

Jun 25, 2025

Spatial Touchstone Standard Operating Procedures (STSOP)

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge54qdg47/v1>



Spatial Touchstone¹

¹SJ-UoA-WCM



Spatial Touchstone

SJ-UoA-WCM

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Protocol status: Working

We use this collection and it's working

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Keywords: comprehensive assessment of imaging based spatial transcriptomic, imaging based spatial transcriptomic, spatial touchstone dataset, spatial transcriptomic, spatial touchstone, project spatial touchstone, spatial touchstone project, cell profiling, cancer tissue, transcriptomic, cell type annotation, multiplexing in situ hybridization, evaluating imaging, situ hybridization

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Abstract

This collection of protocols pertains to the project *Spatial Touchstone: A Comprehensive Assessment of Imaging Based Spatial Transcriptomics, Reproducibility and Best Practices*.

Spatial transcriptomics is a rapidly advancing field, yet it lacks standardized metrics for evaluating imaging-based multiplexing in situ hybridization (ISH) technologies. The Spatial Touchstone Project aims to establish these standards by compiling the largest datasets across normal and cancer tissues. Our Spatial Touchstone dataset, generated from eight tissue types across three global sites, evaluates reproducibility, sensitivity, dynamic range, signal-to-noise ratio, false discovery rates, cell type annotation, and congruence with single-cell profiling.

Materials

⊗ Xylene **Merck MilliporeSigma (Sigma-Aldrich) Catalog #214736**

⊗ Ethanol, 200 proof **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023**

⊗ RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**

⊗ Liberase™ TH Research Grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #5401151001**

⊗ RiboLock RNase Inhibitor **Thermo Scientific Catalog #EO0381**

⊗ Nuclei EZ lysis buffer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #EZ PREP NUC-101**

⊗ MACS BSA Stock Solution **Miltenyi Biotec Catalog # 130-091-376**

⊗ mini-strainer 70µm **pluriSelect Life Science Catalog #43-10070-50**

⊗ 10x Phosphate Buffered Saline **Gibco - Thermo Fisher Scientific Catalog #70011044**

⊗ PluriStrainer Mini 40µm **pluriSelect Life Science Catalog #43-10040-60**

⊗ Luna-FX7 using Acridine Orange/Propidium Iodide Stain (Logos) **Logos Biosystems Catalog #F23011**

<https://intelixbio.com/product/acridine-orange-propidium-iodide-stain-sterile-filtered-2-x-0-5-ml-f23011-lgbd10066/>

Chromium Fixed RNA Profiling Reagent Kits for multiplexed samples

(⊗ Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 4 BC **10x Genomics Catalog #1000475** ;

⊗ Chromium Next GEM Chip Q Single Cell Kit, 48 rxns **10x Genomics Catalog #1000418** ;

⊗ Dual Index Kit TS Set A, 96 rxns **10x Genomics Catalog #1000251**)

0% Glycerol and 0.1X Enhancer (PN-2000482, 10x Genomics)

⊗ 5200 Fragment Analyzer **Agilent Technologies** and a

⊗ Qubit™ Flex Fluorometer **Thermo Fisher Scientific Catalog #Q33327**

⊗ Superfrost Plus Micro Slide **VWR International (Avantor) Catalog #48311-703**

3 ug/mL Proteinase K (CosMx FFPE slide preparation kit, RNA)

CosMx Human Universal Cell Characterization RNA Panel (CMX-H-USCP-1KP-R)

CosMx SMI slide preparation manual MAN-10185-01-1 (NanoString)

⊗ Xenium slides **10x Genomics Catalog #3000941**

Xenium Analyzer User Guide (CG000584 Rev D and Rev E for Multi-modal Segmentation stained slides, 10X Genomics)

Xenium Decoding Consumables Kit, (PN-1000487, 10X Genomics)

⊗ Sodium hydrosulfite **Merck MilliporeSigma (Sigma-Aldrich) Catalog #157953**



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Files

 SEARCH

Protocol

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Single Nucleus RNA-Seq using snPATHO-Seq

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CosMx RNA Slide Preparation

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CosMx Protein slide preparation

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From Xenium RNA to CosMx Protein

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Hematoxylin and Eosin (H and E) Staining and Imaging

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