Chromium Fixed RNA Profiling – Protocol Planner

Introduction

Chromium Fixed RNA Profiling offers comprehensive scalable solutions to measure gene and protein expression in samples that are fixed with formaldehyde. This document provides workflow overviews, document resources, reagents, and consumables for the Chromium Fixed RNA Profiling protocols. This document also provides guidance on selecting the appropriate sample preparation and library construction protocols for different workflows. An overview of the Chromium Fixed RNA Profiling data analysis is also included in this document. The 10x Genomics Chromium Fixed RNA Profiling Reagent Kits are not listed in this document.

High-Level Workflow Overview – Chromium Fixed RNA Profiling

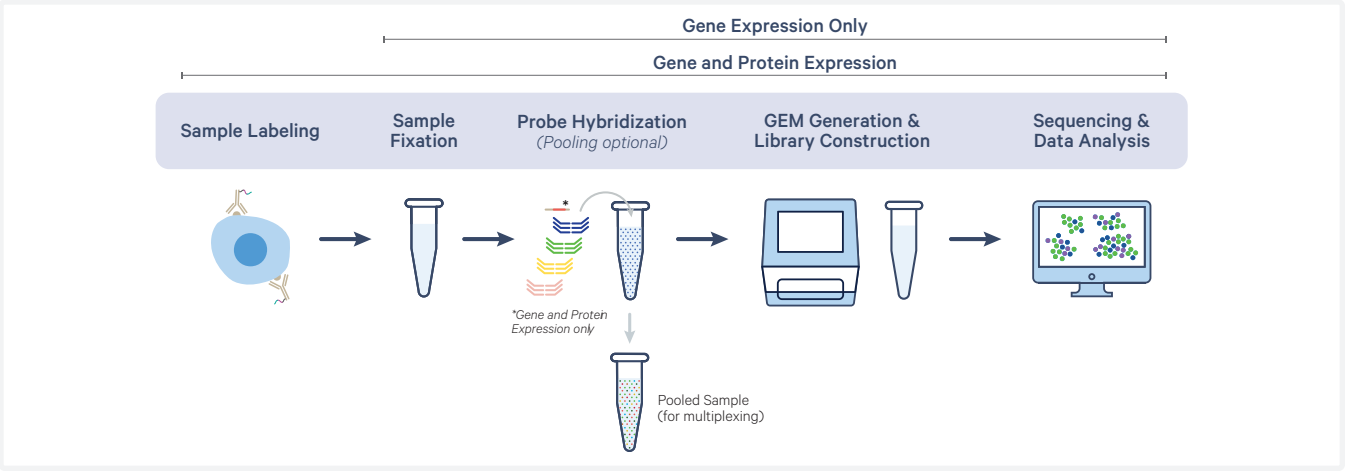


Figure 1. Samples are either first labeled with antibody-oligonucleotide conjugates and then fixed (gene and protein expression workflows) or are directly fixed (gene expression only workflows). Fixed samples are then hybridized to probe sets. For gene & protein expression workflows, additional barcodes are added. The hybridized samples may be processed individually (singleplex workflow) or pooled with up to 16 samples (multiplex workflow). During GEM generation, the probe sets are ligated and extended to incorporate unique barcodes. Sequencing libraries are then prepared, sequenced, and analyzed.

Contents

2 Chromium Fixed RNA Profiling Protocol Selection
3 Workflow Overviews and Document Resources
8 Protocol Steps & Timing
9 Kits, Reagents & Equipment – Sample Preparation
15 Kits, Reagents & Equipment – Library Construction
16 Recommended Thermal Cyclers
17 Recommended Pipette Tips
18 Analysis of Chromium Fixed RNA Profiling Data

Chromium Fixed RNA Profiling Protocol Selection

This section provides guidance on choosing the appropriate documents for a specific Chromium Fixed RNA Profiling Workflow.

Gene Expression Only (Workflows 1 and 2)

	Sample Labeling	Sample Fixation	Probe Hybridization, GEM Generation, & Library Construction
Gene Expression Only	n/a	• Fix Cells/Nuclei Suspension (CG000478) • Tissue Fixation & Dissociation (CG000553) • FFPE - Cell Isolation (CG000632)	**Singleplexing Workflow** • Chromium Fixed RNA Profiling – Singleplexed Samples (CG000691) **Multiplexing Workflow** • Chromium Fixed RNA Profiling – Multiplexed samples (CG000527)
	–	Variable	~2 days

Gene and Protein Expression using Barcode Oligo Capture (Workflows 3 and 4)

