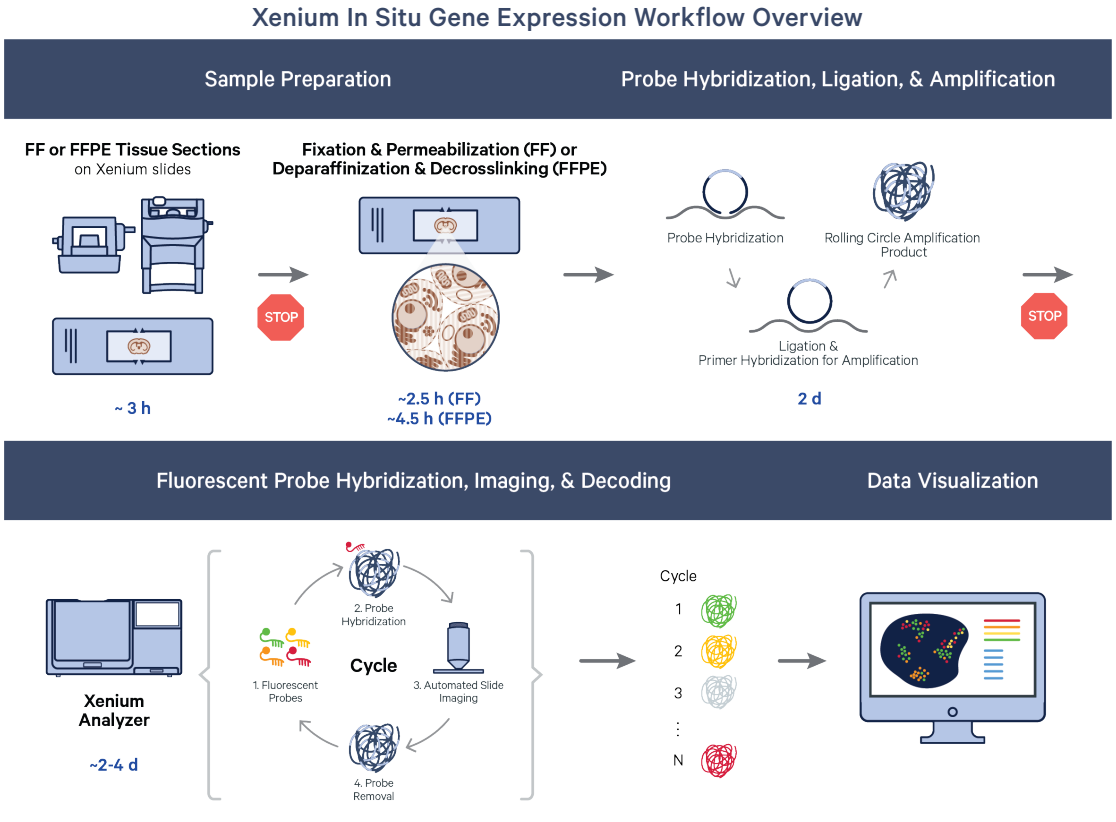
Xenium In Situ Gene Expression – Protocol Planner

Introduction

Xenium In Situ measures gene expression in tissue sections derived from either formalin fixed and paraffin embedded (FFPE) or fresh frozen (FF) tissue samples placed on Xenium Slides. This Protocol Planner provides an overview of the workflow along with the Xenium Analyzer overview. To enable efficient planning, a breakdown of key protocol steps and times, list of user-acquired reagents and consumables, and information about supporting documentation that will be available for executing the Xenium Gene Expression workflow is also provided.

10x Genomics Xenium Reagent Kits are not listed in this document.



Contents

Introduction

The protocol planner provides resources and guidelines to prepare a laboratory for seamless planning and execution of the Xenium In Situ Gene Expression workflow. The keys topics covered in this document are highlighted below.

Xenium Analyzer Overview

The document provides a high level overview of key instrument dimensions, delivery, installation, training along with guidelines for site preparation.

Workflow Documents

A list of documents to support various steps of the in situ gene expression workflow is provided for formalin-fixed & paraffin-embedded (FFPE) and fresh frozen (FF) and tissue samples. These documents, along with many additional resources, will be available on the 10x Genomics Support website once the Xenium Analyzer is installed and ready to use.

Key Protocol Steps & Timing

A breakdown of the off-instrument and on-instrument workflow steps, the time required to perform each step, and safe stopping points are provided.

Reagents & Consumables

(not supplied by 10x Genomics)

The reagents & consumables for various steps of the Xenium In Situ Gene Expression workflow are listed in this document. The Appendix also includes a list of items for optional H&E staining along with post-run quencher removal (only if staining slides after the instrument run). The listed items have been tested by 10x and perform optimally with the assay. These items will not be supplied by 10x Genomics and should be acquired from the indicated vendors. Refer to the manufacturer's website for regional part numbers. For items with multiple options, choose one based on availability and preference. **Substituting materials may adversely affect system performance.** This list may not include some standard laboratory equipment.

Note that some reagents and consumables, such as PBS, Tween, ice buckets etc., are common across multiple steps of the workflow and need not be bought individually for each step.

Reagents & Consumables *(not supplied by 10x Genomics)*

Approximate volumes of bulk (≥100 ml) reagents used for preparing and processing samples on two Xenium Slides per Xenium instrument run are listed below.

For precise volumes of all reagents, consult relevant workflow protocols.

