Chromium Gene Expression Flex (Fixed RNA Profiling)

Welcome and Site Preparation Discussion

CG000563 Rev E

Welcome and Site Preparation Discussion

- Gene Expression Flex Solution Overview
- Considerations for Sample Preparation and Sequencing
- Required Materials and Equipment
- Cell Ranger Analysis Pipeline overview
- Training Next Steps

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Chromium Gene Expression Flex Solution Overview

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Chromium Gene Expression Flex

Solution Highlights

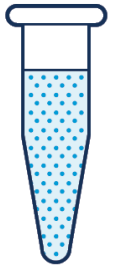
- Sample fixation locks in cells states and preserves fragile samples.
- Enables large, longitudinal studies.
- Removes constraints from transporting and storing precious samples.
- Enables transport of samples around the globe for processing.
- Minimizes variability by matching samples.
- Allows sample multiplexing with unique sample barcodes.
- Neutralizes infectious agents.



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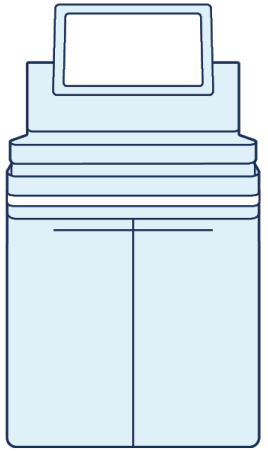
Gene Expression Flex

Input	Sample fixation & Library creation	Sequencing	Data analysis and visualization











Single Cell or Nuclei Suspensions

Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit

Fixed RNA Feature Barcode Kit (Optional)

Chromium Fixed RNA Profiling Reagents:

- Chromium Fixed RNA Kit, Human or Mouse Transcriptome
- Chromium Next GEM Chip Q Single Cell Kit
- Dual Index Kit TS Set A
- Dual Index Kit TT Set A (Optional)

Sequencer

Cell Ranger 7.0 Analysis Pipeline Loupe Bowser

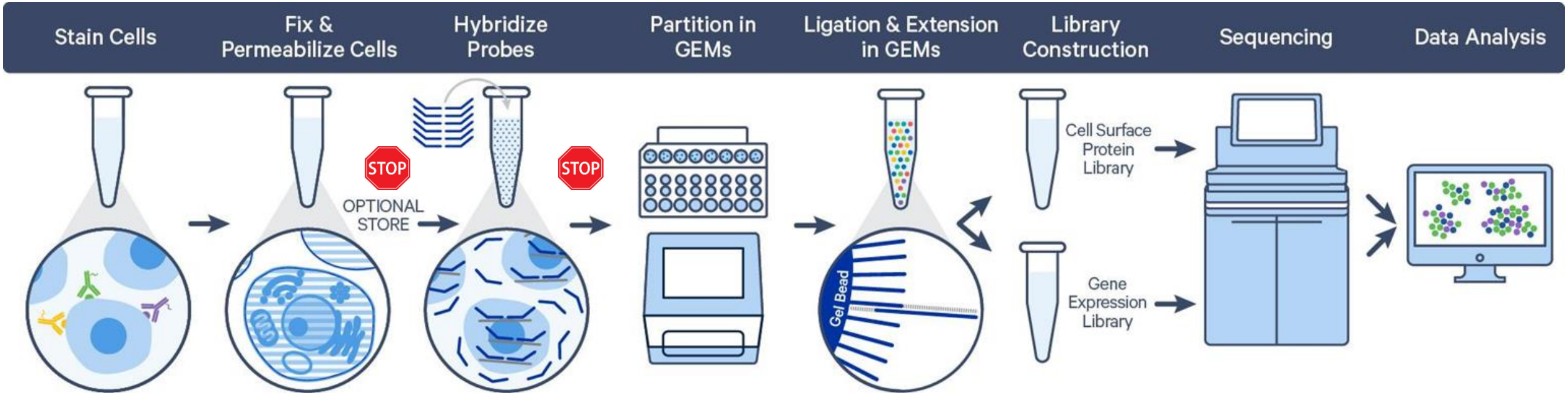
- FASTQ Processing
- Expression Analysis
- Cell Clustering
- Visualization

Assay is only compatible with Chromium X series.

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Gene Expression Flex Workflow

Singleplex workflow overview - compatible with Feature Barcode Technology



*optional: Cell Surface Protein Labeling prior to fixation

- Each samples has a probe barcode.
- No multiplexing.

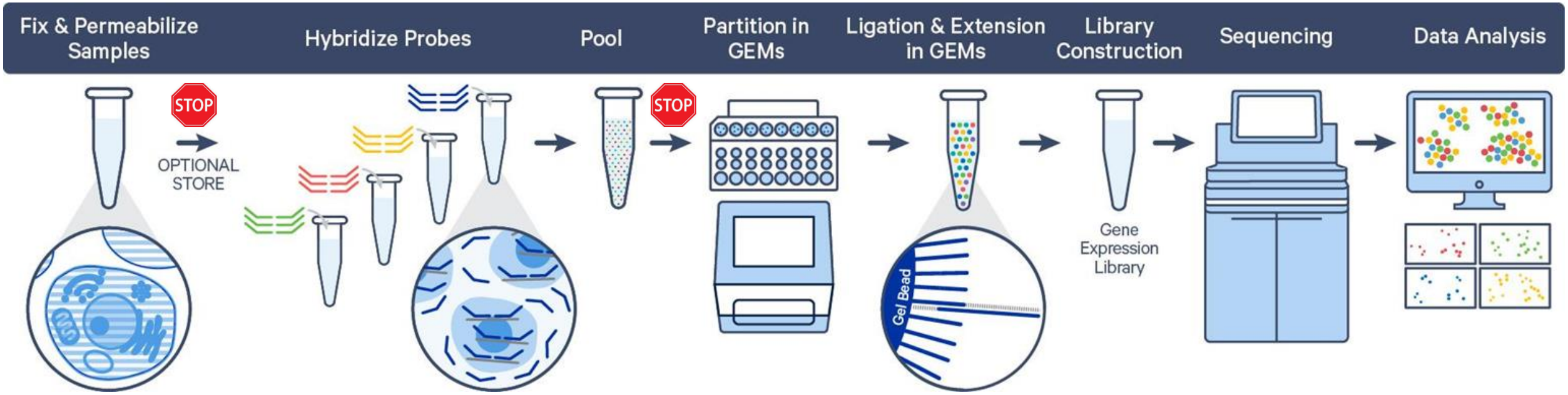
Optional Sample Prep storage:

- Validated stopping points during sample preparation - Post-Fix and Post-Hyb.

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Gene Expression Flex Workflow

Multiplex workflow overview



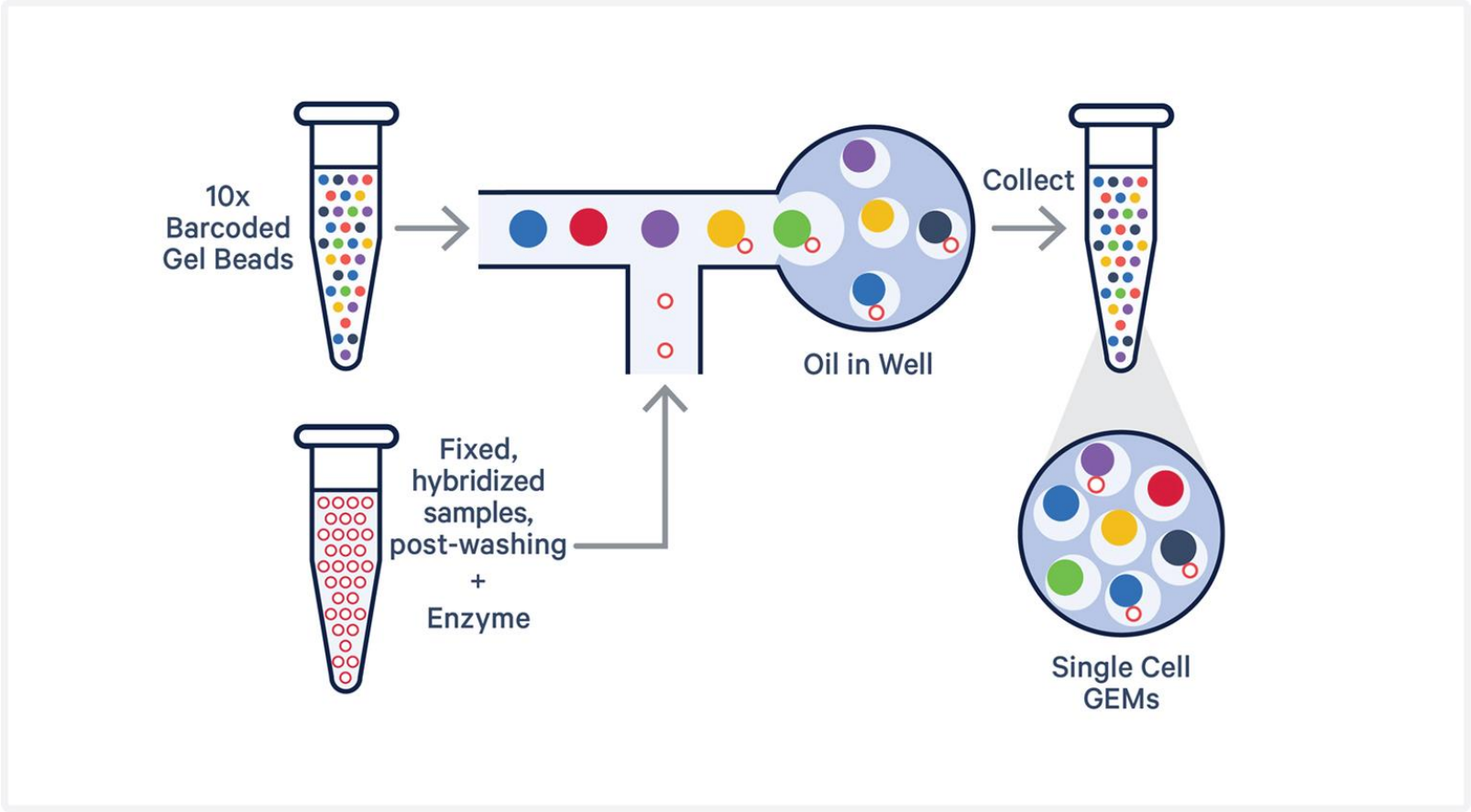
- Multiplex up to 4 or 16 samples.
- Each sample has a unique probe barcode.

STOP Optional Sample Prep storage:

- Validated stopping points during sample preparation - Post-Fix and Post-Hyb.

