Bioinformatics Lab

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Institute for Computational Genomics
RWTH AACHEN UNIVERSITY

Objectives

- Hands on introduction to bioinformatics programming
- Review basic biological/computational aspects
    1. basics of molecular biology
    2. basics of sequencing
    3. basics bioinformatics problems
        - short sequences read alignment
        - gene expression matrix
        - clustering and interpretation

Objectives

- **Introduction to Bioinformatics Frameworks/Tools**

  1. **biological sequence data formats/handling**
     - **Biopython, Pysam, R/bioconductor**
  2. **bioinformatics tools**
     - **BWA (aligner), Seurat, Cell Ranger, …**

Grading/Online material

Evaluation:

- **20% prototypes**
- **60% final project**
- **20% presentation**

Extra-work for media informatics:

- **research report**

References/Courses Online

http://costalab.org/teaching/bioinformatics-software-lab-2019/

Introduction to Molecular Biology

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Understanding Live in a Molecular Level

How is genetic information inherited?

How the genetic information influence cellular processes?

How genes work together to promote particular molecular functions?

Genetic Information - DNA

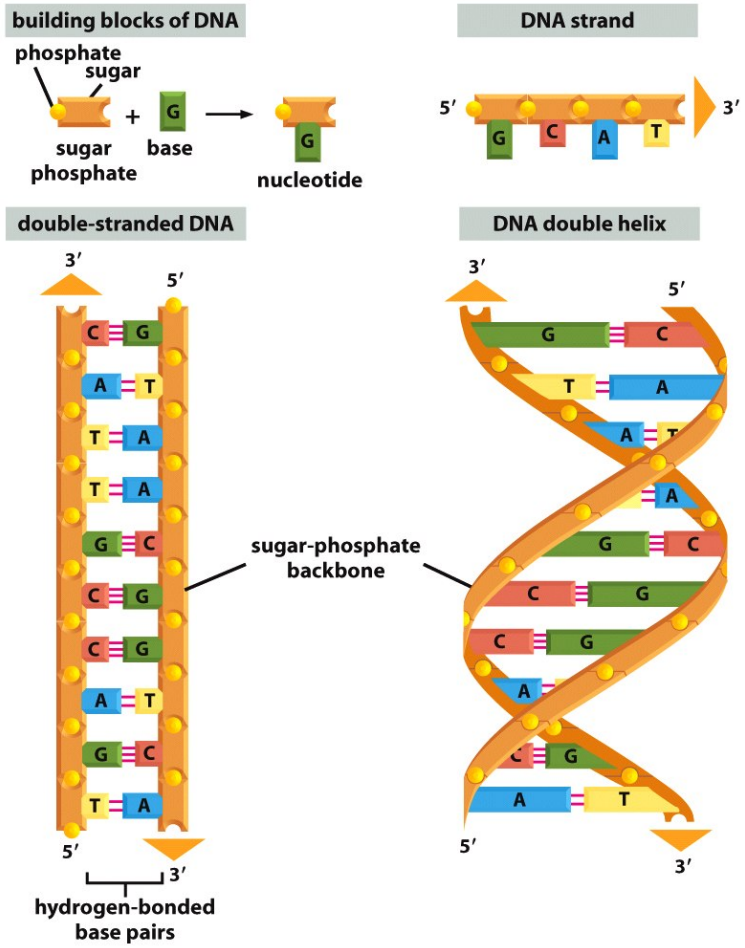
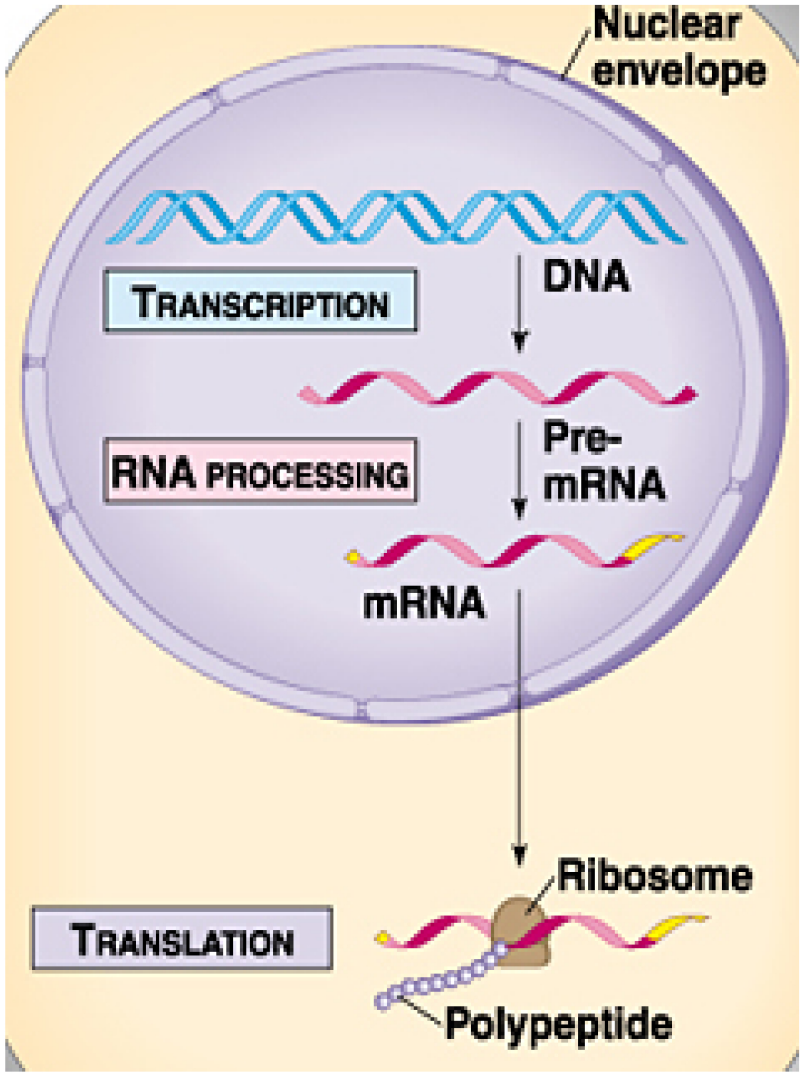


Figure 4-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

DNA *(Deoxyribonucleic)*

- chain of nucleic acids
- 4 bases: A;C;G;T
- forms DNA duplexes with paring A = T e C = G

Central Dogma - Transcription



Transcription

- ***DNA to RNA***

RNA (ribonucleic acid)

- **single stranded**
- **4 bases: A;C;G;U**
- **unstable**
- **transport of information from nucleus to cytoplasm**

Central Dogma - Transcription

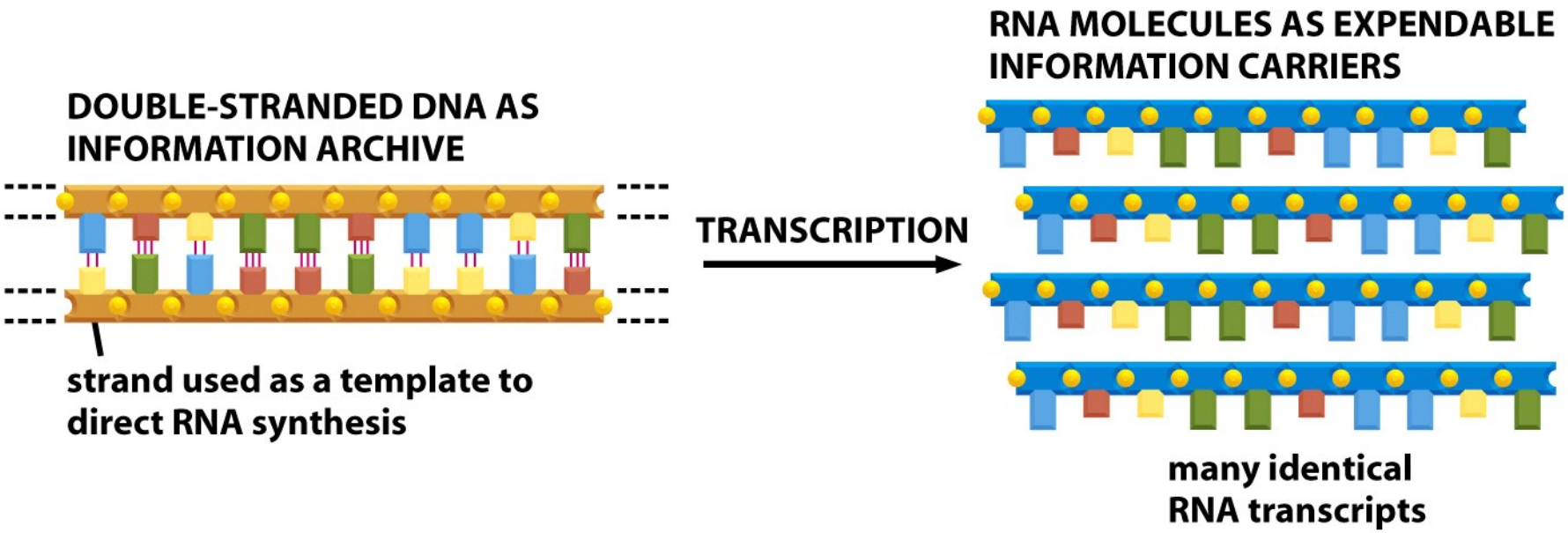
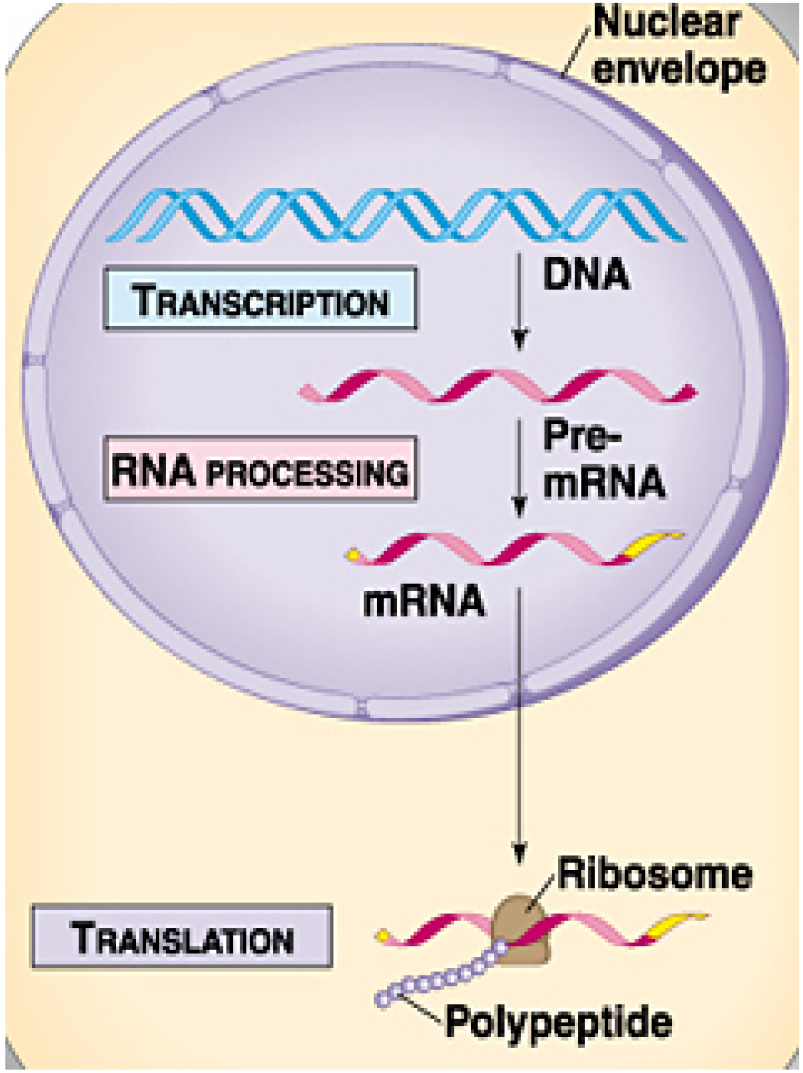


Figure 1-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcription - copy of DNA information to RNA (T to U)

Central Dogma - Translation



Translation

- ***RNA to Protein***
- **performed by the ribosome**
- **follows the genetic code**

Proteins

- **single stranded chain**
- **20 amino acids**
- **assumes 3D structure**
- **main functional entities in the cell**

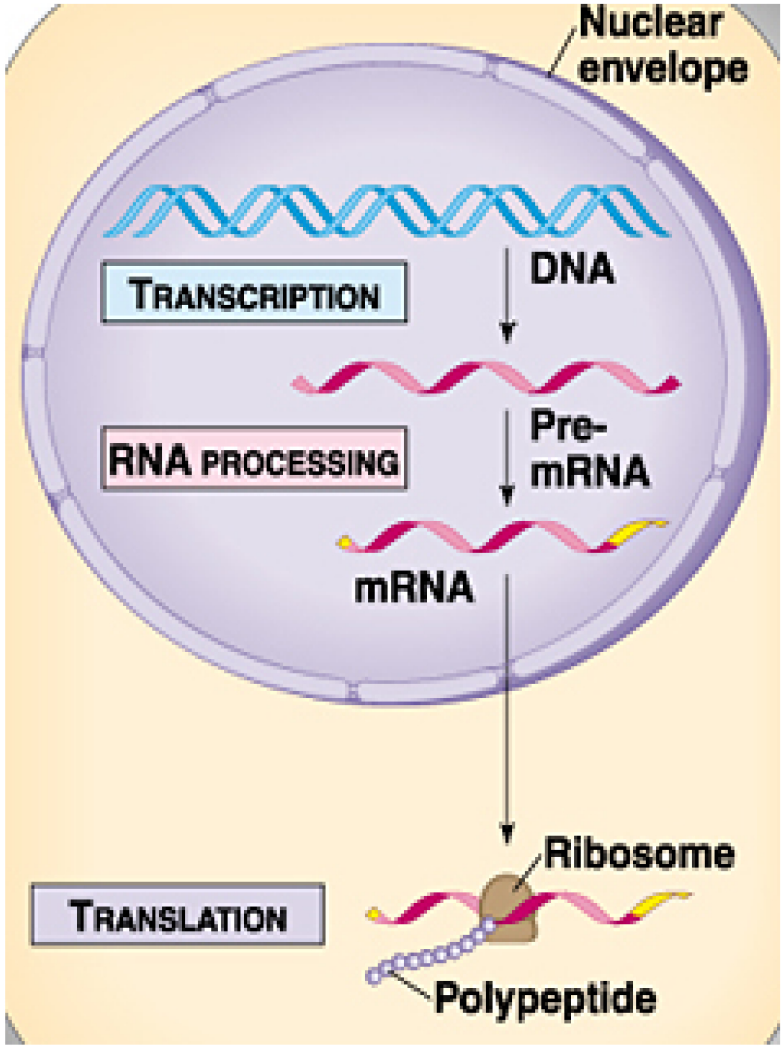
Genetic Code - Translation

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 6-50 Molecular Biology of the Cell 5/e (© Garland Science 2008)

triples of RNA bases encodes a amino acid

Central Dogma



- Dogma: information flux DNA -> mRNA -> Proteins
- Gene: DNA segment coding a protein.
- Transcript: RNA segment associated to a gene.
- Genes is associated to one proteins and one function*

* Genes might be associated to many proteins

Control of Gene Expression

How is the expression of genes controlled?

