Sample Preparation for Single Cell Analysis

A How To Guide: Considerations and Best Practices

The technology of 10X Genomics

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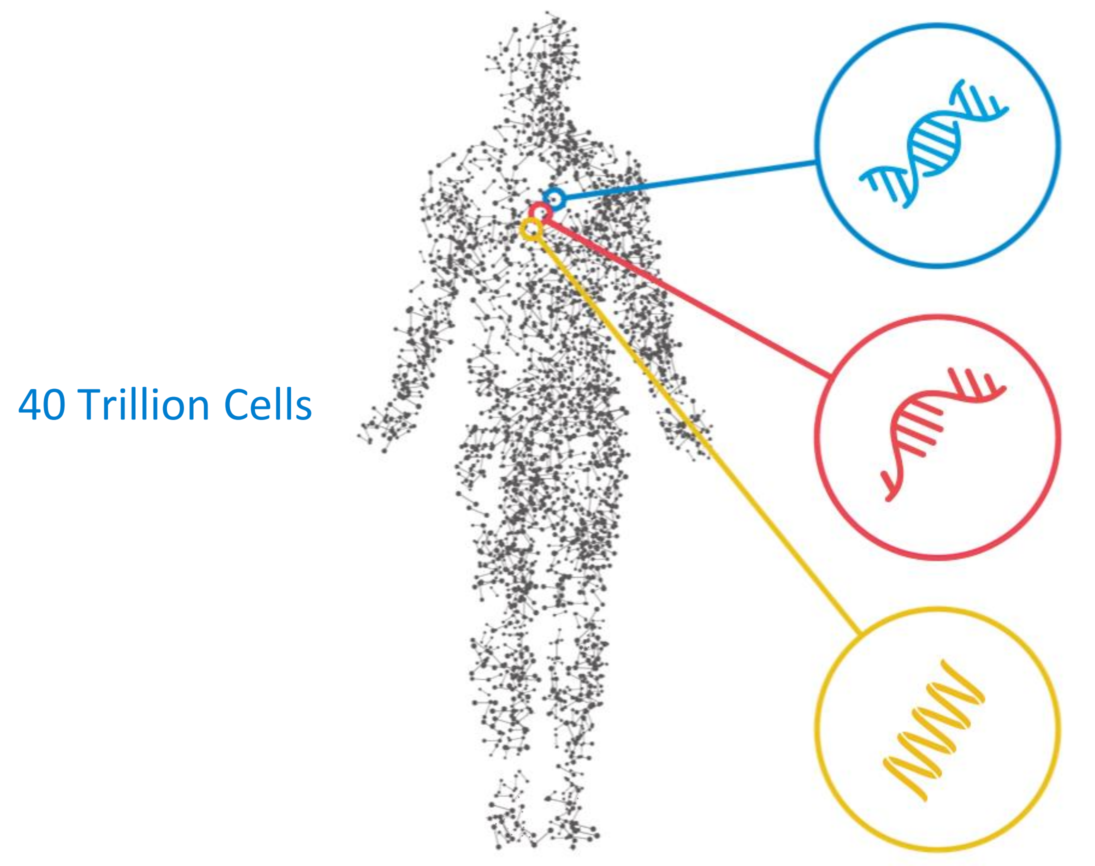
Biology is Immensely Complex



40 Trillion Cells

6 billion DNA bases in the genome
Each cell with its own epigenetic program

Transcribed into over 50,000 mRNA molecules
Each cell with a unique set of expressed genes

Translation into tens of thousands of proteins
Each immune cell with its own unique antibody

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Replacing the legacy toolkit across biology

CRISPR screening
- Liquid handlers
- High content imaging

High throughput CRISPR functional genomics

Chromatin

ChIP- and NGS-based methods

Epigenomics (ATAC-seq)

RNA
- Microarrays
- Microscopy
- qPCR / PCR

Gene expression

RNA + T- and B-cell receptors
- Spectratyping
- NGS methods

High throughput immune cell profiling

Proteins
- Western blots
- Affinity columns
- Protein microarrays
- FACS
- CyTOF

High throughput protein screening

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10x Genomics core single cell technology for molecular barcoding

10x Barcoded Gel Beads

Cells
Enzyme

Oil in Well

Collect

Pool

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Sample considerations – general recommendations

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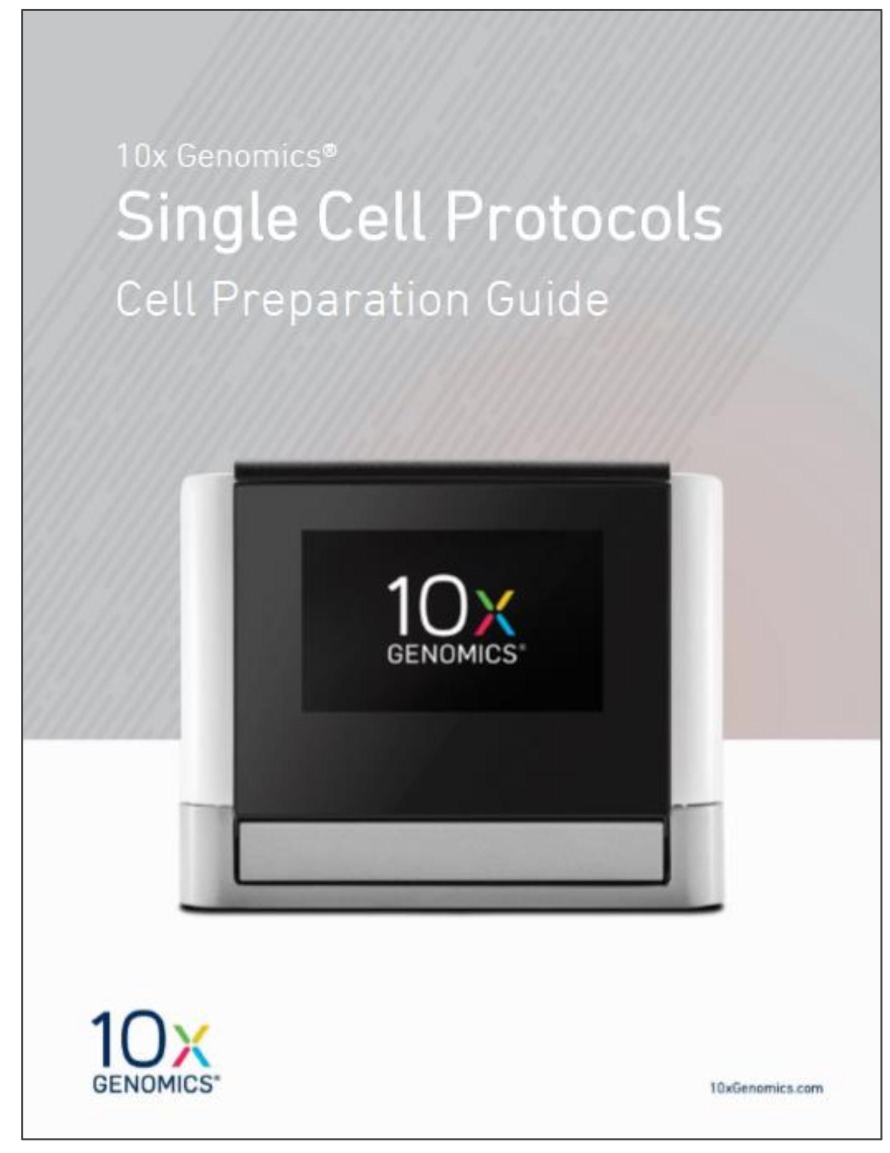
Cell Preparation

- Cell prep guide includes recommendations/tips for preparing cells.
- Demonstrated Protocols available on 10x support website.
  (e.g. fresh frozen PBMCs, neural tissue dissociation)
- Cultured cell lines
- Primary cells
  - FACS-sorted
  - Magnetic-bead purified (e.g. Miltenyi Microbeads)
  - Gradient-purified (e.g. Percoll, Optiprep, Apheresis)
- Solid tissue



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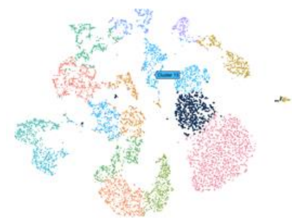
General Cell Handling Recommendations

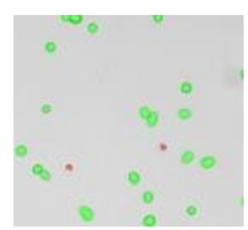
Analysis of Single Cell Transcriptomes

- Requires a fully dissociated, **single cell suspension.**
- Minimizing the presence of cellular aggregates, dead cells, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtaining high quality data.
- Suspension cell lines, bead-enriched and flow-sorted cells can be used directly after washing.



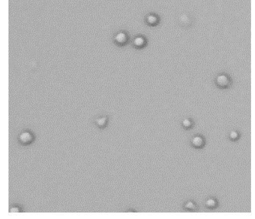
Importance of Input Cell Quality

- Ideally, input cell suspensions should contain more than **90% viable cells**.
- The presence of a high fraction of non-viable or dying cells may decrease recovery.
  - The presence of ambient RNA and cellular debris may impact application performance and negatively impact quality metrics reported by Cell Ranger.



Cell Handling

- It is important to **treat cells *gently*** to minimize cell lysis and loss:
  - When cells lyse, the released ambient mRNA will contaminate other GEMs
  - Wash cells twice using a wide-bore pipette tip to remove ambient RNA and contaminants.
  - Wash and resuspend in PBS + 0.04% non-acetylated BSA to minimize cell loss during handling.



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Rough Pipetting Leads to Cell Lysis and Lower Reads in Cells

Application Performance Metrics Reflect Rough Cell Handling

Metric	Control	Wide Bore (Rough)	Narrow Bore (Rough)	Vortex 5s
Number of Cells	1,118	846	1,012	983
Reads per Cell	50,000	50,000	50,000	50,000
Fraction Reads in Cells	79.40%	72.80%	54.00%	63.10%
Median Genes per Cell	3,137	3,180	2,833	2,934
Median UMI counts per Cell	10,726	11,053	8,832	9,503

- If cells are handled too roughly, many will lyse, releasing mRNA into the cell suspension buffer.
- The ambient RNA will be incorporated into the sequenced library, but will not be associated with cell-containing GEMs. This effectively increases the background, while decreasing the Fraction of Reads in Cells.

