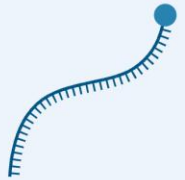
Single-cell RNA sequencing & Hearing Loss

Julia Carey & Michelle Conte

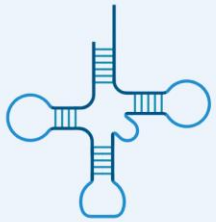
What is the transcriptome?

mRNA



Encodes proteins

tRNA

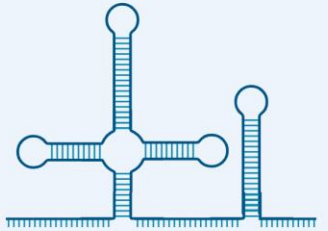


Acts as adaptor between mRNA and amino acids

rRNA

Forms the ribosome

snRNA

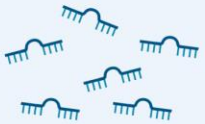


Functions in various nuclear processes (e.g. splicing)

snoRNA

Facilitates chemical modification of RNAs

miRNA

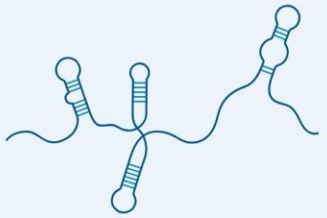


Regulates gene expression

siRNA

Silences gene expression

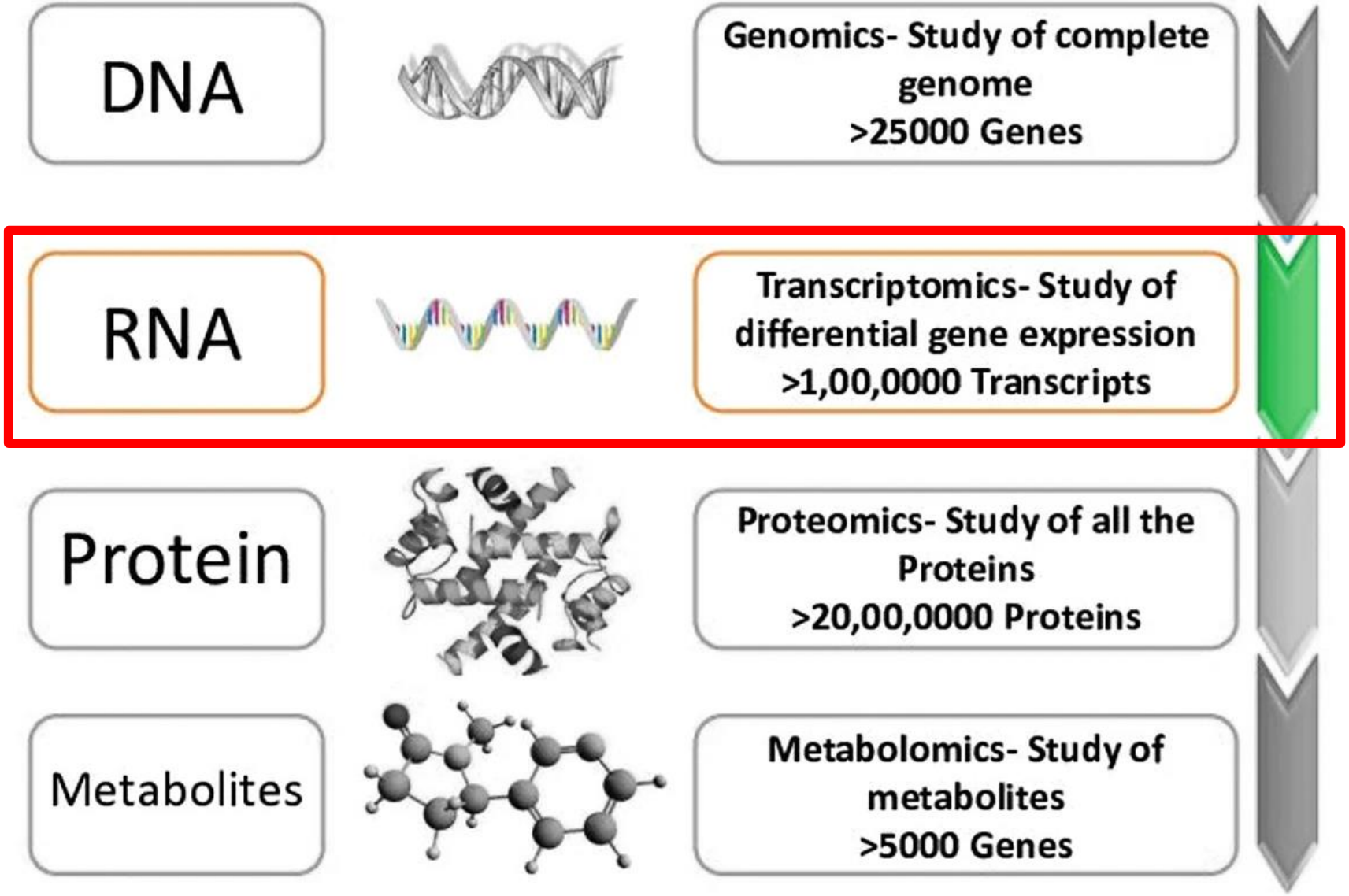
lncRNA



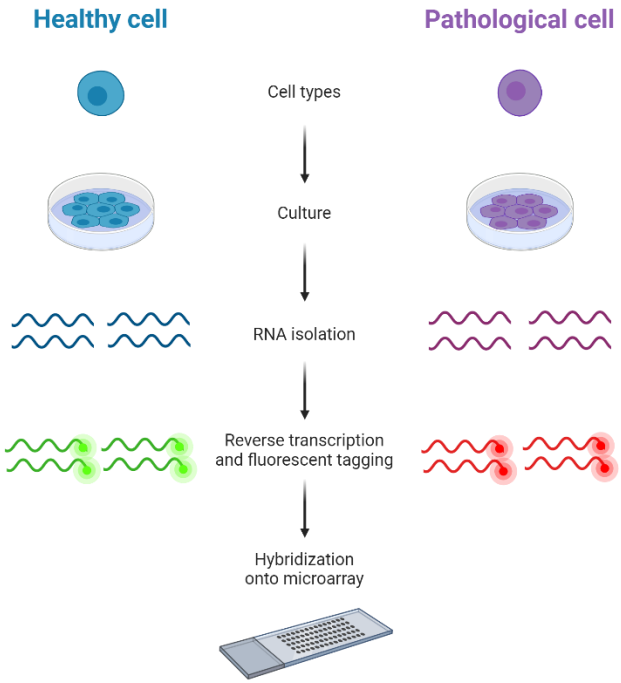
Regulates gene expression

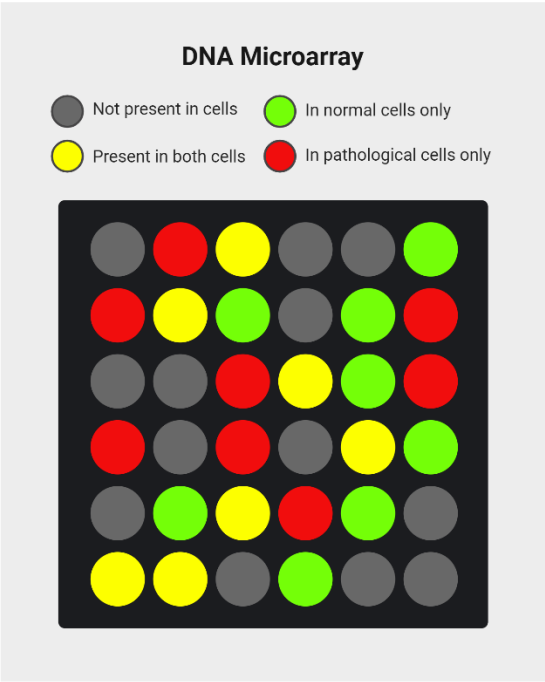
Set of all RNA within a cell

Transcriptomics is the study of RNA in a cell

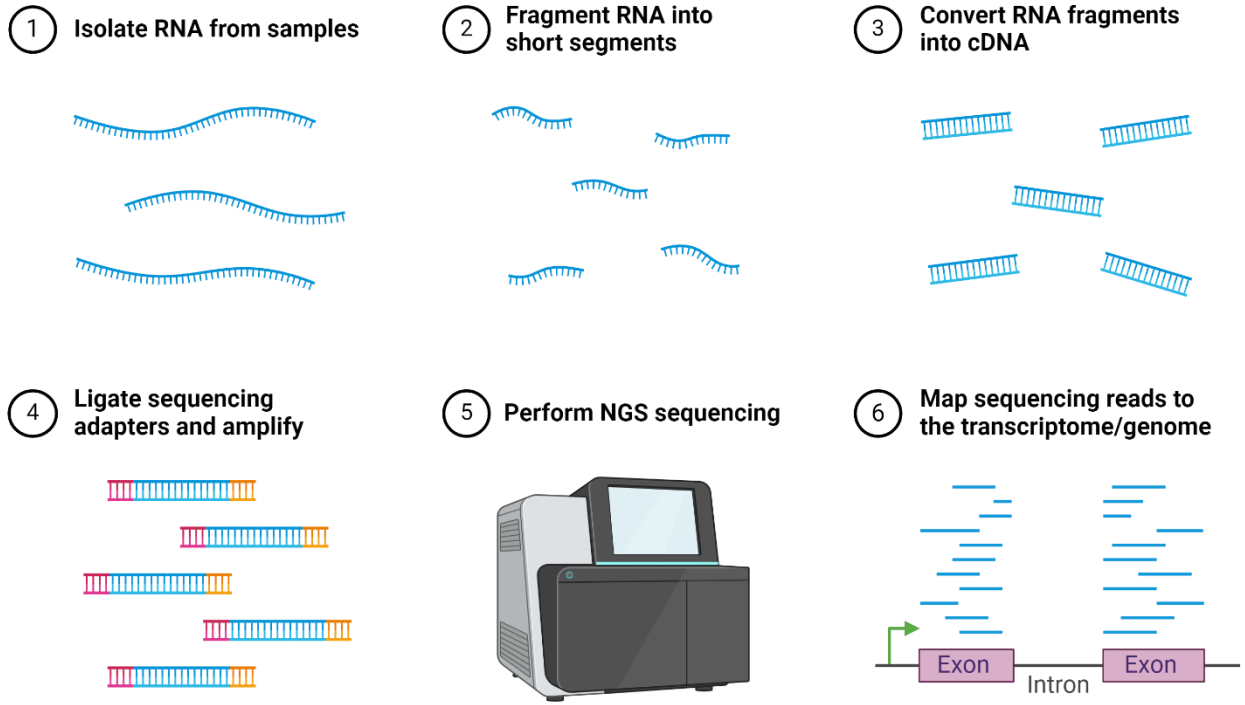


Two main transcriptomics techniques:



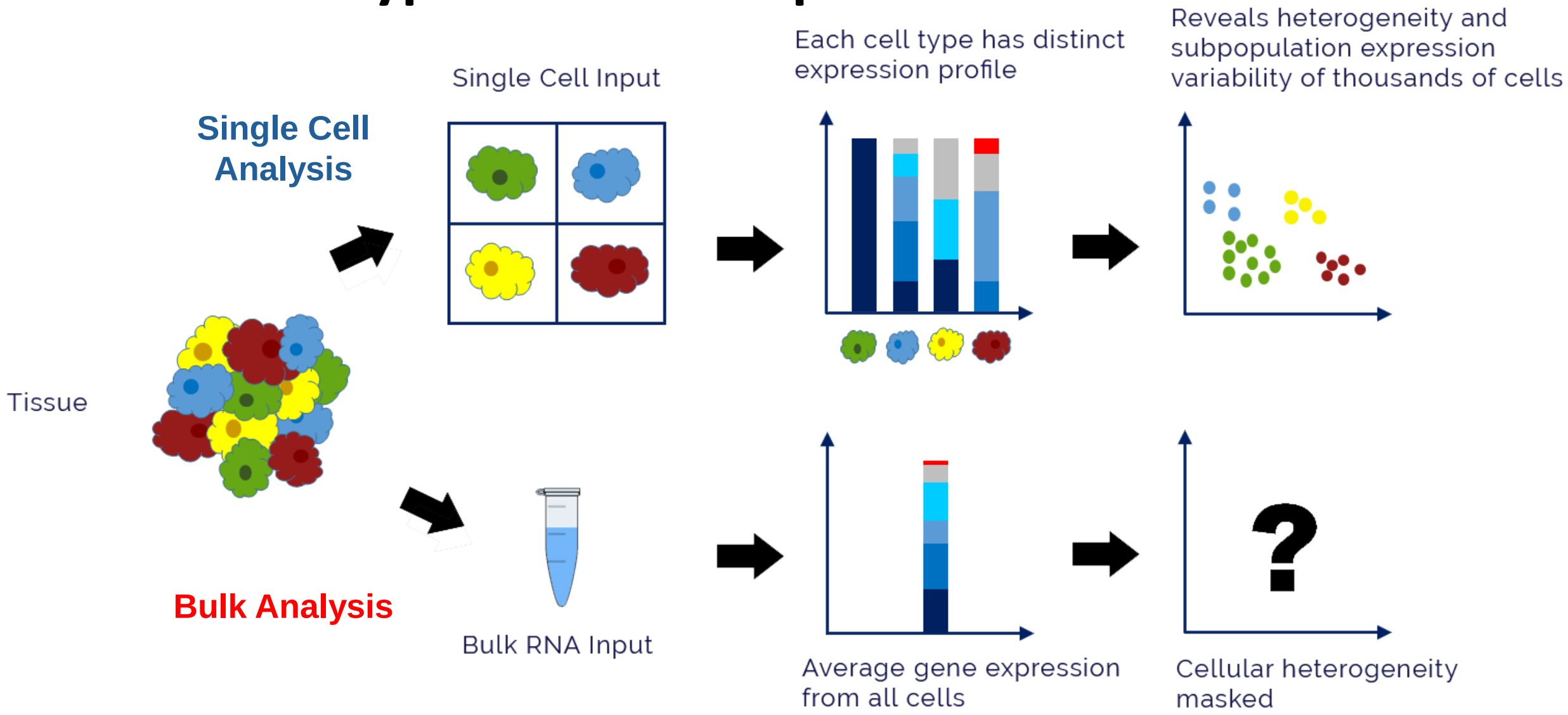


Microarray



RNA sequencing

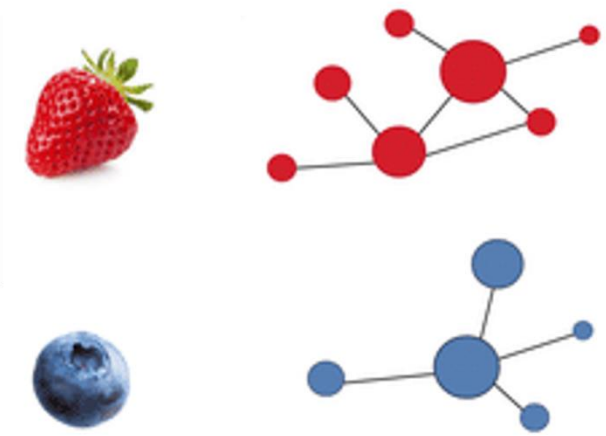
What are the types of RNA-seq?



Bulk RNA-seq can be used to get a global idea of gene expression differences between samples.

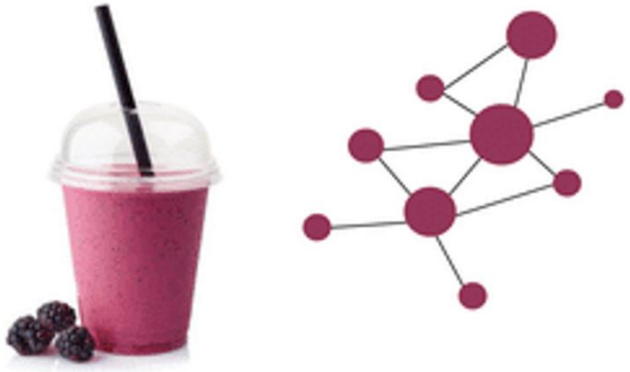
scRNA-seq gives us cell-specific information

organ/ tissue



Single-cell RNA-seq

Bulk RNA-seq

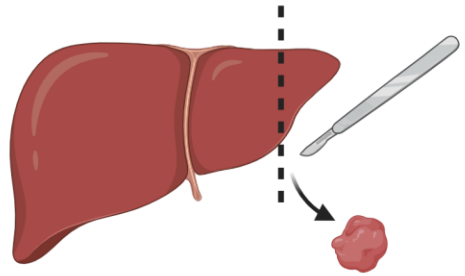


We can get information about each individual berry (cell) vs. all the cells/signals blended together in a smoothie.

