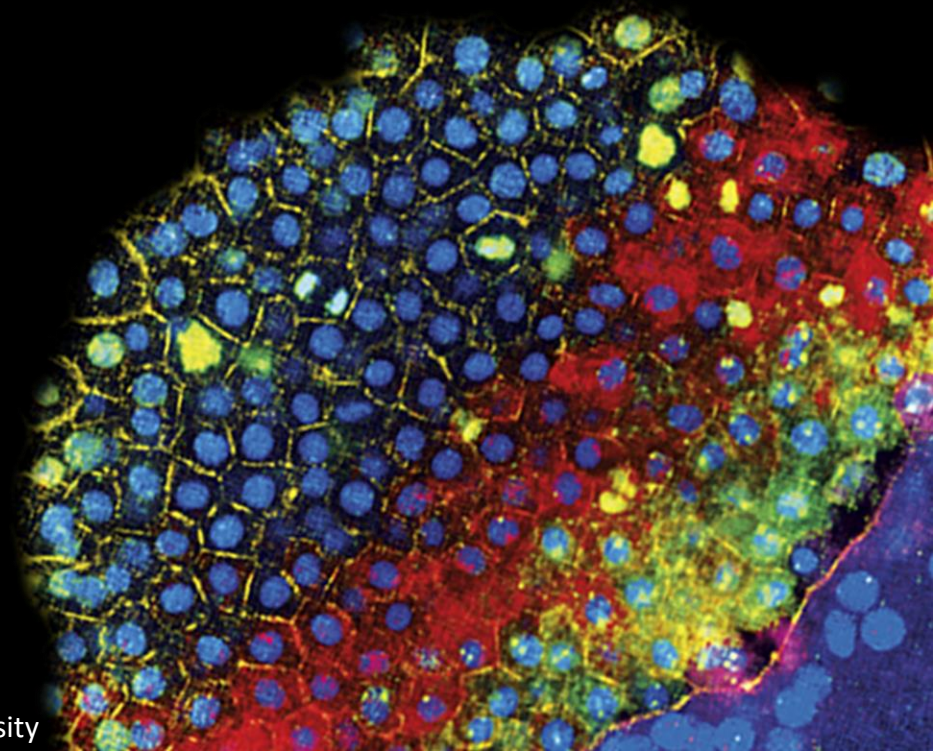


Single-cell RNA sequencing technologies and bioinformatics pipelines

Lucas Bauer & Abigail Olsen

March 2nd, 2023

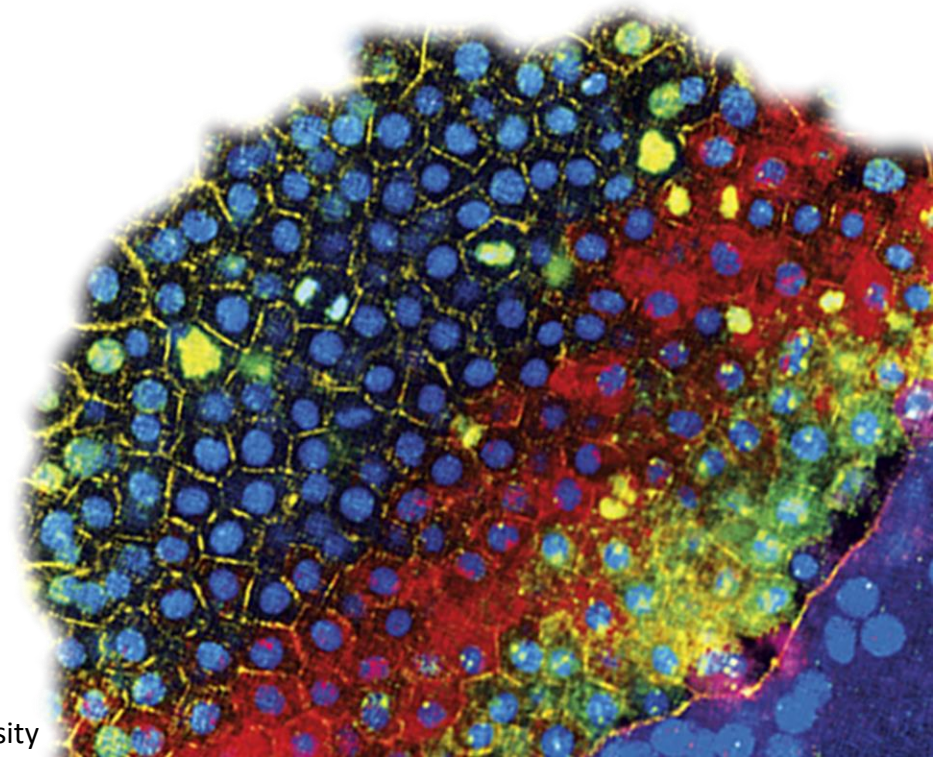


Single-cell RNA sequencing technologies and bioinformatics pipelines

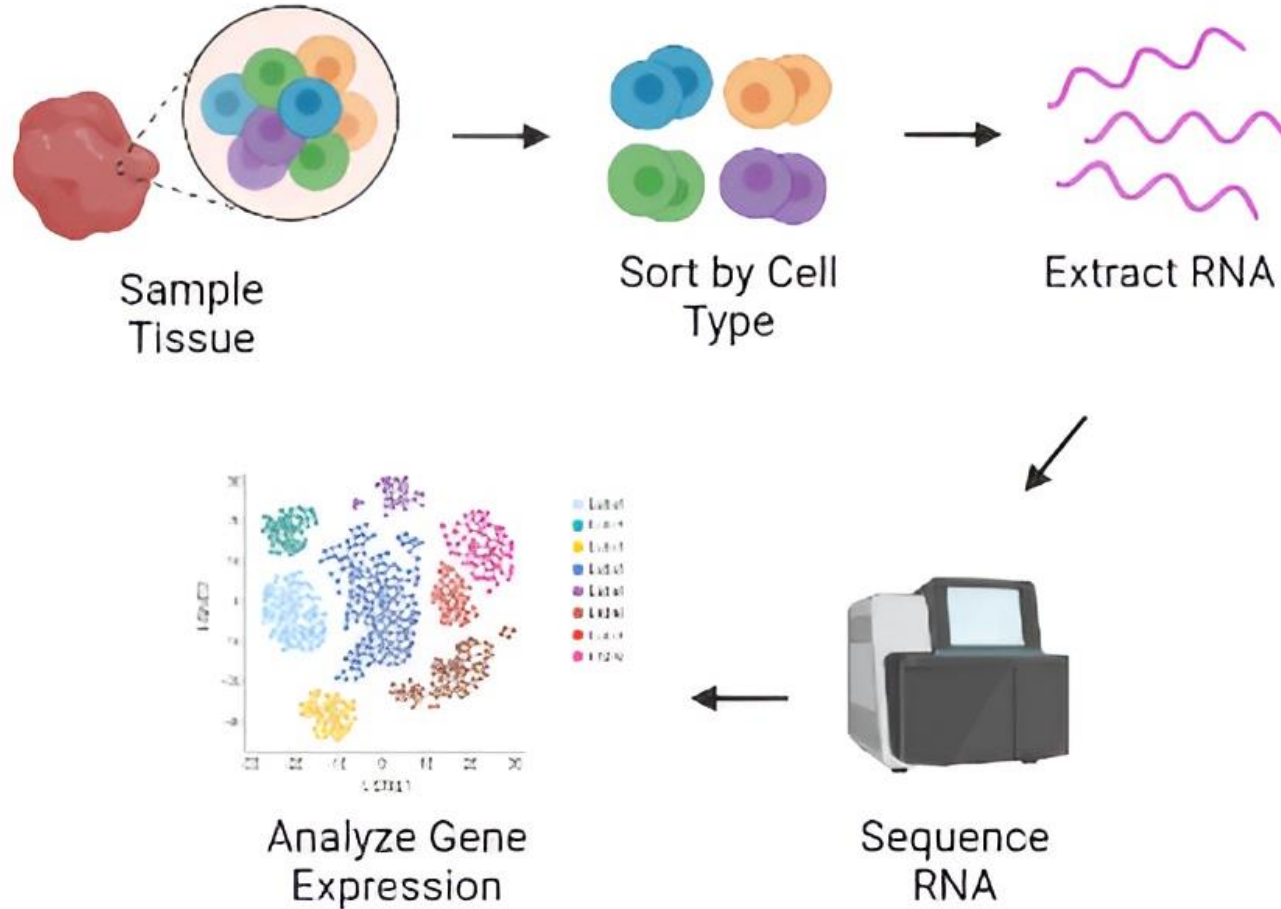
Byungjin Hwang¹, Ji Hyun Lee^{2,3} and Duhee Bang¹

Review from 2018

Lucas Bauer

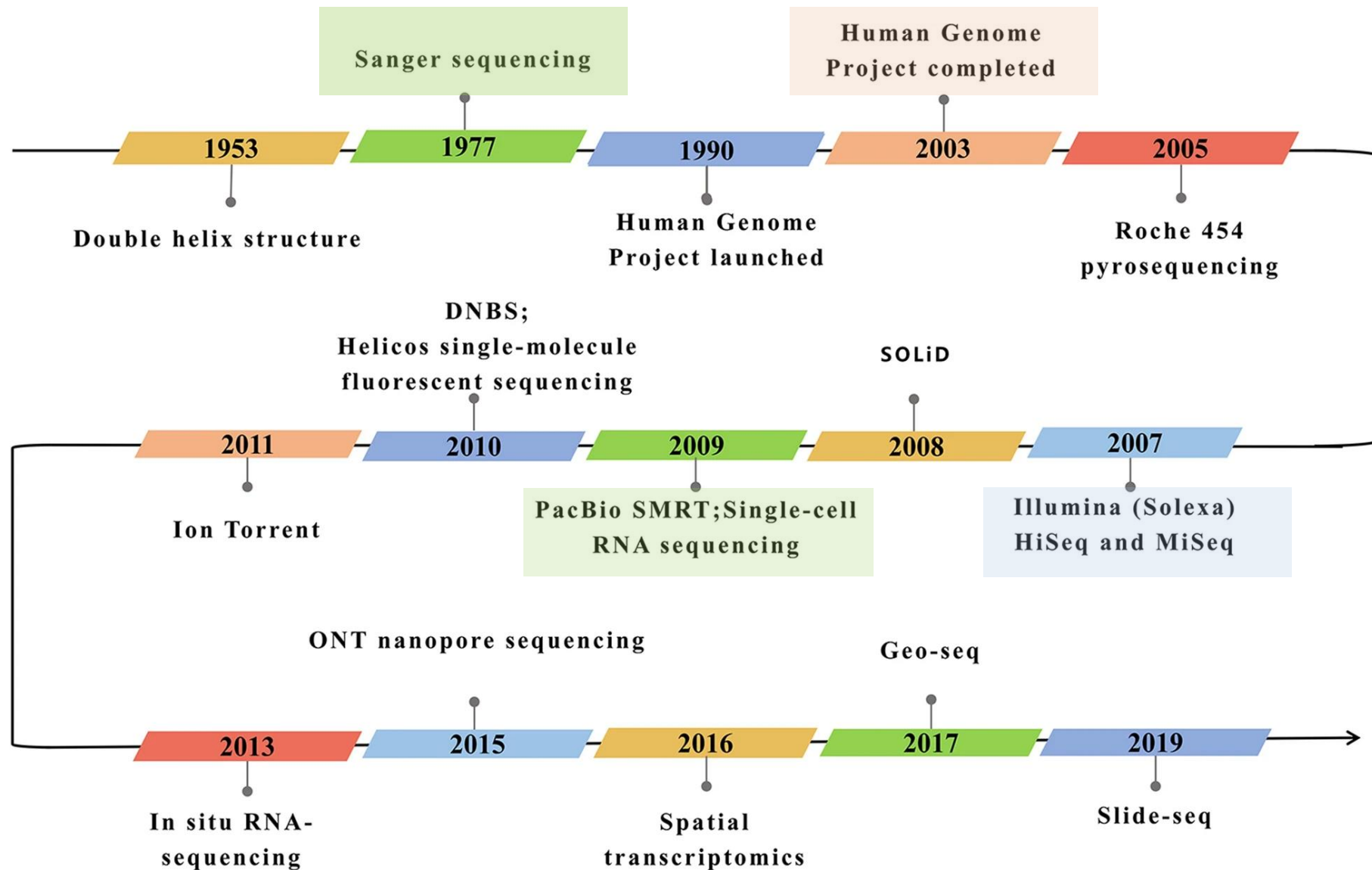


What is single-cell RNA sequencing?

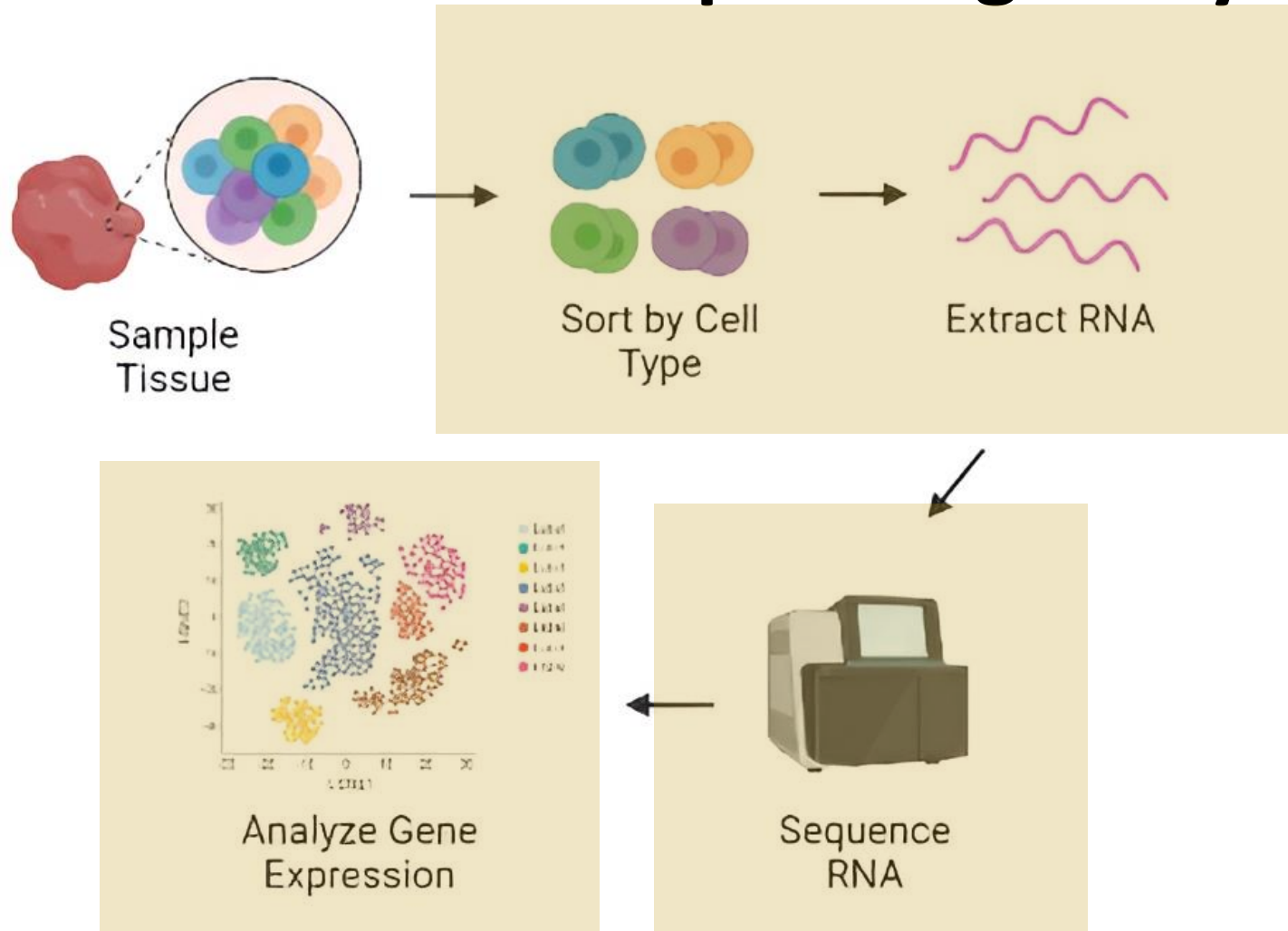


Isolating cells and sequencing their individual transcriptomes

When did scRNA sequencing start?

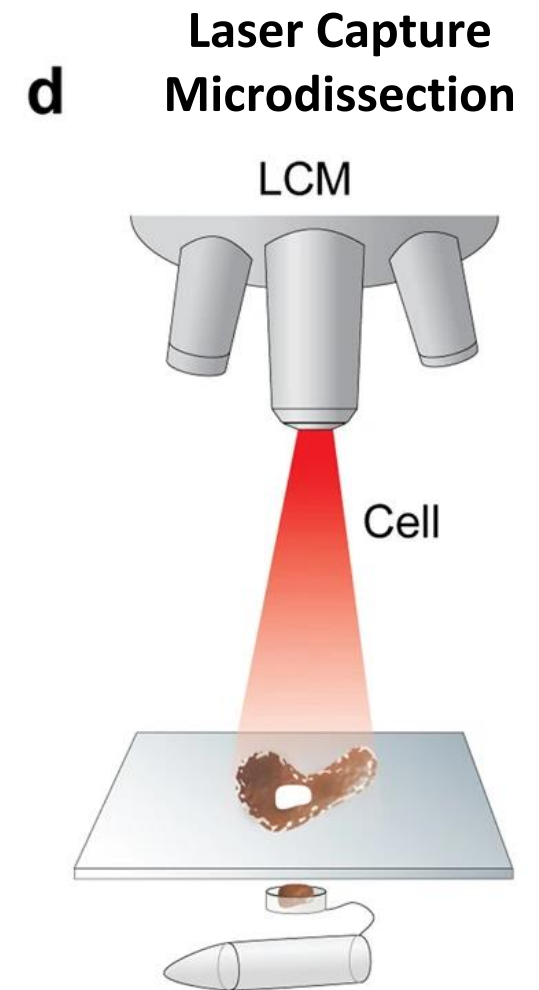
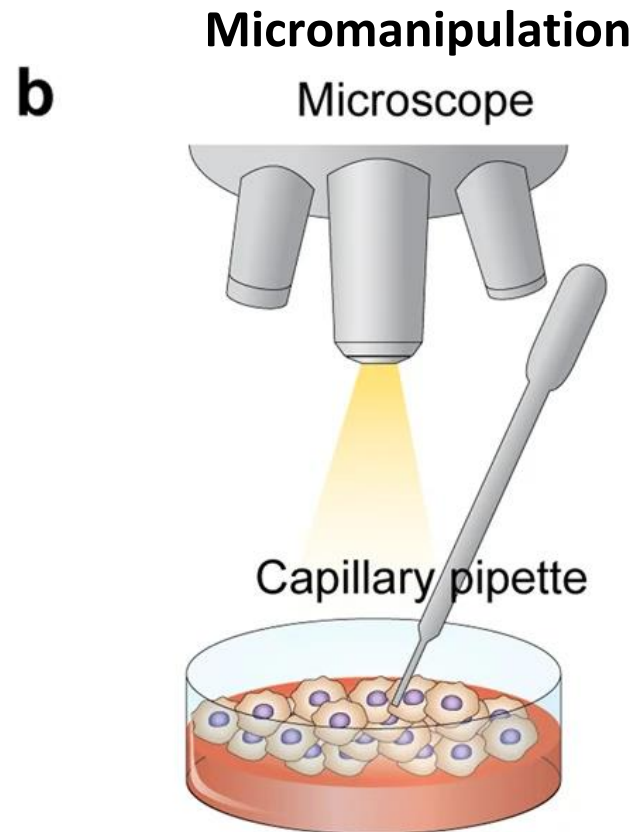
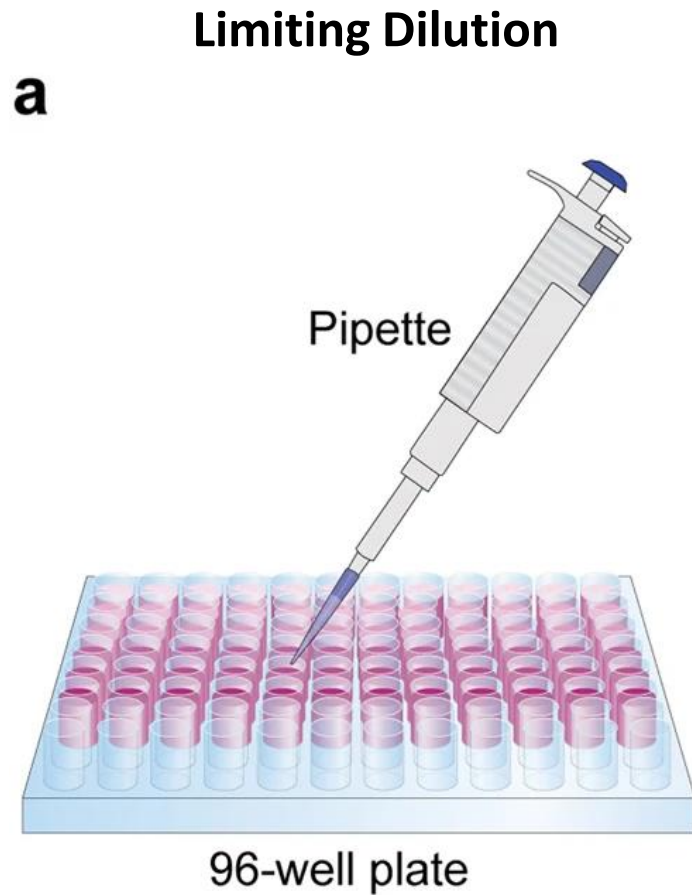


How does the scRNA sequencing assay work?



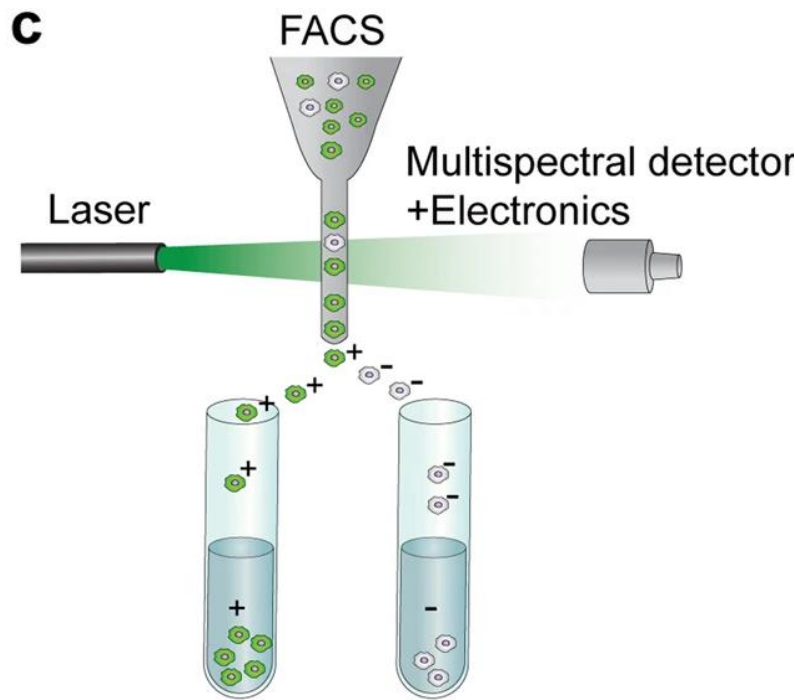
Isolating cells and sequencing their individual transcriptomes

What are ways to isolate cells using manual methods?

