

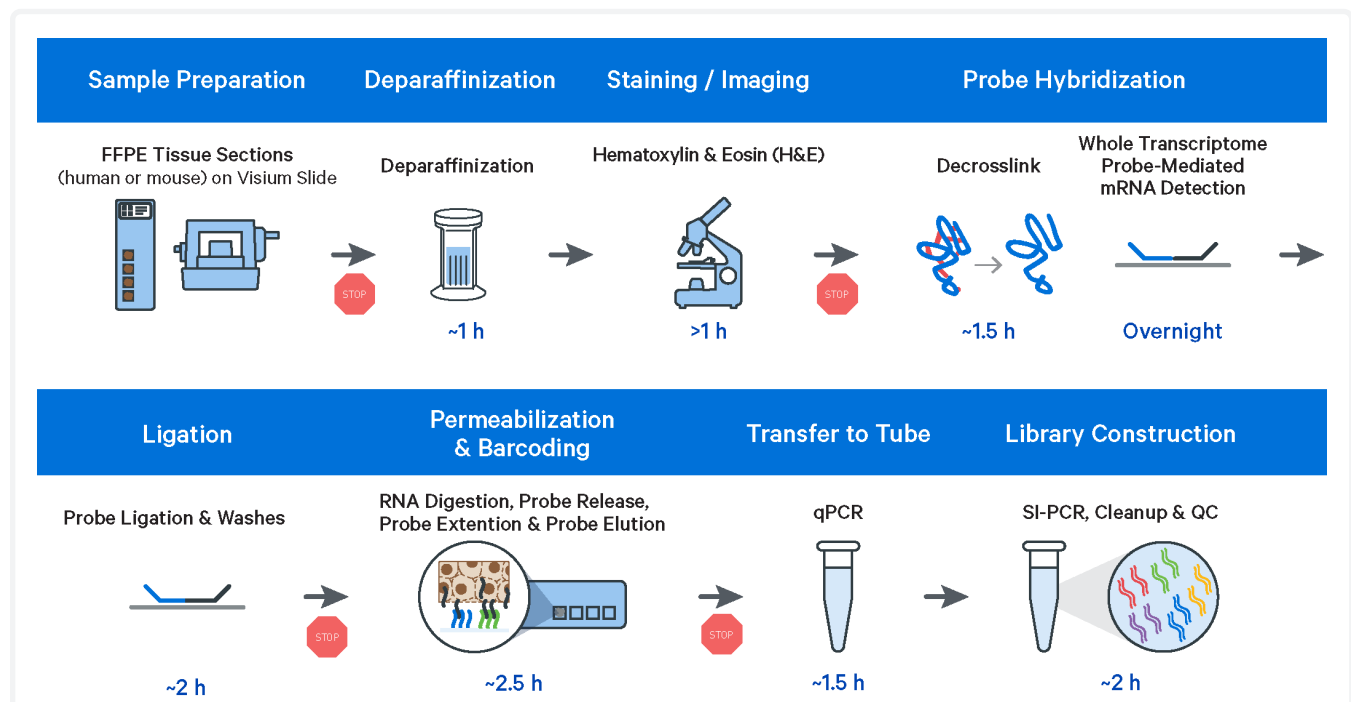
Visium Spatial Gene Expression for FFPE – Protocol Planner

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 the workflow overview and 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

Vendor	Item	Part Number
Fisher Scientific	Epredia HM 355S Automatic Microtome <i>Or any standard histology grade microtome</i>	23-900-672
	Epredia MX35 Premier Disposable Microtome Blades, Low Profile	3052835
	Thermo Scientific Cool-Cut, <i>Optional</i>	77-112-0
	Thermo Scientific Section Transfer System (STS), <i>Optional - If using Section Transfer System</i>	771200
	Fisherbrand Fine Precision Probe	12-000-153
	Fisherbrand Curved Medium Point General Purpose Forceps	16-100-110
	Fisherbrand Premium Plain Glass Microscope Slides	12-544-4
	Epredia Digital Round Tissue Section Water Bath <i>Or any equivalent water bath</i>	A84600061
	Epredia High Capacity Section Dryer <i>Or any equivalent product</i>	A84600051
Ted Pella	Camel Hair Brushes <i>Or any equivalent paintbrush</i>	11859
Additional Materials		
-	Razor Blades	-
-	Biopsy Punch, 5 mm	-
-	Ice Bucket	-
-	Milli-Q Water	-