Certain proteins (transcription factors) bind to DNA and initiate transcription

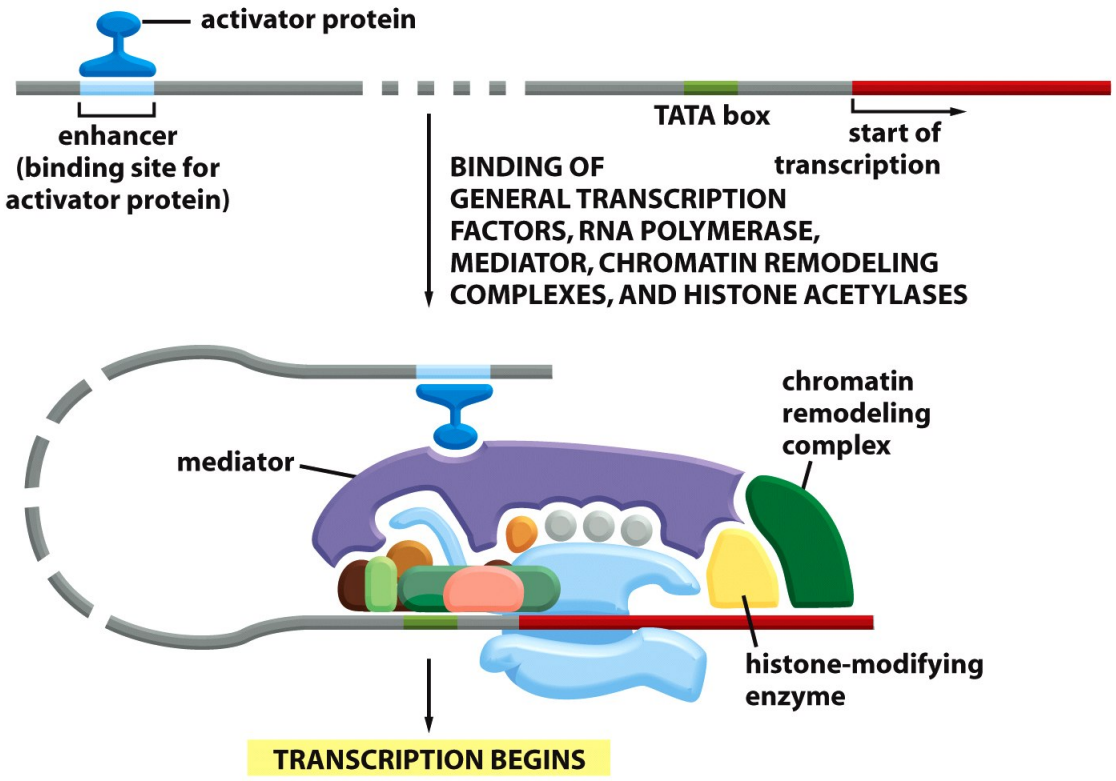


Figure 6-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Gene Expression

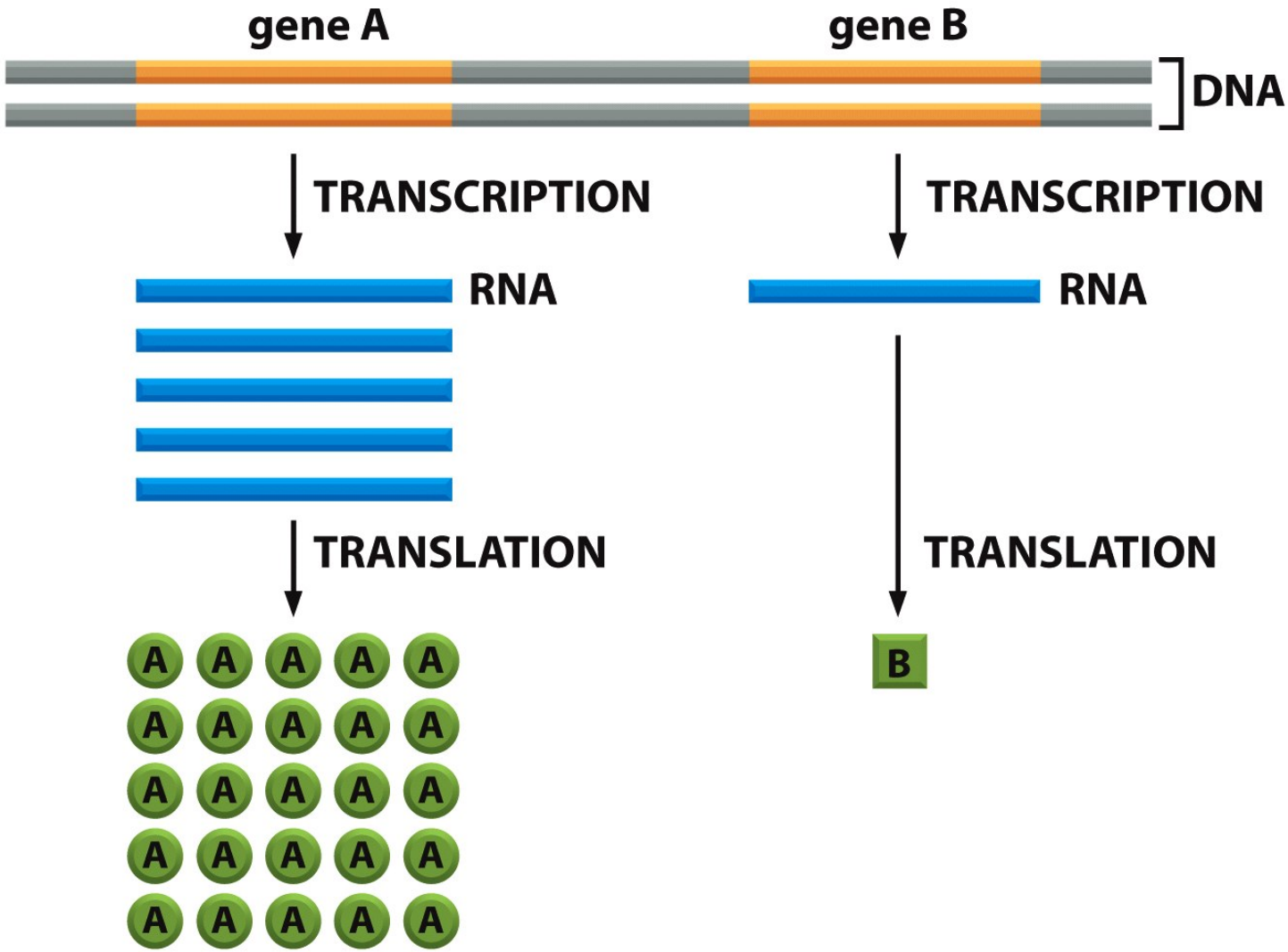
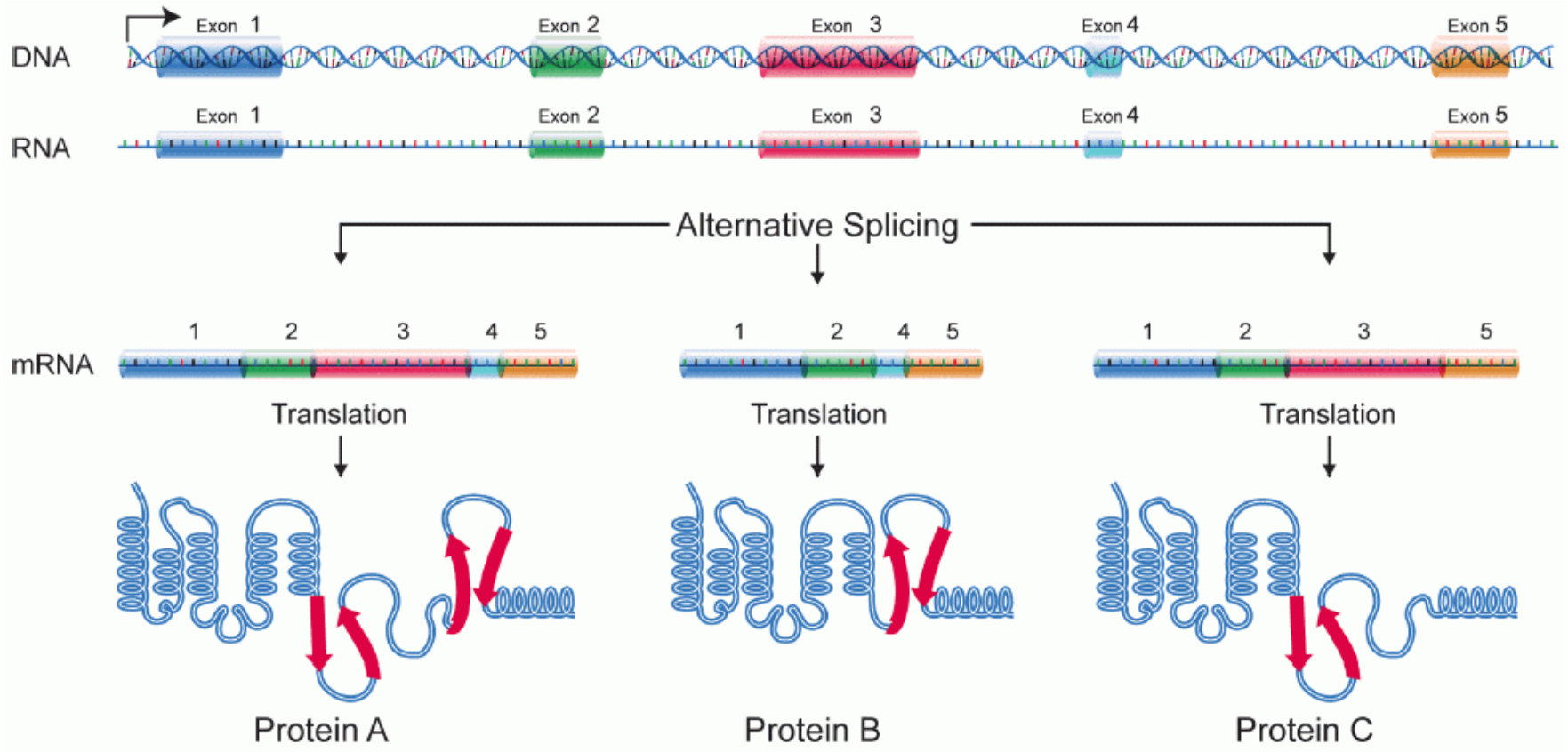


Figure 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Gene / Alternative Splicing



Cellular Complexity

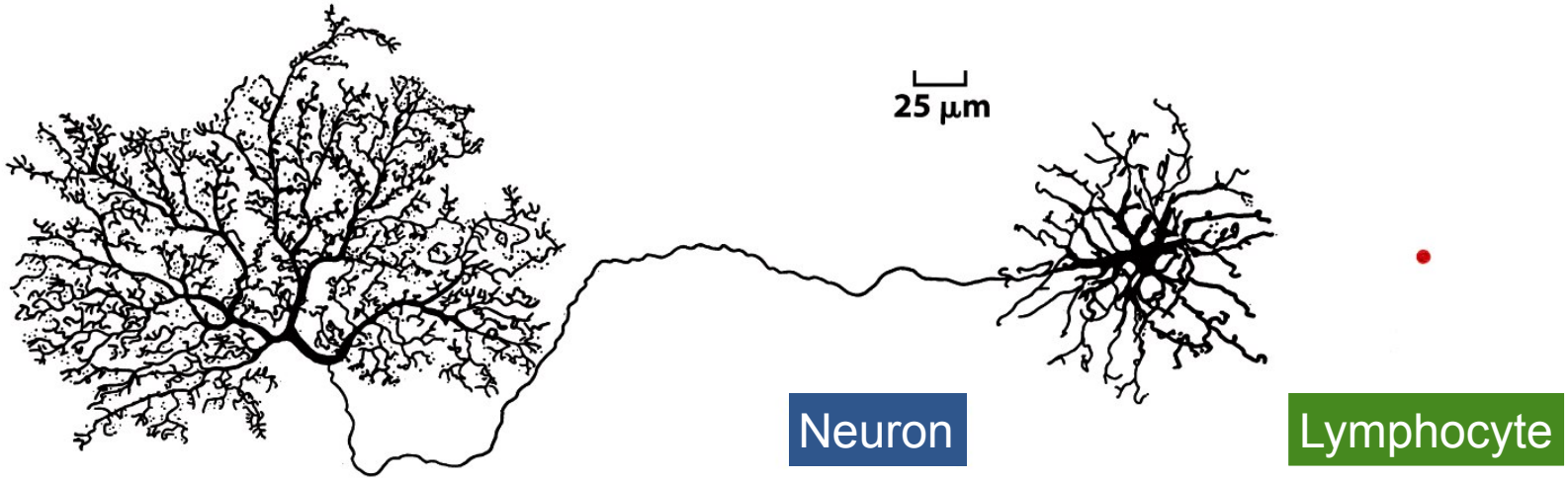


Figure 7-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Two cells of a organism have exactly* the same DNA

How does this differences arise?

How is cell fate remembered?

*** with exception of somatic mutations and rearrangements of immunological loci**

Cellular Complexity & Gene Expression

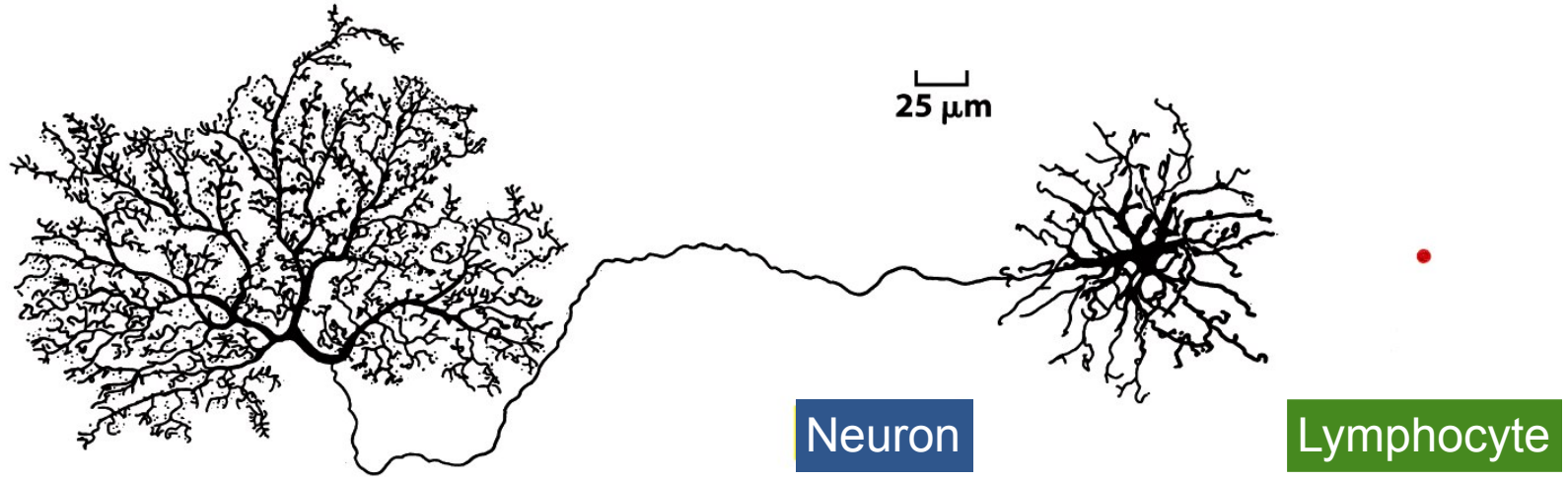
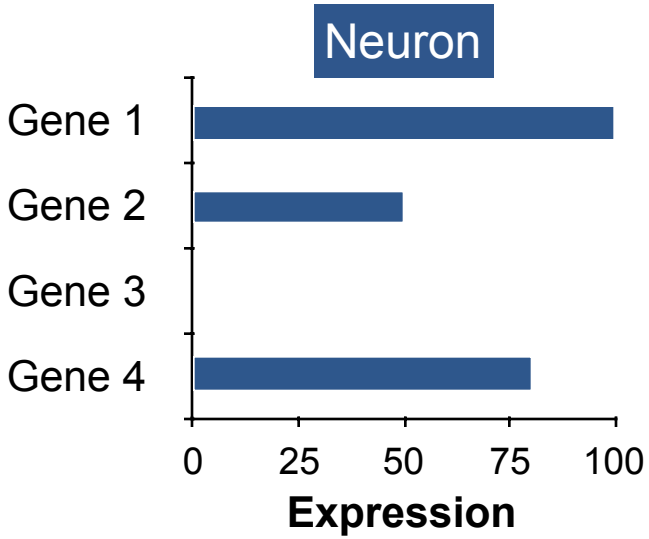
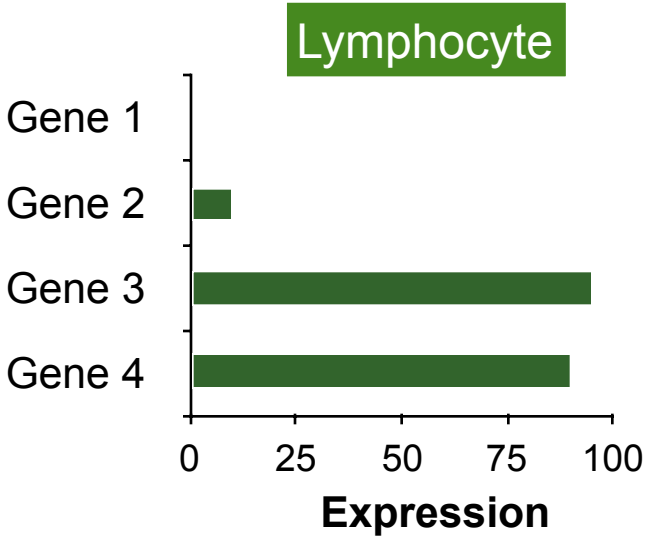


Figure 7-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)





Sequencing

Sequencing

Read the bases of a particular DNA/RNA sequence

Applications:

- sequence DNA of known and unknown organism
- detect variants on patients
- sequence the RNA of a cell
- detect location of proteins interacting with DNA

Problem:

- only short DNA sequences (<1.000 bs) can be read

Solution:

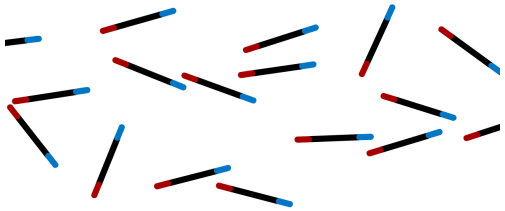
break DNA in several small pieces and use **bioinformatics**

Next Generation Sequencing

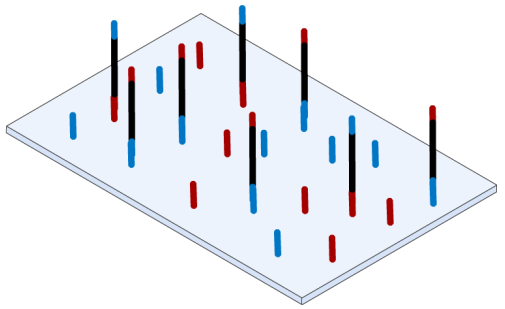
- NGS take advantage of parallelization
  - reads millions/billions of reads for a time
  - short reads (50-100 bps)
  - moderate error rates (0.1%)
- commercial products:
  - 454
  - SOLiD
  - Solexa (Illumina)

Illumina Flow Cell - NGS Sequencing

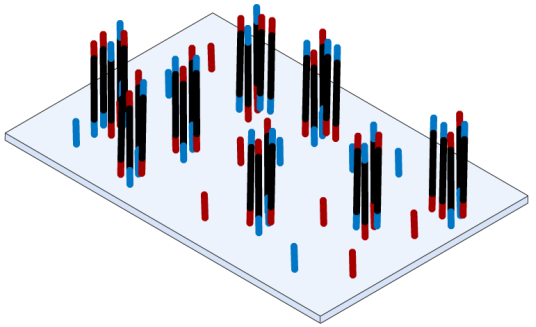
1- fragment sample DNA, insert adapters, attach to flow cell



2- use (bridge) PCR to copy fragments (close to origin)



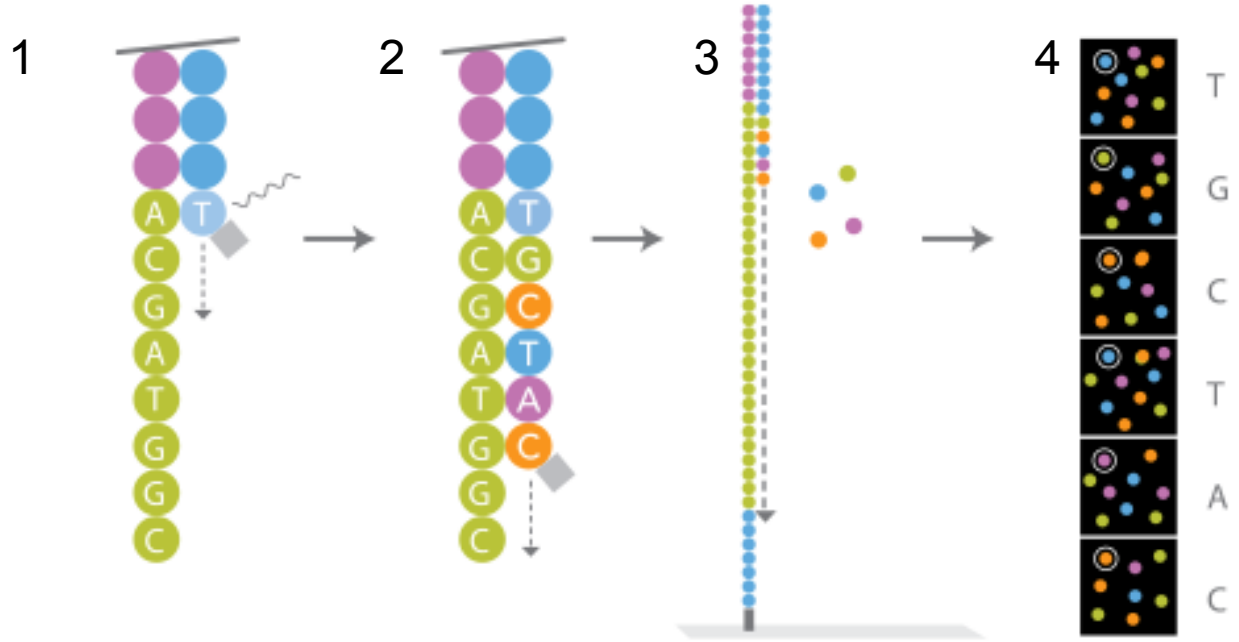
3- clusters of single stranded DNA (200m clusters with 2k DNA strands