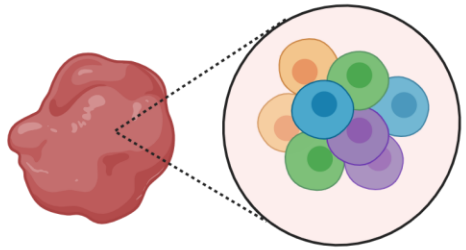
Steinheuer et al., 2021

scRNA-seq allows us to sequence specific cells from tissue

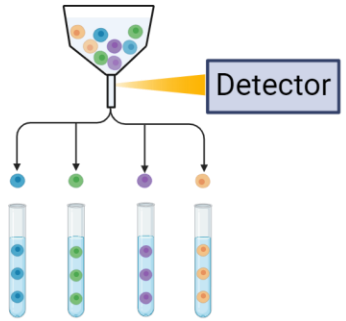
1 Tissue dissection



2 Cellular composition of the dissected tissue

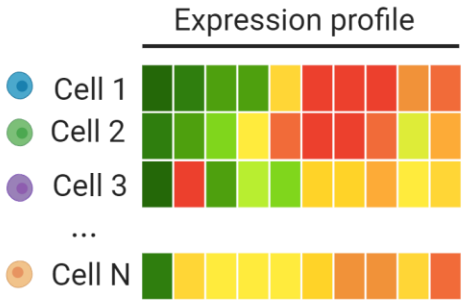


3 Single-cell sorting by FACS

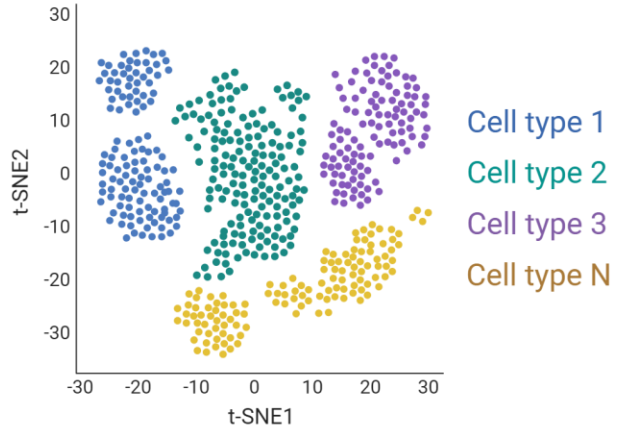


4 Single-cell sequencing

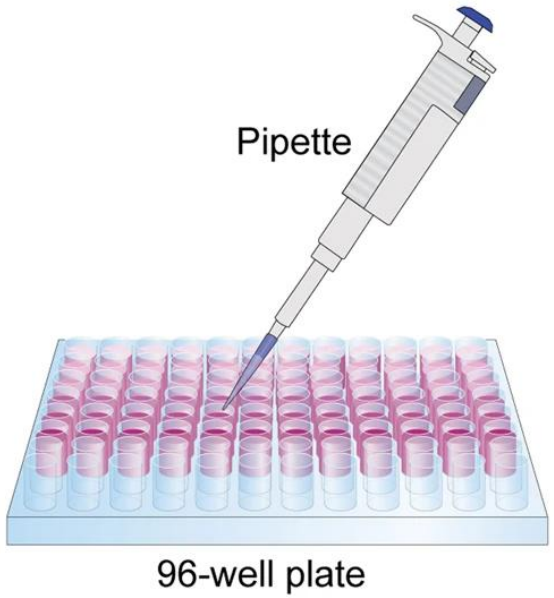
5 Single-cell expression profile



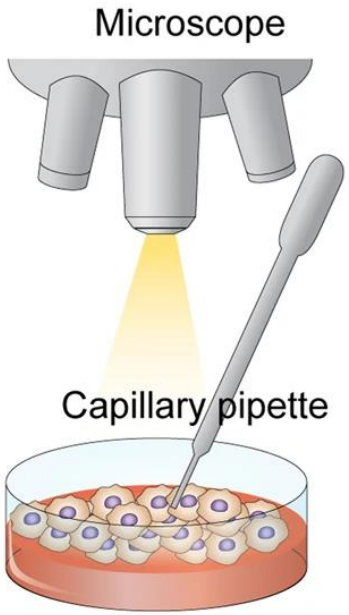
6 Clustering & cell type identification



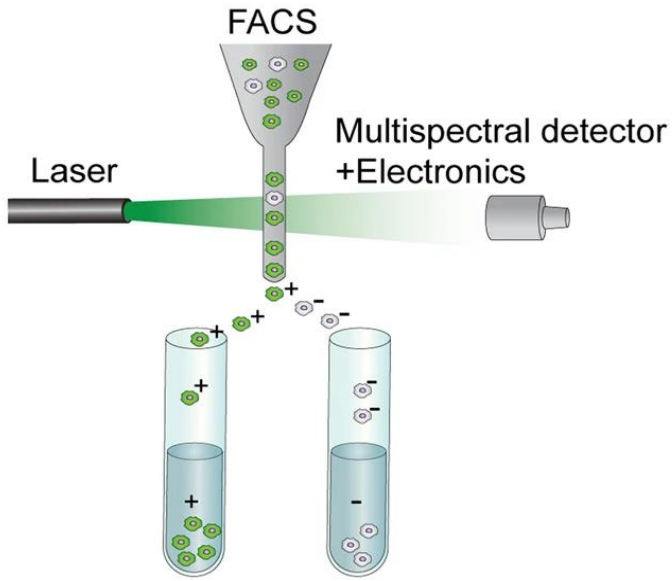
We can sort cells using techniques from pipette to laser



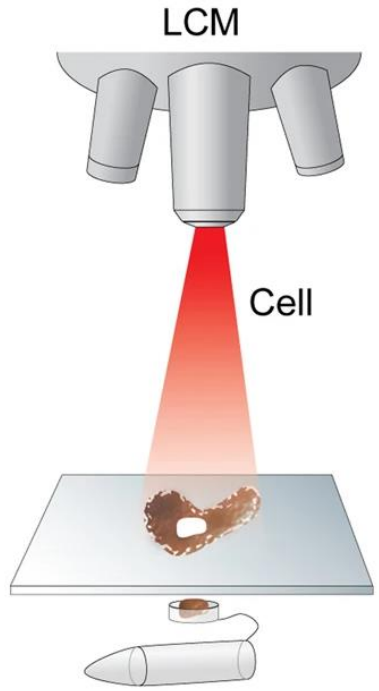
Limiting Dilution



Micromanipulation



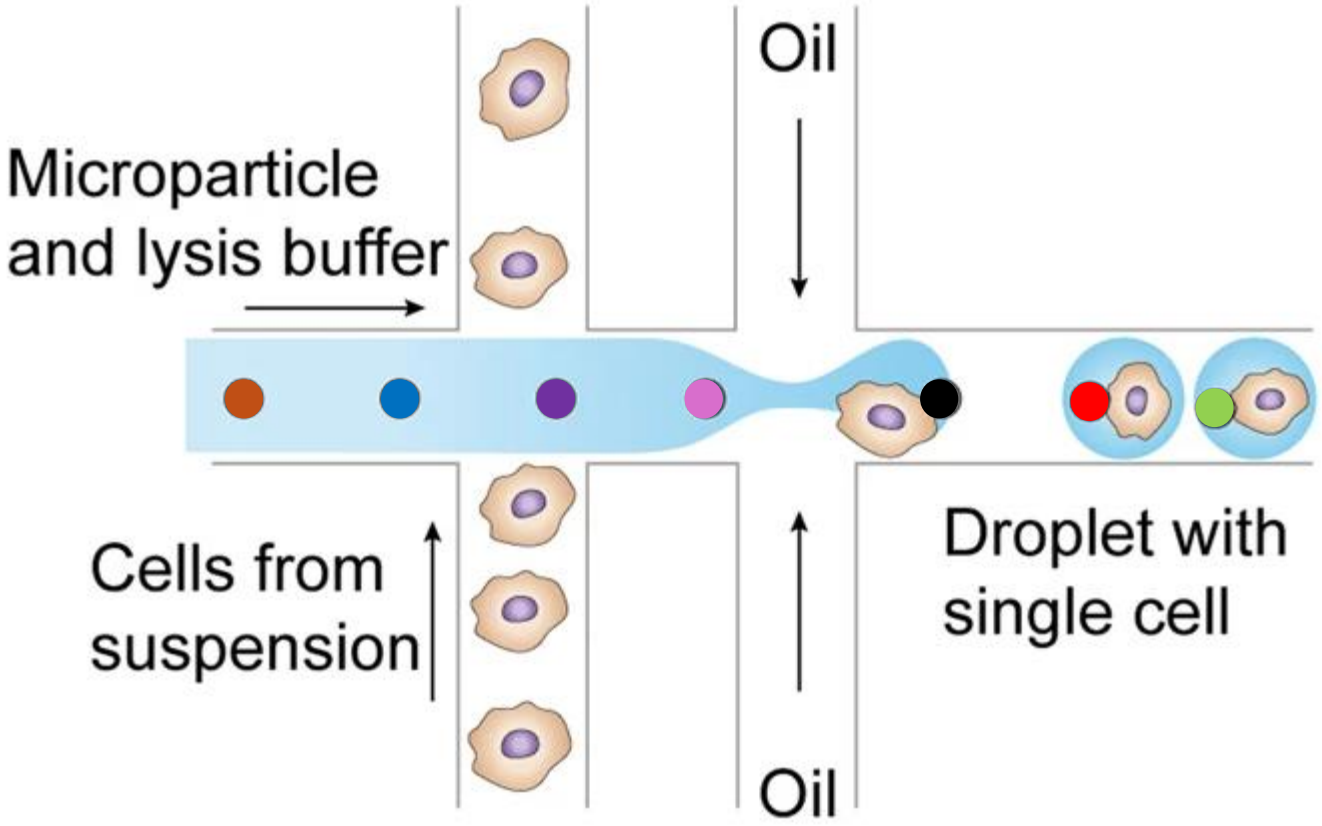
Fluorescent-activated Cell Sorting (FACS)

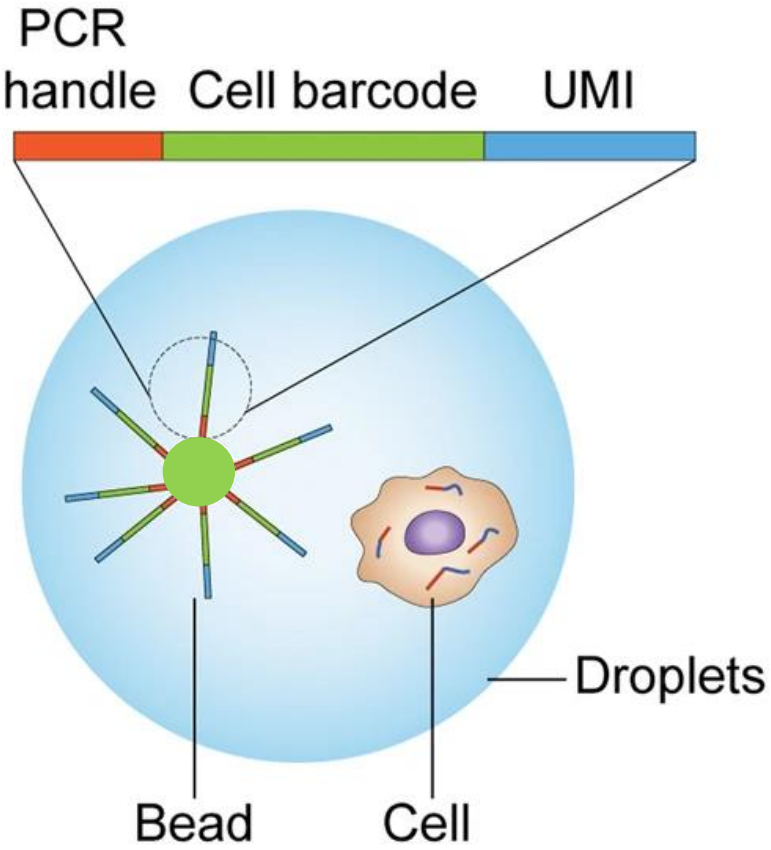


Laser Capture

Hwang et al., 2021

Step 1: Microfluidics are used to isolate cells

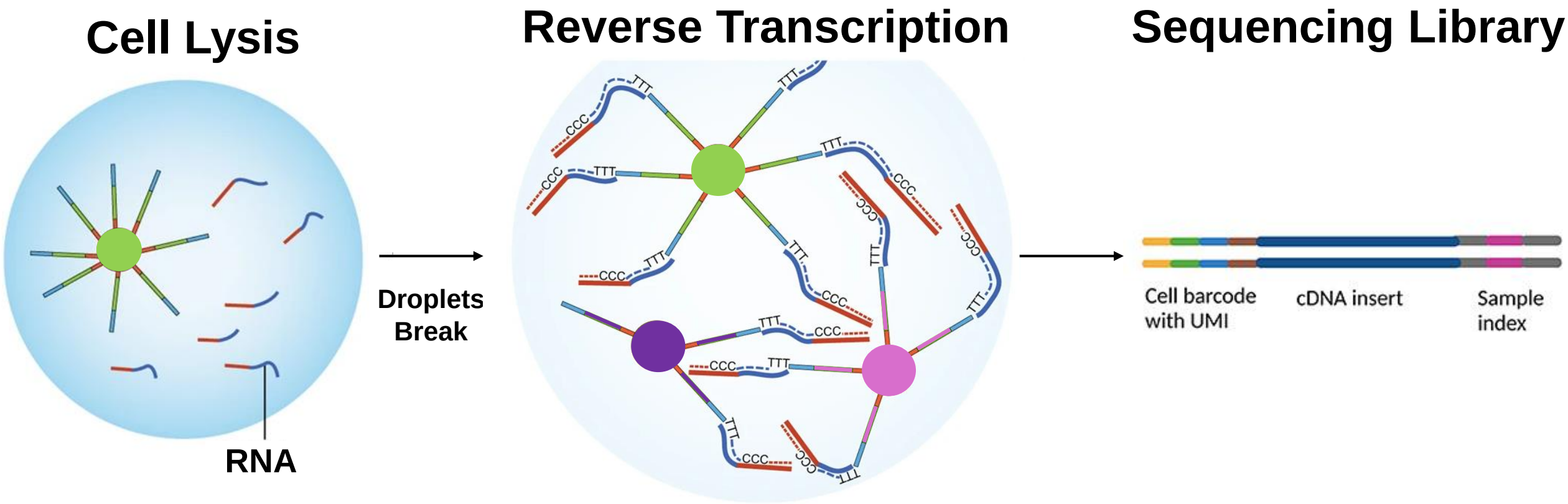




Barcoded beads are used to identify mRNA specific to each cell

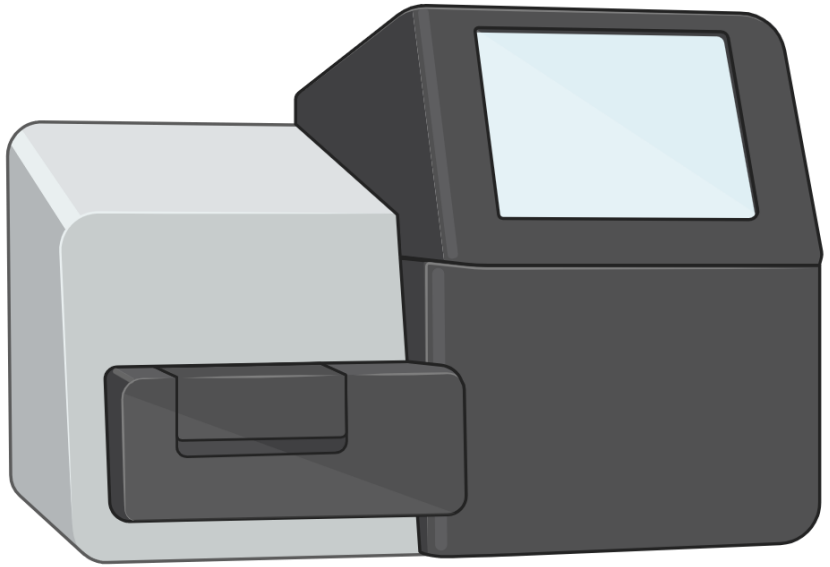
Hwang et al., 2021

Step 2: After cell lysis, you make a cDNA library from RNA



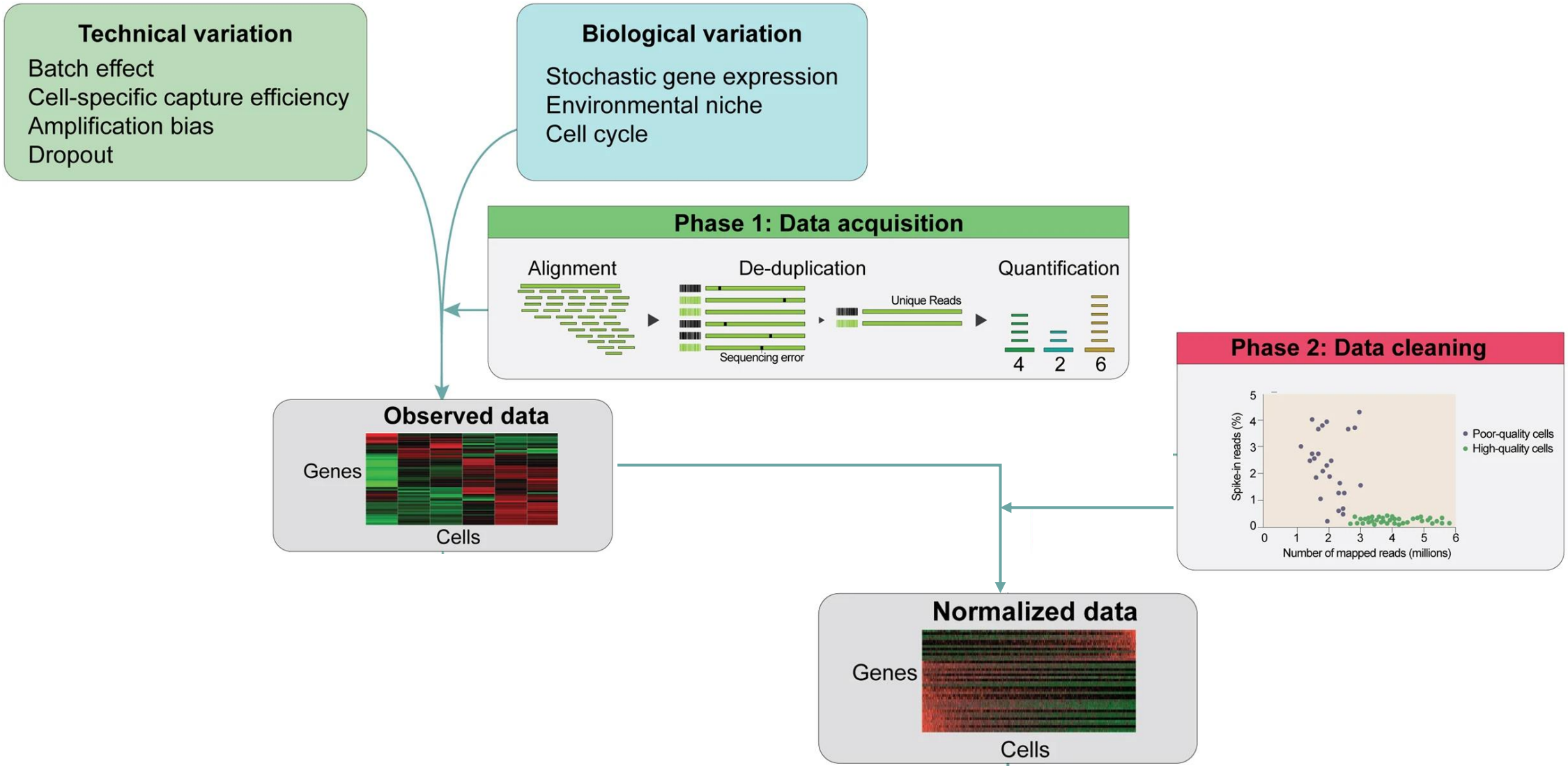
Step 3: Amplify and sequence the cDNA library

Polymerase Chain Reaction



Illumina Next-Gen Sequencing

Step 4: Preprocessing and data normalization



Use bioinformatics tools to eliminate potential technical and biological variation, background noise, low quality cells.