	Sample Labeling	Sample Fixation	Probe Hybridization, GEM Generation, & Library Constructions
Gene & Protein Expression using Barcode Oligo Capture	• Cell Surface Protein Labeling (CG000529) using **TotalSeq™-C** style Antibody-Oligonucleotide Conjugates	• Fix Cells/Nuclei Suspension (CG000478)	**Singleplexing Workflow** • Chromium Fixed RNA Profiling – Singleplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture (CG000674) **Multiplexing Workflow** • Chromium Fixed RNA Profiling – Multiplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture (CG000673)
	~2–3 hours	Variable	~2 days

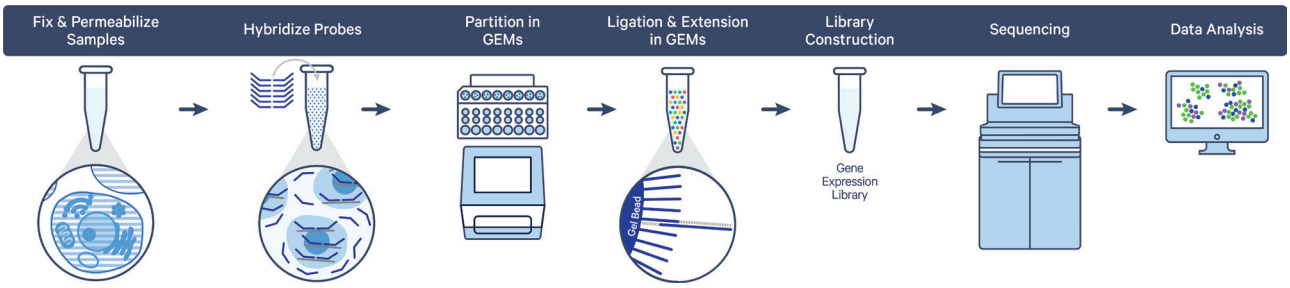
Gene and Protein Expression (Workflow 5)

	Sample Labeling	Sample Fixation	Probe Hybridization, GEM Generation, & Library Constructions
Gene & Protein Expression	• Cell Surface Protein Labeling (CG000529) using **TotalSeq™-B** style Antibody-Oligonucleotide Conjugates	• Fix Cells/Nuclei Suspension (CG000478)	**Singleplexing Workflow** • Chromium Fixed RNA Profiling – Singleplexed Samples with Feature Barcode technology for Protein (CG000477) *Not compatible with multiplexing workflow*
	~2–3 hours	Variable	~2 days

Chromium Fixed RNA Profiling Protocols – Workflow Overviews & Document Resources

Workflow 1: Fixed RNA Profiling of Singleplexed Samples for Gene Expression

Workflow Overview



Document Resources

Sample Preparation

Detailed guidance for sample fixation or isolation of cells from fixed sections.

Sample Fixation
Fix single cell & nuclei suspensions

Demonstrated Protocol CG000478

Tissue Fixation
Fix & dissociate tissues

Demonstrated Protocol CG000553

FFPE Samples - Cell Isolation
Isolate cells from FFPE sections

Demonstrated Protocol CG000632

Library Construction

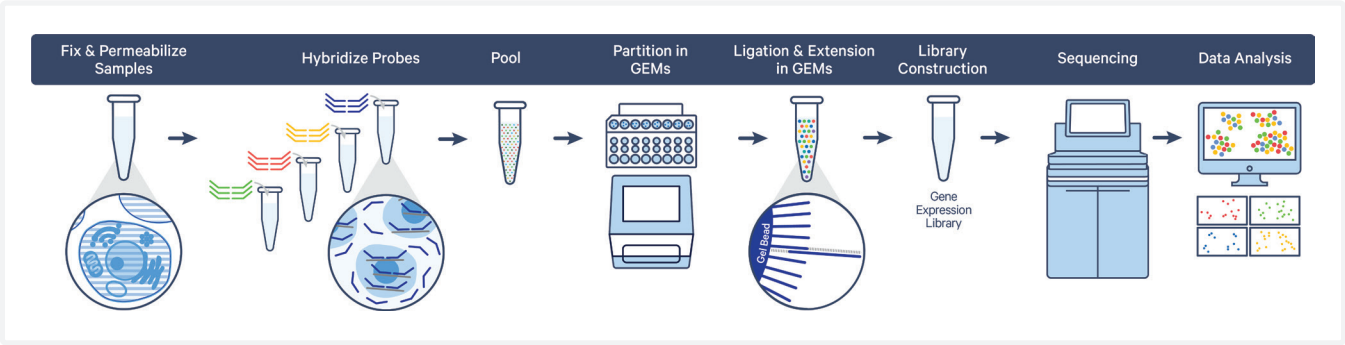
Detailed steps & guidance on probe hybridization, GEM generation & library construction.

Library Construction
Construct Fixed RNA - Gene Expression libraries for singleplexed samples

User Guide CG000691

Workflow 2: Fixed RNA Profiling of Multiplexed Samples for Gene Expression

Workflow Overview



Document Resources

Sample Preparation

Detailed guidance for sample fixation or isolation of cells from fixed sections.

