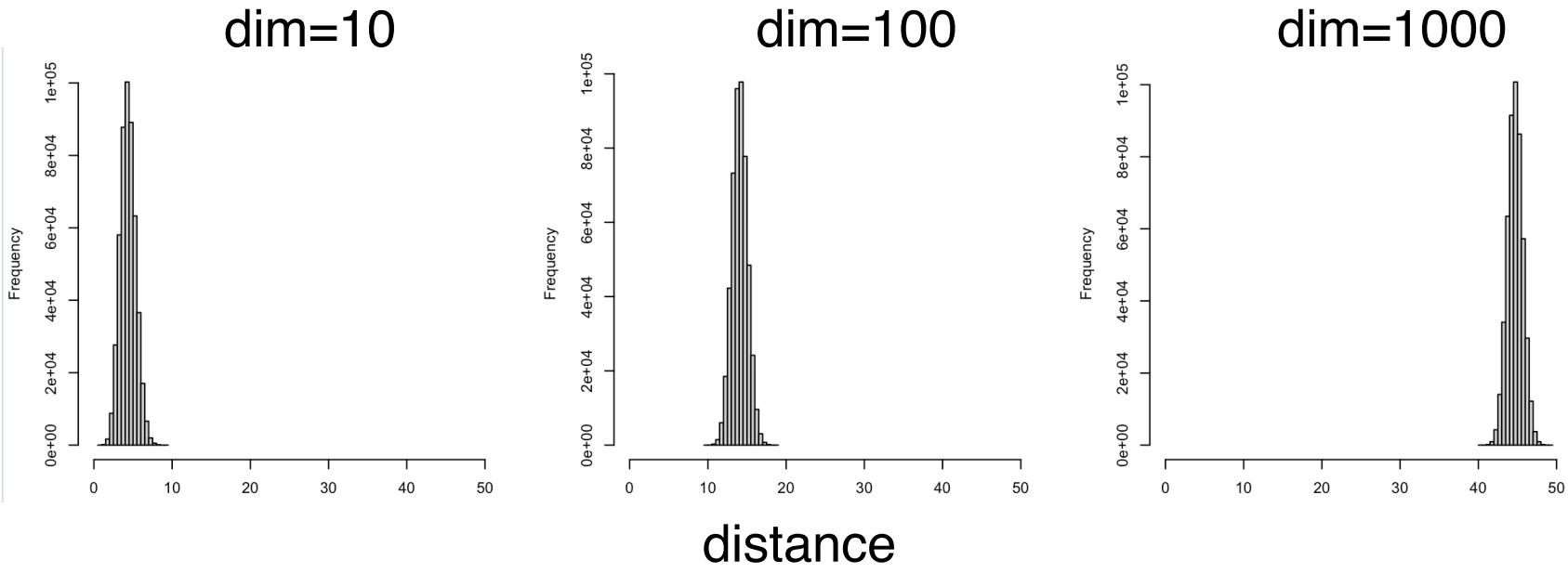
- Hastie, Tibshirani and Friedman, The Elements of Statistical Learning, Chapter 14
- Video lecture: https://www.youtube.com/watch?v=Qa6k7RIwltg

Dimension Reduction

- Distances lose meaning at high dimensional space (curse of dimensionality)

$$\frac{D_{\max} - D_{\min}}{D_{\min}} \to 0.$$

- Example: distance between points sampled from a normal distribution



Dimension Reduction

- Distances lose meaning at high dimensional space (curse of dimensionality)

- Unspecific Filtering (without class labels):
  - Keep variables with highest variance (high variable genes)
    - ***Rationale**:* important features change values across groups

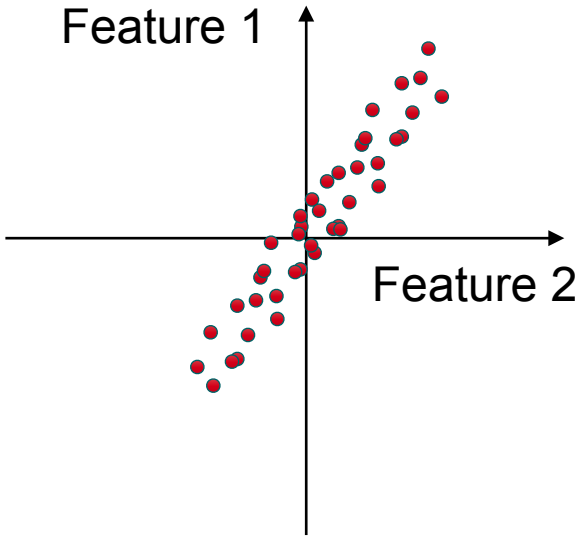
- Dimensionality Reduction by Transformation:
  - linear: principal component analysis (PCA)
  - Non-linear / manifold learning: t-SNE & UMAP (for visualisation)

Principal Component Analysis

- For a data *X*, find linear combination of features (*w)* capturing most of data variance

$$\mathbf{w}_{(1)} = \arg\max_{\|\mathbf{w}\|=1} \{\|\mathbf{X}\mathbf{w}\|^2\}$$

- Can be solved by linear algebra / eigenvector decomposition



Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

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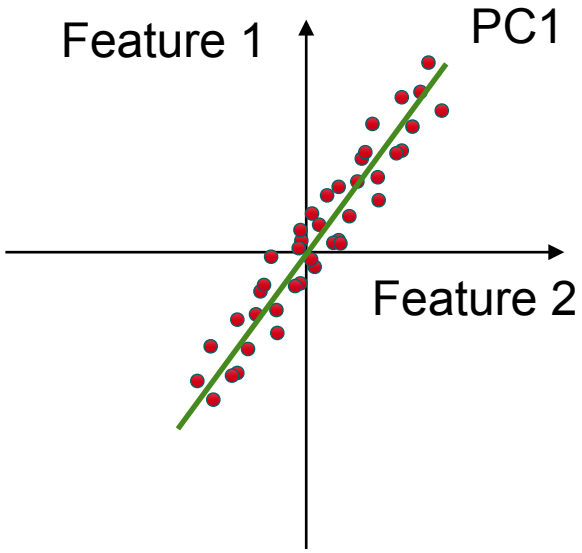

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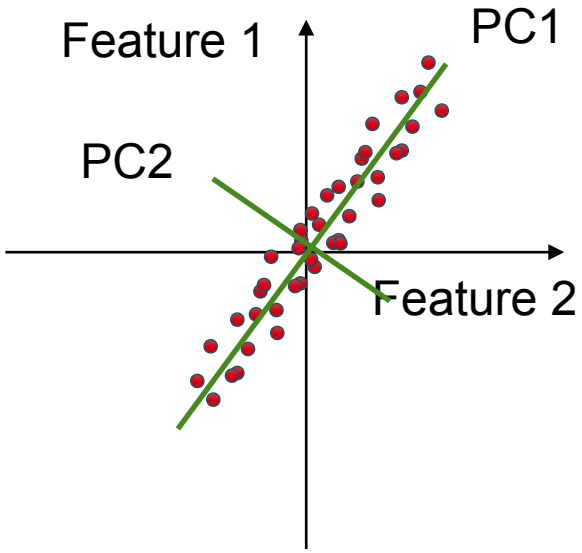

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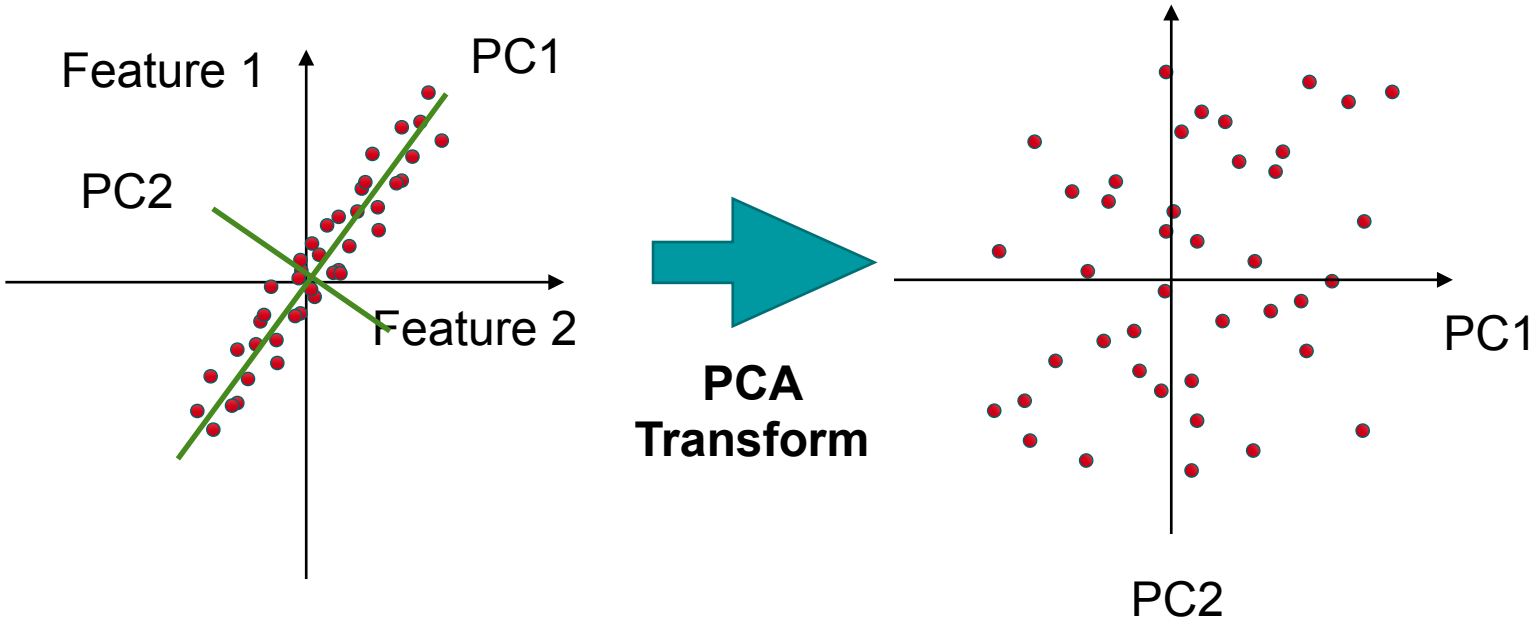