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General Cell Handling Recommendations

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
- GEM generation occurs in microfluidic channels that are narrower than the typical human hair (i.e. < 100 µm) and the presence of cell debris or large aggregates may clog or wet the chip

Cell Counting

- Quantitate cells accurately before loading into the system
  - Approximately 65% loaded cells will be recovered
  - To maximize the likelihood of achieving the desired recovery target, the optimal input cell concentration is 700-1200 cells/µl
  - Recommended range: 500 to 10,000 recovered cells
  - Under- or over-loading may impact application performance

Storage of Single Cell Suspensions

- Cell suspensions should always be kept on ice and where possible proceed with cell loading immediately after sample preparation
  - Ideally incubation time should be kept to a minimum (< 30 min)
- Some cell types are more fragile and cell viability may decrease significantly if not processed and loaded immediately

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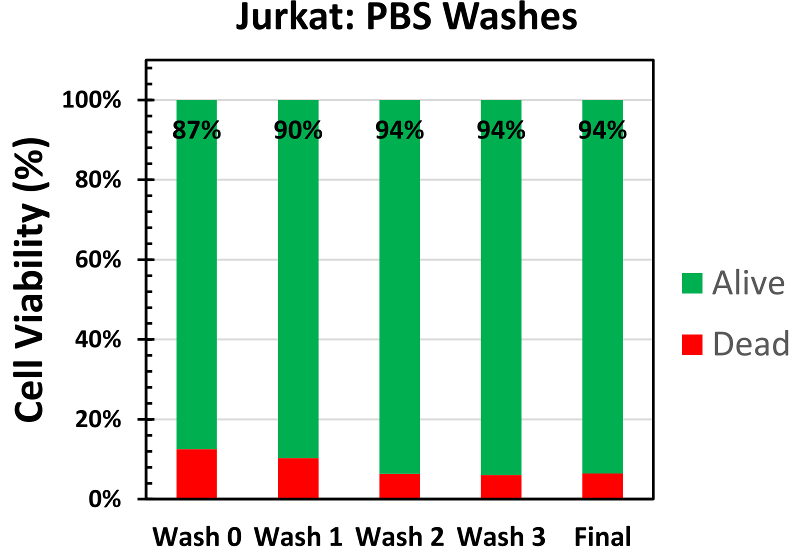
Cell Washing

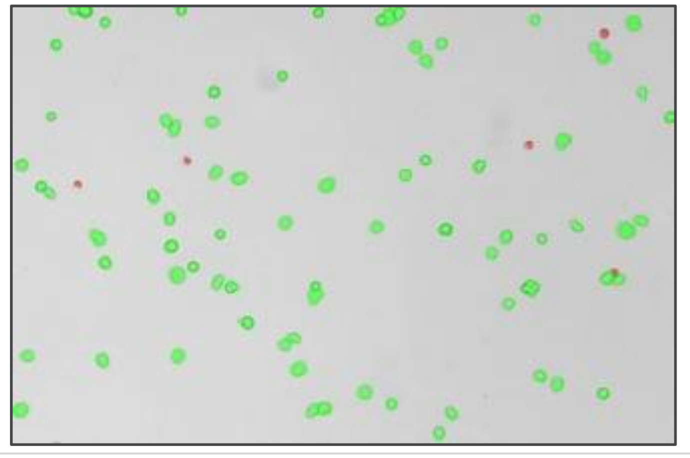
Washing isolated cells

- Transfer cells in media to a 2 mL Eppendorf tube
- Spin down cells to form pellet
  - Depending on cell size and concentration, pellet size varies
- Remove supernatant
- Gently add 1x PBS + 0.04% BSA away from cell pellet
- Gently pipette mix with Wide Bore pipette tip
- Repeat the wash one more time
- Spin down cells to form pellet
- Remove supernatant
- Resuspend cells in 1x PBS + 0.04% BSA with gentle pipette mix
  - **For accurate cell counting, do not invert tubes**
- Adjust to desired cell concentration

Note: PBS can be replaced with most common cell culture buffers and media if cells are unstable in PBS



Live/Dead Staining of Final Suspension



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Alternative Buffer and Media

Tested in-house

- Tested input volume: 2.5 and 33 µl
- **Alternative Buffer**: no influence on performance
  - Dulbecco's Phosphate-Buffered Saline (DPBS)
  - Hank's Balanced Salt Solution (HBSS)
- **Alternative Media**: minimal reduction to no loss in performance
  - Eagle's Minimum Essential Medium (EMEM) + 10% FBS
  - Dulbecco's Modified Eagle Medium (DMEM) + 10% FBS
  - Iscove's Modified Eagle Medium (IMEM) + 10% FBS
  - Roswell Park Memorial Institute (RPMI) + 10% FBS
  - Ham's F12 + 10% FBS
  - 1:1 DMEM/F12 +10% FBS
  - M199

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Recommendations for Limited Samples

Samples with low starting numbers of cells

- If using FACS, directly sort cells into the optimal media for the cell type
- Count cells before washing to estimate the approximate cell number
  - This step will minimize cells lost to counting
  - This step will allow one to add appropriate volume of PBS for resuspension to achieve a target concentration
- Spin down cells in 2mL Round bottom LoBind tubes
  - Inefficient centrifugation may lead to further loss of cells
  - Smaller cells: use higher speed and longer time for centrifugation
  - Important: know the expected position of the pellet as pellet may be invisible to naked eyes
- Washing may be skipped if the number of available cells are very small
  - Recommend washing cells once.
  - Centrifuge once, remove supernatant but ~ 50 µl, and resuspend cells in the leftover supernatant.
  - Important to remove residual Mg^{2+} and EDTA. A 2-fold change up or down in Mg^{2+} concentration will affect the efficiency of the RT step.

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Sample considerations

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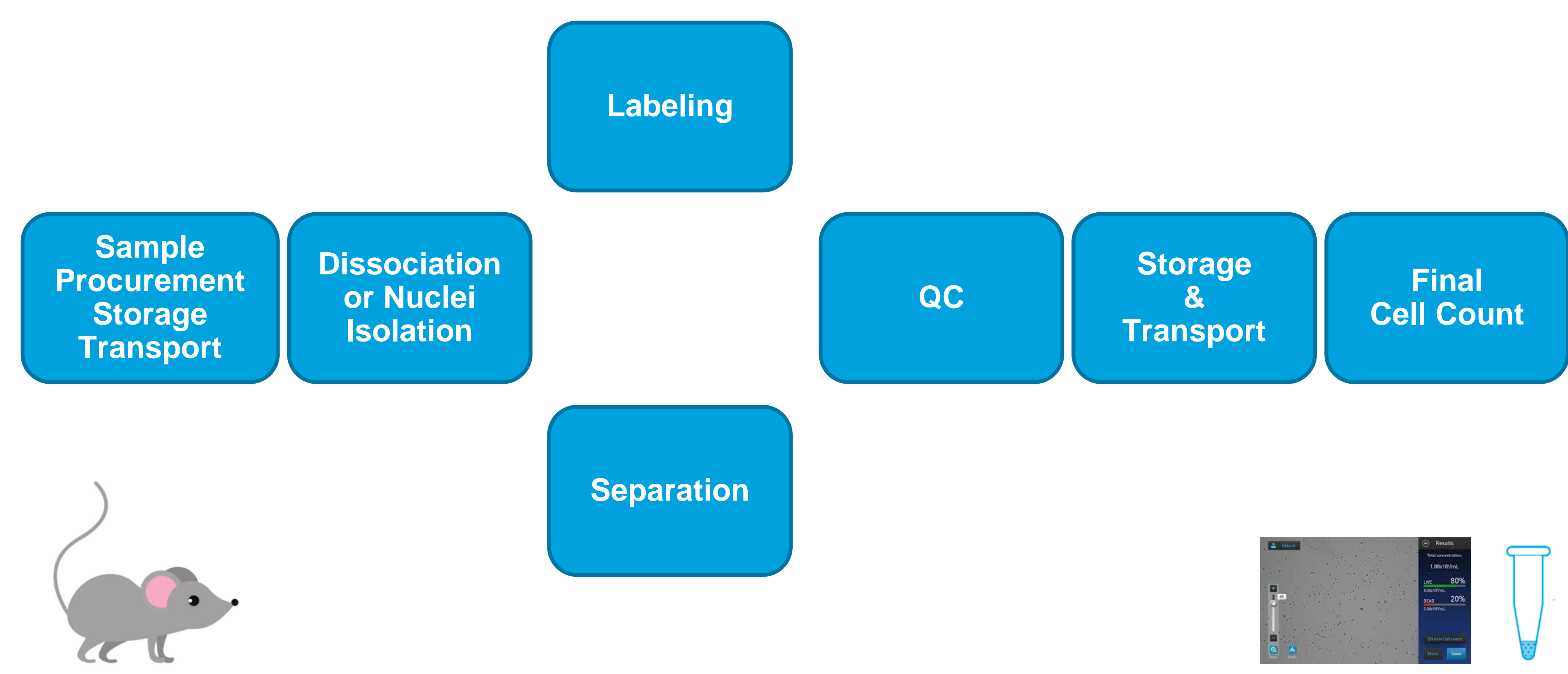
It's a workflow. A set of decisions.



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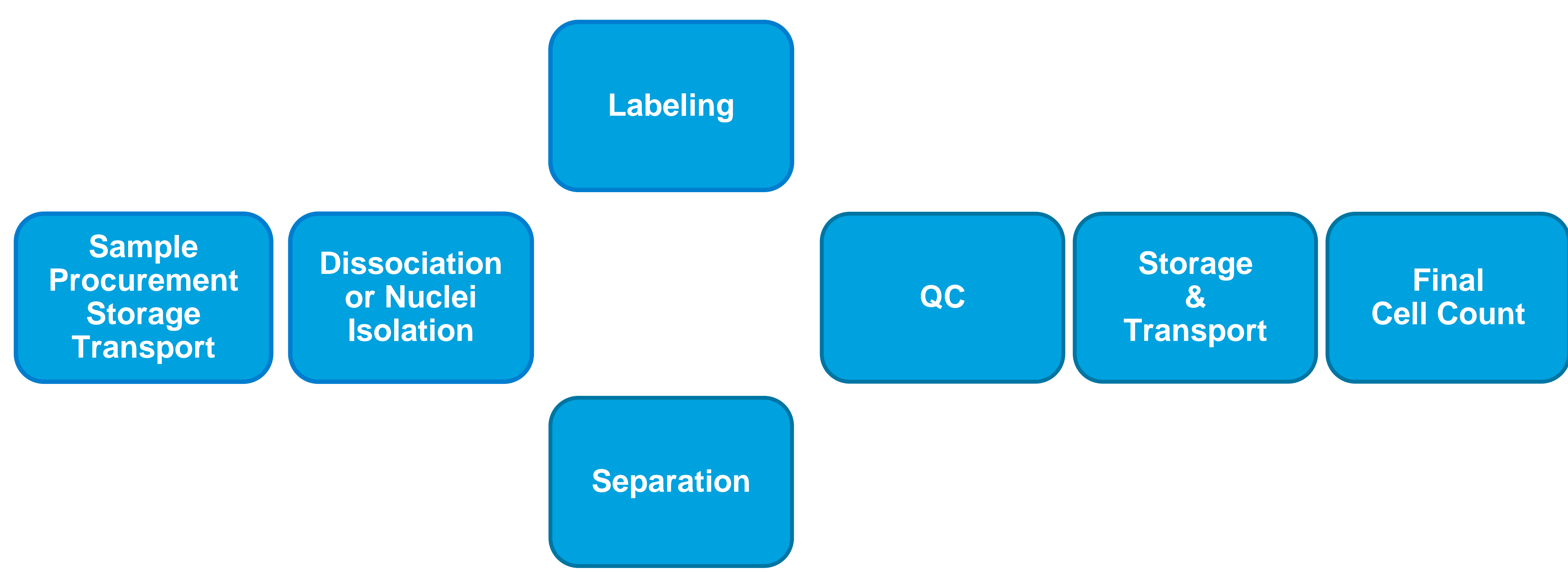
Single Cell GEX sample



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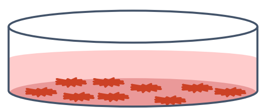
How many samples?

Depends on your question!

