

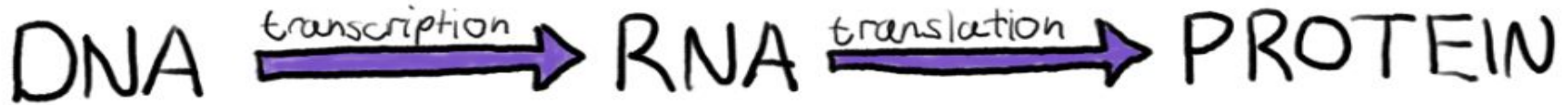
Single Cell RNA Sequence Tutorial

CTSI STEM Project-Adithya Ayanam

The Central Dogma

Central Dogma : Describes the flow of information within a cell, from DNA-> RNA->Protein.

The central dogma



DNA : Deoxyribonucleic acid is the long term data storage of cell and has a double helix structure.

Nucleotides: Each strand of the double helix consists of a series of nucleotide molecules linked by phosphate groups .

Subunits of Nucleotides: A Nitrogenous base, a deoxyribose sugar and a phosphate group.

<https://lazappi.github.io/phd-thesis/1-introduction.html>

The Central Dogma

DNA Nucleotides: Adenine , cytosine, thymine and guanine.

Backbone of DNA: Sugar and phosphate group form the backbone of each DNA strands and the 2 strands are bound together by H bonds between matching base pairs

Guanine: Form 3 hydrogen bonds with cytosine

Adenine :Form 2 hydrogen bonds with thymine.

The Central Dogma

The central dogma of molecular biology suggests that

DNA maintains the information to encode all of our proteins,

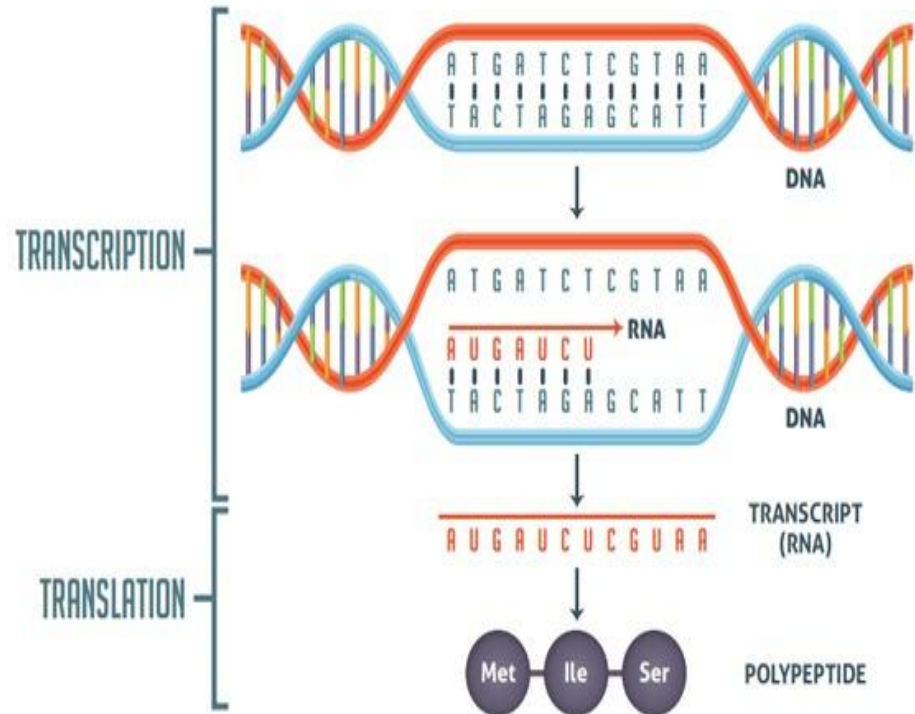
and that three different types of RNA rather passively convert this code into polypeptides.

Messenger RNA (mRNA) carries the protein blueprint from a cell's DNA to its ribosomes, Transfer RNA (tRNA) then carries the appropriate amino acids into the ribosome for inclusion in the new protein.

Meanwhile, the ribosomes themselves consist largely of ribosomal RNA (rRNA) molecules.

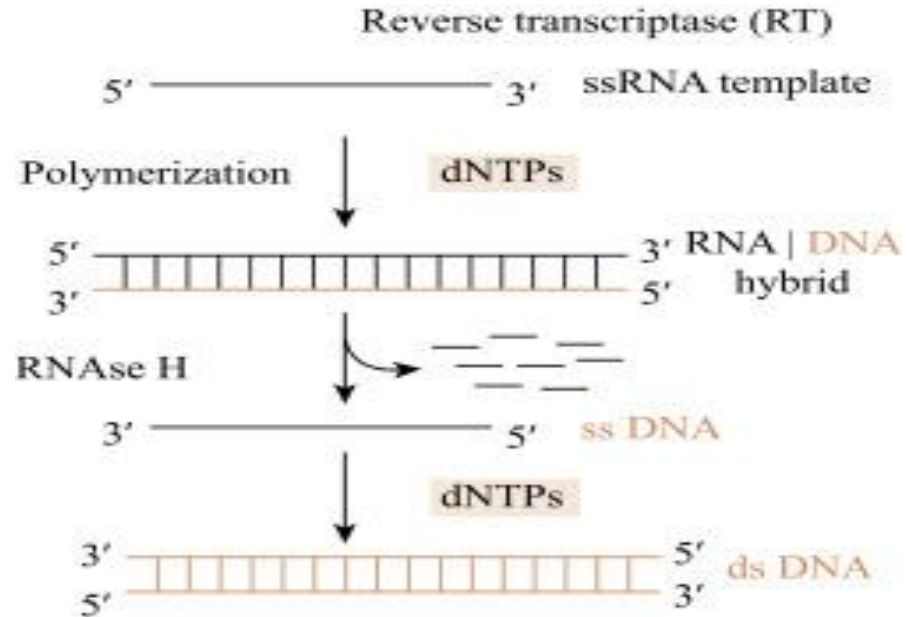
Key Terms- Transcription

Is the process through which DNA converts to RNA. RNA is similar to a single strand of DNA except that the deoxyribose sugar is replaced with ribose and thymine is replaced with Uracil.



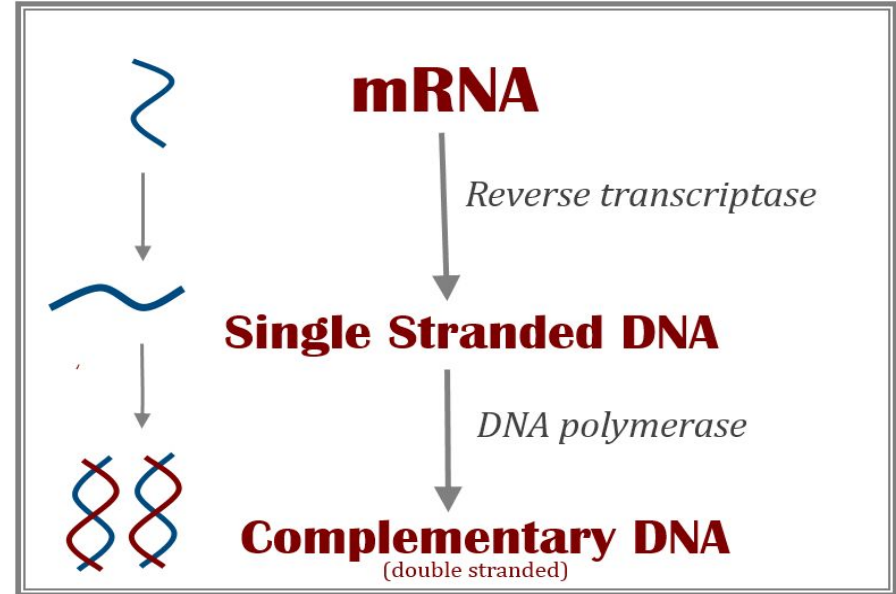
Key Terms -Reverse Transcription

RNA converted to
complementary DNA
or cDNA



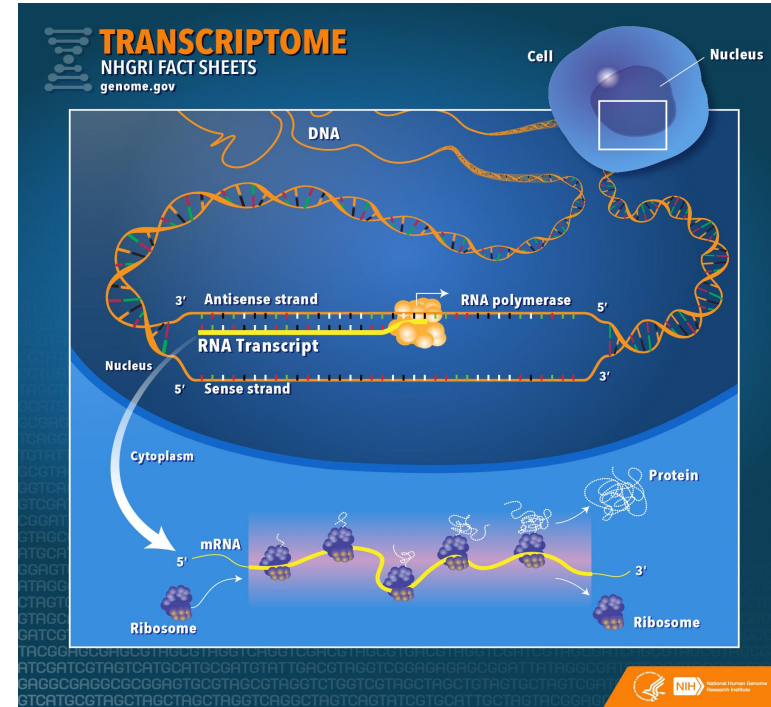
Key terms-Complementary DNA

The DNA that is formed due to reverse transcription.



