

# Spatial Transcriptomics and Lineage Analysis for Craniofacial Mesenchymal Fate Determination

Jifan Feng, Eva Janečková, Heliya Ziaei, Tingwei Guo, Mingyi Zhang, Jessica Junyan Geng, Sa Cha, Angelita Araujo-Villalba, Mengmeng Liu, Thach-Vu Ho, and Yang Chai

Center for Craniofacial Molecular Biology, Herman Ostrow School of Dentistry, USC

## INTRODUCTION

differentiation The post-migratory of cranial neural crest cells (CNCCs) into distinct genetically spatially and mesenchymal subpopulations is critical In this for craniofacial development. study, using palate development as a model, we aim to establish a real-time, high-resolution spatial transcriptomic and cell-type atlas to reveal the diversification of CNCC-derived mesenchyme during craniofacial development.



### MATERIALS AND METHODS

RNA Single-cell sequencing was conducted on mouse palatal shelves at E12.5, E13.5, E14.5, E15.5, and E18.5. SeqFISH spatial genomics was performed on cryosections from E12.5, E13.5, and E15.5 using a custom gene panel. For cell lineage tracing, all pregnant female mice received tamoxifen at a dosage of 1.0 mg/10 g or 1.5 mg/10 g body weight.



Fig. 1. Spatial mapping of mesenchymal subpopulations in the E15.5 hard palate region using seqFISH.

Fig. 6. SeqFISH analysis reveals expression patterns of early mesenchymal lineage markers at E12.5.



Fig. 3. Spatial palatal region using seqFISH.

Fig. 2. Spatial mapping of mesenchymal subpopulations in the E15.5 soft palate region using seqFISH.





Fig. 7. seqFISH maps Sox9+ subsets at E12.5, with *Tfap2b* marking odontogenic and *Hic1* labeling perimysial cells, confirmed by lineage tracing.

# CONCLUSION

Our findings reveal the dynamic cellular positioning and gene expression patterns during the specification of CNCCderived mesenchymal subpopulations throughout palatogenesis.

### RESULTS

mapping of markers for mesenchymal subpopulations in the E15.5



genomics data.

a			
	A Resource For Craniofacial Researchers	DATA - RESOURCES - CONTRIBUTE - POLICIES - COMMUNITY - HELP -	
		E Show e	mpty sections
	Dataset: Hig at E12.5, E1	h-Resolution Spatial Profiling and Cell Lineage Anal 3.5, and E15.5	ysis of Mo
	Show side panel		
	Identifiers ①	Record ID: 62-Y0VT Accession: FB00001382 DOI: 10.25550/62-Y0VTC (Released: 2025-04-10)	
	Description	Spatial Genomics performed SeqFISH-based spatial genomics imaging using a customized gene panel on cryosections o their Gene Positioning System (Spatial Genomics). Spatial plots and seqFISH gene expression patterns were visualized us	i E12.5, E13.5, and E15 ing SGNlite software (Sp
	Project	TGF-β Signaling and Craniofacial Morphogenesis	
	Contributor(s) ①	Jifan Feng, Yang Chai	E
	Protocol(s) ①	FrozenTissueSamplePrep_Spatial Genomics	Œ
	Related Dataset(s)	FACEBASE:62-QZ1A	E
	Protected Human Subjects <sup>①</sup>	No	
	Experiment Type ①	Spatial Genomics	E
	Species ①	Mus musculus	
	163.3 um	Carling and the second	

are available in the FaceBase database.



Fig. 8. The raw and processed sequencing-FISH data for embryonic heads at E12.5, E13.5, and E15.5

# ACKNOWLEDGMENTS

Funding support by NIDCR/NIH R01 DE012711 and U01 DE028729.