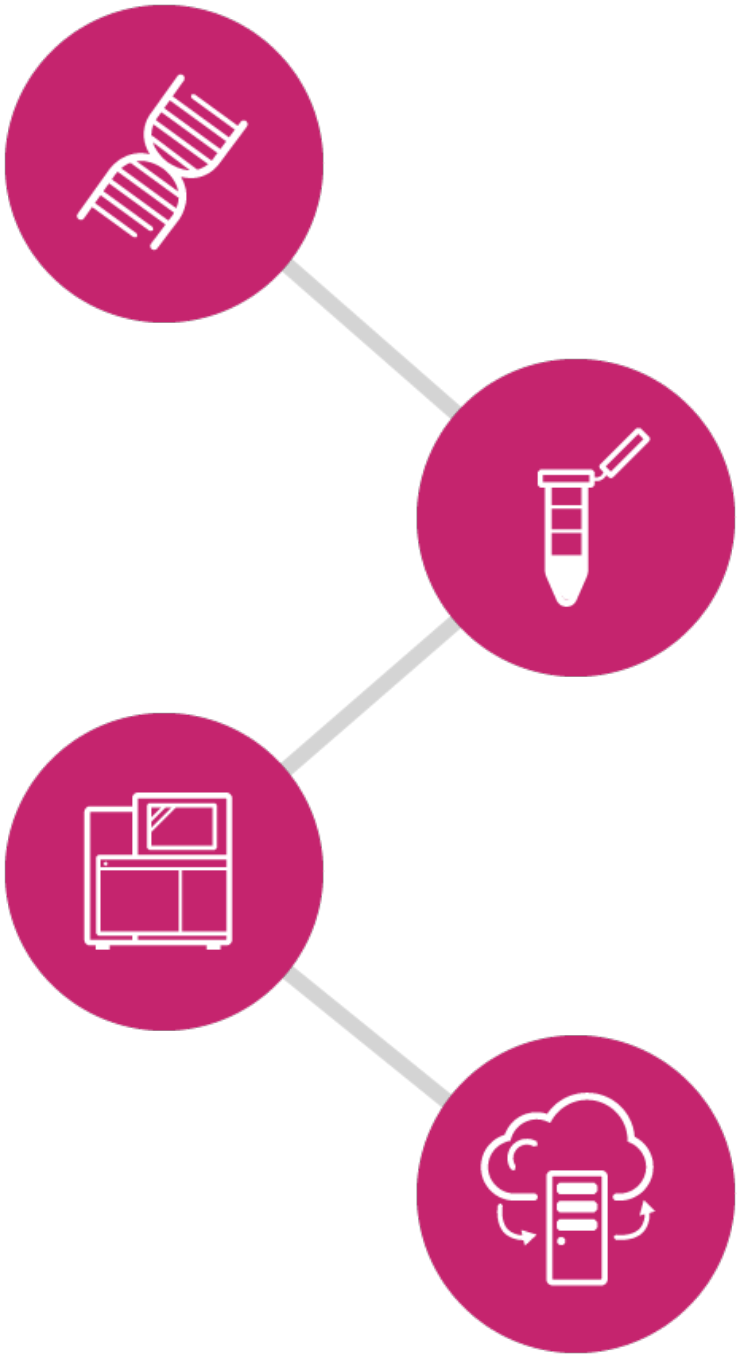
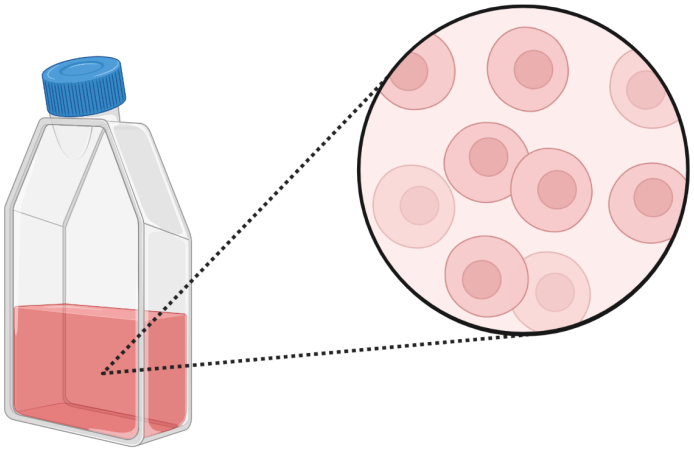


