

Single-Cell RNA Sequencing Reveals Age-Associated Patterns of Hair Bundle Gene Expression

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Introduction

The hair bundle of cochlear hair cells is responsible for mechanosensation of the sound pressure wave. Many genes associated with human hearing loss encode protein products that localize to the hair bundle. Patterns of expression of these genes have not been thoroughly examined beyond the onset of hearing. Our study examines patterns of expression from the onset of hearing to middle age.

Single-Cell Isolation

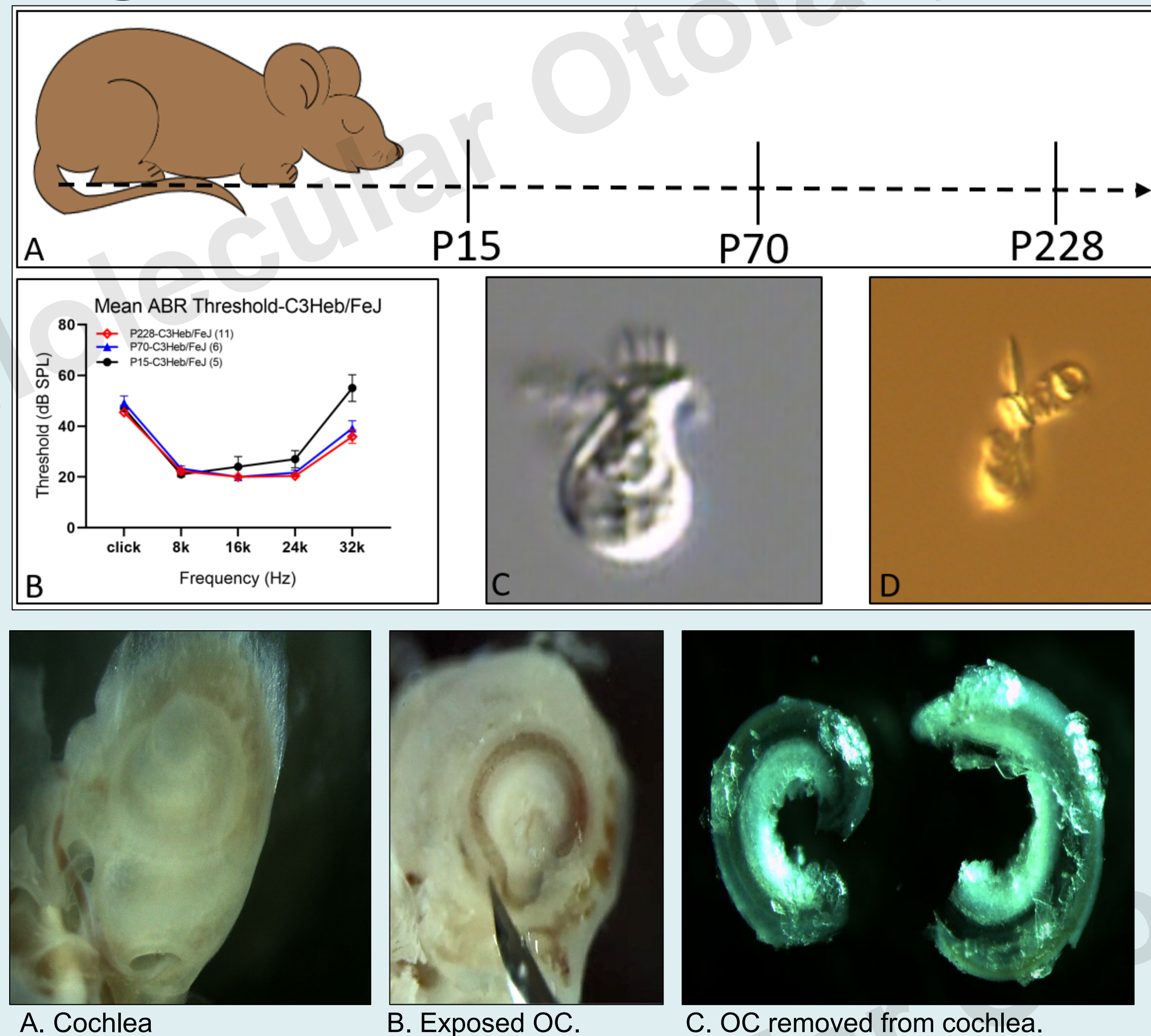


Figure 1: Summary of Experimental Timeline. Inner (C) and outer (D) hair cells were isolated from C3Heb/FeJ mice at the onset of hearing (P15), maturity (P70), and middle age (P228) (A). C3Heb/FeJ mice were selected for this study as they do not show age-related hearing loss across the measured time points (B). For this analysis, we focused on the outer hair cells (OHCs).

Figure 2: Single cell isolation. (A&B) Cochlea are harvested from wild-type mice and the boney covering is removed. (C) The Organ of Corti (OC) is dissected from the cochlea. (D) Hair cells are then dissociated from the OC using a combined enzymatic and manual micropipetting technique.

