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Xenium – Accelerating the Mastery of Biology

Josh Talboom, Ph.D.
Science & Technology Advisor (STA)
San Diego & OC, CA

UCI Xenium Launch Party
Presented by 10x Genomics & UCI GRT Hub
01/11/2024
Biology Is Immensely Complex

40 trillion cells in the human body
Each cell has its own set of expressed genes

Enormous complexity within each cell
Interactions of millions of different molecules and molecular machines

Cells form tissues, which form organs
Each tissue has a vast diversity of cell types and states
Spatial Organization of Cell Types and States
Drives Complex Biological Processes

Slide-level analysis
Cancer classification
Tumor segmentation
Survival prediction

Region-level analysis
Tumor heterogeneity
Immune cell invasion
Tumor vascularization

Cell-level analysis
Cell type classification
Nuclear abnormalities
Virtual staining

Imaging and Molecular Analysis Have Been Separate Worlds
Tremendous Promise for Improving Human Health

By combining spatial and molecular information

- How cell-to-cell interactions impact the microenvironment
- How immunological conditions are created and maintained
- How spatial context influences development
• Reveal the relationship between cellular structure and function with single base-pair resolution
• End-to-end in situ platform with imaging readout (no NGS required)
• Hundreds of RNA targets with subcellular resolution
• Highly specific and sensitive
• High throughput, fast time to results
• Compatible with Fresh Frozen and FFPE
Xenium – Customer Preprint
Disruption of ATM Protein Sensitizes Certain DMG Tumors to Radiation Therapy

- “High-resolution spatially resolved transcriptomic profiling was especially critical in this model since vasculature and neoplastic compartments within the tumor play distinct roles in therapeutic response.”
  - Authors

- Xenium mouse brain panel (247 genes) with custom add-on of 51 genes for a total of 298 genes

- 790,374 individual cells across four tumor-bearing brains

- Identified as many as 29 clusters of neoplastic cells per specimen

New Results

*Ataxia-telangiectasia mutated (Atm) disruption sensitizes spatially-directed H3.3K27M/TP53 diffuse midline gliomas to radiation therapy*

Avani Mangoli, Sophie Wu, Harrison Q Liu, Michael Aksu, Vaibhav J Jain, Bronwen E Foreman, Joshua A Regal, Loren B Weidenhammer, Connor E Stewart, Maria E Guerra Garcia, Emily Hocke, Karen Abramson, Nerissa T Williams, Lixia Luo, Katherine Deland, Laura Attardi, Kouki Abe, Rintaro Hashizume, David M Ashley, Oren J Becher, David G Kirsch, Simon G Gregory, Zachary J Reitman

doi: https://doi.org/10.1101/2023.10.18.562892
Xenium – Customer Preprint

Xenium neighborhood analysis revealed altered immune-neoplastic interactions after treatment

- Estimated the mean distance between neoplastic cells and other cell types
- Identified increased proximity of neoplastic cells and immune cells, after Atm loss and after treatment with irradiation
- Confirmed most co-localization occurred in the irradiated, Atm-null tumor

Figure 4

- Estimated the mean distance between neoplastic cells and other cell types
- Identified increased proximity of neoplastic cells and immune cells, after Atm loss and after treatment with irradiation
- Confirmed most co-localization occurred in the irradiated, Atm-null tumor

doi: https://doi.org/10.1101/2023.10.18.562892
"The Xenium platform is providing us with unprecedented insight into the molecular pathology of disease at an incredible resolution."

Dr. Simon Gregory
Duke University

"It works like a charm, does the job as per specs and beyond. As I said it before, it's a cut above the rest."

Luciano Martelotto, PhD
University of Adelaide
Xenium – A Simple Workflow with 3-4hrs of Hands-On Time
From sections to instrument start in 2-3 days

Sample Preparation | Probe hybridization, ligation & amplification | Fluorescent probe hybridization, imaging & decoding | Data visualization

FF or FFPE tissue sections on Xenium slides | Fixation & permeabilization (FF) or Deparaaffinization & decrosslinking (FFPE) | Probe hybridization | Rolling circle amplification product | Xenium Analyzer | 2. Probe hybridization | 3. Automated slide imaging | 4. Probe removal | Cycle

Simple ~4 hours hands on time workflow | Fully automated decoding and analysis
Xenium Gene Expression
Protocol steps and timing – FFPE