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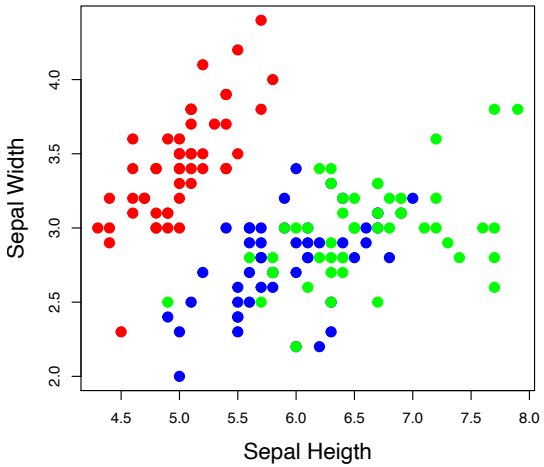
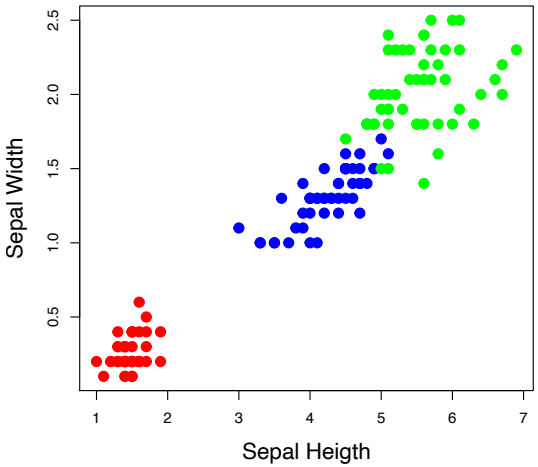
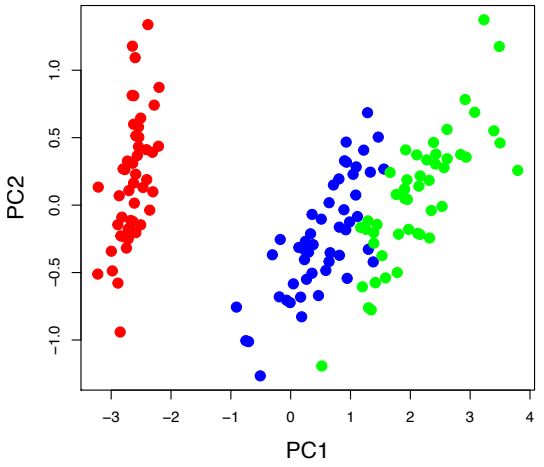
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

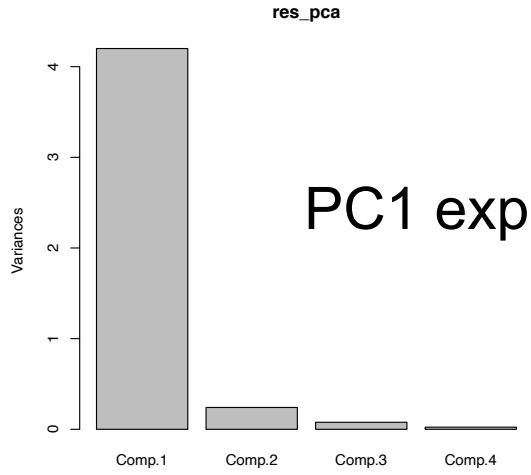
PCA - Iris

- Original iris data had 4 variables







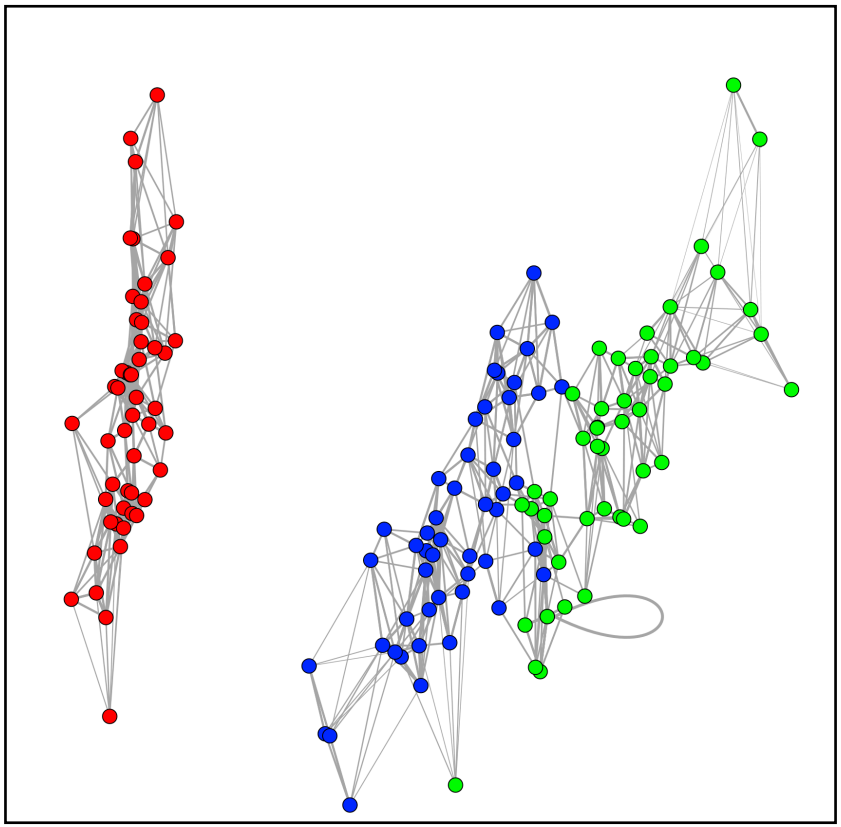


PC1 explains most of variance

Clustering on PCA space

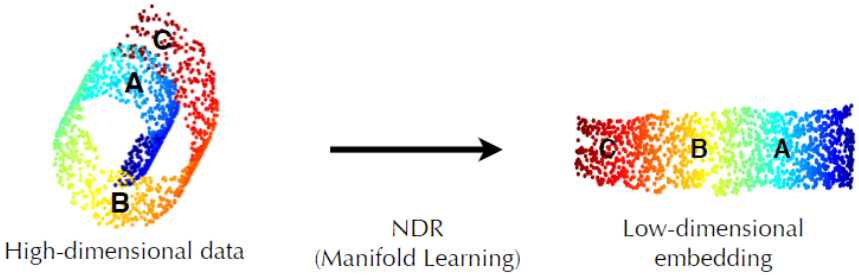
- For single cell data it is usually cluster in PCA space
  - This is crucial for high-dimensional data !

KNN graph of IRIS in PCA space

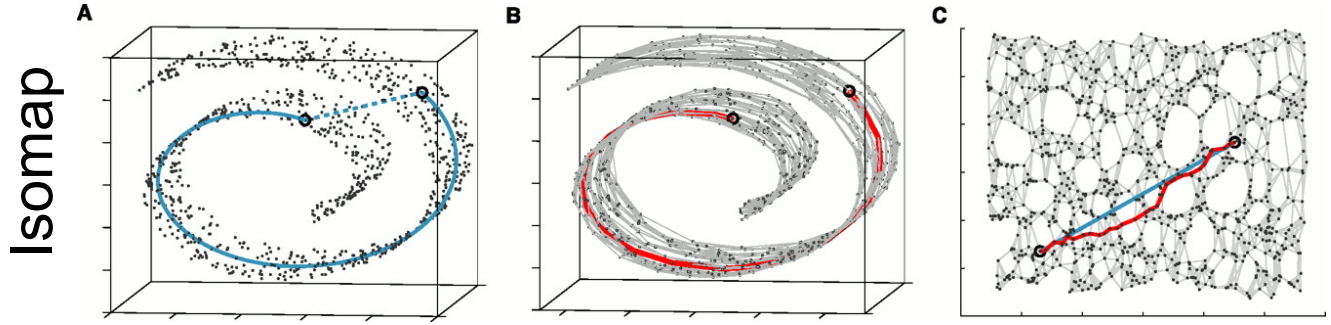


Non-linear / Manifold methods

- Data might be distributed at particular regions of a high dimensional space



- Manifold methods use topological distance (nearest neighbour graphs)



