Multiomics project
Diagonal integration

Group 1

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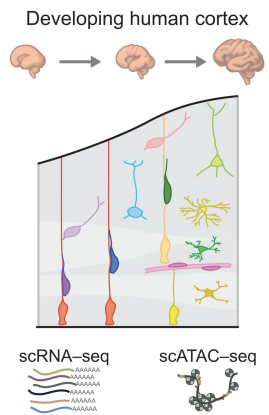
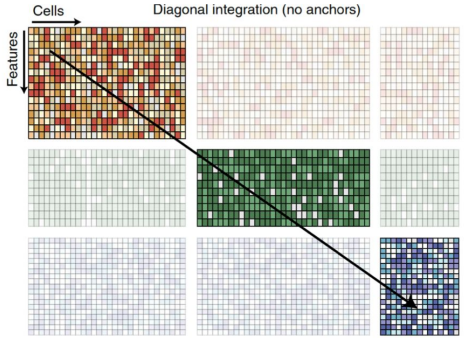
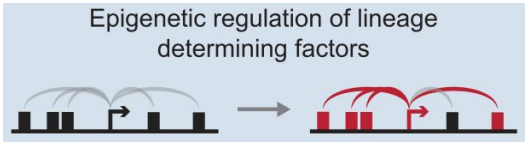
Introduction



Data from different cells, comprising a single cell atlas of
- Gene expression (scRNA-seq)
- Chromatin accessibility (scATAC-seq)

Aims of the analysis:
1. To perform diagonal integration of unmatched scRNA-seq and scATAC-seq data.
2. To associate gene expression to accessibility in the developing human cortex.





Trevino, et al., Cell, 2021

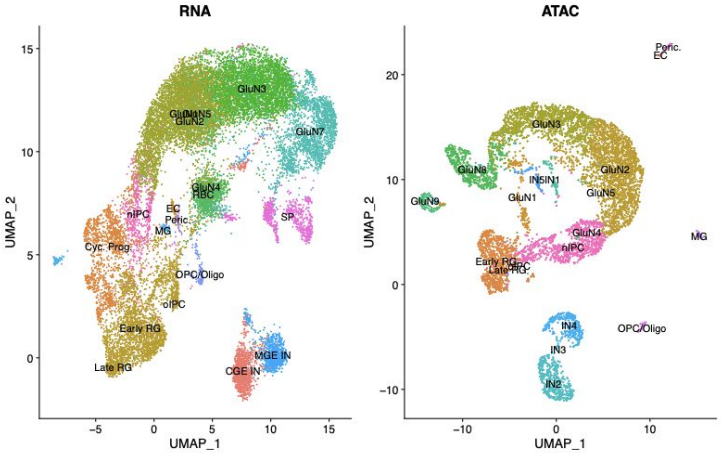
Pre-processing of separate datasets

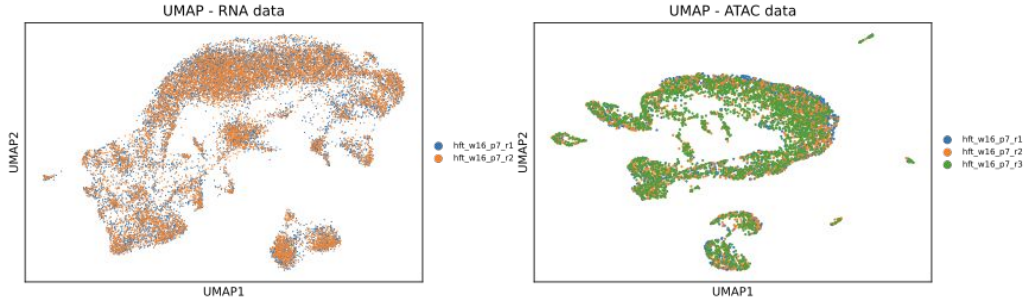
scRNA-seq data:

- Cells were already filtered by % of mito. and total counts.
- Filtered genes (min cells = 3).
- Normalized and log-transformed the raw counts.
- Identified highly variable genes for dimensionality reduction.
- Performed dimensionality reduction with PCA and computed a KNN graph.
- The clusters were previously defined.

scATAC-seq data:

- Some QC metrics were already calculated and used for filtering cells.
- Binarized the data matrix due to sparseness.
- Filtered peaks accessible in < 10 cells.
- Performed dimensionality reduction with Latent Semantic Indexing (LSI) and computed a KNN graph.
- The clusters were previously defined.



