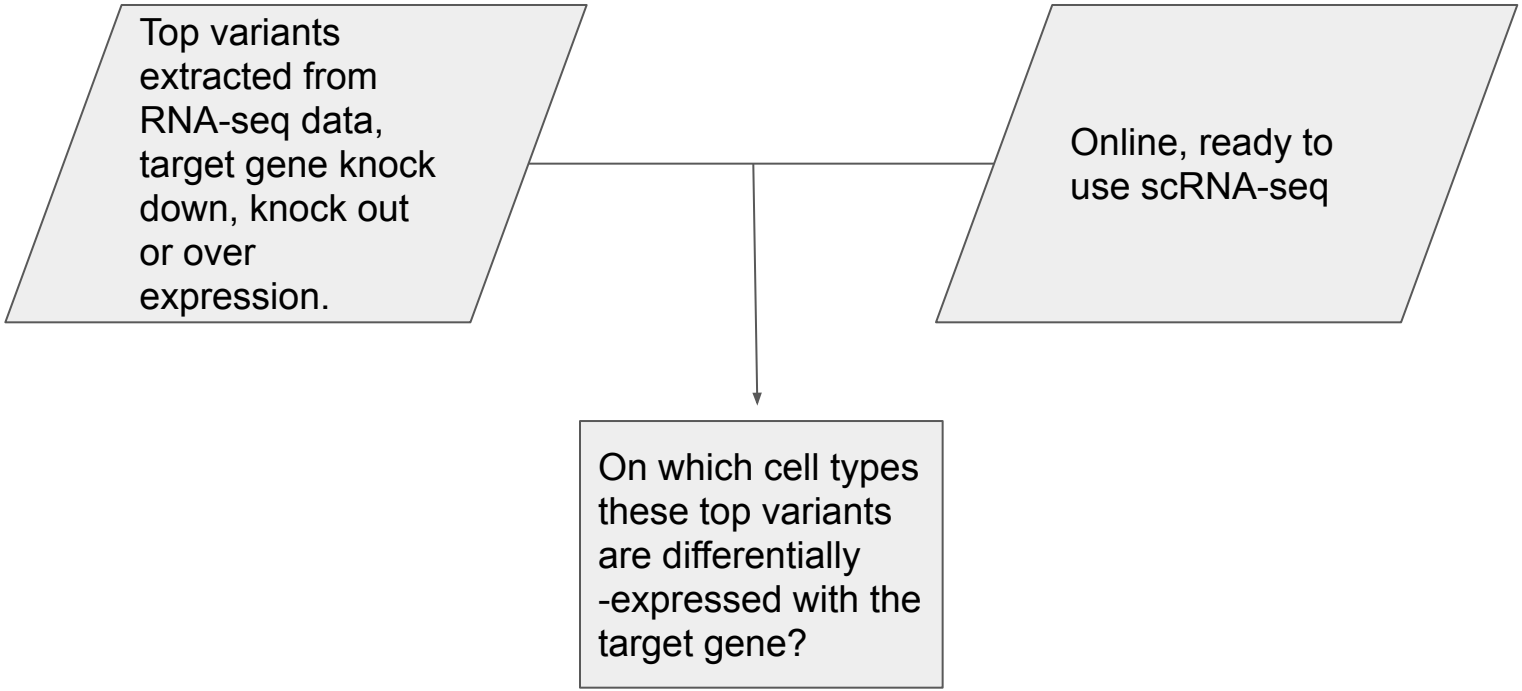
Gene Expression Deconvolution

Maral Dicle Maral
Görkem Durmaz
Ezgi Taş

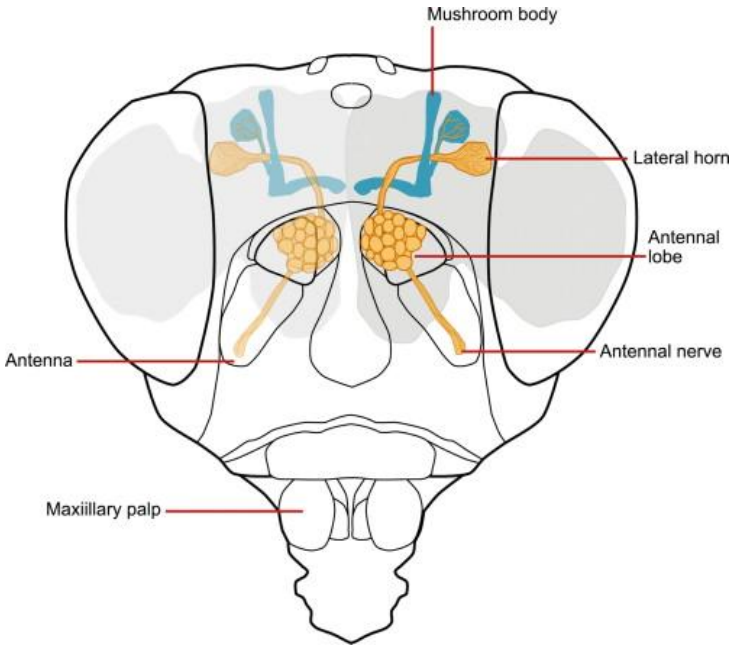
Gene-Expression Deconvolution/Decomposition



Transcriptome Analysis of *IROC* Genes in Fruitfly Olfactory System

- *Role of iroC* transcription factors in olfactory system (*Araucan, caupolican and mirror)*
- Bulk RNA sequencing for *iroC* mutant and wild-type organisms.

In which cell types these genes are differentially expressed?



Differentially Expressed Genes

OLFACTORY RECEPTOR	MEAN COUNT
OR47A	1236,71
OR43B	1474.46
OR33C	251.53
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OR85A	787,40
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OLFACTORY RECEPTOR	MEAN COUNT
ORCO	54059.51
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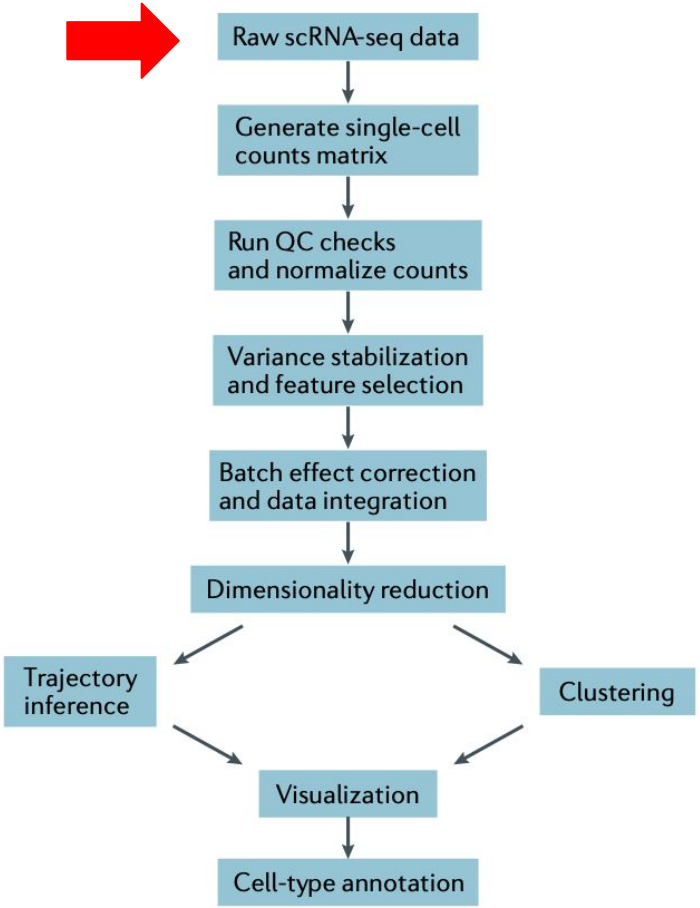
Differential expression analysis done by Ibrahim Ihsan Taskiran, Arzu Celik's Neuroscience Laboratory

