

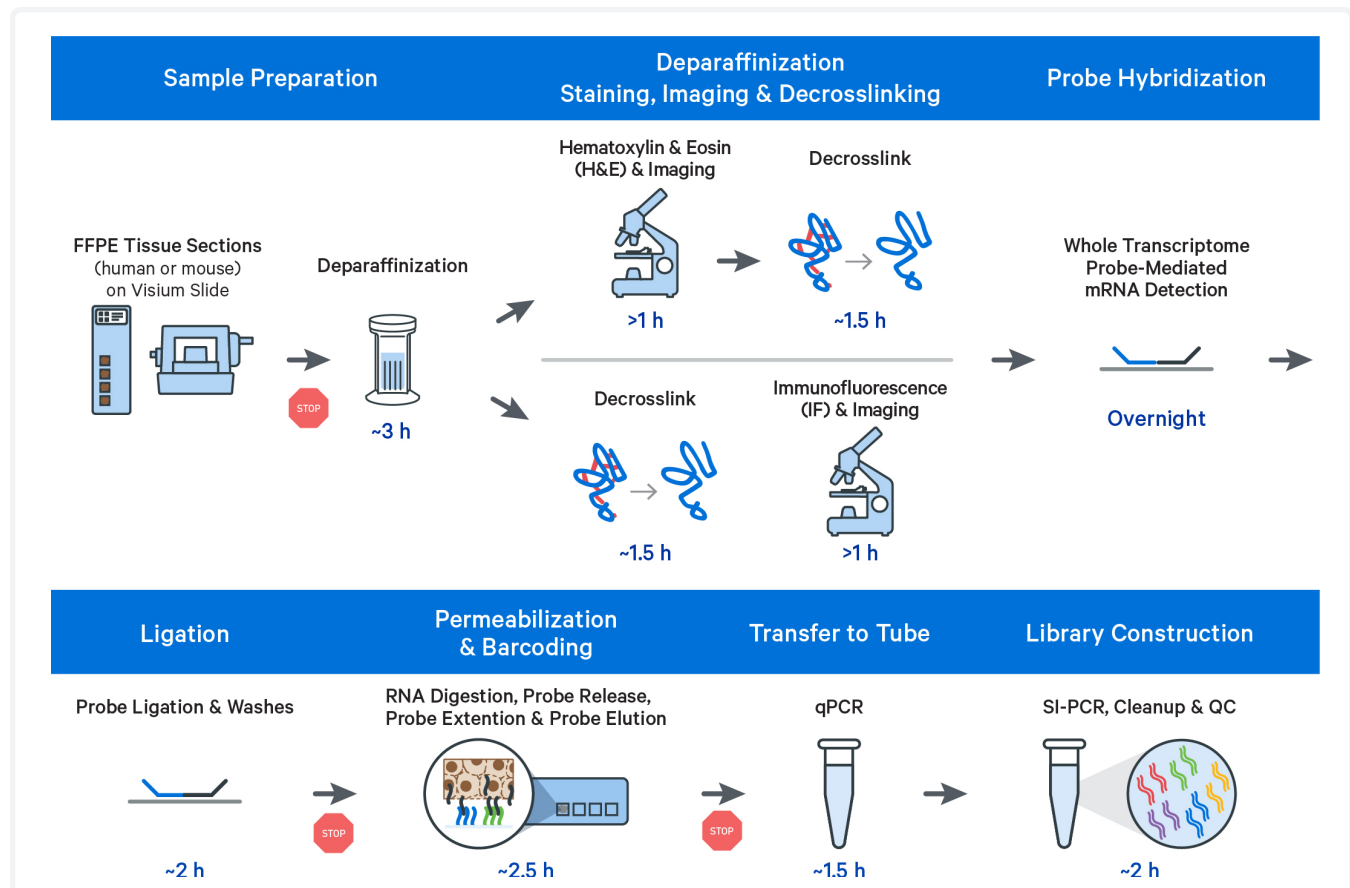
# Visium Spatial Gene Expression for FFPE – Protocol Planner

## Introduction

Visium Spatial Gene Expression for FFPE assays RNA levels by using probes against the transcriptome in intact formalin fixed paraffin embedded (FFPE) tissue sections and maps the location(s) where gene activity is occurring. This document provides additional equipment, kits, and reagents for the Visium Spatial Gene Expression for FFPE protocols. The 10x Genomics Visium Spatial Reagent Kits for FFPE are not listed in this document.

## Workflow Overview

An overview of the Visium Spatial Gene Expression for FFPE workflow is provided below.