Single-Cell RNA Sequencing & Bioinformatics

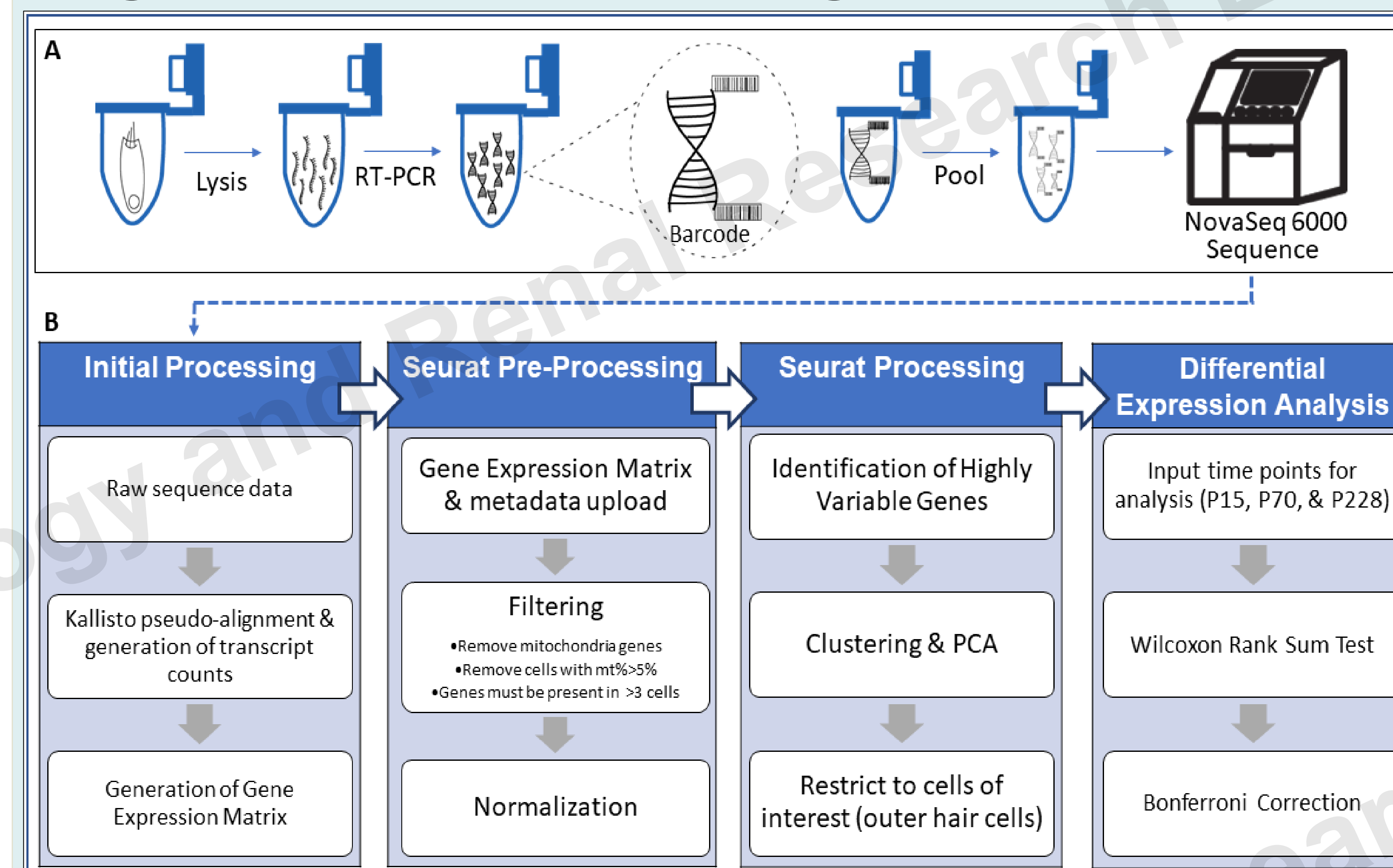


Figure 3: Single-cell RNA sequencing Workflow. (A) Processing of single-cell samples for sequencing. (B) Bioinformatic processing and analysis workflow.