Volume of Bulk Reagents (≥100 ml)			
Item	**~Volumes for two Xenium Slides (ml)**		
	Sample Prep + Probe Hyb.	**Instrument Run Only**	**Total**
Nuclease-free Water	110	1,140*	1,250
Ultrapure Water/Milli-Q Water	-	1,000	1,000
10X PBS	10	100	110
100% DMSO	-	150	150
Xylene *(only for FFPE samples)*	100	-	100
Ethanol *(only for FFPE samples)*	285	-	285

**For instrument run ONLY: nuclease-free water can be substituted with nuclease-free Ultrapure/ Milli-Q water.*

For instrument run during installation and also during training, twice the amount of indicated reagent volumes are needed.

Gene Panel Selection

Prior to executing the Xenium In Situ Gene Expression workflow, ensure that a compatible gene panel has been selected. 10x Genomics provides the option of using pre-designed gene panels. Additionally, the pre-designed panel may be customized by adding genes of interest.

- Visit the 10x Genomics Support website for information regarding all available panels.
- Custom gene panels: Contact 10x Genomics via email at customerservice@10xgenomics.com for information about designing custom gene panels that are compatible with pre-designed panels. The lead time for acquiring custom panels is ~4 weeks (~1 week for gene selection, 3 weeks for ordering and shipping).

Visit the 10x Genomics website for the most current information.

1.0 Xenium Analyzer Overview

Xenium is an end-to-end platform from 10x Genomics that provides highly sensitive, targeted gene expression information at sub-cellular resolution. This platform is powered by the Xenium Analyzer, a versatile instrument for fully automated high-throughput in situ analysis.

1.1 Dimensions

Dimensions	L	W	H
Xenium Analyzer	52.5”/133.3 cm	27”/68.5 cm	31”/ 78.7 cm *59”/149.8 cm - door open*
Xenium Analysis Computer	7”/17.8 cm	26.5”/67.3 cm	18”/ 45.7 cm
Vibration Isolation Table	53”/134.6 cm 3.4”/8.5 cm	30”/76.2 cm 25”/63.5 cm	29”/73.6 cm 17”/43.2 cm
UPS *(APC SRT3000XLT* or similar; not provided by 10x Genomics)*			

**Use equivalent regional models*

Xenium Analyzer on the Vibration Isolation Table

For detailed specifications, consult the Xenium Analyzer Site Preparation Survey (CG000587). Specifications also be available in the Xenium Analyzer User Guide (CG000584).

1.2 Xenium Analyzer - Installation & Training

An overview of the delivery and installation process is provided below.

1.3 Xenium Analyzer - Site Preparation

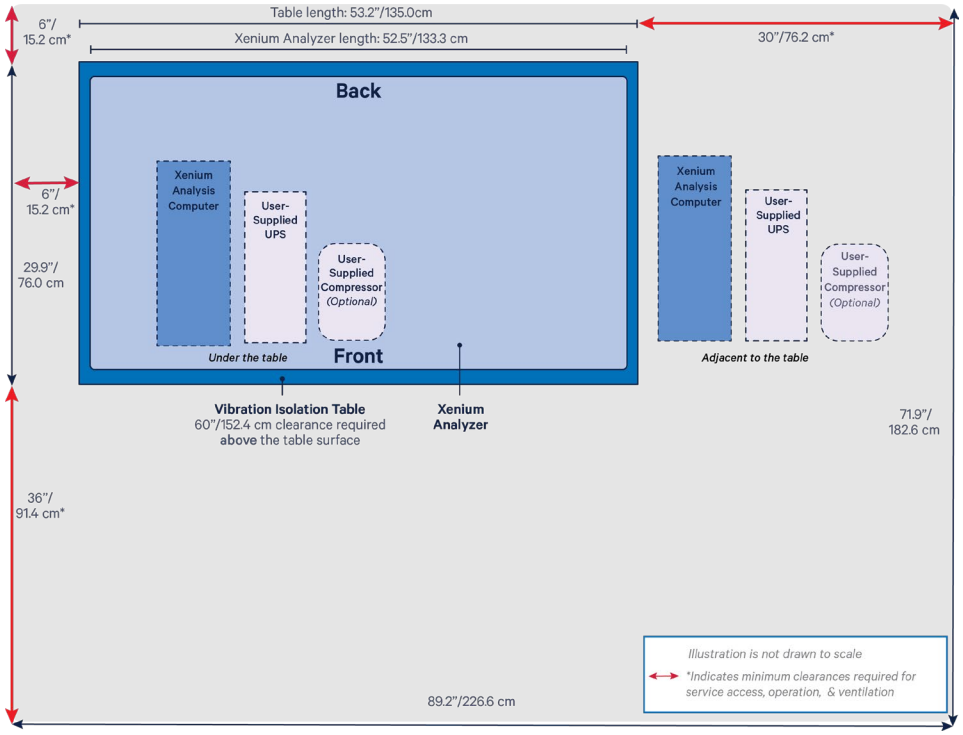
Space Requirements

It is critical to install the instrument in a location away from any vibration sources, such as equipment with compressors (refrigerators, freezers, etc.), motors (centrifuges, shakers, etc.), doors, and busy walkways. Additionally, the installation space should not have soft floor types, such as linoleum or carpet. The instrument should not be placed in direct sunlight or next to other heat generating sources.

The illustration below provides the dimensions and configuration of the space required for installing the Xenium Analyzer. Note that the clearances specified are required for instrument installation, operation, service access, and ventilation. The Xenium Analysis Computer, user-acquired UPS, and compressor (optional) maybe placed under the Vibration Isolation Table or adjacent to it with the indicated clearances.