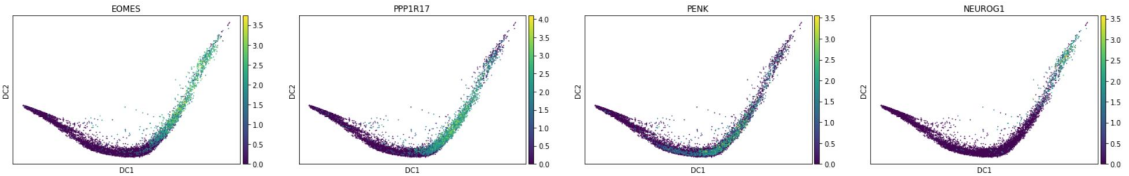


Diffusion pseudotime

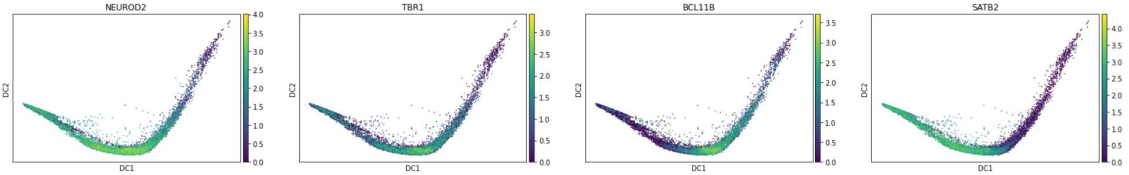
Aim: estimate the order of excitatory neurons along a differentiation trajectory with diffusion pseudotime.

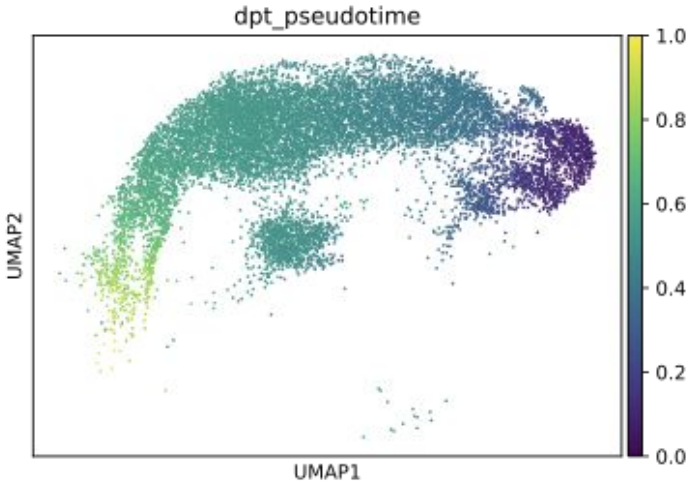
- Subsetted the data to include glutamatergic neurons (GluN) only.
- Performed dimensionality reduction with PCA and computed KNN graph.
- Checked the expression of marker genes along the diff. traj.
- Defined a putative root cell (max of DC1) and plotted diffusion trajectory for GluNs.

Neuronal precursor markers:



GluN markers:





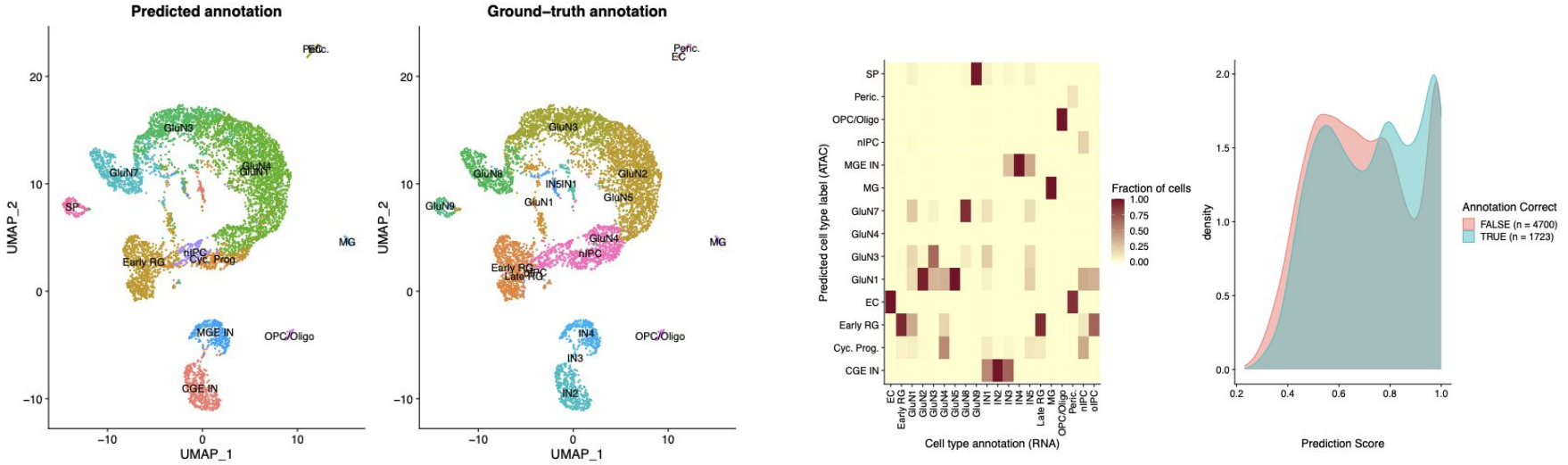
Co-embedding

Aim: annotate cells from the ATAC dataset exploiting the labels of RNA data

How: We infer the cell type annotations for “ATAC cells” through Seurat CCA approach

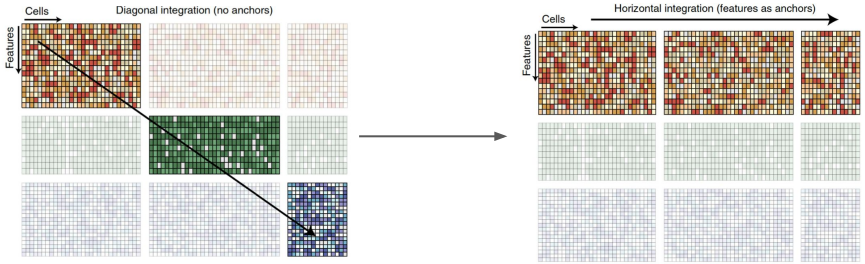
- Estimation of the gene activity based on ATAC data
- Identification of the anchors
- Transfer the metadata (cell annotations)

Comparison between the estimated cell types and the true annotation

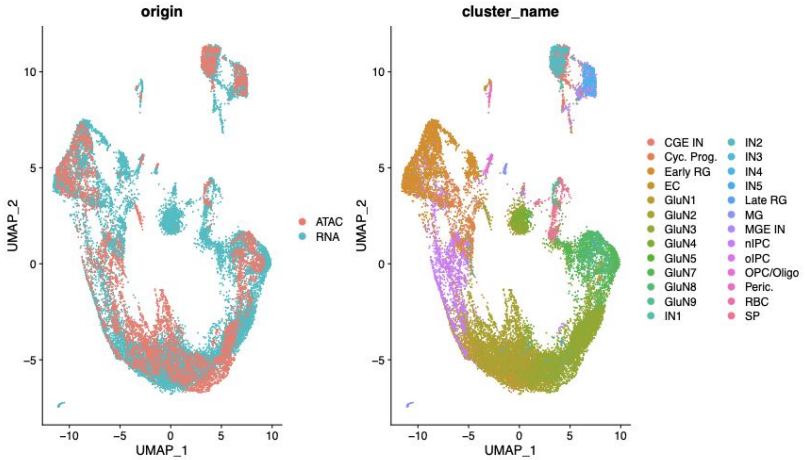


Co-embedding: from diagonal to horizontal configuration

Aim: horizontally integrate the data to have a common embedding space, thus to have gene expression values also for ATAC cells