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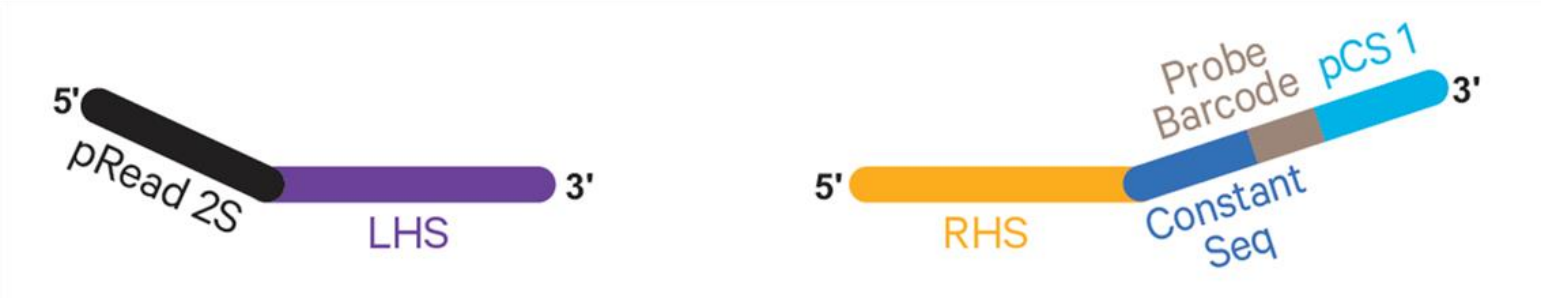
Gene Expression Flex - Creating Gel Beads in Emulsions (GEMs)



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Chromium Gene Expression Flex

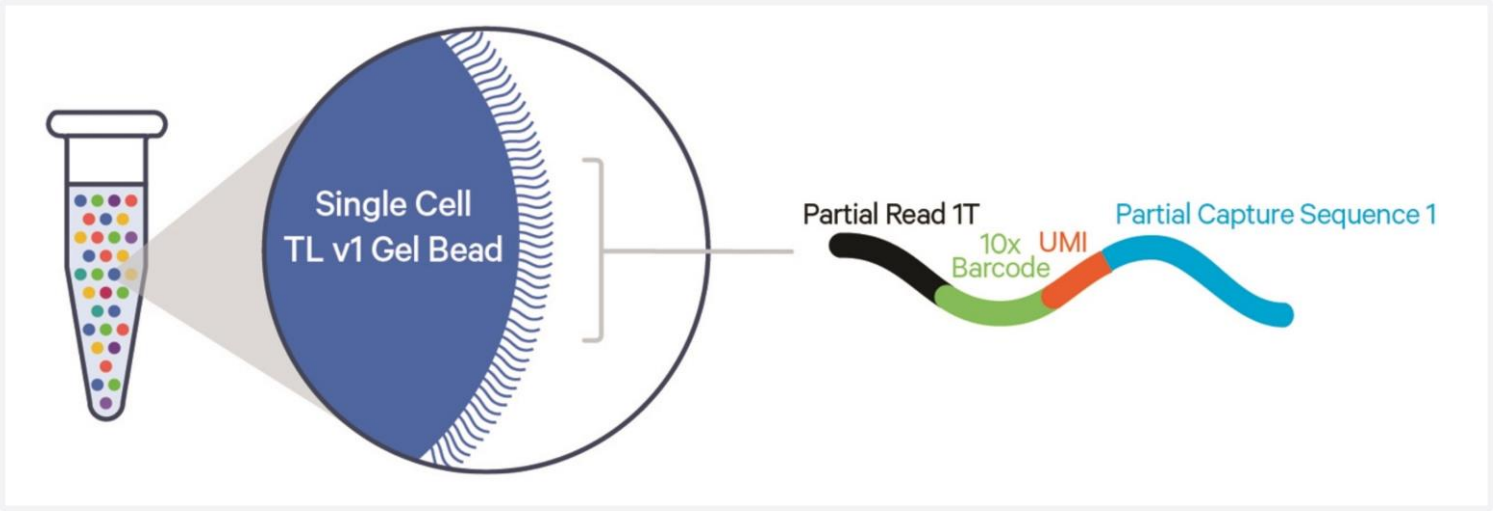
- Samples are fixed in **4% Formaldehyde**, which enables storage before processing.
- Compatible with single cell or nuclei suspensions.
- Assay uses **probe-based chemistry** that covers over **18,000 human genes** and **19,000 mouse genes.**
- All probes target exons of protein coding genes.
- Probes to mitochondrial genes are included, to help assess sample quality.
- Different probe sets than Visium Spatial Gene Expression Probe-based assays.



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Chromium Gene Expression Flex Gel Beads

Capture via single cell TL Gel Beads



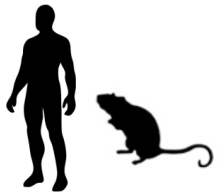
- Partial Read 1
  - Partial Illumina primer sequence
- 10x Barcode
  - 16 nt 10x Genomics Barcode
  - 737k defined barcode sequences
- UMI
  - 12 nt unique molecular identifier
- Partial Capture Sequence 1*
  - Truncated to 12 nt
  - Enables capture of ligated probe pair

*Feature Barcode technology is also enabled via partial Capture Sequence 1. It is the reverse complement of the sequence included in the DNA-based Feature Barcode sequence.

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Chromium Gene Expression Flex reagents





Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit

- Contains reagents for sample fixation and storage

Chromium Fixed RNA Kit, Human Transcriptome

- Contains probes, hybridization reagents, GEM reagents with gel beads, and library construction reagents

Fixed RNA Feature Barcode Kit (*optional; singleplex only)

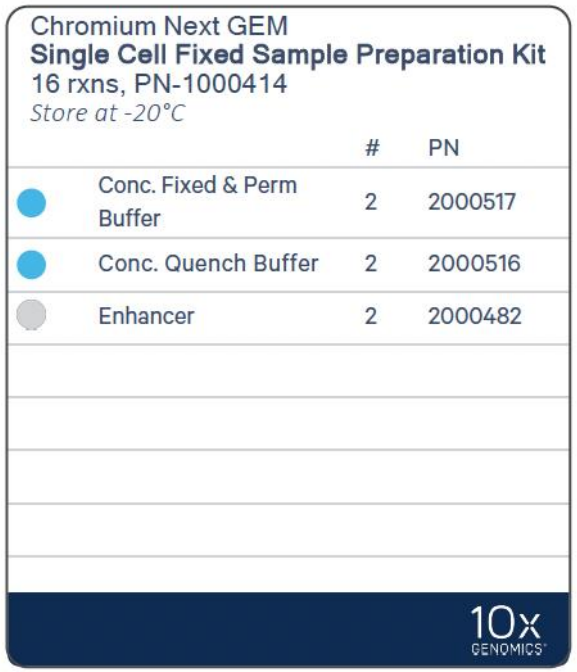
- Contains Feature primers and library construction reagents

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Chromium Gene Expression Flex reagents

Single Cell Fixed RNA Sample Prep Kit





Additional reagents and consumables

Millipore Sigma	Bovine Serum Albumin In DPBS (10%) *Alternative to Thermo Fisher product*	A1595
	Glycerol for molecular biology, ≥99.0%	G5516-100ML
Acros Organics	Glycerol, 99.5%, for molecular biology, DNAse, RNAse and Protease free *Alternative to Millipore Sigma product*	327255000
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
VWR	Seradigm Premium Grade Fetal Bovine Serum (FBS)	97068-085
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
	Corning RPMI 1640 1X with L-Glutamine	10-040-CM

For Sample Filtration		
Miltenyi Biotec*	Pre-Separation Filters (30 µm)	130-041-407
Sysmex*	Sterile single-pack CellTrics filters	04-004-2326
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	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Trypan Blue Stain (0.4%)	T10282
	Ethidium Homodimer-1	E1169
Additional Materials		
Eppendorf	DNA LoBind Tubes 2.0 ml	022431048

**Choose either Miltenyi Biotec or Sysmex filter.*

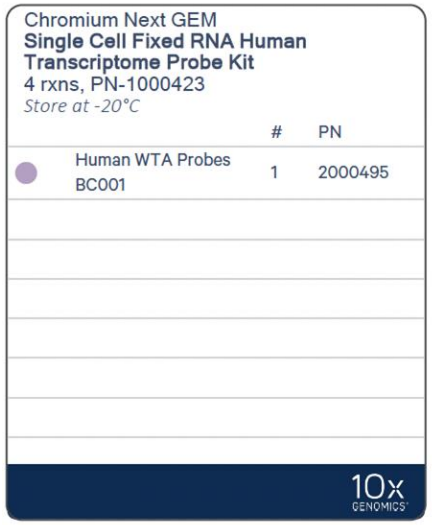
- Items in the table have been validated by 10x Genomics and are highly recommended for Single Cell Fixed Sample Preparation.
- Any Molecular Biology Grade stock solution of 37% formaldehyde that is ~10% methanol stabilized with little to no precipitant may be substituted.