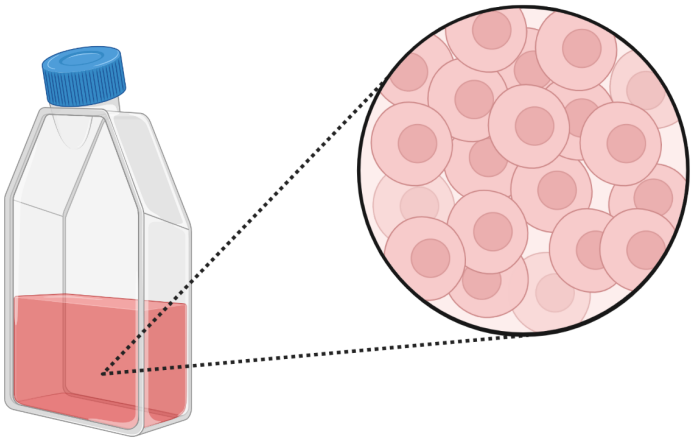
Intro to RNA-Seq

Dr. Princess Rodriguez

MMG232
Spring 2023



normal

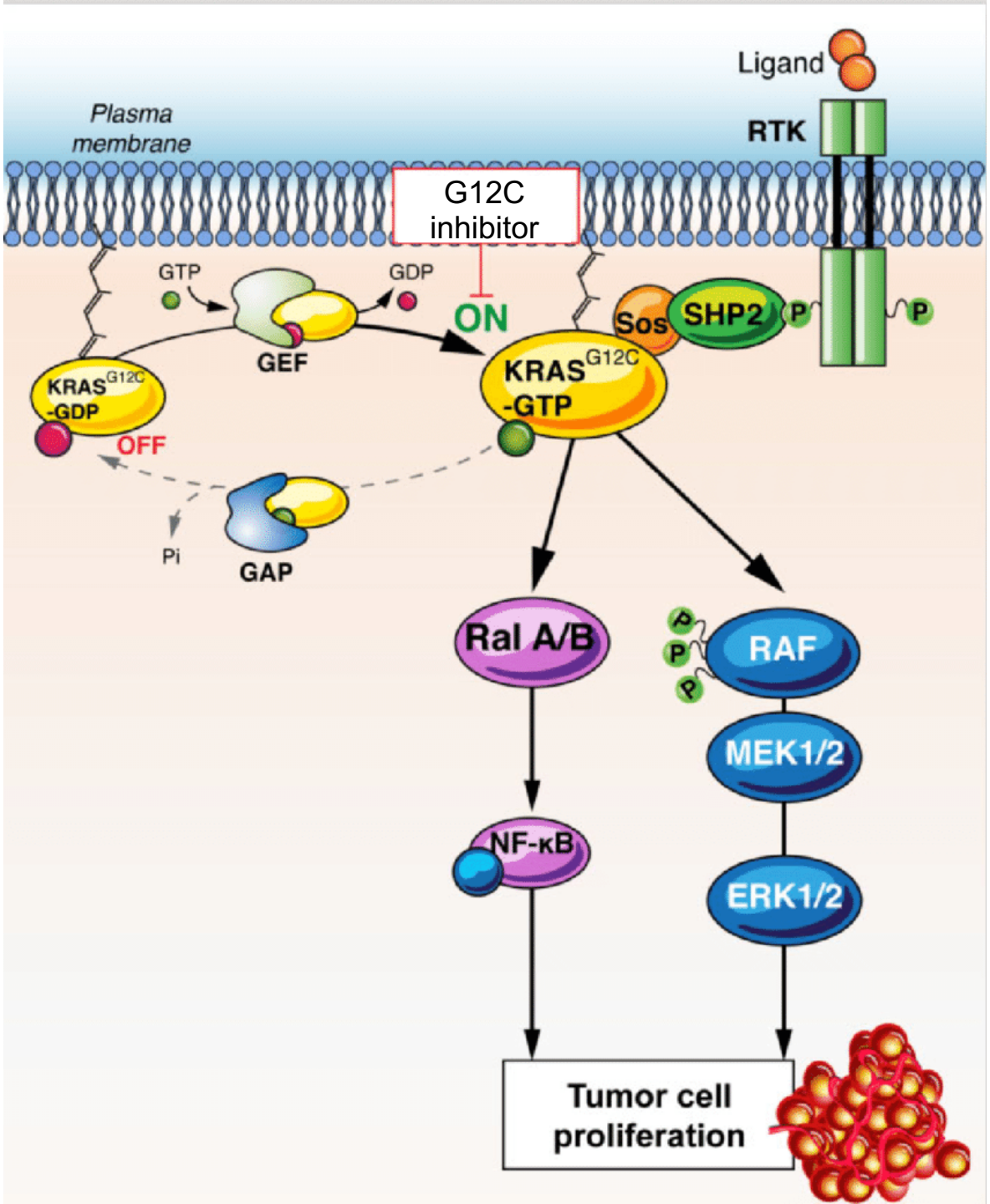


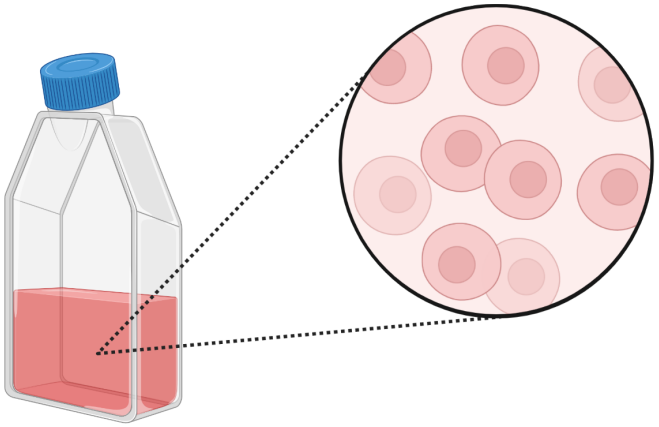
mutation in
Kras^{G12C}

You have been given cells to culture:

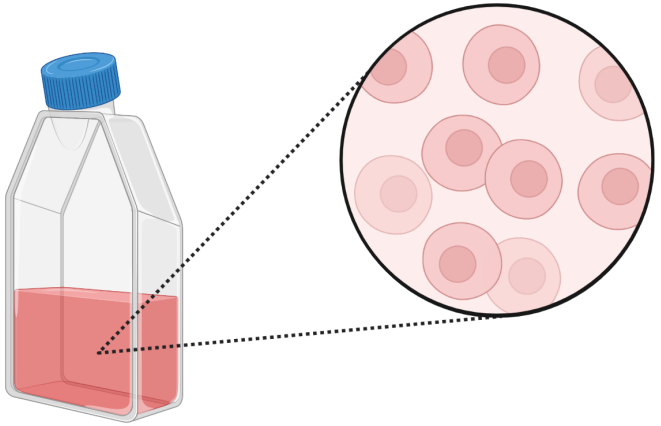
Normal cells need their media changed every 3 days

Other cells need their media changed every day!

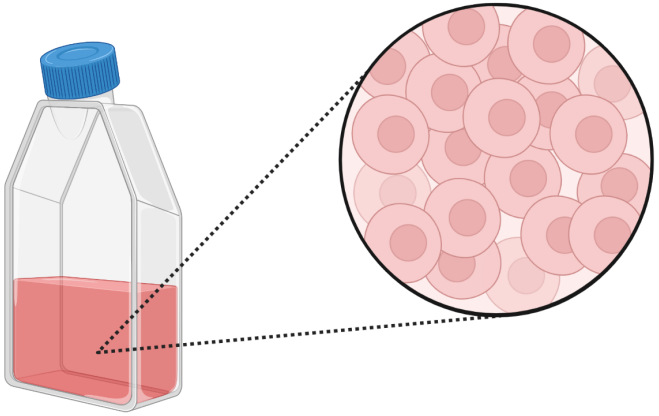

Ligand
RTK
Plasma membrane
G12C inhibitor
GTP
GDP
GEF
ON
Sos
SHP2
P
P
KRAS G12C -GDP
OFF
KRAS G12C -GTP
Pi
GAP
Ral A/B
RAF
P
P
P
MEK1/2
NF-κB
ERK1/2
Tumor cell proliferation