RNA Quality Assessment Using DV200

Vendor	Item	Part Number	
Qiagen	RNeasy FFPE Kit (50)	73504	
	Deparaffinization Solution	19093	
Thermo Fisher Scientific	RNaseZap RNase Decontamination Solution	AM9780	
	Nuclease-free Water (not DEPC-Treated)	AM9937	
	Qubit RNA BR Assay Kit	Q10210	
	Qubit Assay Tubes	Q32856	
	Qubit 4 Fluorometer	Q33238	
	Nanodrop 2000c Spectrophotometers <i>Or any equivalent Nanodrop Alternative to Qubit Fluorometer</i>	ND-2000C	
Eppendorf	DNA LoBind Tubes, 1.5 ml	022431021	
Agilent	RNA 6000 Pico Kit	5067-1513	
	2100 Bioanalyzer Laptop Bundle	G2953CA	
	4200 TapeStation	Choose Bioanalyzer or TapeStation based on availability & preference.	G2991AA
	High Sensitivity RNA ScreenTape		5067-5579
	High Sensitivity RNA ScreenTape Ladder		5067-5581
	High Sensitivity RNA ScreenTape Sample Buffer		5067-5580

Deparaffinization & H&E Staining

Only one xylene, eosin solution, and hematoxylin solution are required to perform deparaffinization and H&E staining. A number of options are listed.

Vendor	Item	Part Number
Millipore Sigma	Xylene, Reagent Grade	214736
	Xylene, Histological Grade <i>Alternative to reagent grade xylene</i>	534056
	Ethyl Alcohol, 200 Proof <i>Alternative to VWR product</i>	E7023
	Eosin Y-solution, Alcoholic <i>Alternative to Abcam product</i>	HT110116
	Hematoxylin Solution, Mayer's <i>Alternative to Agilent product</i>	MHS16
	Hematoxylin Solution According to Mayer <i>Alternative to Agilent product</i>	51275
	Glycerol Solution <i>Alternative to Acros Organic product</i>	49781
Agilent	Hematoxylin, Mayer's <i>Alternative to Millipore Sigma product</i>	S330930-2
	Bluing Reagent, Dako <i>Alternative to Fisher Scientific product</i>	CS70203-2
Acros Organics	Glycerol <i>Alternative to Millipore Sigma product</i>	327255000
Abcam	Eosin Y Solution (Modified Alcoholic) <i>Alternative to Millipore Sigma product</i>	ab246824
Fisher Scientific	Thermo Scientific Shandon Bluing Reagent <i>Alternative to Agilent product</i>	6769001
	Epredia High Capacity Section Dryer	A84600051
	Fisherbrand Cover Glasses: Rectangles <i>Alternative to VWR product</i>	12-544-EP
VWR	Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only) <i>Alternative to Millipore Sigma product</i>	83813.360DP
	Coplin Jar <i>Or any equivalent product</i>	100500-232
	Staining Dishes <i>Alternative to Coplin Jar</i>	25608-906
	Cover Glasses, Rectangular <i>Alternative to Fisher Scientific product</i>	16004-322
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 μm /pixel minimum capture resolution

Exposure times 2-10 milli sec

Visium Spatial Gene Expression for FFPE

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Supplier	Description	Part Number	
Plastics			
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	
Kits & Reagents			
Thermo Fisher Scientific	Nuclease-free Water (not DEPC-Treated)	AM9937	
	Tris 1M, pH 7.0, RNase-free	AM9850G	
	Fisherbrand Premier Plain Glass Microscope Slides, Optional	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
Kits & Reagents		
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>	-
Equipment		
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
Quantification & Quality Control		
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

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