Hwang et al., 2021

Confounding factors that must be accounted for

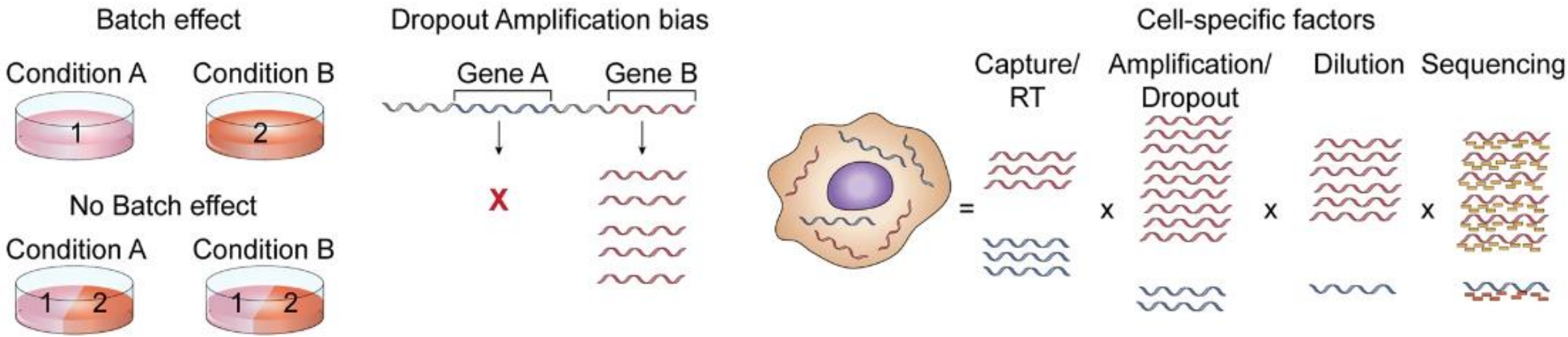
Technical variation

Batch effect
Cell-specific capture efficiency
Amplification bias
Dropout

Biological variation

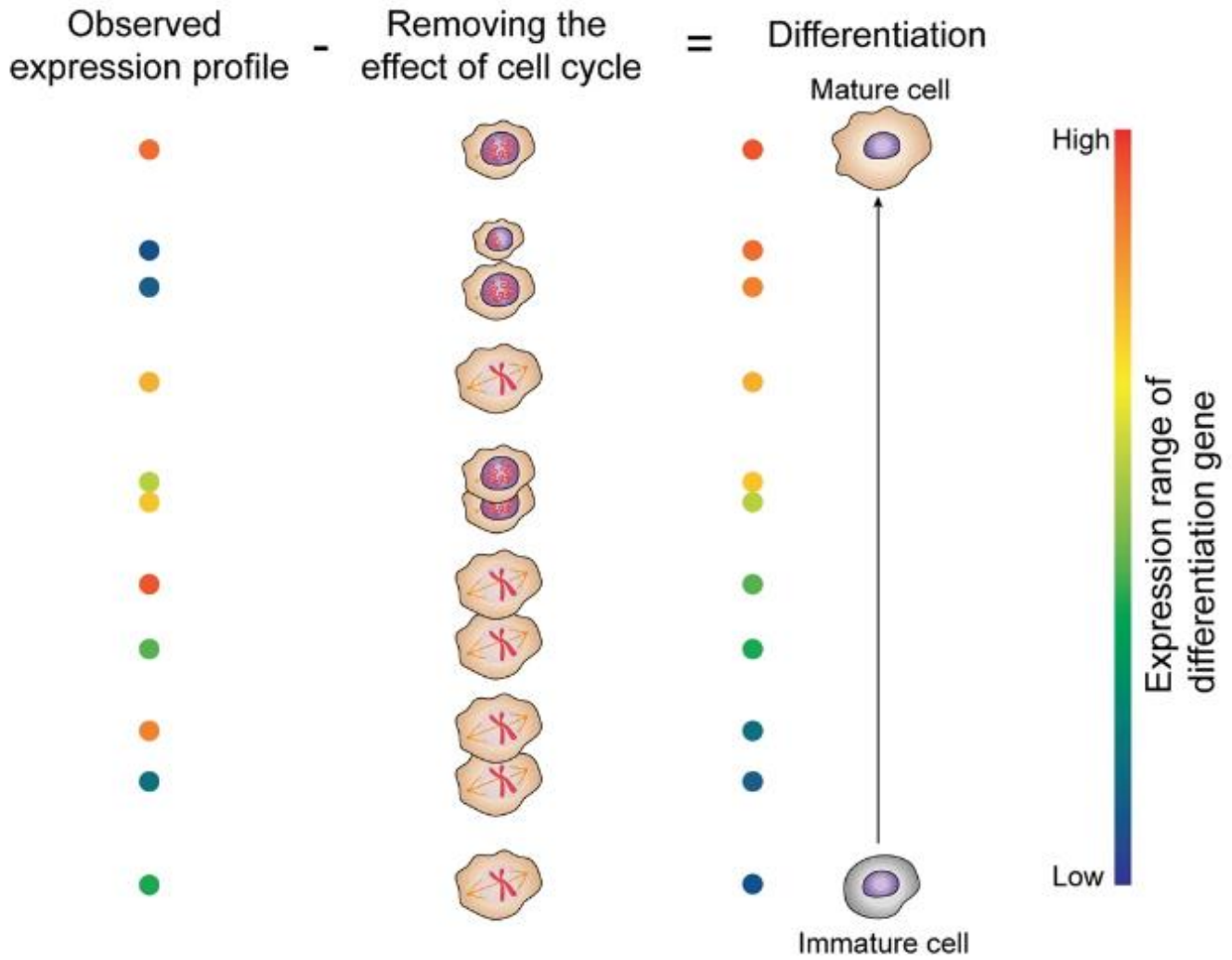
Stochastic gene expression
Environmental niche
Cell cycle

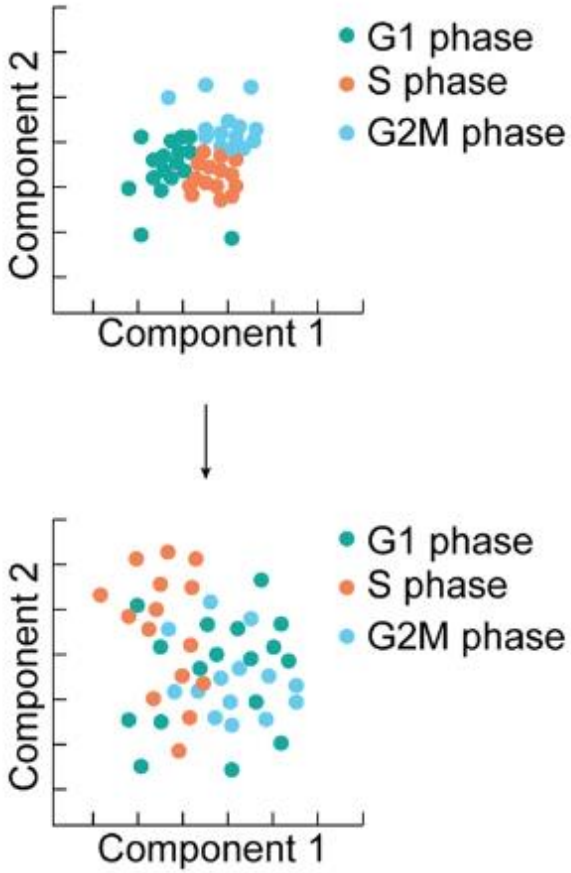
Technical variations to be removed before analysis



Hwang et al., 2021

Cell cycle effect: differential gene expression during the cell cycle

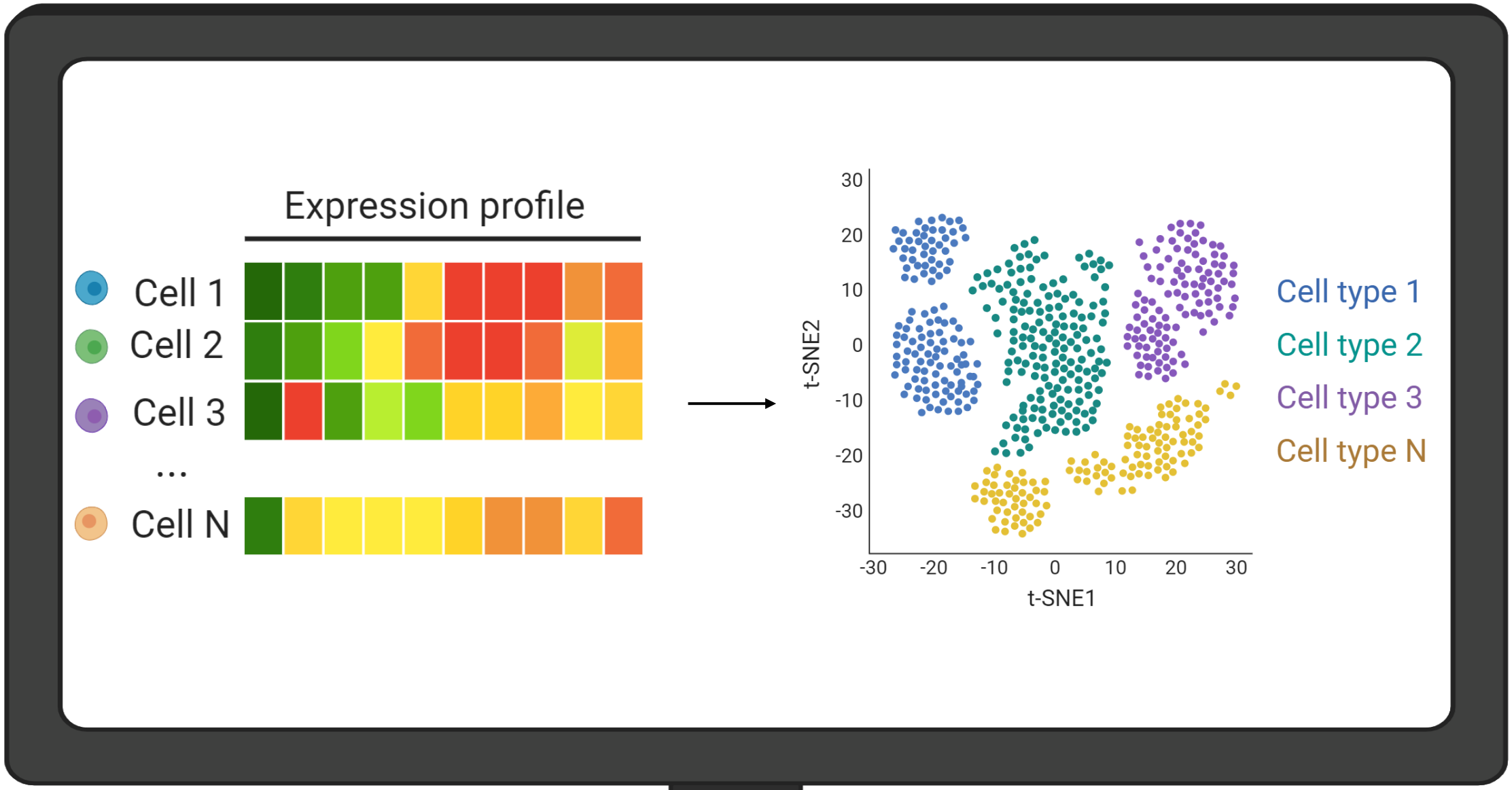




Can be removed by single-cell latent variable model (scLVM)

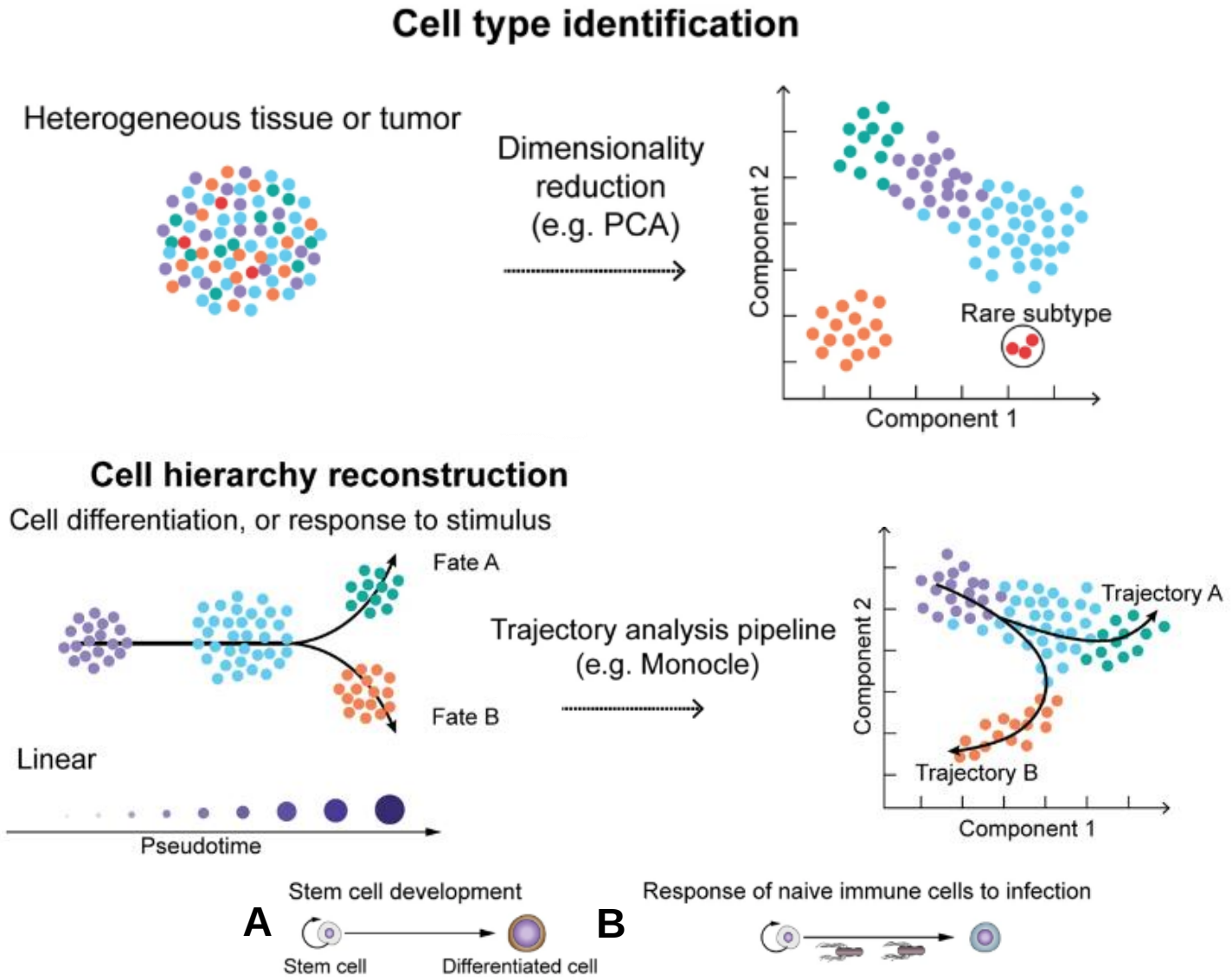
Hwang et al., 2021

Step 5: Analyze the normalized data



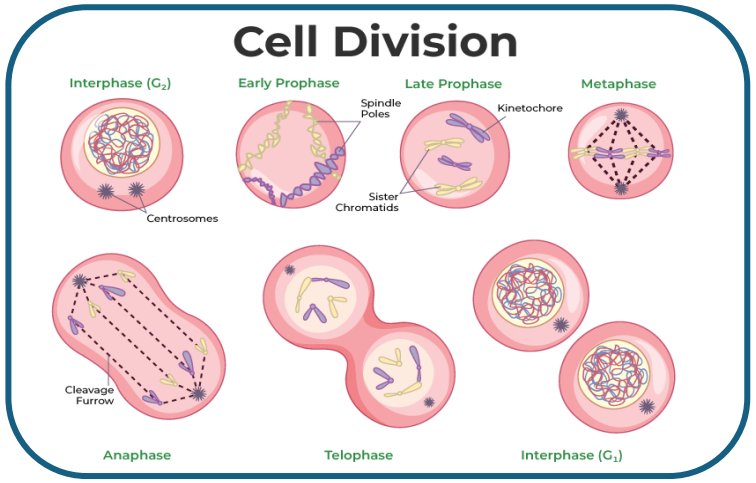
Cluster cells with similar transcriptional profiles.

At the cell-level we can identify cell type and cell differentiation



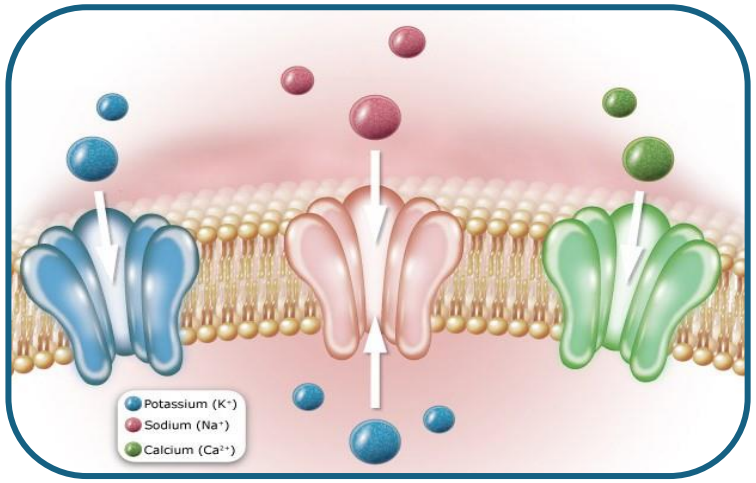
Hwang et al., 2021

We can use scRNA-seq to find gene ontology information



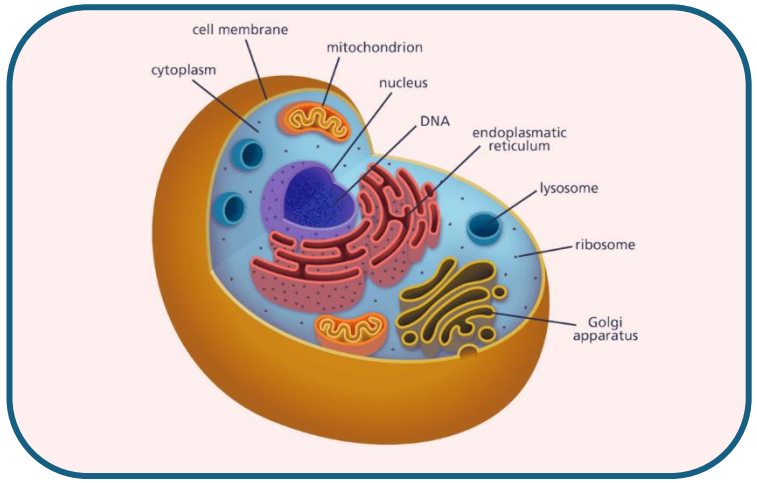
Biological Process

Pathway Involvement



Molecular Function

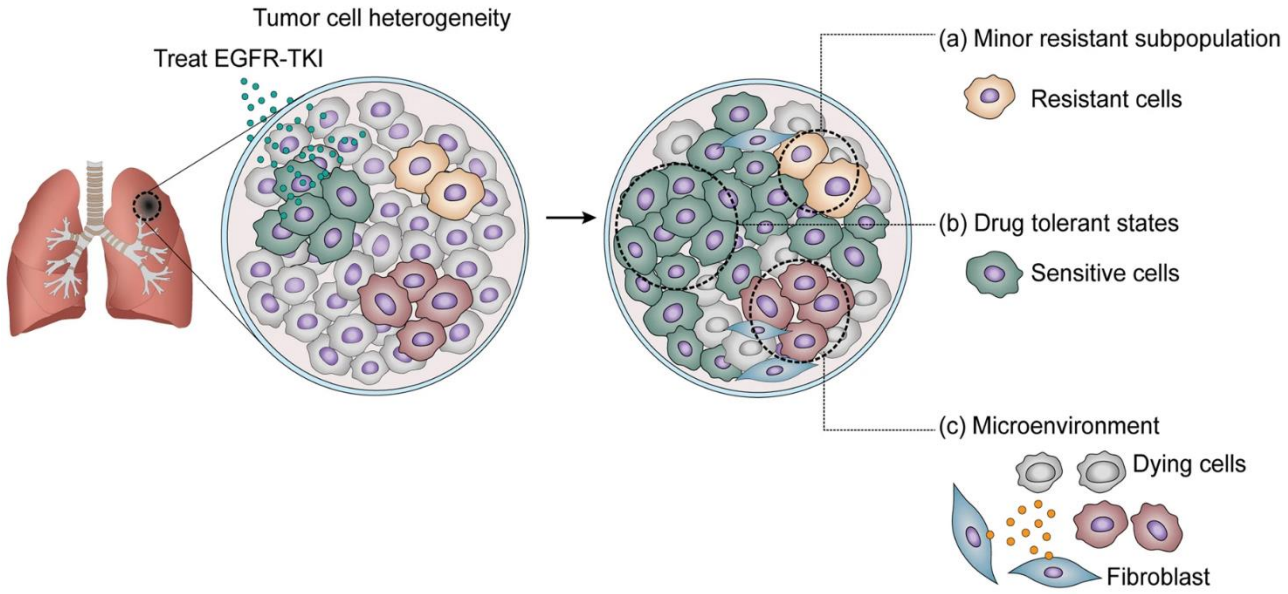
Elemental Task



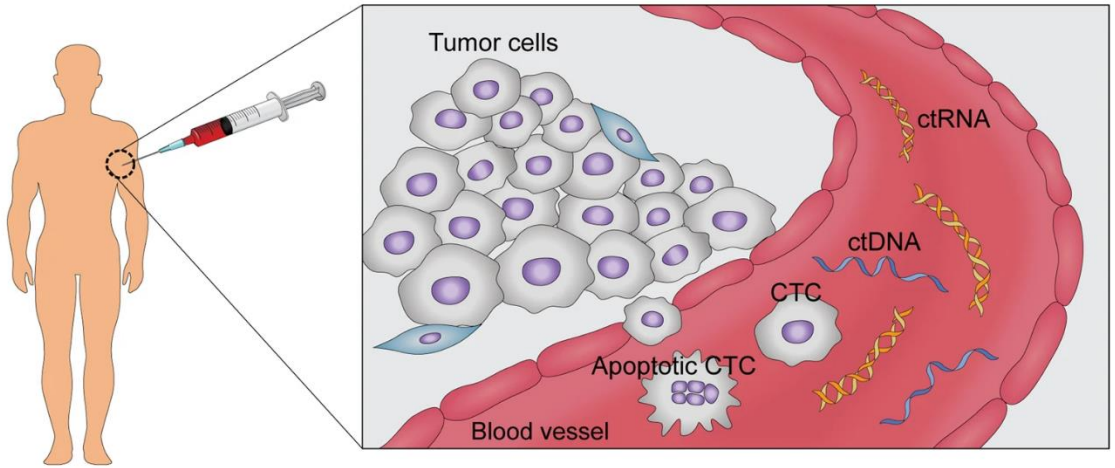
Cellular Component

Location in Cell

How can we use scRNA-seq data in a clinical setting?