Workflow of Single Cell Seq Data Analysis

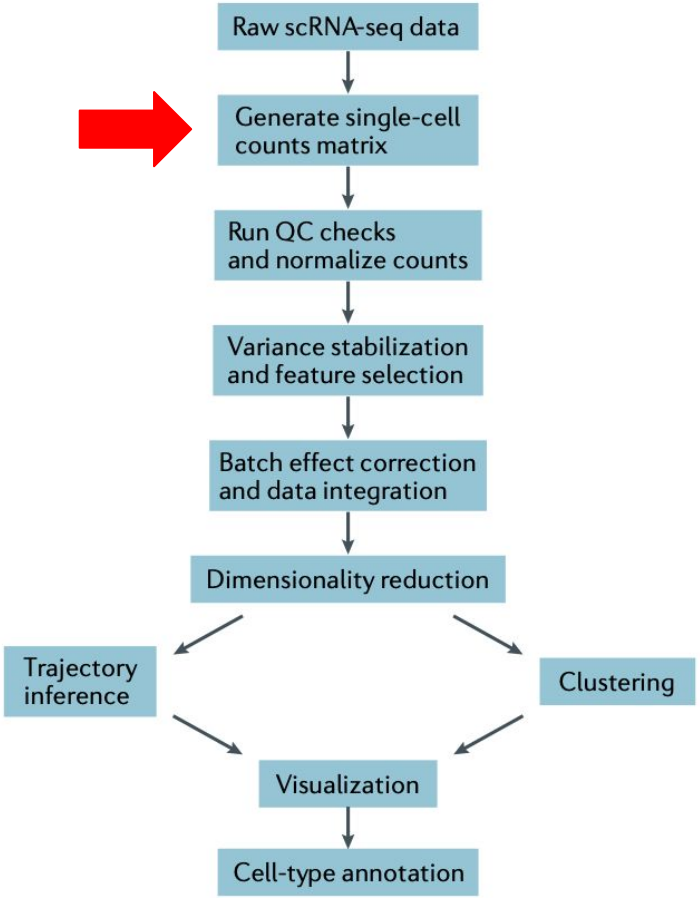


Data Pre-processing

Raw Input

- RNA Seq Read consisting of complementary DNA (cDNA)
- Cell Barcode
- Unique Molecular Index (UMI)

Workflow of Single Cell Seq Data Analysis



Generating a Gene Expression Matrix

- Alignment of cDNA sequences to a reference genome
- Assigning reads to cell of origin

- CellRanger , dropEst, Kallisto - BUStools, StarSolo

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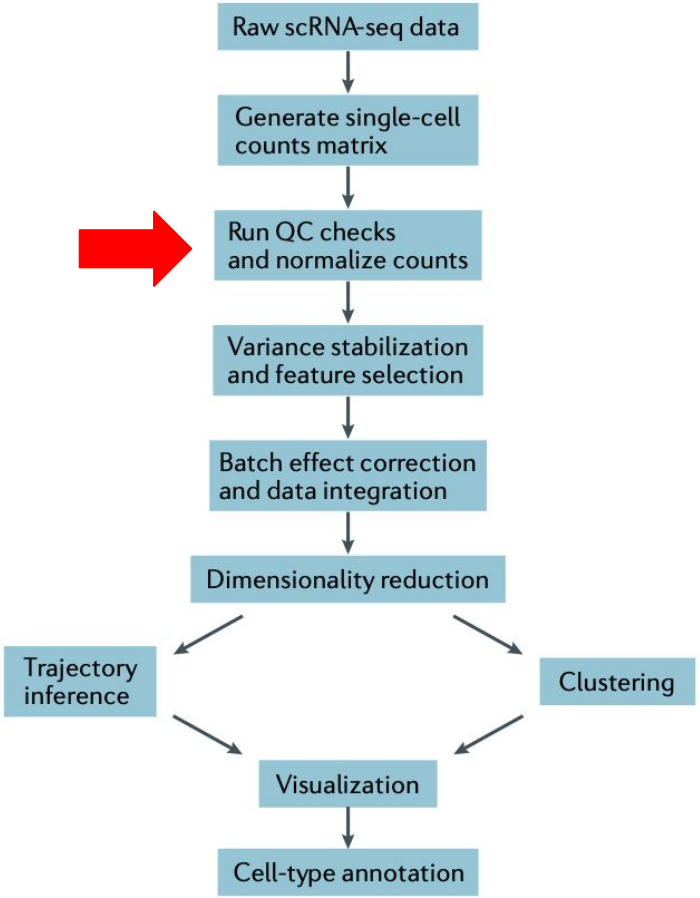
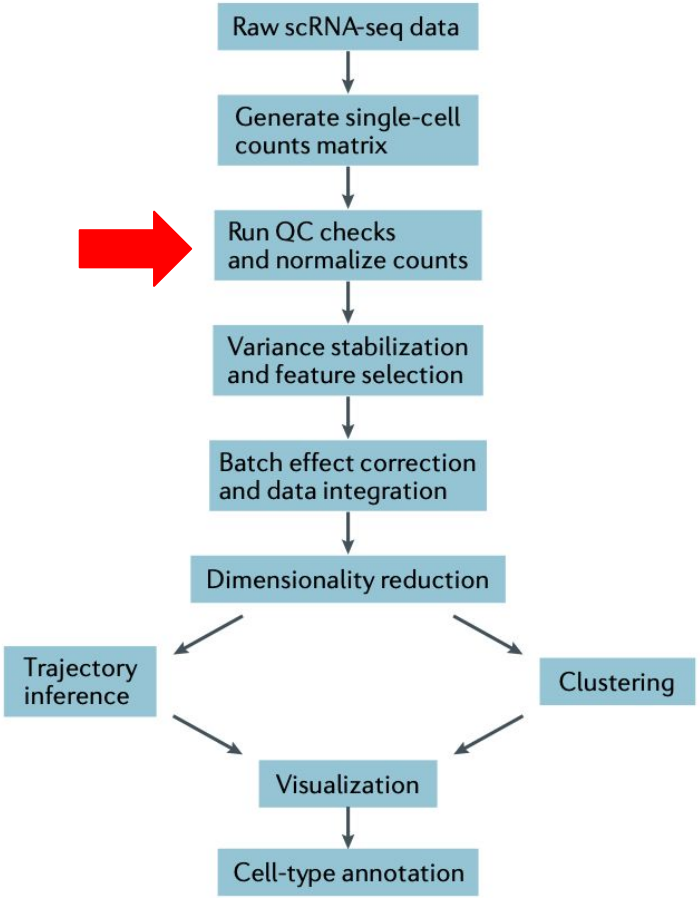


Quality Control and Doublet Detection

- Doublets
- Biases & Noise
- Seurat, SCANPY, Scater

Workflow of Single Cell Seq Data Analysis



Data Normalization

- Normalization is therefore crucial to revealing the true biological heterogeneity of a dataset.