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Chromium Gene Expression Flex reagents

Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 1 BC (PN- 1000474), 4 rxns









- 1 BC per sample
- Barcode is supplied as 4 rxns
- Target up to 10,000 cells per BC
- Loaded into one well

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Chromium Gene Expression Flex reagent kits

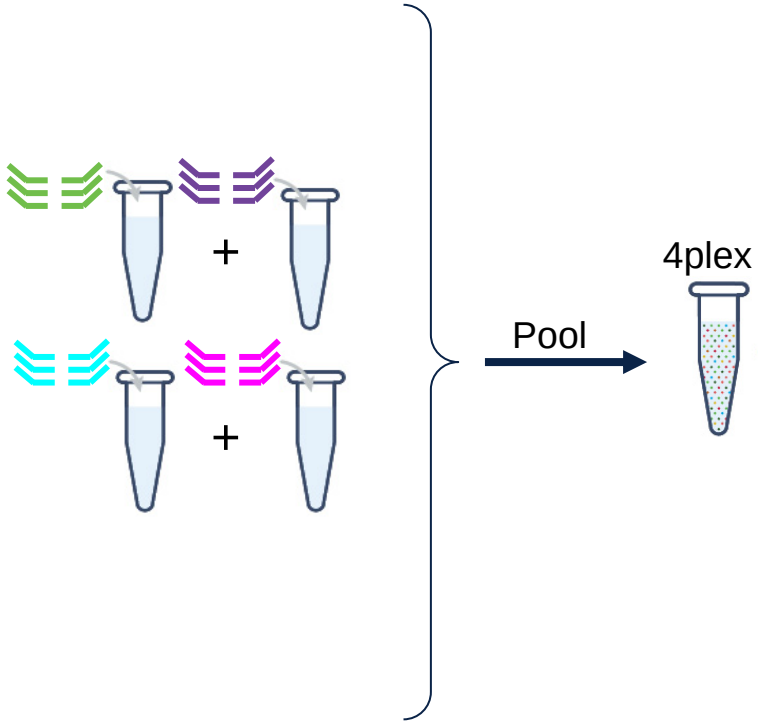
Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 4 BC (PN- 1000475), 16 rxns



Chromium Next GEM
Single Cell Fixed RNA Human Transcriptome Probe Kit
16 rxns, PN-1000420
Store at -20°C

		#	PN
●	Human WTA Probes BC001	1	2000495
●	Human WTA Probes BC002	1	2000496
●	Human WTA Probes BC003	1	2000497
●	Human WTA Probes BC004	1	2000498
●	Hyb Buffer B	1	2000485
○	Enhancer	2	2000482
●	Conc. Post-Hyb Buffer	2	2000533

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- 1 BC per sample
- Each Barcode is supplied as 4 rxns
- Target up to 10,000 cells per BC
- 4 BC pooled = 4plex
- Loaded into one well
- Target up to 40k cells per lane

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Chromium Gene Expression Flex reagent kits

Chromium Fixed RNA Kit, Human Transcriptome, 4 rxns x 16 BC (PN- 1000476), 64 rxns





Chromium Next GEM **Single Cell Fixed RNA Human Transcriptome Probe Kit**
64 rxns, Module 1 PN-1000456
Store at -20°C

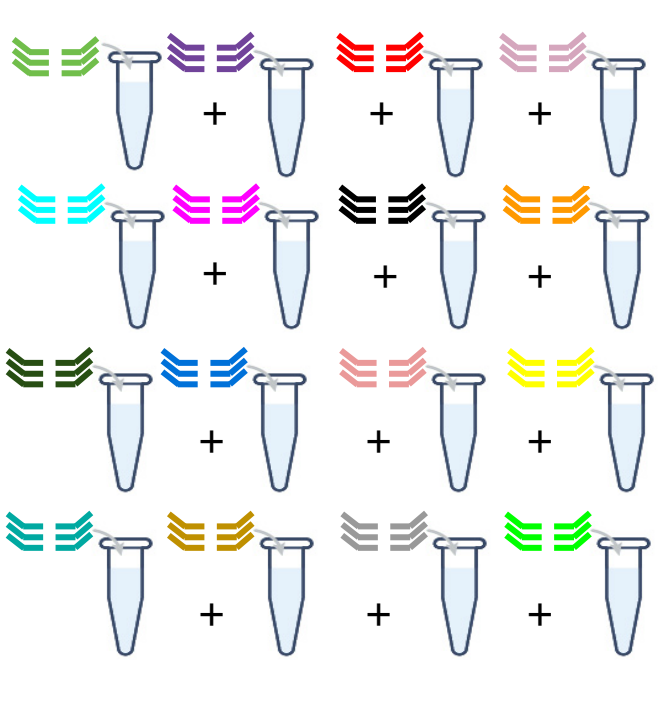
	#	PN
Enhancer	4	2000482
Human WTA Probes BC001	1	2000495
Human WTA Probes BC002	1	2000496
Human WTA Probes BC003	1	2000497
Human WTA Probes BC004	1	2000498
Human WTA Probes BC005	1	2000499
Human WTA Probes BC006	1	2000500
Human WTA Probes BC007	1	2000501
Human WTA Probes BC008	1	2000502

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Chromium Next GEM **Single Cell Fixed RNA Human Transcriptome Probe Kit**
64 rxns, Module 2 PN-1000456
Store at -20°C

	#	PN
Enhancer	4	2000482
Human WTA Probes BC009	1	2000503
Human WTA Probes BC010	1	2000504
Human WTA Probes BC011	1	2000505
Human WTA Probes BC012	1	2000506
Human WTA Probes BC013	1	2000507
Human WTA Probes BC014	1	2000508
Human WTA Probes BC015	1	2000509
Human WTA Probes BC016	1	2000510

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Pool → 16plex



- 1 BC per sample
- Each Barcode is supplied as 4 rxns
- Target up to 8,000 cells per BC
- 16 BC pooled = 16plex
- Loaded into one well
- Target up to 128k cells per lane
  - Higher cell loads may cause clogs or microfluidic failures.

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Chromium Gene Expression Flex reagent kits



Chromium Next GEM Chip Q Single Cell Kit
48 rxns (PN- 1000418)
16 rxns (PN-1000422)
(shipped and stored at ambient temperature)

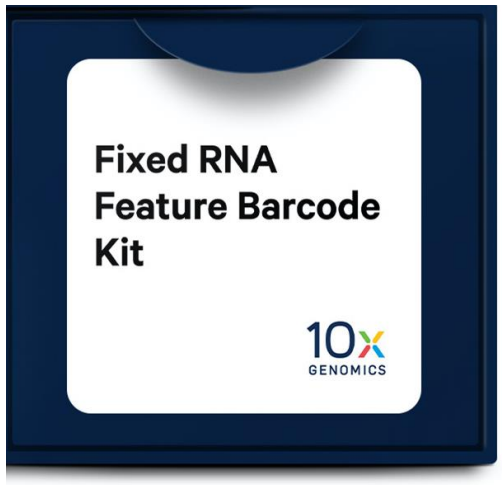


Dual Index Kit TS Set A
(PN-1000251)
(shipped on dry ice; store at -20°C)

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Chromium Gene Expression Flex reagent kits

Feature Barcode Technology for Cell Surface Protein







Dual Index Kit TT Set A
(PN-1000215)
(shipped on dry ice; store at -20°C)
*optional: Singleplex only FB CSP libraries

*Compatible with Singleplex Samples only

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Chromium Next GEM Chip Q





- New Chip architecture allows for faster runtimes
- Up to 8 channels processed in parallel
- Undetected multiplet rate: 0.8% per 1000 cells
- Target 500-10,000 cells per probe per sample per channel
- 4plex: Target up to 40k cells per channel
  - (up to 10,000 cells per BC)
- 16plex: Target up to 128k cells per channel
  - (up to 8,000 cells per BC)
- ~5.5 min Chromium X/iX run time (per chip)

Firmware FW 1.1.0 is required for running this assay

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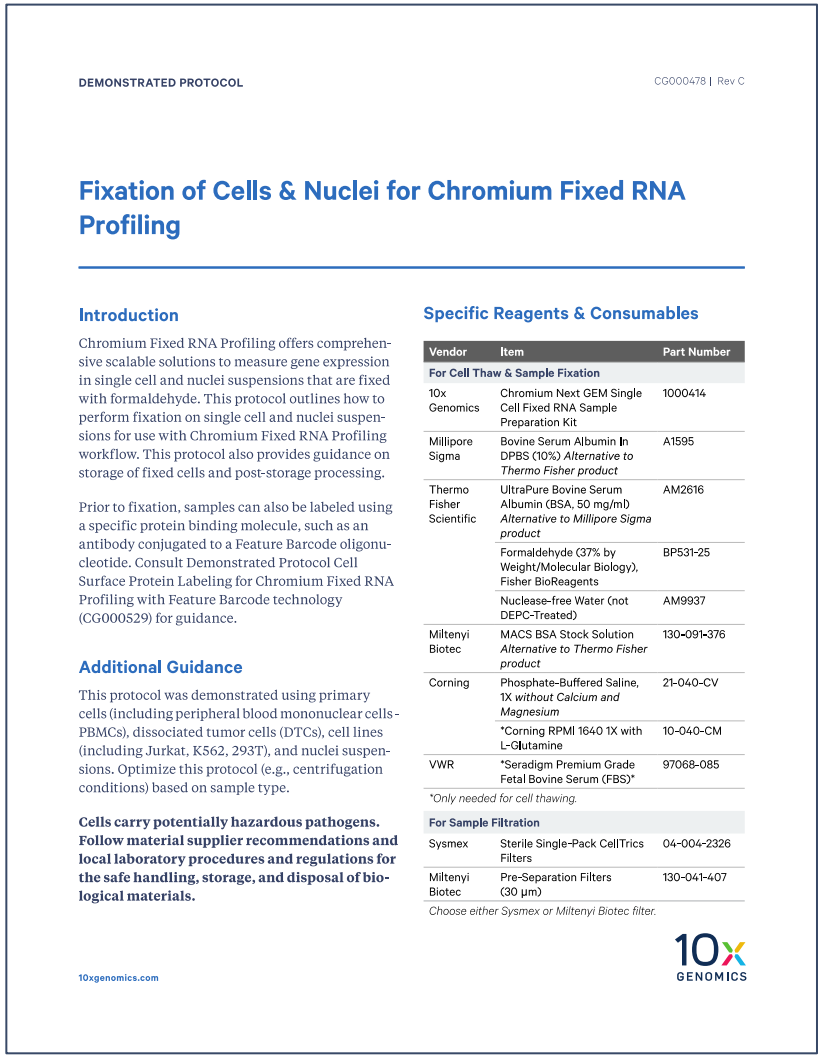
Sample Preparation

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Gene Expression Flex is compatible with single cell and nuclei suspensions

Sample Fixation Demonstrated Protocol

- Validated using primary cells (including PBMCs), dissociated tumor cells (DTCs), dissociated tissue, cell lines, and nuclei suspensions.
- This protocol has been demonstrated using 300,000-10x10^6 cells.
  - Requires ≥300,000 cells or ≥500,000 nuclei.
- Specific Reagents & Consumables
- Tips & Best Practices
- Sample Fixation Conditions
- Recommendations for Fixed Cell/Nuclei Counting
  - Accurate cell/nuclei counts: Fluorescent Dye with automated cell counters
- Sample fixation time and conditions should be kept constant within a study
- Appendix provides guidance for the following:
  - Short-term and long-term storage
  - Post storage processing
  - Fixed Sample Shipping Guidance



DEMONSTRATED PROTOCOL

CG000478 | Rev C

Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling

Introduction

Chromium Fixed RNA Profiling offers comprehensive scalable solutions to measure gene expression in single cell and nuclei suspensions that are fixed with formaldehyde. This protocol outlines how to perform fixation on single cell and nuclei suspensions for use with Chromium Fixed RNA Profiling workflow. This protocol also provides guidance on storage of fixed cells and post-storage processing.