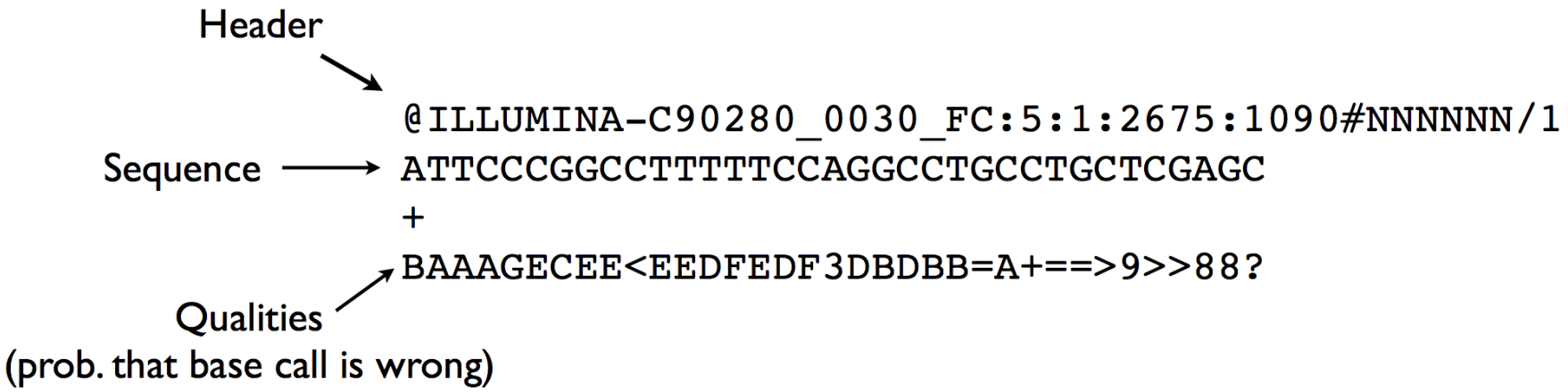
See video http://www.wellcome.ac.uk/Education-resources/Education-and-learning/Resources/Animation/WTX056051.htm

Illumina Flow Cell - NGS Sequencing

- **Iterative evaluation process:**
  1. **add RT-bases, polymerases integrate them**
  2. **wash away all not integrated elements**
  3. **take picture of flow cell to determine current base by dye**
  4. **derive reads from pictures**



Sequencing Results



One character encodes a number using ascii table (0-255)

This number (*Q*) can be converted to *P*

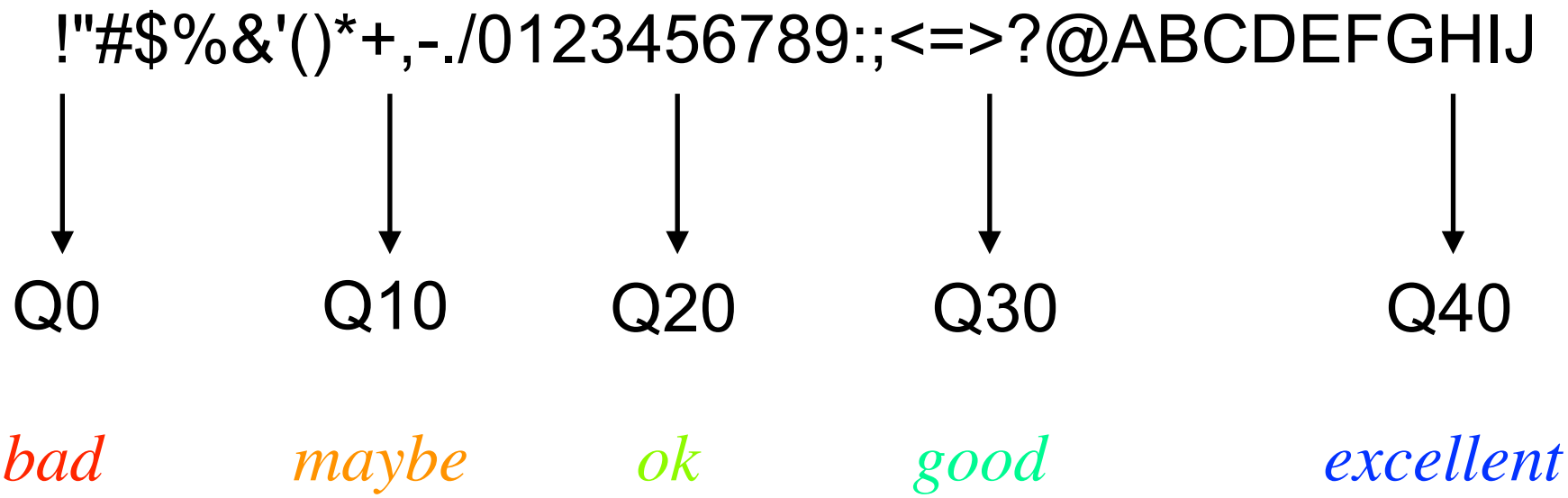
Phred-scale

Q = -10 * log10 P

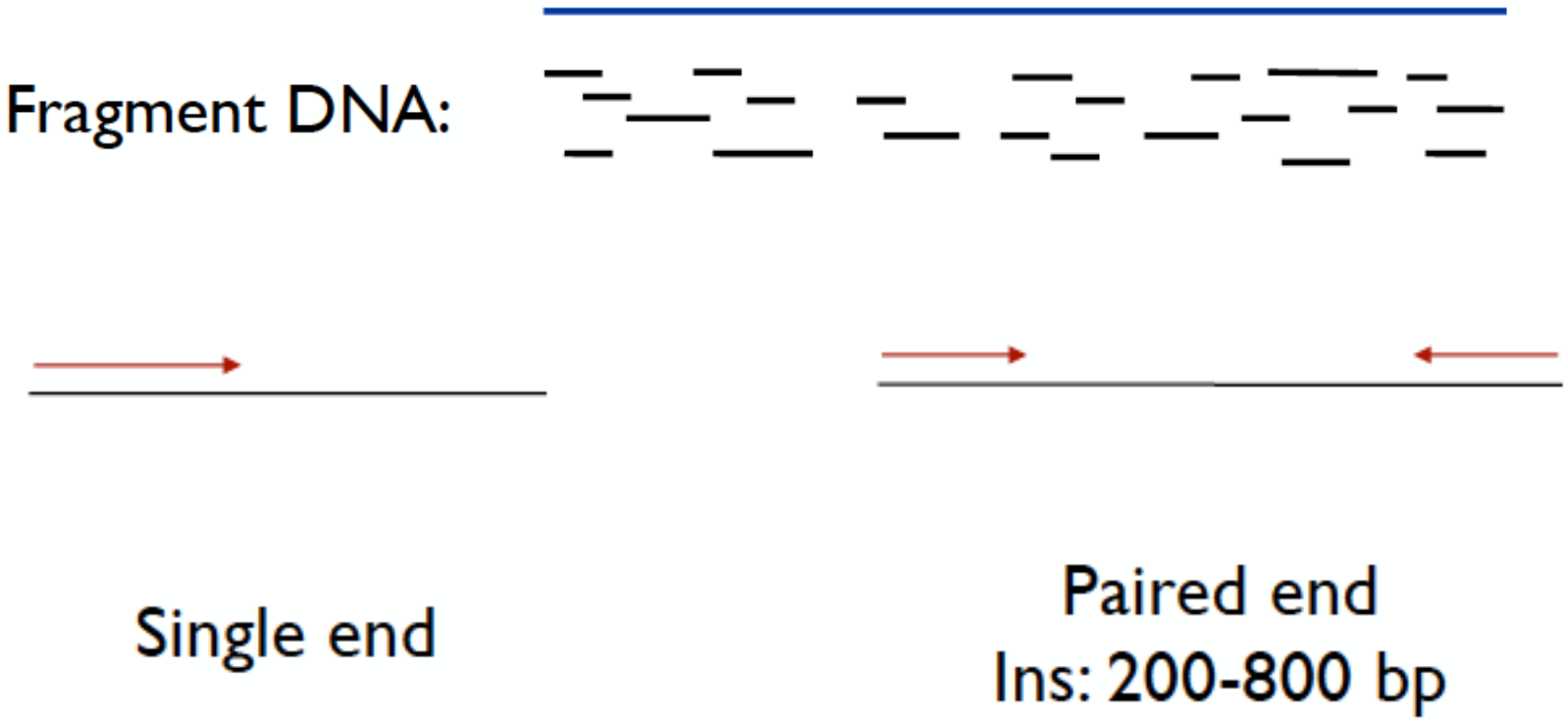
P = 10^(-Q/10)

Sequencing Results / Phred scores

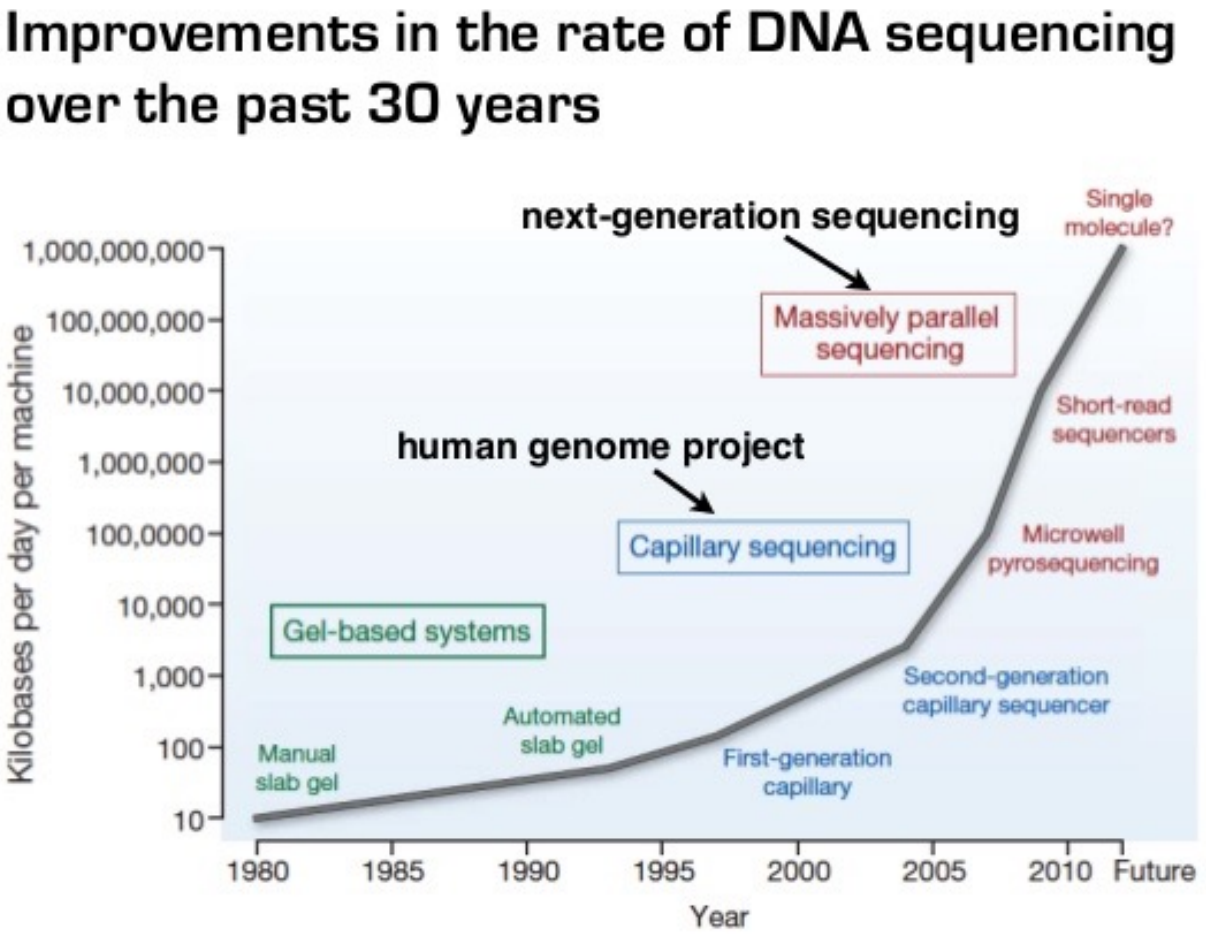
Uses letters/symbols to represent numbers:



Read Types

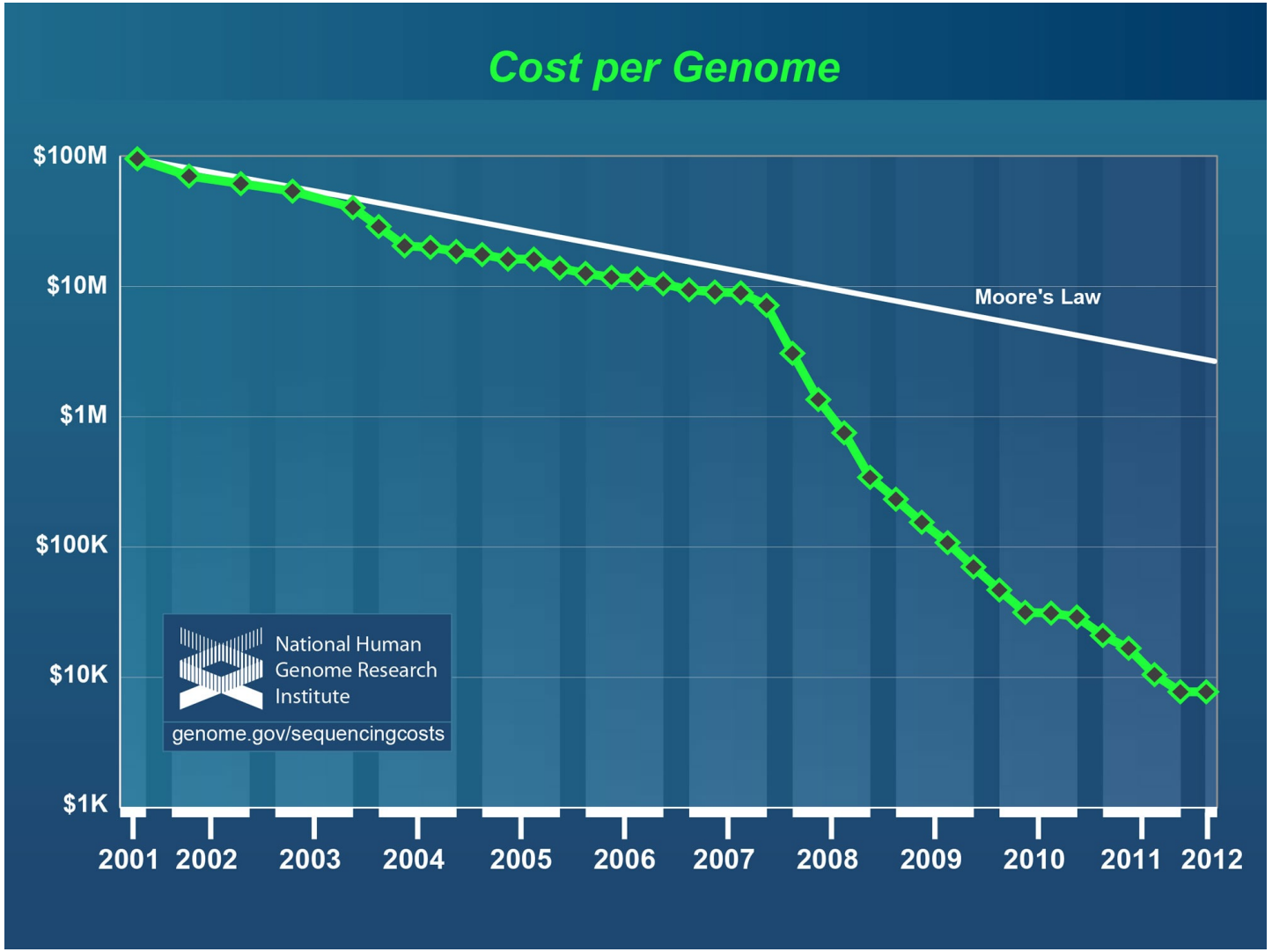


Next Generation Sequencing



Stratton, M. R., Campbell, P. J. & Futreal, P. A. The cancer genome. *Nature* **458**, 719–724 (2009).

Sequencing Costs



Sequence Alignment

Sequence Alignment

NGS

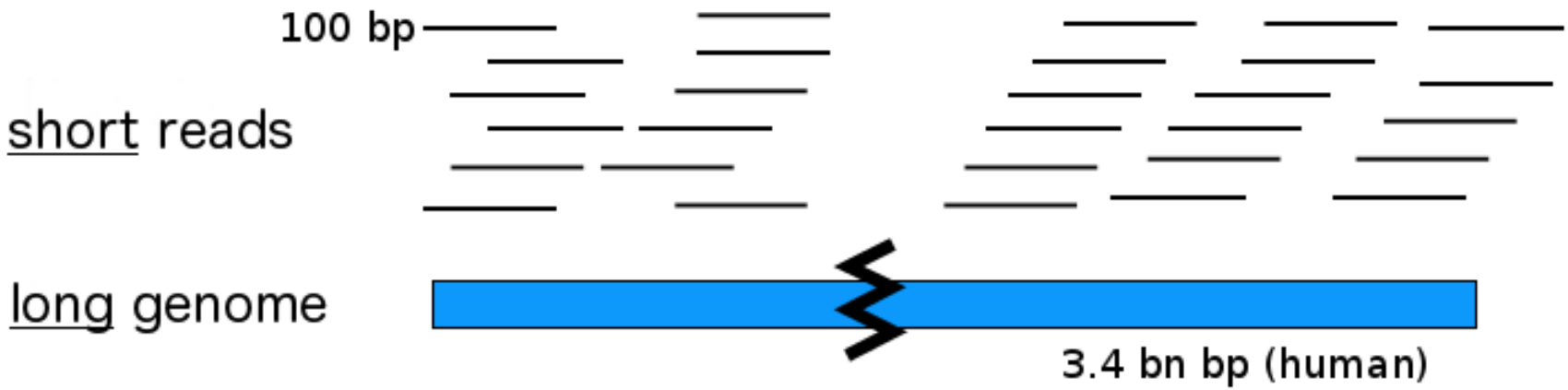
- reads from DNA fragments
- position in genome is unknown
- solution: alignment

DNA Sequencing

- de-novo assembly
  - construct unknown reference sequence from scratch
- resequencing / mapping
  - reference sequence given (applies to human- and mouse-studies)
  - build sequence that is similar but not necessarily identical to reference sequence

Alignment Problem

- a large reference sequence is given (genome)
  - up to billions of base pairs
- millions of short reads (<200bps)
- find most probable position of the read in the genome (by inexact string matching)



Pitfals

- (Unknown) divergent of sample and reference genome
- Repeats in the genome (larger than read size)
- Recombinations
- Poor genome reference quality
- Sequencing/read errors

Algorithms - Alignment

Alignment/Mapping is a typical inexact string match problem

Algorithmic Solutions: ?