**Day 0 (variable)**
- QC FFPE Blocks (H&E)
- Sectioning & Placement (variable)
- Drying at 42°C (~3 h)
- Desiccator (overnight)

**Day 1 (~4 h)**
- Baking at 60°C (~2 h)
- Deparaffinize (~50 min)
- Decrosslinking (~45 min)
- Probe Hybridization (16 h; overnight)

**Day 2 (~6 h)**
- Probe Wash (~35 min)
- Ligation (~2 h 10 min)
- Rolling Circle Amplification (~2 h)
- Autofluorescence Treatment (~45 min)
- Nuclei Staining (~10 min)

**Day 3-6 (variable)**
- Prepare instrument run (1 hr)
- Instrument Run (2-4 days)
- Instrument Unloading (30 min)
- Optional H&E Staining
- Analysis

**Pause:** RT* for ≤1 month in desiccator

**Pause:** 4C** for ≤4 days

**STOP**

**Indicates storage in PBS-T with cassette lid on in the dark**
Xenium Gene Expression
Protocol steps and timing – Fresh Frozen

Day 0 (variable)
- QC FF Blocks (H&E)
- Sectioning & Placement (variable)
- -80C storage until use

Day 1 (~2 h)
- Fixation (~1 h)
- Permeabilization (~1 h)
- Probe Hybridization (16 h; overnight)

Day 2 (~6 h)
- Probe Wash (~35 min)
- Ligation (~2 h 10 min)
- Rolling Circle Amplification (~2 h)
- Autofluorescence Treatment (~45 min)
- Nuclei Staining (~10 min)

Day 3-6 (variable)
- Prepare instrument run (1 hr)
- Instrument Run (2-4 days)
- Instrument Unloading (30 min)
- Optional H&E Staining
- Analysis

**Indicates storage in PBS-T with cassette lid on in the dark

---

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Xenium In Situ Gene Expression

General Workflow Overview

**Sample preparation**
1. Section
2. Fresh Frozen -> fixation and permeabilization
   FFPE -> deparaffinization and decrosslinking

**Assay Workflow**
Targeted barcoding and rolling circle product (RCP) generation

**On-Instrument Operation / Analysis**
RCP readout and data processing

**Data Visualization**
Visualization of data on Xenium Explorer

---

**Benchtop**

**Instrument**

**Computer**
Xenium In Situ Gene Expression

Overview

Sample Preparation

- Tissue placement is the first workflow step
- Improved Xenium Slide design minimizes likelihood of tissue detachment
- Fresh Frozen (FF) samples ➔ fixation and permeabilization
- FFPE samples ➔ deparaffinization and decrosslinking
Xenium In Situ Gene Expression
Tissue Placement Guidelines

Fiducial Guidelines
- Image registration depends on fiducial finding
- Fiducial frame is inside the imageable area
- If a certain number of ‘+’ are covered, instrument run will fail to initiate
  - It is critical to avoid top and bottom ‘+’ of frame (in red)

Best practice: avoid covering fiducials
Overview of H&E Quality Check

**Xenium Sample Preparation – FFPE & FF**

### Tissue stained with H&E

*Slides in images are representative*

### H&E staining

- Staining performed on blank slides
  - No Xenium slide required for QC
  - Section FFPE to 5 µm
  - Section FF to 10 µm

- Remove wax prior to H&E staining

- Any H&E protocol should be fine for QC
  - Protocol provided in the FFPE Tissue Preparation (CG000578)
  - Protocol provided in the FF Tissue Preparation Guide (CG000579)
Xenium Sample Preparation – FF & FFPE

Morphology check of FFPE section prior to starting Xenium workflow

Wrinkle

Folds

Shattering

A few examples of low quality tissue and/or sectioning or rehydration artifacts
Xenium – Leading Analyzable Area for Maximum Flexibility

2 sections
10.5 x 22.5mm

6 mouse brains
6 x 10mm

196 biopsies
1mm TMA
Example 1cm² cohorts

Xenium – Industry Leading Throughput and Speed

Annual Throughput = 672 sections

*Approximate 55 hr run time; data on file at 10x Genomics
Xenium – Panel and Custom Menu Offers Maximum Flexibility