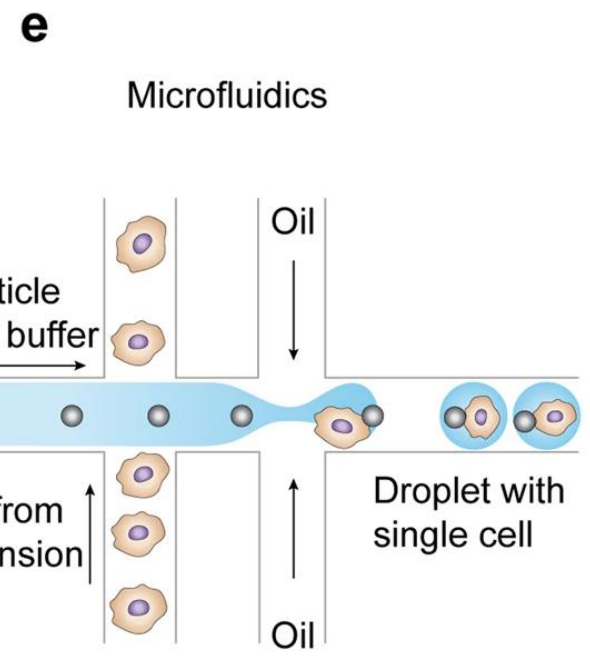


What are ways to isolate cells using High Throughput methods?

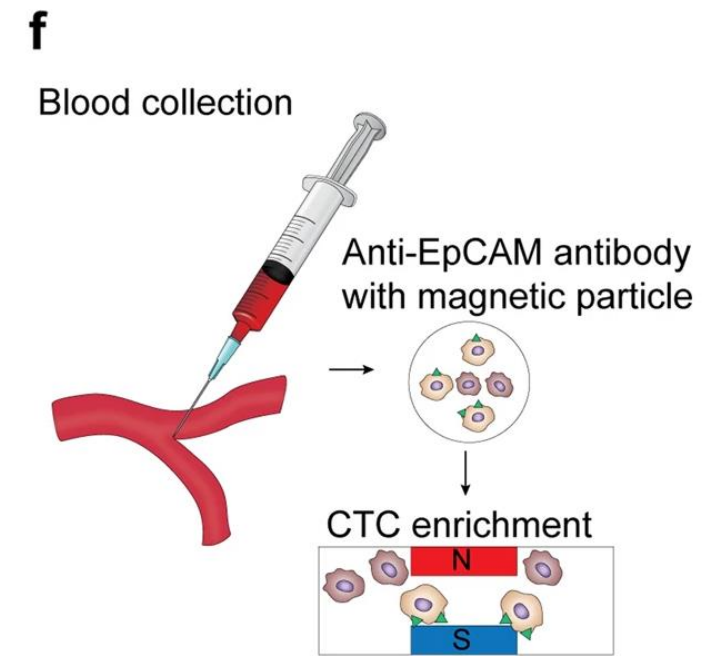
Fluorescence-Activated Cell Sorting



Microfluidics



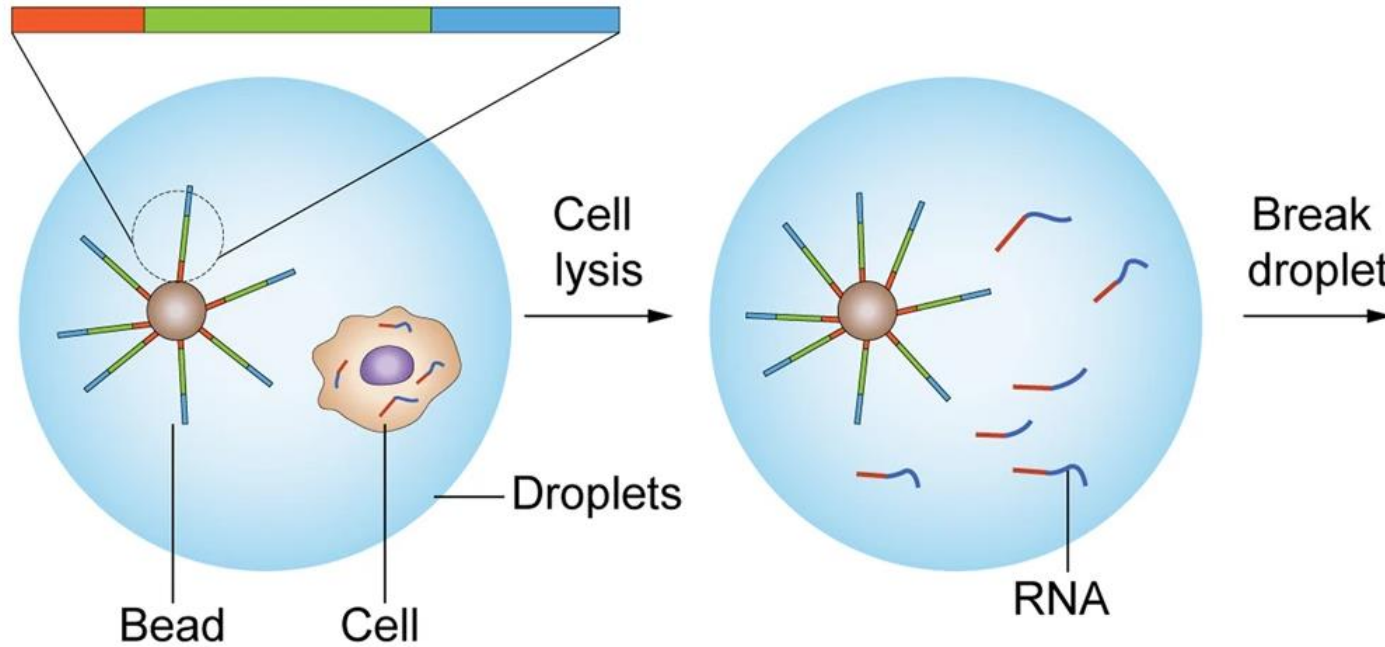
CellSearch System



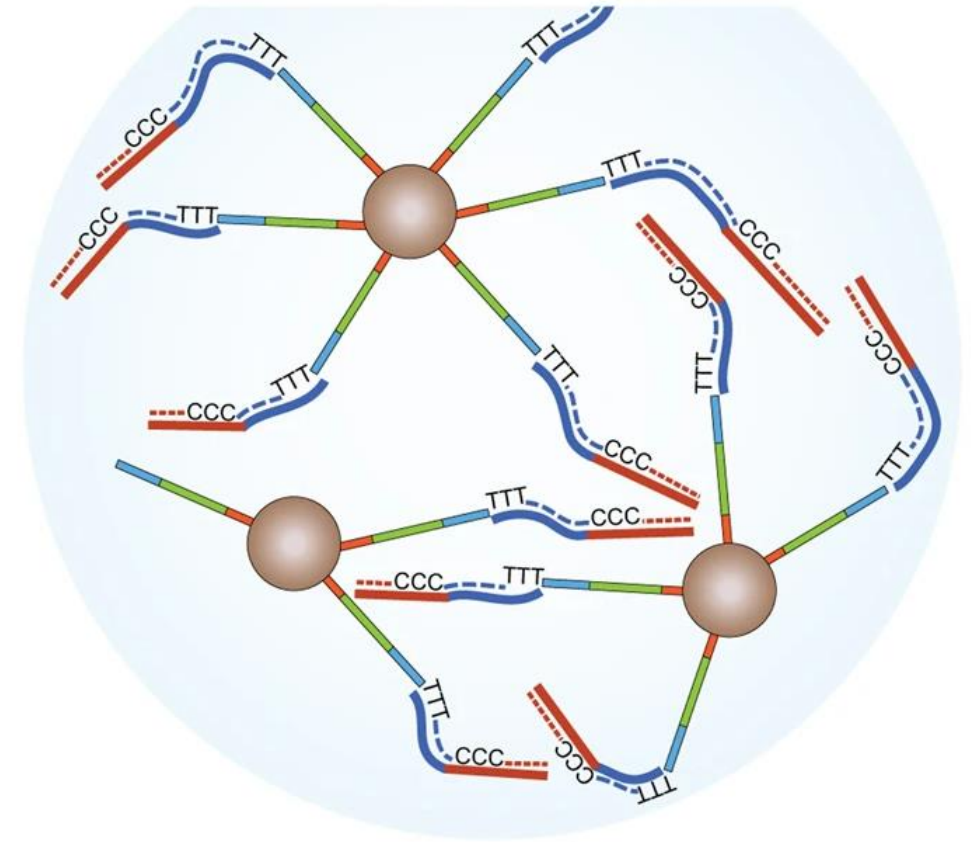
How do you add Barcodes to cells?

Structure of the barcode primer bead

PCR
handle Cell barcode UMI

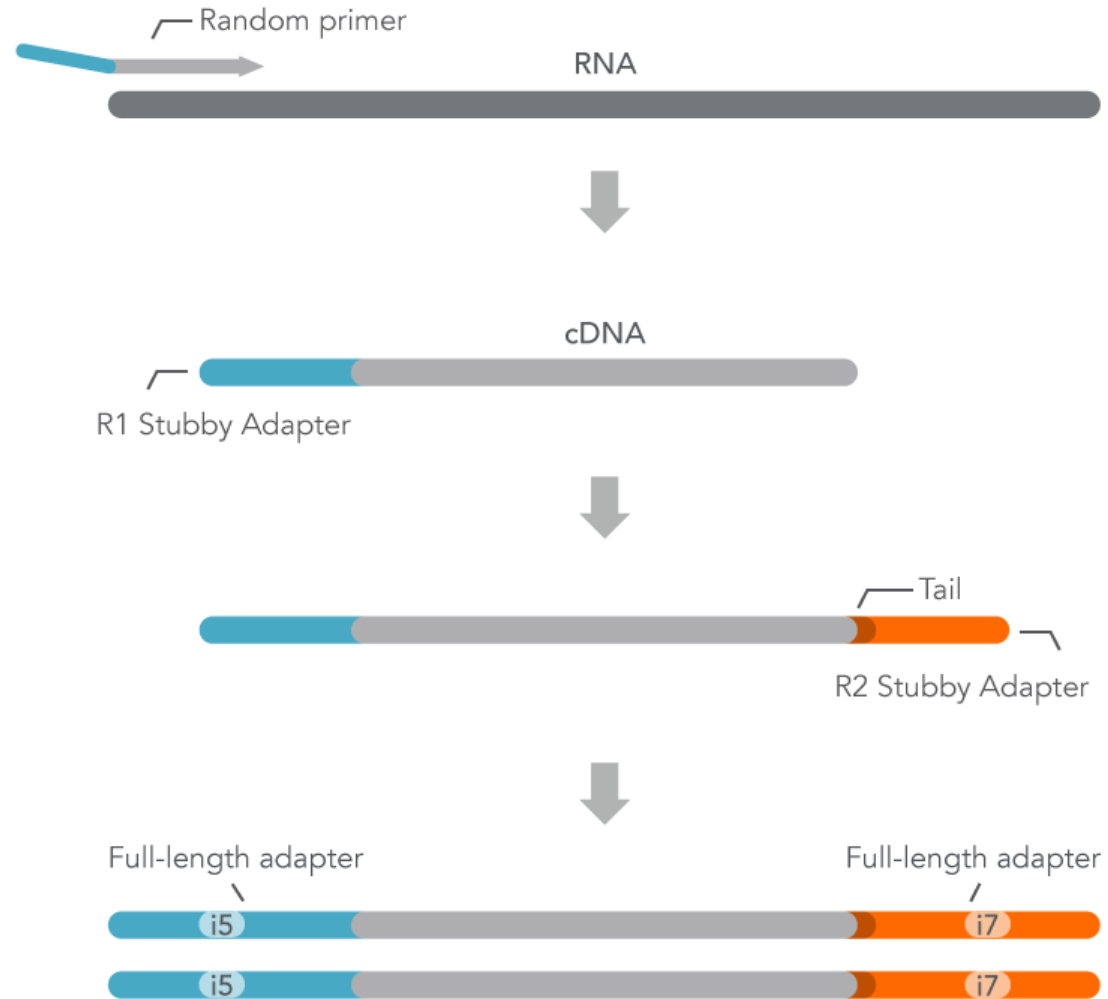


Reverse transcription with template switching



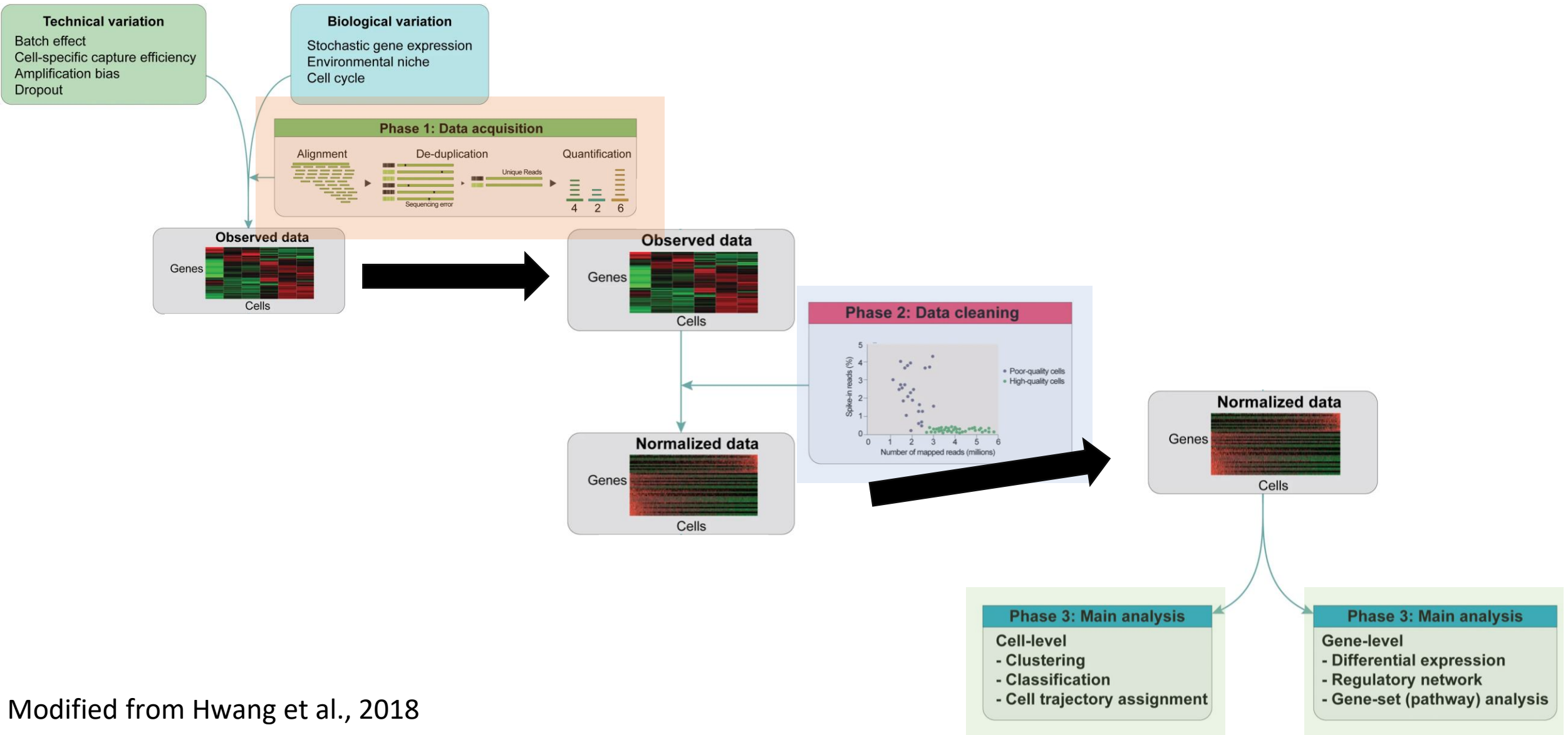
Label cells in the sequencing data

Can you sequence RNA directly?



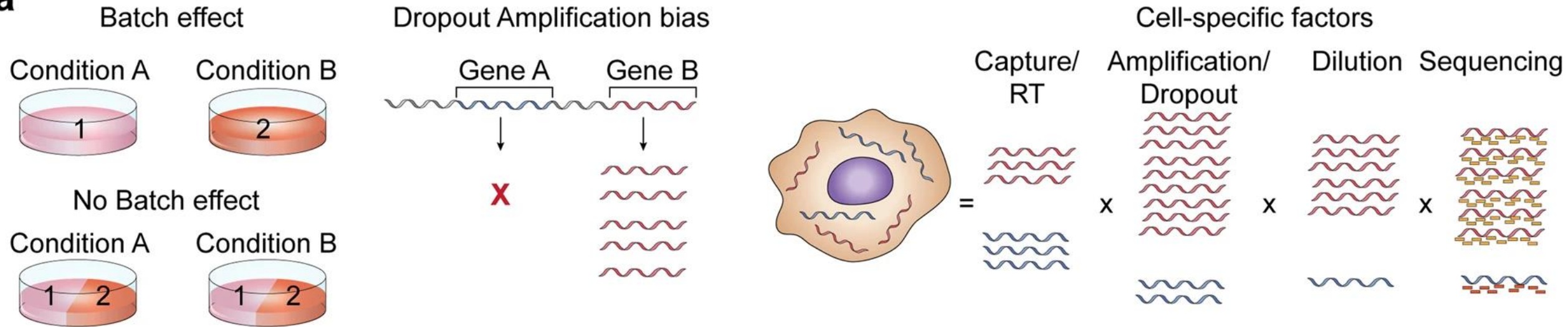
Produce adaptor-bearing cDNA molecules from RNA for sequencing

How do we go from the sequences to data?

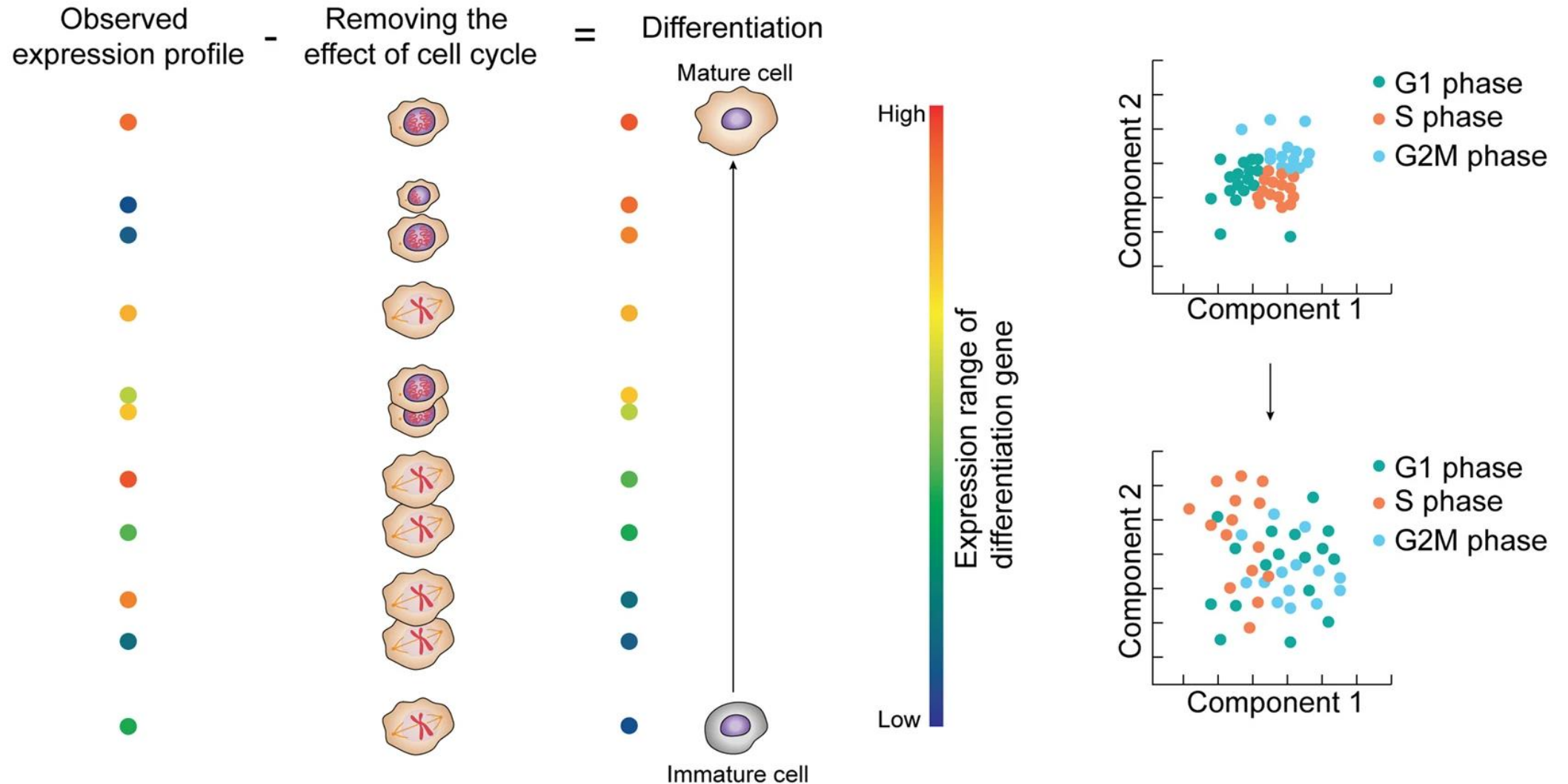


What are the common problems with scRNA sequencing?

a

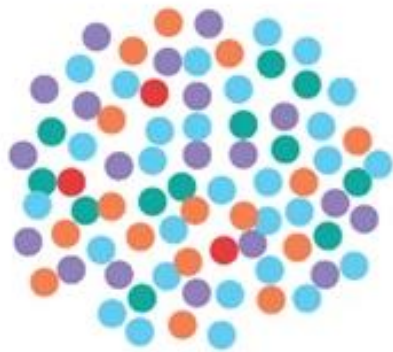


How does cell cycle stage impact scRNA sequencing?

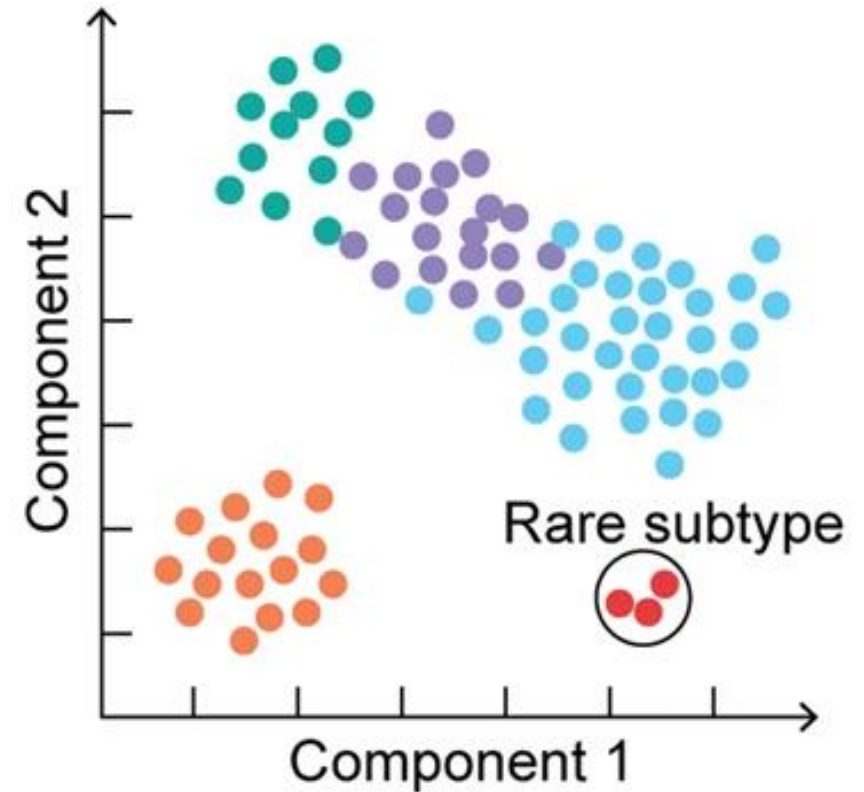


How do you determine if certain cells relate to another?

