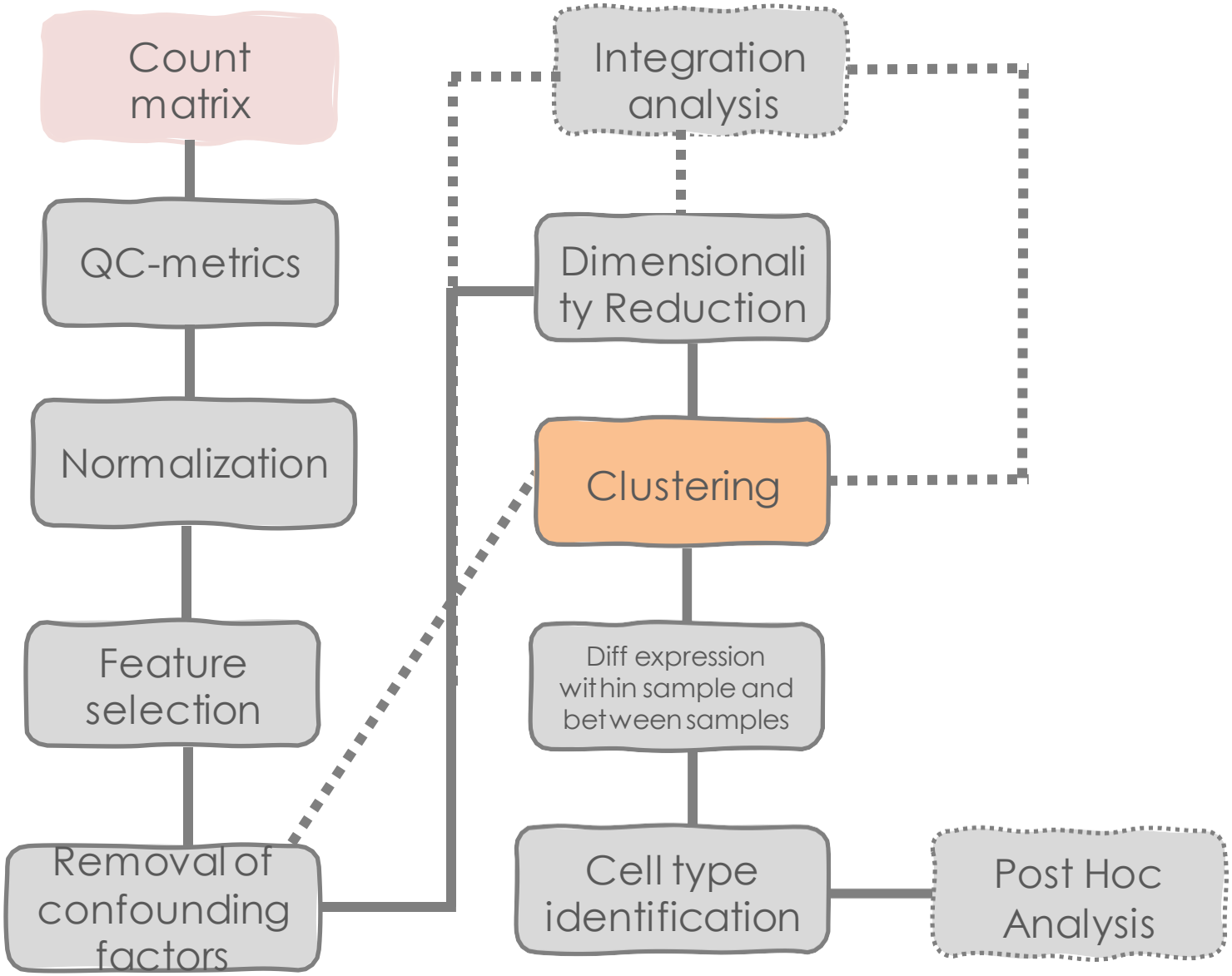
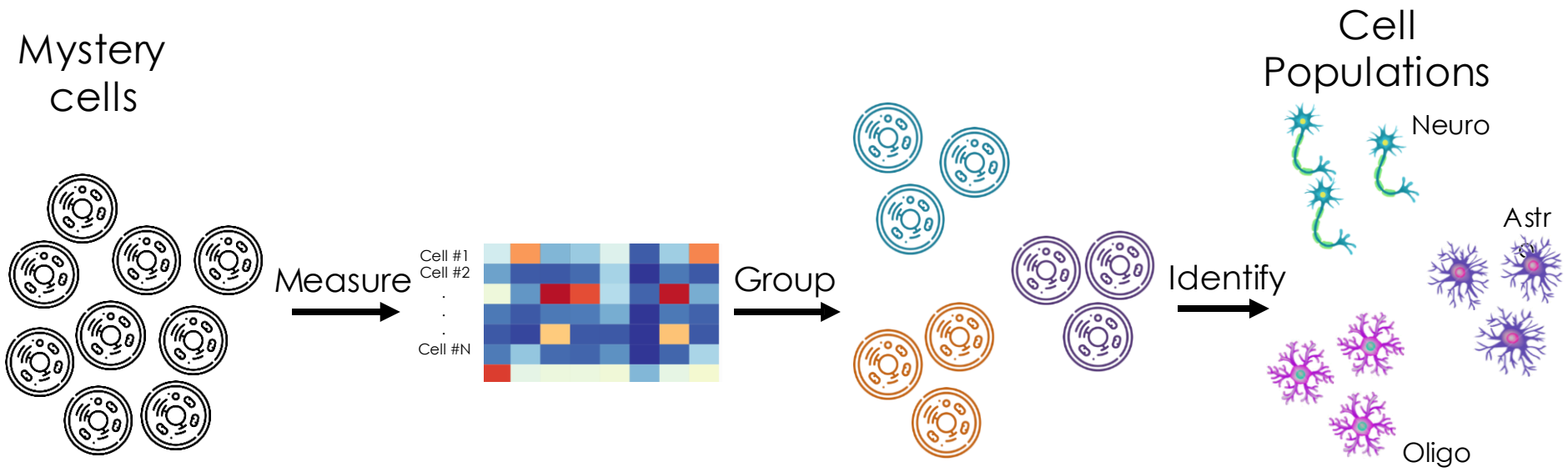