Sample Fixation
Fix single cell & nuclei suspensions

Demonstrated Protocol CG000478

Tissue Fixation
Fix & dissociate tissues

Demonstrated Protocol CG000553

FFPE Samples - Cell Isolation
Isolate cells from FFPE sections

Demonstrated Protocol CG000632

Library Construction

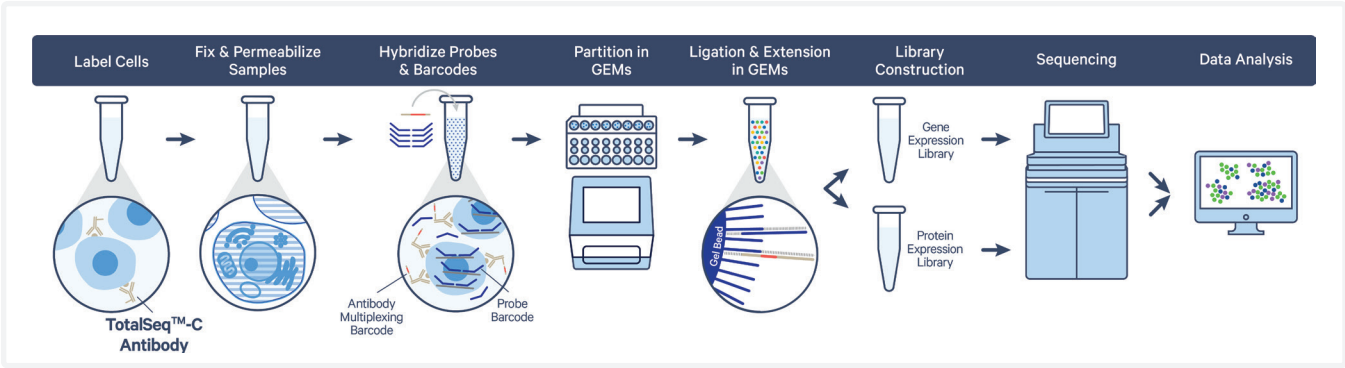
Detailed steps & guidance on probe hybridization, GEM generation & library construction.

Library Construction
Construct Fixed RNA - Gene Expression libraries for multiplexed samples

User Guide CG000527

Workflow 3: Fixed RNA Profiling of Singleplexed Samples for Gene & Protein Expression using Barcode Oligo Capture

Workflow Overview



Document Resources

Sample Preparation

Detailed guidance for sample labeling and fixation.

Cell Surface Protein Labeling
Label cells with antibody-oligonucleotide conjugates*

Demonstrated Protocol CG000529

Use **TotalSeq™-C antibody-oligonucleotide conjugates for labeling*

↓

Sample Fixation
Fix single cell & nuclei suspensions

Demonstrated Protocol CG000478

Library Construction

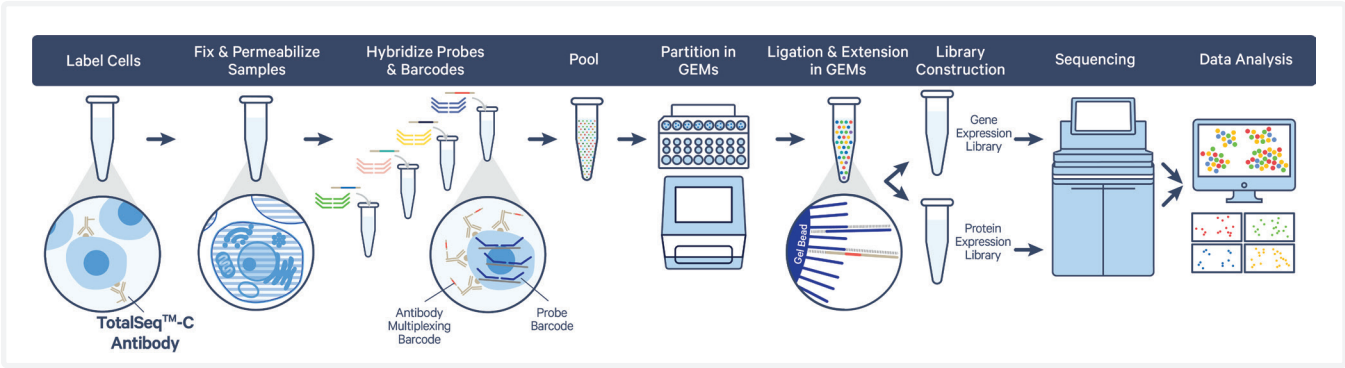
Detailed steps & guidance on probe hybridization, GEM generation & library construction.

Library Constructions
Construct Fixed RNA - Gene and Protein Expression libraries for singleplexed samples

User Guide CG000674

Workflow 4: Fixed RNA Profiling of Multiplexed Samples for Gene & Protein Expression using Barcode Oligo Capture

Workflow Overview



Document Resources

Sample Preparation

Detailed guidance for sample labeling and fixation.

