

## PLANNER

# Visium Spatial Gene Expression

## Protocol Planner

This document provides the time planners and additional equipment, kits, and reagents for the Visium Spatial Gene Expression and Tissue Optimization protocols. The 10x Genomics Visium Spatial Reagent Kits are not listed in this document.

### Visium Spatial Tissue Optimization Protocol Steps & Timing

4 h







Steps		Timing
<b>Step 1 – Tissue Staining &amp; Imaging</b>		
1.1	Tissue Fixation	35 min
1.2	Tissue Staining	30 min
1.3	Tissue Imaging*	Variable
<b>Step 2 – Permeabilization &amp; cDNA Synthesis</b>		
2.1	Tissue Permeabilization	45 min
2.2	Fluorescent cDNA Synthesis	60 min
<b>Step 3 – Tissue Removal</b>		
3.1	Tissue Removal	70 min
3.2	Slide Imaging*	Variable

\*~4 h workflow, excluding imaging steps

## Visium Spatial Gene Expression Protocol Steps & Timing

1-1.5 days



Steps	Timing	Stop & Store
<b>Tissue Staining &amp; Imaging</b>		
Tissue Fixing	35 min	
Tissue Staining	30 min	
Tissue Imaging	Variable	
<b>cDNA Synthesis</b>		
Tissue Permeabilization	15 min	
Reverse Transcription	65 min	
<b>Second Strand Synthesis &amp; Denaturation</b>		
Second Strand Synthesis	25 min	
cDNA Denaturation	15 min	
<b>cDNA Amplification &amp; QC</b>		
Cycle Number Determination – qPCR	45 min	
cDNA Amplification	45-60 min	 4°C ≤72 h or -20°C ≤1 week
cDNA Cleanup – SPRIselect	20 min	 4°C ≤72 h -20°C ≤4 weeks
cDNA QC & Quantification	50 min	
<b>Visium Spatial Gene Expression Library Construction</b>		
Fragmentation, End Repair & A-tailing	50 min	
Post Fragmentation, End Repair & A-tailing Double	30 min	
Sided Size Selection – SPRIselect		
Adaptor Ligation	25 min	
Post Ligation Cleanup- SPRIselect	20 min	
Sample Index PCR	40 min	 4°C ≤72 h
Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	 4°C ≤72 h or -20°C long term
Post Library Construction QC	50 min	

## Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

Supplier	Description	Part Number (US)
<b>Plastics</b>		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml (when processing more than 2 slides)	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8 -Cap Strip, clear Simport Scientific LockMailer Tamper Evidence Slide Mailer (alternatively, use a 50-ml centrifuge tube)	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips. N8010580 N8010535 22-038-399
Corning	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile Corning 250 mL Vacuum System, 0.2 µm Pore 19.6cm <sup>2</sup> NY Membrane	430921 430771
Bio-Rad	Hard-shell PCR Plates 96-well, thin wall (pkg of 50) (alternatively, use any compatible PCR Plate) Microseal 'B' PCR Plate Sealing Film, adhesive (alternatively, use any PCR Plate sealing adhesive)	HSP9665 MSB1001
Rainin	Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR Tips LTS 20UL Filter RT-L10FLR	30389240 30389213 30389226
VWR	Divided Polystyrene Reservoirs	41428-958
<b>Kits &amp; Reagents</b>		
Agilent	Hematoxylin, Mayer's (Lillie's Modification) Bluing Buffer, Dako Eosin, Dako (alternative to Millipore Sigma product)	S330930-2 CS70230-2 CS70130-2
Thermo Fisher Scientific	Nuclease-free Water Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) Tris Base (White Crystals or Crystalline Powder/Molecular Biology) Tris 1M, pH 7.0, RNase-free Shandon Bluing Reagent (alternative to Agilent product) Universal Mouse Reference RNA* (Optional. Alternatively, use any bulk Total RNA. 1µg/µl, RIN ≥ 7)	AM9937 12090-015 BP152-500 AM9850G 6769001 QS0640
Fisher Chemical	Hydrochloric Acid Solution, 0.1N	SA54-1
Electron Microscopy Services	Mayer's Hematoxylin (alternative to Agilent product)	2638102
KAPA Biosystems	KAPA SYBR FAST qPCR Master Mix (2X)	KK4600
Beckman Coulter	SPRIselect Reagent Kit	B23318

