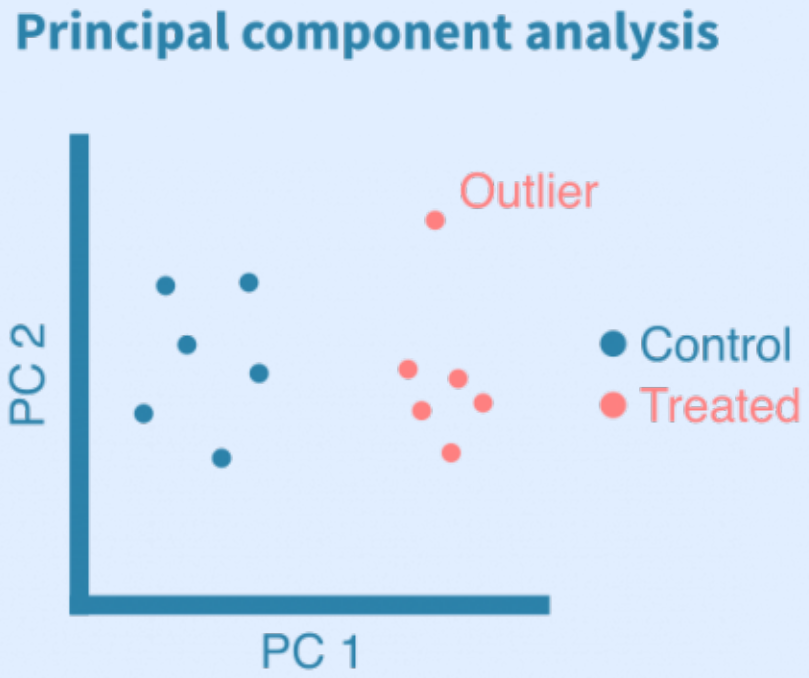
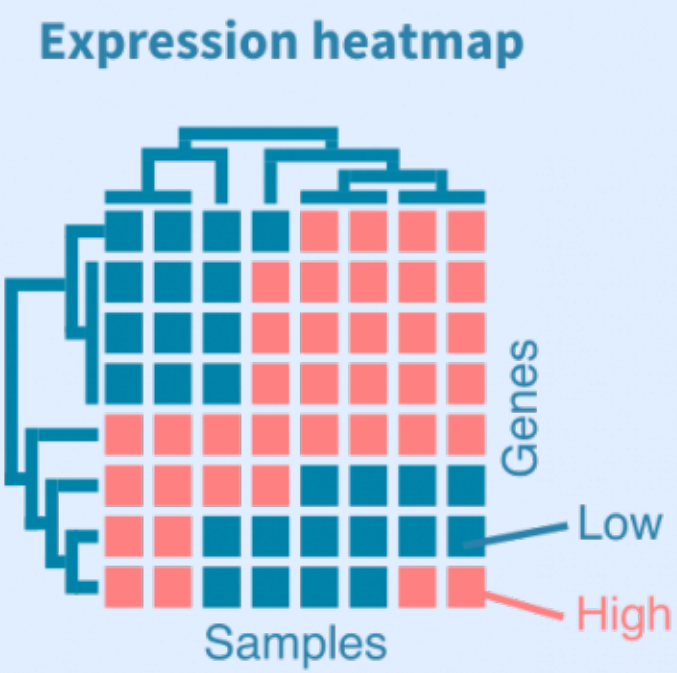



# Quick intro to RNA-Seq

**Princess Rodriguez, PhD**

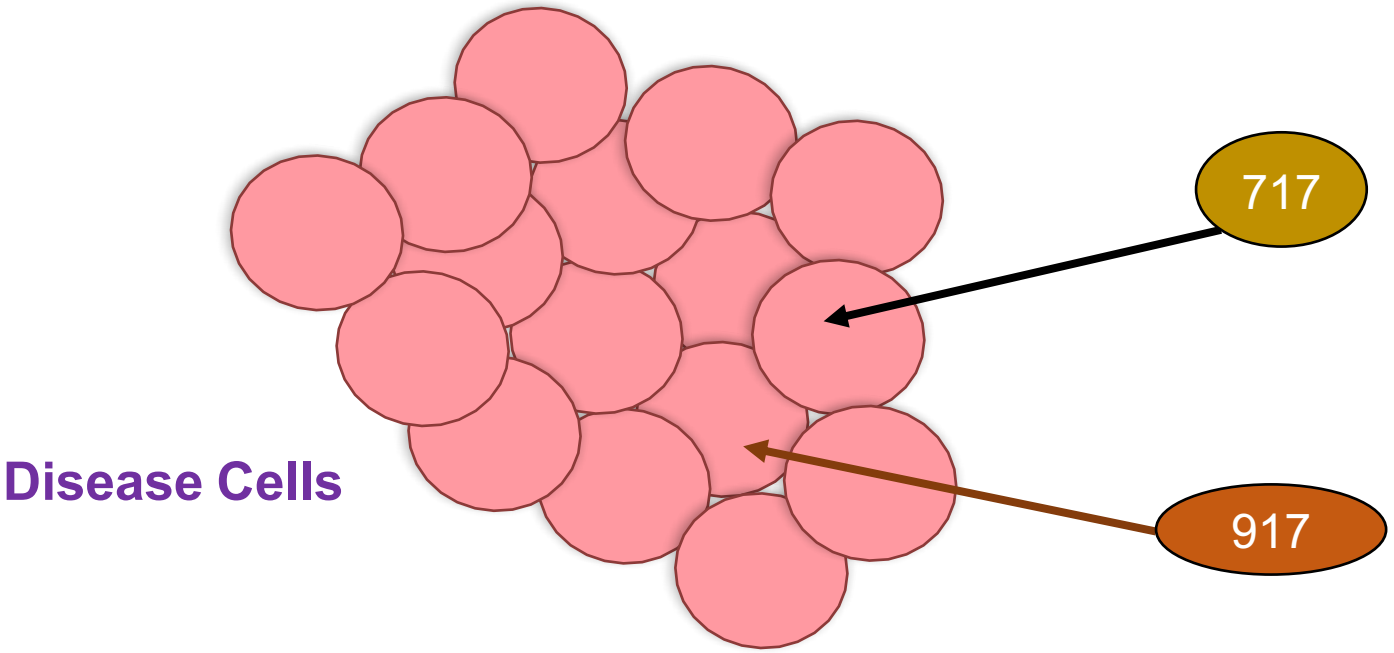
**MMG232**

**Spring 2023**

# Typical RNA-Seq Vignette


- Your lab has introduced a mutation in cells that now causes the cells to behave ***differently*** than the normal cells