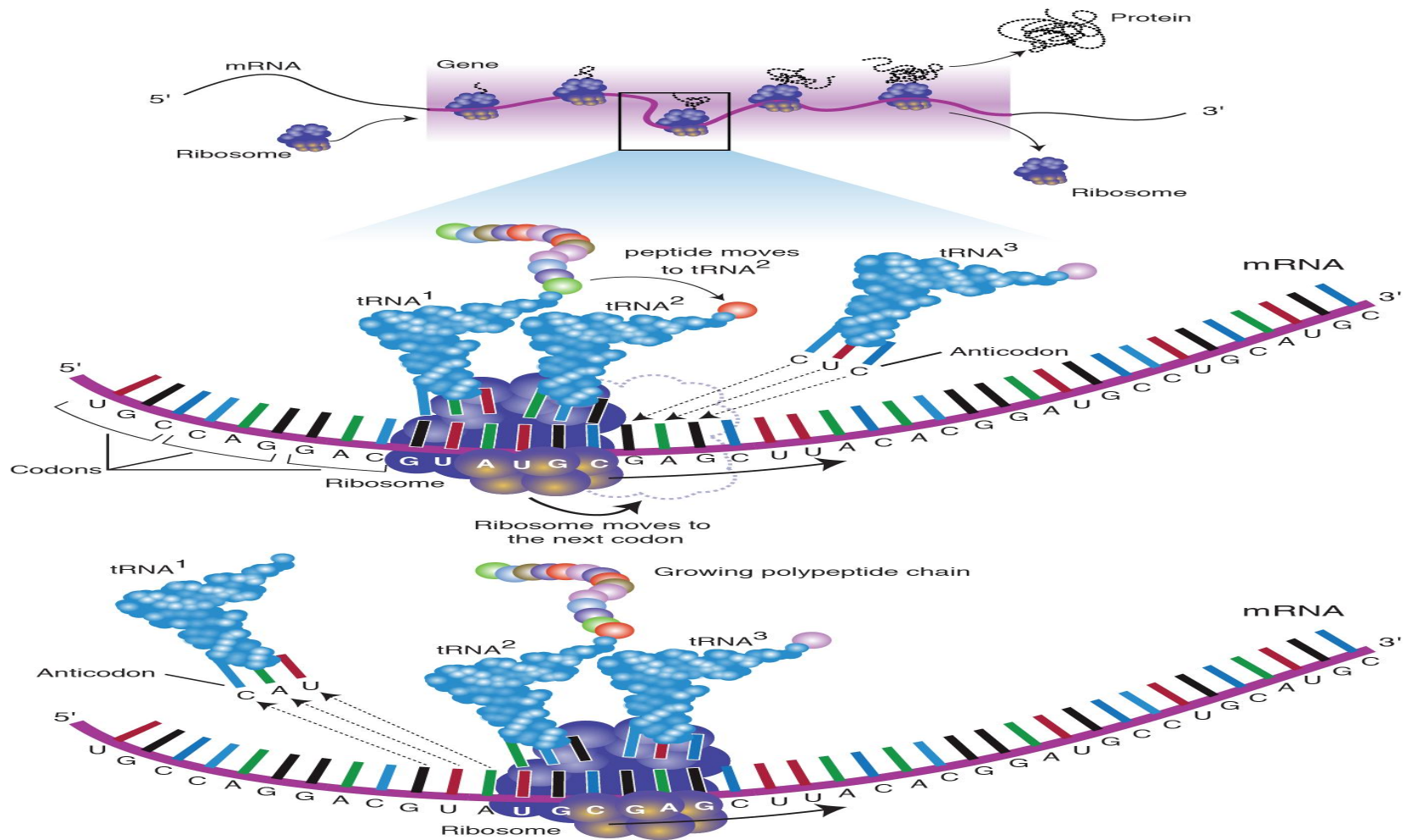
Key Terms-Transcriptome`1

Refers to all of the RNA content of a cell present at a certain point of time.



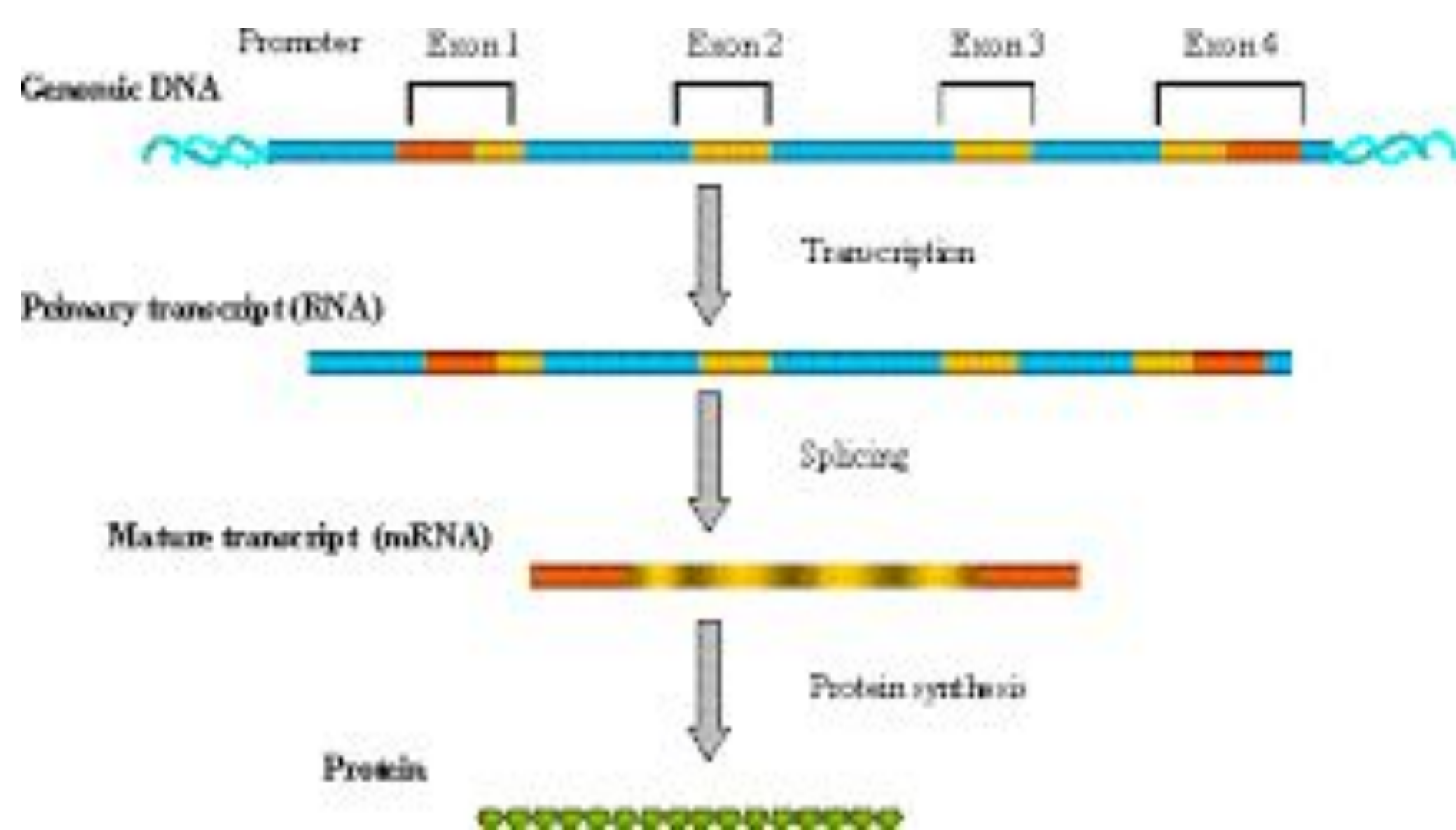
Key Terms -Genome

The haploid set of chromosomes in a gamete or microorganism, or in each cell of a multicellular organism.