To float the instrument on the Vibration Isolation Table, on-site CDA (compressed dry air) is highly recommended. Alternatively, a low noise/vibration compressor may be used (such as, Air Compressor, Low Noise, 3.5 Liter Capacity, 110 VAC, Model ACGP from Newport or equivalent).

Space Requirements: Xenium Analyzer on the Vibration Isolation Table



Fill and share the Xenium Analyzer Site Preparation Survey (CG000587) with 10x Genomics which will be followed by more in-depth discussion with a 10x Representative.

1.3 Xenium Analyzer - Site Preparation *contd.*

Power Supply

The Xenium Analyzer and the Xenium Analysis Computer require uninterrupted power supply for a successful run (~2-4 days/run). Standard emergency generator-backed power is often not uninterruptible and a brief power outage is typical before power resumes. Any interruption in the power supply will terminate the run, resulting in the potential loss of samples, reagents, and data that cannot be replaced/recovered by 10x Genomics.

A user-supplied uninterruptible power supply (UPS) is highly recommended but not required during installation. It is recommended that the instrument should be connected to a UPS during runs (provides ~5 min backup power for 2,000 W). Additionally, connecting the UPS to an emergency generator-backed power supply is recommended.

UPS

The recommended UPS specifications are provided below.

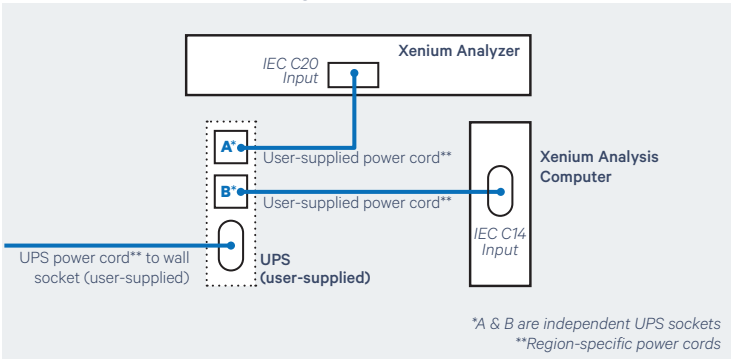
- **Minimum UPS power rating (VA):** 3,000 VA
- **Minimum UPS power rating (W):** 2,700 W
- **Nominal Input Voltage:** 208 V
- **UPS design topology type:** On Line
- **Minimum backup run time for 2,000 W:** ~5 min

APC SRT3000XLT or similar UPS may be used.
Follow manufacturer's instructions for UPS setup and ensure that the battery is connected.

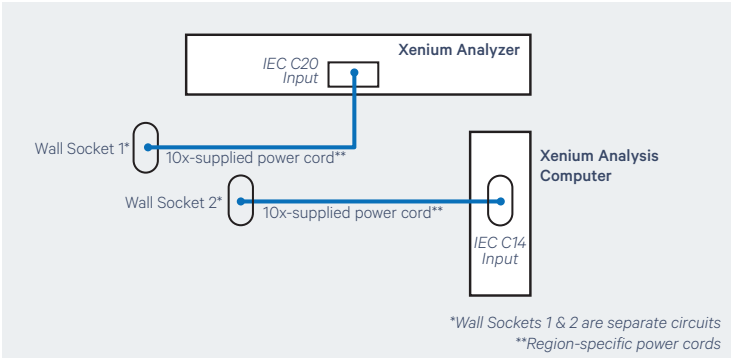
1.3 Xenium Analyzer - Site Preparation *contd.*

The configurations of the Xenium Analyzer and the Xenium Analysis Computer with a UPS (recommended) and without a UPS are illustrated below.

Xenium Analyzer Configuration with UPS (recommended)



Xenium Analyzer Configuration without UPS



UPS should be plugged into an independent circuit. Refer to the user-acquired UPS installation documentation for UPS input circuit requirements.

Power Cords

Refer to the 10x Genomics Support website for the most current information regarding region-specific power cords.

10x Genomics will ship two region-specific power cords that are compatible with the regional wall sockets and the Xenium Analyzer IEC C20 input and the Xenium Analysis Computer IEC C14 input. If the instrument is connected to a UPS, region-specific compatible power cords in compliance with the local standards need to be acquired by the user.

Network Connectivity

Networking capabilities allow for egress of output files to shared network drives and enable faster troubleshooting via remote support of the Xenium Analyzer. Users will have the ability to enable and disable remote access to their instrument directly. The user needs to inform the IT department of their institution regarding the network/ Internet access.

Refer to the Xenium Analyzer Network Connectivity Guidelines Technical Note (CG000645) for comprehensive information regarding remote performance monitoring and remote support along with additional technical details.

Contact support@10xgenomics.com for additional information.

1.4 Items for Installation & Training *(not supplied by 10x Genomics)*

Review the items listed below that should be available during on-site installation by 10x Genomics representative. Refer to the manufacturer's website for regional part numbers.