# Typical RNA-Seq Vignette




- Or your lab noticed that the addition of **Drug 717** caused the disease cells to grow more slowly while the addition of **Drug 917** had no effect

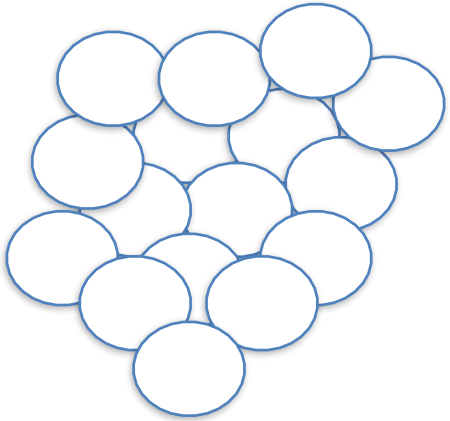
# Typical RNA-Seq Vignette

For either case: you, your PI, and committee decide that an aim for your project should be to perform RNA-Seq to uncover a potential mechanism

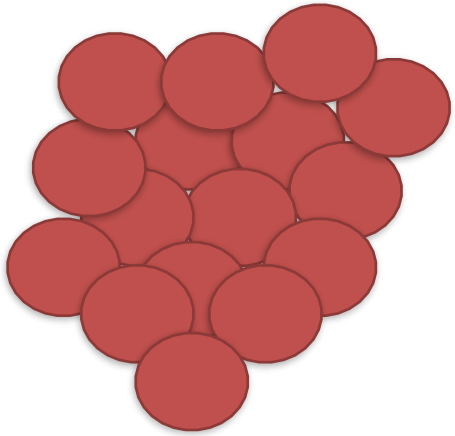
# What is RNA-Seq?

- Technique used to explore and/or quantify gene expression within or between conditions of an organism