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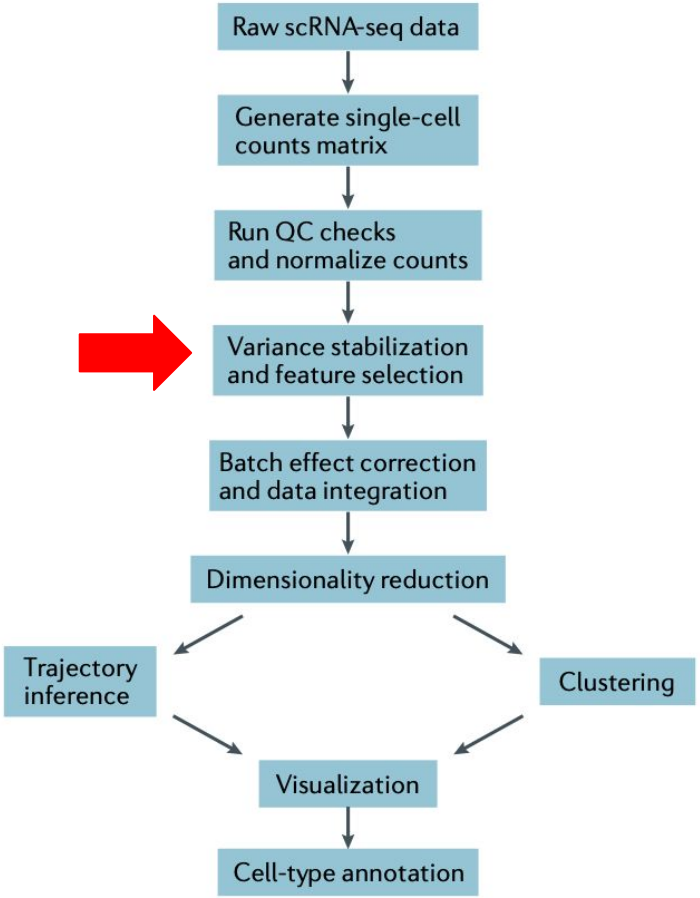
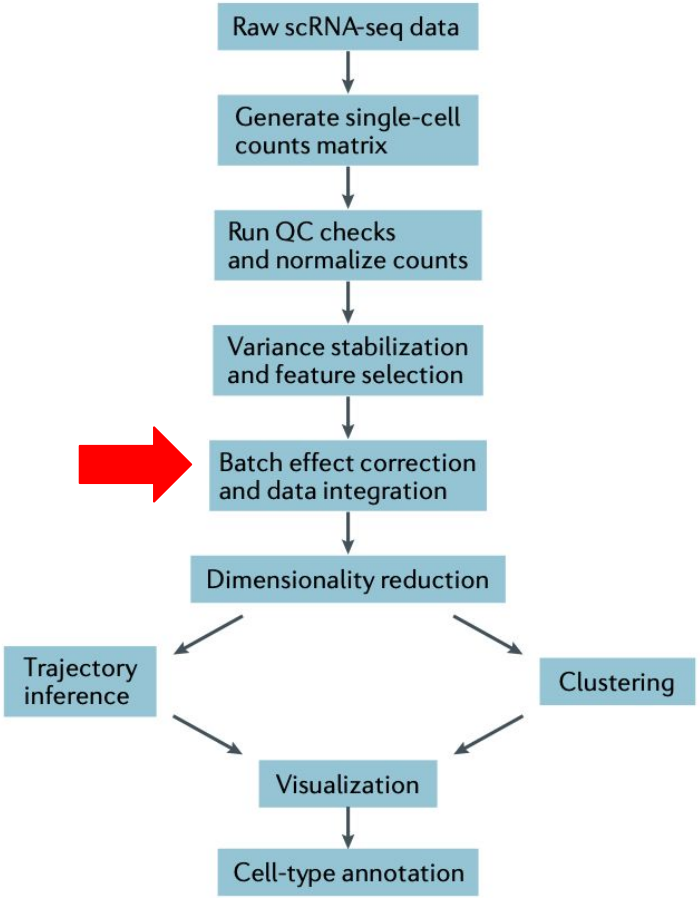


Variance Stabilization

- Enhances the contribution of biologically relevant genes.
- Log-transformation of normalized counts

- Seurat, Pagoda2, SCANPY, ZINB-Wave

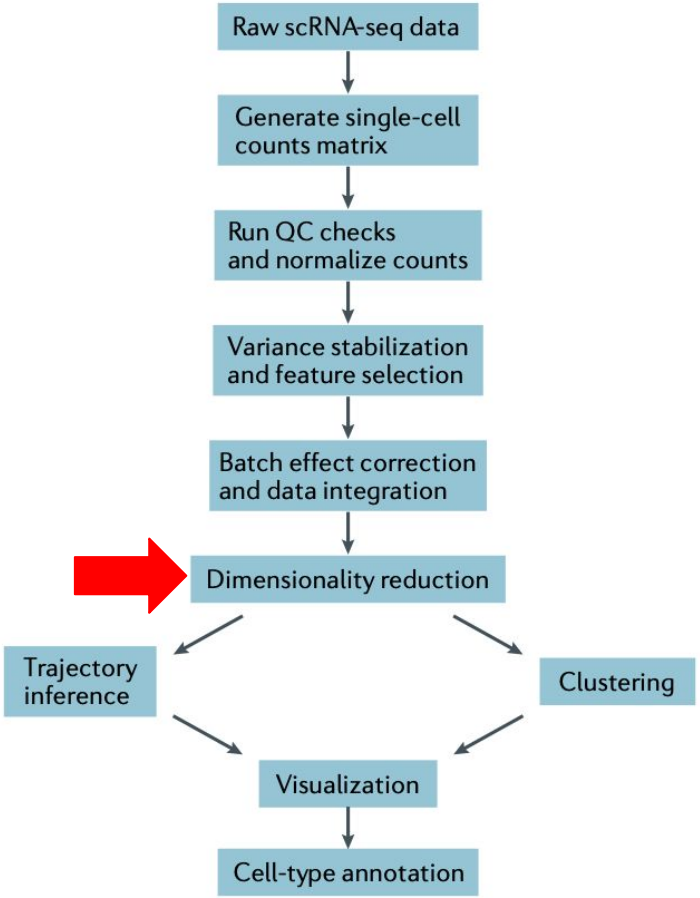
Workflow of Single Cell Seq Data Analysis



Batch Effects and Data Integration

- Integration can help us detect rare cell phenotypes.
- Regression model to eliminate batch differences
- Mutual Nearest Neighbors (MNNs)

Workflow of Single Cell Seq Data Analysis

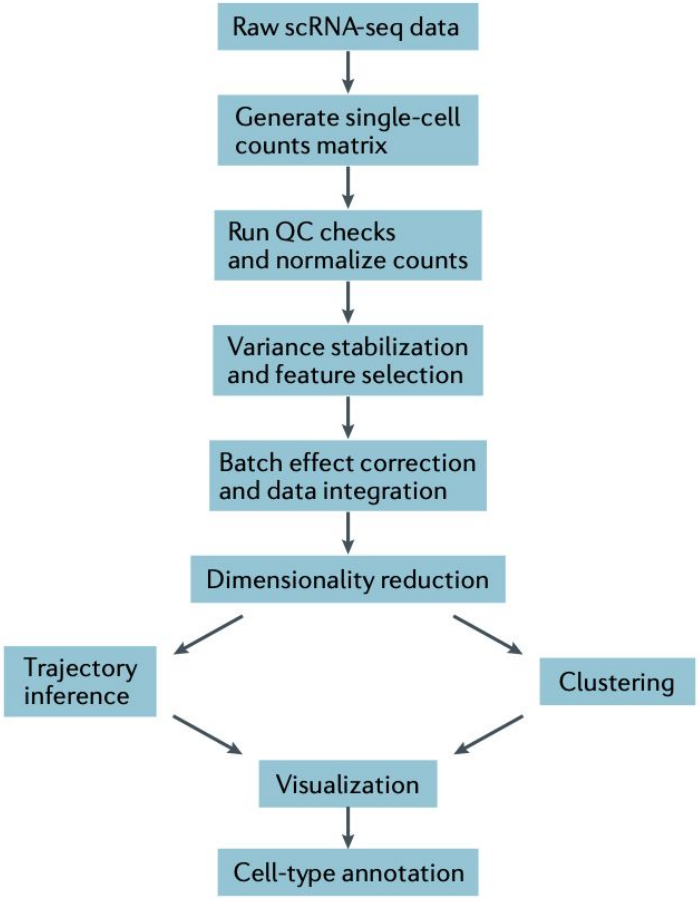


Downstream Analyses

Dimensionality Reduction and Imputation

- A more compact and interpretable data is produced.
- PCA & SCVI

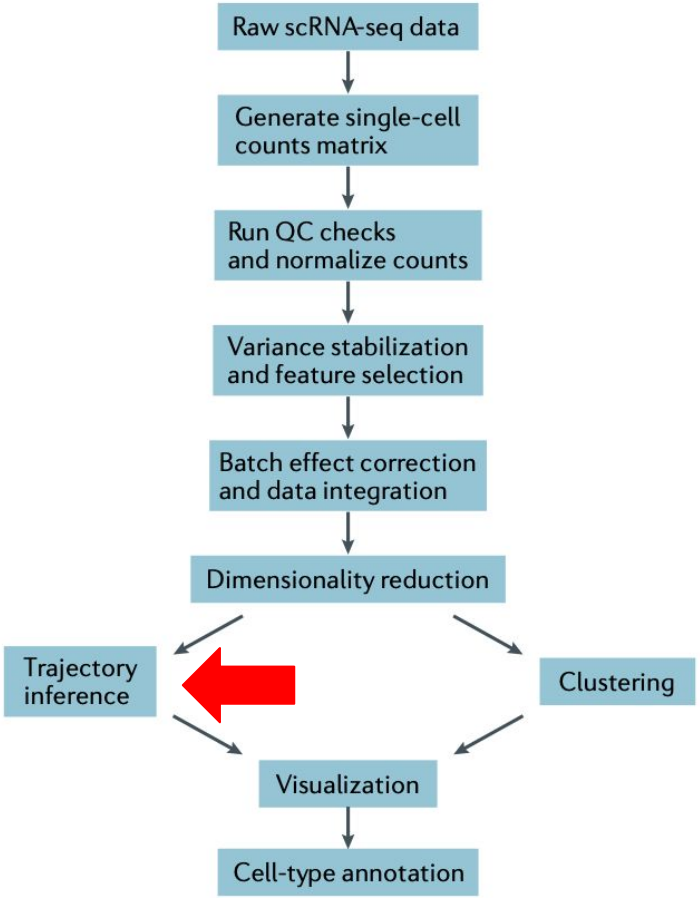
Workflow of Single Cell Seq Data Analysis



