THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Introduction to Single Cell RNA-Seq (scRNA-seq)

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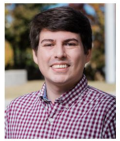

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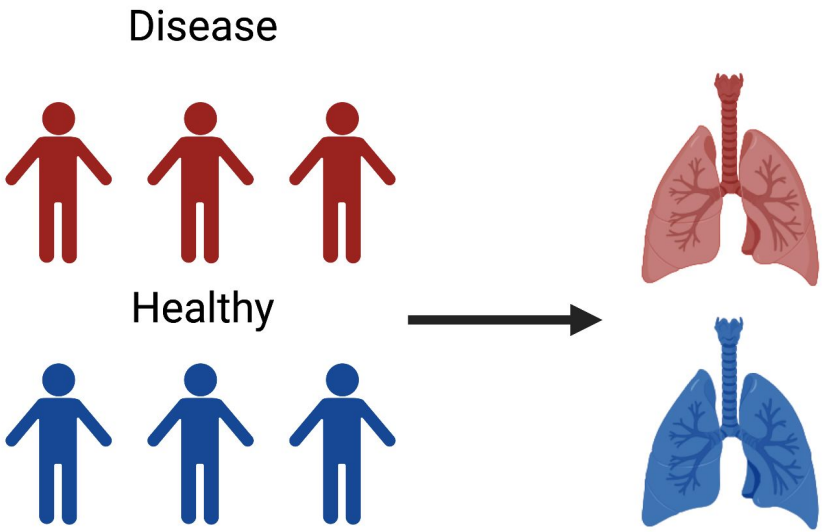
Overview

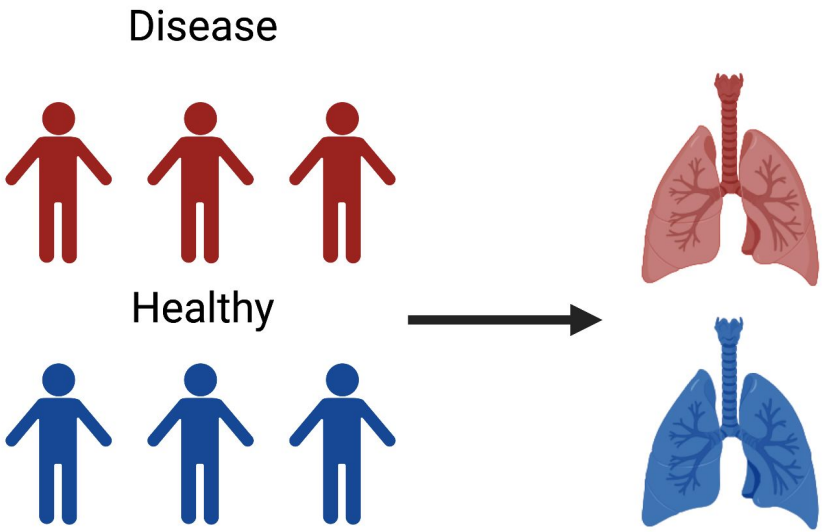
- **What is Single-Cell RNA-Seq?**
  - Most commonly used technologies/platforms
- **Overview of Computational Biology Methods**
  - *Secondary Analysis*
    - 10X Cell Ranger (commercially based pipelines)
    - STARSolo, Alevin (Alevin-fry), kallisto bustools (scientific community)
  - *Tertiary Analysis*
    - Seurat/Scanpy
    - Steps to analyze data
      - Quality control, normalization, dimensionality reduction, cluster identification, data projection, differential expression

Let’s Pretend…



- We’re researchers looking to develop new therapeutics to target an inflammatory lung disease
- Cohort of six individuals *(3 healthy, 3 disease)*

Let's Pretend...



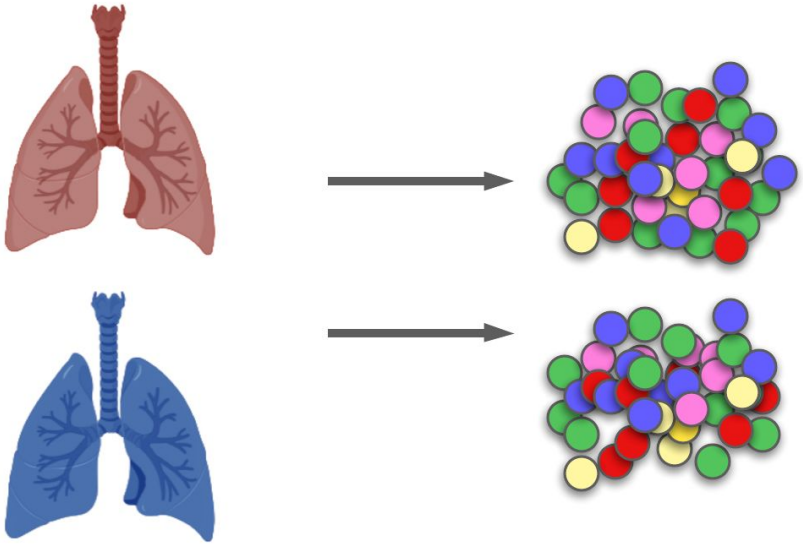
- We have the option of running a single-cell RNA seq analysis or bulk RNA-seq
- We have chosen to go with single-cell…

But why?

Recall from Thursday

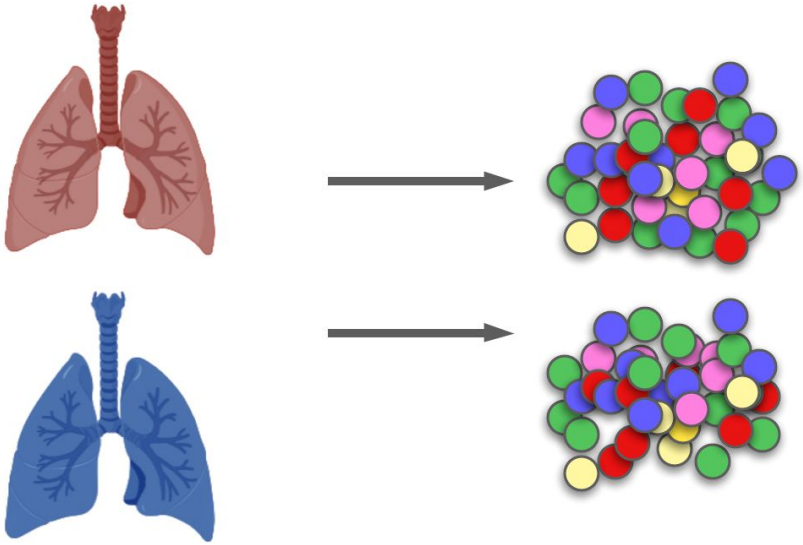
- Bulk RNA-Seq *(or simply "RNA-Seq")*
  - Gives us the average expression across ***all*** cells within a tissue type
  - Confounds any variability within cell-types
  - Gives a high-level picture of the transcriptional landscape
  - Cheaper than single-cell