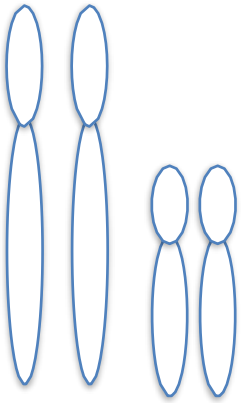
## Normal Cells



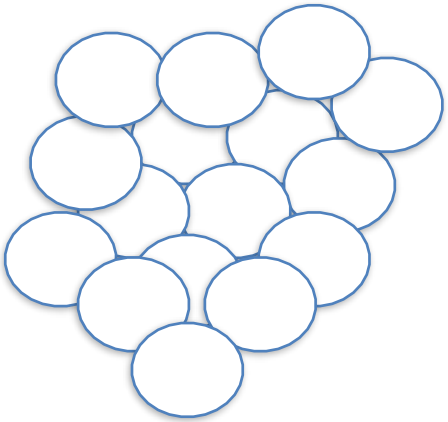
## Mutated Cells



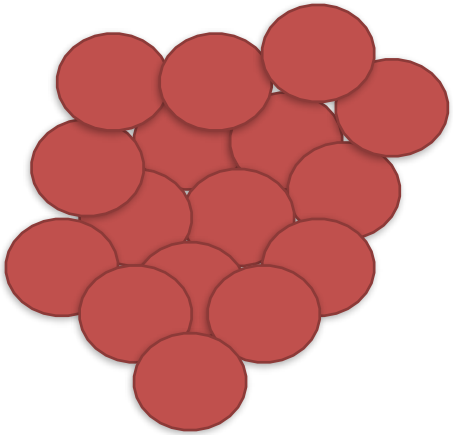
Each cell has a bunch of chromosomes

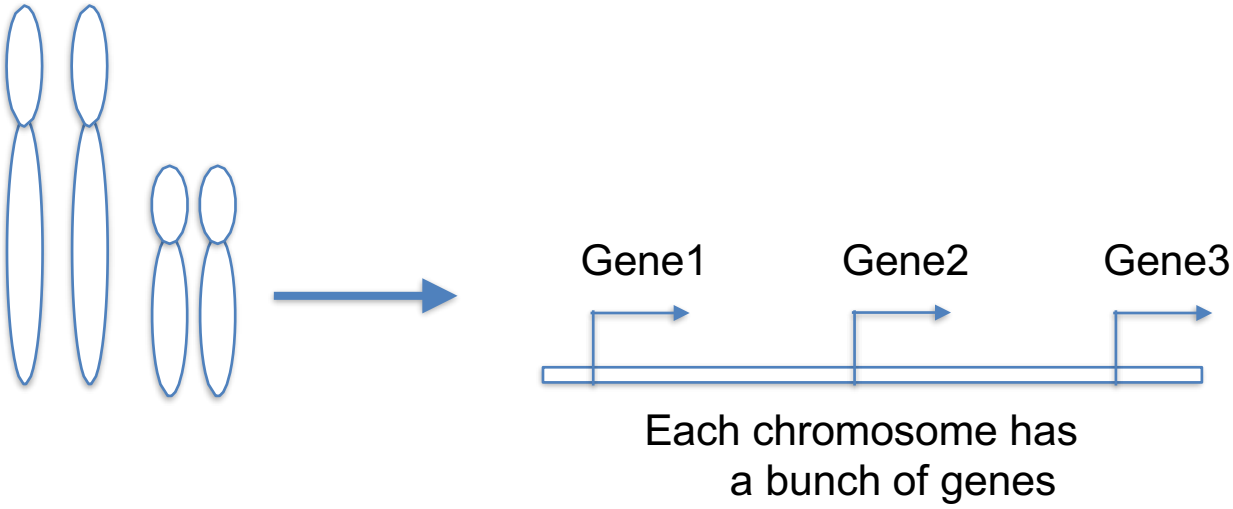


## Normal Cells



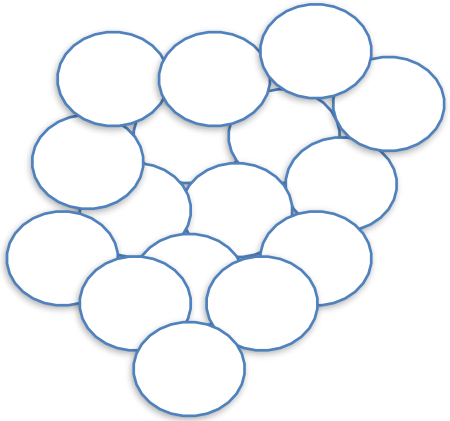
## Mutated Cells



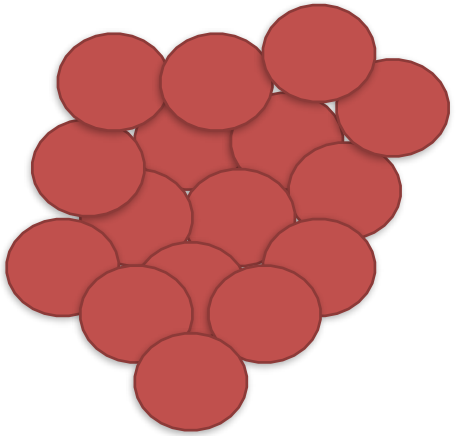