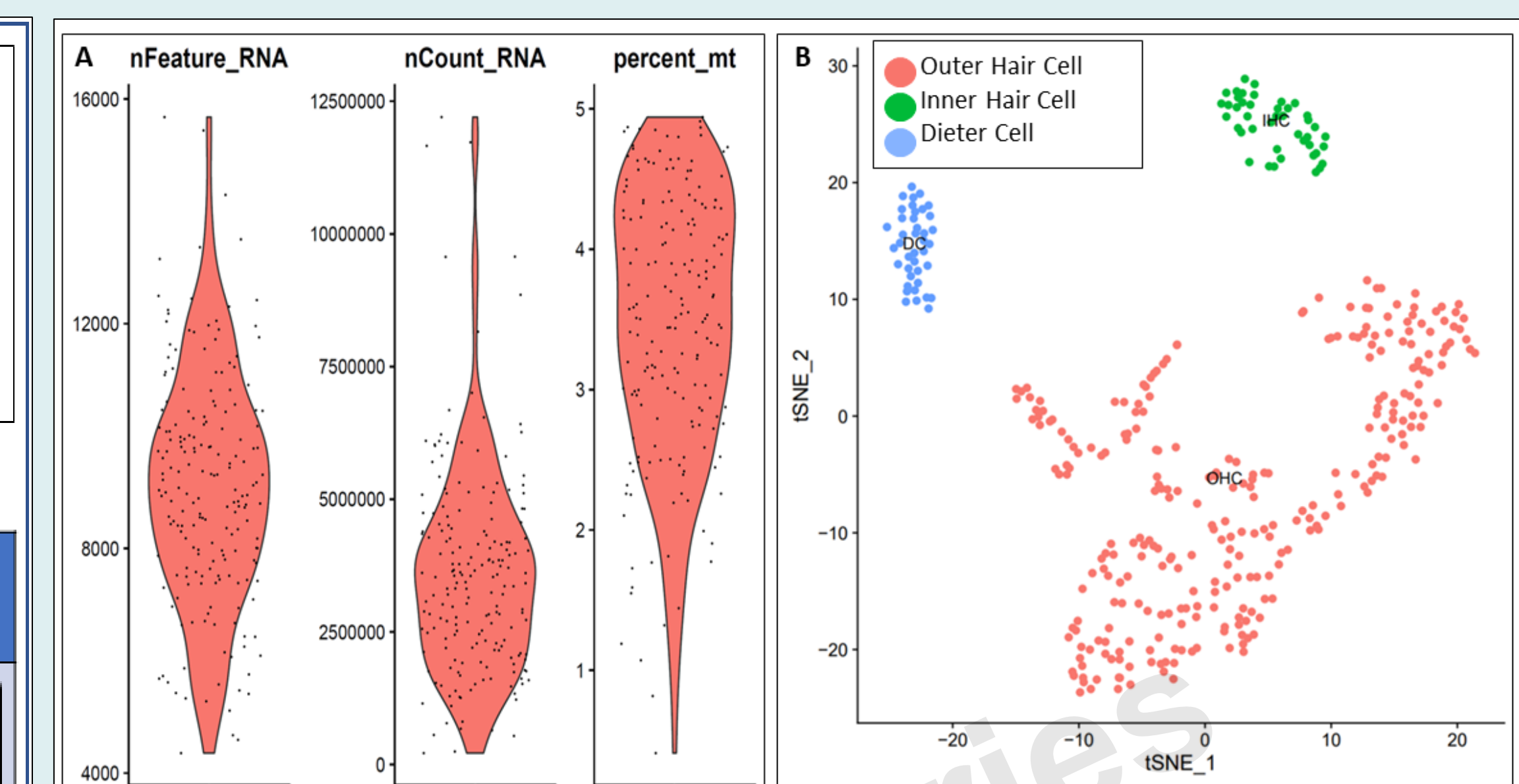


Figure 4: Quality filtering and cell clustering. (A) Number of unique genes/features, read counts, and mitochondrial gene percent of each cell. (B) Clustering