Nov 26, 2025

TDA-MIT: In situ sequencing for mouse skin and mouse lung

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl88ybdl2w/v1

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Cellular Senescence Net...

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Protocol Citation: Ke Zhang 2025. TDA-MIT: In situ sequencing for mouse skin and mouse lung. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl88ybdl2w/v1

Protocol status: Working
We use this protocol and it's working

Created: November 26, 2025

Last Modified: November 26, 2025

Protocol Integer ID: 233528

Keywords: TDA, in situ seqeuencing, profiling gene expression, gene expression in intact tissue section, mouse lung this protocol, mouse lung, mouse skin, tda, iterative fluorescence

Abstract

This protocol describes an in situ sequencing (ISS) workflow for spatially profiling gene expression in intact tissue sections, using padlock probe hybridization, rolling circle amplification, and iterative fluorescence imaging for barcode decoding.

Materials

	Poly-D-lysine solution	Thermofisher Scientific	Cat# A3890401
	16% Paraformaldehyde, PFA	Electron Microscope Sciences	Cat# 15710-S
	1X PBS, 7.4	Gibco	Cat# 10010-023
	10X PBS, 7.4	Gibco	Cat# 70011-044
	SUPERase In RNase inhibitor	Invitrogen	Cat# AM2696
	1M Tris pH 7.5	Thermofisher Scientific	Cat# 15567027
	Tween-20	Calbiochem	Cat# 655206
	20X SSC buffer	Sigma-Aldrich	Cat# S6639
	OminiPur Formamide	Calbiochem	Cat# 75-12-7
	10X Antarctic Phosphatase Buffer	New England Biolabs	Cat# B0289S
	Antarctic Phosphatase	New England Biolabs	Cat# M0289S
	Ribonucleoside vanadyl complex, RVC	New England Biolabs	Cat# S1402S
	10mg/mL Yeast tRNA	Fisher Scientific	Cat# AM7119
	T4 DNA ligase, 5Weiss U/uL	Thermo Scientific	Cat# EL0011
	BSA, molecular biology grade	New England Biolabs	Cat# B9000S
	Phi29 DNA polymerase	Thermo Scientific	Cat# EP0094
	10 mM dNTP mix	Invitrogen	Cat# 18427088
	5-(3-aminoallyl)-dUTP	Invitrogen	Cat# AM8439

	Methacrylic acid NHS ester	Sigma-Aldrich	Cat# 730300
	Acrylamide, solution 40%	Bio-Rad	Cat# 161-0140
	Bis-acrylamide solution, 2%	Bio-Rad	Cat# 161-0142
	Ammonium persulfate, APS	Sigma-Aldrich	Cat# A3678
	Tetramethylethylenediamine, TEMED	Sigma-Aldrich	Cat# T9281
	Gel-Slick Solution	Lonza	Cat# 50640
	Proteinase K, RNA grade	Invitrogen	Cat# 25530049
	OminiPur SDS, 20%	Calbiochem	Cat# 7991
	5M NaCl	Thermofisher Scientific	Cat# AM9759
	Shrimp Alkaline Phosphatase	New England Biolabs	Cat# M0371L
	CutSmart buffer	New England Biolabs	Cat# B6004S
	Triton-X-100, 10% solution	Sigma-Aldrich	Cat# 93443
	AEBSF	Sigma-Aldrich	Cat# A8456-25MG
	DAPI	Sigma-Aldrich	Cat# 10236276001
	100% Ethanol	VWR Life Science	Cat# 89125-170

Sample preparation

1 A quartz-bottom culture dish was coated with a poly-D-lysine solution. OCT-embedded mouse lung and skin tissues were sectioned into 14 µm slices, then fixed with 4% PFA in PBS for 15 minutes, followed by three washes with PBSR (PBS + RNase Inhibitor). Fixed samples submerged with PBSR underwent Raman imaging before being permeabilized with pre-chilled methanol at -20°C for 20 minutes, followed by hybridization.