All Cells Are Not Created Equal



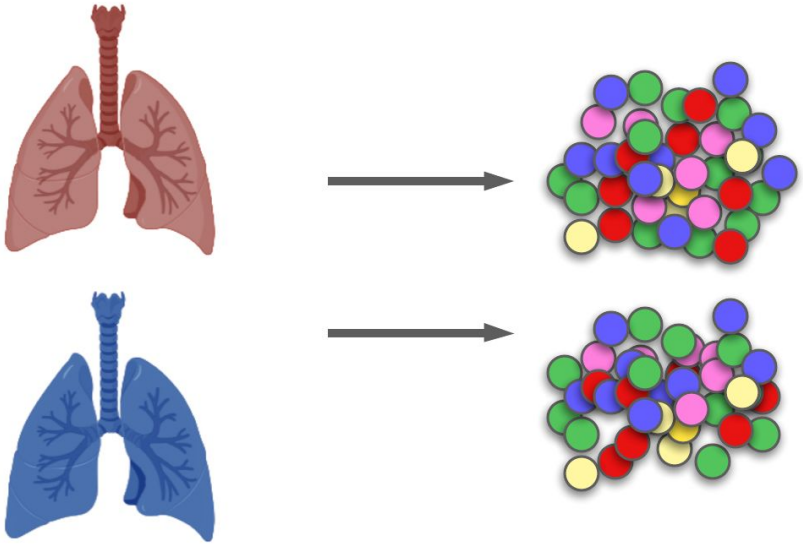
- Lung tissue is comprised of multiple different cell types (over 40..)
- Bulk RNA-Seq doesn’t tell us which cells are the primary drivers of inflammatory gene expression
- Identifying cell-specific expression can lead to a more targeted therapeutic

All Cells Are Not Created Equal



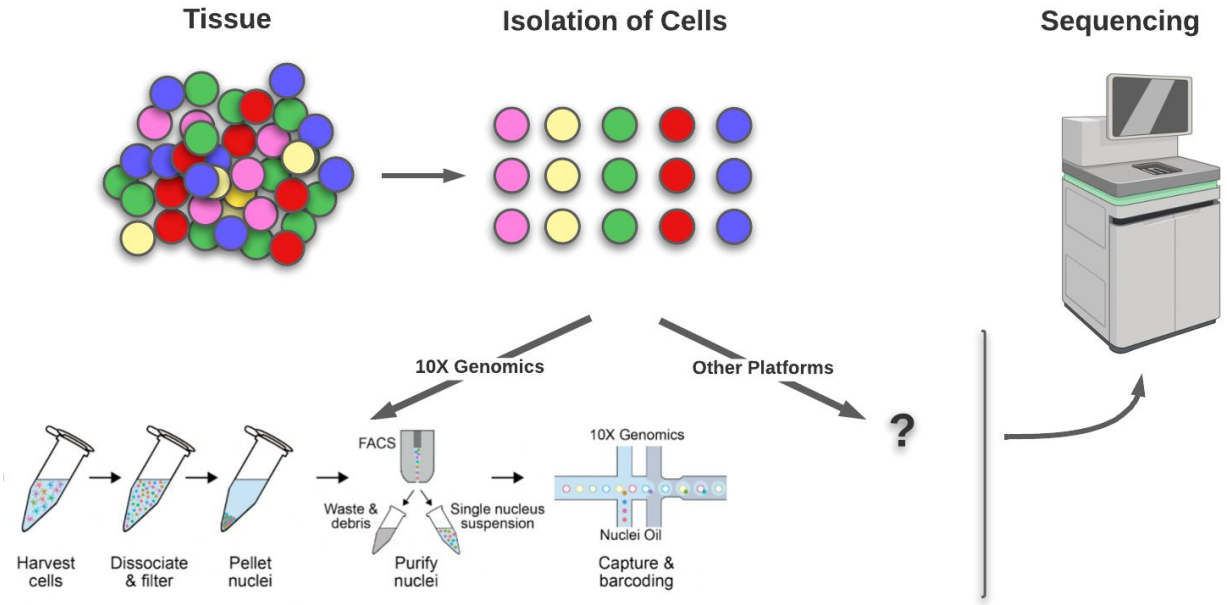
- Through a method called ***Pseudobulk***, we can still answer fundamental question of disease/non-disease and emulate a bulk RNA-seq using single-cell
- We'll touch on this later…

Some More Questions scRNA-Seq Can Answer...



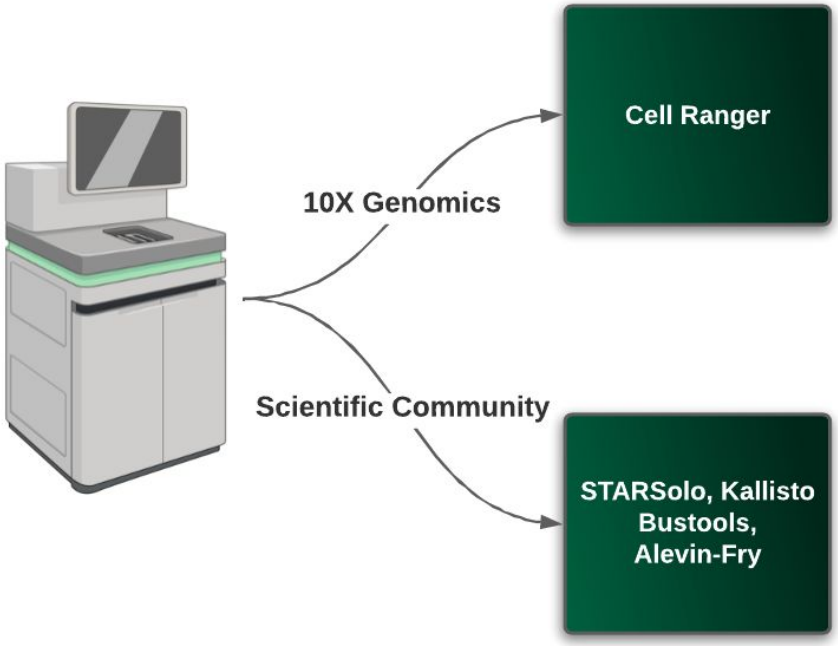
- How does the cellular landscape of healthy individuals differ?
- Are there novel inflammatory cell populations in the disease group?