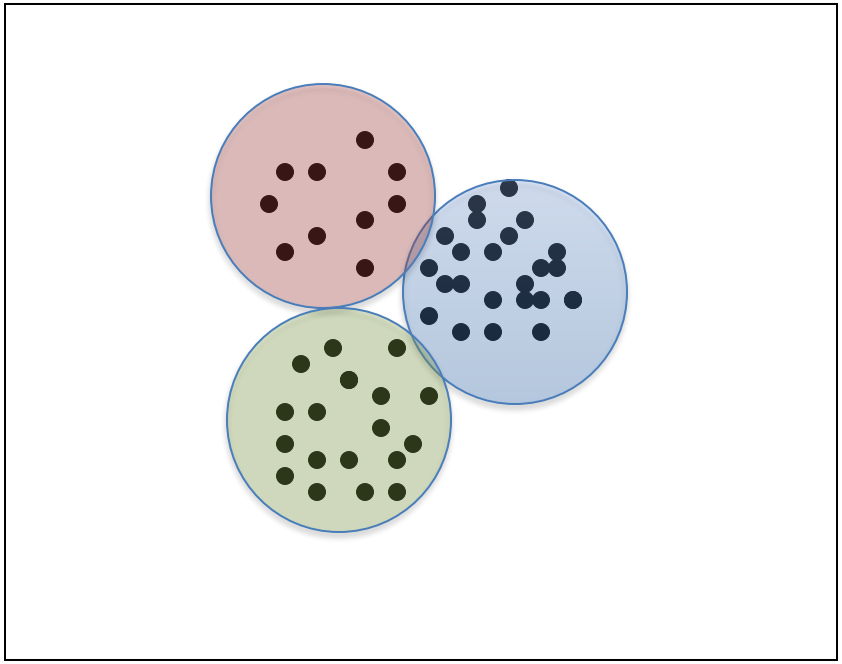