Algorithms - Alignment

Alignment/Mapping is a typical inexact string match problem

Algorithmic Solutions:

- **Smith & Waterman - dynamic programming (quadratic time/memory)**

Algorithms - Alignment

Alignment/Mapping is a typical inexact string match problem

Algorithmic Solutions:

- **Smith & Waterman - dynamic programming (quadratic time/memory)**
- **Blast - k-mer search for seeding followed by dynamic programming**
  - **large memory requirement**
  - **local alignment**

11th and 13th most cited papers ever!!!

Algorithms - Alignment

Short read alignment is a special problem

- **reference sequence is large and fixed**
- **query sequence (reads) are short and many**

Solution: ?

Algorithms - Alignment

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- **reference sequence is large and fixed**
- **query sequence (reads) are short and many**

Solution: ?

1. Use a data structure to represent reference

- **k-mer hash table (>40GB for k=8)**
- **suffix trees (> 4GB)**

Algorithms - Alignment

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- **k-mer hash table (>40GB for k=8)**
- **suffix trees (> 4GB)**

2. Find candidate (k-mer) hits on genome (>100)

Algorithms - Alignment

Short read alignment is a special problem

- **reference sequence is large and fixed**
- **query sequence (reads) are short and many**

Solution: ?

1. Use a data structure to represent reference

- **k-mer hash table (>40GB for k=8)**
- **suffix trees (> 4GB)**

2. Find candidate (k-mer) hits on genome (>100)

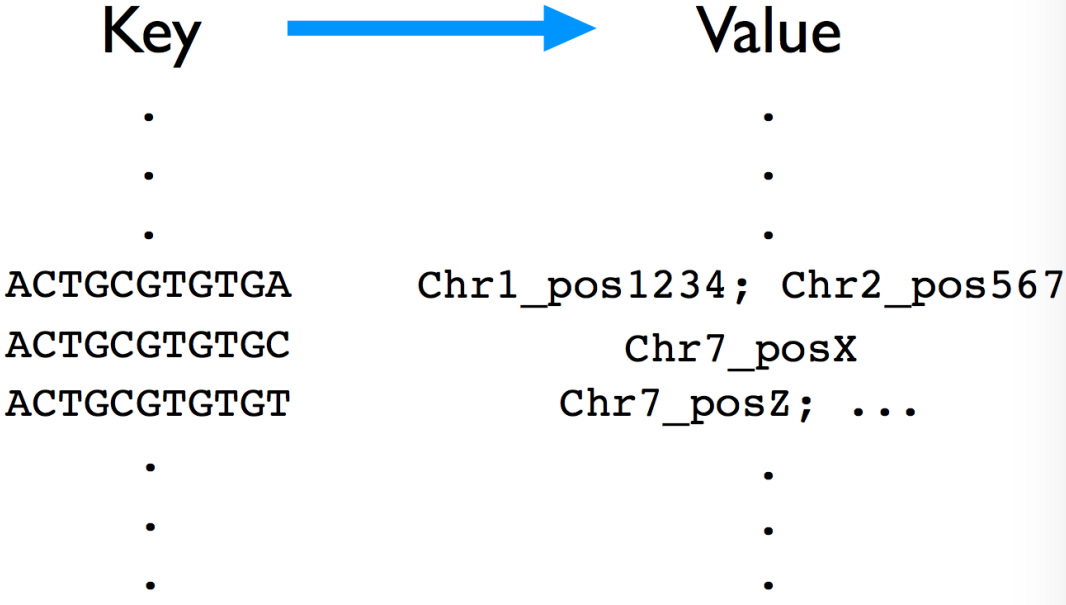
3. Improve alignment with Smith-Waterman

Methods work on linear time (query sequence)

Hash based algorithm

Lookups in hashes are *fast!*

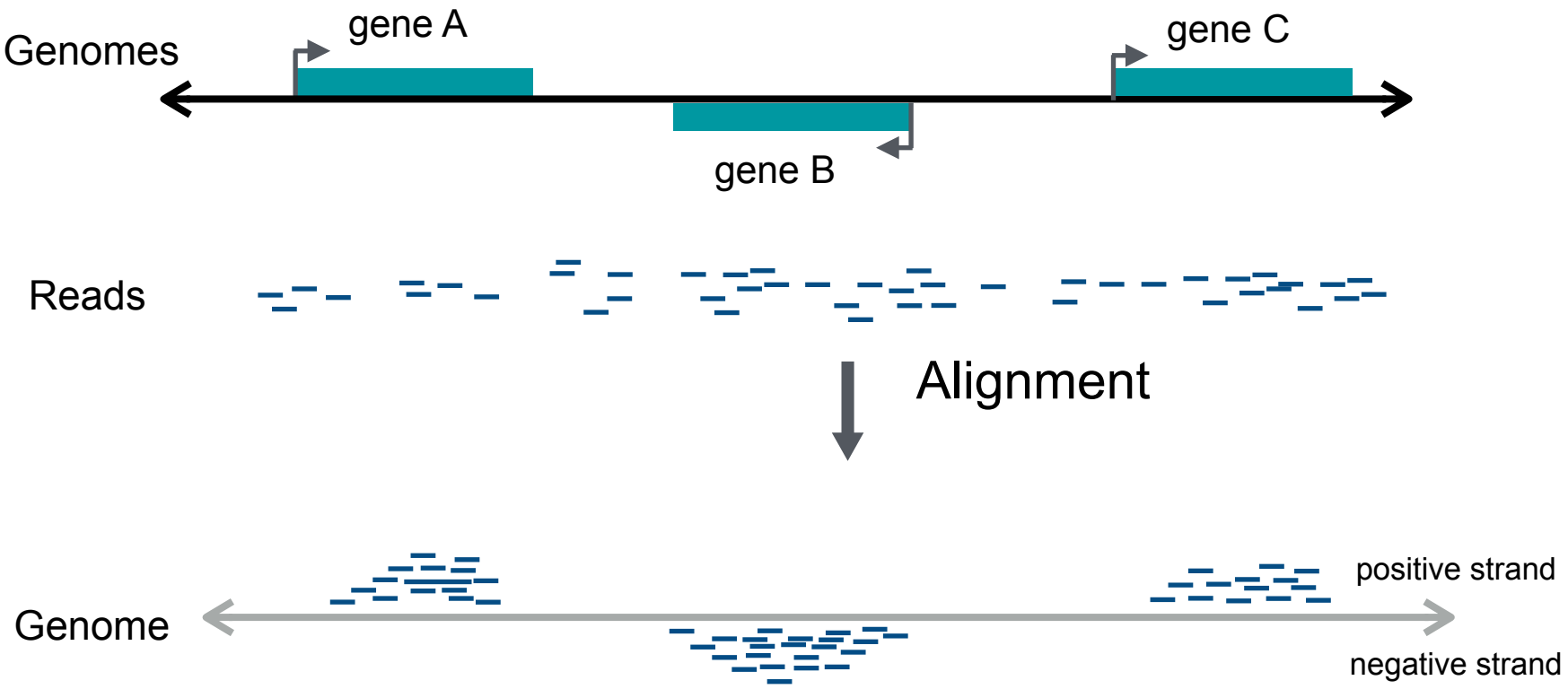
1. Index the reference using *k*-mers.
2. Search reads vs. hash *k*-mers
3. Perform alignment of entire read around seed
4. Report best alignment



Also known as *Seed and extend*

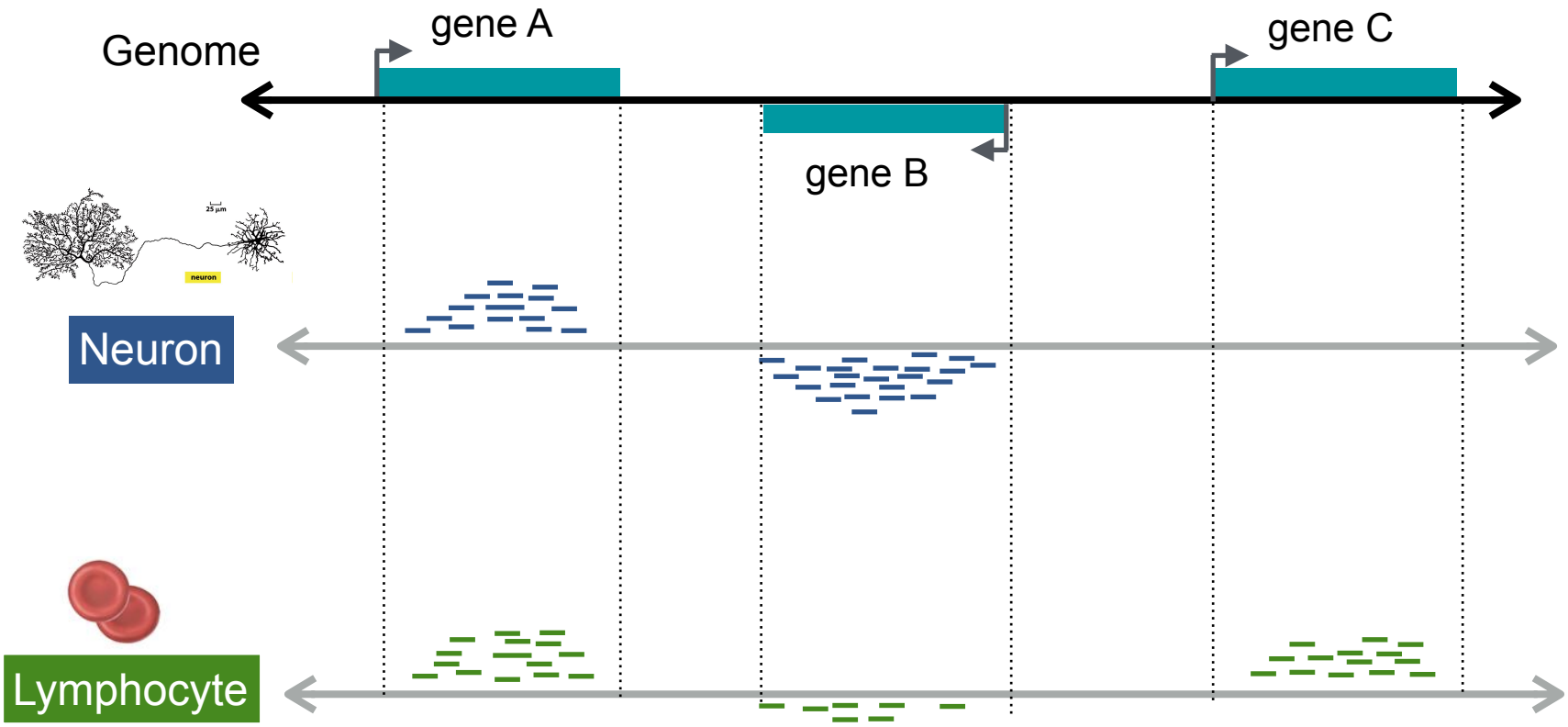
Alignment Results

- Position and strand of reads aligned to the genome



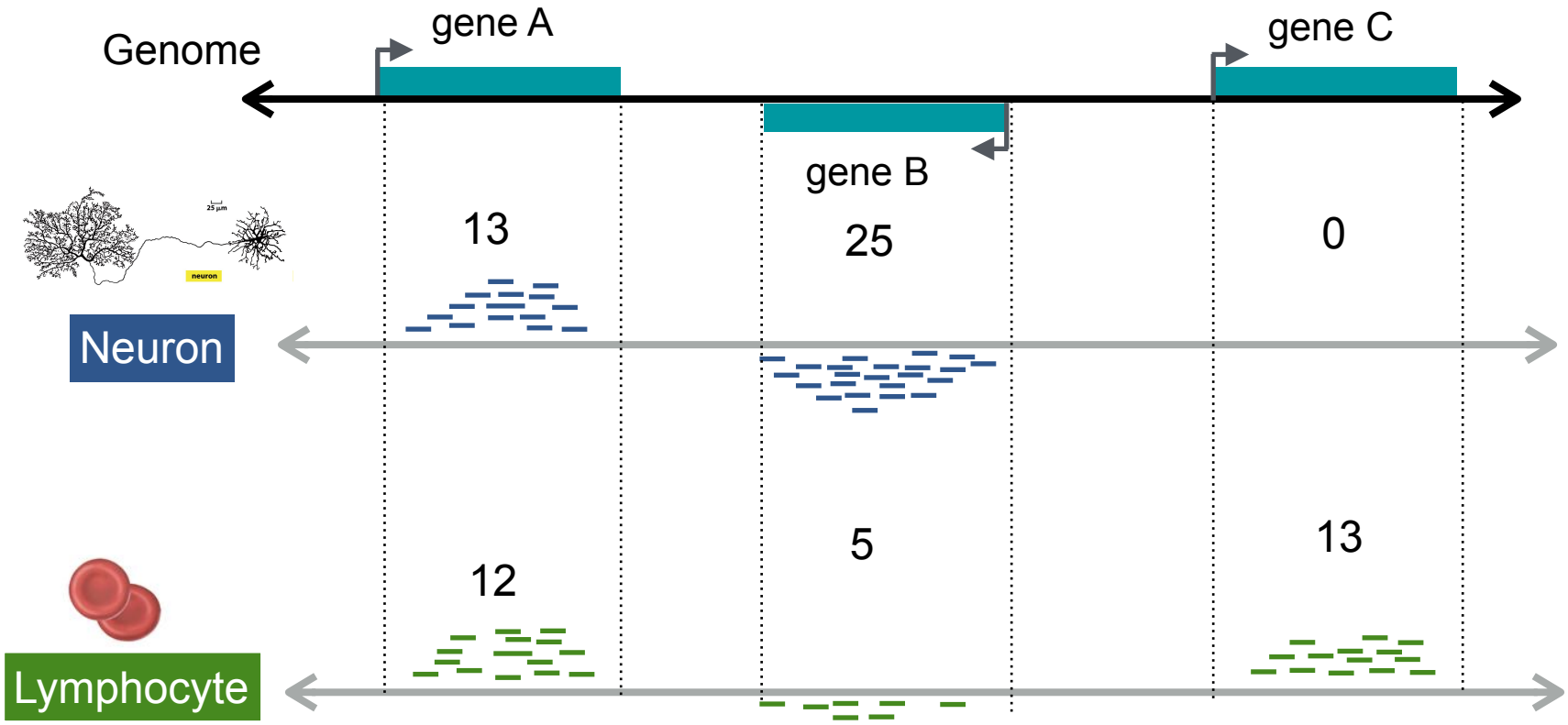
Gene Quantification

- Perform sequencing for each cell (neuron, lymphocyte)
- Align reads to genome

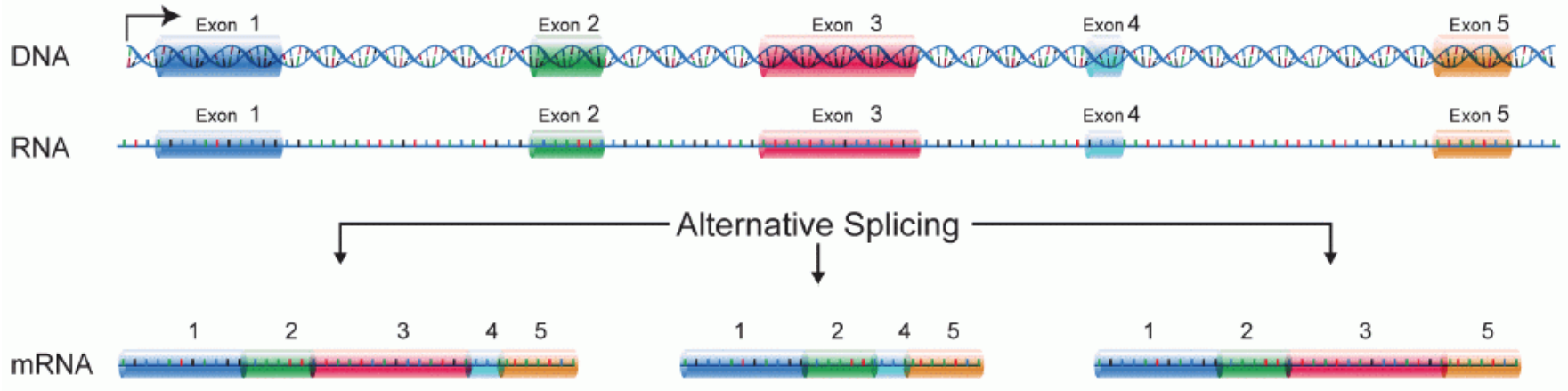


Gene Quantification

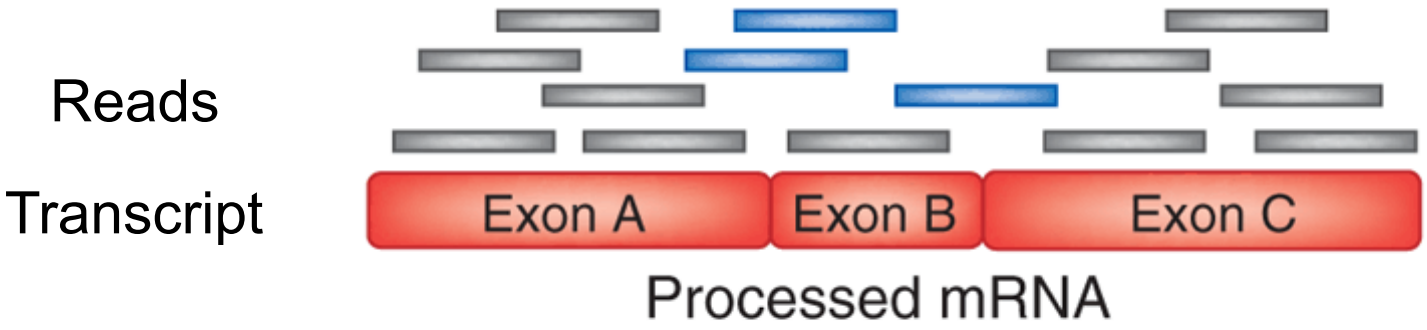
- Perform sequencing for each cell (neuron, lymphocyte)
- Align reads to genome
- Count number of reads inside genes (using known genes annotation)



