Construction of a Spatiotemporal Transcriptomic Atlas of Zebrafish Embryogenesis using the DNBSEQ-T7 Sequencer

This application briefly describes how a research team constructed a spatiotemporal transcriptomic atlas of zebrafish embryogenesis using the STOmics gene expression reagent kit (BGI Research) based on DNBSEQ-T7 platform. The results allowed the group to identify the spatial distribution of co-expressed gene modules and ligand-receptor pairs in specific tissues and then construct spatially resolved cell development trajectories using the DNBelab C4 portable single-cell system[1].

Recommended application: Frontier technology – spatiotemporal omics

Recommended model: DNBSEQ-T7RS

- ## Construction of the spatiotemporal transcriptomic atlas

  A spatiotemporal transcriptomic atlas of zebrafish embryogenesis can be constructed using the STOmics gene expression reagent kit S1 and the DNBSEQ-T7 sequencing platform.

- ## Excellent performance in many dimensions

  Transcriptome capture chips were characterized by nanoscale spatial resolution, a centimeter-level panoramic field of view, and high capture efficiency and sensitivity.

- ## One-stop service for experiment and data analysis

  The short experimental process took only 8 hours from tissue section to sequencing library, and the resulting sequencing data was analyzed using the integrated cloud analysis platform.

Background

Embryogenesis in vertebrates is an intricate and dynamic process. During embryogenesis, changes in gene expression can be very intense, and transitions in cellular states can occur frequently within a short time period. Transcription factors, morphological factors, signaling pathways, signals from the extracellular matrix, and other factors play a key role in determining cellular growth and differentiation[2-4]. How these regulators interact spatially to accurately induce cells to take on different forms, reside in different locations, and realize different functions to give rise to complex invertebrate embryos is a fundamental question in embryogenesis that requires further research.

Zebrafish are a model organism commonly used to study vertebrate embryogenesis, with advantages conducive to research such as rapid development, high embryo transparency, and accessibility to physical and genetic manipulation. Today, progress in sequencing techniques has made it possible to assemble a single-cell atlas during the development of a model organism, thereby enabling the multi-modal information analysis of the whole genome, including the gene expression, epigenetic state, and protein levels of a single cell[5,6]. Nevertheless, how transcriptome states of the developing zebrafish relate temporally and spatially remains unknown. Plus, limitations of spatial transcriptome techniques prevent assessing the spatial relationship between different cell types in complex tissues.

Study description

At present, *in situ* hybridization allows us to explore the expression atlas of specific genes during development but not the changes in the overall transcriptome[7]. In contrast, with Tomo-seq, a technique based on overall section sequencing, the spatial information of the tissue section is lost[8]. BGI Research offers the STOmics gene expression reagent kit, a spatiotemporal omics product based on their independently developed Stereo-seq technique used for *in situ* RNA capture and enables simultaneous analysis of genes and images[9]. Compared to other current technologies, Stereo-seq has a more sensitive and stronger mRNA capture capacity with the same precision. As a molecular "microscope" in the new era, this technology is a foundational tool to determine organ structure, life development, special evolution, and human diseases. It promises to user in the third scientific and technological revolution in life science after microscopy and DNA sequencing. Leveraging these advantages, a research team used Stereo-seq to study zebrafish embryonic cells. Obtaining related sequencing data with the DNBSEQ-T7 sequencer, they could construct a spatiotemporal transcriptomic atlas, determine subcellular types and spatial gene modules, and elucidate the spatial distribution of ligand-receptor pairs.

Materials and Methods

Sample preparation

With tweezed egg membranes, zebrafish embryos were preserved overnight in a paraformaldehyde solution at 4°C. They were then dehydrated with methanol at -20°C for at least 2 hours. The dehydrated tissue samples were embedded in OCT embedding medium and sectioned sagittally for Stereo-seq library preparation and sequencing.

Stereo-seq library preparation and sequencing

The Stereo-seq library was prepared using the STOmics gene expression reagent kit S1. The general process was as follows: tissue sections were attached to Stereo-seq chips and incubated in -20°C methanol for 30 min for nucleic acid staining and imaging. To increase cell permeability, tissue sections were permeabilized for 3 min at 37°C if obtained within 18 h of fertilization or for 5 min if obtained within 18~24 h of fertilization.

