

Methods to Enable Spatial Transcriptomics of Bone Tissues

Luigi Mancinelli^{1,2}, Karen Elizabeth Schoedel^{3,4}, Kurt Richard Weiss^{5,6,7}, Giuseppe Intini^{1,2,8,9,10}

¹Department of Periodontics and Preventive Dentistry, University of Pittsburgh School of Dental Medicine ²Center for Craniofacial Regeneration, University of Pittsburgh School of Dental Medicine ³UPMC Presbyterian ⁴UPMC Shadyside ⁵Musculoskeletal Oncology Laboratory, Department of Orthopaedic Surgery, University of Pittsburgh ⁶UPMC Hillman Cancer Center ⁷Departments of Anatomic Pathology and General Surgical Oncology, University of Pittsburgh ⁸Department of Medicine, University of Pittsburgh School of Medicine ⁹University of Pittsburgh UPMC Hillman Cancer Center ¹⁰McGowan Institute for Regenerative Medicine, University of Pittsburgh

Corresponding Author

Luigi Mancinelli
lum39@pitt.edu

Citation

Mancinelli, L., Schoedel, K.E., Weiss, K.R., Intini, G. Methods to Enable Spatial Transcriptomics of Bone Tissues. *J. Vis. Exp.* (207), e66850, doi:10.3791/66850 (2024).

Date Published

May 3, 2024

DOI

10.3791/66850

URL

jove.com/video/66850

Materials

Name	Company	Catalog Number	Comments
Advanced orbital shaker	VWR	76683-470	Use to keep tissues under agitation during incubation as reported in the method instructions.
Camel Hair Brushes	Ted Pella	11859	Use to handle FFPE sections as reported in the guidelines.
Dual Index Kit TS Set A 96 rxns	10X Genomics	PN-1000251	Use to perform spatial transcriptomics.
Ethanol 200 Proof	Decon Labs Inc	2701	Use to perform tissue dehydration as reported in the method instructions.
Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA)	Thermo Fisher Scientific	S312-500	Use to prepare EDTA 20% pH 8.0.
Fisherbrand Curved Medium Point General Purpose Forceps	Fisher Scientific	16-100-110	Use to handle FFPE sections as reported in the guidelines.
Fisherbrand Fine Precision Probe	Fisher Scientific	12-000-153	Use to handle FFPE sections as reported in the guidelines.
Fisherbrand Superfrost Plus Microscope Slides	Fisher Scientific	12-550-15	Use to attach sectioned scrolls as reported in the guidelines.
High profile diamond microtome blades	CL Sturkey	D554DD	Use to section FFPE blocks as reported in the guidelines.
Novaseq 150PE	Novogene	N/A	Sequencer.
Paraformaldehyde (PFA) 32% Aqueous Solution EM Grade	Electron Microscopy Sciences	15714-S	Dilute to final concentration of 4% with 1x PBS to perform tissue fixation.
Phosphate buffered saline (PBS)	Thermo Fisher Scientific	10010-049	Ready to use. Use to dilute PFA and to perform washes as reported in the method instructions.
Premiere Tissue Floating Bath	Fisher Scientific	A84600061	Use to remove wrinkles from FFPE sections as reported in the guidelines.
RNase AWAY Surface Decontaminant	Thermo Fisher Scientific	7002	Use to clean all surfaces as reported in the method instructions.

RNeasy DSP FFPE Kit	Qiagen	73604	Use to isolate RNA from FFPE sections once they have been generated as reported in the guidelines.
Semi-Automated Rotary Microtome	Leica Biosystems	RM2245	Use to section FFPE blocks as reported in the guidelines.
Sodium hydroxide	Millipore Sigma	S8045-500	Prepare 10 N solution by slowly dissolving 400 g in 1 liter of Milli-Q water.
Space Ranger	10X Genomics	2.0.1	Use to process sequencing data output .
Surgipath Paraplast	Leica Biosystems	39601006	Use to perform tissue infiltration and embedding as reported in the method instructions.
Visium Accessory Kit	10X Genomics	PN-1000194	Use to perform spatial transcriptomic experiments.
Visium Human Transcriptome Probe Kit Small	10X Genomics	PN-1000363	Use to perform spatial transcriptomic experiments.
Visium Spatial Gene Expression Slide Kit 4 rxns	10X Genomics	PN-1000188	Use to place the sections if performing spatial transcriptomic experiments.
Xylene	Leica Biosystems	3803665	Use to perform tissue clearing as reported in the method instructions.