- t-SNE and UMAP are newer/widely used methods

Adapted from Tenembaum, et al. 2000

t-distributed stochastic neighbor

- For a given kernel ***D*** learn a ***I*** dimensional map ***Y***

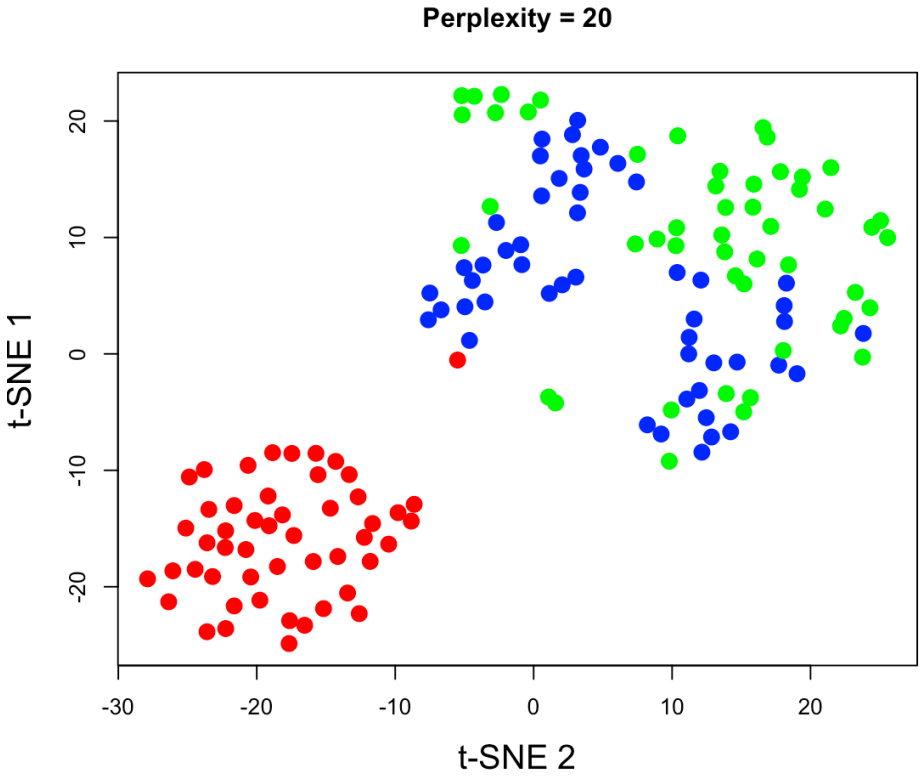
$$KL(D \mid Q) = \sum d_{ij} log(\frac{d_{ij}}{q_{ij}}) \qquad \text{where} \qquad q_{ij} = \frac{|y_i - y_j|^2}{\sum_k \sum_l |y_k - y_l|^2}$$

See for more details: https://www.youtube.com/watch?v=9iol3Lk6kyU&t=350s

t-distributed stochastic neighbor

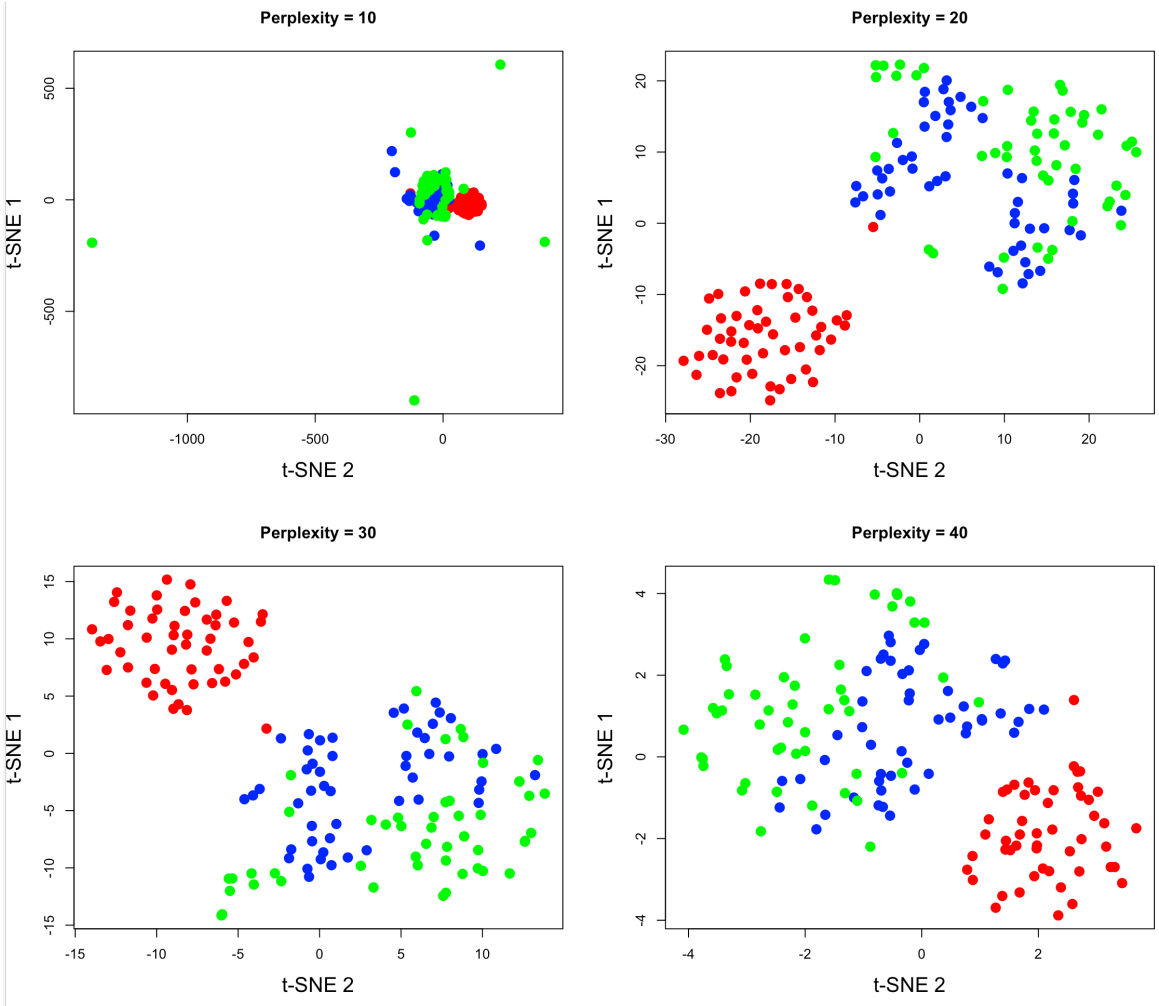
- For a given kernel ***D*** learn a ***I*** dimensional map ***Y***

$$KL(D \mid Q) = \sum d_{ij} log(\frac{d_{ij}}{q_{ij}}) \qquad \text{where} \qquad q_{ij} = \frac{|y_i - y_j|^2}{\sum_k \sum_l |y_k - y_l|^2}$$



See for more details: https://www.youtube.com/watch?v=9iol3Lk6kyU&t=350s

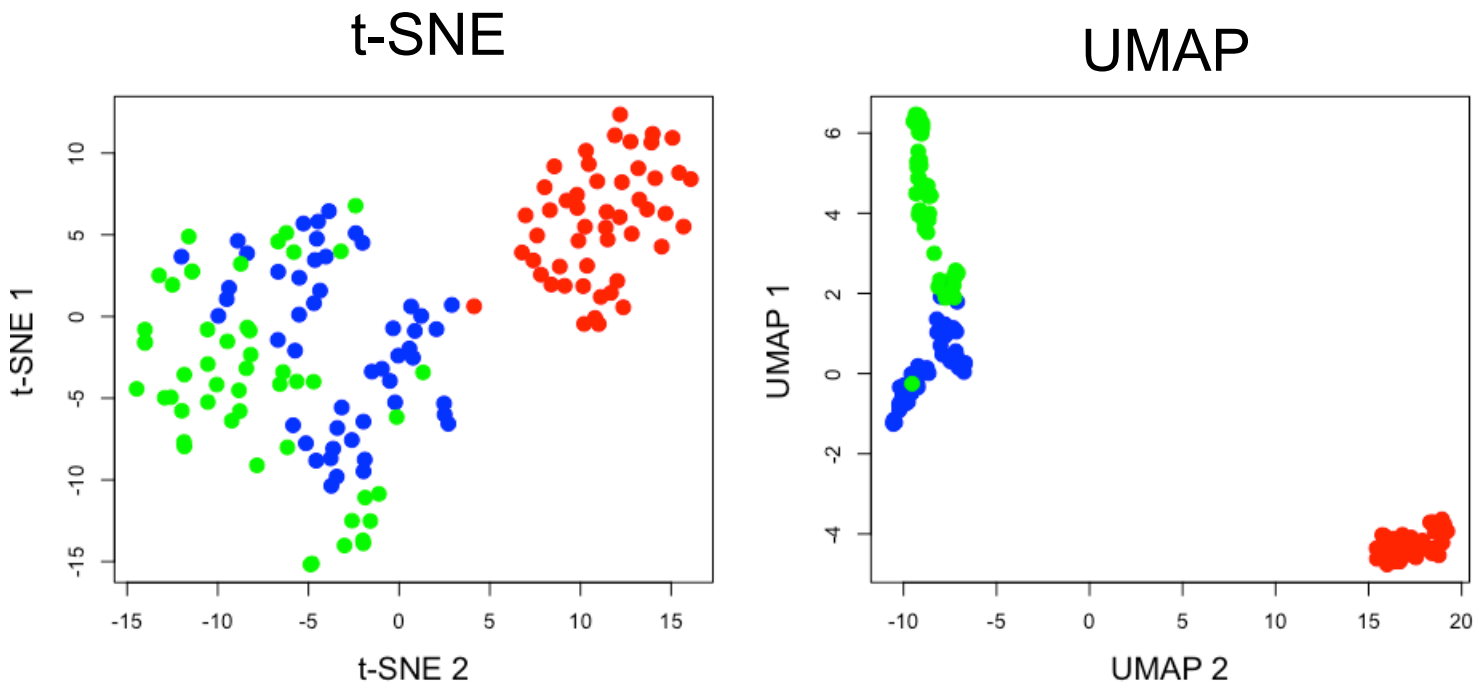
t-distributed stochastic neighbor



- Sensitive to distinct starts and parametrisation
  - Perplexity ~ neighbourhood size

See for more details: https://www.youtube.com/watch?v=9iol3Lk6kyU&t=350s

Manifold learning and IRIS



- Nice low dimensional visualisation of the data
- Caution: These methods fail capturing global structures (distance between clusters!)