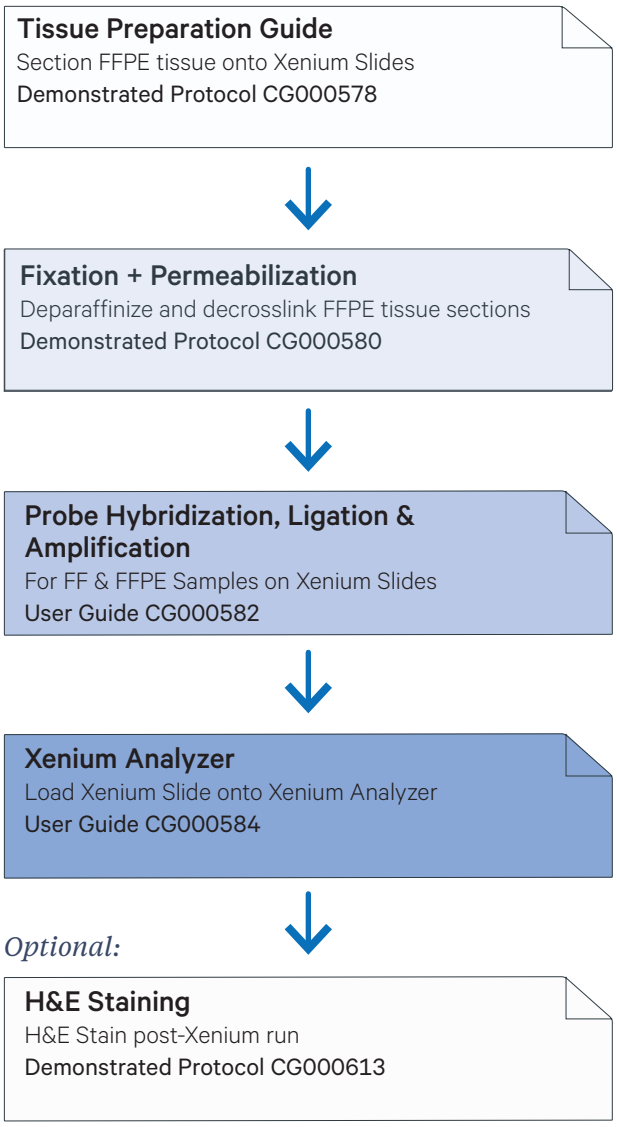
For Installation				
	Item	**Description**	**Vendor**	**Part Number**
☐	Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
		Nuclease-free Milli-Q water (Biopak® Polisher) *(select one based on availability)*	Millipore Sigma	CDUFBI0A1
☐	PBS-T	Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4	Millipore Sigma	P3563-10PAK
		Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 *(select one based on availability)*	Millipore Sigma	PPB005-20PAK
☐	PBS *Alternate for making PBS-T*	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404
☐	100% DMSO	Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	41639-100 ML
		Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	41639-500 ML
		Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	D8418-1L
		Dimethyl sulfoxide, Fisher BioReagents (>99.7%)	Fisher Scientific	BP231-1
		Dimethyl sulfoxide (for molecular biology, 99.5+%) *(select one based on availability)*	Fuji Film	043-29355 500 ml
☐	KCl	Potassium Chloride (KCl, sterile), 500 ml	Teknova	P0330
		Potassium Chloride (KCl, sterile), 1L	Teknova	P0335
		KCl (2 M), RNase-free *(conc. in working solution will be 50 mM; select one based on availability)*	Invitrogen	AM9640G
Additional Materials				
☐	Centrifuge	Allegra X-14 Series Benchtop Centrifuge 120 V *Or equivalent; fits deep-well 96 well plates (~2 ml vol.)*	Beckman Coulter Coulter	-
☐	Serological Pipettes	10 ml, 25 ml, 50 ml, 100 ml		
☐	Serological Pipette Controller	Compatible with 10, 25, 50 & 100 ml serological pipettes		
☐	Graduated Cylinders	500 ml and 1 L		
☐	Pipette Tips	Tips LTS 1ML Filter RT-L1000FLR *Or equivalent*	Rainin	30389213
☐	Pipettes	Pipet-Lite LTS Pipette L-1000XLS+ *Or equivalent*	Rainin	17014382
☐	Glass Bottles with Cap	Pyrex Reusable Media Storage Bottles (500 ml and 1 l) *Or equivalent*		
☐	Compressed Canned Air for cleaning			
☐	Lens-cleaning Paper or Lint-free Laboratory Wipes			

Additional Materials	
☐	Plate seals
☐	70% Isopropanol
☐	Laboratory Balance
☐	Ultrapure/Milli-Q water, *from Milli-Q Integral Ultrapure Water System or equivalent*
☐	A fume hood is available during installation with appropriate procedures in place for handling volatile and hazardous chemicals in compliance with your institutional guidelines
☐	A liquid waste disposal system is available in compliance with your institutional guidelines
☐	If the Ethernet port on site cannot be reached using the 8 ft cable (10x-supplied), a CAT6 or higher Ethernet cable is available to connect to the port

This list may not include some standard laboratory equipment.

2.0 FFPE Samples

2.1 Workflow Documents



Documents will be available on the 10x Genomics Support website.