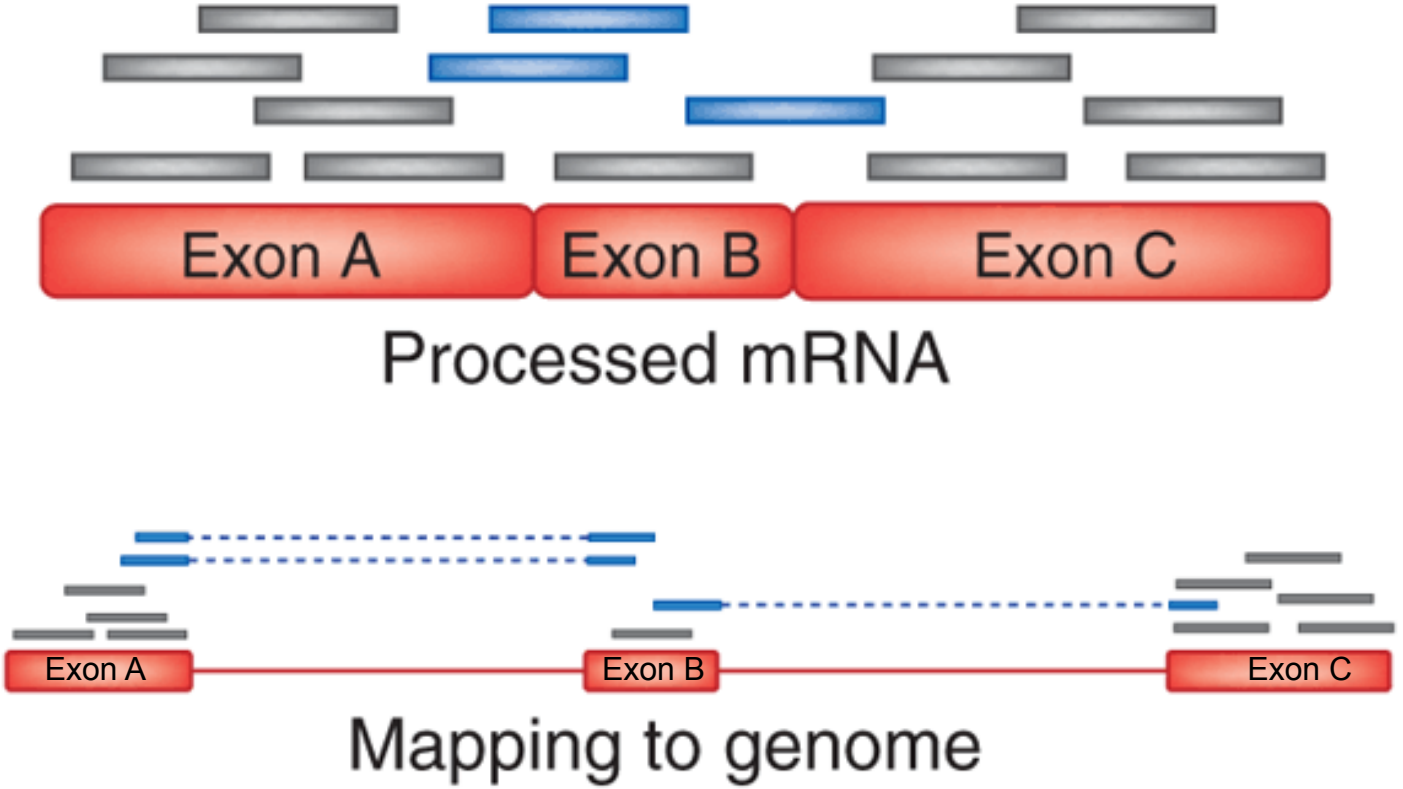
Gene Quantification - Transcripts



Transcripts

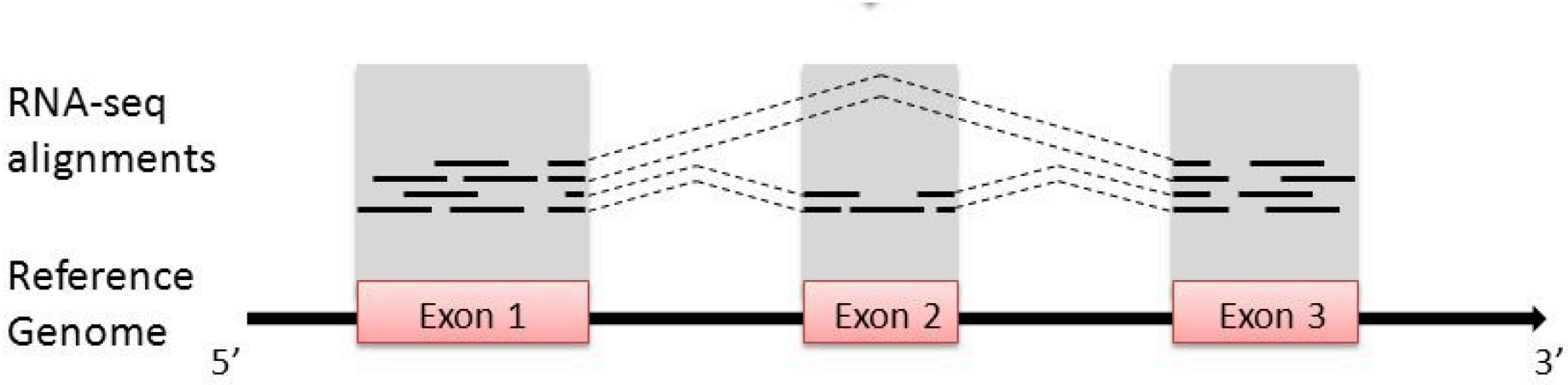


Alignment - Split Read Mapping (RNA-Seq)



- **reads needs to be split within intros when mapped to genome (special aligners / STAR)**

Quantification - Gene vs. Transcript vs. Exon



Counting Strategies

Gene Level - 17 reads
Exon level - exon 1 (8 reads), exon 2 (3 reads), exon 3 (6 reads)
Transcript Level - Exons 1,2 & 3 (10 reads) and exon 1 & 3 (7 reads) *
* complex computational methods required (TopHAT)

Quantificaiton - Normalization

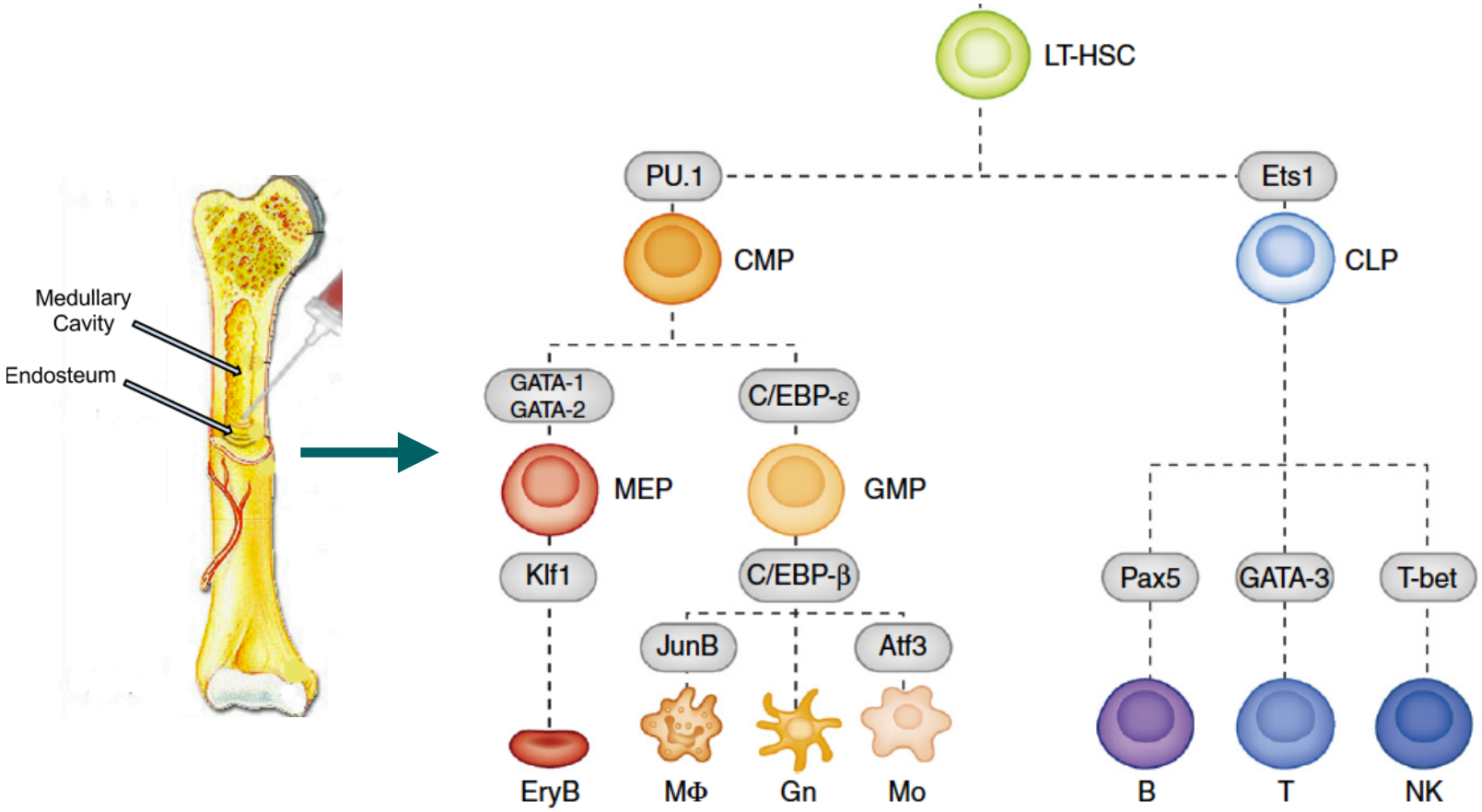
- Correct for:
  - Genes having distinct size
  - Sequencing efficiency differs between cell (usually same RNA quantity provided for sequencing)

	Cell A	Cell B	…
GeneA (1kb)	20	15	30
GeneB (2kb)	100	300	10
GeneC (1.5kb)	10	20	100
Gene D (3kb)	300	200	100
Total Library	430	535	240

$$\text{Reads per kilobase million (RPKM)} = \#reads * \frac{gene\ size}{1.000} * \frac{total\ library}{1.000.000}$$

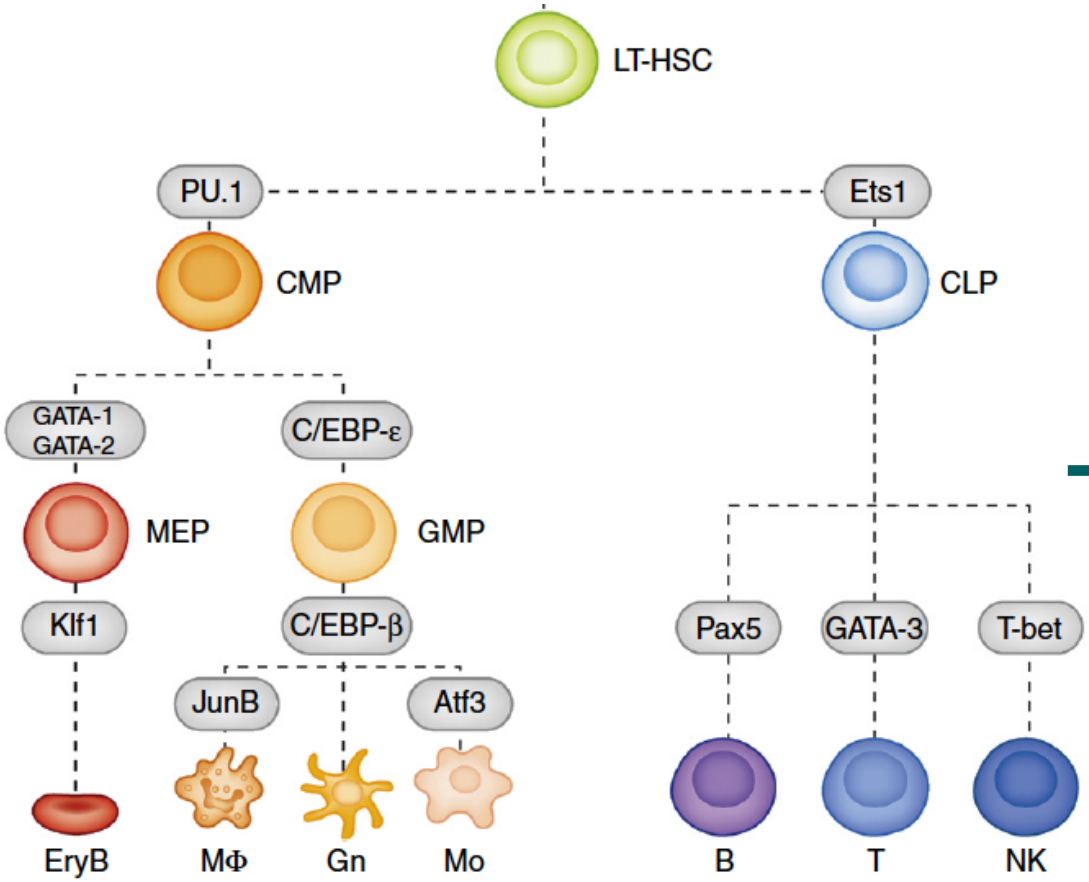
Expression at Single Cell Level

Cell Differentiation



Source: Amit (2016), *Nature Immunoloy.*

Cell Differentiation & Gene Expression

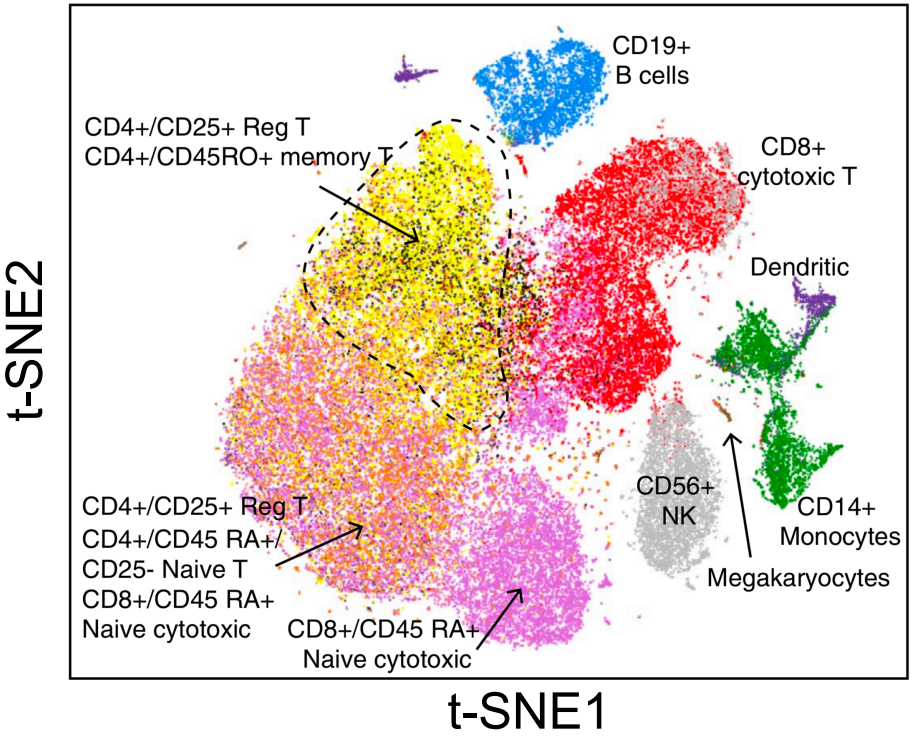


	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

Source: Amit (2016), *Nature Immunoloy.*

Gene Expression of Lymphoid Cells

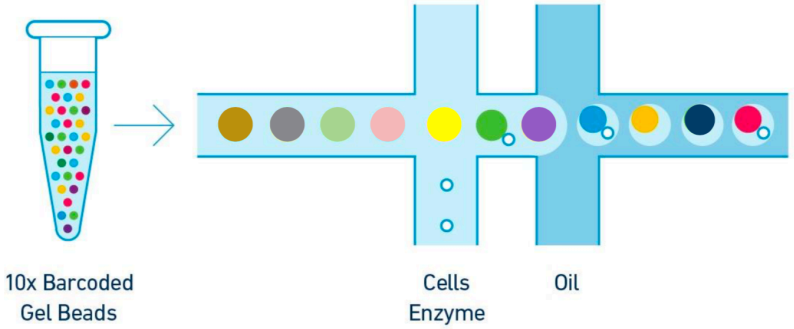
PBMCs from Humans

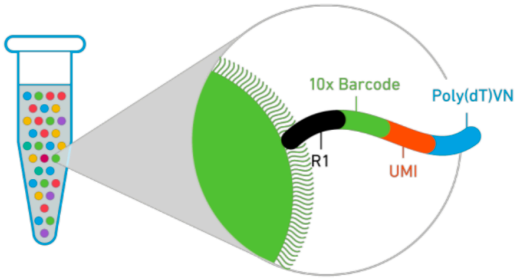


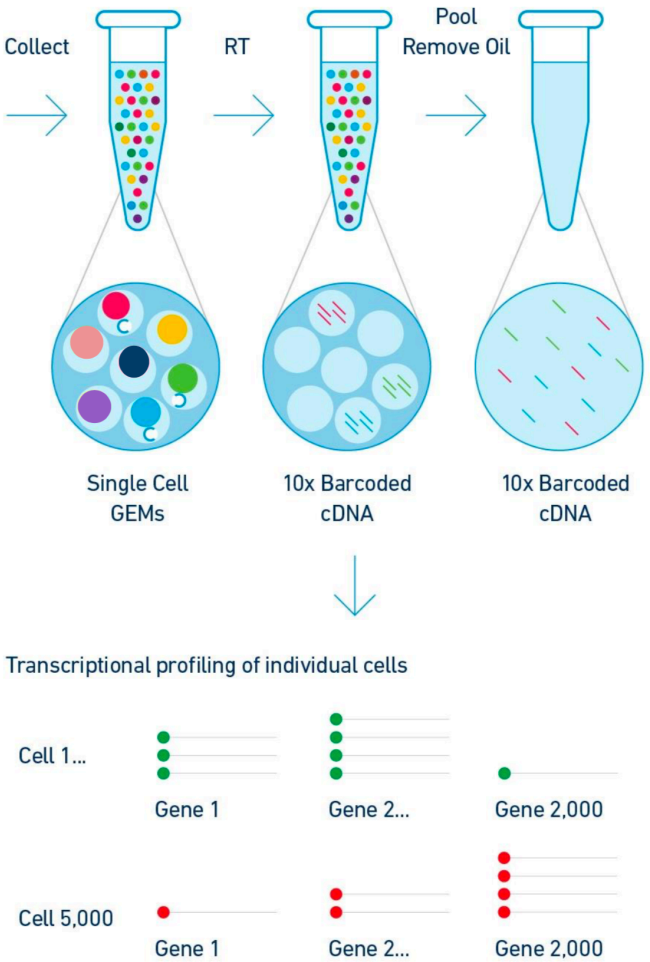
Single cell RNA-seq from 68k cells

Source: Zheng et al. 2017 & Buenrostro et al. 2018

Droplet based RNA single cell sequencing





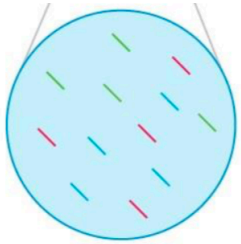


- Input: Single cells in suspension + 10x Gel Beads and Reagents
- Output: Digital gene expression profiles from every partitioned cell