Cell Surface Protein Labeling
Label cells with antibody-oligonucleotide conjugates*

Demonstrated Protocol CG000529

Use **TotalSeq™-C antibody-oligonucleotide conjugates for labeling*

Sample Fixation
Fix single cell & nuclei suspensions

Demonstrated Protocol CG000478

Library Construction

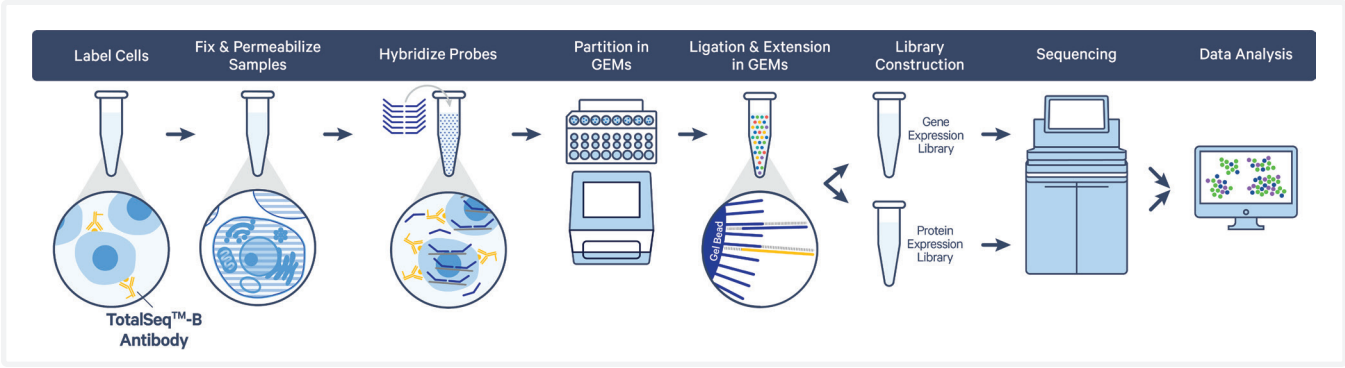
Detailed steps & guidance on probe hybridization, GEM generation & library construction.

Library Constructions
Construct Fixed RNA - Gene and Protein Expression libraries for multiplexed samples

User Guide CG000673

Workflow 5: Fixed RNA Profiling of Singleplexed Samples for Gene & Protein Expression

Workflow Overview



Document Resources

Sample Preparation

Detailed guidance for sample labeling and fixation.

Cell Surface Protein Labeling
Label cells with antibody-oligonucleotide conjugates*

Demonstrated Protocol CG000529

Use **TotalSeq™-B antibody-oligonucleotide conjugates for labeling*

↓

Sample Fixation
Fix single cell & nuclei suspensions

Demonstrated Protocol CG000478

Library Construction

Detailed steps & guidance on probe hybridization, GEM generation & library construction.

Library Constructions
Construct Fixed RNA - Gene and Protein Expression libraries for singleplexed samples

User Guide CG000477

Protocol Steps & Timing

Steps	Timing	Stop & Store
Sample Labeling *(For Gene & Protein Expression)*	2–3 h	
Sample Fixation	Variable STOP	4°C ≤1 week/-80°C ≤6 months
Library Construction Protocol	2 days (see below for details)	

Protocol Steps & Timing for Chromium Fixed RNA Profiling Library Construction Workflows

The table below provides a general overview of the timing and stop options for the library construction steps. These steps may slightly differ depending upon the workflow followed. For detailed guidance, consult the appropriate User Guide.

Steps	Timing	Stop & Store
Step 1: Probe Hybridization		
1.1 Probe Hybridization	16–24 h	
Step 2: GEM Generation & Barcoding		
2.1 Post-Hybridization Pool* & Wash	60–90 min STOP	-80°C ≤6 months
2.2 Prepare Master Mix + Sample Dilution	30 min	
2.3 Load Chromium Next GEM Chip Q	10 min	
2.4 Run the Chromium X/iX	5.5 min	
2.5 Transfer Gel Beads-in-emulsion (GEMs)	5 min	
2.6 GEM Incubation	125 min STOP	4°C ≤1 week (GEMs) or -80°C ≤6 months (washed undiluted sample)
Step 3: GEM Recovery and Pre-Amplification		
3.1 Post-GEM Incubation – Recovery	10 min	
3.2 Pre-Amplification PCR	55 min STOP	4°C ≤72 h/-20°C ≤1 week
3.3 DNA Cleanup – SPRIselect	30 min STOP	4°C ≤72 h/-20°C ≤4 weeks
Step 4: Fixed RNA – Gene Expression Library Construction		
4.1 Sample Index PCR	40 min STOP	4°C ≤72 h
4.2 Post Sample Index PCR Size Selection – SPRIselect	30 min STOP	4°C ≤72 h/-20°C long term
4.3 Post Library Construction QC	60 min	
Step 5: Fixed RNA – Protein Expression Library Construction**		
5.1 Sample Index PCR	40 min STOP	4°C ≤72 h
5.2 Post Sample Index PCR Size Selection – SPRIselect	30 min STOP	4°C ≤72 h/-20°C long term
5.3 Post Library Construction QC	60 min	