2.2 FFPE Samples - Key Protocol Steps & Timing

» Tissue Sectioning & Section Placement (off-instrument; ~3 h)

Demonstrated Protocol CG000578

STOP *Sections placed on the Xenium slide can be stored at room temperature in a desiccator for up to 4 weeks.*

» Tissue Section Deparaffinization & Decrosslinking (off-instrument; ~4.5 h)

Demonstrated Protocol CG000580

Steps		Timing
1.1	Buffer Preparation	30 min
1.2	Deparaffinization	3 h (includes 2 h baking step at 60°C)
1.3	Cassette Assembly	10 min
1.4	Decrosslinking	45 min
		Proceed **immediately** to Probe Hybridization, Ligation & Amplification

» Probe Hybridization, Ligation & Amplification (off-instrument; ~2 days)

Refer to the Probe Hybridization, Ligation & Amplification section for details.
User Guide CG000582

» Xenium Analyzer (on-instrument; ~2-4 days)

Refer to the Xenium Analyzer section for details.
User Guide CG000584

2.3 FFPE Samples - Reagents & Consumables *(not supplied by 10x Genomics)*

FFPE Tissue Sectioning & Section Placement

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For FFPE Tissue Sectioning & Section Placement				
	Item	**Description**	**Vendor**	**Part Number**
☐	Microtome	Epredia HM 355S Automatic Microtome *Or any standard histology grade microtome*	Fisher Scientific	23-900-672
☐	Microtome blade	Epredia MX35 Premier Disposable Microtome Blades, Low Profile	Fisher Scientific	3052835
☐	Cool-Cut, *Optional*	Thermo Scientific Cool-Cut	Fisher Scientific	77-112-0
☐	Section transfer system (STS) *Optional*	Thermo Scientific Section Transfer System (STS),	Fisher Scientific	771200
☐	Probes	Fisherbrand Fine Precision Probe	Fisher Scientific	12-000-153
☐	Forceps	Fisherbrand Curved Medium Point General Purpose Forceps	Fisher Scientific	16-100-110
☐	Blank Slides *Optional, for sectioning practice*	Superfrost Plus Microscope Slides	Geyer	194242
☐	Water bath	Tissue Floating Bath, Lighted *Or equivalent*	Fisher Scientific	A84600061
		Epredia Digital Round Tissue Section Water bath *If using optional Section Transfer System*	Fisher Scientific	A84600061
☐	Section dryer oven *Optional, but recommended*	Epredia High Capacity Section Dryer *Or equivalent. Thermal cycler may also be used for section drying*	Fisher Scientific	A84600051
☐	Brushes	Camel Hair Brushes *Or equivalent paintbrush*	Ted Pella	11859
☐	Fan *For drying slides*	Personal Rechargeable Fan *Or equivalent*	Holmes	085-01-0117
☐	Cutting Mat	WellTech Cutting Mat	WellTech Precision Lab	-
☐	Wax Trimmer *Optional*	Electronic Microscopy Sciences Paraffin Block Trimmer Wax Trimmer, 115 VAC	Fisher Scientific	NC0310844
Additional Materials				
☐	Razor blades			
☐	Ice bucket (4-5 L)			
☐	Ultrapure/Milli-Q Water for Water Bath, *from Milli-Q Integral Ultrapure Water System or equivalent*			

This list may not include some standard laboratory equipment.

FFPE Tissue Sections: Deparaffinization & Decrosslinking

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

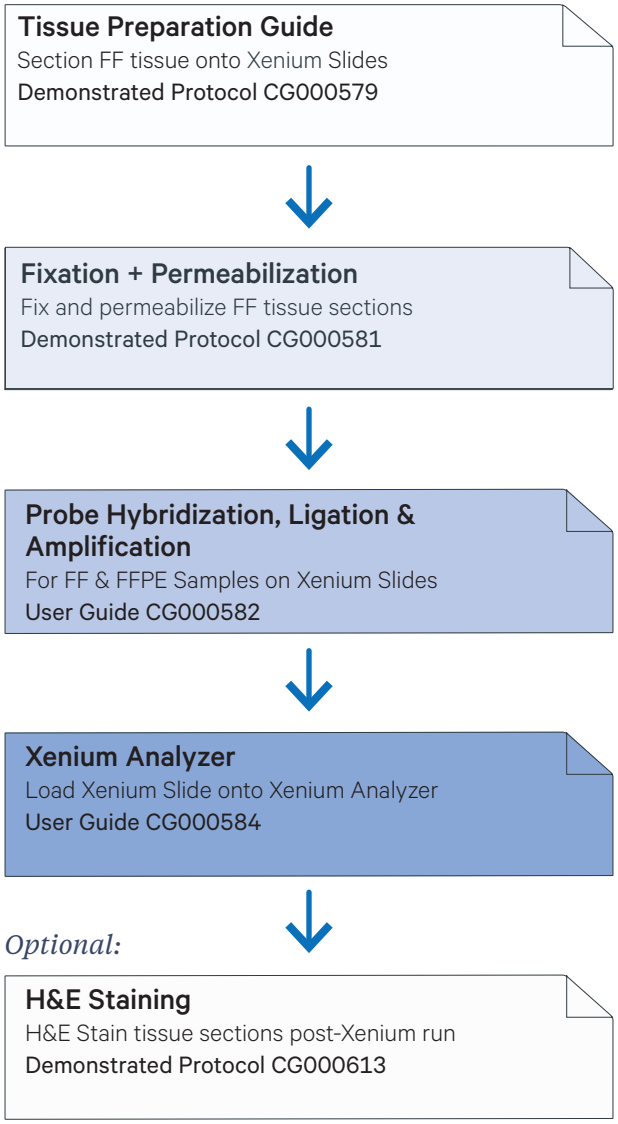
For FFPE Tissue Sections: Deparaffinization & Decrosslinking				
	Item	**Description**	**Vendor**	**Part Number**
☐	Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
☐	or	Xylene, Histological Grade	Millipore Sigma	534056
☐	Neo-clear	Neo-clear Xylene Alternative Substitute	Millipore Sigma	1098435000
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
☐		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP
☐	Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
☐	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Urea	Urea Solution, 8M	Millipore Sigma	51457
☐	10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution; *not 100% Tween diluted to 10%*)	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404
☐	Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
☐	Staining jar/dishes	Coplin Jar	VWR	100500-232
		Staining Dishes	VWR	25608-906
☐	Section dryer oven	Epredia High Capacity Section Dryer *Or equivalent. Thermal cycler may also be used for section drying*	Fisher Scientific	A84600051
Additional Materials				
☐	Water Bath *Alternatively, Eppendorf Thermomixer C (5382000023) with SmartBlock -2.0 mL (5362000035) or equivalent may be used*			
☐	Slide drying rack			
☐	Fume Hood			
☐	Vortex			
☐	Ultrapure/Milli-Q Water for Water Bath, *from Milli-Q Integral Ultrapure Water System or equivalent*			