Heterogeneous tissue or tumor

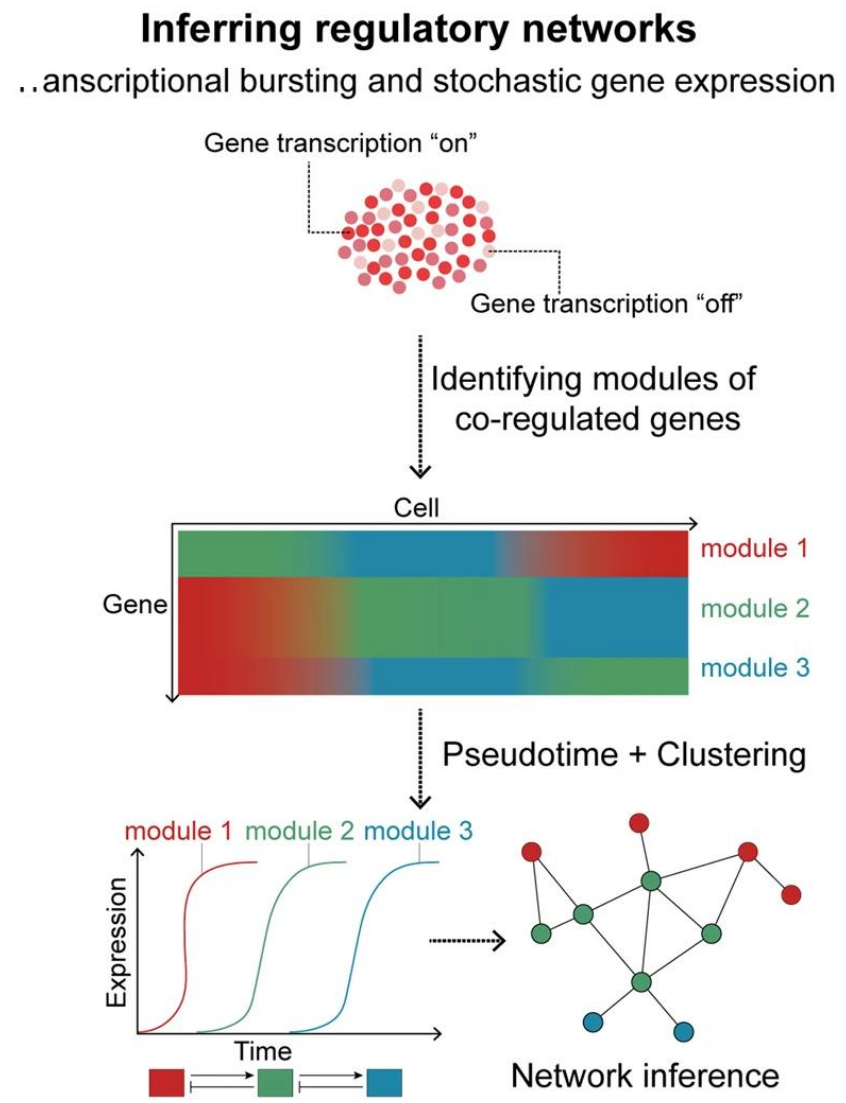
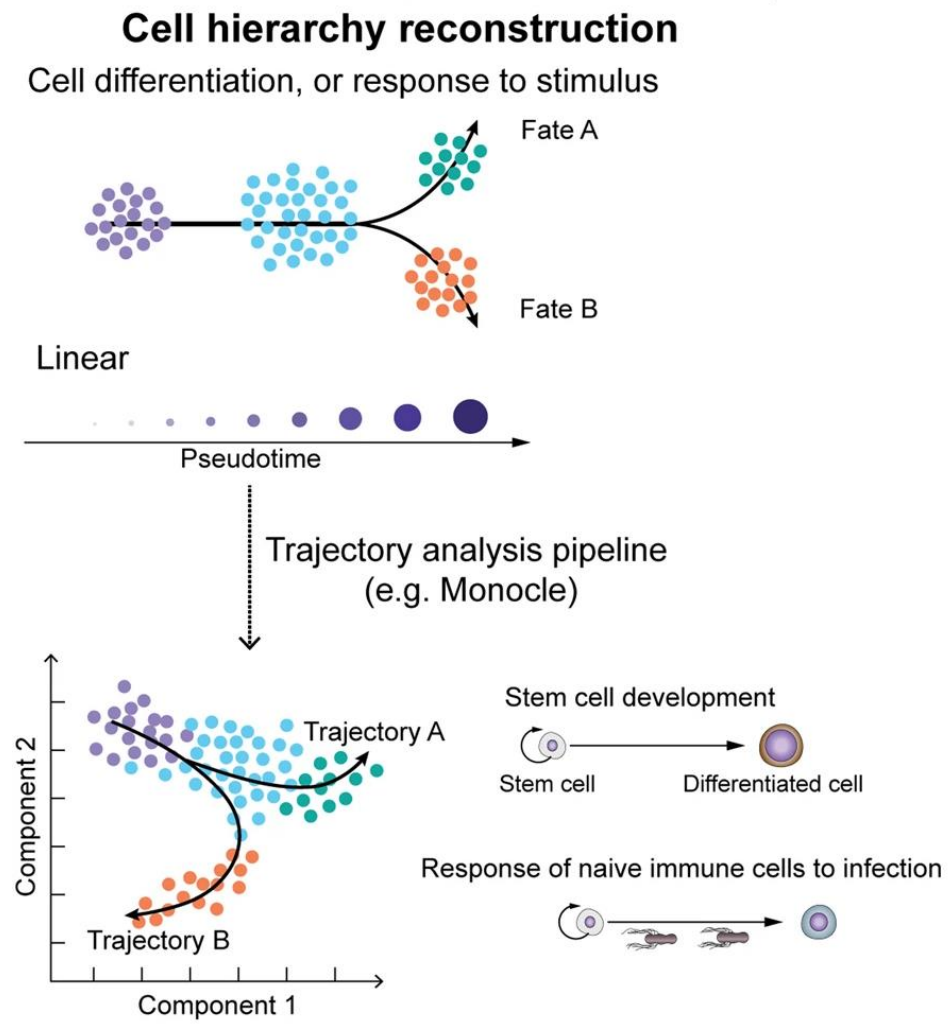


Dimensionality
reduction
(e.g. PCA)

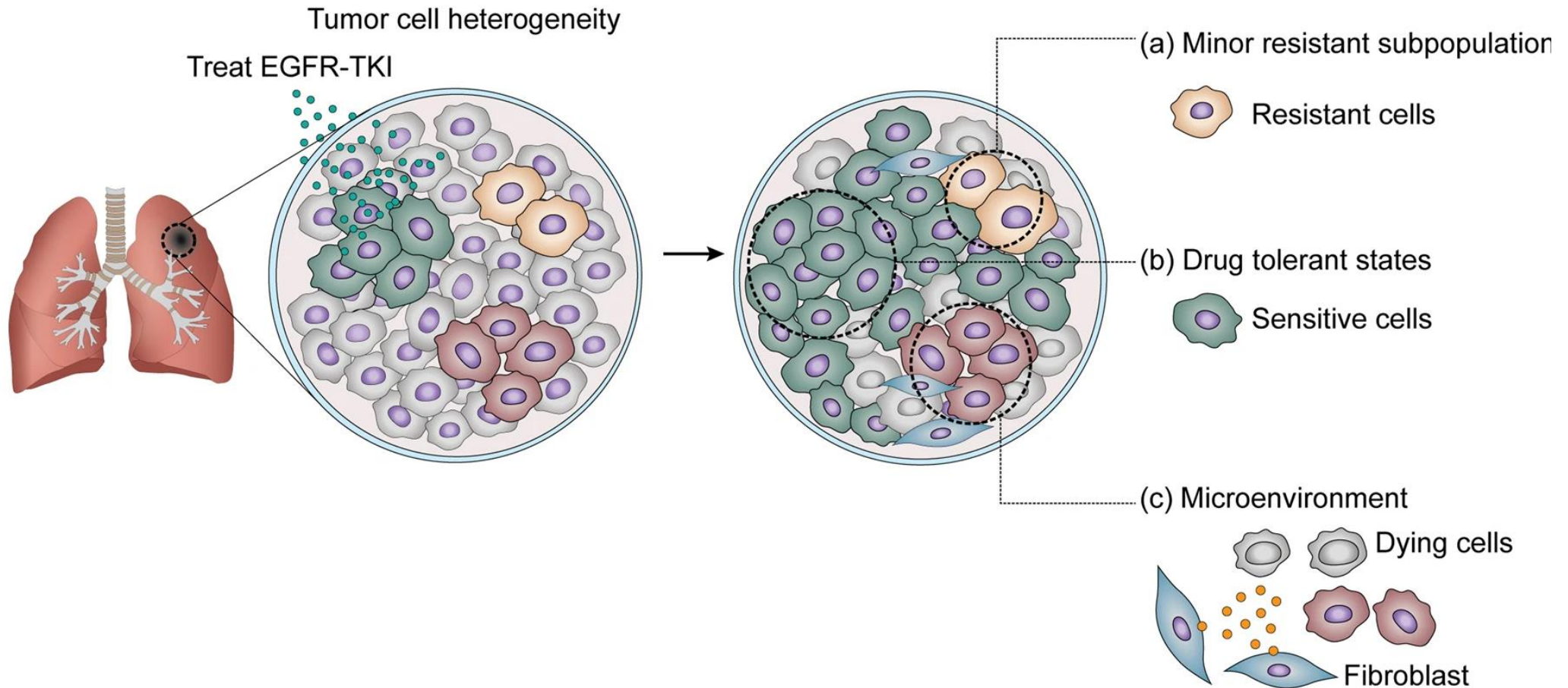


Cluster cell types from transcript abundance data

What do the cell clusters mean?



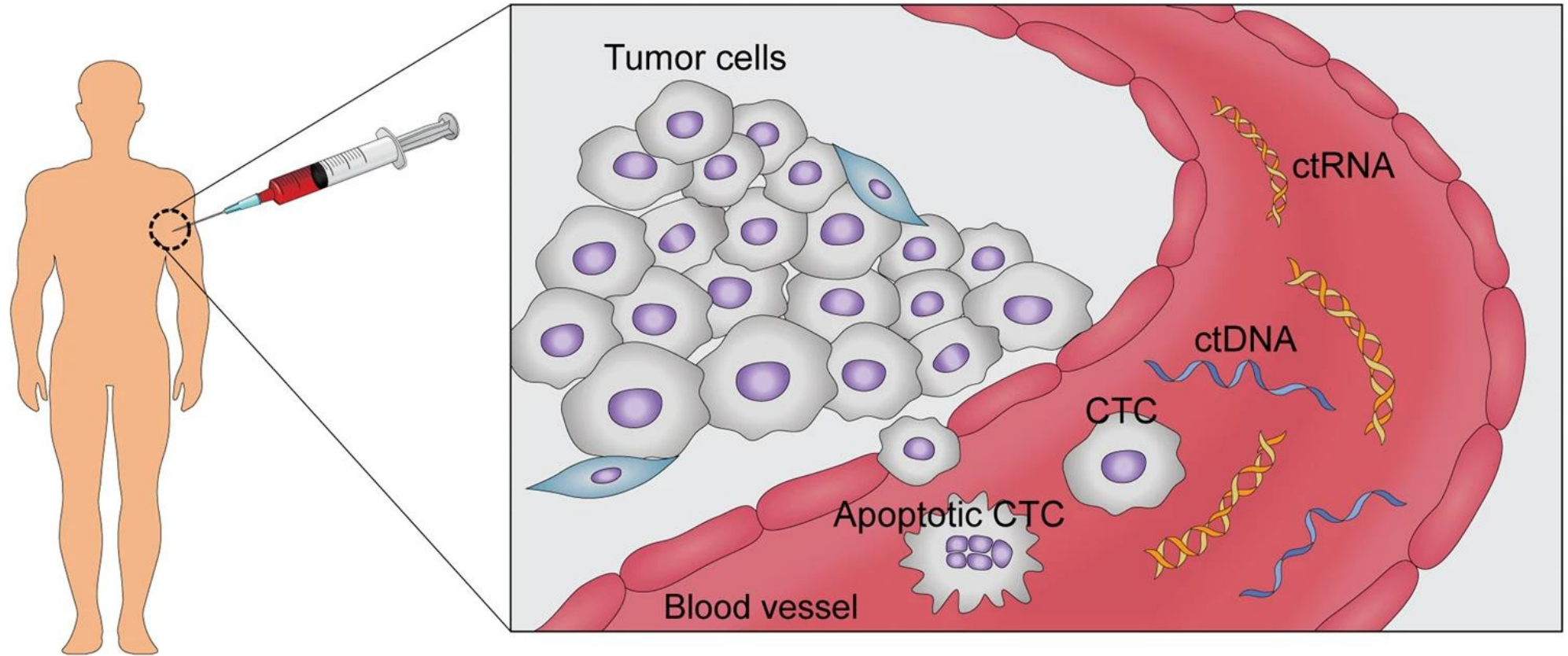
1. How can scRNA sequencing be applied?



Characterize cell lineages in tumors.

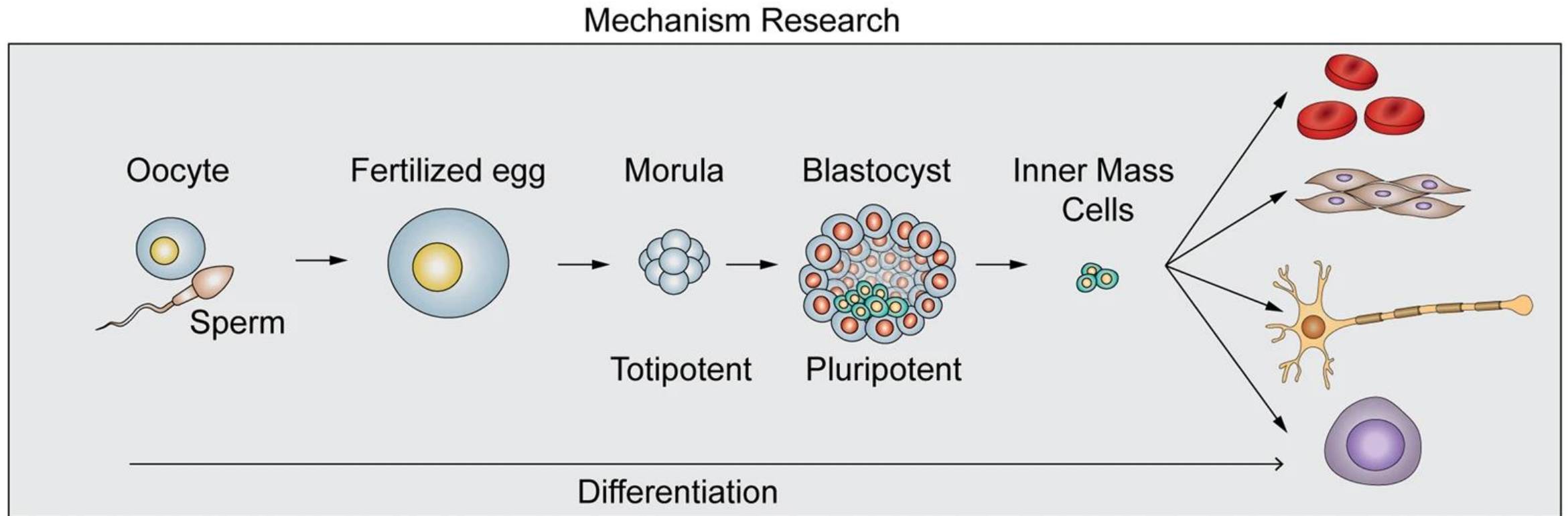
2. How can scRNA sequencing be applied?

b. Non-invasive biopsy diagnosis



Identify disease biomarkers in discovery and **clinical diagnostics**.

3. How can scRNA sequencing be applied?



Investigate specific **cell differentiation** mechanisms.

Liu Dong Lab: Application to Hearing Loss



Liu Dong



Liu Dong Lab

Professor: Nantong University

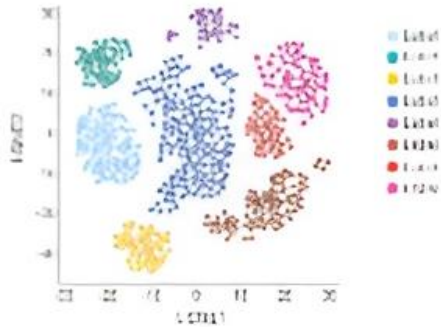


"To identify novel regulators of angiogenesis during embryonic development and tissue regeneration."

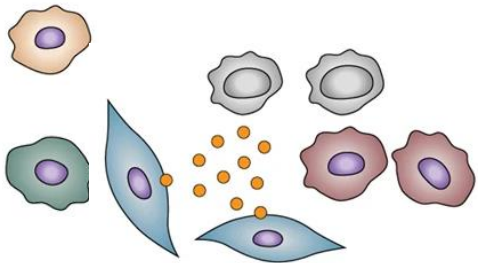
Summary: scRNA Sequencing



Separate and individually sequence unique cells.

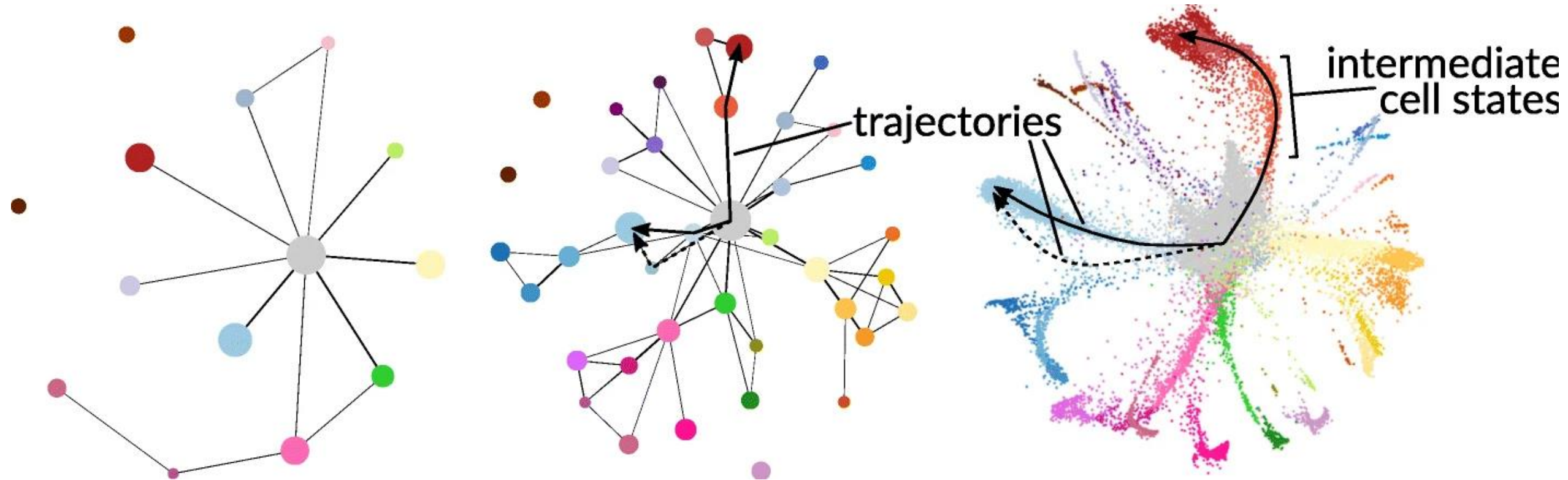


Perform informative analyses collected data which directly biological questions.



Use information to inform clinical decisions regarding treatment of disease.

Question?





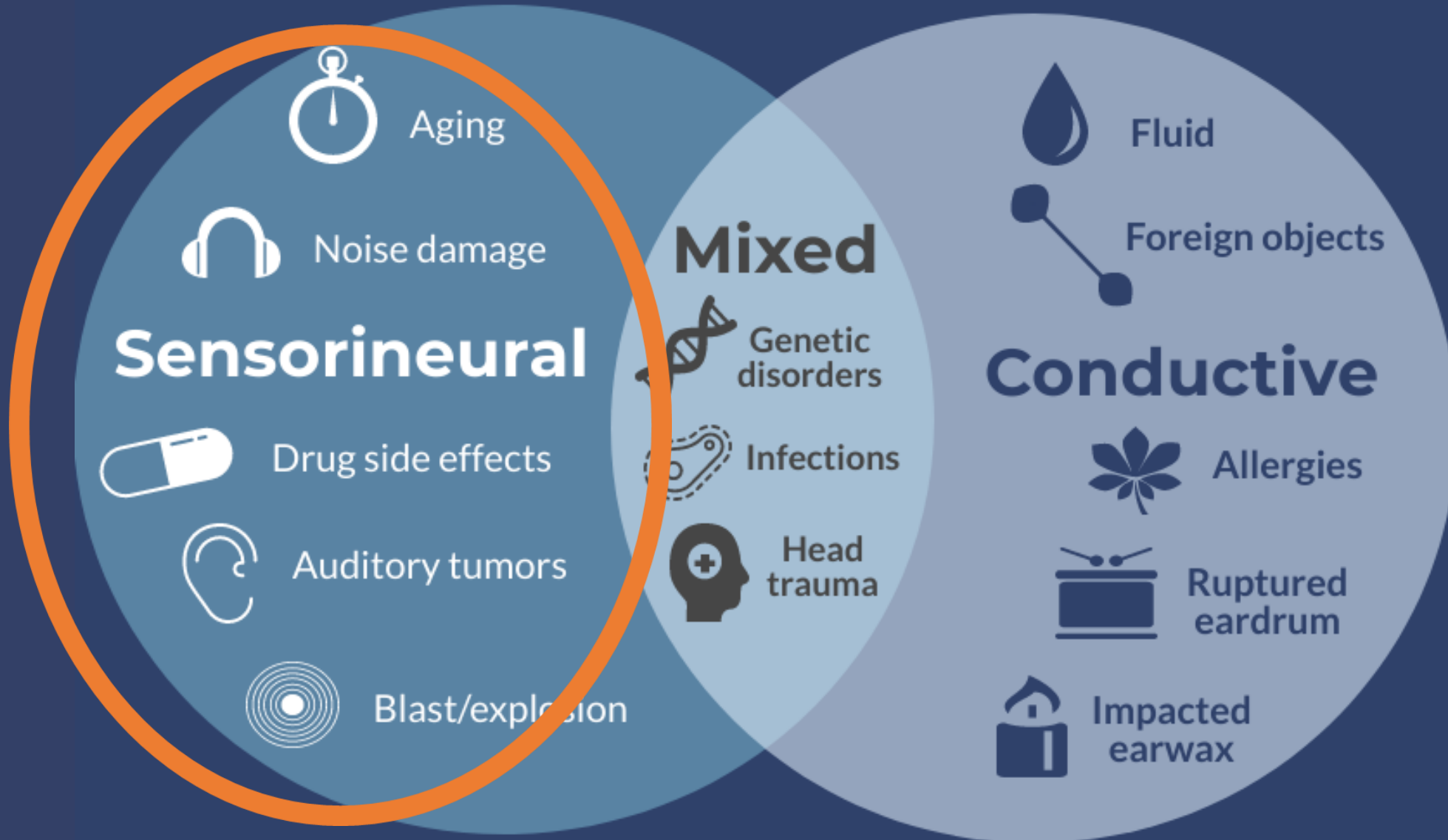
Single-cell RNA-sequencing of zebrafish hair cells reveals novel genes
potentially involved in hearing loss

Qian, et al, 2022

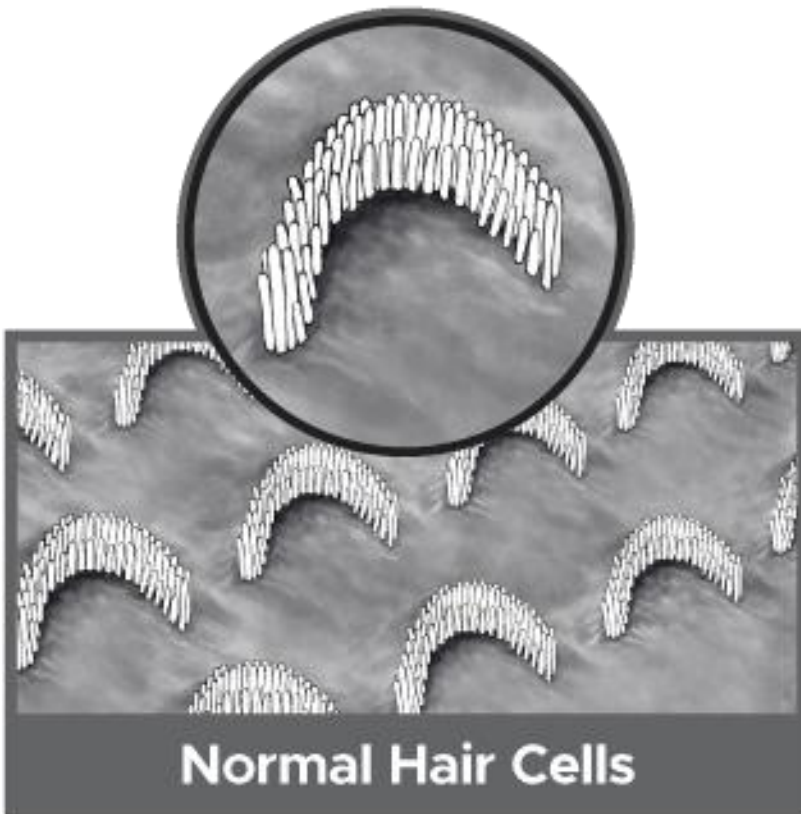
Why is hearing important?



