AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 03, Issue: 02 (February, 2026)
Available online at http://www.agrimagazine.in

Single-Cell RNA Sequencing and Its Applications in Fisheries

*Shasti Risha K, A Chandrasekhara Rao, Dheeran P, Shivkumar, Bheemeswararao and Suguna T
Fisheries Research Station, Andhra Pradesh Fisheries University, Undi, India
*Corresponding Author's email: shastirishak@gmail.com

Single-cell RNA sequencing (scRNA-seq) enables gene expression analysis at the individual cell level, which surpasses traditional bulk RNA sequencing because it shows both cellular heterogeneity and the existence of rare cell types. The method starts with single cell isolation which leads to RNA capture and reverse transcription before cDNA amplification and sequencing occur and finally bioinformatic analysis identifies different cell populations along their developmental pathways. The use of scRNA-seq in fisheries research has discovered immune cell differences between species such as sharks and zebrafish and largemouth bass which helps improve disease control methods. The system reconstructs cell lineage relationships which show craniofacial development of pipefish and hematopoietic development across different animal species. The method establishes gene regulatory networks while it reveals immune response patterns to environmental stress factors which assist in diagnosing diseases. The system uses scRNA-seq technology to study how cells react to drugs while their resistance mechanisms develop and it studies endangered species by examining their genetic variation and reproductive behavior. The system uses scRNA-seq to give researchers detailed insight into cellular systems which helps their research in immunology and development and environmental studies and drug development and conservation efforts.

Introduction

Transcriptome analysis studies the complete set of RNA transcripts which an organism's genome generates. The first method for conducting whole transcriptome studies identifies all transcript types present in a sample which includes both siRNA and lncRNA regulatory transcripts, but it restricts researchers from testing single cell samples. In 2009, the first scientific and technological advancement of single cell RNA sequencing technology arose, upon sequencing the blastomere and oocytes transcriptome. Single-cell RNA sequencing (scRNA-seq) has brought about a major transformation to our knowledge of cellular diversity through its ability to provide detailed transcriptome data. ScRNA-seq enables researchers to study gene expression patterns in single cells, which distinguishes it from traditional bulk RNA sequencing methods that offer average gene expression data for groups of cells. The advanced method enables scientists to study intricate biological structures such as tissues and organs, which lets them identify various cell types and their different developmental stages. Scientists use scRNA-seq technology to study single cell transcriptomes, which enables them to uncover basic biological mechanisms that drive cell differentiation and disease development and treatment response.

RNA-Seq Vs. Sc-RNA Seq

Particulars	RNA-Seq	Sc-RNA-Seq
Sample size	Gene expression profiles are analyzed from bulk samples containing thousands to millions of cells eg. Liver	Analyzes gene expression profiles at the level of individual cells eg. hepatocytes

Resolution	Unable to distinguish between different cell types or states present within the sample.	Offers single-cell resolution, identify and characterize cellular subpopulations within heterogeneous samples
Data Complexity	Straightforward, consisting of aggregated gene expression profiles for the entire sample.	More complex, as they comprise gene expression profiles from individual cells
Experimental Design	Require less sample preparation and sequencing depth	Require specialized protocols for single-cell isolation, library preparation, and data analysis
Applications	Compare expression patterns between different experimental conditions or sample types	Studying cellular heterogeneity, cell lineage dynamics, cell state transitions