## Kits, Reagents & Equipment

The items in the tables below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits for FFPE. 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.

### Sample Preparation

For FFPE Tissue Sectioning & Section Placement			
Item	Alternatives/Options	Vendor	Part Number
Microtome	Epredia HM 355S Automatic Microtome <i>Or any standard histology grade microtome</i>	Fisher Scientific	23-900-672
Microtome blade	Epredia MX35 Premier Disposable Microtome Blades, Low Profile	Fisher Scientific	3052835
Cool-Cut, <i>Optional</i>	Thermo Scientific Cool-Cut	Fisher Scientific	77-112-0
Section transfer system (STS), <i>Optional</i>	Thermo Scientific Section Transfer System (STS), <i>Section Transfer System</i>	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
Microscope slides	Fisherbrand Premium Plain Glass Microscope Slides	Fisher Scientific	12-544-4
Water bath	Premiere Tissue Floating Bath, Lighted <i>Or any equivalent water bath</i>	Geyer	194242
Section dryer oven	Epredia High Capacity Section Dryer	Fisher Scientific	A84600051
Brushes	Camel Hair Brushes, <i>Or any equivalent paintbrush</i>	Ted Pella	11859
Additional Materials			
Razor blades	-	-	-
Ice bucket	-	-	-
Ultrapure/Milli-Q Water, <i>from Milli-Q Integral Ultrapure Water System or equivalent</i>	-	-	-

## RNA Quality Assessment Using DV200

For RNA Quality Assessment			
Ethanol	Ethyl Alcohol, Pure (200 Proof, anhydrous)	Millipore Sigma	E7023-500ML
RNA extraction kit	RNeasy FFPE Kit (50)	Qiagen	73504
Deparaffinization solution	Deparaffinization Solution	Qiagen	19093
RNase decontamination solution	RNaseZap RNase Decontamination Solution	Thermo Fisher Scientific	AM9780
Nuclease-free water	Nuclease-free Water (not DEPC-Treated)		AM9937
1.5-ml centrifuge tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
Nanodrop/Qubit fluorometer	Nanodrop 2000c Spectrophotometers <i>Or any equivalent Nanodrop</i>	Thermo Fisher Scientific	ND-2000C
	Qubit 4 Fluorometer	Thermo Fisher Scientific	Q33238
	Qubit RNA BR Assay Kit	Thermo Fisher Scientific	Q10210
	Qubit Assay Tubes	Thermo Fisher Scientific	Q32856
Bioanalyzer/Tapestation	RNA 6000 Pico Kit	Agilent	5067-1513
Choose Bioanalyzer or TapeStation based on availability & preference.	2100 Bioanalyzer Laptop Bundle	Agilent	G2953CA
	4200 TapeStation	Agilent	G2991AA
	High Sensitivity RNA ScreenTape	Agilent	5067-5579
	High Sensitivity RNA ScreenTape Ladder	Agilent	5067-5581
	High Sensitivity RNA ScreenTape Sample Buffer	Agilent	5067-5580

## Deparaffinization, H&E Staining, Imaging & Decrosslinking

For each item, a number of vendor options are listed. Choose item based on availability and preference.

Item	Alternatives/Options	Vendor	Part Number
Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	Xylene, Histological Grade	Millipore Sigma	534056
Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
Eosin	Eosin Y-solution, Alcoholic	Millipore Sigma	HT110116
	Eosin Y Solution (Modified Alcoholic)	Abcam	ab246824
	Eosin Y with Phloxine 1% alcoholic solution	VWR	10143
Hematoxylin	Hematoxylin Solution, Mayer's	Millipore Sigma	MHS16
	Hematoxylin Solution According to Mayer	Millipore Sigma	51275
	Hematoxylin, Mayer's	Agilent	S330930-2
Bluing reagent	Bluing Reagent, Dako	Agilent	CS70230-2
	Thermo Scientific Shandon Bluing Reagent	Fisher Scientific	6769001
	Scott's Bluing Agent	Ricca Chemical Company	6697
Glycerol	Glycerol Solution	Millipore Sigma	49781
	Glycerol	Acros Organics	327255000
0.1 N HCl	Hydrochloric Acid Solution, 0.1 N <i>Or any equivalent HCl</i>	Fisher Chemical	SA54-1
TE Buffer (pH 9.0)	TE Buffer (pH 9.0) <i>Alternatively, prepare TE buffer using Tris and EDTA and adjust the pH to 9.0</i>	GeneMed	10-0046
Or	Tris Base <i>For preparing TE buffer (pH 9.0), alternative to Genemed product</i>	Fisher Scientific	BP152-500
Prepare using Tris and 0.5 M EDTA	UltraPure 0.5 M EDTA, pH 8.0 <i>For preparing TE buffer (pH 9.0), alternative to Genemed product</i>	Thermo Fisher Scientific	15575020
	1.0 M HCl <i>For preparing TE Buffer (pH 9.0), alternative to Genemed product</i>	Millipore Sigma	258148
Staining jar/dishes	Coplin Jar	VWR	100500-232
	Staining Dishes	VWR	25608-906
Section dryer oven	Epredia High Capacity Section Dryer <i>Or any equivalent product</i>	Fisher Scientific	A84600051
Slide holders	Slide Holders, 24-place	VWR	25608-868
Coverslips	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
	Cover Glasses, Rectangular	VWR	16004-322
Sealing film	Microseal 'B' PCR Plate Sealing Film, adhesive	Bio-Rad	MSB1001