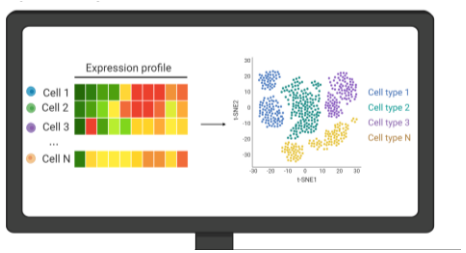
Tumor Heterogeneity & Drug Resistance Identification



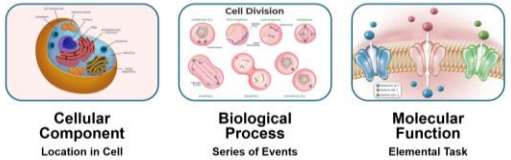
Liquid Biopsy Diagnosis

Hwang et al., 2021

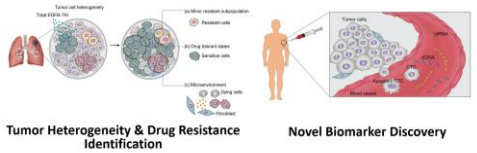
scRNA-seq:



allows for the analysis of individual cells at both the cell- and gene-level.

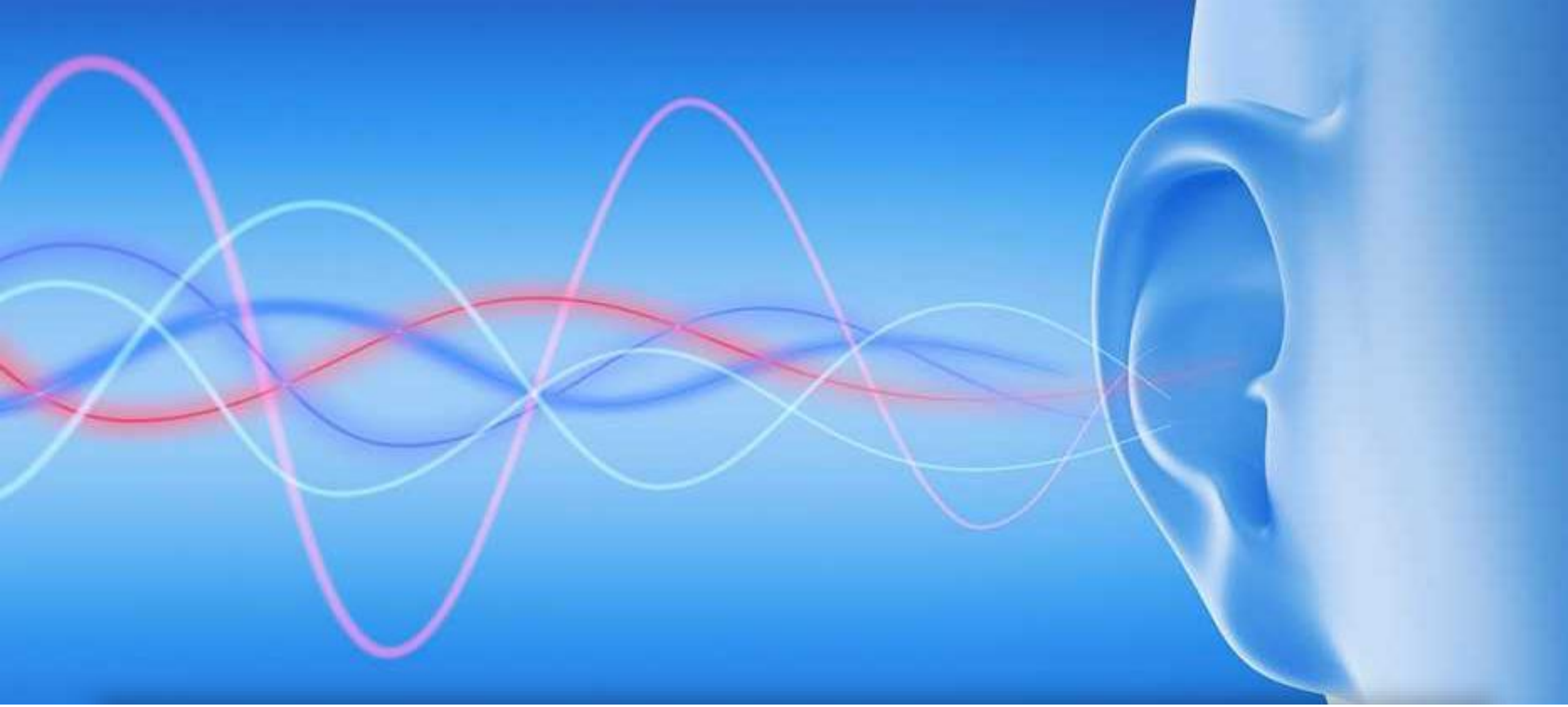


can be used to identify cell types, study cell differentiation and infer gene ontology networks.



has numerous applications to the medical field; such as its use in tumor heterogeneity and liquid biopsy diagnosis.

Questions?



Single-cell RNA-sequencing of zebrafish hair cells reveals novel genes potentially involved in hearing loss – *Qian et. al*

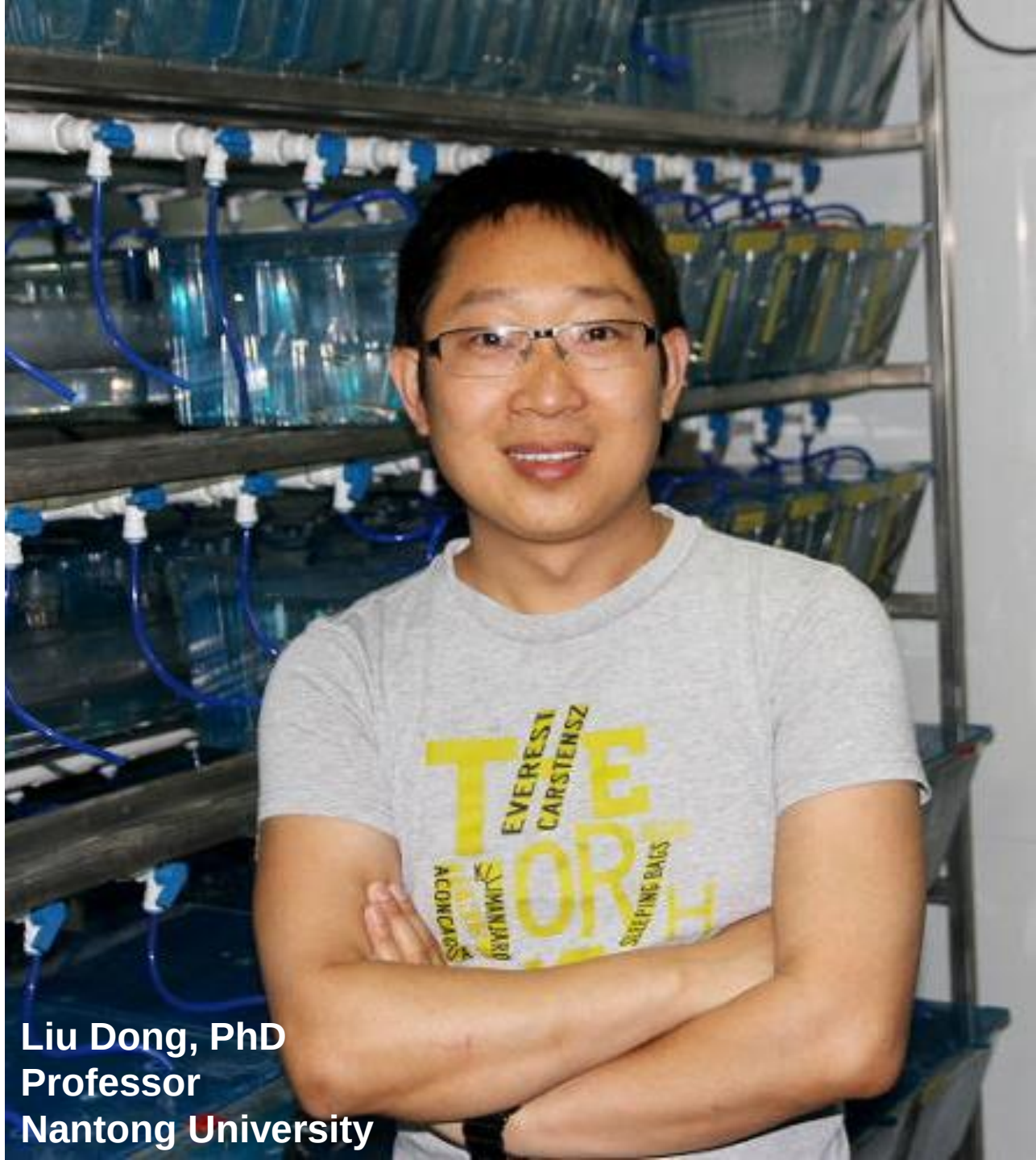
Scientists Behind the Science

First Authors

Fuping Qian

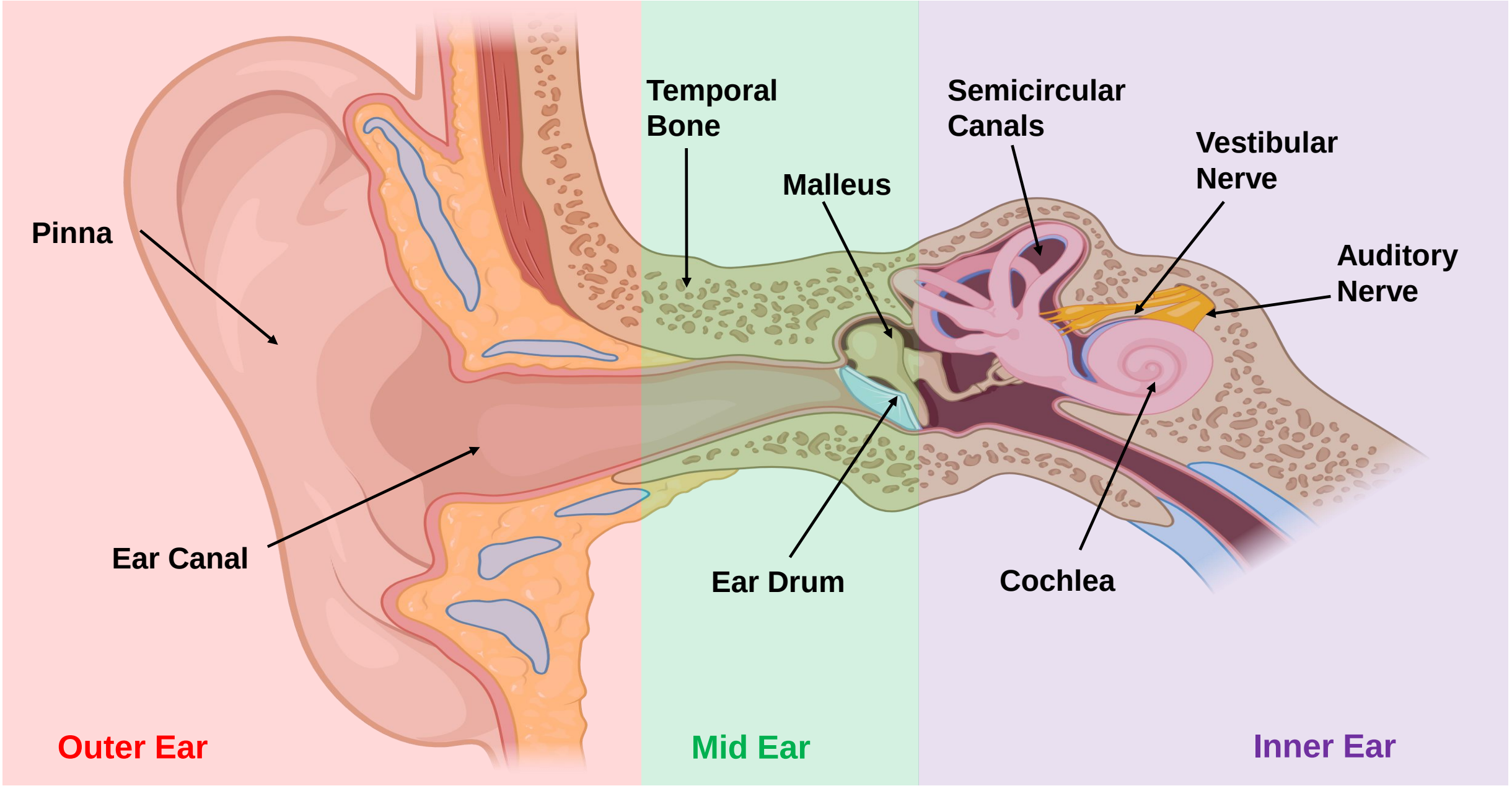
Guanyun Wei, PhD

The Dong lab aims to identify novel regulators of blood vessel formation in embryonic development and tissue regeneration.

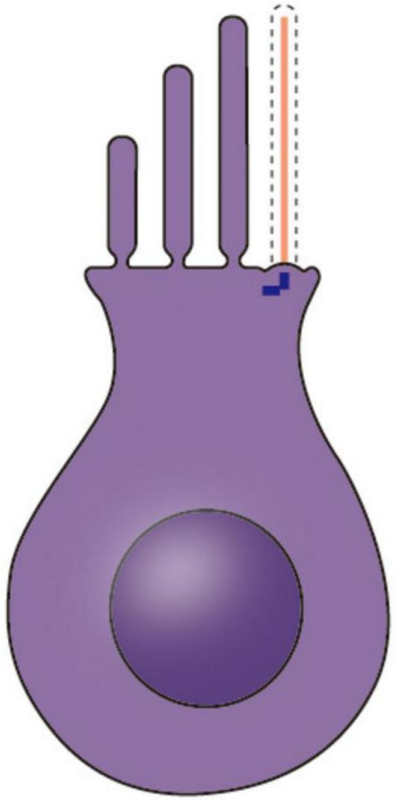


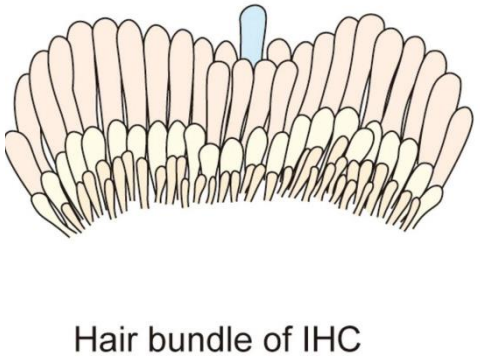
Liu Dong, PhD
Professor
Nantong University

What is the structure of the human ear?

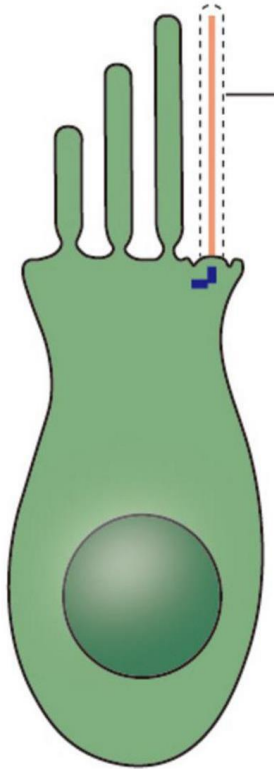


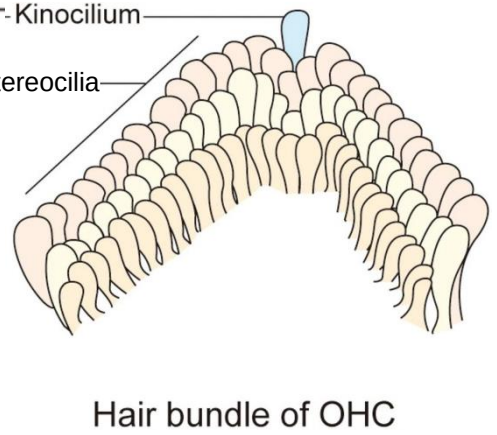
What are the types of hair cells in the human ear?