After reverse transcription of RNA into cDNA, the cDNA was purified using magnetic beads. The single-cell RNA-Seq sequencing library was then prepared according to the reagent kit instructions to complete the double-end sequencing (read1: 50 basic groups, read2: 100 basic groups) using the DNBSEQ-T7 sequencer.

Collection of zebrafish embryos and isolation of single cells

About 250~1,000 zebrafish embryos were transferred to a peri dish containing protease E for culture. After incubation, they were dissociated using a trypsin-EDTA solution and suspended in BSA/PBS to obtain a single-cell solution.

Single-cell RNA library preparation and sequencing

The single-cell suspension was diluted and encased in drops by the MGI-developed high-throughput single-cell library preparation reagent kit (940-000047-00) and the DNBelab C4 portable single-cell system. The cells were then cultured at room temperature, and the mRNA released from the cells was captured. After demulsifying the drops, the mRNA was

recovered using magnetic beads, converted to cDNA, and processed by PCR purification and rolling circle amplification (RCA) to prepare DNA nanoballs (DNBs) for signal amplification. Finally, double-end sequencing was performed on the DNBSEQ-T7 sequencer (read 1: 41 basic groups, read 2: 100 basic groups, sample indexing: 10 basic groups).

Data analysis

Transcripts captured by 15*15DNA nanospheres were combined into a bin 15. This bin was considered a basic analysis unit, whose ID was the spatial coordinates on the capture chip. Sections from the same embryo were integrated into one group. Data analyses included data quality control, unsupervised clustering analysis, marker gene analysis, pseudo-time analysis, gene modules analysis, and ligand-receptor distance analysis. All were conducted successively using Seurat, the R software package, and DNBelab C Series HT-scRNA-analysis software, an analysis software developed based on the high-throughput single-cell platform.

Sample collection	Library preparation and sequencing	Bioinformatics Analysis	Result analysis
Zebrafish embryos	 STOmics gene expression reagent kit S1  DNBSEQ-T7 gene sequencer	Seurat DNBelab C Series HT-scRNA-analysis software	Unsupervised clustering analysis, Marker gene analysis, Pseudo-time analysis, Gene modules analysis, Ligand-receptor distance analysis,

