



# Clustering

Workshop on RNA-Seq

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# Clustering

- What is clustering?
  - Clustering is an approach to classify/group data points.
- Why do we use clustering?
  - For exploring the data
  - To discover patterns in our data set
  - Identify outliers

# Clustering Methods

- Centroid-based
- Density-based
- Distribution-based
- Hierarchical-based

## Steps:

In short all clustering approach follows these steps:

- Calculate distance between data points
- Group | cluster the data based on similarities

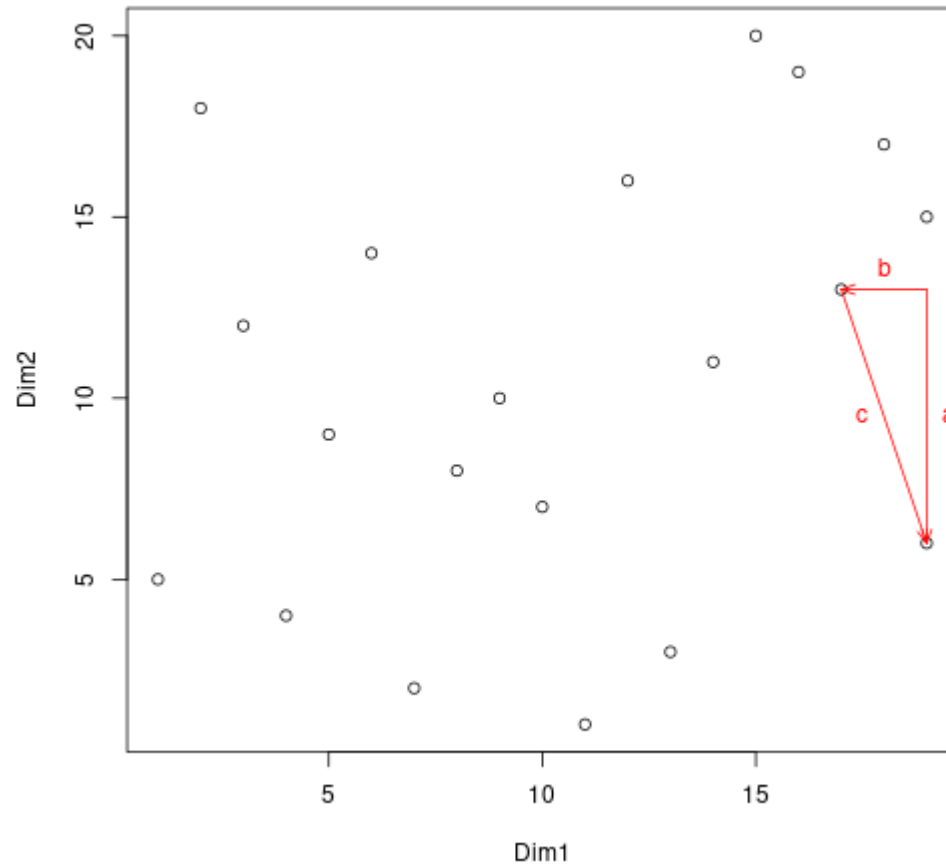
## Distance can be measured in:

- In multidimensional space (raw data)
- In reduced space (i.e. top PCs)