Cochlear Inner Hair Cell (IHCs)

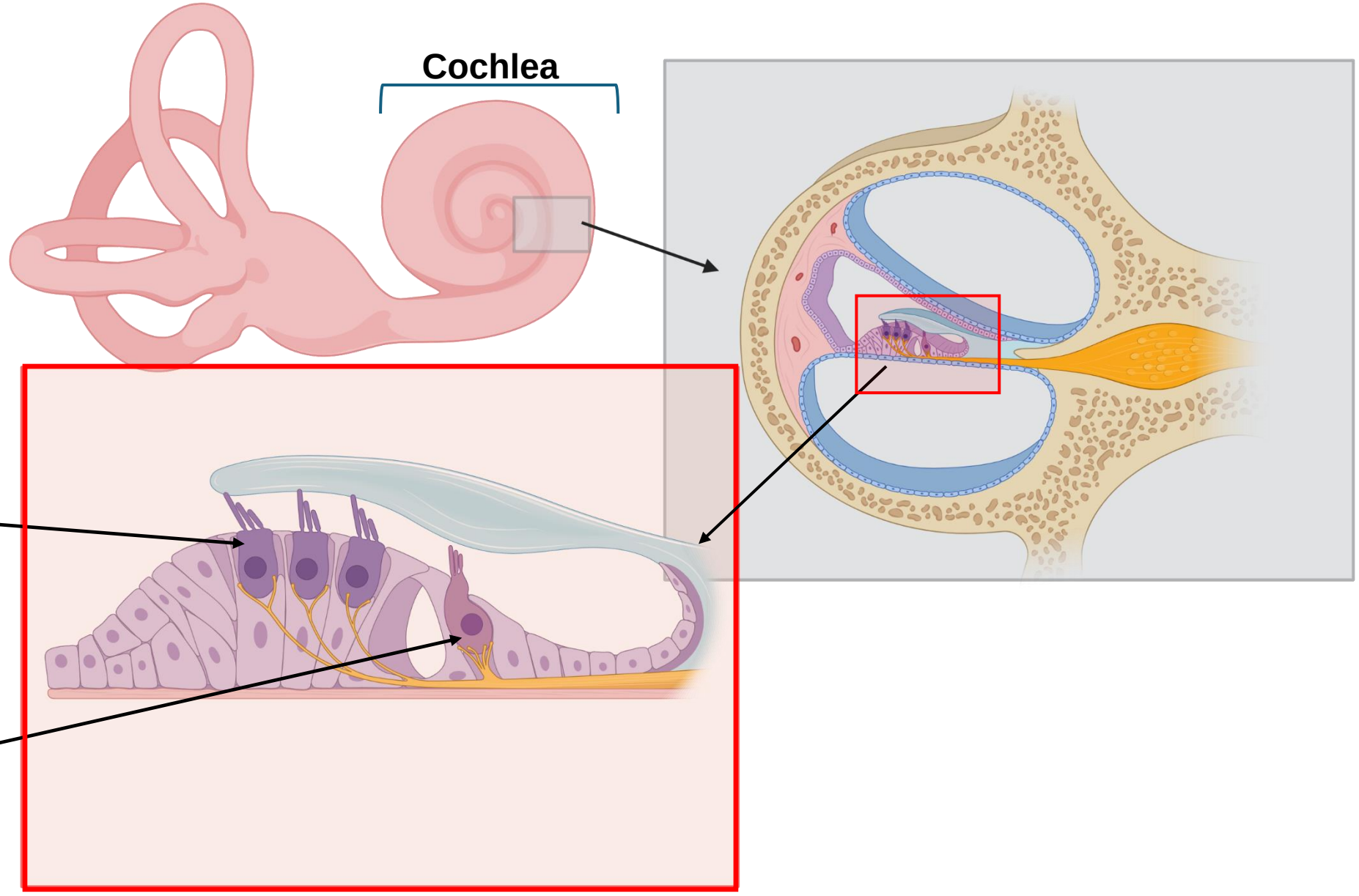




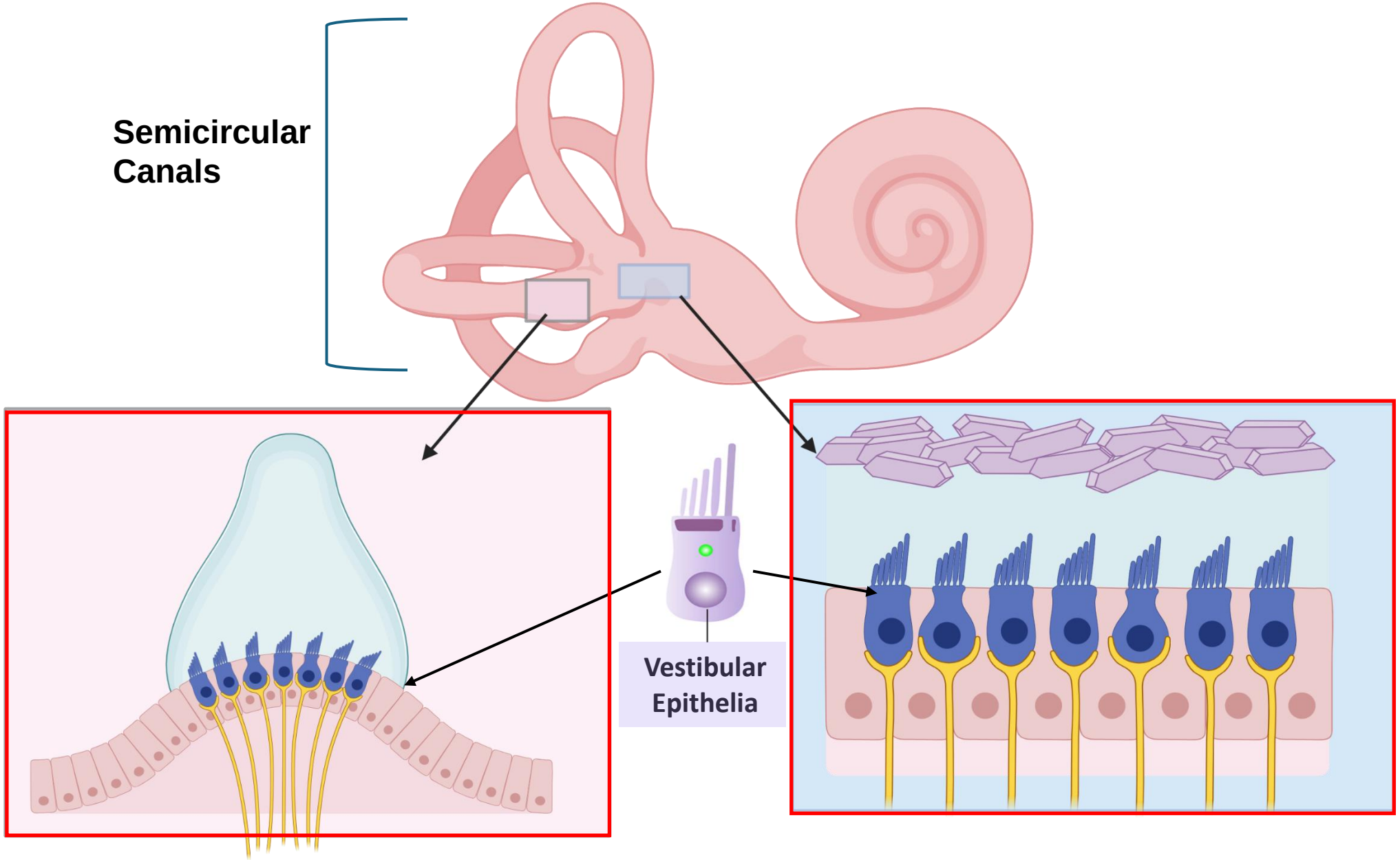
Cochlear Outer Hair Cell (OHCs)

Vestibular Epithelia

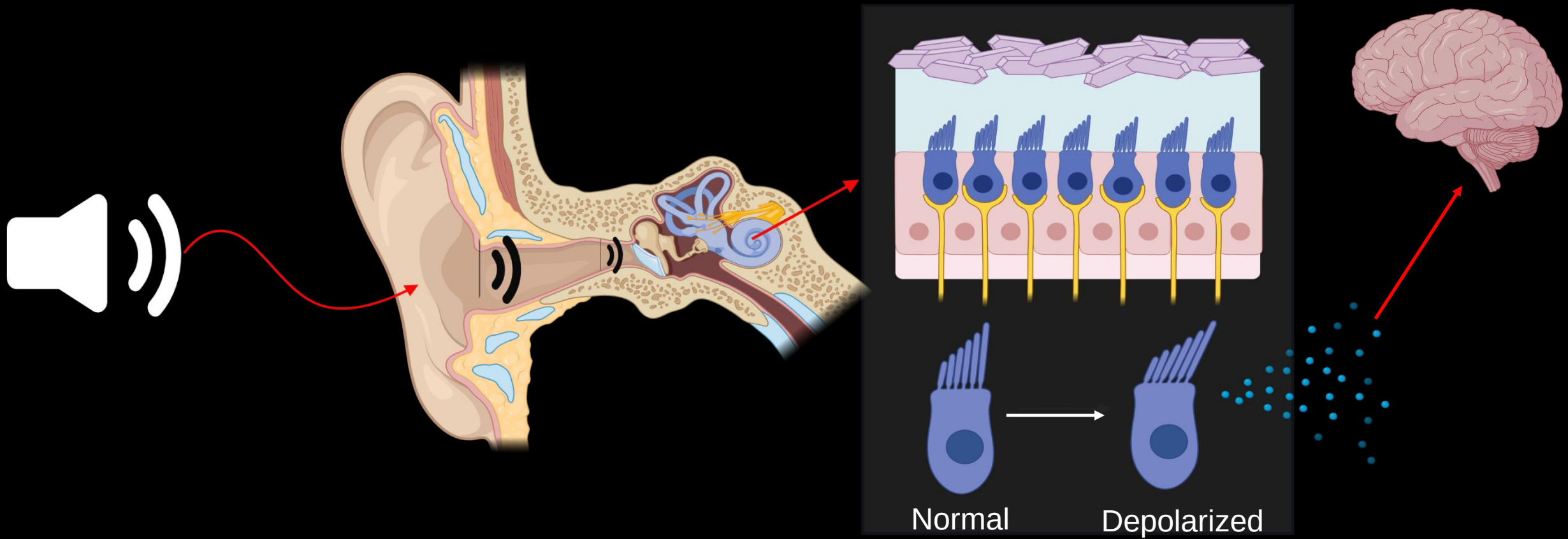
Where are these cells found in the inner ear?



Where are the vestibular cells found in the inner ear?

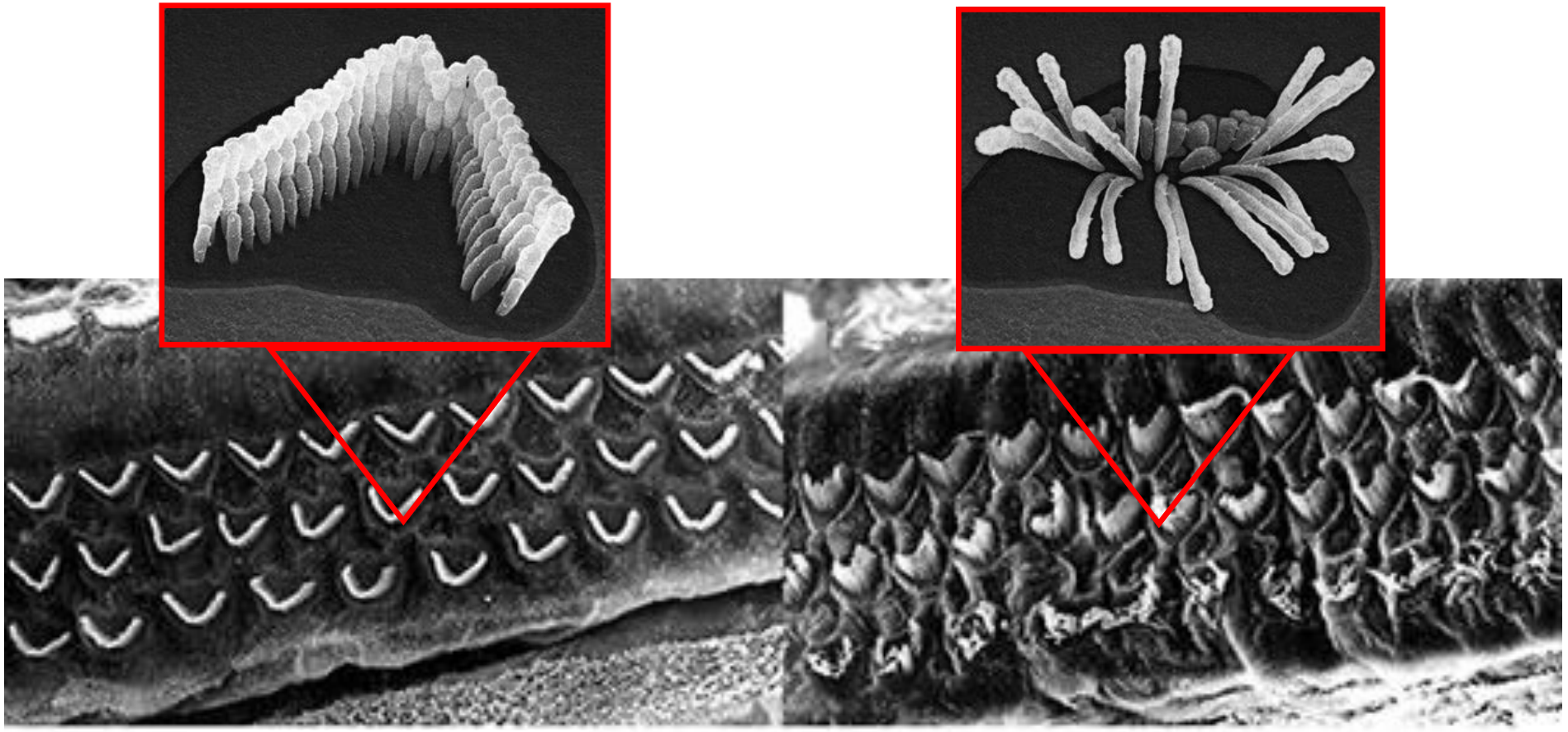


How do hair cells function in hearing?



Cilia detect mechanical vibrations and allow for neurotransmitter release

What causes hearing loss?

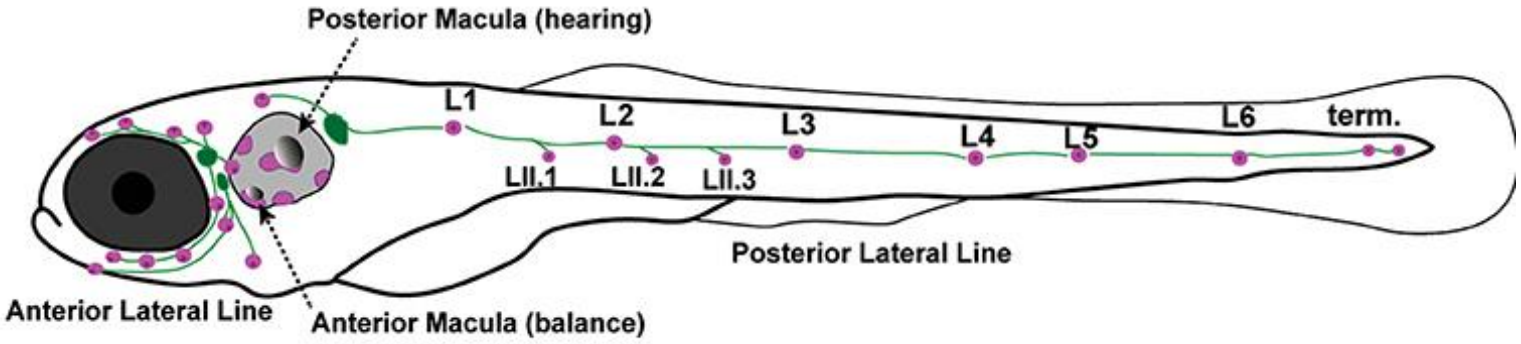


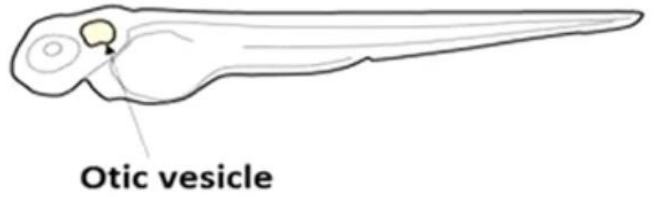
Normal Hair Cells

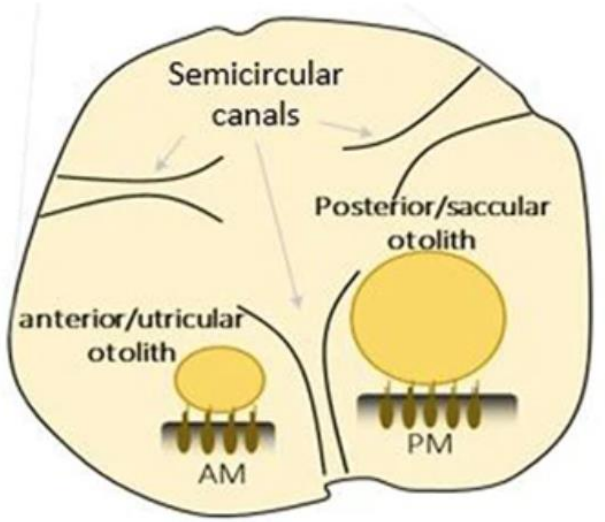
Damaged Hair Cells

Damaged hair cells and cilia dysfunction

Why use zebrafish as the model organism?