Count matrix
QC-metrics
Normalization
Feature selection
Removal of confounding factors
Integration analysis
Dimensionality Reduction
Clustering
Diff expression within sample and between samples
Cell type identification
Post Hoc Analysis

Clustering: Cell Identity



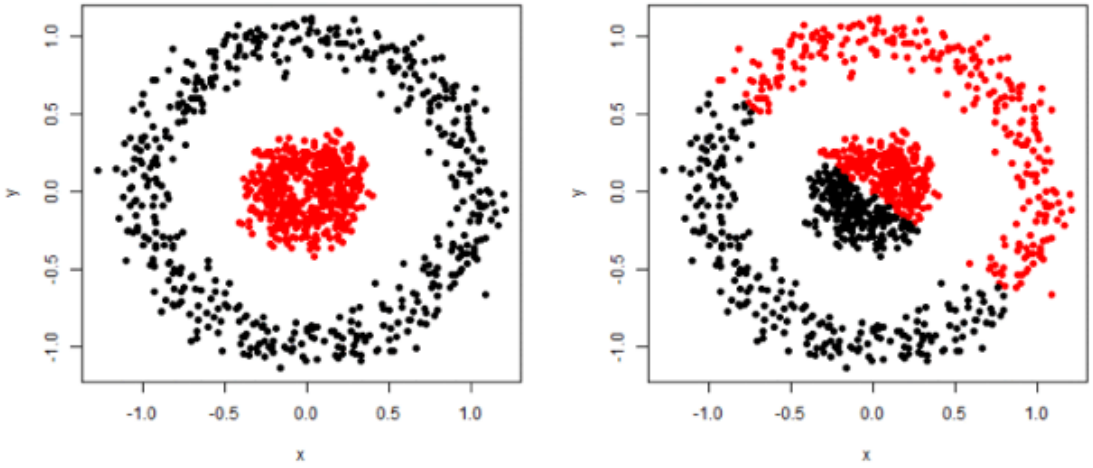
Clustering: Cell Identity



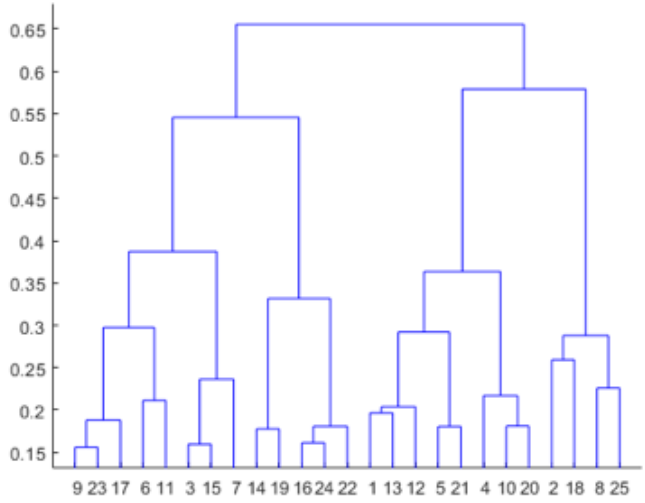
Clustering

Clustering method are divided into two categories* :

Partitioning clustering



Hierarchical clustering



*Handbook of cluster analysis, Hennig C. et al.