of inner hair cells, outer hair cells, and Deiter's cells.

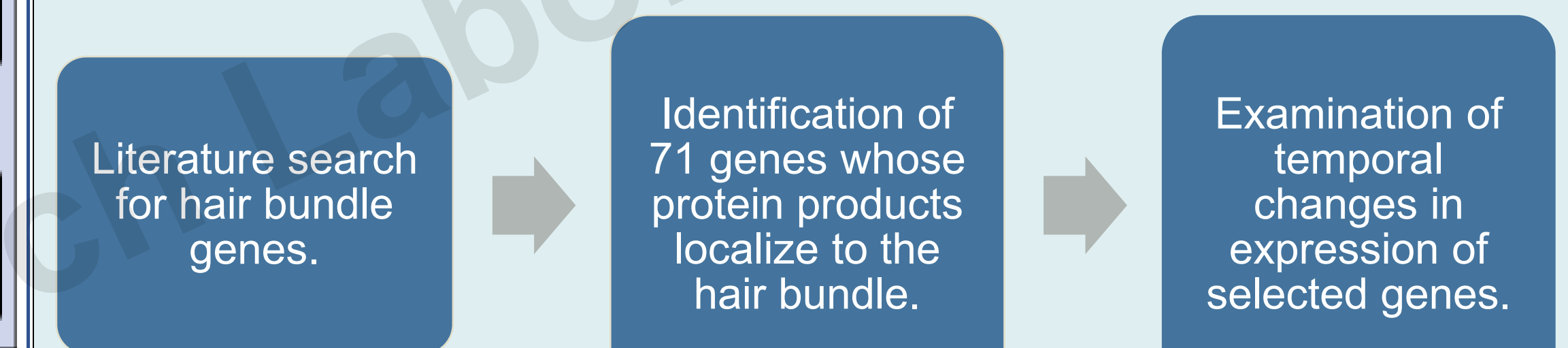


Figure 5: Selection of hair-bundle genes for differential expression analysis.