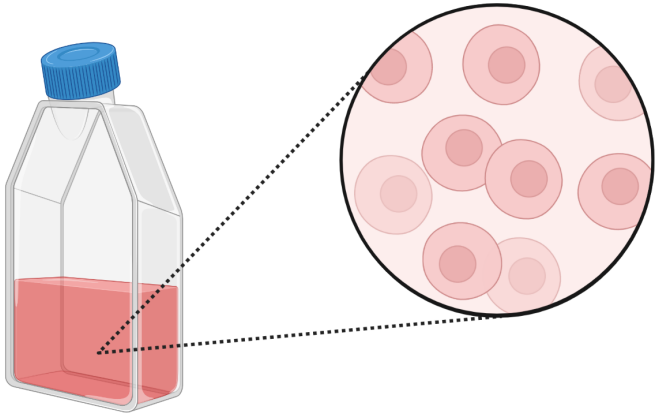
normal



Normal + **G12C inhibitor**



mutation in
Kras^{G12C}



mutation in *Kras*^{G12C}
+ **G12C inhibitor**

Typical RNA-Seq Vignette

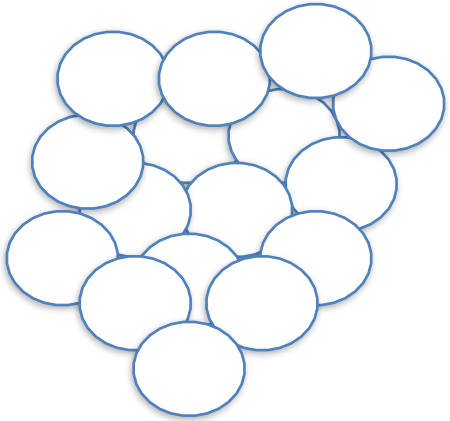
You and your PI decide to uncover the potential mechanism of the **G12C inhibitor** and understand if there are any off-target effects.

The goal is to take the G12C inhibitor to animal studies to see if it works the similarly *in vivo*!

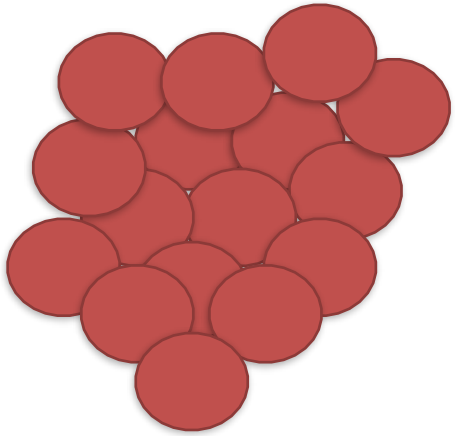
What is RNA-Seq?