Types of hearing loss

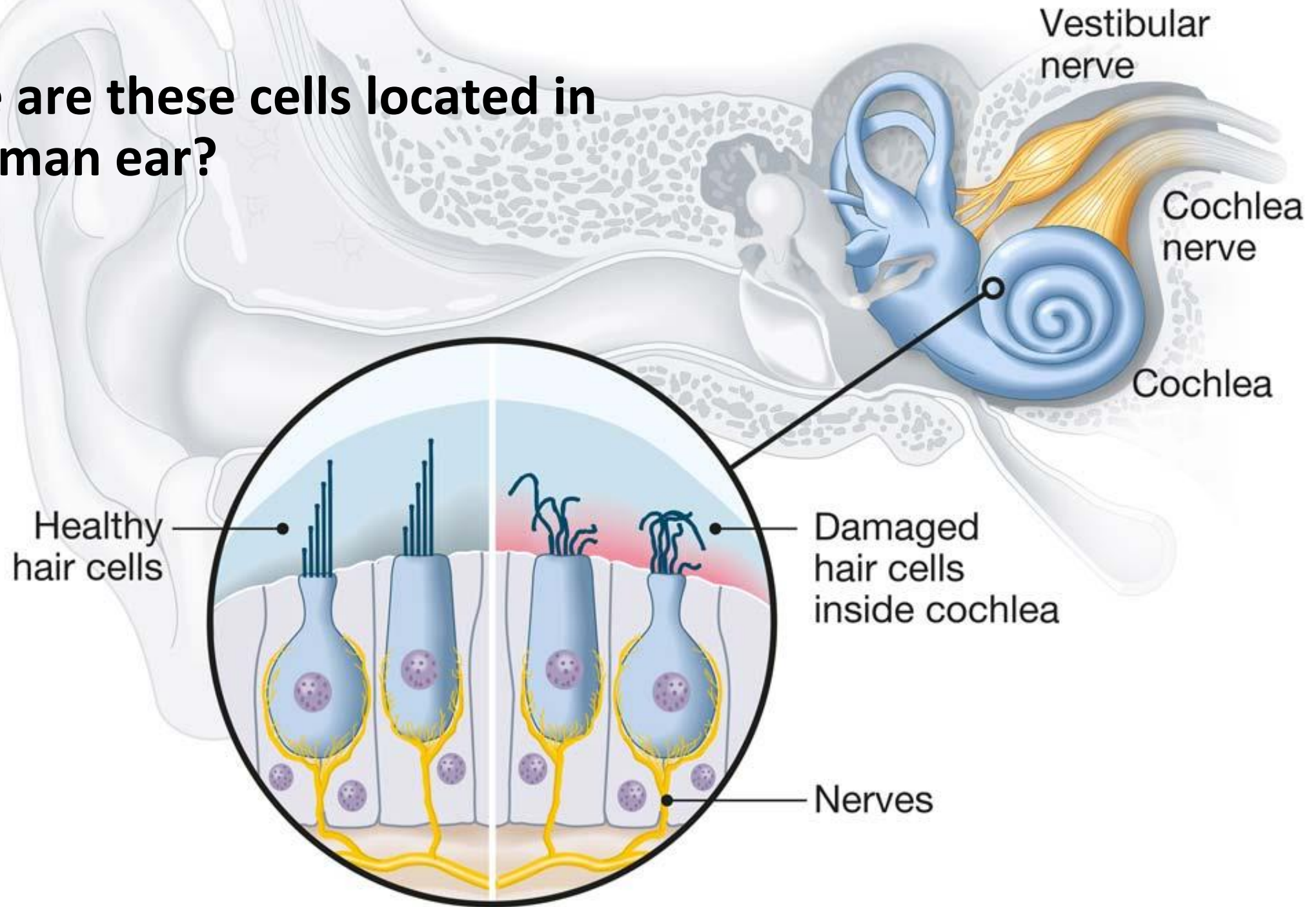


What causes hearing loss?

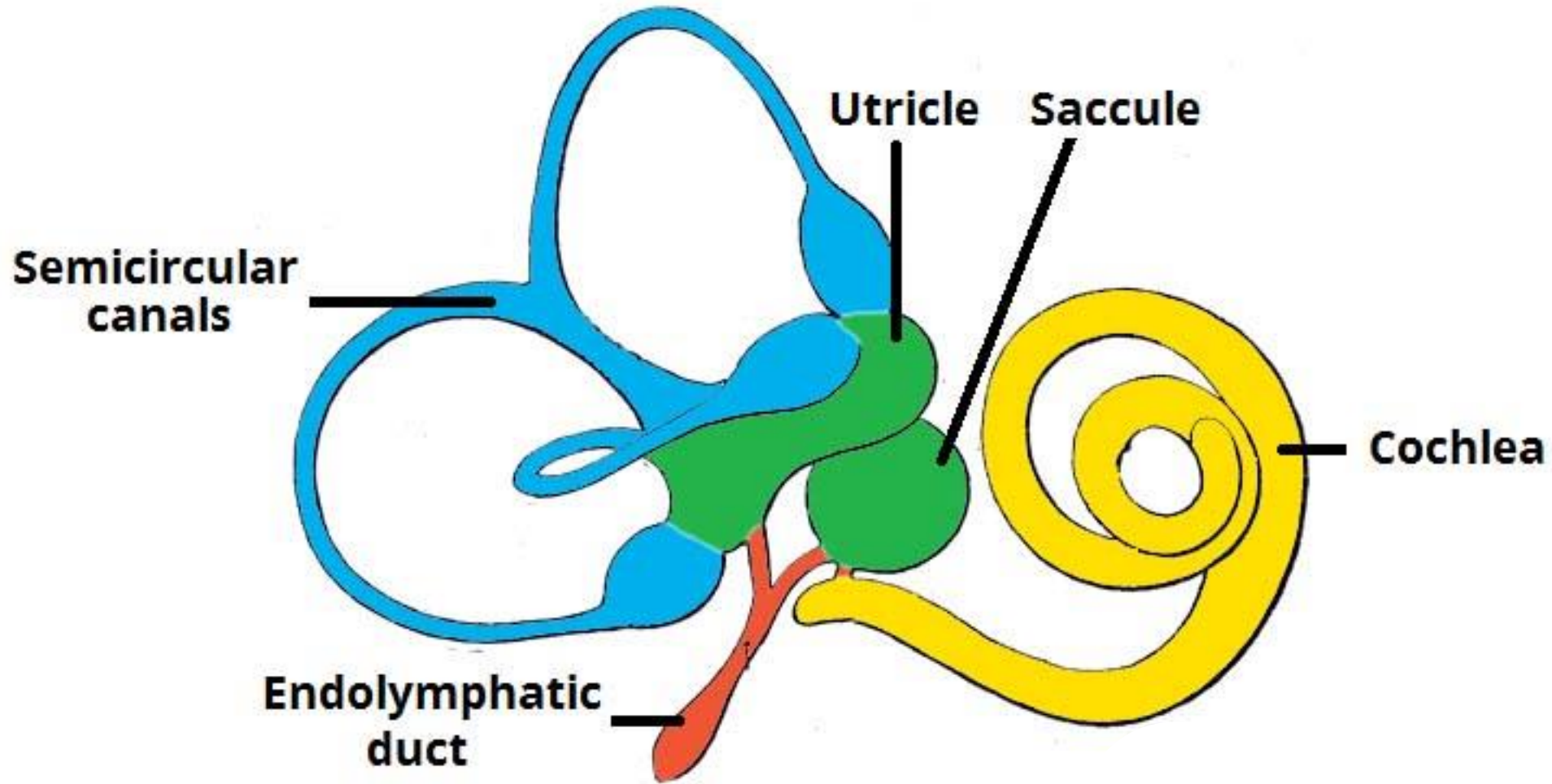


Damaged hair cells

Where are these cells located in the human ear?



What is the structure of the human inner ear?



What is the gap in knowledge?



How are the human genes involved in hearing loss related to zebrafish?

Figure 1A: Zebrafish hear all along their body

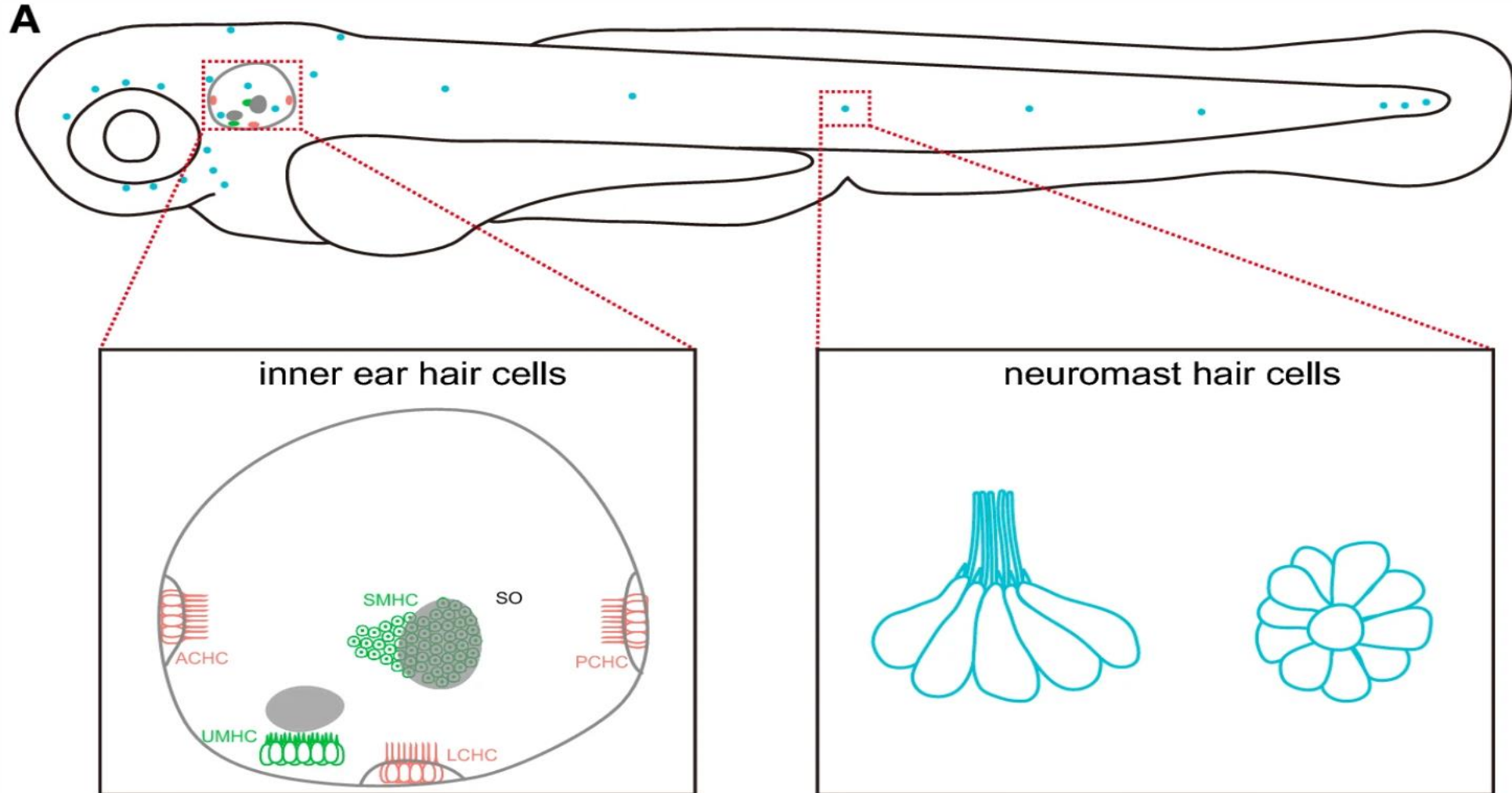


Figure 1B-D: What do the zebrafish hair cells look like?

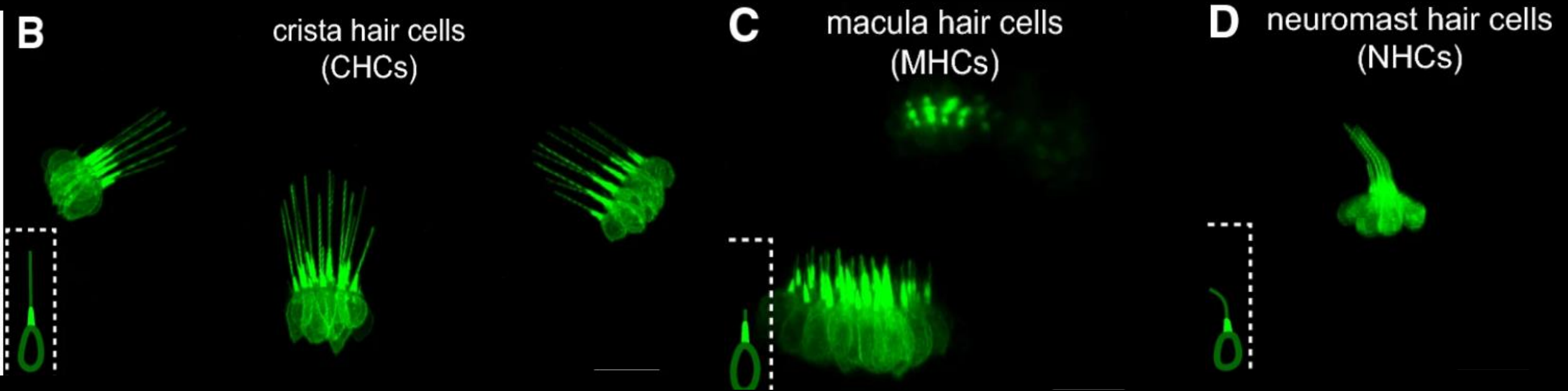
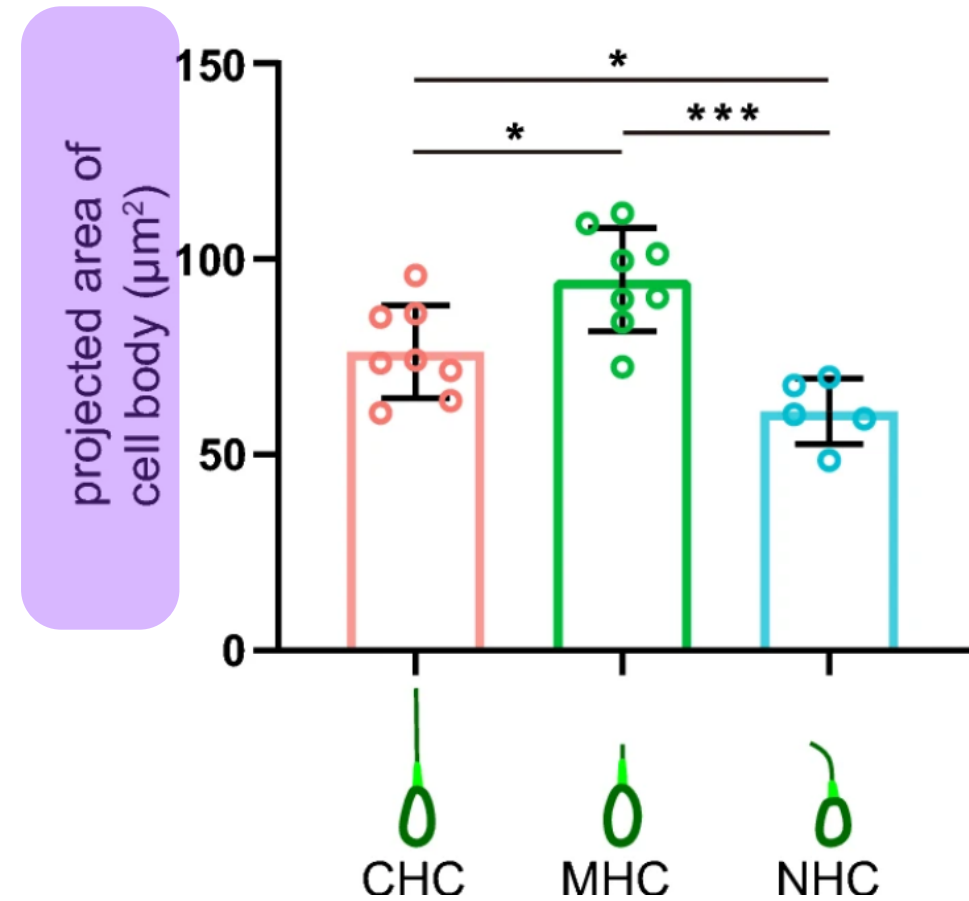
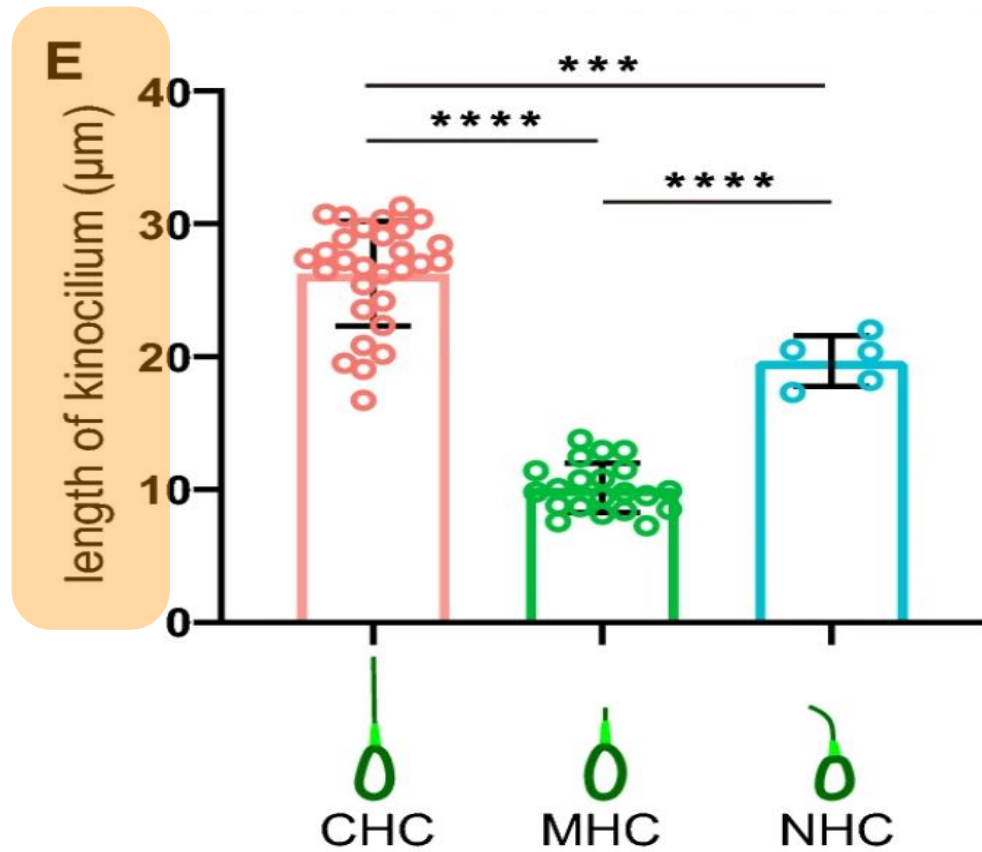
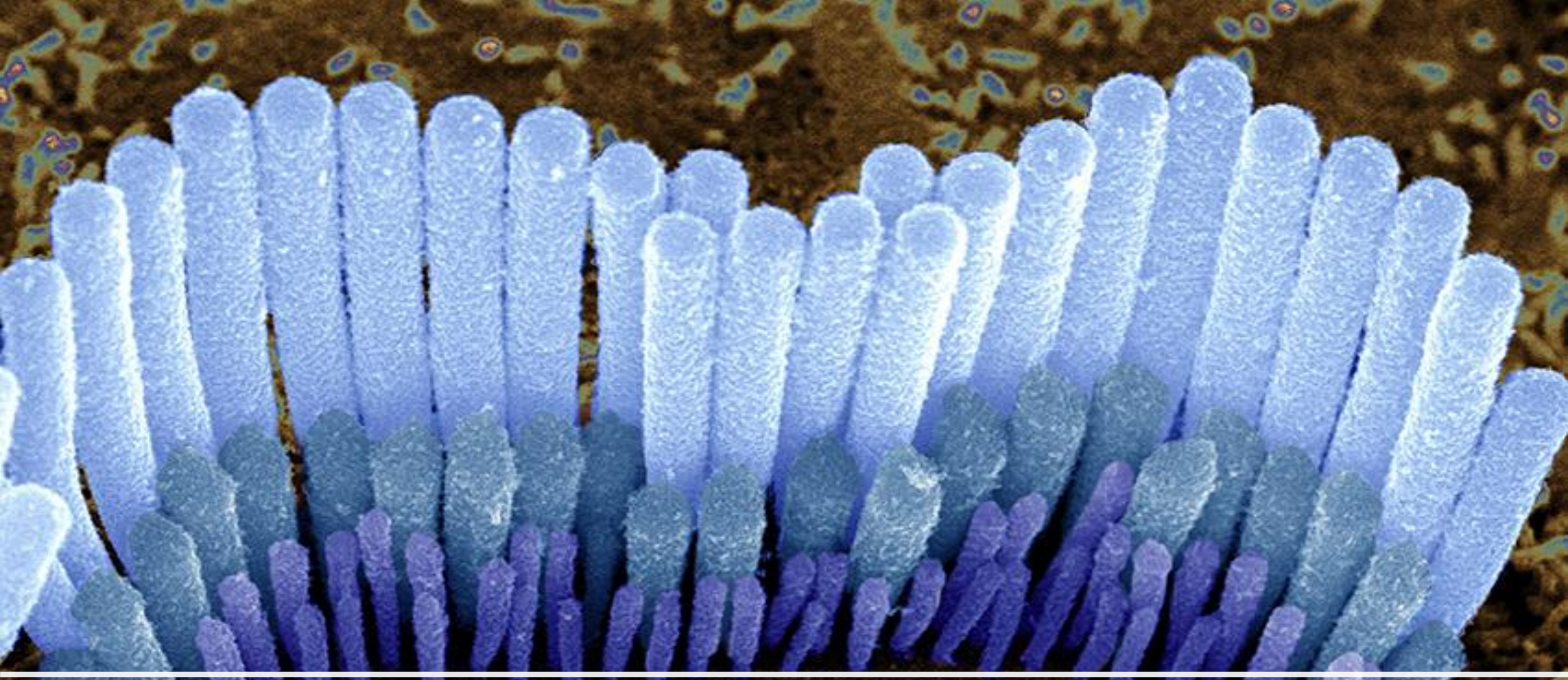


Figure 1E-F: What are the **lengths** and **areas of the cell bodies** of the different zebrafish hair cells?