Clustering

- The goal of clustering is to identify subpopulations.
- Most popular types of clustering are hierarchal, graph-based and k-means clustering.
- Graph-based clustering is the most popular and best performing algorithm.
  - Louvain and Leiden algorithms

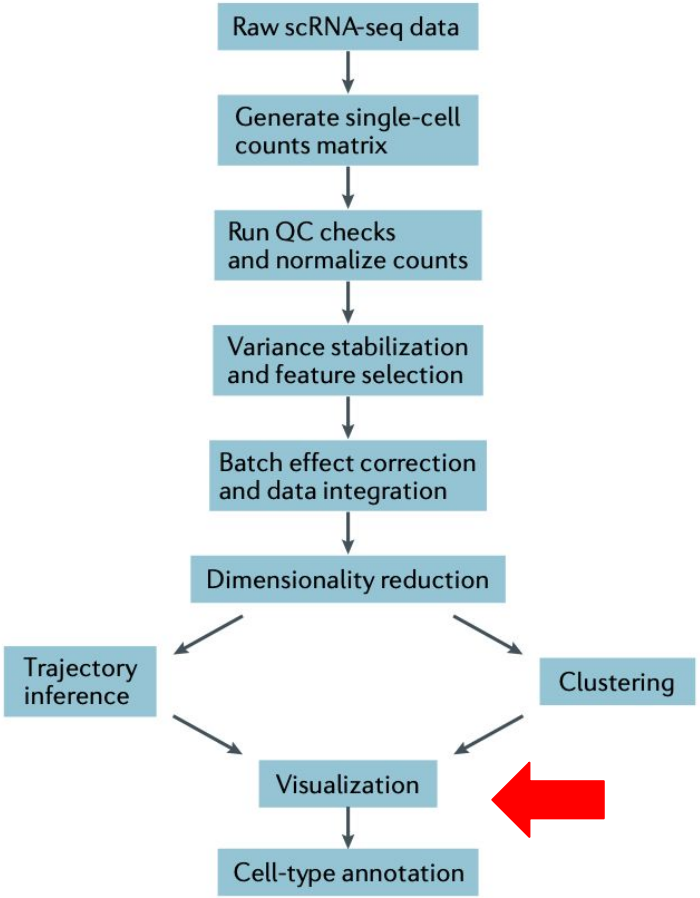
Workflow of Single Cell Seq Data Analysis



Trajectory Inference

- A continuum of gene expression patterns in development

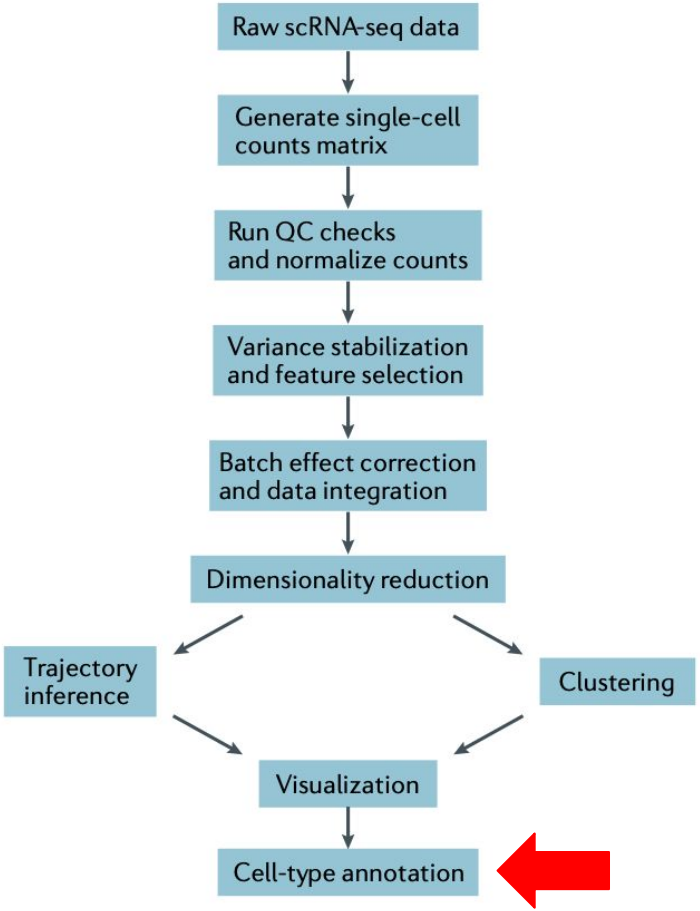
Workflow of Single Cell Seq Data Analysis