The Process

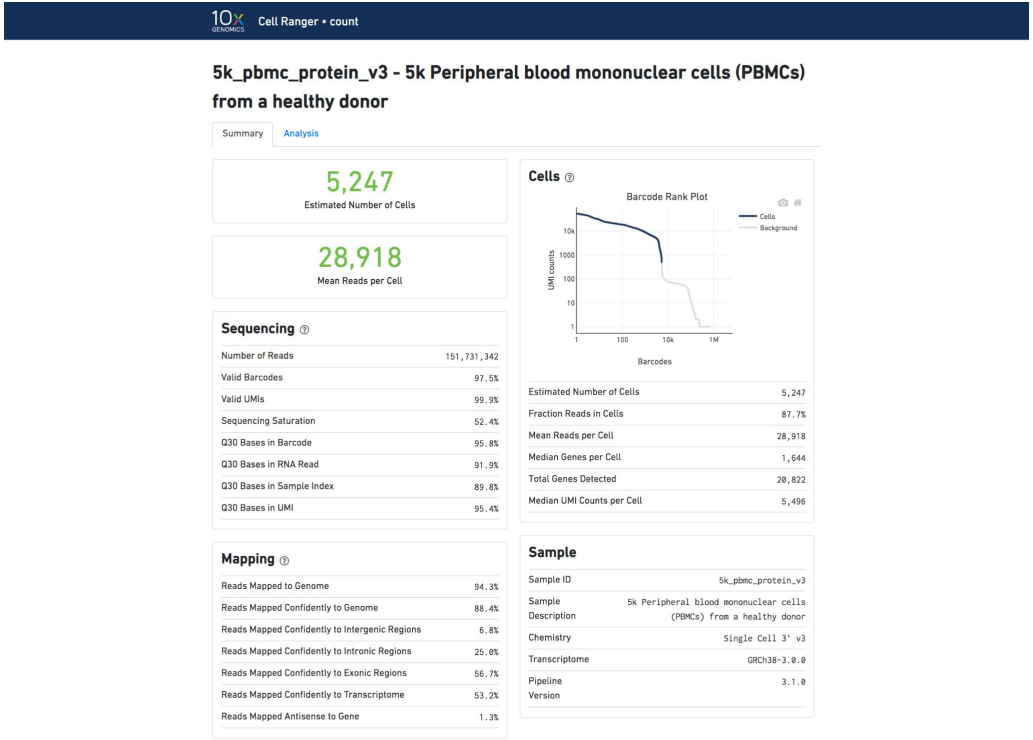


Katherine E. Savell*, Jennifer J. Tuscher*, Morgan E. Zipperly*, Corey G. Duke*, Robert A.Phillips III*, Allison J. Bauman, Saakshi Thukral, Faraz A. Sultan, Nicholas A. Goska, Lara Ianov, Jeremy J. Day (Science Advances, June, 2020). *A dopamine-induced gene expression signature regulates neuronal function and cocaine response* DOI: 10.1126/sciadv.aba4221

The Process



Single-Cell RNA-Seq - 10X Genomics



10x GENOMICS Cell Ranger • count

5k_pbmc_protein_v3 - 5k Peripheral blood mononuclear cells (PBMCs) from a healthy donor

Summary | Analysis

5,247
Estimated Number of Cells

28,918
Mean Reads per Cell

Sequencing

Number of Reads	151,731,342
Valid Barcodes	97.5%
Valid UMIs	99.9%
Sequencing Saturation	52.4%
Q30 Bases in Barcode	95.8%
Q30 Bases in RNA Read	91.9%
Q30 Bases in Sample Index	89.8%
Q30 Bases in UMI	95.4%

Mapping

Reads Mapped to Genome	94.3%
Reads Mapped Confidently to Genome	88.4%
Reads Mapped Confidently to Intergenic Regions	6.8%
Reads Mapped Confidently to Intronic Regions	25.0%
Reads Mapped Confidently to Exonic Regions	56.7%
Reads Mapped Confidently to Transcriptome	53.2%
Reads Mapped Antisense to Gene	1.3%

Cells

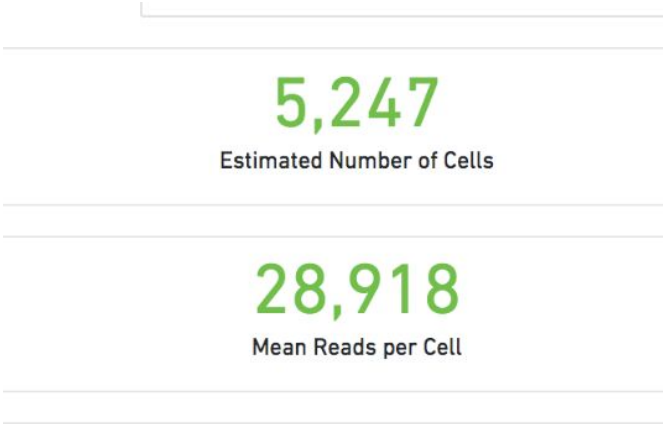
Barcode Rank Plot

Estimated Number of Cells	5,247
Fraction Reads in Cells	87.7%
Mean Reads per Cell	28,918
Median Genes per Cell	1,644
Total Genes Detected	20,822
Median UMI Counts per Cell	5,496

Sample

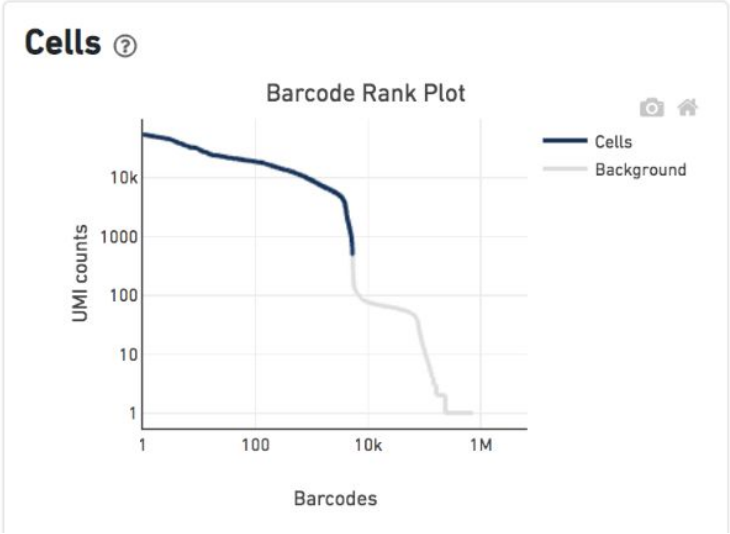
Sample ID	5k_pbmc_protein_v3
Sample Description	5k Peripheral blood mononuclear cells (PBMCs) from a healthy donor
Chemistry	Single Cell 3' v3
Transcriptome	GRCh38-3.0.0
Pipeline Version	3.1.0

Single-Cell RNA-Seq - 10X Genomics



Single-Cell RNA-Seq - 10X Genomics

- Shows distribution of counts and which cells are associated with barcodes.
- We want to see a steep drop from the cells to the background.
- Indicates good separation.