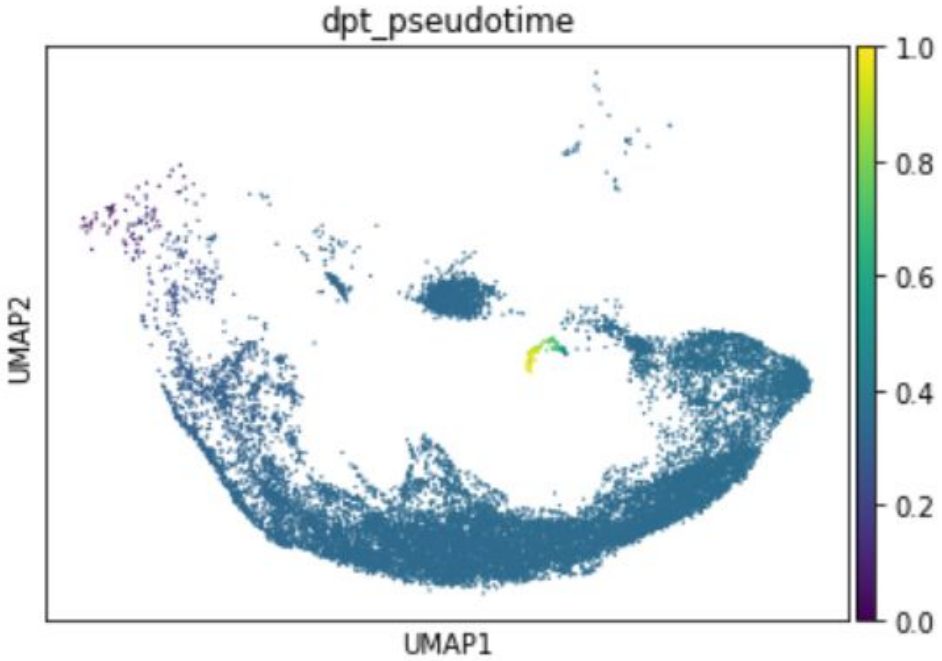
Horizontal integration: Seurat CCA



How: we impute the gene expression value for “ATAC cells” exploiting Seurat CCA approach

- Estimation of the gene activity based on ATAC data
- Identification of the anchors
- Transfer the gene expression values

Co-embedding to define a pseudotime ordering of differentiating glutamatergic neurons from nIPCs



- Once we have a common embedding, we can use **standard similarity-based trajectory inference methods** to order the excitatory neurons in pseudotime.
- We used the Diffusion Pseudotime implementation in sc.tl.dpt.

Selecting features for chromatin accessibility-expression associations

After having identified a common embedding and a common pseudotime axis, we need to **select the features** that we will use to **associate gene expression to chromatin accessibility**.

Why the feature selection step?

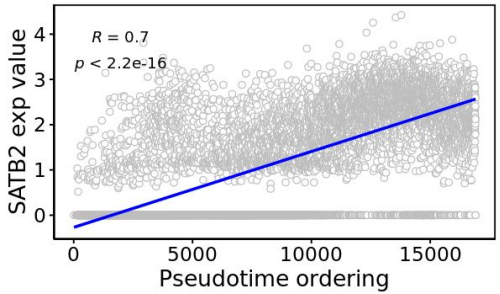
1. Test all the peaks against all the genes is **computationally expensive** + **multiple test burden**
2. **Long range interactions** on the genome are **not very common** (no sense to test for associations between genes and chromatin regions that are extremely far apart e.g. on different chromosomes)

Gene Expression

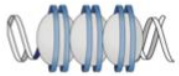
Aim: Select a subset of interesting genes that seem to have a **dynamic behaviour** in the differentiation trajectory