Key Terms- Gene Expression

Gene expression is the process by which the instructions in our DNA are converted into a functional product, such as a protein.



Key Terms- Transcriptomics

The transcriptome is defined as the complete set of transcripts in a cell, and their quantity, for a specific developmental stage or physiological condition

4-5 Multiple Core Tissue

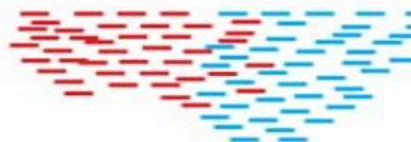
RNA isolation



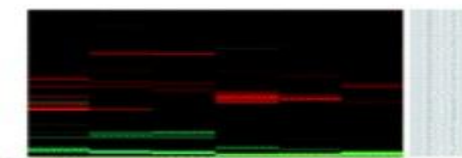
cDNA Libraries



Sequencing Reads



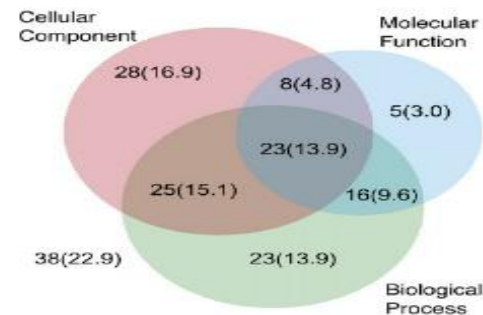
de novo Transcript Assembly



Gene Expression Profiling



Alternative spliced transcript
identification



Functional Annotation

Key Terms- Gene Profiling

Gene profiling is the measurement of the activity of thousands of **genes** at time or to create a complete picture of cellular function

Types of RNA

mRNA: RNA molecules that are transcribed from protein coding genes are known as messenger RNA.

rRNA: Ribosomal RNA which forms part of the ribosome that manufactures protein

tRNA: Transfer RNA carries amino acids to the ribosome

miRNA: Micro RNA that have a role in regulating gene expression

lncRNA: Long non-Coding RNA that are also involved in regulation among other processes

Exons and Introns

Exons: Are regions in the genes that encode Information .

Introns: Are regions in the genes that are of non-coding in nature.

Transcription Stage: RNA undergo transcription that initially contains the intronic sequences

Splicing Stage: The intronic sequences are removed through the RNA Splicing and sequence of adenine nucleotide (aPoly(A) tail) is added where transcription ends.

This ends up as a mature RNA molecules.

This process allows multiple forms of a protein to be produced from a single gene by which exons are retained or removed.

Translation stage: A mature RNA is then converted to proteins and this is called translation where the information encoded by nucleic acids in RNA is translated to information stored as amino acids in the protein.

What is Single Cell RNA Sequence

Sequencing Rna is more difficult than sequencing DNA

Transcriptome of all cell types in a tissue can be studied using single cell RNA sequencing simultaneously to know the distinct characteristic of the cell and discover new cell types.

Why is Single Cell RNA Sequence Important ?

Use detect individual immune **cells**,

Distinguish different groups of immune **cells**,

Discover new immune **cell** populations and their relationships .

Helps to understand the complex immune system and propose new targets for disease treatment.

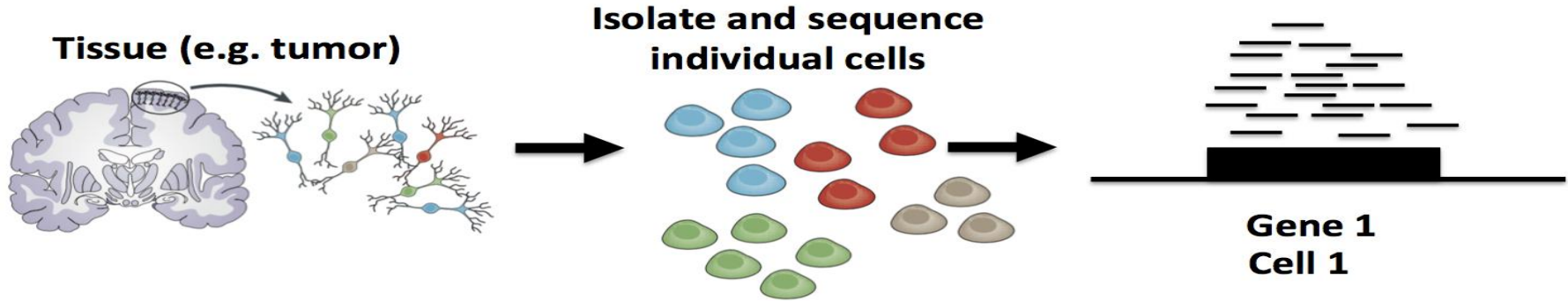
Why is Single Cell RNA Sequence Useful

- Understanding heterogeneous tissues
- Identification and analysis of rare cell types
- Changes in cellular composition
- Dissection of temporal changes

Example of applications:

- Differentiation paths
- Cancer heterogeneity
- Neural cell classification
- Embryonic development
- Drug treatment response

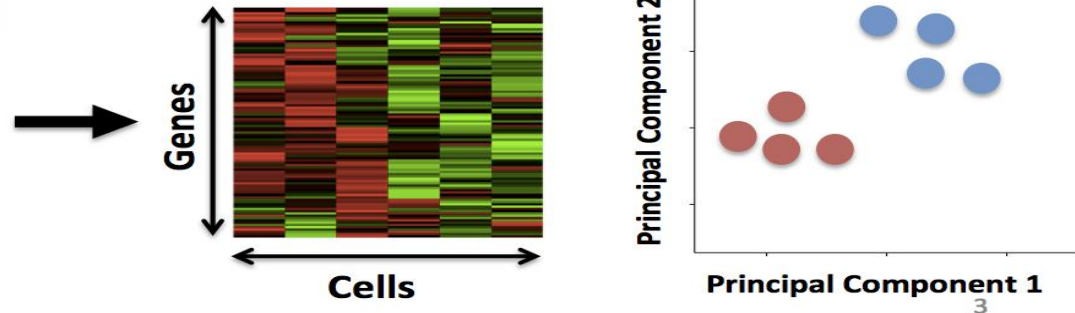
Single-cell RNA-Seq (scRNA-Seq)