Customize any panel or build your own standalone panel

Pre-designed & validated panels

- **Human Breast**
  - 280 genes

- **Human Lung**
  - 289 genes

- **Human Brain**
  - 266 genes

- **Human Colon**
  - 322 genes

- **Human Multi-Tissue & Cancer**
  - 377 genes

- **Human Skin**
  - 260 genes

- **Mouse Brain**
  - 248 genes

- **Mouse Multi-Tissue**
  - 379 genes

Add up to 100 custom targets

Standalone custom

- **480 custom genes**
- **300 custom genes**
- **100 custom genes**
- **50 custom genes**

Coming Soon (Q2)

**5000 Gene Panel**

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Tools for Xenium panel selection and design

**Xenium Panel Selector tool**
(https://store.10xgenomics.com/planners/xenium-panel)

- Helps decide between using pre-designed panels, add-on panels, or standalone custom panels
- Allows you to enter a list of genes and compare the overlap with available pre-designed panels

**Xenium Panel Designer**
(https://cloud.10xgenomics.com/xenium-panel-designer)

- Takes you step-by-step through panel design once you’ve selected a panel strategy and gene list
- Recommends optimizations for panel performance

**Designing Custom Xenium Panels Tech Note**

**Getting Started with Xenium Panel Design**

- Thorough documentation of design considerations and workflow
## Overview of customization options

<table>
<thead>
<tr>
<th>Customization type</th>
<th>Example Applications</th>
<th>Panel Design Process</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Add-on custom for pre-designed human and mouse panels</strong> <em>(Up to 100 add-on genes)</em></td>
<td>• Any application with standard human or mouse genes (e.g., genes in the GRCh38 or mm10 transcriptome)</td>
<td>• End-to-end design in <a href="#">Xenium Panel Designer</a> on 10x Genomics Cloud</td>
</tr>
<tr>
<td><strong>Standalone custom for human and mouse</strong> <em>(Up to 480 genes)</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **Species standalone custom**                           | • Mammals besides human and mouse  
• Other diploids with high-quality assembly and annotations  
• Xenografts                                                                                                                                         | • Submit design request on [Xenium Panel Designer](#) on 10x Genomics Cloud; [panel design through 10x Support](#)  
• See also: [Species Standalone Custom and Advanced Custom Panel Design for Xenium In Situ](#) Tech Note |
| **Advanced custom** *(option for standalone or add-on panels)* | • Isoforms / gene fusions  
• Protein tags, fluorescent reporters, transgenes  
• Bacterial and viral sequences                                                                                                                        | • Submit design request on [Xenium Panel Designer](#) on 10x Genomics Cloud; [panel design through 10x Support](#)  
• Requires selection of your own RNA-binding domain sequences  
• See also: [Species Standalone Custom and Advanced Custom Panel Design for Xenium In Situ](#) Tech Note |
Xenium – Non-Destructive Workflow

Allows for additional assays and gaining additional insights from the same tissue section post run.
Xenium – Tech Note on Post-Run H&E, IF, & Visium CytAssist

Post-Xenium In Situ Applications: Immunofluorescence, H&E, and Visium CytAssist Spatial Gene Expression

Introduction
The Xenium in Situ Expansion module preserves gene expression at nodule-like resolution in both fresh FFPE and formalin-fixed bulky tissues. H&E tissue sections. Gene expression is measured via punctuated, or active protein signals that range hundreds of genes at once per nucleus. This is achieved by using a specific antibody to detect the expression of genes across the entire nucleus, providing insight into the distribution of gene expression and cellular function. In contrast, tissue sections mounted on glass the Xenium in Situ Expansion module captures gene expression and can be used to visualize gene expression.

Contents
1. Introduction
2. Post-Xenium In Situ Applications
3. Immunofluorescence
4. H&E Staining
5. Visium CytAssist Spatial Gene Expression via Xenium Analyzer

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Xenium – Post Run: Both FF and FFPE Samples are Compatible with IF

[Image of tissue sections with fluorescent markers for DAPI, GFAP, NeuN, VIM, α-SMA, and other markers for FF mBrain and FFPE hBreast tissues.]
Xenium – Post Run: Whole-Transcriptome - Visium Cytassist

Human FFPE breast infiltrative ductal carcinoma
Xenium – Usable Data Immediately After Your Run Ends

No additional steps of analysis time required to analyze and visualize your data

- Localized transcripts
- Morphology images
- Cell segmentation
- Per-cell transcript information
- Unsupervised clustering
Xenium – Common File Formats for Ease of Use