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Supplier	Description	Part Number (US)
<b>Kits &amp; Reagents</b>		
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
	Potassium Hydroxide Solution, 8M	P4494-50ML
	Methanol, for HPLC, ≥ 99.9%	34860
	2-Propanol (Isopropanol), ≥ 99.5%	I9516-25ML
	SSC Buffer 20X Concentrate	S66391L
	Sodium dodecyl sulfate (SDS) solution, 10% in water*	71736
	Hematoxylin Solution, Mayer's (alternative to Agilent product)	MHS16-500ML
	Hematoxylin solution according to Mayer (alternative to Agilent product)	51275-100ML
	Blueing Reagent (alternative to Agilent product)	65354-85
	Eosin Y solution, aqueous	HT110216-500ML
	Eosin Y-solution 0.5% aqueous (alternative to HT110216-500ML)	1098441000
	Acetic acid, ≥ 99.9%	A6283
Qiagen	Qiagen Buffer EB	19086
-	Ultrapure/Milli-Q water (from Milli-Q Integral Ultrapure Water System or equivalent)	
<b>Equipment</b>		
Rainin	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
Thermo Fisher Scientific	MYFUGE 12 Mini Centrifuge (alternatively, use any equivalent mini centrifuge)	C1012
<b>Quantification &amp; Quality Control</b>		
Agilent	2100 Bioanalyzer Laptop Bundle	G2943CA
	High Sensitivity DNA Kit	5067-4626
	4200 TapeStation	G2991AA
	High Sensitivity D1000 ScreenTape/Reagents	5067-5592/ 5067-5593
PerkinElmer	High Sensitivity D5000 ScreenTape/Reagents	5067-5584/ 5067-5585
	LabChip GX Touch HT Nucleic Acid Analyzer	CLS137031
	DNA High Sensitivity Reagent Kit	CLS760672
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

\* Only required for Visium Spatial Tissue Optimization protocol

## Cryostat Specifications

The Cryostar NX70 Cryostat with listed features was used by 10x Genomics. Any equivalent system with the listed features may be used.

Component	Features
Main Cryochamber	<ul style="list-style-type: none"> <li>Maintains stable temperatures from -10°C to -20°C</li> </ul>
Blade Holder	<ul style="list-style-type: none"> <li>Separate and adjustable temperature control</li> <li>Maintains stable temperatures from -8°C to -35°C</li> </ul>
Specimen Head	<ul style="list-style-type: none"> <li>Separate and adjustable temperature control</li> <li>Maintains stable temperatures from -8°C to -35°C</li> <li>X-axis and Y-axis adjustment</li> </ul>
Blade Holder Base	<ul style="list-style-type: none"> <li>Adjustable cutting angle</li> <li>Adjustable blade position</li> <li>Cuts 10-50 µM thick sections</li> </ul>
Cryobar	<ul style="list-style-type: none"> <li>Rapid cooling</li> </ul>

## Additional Items for Cryosectioning

Vendor	Item	Part Number (US)
VWR	TissueTek O.C.T. Compound	25608-930
10x Genomics	Visium Spatial Tissue Optimization Slide / Visium Spatial Gene Expression Slide	3000394/ 2000233
Thermo Fisher Scientific	CryoStar NX70 Cryostat, Vacutome, Low Profile Blade Carrier	957020
	30 mm Specimen Chuck	715870-CN
	Shandon ColorFrost Plus Slides (Optional) (alternatively, use any 75 mm x 25 mm x 1 mm microscope slide)	6776214
	Simport Scientific LockMailer Tamper Evident Slide Mailer (case of 500) (Optional)	22-038-399
	MX35 Ultra Microtome Blade (Low-Profile)	3053835
	Anti-Roll Plate (Glass)	1407086
	Flat cryostat brush, 12 mm	334160
	Brush, small beveled	334171
	Magnetic Brush, big	334172

## Recommended Thermal Cyclers

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

## Recommended Real Time qPCR Systems

Supplier	Description	Part Number
Applied Biosystems	QuantStudio 12K Flex system	4471087
Bio-Rad	CFX96 Real-time System	1855196

For complete protocol information, consult the Visium Spatial Tissue Optimization Reagent Kits User Guide (CG000238) and the Visium Spatial Gene Expression Reagent Kits User Guide (CG000239).

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**Contact:**  
[support@10xgenomics.com](mailto:support@10xgenomics.com)  
 10x Genomics  
 6230 Stoneridge Mall Road  
 Pleasanton, CA 94588 USA