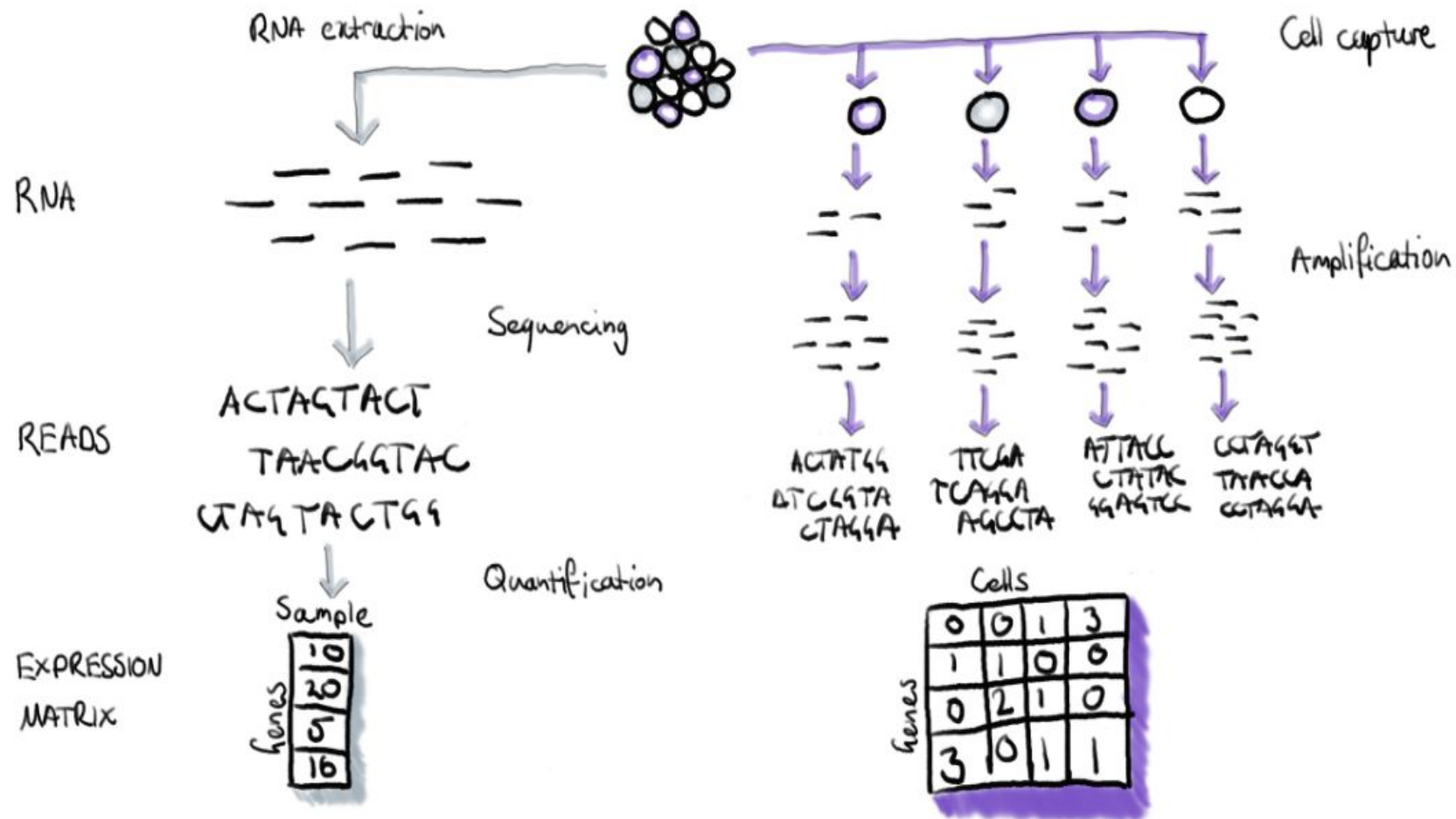


Read Counts

	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

Compare gene expression profiles of single cells





RNA

Rna is more fragile as DNA contains deoxyribose, **RNA** contains ribose, characterised by the presence of the 2'-hydroxyl group on the pentose ring

2 types of RNA sequencing.

- Direct RNA sequencing

- Indirect RNA sequencing

Types of RNA sequencing -Direct RNA sequencing

Extraction of RNA using the normal RNA extraction method from a cell.

Isolation of RNA content is huge as normally the DNA and its contents are the same in all the cells.

RNA by itself has 3 types inside the cell such as mRNA, tRNA, and rRNA.

It is for this reason that we need to isolate the RNA sequence.

Sequencing is the third and final step.

Isolation can be done in 3 different ways.

Isolation of RNA

1. Poly A library for mRNA.
 - a. Because most of the time, we sequence mRNA as mRNA is the actual transcript from where the protein are made.
 - b. We use the polya tail to create a Poly a library and mRNA has the Poly a tail and other RNA's do not have this.
2. Size Exclusion Chromatography
 - a. Small Fragment RNA. Like tRNA, degraded RNA
3. Size Exclusion Magnetic Bead
 - a. Small Fragment RNA. Like tRNA and degraded RNA.

Sequencing of RNA

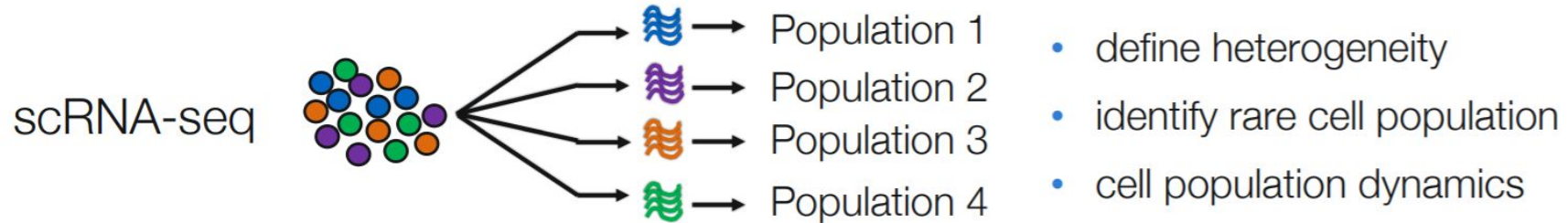
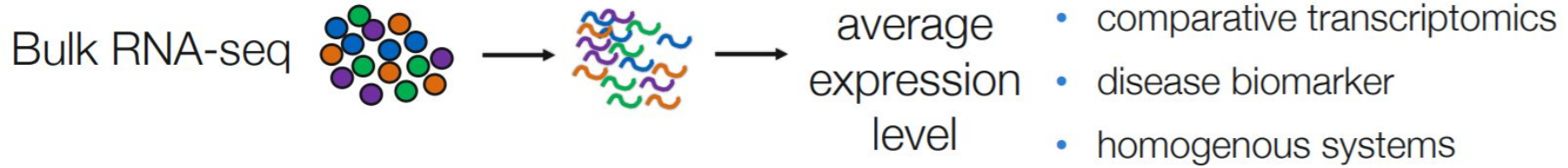
We use the normal sequencing process after isolation of RNA.

The sequencing process used are

- High ThroughPut Sequencing

- Next Generation Sequencing

BULK RNA-Seq VS scRNA-seq



Workflow of RNA Seq

Sequencing Base Call

Short Read

Expression levels of Transcripts

Statistical Analysis

Differentially expressed transcripts

Cellular functional pathway analysis

Microarray

Hybridization.
Scanning images.
Quantification.

Raw intensities

Preprocessing:
Background correction,
Normalization,
Summarization.

Expression levels of
Transcripts (continuous)

Statistical analysis

Differentially
expressed
transcripts

Cellular
functional/pathway
analysis

GO

RNA-Seq

Sequencing.
Base call.

Short reads

Aligned to
reference genome,
known isoform & exon-
junction sequences.

Expression levels of
Transcripts (counts)

Novel
transcripts

Statistical analysis

GO

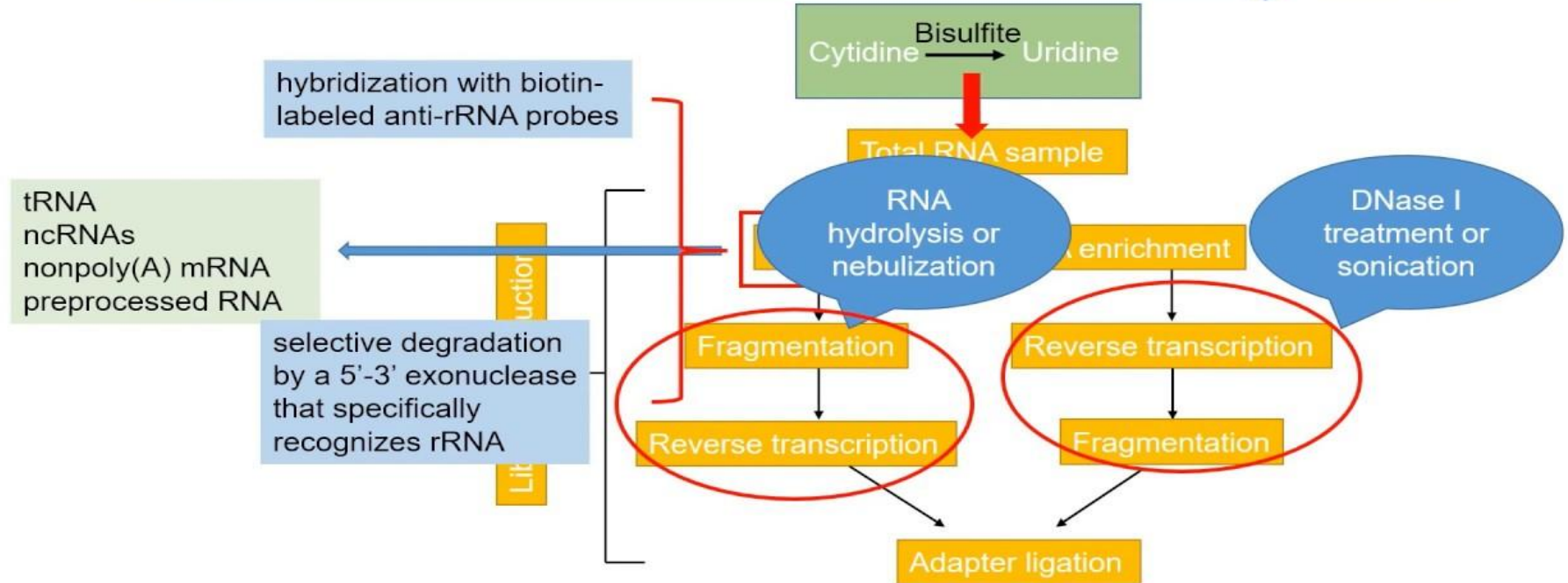
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KEGG

Library Preparation Workflow



Library preparation



Library Preparation Steps:

Introduction

Sample Preparation:

Chemically Lyse the cells by disrupting the structure of the cell wall and release the molecules inside.

Isolate the RNA molecules:

Using a chemical process call phenol/chloroform extraction.

Or by physically separating different types of molecules by passing through a silica column.

Library Preparation

Get the mature mRNA Transcripts :

For this the oligonucleotide probes that bind to the Poly (A) tail is used , but this won't capture immature mRNA or other types of RNA molecules.

An alternate method is depletion of rRNA a different kind of probe that bind to the rRNA is used to remove it.