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

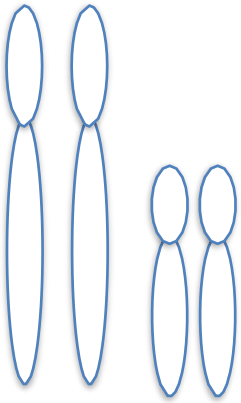
Normal Cells



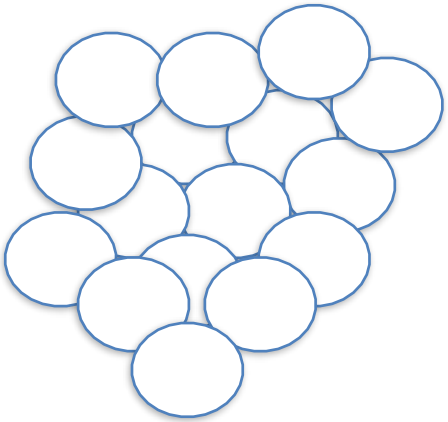
Mutated Cells



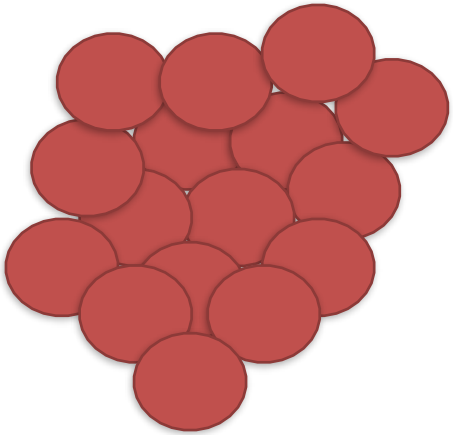
Each cell has a bunch of chromosomes

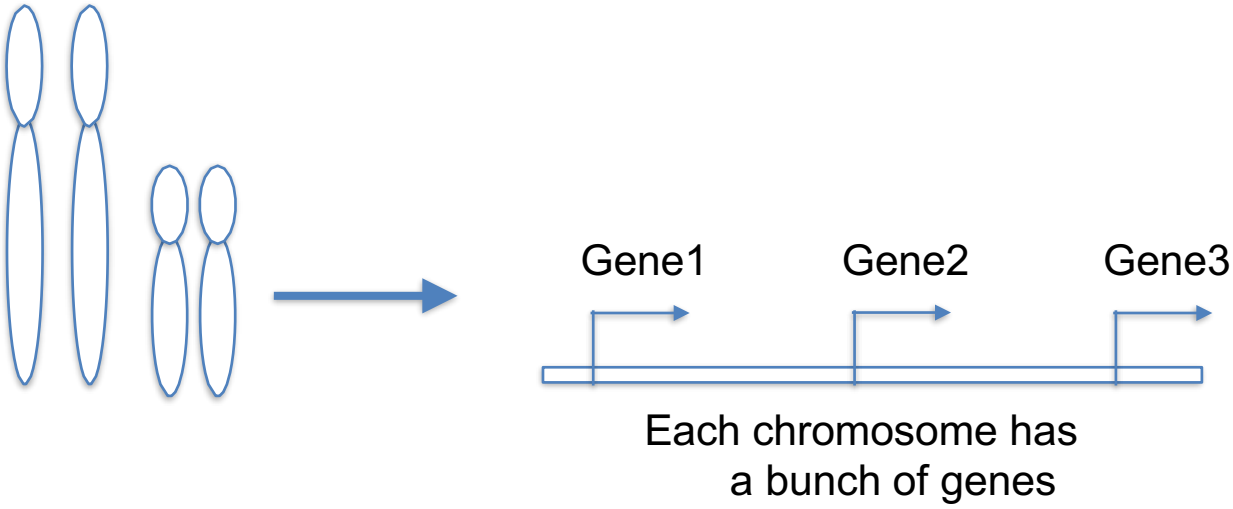


Normal Cells



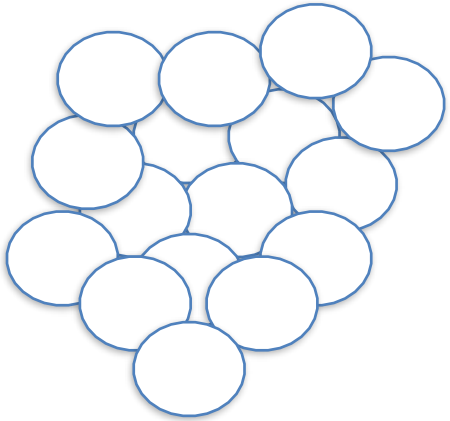
Mutated Cells



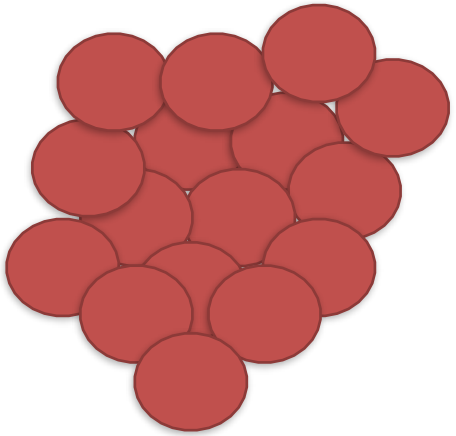


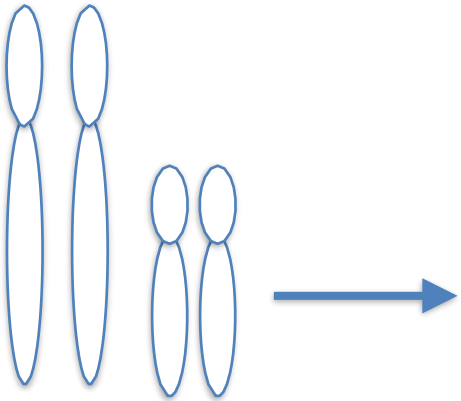
Each chromosome has
a bunch of genes

Normal Cells

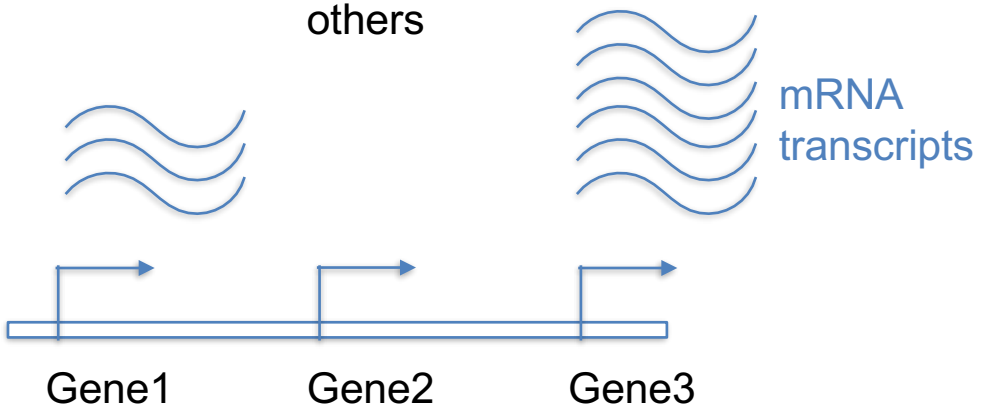