*Pooling is only applicable to multiplexing workflows
**Only applicable to gene and protein expression workflows (CG000673, CG000674, and CG000477)

Kits, Reagents & Equipment – Sample Preparation

The items in the table below have been tested by 10x Genomics and perform optimally with the assay. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, freezers, etc.

For Cell Surface Protein Labeling for Chromium Fixed RNA Profiling with Feature Barcode technology (Demonstrated Protocol CG000529)

Vendor	Item	Part Number
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TruStain FcX (anti-mouse CD16/32) Antibody	156604
	True-Stain Monocyte Blocker	426101
	TotalSeq™ Antibody-Oligonucleotide Conjugates*	-
	Cell Staining Buffer	420201
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Fetal Bovine Serum, qualified, heat inactivated	16140071
Millipore Sigma	Bovine Serum Albumin In DPBS (10%) *Alternative to Thermo Fisher product*	A1595
Miltenyi Biotec	MACS BSA Stock Solution *Alternative to Thermo Fisher product*	130-091-376
Corning	Phosphate-Buffered Saline, *1X without Calcium and Magnesium*	21-040-CV
VWR	Fetal Bovine Serum (FBS) *Alternative to Thermo Fisher product*	97068-085
For Cell Counting		
Thermo Fisher Scientific	Countess II FL Automated Cell Counter *Discontinued*	AMQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Trypan Blue Stain (0.4%)	T10282
Nexcelom	Celleca MX HIgh-throughput Automated Cell Counter	MX-112-0127
	ViaStain AOPI Staining Solution	CS2-0106-5mL

This list may not include some standard laboratory equipment.

**Choose appropriate TotalSeq™ antibody-oligonucleotide conjugates based on the Chromium Fixed RNA Profiling workflow.*

Chromium Fixed RNA Profiling Workflows	TotalSeq™ Antibody-Oligonucleotide Conjugates
Gene & protein expression using barcode oligo capture – singleplexing & multiplexing workflows	TotalSeq™-C antibody-oligonucleotide conjugates
Gene & protein expression – singleplexing workflow	TotalSeq™-B antibody-oligonucleotide conjugates

For Cells & Nuclei Fixation (Demonstrated Protocol CG000478)

Vendor	Item	Part Number
For Cell Thaw & Sample Fixation		
10x Genomics	Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit	1000414
Millipore Sigma	Bovine Serum Albumin In DPBS (10%) *Alternative to Thermo Fisher product*	A1595
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Formaldehyde (37% by Weight/Molecular Biology), Fisher BioReagents	BP531-25
	Nuclease-free Water (not DEPC-Treated)	AM9937
Miltenyi Biotec	MACS BSA Stock Solution *Alternative to Thermo Fisher product*	130-091-376
Corning	Phosphate-Buffered Saline, 1X *without Calcium and Magnesium*	21-040-CV
	*Corning RPMI 1640 1X with L-Glutamine	10-040-CM
VWR	*Seradigm Premium Grade Fetal Bovine Serum (FBS)	97068-085
	**Only needed for cell thawing prior to fixation*	
For Sample Filtration		
Sysmex	Sterile Single-Pack CellTrics Filters	04-004-2326
Miltenyi Biotec	Pre-Separation Filters (30 µm)	130-041-407
	Choose either Sysmex or Miltenyi Biotec filter.	
For Cell Counting		
Nexcelom Biosciences	ViaStain PI Staining Solution	CS1-0109-5mL
	ViaStain AOPI Staining Solution *Alternative to PI Staining Solution.*	CS2-0106-5mL
	†Cellaca MX High-throughput Automated Cell Counter	MX-112-0127
Thermo Fisher Scientific	†Countess II FL Automated Cell Counter *Discontinued* *Requires additional fluorescent filters*	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Trypan Blue Stain (0.4%)	T10282
	Ethidium Homodimer-1	E1169
	†*Choose either Countess or Cellaca.*	
For Storage & Post-Storage Processing		
Acros Organics	Glycerol, 99.5%, for molecular biology, DNAse, RNAse and Protease free *Alternative to Millipore Sigma product*	327255000
Millipore Sigma	Glycerol for molecular biology, ≥99.0% *Alternative to Acros Organics product*	G5516-100ML
	Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free	126615
	Protector RNase Inhibitor	3335402001
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA 50 mg/mL)	AM2616
Additional Materials		
Eppendorf	DNA LoBind Tubes 2.0 ml	022431048
	ThermoMixer C *For fixation at room temperature;* *alternatively, use a heat block or a water bath*	5382000023
VWR	Vortex Mixer	10153-838

Choose Ethidium Homodimer-1, Trypan Blue, or PI Staining Solution based on the presence of debris in the sample.