Part 1: Determining the morphological and molecular structure of hair cells



Single cell RNA sequencing of hair cells

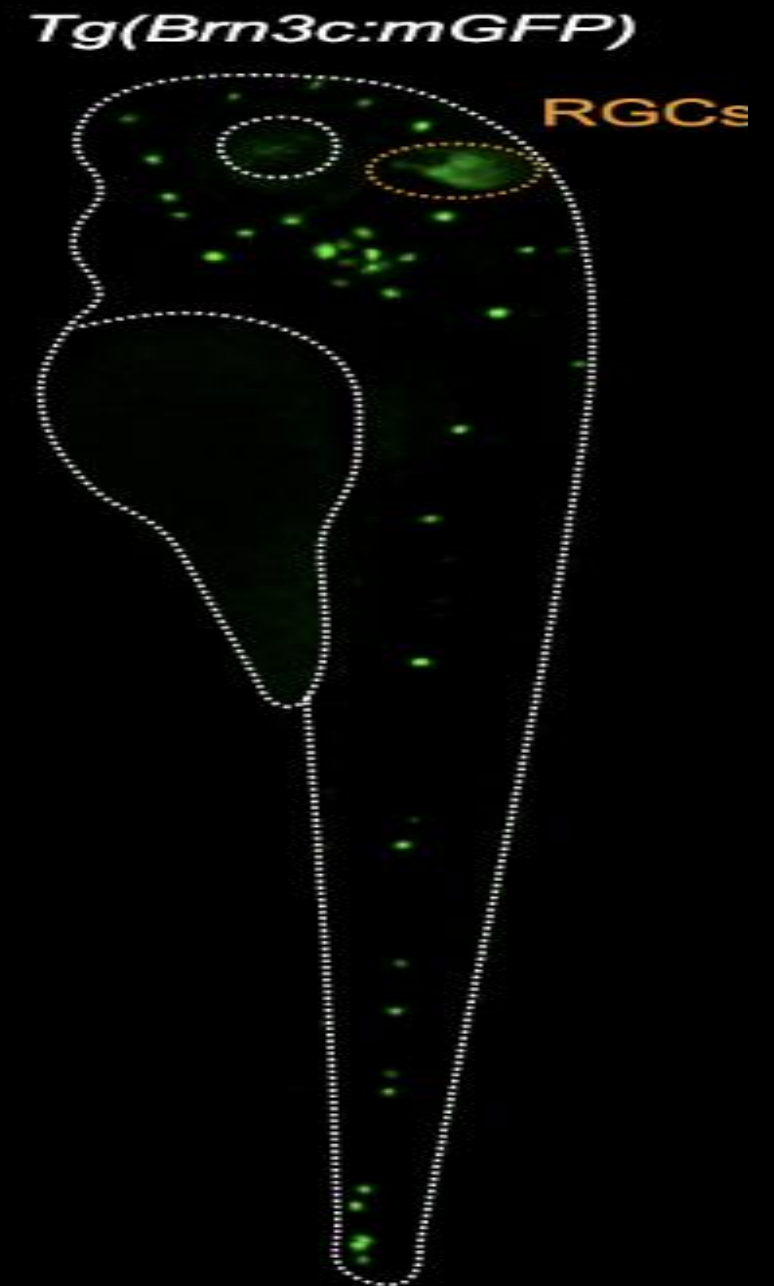
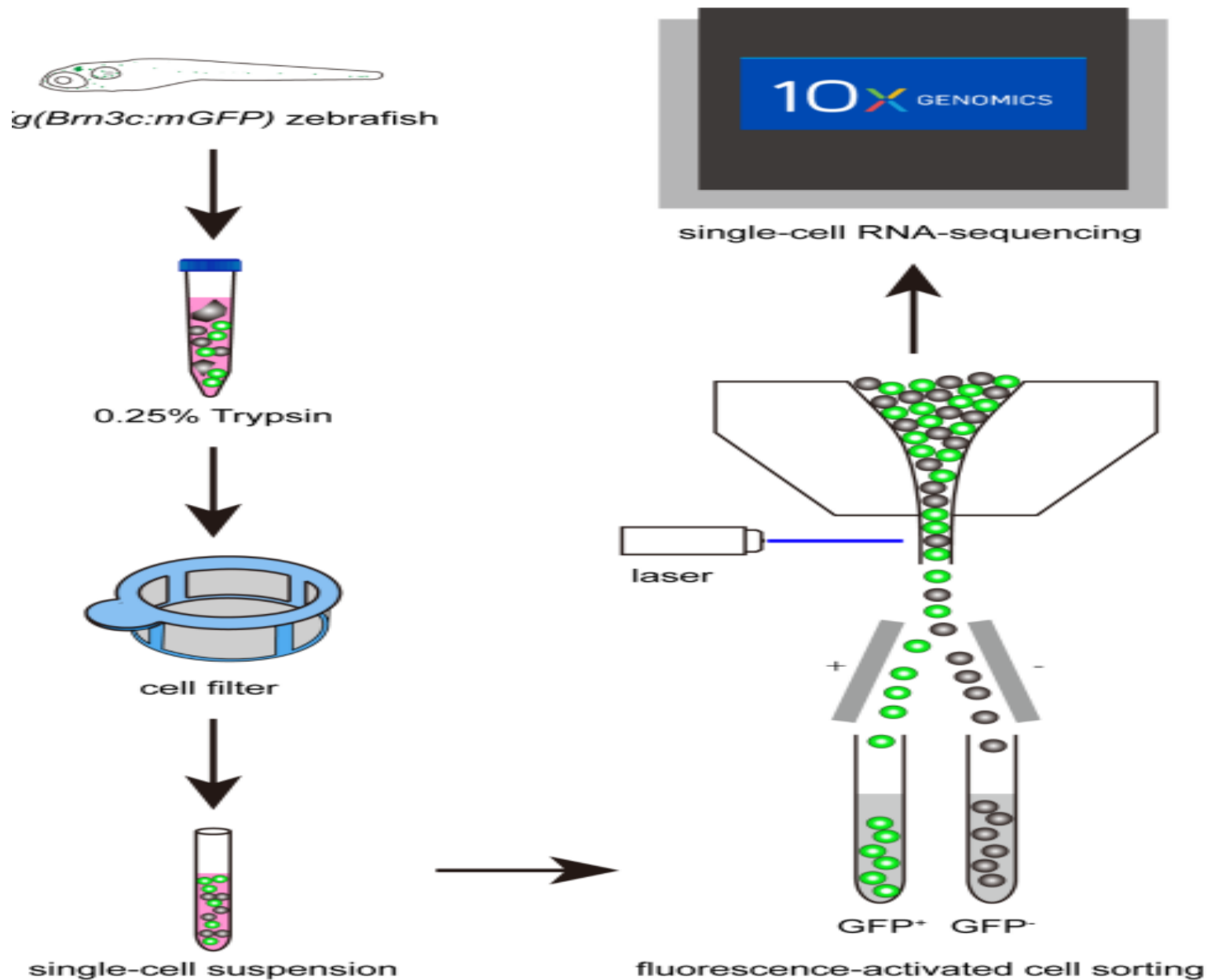
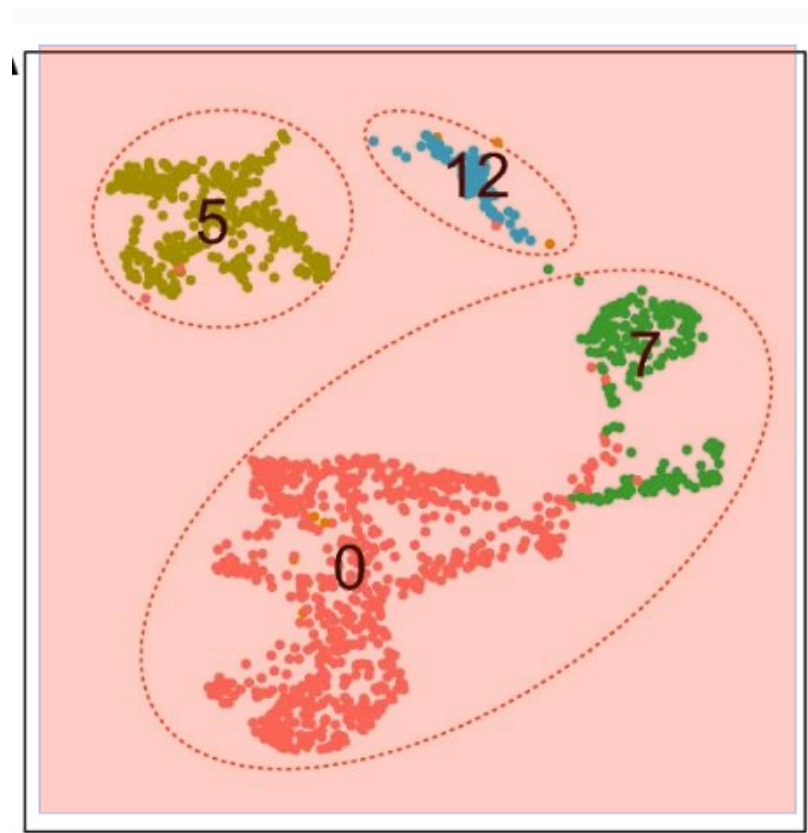
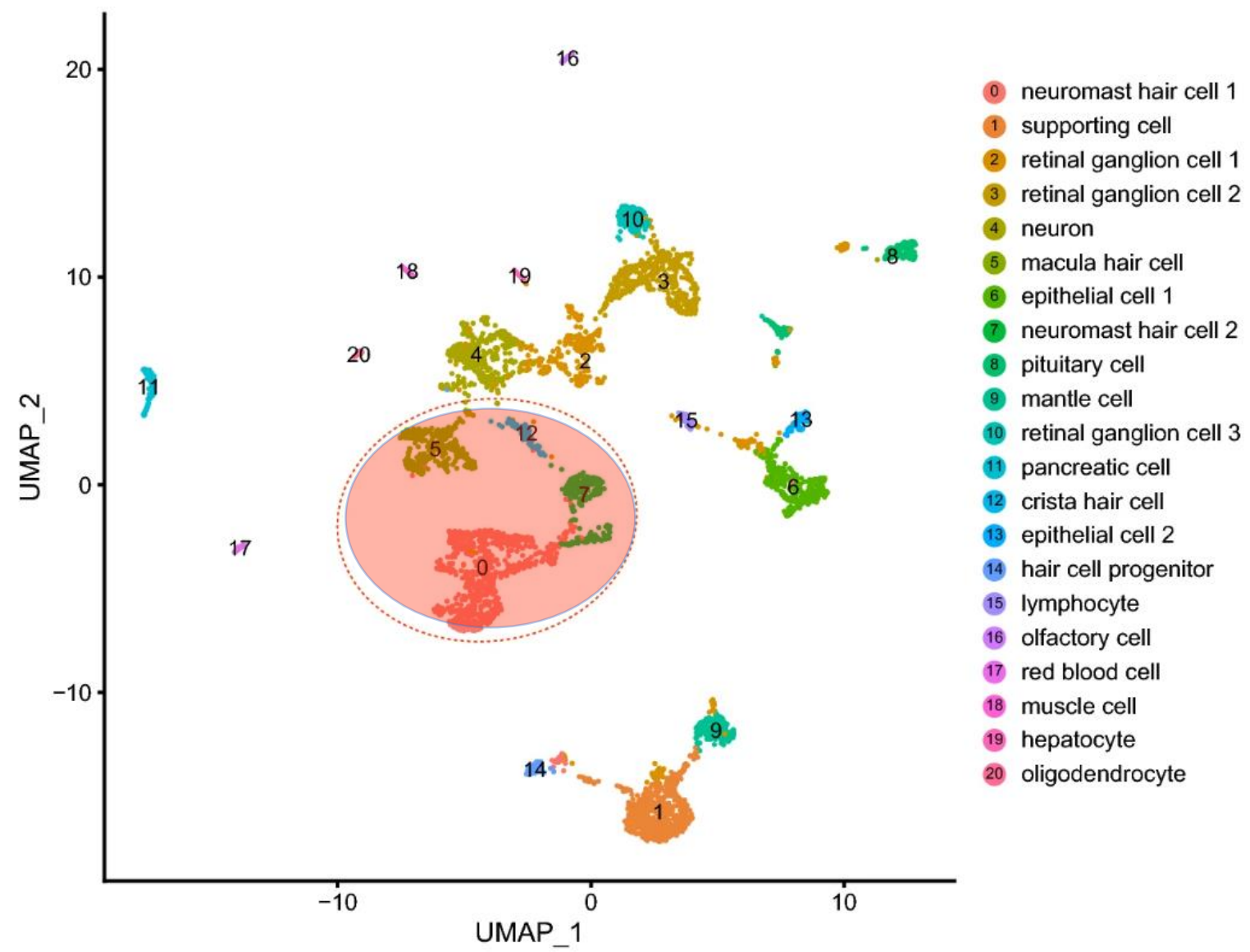


Figure 2B&3A: What are the results of the scRNA seq of zebrafish hair cells?



There are 4 clusters of hair cells

Whole mount in situ hybridization (WISH) confirmed the clusters

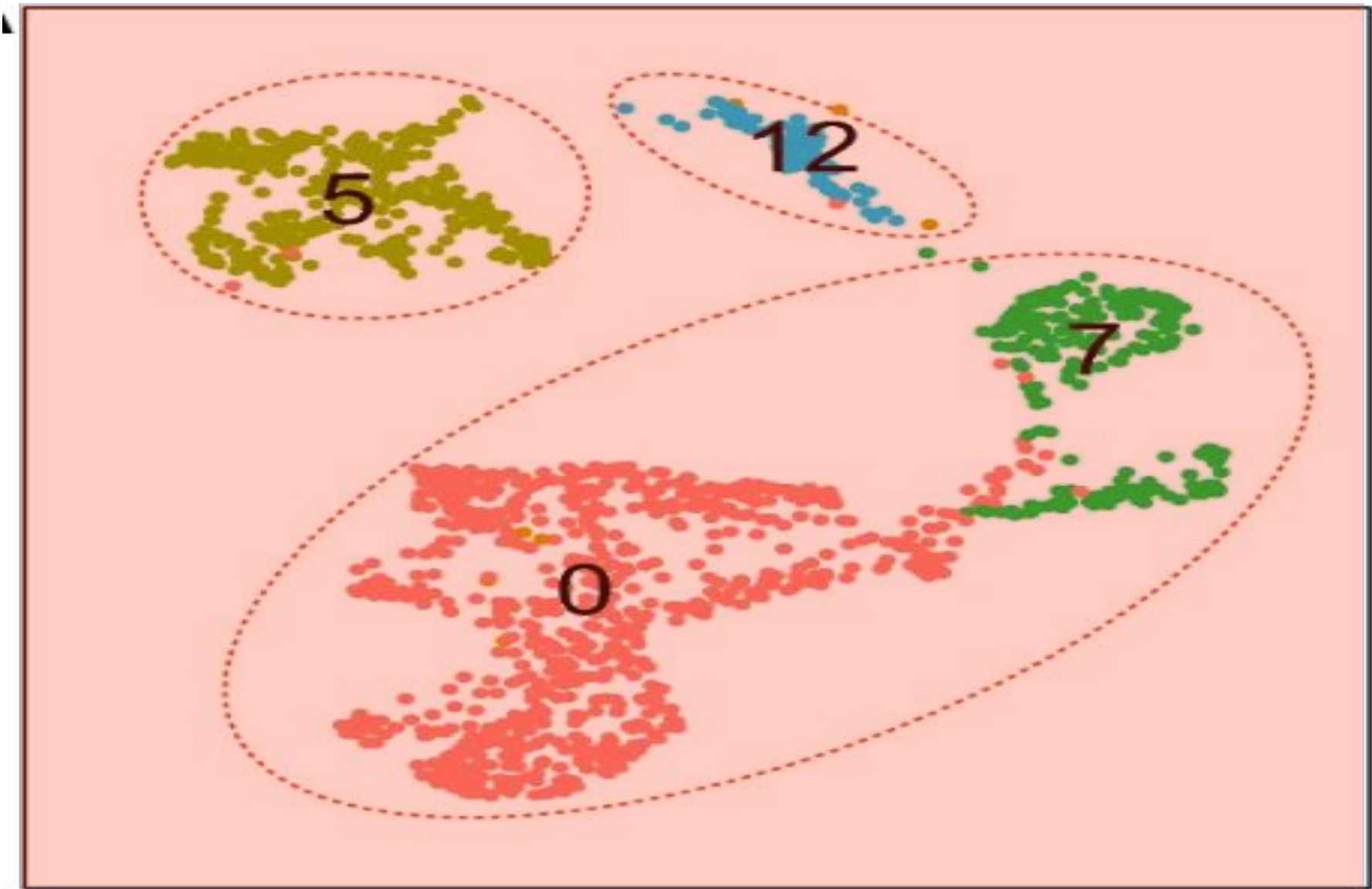


Figure 3G-I: Where do the **RNAs** associated with the specific hair cells localize in the zebrafish embryos?

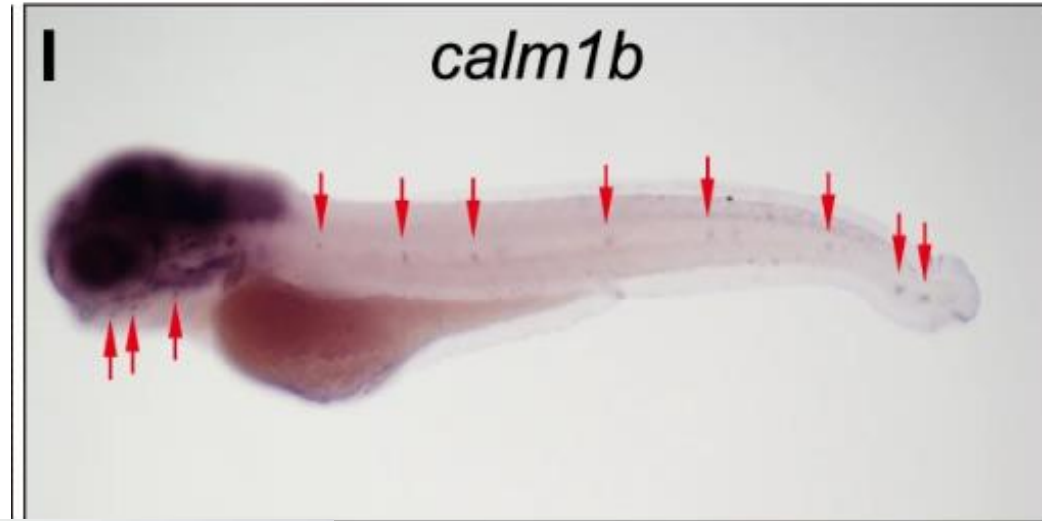
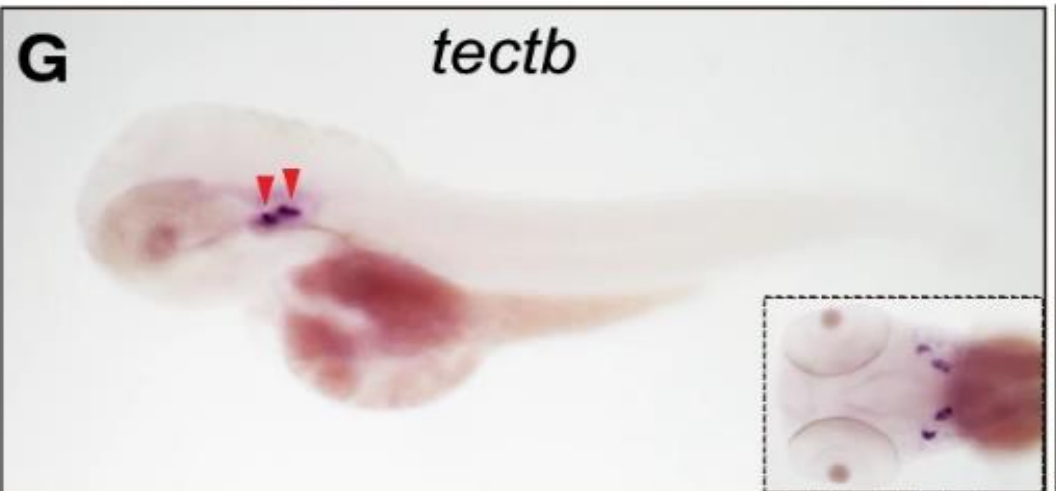


Figure 2C: How many cells were in each cluster?

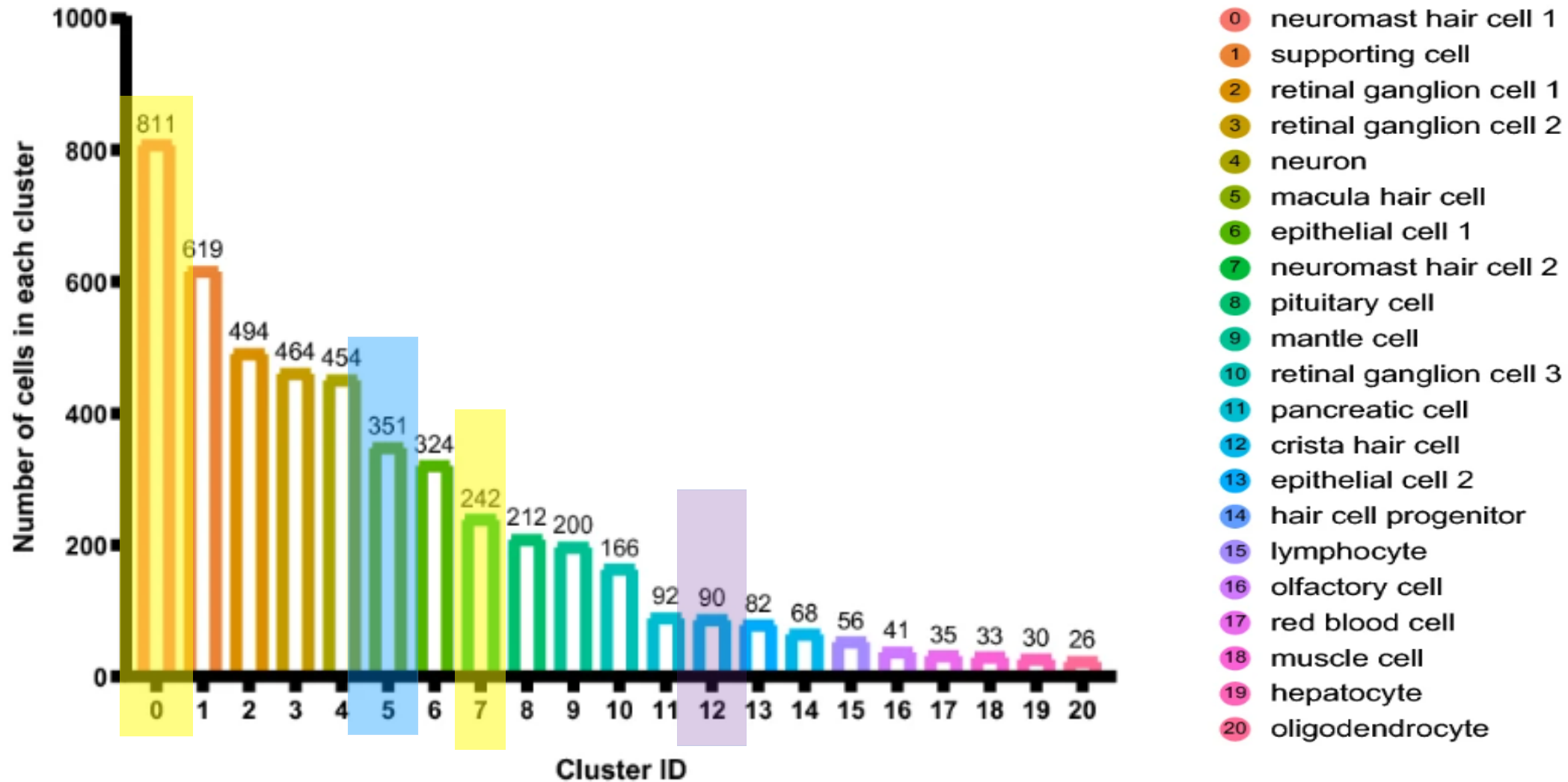


Figure 2D: How many marker genes were in each cluster?