Prior to fixation, samples can also be labeled using a specific protein binding molecule, such as an antibody conjugated to a Feature Barcode oligonucleotide. Consult Demonstrated Protocol Cell Surface Protein Labeling for Chromium Fixed RNA Profiling with Feature Barcode technology (CG000529) for guidance.

Additional Guidance

This protocol was demonstrated using primary cells (including peripheral blood mononuclear cells - PBMCs), dissociated tumor cells (DTCs), cell lines (including Jurkat, K562, 293T), and nuclei suspensions. Optimize this protocol (e.g., centrifugation conditions) based on sample type.

Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage, and disposal of biological materials.

Specific Reagents & Consumables

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) *Alternative to Millipore Sigma product*	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.		
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.		

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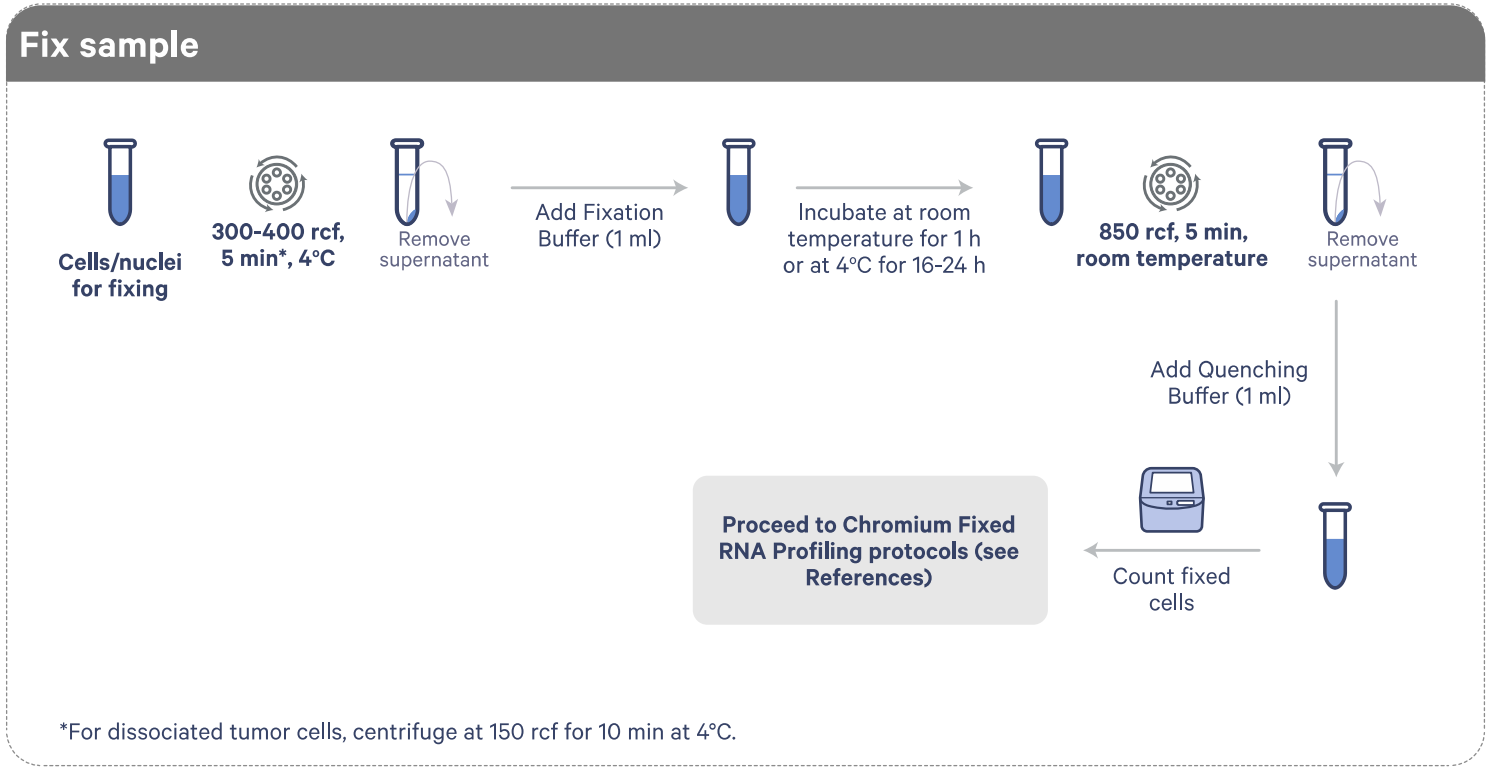
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Sample Fixation for FRP

Overview



Fixation Conditions	
Fixed Sample Use	**Fixation Time & Temperature**
Fixed sample to be processed immediately	1 h at room temperature (20°C)
Fixed sample to be stored subsequently	16-24 h at 4°C

DO NOT mix samples with different fixation times in one experiment.

- If planning to store the fixed samples, it is strongly recommended to perform a 16-24 h incubation at 4°C and store the fixed samples at -80°C for best results.

Fixed Cell Counting
Accurate sample counting is critical for optimal assay performance.
It is strongly recommended that the sample be stained with a fluorescent dye and counted using an automated fluorescent cell counter.

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Sample Preparation Demonstrated Protocols

Optimization may be required

Single Cell Suspensions	
Cultured Cell Lines	Single Cell Suspensions from Cultured Cell Lines for Single Cell RNA Sequencing
PBMCs	Isolation of Leukocytes, Bone Marrow and Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing
	Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing
Dissociated Tumor Cells (DTCs)	Tumor Dissociation for Single Cell RNA Sequencing
	Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing
Dead Cell Removal	Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing

*Single cell isolation protocols that yielded good results for 3’ GEX are expected to perform similarly in Fixed RNA Profiling.

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Nuclei Isolation Demonstrated Protocols & Products

Optimization may be required

Nuclei Isolation	
Nuclei Isolation from Fresh Tissue	Isolation of Nuclei for Single Cell RNA Sequencing
Nuclei Isolation from Frozen Tissue	Chromium Nuclei Isolation Kit Sample Prep User Guide
QC guidance	• Existing QC Guidance • Clean up steps are compatible

*Nuclei isolation protocols that yielded good results for 3’ GEX are expected to perform similarly in Gene Expression Flex. Recommend performing small pilot experiments prior to committing to large scale experiment.

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Demonstrated Protocols for Tissue

Optimization may be required

Fresh Tissue => Dissociation into single cell suspensions	
Dissociated Tumor Cells (DTCs)*	Tumor Dissociation for Single Cell RNA Sequencing
	Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing
Tissue Fixation & Dissociation **(**preferred method)**	Tissue Fixation & Dissociation for Chromium Fixed RNA Profiling

Frozen Tissue =>	
Chromium Nuclei Isolation Kit	Chromium Nuclei Isolation Kit Sample Prep User Guide
Tissue Fixation & Dissociation **(**preferred method)**	Tissue Fixation & Dissociation for Chromium Fixed RNA Profiling

FFPE Tissue =>	
Single-cell FFPE (scFFPE)	Isolation of Cells from FFPE Tissue Sections for Chromium Fixed RNA Profiling

*Tissue dissociation protocols that yielded good results for 3' GEX are expected to perform similarly in Gene Expression Flex.

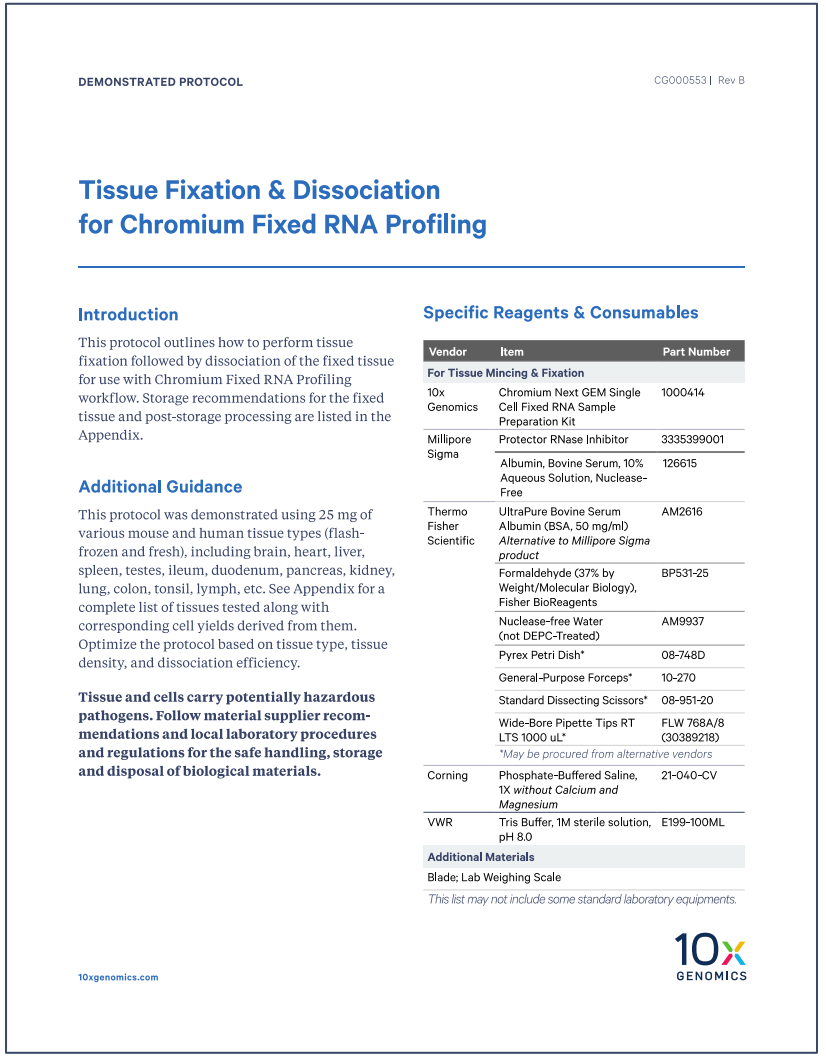
**Tissue Fixation and Dissociation Demonstrated Protocol is the preferred sample preparation method for fresh or frozen tissues.