See for more details: https://www.youtube.com/watch?v=9iol3Lk6kyU&t=350s

Resume / Dimension Reduction

- PCA analysis is a wide spread technique to reduce dimension!
  - Can only capture linear relationships
- Manifold methods
  - Nice low dimensional representation of data
  - Require parametrisation and lose global distance information

Complete course on manifolds/dimension reduction:

https://www.youtube.com/watch?v=evGm6IJKrDI&t=4421s

Cluster Validation

- How to evaluate clustering results? Which is the best method? How many clusters?
- Internal/relative validation:
  - Measure of cluster coherence:
    - Distance within a cluster -> small (compactness)
    - Distance between clusters -> high (separation)
  - Stability measures:
    - Cluster data in part of the data and compare results
- External validation:
  - Compare clusters with class labels (iris data)
    - Not possible in real word problems!

Silhouette - Internal Index

The silhouette for a given object *i* is defined as:

$$s(i) = \frac{b(i) - a(i)}{max(a(i), b(i))}$$

where

a(i) –mean distance of *i* to objects on same cluster **(compactness)**

d(i,k) – mean distance of *i* to objects of cluster *k* (not own)

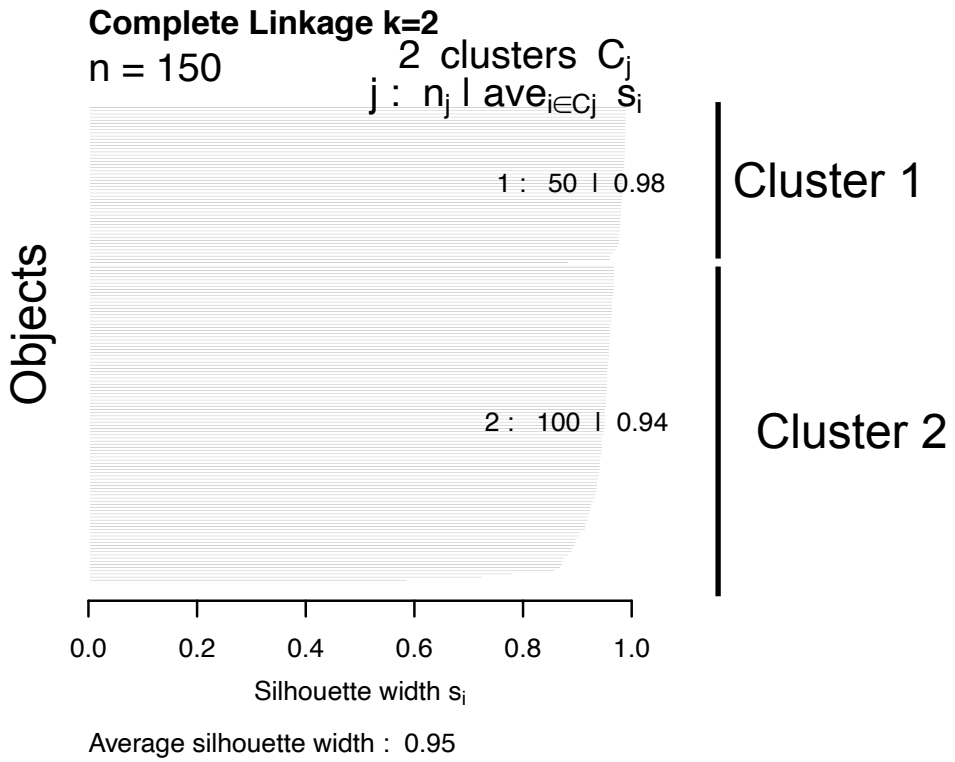
b(i) = min_k *(d(i,k))* **(separation)**

Average of *s(i)* -> quality of all results or clusters

Value of 1 indicate perfect solutions!

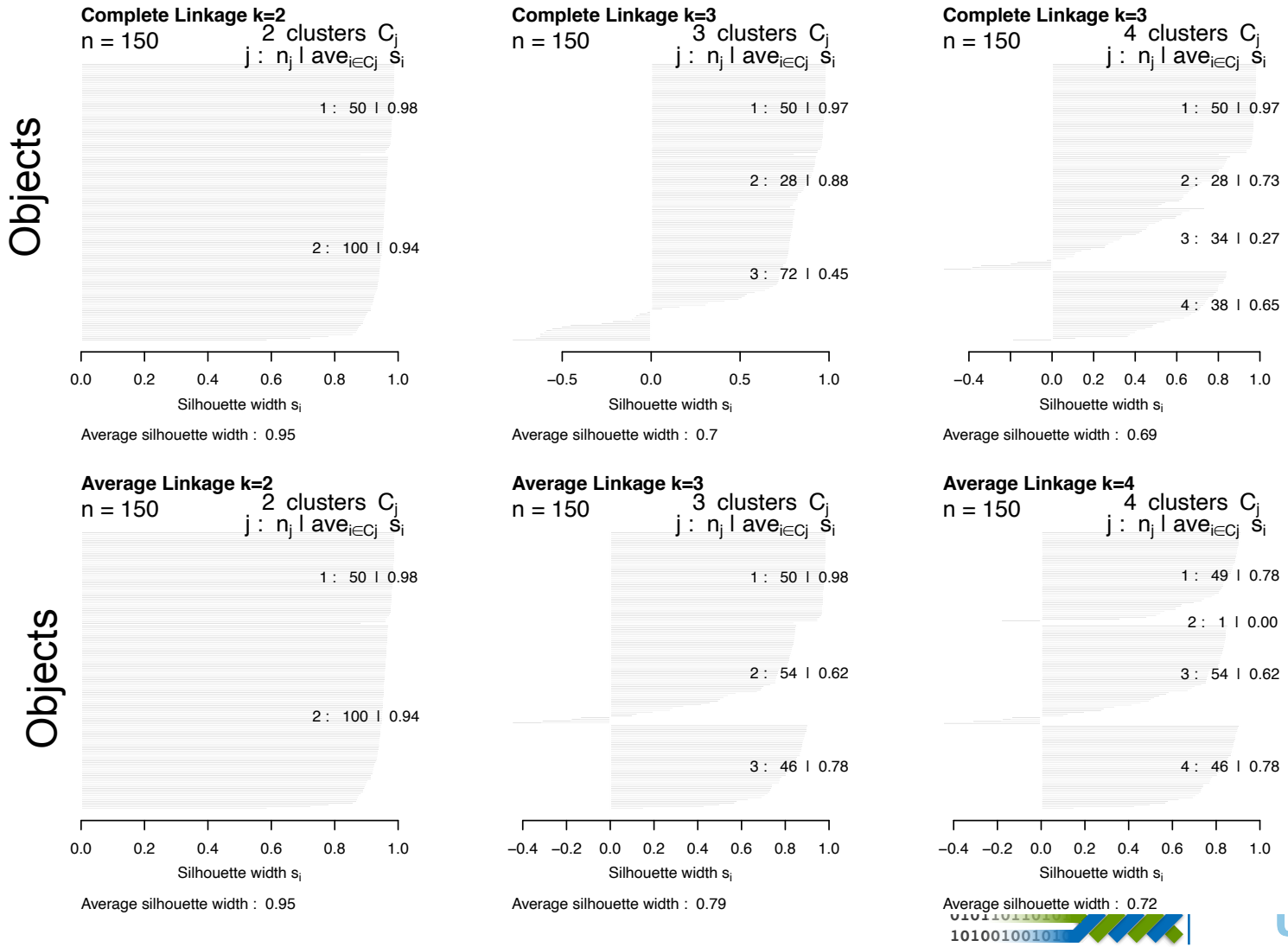
Silhouette - Internal Index / Iris

- silhouette values for hierarchical clustering with Pearson



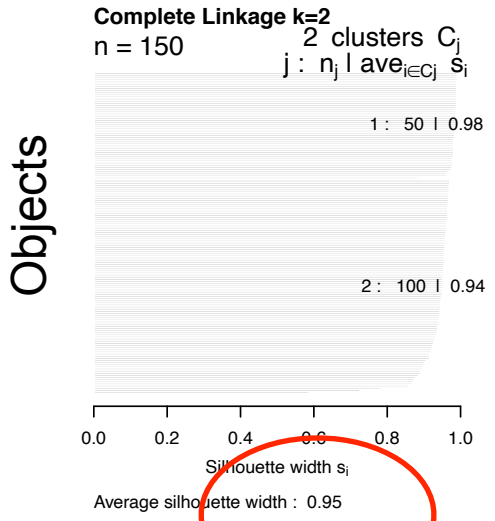
Silhouette - Internal Index / Iris

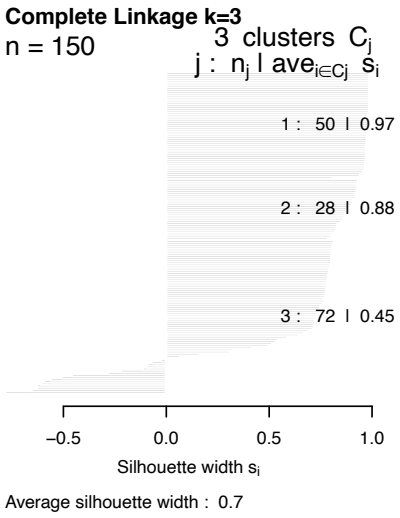
- silhouette values for hierarchical clustering with Pearson