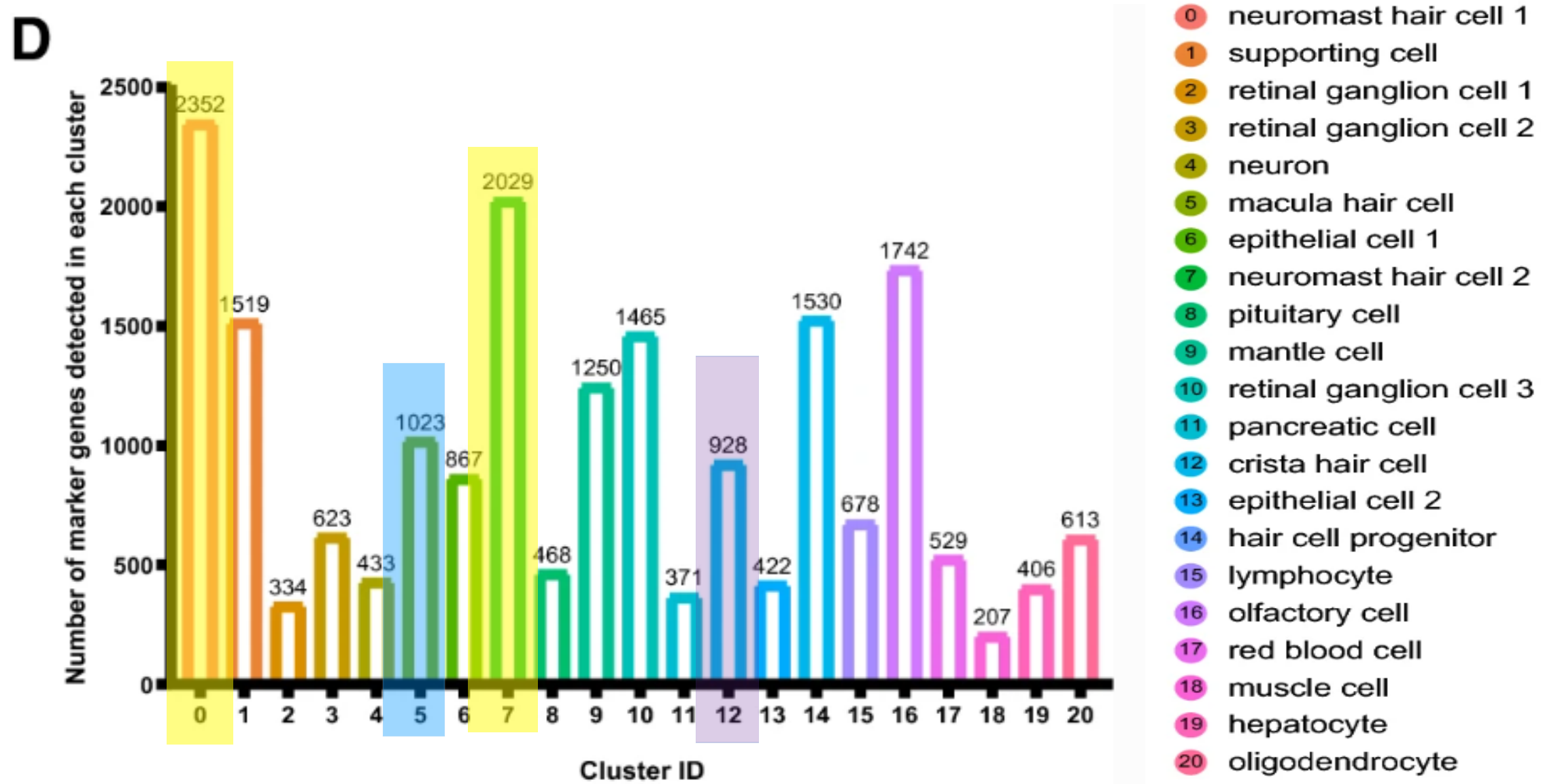
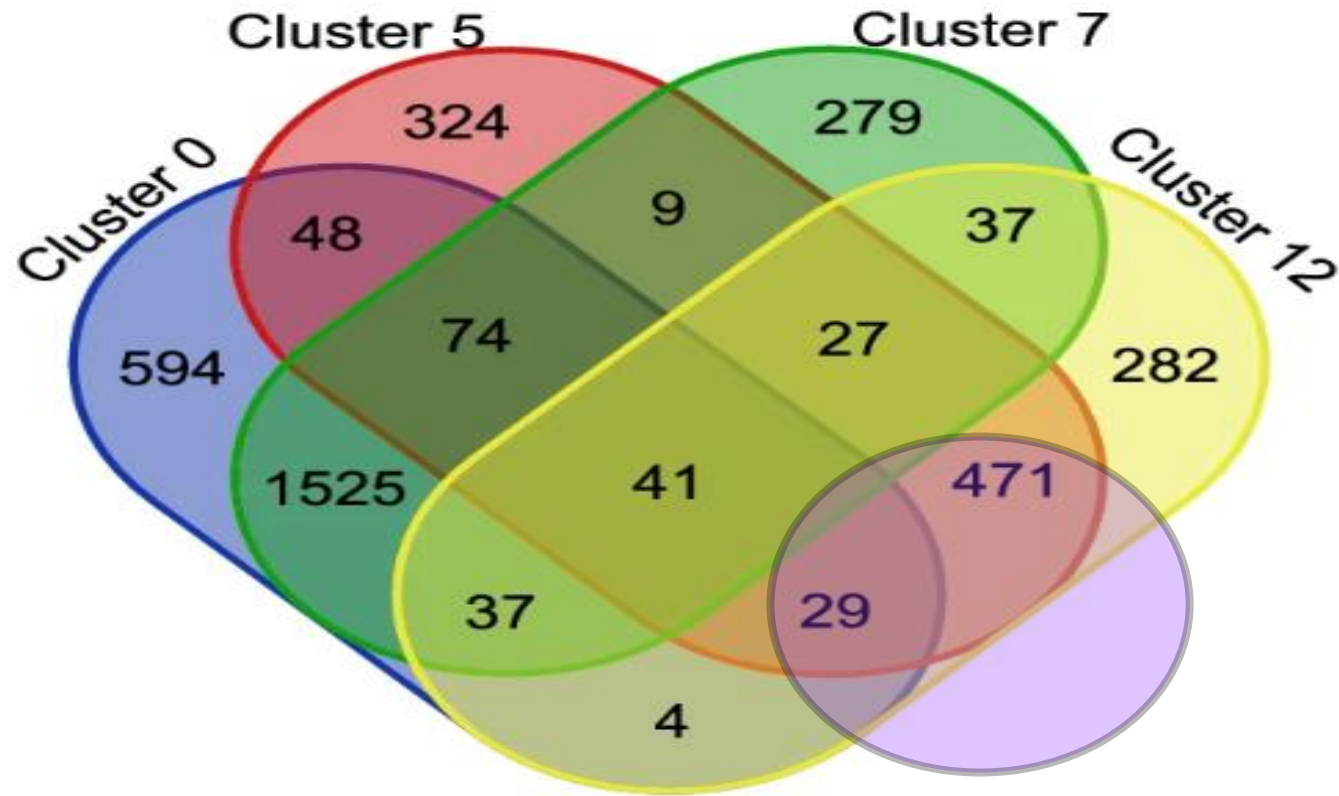


Figure 4A: How do the marker genes in the different hair types compare?

cluster 0=2352
cluster 5 = 1023
cluster 7 =2029
cluster 12 = 928



Cluster 0: mature neuromast hair cells
Cluster 5: macula hair cells
Cluster 7: young neuromast hair cells
Cluster 12: crista hair cells

Quiz: What are the three gene ontologies?

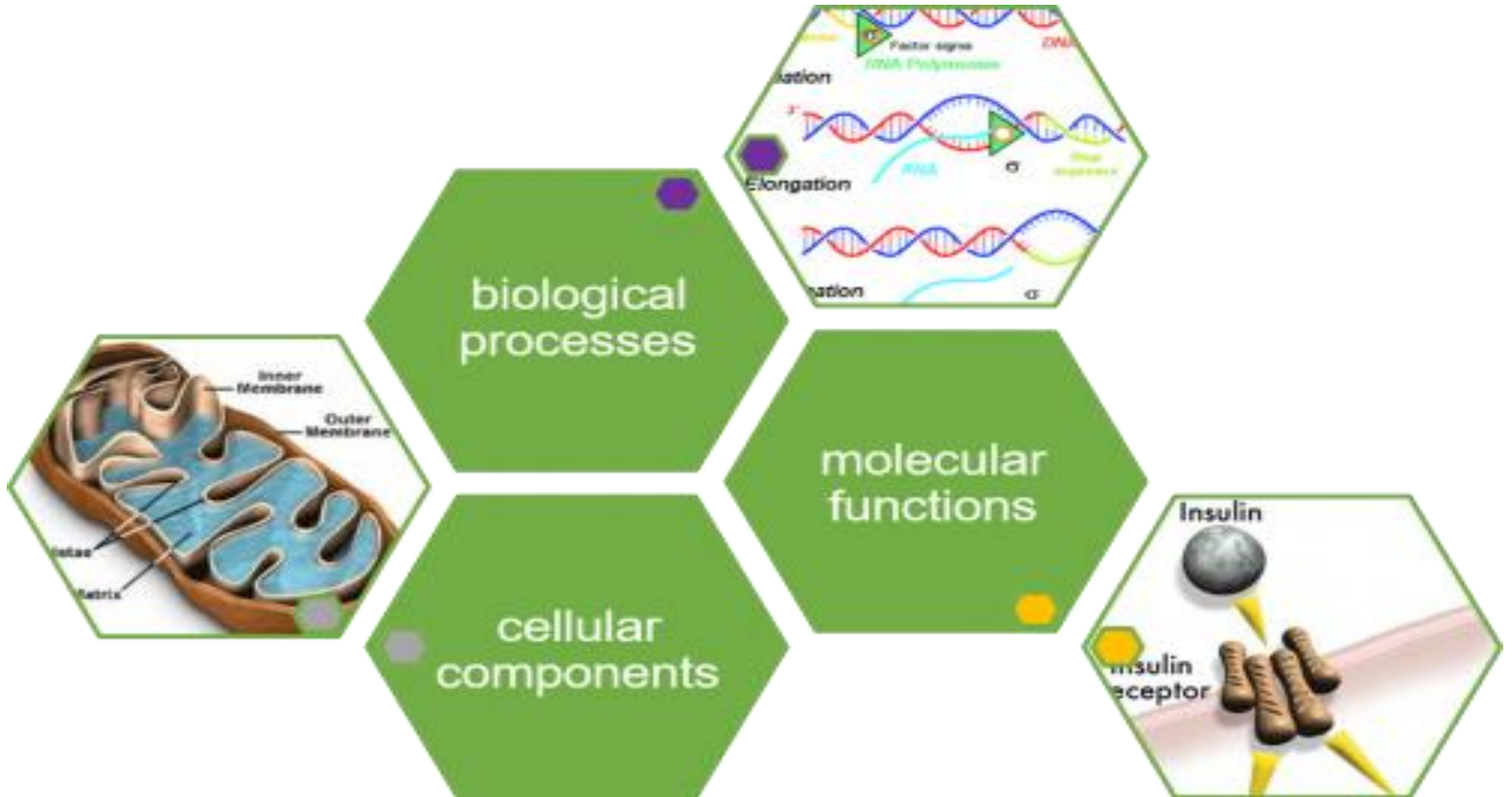


Figure 4C: What are the biological processes of the hair cells?

cluster 0=mature neuromast hair cells
cluster 5=macula hair cells
cluster 7 =young neuromast hair cells
cluster 12 = crista hair cells

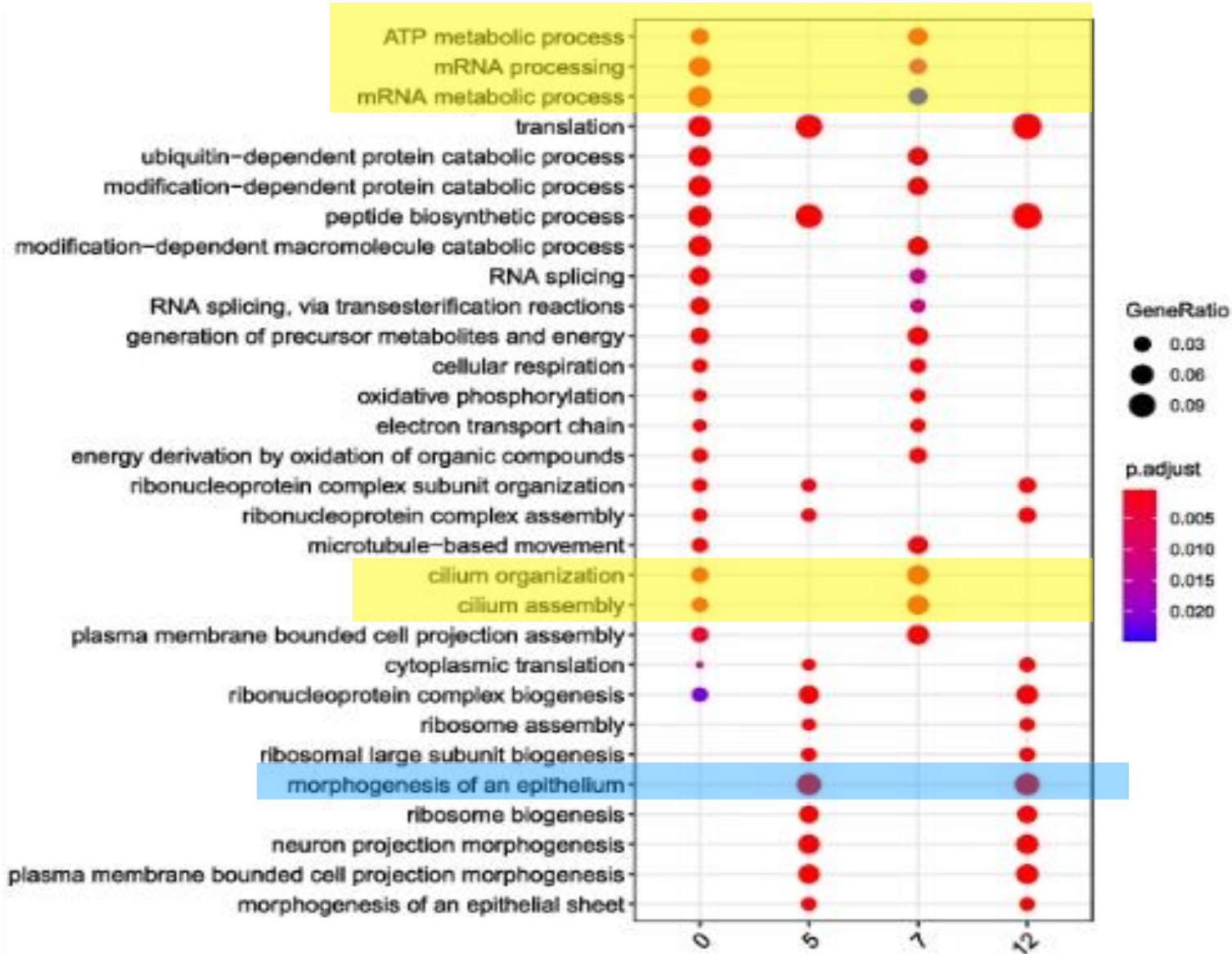


Figure 4D: What is the molecular function of the hair cells?

cluster 0=mature neuromast hair cells
cluster 5=macula hair cells
cluster 7 =young neuromast hair cells
cluster 12 = crista hair cells

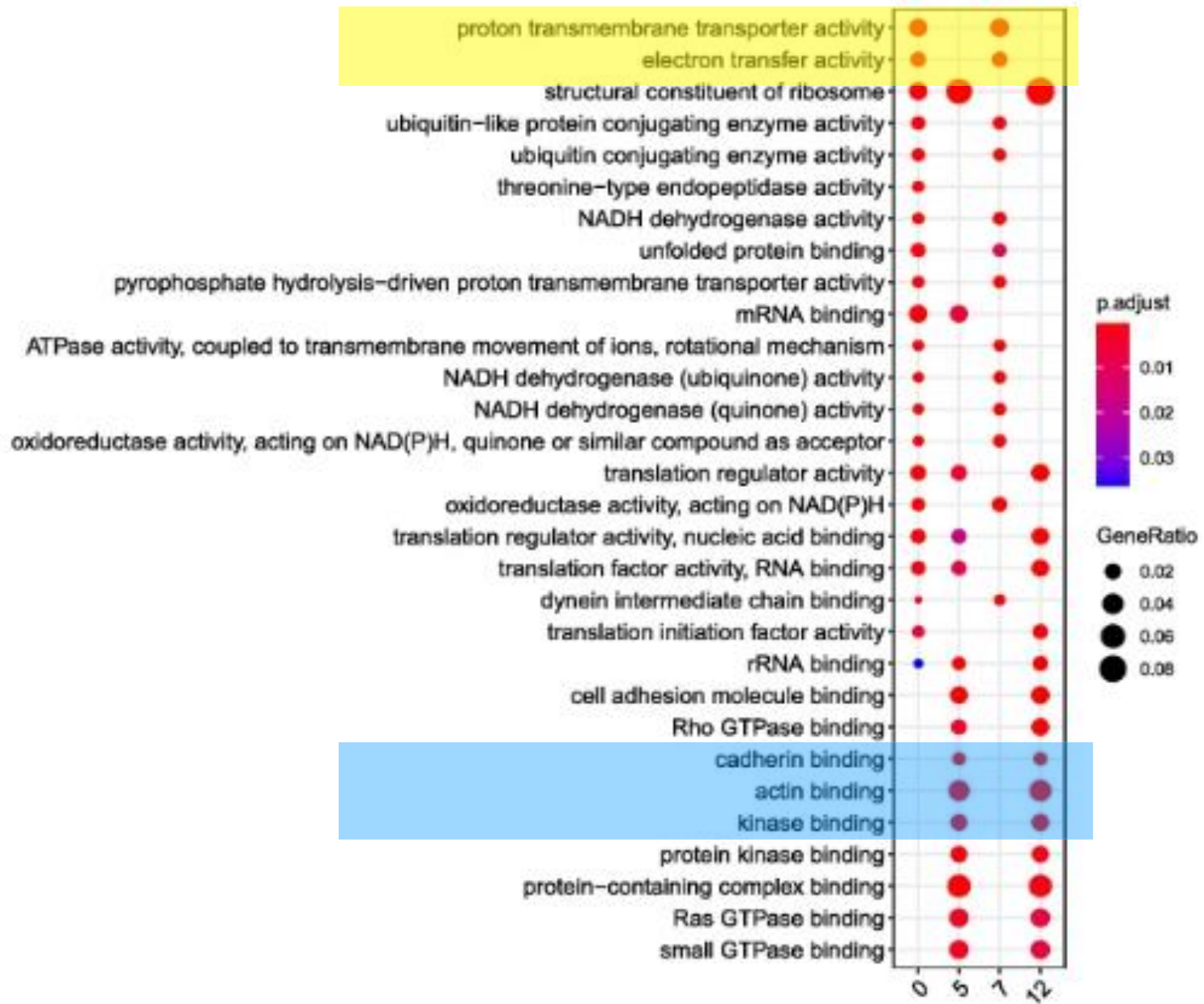
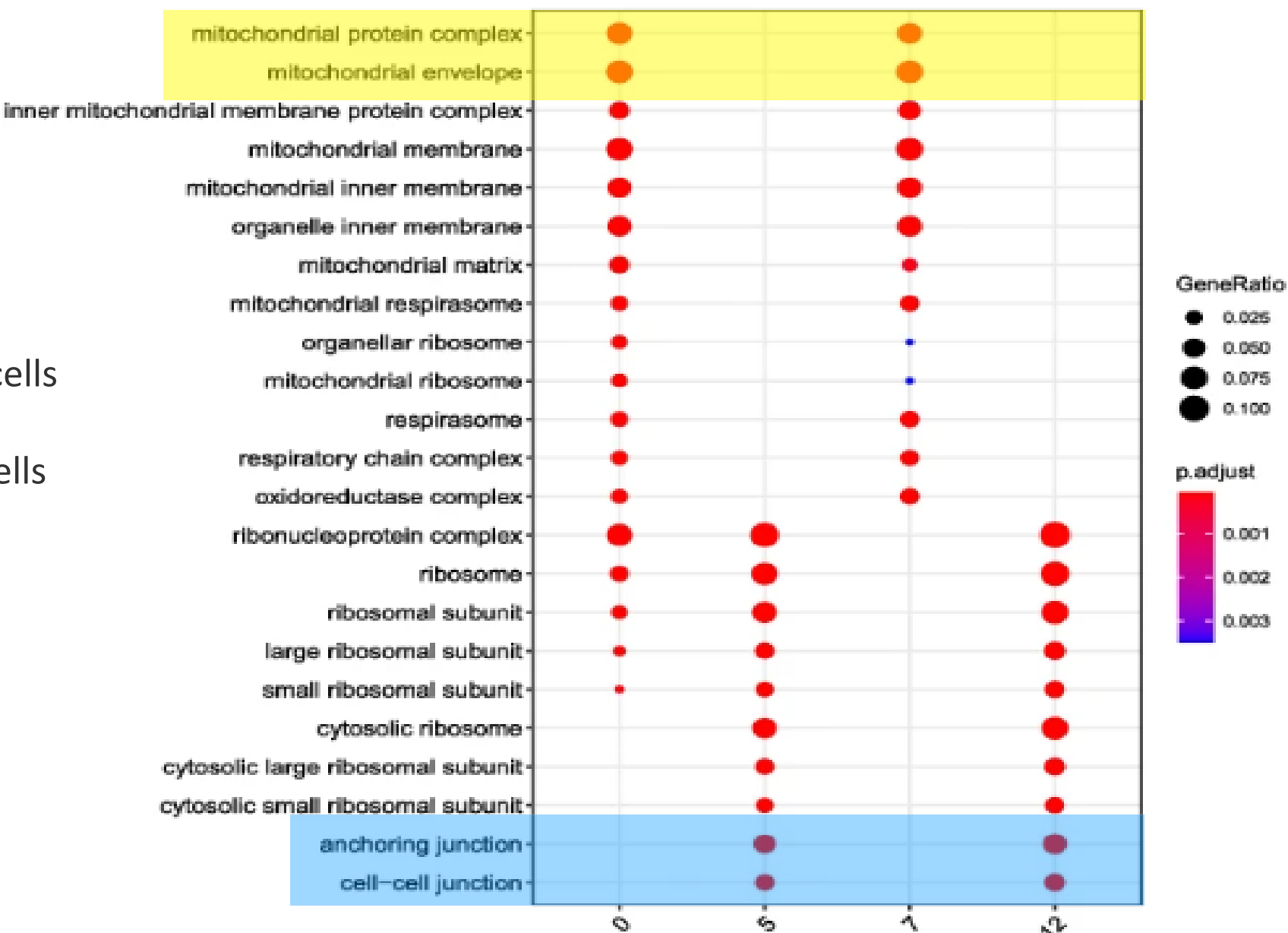
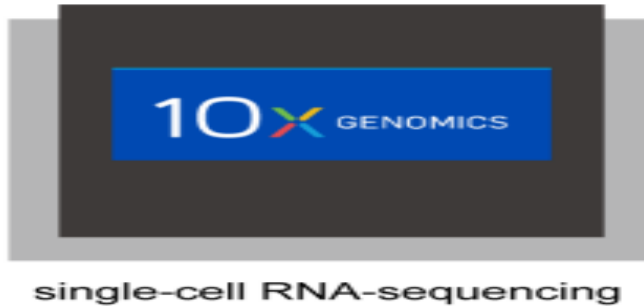


Figure 4E: What are the cellular components of the hair cells?