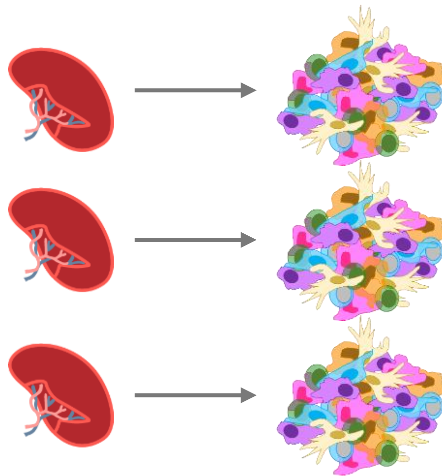
Experiment:
Cell line (homogeneous population) with X treatment

- Each cell could be seen as a replicate
- N > 1 is always best



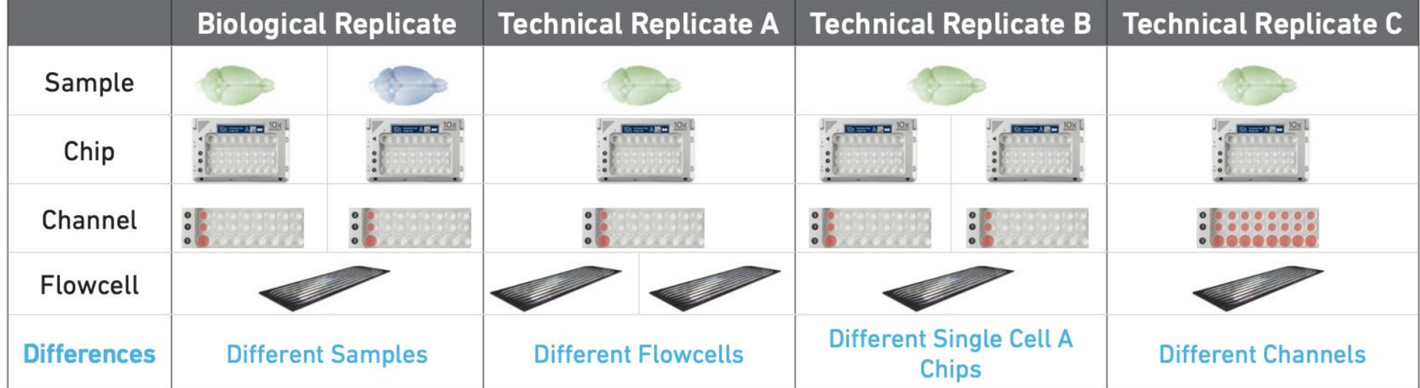
Experiment:
Heterogenous sample with X treatment

- More variability to account for
- The more variance you expect, the replicates you may want to run (N ≥ 3)



Biological & Technical Variation in Single Cell Gene Expression Experiments

- Biological and technical replicates evaluated
  - Across samples, flowcells, chips, channels



	Biological Replicate		Technical Replicate A		Technical Replicate B		Technical Replicate C
Sample							
Chip							
Channel							
Flowcell							
Differences	Different Samples		Different Flowcells		Different Single Cell A Chips		Different Channels

Biological variation exceeded all investigated sources of technical biases

CG000170 | Rev A

TECHNICAL NOTE

Biological & Technical Variation in Single Cell Gene Expression Experiments

Introduction

The Chromium Single Cell 3' v2 Reagent Kits protocol (Document CG00052) produces Single Cell 3' short-read sequencer compatible libraries. Technical and biological variation may be present in the experiment design, and may impact data interpretation. Potential sources of technical variation include running a sample on two separate microfluidic chips or at different well positions on the same chip, and/or technical variation introduced by sequencing libraries on separate Illumina flowcells or sequencing lanes. This Technical Note examines the potential sources of technical and biological variation and their effects on single cell gene expression. These factors need to be considered when designing an experiment to minimize bias and generate reliable single cell gene expression data.

Method

Single cell gene expression profiles from embryonic day E18 mouse (Document LIT000015) were compared. Briefly, combined cortex, hippocampus, and ventricular zone from C57EHCV E18 mice (BrainBits) were processed according to the Demonstrated Protocol – Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing (Document CG00055) to obtain single cell suspensions. Single Cell 3' v2 libraries were generated following the Single Cell 3' v2 Reagent Kits User Guide (Document CG00052). The libraries were sequenced on the Illumina HiSeq 4000 platform. Data were processed with Cell Ranger (v1.3.0) and the "cellranger-aggr" pipeline was applied to combine the individual datasets and normalize read depth across samples to approximately 18,500 reads per cell. The study design to investigate different sources of biological and technical variation is shown in Table 1. The presence of potential batch effects on gene expression introduced by the experimental conditions were assessed using tSNE clustering plots, R^2-values, and Pearson correlation between replicate samples (Figures 1-4).

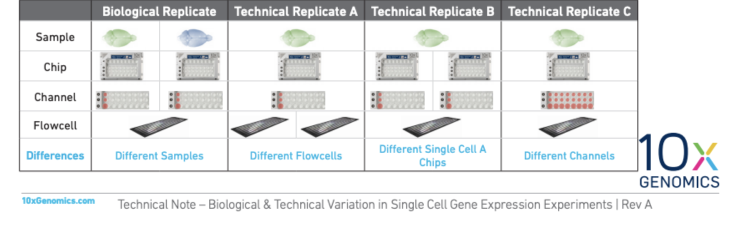
Results

Biological Replicates

To assess gene expression variation between biological replicates, neuronal tissue from two different mice, dissociated on different days (Replicates 1 & 2), were used to prepare single cell suspensions. Two libraries were generated by loading the cell suspensions on the same sample input well but on two separate microfluidic chips. Both libraries were sequenced on two separate lanes of the same Illumina flowcell (Table 1).

Gene expression profiles between the biological replicates, were highly correlated with a Pearson correlation of 0.968 and R^2 of 0.938 (Figure 1A). tSNE clustering showed a high degree of concordance of the cell distribution between the two biological replicates (Figure 1B). In addition, expression signatures for known neuronal marker genes were similarly distributed across the cell clusters in the tSNE plots (Figure 1C).

Table 1: Study design to investigate different sources of technical variation.



	Biological Replicate		Technical Replicate A		Technical Replicate B		Technical Replicate C
Sample							
Chip							
Channel							
Flowcell							
Differences	Different Samples		Different Flowcells		Different Single Cell A Chips		Different Channels

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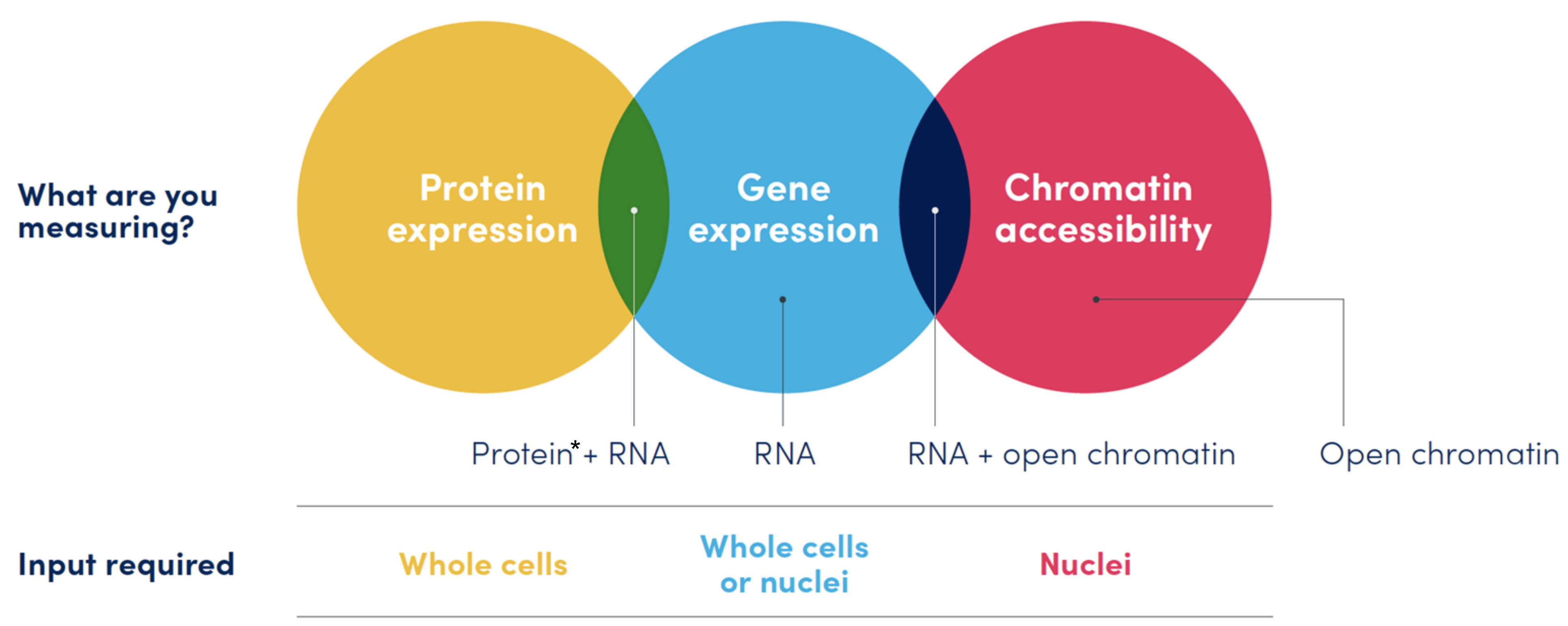
Different assays require different input materials

Consider your experimental goals



*Cell Surface protein

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Sample procurement, storage, transport

ATAC	GEX
Cultured cells, PBMC *Fresh and cryopreserved *10x protocols work as-is	Cultured cells, PBMC *Fresh and cryopreserved *10x protocols work as-is
Fresh tissue *Optimization	Fresh tissue *Significant optimization
Snap-frozen tissue *Optimization	Snap-frozen tissue *Nuclei will be generated *Technically challenging *Significant optimization
Fixation? *Not tested	Fixation? *Methanol is possible, but we prefer cryopreservation

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Gene expression extensions – Additional considerations

	Gene Expression, *3' or Immune Profiling (5')*	TCR or Ig Target Enrichment, *Immune Profiling*	CRISPR Screening, *3' Kit*	Cell Surface Protein, *3' or 5'*. Antigen, *5' Kit*
Fresh cells	✓	✓	✓	✓
Fresh tissue	✓	✓	✓	✓
Cryopreserved cells	✓	✓	✓	✓
Snap-frozen tissue (nuclei)	✓	Very high risk ✗	Not compatible ✗	✗
Methanol-fixed cells	✓	✗	✗	✗