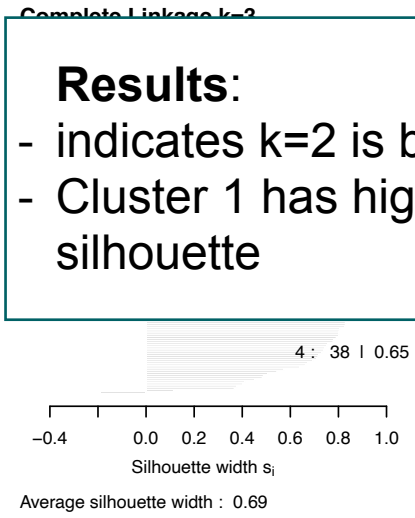


Silhouette - Internal Index / Iris

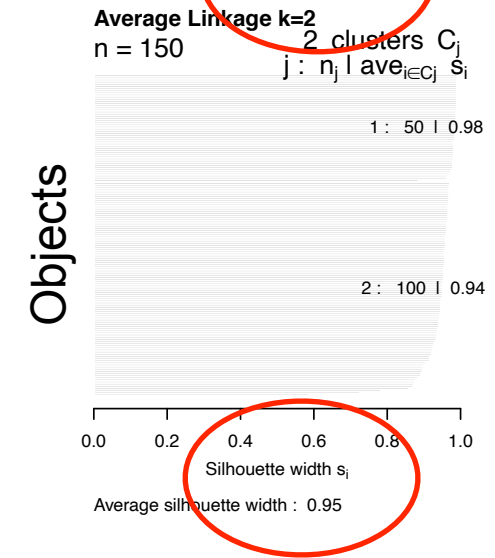
- silhouette values for hierarchical clustering with Pearson

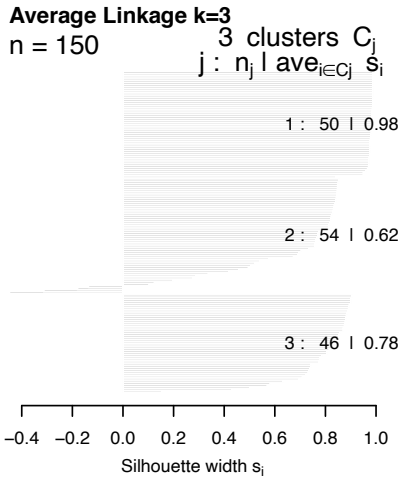


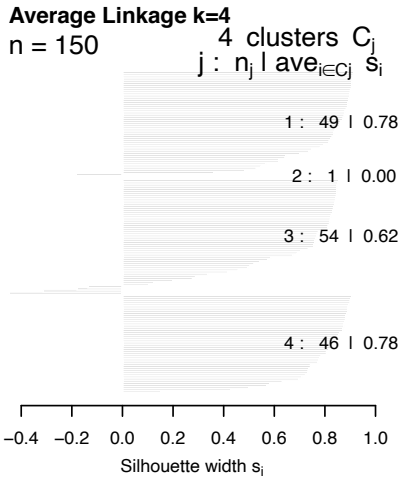




Results:
- indicates k=2 is best
- Cluster 1 has high silhouette







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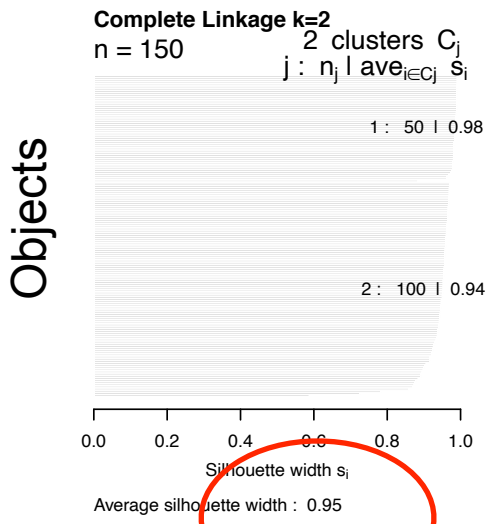
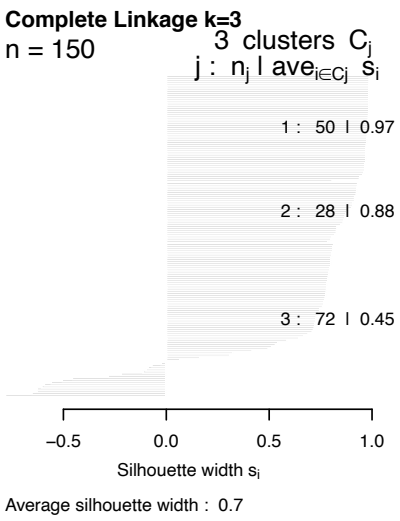
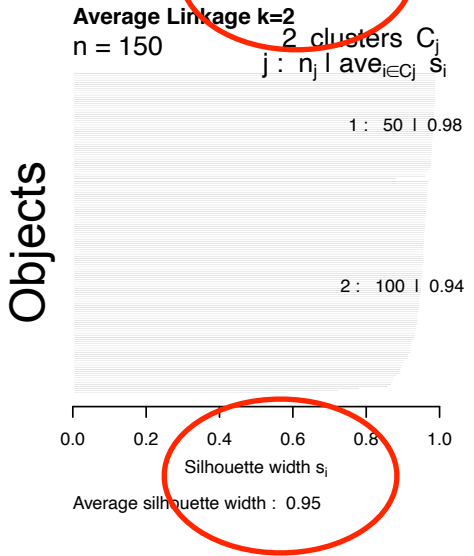
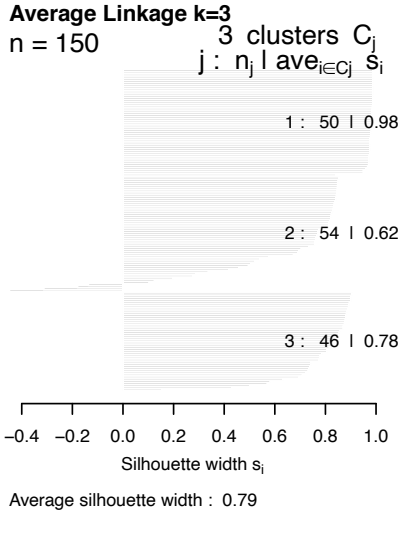
Silhouette - Internal Index / Iris

- silhouette values for hierarchical clustering with Pearson









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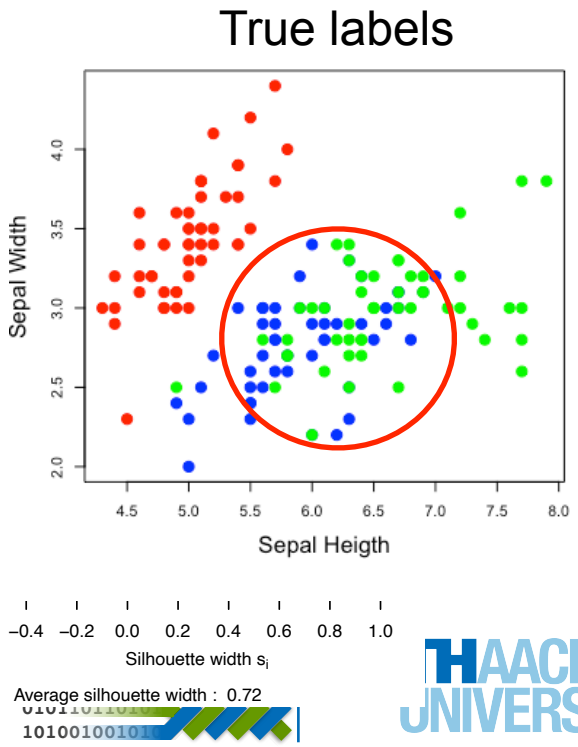