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Tissue Fixation & Dissociation for Fixed RNA Profiling

Tissue Fixation & Dissociation Demonstrated Protocol

- Validated using 25 mg of various tissue types, including human flash frozen and fresh tissue.
- 25-100 mg of tissue is recommended for this protocol.
  - 1 mL Fixation buffer per 25 mg tissue
- Preferred sample preparation method for fresh or frozen tissue for optimal assay performance.
- Specific Reagents & Consumables
  - gentleMACS Octo Dissociator with Heaters
  - Pasteur Pipettes for manual dissociation
- Tips & Best Practices
  - Tissue Quality & Processing
  - Fixed Sample Storage
- Appendix provides guidance for the following:
  - Short-term and long-term storage
  - Post storage processing (Fixed Tissue & Fixed Cells)
  - Fixed Sample Shipping Guidance
  - Fixed Cell Counting Recommendations
    - Accurate cell counts: Fluorescent Dye with automated cell counters



DEMONSTRATED PROTOCOL

CG000553 | Rev B

Tissue Fixation & Dissociation for Chromium Fixed RNA Profiling

Introduction

This protocol outlines how to perform tissue fixation followed by dissociation of the fixed tissue for use with Chromium Fixed RNA Profiling workflow. Storage recommendations for the fixed tissue and post-storage processing are listed in the Appendix.

Additional Guidance

This protocol was demonstrated using 25 mg of various mouse and human tissue types (flash-frozen and fresh), including brain, heart, liver, spleen, testes, ileum, duodenum, pancreas, kidney, lung, colon, tonsil, lymph, etc. See Appendix for a complete list of tissues tested along with corresponding cell yields derived from them. Optimize the protocol based on tissue type, tissue density, and dissociation efficiency.

Tissue and cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

Specific Reagents & Consumables

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		

This list may not include some standard laboratory equipments.

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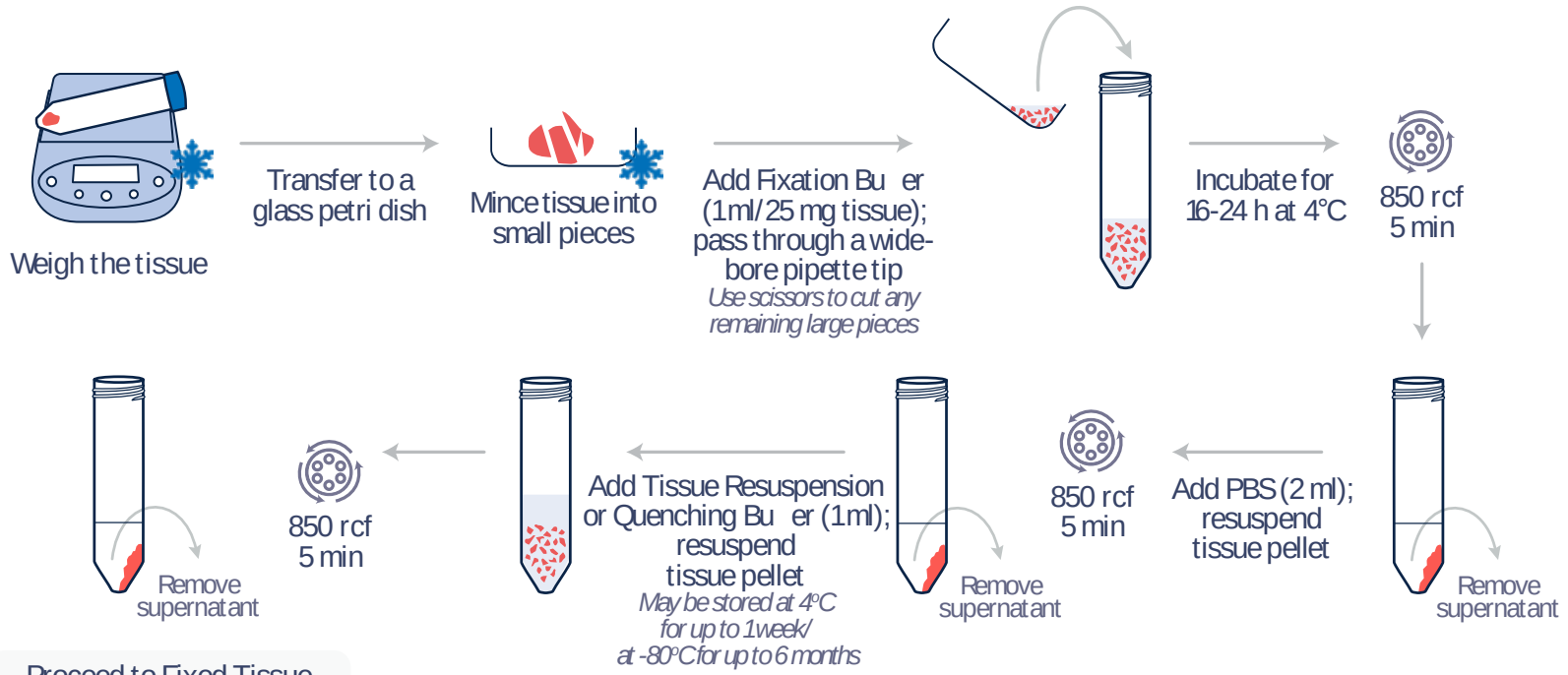
Visit support.10xgenomics.com for the most up-to-date documentation

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Tissue Fixation & Dissociation for Fixed RNA Profiling

Fix Tissue

1 Tissue Fixation



Fix Tissue
1 mL Fixation buffer per 25 mg tissue
Use pre-chilled glass petri dish maintained on ice and using a blade, mince tissue finely (enables passing through a 1 ml wide-bore pipette tip).
If larger tissue pieces fail to pass through the pipette, use dissection scissors to cut the tissue pieces further until they pass through the pipette.

If using frozen tissue, maintain the tissue on dry ice before and after weighing. Use a pre-chilled petri dish placed on ice while mincing the tissue.

DO NOT agitate or mix the sample during incubation.

Fixation time and temperature should be consistent across all samples in an experiment.

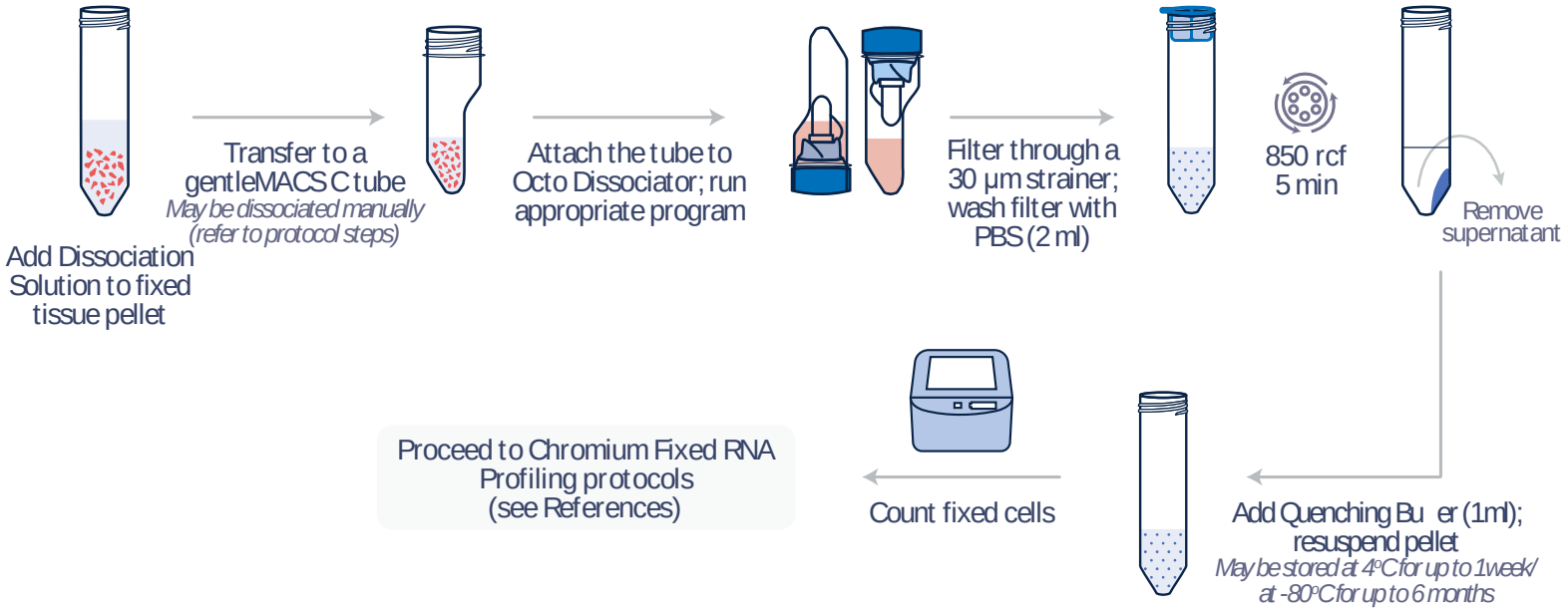
Fixed tissue pieces can be stored at 4°C for up to 1 week or at -80°C for up to 6 months. See Appendix for guidance on short-term storage and post-storage processing of fixed tissue pieces.

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Tissue Fixation & Dissociation for Fixed RNA Profiling

Fixed Tissue Dissociation: Octo Dissociator

2. Fixed Tissue Dissociation **(preferred method)**



Fixed Tissue Dissociation
Using Octo Dissociator is highly recommended.
Octo Dissociator Program is outlined in Demonstrated Protocol.