Mutated Cells

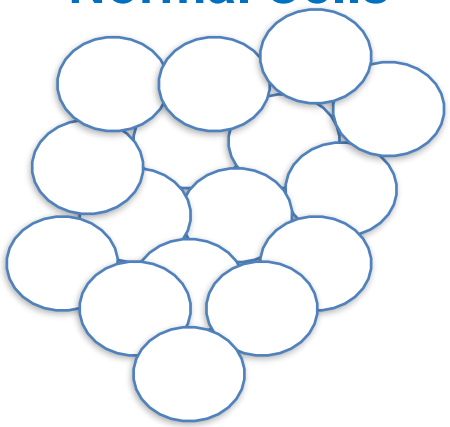




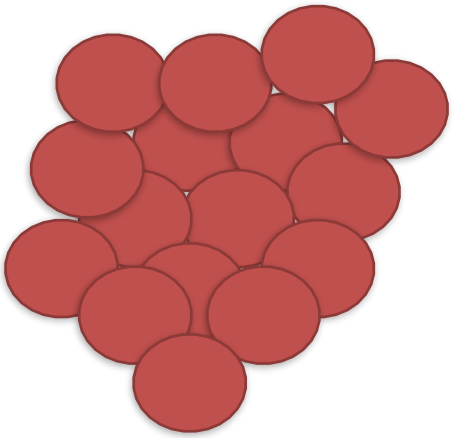
Some genes are active more than others

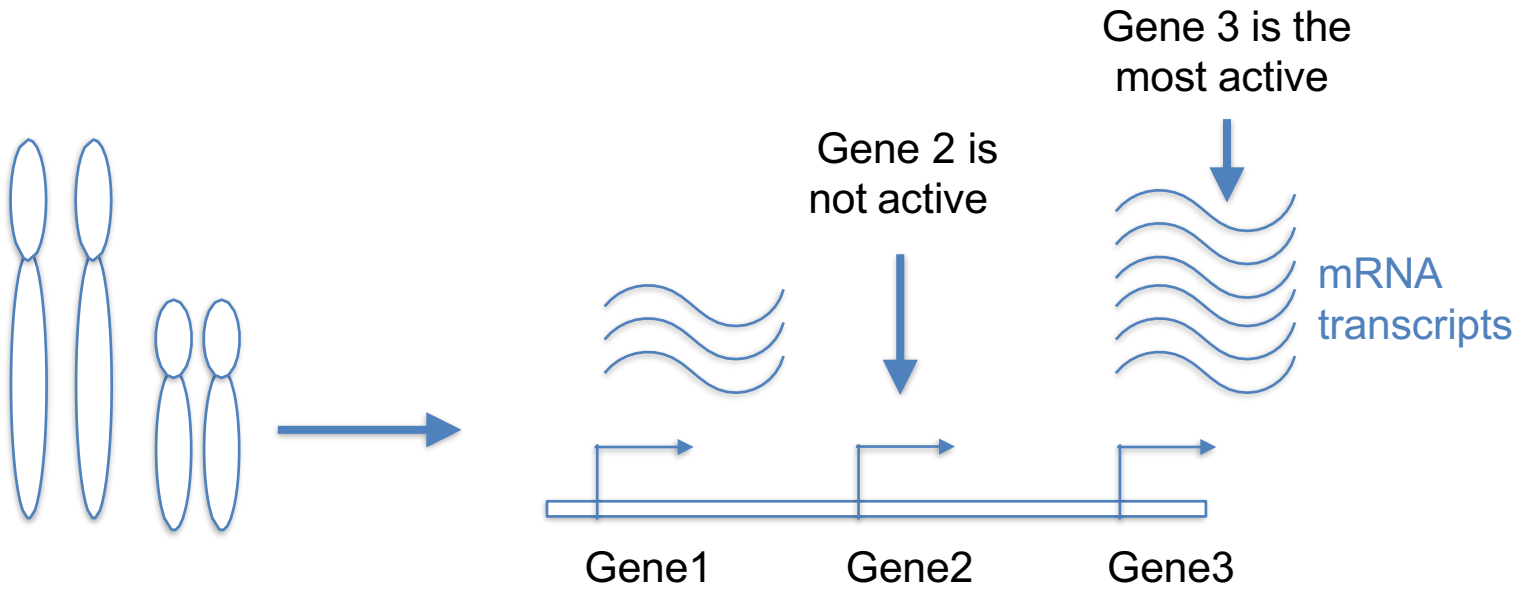


Normal Cells

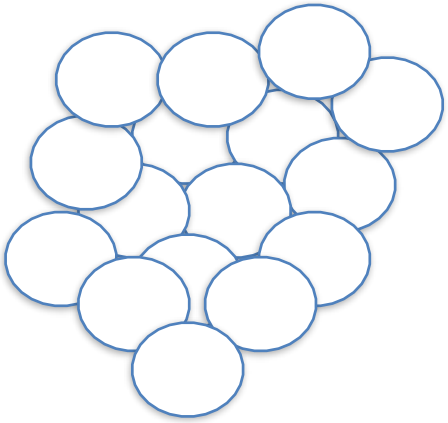


Mutated Cells

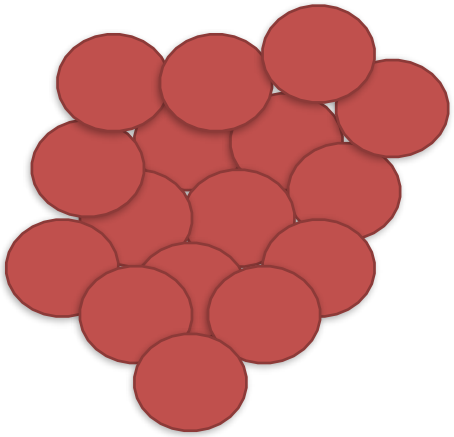




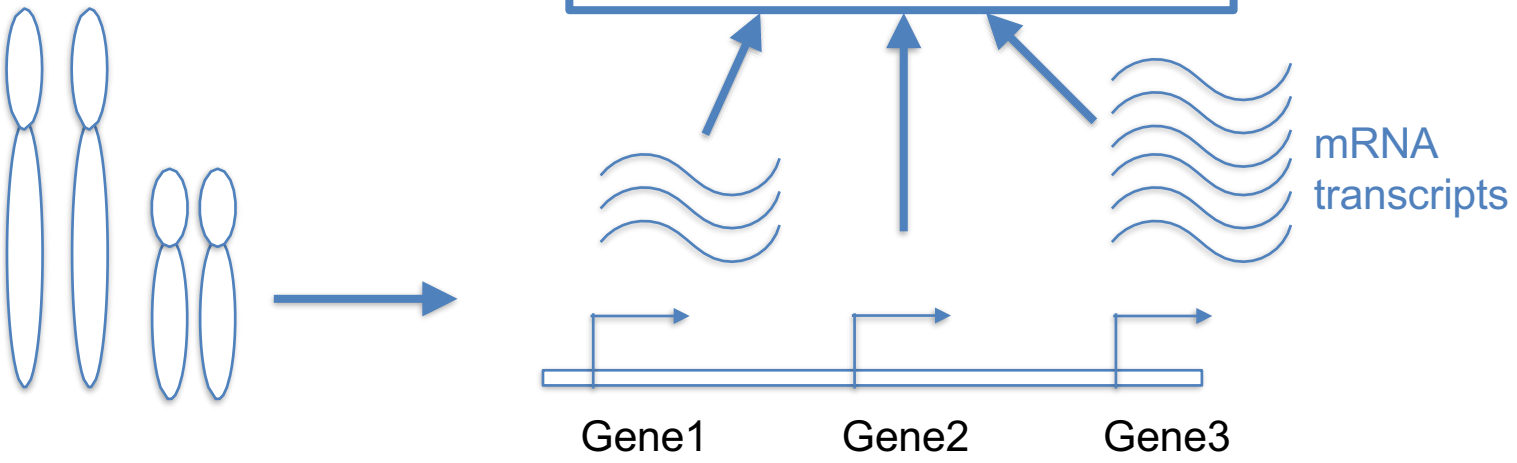
Normal Cells



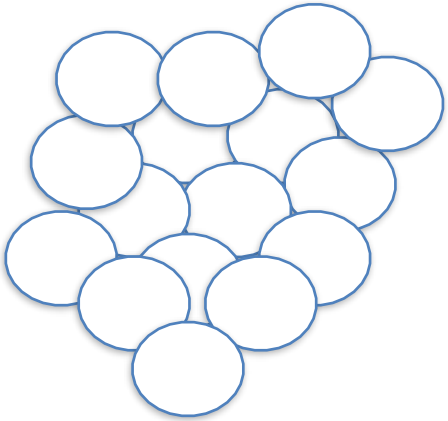
Mutated Cells

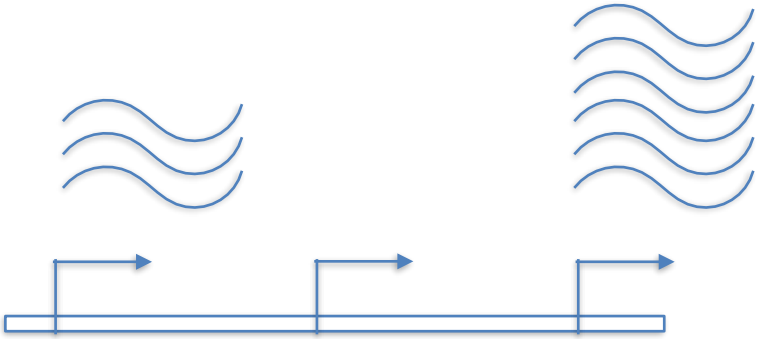


RNA-Seq tells us which genes are active, and how much they are transcribed!