This list may not include some standard laboratory equipment.

Refer to the Probe Hybridization, Ligation & Amplification section and the Xenium Analyzer section for reagents & consumables required. The information in these two sections applies to both FFPE and FF samples.

3.0 Fresh Frozen Samples

3.1 Workflow Documents



Documents will be available on the 10x Genomics Support website.

3.2 Fresh Frozen Samples - Key Protocol Steps & Timing

» Tissue Sectioning & Section Placement (off-instrument; ~3 h)

Demonstrated Protocol CG000579

STOP *Sections placed on the Xenium slide can be stored at -80°C for up to 4 weeks.*

» Tissue Section Fixation + Permeabilization (off-instrument; ~2.5 h)

Demonstrated Protocol CG000581

Steps		Timing
1.1	Buffer Preparation	30 min
1.2	Slide Preparation	5 min
1.3	Fixation Permeabilization	30 min
1.4	Cassette Assembly	65 min
1.5		10 min
		Proceed **immediately** to Probe Hybridization, Ligation & Amplification

» Probe Hybridization, Ligation & Amplification (off-instrument; ~2 days)

Refer to the Probe Hybridization, Ligation & Amplification section for details.
User Guide CG000582

» Xenium Analyzer (on-instrument; ~2-4 days)

Refer to the Xenium Analyzer section for details.
User Guide CG000584

3.3 Fresh Frozen Samples - Reagents & Consumables *(not supplied by 10x Genomics)*

Fresh Frozen (FF) Tissue Sectioning & Section Placement

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For FF Tissue Sectioning & Section Placement				
	Item	**Description**	**Vendor**	**Part Number**
	Tissue Freezing			
☐	Isopentane	Isopentane (2-Methylbutane)	Millipore Sigma	270342
☐	Forceps	Specimen Forceps, Straight, 203 mm (8")	VWR	82027-436
		Specimen Forceps, Straight, 152 mm (6")	VWR	82027-438
	Frozen Tissue Embedding			
☐	Embedding Compound	TissueTek O.C.T. Compound	VWR	25608-930
☐	Embedding Molds	Epredia Peel-A-Way Disposable Embedding Molds	Fisher Scientific	12-20
	Frozen Tissue Sectioning			
☐	Blank Slides *Optional, for sectioning practice*	Superfrost Plus Microscope Slides	Fisher Scientific	12-550-15
☐	Cryostat	CryoStar NX70 Cryostat	Fisher Scientific	957020
☐	Brushes	Flat cryostat brush, 10 mm *Or equivalent*	Fisher Scientific	14-071-00
☐	Specimen Chuck	Thermo Scientific CryoStar NX70 Specimen Chuck	Fisher Scientific	14-071-413
☐	Microtome Blade	MX35 Ultra Microtome Blade, Low Profile	Fisher Scientific	3051835
☐	Slide Mailer	Simport Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
☐	Anti-Roll Plate *Optional*	Glass Anti-Roll Plate	Fisher Scientific	A78930200
	Additional Materials			
☐	Dry Ice			
☐	Razor blades			
☐	Ice bucket			
☐	Aluminum Foil			

This list may not include some standard laboratory equipment.

Fresh Frozen (FF) Tissue Sections: Fixation & Permeabilization

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers

For FF Tissue Sections: Fixation & Permeabilization				
	Item	**Description**	**Vendor**	**Part Number**
☐	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Nuclease-free Water	Nuclease-free water (not-DEPC treated)	Thermo Fisher Scientific	AM9932/ AM9937
☐	Formaldehyde or Paraformaldehyde	Formaldehyde (37% by Weight/Molecular Biology)	Thermo Fisher Scientific	BP531-500
		Formaldehyde solution	Millipore Sigma	252549/ F8775/ 47608
		Paraformaldehyde 16% Aqueous Solution, EM Grade	Electron Microscopy Sciences	15710
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
☐	10% Tween-20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404
☐	Methanol	Methanol, for HPLC	Millipore Sigma	34860
☐	SDS	Sodium dodecyl sulfate solution (for molecular biology, 10% in H2O)	Millipore Sigma	71736
☐	Forceps	Tweezers, 4" Wafer Handling	Excelta Corp	491P-SA-PI
☐	Slide Mailers	Sim port Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399
Additional Materials				
☐	Dry Ice			
☐	10x Genomics has tested only the listed thermal cyclers. Currently no alternate recommendations are available. Use one of the listed thermal cyclers based on preference and availability. • Bio-Rad: C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (1851197) • Analytik Jena: Biometra TAdvanced 96 SG (846-x-070-241 where x=2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz) • VWR: XT^{96}Gradient with 96-well gradient block & standard lid (76452-153) • Marshall Scientific: MJ Research PTC-200 Thermal Cycler (05434-05)			
☐	Slide drying rack			
☐	Serological pipettes			
☐	Fume Hood			
☐	Vortex			
☐	Ice bucket			
☐	Ultrapure/Milli-Q Water for Water Bath, *from Milli-Q Integral Ultrapure Water System or equivalent*			

This list may not include some standard laboratory equipment.