Visualization

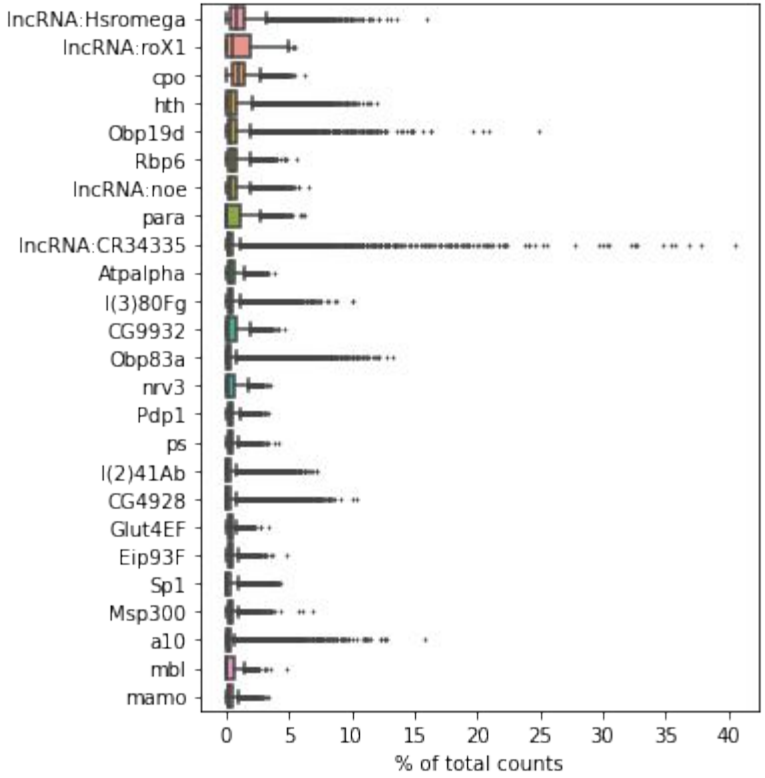
- 2D or 3D scatterplot of cells

Workflow of Single Cell Seq Data Analysis

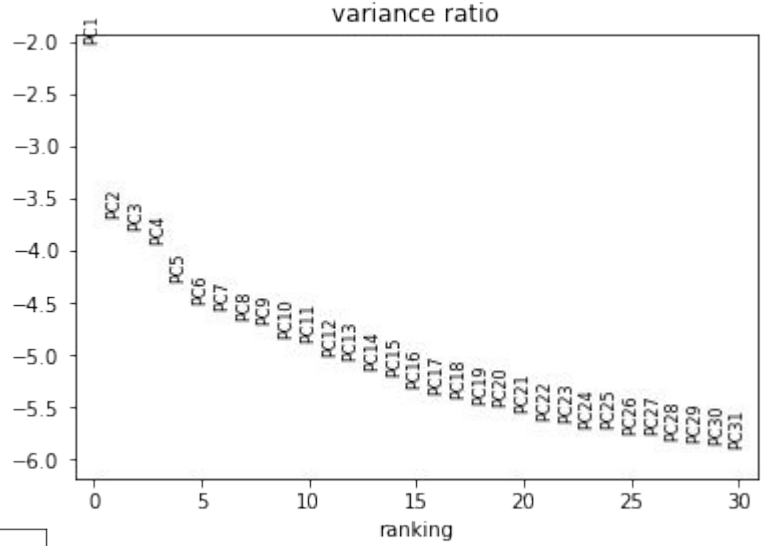


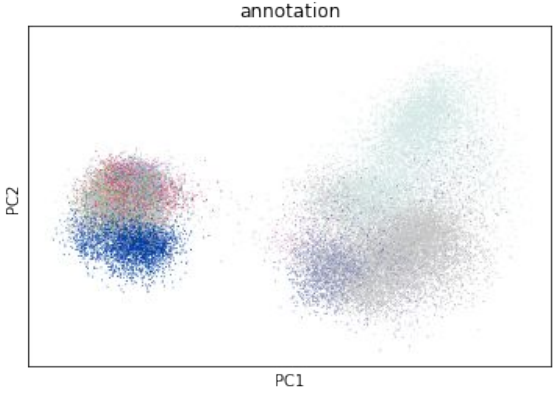
Cell-type Annotation

Preprocessing: Highly expressed genes in all cells



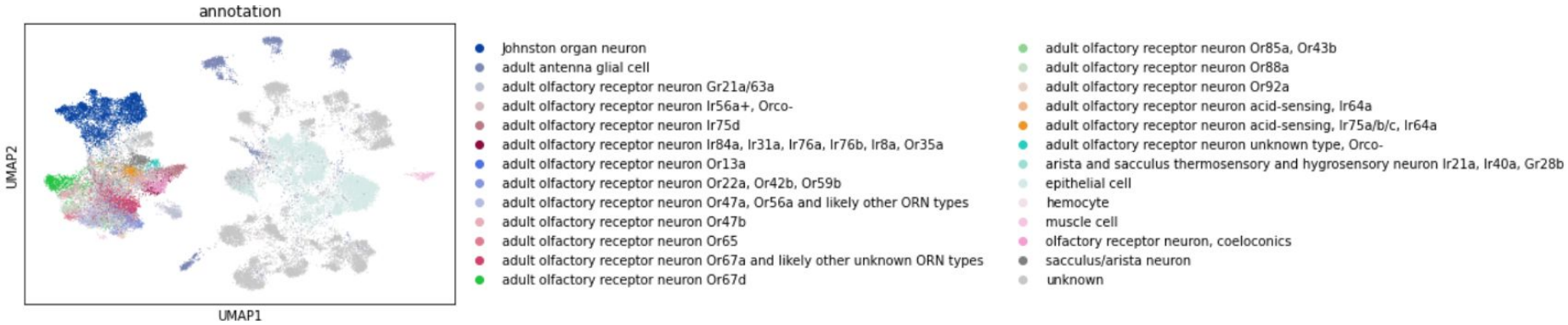
Preprocessing: PCA





- Johnston organ neuron
- adult antenna glial cell
- adult olfactory receptor neuron Gr21a/63a
- adult olfactory receptor neuron Ir56a+, Orco-
- adult olfactory receptor neuron Ir75d
- adult olfactory receptor neuron Ir84a, Ir31a, Ir76a, Ir76b, Ir8a, Or35a
- adult olfactory receptor neuron Or13a
- adult olfactory receptor neuron Or22a, Or42b, Or59b
- adult olfactory receptor neuron Or47a, Or56a and likely other ORN types
- adult olfactory receptor neuron Or47b
- adult olfactory receptor neuron Or65
- adult olfactory receptor neuron Or67a and likely other unknown ORN types
- adult olfactory receptor neuron Or67d
- adult olfactory receptor neuron Or85a, Or43b
- adult olfactory receptor neuron Or88a
- adult olfactory receptor neuron Or92a
- adult olfactory receptor neuron acid-sensing, Ir64a
- adult olfactory receptor neuron acid-sensing, Ir75a/b/c, Ir64a
- adult olfactory receptor neuron unknown type, Orco-
- arista and sacculus thermosensory and hygrosensory neuron Ir21a, Ir40a, Gr28b
- epithelial cell
- hemocyte
- muscle cell
- olfactory receptor neuron, coeloconics
- sacculus/arista neuron
- unknown

Results: Human annotation off cell types



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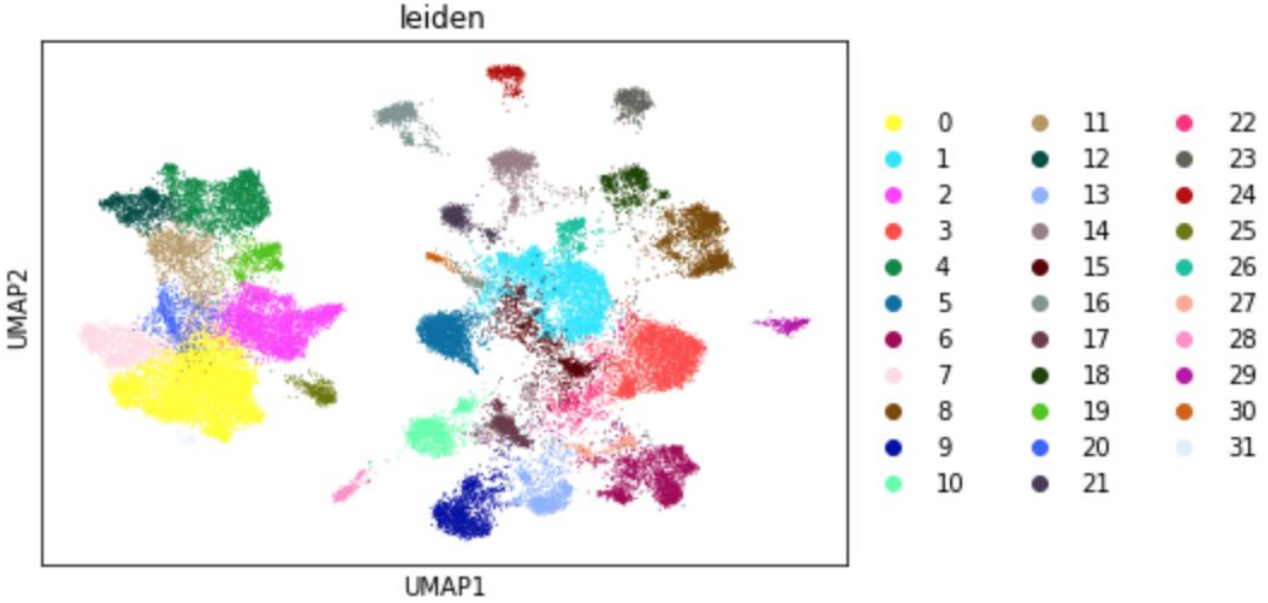
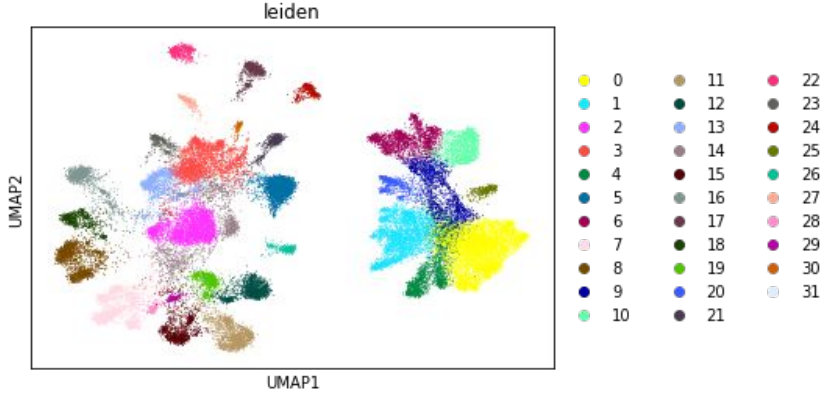
Differential expression analysis done by Ibrahim Ihsan Taskiran, Arzu Celik's Neuroscience Laboratory