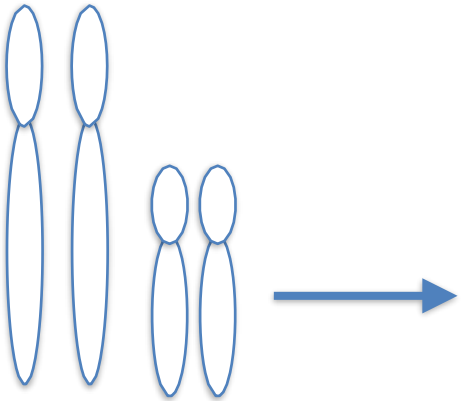

Each chromosome has
a bunch of genes

## Normal Cells

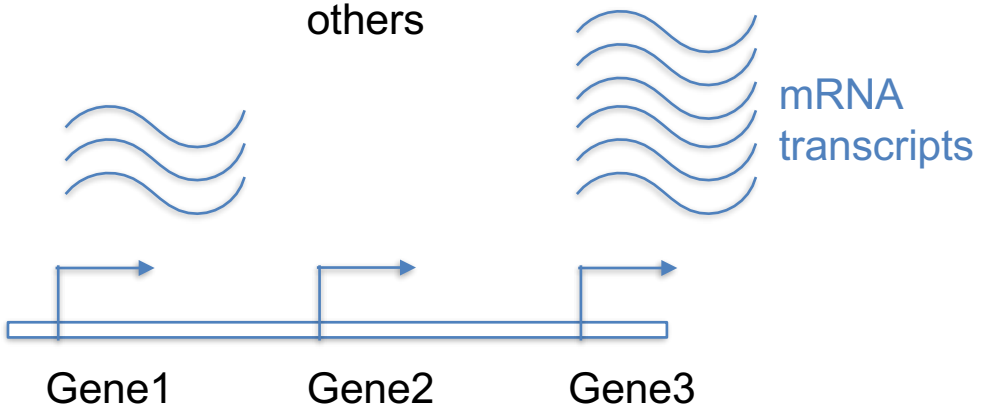


## Mutated Cells

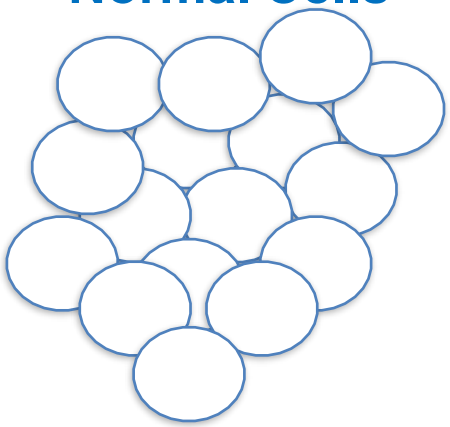




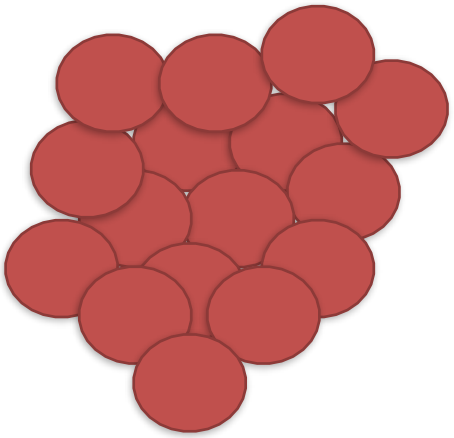
Some genes are active more than others

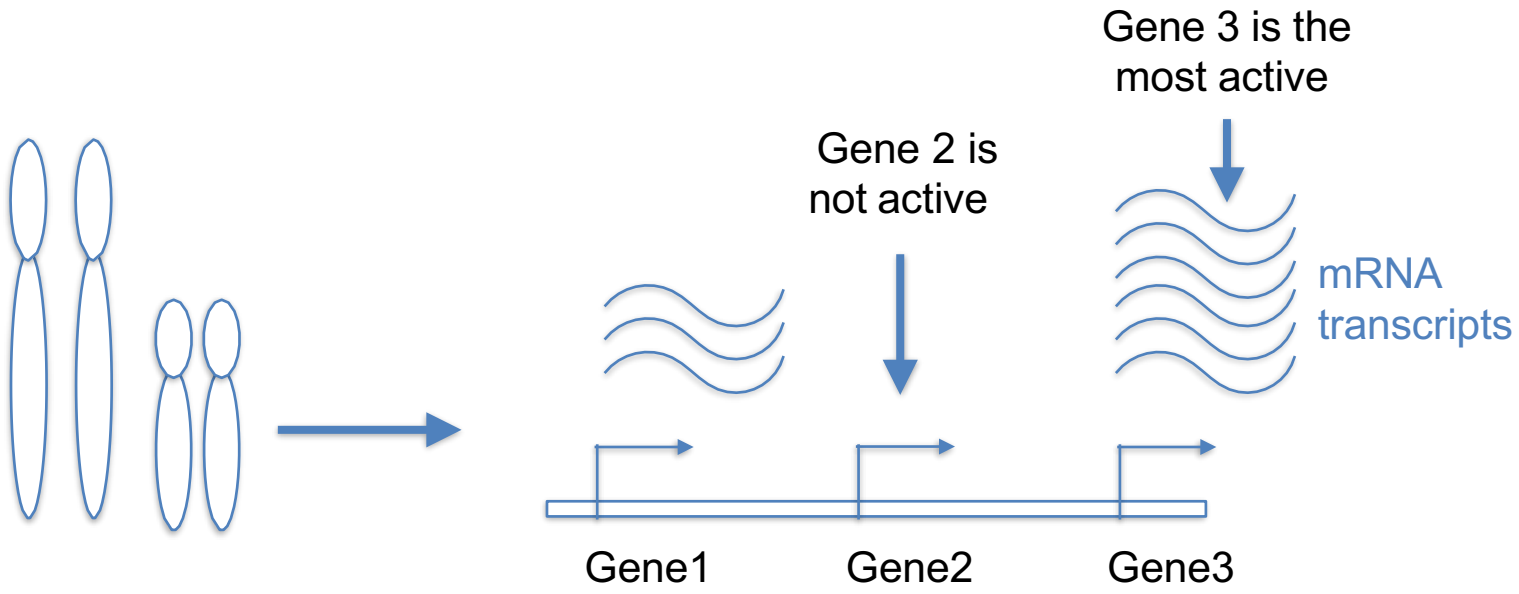