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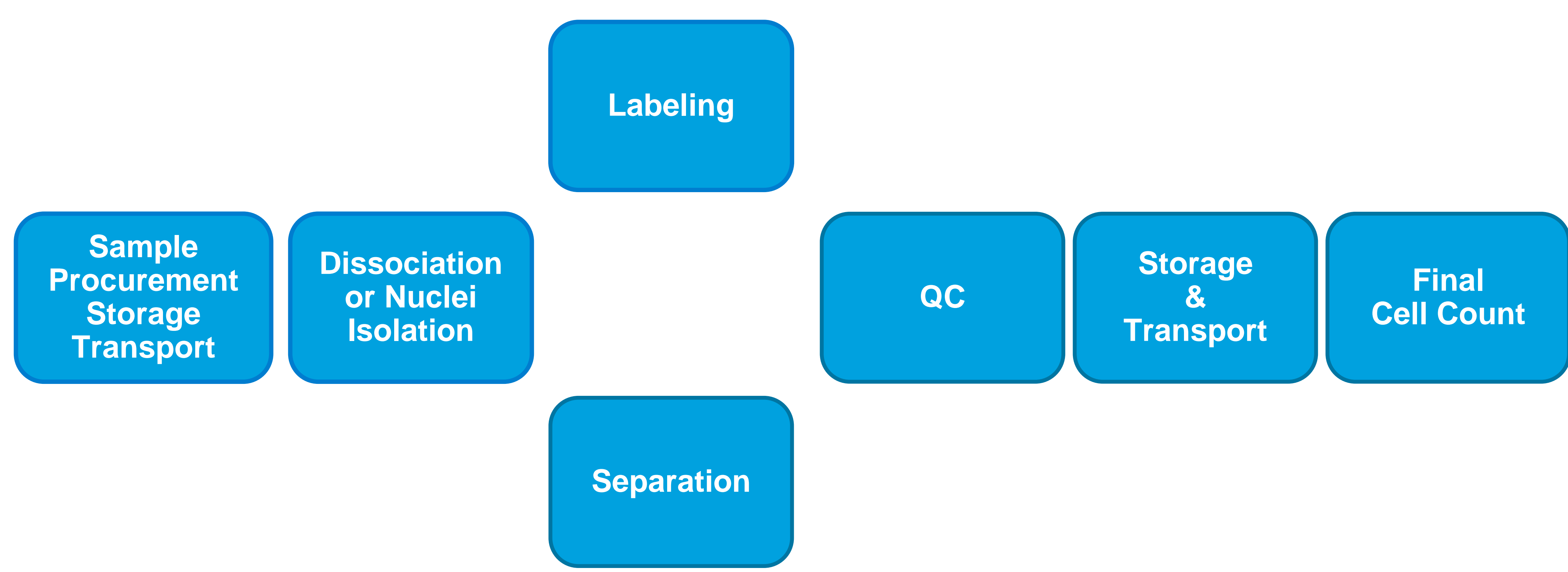
Single Cell GEX sample



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Cells vs nuclei

	Cells	Nuclei
Assay compatibility	• All gene expression and immune profiling solutions	• All gene expression and epigenomics assays, no VDJ or cell surface protein
Sample type	• Requires fresh tissue that can be easily dissociated	• Good for flash-frozen tissue or hard to dissociate tissue • Required input for epigenomics assays
Analytes obtained	• Spliced mRNA and lots of it • Cell surface proteins	• Unspliced RNAs with lots of introns • Chromatin (epigenomics assays) • No cell surface proteins, no cell membrane
Storage	• Cell suspensions can be cryopreserved • Fresh tissue must be dissociated	• Frozen tissue can be transported and stored
Protocols available	• Lots of dissociation protocols, some optimization may be required	• Protocols available, optimization required

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Why Use Nuclei?

- When cells are large and exceed the limits for the microfluidic chip
  - Hepatocytes
  - Neurons
- When cells are of a challenging shape
  - Cardiomyocytes
- When cells are difficult to get into a single cell suspension
  - Sample contains a lot of debris
  - Neurons are highly interconnected and may not efficiently dissociate into single cells after enzymatic treatment
  - Dissociation-resistant tissue samples such as complex tissues/ organs where nuclei (but not whole intact cells) can be isolated

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Why Use Nuclei?

- *Possible* solution for archival (cryopreserved) or damaged samples in which the cell wall is breaking down
  - Laser capture microdissection will physically damage whole cells (cell wall)
  - Nuclei isolation will not rescue damaged cells that are already dying or undergoing apoptosis
- *Possible* solution for experiments aiming to reveal molecular genetic regulatory mechanisms specific to the nucleus
- Sample types that have a cell wall that does not lyse in our assay
  - Various plants, yeast

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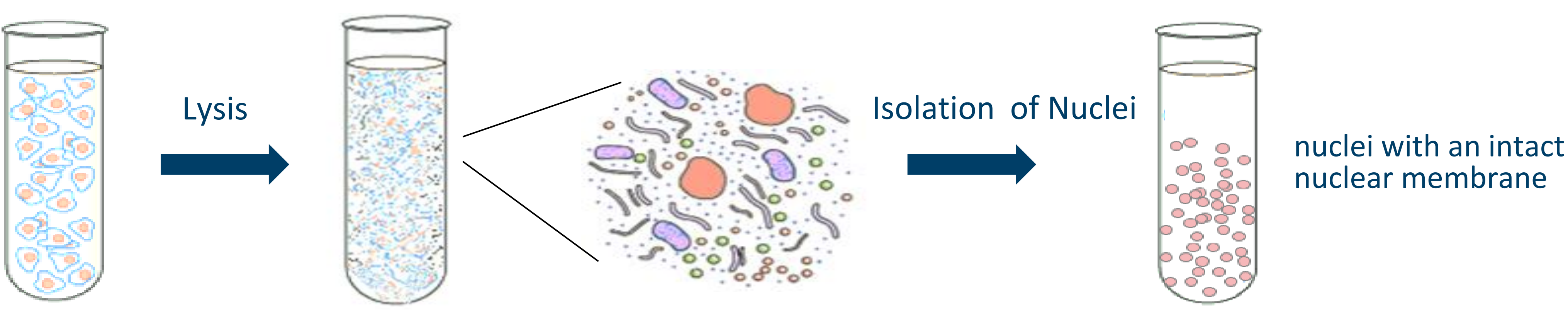
Basics Of Nuclei Isolation

1. Isolation of nuclei from cells or tissues requires disrupting the structural integrity of the mammalian cellular membrane.
   – Detergent-based lysis solubilizes the hydrophobic membrane proteins which releases the cell's cytoplasmic contents *(i.e.* organelles, cytoplasmic nucleic acids, ambient RNA, proteases) without impacting the integrity of the nuclear membrane.

2. Low speed centrifugation and repeated washing steps separate the nuclei from the cell homogenate and debris



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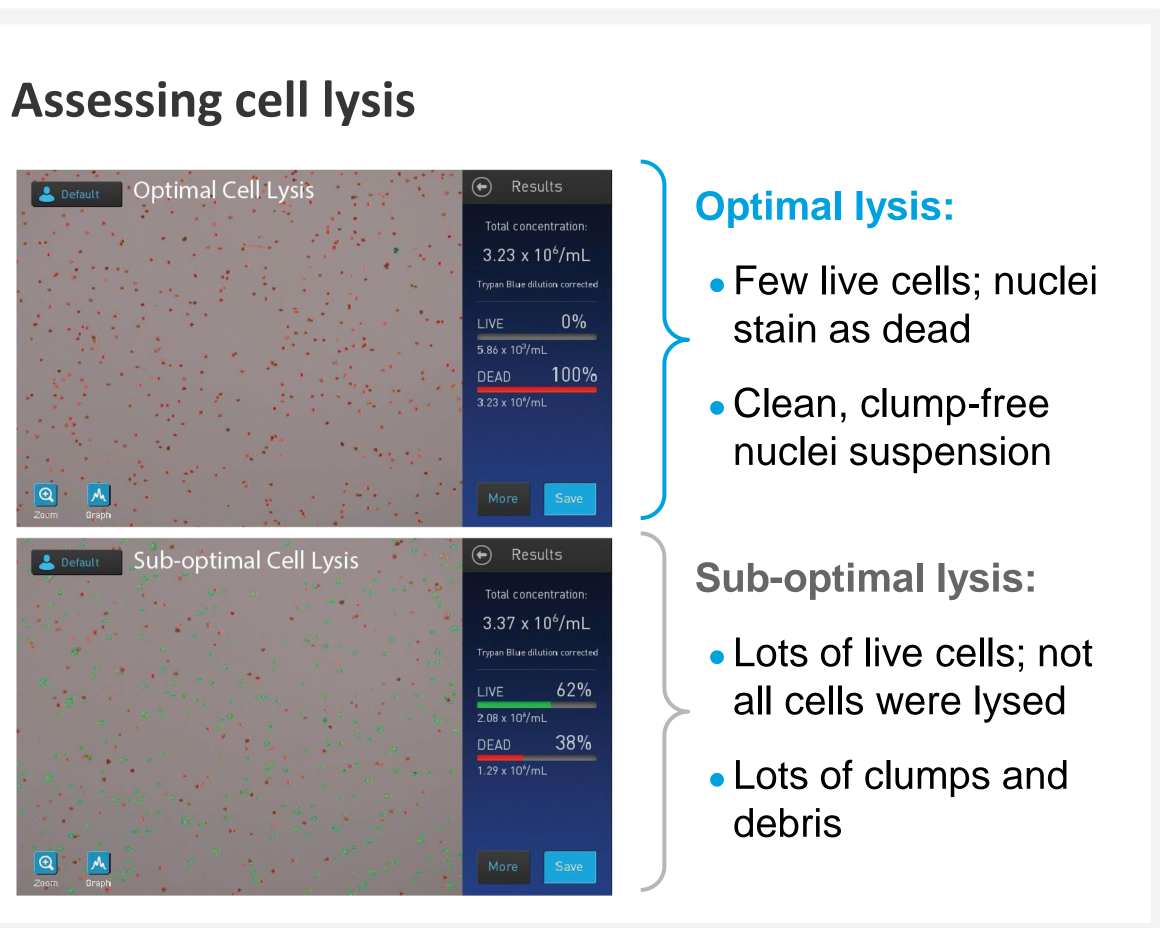
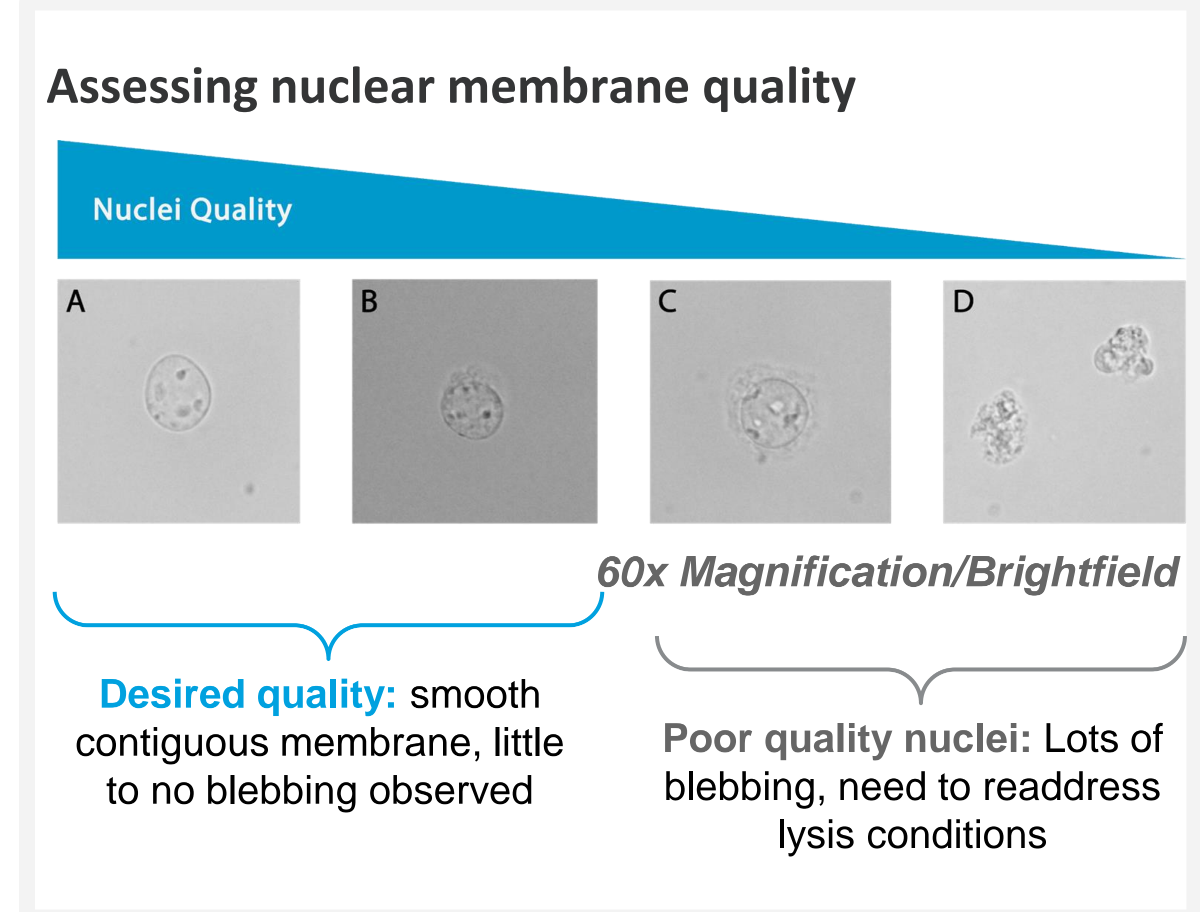
What about nuclei quality?