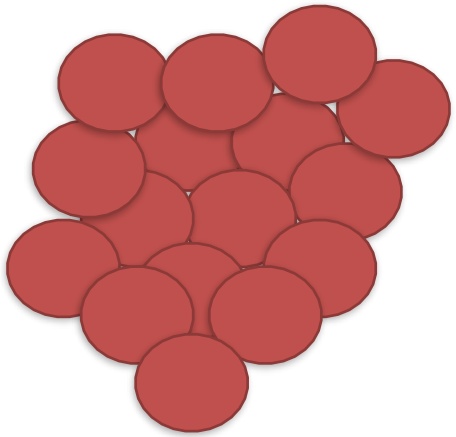
Normal Cells

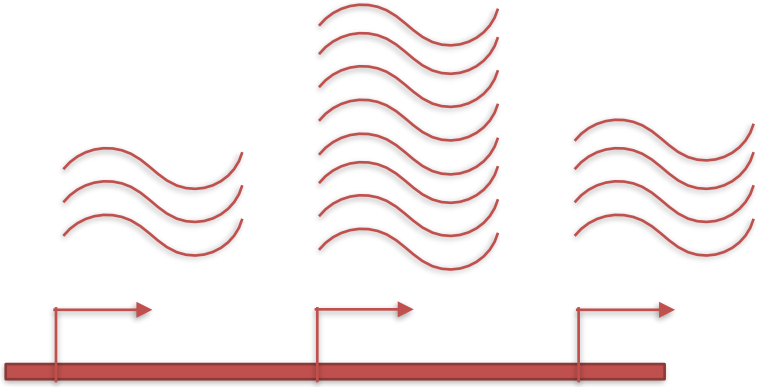




So, we can use RNA-Seq to measure gene expression in normal cells …

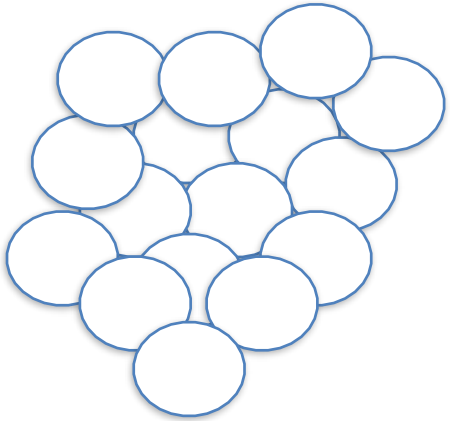
Mutated Cells



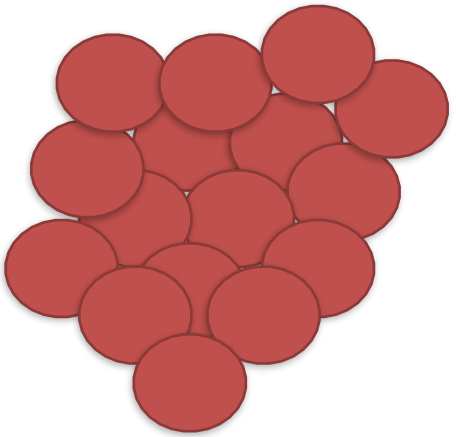


… then use it to measure gene expression in mutated cells

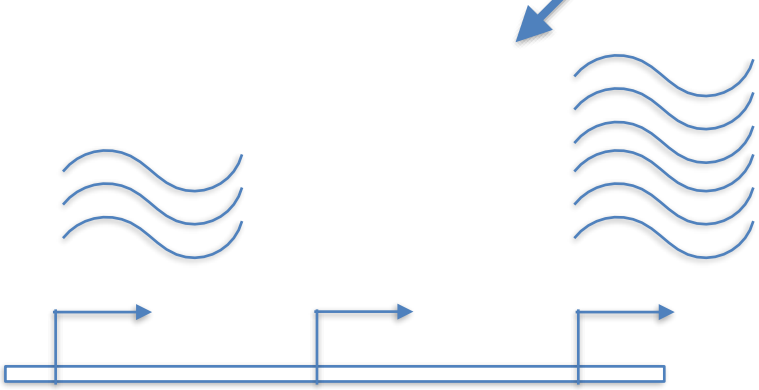
Normal Cells

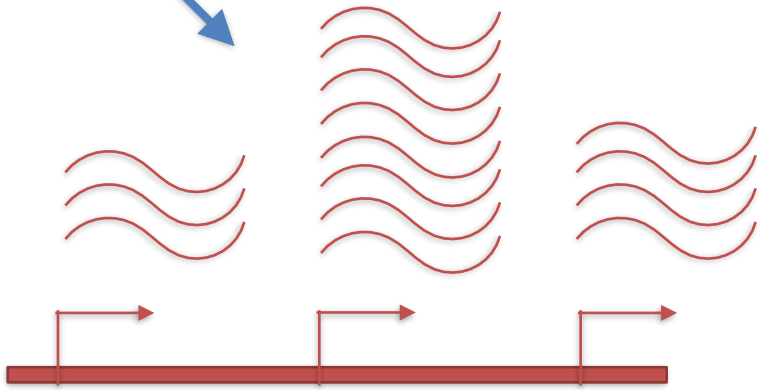