## Normal Cells

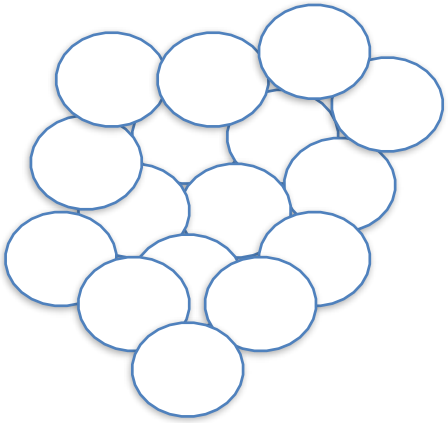


## Mutated Cells

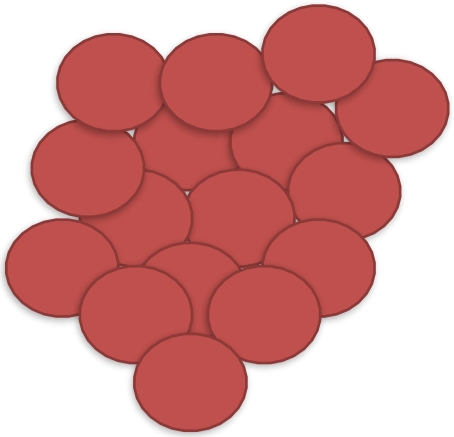




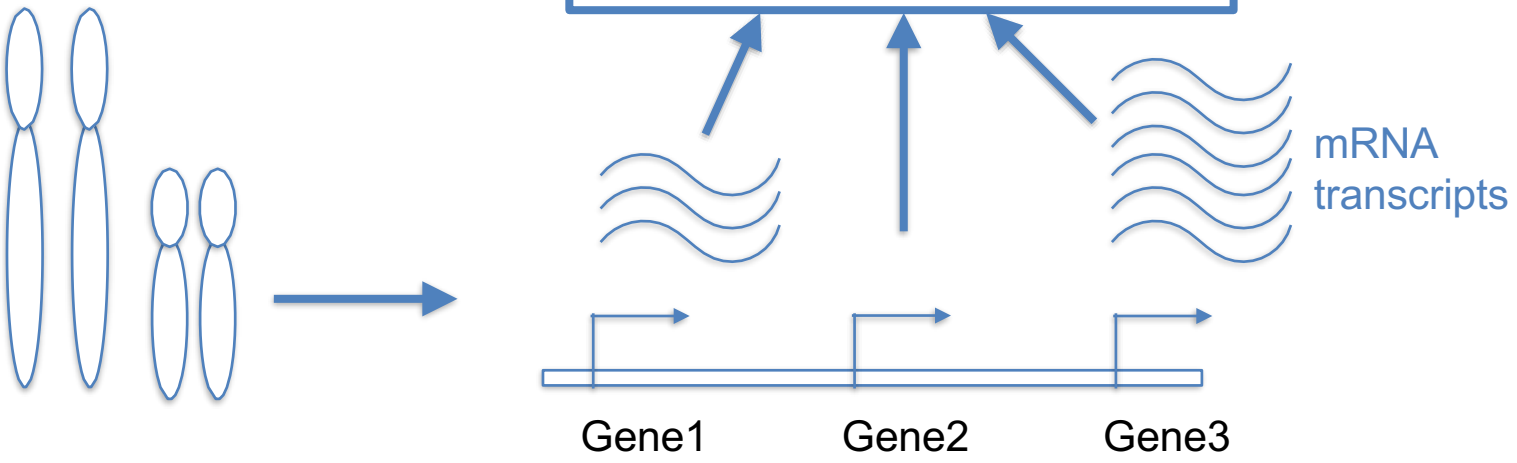
## Normal Cells



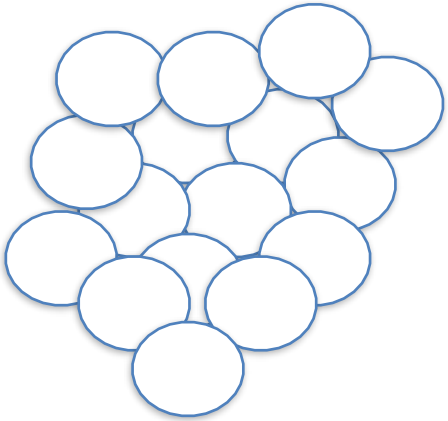
## Mutated Cells

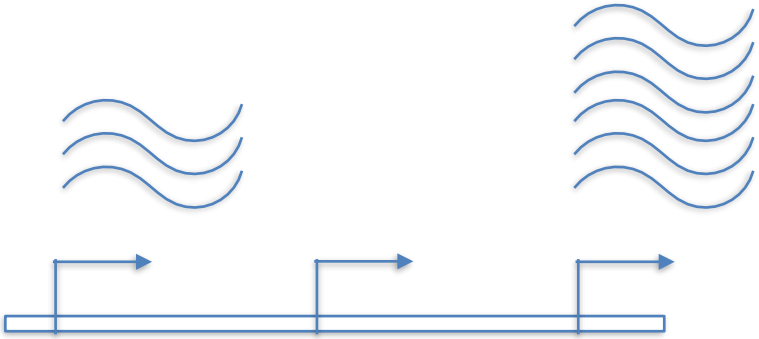


RNA-Seq tells us which genes are active, and how much they are transcribed!