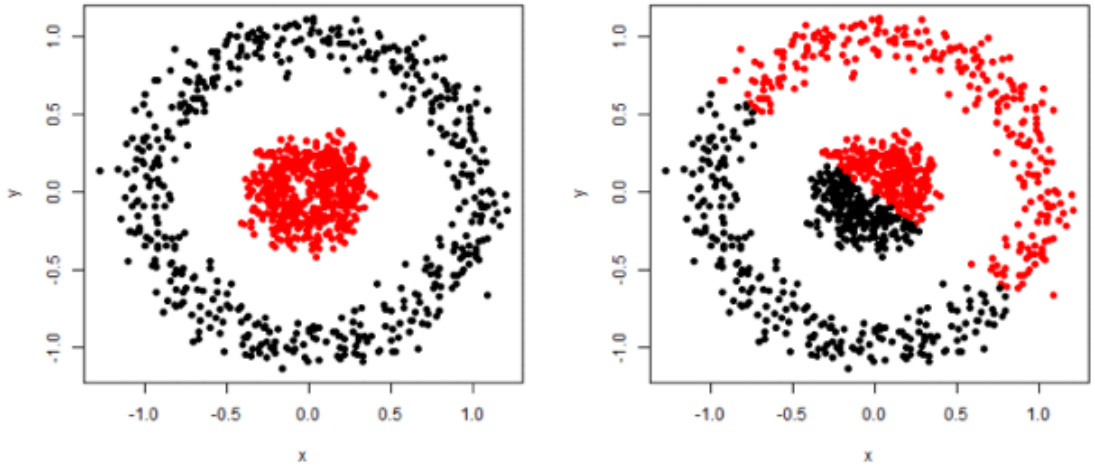
Partitioning clustering

Convex partitioning. Example: K-means

Density based approaches. Example: DBSCAN

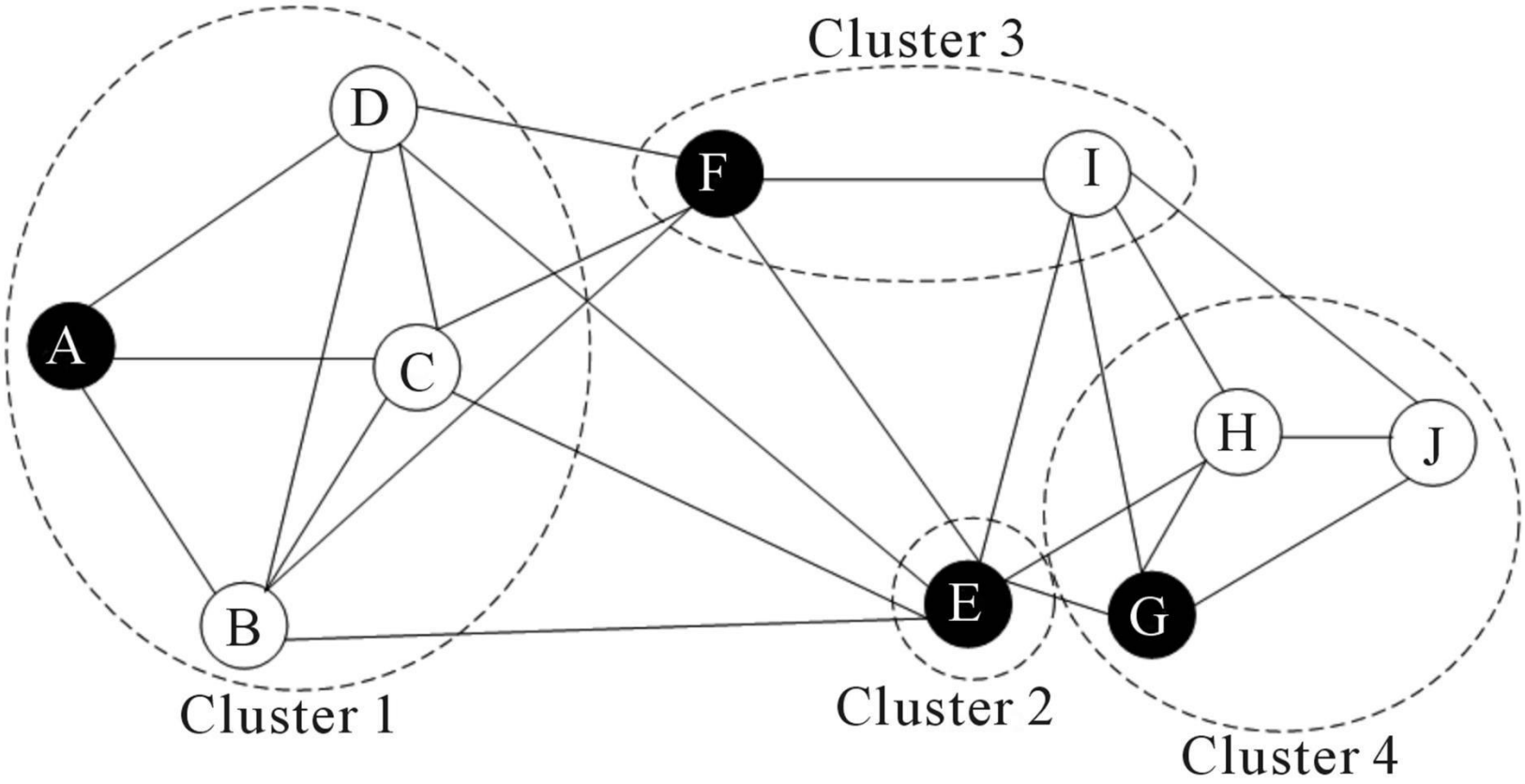
Model-based approaches. Example: Mclust

Graph based approaches : Example to follow



Graph-based

- Nodes -> cells
- Edges -> similarity ()



Graph-based: types

- k-Nearest Neighbor **(kNN)** graph

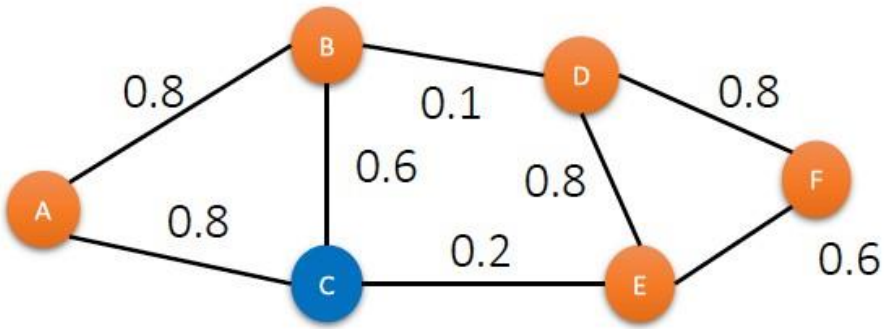
A graph in which two vertices *p* and *q* are connected by an edge, if the distance between *p* and *q* is among the *k*-th smallest distances from *p* to other objects from *P*.