Reverse Transcription:

Sequencing DNA is easier than sequencing RNA. Sample must be first reverse-transcribed using retroviral enzyme to produce single strand of cDNA.

Fragmentation :

To Read the full length of the transcript the mRNA must be fragmented into smaller parts.

Adaptor :

CDNA is attached with adaptor sequences that are used to bind the molecules and initiate sequencing

Multiplexing barcode:

Adaptors contains multiplexing barcodes that tag all molecules in sample

Attaching multiplexing barcode allow multiple samples to be sequenced a once.

Paired-end sequencing:

A section of nucleotides is read form one end of a fragment before it is flipped and the other end read

This process requires additional set of adaptors.

Quality Control steps:

This is performed to ensure high quality cDNA sample is loaded to the sequencing machine.

Unique Molecular Identifiers

These are tags that are used to detect and quantify unique mRNA transcripts.

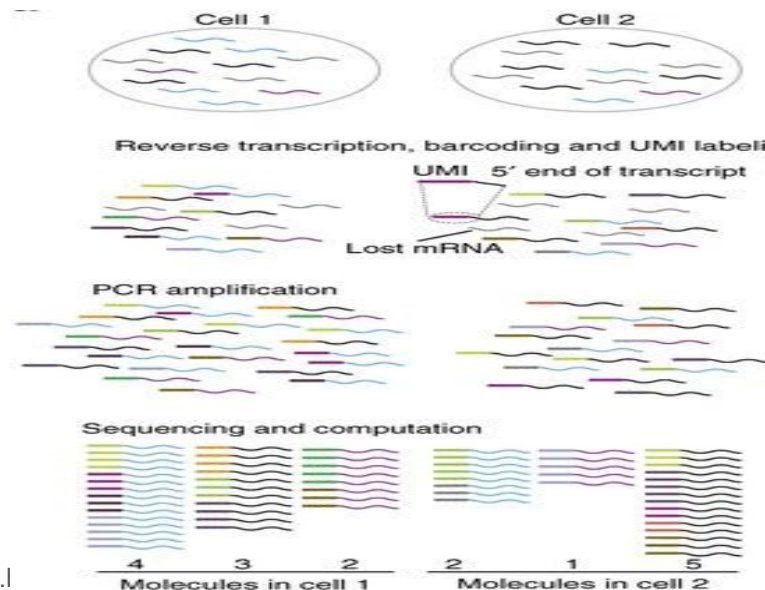
mRNA libraries are generated by fragmentation and reverse-transcribed to cDNA.

Oligo(dT) primers with specific sequencing linkers
cDNA.

Another sequencing linker with a 10bp
label and an index sequence is added to
is amplified and sequenced.

Sequencing allows for high resolution reads,
accurate detection of true variants.

<https://www.illumina.com/science/sequencing-method-explorer/kits-and-arrays/umi.1>



Unique Molecular Identifiers - Pros and Cons

Pros

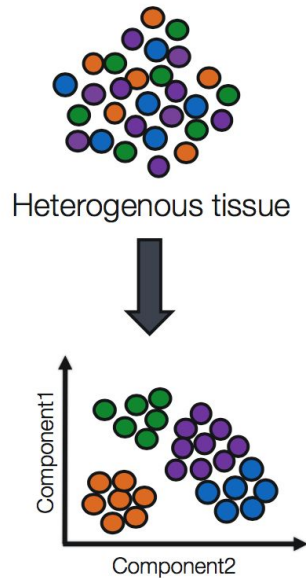
- Can sequence unique mRNA transcripts
- Can detect transcripts occurring at low frequencies
- Transcripts can be quantified based on sequencing reads specific to each barcode
- Can be applied to multiple platforms to karyotype chromosomes

Cons:

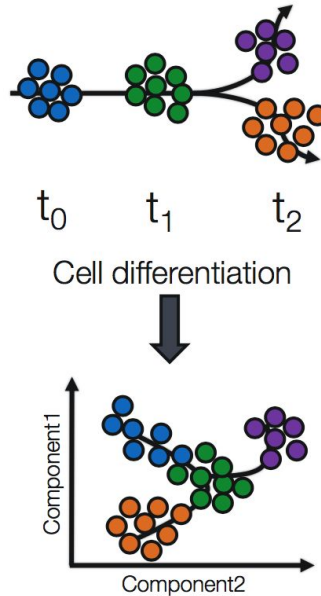
Targets smaller than 500bp are preferentially amplified by polymerases during PCR.

Common Applications of SingleCell RNA seq

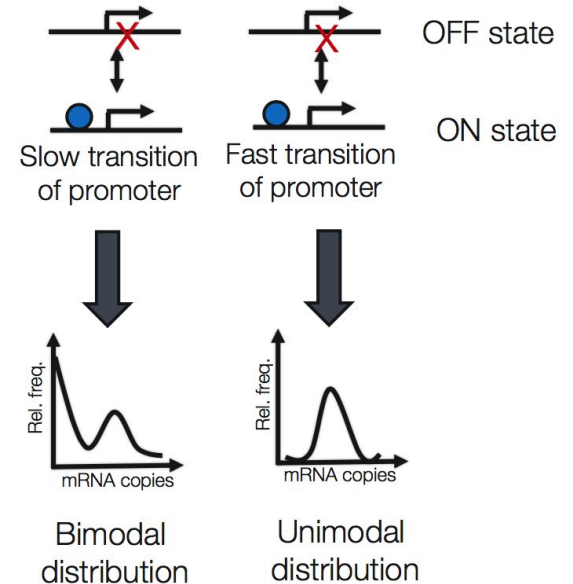
Studying heterogeneity



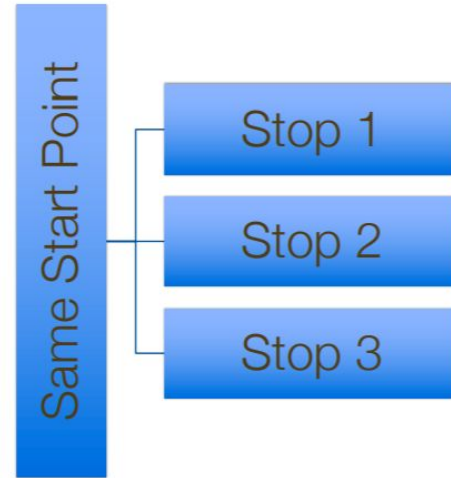
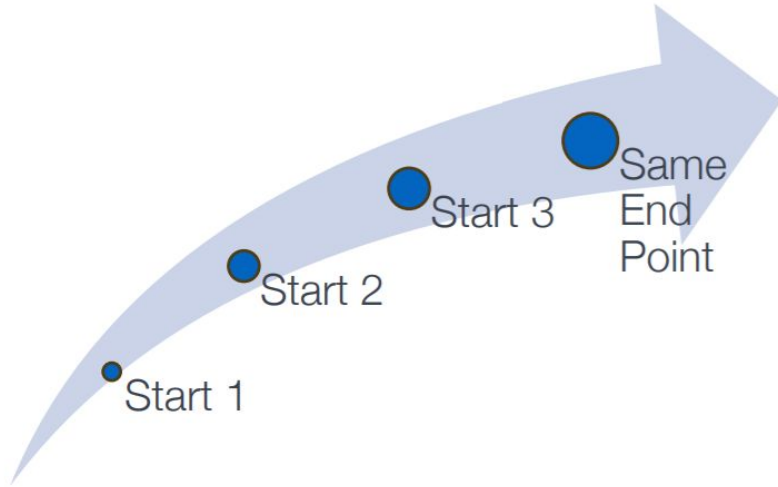
Lineage tracing study



Stochastic gene expression study



Development Experiment



- Collect all samples and prep libraries together in one batch.
- Biological duplicates (at minimum)

More Cells or More Sequencing Reads

- Required number of cells increases with complexity of the sample.
- As the number of genes involved in the biology decrease then the coverage requirements increase (more reads).
- Cell-type classification of a mixed population usually requires lower read depth and can be sequenced at 10,000-50,000 reads per cell.
- Suggest starting with 25,000-55,000 reads per cell. Can always re-sequence your samples.