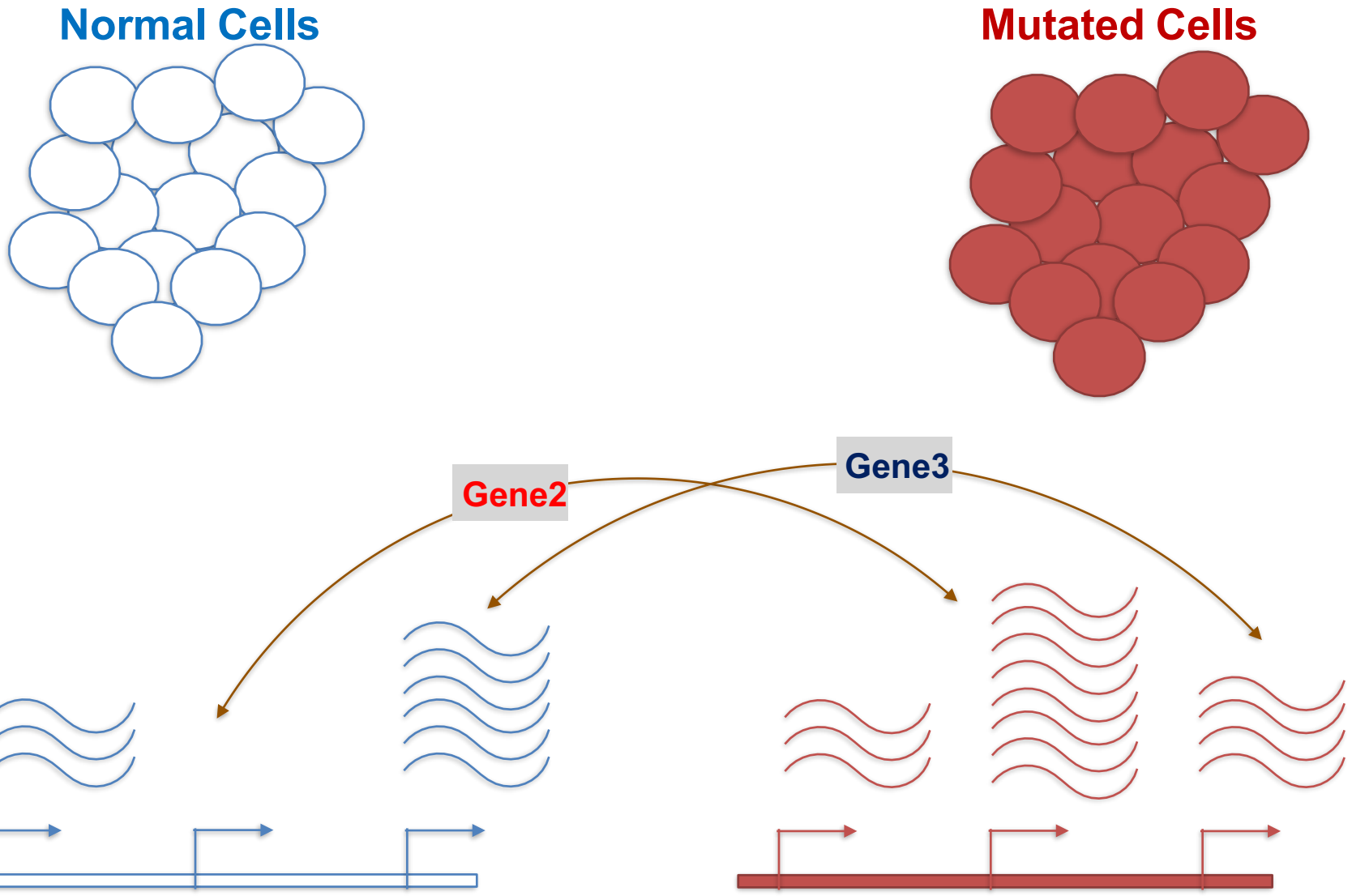
Mutated Cells



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





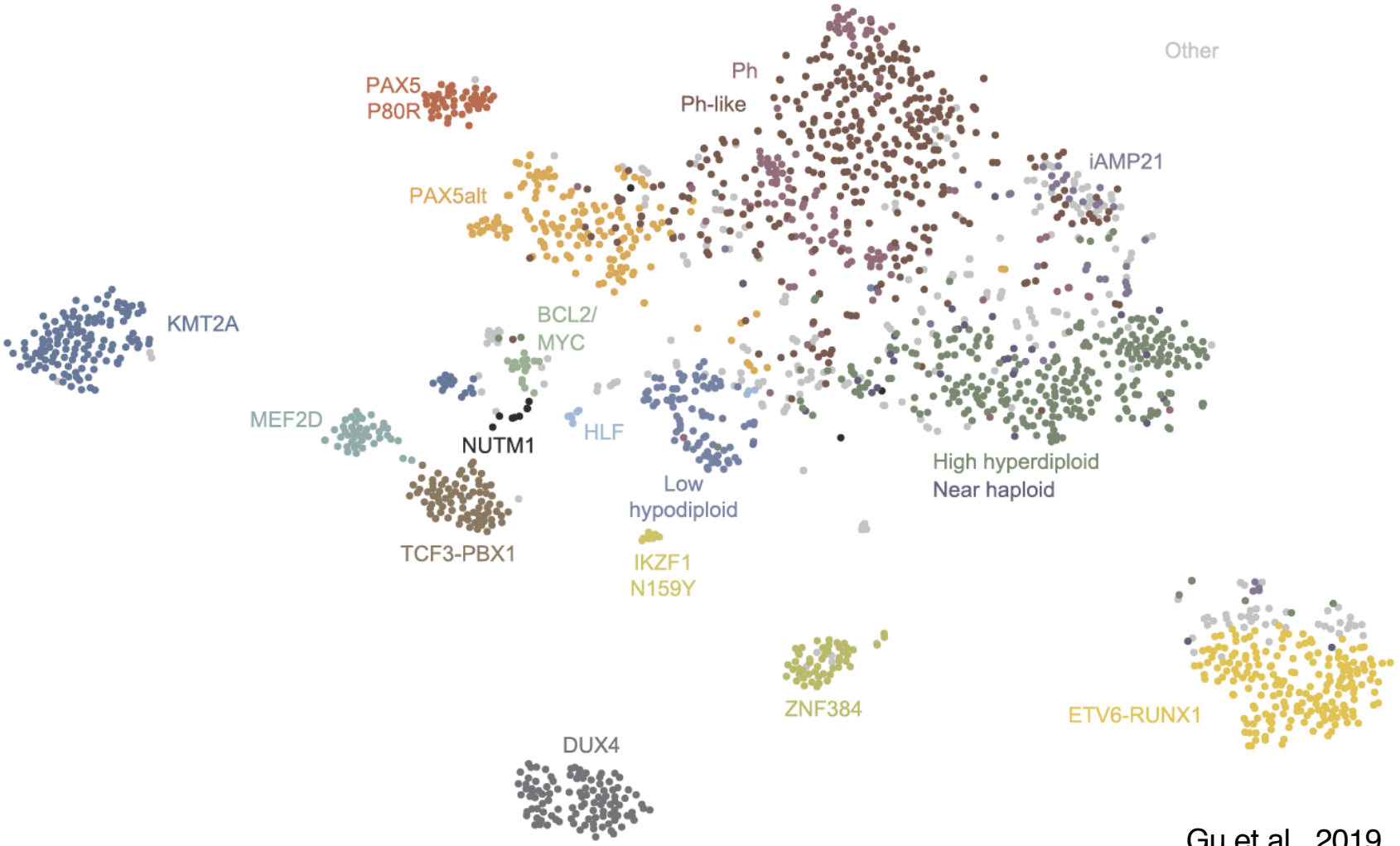


Differences apparent for Gene 2 and
to a lesser extent Gene 3

Other 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

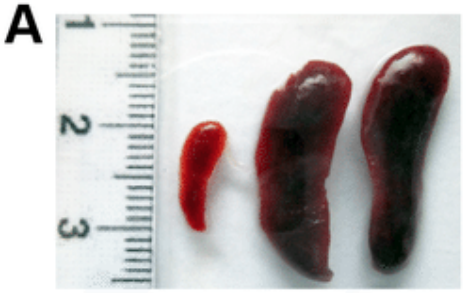
2) Sequence

3) Data analysis

RNA-Seq experimental workflow

1

Samples of interest