- Shared Nearest Neighbor **(SNN)** graph

A graph in which weights define proximity, or similarity between two nodes in terms of the number of neighbors (i.e., directly connected nodes) they have in common.

```
obj <- FindNeighbors(obj)
```

Graph-based: types



adjacency

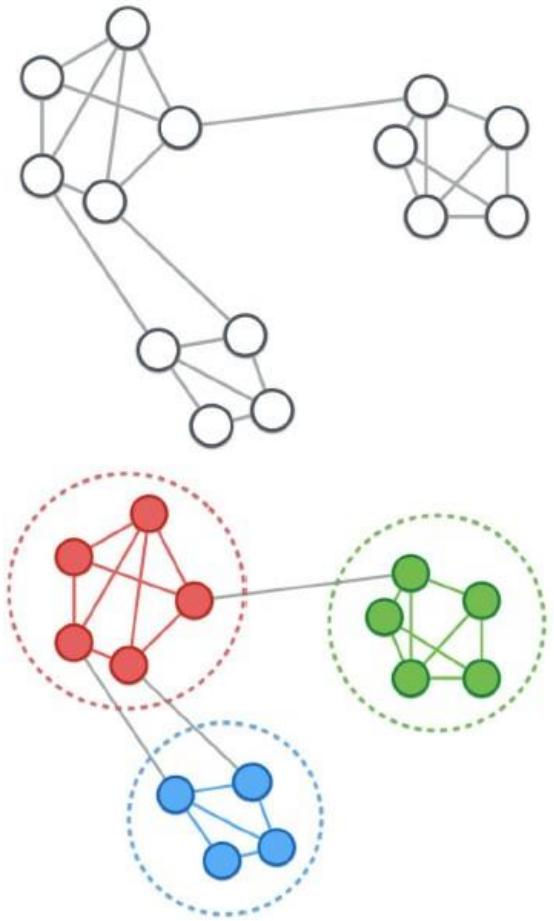
$$A = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0 & 1 & 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 \end{pmatrix} \end{array}$$