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The Process

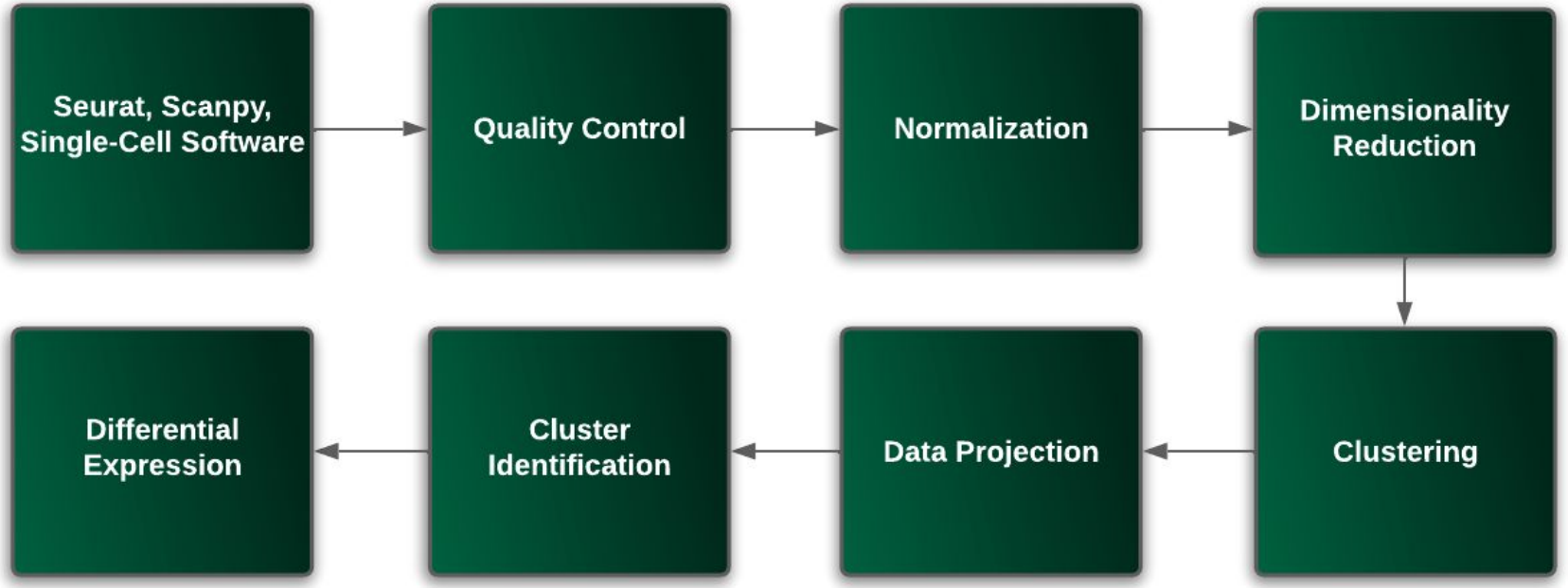
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8
Gene 1	0	12	9	0	0	0	41	30
Gene 2	2	0	0	43	0	0	0	97
Gene 3	0	89	0	0	7	0	0	0
Gene 4	0	0	0	0	0	0	0	0
Gene 5	12	0	0	0	52	0	0	19
Gene 6	0	43	0	77	0	0	60	0
Gene 7	0	25	66	0	0	0	0	0

scRNA-Seq vs Bulk RNA-Seq Gene Matrix

	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8
Gene 1	0	12	9	0	0	0	41	30
Gene 2	2	0	0	43	0	0	0	97
Gene 3	0	89	0	0	7	0	0	0
Gene 4	0	0	0	0	0	0	0	0
Gene 5	12	0	0	0	52	0	0	19
Gene 6	0	43	0	77	0	0	60	0
Gene 7	0	25	66	0	0	0	0	0

Gene	Total Expression
Gene 1	12
Gene 2	18
Gene 3	12
Gene 4	10
Gene 5	23
Gene 6	11