If tissue is tough to triturate and/or solution is not cloudy after trituration, see Troubleshooting guidance

TIPS *Use of swinging-bucket rotor is recommended for higher cell recovery.*

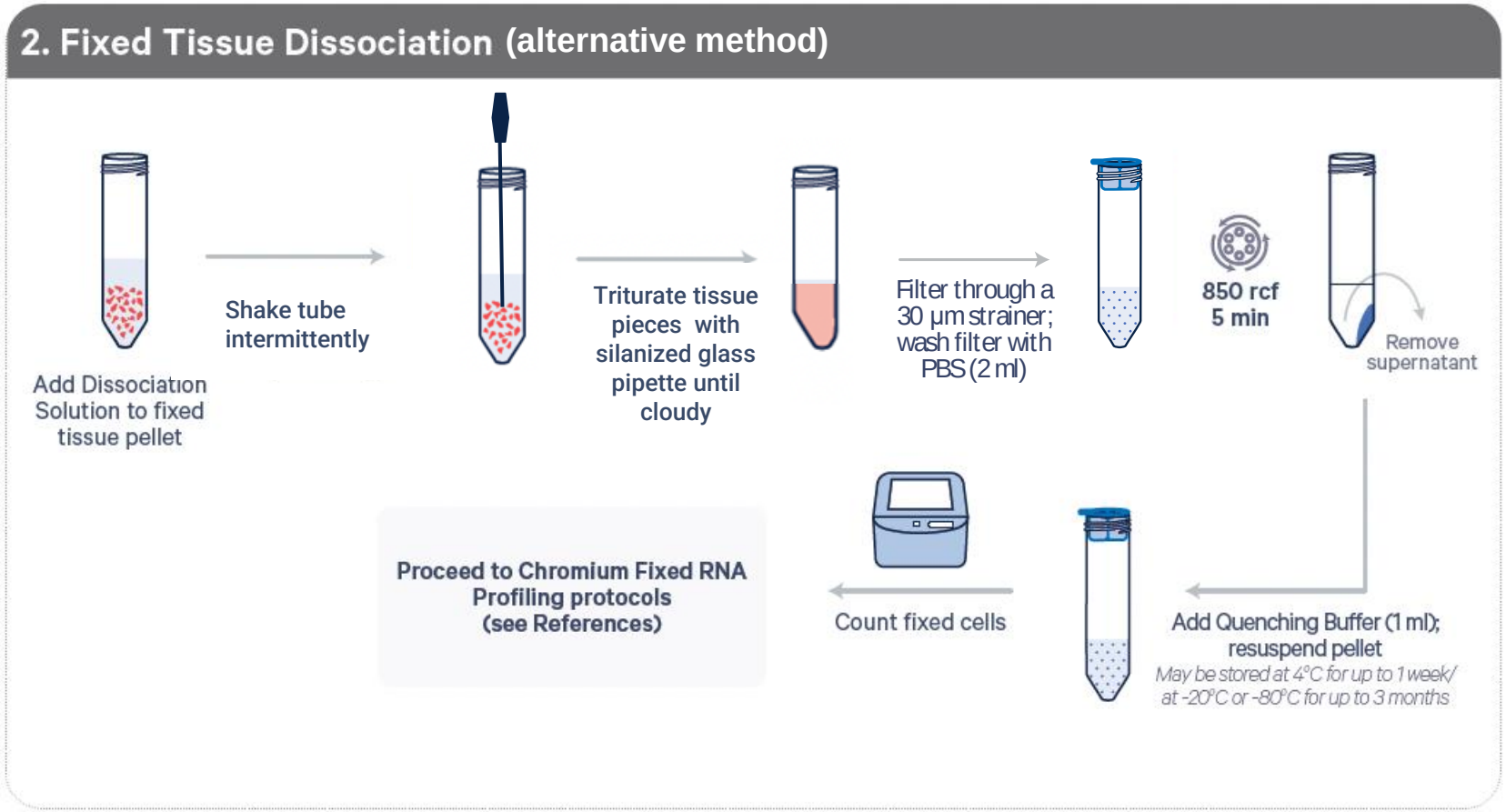
Fixed tissue pieces can be stored at 4°C for up to 1 week or at -80°C for up to 6 months. See Appendix for guidance on short-term storage and post-storage processing of fixed tissue pieces.

Visit support.10xgenomics.com for the most up-to-date documentation

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Tissue Fixation & Dissociation for Fixed RNA Profiling

Fixed Tissue Dissociation: Manual Dissociation



Fixed Tissue Dissociation
Manually shaking tube intermittently
Manual dissociation with silanized glass pipette (15-20X) until solution begins to turn cloudy.

If tissue is tough to triturate and/or solution is not cloudy after trituration, see Troubleshooting guidance

TIPS *Use of swinging-bucket rotor is recommended for higher cell recovery.*

Fixed tissue pieces can be stored at 4°C for up to 1 week or at -80°C for up to 6 months. See Appendix for guidance on short-term storage and post-storage processing of fixed tissue pieces.

Pipette recommendation: https://tissue.transnetyx.com/Fire-Polished-Pipette_2
*silanized 9" pasteur pipette with tip fire polished to an opening of 0.5 mm

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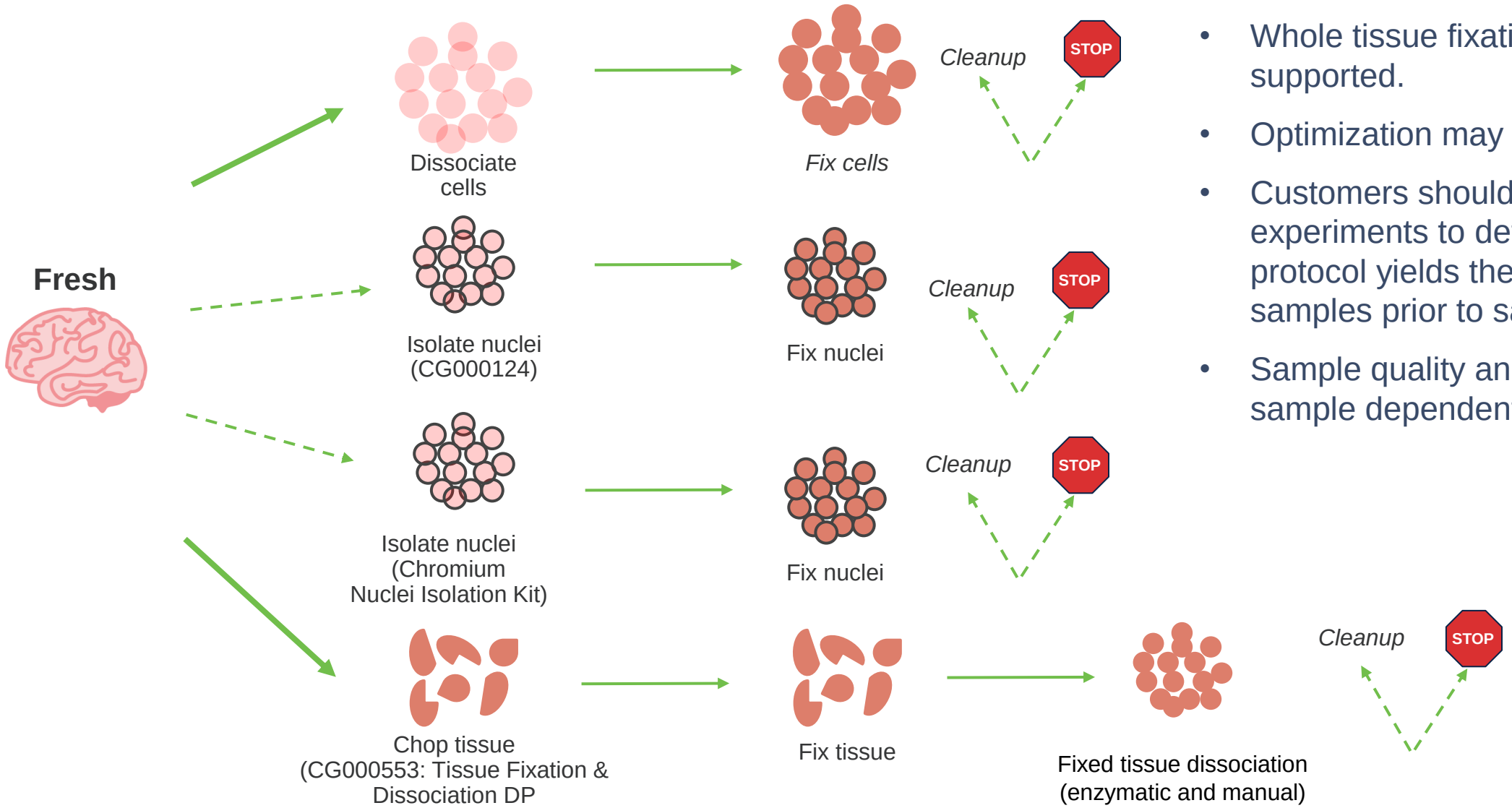
Tissue Quality & Processing

Tips & Best Practices

- Perform pilot experiments to determine if the tissue type is suitable for fixation and/or dissociation for preparing single cell suspension(s).
- Ensure that variation in cell yields from different tissue types is considered when performing the protocol.
- The fresh tissue should be minced only on a glass surface. DO NOT use plastic petri dish for mincing tissue.
- Fixed tissue pieces and cell suspension derived from fixed dissociated tissues can be stored at 4°C for up to 1 week or at -80°C for up to 6 months after resuspending in appropriate reagents.

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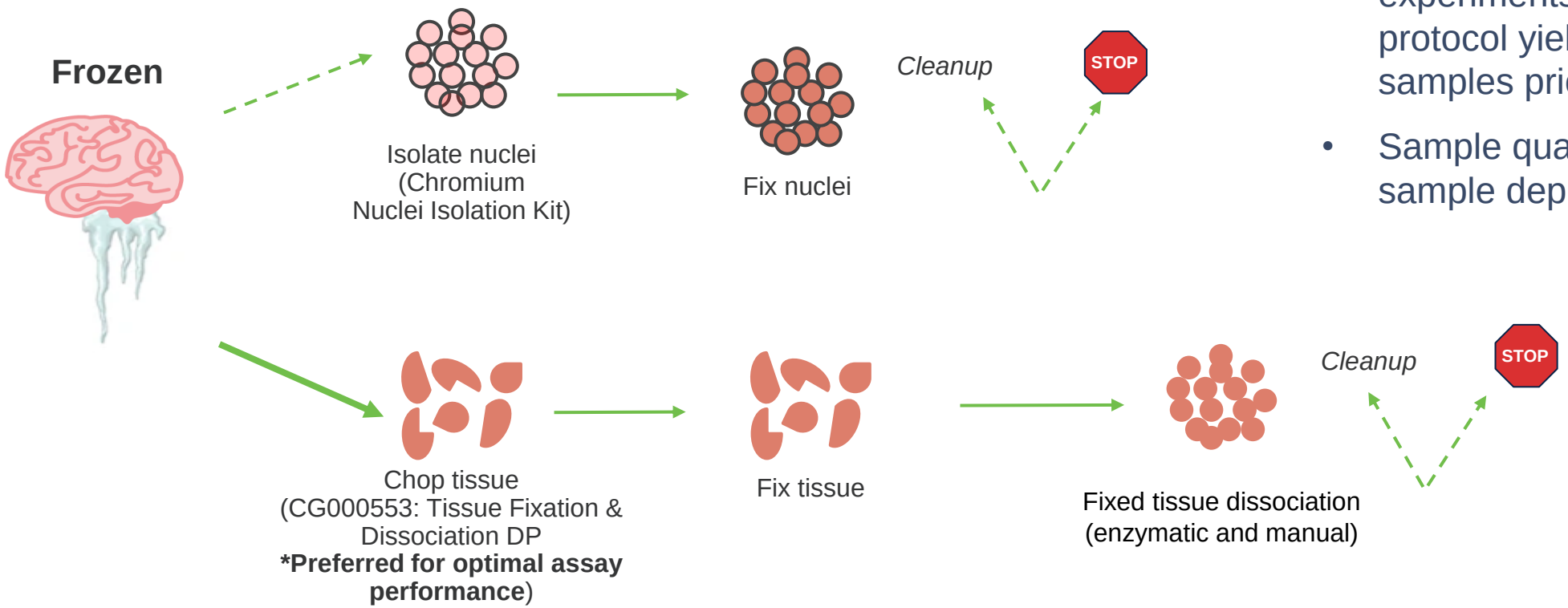
Fresh Tissue Sample Preparation Recommendations



- Whole tissue fixation will not be supported.
- Optimization may be required.
- Customers should perform pilot experiments to determine which protocol yields the highest quality samples prior to sample fixation.
- Sample quality and yield will be sample dependent.