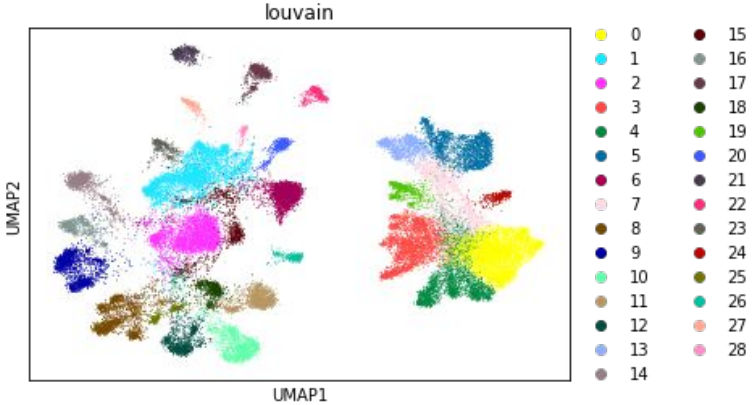
Results: Clustering with leiden algorithm

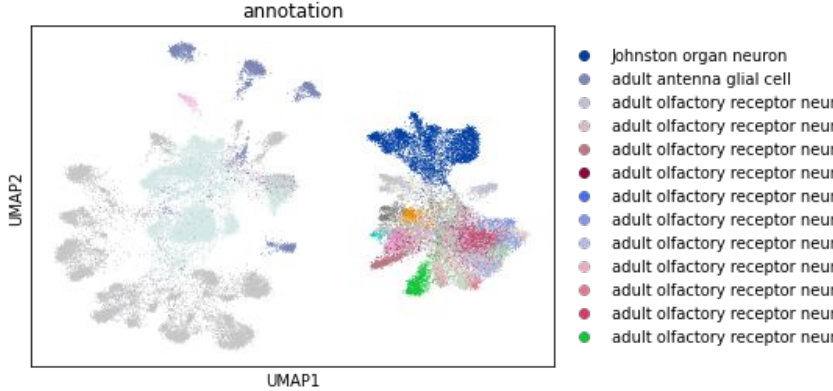


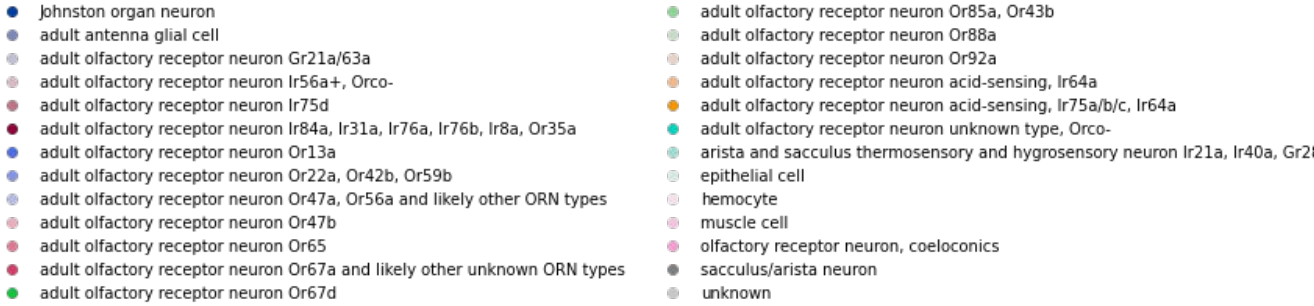
Results: Clustering with different parameters

- Default metrics are used. distance metric is euclidean and number of neighbors is 15. Number of principal components are 0.



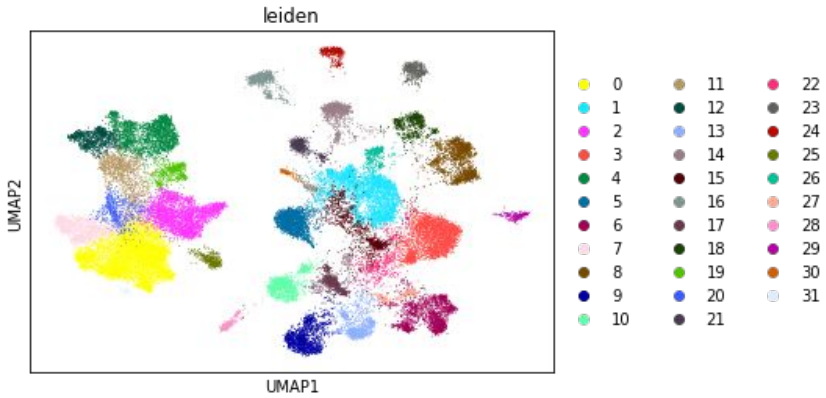


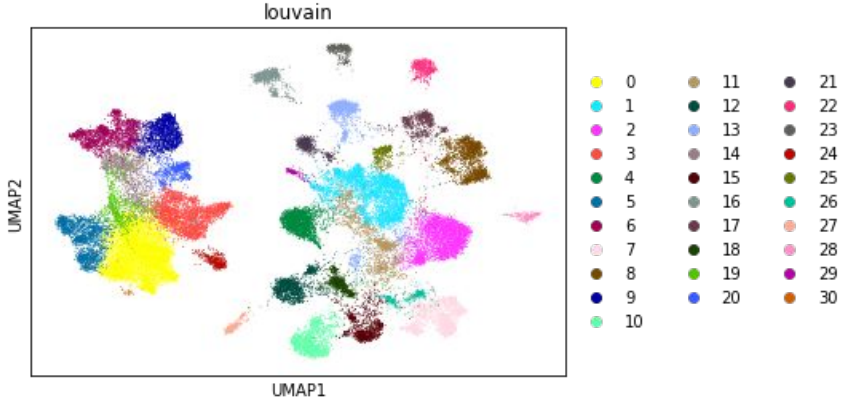


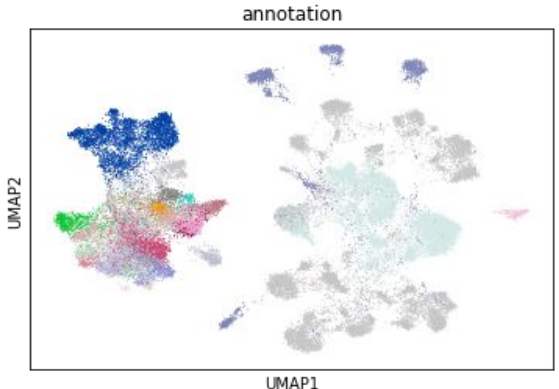


Results: Clustering with different parameters

- Number of neighbors is decreased to 10, number of PCs set to be 31 and metric used is euclidean distance.