Different than cells





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Sample types

Dissociating cultured cell lines, primary cells, and solid tissue

Cultured cell lines

- Enzymatic dissociation
  - Collagenase (e.g. differentiated cells in culture)
  - Dispase (e.g. differentiated cells in culture)
  - Accutase (e.g. iPSCs, hESCs)
  - Accumax (e.g. iPSCs, hESCs)
  - Trypsin-EDTA (e.g. fibroblasts)
  - TrypLE (e.g. fibroblasts)

Primary cells

- FAC-sorted
- Magnetic-bead purified (e.g. Miltenyi Microbeads)
- Gradient-purified (e.g. Percoll, Optiprep, Apheresis)

Solid tissue

- Best practices in 10x Genomics DP for mouse neuronal cells
- Refer to publications (Worthington database)
- Enzymatic dissociation
  - Papain (neurons), Collagenase, Dispase, Accutase, Accumax, Trypsin-EDTA
- Mechanic dissociation (less frequent)
  - Cut, pipette, centrifugal mill

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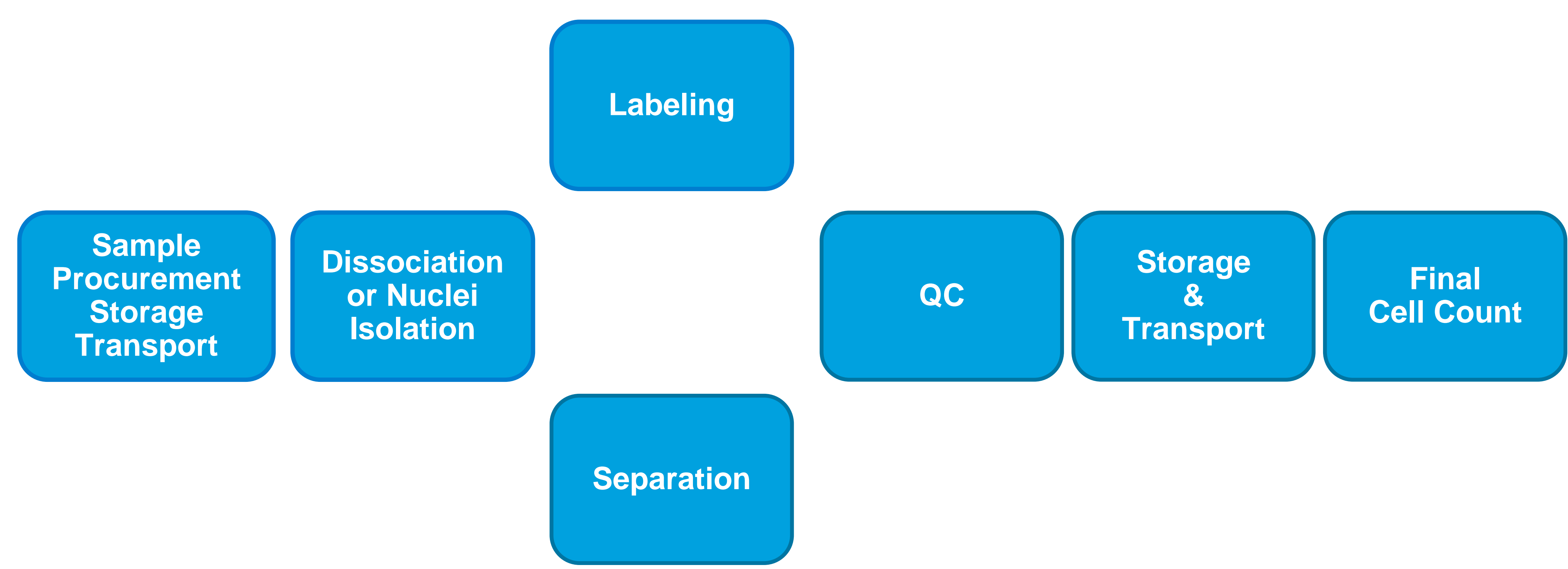
Single Cell GEX sample



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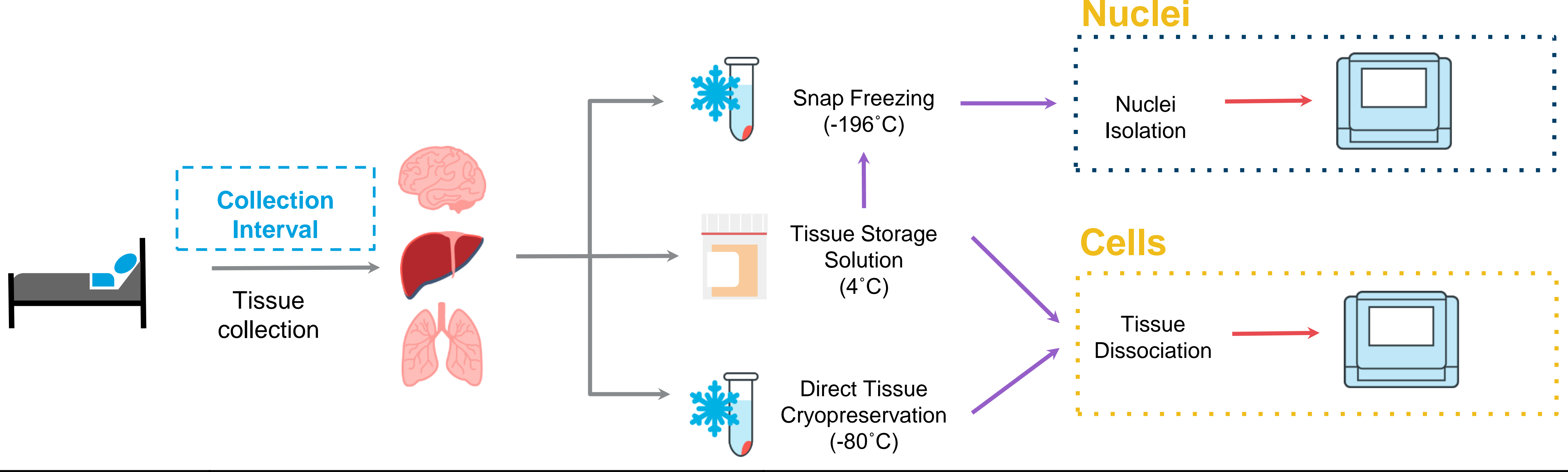
Methods for tissue collection and transport



	When to use	Considerations
Tissue storage solution	• Short term storage <72hrs • Only wet ice/cold packs are available for shipping	• Variability across tissue types—pilot experiment is suggested • Additional cleanup may be necessary
Flash-freeze whole tissue	• Long term storage >72hrs • Dry ice is available for shipping	• Only nuclei can be isolate • No cell surface proteins or VDJ transcripts capture
Cryopreservation in media	• Long term storage >72hrs • Dry ice is available for shipping	• May preserve cell surface proteins • Variability across tissue types—pilot experiment suggested

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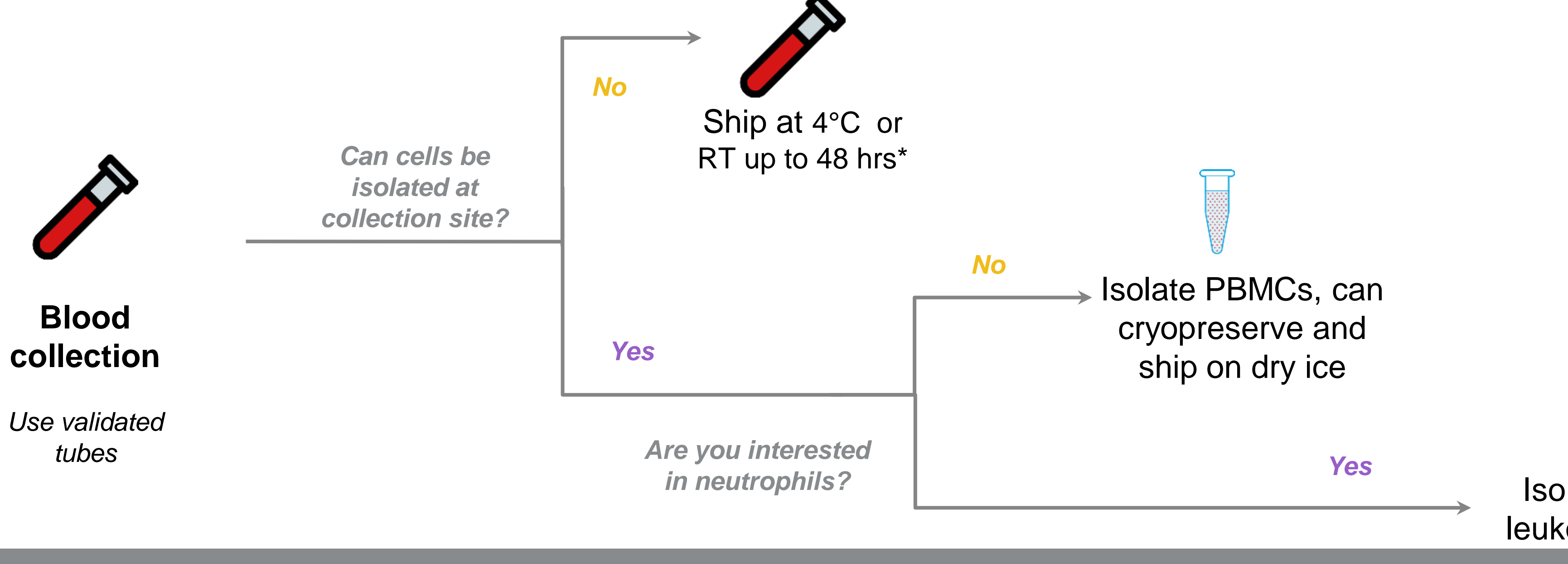
How do I collect, ship and store blood samples?