10x has extensive experience optimizing single cell and spatial data formats

Single-cell tools (filtering, clustering, trajectory analysis) continue to work with Xenium data

Usable data immediately after run ends

Seamless integration with Seurat, Squidpy, stLearn, Giotto, and Voyager
Xenium Onboard Analysis Output Formats

**ONBOARD ANALYSIS**

- Imaging
- Decoding
- Segmentation
- Cell assignment
- Clustering

**Output Formats**

- **Morphology images**
  - OME-TIFF

- **Transcripts w/ calibrated Q-scores**
  - Zarr
  - Parquet
  - CSV

- **Cell segmentation**
  - Zarr
  - Parquet
  - CSV

- **Cell-feature matrix & clustering**
  - Zarr
  - MEX
  - HDF5
Xenium – Intuitive Visualization & Analysis

Begin exploration immediately without the need for off-instrument processing

View transcript density plots, and visualize graph-based clustering results
Increased Plexity and Integrated Protein Coming Soon to Xenium

**Multimodal Segmentation**
- Broad compatibility

**~5000 Plex Panels**
- ~5 day run
- High quality data

**In-line Multiplex Protein**
- Same slide as RNA

**1000-2000 Plex Panels**
- Offers more flexibility

**Shipping Q1 2024**

**Q2 2024**

**Second Half 2024**

*Final product release timelines and configurations subject to change*
Xenium – Multi-modal Segmentation Built to Enable Broad Coverage

- Membrane Antibody Cocktail
- Interior Antibody Cocktail
- Cytoplasmic RNA Stain
- DAPI Nuclear Stain

Four Channel Imaging Inputs for Segmentation

Cell boundary & interior stain

Membrane segmentation

Interior stain with nuclear expansion

Nuclear expansion

Xenium Multi-tissue Stain Mix
Xenium – Developing a Comprehensive Solution for Cell Segmentation

Pathology → Cell Morphology Stain Development → Training and Benchmark Data → Computational Methods → Quantitative Performance Metrics

- Pathology
  - Epithelial tissue
    - Entohelium
    - Glanular
    - Lining
  - Nervous tissue
    - Neurons
    - Microglia
    - Astrocytes
    - Oligodendrocytes
  - Immune cells
    - Bone
    - Blood
    - Cartilage
    - Stromal
  - Connective tissue
  - Muscle tissue
  - Smooth
  - Cardiac
  - Skeletal

- Cell Morphology Stain Development

- Training and Benchmark Data
  - Sample images

- Computational Methods
  - Neural network diagram

- Quantitative Performance Metrics
  - Graphs and charts
Xenium – Tissue Staining and Segmentation Workflow

Sample Prep (manual, ~3 days)
- Slide Prep: 0.5 day
- RNA prep: 1.5 days
- Cell Segmentation Prep: 1 day

Combined sample preparation on same section

- RNA gene expression panel
- Multi-Tissue Stain Mix

Xenium analyzer imaging, readout, and primary data analysis
- RNA readout & decoding
- Cell Segmentation Imaging and Algorithm

- Fully integrated workflow with RNA Assays
- Comprehensive cell segmentation staining in 4 channels
- Segmentation algorithm designed and optimized for Xenium
- Segmentation runs on the Xenium Analyzer instrument
- Deep learning approach trained on Xenium sample data
- Works on FFPE & FF, Validated on Human and Mouse

Slide Prep: 0.5 day
RNA prep: 1.5 days
Cell Segmentation Prep: 1 day
RNA readout & decoding
Cell Segmentation Imaging and Algorithm

Breast
Colon
Kidney
Xenium – Distinct Advantages of 4 Channel Cell Morphology Images

FFPE human colon

- Nuclei
- Interior antibody stain
- Cytoplasmic RNA stain
- Membrane antibody stain

Merge
Xenium – Cell Segmentation, RNA Decoding and Cell Clustering

FFPE human colon

Cells colored based on clustering from hMulti panel
Distinct Advantages of Four Channel Cell Morphology Images

FFPE human kidney

Nuclei

Interior antibody stain

Cytoplasmic RNA stain

Membrane antibody stain

Merge
Xenium – Cell Segmentation, RNA Decoding and Cell Clustering

FFPE human kidney

Cells colored based on clustering from hMulti panel
Xenium Analysis Software enables flexibility to optimize cell segmentation in every sample

Customize Xenium nuclear segmentation

Adjust cell expansion distance or nucleus intensity filter, or resegment previously acquired data with the latest segmentation algorithm.