Results

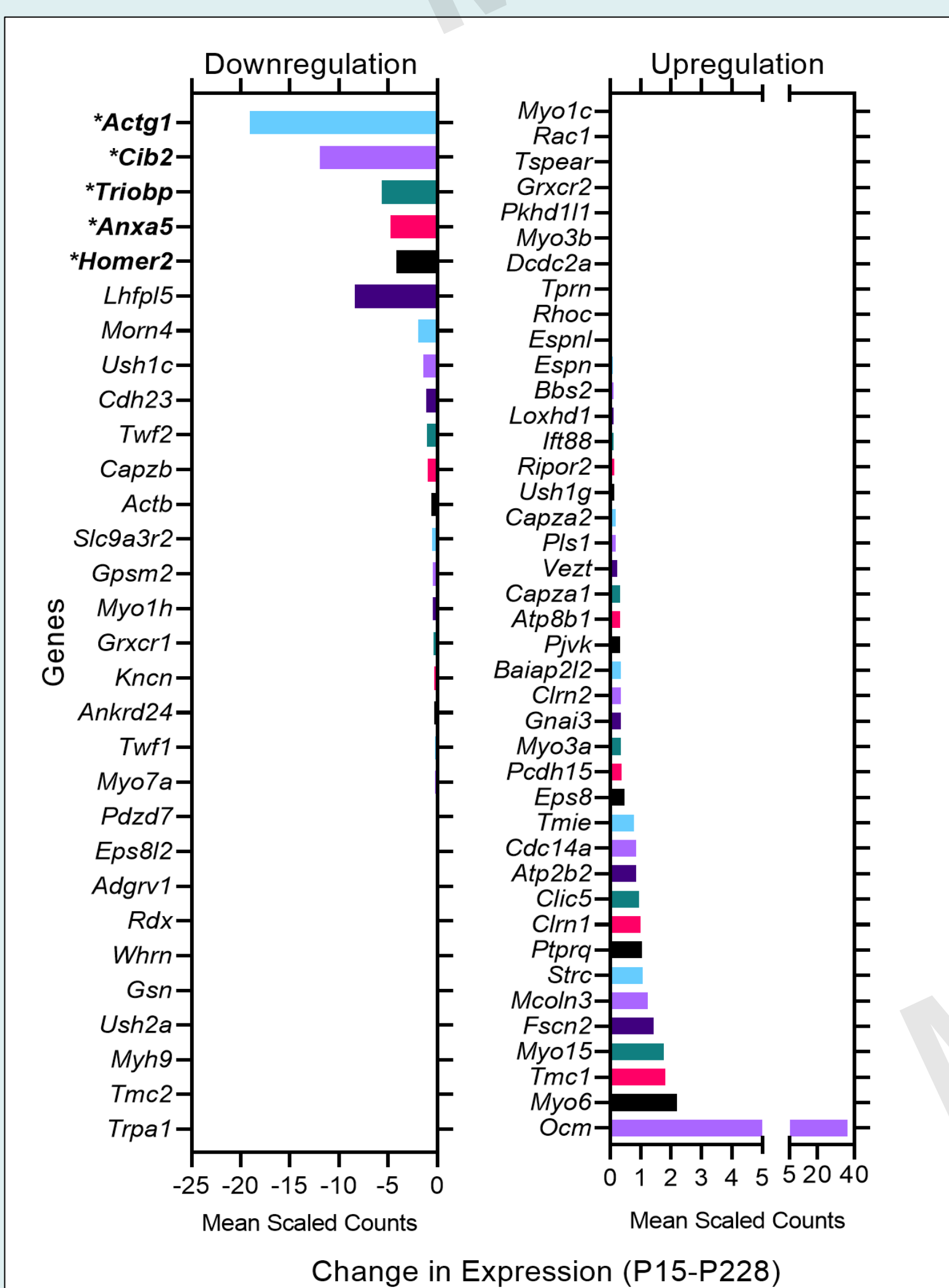


Figure 6: Differential expression analysis. 5 of 71 genes examined showed significant temporal changes in expression after Bonferroni Correction (*).