*4°C storage minimizes cell stress and T cell apoptosis

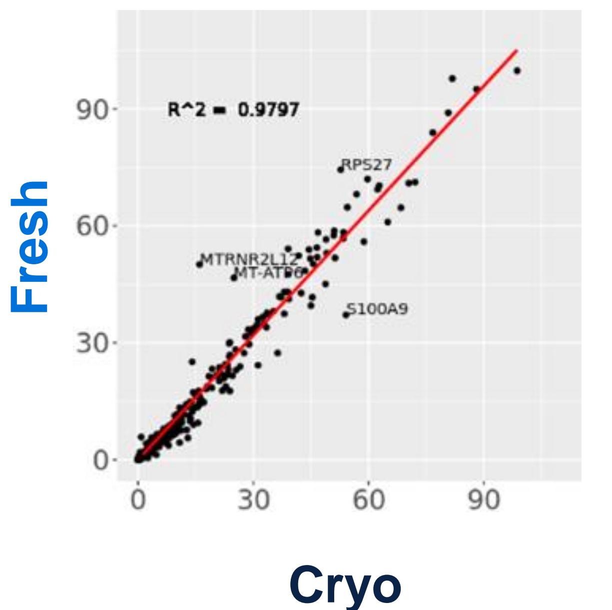
Interested in:	Collection method	Storage Temp and Time	When to use:	Considerations
PBMCs	Vacutainer or CPT tube	4°C up to 48 hrs	• Ice or cold pack is available at collection site • Interested in preserving T cell biology	• Do not let tubes touch cold pack or ice to avoid freezing—make sure tubes are well insulated in styrofoam rack
PBMCs	Vacutainer or CPT tube	RT up to 48 hrs	• Ice or cold pack is not available at collection site • Interested in preserving plasma cells • Ok with some cell stress genes—may be corrected bioinformatically	• Cell stress is observed at RT—may be corrected bioinformatically • Signs of T cell apoptosis appear
PBMCs	Frozen PBMCs in media+DMSO	Liquid nitrogen, long term	• Able to process blood samples at collection site and isolate PBMCs • Access to dry ice for shipping	• Requires skilled tech at collection site • Not interested in neutrophils
Neutrophils	Vacutainer tube only	RT < 24hrs	• Looking to preserve neutrophils	• Process as soon as possible • Work at RT, do not put sample on ice

Cells can be cryopreserved and shipped

Protocols available on the 10x Support Site

PBMCs

- High correlation between fresh and cryopreserved cells is observed



CG00039 • Rev D

DEMONSTRATED PROTOCOL

Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing

Overview

This protocol outlines cryopreservation and thawing of human peripheral blood mononuclear cells (PBMCs) for use with 10x Genomics Single Cell protocols. While this Demonstrated Protocol is specific to PBMCs, the protocol may be used as a basis for handling other primary cells in preparation for use in the 10x Genomics Single Cell protocols.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (Document CG000091) for determining accurate cell counts.

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.

Cell Sourcing

This protocol was demonstrated using fresh human PBMCs from AllCells.

Preparation-Buffers

Cryopreservation	
Media/Buffers	**Composition**
Resuspension Medium (maintain at 4°C)	40% FBS in cell culture media (e.g., IMDM/RPMI)
2X Freezing Medium (maintain at 4°C)	30% DMSO in cell culture media (e.g., IMDM/RPMI) containing 40% FBS
Thawing & Resuspension	
Media/Buffers	**Composition**
Complete Growth Medium (maintain at 37°C)	10% FBS in cell culture media (e.g., IMDM/RPMI)
PBS + 0.04% BSA (maintain at room temperature)	

Specific Reagents & Consumables

Vendor	Item	Part Number
Thermo Fisher Scientific	Gibco IMDM	12440-053
	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616

10xGenomics.com Demonstrated Protocol – Fresh Frozen Human PBMCs for S

CG000233 • Rev A

DEMONSTRATED PROTOCOL

Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing

CG00014 • Rev F

DEMONSTRATED PROTOCOL

Fresh Frozen Human-Mouse Cell Line Mixtures for Single Cell RNA Sequencing

Overview

The ability of the 10x Genomics Single Cell Solutions to partition single cells in a heterogeneous population can be verified by profiling a mixture of cells from two different species. Ideally, all sequence reads from a single Gel Bead-in-emulsion (GEM) will be unambiguously mapped to the transcriptome of only one of the two species. The fraction of GEMs containing a mixture of human and mouse transcripts is used to infer doublet rates (see Appendix).

10x Genomics routinely uses a 1:1 mixture of human and mouse cells to validate the technical performance of the 10x Genomics Single Cell Solutions. This Demonstrated Protocol outlines cryopreservation and thawing of 1:1 mixtures of human and mouse cells in preparation for use in 10x Genomics Single Cell protocols.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (Document CG000091) for determining accurate cell counts.

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.

Cell Sourcing

This protocol was demonstrated by preparing a 1:1 mixture of 293T/17 and NIH/3T3 cells.

Cell Type Used	Species	Supplier
293T/17 (CRL-11268)	Human	ATCC
NIH/3T3 (CRL-1658)	Mouse	ATCC

NIH/3T3 cells will often be the limiting reagent as they grow to a lower density than the 293T/17 cells. Approximately 5 times more NIH/3T3 culture flasks are required to achieve balanced cell numbers (e.g., 4 confluent T75 flasks of 293T/17s will require ~20 nearly confluent T75 flasks of NIH/3T3 cells).

Preparation - Buffers

Cryopreservation (maintain at 4°C)	
Media	**Composition**
Cryopreservation Medium	20% FBS + 10% DMSO in cell culture media (e.g., DMEM)
Thawing (maintain at room temperature)	
PBS + 0.04% BSA	

Specific Reagents & Consumables

Vendor	Item	Part Number
Thermo Fisher Scientific	Gibco DMEM	11965-092
	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Trypan Blue Stain (0.4%)	T10282
	Nunc Biobanking & Cell Culture Cryogenic Tubes, 1.8 ml	368632
	Countess II FL Automated Cell Counter	AMQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
Fisher Scientific	Dimethyl Sulfoxide (DMSO), for molecular biology	ICN19141880
Millipore-Sigma	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin (alternative to Thermo Fisher product)	SRE0036
Miltenyi	MACS SmartStrainers, 30 µm	130-098-458
Eppendorf	DNA LoBind Tubes, 2.0 ml	022431048
VWR	Seradigm Premium Grade Fetal Bovine Serum (FBS)	97068-085
	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 50 ml	82018-050
Biocision	CoolCell FTS30 Cell Freezing Container	BSC-170
Corning	Phosphate-Buffered Saline without Calcium & Magnesium	21-040-CV

10x GENOMICS

10xGenomics.com Demonstrated Protocol – Fresh Frozen Human-Mouse Cell Line Mixtures for Single Cell RNA Sequencing • Rev F

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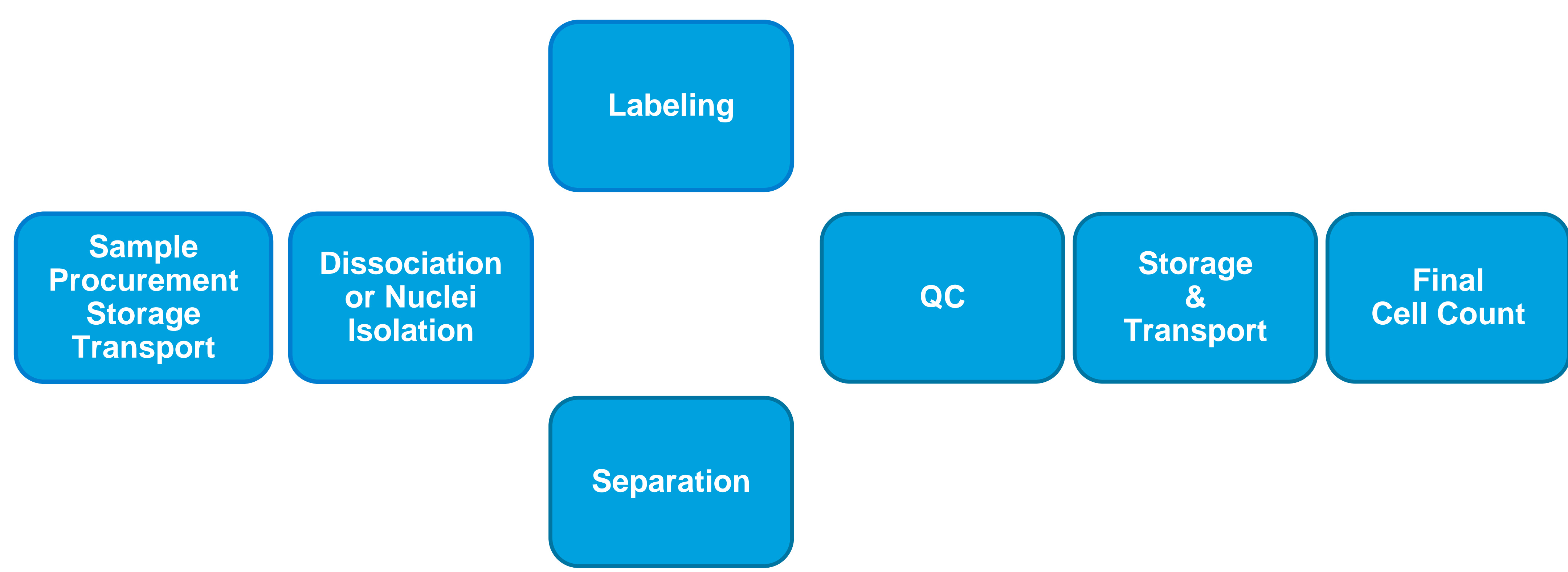
Single Cell GEX sample



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How to choose a dissociation method

Tissue

Frozen

Fresh

Faster protocol

Cell enrichment
GEX Feature Barcode

Mechanical dissagregation

Enzymatic dissociation

Nuclei suspension
ATAC, GEX*

ATAC

Cell suspension
GEX

**Not Compatible: T Cell/B Cell Target Enrichment, Cell Surface Protein, Antigen, CRISPR Screening*

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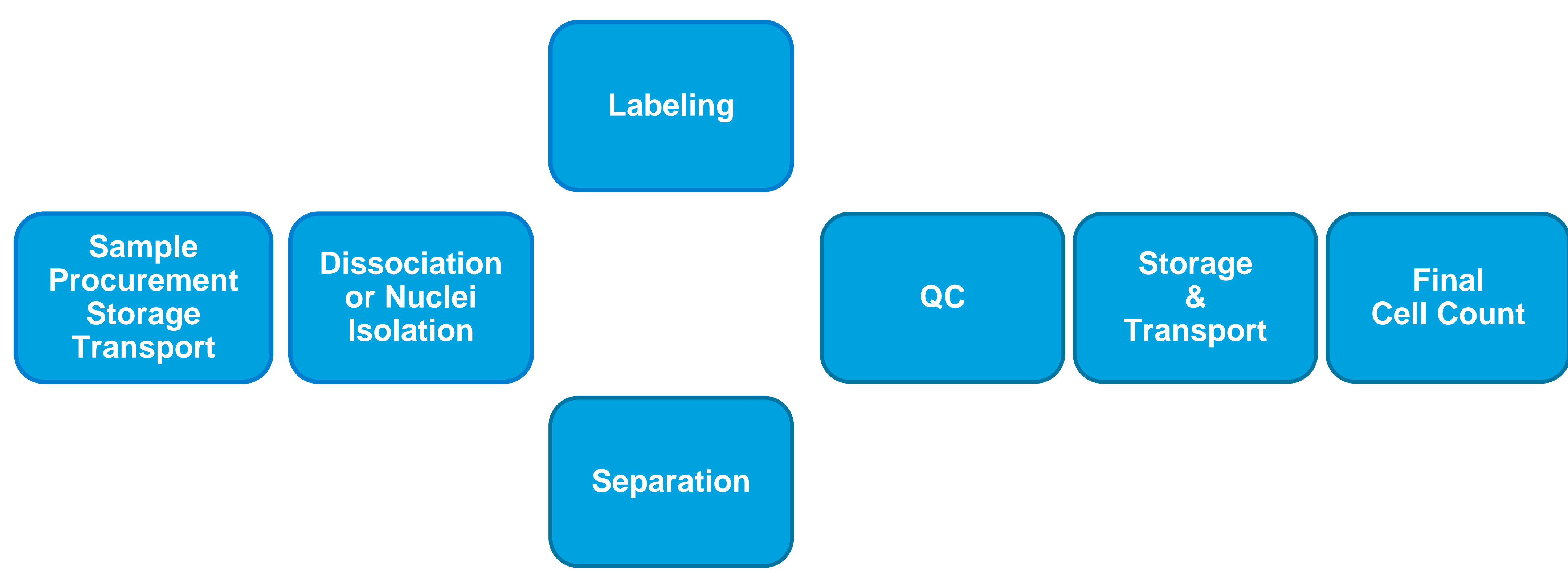
Single Cell GEX sample