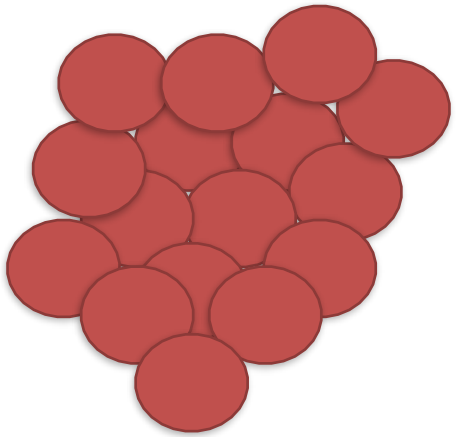
## Normal Cells

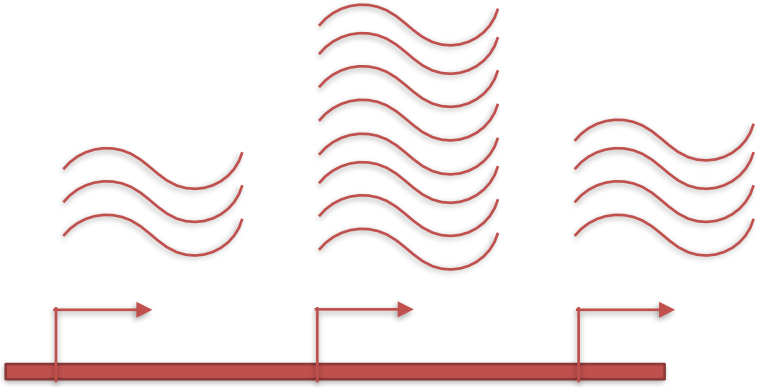




So, we can use RNA-Seq to measure gene expression in normal cells …

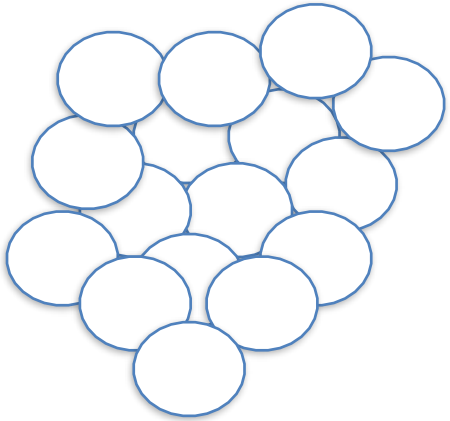
## Mutated Cells



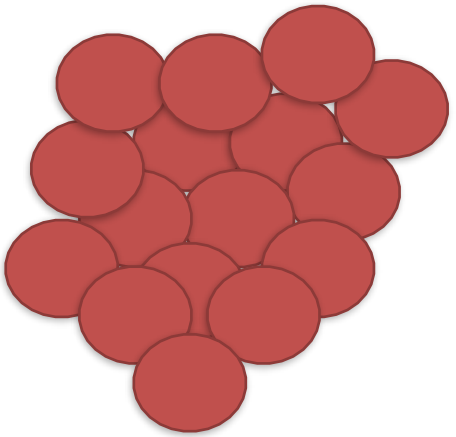


… then use it to measure gene expression in mutated cells

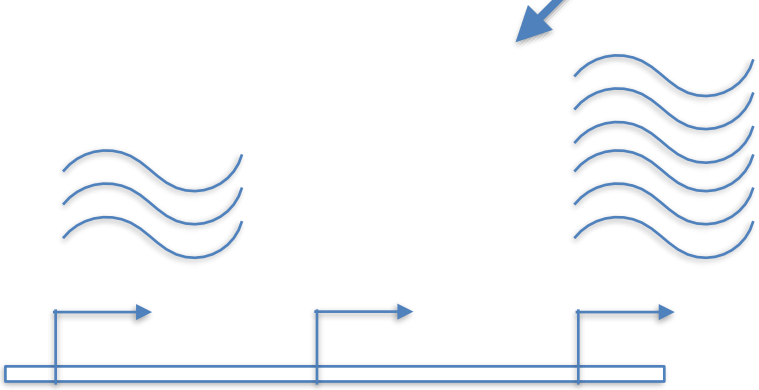
**Normal Cells**

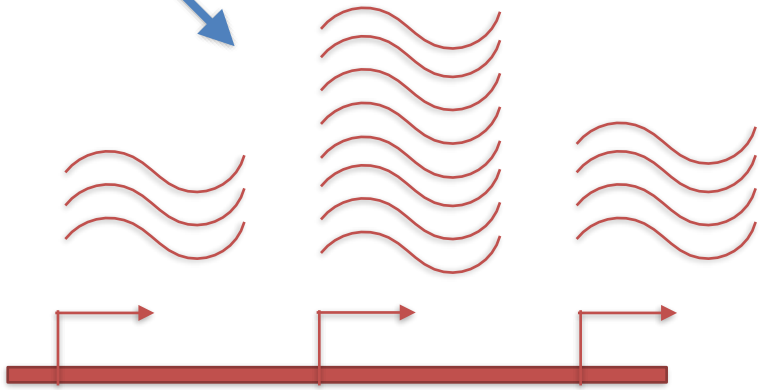


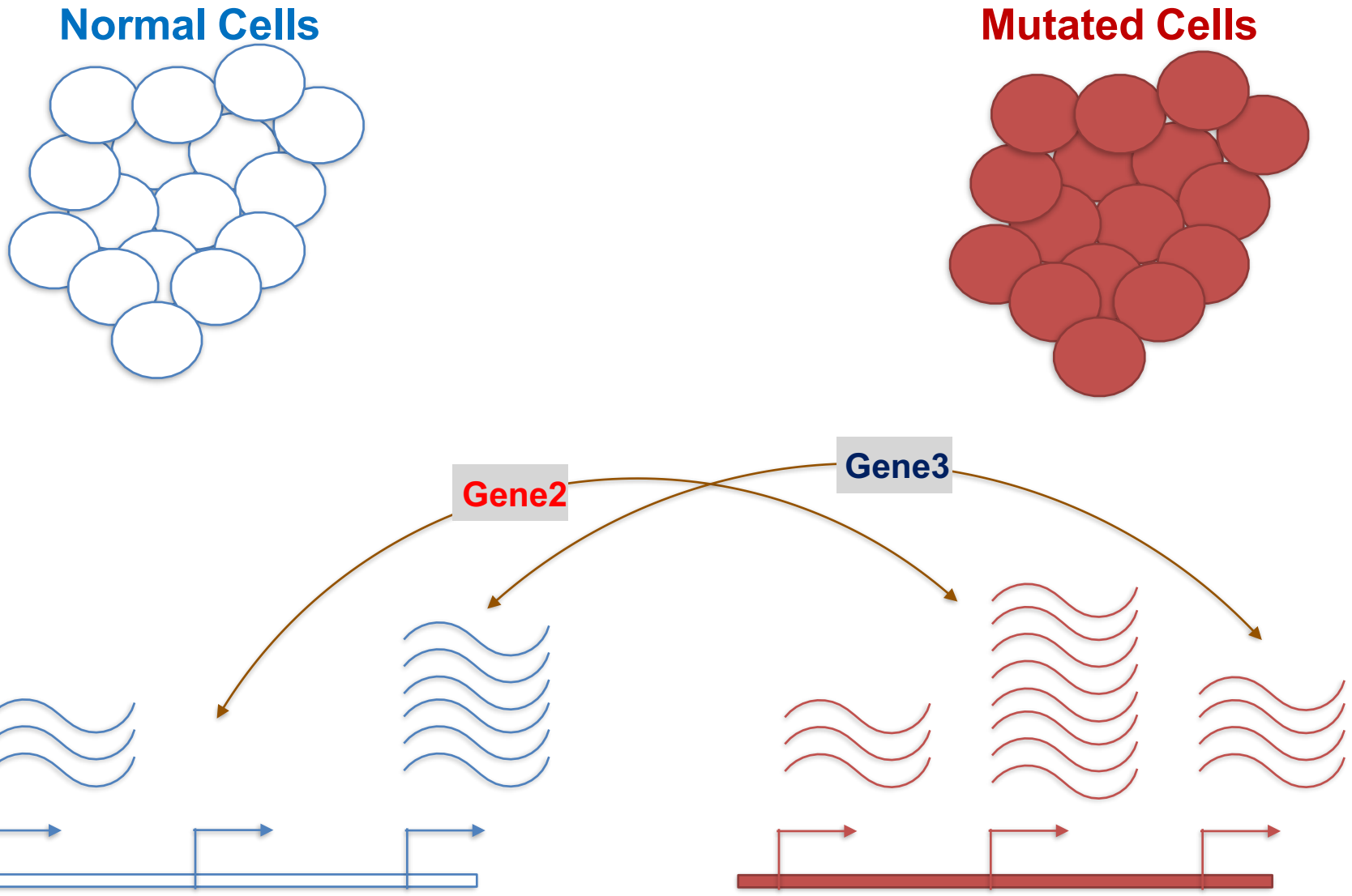
**Mutated Cells**



Then we can compare the two cell types to figure out what is different in the mutated cells!