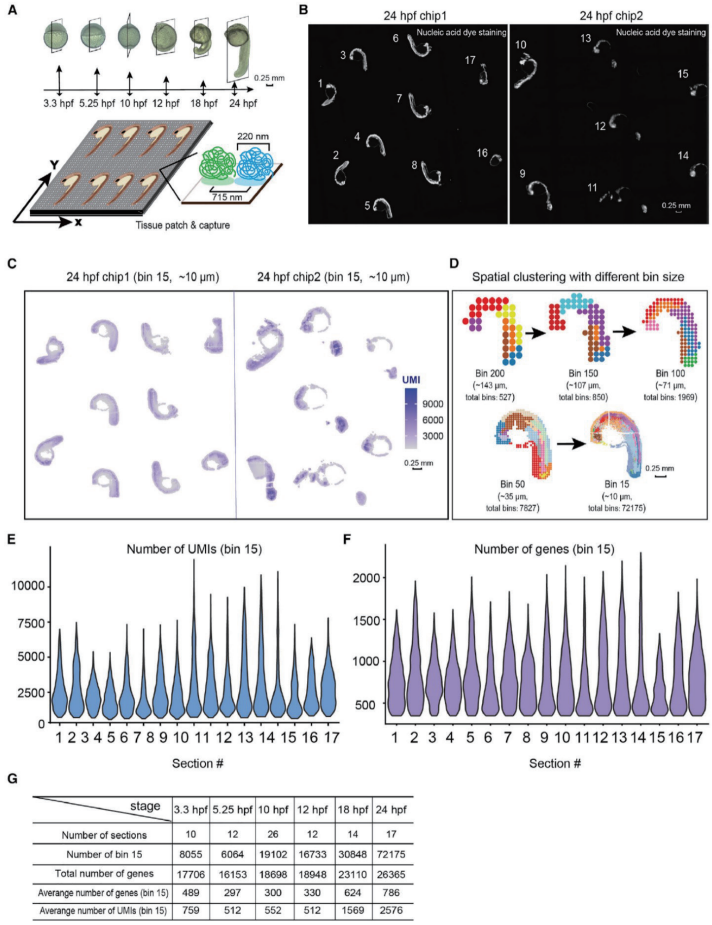
Results

Inspection of capture quality of spatiotemporal transcriptomes

The spatiotemporal transcriptomic sequencing data were quantitatively analyzed and spatial mapped. At a grid size of about 10 μm, the distribution of transcripts captured by spatiotemporal transcriptomes and the original tissue sections was highly consistent (Fig. 1B, C), demonstrating the high capture completeness rate and high spatial resolution of Stereo-seq. The average gene capture number was about ≥ 300, and the average UMI capture number was ≥ 500 (Fig. 1G), meeting the requirements of subsequent analysis.

Display of analysis results of spatiotemporal transcriptomes of zebrafish embryogenesis

Unsupervised clustering analysis of spatiotemporal transcriptomes revealed that the spatial distribution of different tissues during embryogenesis was consistent with the anatomy (Fig. 2A). The captured high-density information enabled deeper clustering analysis to reveal the distribution of different subcellular types, like germinal layer and anterior renal cells, and their marker genes (Fig. 2B-E). Through hotspot analysis of spatiotemporal transcriptomes, co-expressed gene modules during development



stage	3.3 hpf	5.25 hpf	10 hpf	12 hpf	18 hpf	24 hpf
Number of sections	10	12	26	12	14	17
Number of bin 15	8055	6064	19102	16733	30848	72175
Total number of genes	17706	16153	18698	18948	23110	26365
Averange number of genes (bin 15)	489	297	300	330	624	786
Averange number of UMIs (bin 15)	759	512	552	512	1569	2576

Fig. 1. Spatiotemporal transcriptomic capture of zebrafish embryonic section samples during different developmental stages using Stereo-seq.

were explored. These gene modules perform specific functions during development (Fig. 3A) and showed consistency in spatial distribution with all cell groups (Fig. 3B, C).

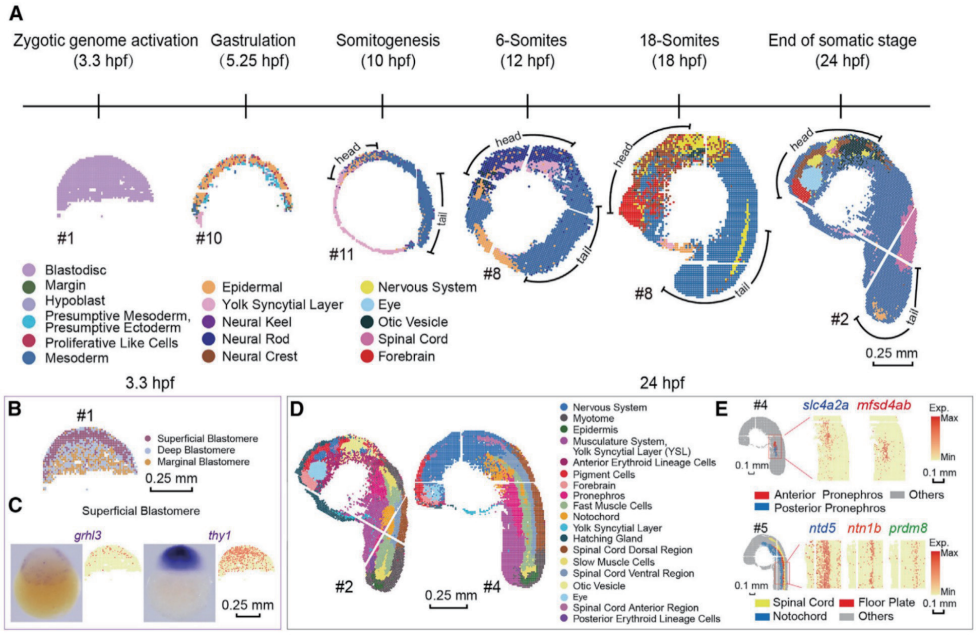


Fig. 2. Spatiotemporal transcriptomic atlas of zebrafish embryogenesis.