For Tissue Fixation & Dissociation (Demonstrated Protocol CG000553)

Vendor	Item	Part Number
For Tissue Mincing & Fixation		
10x Genomics	Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit	1000414
Millipore Sigma	Protector RNase Inhibitor	3335399001
	Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free	126615
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml) *Alternative to Millipore Sigma product*	AM2616
	Formaldehyde (37% by Weight/Molecular Biology), Fisher BioReagents	BP531-25
	Nuclease-free Water (not DEPC-Treated)	AM9937
	Pyrex Petri Dish*	08-748D
	General-Purpose Forceps*	10-270
	Standard Dissecting Scissors*	08-951-20
	Wide-Bore Pipette Tips RT LTS 1000 uL*	FLW 768A/8 (30389218)
	**May be procured from alternative vendors*	
Corning	Phosphate-Buffered Saline, 1X *without Calcium and Magnesium*	21-040-CV
VWR	Tris Buffer, 1M sterile solution, pH 8.0	E199-100ML
Additional Materials		
Blade; Lab Weighing Scale		
For Tissue Dissociation		
Millipore Sigma	Liberase TL Liberase TH *(optional)*	5401020001 5401135001
Corning	Corning RPMI 1640	10-040-CV
Thermo Fisher Scientific	Gibco RPMI 1640	11875093
Choose either Corning or Gibco RPMI		
If using gentleMACS Octo Dissociator		
Miltenyi Biotec	gentleMACS Octo Dissociator with Heaters gentleMACS C Tubes	130-096-427 130-093-237
For Sample Filtration		
Sysmex	Sterile Single-Pack CellTrics Filters (30 µm)	04-004-2326
Miltenyi Biotec	MACS SmartStrainers (30 µm)	130-098-458
Choose either Sysmex or Miltenyi Biotec filter.		
For Cell Counting		
Nexcelom Biosciences	ViaStain AOPI Staining Solution	CS2-0106-5mL
	Cellaca MX High-throughput† Automated Cell Counter	MX-112-0127
Thermo Fisher Scientific	Countess II FL Automated Cell Counter† *Requires additional fluorescent filters*	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Ethidium Homodimer-1	E1169
†Choose either Countess or Cellaca.		

For Storage & Post-Storage Processing		
Acros Organics	Glycerol, 99.5%, for molecular biology, DNAse, RNAse and Protease free *Alternative to Millipore Sigma product*	327255000
Millipore Sigma	Glycerol for molecular biology, ≥99.0% A*lternative to Acros Organics product*	G5516-100ML
	Protector RNase Inhibitor	3335402001
VWR	Vacuum Filter/Storage System (0.2 µm)	29442-936
Additional Materials		
Eppendorf	DNA LoBind Tubes 2.0 ml	022431048
	ThermoMixer C *Recommended for manual dissociation*	5382000023

This list may not include some standard laboratory equipment.

For Isolation of Cells from FFPE Tissue Sections (Demonstrated Protocol CG000632)

Vendor	Item	Part Number
For Tissue Section Transfer & Deparaffinization		
Millipore Sigma	Xylene, Reagent Grade	214736
	Ethyl Alcohol, 200 Proof, anhydrous	E7023
VWR	Ethanol absolute ≥99.5%, TechniSolv, pure (for Europe)	83813.360DP
GREENFIELD GLOBAL	Ethyl Alcohol Anhydrous, USP (for Canada)	P006EAAN
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
	15 ml PP Centrifuge Tubes	430791
Thermo Fisher	Nuclease-free Water (not DEPC-Treated)	AM9937
If using gentleMACS Octo Dissociator		
Miltenyi Biotec	gentleMACS C Tubes	130-093-237

Additional Materials
Disposable plastic Pasteur pipette Nuclease-free Water Water bath Centrifuge

For Tissue Section Dissociation		
Millipore Sigma	Liberase TH	5401151001
Corning	RPMI	10-040-CV
If using pellet pestle:		
Fisher Scientific	RNase-Free Disposable Pellet Pestles	12-141-364
Fisher Scientific	BD Luer-Lok PrecisionGlide Disposable Syringes with Detachable Needles *OPTIONAL*	14-823-37
If using gentleMACS Octo Dissociator:		
Miltenyi Biotec	gentleMACS Octo Dissociator with Heaters	130-096-427
If using Tissue Resuspension Buffer:		
Millipore Sigma	Protector RNase Inhibitor	3335399001
	Albumin, Bovine Serum*, 10% Aqueous Solution, Nuclease-Free	126615
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin* (BSA, 50 mg/ml)	AM2616
**Choose either Millipore or Thermo Fisher Scientific BSA.*		
VWR	Tris Buffer, 1M sterile sol., pH 8.0	E199-100ML
If using Quenching Buffer:		
10x Genomics	Conc. Quench Buffer**	2000516