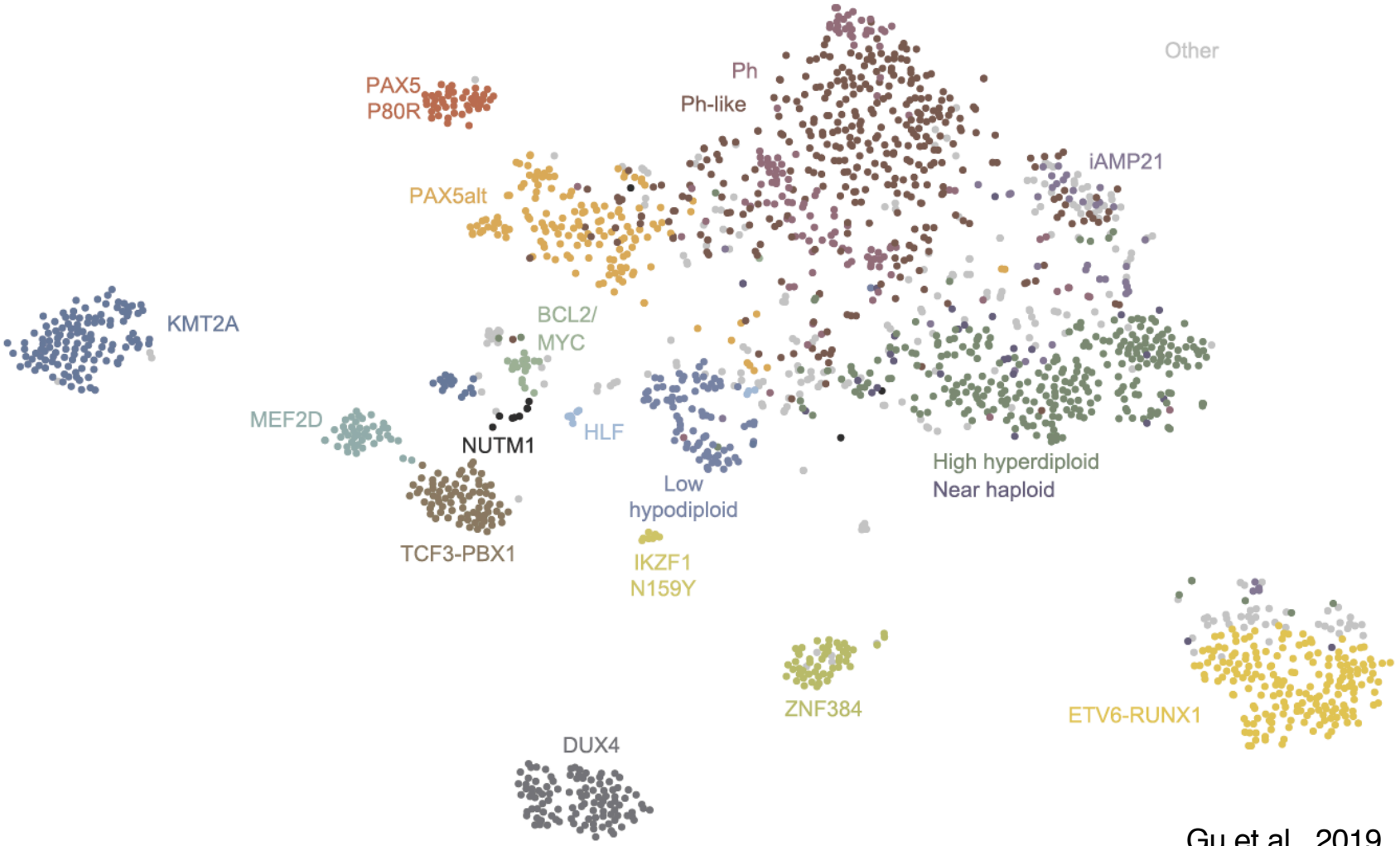



Differences apparent for Gene 2 and
to a lesser extent Gene 3

# Common uses of RNA-Seq

- Expression variation in response to environmental stimuli
- Which genes are expressed in which tissues & how much?
- Study of genotype ↔ phenotype mapping
- Discovery/annotation of genes & transcripts
- Gene regulatory networks (co-regulated genes)
- Medical diagnostic

# Transcriptomics has led to the identification of new subtypes in ALL




Gu et al., 2019

# There are 3 Basic Steps in performing RNA-Seq yourself

1) Prepare a sequencing library

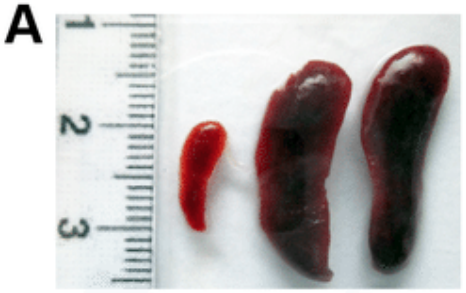
2) Sequence

3) Data analysis

# RNA-Seq experimental workflow

1

Samples of interest

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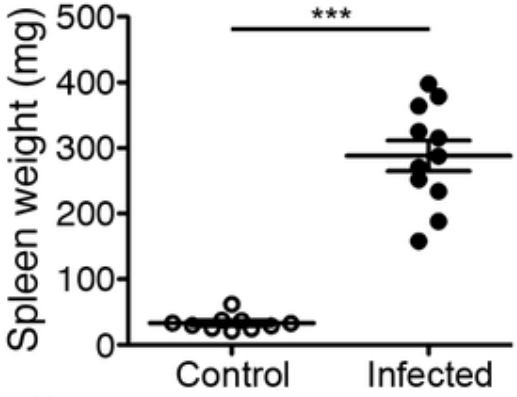
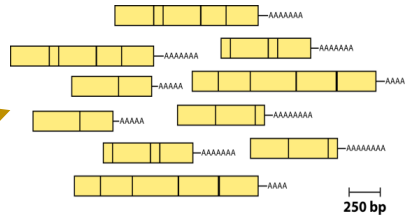