Import segmentation with Xenium Ranger

Reassign transcripts in Xenium Ranger using segmentation results produced by a 3rd party tool and visualize in Xenium Explorer.

Align an import segmentation on same section images

Align and import same section H&E images in Xenium Explorer (IF image support coming Fall 2023). Import alignment in Xenium Ranger to leverage segmentation on post-Xenium images.

Learn more at [10xgenomics.com/products/xenium-analysis](http://10xgenomics.com/products/xenium-analysis)
Xenium – 5000-Plex Multi-Tissue Gene Panel

Seven Human FFPE Tissues

- Brain
- Lung
- Skin
- Colon
- Pancreas
- Liver
- Kidney
Xenium – Multiplexed Protein & RNA from the Same Section

- FABP4
- aSMA
- PANCK
- HH3
- MUC
- VIM
- PCNA
- ERK1/2
- IBA1
- HLA-DR
- CD68
- CD20
From Discovery to Focused – Complementary Workflows

Discovery

- Chromium Single Cell
  - Entire transcriptome measurement

Focused

- Xenium In Situ
  - Targeted measurement
  - Subcellular resolution
  - High sensitivity
  - High throughput

- Visium Spatial
Visium

EXPLORE THE SPATIAL ARCHITECTURE OF GENE & PROTEIN EXPRESSION

• Histological & whole transcriptomic information from an entire tissue section
• Slide contains arrayed barcoded oligos representing spatial locations
• Leverages NGS while preserving spatial location
• Unbiased gene & protein expression in tissue context
• Compatible with Fresh Frozen, Fixed, & FFPE
Multimodal Analysis of Composition and Spatial Architecture

Human squamous cell carcinoma - Ji et al, Cell, 2020

- Chromium + Visium + Proteomics + CRISPR in human skin cancer and a mouse model
- Provided a compelling case for the multiomic study in oncology:
  - Integrated single cell, spatial, proteomic, and CRISPR techniques/data
  - Gave insights on tumorigenesis, cell signaling, and immune cell infiltration in skin cancer
Multimodal Analysis of Composition and Spatial Architecture
Human squamous cell carcinoma - Ji et al, Cell, 2020

What's happening at the edges of the pathology?
**Visium – How it Works**

Allows Discovering Molecular Data at an Enhanced Resolution

- **Visium HD Slide**, 6.5 mm
- **Capture Area with continuous lawn of oligos**, 6.5 mm x 6.5 mm
- **Fiducial frame**, 8 mm x 8 mm
- **Grid of 2 x 2 μm barcoded squares**, binned to 8 x 8 μm
Visium CytAssist – Workflow Overview

Sample Input
Library Preparation
Sequencing
Data Analysis

Sample Preparation
User-supplied human samples
Freshly Placed FFPE Sections
Archived H&E Slides

Library Preparation
Visium CytAssist 10x Genomics Kits
User-supplied third-party reagents

Sequencing
10x Genomics library-compatible sequencer
(e.g., Illumina sequencer)

Analysis
Space Ranger analysis pipeline
Loupe Browser visualization tool
Visium HD – Resolution

Putting Visium HD in perspective

**Visium**

- **55 µm** spots
  - Hexagonally arranged
  - with **45 µm gaps**

**5,000** Features

**Visium HD**

- **8 µm** Square bins
  - Continuous grid-pattern
  - with **No Gaps**

**11,000,000** Features

**Actual Feature size**

- **2 µm** Squares
- Continuous Oligo Lawn
- with **No Gaps**
Visium HD – Comparison to Standard Visium

Visium

Visium HD
Visium HD – Achieve High-Quality Data with Confidence

Accurate and precise transcript localization enabled by CytAssist

Mouse intestine
Graph-based clusters

- Crypts containing paneth cells (Lyz1 + Defa21 expression expected)
- Non-epithelial layers below the crypt (Lyz1 + Defa21 expression not expected)

95% of Lyz1 and Defa21 transcripts localized in the crypt region
Visium HD – Easy-To-Use Analysis Tools Hastens Discovery
Visium HD – Pre-Orders Available Now

• **Unparalleled Spatial Discovery:** Whole transcriptome gene expression analysis for FFPE

• **Resolved at Single Cell Scale:** Capture Area with continuous lawn of 2 x 2 µm barcoded squares