***Included in the 10x Genomics Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit, 16 rxns (PN-1000414); also includes Enhancer (PN-2000482) if storing fixed cells.*

For Sample Filtration		
Sysmex	Sterile Single-Pack CellTrics Filters***(use 30 µm)	04-004-2326
Miltenyi Biotec	MACS SmartStrainers*** (30 µm)	130-098-458
	OR	
	Pre-Separation Filters***(30 µm)	130-041-407
****Choose either Sysmex or Miltenyi Biotec filter.*		
For Cell Counting		
Nexcelom Biosciences	ViaStain PI Staining Solution	CS2-0109-5mL
	ViaStain AOPI Staining Solution *Alternative to PI Staining Solution*	CS2-0106-5mL
	Cellaca MX High-throughput† Automated Cell Counter	MX-112-0127
Thermo Fisher Scientific	Countess II FL Automated Cell Counter† *Requires additional fluorescent filters*	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Ethidium Homodimer-1	E1169
†Choose either Countess or Cellaca.		

This list may not include some standard laboratory equipment.

Kits, Reagents & Equipment – Library Construction

(User Guides – CG000691, CG000527, CG000673, CG000674 & CG000477)

The items in the table below have been tested by 10x Genomics and perform optimally with the assay. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, pH meters, freezers, etc.

Supplier	Description	Part Number
Plastics		
Eppendorf	DNA LoBind Tubes, 1.5 ml	022431021
	DNA LoBind Tubes, 5.0 ml	0030108310
	PCR Tubes 0.2 ml 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
Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.		
Corning	Corning Centrifuge Tubes with CentriStar Cap (15 ml), sterile	430790
	Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile	430921
Sysmex	Sterile single-pack CellTrics filters	04-004-2326
Miltenyi Biotec	Pre-Separation Filters (30 µm) *Alternative to Sysmex product*	130-041-407
Choose either Sysmex or Miltenyi Biotec filter.		
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
	Tween 20 Surfact-Amps Detergent Solution (10% solution)	28320
Beckman Coulter	SPRIselect Reagent Kit	B23318
Millipore Sigma	Ethyl Alcohol, Pure (200 Proof, anhydrous)	E7023-500ML
	Glycerol for Molecular biology, ≥99.0%	G5516
Acros Organics	Glycerol, 99.5%, for molecular biology, DNAse, RNAse and Protease free *Alternative to Millipore Sigma product*	327255000
Qiagen	Qiagen Buffer EB	19086
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Cell Counting		
Thermo Fisher Scientific	Ethidium Homodimer-1	E1169
	Trypan Blue Stain (0.4%)	T10282
	Countess II FL Automated Cell Counter *Discontinued* *Requires additional fluorescent filters*	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
Nexcelom Biosciences	Cellaca MX High-throughput Automated Cell Counter	MX-112-0127
	ViaStain PI Staining Solution	CS1-0109-5mL
	ViaStain AO/PI Staining Solution *Alternative to PI Staining Solution*	CS2-0106-5mL
Choose Ethidium Homodimer-1, Trypan Blue, or PI Staining Solution based on the presence of debris in the sample.		

Equipment		
VWR	VWR Mini Centrifuge *Or any equivalent mini centrifuge*	76269-064
	Divided Polystyrene Reservoirs	41428-958
	Vortex Mixer	10153-838
Eppendorf	Eppendorf ThermoMixer C	5382000023
	Eppendorf ThermoTop with condens.protect technology *Required for overnight hybridization step when using 1.5-ml microcentrifuge tubes*	5308000003
	Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessels	5360000038
	Eppendorf SmartBlock 2.0 ml, Thermoblock for 24 reaction vessels	5362000035
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle	G2953CA
	High Sensitivity DNA Kit	5067-4626
	4200 TapeStation	G2991AA
	High Sensitivity D5000: ScreenTape/ Reagents	5067-5592/ 5067-5593
	High Sensitivity D1000: ScreenTape/ Reagents	5067-5584/ 5067-5585
PerkinElmer	LabChip GX Touch HT Nucleic Acid Analyzer	CLS137031
	DNA High Sensitivity Reagent Kit	CLS760672
Choose Bioanalyzer, TapeStation or LabChip based on availability & preference.		
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

Recommended Thermal Cyclers

Supplier	Description	Part Number
BioRad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module *Discontinued*	1851197
	PTC Tempo Deepwell Thermal Cycler	12015392
Eppendorf	Mastercycler X50s*	North America 6311000010
Analytik Jena	Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function*	846-x-070-241

*For these thermal cyclers, the ramp rates should be adjusted for all the steps as described below:

- Eppendorf MasterCycler X50s: 3°C/sec heating and 2°C/sec cooling
- Analytik Jena Biometra TAdvanced: 2°C/sec heating and cooling

Recommended Pipette Tips

10x Genomics recommends using only validated emulsion-safe pipette tips for all Single Cell protocols. Rainin pipette tips have been extensively validated by 10x Genomics and are highly recommended for all single cell assays. If Rainin tips are unavailable, any of the listed alternate pipette tips validated by 10x Genomics may be used.