In situ sequencing library construction

2 SNAIL probes were designed, and the probe library was constructed according to Wang et al.[1]. The probes were dissolved and pooled in ultrapure RNase-free water. The probe mixture was heated to 40°C for 15 minutes and then equilibrated to 37°C. Tissue samples were removed from -20°C storage, equilibrated to room temperature, and treated with 10 mM Tris pH 7.5 for 10 minutes. The samples were washed with PBSTR (0.1% Tween-20, 0.1 U/µL SUPERase•In in PBS) and incubated in hybridization buffer (2X SSC, 10% formamide, 1% Tween-20, 20 mM ribonucleoside vanadyl complex, 0.1 mg/mL yeast tRNA, 0.1 U/µL SUPERase•In, SNAIL probes (4 nM per probe for STARmap-ISS, 10nM per probe for STARmap-ISH) at 40°C with gentle shaking for 48 hours. The samples were washed twice with PBSTR for 20 minutes at 37°C and then with 4X SSC in PBSTR for 20 minutes at 37°C, followed by a rinse with PBSTR at room temperature. The SNAIL padlock probes annealed to the samples were ligated by incubating with a T4 DNA ligation mixture (1:10 dilution of T4 DNA ligase, 0.2 mg/mL BSA, 0.5 U/µL SUPERase•In) for 2 hours at room temperature with gentle agitation, followed by two 5-minute washes with PBSTR. The samples were then incubated in the RCA mixture (1:50 dilution of Phi29 DNA polymerase, 250 µM dNTP, 20 µM 5-(3-aminoallyl)-dUTP, 0.2 mg/mL BSA, 0.2 U/µL SUPERase•In) for 2 hours at 30°C with gentle agitation, followed by two 5-minute washes with PBSTR. Subsequently, the samples were treated with 25 mM acrylic acid NHS ester for 2 hours at room temperature with agitation, rinsed with PBST (0.1% Tween-20 in PBS) once, and incubated with monomer buffer (4% acrylamide, 0.2% bis-acrylamide, 2X SSC in H2O) for 15 minutes for polymerization pre-treatment. The buffer was removed, and 30 µL of monomer mixture (0.1% ammonium persulfate, 0.1% tetramethylethylenediamine in monomer buffer) was directly added to the center of each sample, which was immediately covered with a coverslip (coated with Gel-Slick Solution according to the manufacturer's instructions) and allowed to polymerize at room temperature for 1 hour. The tissue-gel hybrid was washed with PBST twice and cleared by proteinase K digestion mixture (50 mM Tris pH 7.5, 100 mM NaCl, 1% SDS, 0.2 mg/mL proteinase K in H2O) at 37°C overnight. On the following day, the samples were treated with a dephosphorylation mixture (1:100 dilution of shrimp alkaline phosphatase, 0.2 mg/mL BSA, 1:10 dilution of CutSmart buffer in H2O) and rinsed with PBST.

Imaging and sequencing

3 For *in situ* hybridization detection, the 19-nt fluorescent oligo complementary to the DNA amplicon was diluted to 100 nM in 1X SSC dissolved in PBST, and samples were incubated at room temperature for 30 minutes, then washed with PBST three times for 5 minutes each before imaging. For *in situ* sequencing detection, the protocol was followed according to Wang et al.[1]. Each sequencing cycle started with treatment with stripping buffer (60% formamide, 0.1% Triton-X-100) for 5 minutes and triple-washing with PBST for 5 minutes. The samples were then incubated with a sequencing mixture (0.2 mg/mL BSA, 10 µM reading probe, 5 µM fluorescent decoding probe, 1:25 dilution of T4 DNA ligase) for 3 hours at room temperature. Subsequently, the samples were triple washed with washing and imaging buffers (2X SSC, 10% formamide) for 10 minutes before imaging. DAPI staining was performed after Cycle 6 of imaging for cell segmentation. Images were acquired using a Leica Stellaris5 confocal microscope with a 405 nm diode, white light laser, and a 20x air objective (NA 0.75).

Protocol references

1. Wang, X. *et al.* Three-dimensional intact-tissue sequencing of single-cell transcriptional states. *Science* **361**, (2018).