Single Cell Methods

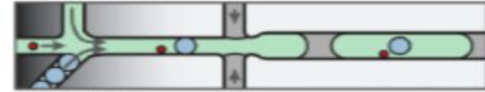
CELL-seq
MARS-seq
SMART-seq
SCRB-seq



Chromium (10x)



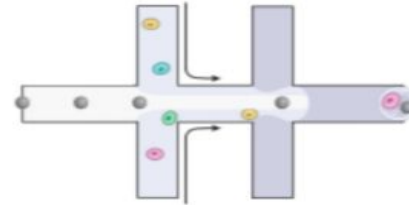
inDrops



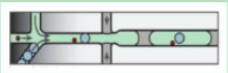




Seq-Well



Drop-seq



Comparison of Single Cell Methods

	inDrops	10x Genomics	Drop-seq	Seq-well (Honeycomb)	SMART-seq
Cell capture efficiency	~70-80%	~50-70%	~10%	~80%	~80%
Time to capture 10k cells	~30min	10min	1-2 hours	5-10min	--
Encapsulation type	Droplet 	Droplet 	Droplet 	Nanolitre well 	Plate-based 
Library prep	CEL-seq Linear amplification by IVT	SMART-seq Exponential PCR based amplification	SMART-seq Exponential PCR based amplification	SMART-seq Exponential PCR based amplification	SMART-seq Exponential PCR based amplification
Commercial	Yes	Yes	--	Yes (Summer 2020)	Yes
Cost (~\$ per cell)	~0.06	~0.2	~0.06	~0.15	1
Strengths	<ul style="list-style-type: none"> • Good cell capture • Cost-effective • Real-time monitoring • Customizable 	<ul style="list-style-type: none"> • Good cell capture • Fast and easy to run • Parallel sample collection • High gene / cell counts 	<ul style="list-style-type: none"> • Cost-effective • Customizable 	<ul style="list-style-type: none"> • Good cell capture • Cost-effective • Real-time monitoring • Customizable 	<ul style="list-style-type: none"> • Good cell capture • Good mRNA capture • Full-length transcript • No UMI
Weaknesses	Difficult to run	Expensive	Difficult to run & low cell capture efficiency	Available Soon	Expensive

Best Practices to Obtain High Quality Sample

- Optimize a dissociation protocol that is best-suited for your cell type of interest.
- Short sample prep time.
- Maintaining low temperature.
- Gentle treatment
 - gentle lysis condition (low temp, short time)
 - short FACS time, slow sorting, bigger nozzle (in certain cases)
 - Gentle centrifugation (300-500xg) and resuspension
 - Removing debris by filter or density medium
- Include BSA (up to 1%) or FBS (up to 2%) in final buffer.

Best Practices for Experimental Design

- Include biological replicates.
- Perform drug/treatment/model vs control on the same day.
- Randomize the order of samples run on different days.
- Use same sex littermates as controls in mouse experiments.
- Our experience is that library prep is the largest source of batch effect.
 - Collect all your samples in one study together then prep as one large library group.

Best practices for Experimental Design

Pilot Study Experimental Design

- Control vs diseased animal
- Each sample requires pooling several animals.
 - Control and diseased littermates pooled for a single sample.
- Both control and diseased samples run on same day.
- The entire experiment is repeated on a second day running samples.
 - Load samples in opposite order.
- Libraries from the four single cell samples are prepared as one batch.
- Sequencing and analysis performed
 - Check that sample prep was of good quality.
 - Determine that the desired information can be obtained from the experiment.

Data Analysis

Cell capture technologies and scRNA-seq protocols have developed rapidly but the data they produce still presents a number of challenges.

Use zero inflated versions of common distributions.

Bulk RNA seq experiments usually involve predefined groups of samples for example, cancer cells and normal tissue, different tissue types or treatment and control groups.

Data Analysis

- Single-cell transcriptome sequencing is used to measure gene expression at the single-cell level
- it provides a higher resolution of cellular differences than bulk RNA-seq.
- With more detailed and accurate information, scRNA-seq will greatly promote the understanding of cell functions, disease progression, and treatment response.
- Although the scRNA-seq experimental protocols have been improved very quickly, many challenges in the scRNA-seq data analysis still need to be overcome.
- scRNA Data normalization and cluster analysis, are the two most important challenges in the scRNA-seq data analysis.
- present a protocol to discover and validate cancer stem cells (CSCs) using scRNA-seq.

Phases of scRNA-seq Analysis

PHASES OF ANALYSIS

1. Data acquisition  Alignment
Quantification

3. Cell assignment  Clustering
Classification
Ordering

2. Data cleaning  Quality control
Normalisation

4. Gene identification  Differential expression
Expression patterns

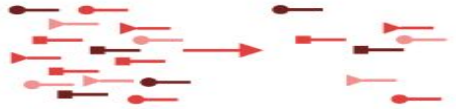
Phases of scRNA -Seq Analysis

Phase 1 - Data acquisition

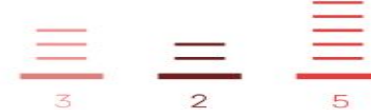
Alignment



De-duplication



Quantification

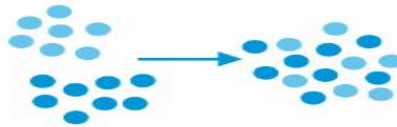


Phase 2 - Data cleaning

Quality control




Normalisation



Imputation

0	1	0	0
0	0	0	0
0	0	2	0
0	1	0	0



1	1	0	1
0	0	0	0
0	1	2	2
0	1	1	0

Phase 3 - Cell assignment

Clustering



Unknown groups

Classification



Known groups

Ordering



Trajectory

Phase 4 - Gene identification

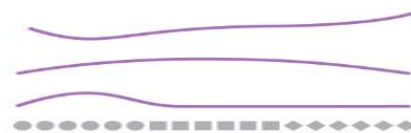
Differential expression



Marker genes

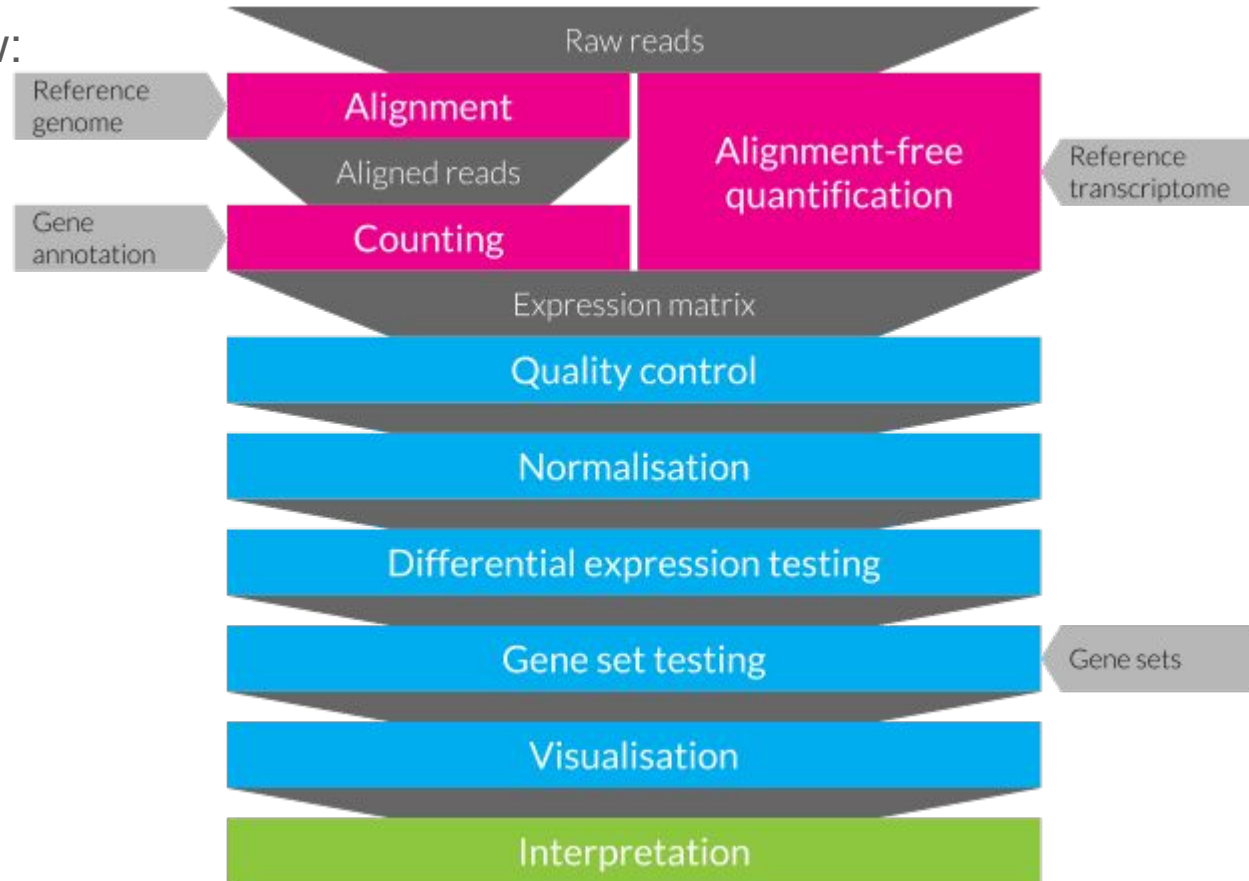


Expression patterns



Analysis of RNA -Seq Data

Expression Testing Workflow:



RNA -seq differential expression testing Workflow

An expression matrix is created from raw reads either by aligning them to a reference genome and counting those that overlap annotated genes or by alignment free quantification .(the steps in pink)

Data Analysis consists of several steps including quality control of sample and features, normalization to remove technical differences between samples , testing for differential expression, gene set testing to identify enriched signatures and visualization. (steps in blue)

Results must be carefully interpreted to extract meaning (Green)

Phases of scRNA-seq Analysis

Phase 1 : Data Acquisition

Alignment , De-Duplication, Quantification

Phase 2:Data Cleaning

Quality control , Normalization , Imputation

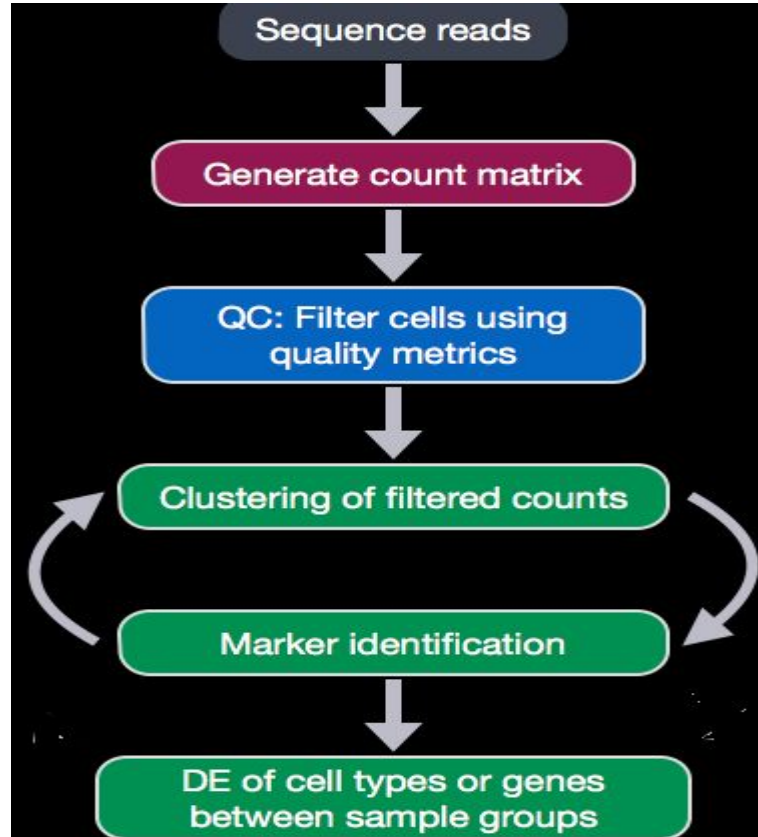
Phase 3:Cell assignment

Clustering, Classification , Ordering

Phase 4:Gene Identification

Differential expression , Marker genes and Expression patterns.

General Workflow for Analyses.



"Differential Gene Expression (DGE) Analysis." *Training*, [hbctraining.github.io/Training-modules/planning_successful_rnaseq/lessons/sample_level_QC.h](https://hbctraining.github.io/Training-modules/planning_successful_rnaseq/lessons/sample_level_QC.html)

Raw Sequencing Data to counts.

scRNA-seq method is used for

- analyzing differences in cellular gene expression,
 - For tissue heterogeneity analyses
 - Lineage tracing
 - Cell population dynamics

Complexity of scRNA-seq data, characterized as

- Large volume of data- representing thousands of cell
- Low depth of sequencing per cell- resulting in large number of genes without any corresponding reads.
- Makes analysis of data more involved of Bulk-RNA seq

“Differential Gene Expression (DGE) Analysis.” *Training*,

hbctraining.github.io/Training-modules/planning_successful_rnaseq/lessons/sample_level_QC.html

Goal of Analysis

The analysis goal can be

- Marker Identification
- Lineage Tracing
- Custom Analysis

Our project goal is marker identification and cell type classification.

Steps in scRNA seq Method

Generation of count Matrix

Assess raw counts to filter out

- poor quality cells with low number of genes or UMIs

- High mitochondrial expression of indicative of dying cells

- Low num of genes per UMI

The cells are clustered based on similarities in transcriptional activity with the idea that different cell types separate into different clusters.

Explore genes that are markers for different clusters that help identify the cell types for each cluster.

Generation of Count Matrix

The sequencing facility will either output

Raw sequencing data as BCL format or FASTQ.

If reads are in BCL format, convert into FASTQ format.

The steps involved in the generation of Count Matrix are

1. Formatting READS and filtering noisy cellular barcodes
2. Demultiplexing the samples
3. Pseudo-mapping to cDNAs
4. Counting molecule identifiers.

Formatting Reads and Filtering Noisy Cellular Barcodes

FASTQ files parse out cell barcodes, UMIs, and sample barcodes.

The cellular barcodes match a low number of reads less than thousand reads due to encapsulation of free floating RNA from dying cells, small cells, or set off cells that failed for some reason.

Excess barcodes need to be filtered out of the sequence data prior to read alignment.

Not all cellular barcodes identified will be real. Some will be low abundance barcode that do not represent an actual cell.

Others will be barcodes that do not come from a set of known barcodes.

Unknown barcodes will be dropped.

Demultiplexing sample reads

Sequencing more than single Sample requires demultiplexing the sample.

We need to parse the reads to determine the sample barcodes associated with each cell.

Pseudomapping to cDNA

This is done by pseudo-aligners either Kallisto or RapMap.

The SAM or BAM file output from these tools need to be saved.

Counting molecular identifiers

Final Step is to infer which cDNA was the origin of the tag a UMI was attached to. We use pseudo-alignments to the cDNAs and consider a tag assigned to a cDNA as a partial evidence for a cDNA, UMI pairing.

For actual counting, count only unique UMIs for gene, UMI pairings.

At this point, duplicate UMIs will be collapsed for the counting of the identifiers.

Now, we have count matrix containing counts per gene for each cell, which we can use to explore our data for quality information.

Quality Control `

Get Data from single cell RNA seq experiment

Construct and Evaluate the QC metrics and filter out the low quality cells

Figure out the data set

The data set used is peripheral blood mononuclear cells(PBMC) that has 2,700 cells taken from a healthy donor sequenced on illumina NextSeq 500.

We use the Seurat tutorial

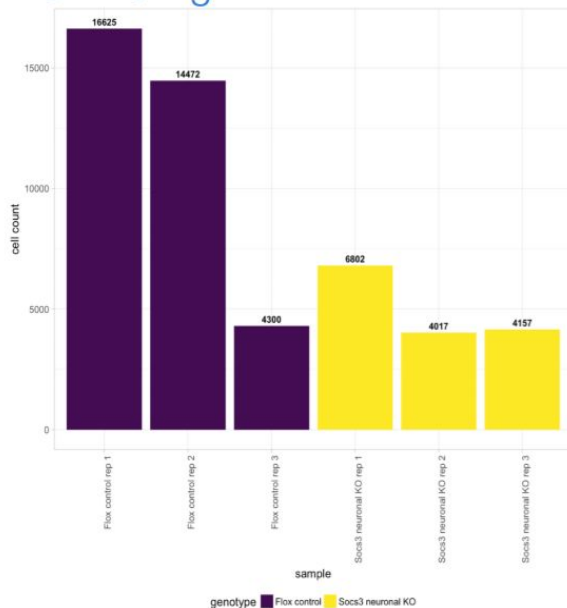
For this data set, we will be analyzing the workflow from Count Matrix to marker identification

Quality Control QC Metrics

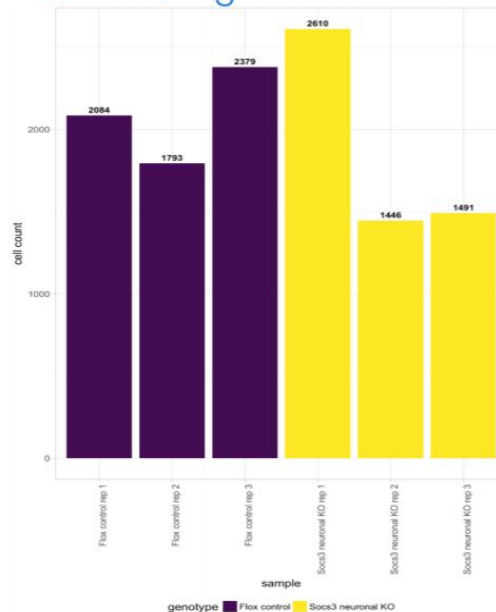
- Reads /Cell: The no of reads to a assigned cell barcode
- UMI / Cell: genes per UMI
- Genes Detected : Genes with a non zero count per cell
- Mitochondrial count ratio: This is the biomarker for the stressed out cells