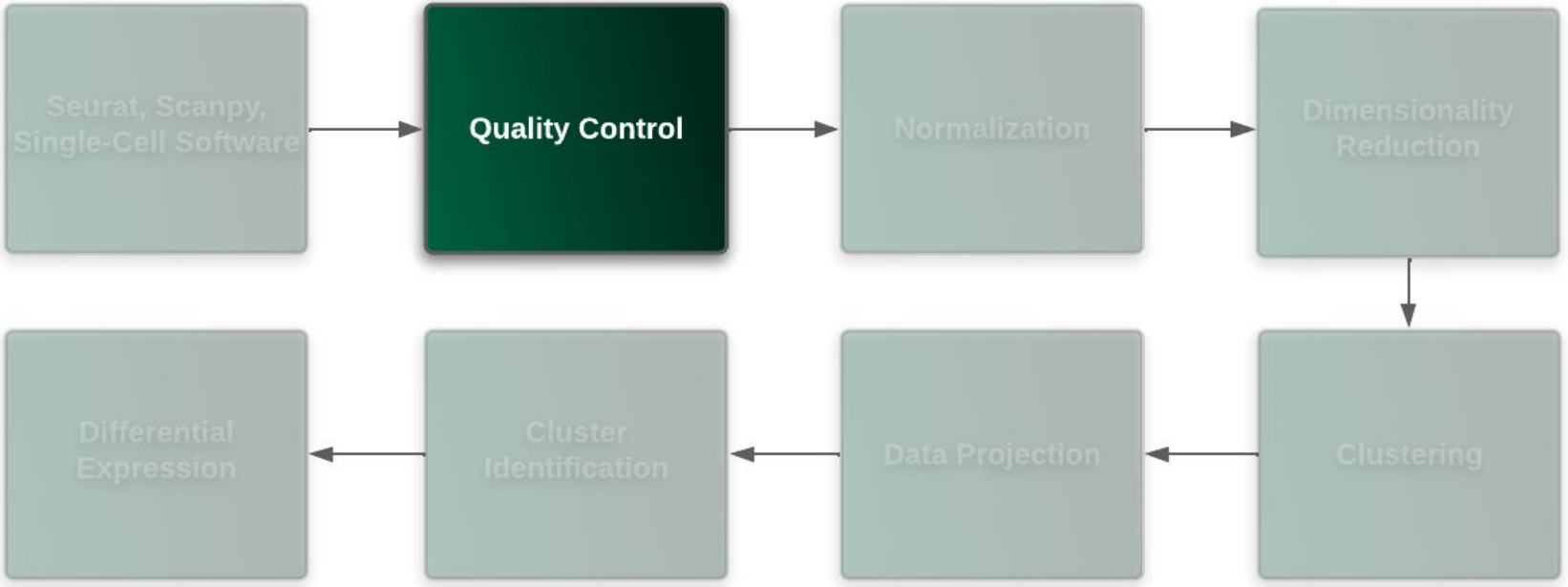
Roadmap



Tertiary Analysis

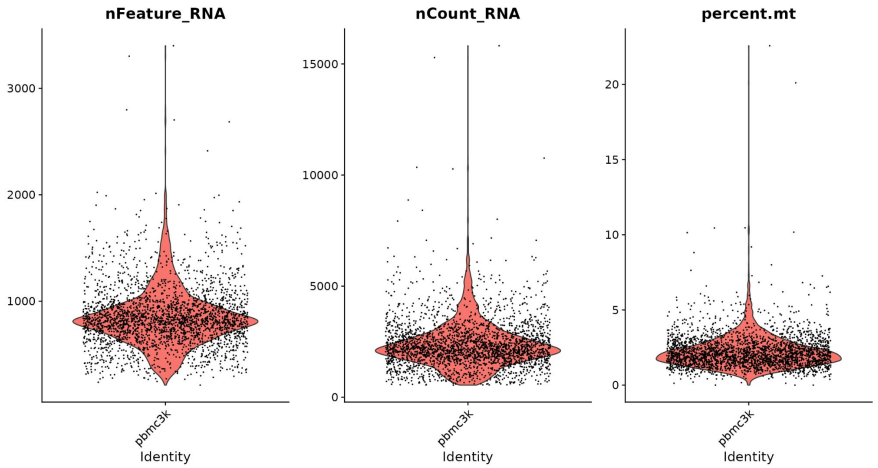
- **Seurat:** R based package for analyzing single-cell data.
- **Scanpy:** Python based package for analyzing single-cell data
- Both employ similar methods,
  - Slight differences in certain algorithms/methods that some their functions use.