How: We **correlated** the log-normalized gene expression to the value of pseudotime (Spearman correlation)

SATB2: known marker of glun differentiation - highest correlation value



Chromatin Accessibility



Aim: Select a subset of peaks that could probably be involved in gene expression regulation

How: We subset the possible gene-region pairs to regions within a certain range of the gene (100000 base pairs)

List of gene-peak pairs

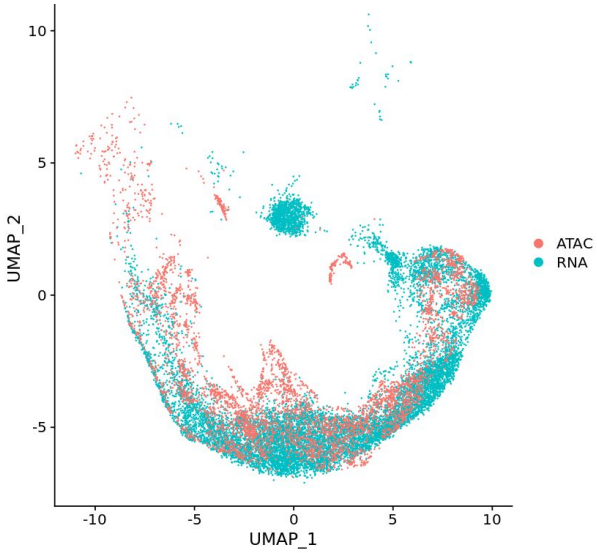
Aggregating expression/accessibility profiles from multiple cells

Why this step?

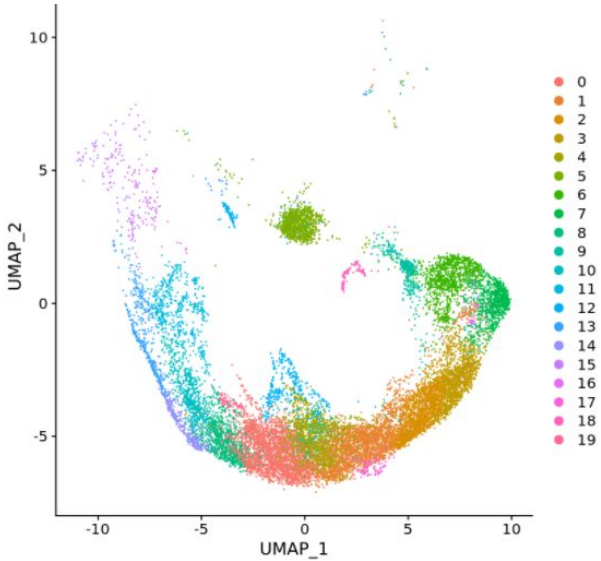
1. To associate gene expression to accessibility (last step) we need the same cell-level unit
2. To deal with the high sparsity of the scATAC profiles
3. To prioritize the most robust associations
4. To reduce the computational burden of testing for associations

Common embedding

(only cells belonging to glutamatergic neuron differentiation trajectory)



Clustering on the co-embedding



For each cluster, summarize expression/ATAC counts

(AggregateExpression function from Seurat package)