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Frozen Tissue Sample Preparation Recommendations



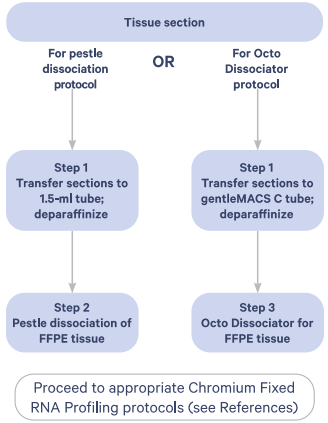
- Whole tissue fixation will not be supported.
- Optimization may be required.
- Customers should perform pilot experiments to determine which protocol yields the highest quality samples prior to sample fixation.
- Sample quality and yield will be sample dependent.

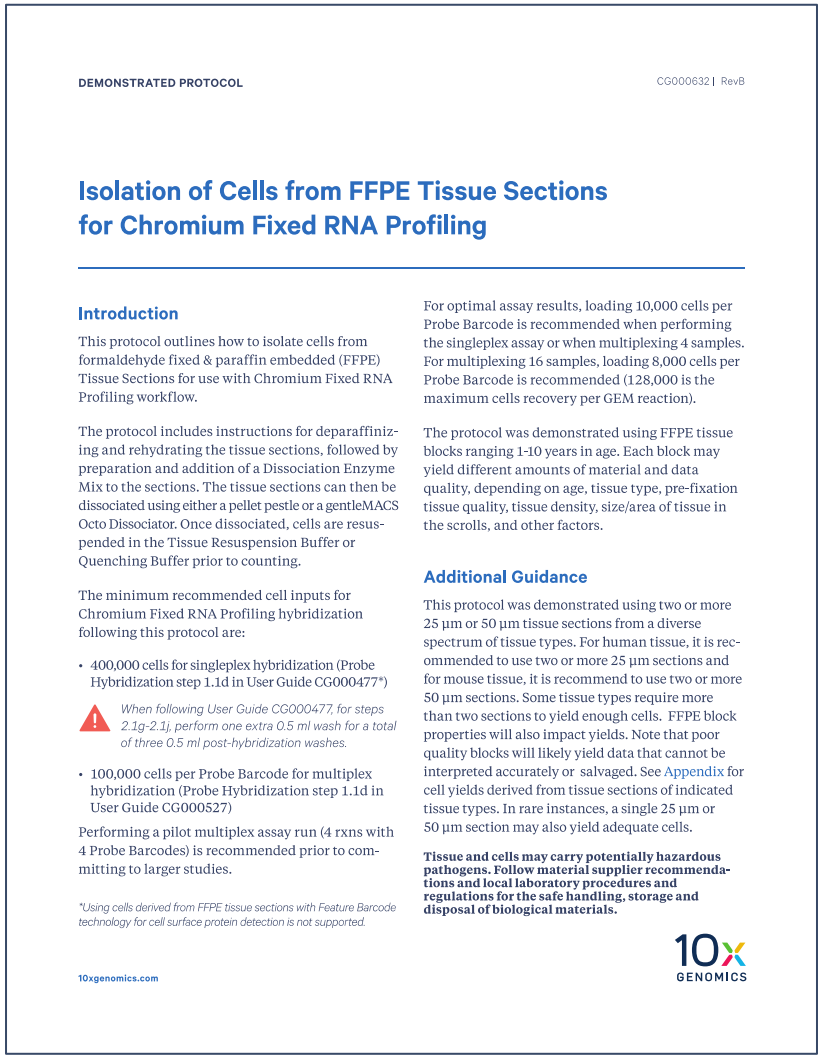
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Isolation of Cells from FFPE Tissue Sections for Chromium Fixed RNA Profiling

FFPE Tissue Dissociation Demonstrated Protocol

- Validated using various human and mouse FFPE tissues.
- Specific Reagents & Consumables
- Tips & Best Practices
  - FFPE scrolls should be kept intact throughout protocol.
- Utilize either pellet pestle or Octo Dissociator for tissue dissociation
- Recommendations for Fixed Cell/Nuclei Counting
  - Accurate cell/nuclei counts: Fluorescent Dye with automated cell counters.
- Appendix provides guidance for the following:
  - Cell/Nuclei Counting
  - Cell Yields from FFPE Tissue Sections
  - Troubleshooting
  - Short and long-term storage





DEMONSTRATED PROTOCOL CG000632 | RevB

Isolation of Cells from FFPE Tissue Sections for Chromium Fixed RNA Profiling

Introduction

This protocol outlines how to isolate cells from formaldehyde fixed & paraffin embedded (FFPE) Tissue Sections for use with Chromium Fixed RNA Profiling workflow.

The protocol includes instructions for deparaffinizing and rehydrating the tissue sections, followed by preparation and addition of a Dissociation Enzyme Mix to the sections. The tissue sections can then be dissociated using either a pellet pestle or a gentleMACS Octo Dissociator. Once dissociated, cells are resuspended in the Tissue Resuspension Buffer or Quenching Buffer prior to counting.

The minimum recommended cell inputs for Chromium Fixed RNA Profiling hybridization following this protocol are:

- 400,000 cells for singleplex hybridization (Probe Hybridization step 1.1d in User Guide CG000477*)

When following User Guide CG000477, for steps 2.1g-2.1j, perform one extra 0.5 ml wash for a total of three 0.5 ml post-hybridization washes.

- 100,000 cells per Probe Barcode for multiplex hybridization (Probe Hybridization step 1.1d in User Guide CG000527)

Performing a pilot multiplex assay run (4 rxns with 4 Probe Barcodes) is recommended prior to committing to larger studies.

**Using cells derived from FFPE tissue sections with Feature Barcode technology for cell surface protein detection is not supported.*

For optimal assay results, loading 10,000 cells per Probe Barcode is recommended when performing the singleplex assay or when multiplexing 4 samples. For multiplexing 16 samples, loading 8,000 cells per Probe Barcode is recommended (128,000 is the maximum cells recovery per GEM reaction).

The protocol was demonstrated using FFPE tissue blocks ranging 1-10 years in age. Each block may yield different amounts of material and data quality, depending on age, tissue type, pre-fixation tissue quality, tissue density, size/area of tissue in the scrolls, and other factors.

Additional Guidance

This protocol was demonstrated using two or more 25 µm or 50 µm tissue sections from a diverse spectrum of tissue types. For human tissue, it is recommended to use two or more 25 µm sections and for mouse tissue, it is recommend to use two or more 50 µm sections. Some tissue types require more than two sections to yield enough cells. FFPE block properties will also impact yields. Note that poor quality blocks will likely yield data that cannot be interpreted accurately or salvaged. See Appendix for cell yields derived from tissue sections of indicated tissue types. In rare instances, a single 25 µm or 50 µm section may also yield adequate cells.

Tissue and cells may carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

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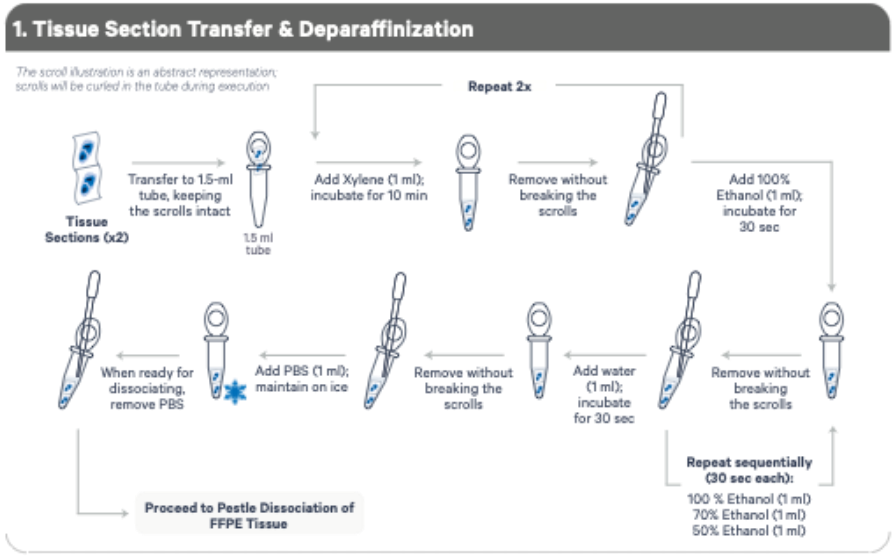
Visit support.10xgenomics.com for the most up-to-date documentation

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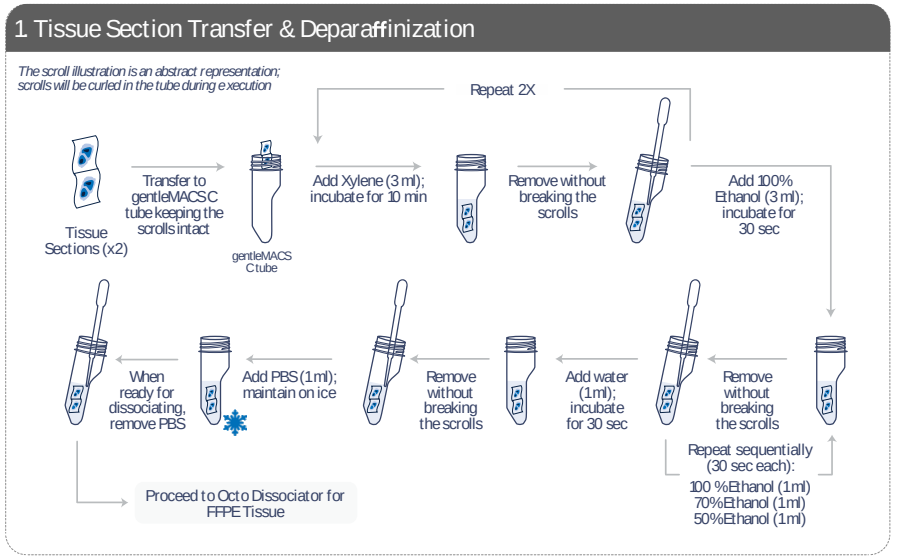
Isolation of Cells from FFPE Tissue Sections for Chromium Fixed RNA Profiling

Deparaffinization and rehydration

Pellet pestle method



Octo Dissociator method



FFPE Tissue Dissociation
Gently transfer scrolls, add and remove reagents
Pellet and Octo Dissociator methods generated comparable results

Ensure that the scrolls stay intact during transfer and through steps 1a-1m. Add reagents along the side of the tube and not directly onto the scrolls. Intact sections enable easier liquid aspiration and minimize tissue loss. If scrolls break, see guidance in Troubleshooting section. Proceed with the protocol and based on cell yields process more sections if needed.