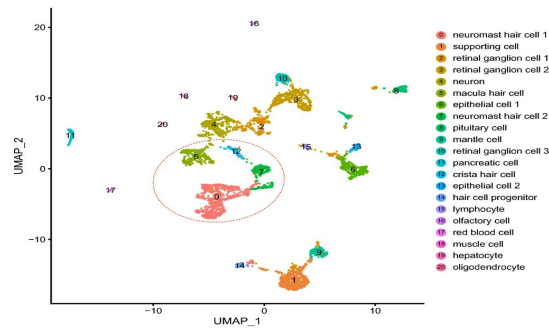
cluster 0=mature neuromast hair cells
cluster 5=macula hair cells
cluster 7 =young neuromast hair cells
cluster 12 = crista hair cells



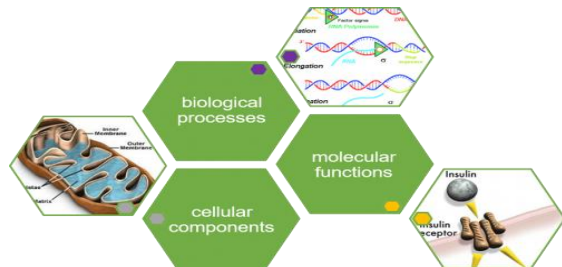
Part 1: Recap



Single cell RNA sequencing to analyze cells of mutant zebrafish Brn3C-mGFP



Clustering of specific cells using UMAP



Gene ontologies of the hair cells

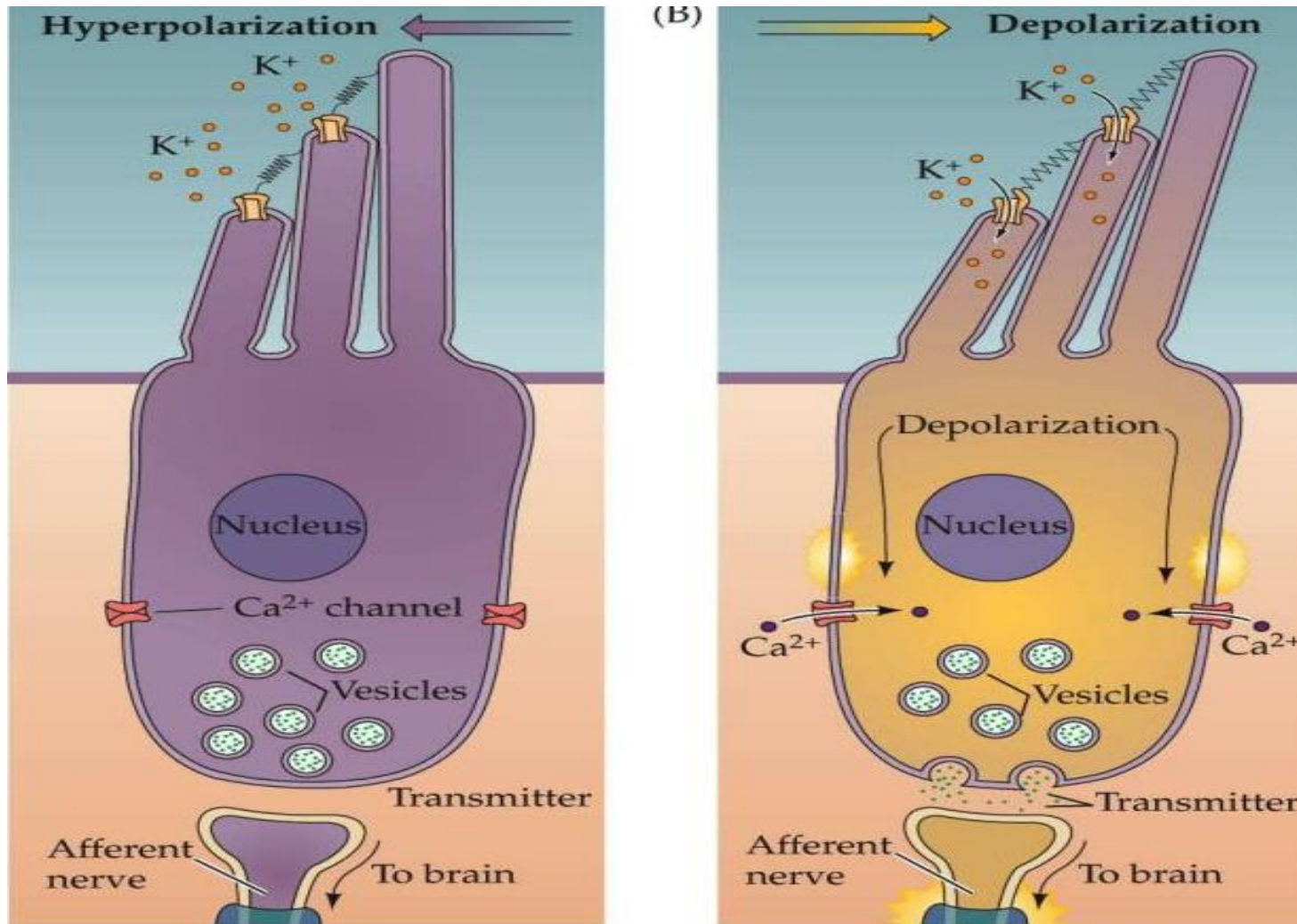


Part 2: Hearing and hearing loss

How do the hair cells transmit sounds to the brain?



What is mechanoelectrical transduction?



Hair cell depolarization sends auditory information to the brain

Figure 5G: Do the hair cells have **cdh23** for the mechanoelectrical transduction channels?

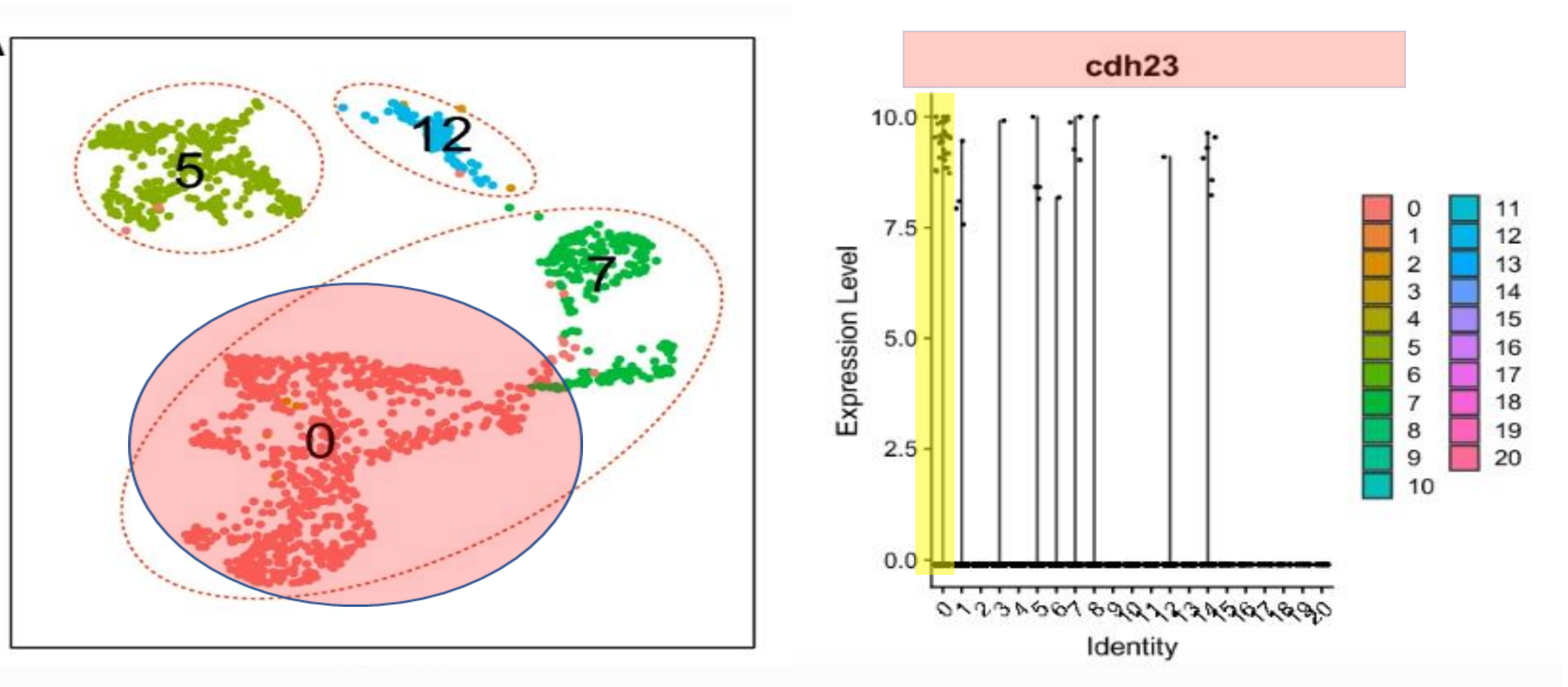


Figure 5H: Do the hair cells have *pcdh15a* for the mechanoelectrical transduction channels?

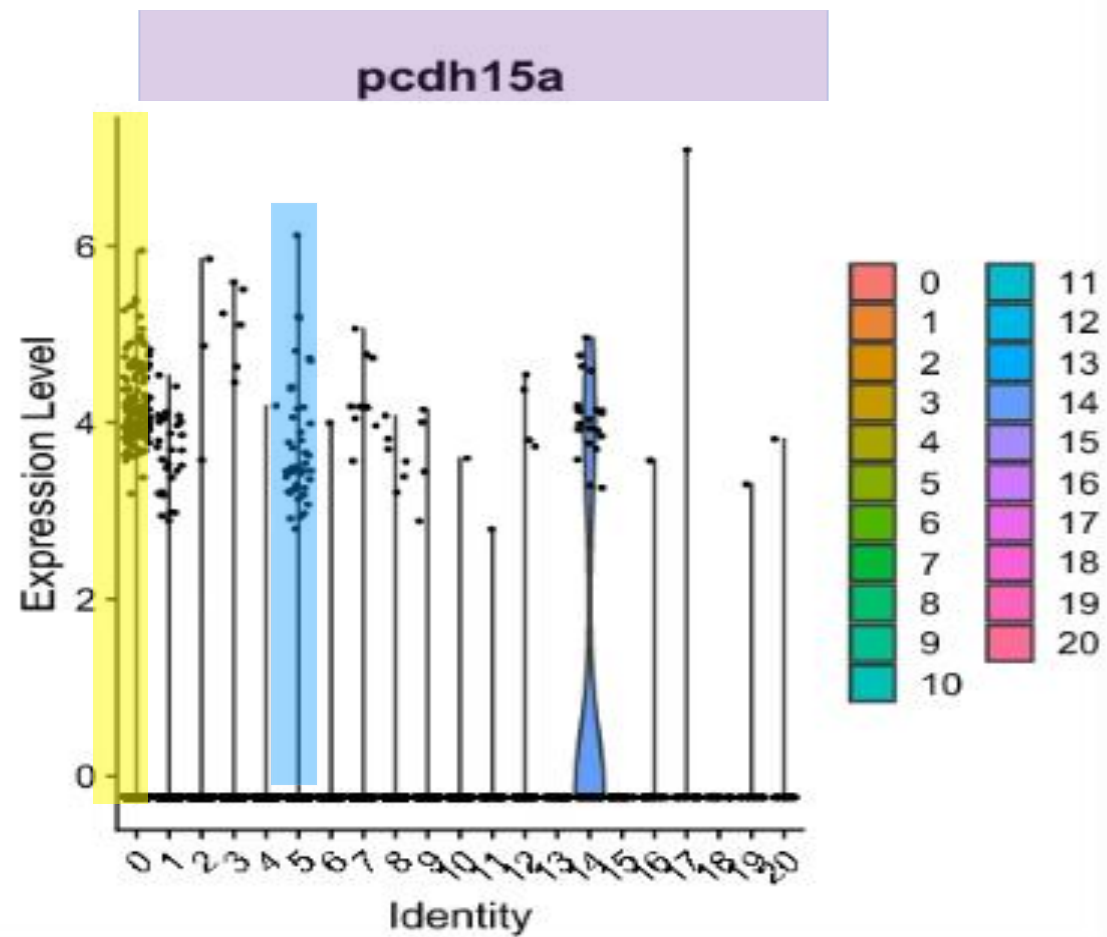
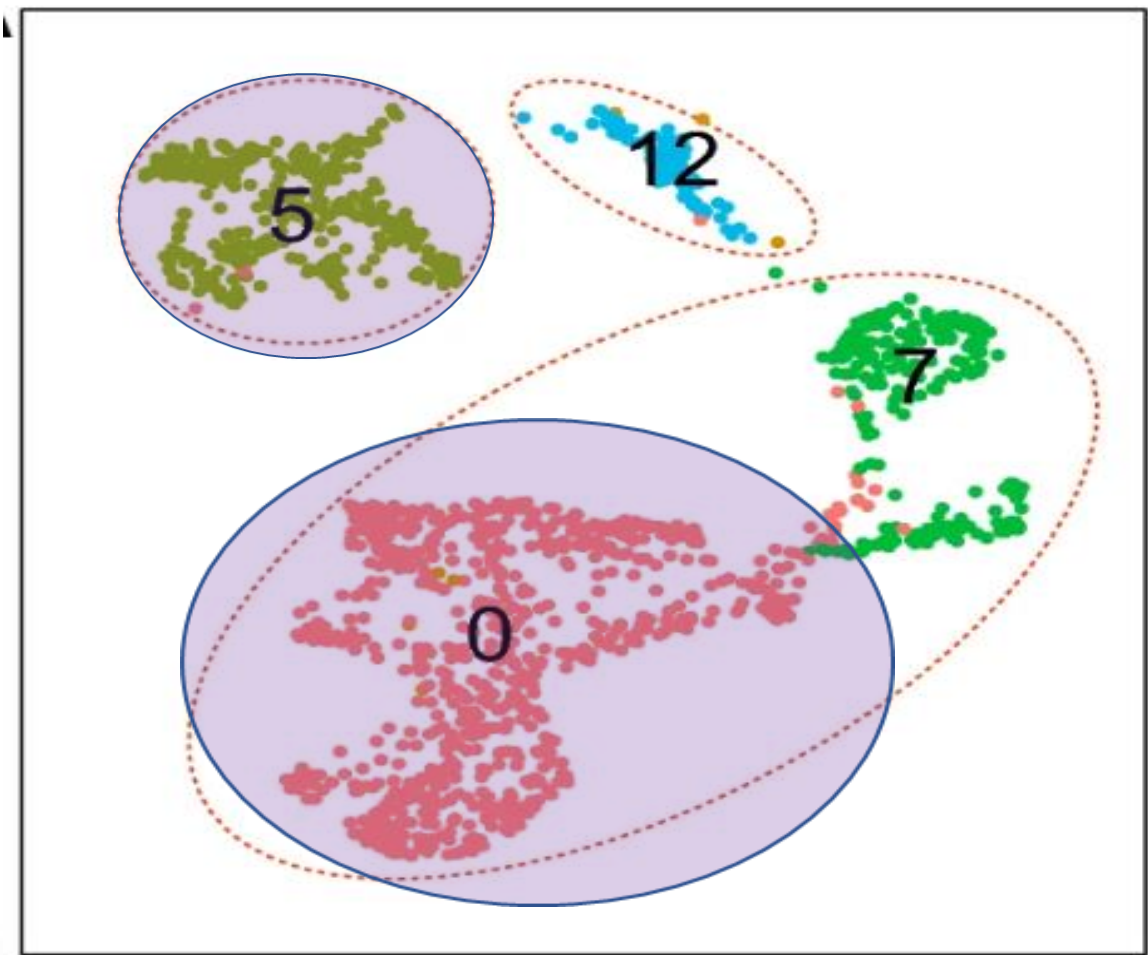


Figure 6A: What's the connection to hearing loss?

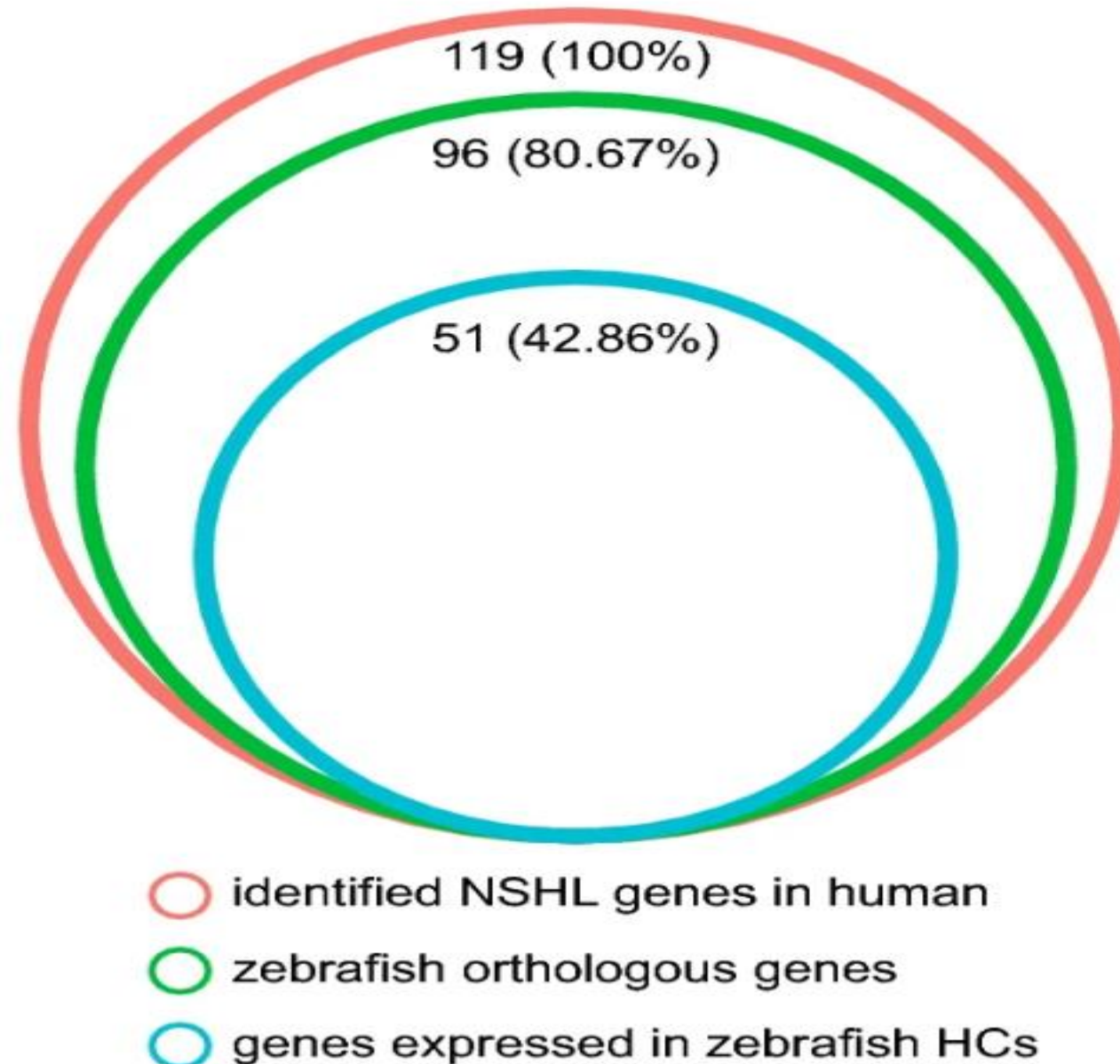


Figure 6B: What **zebrafish** genes correspond to known **human** hearing loss gene?

Human NSHL Genes	Zebrafish Orthologous Genes	UMAP-Cluster			
<i>AIFM1</i>	<i>aifm1</i>	0,7	<i>LRTOMT</i>	<i>tomt</i>	7,14
<i>CDC14A</i>	<i>cdc14aa; cdc14ab</i>	0,14; 0,7,14	<i>MARVELD2</i>	<i>marveld2a</i>	5,9
<i>CIB2</i>	<i>cib2</i>	0,7,14	<i>MET</i>	<i>met</i>	1,12
<i>CLIC5</i>	<i>clic5a; clic5b</i>	0,7; 0,7,14	<i>MSRB3</i>	<i>msrb3</i>	0,5,7,14
<i>COCH</i>	<i>coch</i>	5,12	<i>MYH14</i>	<i>myh14</i>	0,7,14
<i>COL11A1</i>	<i>col11a1a</i>	5,12	<i>MYO15A</i>	<i>myo15aa</i>	0,7,14
<i>COL11A2</i>	<i>col11a2</i>	5	<i>MYO6</i>	<i>myo6b</i>	0,7,14
<i>COL4A6</i>	<i>col4a6</i>	5,12	<i>MYO7A</i>	<i>myo7aa</i>	0,7,14
<i>DMXL2</i>	<i>dmxl2</i>	0,7,14	<i>OSBPL2</i>	<i>osbpl2b</i>	0,7
<i>EPS8L2</i>	<i>eps8l2</i>	0,14	<i>OTOF</i>	<i>otofa; otofb</i>	0,7,14
<i>ESPN</i>	<i>espn</i>	0,5,7,14	<i>OTOG</i>	<i>otog</i>	0,5,12
<i>EYA4</i>	<i>eya4</i>	0,5,7,4	<i>OTOGL</i>	<i>otogl</i>	5
<i>GAB1</i>	<i>gab1</i>	5,12	<i>PLS1</i>	<i>pls1</i>	0
<i>GIPC3</i>	<i>gipc3</i>	0,7,14	<i>POU4F3</i>	<i>pou4f3</i>	0,7,14
<i>GJB2</i>	<i>cx30.3</i>	5	<i>SIX1</i>	<i>six1a; six1b</i>	0,5,7; 0,5,7,14
<i>GJB6</i>	<i>cx30.3</i>	5	<i>SLC17A8</i>	<i>slc17a8</i>	0,7
<i>GPSM2</i>	<i>gpsm2</i>	0,1,14	<i>SLC26A5</i>	<i>slc26a5</i>	5
<i>GRXCR1</i>	<i>grxcr1a; grxcr1b</i>	0,7,14	<i>SMPX</i>	<i>smpx</i>	0,7,14
<i>KARS</i>	<i>kars1</i>	0	<i>SPNS2</i>	<i>spns2</i>	5
<i>KITLG</i>	<i>kitlga; kitlgb</i>	1,5,12; 1,12	<i>STRC</i>	<i>strc</i>	0,7
<i>LHFPL5</i>	<i>lhfp15a</i>	0,5,7	<i>TECTA</i>	<i>tecta</i>	5
<i>LOXHD1</i>	<i>loxhd1a</i>	0,7	<i>TMC1</i>	<i>TMC1</i>	0,7
			<i>TMIE</i>	<i>tmie</i>	0,7,14
			<i>TMPRSS3</i>	<i>tmprss3a</i>	0,7,14
			<i>TNC</i>	<i>tnc</i>	0,5
			<i>TRIOBP</i>	<i>triobpa; triobpb</i>	0,7,14; 0,14
			<i>USH1C</i>	<i>ush1c</i>	0,7
			<i>WBP2</i>	<i>wbp2</i>	0,7,14
			<i>WHRN</i>	<i>whrna</i>	0,7,14

Figure 6D: Both *capgb* and *mb* were the focus of the research

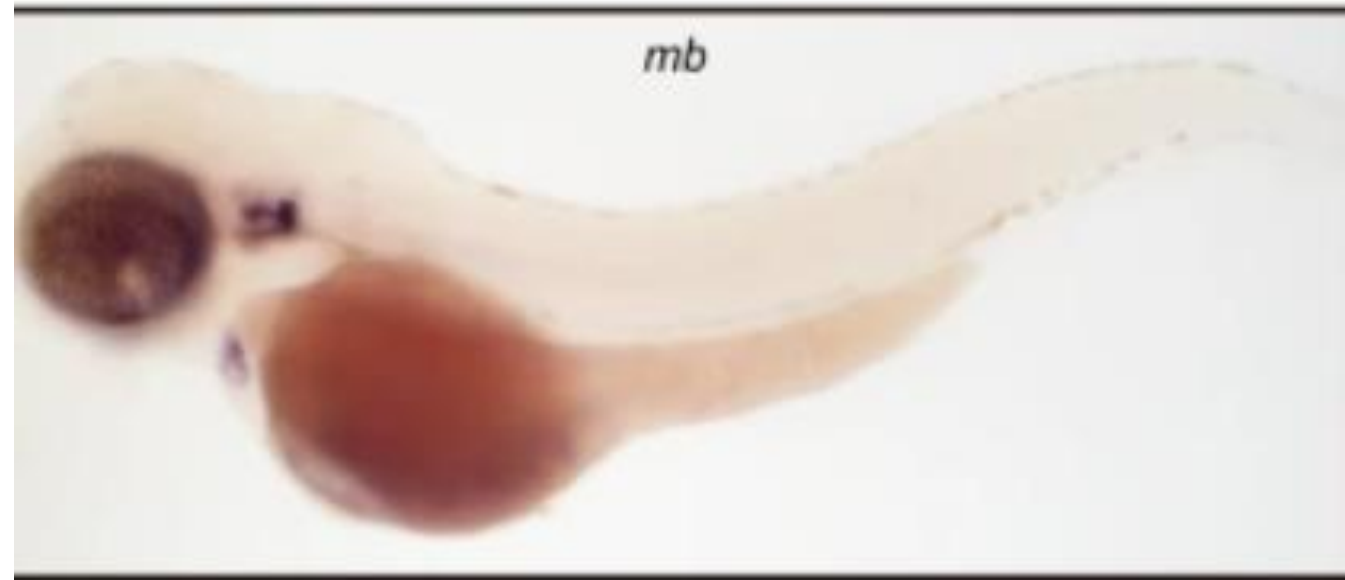


Figure 7A: What happened when the *capgb* gene was knocked down?