Filtering and Correction

Pre-Filtering



Post-Filtering



Libraries were of 3,000 cells. Post-filtering retains 50-80% of cells

Count Normalization and Principal Component Analysis

. Goal of clustering

- separate different cell types into unique clusters of cells.
- Determine the genes that are most different in their expression between cells.
- Determine which correlated genes sets are responsible for the largest differences in expression between cells.
- Normalization is the process of scaling raw count values to account for the “uninteresting” factors

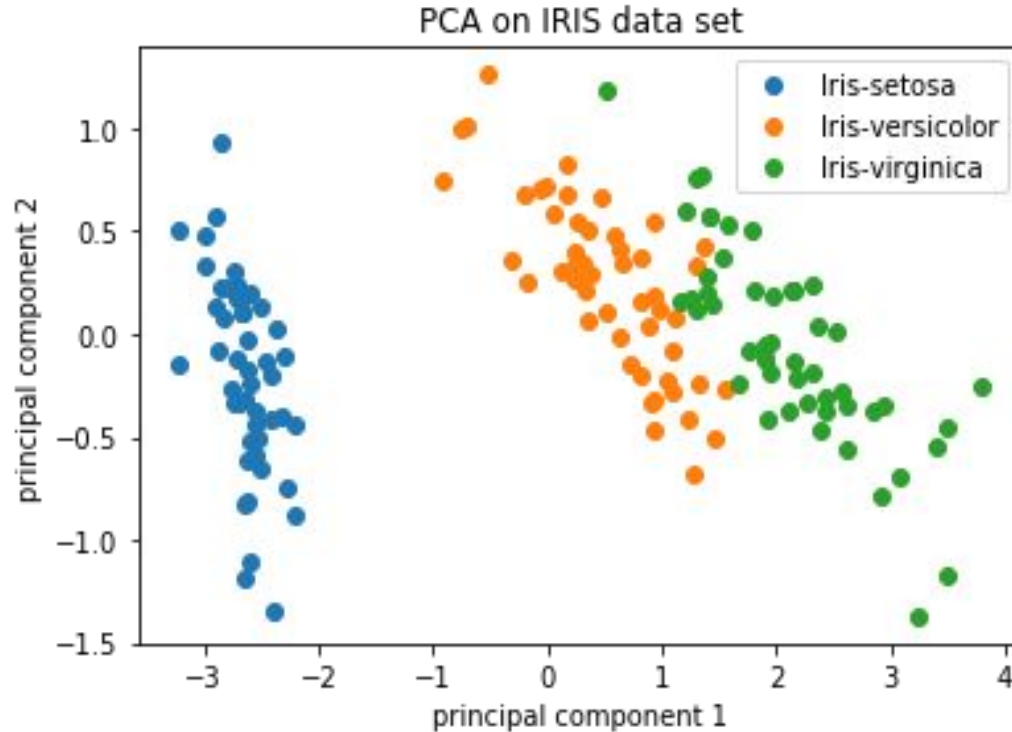
“Differential Gene Expression (DGE) Analysis.” *Training*,

hbctraining.github.io/Training-modules/planning_successful_rnaseq/lessons/sample_level_QC.html.

Principal Component Analysis

- A technique used to emphasize variation as well as similarity, and to bring out strong patterns in a dataset (dimensionality reduction).
- use between 10-100 PC scores to compare the cells, which will highlight the greatest sources of variation present in our dataset.
- We will use these PCs to cluster our cells based on similarity in expression, in addition to exploring the sources of variation associated with these highest PCs

Principal Component Analysis



Clustering Of Cells

To identify clusters, the following steps will be performed:

1. **Normalization, variance stabilization**, and **regression of unwanted variation** (e.g. mitochondrial transcript abundance, cell cycle phase, etc.) for each sample
2. **Integration** of the samples using shared highly variable genes (optional, but recommended to align cells from different samples/conditions if cell types are separating by sample/condition)
3. **Clustering cells** based on top PCs (metagenes)
4. Exploration of **quality control metrics**: determine whether clusters are unbalanced wrt UMIs, genes, cell cycle, mitochondrial content, samples, etc.
5. Searching for expected cell types using **known cell type-specific gene markers**

“Differential Gene Expression (DGE) Analysis.” *Training*,
hbctraining.github.io/Training-modules/planning_successful_rnaseq/lessons/sample_level_QC.html.

Points to Consider While Clustering Cells

Cluster appears to contain two separate cell types,

- Increase cluster resolution to properly subset the clusters.
- Alternatively, if we still can't separate out the clusters using increased resolution, then it's possible that we had used too few principal components such that we are just not separating out these cell types of interest.
- To inform our choice of PCs, we could look at our PC gene expression overlapping the UMAP plots and determine whether our cell populations are separating by the PCs included.

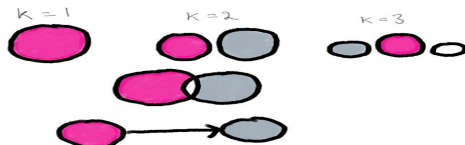
Points to Consider While Clustering Cells

4. CLUSTERING TREES

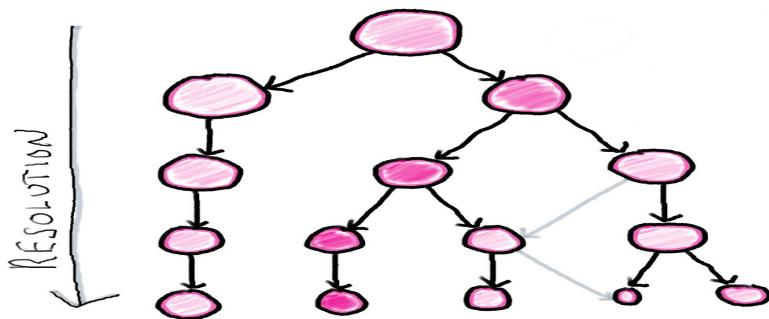
How many clusters to use?

A tree of clusters!

1. Cluster at multiple resolutions
2. Calculate overlap between clusters
3. Build a graph
4. Weight edges
5. Visualise



$$\text{in prop} = \frac{\text{edge samples}}{\text{high res samples}}$$



Structure can
show stability

Show information
across resolutions

Marker Identification

1. **Identification of all markers for each cluster:** this analysis compares each cluster against all others and outputs the genes that are differentially expressed/present.
 - *Useful for identifying unknown clusters and improving confidence in hypothesized cell types.*
2. **Identification of conserved markers for each cluster:** This analysis looks for genes that are differentially expressed/present within each condition first, and then reports those genes that are conserved in the cluster across all conditions. These genes can help to figure out the identity for the cluster.
 - *Useful with more than one condition to identify cell type markers that are conserved across conditions.*
3. **Marker identification between specific clusters:** this analysis explores differentially expressed genes between specific clusters.
 - *Useful for determining differences in gene expression between clusters that appear to be representing the same celltype (i.e with markers that are similar) from the above analyses.*

Final Step

Now that we have our clusters defined and the markers for each of our clusters, we have a few different options:

- Experimentally validate intriguing markers for our identified cell types.
- Perform differential expression analysis between conditions `ctrl` and `stim`
 - Biological replicates are **necessary** to proceed with this analysis
- Trajectory analysis, or lineage tracing, could be performed if trying to determine the progression between cell types or cell states. For example, we could explore any of the following using this type of analysis:
 - Differentiation processes
 - Expression changes over time
 - Cell state changes in expression

Useful Links for reference - Sources Cited from

<https://lazappi.github.io/phd-thesis/1-introduction.html>

<https://learn.gencore.bio.nyu.edu/ngs-file-formats/how-sequencing-works/>

https://satijalab.org/seurat/v3.0/interaction_vignette.html

https://broadinstitute.github.io/2019_scWorkshop/

<https://scrnaseq-course.cog.sanger.ac.uk/website/introduction-to-single-cell-rna-seq.html>

www.biostars.org

https://hbctraining.github.io/scRNA-seq/lessons/01_intro_to_scRNA-seq.html

Youtube links

<https://www.youtube.com/watch?v=ubgY58azZW0>

https://www.youtube.com/watch?v=U1uQ_wBcO4U

https://www.youtube.com/watch?v=MFRkwXq6v_I

<https://www.youtube.com/watch?v=BfxDfL1GBzk>

<https://www.youtube.com/watch?v=tlf6wYJrwKY>

<https://www.youtube.com/watch?v=ReBYbe4FyK0>

<https://www.youtube.com/watch?v=Ji9nFCYl7Bk>

<https://www.youtube.com/watch?v=jFCD8Q6qSTM>

<https://www.youtube.com/watch?v=-Ud7lXpgZqw>

Final Thoughts on Single Cell Seq RNA

- Practice your sample prep protocol. KEY to SUCCESS
- Start with a pilot sample set to ensure your protocol is working.
- Do not make your scRNA-seq run day the first day you run through the whole protocol.
- Be sure sequencing core understands the specific sequencing parameters needed for your scRNA-seq library.

qPCR

Precise quantitation is key to good clustering / sequencing