Workflow of Sc-RNA Sequencing

- **Cell Isolation**: The process begins with the isolation of single cells from the biological sample of interest, which could range from tissue to cultured cells. Various techniques such as fluorescence-activated cell sorting (FACS), microfluidics, or manual picking are employed to ensure the isolation of individual cells while maintaining their viability and integrity.
- **Cell Lysis and RNA Capture**: Once isolated, the individual cells undergo lysis to release their RNA content. This RNA is then captured using oligo-dT primers or other methods, allowing for the preservation of the mRNA transcripts.
- **Reverse Transcription**: The captured RNA molecules are reverse transcribed into complementary DNA (cDNA) using reverse transcriptase enzymes. This step is crucial for converting RNA into a stable form suitable for subsequent amplification and sequencing.
- **Amplification**: The cDNA undergoes amplification to increase the amount of material available for sequencing. This amplification step can introduce biases and artifacts, so various methods, such as polymerase chain reaction (PCR) or in vitro transcription (IVT), are employed to minimize these effects.
- **Library Preparation**: The amplified cDNA is then prepared into sequencing libraries, typically by adding sequencing adapters and barcodes that enable multiplexing of samples. This step ensures that each cell's transcriptome can be uniquely identified during downstream analysis.
- **Sequencing:** The prepared libraries are sequenced using high-throughput next-generation sequencing platforms, such as Illumina or 10x Genomics. This step generates millions of short sequence reads corresponding to the transcripts present in each individual cell.
- **Data Analysis**: Finally, the raw sequencing data undergoes bioinformatic analysis to infer gene expression profiles for each cell. This analysis involves several steps, including read alignment, quantification of gene expression levels, identification of cell clusters, and characterization of cellular heterogeneity. Advanced computational methods and specialized software packages are often employed for this purpose.

Sc-RNA Sequencing data analysis

- Pre-processing and visualization: Raw data generated by sequencing machines are processed to obtain matrices of molecular counts (count matrices) or, alternatively, read counts (read matrices), depending on whether unique molecular identifiers (UMIs)
- Quality control: The process of assessing the quality and reliability of individual cells' gene expression profiles before downstream analysis. The distributions of these QC covariates are examined for outlier peaks that are filtered out by thresholding. Datasets that contain heterogeneous mixtures of cell types may exhibit multiple QC covariate peaks.
- Normalization: Normalization is the process of adjusting gene expression measurements to account for technical biases and differences across cells. Technical bias includes

differences in sequencing depth (the total number of reads obtained for each cell), cell size, RNA quality, and capture efficiency. Total count normalization refers to dividing the expression counts of each gene by the total count for that cell. Eg. DESeq2 Normalization, Scran Normalization in R package

- Data correction: Normalized data may still contain unwanted variability. Data correction targets further technical and biological covariates such as batch, or cell cycle effects. Available algorithm for batch effect correction- HarmonyIntegration(), and cell cycle scoring - CellCycleScoring().
- Data integration: Process of combining information from multiple scRNA-seq datasets or experiments to enable joint analysis and comparison of cell populations. Data integration methods includes: Mutual Nearest Neighbours, Canonical Correlation Analysis.