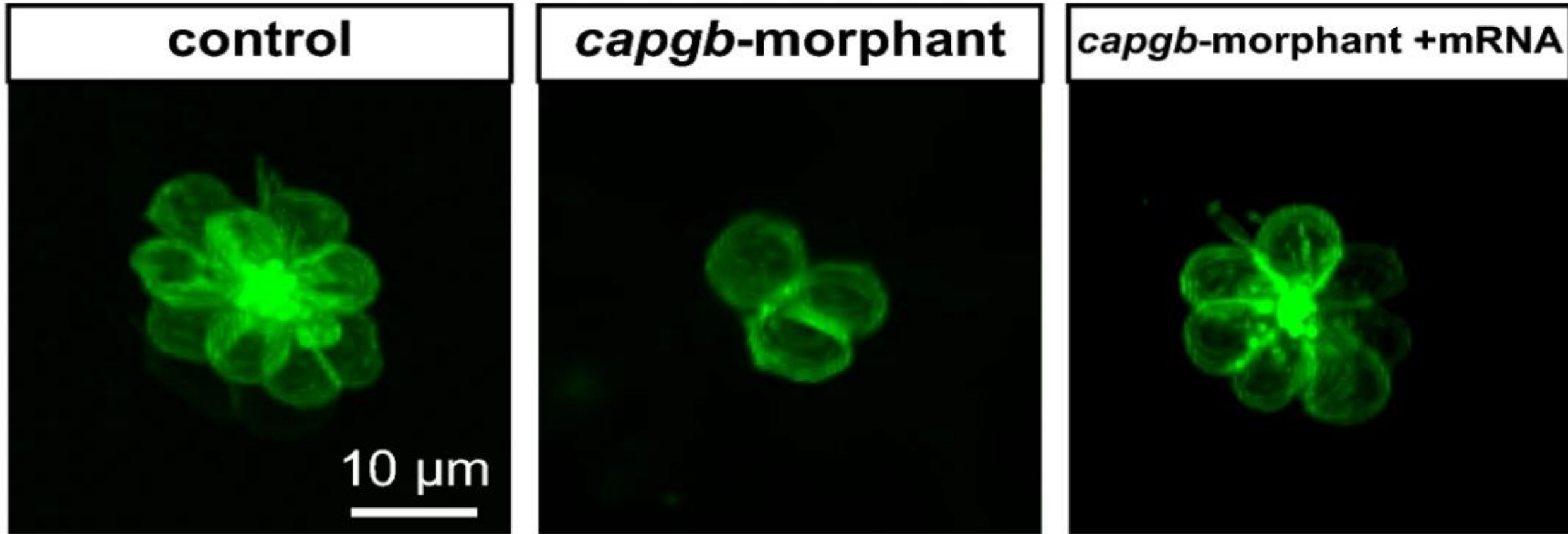


Figure 7B: How did the Capgb knockdown affect the number of neuromast hair cells?

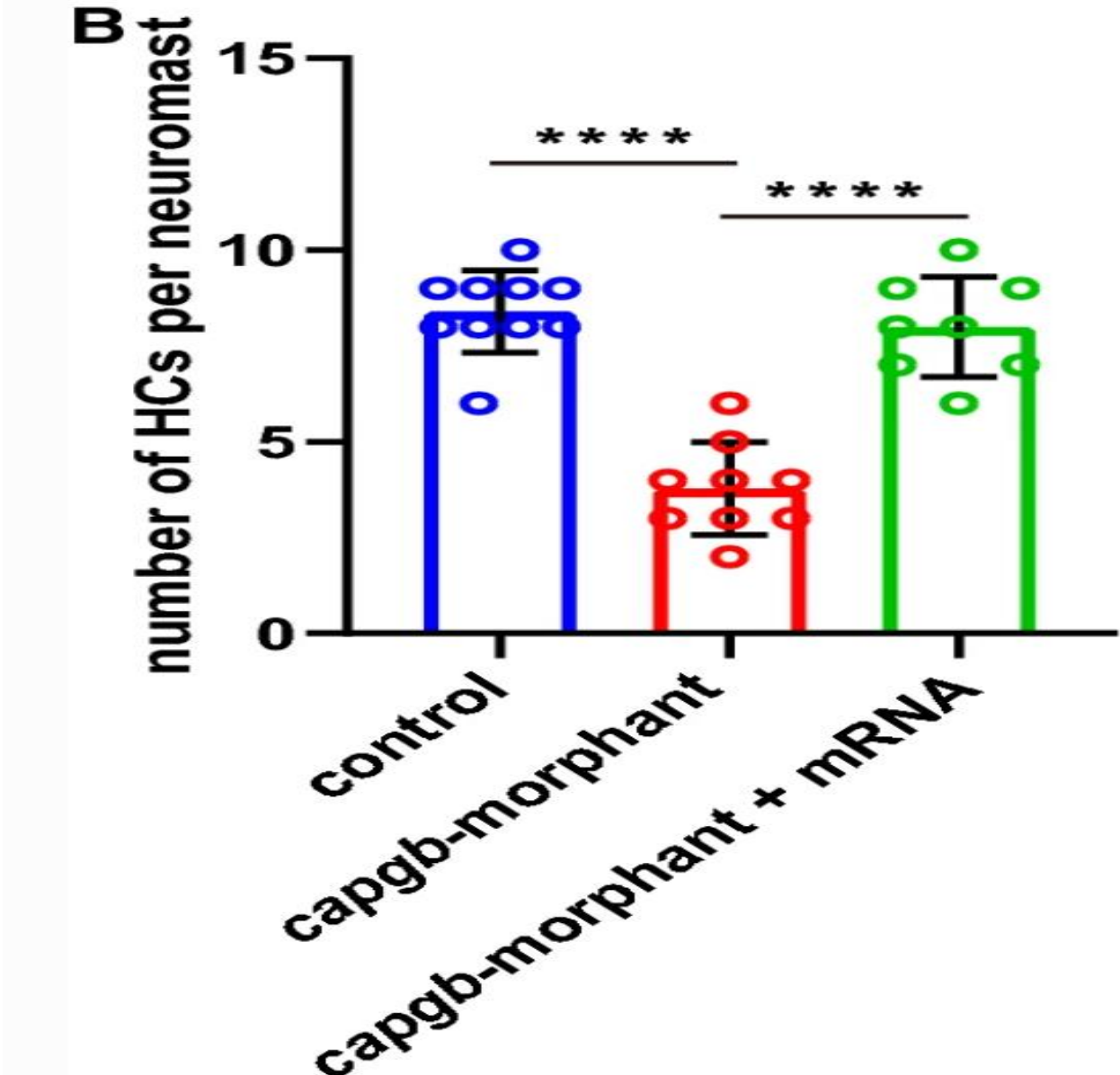


Figure 7C-D: How did the *capgb*-morphants respond to acoustic stimuli?

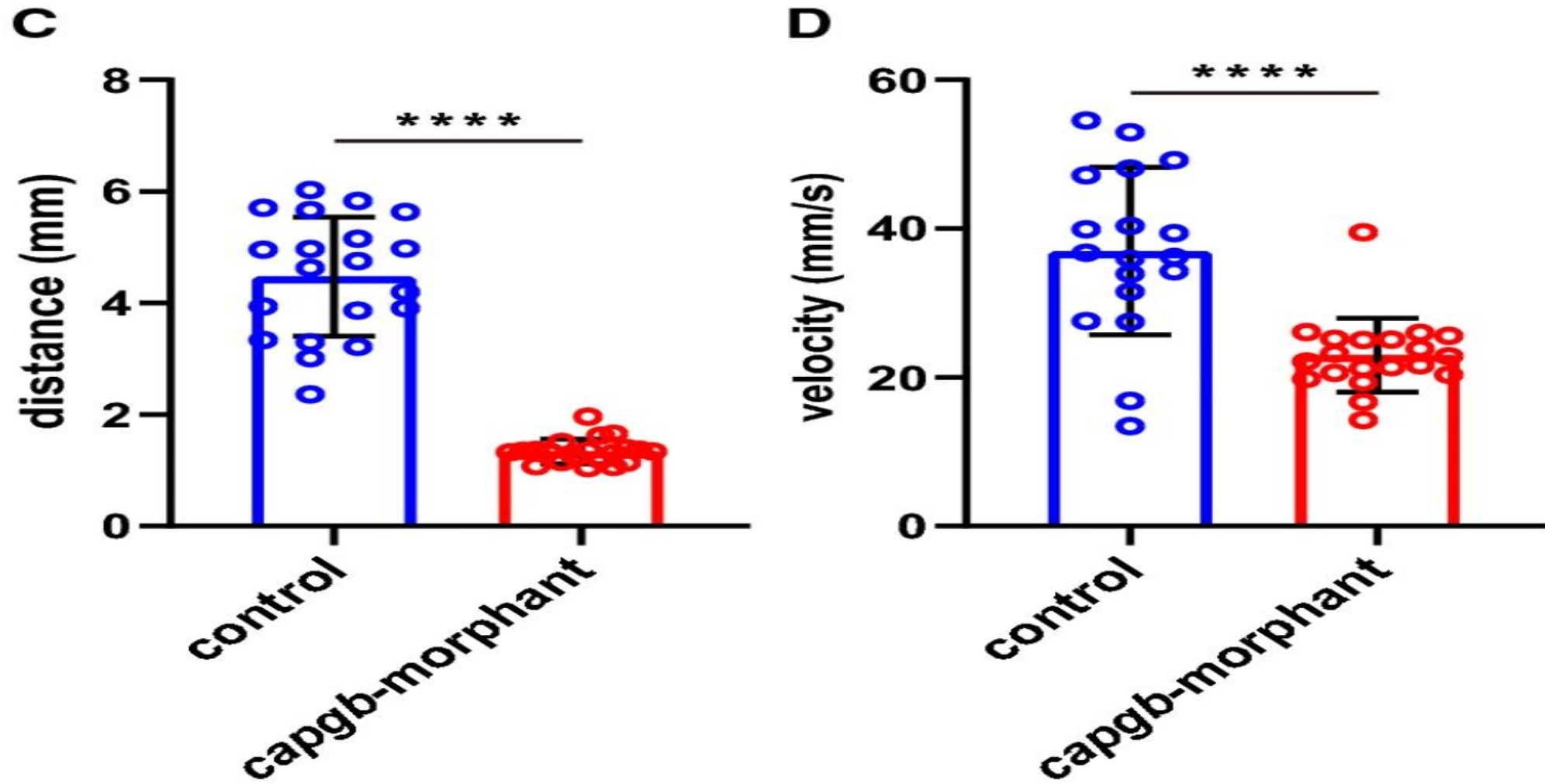


Figure 7E: What happened when the *mb* gene was knocked down?

control

***mb*-morphant**

***mb*-morphant + mRNA**

10 μm




Figure 7F: How did the Mb gene knockdown affect the number of macula hair cells?

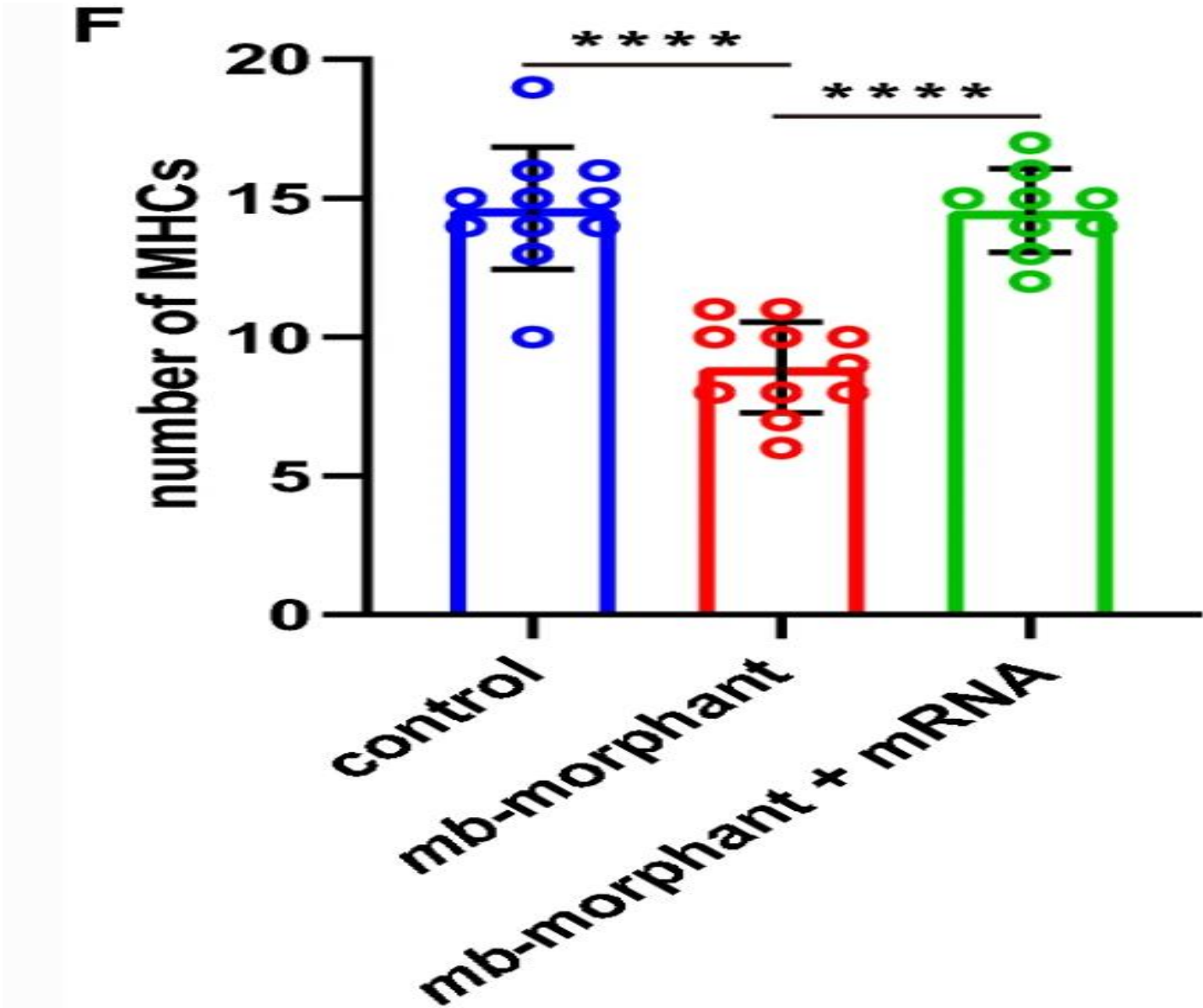
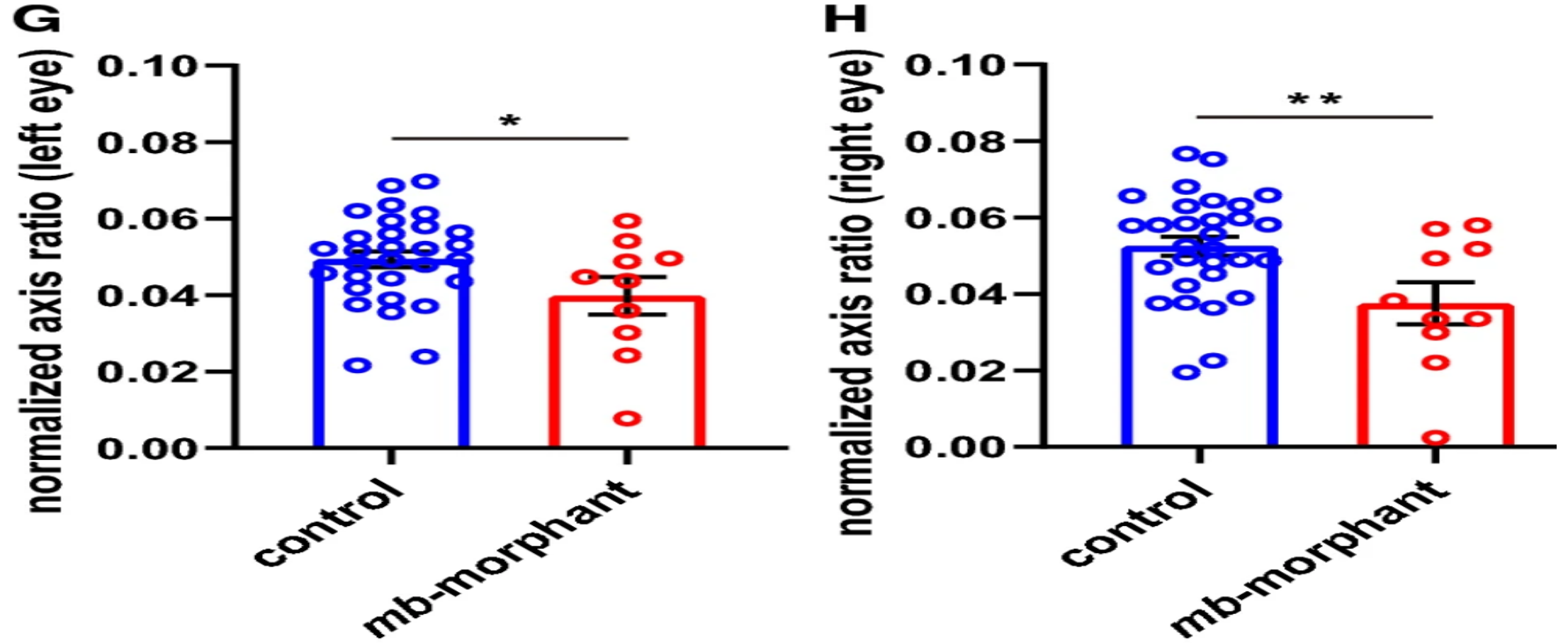
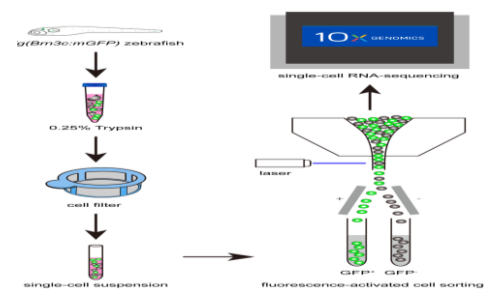


Figure 7G-H: How did the *mb*-morphants respond to vestibulo-ocular reflex test?



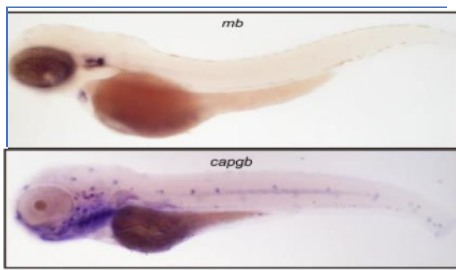
Summary



Using scRNA seq and gene ontologies to distinguish macula, crista, and neuromast hair cells

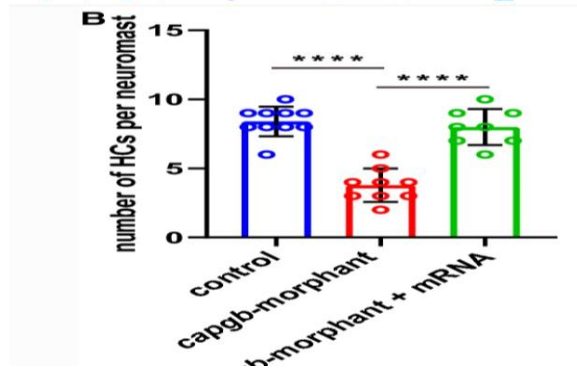
Human NSHL Genes	Zebrafish Orthologous Genes	UMAP-Cluster
AIFM1	atf1	0,7
CDC14A	cdc14aa; cdc14ab	0,14; 0,7,14
CIE2	cb2	0,7,14
CLIC5	clic5a; clic5b	0,7; 0,7,14
COCH	coch	5,12
COL11A1	col11a1a	5,12
COL11A2	col11a2	5
COL4A6	col4a6	5,12
DMXL2	dmx2	0,7,14
EPSSB2	epsb2	0,14
ESPN	espn	0,5,7,14
EYA4	eya4	0,5,7,4
GAB1	gab1	5,12
GPC3	gpc3	0,7,14
GJB2	cx30.3	5
GJB6	cx30.3	5
GPRM2	gpram2	0,1,14
GRXCR1	grxcr1a; grxcr1b	0,7,14
KARS	kars1	0
KITLG	kitlg; kitlgb	1,5,12; 1,12
LHFPL5	lhfpl5a	0,5,7
LOXHD1	loxhd1a	0,7

Genes/orthologs associated with hearing loss



Capgb and Mb gene knockdown relation to hearing and balance

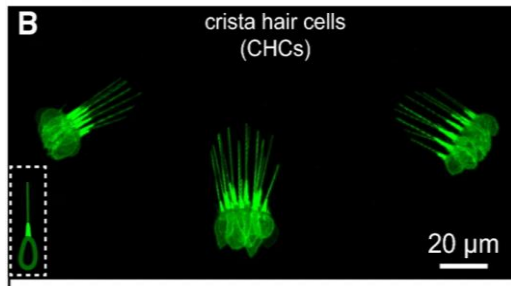
What are the future directions for this research?



What is capgb's role in hair development?



What other roles does the mb gene play in hearing loss?



How are crista hair cells involved in hearing loss?

Any More Questions?



References: Part 1

Papers

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Images

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