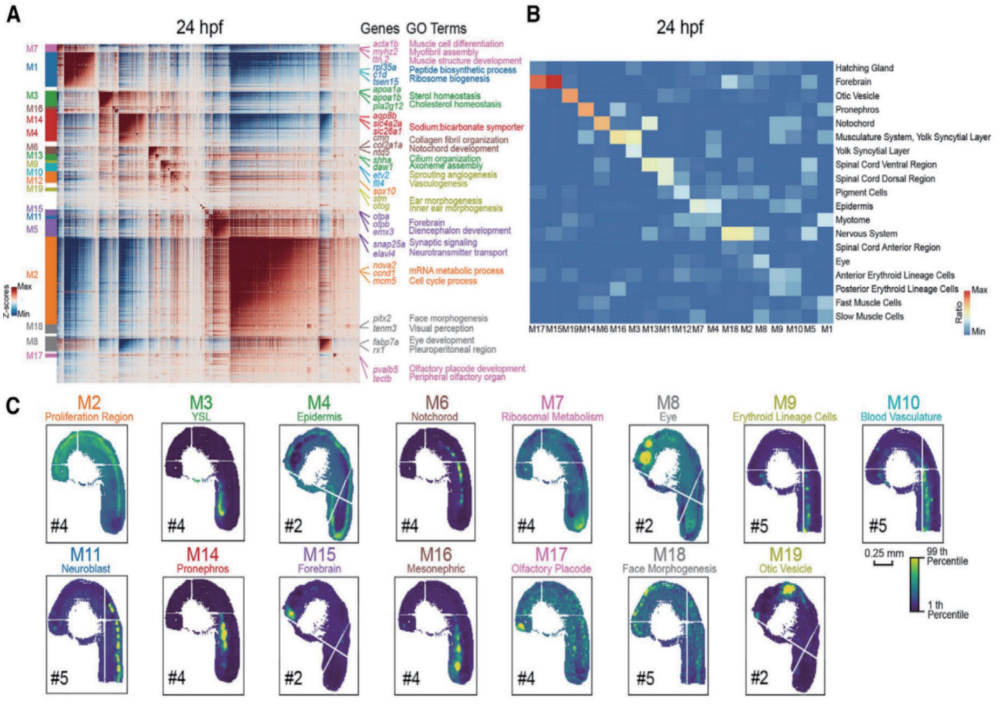


Fig. 3. Function of gene modules and their relationship with the spatial distribution of cell groups.

Based on the single-cell transcriptome sequencing technique and pseudo-time analysis, the spatiotemporal transcriptomes were used to construct the spatiotemporal development trajectories of the embryonic cells (Fig. 4A). Fig. 4B shows the development trajectories of the central nervous system branch and chromocyte branch of zebrafish embryos, which were mapped to spatiotemporal transcriptomes to construct their spatially resolved cell development trajectory atlas (Fig. 4C). Spatiotemporal transcriptomes also permitted calculating the relative spatial distance of ligand-receptor pairs (Fig. 5A, B). The analysis revealed the cell types involved in interactions between these ligand-receptor pairs (Fig. 5C), established the time window of interactions, and allowed exploring potential receptors of ligands.