Source: 10x genomics

Basics Bioinformatics - single cell RNA-seq

Sequences



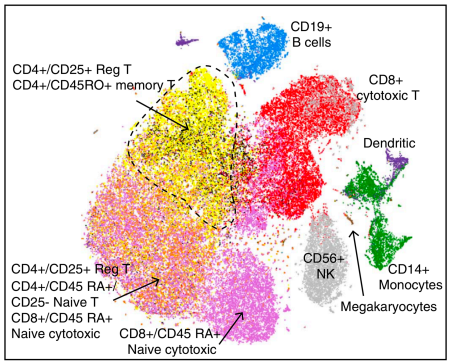
10x Barcoded cDNA

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering

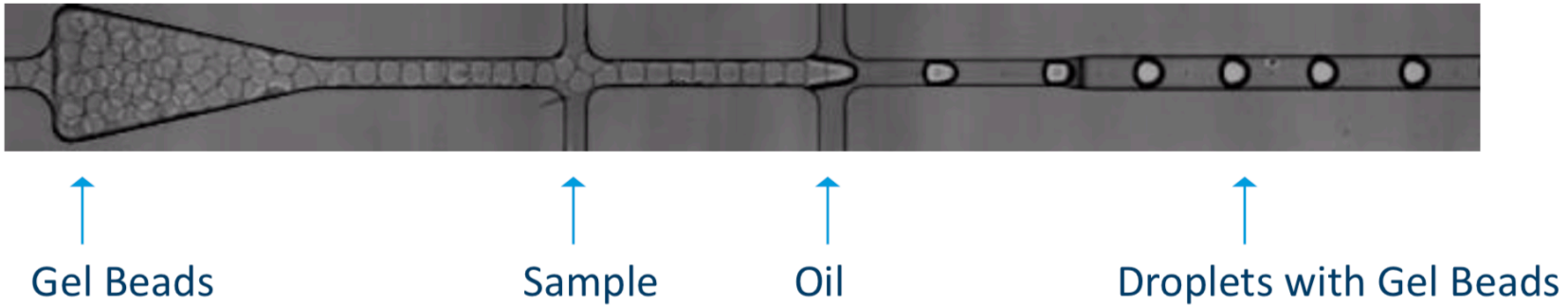


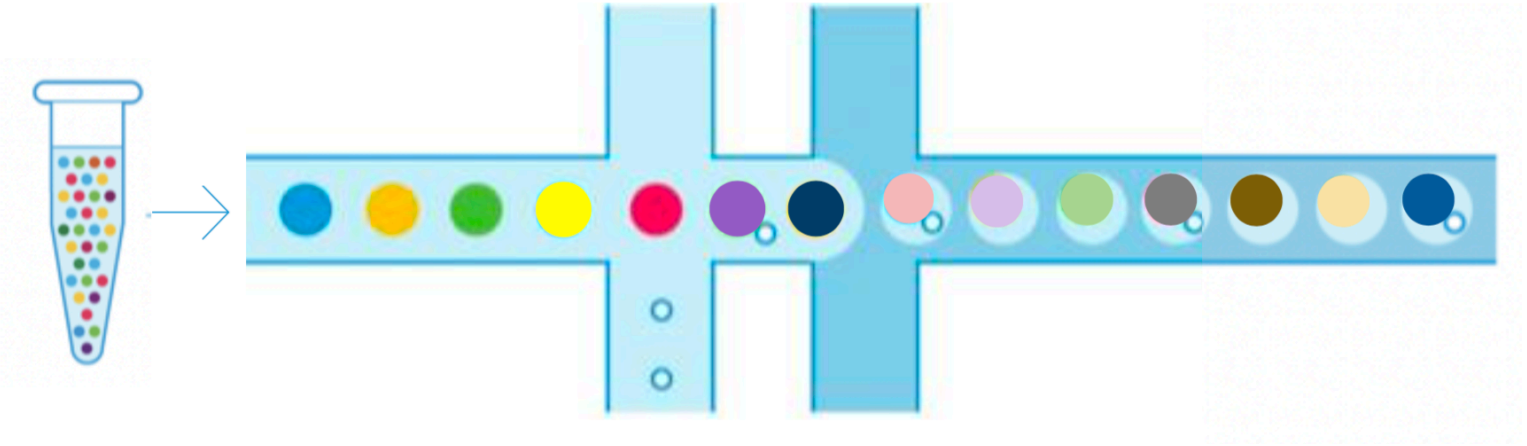
1. Alignment / Transcript count
2. Cell filtering
3. Removal of biological variation
4. Dimension reduction / cell clustering

cell ranger 10x genomics

Seurat -R

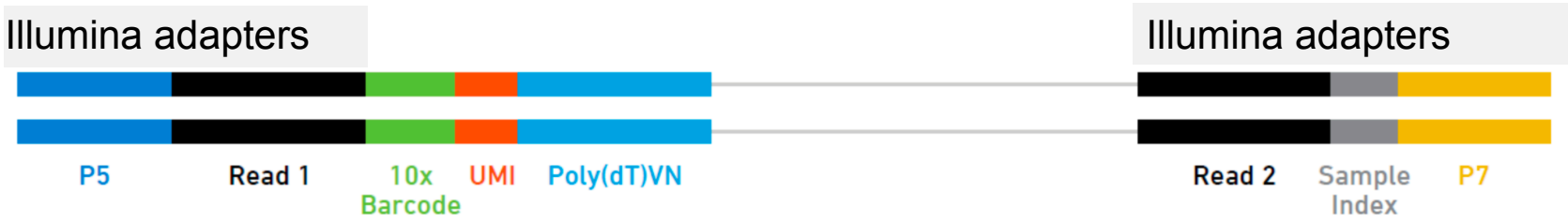
Droplet based RNA single cell sequencing





Source: 10x genomics

Basics Bioinformatics - Transcript Counts



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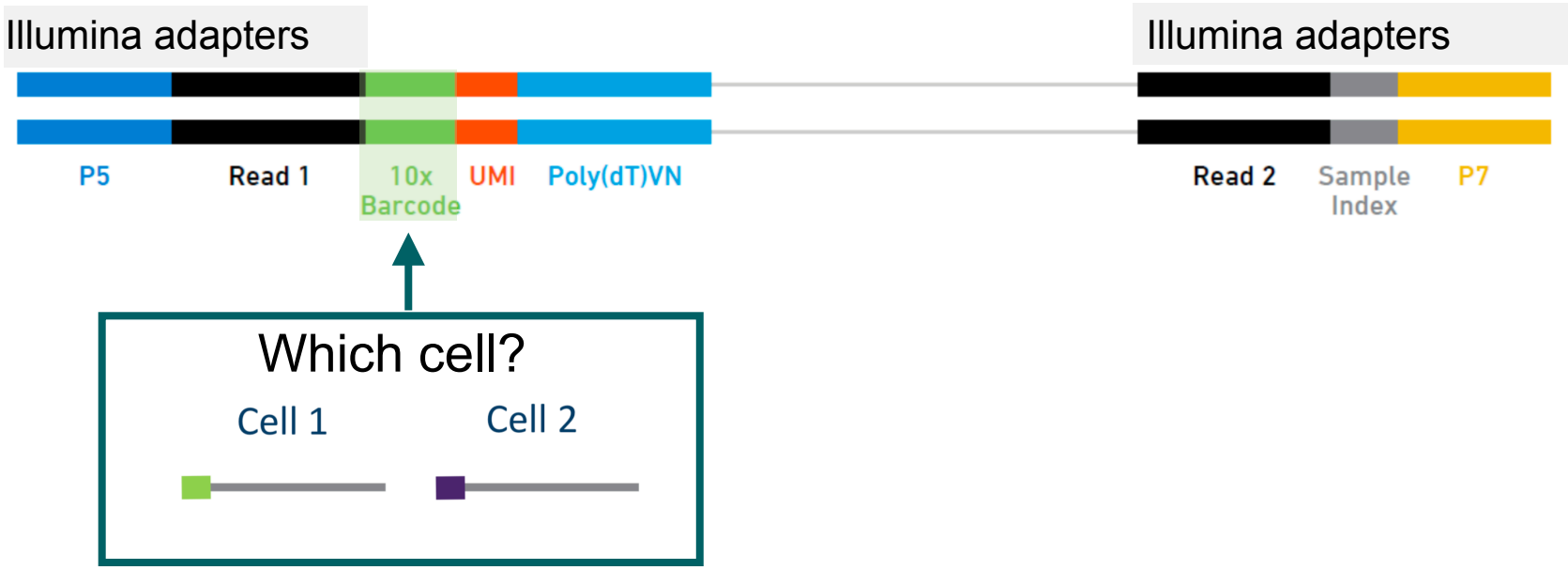

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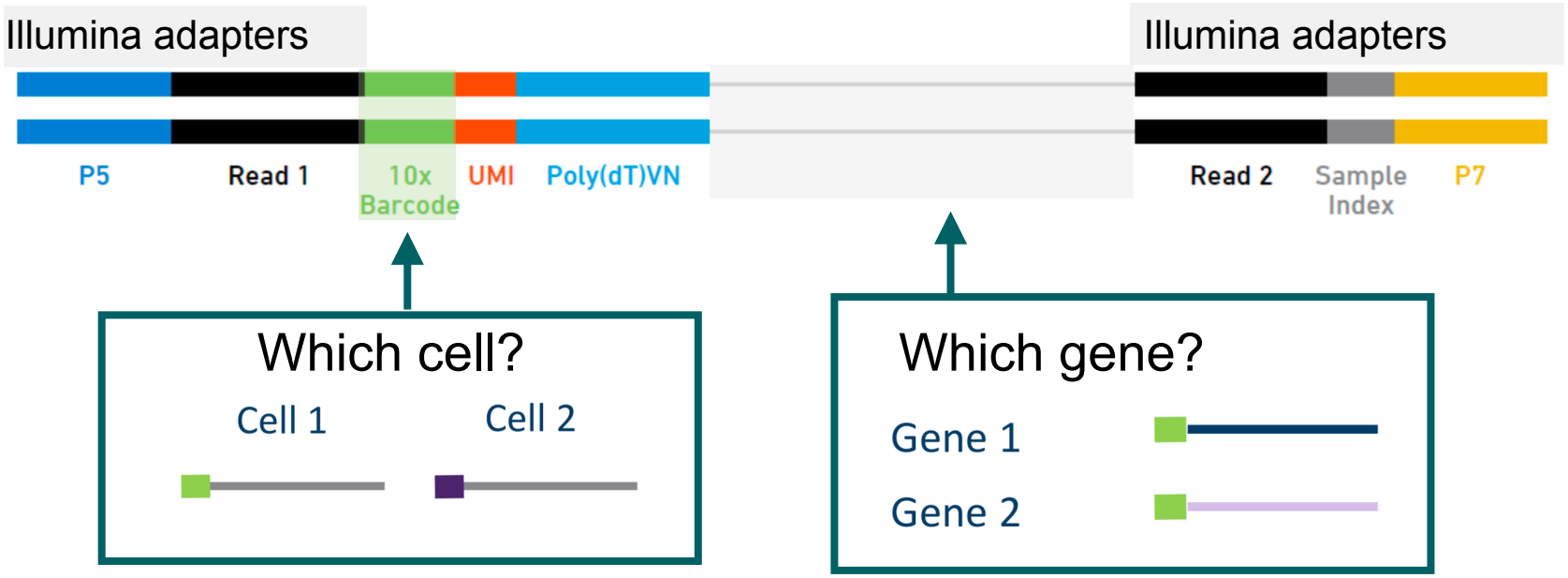
Basics Bioinformatics - Transcript Counts



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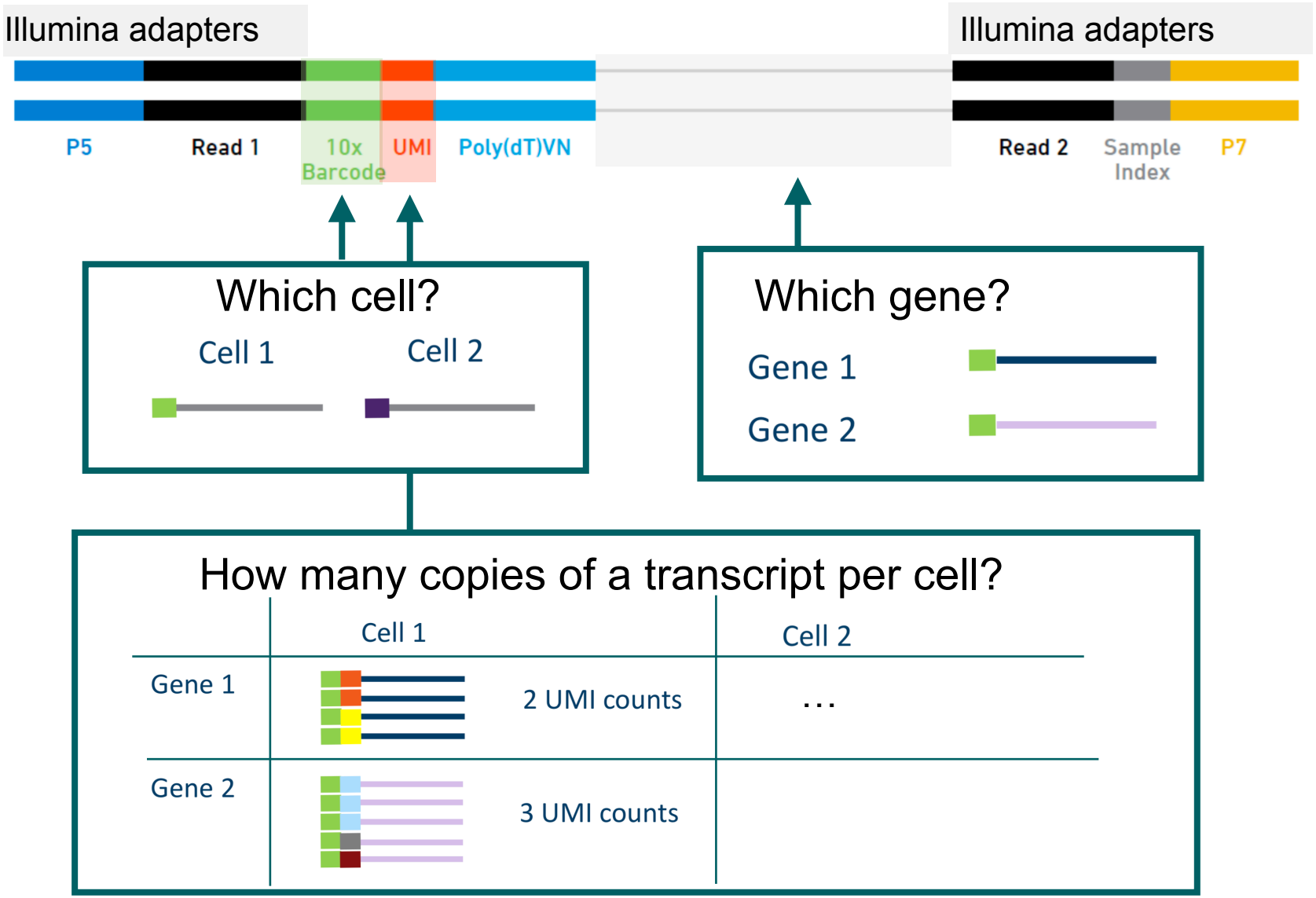
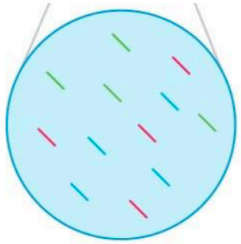


Source: 10x genomics

Basics Bioinformatics - single cell RNA-seq

Sequences

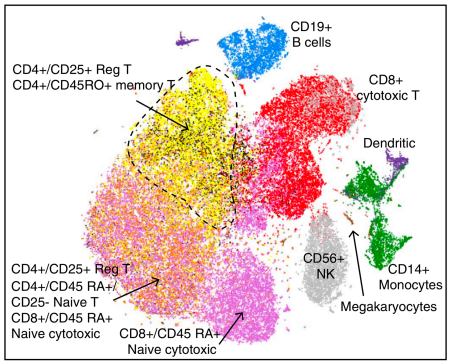


Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering



1. Alignment / Transcript count

2. Cell filtering

3. Removal of biological variation

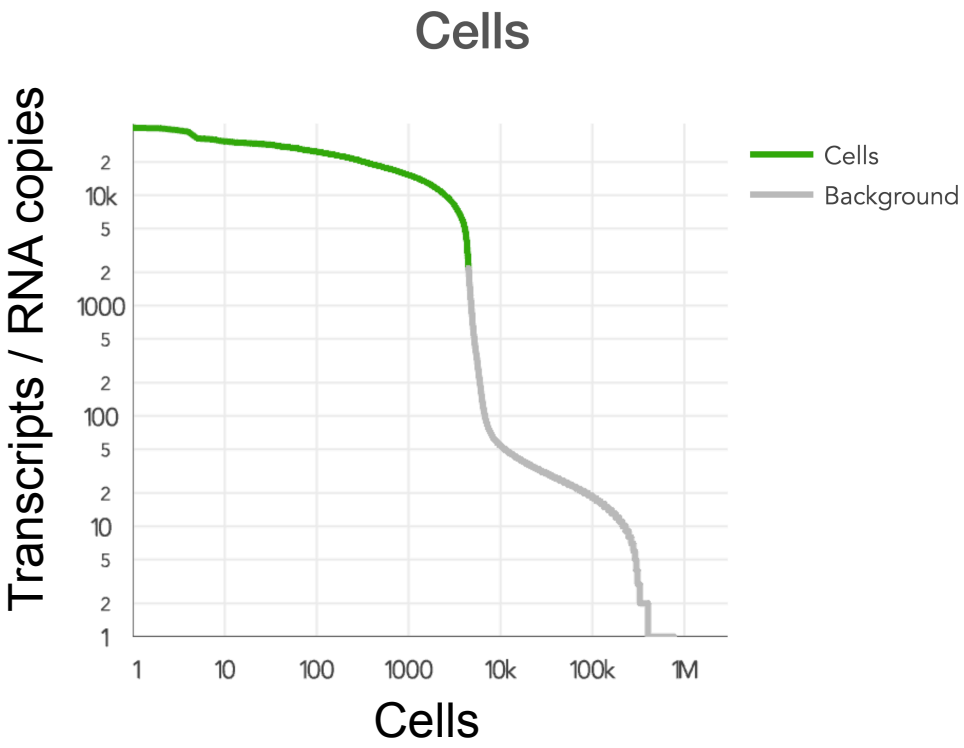
4. Dimension reduction / cell clustering

cell ranger
10x genomics

Seurat -R

Basics Bioinformatics - Cell Filtering

1. sum UMIs (copy of transcripts) per cell
2. consider cells with total UMI count > 99th of expected recovered cells