# Euclidean distance

- Euclidean distance is a straight line between two points

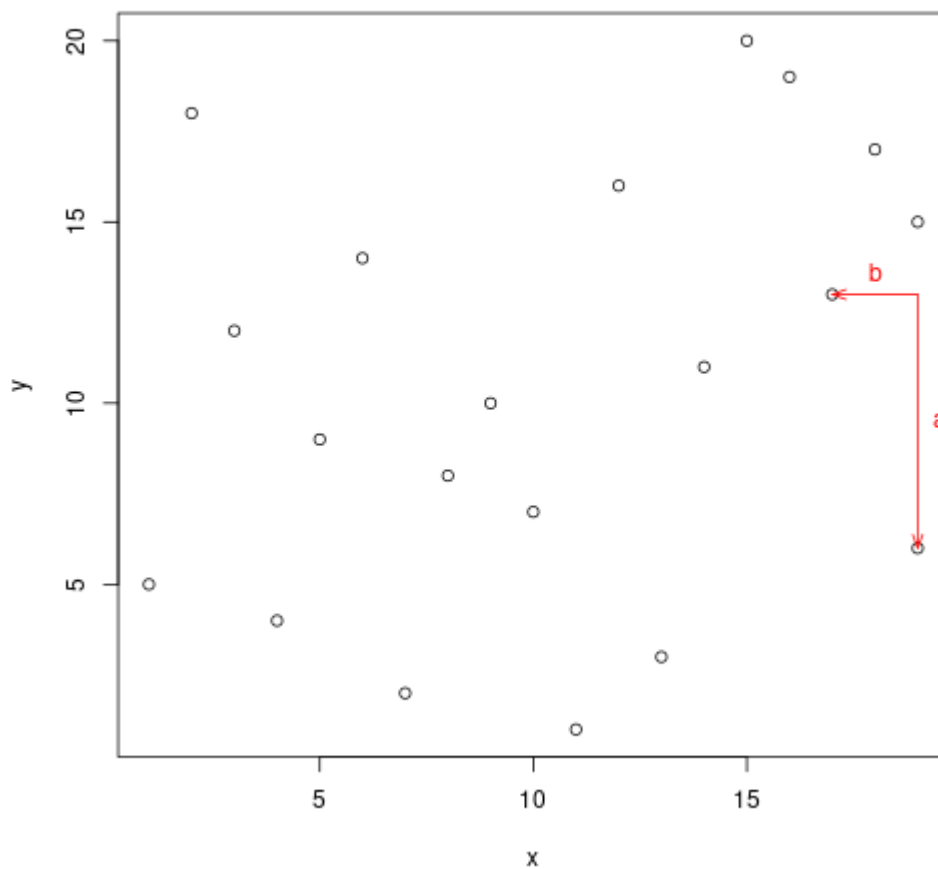
$$c^2 = a^2 + b^2$$



# Manhattan distance

- Manhattan distance