TIPS *Always use fresh/freshly prepared reagents for the deparaffinization steps.*
Ensure the scrolls are fully submerged in regents in the following steps.

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Debris Removal

Filtering cell and nuclei suspensions

- Strainers with appropriate pore sizes should be used to allow cells to pass through the filter while cellular debris and aggregates are retained.

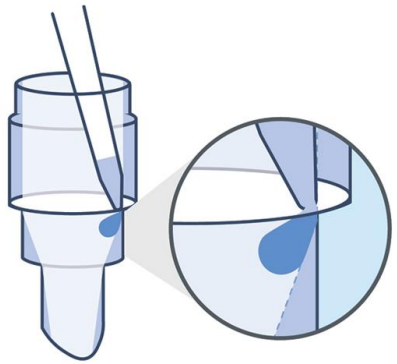
Filtration using 30 µm filters



Pre-Separation Filters from Miltenyi Biotec



Celltrix Filters from Sysmex



Lightly touch the filter membrane with the tip where the filter meets the wall

For FFPE samples, as there's a wash step of the filter, apply suspension to the center of the filter

Maximize Recovery After Filtration



Pre-Separation Filters from Miltenyi Biotec



Celltrix Filters from Sysmex

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Sample Storage

Validated stopping points during sample preparation - Post-Fix and Post-Hyb [Wash]

Single cell/nuclei suspension → Fix cells → Probe Hyb 16-24 h at 42°C → Pool & Wash



Fix cells → Quench, Add Enhancer & Glycerol, and Store Cells → STOP

4°C for 1 week; -80°C for up to 6 months

Pool & Wash → Add Enhancer & Glycerol, and Store Cells → STOP

-80°C for up to 6 months

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Considerations for sample preparation and sequencing

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Sample Quality for Fixed RNA Profiling

Fresh/frozen single cell or nuclei suspensions

Sample Quality
• Use high-quality single cell or nuclei suspensions that can withstand the fixation steps.
• Perform pilot experiments to determine if the sample type is suitable for the fixation.
• >80% viability will have the greatest sensitivity and cell recovery. However, the Fixed RNA Profiling assay is robust to samples at much lower viability, with successful results demonstrated even with low viability samples (50% or lower). Low viability samples may have more variable cell calling and lower sensitivity.
• Samples should have minimal debris for best results; debris can have associated RNA that is detected outside cells.

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General cell handling recommendations

Fresh/frozen single cell or nuclei suspensions

Analysis of Fixed RNA Profiling transcriptomes

- ❑ Input cell suspensions should contain more than 80% viable cells, greatest sensitivity and cell recovery.
- ❑ However, the Fixed RNA Profiling assay is robust to samples at much lower viability, with successful results demonstrated even with low viability samples (50% or lower).
- ❑ Low viability samples may have more variable cell calling and lower sensitivity.

Importance of input cell quality

- ❑ Requires a fully dissociated, single cell or nuclei suspension.
- ❑ Minimizing the presence of cellular aggregates, dead cells, and debris is critical to obtaining high quality data and optimal cell recovery.
- ❑ Suspension cell lines, bead-enriched and flow-sorted cells can be used directly after washing.

Cell handling

- ❑ It is important to treat cells gently to minimize cell lysis and loss prior to sample fixation.
- ❑ Use of swinging bucket rotor is recommended for higher cell/nuclei recovery.
- ❑ Centrifugation speed and time may need to be optimized depending upon the sample type.

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General cell handling recommendations

Fresh/frozen single cell or nuclei suspensions

Debris/aggregate removal

- ❏ Use a cell strainer to remove aggregates or debris from washed cells.
- ❏ The presence of cell aggregates, debris and/or fibers can result in inaccurate cell counts.



Cell Counting

- ❏ Quantitating cell numbers accurately throughout the workflow is critical for optimal assay performance.
- ❏ Strongly recommend cell/nuclei suspensions are stained with a fluorescent dye and counted using an automated fluorescent cell counter.
- ❏ Under- or over-counting cells may impact application performance.



Storage of single cell or nuclei suspensions prior to fixation

- ❏ Cell/nuclei suspensions should always be kept on ice and where possible proceed with sample fixation immediately after sample preparation.
- ❏ Some cell types are more fragile and cell viability may decrease significantly if not processed and fixed as soon as possible.



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Sample Quality for Fixed RNA Profiling

FFPE single cell suspensions

Sample Quality
• FFPE blocks will yield different amounts of material and will generate variable quality data because of age, tissue type, pre-fixation tissue quality, tissue density, size/ area of tissue in the scrolls, and other block properties.
• Perform pilot experiments to determine if the FFPE block is suitable for single cell analysis using the Fixed RNA Profiling assay.
• Samples should have minimal debris for best results; debris can have associated RNA that is detected outside cells.

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General cell handling recommendations

FFPE single cell suspensions

Analysis of Fixed RNA Profiling transcriptomes

- ❑ Low-quality FFPE blocks may have more variable cell calling and lower sensitivity.

Sample quality and handling

- ❑ Requires a fully dissociated, single cell or nuclei suspension.
- ❑ Minimizing the presence of aggregates and debris is critical to obtaining high quality data and optimal cell recovery.
- ❑ Use of swinging bucket rotor is recommended for higher sample recovery.
- ❑ Centrifugation speed and time may need to be optimized.

Scroll handling

- ❑ If needing to transfer FFPE scroll to a different tube, tap the tube to promote transfer- do NOT use forceps for transfer
- ❑ Pipette deparaffinization reagents on wall of tube, not onto the FFPE scroll.
- ❑ Ensure the FFPE scroll remains intact to maximize cell/nuclei recovery.

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Libraries are compatible with Illumina Sequencers



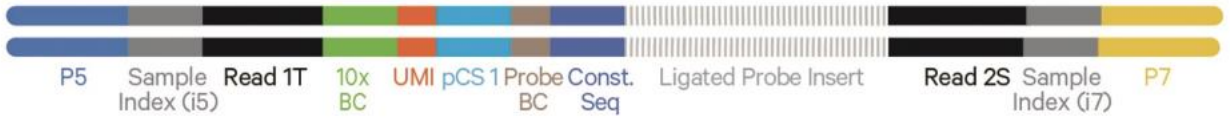


	Read 1	i7 Index	i5 Index	Read 2
Purpose	10x barcode & UMI	Sample Index	Sample Index	Transcript
Length	28	10	10	90

	Fixed RNA Profiling Libraries	Feature Barcode Libraries
Raw Reads Per Cells	minimum 10,000	minimum 5000

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Libraries are compatible with Illumina Sequencers






iSeq


MiSeq


NextSeq 500/550


NextSeq 1000/2000


NovaSeq

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Sequencing Recommendations

The following libraries can be pooled:

- Single Cell 3’ v3.1 Dual Index Expression Libraries
- Single Cell 3’ v3.1 Dual Index Cell Surface Protein or CRISPR Screening Libraries
- Single Cell 5’ v2 Gene Expression or V(D)J Enriched Libraries*
- Single Cell 5’ v2 Cell Surface Protein or CRISPR Screening Libraries*
- Spatial Gene Expression Libraries

The following libraries *cannot* be pooled:

- Single Cell Multiome ATAC libraries
- Single Cell ATAC libraries
- Spatial Gene Expression Probe-based Libraries

*If run with 28 cycles for Read 1

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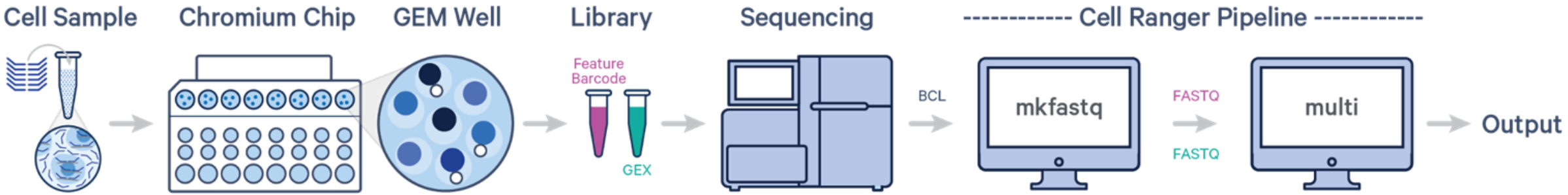
Cell Ranger Analysis Pipeline Overview

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Analyzing Gene Expression Flex data

Laboratory and analysis workflow overview

Singleplex (optional: Cell Surface Protein)



Multiplex



- **FRP data analysis is only supported starting from Cell Ranger 7.0, using *cellranger multi***

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Options for running Cell Ranger pipelines

	10x Cloud Analysis (US and Canada only)	Cluster Mode	Single Server
Recommended for:	Most users*	Organizations using an HPC with SGE or LSF	Users with access to Linux machines
Compute details:	Splits each analysis across multiple compute nodes to decrease run time	Splits each analysis across multiple compute nodes to decrease run time	Runs each analysis directly on a single machine
Requirements:	Internet connected laptop or desktop	• HPC with SGE or LSF • NFS file system • Other single server requirements	• Linux computer with supported operating system • 8 cores & 64 GB RAM (minimum) • 1TB free disk space

* Not compatible with BCL files. In order to use Cloud Analysis, we recommend that you request FASTQ files from your sequencing provider.

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Reference materials and resources

Available at support.10xgenomics.com



Specifications



User guides



Product sheet



Technical notes



Public data sets



How-to-videos



Demonstrated protocols

Contact us: support@10xgenomics.com

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