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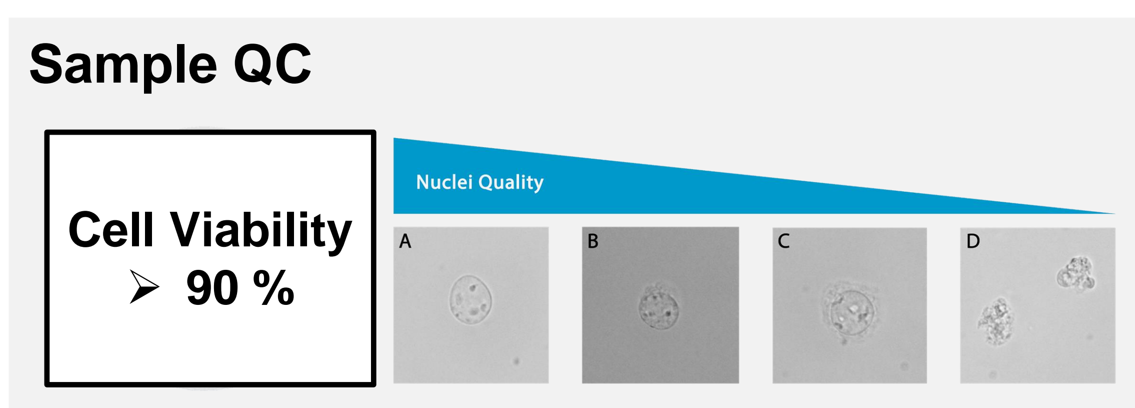
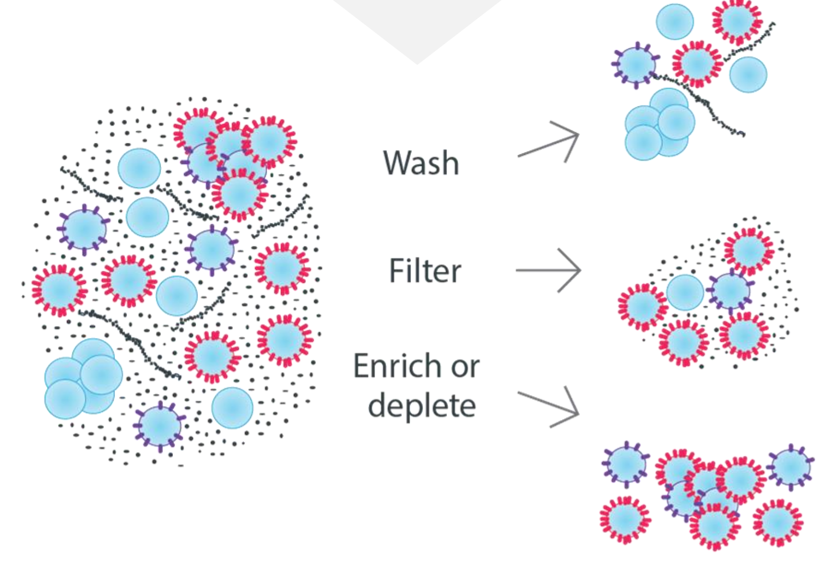
What if my sample doesn't meet the criteria?

After cell or nuclei isolation, sample separation or enrichment may be desired

- Remove dead cells/enrich for live cells
- Clean up cellular debris/Enrich for cells or nuclei
- Remove ambient RNA
- Enrich or deplete for a specific cell type
  - Only interested in a specific cell type—looking for subpopulations
  - Cell type of interest only makes up for a small proportion of total cell population
  - Large number of "undesired" cells in total population (i.e. granulocytes, xenografts)





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Separation

Separate intact cells and nuclei from

- Aggregates/clumps
- Debris
- Free-floating mRNA
- Dead Cells
- Enrichment/Depletion

Challenges with separation

- Samples are fragile
- Physical stress
- Buffers
- Time
- Yield

Want the minimum manipulation required to deliver application performance.

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Debris removal

Filtering cell suspensions

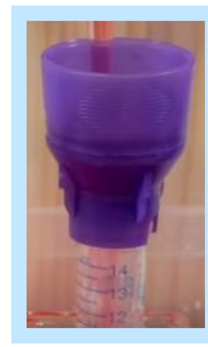
- Strainers with appropriate pore sizes should be used to allow cells to pass through the filter while cellular debris and aggregates are retained

Flowmi™ Cell Strainer (Bel-Art Products)

Pro: required sample volume is low

Con: sample concentration is decreased by 20 to 40% after straining

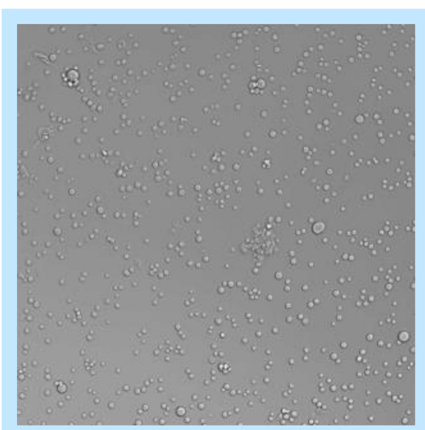
MACS® SmartStrainer (Miltenyi Biotec)



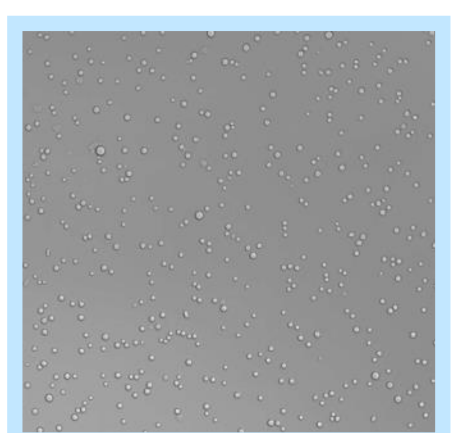
Pro: minimal change in sample concentration

Con: required minimal volume is ~ 500 µL + loss of sample by 100 -150 µL

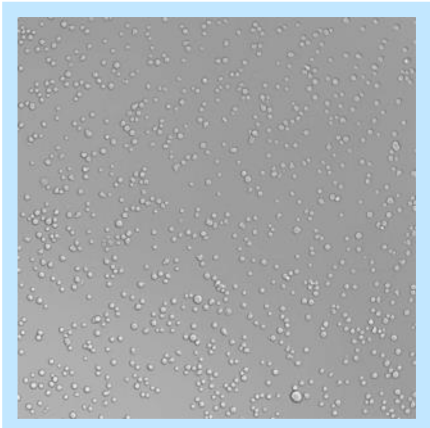
Unfiltered Jurkat cells



Jurkat cells filtered with FlowMi (40 µm)



Jurkat cells filtered with MACS (30 µm)



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Basic Methods for Sample Separation

Method	Thorough centrifugation (e.g. 3x with PBS + 0.04% BSA)	Gentle centrifugation (e.g. 1x with media)	Magnetic beads	Density Gradient	FACS
10x Protocol Example	PBMC (CG000039)	Cell Prep Guide (CG000053)	Dead Cell Removal (CG000093)	Nuclei Isolation (CG000124)	Customer Developed Protocol (Martelotto)
Sample Size	Abundant	Limited	Abundant	Abundant	Limited
Benefits	Thorough	**Gentle**	Specific, easily accessible, scalable	Removes Debris	**Versatile, quick**
Possible Challenges	Yield, Harsh	Less thorough	Yield	Yield, Harsh, Time	Expensive, Harsh

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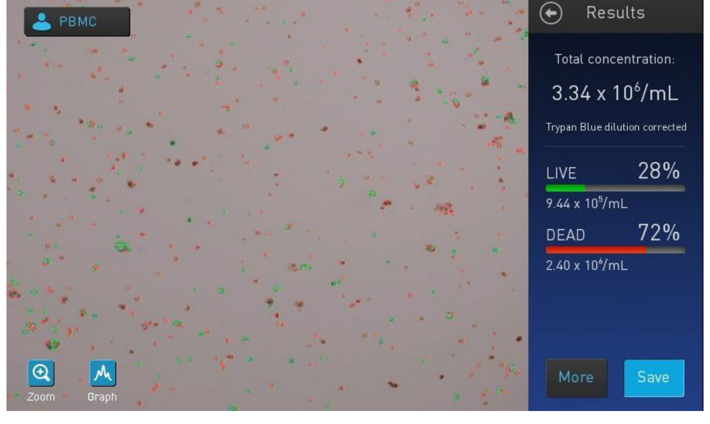
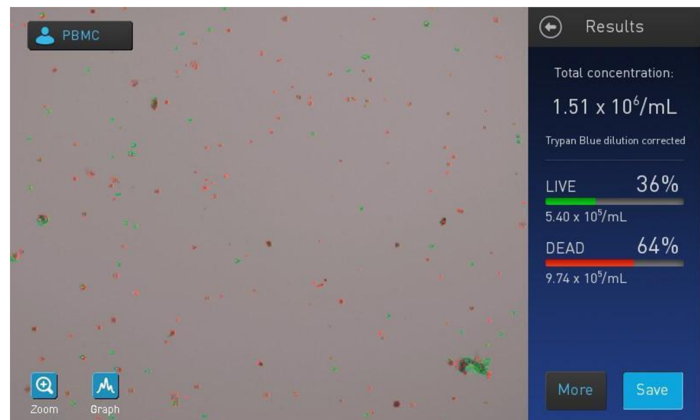
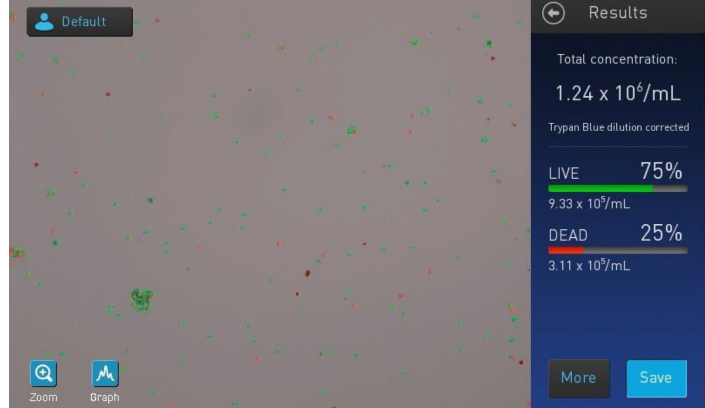
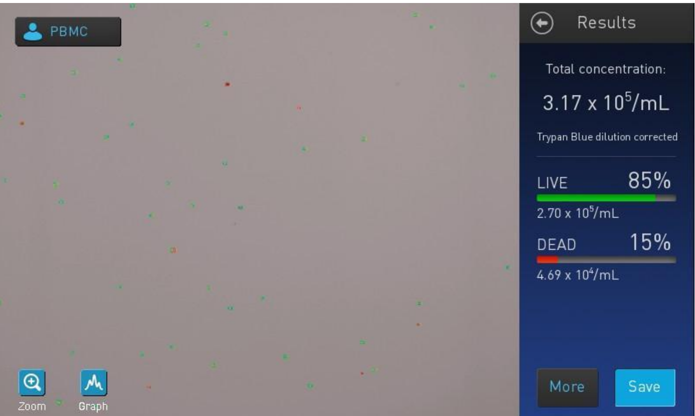
Low Speed Centrifugation Can Remove Dead Cells

	Clear Cell Renal Carcinoma	Colorectal Cancer
Before		
After filtering and dead cell removal spins		

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Recommendations for FACs

- Pre-sorting:

- Ensure that the sheath fluid and collection buffer is compatible with the 10x workflow--importantly, it does not contain EDTA or excessive amounts of Mg2+ (should be less than 0.1 mM EDTA and less than 3 mM Mg2+).