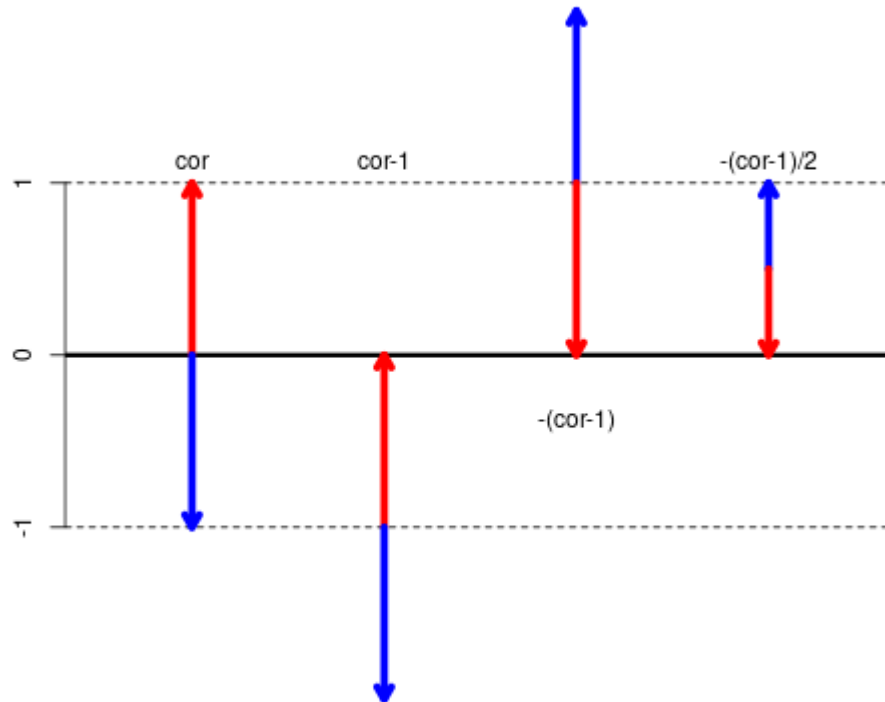
$$a + b$$



# Inverted pairwise correlations

- Inverted pairwise correlations

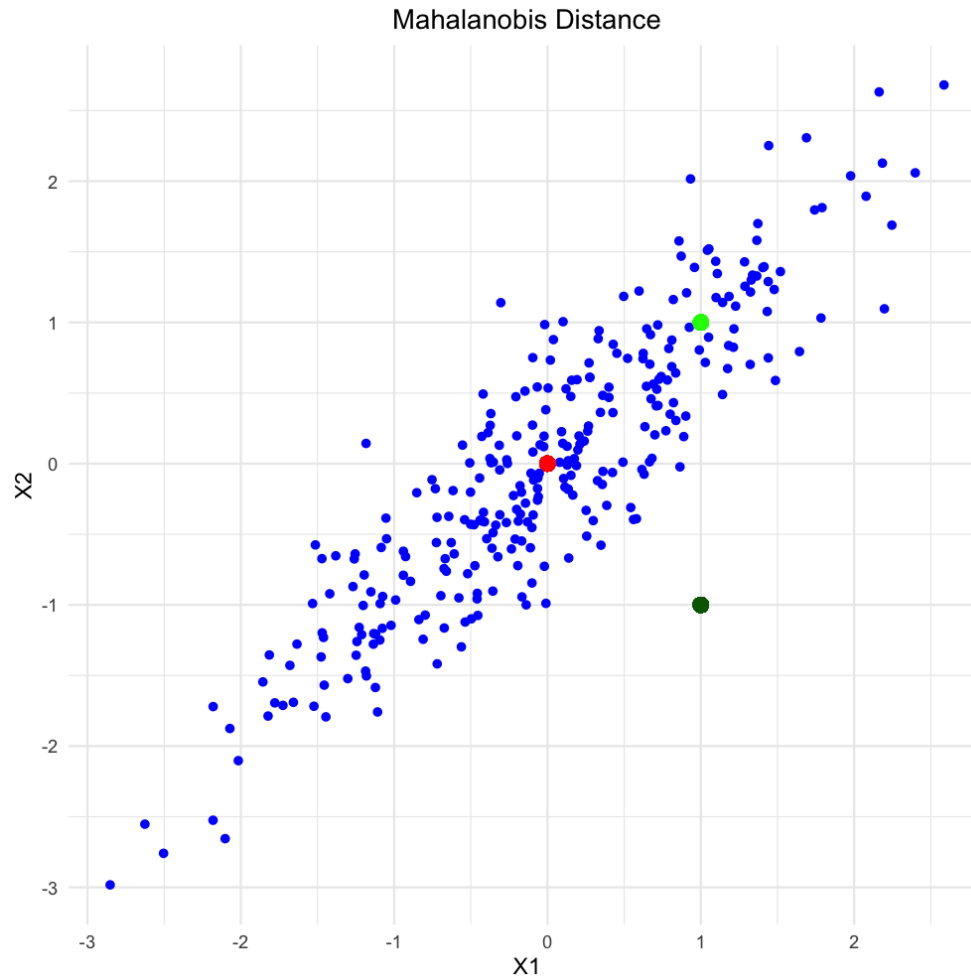
$$dist = -(cor - 1)/2$$





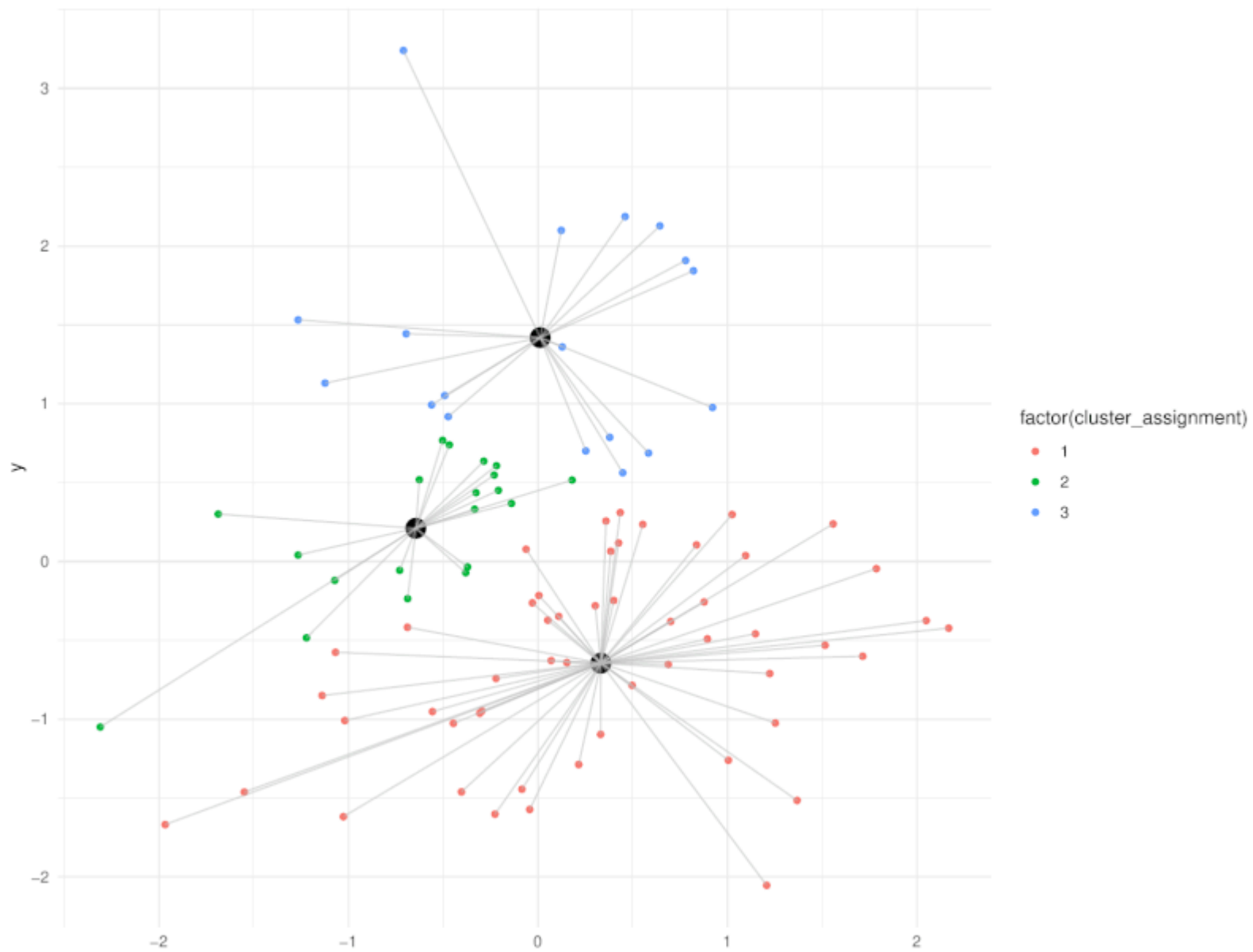
# Mahalanobis Distance

- Despite of previous approach which was based on distance between data points, this method measures the distance between a data point and a distribution.