Estimated Number of Cells
4,495

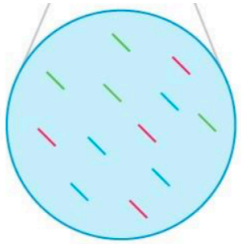
Post-Normalization Mean Reads per Cell
89,289

Median Genes per Cell
2,504

cell ranger - 10x genomics

Basics Bioinformatics - single cell RNA-seq

Sequences



10x Barcoded cDNA

Expression matrix

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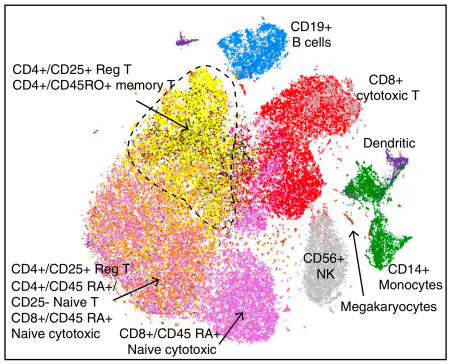

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cell ranger 10x genomics

Seurat -R

Basics Bioinformatics - Dimension Reduction

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

- High dimension matrix:
  - 4945 cells vs. 17328 genes
- Sparse matrix:
  - 50% zeros (90k reads per cell)

Data: dendritic cells of Peyer patches with Torrow & Hornef UK Aachen

Basics Bioinformatics - Dimension Reduction

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Reduction with t-SNE or PCA

t-SNE matrix

t-SNE Scores

	Cell 1	Cell 2	...
t-SNE1	3.1	0.3	
t-SNE2	-2.1	2.1	

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t-SNE - local neighbourhood preserving
PCA - distance preserving

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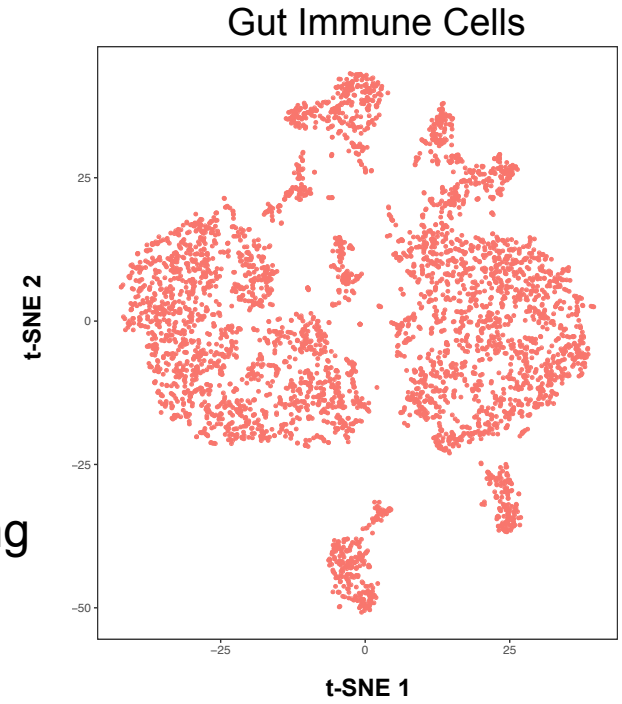
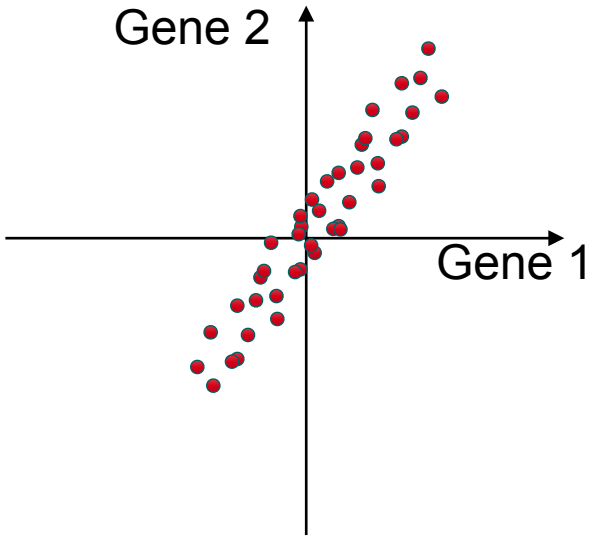


t-SNE - local neighbourhood preserving
PCA - distance preserving

Principal Component Analysis

- **method for dimension reduction**
  - **find combination of genes explaining cells with distinct expression**
- **For a expression matrix (X) -> find directions (w) with highest variance**

$$\mathbf{w}_{(1)} = \underset{\|\mathbf{w}\|=1}{\arg\max} \{ \|\mathbf{X}\mathbf{w}\|^2 \}$$



Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

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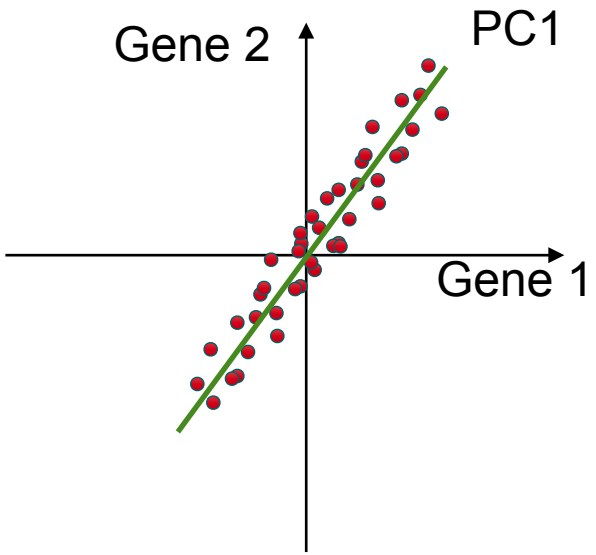

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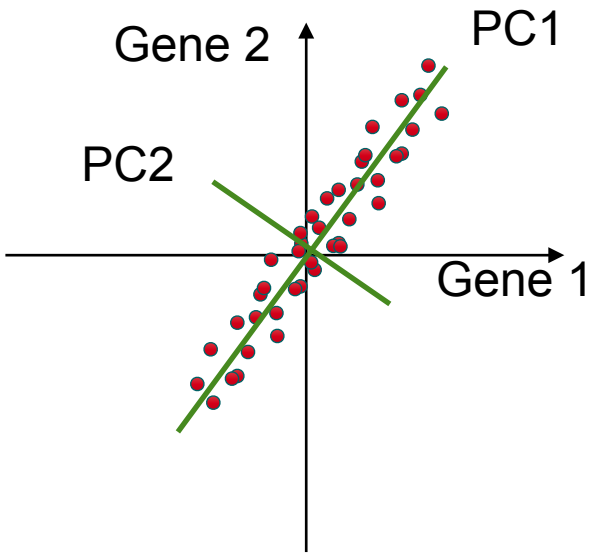

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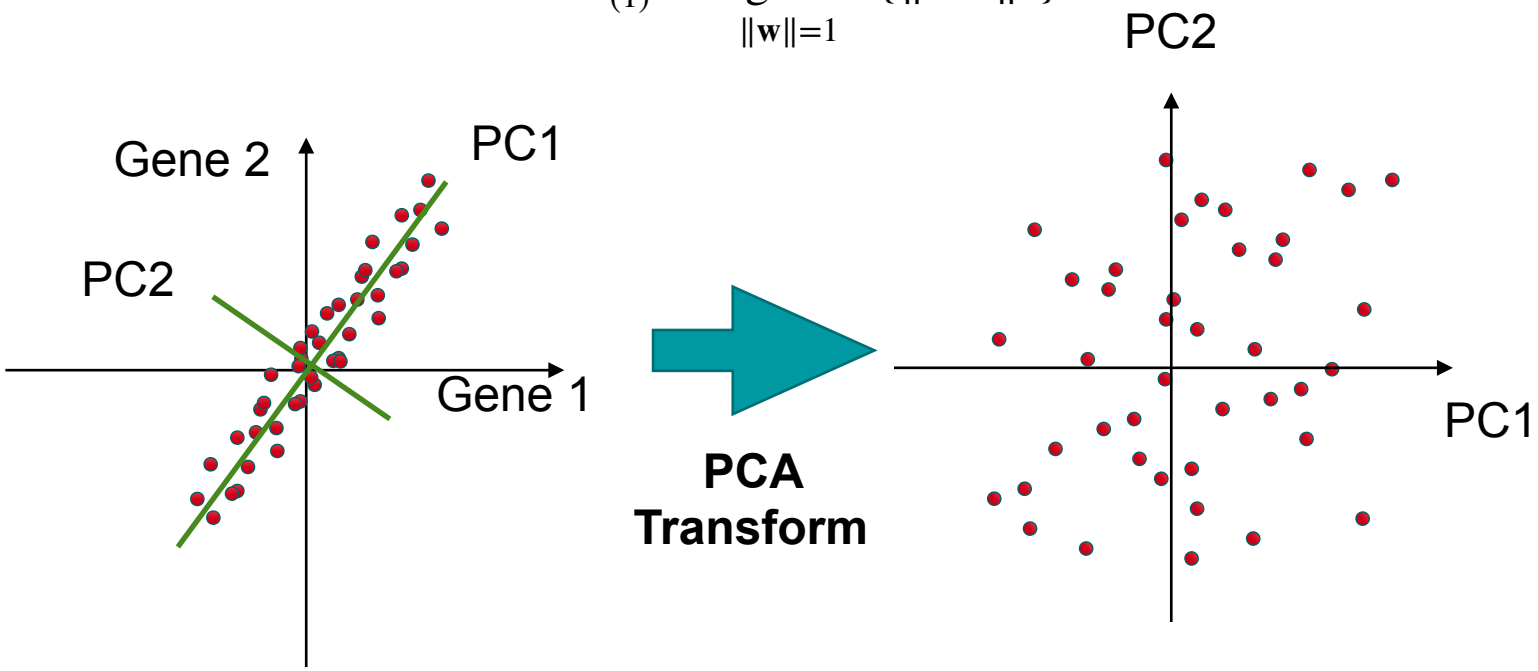

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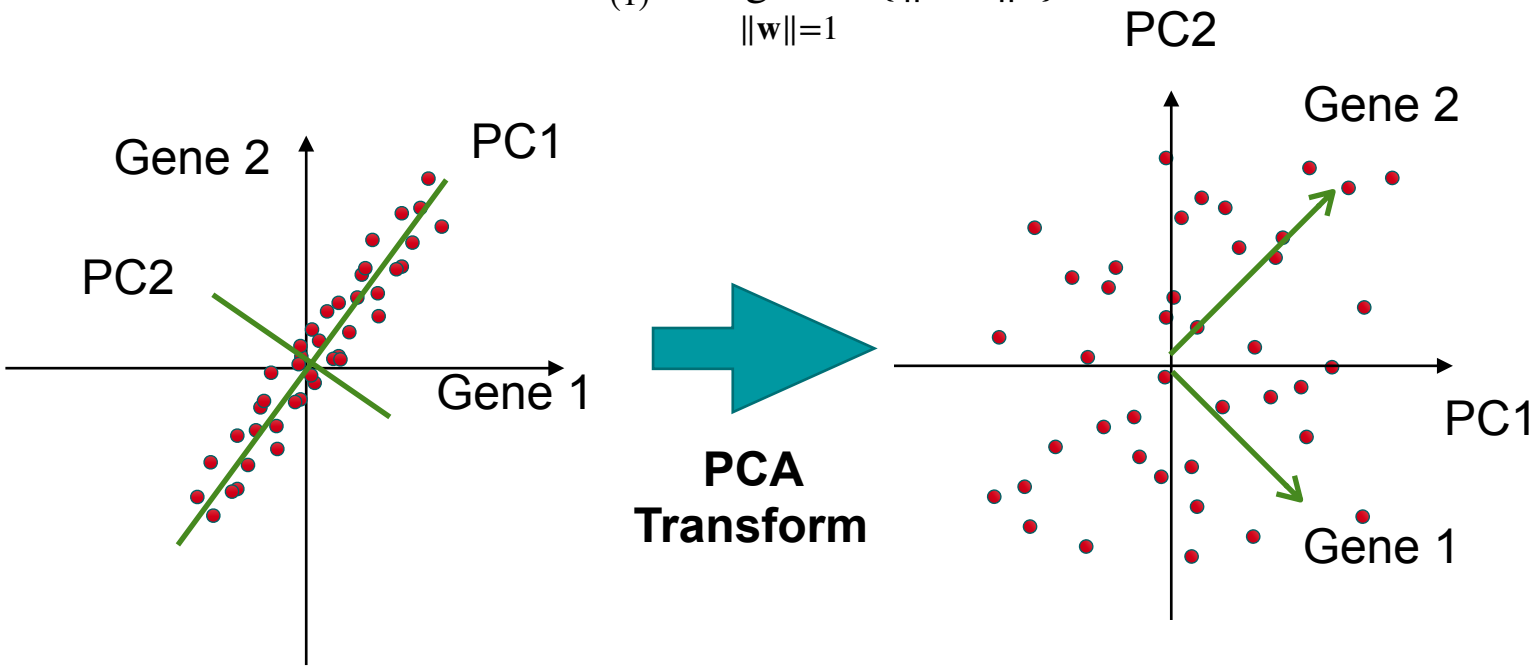
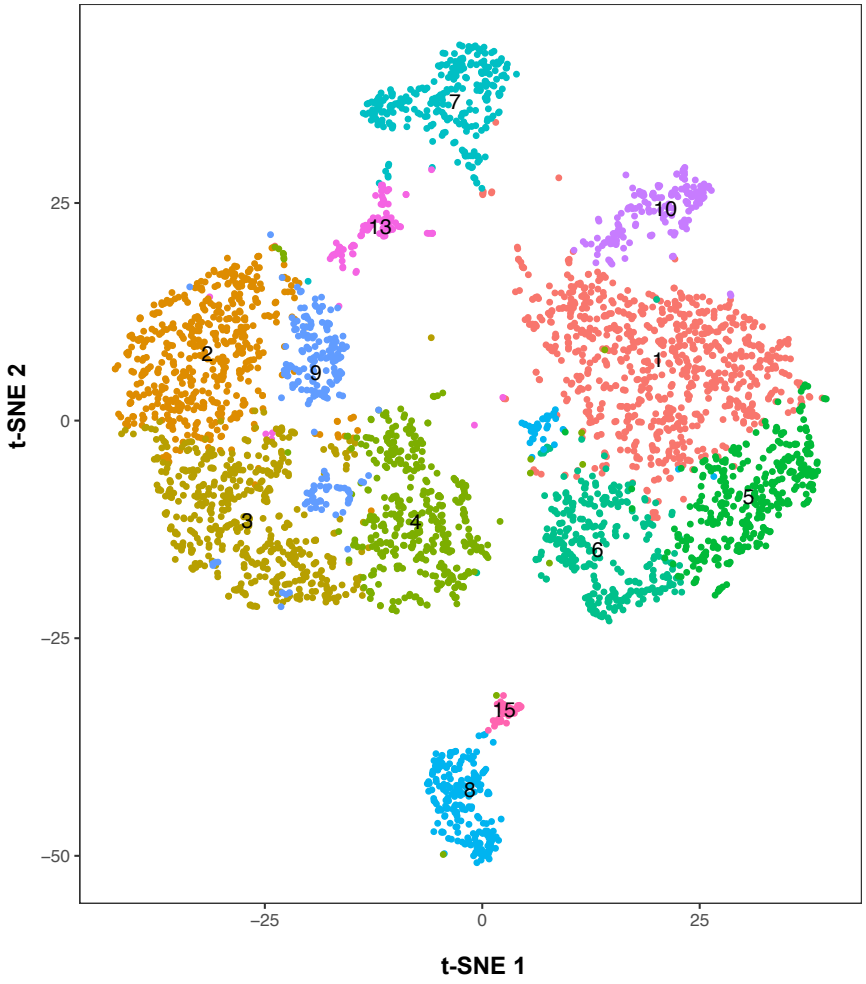


Basics Bioinformatics - Clustering

Gut Immune Cells - 12 groups

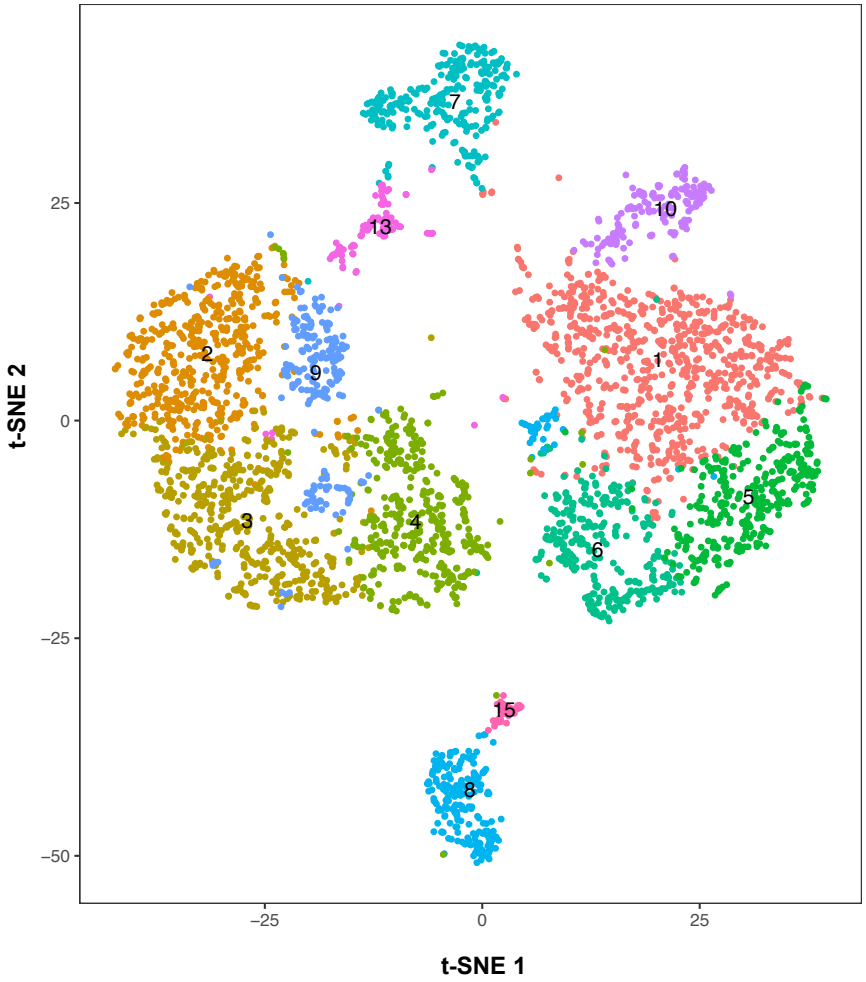


Clustering - identify cells with similar expression patterns
- based on PCA (20 dimension)

How to identify cell types?