Roadmap *(Quality Control)*



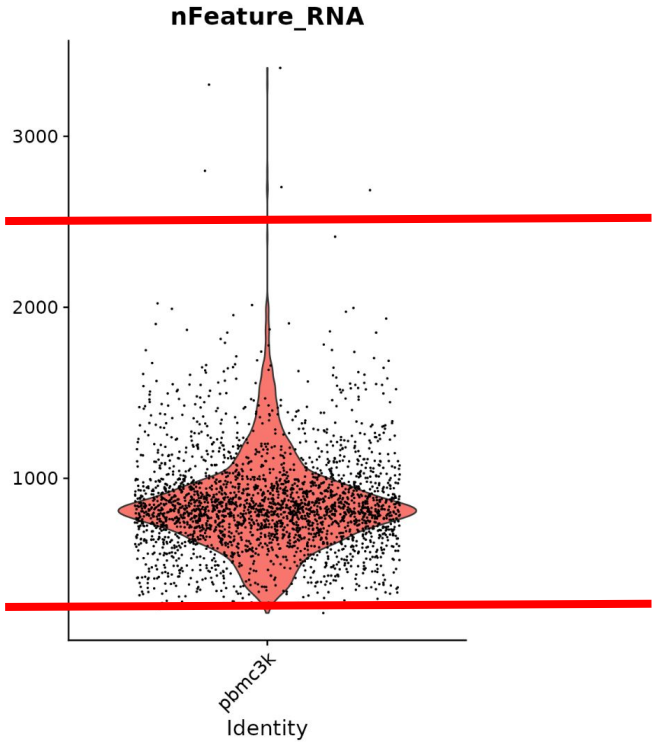
Roadmap *(Quality Control)*

- QC for single-cell is data-driven
- Variable depending on data-type



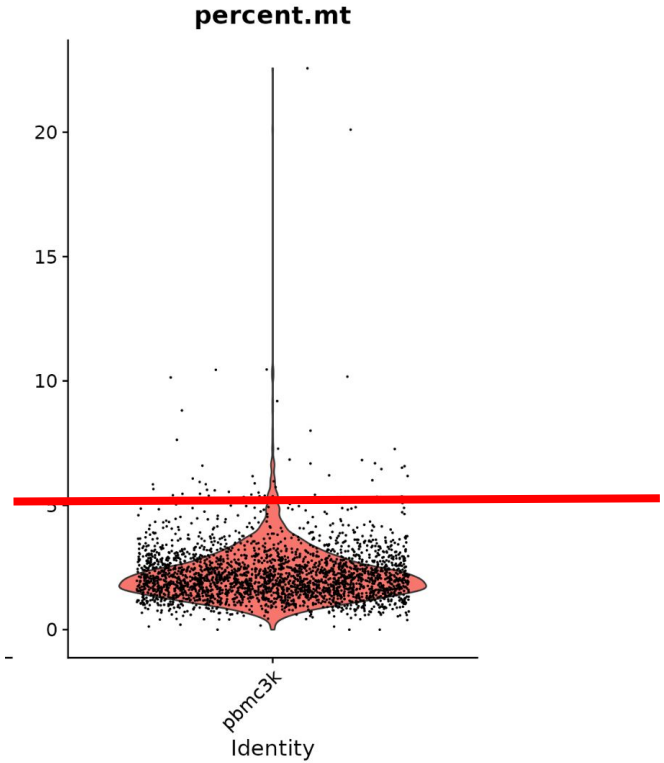
Roadmap *(Quality Control)*

- > 2500 features
- < 200 features

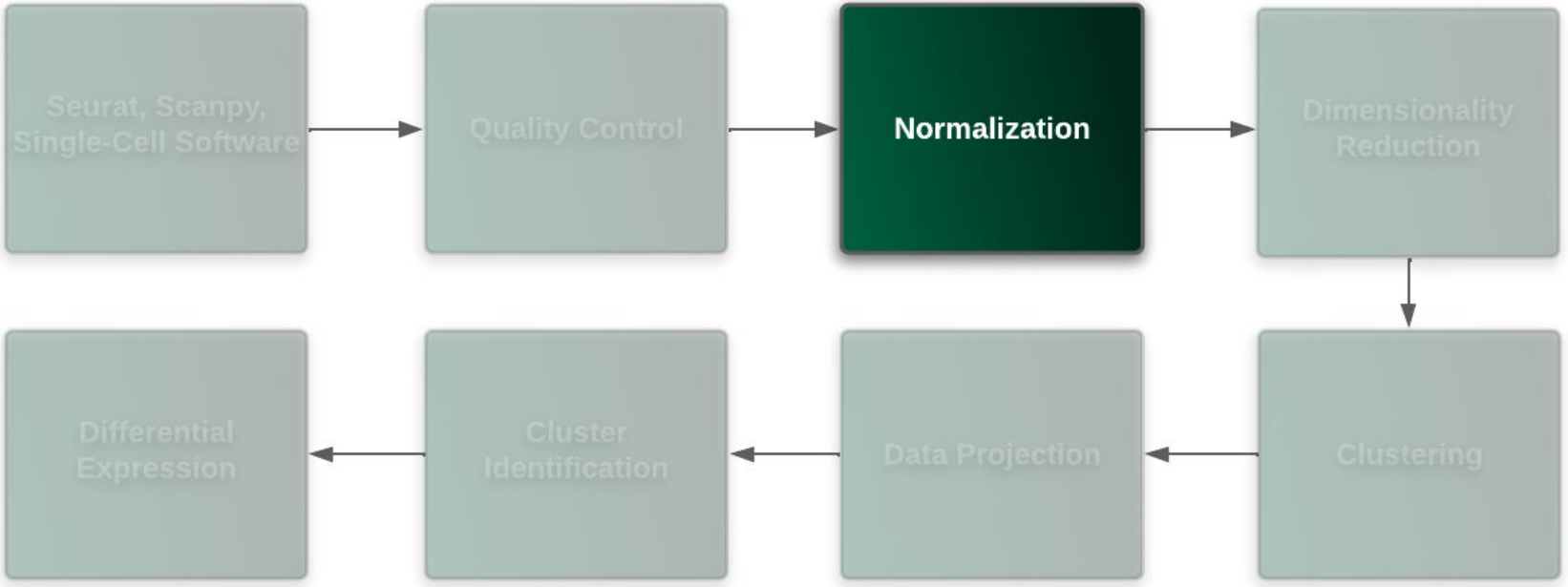


Roadmap *(Quality Control)*

- > 5% MT
- Again variable, cardiomyocytes will have higher percentage MT, etc
- Note: more QCs should be performed beyond what is shown here



Roadmap *(Normalization)*



Roadmap *(Normalization)*

- Inherent bias present.
- Sequencing depth, gene length, etc.
- Normalization removes this bias so that the differences we see from the cells are biological differences.

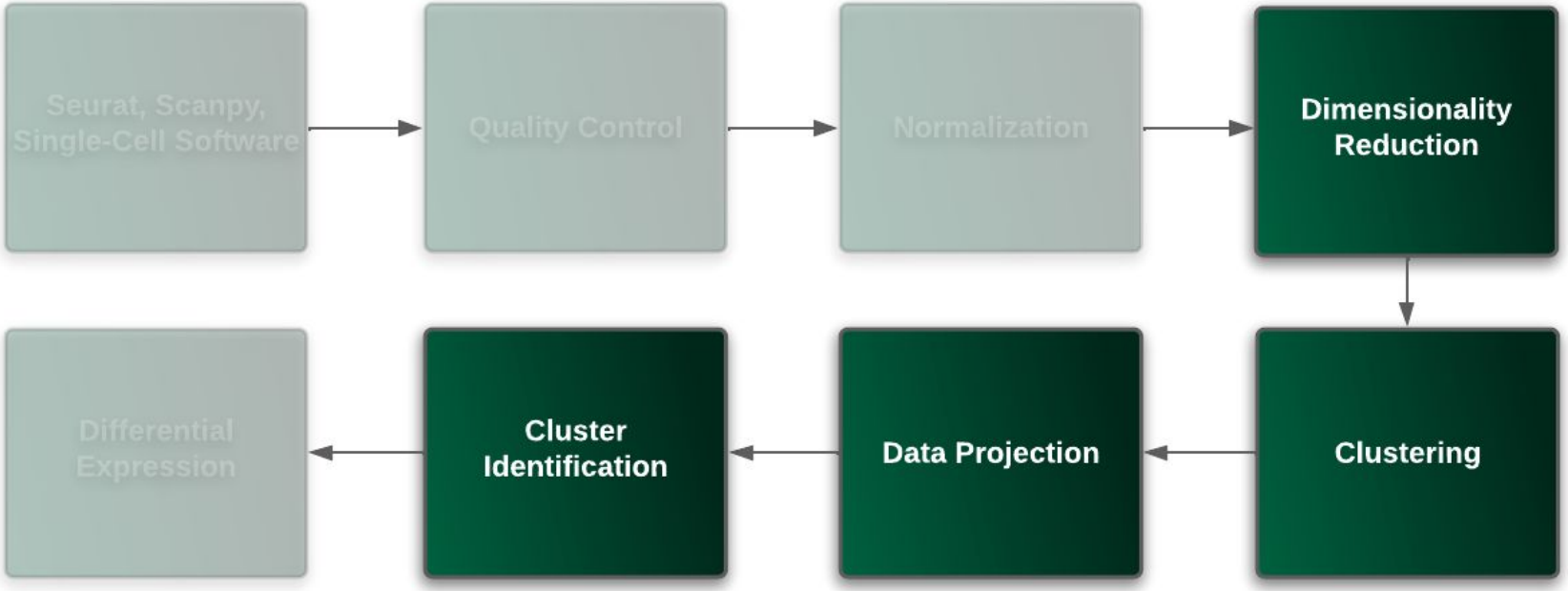
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8
Gene 1	0	12	9	0	0	0	41	30
Gene 2	2	0	0	43	0	0	0	97
Gene 3	0	89	0	0	7	0	0	0
Gene 4	0	0	0	0	0	0	0	0
Gene 5	12	0	0	0	52	0	0	19
Gene 6	0	43	0	77	0	0	60	0
Gene 7	0	25	66	0	0	0	0	0

Roadmap *(Normalization)*

- **Log-Normalization**
  - Counts for each cell are divided by total cell counts, and multiplied by a scale factor. They are then log-transformed.

- **SCTransform (“V2 flavor”)**
  - Normalization Using a Regularized Negative Binomial Model
  - Most commonly used normalization method.