weight

$$W = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0 & 0.8 & 0.8 & 0 & 0 & 0 \\ 0.8 & 0 & 0.6 & 0.1 & 0 & 0 \\ 0.8 & 0.6 & 0 & 0 & 0.2 & 0 \\ 0 & 0.1 & 0 & 0 & 0.8 & 0.8 \\ 0 & 0 & 0.2 & 0.8 & 0 & 0.6 \\ 0 & 0 & 0 & 0.8 & 0.6 & 0 \end{pmatrix} \end{array}$$

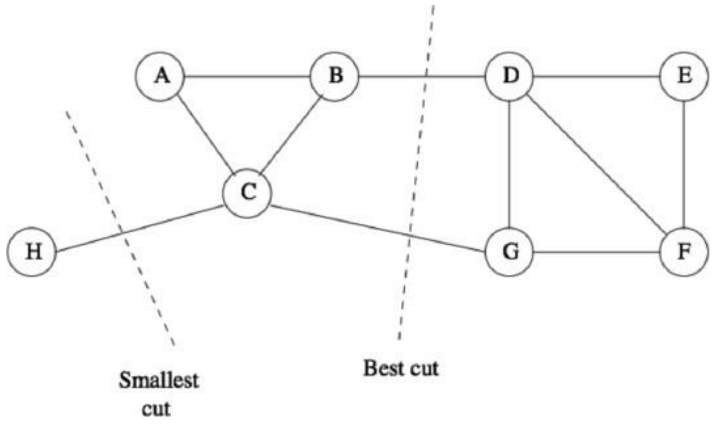
Graph-based: communities

- Communities (clusters):
  - groups of nodes **with higher probability of being connected** to each other than to members of other groups
- Community detection:
  - find a group (community) of nodes with **more edges inside** the group than edges linking nodes of the group with the rest of the graph.