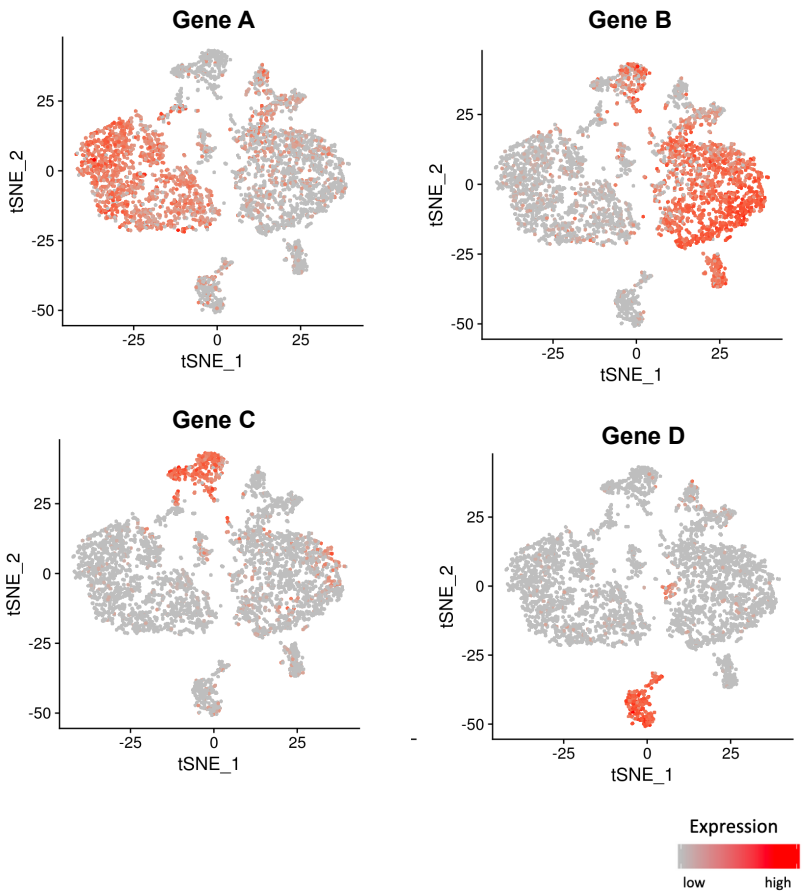
Cell Identity with an Expert

Gut Immune Cells - 12 groups



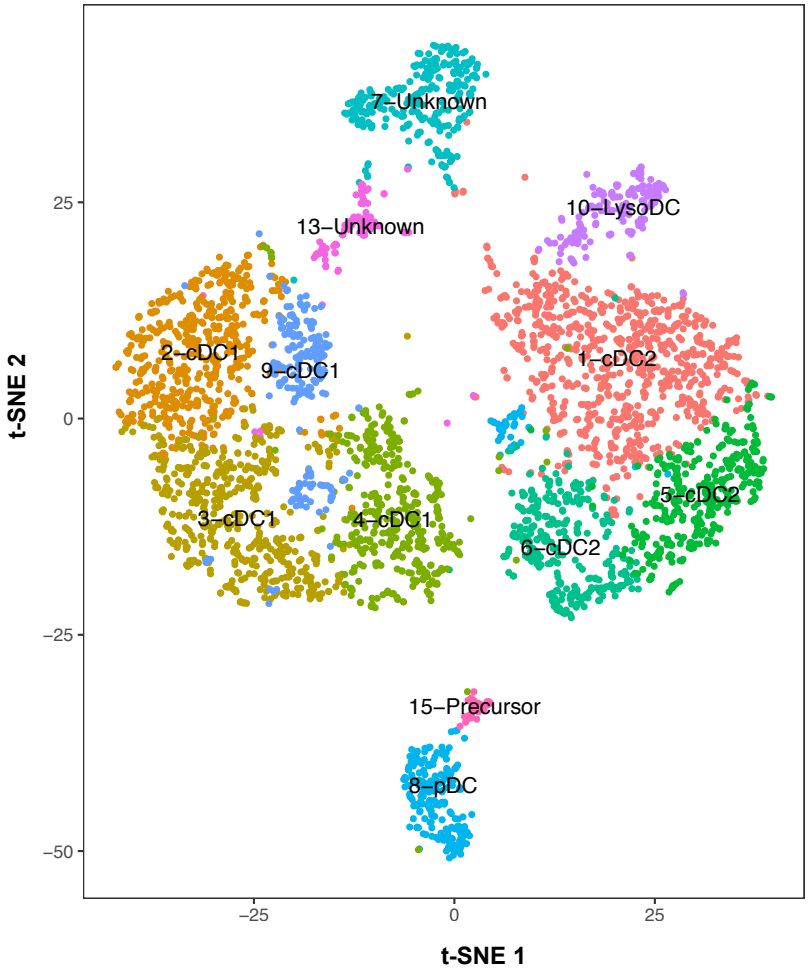
Check expression of:

1. known genes



Cell Identity with an Expert

Gut Immune Cells - 12 groups



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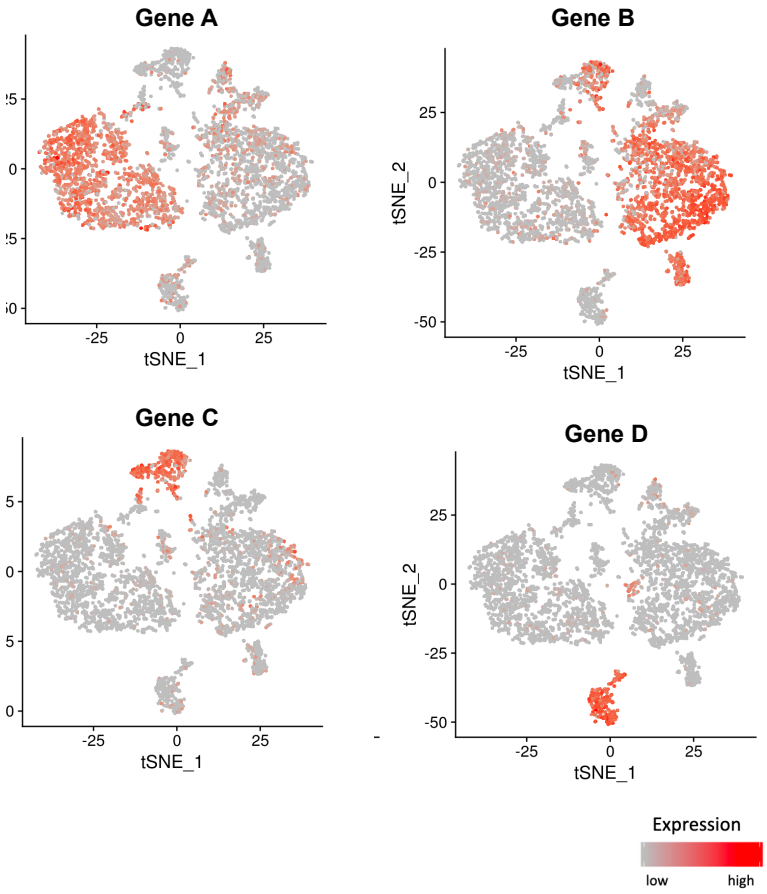

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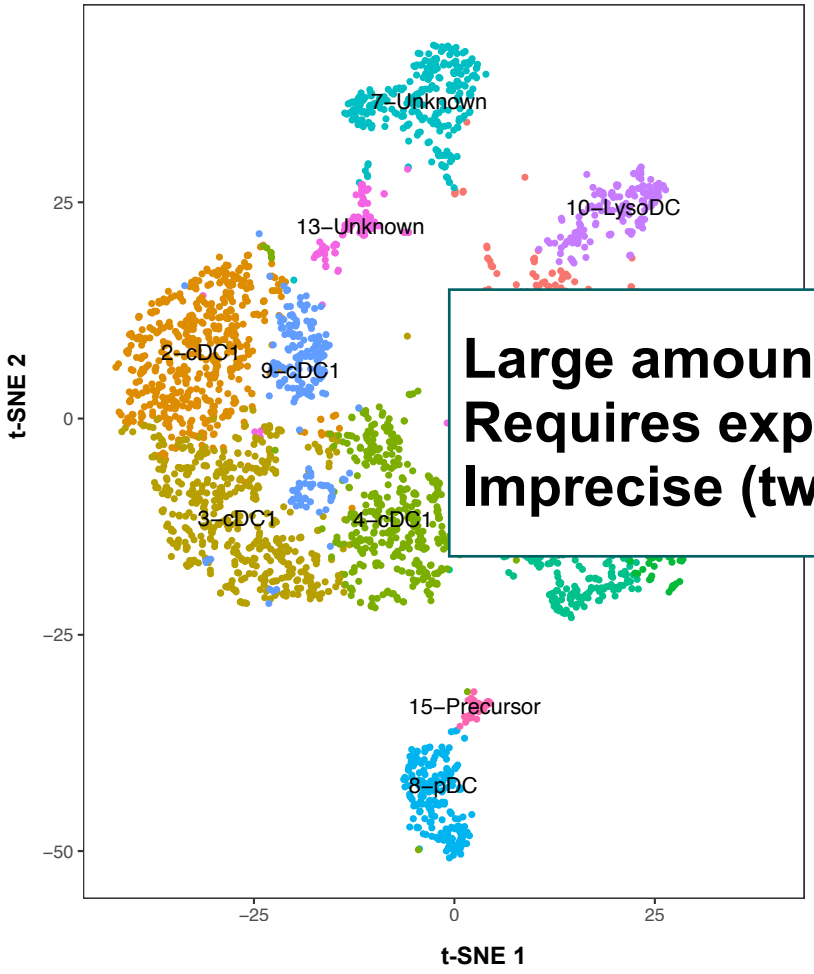
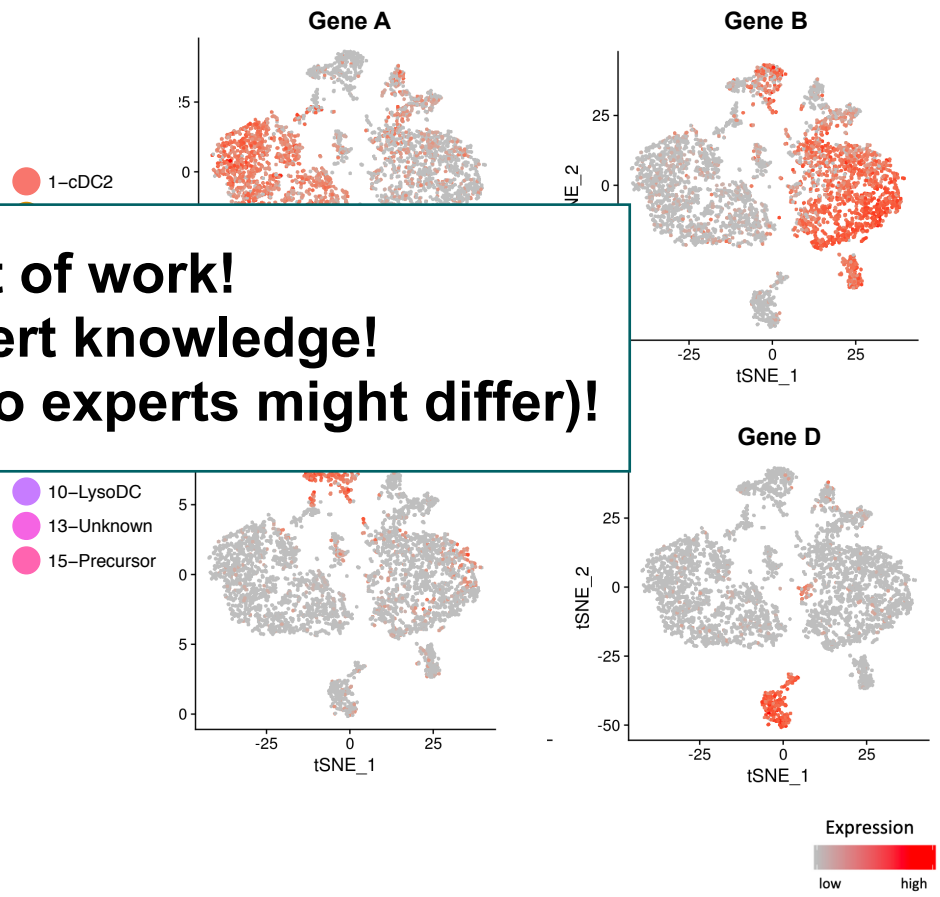


Check expression of:

1. known genes

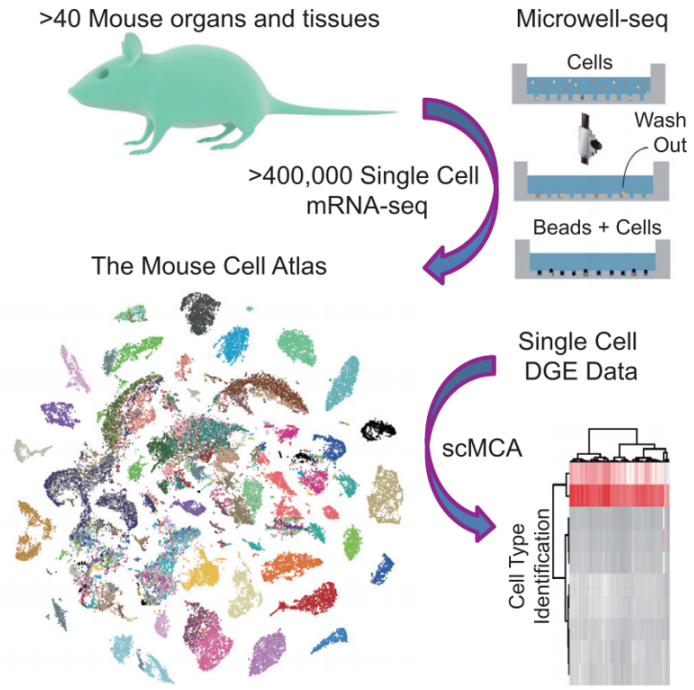
Large amount of work!
Requires expert knowledge!
Imprecise (two experts might differ)!



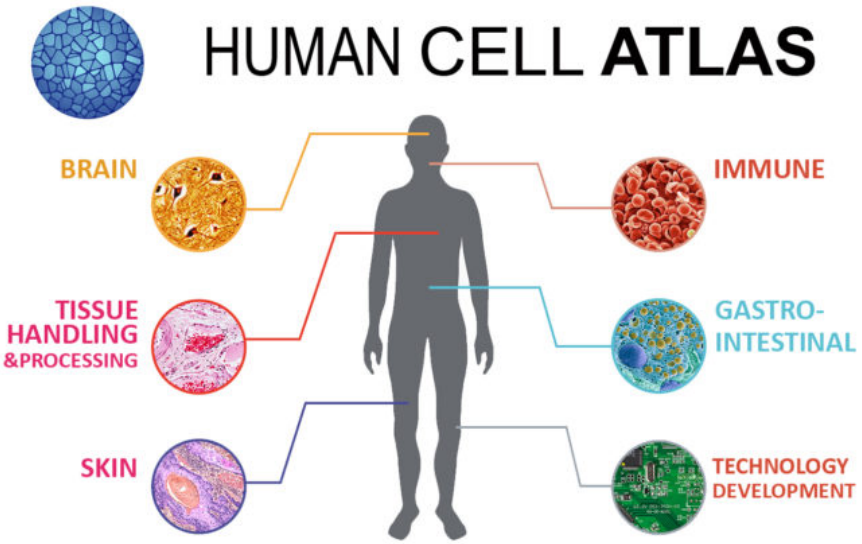
Automatic Cell Identification

Large consortia are sequencing and annotating cell types.

Mouse cell atlas



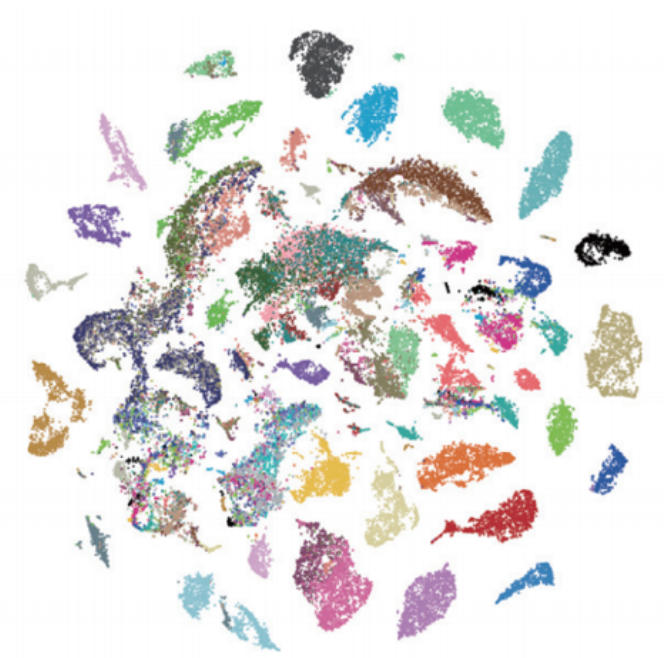
400.000 cells on 40 tissues



In construction !!!
by Chan-Zuckerberg Initiative

Automatic Cell Identification

Mouse cell atlas



400.000 cells on 40 tissues

Use pre-annotated cells to build classifiers to annotate novel data

Challenges:

- use mouse data to annotate humans?
- Indicate unknown cells?
- Build across tissue classifiers?
- Feature selection (relevant genes)?

Calendar

29.04.2019 –Introduction to Bioinformatics, Next Generation Sequencing and Single Cell Sequencing

06.05.2019 – Practical Course in NGS data analysis

13.05.2019 – Introduction to HPC clusters and GPUs

20.05.2019 – Project Description

27.05.2019 to 8.07.2019 – Project Development

15.07.2019 – Project Presentation

Thank you!