

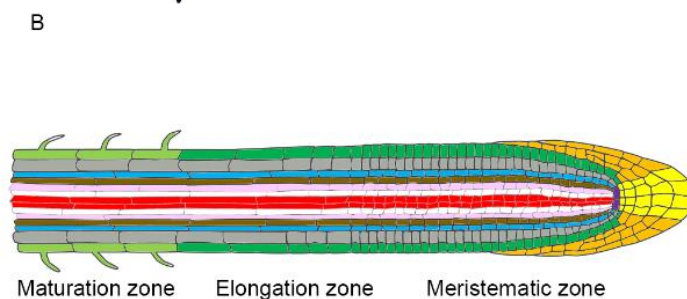
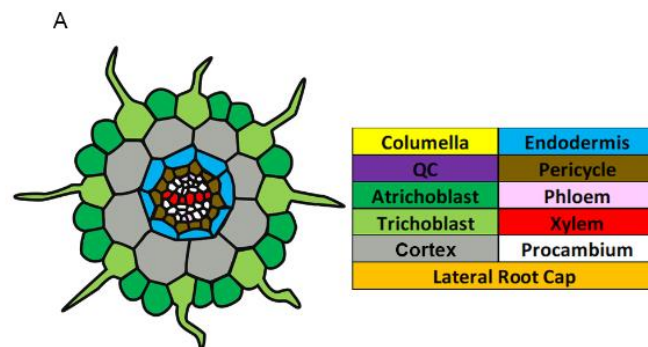
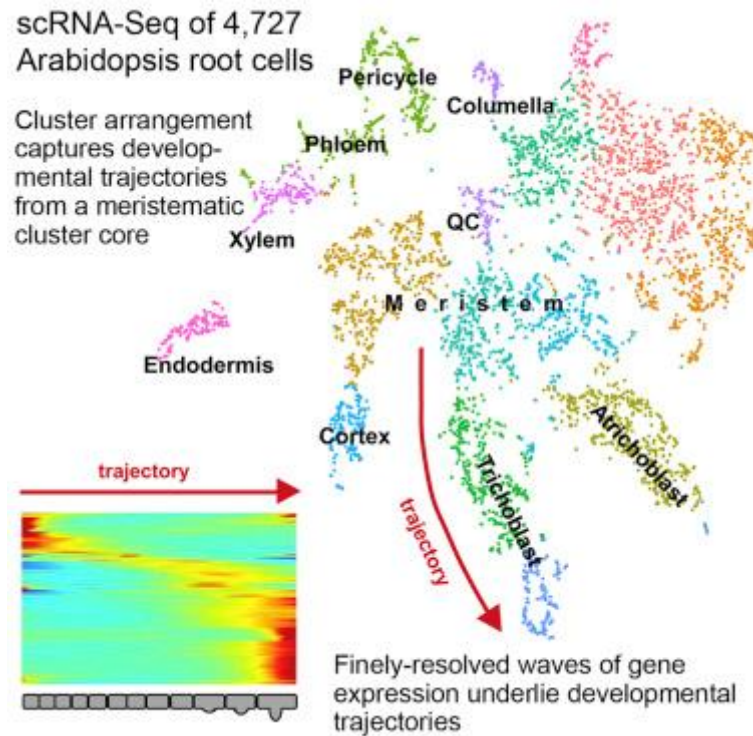
Planning

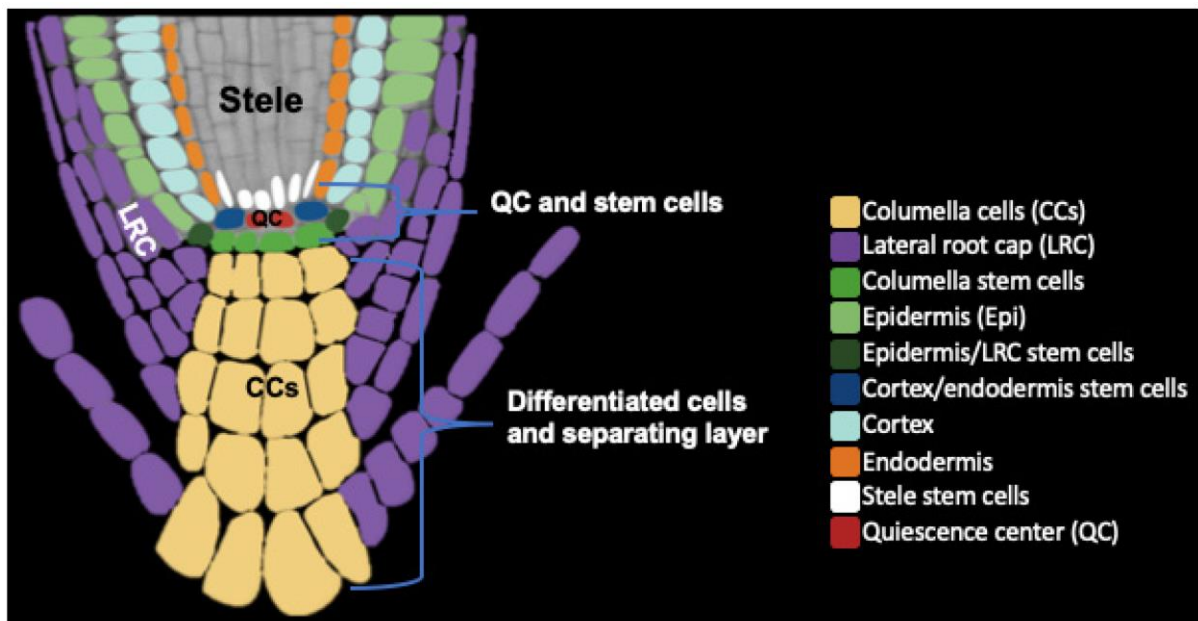
Week	Topic
1	Introduction to RStudio/github and installation, Introduction to Seurat: data exploration of an <i>Arabidopsis</i> single-cell (Denyer et al., 2019) root dataset, dimensionality reduction methods and clustering
2	Cluster identification and data simulation tools
3	Introduction to trajectory inference (slingshot) and differential expression (tradeseq) on simulated data
4	Trajectory inference and differential expression on <i>Arabidopsis</i> data
5	Go enrichment and visualization, exploration of specific topic
6	Exploration of specific topic