Gap statistic - Internal Index

For a given solution with *K clusters*

$$W_K = \sum_{k=1}^{K} \sum_{y_i=k} \sum_{y_j=k} ||x_i - x_j||^2$$

W_K - measures cluster compactness
W_K - tends to 0 for increasing K

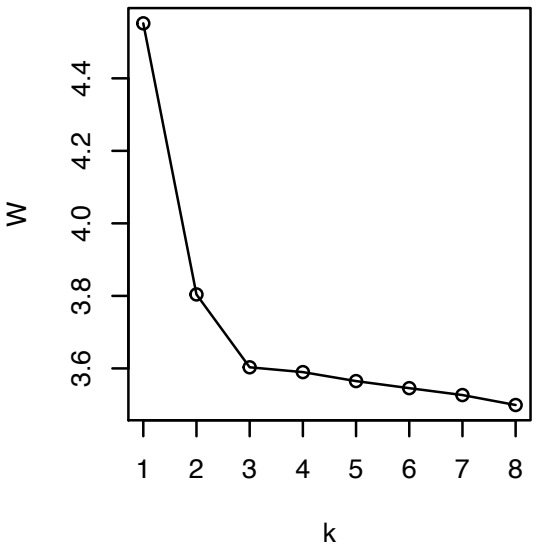
The Gap Statistic consider clustering of random data *W**

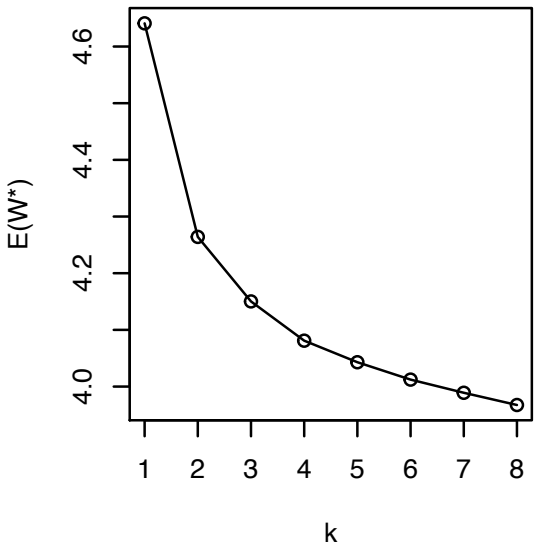
$$GAP(k) = E_r[logW_K^*] - logW_K$$

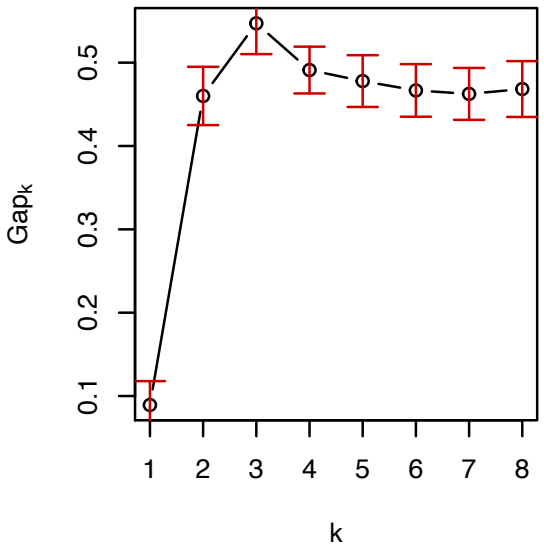
where *W** estimated from clustering random points at the same data space of *X*

Gap statistic - Iris

- GAP statistics for Iris / Average Linkage with Pearson



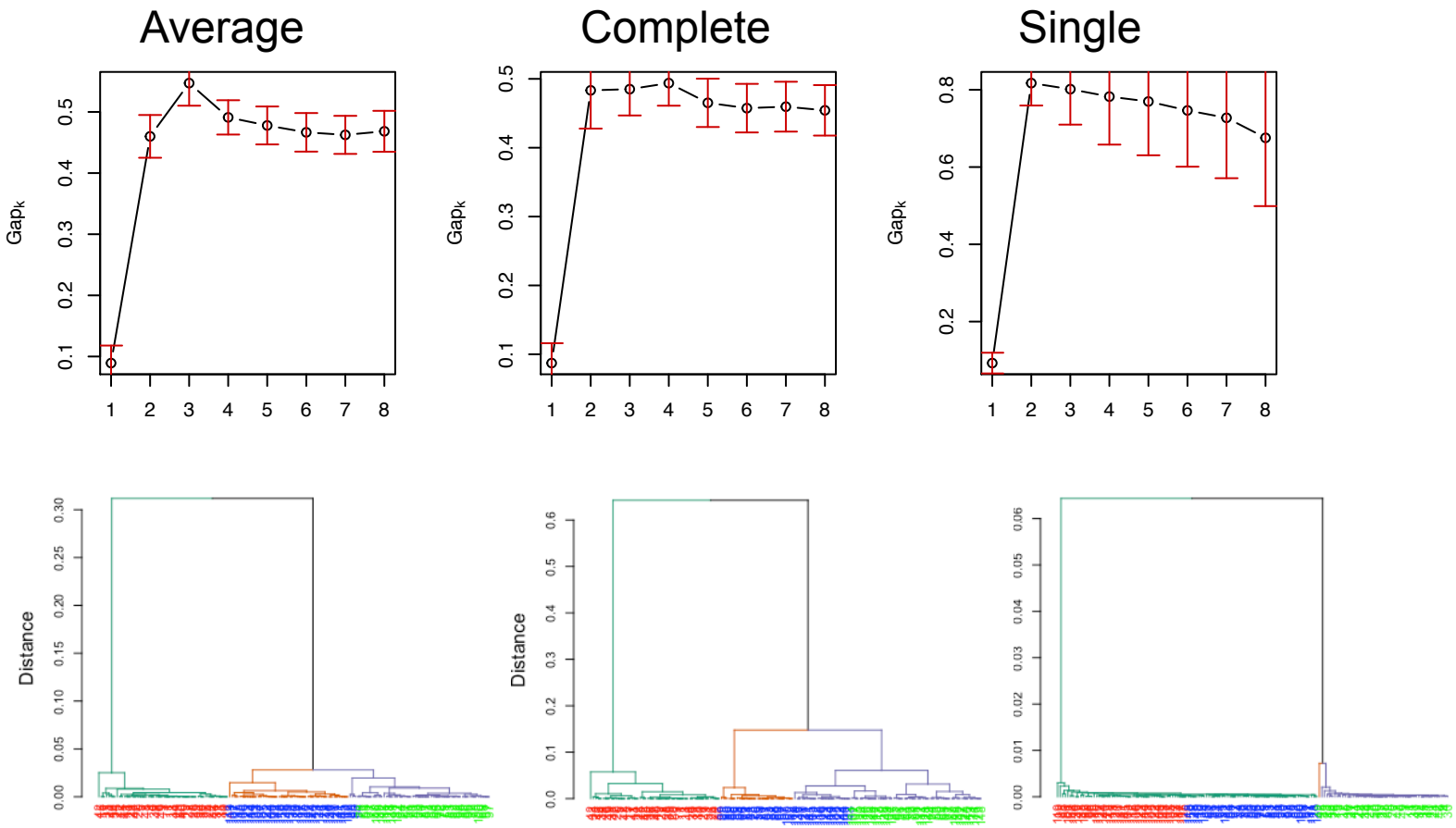




3 clusters has highest Gap !!!

Gap statistic - Iris

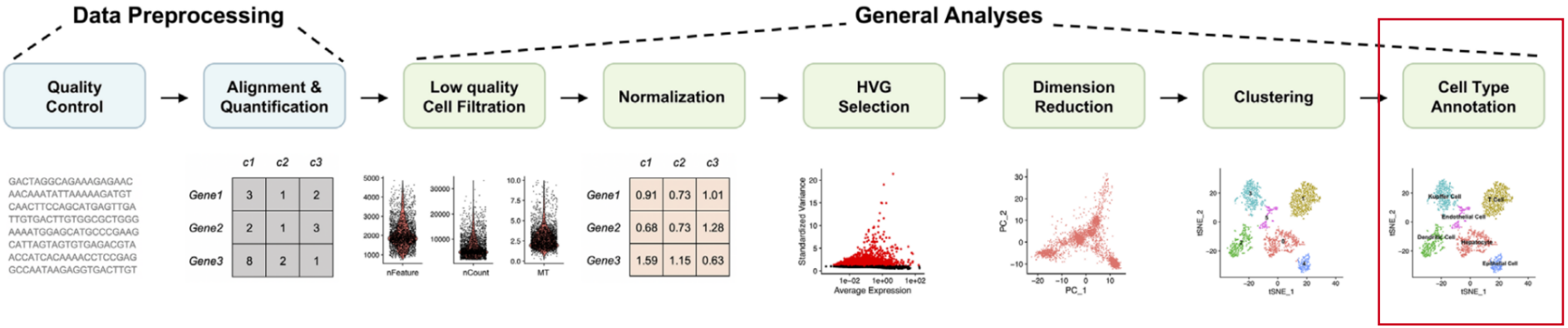
- GAP statistics for distinct linkage methods