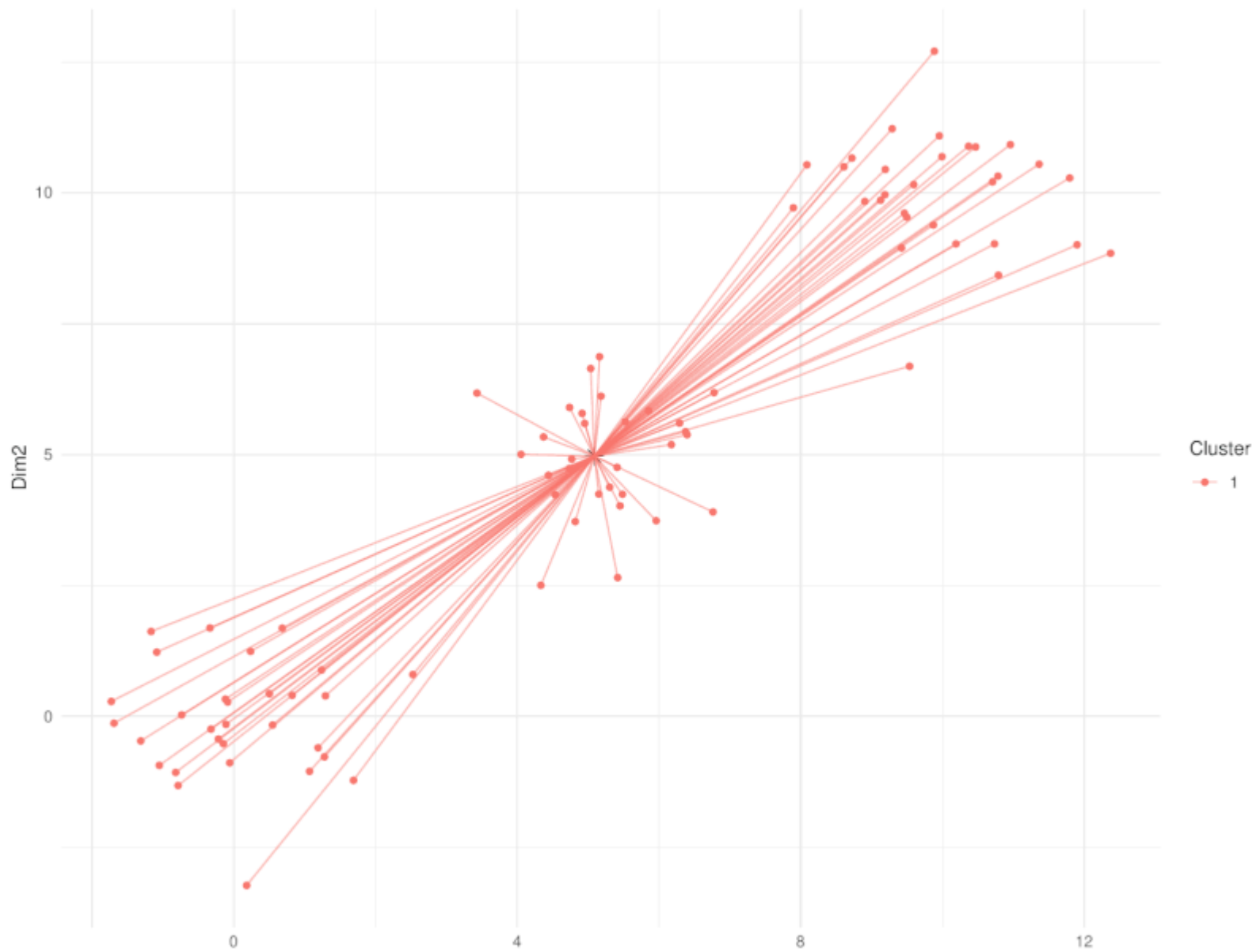
## Centroid-based: K-means clustering

- One of the most commonly used clustering methods
- In this method the distance between data points and centroids is calculated
- Each data point is assigned to a cluster based on Euclidean distance from centroid.
- Dependent on number of K (clusters) new centroids are created