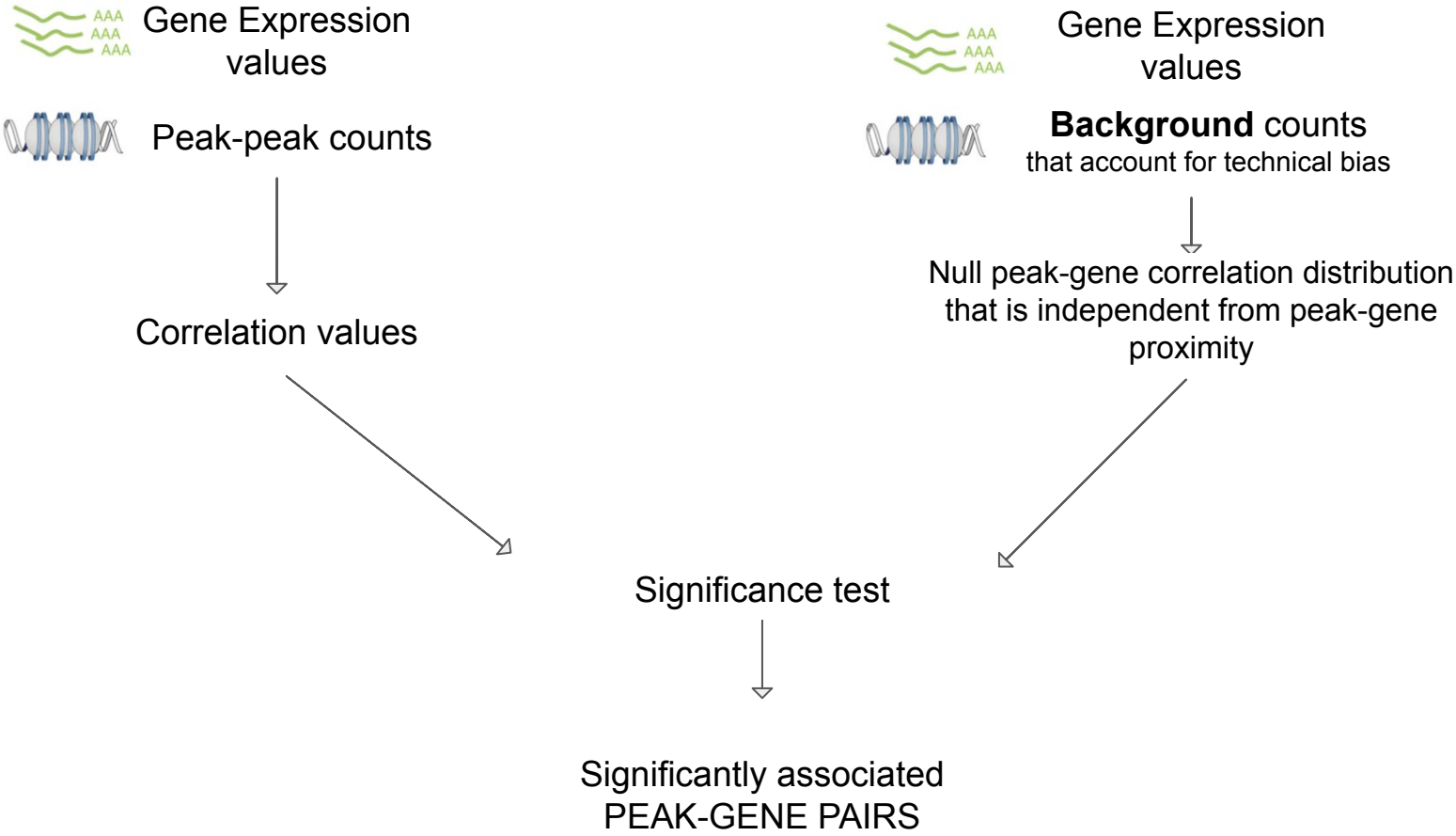
RNA assay

	Cluster 1	Cluster 2	Cluster ...	Cluster M
Gene1	Exp value			
Gene2				
...				
GeneN				

ATAC assay

	Cluster 1	Cluster 2	Cluster ...	Cluster M
Peak1	ATAC count			
Peak2				
...				
PeakN				