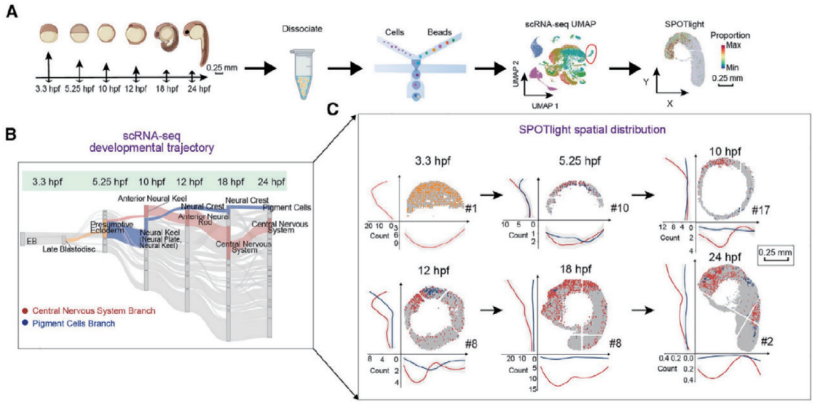


Fig. 4. Construction of spatially resolved embryonic cell developmental trajectory.

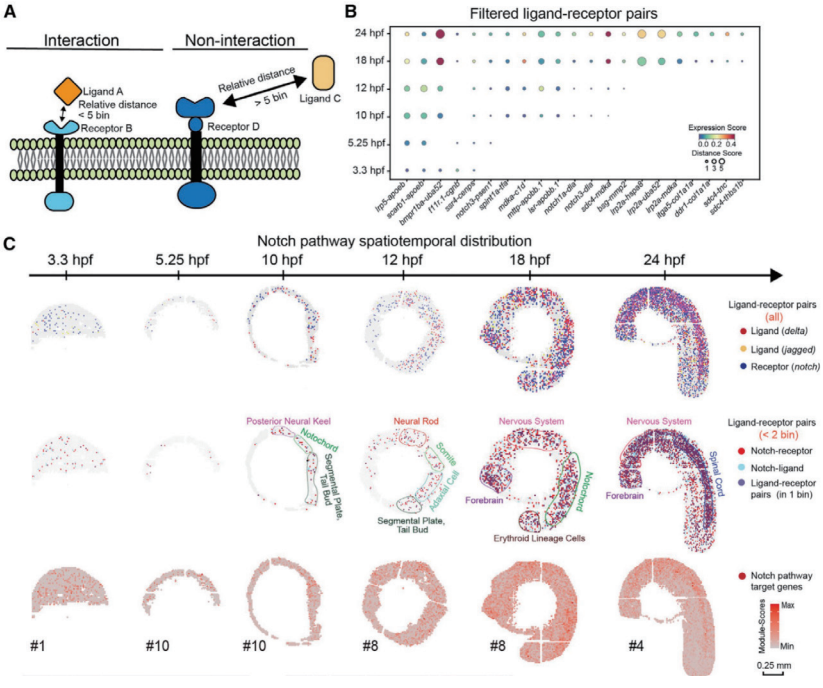


Fig. 5. Relative spatial distance of ligand-receptor pairs and their relationship to a signaling pathway.

Summary

This application briefly describes the construction of a spatiotemporal transcriptomic atlas of zebrafish embryogenesis. The researchers who carried out this work also explored the cell types involved, identified spatial cell modules, and characterized the spatial distribution of ligand-receptor pairs. Finally, they constructed spatially resolved cell development trajectories based on single-cell sequencing. The STOmics gene expression reagent kit and DNBelab C4 portable single-cell system independently developed by BGI Research, combined with the DNBSEQ-T7 sequencer, completed the high-precision and high-density capture of spatiotemporal transcriptomes and the capture and sequencing of single-cell transcriptomes needed to study the cellular and molecular development mechanisms of zebrafish embryogenesis.

DNBSEQ-T7RS Genetic Sequencer

Reference

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	DNBSEQ-T7RS Genetic Sequencer	900-000128-00
Software	Data Center Appliance	900-000444-00
Library Prep Reagents	DNBelab C Series High-throughput Single-cell RNA Library Preparation Set V2.0（16 RXN）	940-000519-00
Sequencing Reagents	DNBSEQ-T7RS High-throughput Sequencing Set（FCL PE100）V2.0	1000028455

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2. For HotMPS sequencers: This sequencer is only available in selected countries, and its software has been specially configured to be used in conjunction with MGI's HotMPS sequencing reagents exclusively.

3. For HotMPS reagents: This sequencing reagent is only available in selected countries.