• **Data Quality You Can Trust:** Accurate transcript localization enabled by Visium CytAssist

• **Enhanced histology:** Same section H&E and IF
Unlock the Full Spectrum of Spatial Biology

**Visium HD is Unbiased Discovery**
- Whole transcriptome
- Sequencing based
- Transcripts assigned to multi-micron areas

**Xenium is Precision Insights**
- 100s–1000s of transcripts
- High-resolution imaging based
- Transcripts assigned to cells
Chromium
Single Cell
• Partitions and barcodes cells in minutes
• Analyzes 100s to millions of cells
• Leverages scale of NGS while preserving cellular identity
• Fixed & FFPE compatible (Gene Expression Flex)
• Perform Single Cell:
  • Gene expression
  • Protein expression
  • Functional genomics - CRISPR
  • Immune repertoire & corresponding antigens
  • Epigenetics
Chromium X – Instrument

Chromium X
Next generation single cell system that runs all standard 80K cells/run assays and HT & Fixed assays with up to 1M cells/run

Chromium Flex Chip Q
4 x 16 Kit – up to 128K cells/channel

- Recovery well
- Gel bead well
- Sample well
Key Takeaways:

- Fresh and FFPE samples generated high-quality single cell data
- Highly consistent behavior between samples
- Flex made single-cell profiling easier
- Gene expression data mirrored tumor differences in both fresh and FFPE samples
  - Alveolar vs. undifferentiated signature
- Flex enabled single-cell profiling of FFPE tissues & reduced cost:
  - Retrospective analysis annotated with long-term clinical follow-up data
  - Easy integration of single cell and spatial data
  - Facilitates routine insights utilizing histological & molecular features

https://doi.org/10.1101/2023.04.25.538273
Flex – Multiple Sample Types, Ease of Scale, & Run Options

Broad Sample Compatibility
- Human
- Mouse
- Cell lines
- Primary cells
- Nuclei
- Fresh tissue
- PFA fixed tissue
- FFPE tissue

Innovative Probe Design
- Sample-specific barcode
- Compatibility with low quality samples
  - Only requires 50nt transcript sequence
- De-identification of patient information
  - Indirect transcript readout
- Maximum sensitivity
  - 3 probe pairs per gene
- Scale at ease
  - Built in multiplexing

Flexible Sample & Cell Input
- Up to 1M cells and 128 total samples per chip
  - 16 samples per lane with the 4 x 16 (64 rxn) multiplex Kit
Flex – How it Works

10x Barcoded Gel Beads

Oil

Cells Enzyme

Collect

Reverse Transcription

Pool Remove Oil

Single Cell GEMs

10x Barcoded cDNA and Feature Barcode DNA

10x Barcoded cDNA and Feature Barcode DNA

Single Cell TL v1 Gel Bead

Partial Read 1T

Partial Capture Sequence 1

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Flex – Multiplex Workflow

Built-in 4 or 16 sample multiplexing - requiring no extra reagents or steps

- Each set of probes contains a **unique barcode**; each individual molecule can be assigned back to its sample of origin
- Enables pooling of **up to 16 samples** and **up to 128,000 cells** in one channel
- Reduces cost per sample, and allows for higher throughput and batching
- No need to decide up front which samples to pool together
Flex – Scales Across Gene and Protein Expression

Multiplexed mouse splenocyte sample sub-pooled with 16 barcodes

Gene Expression: *CD14*

Antibody: CD14

Multiplex up to 16 samples using TotalSeq™-C feature barcode oligo

Simultaneous and robust gene and protein expression
MultiPro™ Human Fixed Cell Immune Profiling Antibody cocktail

The MultiPro™ Fixed Cell Immune Profiling Antibody Cocktail contains 58 antibodies. 53 antibodies against cell surface and intracellular proteins plus 5 isotype control antibodies. Fully compatible with the 10x Genomics Single Cell Gene Expression Flex with Feature Barcodes and Sample Multiplexing product.