### Additional Materials

Beakers	-	-
Ultrapure/Milli-Q Water, <i>from Milli-Q Integral Ultrapure Water System or equivalent</i>	-	-

## Deparaffinization, Decrosslinking, IF Staining & Imaging

For each item, a number of vendor options are listed. Choose item based on availability and preference.

Item	Alternatives/Options	Vendor	Part Number
Xylene	Xylene, Reagent Grade	Millipore Sigma	214736
	Xylene, Histological Grade	Millipore Sigma	534056
Ethanol	Ethyl Alcohol, 200 Proof	Millipore Sigma	E7023
	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
TE Buffer (pH 9.0)	TE Buffer (pH 9.0) <i>Alternatively, prepare TE buffer using Tris and EDTA and adjust the pH to 9.0</i>	GeneMed	10-0046
Or	Tris Base <i>For preparing TE buffer (pH 9.0), alternative to Genemed product</i>	Fisher Scientific	BP152-500
Prepare using Tris and 0.5 M EDTA	UltraPure 0.5 M EDTA, pH 8.0 <i>For preparing TE buffer (pH 9.0), alternative to Genemed product</i>	Thermo Fisher Scientific	15575020
	1.0 M HCl <i>For preparing TE Buffer (pH 9.0), alternative to Genemed product</i>	Millipore Sigma	258148
PBS	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
BSA	Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free	Millipore Sigma	126615-25ML
10% Tween-20	Tween 20 Surfact-Amps Detergent Solution (10% solution)	Thermo Fisher Scientific	28320
Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9937
DAPI	DAPI Solution (1 mg/ml)	Thermo Fisher Scientific	62248
Antibody	-	-	-
RNase inhibitor	Protector RNase Inhibitor (40 U/μl)	Millipore Sigma	3335399001
	RNase Inhibitor, Murine (40 U/μl)	New England Biolabs	M0314
	RiboLock RNase Inhibitor (40 U/μl)	Thermo Scientific	EO038
Mounting medium	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only)	VWR	83813.360DP
Section dryer oven	Epredia High Capacity Section Dryer	Fisher Scientific	A84600051
Slide holders	Slide Holders, 24-place	VWR	25608-868
Coverslips	Fisherbrand Cover Glasses: Rectangles	Fisher Scientific	12-544-EP
	Cover Glasses, Rectangular	VWR	16004-322
Staining jar/dishes	Coplin Jar	VWR	100500-232

### Additional Materials

Beakers	-	-
Ultrapure/Milli-Q Water, <i>from Milli-Q Integral Ultrapure Water System or equivalent</i>	-	-

## Imaging for FFPE

A **brightfield imaging system** with a scanning stage that can accurately capture and stitch together large tiled images is required to run Visium Spatial for FFPE. The microscope objective should be dry and free of immersion oil. Low magnification, high Numeric Aperture (NA) microscopes, e.g., 10x/0.4NA or 20x/0.8NA, are recommended to balance high resolution and high acquisition speed. Visium slides are loosely mounted with glycerol and a coverslip and therefore should not be inverted for imaging. If using an inverted microscope, perform imaging through the slide rather than the coverslip and use an objective (with a correction collar) designed to image through microscope slides.