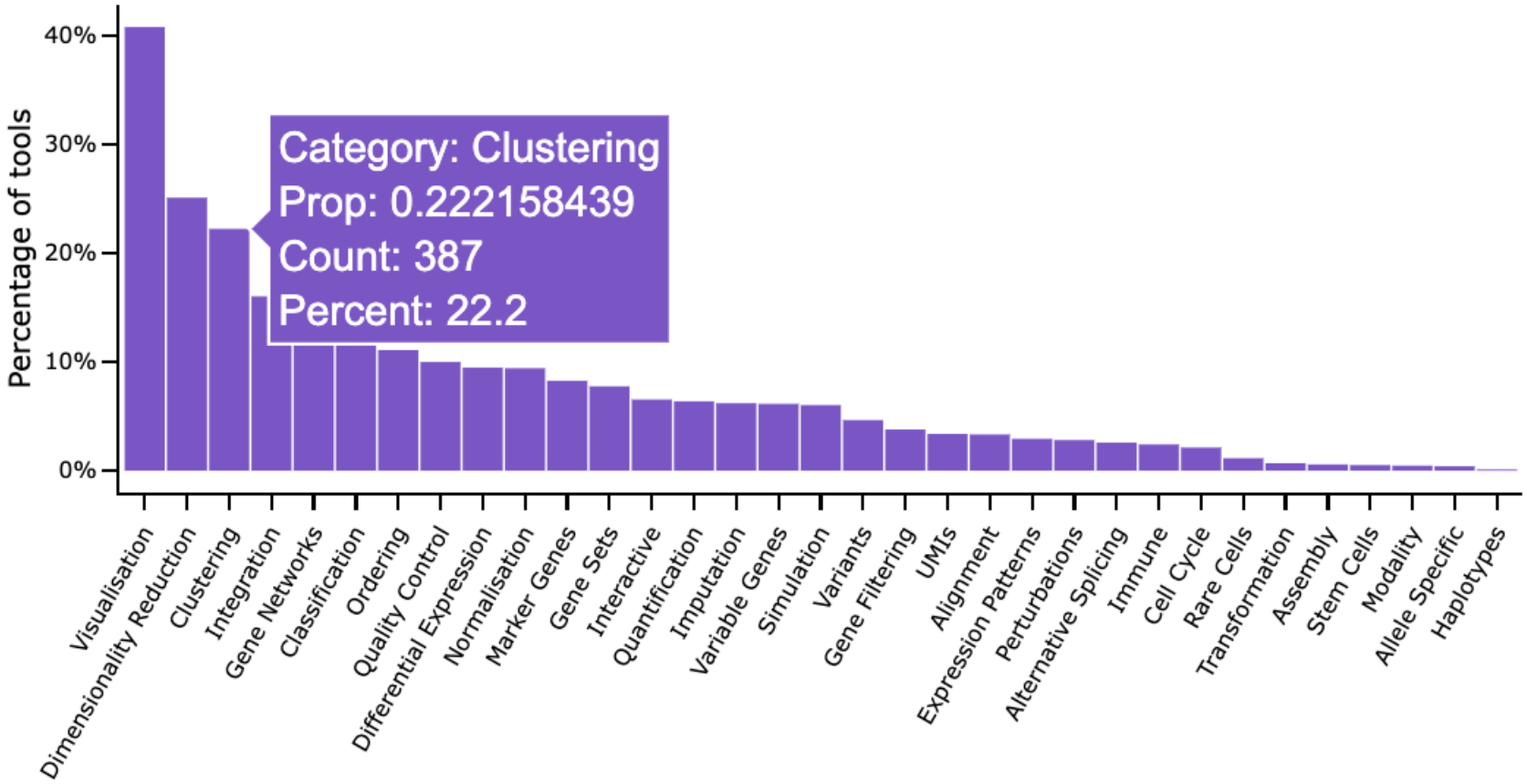
Graph-based: Cuts

- Graph cut partitions a graph into subgraphs
- Cut size is the number of cut edges
- Clustering by graph cuts: find the smallest cut that bi-partitions the graph
- The smallest cut is not always the best cut
- NP-hard
  - Heuristic methods applied e.g. Louvain



*Shi & Malik (IEEE PAMI 2000)

scRNA-seq clustering methods



https://www.scrna-tools.org/

Seurat

1. Construct SNN graph based on the Euclidean distance in PCA space.

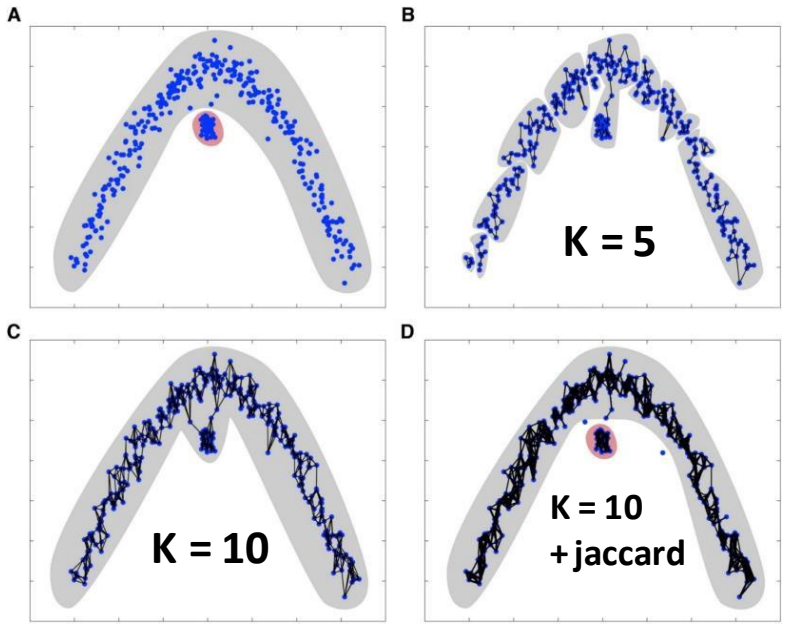
(Default, but could be also kNN)

2. Refine the edge weights between any two cells based on the shared overlap in their local neighborhoods (Jaccard index).

3. Cluster cells by optimizing for modularity (cuts) (Louvain algorithm)

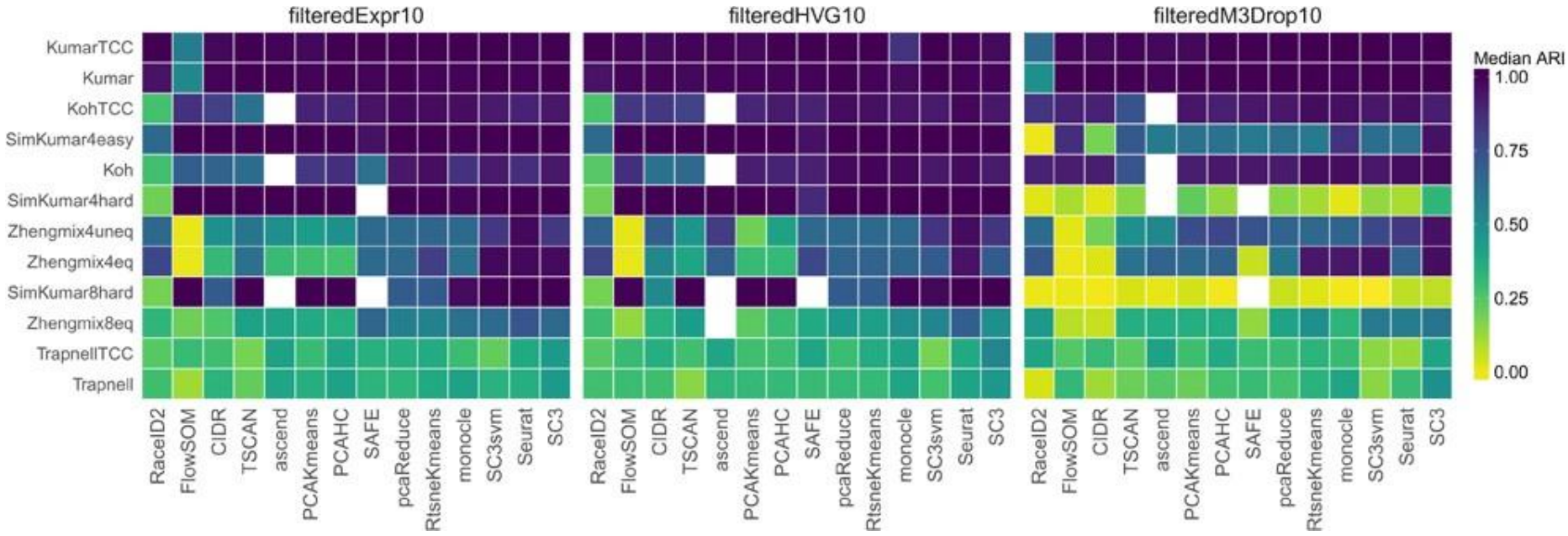
(Modularity is a cost function, resolution is a parameter used to calculate the modularity)

```
obj <- FindClusters(obj)
```



Xu and Su (https://doi.org/10.1093/bioinformatics/btv088)
Levine et al. (https://doi.org/10.1016/j.cell.2015.05.047)

Benchmarking



Duò et al. (https://doi.org/10.12688/f1000research.15666.2)

Clustering: Challenges

- What is a cell type?
- What is the number of clusters *k*?
- Check QC after clustering to see if no biases are constituting your clusters
- Clustering is subjective – No ground truth
- How stable are the clusters
- How dependent are the clusters on the surrounding cells

•Scalability: in the last few years the number of cells in scRNA-seq experiments has grown by several orders of magnitude from ~10^2 to ~10^6