Applications of ScRNA sequencing in fisheries

- **Cellular Heterogeneity** - ScRNA-seq enables the identification and characterization of rare cell populations and subtypes within complex tissuesIn white-spotted bamboo shark (*Chiloscyllium plagiosum*), zebrafish (*Danio rerio*), and Chinese tongue sole (*Cynoglossus semilaevis*), scRNA-seq identified rare immune subsets like phagocytic B cells (B-4 cluster with *mmp9*, *prdx6*) and immature B cells, revealing species-specific proportions (e.g., 52% immature B cells in shark) (Schneider et al., 2024). In largemouth bass (*Micropterus salmoides*) head kidney, it characterized macrophage and lymphocyte diversity for aquaculture disease management (Wang et al., 2025).
- **Cell hierarchy reconstruction** - ScRNA-seq can capture dynamic changes in gene expression associated with cellular state transitions, such as differentiation, reprogramming, or response to environmental stimuli. Gulf pipefish (*Syngnathus scovelli*) embryo scRNA-seq (35,785 cells) traced craniofacial development trajectories, identifying *scpp1*-regulated tooth loss and dermal armor lineages for breeding body shape traits (Small et al., 2024). Shark, zebrafish, and sole immune scRNA-seq reconstructed hematopoietic stem cell (HSC, *meis1b*+) differentiation to T/B cells, showing conserved trajectories (Schneider et al., 2024).
- **Inferring regulatory networks** - Sc-RNA seq reveals the interactions between genes that control gene expression patterns within individual cells or across cell populations. Bamboo shark B cell subsets revealed networks like protein glycosylation (*ost4*, *ddost*) in plasma cells (B-6) and phagocytosis pathways (*rhoh*, *met*) upregulated versus bony fish, inferring divergent regulation via shared DEGs (Schneider et al., 2024). Pipefish atlases inferred *runx2b*-driven skeletal networks from mesenchymal cells (Small et al., 2024)
- **Disease diagnostics and immune profiling** - ScRNA seq reveals the specific cell types involved in disease pathogenesis. Tilapia (Oreochromis niloticus) gill scRNA-seq profiled heterogeneity post-microplastic exposure, identifying ionocyte and pillar cell responses for pollution diagnostics in farmed fish (Yan et al., 2024). Cavefish (Astyanax mexicanus) LPS stimulation showed IFNG+ T cells and FOXO1B+ RAG+ B cells, profiling cytokine and antigen roles (Forberg et al., 2022).
- **Drug Discovery and Development** - ScRNA-seq can be used to assess cellular responses to drug treatments. Uncover mechanisms of drug resistance by profiling individual cells from drug-resistant tumors or cell lines. Largemouth bass immune atlas supports screening drug responses in leukocyte subsets, capturing resistance signatures like upregulated stress genes (Wang et al., 2025). Zebrafish models infer combinatorial therapies targeting conserved T cell cytokines (*ifng*, *il12*) (Schneider et al., 2024).
- **Conservation genomics** - ScRNA-seq enables researchers to assess genetic diversity within and between populations of endangered or threatened species at the single-cell level. Yangtze sturgeon (*Acipenser dabryanus*) scRNA-seq atlas profiled gamete diversity for fertility monitoring in this endangered species, identifying reproductive regulatory variants (Zhang et al., 2025). Pipefish brood pouch cells showed endocytosis networks (*C-type lectins*) for population resilience assessment (Small et al., 2024).

Conclusion

Single-cell RNA sequencing (scRNA-seq) has transformed fisheries research by revealing cellular heterogeneity, developmental pathways, and immune responses with high precision. The technology benefits disease control and medication development and environmental protection efforts through its ability to detect unusual cell types and active biological systems. Future advances integrating scRNA-seq with multi-omics and spatial transcriptomics, alongside expanded species studies and enhanced computational tools, will deepen biological insights and support sustainable aquaculture, health monitoring, and conservation genomics, establishing scRNA-seq as essential in fisheries science.

References

1. Daniels, R. R., Taylor, R. S., Robledo, D., & Macqueen, D. J. (2023). Single cell genomics as a transformative approach for aquaculture research and innovation. Reviews in Aquaculture, 15(4), 1618–1637. https://doi.org/10.1111/raq.12806
2. Forberg, T., et al. (2022). RNA-Seq of single fish cells – Seeking out the leukocytes mediating immunity in teleost fishes. Frontiers in Immunology, 13, Article 798712. https://doi.org/10.3389/fimmu.2022.798712
3. Schneider, R. F., Singh, P., & Meyer, A. (2024). Single-cell RNA sequencing illuminates the ontogeny, conservation and divergence of immune cells in cartilaginous and bony fishes. Nature Communications, 15, Article 7607. https://doi.org/10.1038/s41467-024-51761-4
4. Small, C. M., Harlin-Cognato, A. D., Allen, J. M., Ryan, T. M., & Cresko, W. A. (2024). Single cell RNA sequencing provides clues for the developmental genetic basis of evolutionary novelty in Syngnathidae. eLife. https://elifesciences.org/reviewed-preprints/97764v1
5. Wang, Y., et al. (2025). Single-cell RNA sequencing reveals the immune cell landscape of largemouth bass (Micropterus salmoides) head kidney. Aquaculture. https://doi.org/10.1016/j.aquaculture.2025.500804
6. Yan, Y., et al. (2024). Single-cell RNA sequencing profiling cellular heterogeneity and distinct responses of tilapia gills to microplastics and nanoplastics. Environmental Science & Technology, 58(15), 6788–6799. https://doi.org/10.1021/acs.est.3c10338
7. Zhang, et al. (2025). Single-cell atlas in the living fossil Yangtze sturgeon provides insights into cell types and evolutionary conservation. Science China Life Sciences.