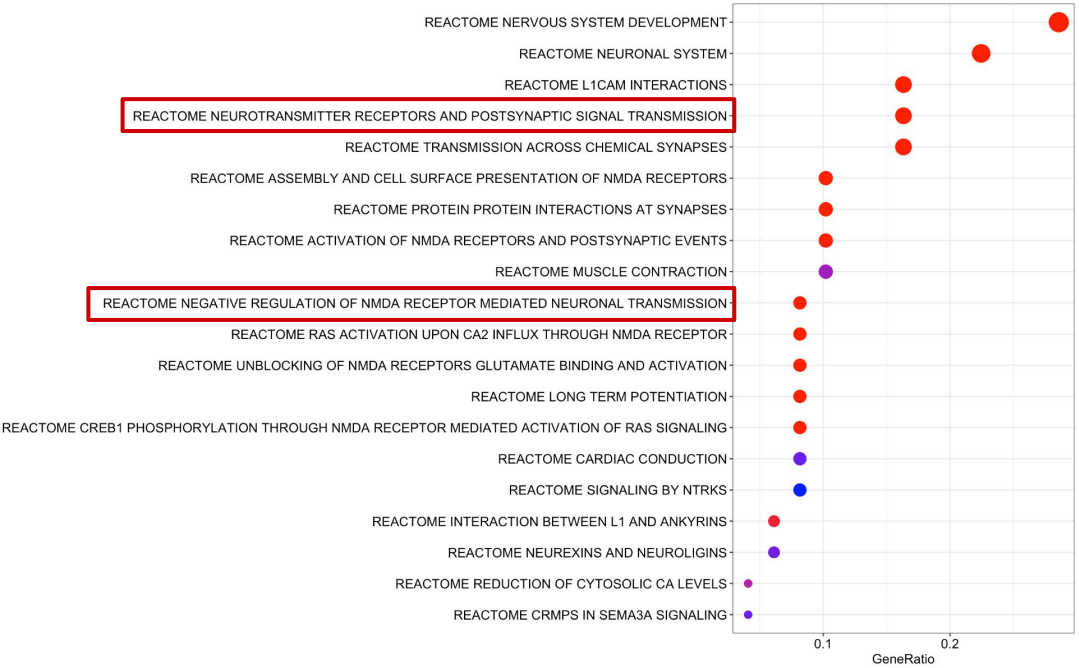
Associating gene expression to accessibility



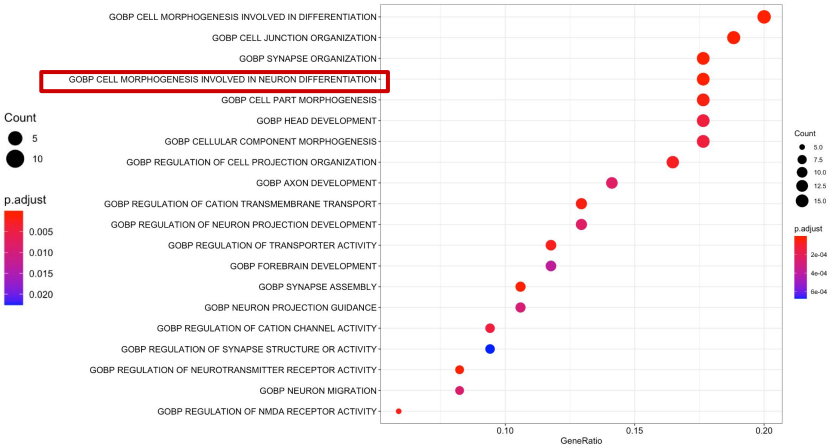
Functional pathway analysis

Over-representation analysis of genes with significant correlations to accessible peaks.

Reactome gene sets



GO: Biological process gene sets



Summary of analysis steps

- Preprocessing of RNA-seq and ATAC-seq data separately
- Co-embedding - from diagonal to horizontal integration (CCA)
- Order excitatory neurons along a differentiation trajectory (dpt)
- Feature selection:
  - Genes - based on correlation to the trajectory
  - Peaks - subset the possible gene-region pairs to regions within 100000 bp of the gene
- Aggregate expression/accessibility profiles (computational intensity reduction and dealing with ATAC sparsity)
- Identify significant gene expression to accessibility associations

Challenges

- ATAC-seq data is large - lots of memory trouble
- Deciding which method and parameters to choose for:
  - Co-embedding (e.g. how to count summarize ATAC-seq signal over genes)
  - Feature selection (variable genes/correlation to pseudotime, chromVAR/Cicero…)
  - Aggregation (clustering, subsampling)
- Interoperability between AnnData, SCE, and Seurat

Thank you for your kind attention

and

Thanks to Emma and Charlotte