Resume / Validation

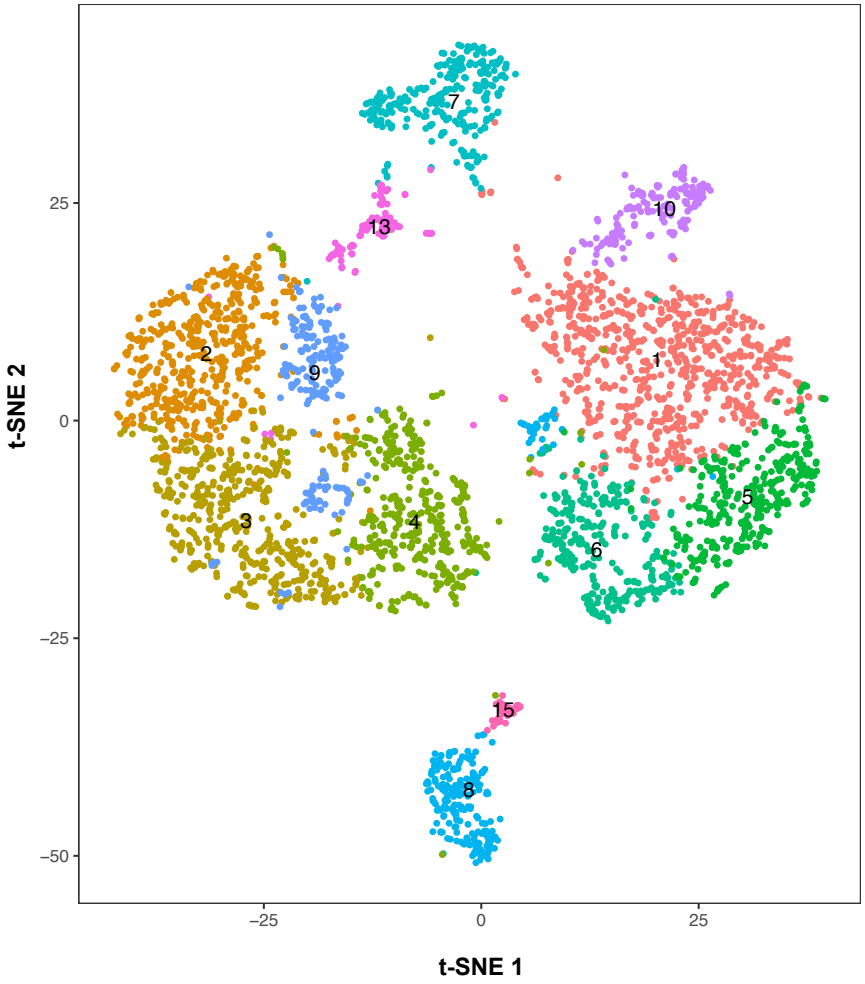
- Help detection of number of clusters / real clusters
  - Do not work perfectly!
- GAP statistics is widely used
  - Requires *r* data randomisations
    - high computational costs
    - random datasets uniformly distributed (unreal assumption)
- Expert interpretation is important!

Basics Bioinformatics - single cell RNA-seq



Basics Bioinformatics - Clustering

Gut Immune Cells - 12 groups

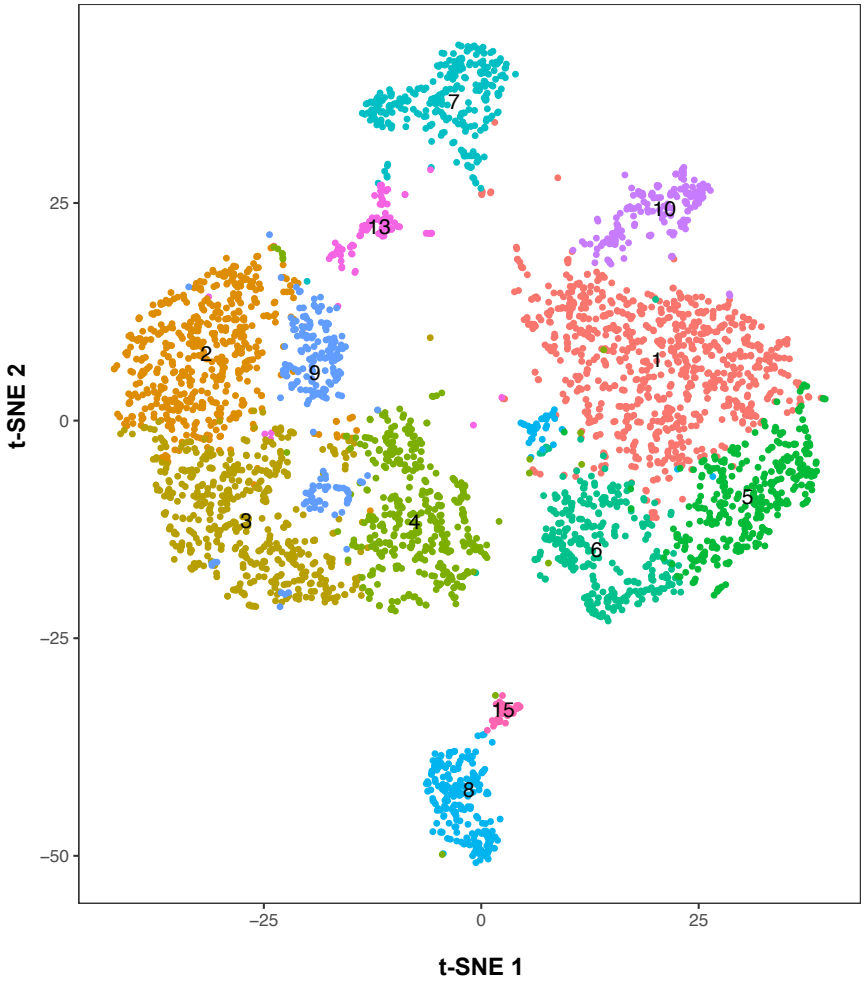


Clustering - identify cells with similar expression patterns
- based on PCA (20 dimension)

How to identify cell types?

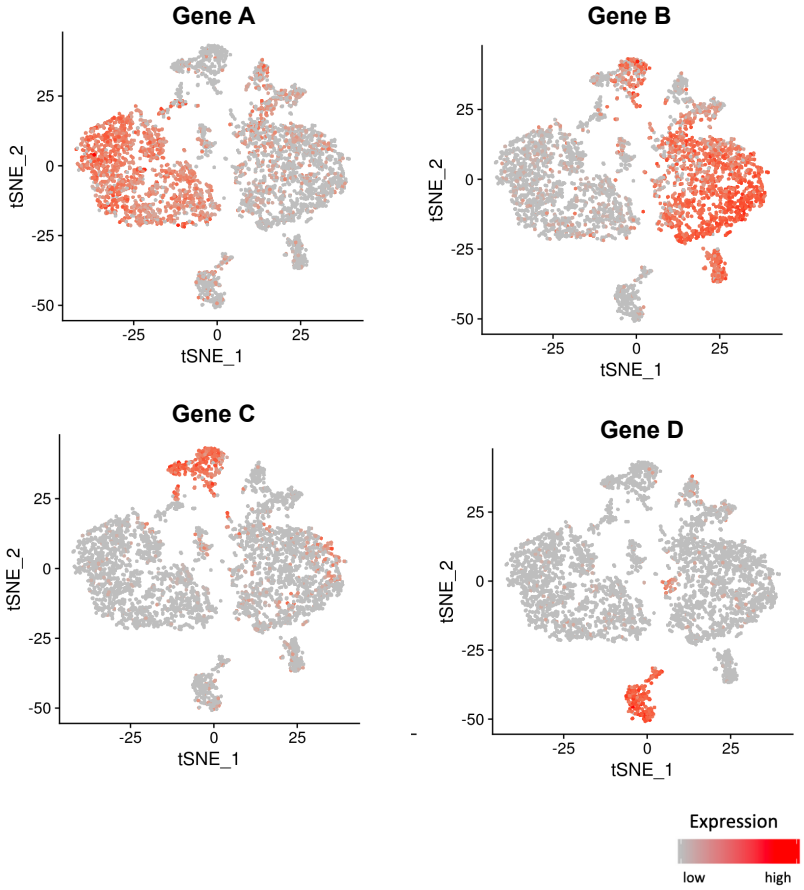
Cell Identity with an Expert

Gut Immune Cells - 12 groups



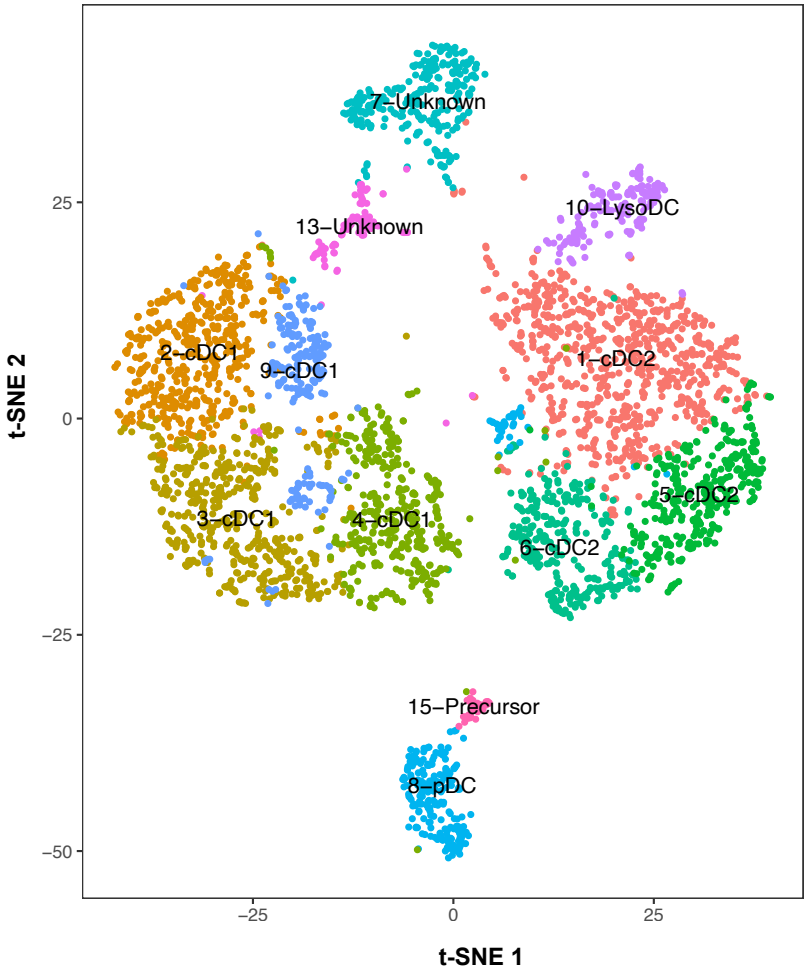
Check expression of:

1. known genes



Cell Identity with an Expert

Gut Immune Cells - 12 groups



Check expression of:

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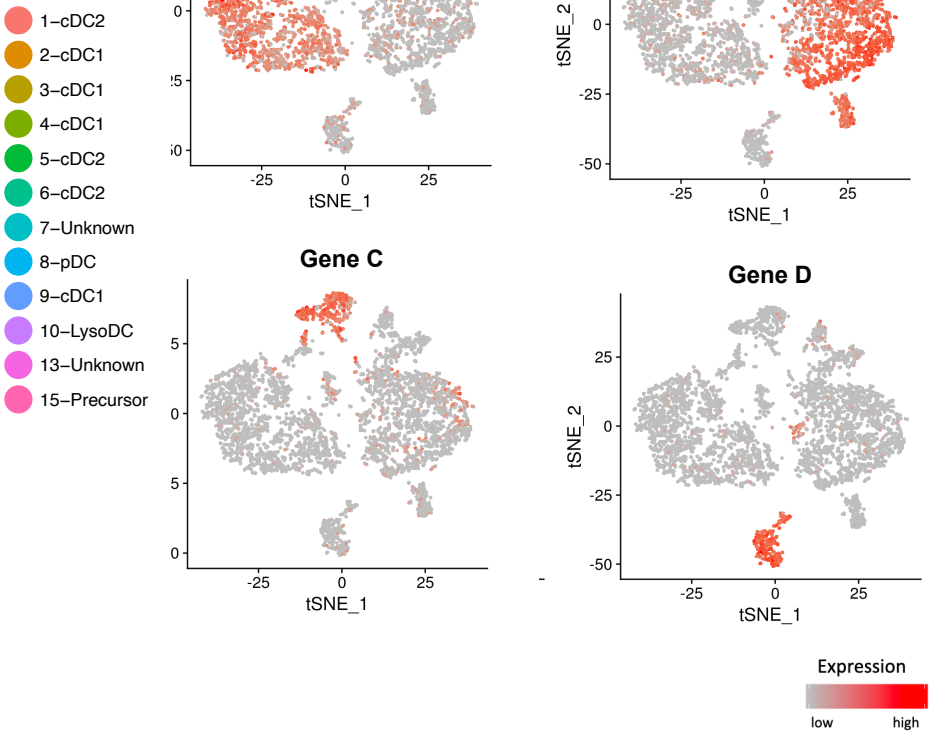
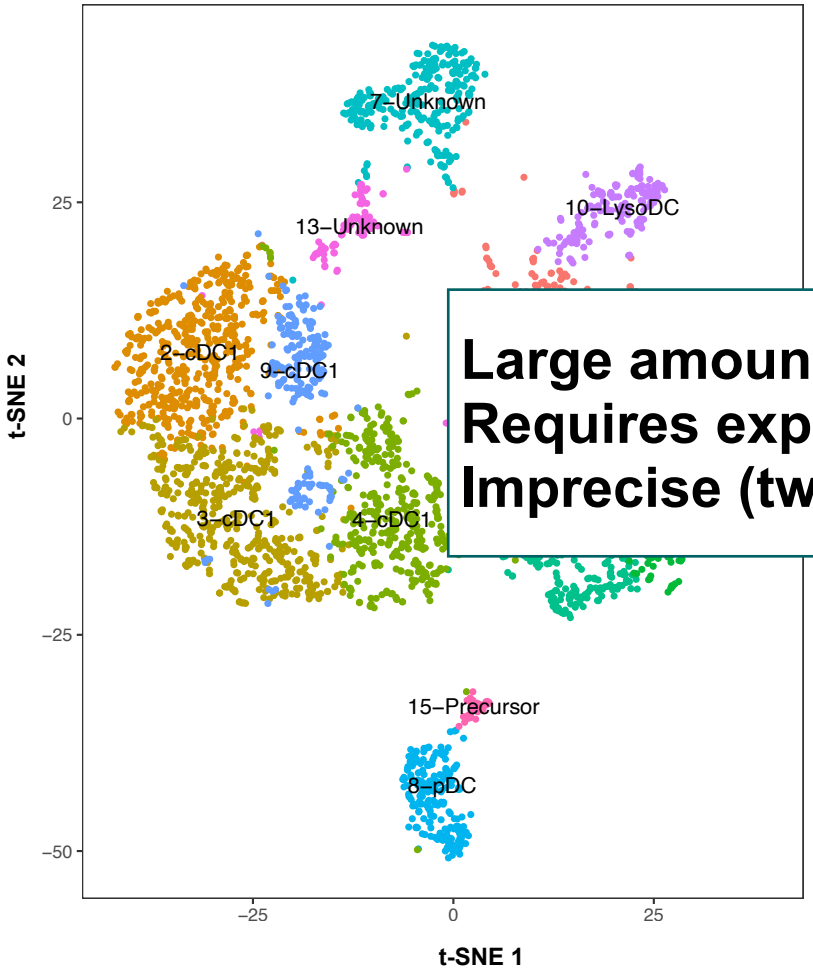


Cell Identity with an Expert

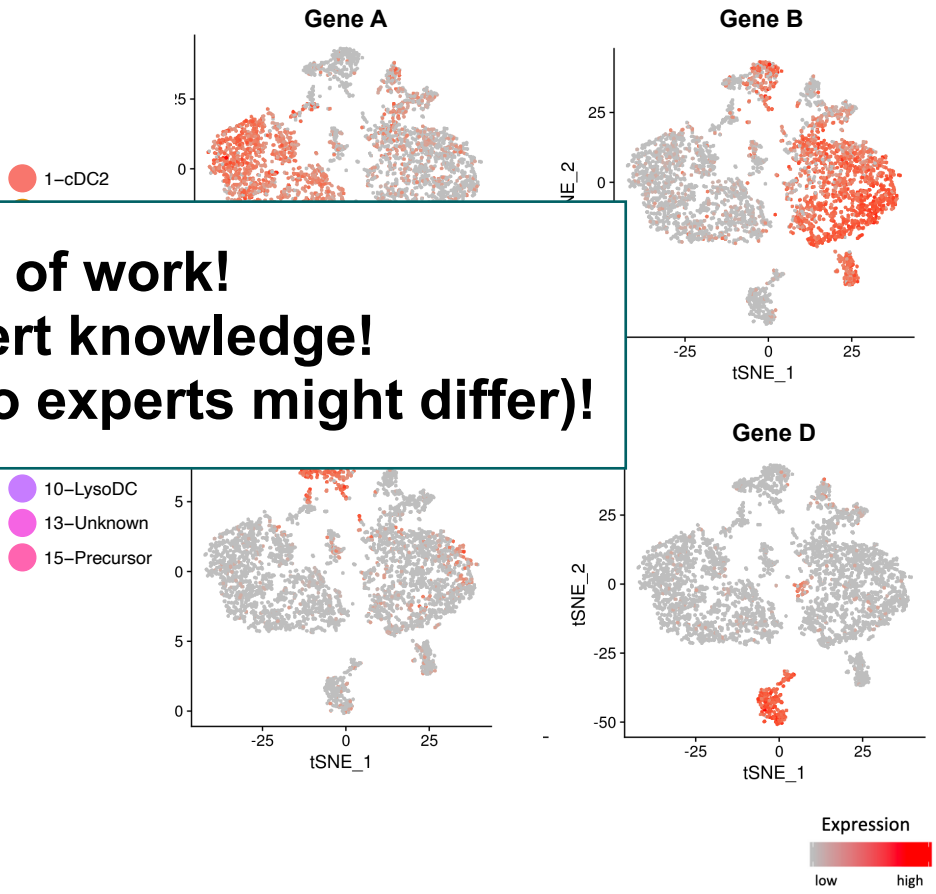
Gut Immune Cells - 12 groups



Check expression of:

1. known genes

Large amount of work!
Requires expert knowledge!
Imprecise (two experts might differ)!



Resume / Single cell clustering

- Finding groups of single cells require complex pipeline:
  - Cell filtering
  - Normalisation
  - Artefact removal
  - **Dimension reduction**
  - **Integration**
  - **Clustering**
  - **Cell annotation / visualisation**
- Open points:
  - How to deal with large data sets (millions of cells)?
  - How to detect cells of rare populations?

Calendar

Today – Introduction to Bioinformatics, Next Generation Sequencing, Single cell Analysis

2.05.2022 – Single cell Analysis Practical

8.05.2022 – Computational Epigenomics / Project Description / Using RWTH HPC/GPU cluster

15.05.2022 – 4.7.2022 – Project development

11.07.2022 – Project Presentation

Communication/discord channel: https://discord.gg/hmGxznNpZH .

Thank you!