Supplier	Description	Part Number (US)
Recommended Pipettes & Pipette tips		
Rainin	**Pipettes**	
	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	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
	Pipette Tips	
	Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
Alternate Recommendations *(If Rainin pipette tips are unavailable, any of the listed pipette tips may be used)*		
Eppendorf	**Pipettes**	
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 0.5 – 10 µL	3125000010
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 10 – 100 µL	3125000036
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 30 – 300 µL	3125000052
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.1 – 2.5 µL	3123000012
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.5 – 10 µL	3123000020
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 20 µL	3123000039
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 200 µL	3123000055
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 100 – 1000 µL	3123000063
	Pipette Tips (compatible with Eppendorf pipettes only)	
	ep Dualfilter T.I.P.S., 2-20 µL	0030078535
	ep Dualfilter T.I.P.S., 2-200 µL	0030078551
	ep Dualfilter T.I.P.S., 2-1,000 µL	0030078578
Labcon*	ZAP SLIK 20 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1143-965-008
	ZAP SLIK 200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1144-965-008
	ZAP SLIK 1200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1145-965-008
Biotix*	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 0.1-20uL	63300931
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 200uL	63300001
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 1200uL	63300004

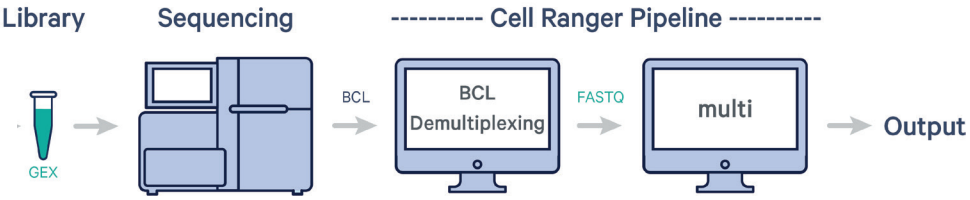
**Compatible with Rainin pipettes*

Analysis of Chromium Fixed RNA Profiling Data

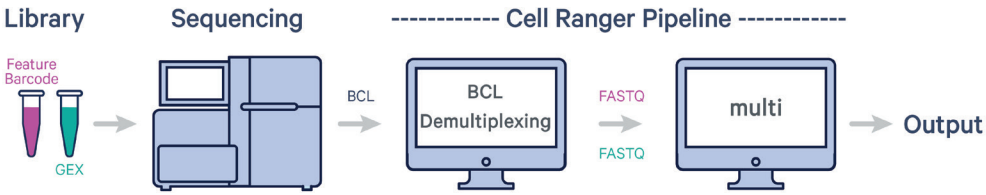
For detailed information on analysis of Chromium Fixed RNA Profiling data, refer to 10x Genomics Software Support site.

Data Analysis Overviews

Chromium Fixed RNA Profiling for Gene Expression Only (Singleplexing & Multiplexing Workflows)



Chromium Fixed RNA Profiling for Gene & Protein Expression (Singleplexing & Multiplexing Workflow)



The following key information should be collected and provided to the data analyst/bioinformatician to ensure that the parameters are set appropriately for Cell Ranger to analyze the data:

- Target number of cells to be recovered per sample
- Sample and library name to be appended to the FASTQ file name and the corresponding library type
- The sample indices used for each Chromium Fixed RNA Profiling – Gene Expression or Protein Expression library
- The probe set used (human, mouse, custom)
- The Probe Barcodes used for each sample in a multiplexing experiment
- The Probe Barcodes and Antibody Multiplexing Barcodes used for each sample in a multiplexing experiment with Protein library
- For Protein libraries, the antibody barcode sequence associated with each feature (for the Feature Reference CSV file)

Document Revision Summary

Document Number	CG000528
Title	Chromium Fixed RNA Profiling – Protocol Planner
Revision	Rev C to Rev D
Revision Date	August 2023
General Changes	Updated for general minor consistency of language, format, and terms throughout
Specific Changes	Added new section on high-level protocol overview on page 1
	Added Chromium Fixed RNA Profiling Protocols Selection section on page 2
	Added Workflow Overviews and Document Resources for all the workflows
	Updated Data Analysis section

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