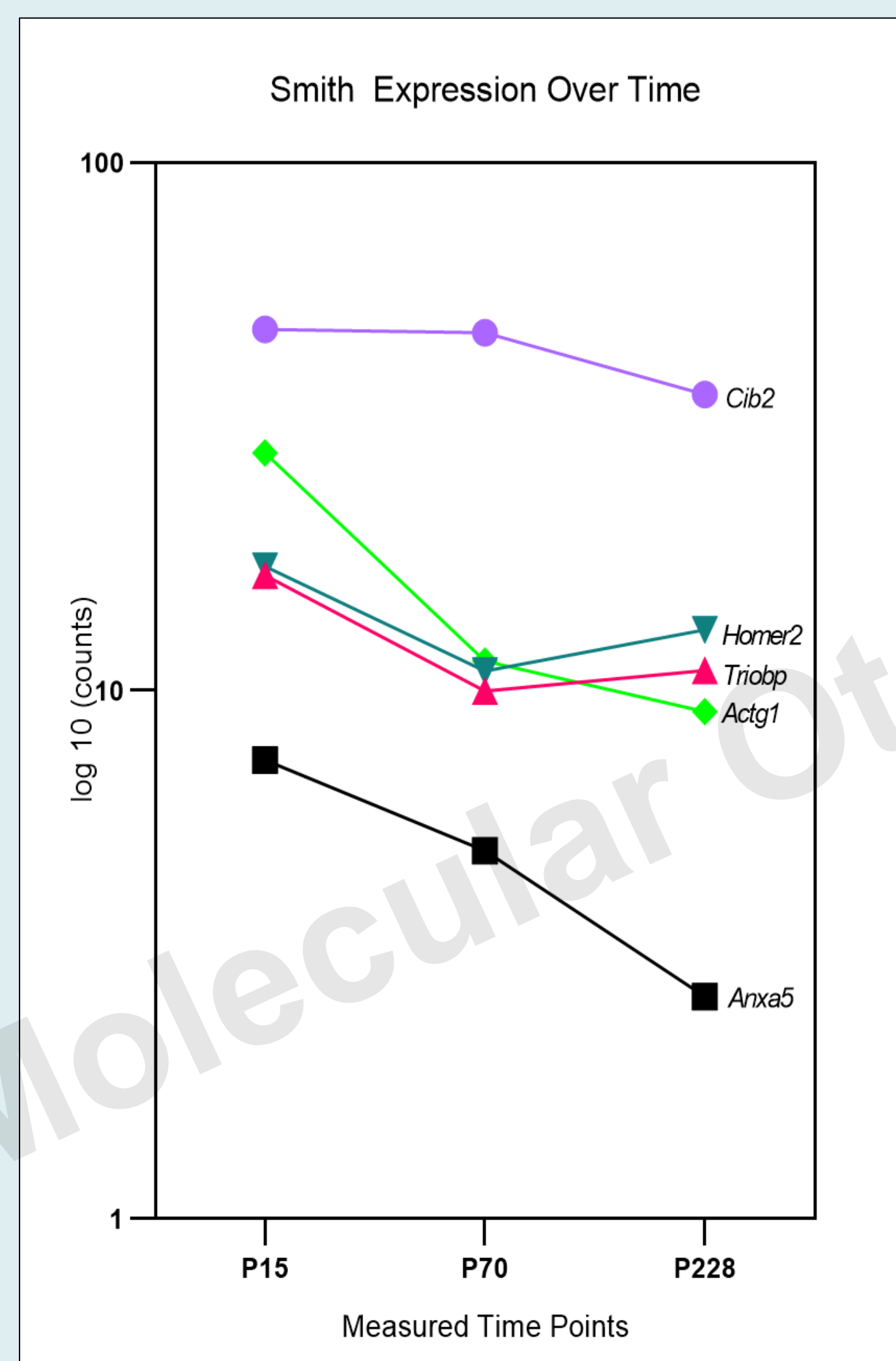


Figure 7: Expression levels of the 5 differentially-expressed genes across measured time points.