Refer to the Probe Hybridization, Ligation & Amplification section and the Xenium Analyzer section for reagents & consumables required for these steps. The information applies to both FF and FFPE samples.

4.0 Probe Hybridization, Ligation & Amplification

4.1 Key Protocol Steps & Timing *(off-instrument; for both FFPE & FF samples)*

» Probe Hybridization, Ligation & Amplification (off-instrument; ~2 days)

User Guide CG000582

Steps		Timing	Stop & Store
Day 1 **Step 1: Probe Hybridization**			
1.1	Buffer Preparation	20 min	
1.2	Probe Hybridization	16-24 h (overnight)	
Day 2 **Step 2: Post Hybridization Wash**			
2.1	Post Hybridization Wash	35 min	
Step 3: Ligation			
3.1	Ligation	~2 h	
Step 4: Amplification			
4.1	Amplification	~2 h	
4.2	Post-Amplification wash	15 min	STOP 4°C overnight or ≤4 days (in the dark)
Step 5: Autofluorescence Quenching			
5.1	Autofluorescence Quenching	45 min	STOP 4°C overnight or ≤4 days (in the dark)
5.2	Nuclei Staining	20 min	STOP 4°C overnight or ≤4 days (in the dark)*

**Alternatively, slides can be stored for up to 24 days after Nuclei Staining. For long-term storage, the Xenium Cassette Lid should be replaced with a slide seal and slides should be stored at 4°C in the dark. PBS-T storage buffer should be exchanged with fresh PBS-T every 3-4 days.*

Storing slides for more than 24 days may carry the risk for a lower number of genes or transcripts detected per cell, changes in tissue morphology over time, and microbe growth. These risks are dependent on many factors including input tissue quality and how nuclease-free/microbe-free the workflow is.

4.2 Probe Hybridization, Ligation & Amplification - Reagents & Consumables *(not supplied by 10x Genomics)*

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

	For Probe Hybridization, Ligation & Amplification			
	Item	**Description**	**Vendor**	**Part Number**
☐	Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9932/ AM9937
		Nuclease-free Milli-Q water (Biopak® Polisher) *(select one based on availability)*	Millipore Sigma	CDUFBI0A1
☐	TE Buffer	TE Buffer, TRIS-EDTA, 1X Solution, pH 8.0	Thermo Fisher Scientific	BP24731
☐	PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023-500ML
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP
☐	1.5 ml tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
		Low DNA Binding Tubes, 1.5 ml	Sarstedt	72.706.700
☐	2.0 ml tubes	DNA LoBind Tubes, 2.0 ml	Eppendorf	022431048
		Low DNA Binding Tubes, 2.0 ml	Sarstedt	72.695.700
☐	15 ml tubes	15 ml PP Centrifuge Tubes	Corning	730791
☐	50 ml tubes	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile	Corning	430921
☐	Pipette tips	Tips LTS 200UL Filter RT-L200 FLR	Rainin	30389240
		Tips LTS 1ML Filter RT-L1000 FLR	Rainin	30389213
		Tips LTS 20UL Filter RT-L20 FLR	Rainin	30389226
☐	Pipettes	Pipet-Lite LTS Pipette L-20XLS+	Rainin	17014392
		Pipet-Lite LTS Pipette L-100XLS+	Rainin	17014384
		Pipet-Lite LTS Pipette L-200XLS+	Rainin	17014391
		Pipet-Lite LTS Pipette L-1000XLS+	Rainin	17014382
Additional Materials				
☐	Water Bath *Alternatively, Eppendorf Thermomixer C (5382000023) with SmartBlock -2.0 mL (5362000035) or equivalent may be used*			
☐	Mini centrifuge			
☐	Vortex			
☐	Ice Bucket			
☐	Ultrapure/Milli-Q Water for Water Bath, *from Milli-Q Integral Ultrapure Water System or equivalent*			

This list may not include some standard laboratory equipment.

5.0 Xenium Analyzer

5.1 Key Protocol Steps & Timing *(on-instrument; for both FFPE & FF samples)*

» Xenium Analyzer (on-instrument; ~2-4 days)

User Guide CG000584

Steps	Timing	
	Hands-on Time	Total Time
Day 1		
Thaw Decoding Reagents	5 min	16-24 h (overnight)
Day 2		
Prepare Buffers	1 h	1 h
Initialize Instrument	-	5-10 min
Input Experimental Details	5-10 min	5-10 min
Load Instrument	~5 min	~5 min
Overview Scan	-	1 h
Select Region & Initiate Run	~10 min	~10 min
Day 4-6		
Run Time	-	2-4 days
Post-Run Cleanup	5 min	10 min