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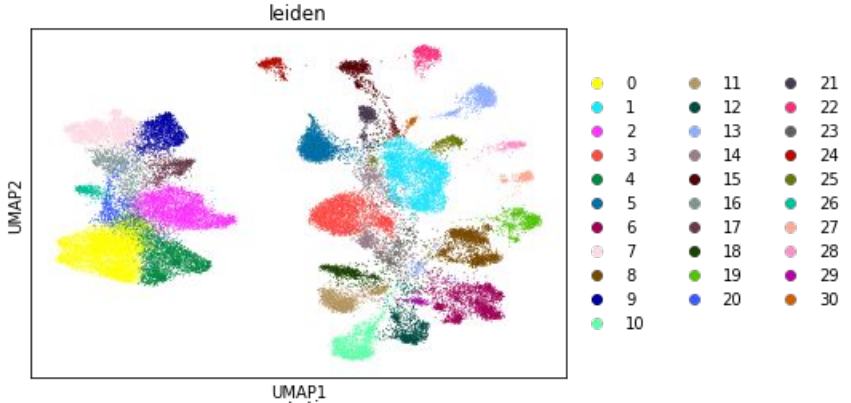
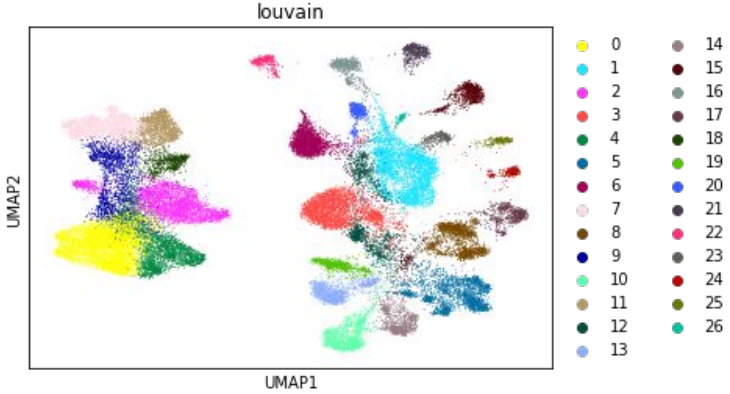
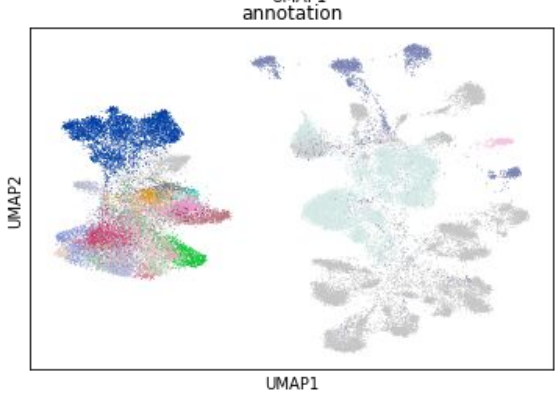
epithelial cell
hemocyte
muscle cell
olfactory receptor neuron, coeloconics
sacculus/arista neuron
unknown

Results: Clustering with different parameters

- Number of neighbors is 15, number of PCs set to be 31 and metric used is euclidean distance.

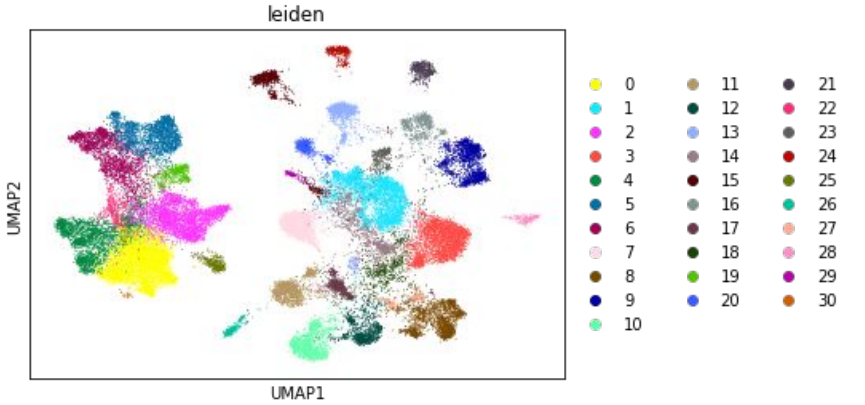


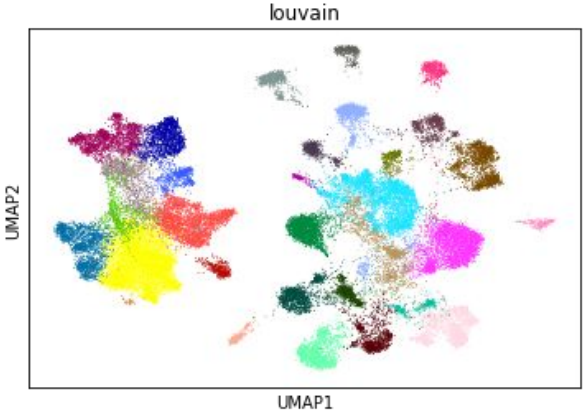


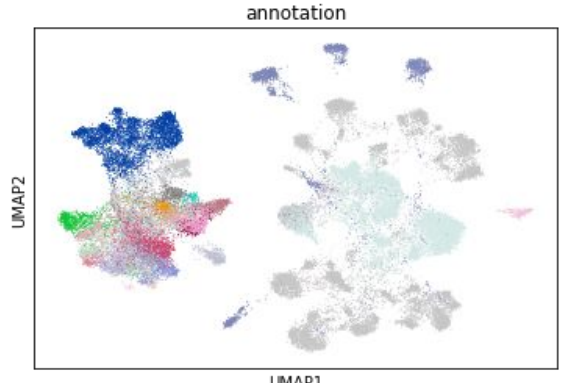


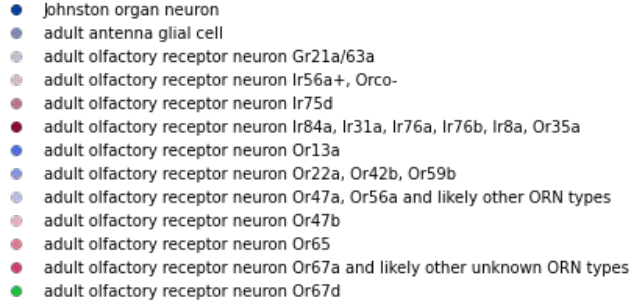
Results: Clustering with different parameters

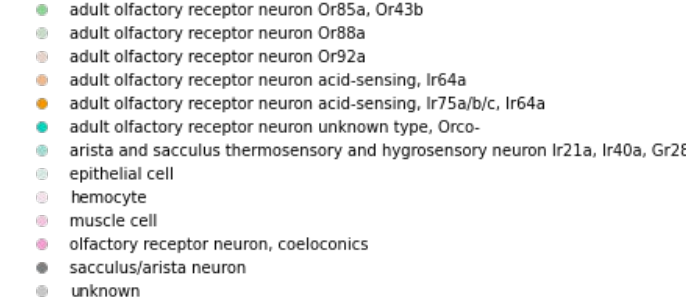
- Number of neighbors is 15, number of PCs set to be 31 and metric used is correlation distance.