Host / Isotype: Rabbit polyclonal IgG, Mouse IgG2b, Mouse IgG3, Mouse IgG2a, Mouse IgG1
Reactivity: Human

Cat No : G900004

Overview
Tested Applications
Product Information
Targets
Protocols
Sequencing Parameters
Feature Barcode Reference File Download

Validation Data Gallery

Inquire for pricing/ 4wks
Cat No. G900004
In stock for next day delivery.
Freight/Packing: $40.00

County/Region
United States

Size/Concentration Quantity
4wks 1

Request bulk or custom quote
# Flex – Proteintech’s Human Immune Cell Profiling Cocktail

List of human immune cell protein targets – 53 total antibodies + 5 IgG Xx mouse & rabbit isotype controls

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<th>NUCLEAR</th>
<th>CELL SURFACE</th>
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</thead>
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<tr>
<td>AHNAK, DCs, T cells, basophils</td>
<td>CD13, Granulocytes, Monocytes</td>
</tr>
<tr>
<td>BACH1, Neutrophils, APCs, NK cells</td>
<td>CD16, NK cells, Monocytes, Neutrophils</td>
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<tr>
<td>IRF4, T cells, APCs</td>
<td>CD18, Leukocytes</td>
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<tr>
<td>MAFB, Monocytes/Macrophages</td>
<td>CD19, B cells</td>
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<tr>
<td>NFKB2, Ubiquitous</td>
<td>CD2, NK cells, T cells</td>
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<tr>
<td>POU2AF1, B cells, Airway epithelial cells</td>
<td>CD21, B cells</td>
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<td>SPI1, Granulocytes, Monocytes, B cells, NK cells</td>
<td>CD27, Treg, T cells, B cells</td>
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<td>T-bet, NK cells, T cells, B cells</td>
<td>CD28, T cells, NK cells</td>
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<td>CYTOSOL, APCs, T cells</td>
<td>CD35, B cells, T cells, Monocytes, Granulocytes</td>
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<td>VIM, Mesenchymal cells</td>
<td>CD44, Ubiquitous</td>
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<td>ZAP70, NK cells, T cells, B cells</td>
<td>CD45, Lymphocytes</td>
</tr>
<tr>
<td>SEERATED, T cells, APCs, fibroblasts</td>
<td>CD45RA, B and T cells</td>
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<tr>
<td>IFNG, T cells, NK cells, APCs, ILCs</td>
<td>CD5, T cells</td>
</tr>
<tr>
<td>IL-1B, Neutrophils, APCs</td>
<td>CD12, Monocytes, NK cells, DCs</td>
</tr>
<tr>
<td>IL-17A, Th17 cells, ILCs, NK cells</td>
<td>CD63, Basophils, Monocytes, DCs, NK cells</td>
</tr>
<tr>
<td>IL-6, T cells, APCs, fibroblasts</td>
<td>CD64, Monocytes, DCs</td>
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<tr>
<td>IL-8, APCs, Epithelial cells, T cells, APCs</td>
<td>CD7, NK cell, T cell</td>
</tr>
<tr>
<td>S100A4, Leukocytes</td>
<td>CD71, Monocytes, B cells</td>
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<tr>
<td>TGFB1, Leukocytes</td>
<td>CD81, Leukocytes</td>
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<tr>
<td>TNFa, APCs, T cells, NK cells</td>
<td>CD83, APC B cells</td>
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<tr>
<td>ANXA2, Basophils, Monocytes, T cells, DCs</td>
<td>CD86, Monocytes, B cells</td>
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<tr>
<td>CD11a, Leukocytes</td>
<td>CD8A, T cells</td>
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<tr>
<td>CD11b, Granulocytes, Monocytes/Macrophages</td>
<td>CD88, APCs</td>
</tr>
<tr>
<td>CD11c, DCs, B cells</td>
<td>CD89, APCs</td>
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</tbody>
</table>

**+ 5 Mouse & Rabbit IgG Controls**

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Flex – Proteintech’s Human Immune Cell Profiling Cocktail

Nuclear – example gene & protein expression data from cells profiled via Chromium Gene Expression Flex

Nuclear Example = IRF4

```
<table>
<thead>
<tr>
<th>Cell type</th>
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<tbody>
<tr>
<td>B</td>
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<tr>
<td>DC</td>
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<tr>
<td>Mono</td>
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<tr>
<td>NK</td>
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<tr>
<td>Others</td>
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</table>

Rest: PBMC

IRF4(PROT) | IRF4(RNA) | Cell type |
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<td>Mono</td>
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<td>Others</td>
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LPS: 4H-Ti

IRF4(PROT) | IRF4(RNA) | Cell type |
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</table>

PMA: 4H-Ti

IRF4(PROT) | IRF4(RNA) | Cell type |
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<td>Others</td>
</tr>
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<td></td>
<td>T</td>
</tr>
</tbody>
</table>
```
Flex – Proteintech’s Human Immune Cell Profiling Cocktail