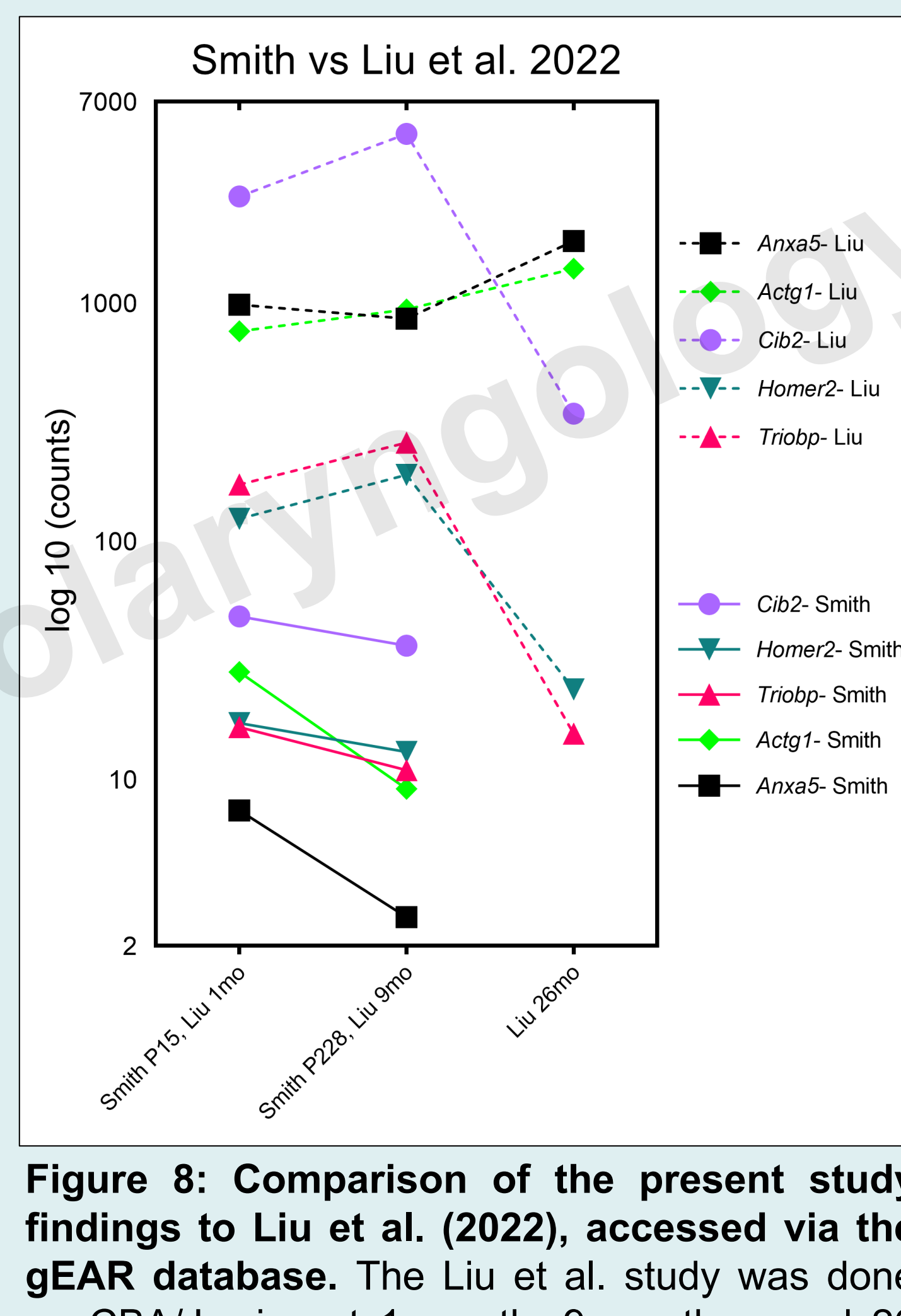


Figure 8: Comparison of the present study findings to Liu et al. (2022), accessed via the gEAR database. The Liu et al. study was done on CBA/J mice at 1 month, 9 months, and 26 months of age. Liu et al. did not show the same patterns of differential expression between P15/1mo to P228/9mo.