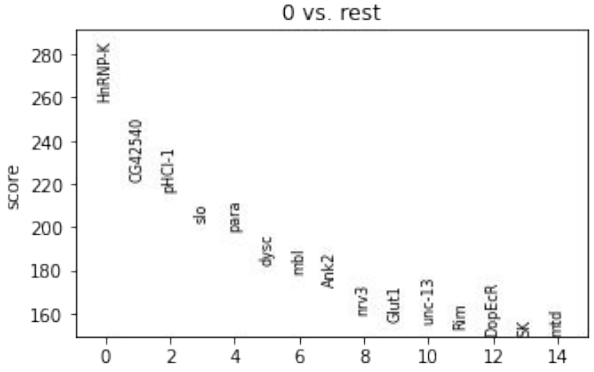


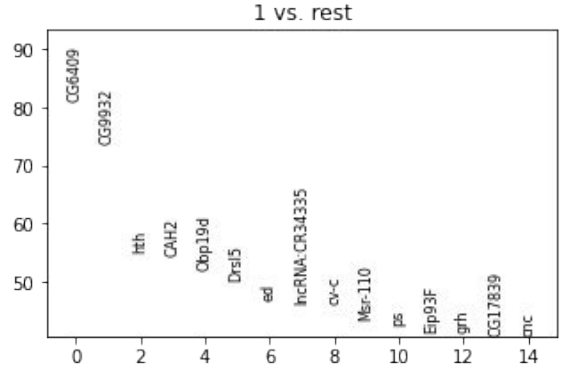


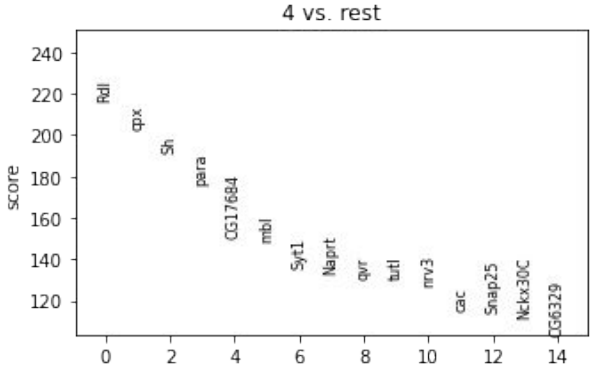


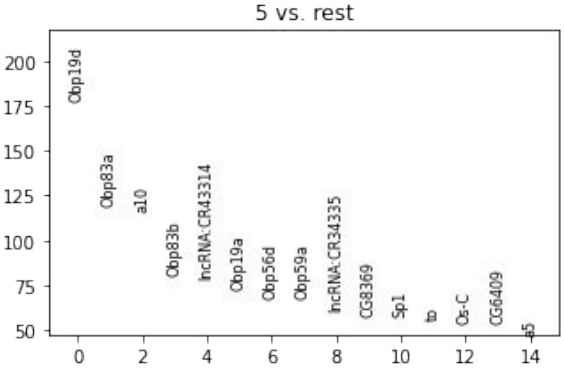


Results: Marker Genes

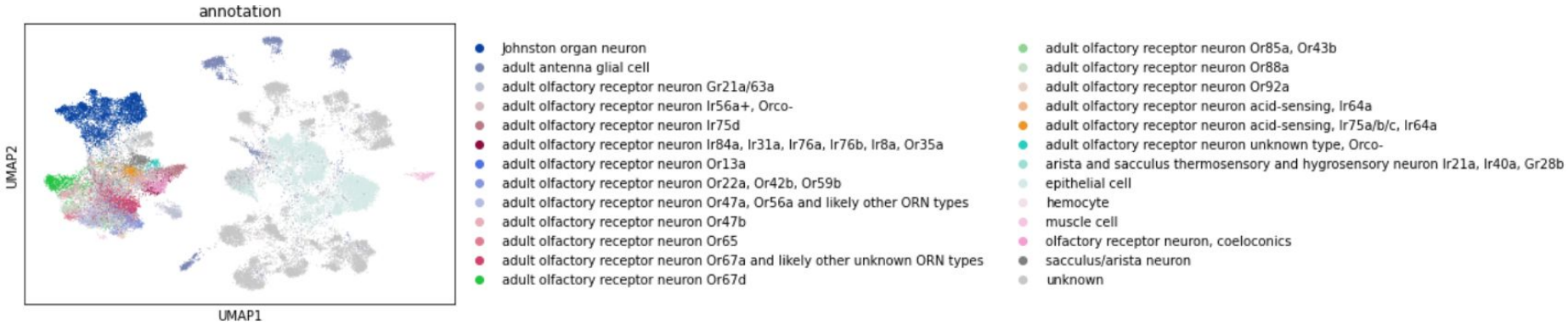




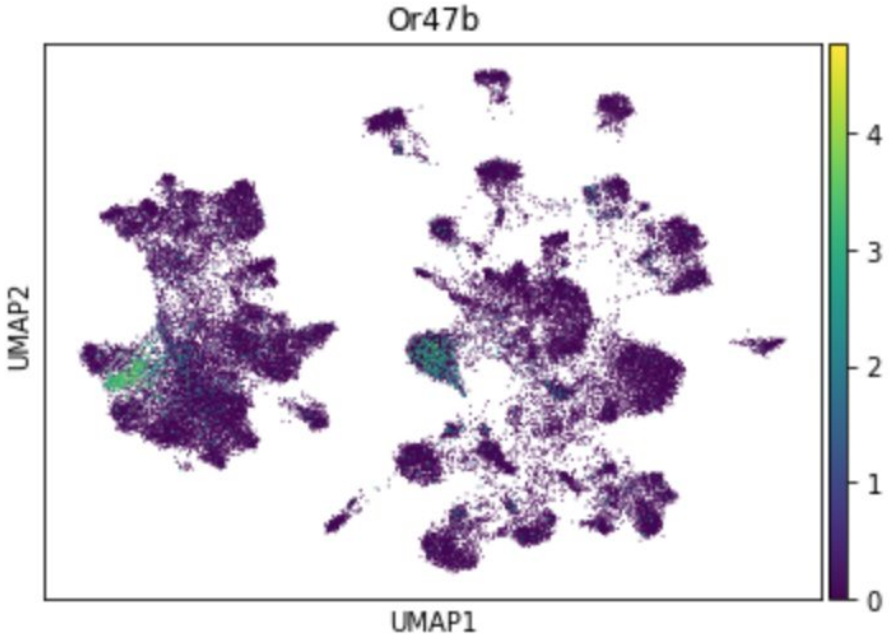




Results: Human annotation off cell types



Results: Expression pattern of Or47b gene across all the clusters



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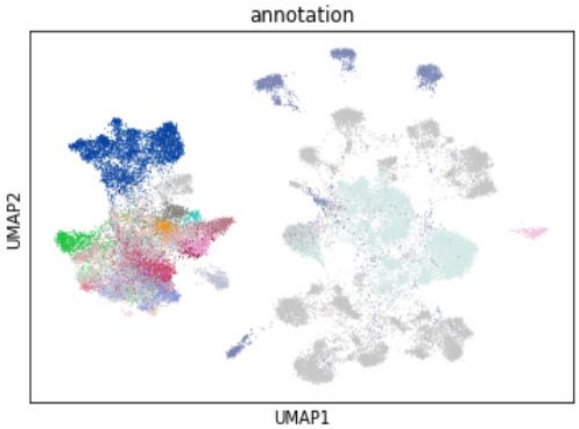
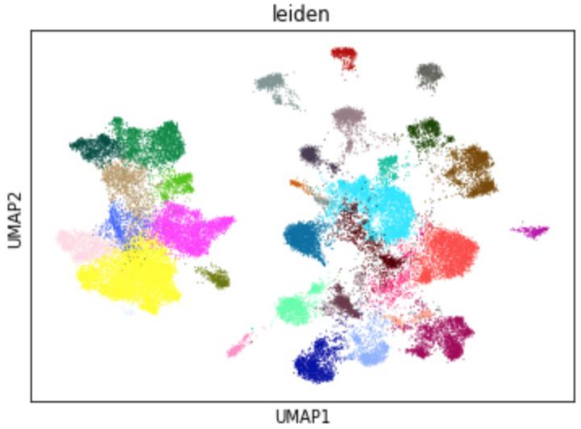
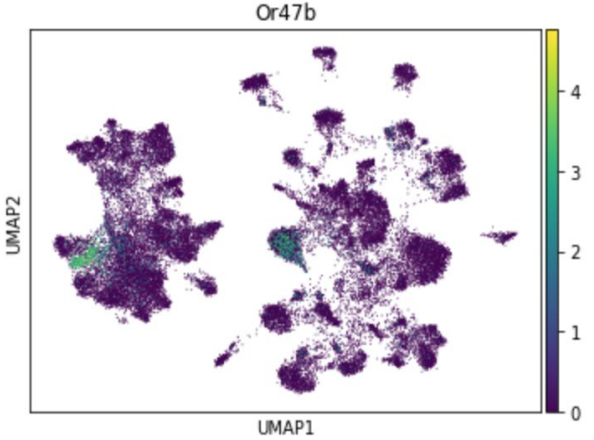
Results: Comparison of Or47b expression with clusters

Leiden Algorithm, number of neighbors=10, metric used is euclidean distance.







Results: Number of various cell types in the clusters

	Cluster 0	Cluster 1	Cluster 2
adult olfactory receptor neuron Or22a, Or42b, Or59b	649	0	0
adult olfactory receptor neuron Or47a, Or56a and likely other ORN types	554	0	2
adult olfactory receptor neuron Or47b	8	0	0
adult olfactory receptor neuron Or67a and likely other unknown ORN types	1181	0	22
adult olfactory receptor neuron Or85a, Or43b	161	0	0

Conclusion and Future Work

- We might try **different approaches for cell type annotation** (ML approaches or different human annotations for the cluster marker genes)

- **Subclustering** the interested clusters

- **Adjusted Rand Index** to quantify cluster similarity

- **Bootstrapping** to improve confidence in clusters

References

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THANK YOU!