Cytosolic – example gene & protein expression data from cells profiled via Chromium Gene Expression Flex

Cytosolic Example = SRC

[Diagram showing gene expression data for SRC in different cell types]
Flex – Proteintech’s Human Immune Cell Profiling Cocktail

Cell Surface – example gene & protein expression data from cells profiled via Chromium Gene Expression Flex

Cell Surface Example = CD4
Flex – Proteintech’s Human Immune Cell Profiling Cocktail

Secreted – example gene & protein expression data from cells profiled via Chromium Gene Expression Flex

Secreted Example = IL1B

[Image showing heatmaps and cell type distributions for IL1B in different conditions]
Conclusion
10x Toolkit  – Research Use Case
Shared sample compatibility, technologies, workflows, & software in FFPE human ductal carcinoma tissue

nature communications

High resolution mapping of the tumor microenvironment using integrated single-cell, spatial and in situ analysis

Received: 15 November 2022
Accepted: 9 November 2023
Published online: 19 December 2023

Amanda Janesick1, Robert Shelansky1, Andrew D. Gottscho1, Florian Wagner1, Stephen R. Williams1, Morgane Rouault1, Ghezal Belyakoff1, Carolyn A. Morrison1, Michelli F. Oliveira1, Jordan T. Sicherman1, Andrew Kohtwa1, Jawad Abousoud1, Tingsheng Yu Drennon1, Seayar H. Mohabat1, 10x Development Teams1 & Sarah E. B. Taylor1
10x Toolkit – Use Case
Janesick et al., Nature Communication, 2023

10k cells target

FFPE tissue dissociation

Serial FFPE sections

Vlsium Spatial

Chromium Single Cell Gene Expression Flex

Whole transcriptome

In Situ gene expression

Xenium In Situ
Chromium – Reveals molecularly Distinct Cells
Janesick et al., Nature Communication, 2023

- Identified 17 “normal” cell clusters that matched the clusters expected in human breast tissue
- Chromium Flex enabled single-cell resolution of the FFPE tissue, revealing molecularly distinct cancer cell populations/clusters

Single-Cell Whole Transcriptome for Unbiased Discovery

A Chromium single cell FFPE sequencing (scFFPE-seq)

![tSNE visualization with cell clusters](image)
Visium – Resolves Spatial Components Tissue
Janesick et al., Nature Communication, 2023

- Visium pinpointed the spatial location of three scFFPE cancer cell types

- Intact sections profiled delicate/difficult cells lost in single-cell processing

- scFFPE deconvolved cell type & proportions were inconclusive across several spatial clusters due to cell proximity and gene homogeneity
Xenium – Localizes Specific Cell Subtypes
Janesick et al., Nature Communication, 2023

• Xenium localized each cell belonging to a cancer cell type identified by Chromium and Visium

• Found major differences in the cell type composition between “DCIS” & “Invasive Tumor” regions

• Subcellular resolution revealed molecularly distinct cells related with different stages of cancer

Targeted Spatial with Sub-Cellular Resolution for Focused Research

Xenium Cell Annotations

Selection Cancer Regions
Summary – The Power of the 10x Toolkit
Janesick et al., Nature Communication, 2023

- Pathologist only annotated double-positive regions in the FFPE ductal carcinoma tissue
- Xenium’s subcellular resolution revealed a small previously unknown triple-positive region
- The integrated Chromium & Visium whole-transcriptome data confirmed the triple-positive region
Biology’s Most Comprehensive Toolkit Backed By #1 Support

Support a large diversity of samples

Provide robust pipelines and understandable data formats

Sample prep

Data analysis
Xenium – Exceptional Launch Year

Early customer success, from install to insight
Thank you!
Please attend our Xenium Workshop
Sprague Hall 105 - Jan 18th - 10:30 AM – 12 PM

Josh Talboom, PhD
Science & Technology Advisor
joshua.talboom@10xgenomics.com

Christine Kao
Sales Executive
christine.kao@10xgenomics.com
Xenium – Chemistry Exclusive - Expressed SNVs

Distinguishing between normal/WT and cancer-associated/MUT cells

Cell Co-Culture: A549 + ME180

**KRAS MUT c.34G>A**

**KRAS WT c.34G**

MUT Cells

WT Cells