- Include a dead cell marker in the sorting scheme to exclude all dead cells.

- During sorting:

- Use a larger flow nozzle (such as a 100um nozzle, if using a sorter with a nozzle), or use lower pressure when sorting your cells. Using lower pressure during the sorting process will help preserve cell health and viability.

- Post-sorting:

- Always count your cells after sorting. Counts from cell sorters tend to be inaccurate and highly variable depending on the sorter, so we always recommend recounting before loading onto the 10x chip.

- **What are the best practices for flow sorting cells for 10x Genomics assays?**

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Labeling: Feature Barcoding Technology

Cell Surface Protein

CG000149 • Rev C

DEMONSTRATED PROTOCOL

Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols
with Feature Barcode technology

Overview

Cell surface proteins can be labeled using a specific protein binding molecule, such as an antibody conjugated to a Feature Barcode oligonucleotide. This protocol provides guidance for antibody-oligonucleotide conjugation and outlines cell surface protein labeling for use with Single Cell RNA sequencing protocols with Feature Barcode technology for cell surface protein. This protocol also provides guidance for enriching labeled cells using Fluorescence Activated Cell Sorting (FACS).

Consult Demonstrated Protocol Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols with Feature Barcode technology (CG000391) for guidance on cell surface protein and Cell Multiplexing Oligo labeling.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines on Accurate Target Cell Counts (Document CG000091) for determining accurate cell counts.

For Cell Surface Protein Labeling		
Vendor	Item	Part Number
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TotalSeq™ Antibody-Oligonucleotide Conjugates*	-
	Cell Staining Buffer	420201
	Antibodies (Fluorophore)†	-
	If using FACS for enriching labeled cells	
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Trypan Blue Stain (0.4%)	T10282
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

Antigen Specificity

CG000203 • Rev C

DEMONSTRATED PROTOCOL

Cell Labeling with dCODE™ Dextramer® Reagents for Single Cell RNA Sequencing Protocols
with Feature Barcode technology

Overview

Multimeric MHC peptide complexes, such as dCODE™ Dextramer® reagents, bind to T-cell receptors (TCRs) with high affinity, which can enable detection of TCR antigen specificity. This protocol provides guidance for labeling cells with dCODE™ Dextramer® reagents (dCODE™ Dextramer® MHC-Feature Barcode oligonucleotide conjugate) along with TotalSeq-C antibody-oligonucleotide conjugates. This document also provides guidance for enriching dCODE™ Dextramer®+ T cells by Fluorescence Activated Cell Sorting (FACS). These dCODE™ Dextramer® reagents and TotalSeq-C antibody-oligonucleotide conjugate labeled cells can be used for generating Chromium Single Cell libraries as described in the User Guide for Chromium Single Cell Immune Profiling Solution with Feature Barcode technology (CG000186, CG000208, CG000330, and CG000424).

To obtain more accurate cell calling of the analysis, cells should be labeled with both antibody-oligonucleotide conjugates and dCODE™ Dextramer® reagents. dCODE™ Dextramer® reagent only type of analyses are not supported currently.

Additional Guidance

Specific Reagents & Consumables

Vendor	Item	Part Number
Immudex	dCODE™ Dextramer® Reagents	-
	dCODE™ Dextramer® Reagents Controls	-
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TotalSeq™-C Antibody-Oligonucleotide Conjugate (see Appendix for a list of recommended antibody-oligonucleotide conjugates)	-
	Antibodies (Fluorophore)* If enriching dCODE™ Dextramer®+ cells through FACS	-
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616

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Summary

- ✓ Requires a fully dissociated, **single cell suspension.**
- ✓ Ideally, input cell suspensions should contain more than **90% viable cells**.
- ✓ the optimal input cell concentration is 700-1200 cells/µl

When our standard guidance isn't applicable:

- Treat cells gently and minimize decomposition
- Work quickly
- *Consider the benefits and drawbacks of every different technique*

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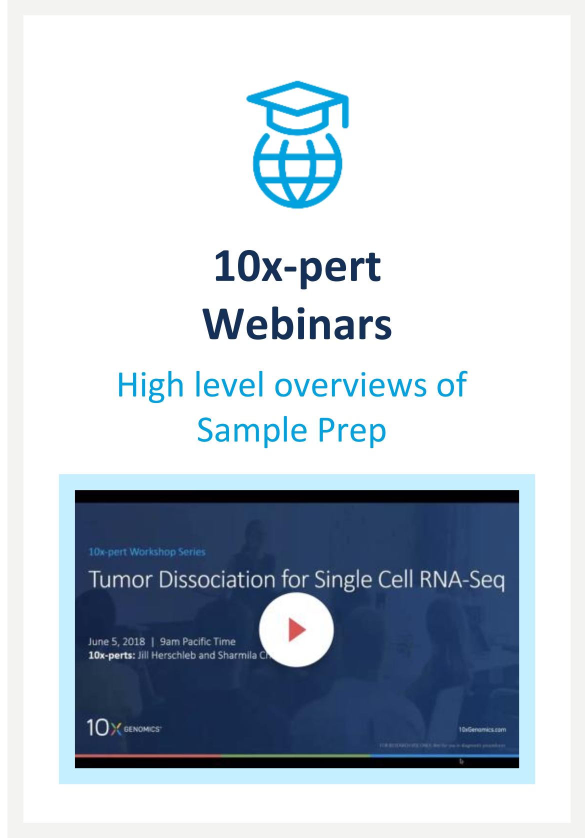
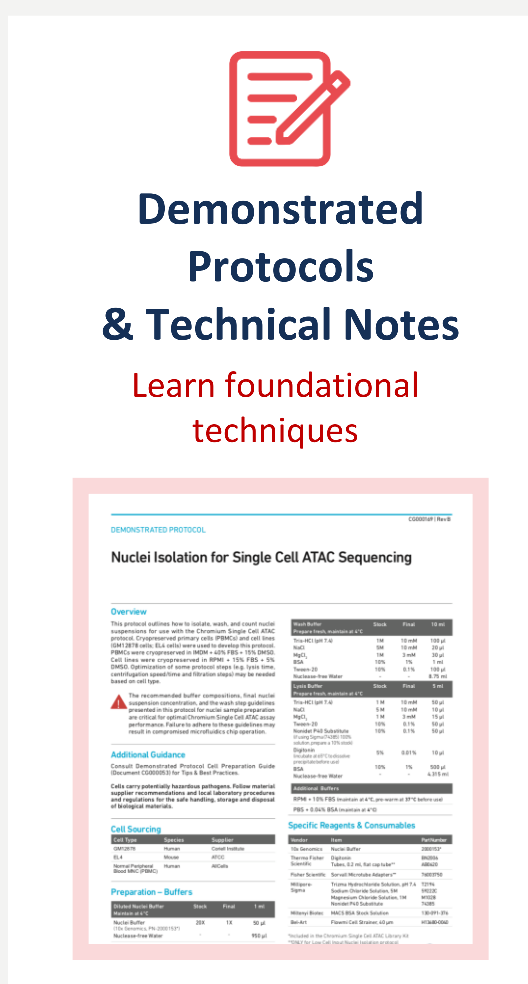
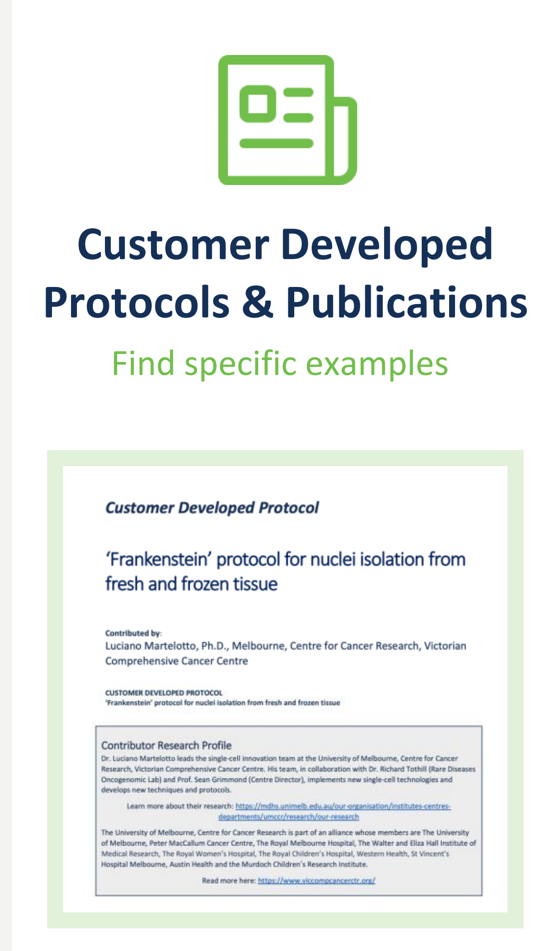
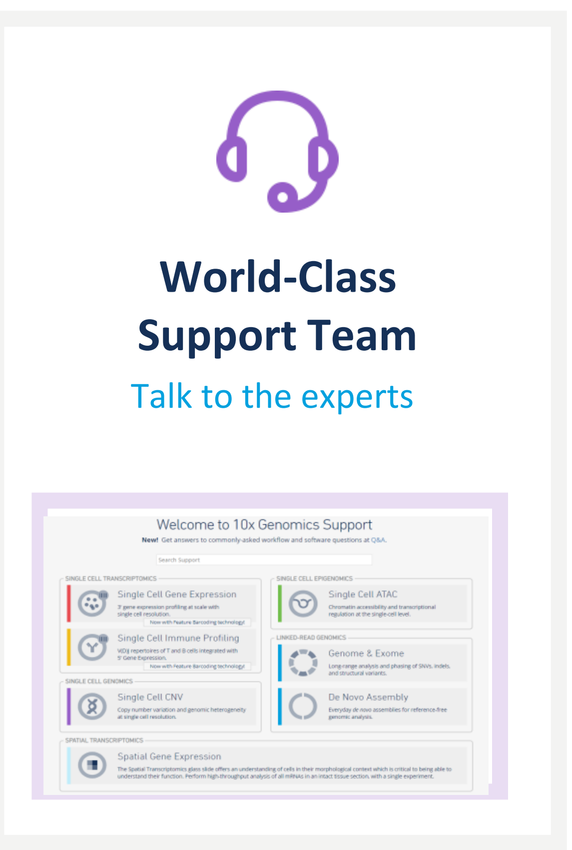
Sample Prep Support









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Thank You!

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