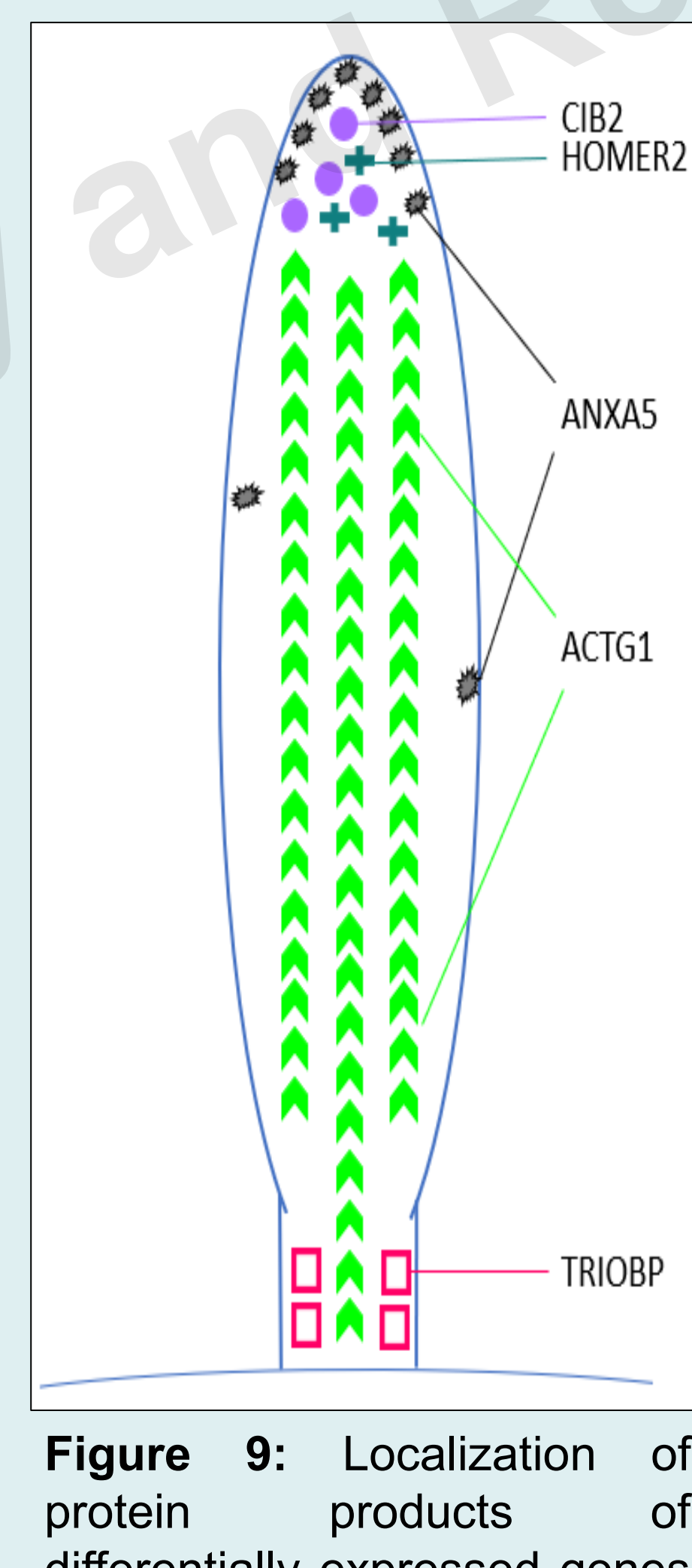


Figure 9: Localization of protein products of differentially expressed genes within the stereocilia.

Conclusions

- Expression of five genes (*Actg1*, *Anxa5*, *Cib2*, *Homer2*, and *Triobp*) whose protein products localize to the hair bundle decreased over time even prior to the onset of age-related hearing loss.
- Our data set shows normal changes in expression over time in the absence of detectable hearing loss.
- Cataloging changes in hair cell gene expression that occur prior to the onset of age-related hearing loss provides context to transcriptomic studies in models of age-related hearing loss.
- When comparing scRNA-seq datasets, information regarding the genetic background of the model used, single-cell isolation technique, library preparation method, bioinformatic processing pipeline, and batch bias must all be taken into consideration.

References

- Full list of references available via the QR code.
- Liu H, Giffen KP, Chen L, et al. Molecular and cytological profiling of biological aging of mouse cochlear inner and outer hair cells. *Cell Rep.* 2022;39(2):110665. doi:10.1016/j.celrep.2022.110665
 - Orvis J, Gottfried B, Kancherla J, et al. gEAR: Gene Expression Analysis Resource portal for community-driven, multi-omic data exploration. *Nat Methods.* 2021;18(8):843-844. doi:10.1038/s41592-021-01200-9
 - Richardson GP, Petit C. Hair-Bundle Links: Genetics as the Gateway to Function. *Cold Spring Harb Perspect Med.* 2019;9(12):a033142. Published 2019 Dec 2. doi:10.1101/cshperspect.a033142



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