Supplier	Model	Configuration
Thermo Fisher Scientific	EVOS M7000	Inverted
Leica	Aperio Versa 8	Upright
	Leica DMI8	Inverted
MetaSystems	Metafer	Upright
Nikon	Nikon Eclipse Ti2	Inverted
BioTek	Cytation 7	Inverted or Upright
Keyence	Keyence BZX800	Inverted

### Brightfield Recommended Configuration

Color camera (3 x 8 bit, 2424 x 2424 pixel resolution)

White balancing functionality

2.18  $\mu\text{m}$ /pixel minimum capture resolution

Exposure times 2-10 milli sec

## Visium Spatial Gene Expression for FFPE

The items in the table below have been validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits for FFPE. 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	
<b>Plastics</b>			
Eppendorf	DNA LoBind Tubes, 1.5 ml	022431021	
	DNA LoBind Tubes, 2.0 ml <i>when processing more than two slides</i>	022431048	
	PCR Tubes 0.2 ml 8-tube strips	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.	951010022
USA Scientific	TempAssure PCR 8-tube strip		1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml		N8010580
	MicroAmp 8-Cap Strip, clear	N8010535	
	Simport Scientific LockMailer Tamper Evidence Slide Mailer	22-038-399	
Corning	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile <i>Alternative to slide mailer</i>	430921	
Bio-Rad	Hard-shell PCR Plates 96-well, thin wall (pkg of 50) <i>Or any compatible PCR Plate</i>	HSP9665	
	Microseal 'B' PCR Plate Sealing Film, adhesive <i>Or any PCR Plate sealing adhesive</i>	MSB1001	
Rainin	Tips LTS 200UL Filter RT-L200FLR	30389240	
	Tips LTS 1ML Filter RT-L1000FLR	30389213	
	Tips LTS 20UL Filter RT-L10FLR	30389226	
VWR	Divided Polystyrene Reservoirs	41428-958	
<b>Kits &amp; Reagents</b>			
Thermo Fisher Scientific	Nuclease-free Water (not DEPC-Treated)	AM9937	
	Tris 1M, pH 7.0, RNase-free	AM9850G	
	Fisherbrand Premier Plain Glass Microscope Slides, <i>Optional</i>	12-544-4	
	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	AM9624	
	Tween 20 Surfact-Amps Detergent Solution (10% solution)	28320	
	UltraPure 0.5 M EDTA, pH 8.0 <i>For preparing TE Buffer (pH 9.0)</i>	15575020	
Fisher Chemical	Hydrochloric Acid Solution, 0.1N <i>Or any equivalent HCl</i>	SA54-1	
KAPA Biosystems	KAPA SYBR FAST qPCR Master Mix (2X)	KK4600	
Beckman Coulter	SPRIselect Reagent Kit	B23318	

Supplier	Description	Part Number
<b>Kits &amp; Reagents</b>		
Millipore Sigma	Ethyl Alcohol, Pure (200 Proof, anhydrous)	E7023-500ML
	Potassium Hydroxide Solution, 8M	P4494-50ML
	SSC Buffer 20X Concentrate	S6639-1L
	Hydrochloric Acid Solution, 1.0N <i>Alternative to Fisher Chemical product</i>	H9892
Qiagen	Qiagen Buffer EB	19086
Genemed	TE Buffer (pH 9.0) <i>Alternatively, prepare using Tris base and EDTA solution and adjust the pH to 9.0</i>	10-0046
Fisher Scientific	Tris Base	BP152-500
-	Ultrapure/Milli-Q water, <i>from Milli-Q Integral Ultrapure Water System or equivalent</i>	-
<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
VWR	VWR Mini Centrifuge <i>Or any equivalent mini centrifuge</i>	76269-064
<b>Quantification &amp; Quality Control</b>		
Agilent	2100 Bioanalyzer Laptop Bundle	G2953CA
	High Sensitivity DNA Kit	5067-4626
	4200 TapeStation	G2991AA
	High Sensitivity D1000: ScreenTape/ Reagents	5067-5592/ 5067-5593
	High Sensitivity D5000: ScreenTape/ Reagents	5067-5584/ 5067-5585
PerkinElmer	LabChip GX Touch HT Nucleic Acid Analyzer	CLS137031
	DNA High Sensitivity Reagent Kit	CLS760672
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

Choose Bioanalyzer, TapeStation or Lab Chip based on availability & preference.



## Recommended Thermal Cyclers

Supplier	Description	Part Number
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro (discontinued)	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
Bio-Rad	CFX96 Real-time System	1855096
Bio-Rad	CFX384 Real-time System	1855384

## Document Revision Summary

<b>Document Number</b>	CG000404
<b>Title</b>	Visium Spatial Gene Expression for FFPE – Protocol Planner
<b>Revision</b>	Rev A to Rev B
<b>Revision Date</b>	October 2021
<b>General Changes</b>	Updated for general minor consistency of language and terms throughout
<b>Specific Changes</b>	Added reagent list for Deparaffinization & IF Staining

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