## Centroid-based: K-means clustering

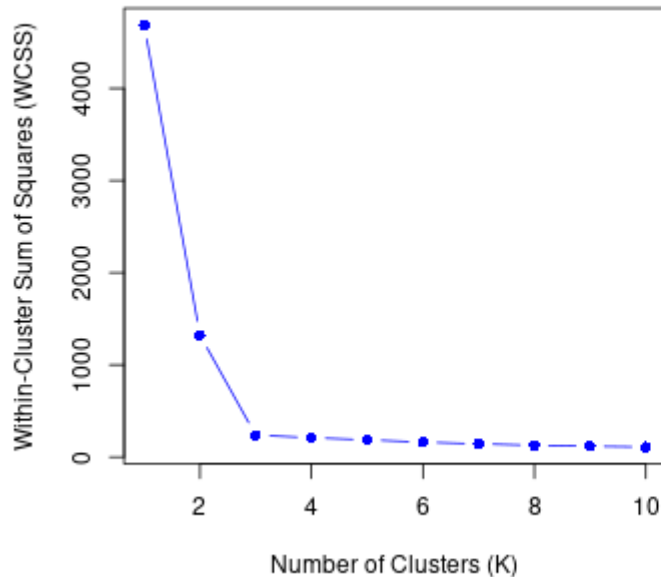
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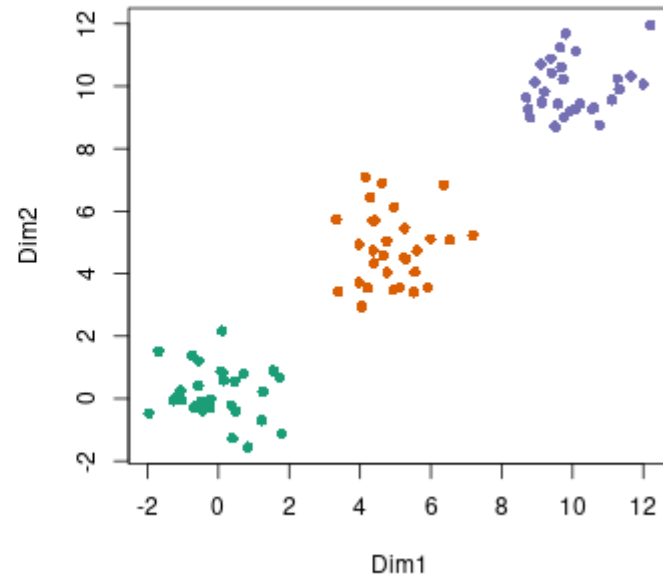
# What is optimal K?

- The user needs to define the number of clusters:
  - **Elbow method.**
  - Gap statistics.
  - Average Silhouette method

**Elbow Method for Optimal K**



**K-Means Clustering (K = 3)**



## Density-based clustering: DBSCAN

- This method identifies regions within your data distribution that exhibits high density of data points.

## Distribution-based clustering: Gaussian Mixture Model (GMM)



- It involves modeling the data points with probability distribution.
- In this method prior knowledge on distribution of your data is required. If you do not know the distribution of your data try another approach.
- You need to specify number of clusters.

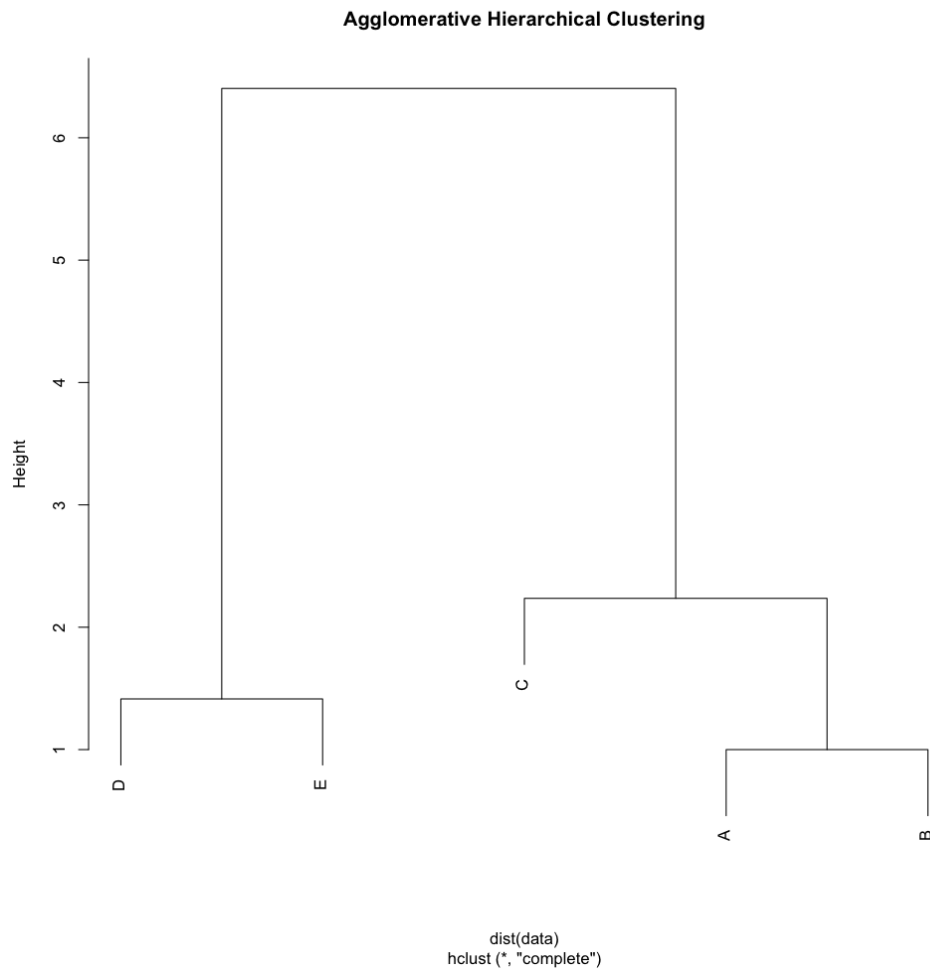


# Hierarchical-based clustering

- This approach creates a tree of clusters.
- Well suited for hierarchical data (e.g. taxonomies).
- Final output is a dendrogram representing the order decisions at each merge/division of clusters.
- Two approaches:
  - Agglomerative (Bottom-up): All data points are treated as clusters and the joins similar ones.
  - Divisive (Top-down): All data points are in one large clusters and recursively splits the most heterogeneous clusters.
- Number of clusters are decided after generating the tree.

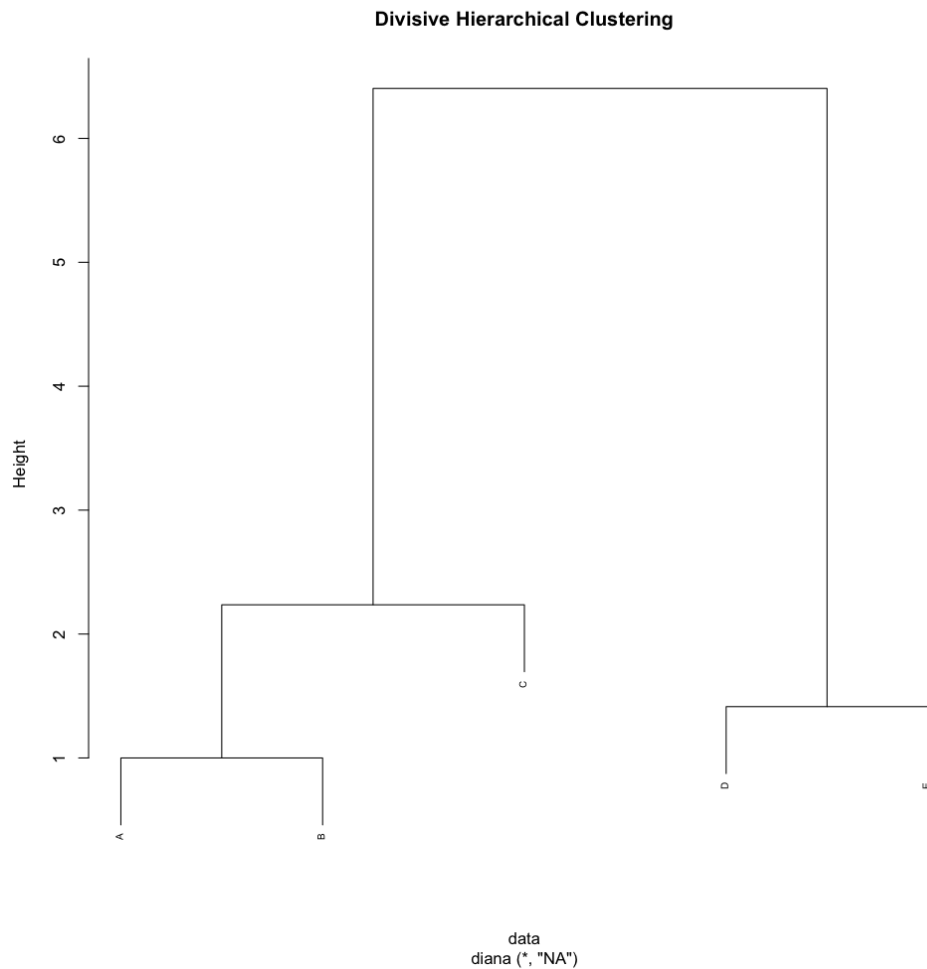
# Hierarchical-based clustering

- Agglomerative clustering



# Hierarchical-based clustering

- Divisive clustering

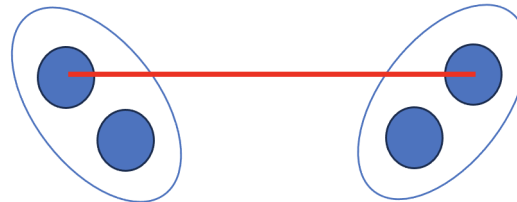


# Linkage methods.

To combine clusters, it's essential to establish their positions relative to one another. The technique used to determine these positions is known as **Linkage**.

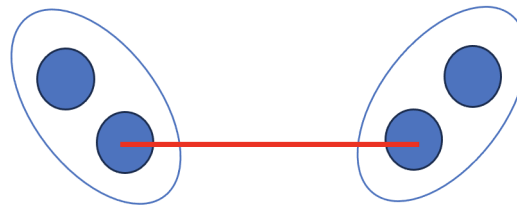
## Complete Linkage

*Maximum distance*



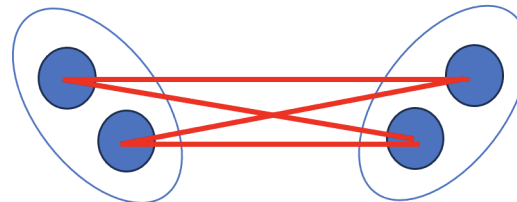
## Single Linkage

*Minimum distance*



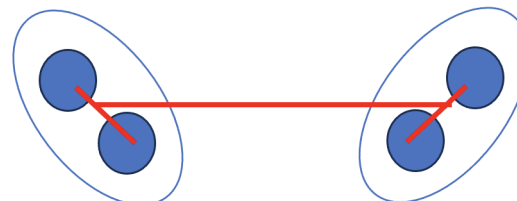
## Average Linkage

*Average of all points*



## Ward's Linkage

*Minimize within cluster variance*



# Summary

- For bulk RNASeq you can perform clustering on raw or Z-Score scaled data.
- For the sample size is large ( $>10,000$ ) you can perform clustering on PC. For instance in scRNASeq data.
- You always need to tune some parameters.
- K-means performs poorly on unbalanced data.
- On hierarchical clustering, some distance metrics need to be used with a certain linkage method.
- Checking clustering Robustness (a.k.a Ensemble perturbations):
  - Most clustering techniques will cluster random noise.
  - One way of testing this is by clustering on parts of the data (clustering bootstrapping)
  - Read more in [Ronan et al \(2016\) Science Signaling](#)).

Thank you. Questions?