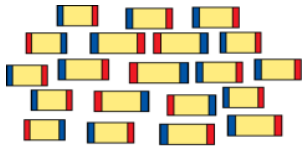

2

Isolate RNAs



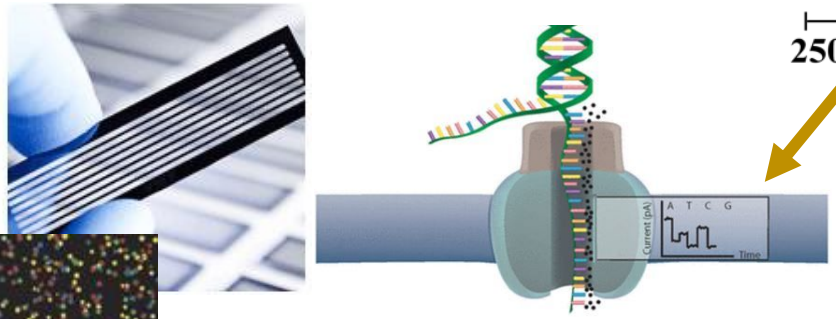

3

Library build

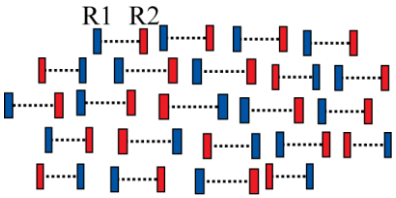



4

Illumina sequencing versus ONP

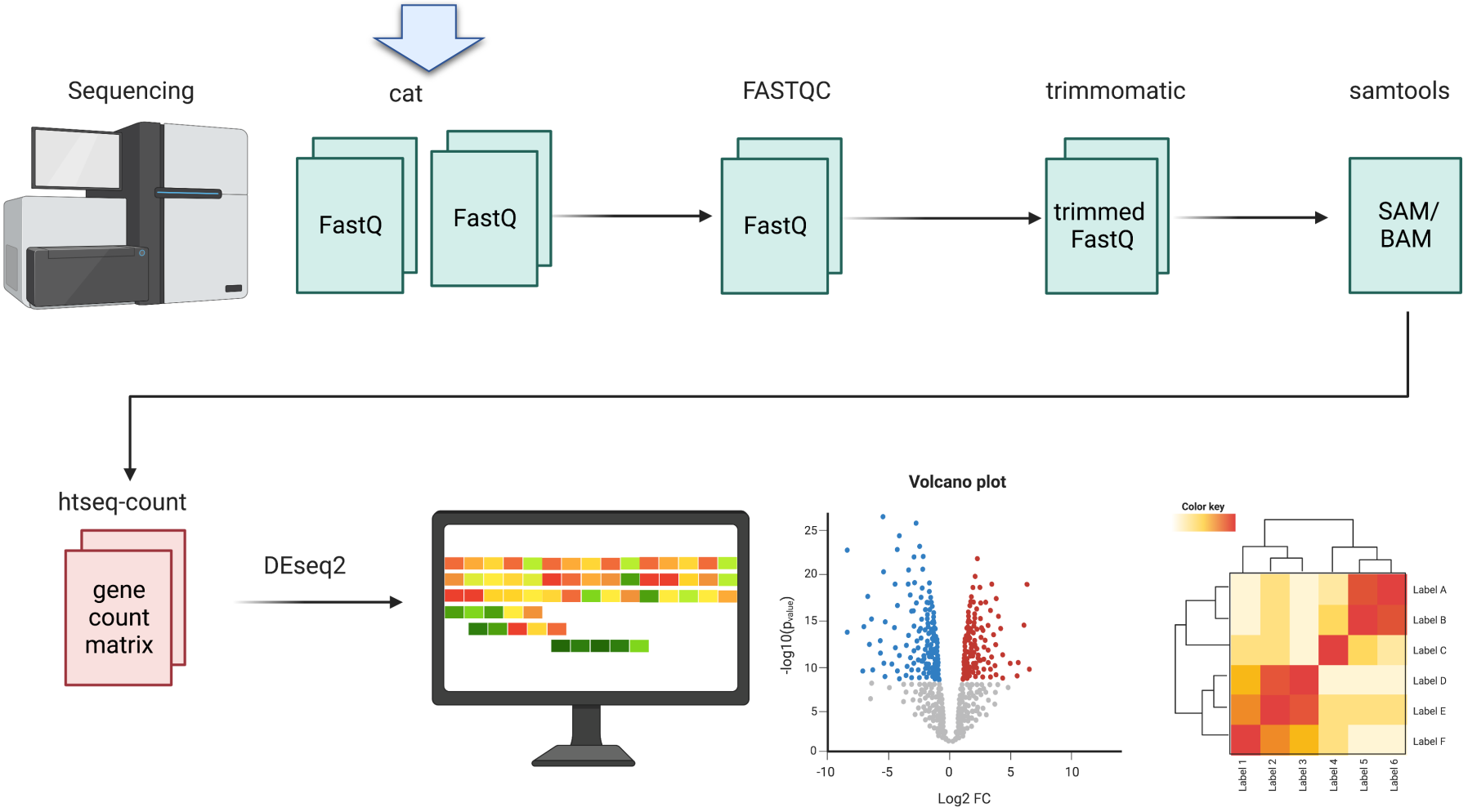


5




Reads (R1 and R2) generated

# RNA-Seq processing



# What is a FASTQ?

```
@NS500177:196:HFTTTAFXX:1:11101:10916:1458 2:N:0:CGCGGCTG
ACACGACGATGAGGTGACAGTCACGGAGGATAAGATCAATGCCCTCATTAAAGCAGCCGGTGTAA
+
AAAAAEEEEEEEEEEE//AEEEAEEEEEEEEEEE/EE/<<EE/AAEEAEE//EEEEAEEEAEA<
```

1
2
3
4

**Each sequencing "read" consists of 4 lines of data :**

1. The first line (which always starts with '@') is a unique ID for the sequence that follows
2. The second line contains the bases called for the sequenced fragment
3. The third line is always a "+" character
4. The fourth line contains the quality scores for each base in the sequenced fragment