Roadmap *(Dimensionality Reduction, Cluster Identification)*



Roadmap *(Dimensionality Reduction, Cluster Identification)*

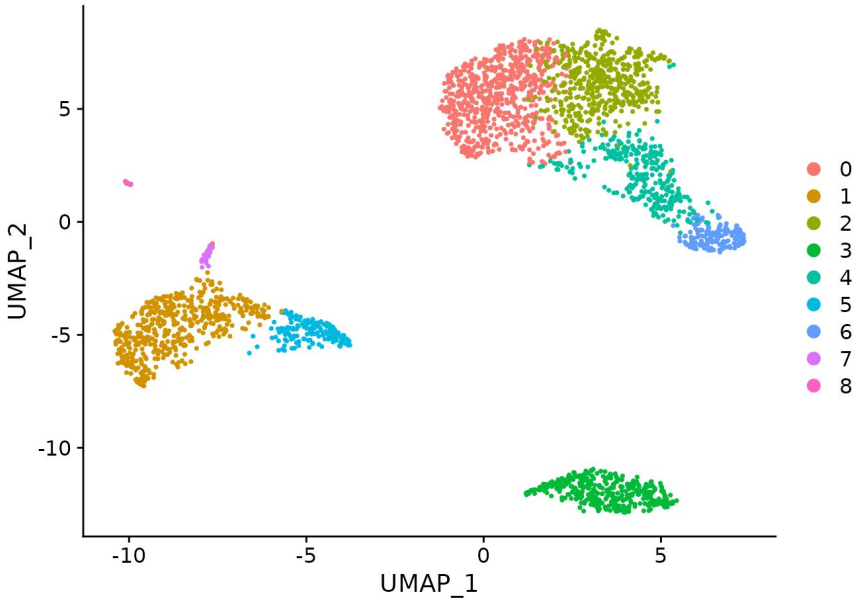
- **Dimensionality Reduction:** Capturing the core variability within the data-set.
- **Clustering:** Finding patterns with cells.
- **Data Projection/Cluster Identification:** Visualizing cell populations and identifying marker genes in each cluster that represent a cell-type.

RoadMap *(Data Projection - UMAP)*

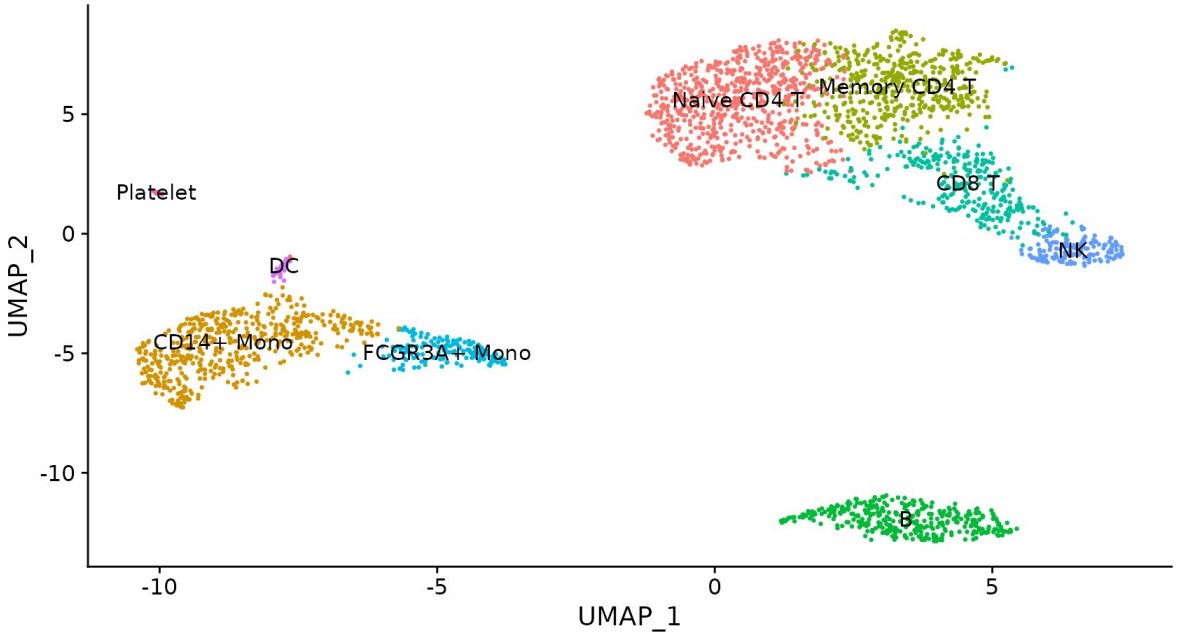
- **UMAP**
  - “data projection.”
  - Non-linear dimensionality reduction technique.
  - Visualizes high-dimensional data in low dimensional space.

- **TSNE**
  - Doesn’t preserve the data as well
  - Computational speed, etc..

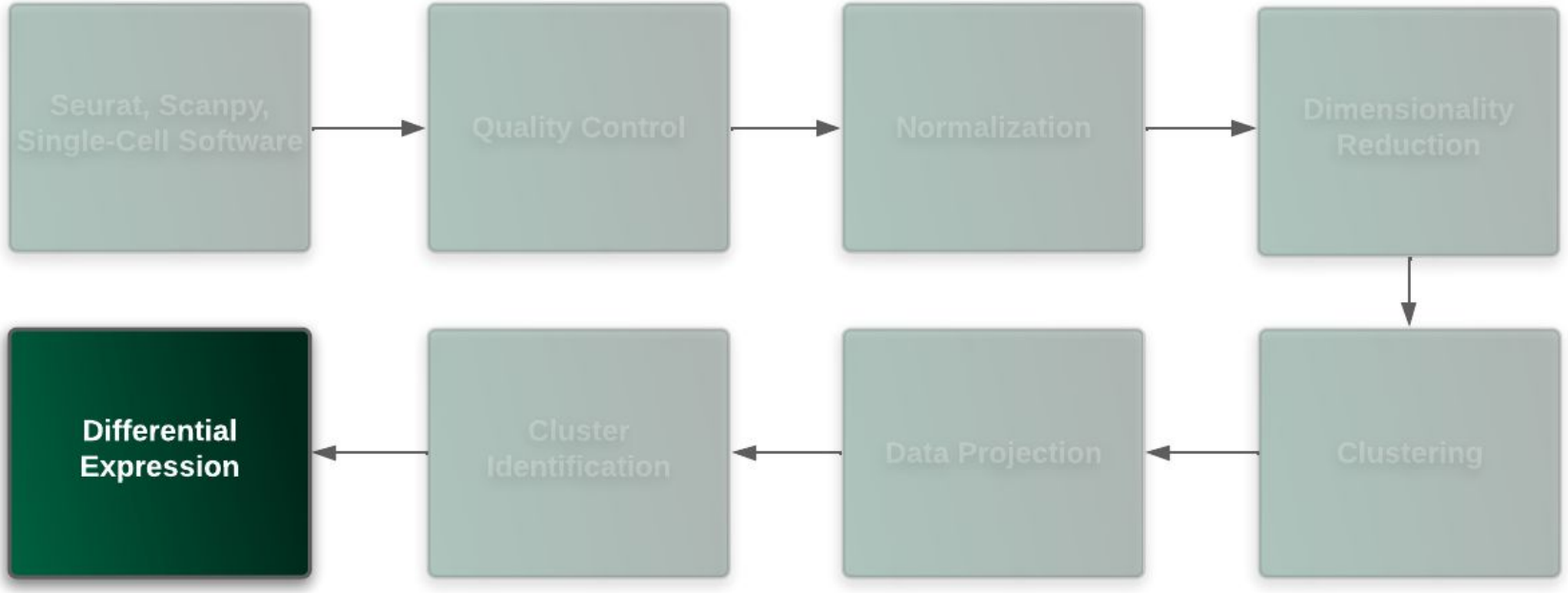
https://pair-code.github.io/understanding-umap/