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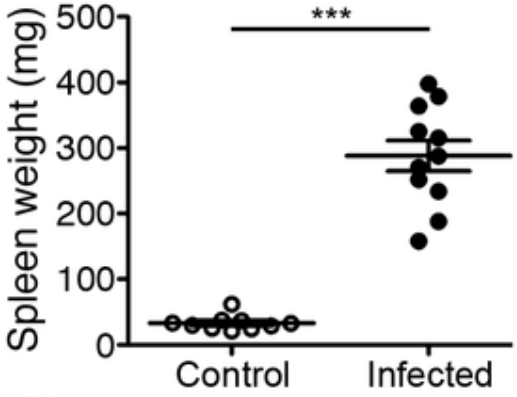
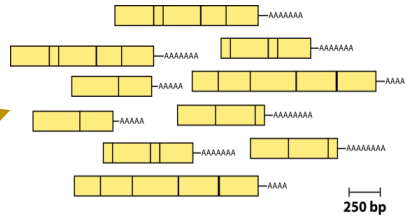


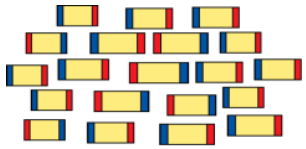
2

Isolate RNAs



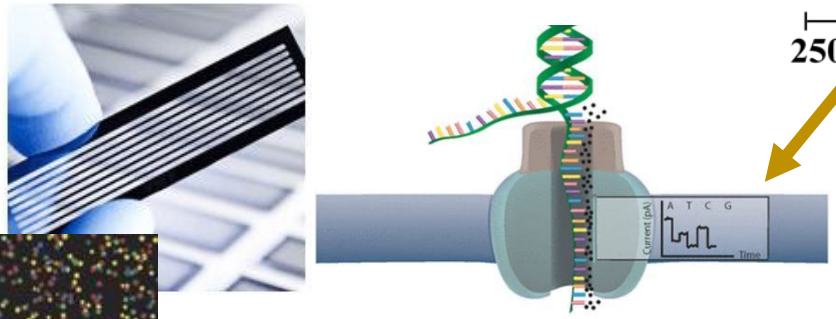
3

Library build

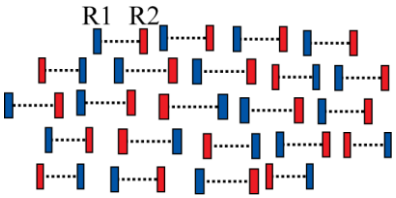


4

Illumina sequencing versus ONP

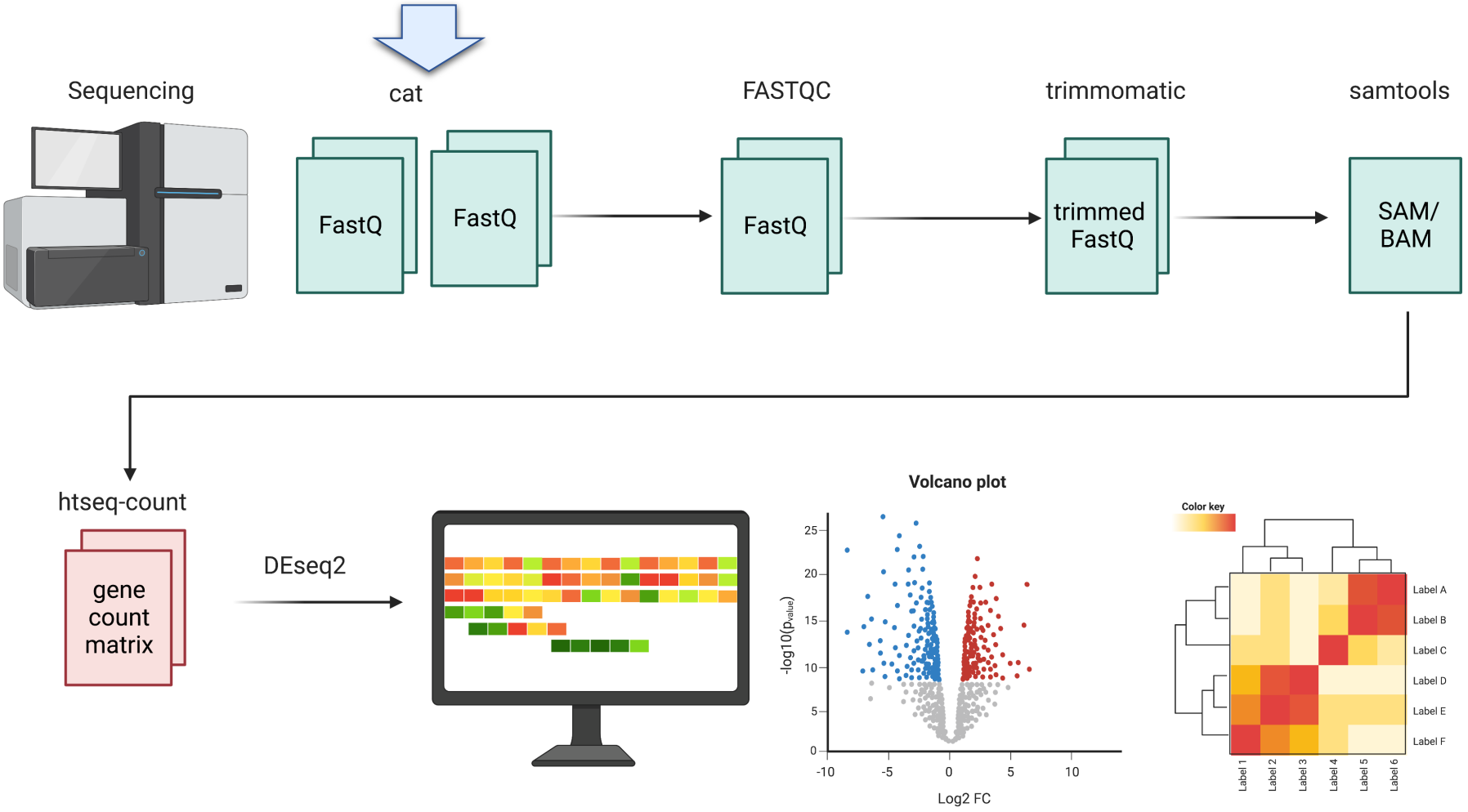


5



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