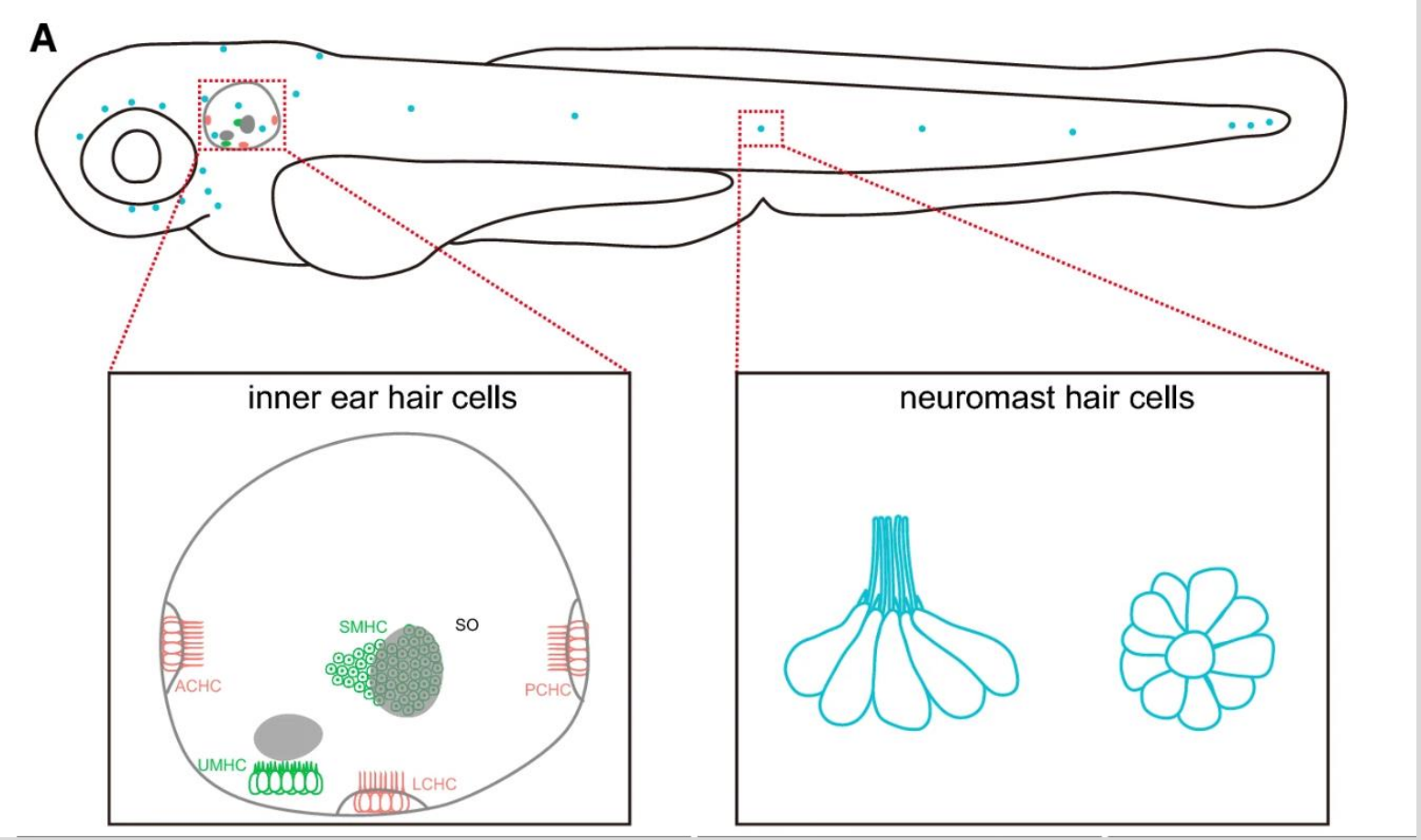






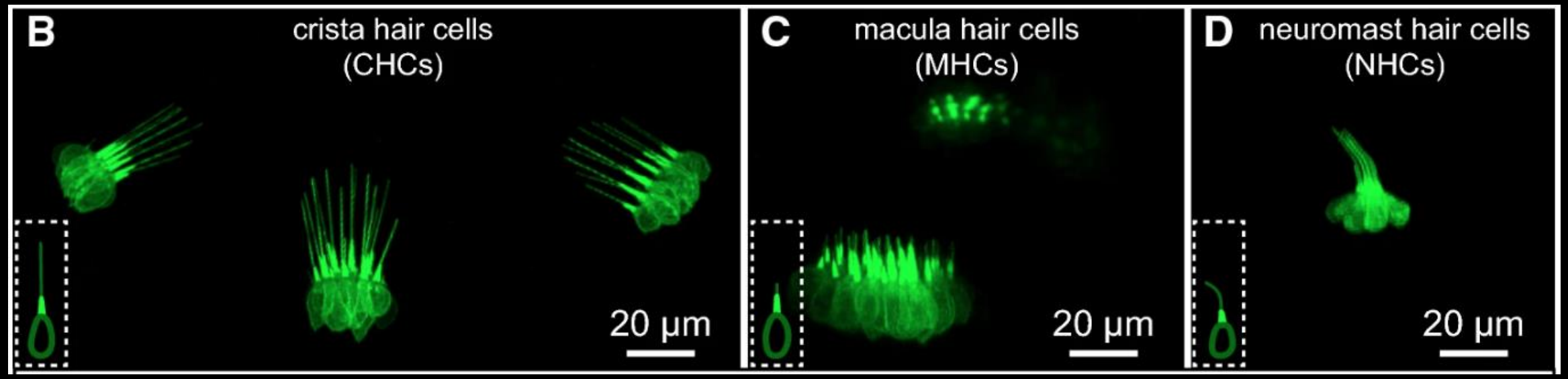
Abundance of hair cells with similar functions to mammals

What are the types of hair cells in zebrafish?

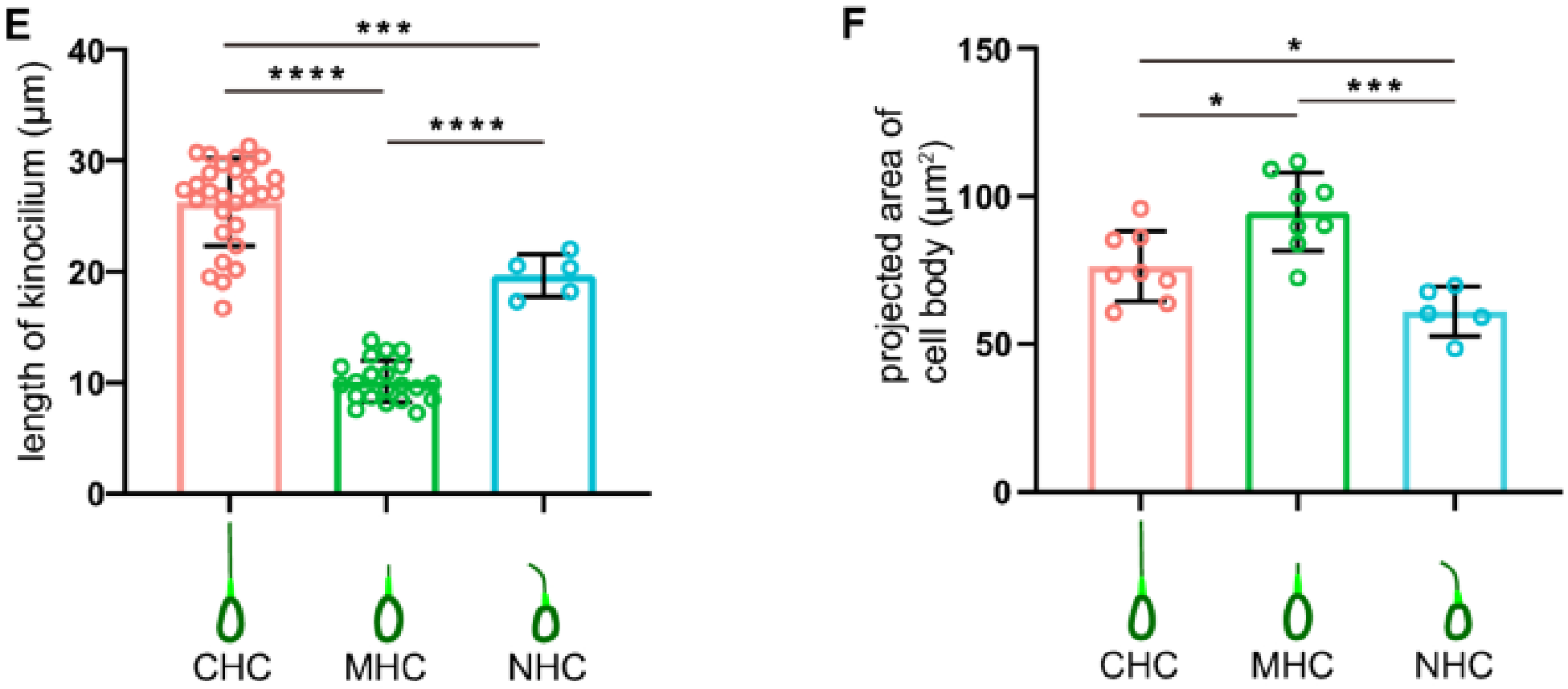


Crista, Macula, and Neuromast Cells

Inner ear and Neuromast cells differ in ***shape***

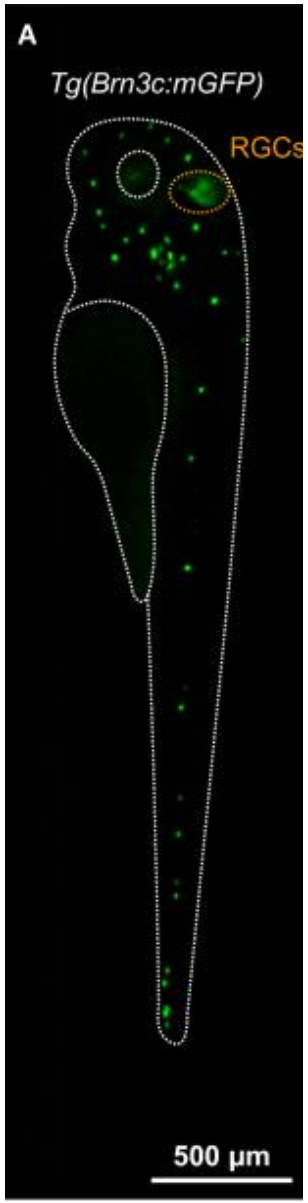


Inner ear and Neuromast cells differ in ***size***

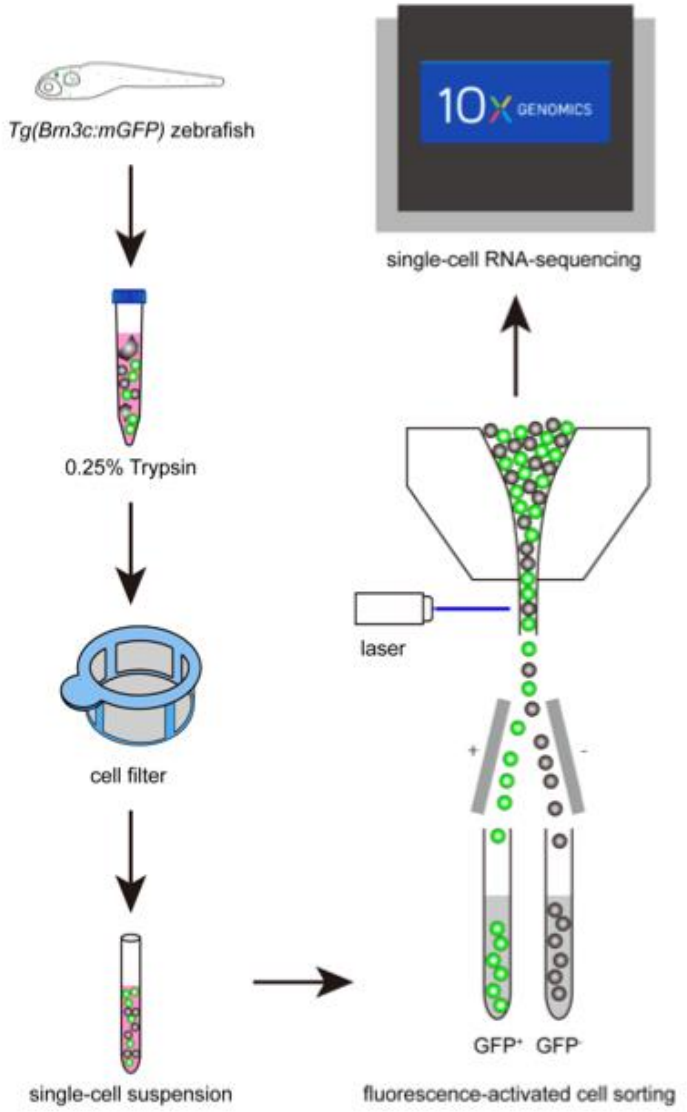


Finding the molecular function of each cell type: Step 1

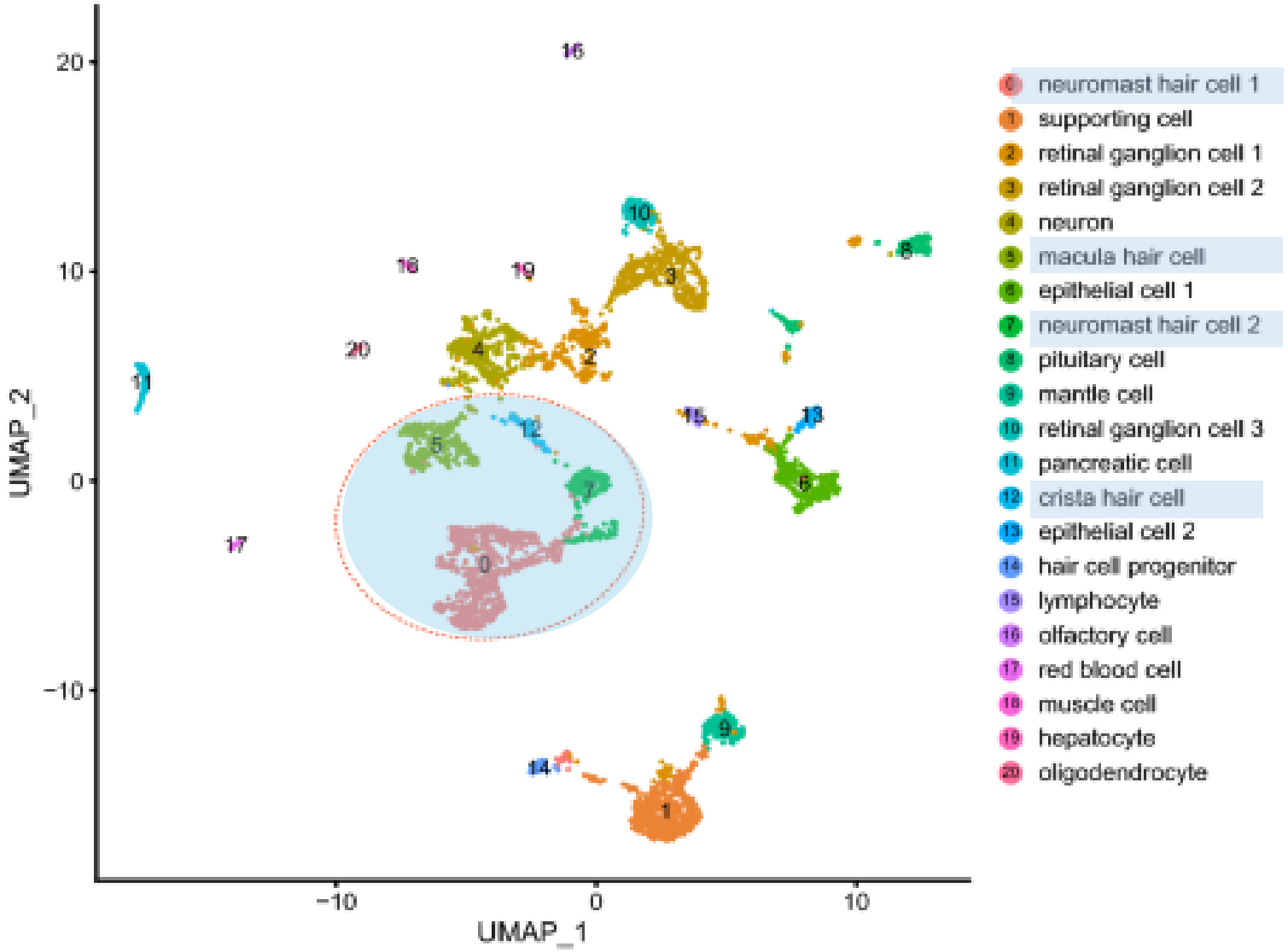
mGFP Labeling



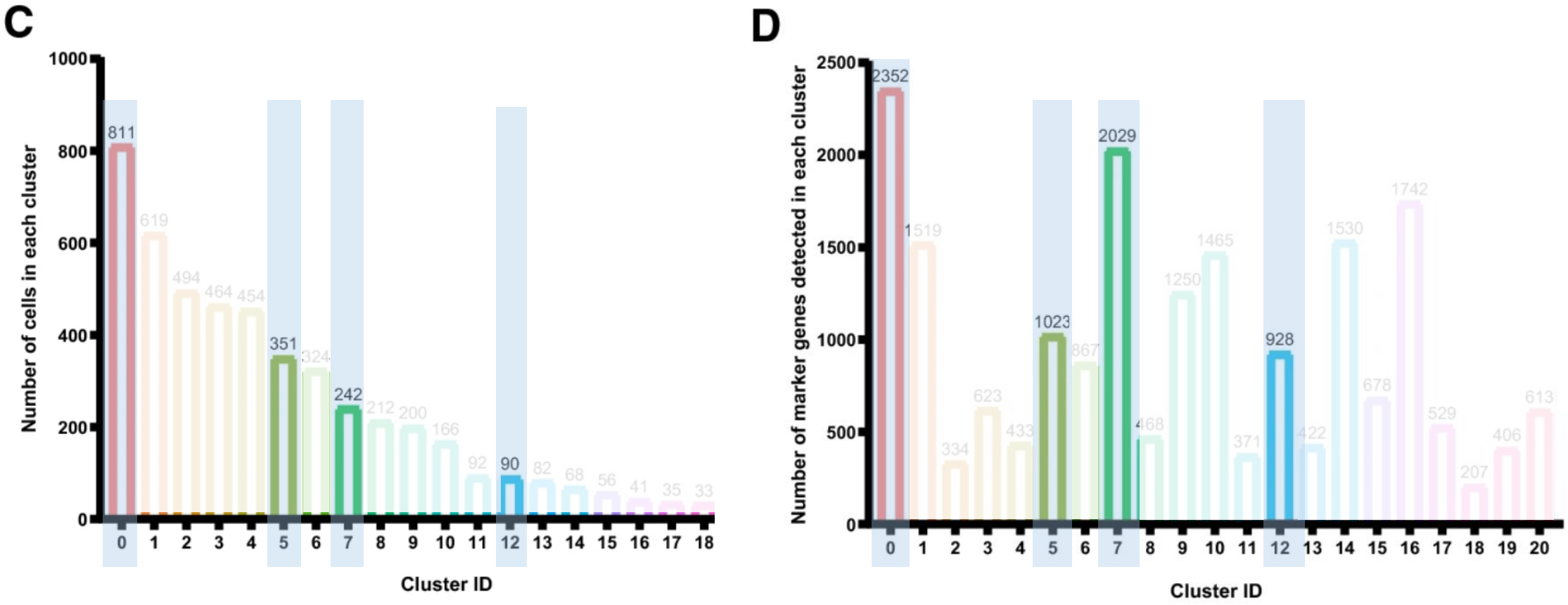
FACS Sorting



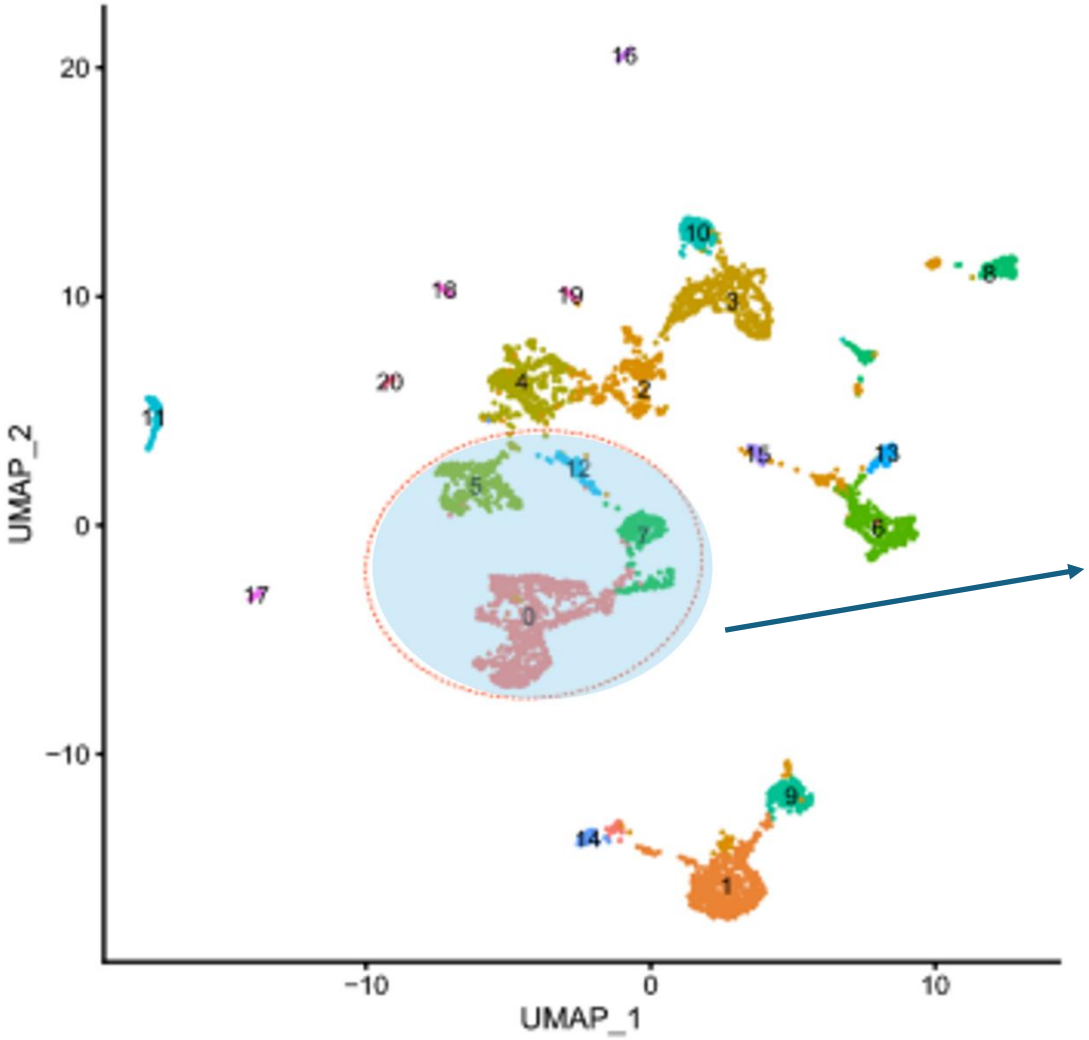
What did the scRNA-seq reveal?



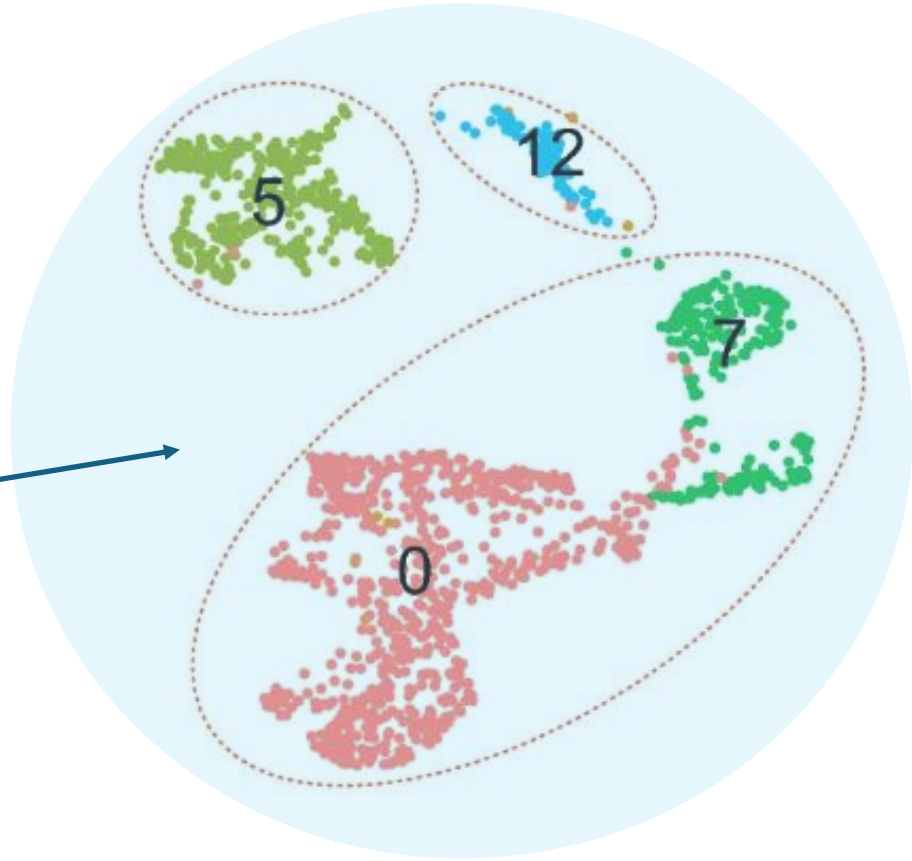
What did the scRNA-seq reveal?



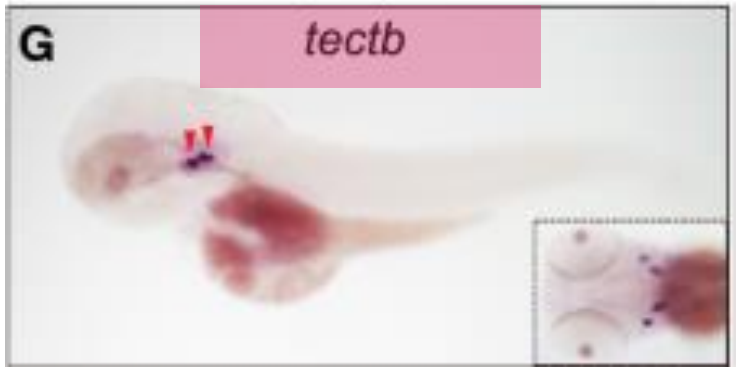
Finding the molecular function of each cell type: *Step 2*



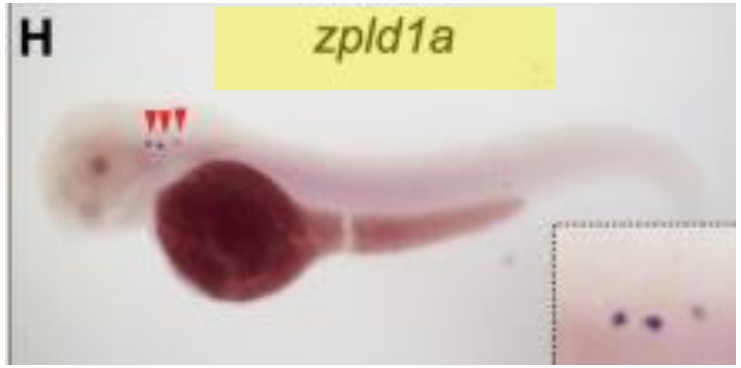
Whole mount in-situ Hybridization (WISH)



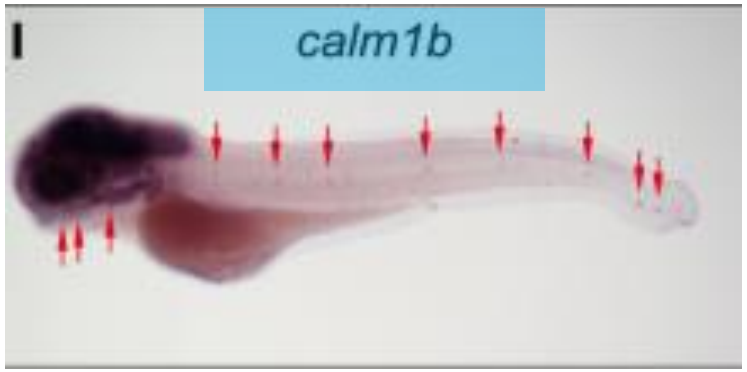
Whole Mount In-Situ Hybridization (WISH) results



Tectb gene **was localized primarily in macula cells**

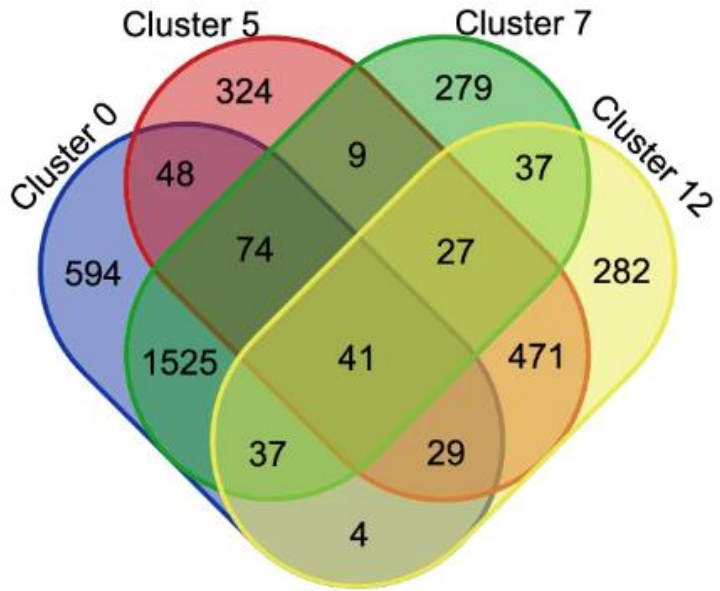


Zpld1a gene **was localized primarily in crista cells**



Calm1b gene **was localized primarily in neuromast cells**

What was determined from the molecular analysis?



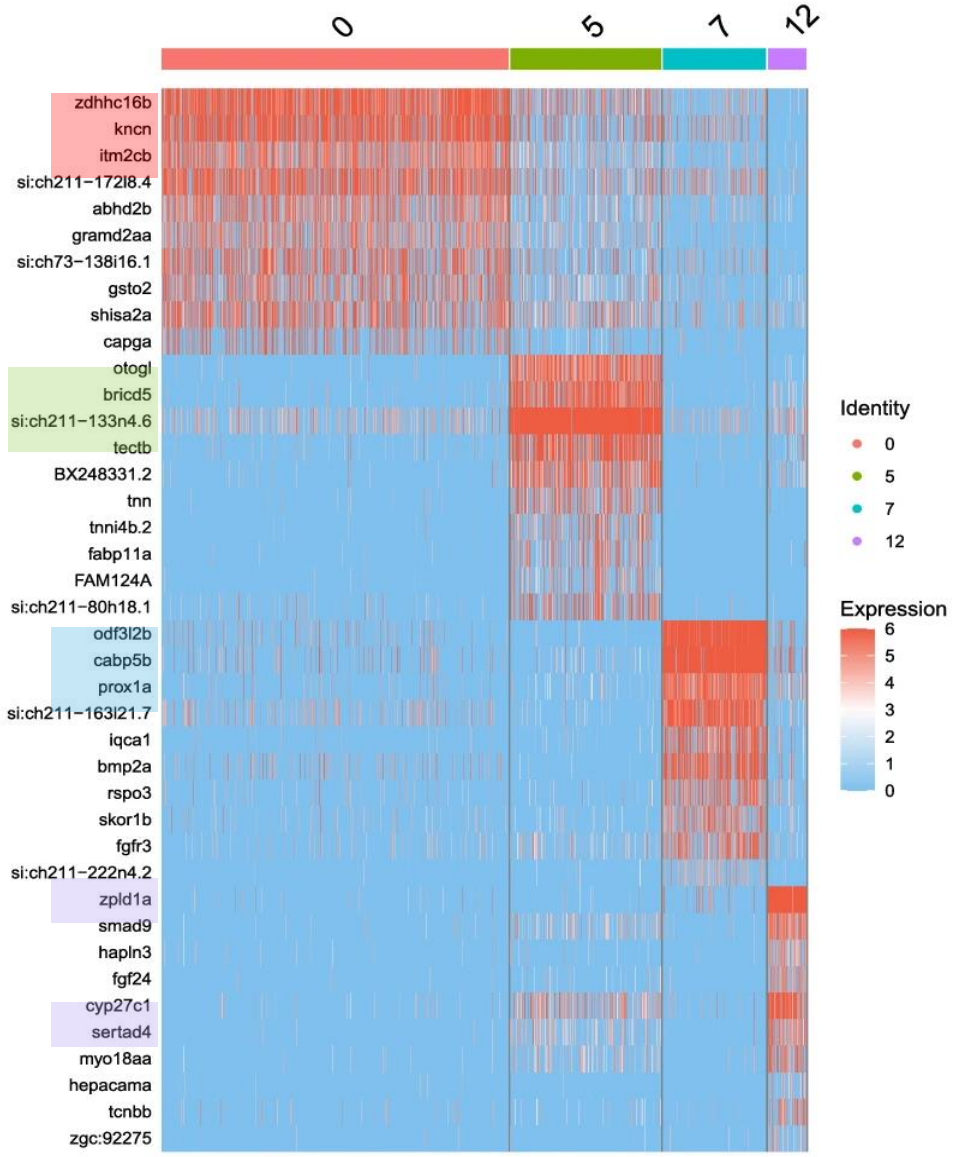
Cluster 0: mature neuromast hair cells
Cluster 5: macula hair cells
Cluster 7: young neuromast hair cells
Cluster 12: crista hair cells

Total Marker Genes:

Cluster 0:	2352
Cluster 5:	1023
Cluster 7:	2029
Cluster 12	:928

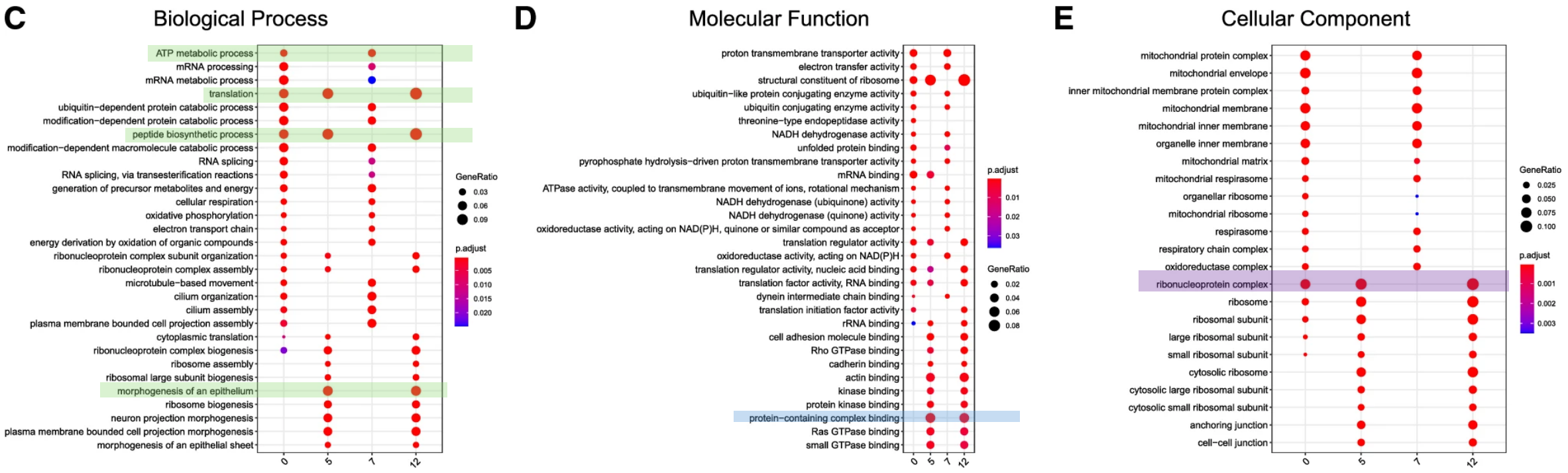
Genes potentially related to hearing loss

What was determined from the molecular analysis?



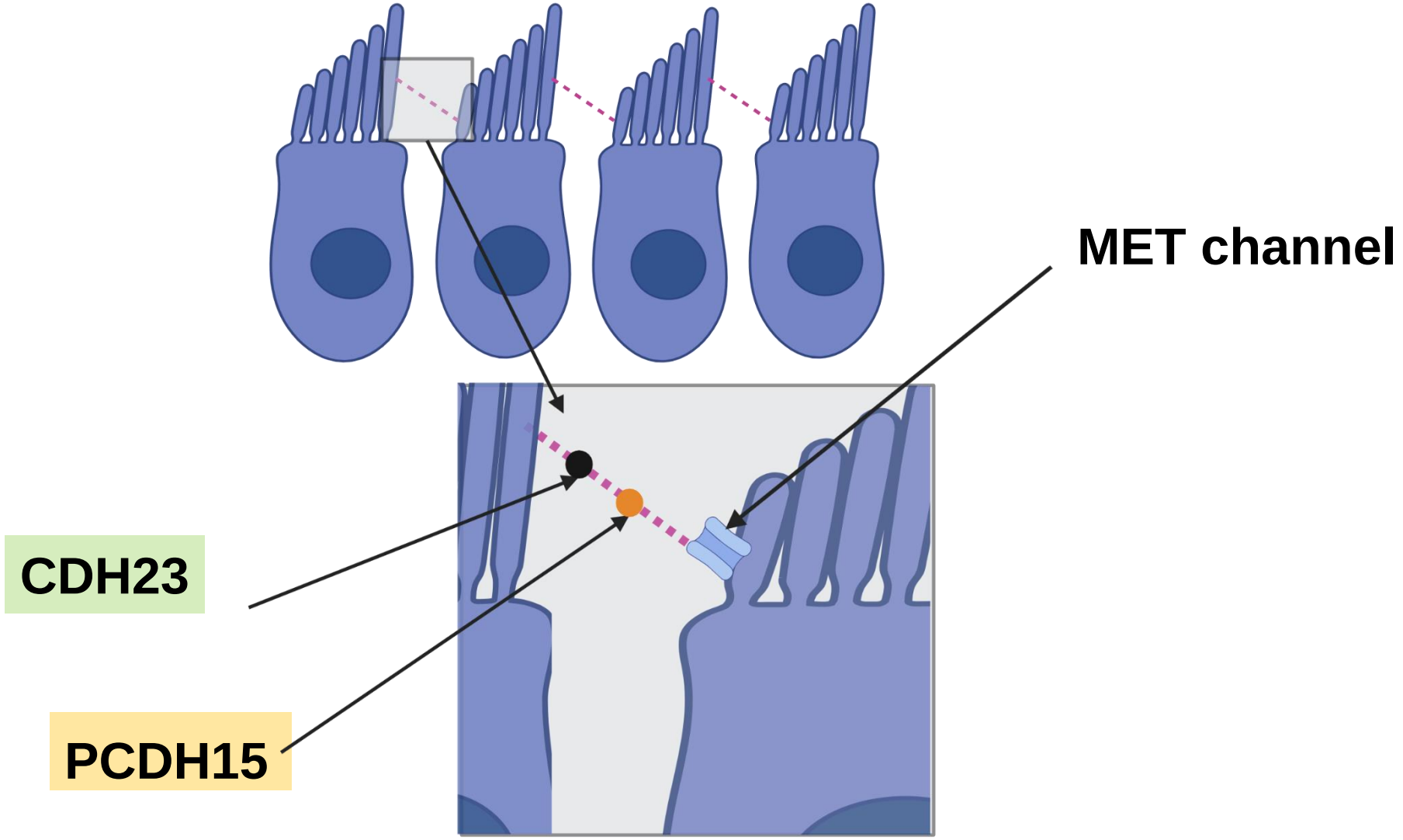
The marker genes in each cell cluster differ

What is the gene ontology of the different hair cells?



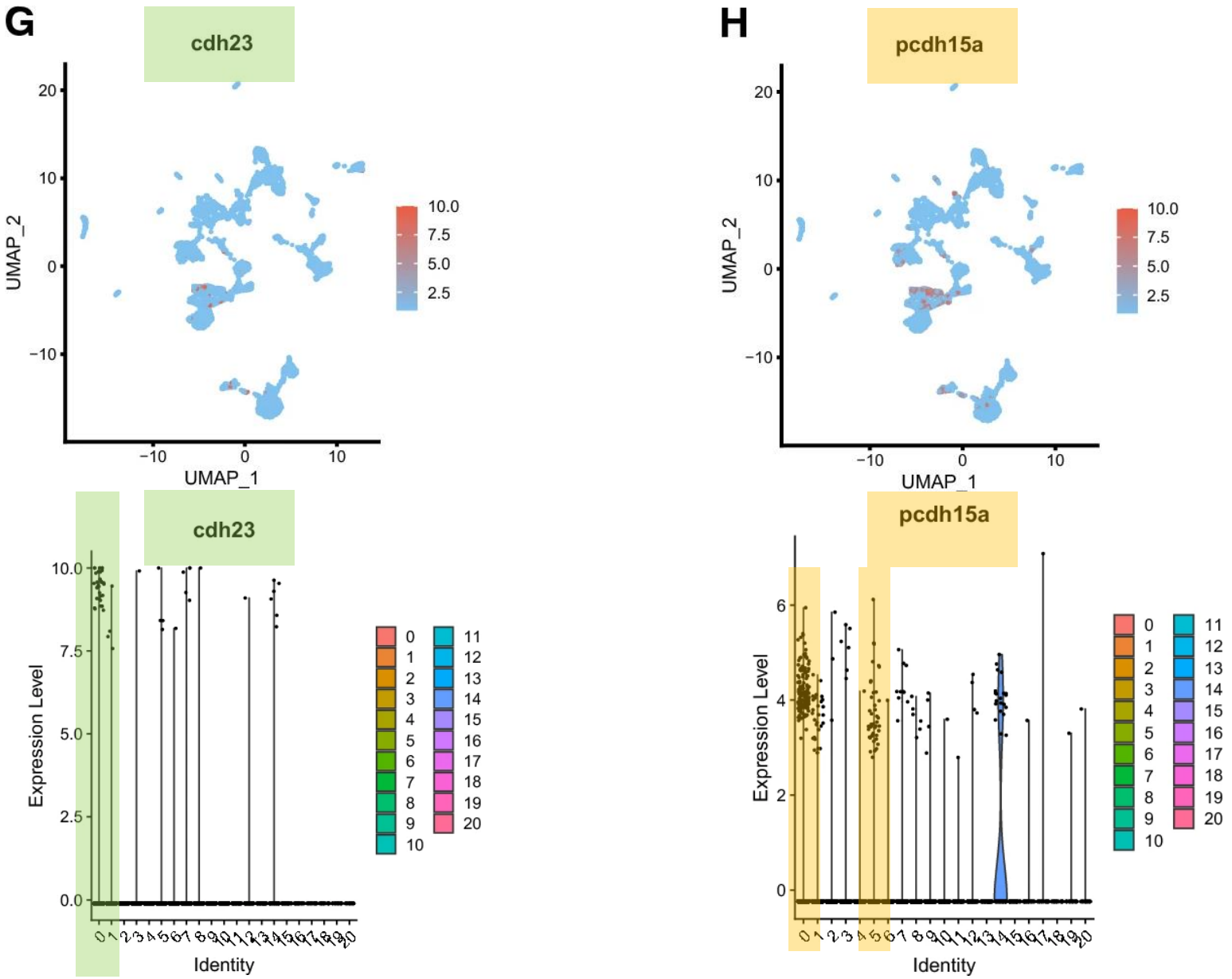
Cluster 0: mature neuromast hair cells
Cluster 5: macula hair cells
Cluster 7: young neuromast hair cells
Cluster 12: crista hair cells

What are indications of mammalian hair cell function?



MET channels that are essential for hearing

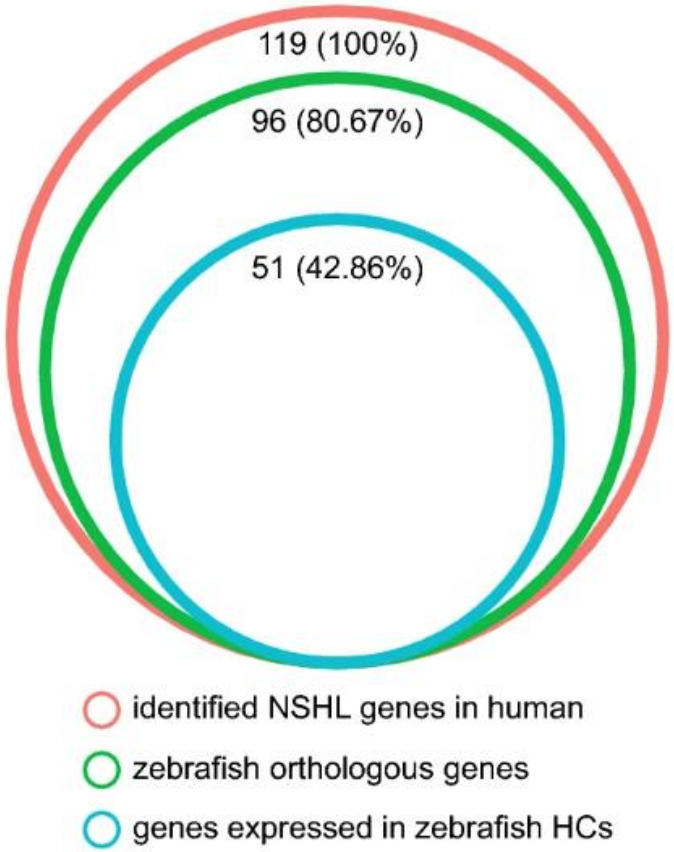
Zebrafish hair cell's expression of MET components

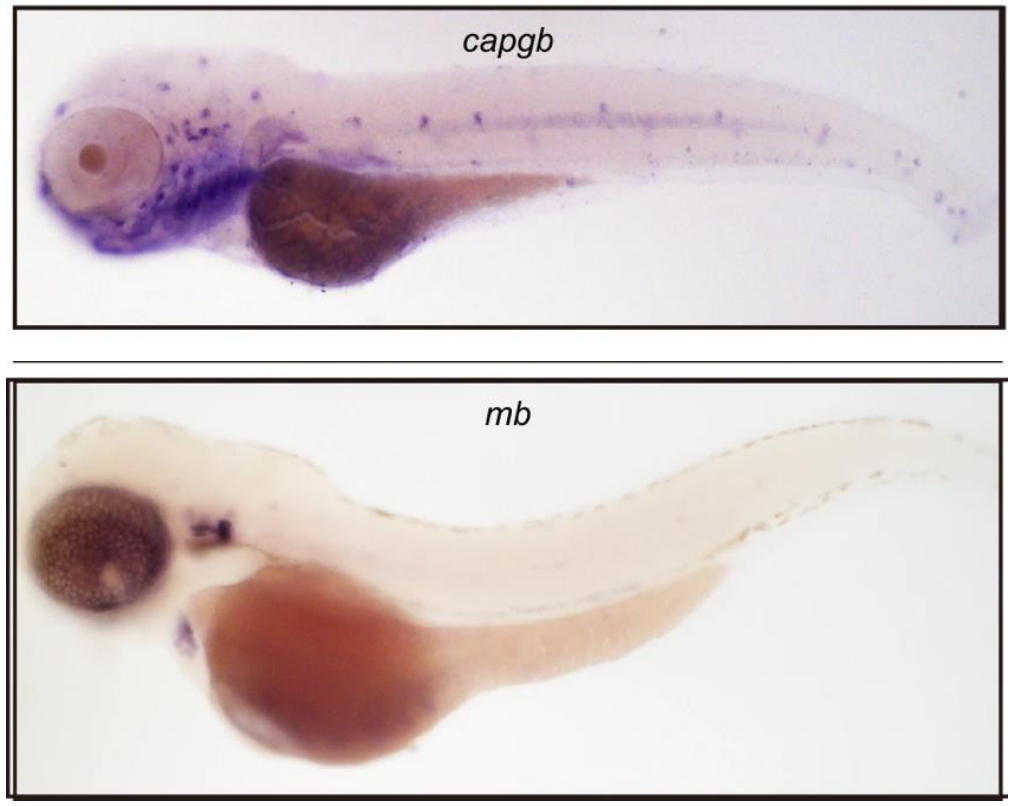


Cluster 0: CDH23 and PCDH15 expression

Cluster 5: PCDH15 expression

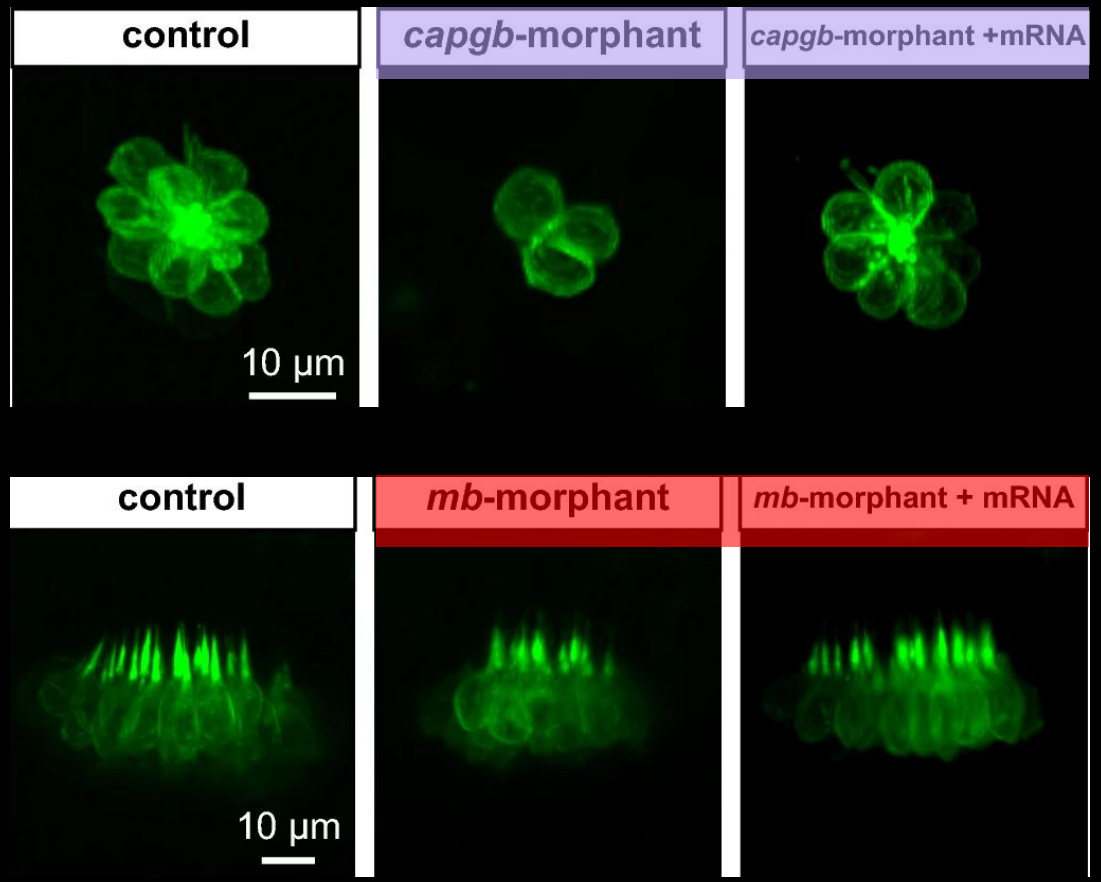
How can we use this scRNA-seq data?





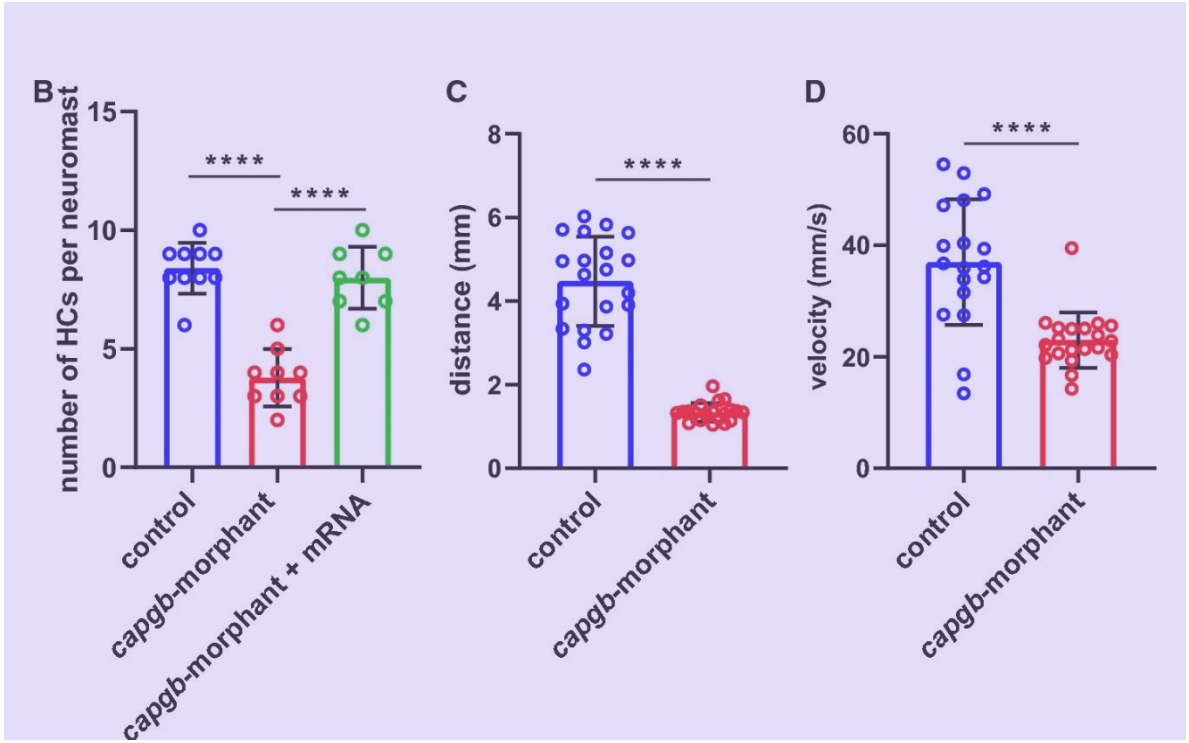
It can lead to genes of interest

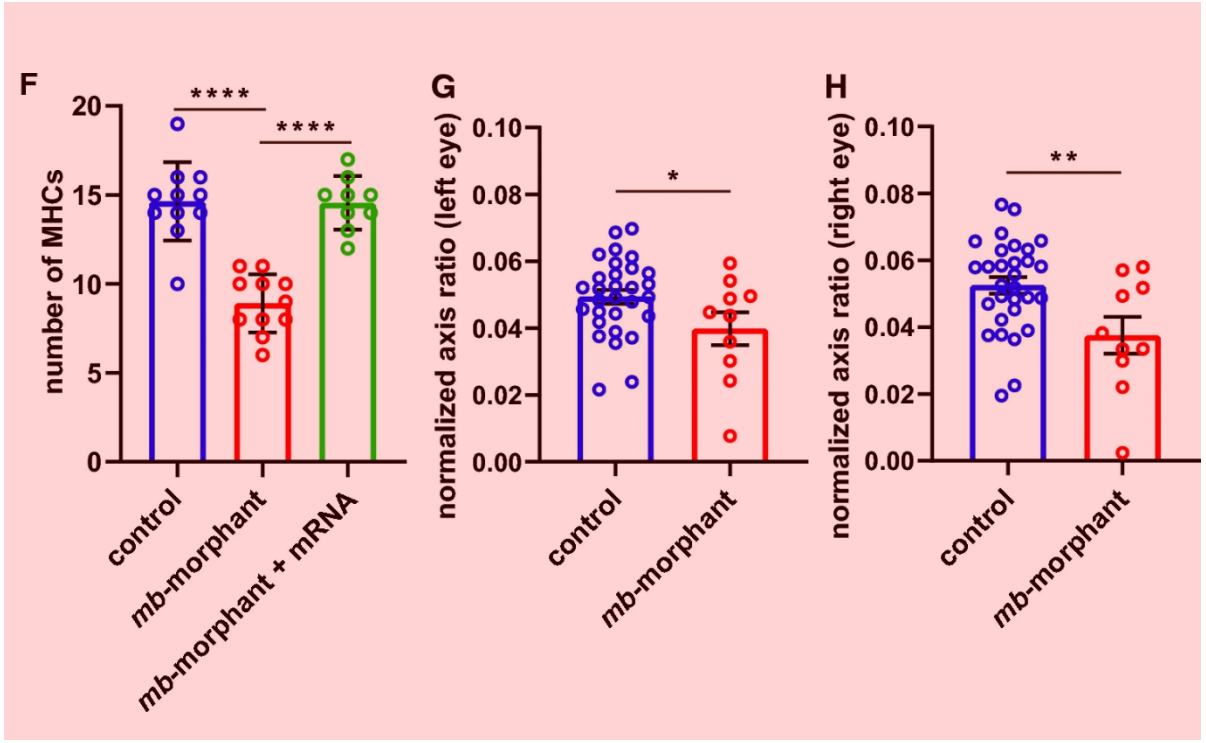
What did analysis of capbg and mb genes provide?



Down-regulation of both genes resulted in loss of hair cells

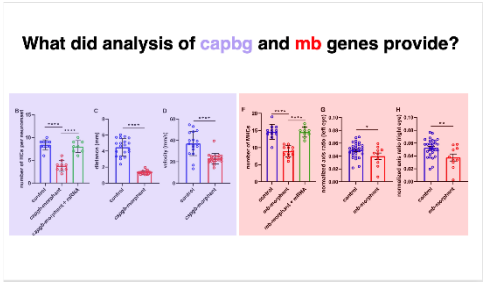
What did analysis of capbg and mb genes provide?



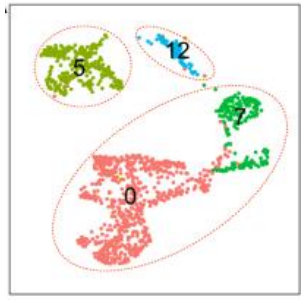


What is the future direction of this study?

Looking at the role of down-regulating capbg and mb genes in hair cell activity as a whole

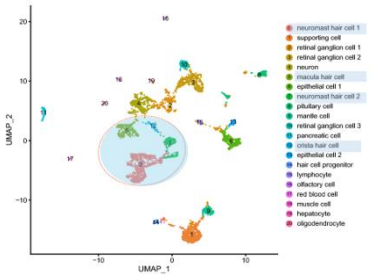


What are the mechanisms of these genes in hearing loss?

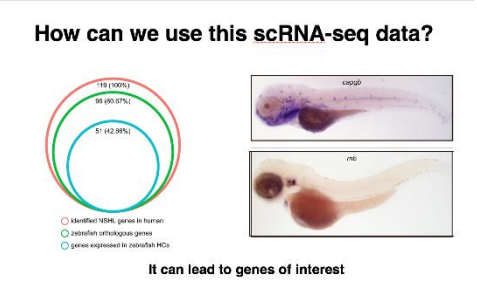


How does each cell type individually affect hearing loss?

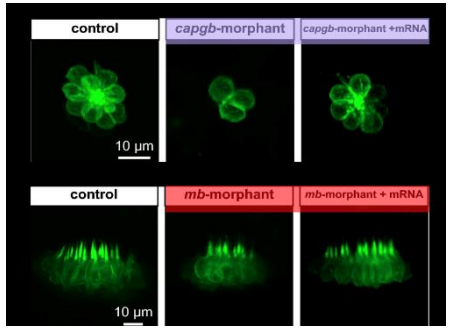
Summary



scRNA sequencing provides insight into the different hair cell types of zebrafish



Gene orthologs associated with hearing functions identified



Correlation between down-regulation of Capgb and Mb genes and hearing function

Questions?

References

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[3] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009 Jan;10(1):57-63. doi: 10.1038/nrg2484. PMID: 19015660.

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