Roadmap *(Cluster Identification)*



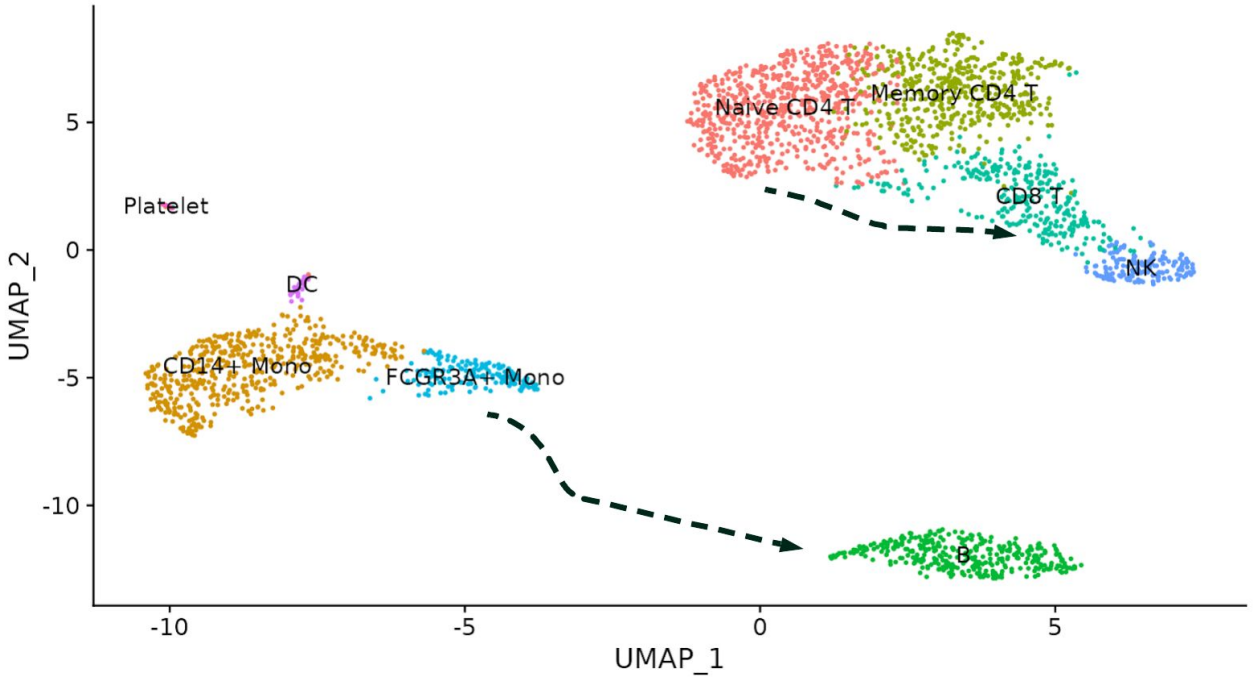
Roadmap *(Differential Expression)*



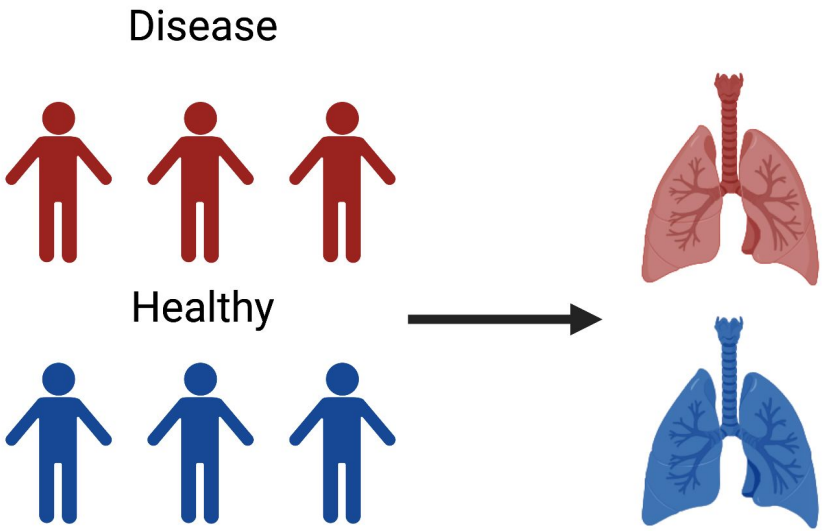
Roadmap *(Differential Expression)*

Differential expression in single-cell can proceed in two ways..

Cluster-Specific DE



Differential Expression Between Phenotypes/Conditions



Pseudobulk

- Aggregation of single-cell counts by cell type for comparison across conditions.
- Allows you to conduct phenotype comparisons (disease vs normal.)
- Useful for situations with lower counts.
- Able to assess biological variation to a greater extent.

Recap on scRNA-Seq

- scRNA-seq allows for novel identification of cell populations, a look into the cell-specific expression landscape, and ability to look at tissue-wide differences (pseudobulk.)
- Single-Cell RNA-Seq is ***evolving fast***.
- Multimodal approach - simultaneously measure transcriptomics and cell-surface protein, chromatin accessibility, etc.

Links To Sample Data

- 10X Genomics Data
- Seurat Data
- Seurat Data Package

U-BDS

Data Science Office Hours

Thursdays 3:30-4:00

Slack ID: C0118620WC8

Training Guides

https://github.com/U-BDS/training_guides