Specific topics: Polyploidy mapping, Dynamic gene regulatory network inference, integration of different datasets, trajectory alignment between different datasets, probabilistic pseudotime inference

Spatiotemporal Developmental Trajectories in the Arabidopsis Root Revealed Using High-Throughput Single-Cell RNA Sequencing

<https://doi.org/10.1016/j.devcel.2019.02.022> (Denyer et al., 2019)





Columella cells contain starch-filled plastids, and the settling of these amyloplasts has been proposed to **constitute the initial act of gravity perception in plants.**

The plant stele consists of the primary vascular system of the plant axis.

Some other Arabidopsis root single-cell datasets:

Wendrich et al. (2020), <https://doi.org/10.1126/science.aay4970>

Ryu et al. (2019) : <https://doi.org/10.1104/pp.18.01482>

Zhang et al. (2019) : <https://doi.org/10.1016/j.molp.2019.04.004>

General workflow

https://satijalab.org/seurat/articles/pbm3k_tutorial.html

Analysis on Denyer et al., 2019 (Python) : <https://training.galaxyproject.org/training-material/topics/single-cell/tutorials/scrna-plant/tutorial.html>

QC, normalization, feature selection

<http://bioconductor.org/books/3.13/OSCA.basic/quality-control.html>

Dimensionality reduction

General introduction on dimensionality reduction for single-cell data:

<http://bioconductor.org/books/3.13/OSCA.basic/dimensionality-reduction.html>

“One might wonder whether the results of such extreme data compression can be trusted. Indeed, some of our more quantitative colleagues consider such visualizations to be more artistic than scientific, fit for little but impressing collaborators and reviewers!”

	Local structure	Global structure
Linear		PCA
Non-linear	t-SNE, UMAP	Diffusion map, Advanced manifold methods (Pacmap)

Understanding UMAP: <https://pair-code.github.io/understanding-umap/>

PaCMAP: <https://towardsdatascience.com/why-you-should-not-rely-on-t-sne-umap-or-trimap-f8f5dc333e59>

“UMAP has a lot of advantages over t-SNE in that it better preserves global structure, is faster, and has slightly more interpretable hyper parameters, but at its core it uses distances between nearest neighbors in the construction of the graph, and as such focuses on *local* structure.”

Storing a custom dimensionality reduction calculation:
https://satijalab.org/seurat/archive/v3.0/dim_reduction_vignette.html

Cell-cycle assignment

<http://bioconductor.org/books/3.16/OSCA.advanced/cell-cycle-assignment.html>

For an example in *Arabidopsis* see <https://doi.org/10.1016/j.devcel.2021.02.021>

Clustering

Seurat implements a graph-based clustering approach:
<http://bioconductor.org/books/3.13/OSCA.basic/clustering.html#clustering-graph>

“The main drawback of graph-based methods is that, after graph construction, no information is retained about relationships beyond the neighboring cells¹.”

Cluster annotation

<http://bioconductor.org/books/3.16/OSCA.basic/cell-type-annotation.html#assigning-cluster-labels-from-markers>

Also look into <https://bioconductor.org/packages/3.16/bioc/html/SingleR.html>

<https://bioconductor.org/packages/release/bioc/vignettes/SingleR/inst/doc/SingleR.html>

<https://bioit3.irc.ugent.be/plant-sc-atlas/root>

<https://www.arabidopsis.org/>

GO-enrichment of clusters

Visualization

UMAP,...

Immediate integration of WT and mutant or after clustering?

https://satijalab.org/seurat/archive/v3.0/immune_alignment.html