5.2 Xenium Analyzer - Reagents & Consumables *(not supplied by 10x Genomics)*

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For Reagent Bottle Buffer Preparation				
	Item	**Description**	**Vendor**	**Part Number**
☐	Nuclease-free Water	Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific	AM9932/ AM9937
☐	PBS-T	Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4	Millipore Sigma	P3563-10PAK
		Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 *(select one based on availability)*	Millipore Sigma	PPB005-20PAK
☐	PBS *Alternate for making PBS-T*	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	10% Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution) *(use one ampule per use)*	Thermo Fisher Scientific	28320
		10% Tween-20	Bio-Rad	1662404
☐	100% DMSO	Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	41639-100 ML
		Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	41639-500 ML
		Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	D8418-250ML
		Dimethyl sulfoxide (molecular biology grade)	Millipore Sigma	D8418-1L
		Dimethyl sulfoxide, Fisher BioReagents (>99.7%)	Fisher Scientific	BP231-1
		Dimethyl sulfoxide (for molecular biology, 99.5+%) *(select one based on availability)*	Fuji Film	043-29355 500 ml
☐	KCl	Potassium Chloride (KCl, sterile), 500 ml	Teknova	P0330
		Potassium Chloride (KCl, sterile), 1L	Teknova	P0335
		KCl (2 M), RNase-free *(conc. in working solution will be 50 mM; select one based on availability)*	Invitrogen	AM9640G
Additional Materials				
☐	Centrifuge	Allegra X-14 Series Benchtop Centrifuge 120 V Or equivalent; fits deep-well 96 well plates (~2 ml vol.)	Beckman Coulter Coulter	-
☐	Serological Pipettes	10 ml, 25 ml, 50 ml, 100 ml		
☐	Serological Pipette Controller	Compatible with 10, 25, 50 & 100 ml serological pipettes		
☐	Graduated Cylinders	100 ml and other volumes as needed		
☐	Pipette Tips	Tips LTS 1ML Filter RT-L1000FLR *Or equivalent*	Rainin	30389213
☐	Pipettes	Pipet-Lite LTS Pipette L-1000XLS+ *Or equivalent*	Rainin	17014382
☐	Glass Bottles with Cap	Pyrex Reusable Media Storage Bottles (500 ml and 1 l) *Or equivalent*		
☐	Compressed Canned Air for cleaning			
☐	Lens-cleaning Paper or Lint-free Laboratory Wipes			
☐	Plate seal			
☐	70% Isopropanol			
☐	Laboratory Balance			
☐	Ultrapure/Milli-Q water, from Milli-Q Integral Ultrapure Water System or equivalent			

This list may not include some standard laboratory equipment.

Appendix

Quencher Removal & H&E Staining - Reagents & Consumables
(not supplied by 10x Genomics)

Optional; Only if following 10x Genomics H&E protocol

For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For Quencher Removal *Post-run quencher removal is required only if staining slides after the instrument run*				
	Item	**Description**	**Vendor**	**Part Number**
☐	Sodium Hydrosulfite	Sodium hydrosulfite, technical grade	Sigma Aldrich	157953
☐	Forceps	Tweezers, 4' Water Handling	Excelta Corp	491P-SA-PI
☐	PBS *(optional)*	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Slide Mailers	Sim port Scientific LockMailer Tamper Evident Slide Mailer	Fisher Scientific	22-038-399

For H&E Staining				
	Item	**Description**	**Vendor**	**Part Number**
☐	Hematoxylin	Hematoxylin Solution, Mayer's	Sigma Aldrich	MHS16
☐	Eosin	Eosin Y Solution, Alcoholic	Leica	3801615
☐	Bluing Reagent	Bluing Solution	Dako	CS702
☐	Mounting Media	Surgipath SUB-X Mounting Media *Discontd.* *Cytoseal or equivalent mounting media can be used*	Leica	3801741
☐	Ethanol	Ethyl Alcohol, 200 Proof, anhydrous	Millipore Sigma	E7023
		Ethanol absolute ≥99.5%, TechniSolv, pure (Europe)	VWR	83813.360DP
☐	Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
		Xylene, Histological Grade	Millipore Sigma	534056
☐	Forceps	Tweezers, 4' Water Handling	Excelta Corp	491P-SA-PI
☐	Filter Paper	Fisherbrand Qualitative Grade Plain Filter Paper Circles *Or equivalent*	Fisher Scientific	09-795-H
☐	Coverslips	Fisherbrand Cover Glasses: Rectangles *Discontinued*	Fisher Scientific	12-544-EP
		Cover Glasses, Rectangles	VWR	16004-322

Additional Materials	
☐	Vortex
☐	Staining jar/dishes
☐	Wide-bore pipette tips
☐	Ultrapure/Milli-Q water, *from Milli-Q Integral Ultrapure Water System or equivalent*

This list may not include some standard laboratory equipment.

Document Revision Summary

Document Number	CG000601
Title	Xenium In Situ Gene Expression - Protocol Planner
Revision	Rev C to Rev D
Revision Date	July 2023

Specific Changes

- Included table specifying volume of bulk reagents needed for the workflow (page 3)
- Includes hyperlink to Xenium Network Connectivity Technical Note (page 8)
- Included new section 1.4 Items Needed for Installation (pages 9 10)
- Corrected to specify -80°C as the recommended temperature for storing sections placed on the Xenium slide (pages 12, 16)
- Updated list of tested thermal cyclers (page 18)
- Included additional vendors for procuring DMSO (pages 10, 22)
- Updated Bio-Rad as additional vendor for procuring 10% Tween 20 (pages 9, 18, 22)
- Updated storage guidance after nuclei staining (page 19)

General Changes

Updated for general minor consistency of format, language, and terms throughout

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA