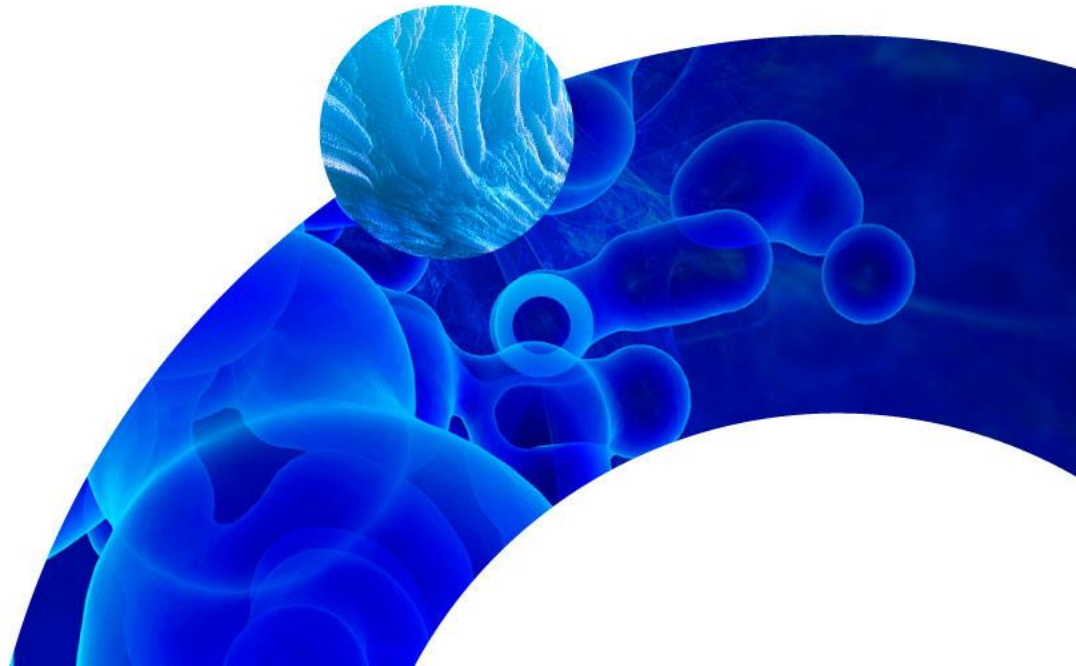
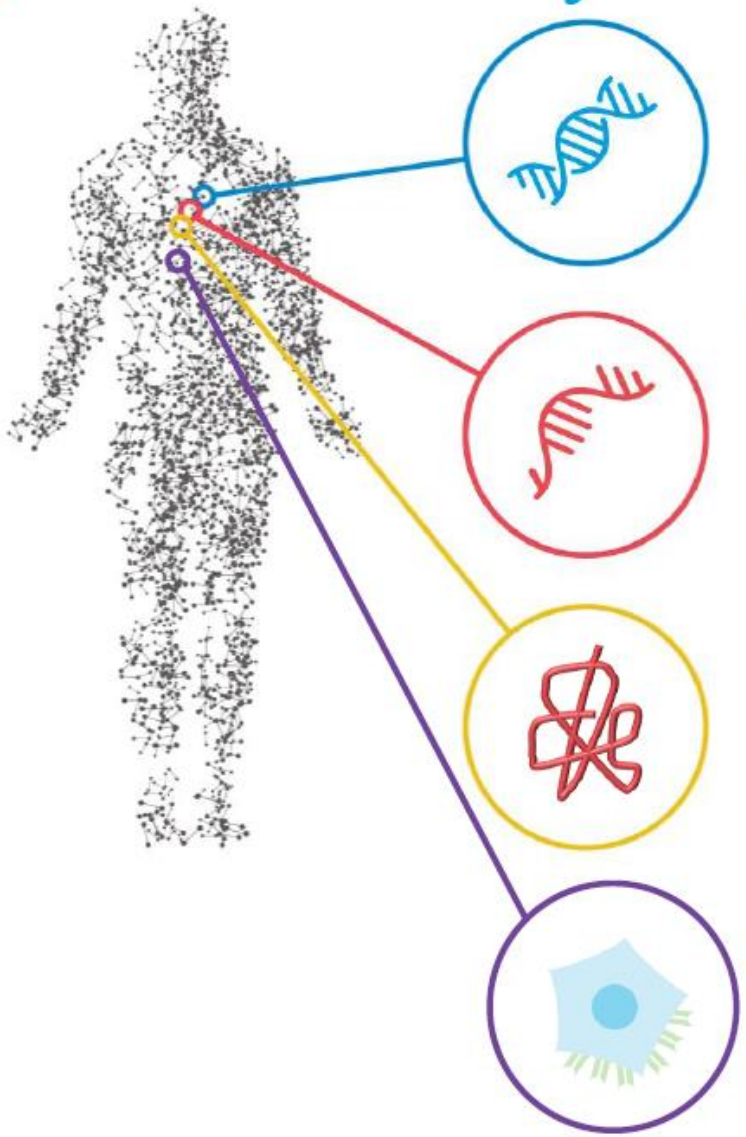
Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Workflow training



CG000214 Rev D

Biology is Immensely Complex



6 billion DNA bases in the genome

Genomic variants
Epigenetic regulations
Transcription regulations

Transcribed into over 50,000-300,000 mRNA molecules

Post-transcriptional regulations
Translational regulations

Translation into tens of thousands of proteins

Post-translational regulations
Protein activity regulations

~37.2 x 10^{12} cells

~ 200 different cell types
~20 different types of structures or organelles

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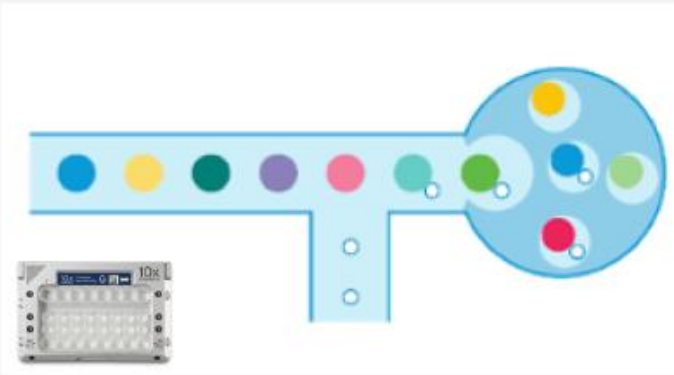
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10x Develops Tools to Resolve Complexity

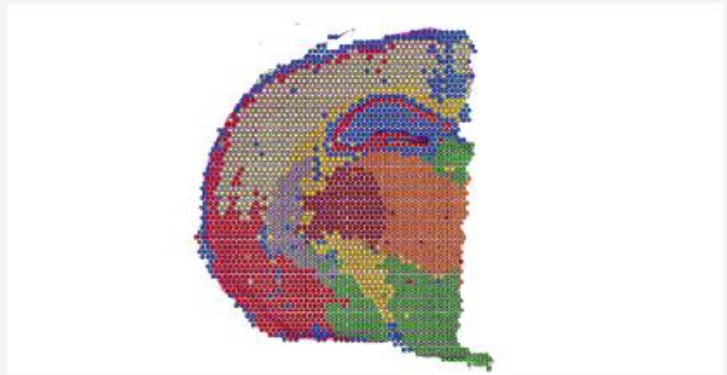
Instruments



Microfluidic Partitioning & Barcoding



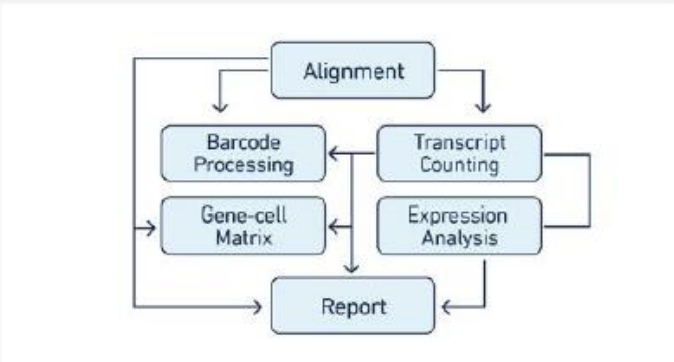
Spatial Barcoding



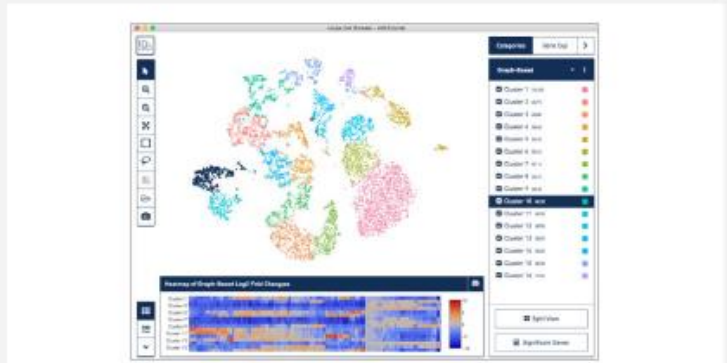
Novel Assays & Biochemistry



Analysis Software



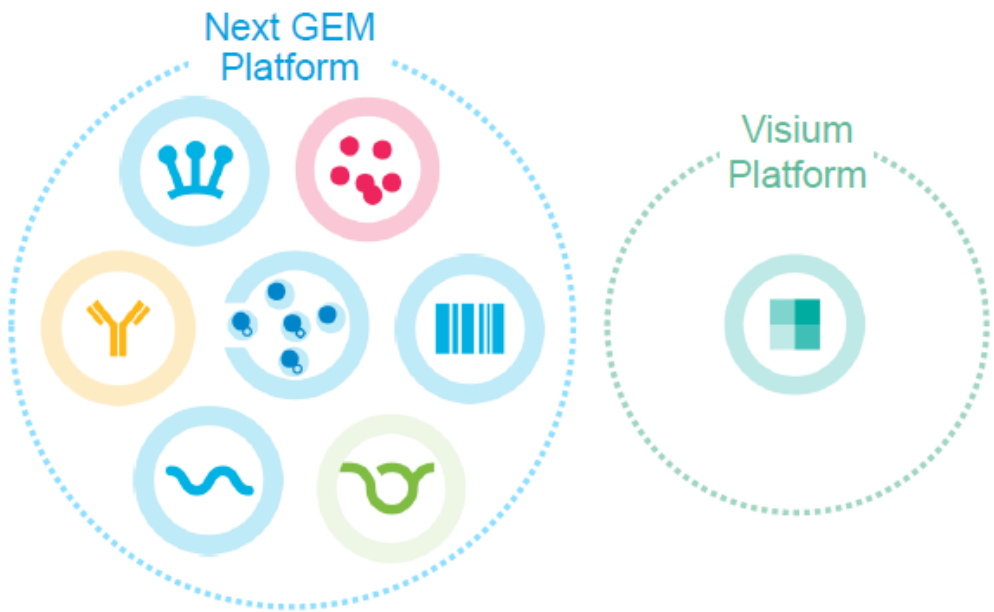
Visualization Software



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10x Genomics innovation engine



2015
- Linked-Read Genome Sequencing

2016
- Single Cell Gene Expression

2017
- High Definition Immunology

2018
- Single Cell CNV
- Single Cell ATAC
- (v3) Single Cell Gene Expression

2019
- Cell Surface Proteins
- CRISPR Profile
- Antigen Specificity

Two successful acquisitions

EPINOMICS

SPATIAL TRANSCRIPTOMICS

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Agenda

Chromium Platform

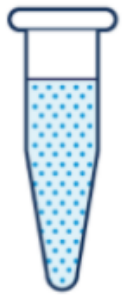


Visium Platform

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Chromium Single Cell Gene Expression Workflow

Input → **Library Creation** → **Sequence** → **Data Analysis and Visualization**

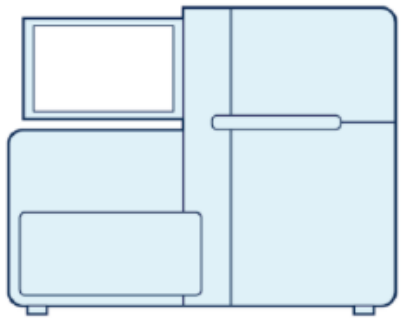


User-Supplied Cells

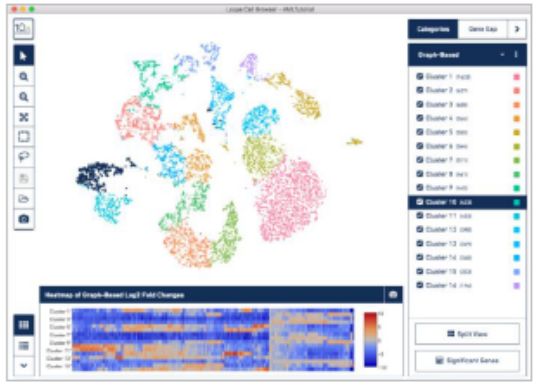


Chromium Controller and Single Cell Gene Expression Reagents

- Gel Bead and Library Reagents
- Feature Barcoding Library Kit
- Chip and Sample Indexes



Sequence



Cell Ranger Pipeline Loupe Cell Browser

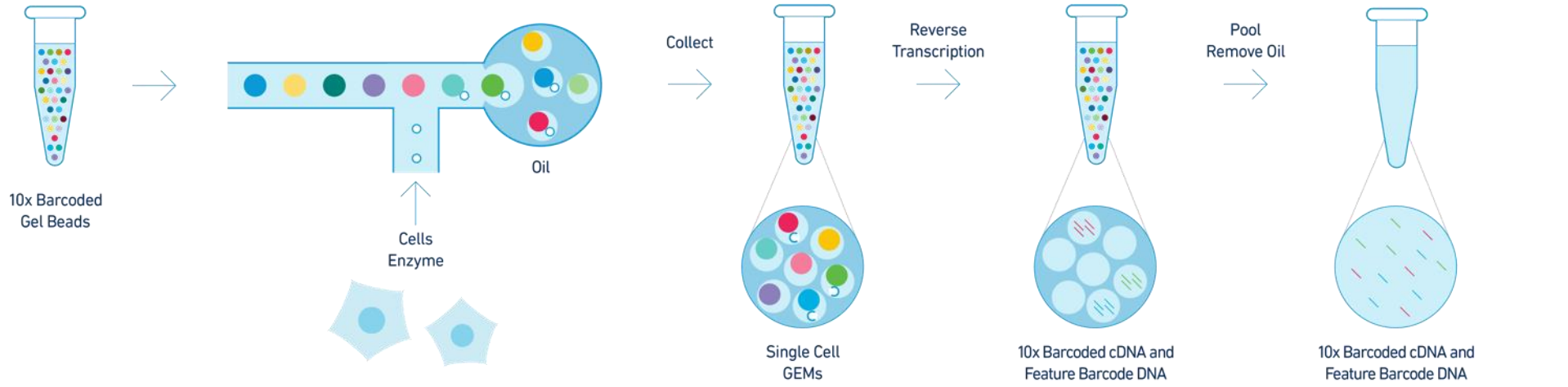
- Gene/cell matrix
- Unsupervised clustering
- Feature Barcoding analysis
- Heatmaps

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Workflow overview



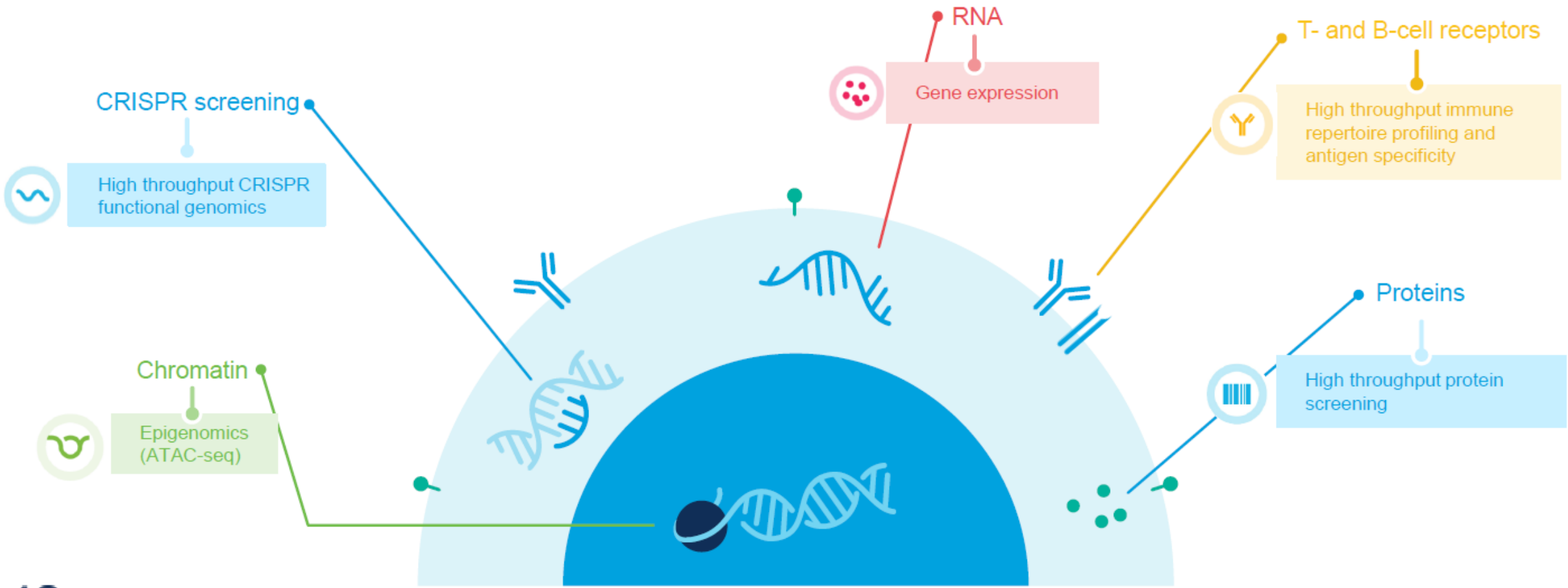
Inputs:
10x Genomics gel beads, reagents and single cells in suspension

Outputs:
Digital gene expression and cell surface protein expression or CRISPR perturbation profiles from every partitioned cell

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Single cell multiomic cytometry

Simultaneous measurements of different cellular analytes



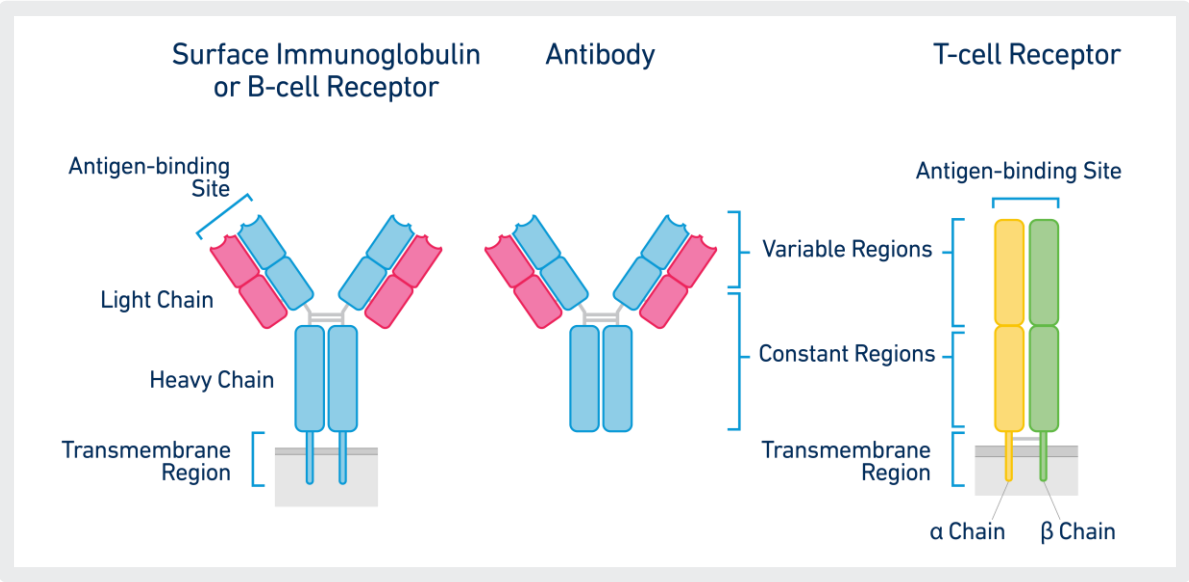
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Key components of the adaptive immune system

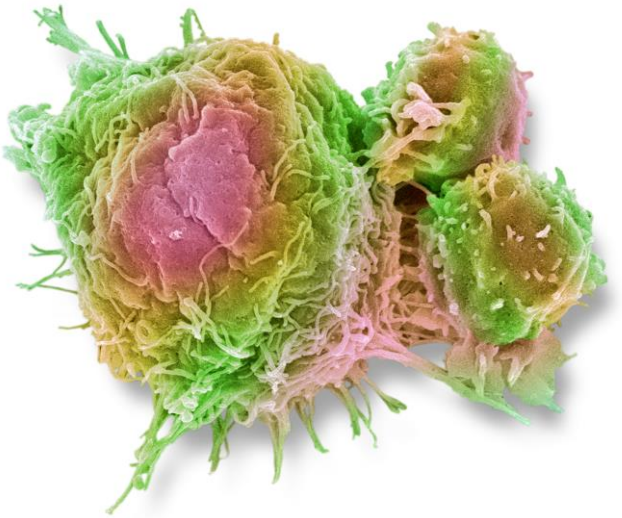
Diverse immune receptors provide broad surveillance

Determinants of antigen specificity



- In both T and B cells specificity is determined by two distally encoded, co-expressed genes
- Diversity is generated by V(D)J recombination and somatic hypermutation

T cells attacking a tumor cell



- Paired characterization of the TCR α and β transcripts in each T cell is critical to dissecting cellular interactions

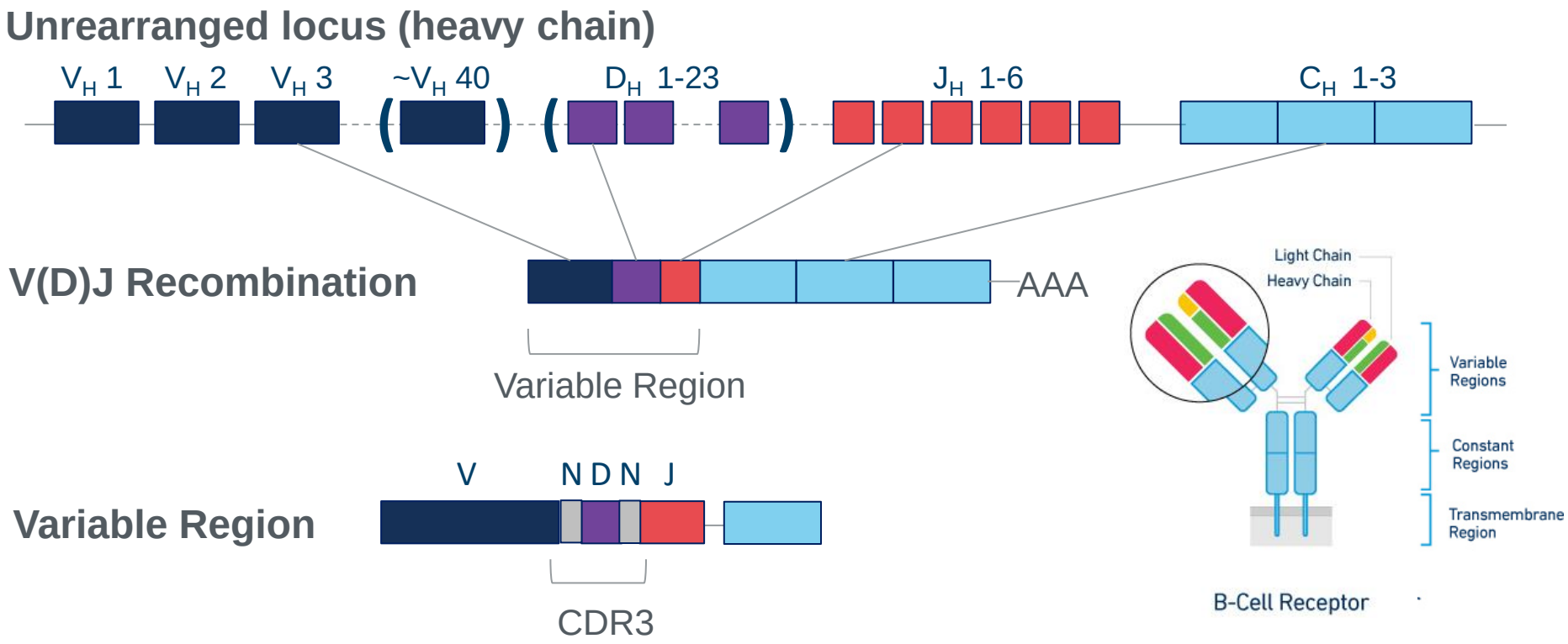
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V(D)J recombination generates immune repertoire diversity

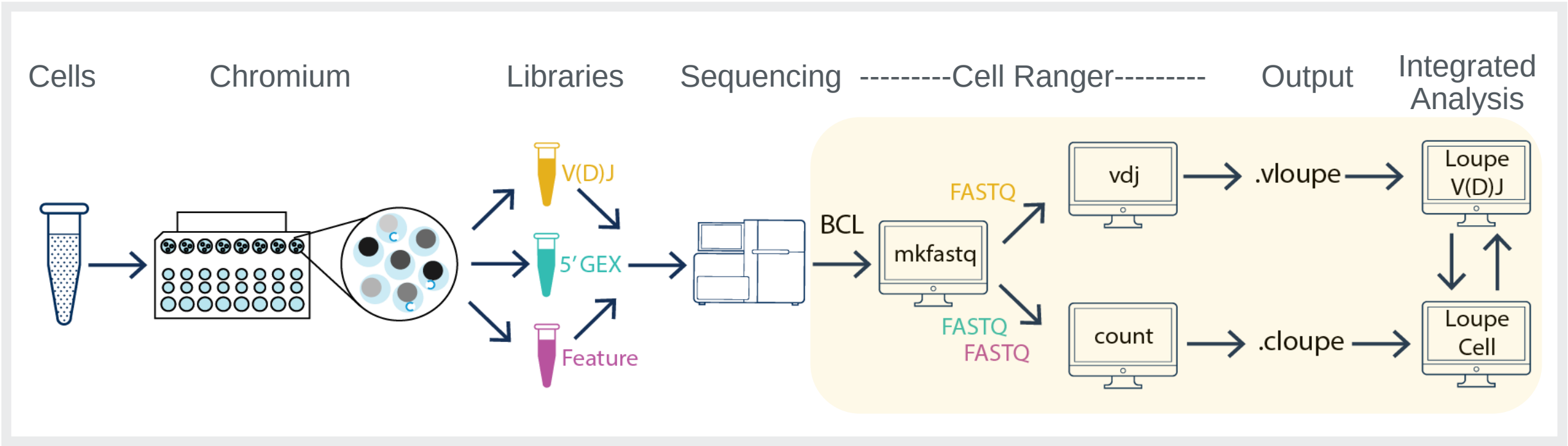
Enormous diversity of T and B cell antigen-specific receptors

- Such diversity is generated by V(D)J recombination + N nucleotide addition or deletion
- Full-length sequencing of the paired heavy and light chains (B cells) or α and β chains (T cells) is critical to dissecting these interactions



Analyzing immune profiling data

Overview



Laboratory

Analysis

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The Chromium Single Cell ATAC Solution

Comprehensive Scalable Solution for Single Cell Open Chromatin Profiling



- **Chromatin Profiling Benefits**
  - Capture genome-wide regulatory circuitry, including open enhancers, promoters, repressors, insulators
  - Interrogate multiple transcription factors' binding sites simultaneously
- **Solution Features**
  - Ready-to-use, robust workflow, including demonstrated protocols for various sample types and turn-key software tools
  - Flexible for your experimental needs: Profile 500-10,000 nuclei per sample, up to 8 samples in parallel
  - High quality and performance: Capture up to 65% of nuclei with low multiplet rates and low mitochondrial reads
  - Now on Next GEM technology[1]

[1] Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents

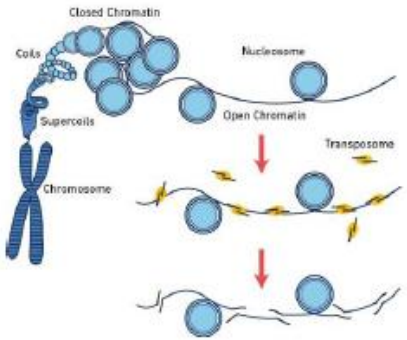
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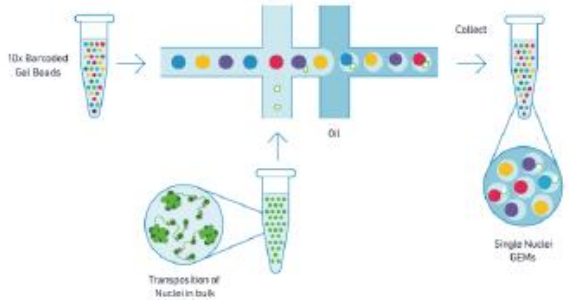
Single Cell ATAC

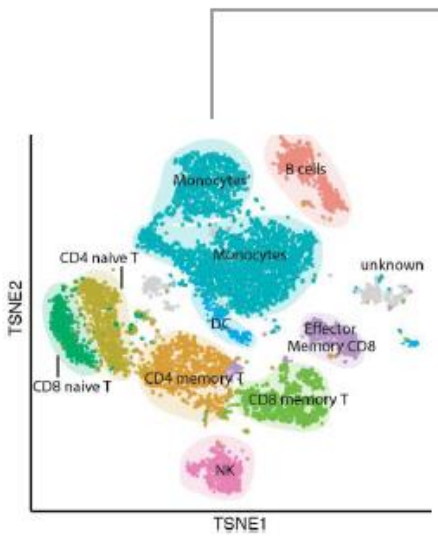
Uncovers Cell Type-Specific Epigenetic Regulation

Profiling Chromatin Accessibility

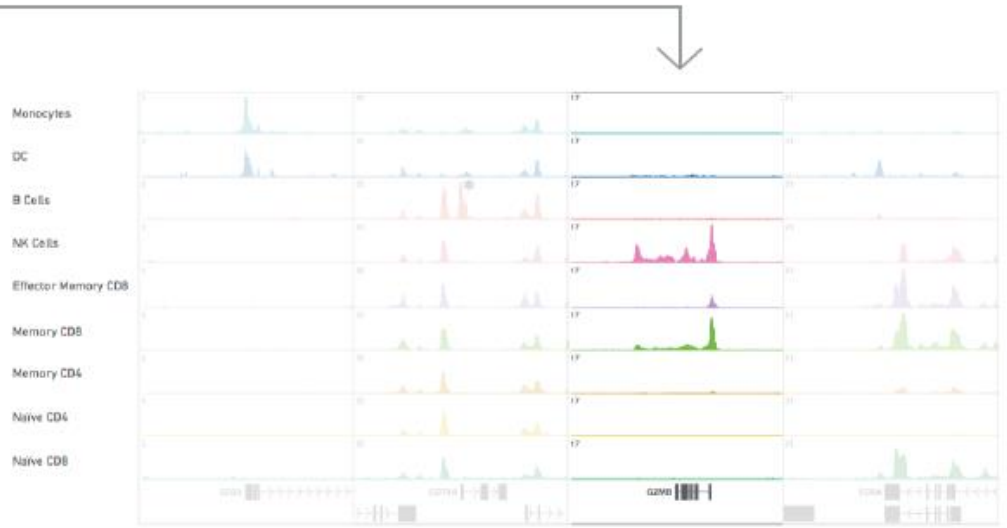


Single Nuclei Barcoding and Library Generation





9,542 PBMCs

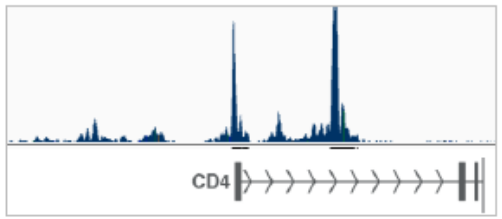


Cell-type specific epigenomic profiles

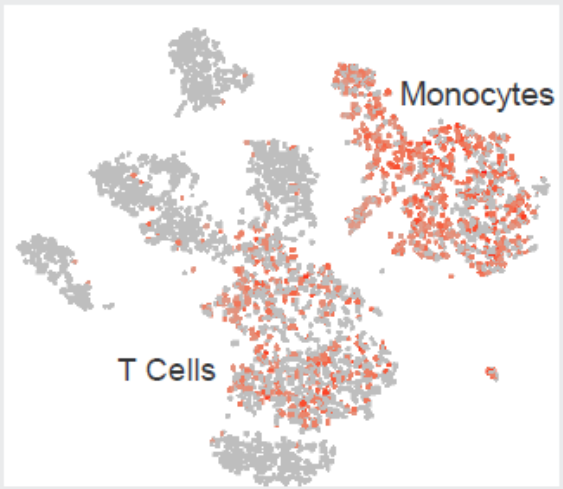
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Uncover Cell Type-Specific Gene Regulation

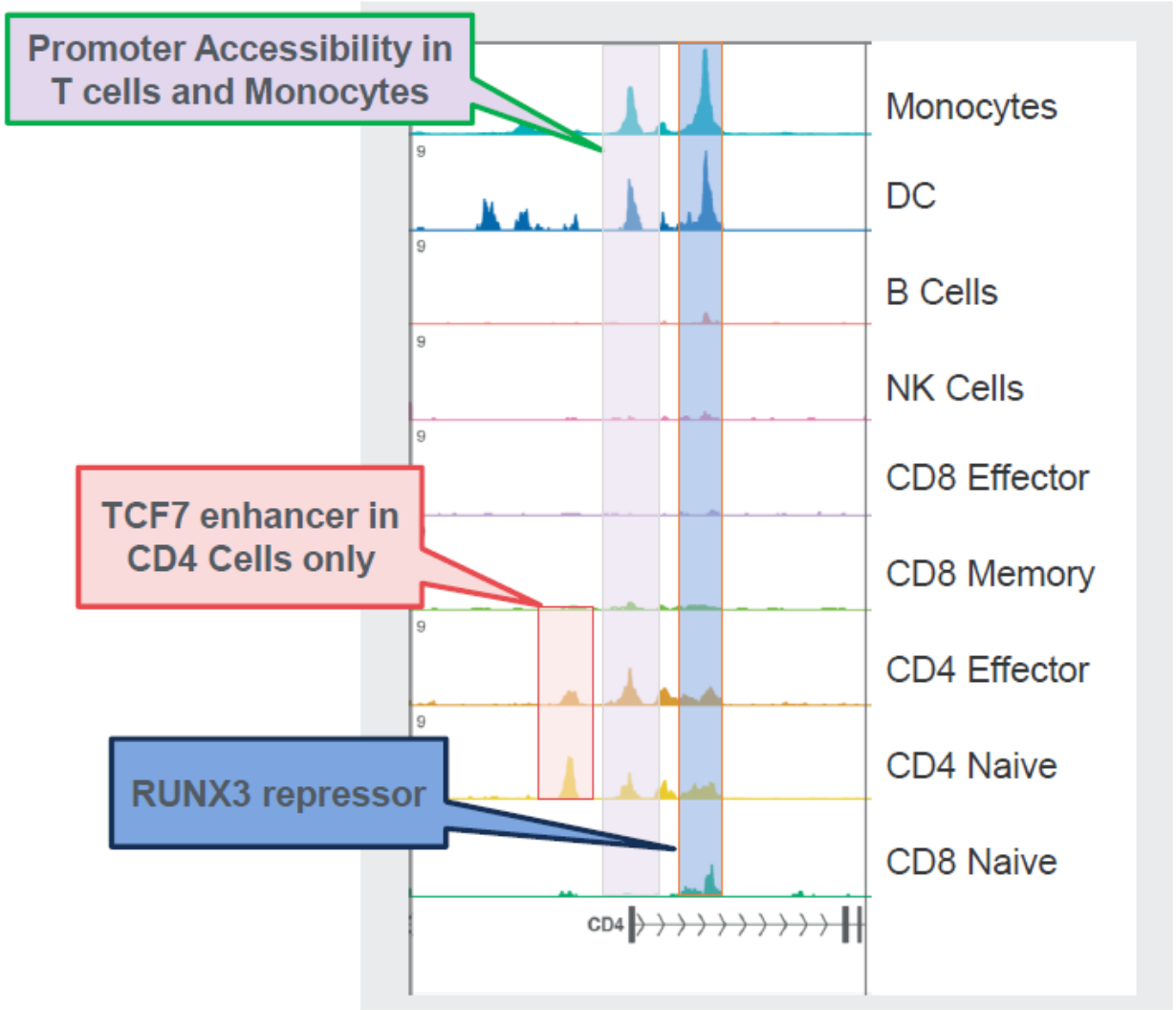
Bulk ATAC-seq from PBMCs



CD4 RNA Expression



Single Cell ATAC-seq



Bulk ATAC-seq misses CD4 enhancer critical for T cell identity

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Chromium Single Cell Multiome ATAC + Gene Expression

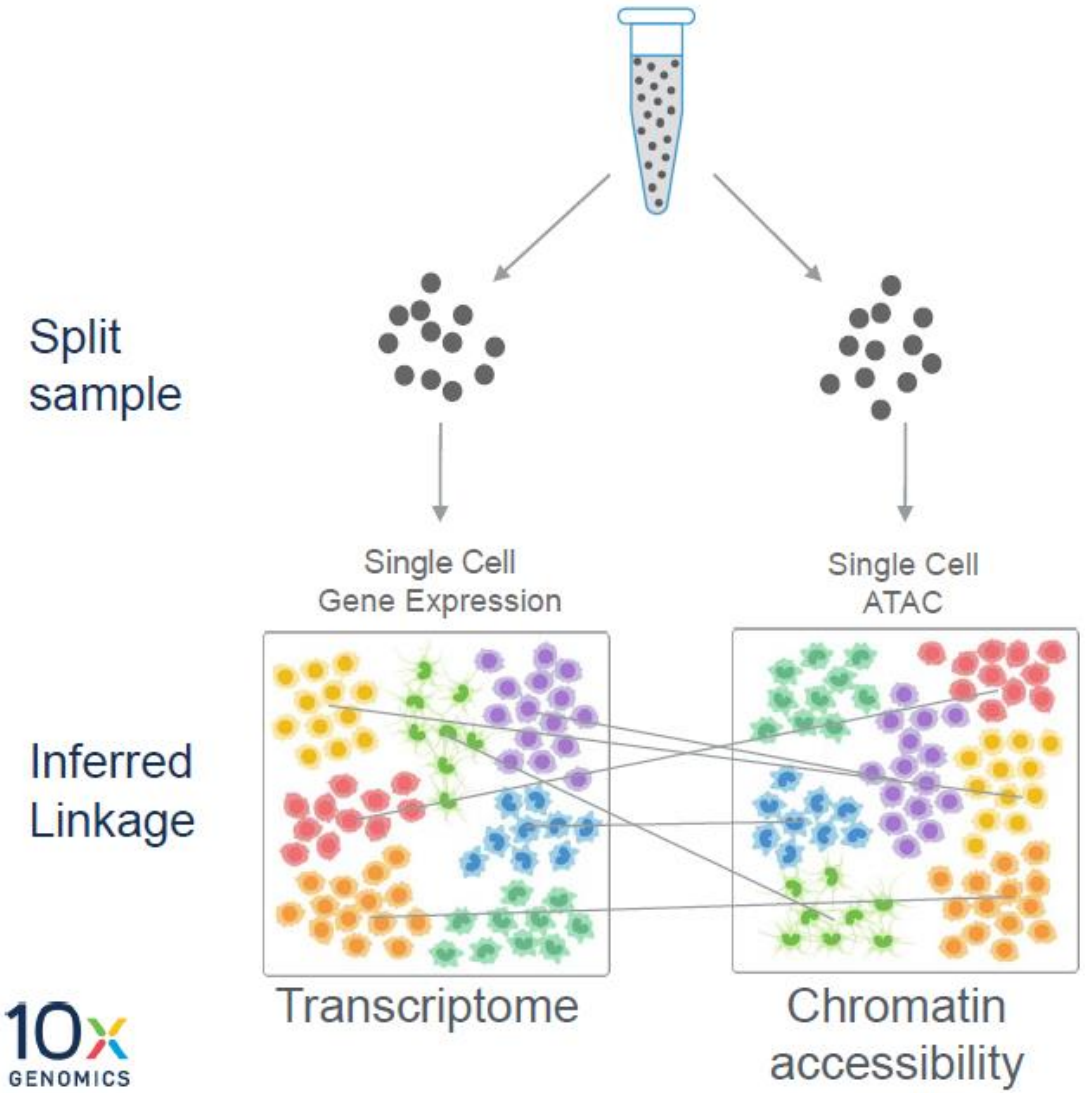
Introducing a new single cell multiomic capability



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Current approach to integrate single cell transcriptomic and epigenomic data



Potential downsides:

- Chromatin accessibility is assumed to be positively correlated with gene expression
- Chromatin changes can proceed gene expression (lineage priming)
- Open chromatin peaks not associated with promoters is discarded (rich in cell type information)

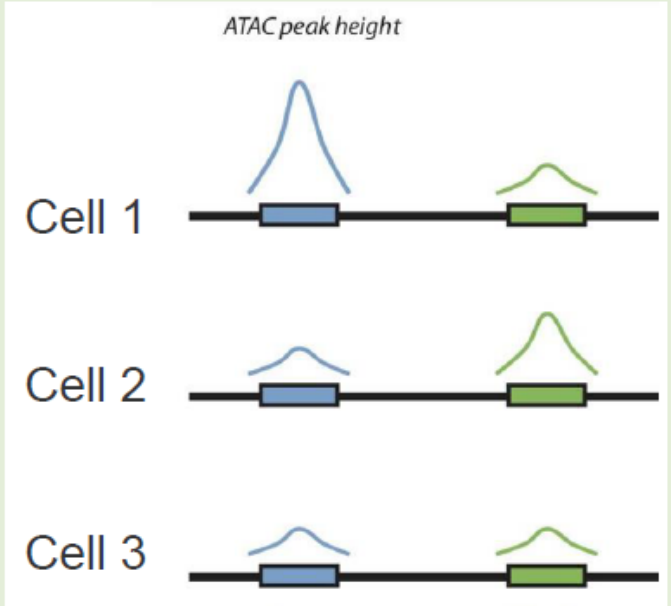
Cell types may be misassigned
Rare cells may be difficult to link

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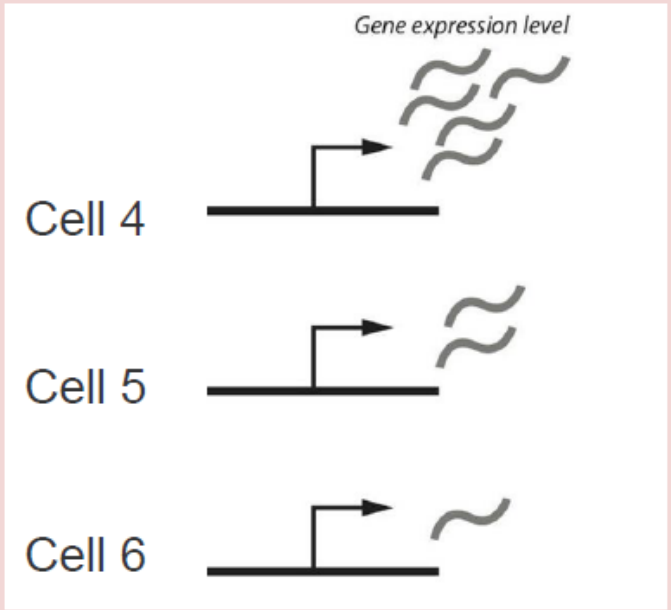
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Capturing both modalities together allows direct interrogation of gene regulation

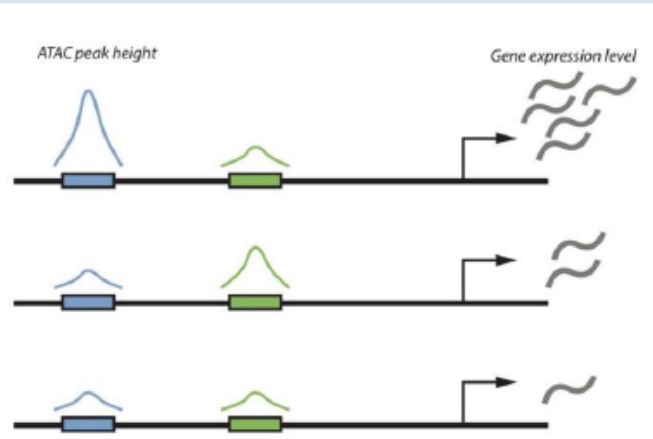
Chromatin accessibility (input)



Gene expression (output)



Dual assay



Directly link gene expression to chromatin accessibility

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10x Genomics Feature Barcode technology

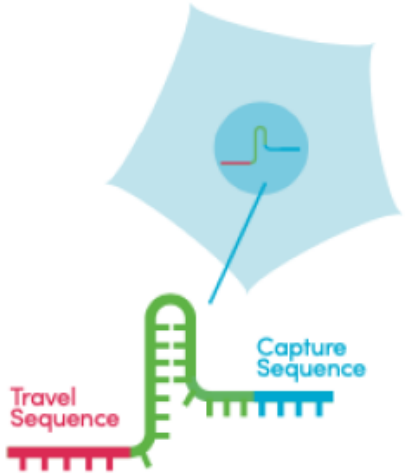
Using DNA barcodes to label additional cellular features



Antibodies for cell surface protein analysis



Peptide-MHC multimers to measure antigen specificity



Specific CRISPR perturbations for functional genomics screens

Compatible PRODUCTS | 10x GENOMICS

BioLegend® Enabling Legendary Discovery™

IMMUDEX

MilliporeSigma

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Advances in Spatial Genomics

Chromium Platform



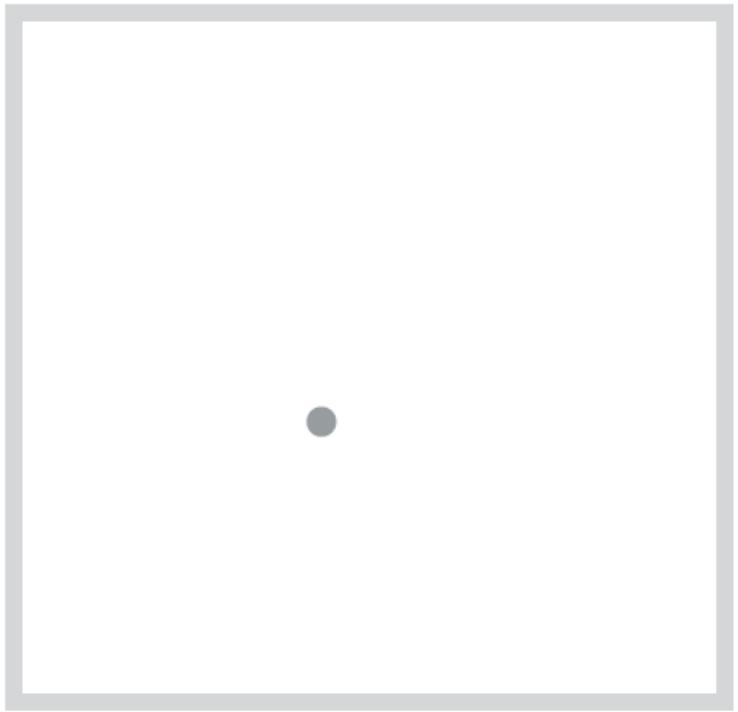
Visium Platform



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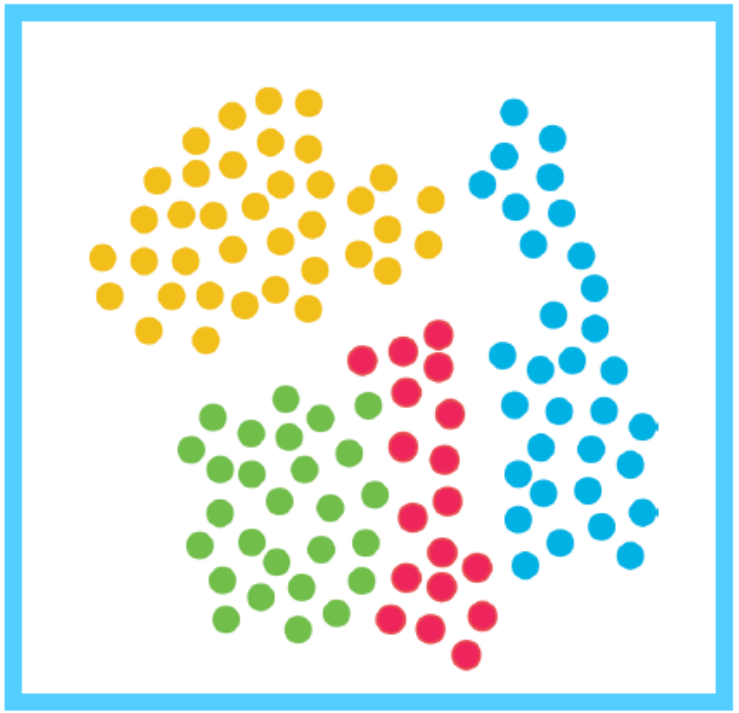
Product platforms

Traditional Platforms



Bulk

Chromium



Single Cell

Visium

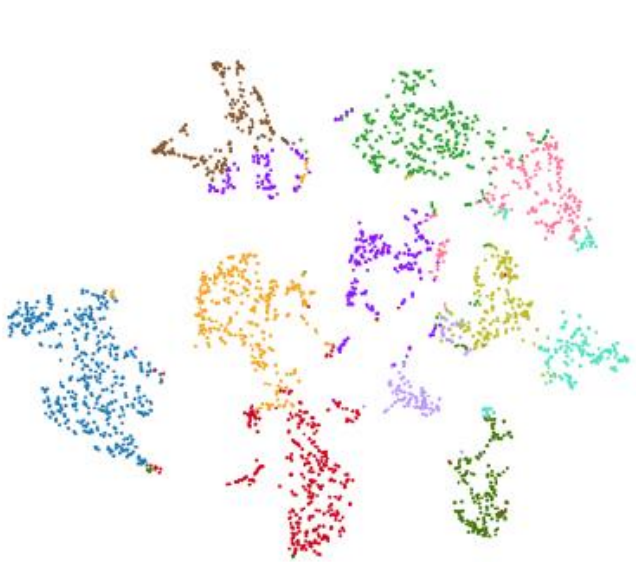


Spatial

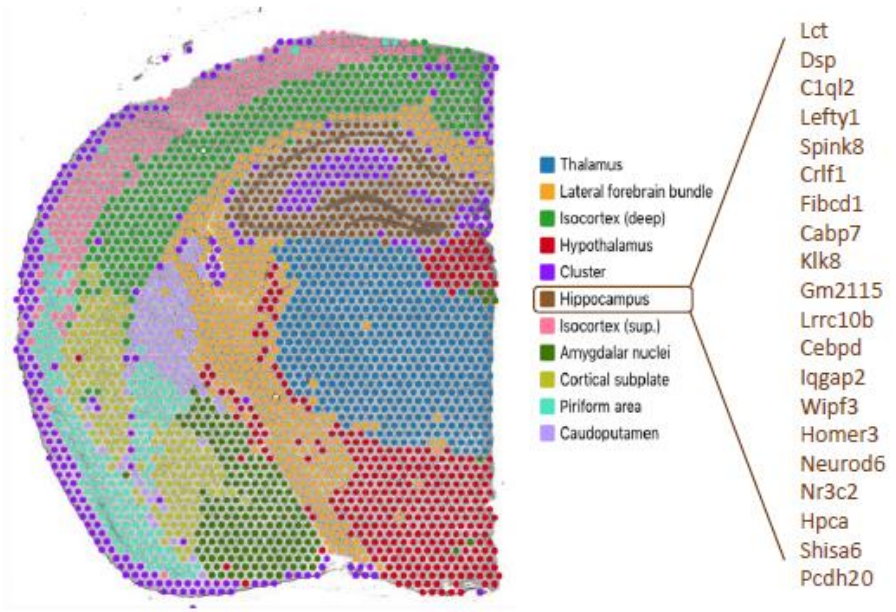
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Cluster or Image Driven Analysis of Spatial Data

Start With the Gene Expression Data or microscopy images of the same section



Clustering/classification of glass slide spots based on their gene expression profile



Annotation directly on H&E image



Captured H&E image from the microscope

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How to Learn More?



10x Community

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Agenda

- Chromium Single Cell Gene Expression Solution with Feature Barcode technology overview
- The biochemistry behind the Single Cell Gene Expression Solution
- Sample preparation recommendations
- Single Cell Gene Expression workflow
- Sequencing specifications

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology overview

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Solution highlights



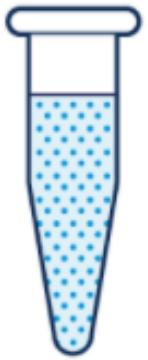
- Unbiased single cell transcriptome 3’ gene expression that enables discovery research
  - Simultaneously assess perturbation phenotypes, protein abundance and gene expression from the same cell
  - Compatible with targeted gene expression
- Ready-to-use, robust workflow, including demonstrated protocols for various sample types
  - Compatible with whole cells and nuclei
- Now on Next GEM technology* and incorporating dual indexing
- Easy-to-use and convenient software with Cell Ranger Analysis Pipeline and Loupe Browser visualization tools

*Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Input | **Library Creation** | **Sequence** | **Data Analysis and Visualization**

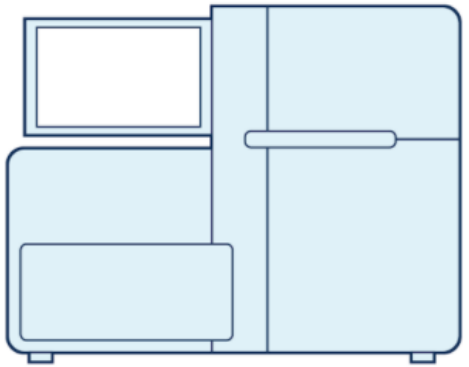


User-supplied
Cells or nuclei

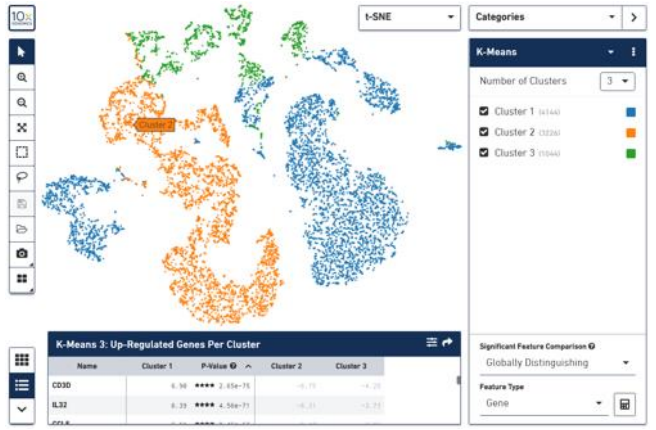


Chromium Controller and Next GEM Single Cell 3' Reagents

- Chromium Next GEM Single Cell 3' Kit v3.1
- 3' Feature Barcode Kit
- Chromium Next GEM Chip G Single Cell Kit
- Dual Index Kit TT Set A
- Dual Index Kit NT Set A



Sequencer

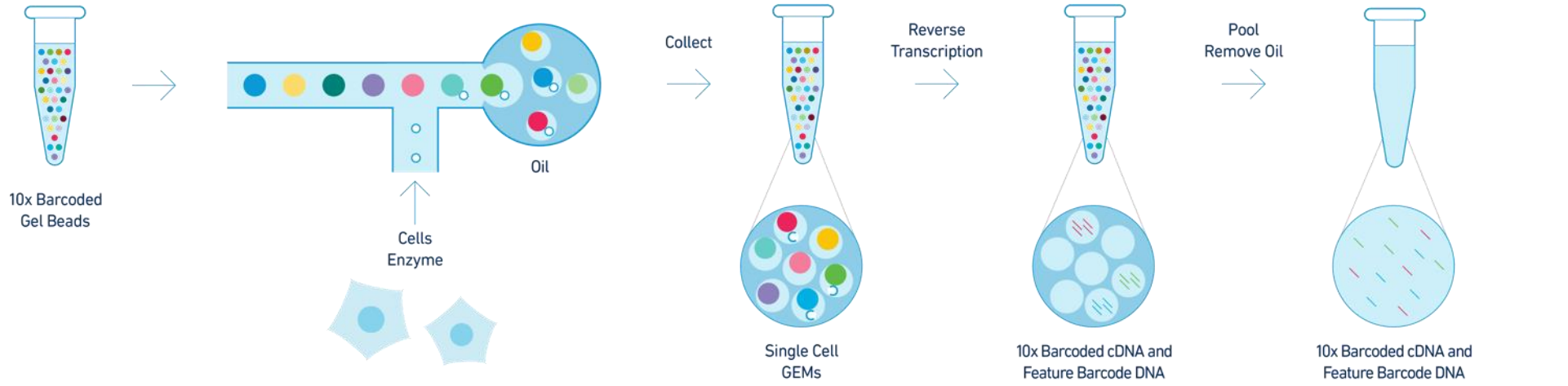


Cell Ranger Pipeline
Loupe Browser

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Workflow overview



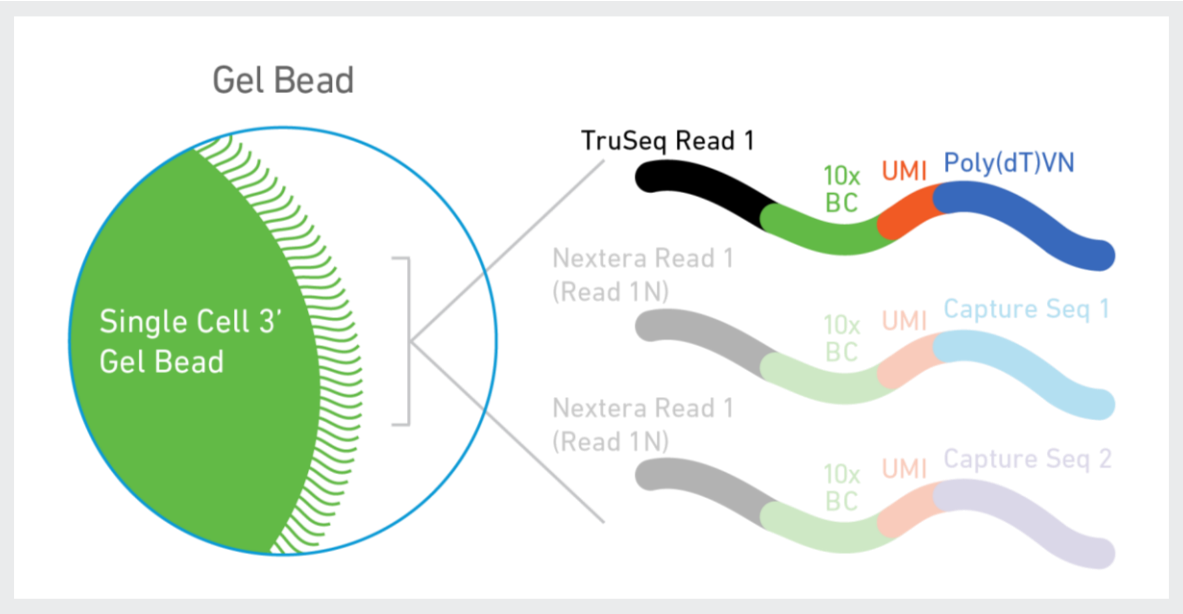
Inputs:
10x Genomics gel beads, reagents and single cells in suspension

Outputs:
Digital gene expression and cell surface protein expression or CRISPR perturbation profiles from every partitioned cell

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Feature Barcode technology enabled via Single Cell 3’ Gel Beads



i. TruSeq Read 1
22 nt Partial Illumina TruSeq Read 1 sequence

ii. 10x Genomics Barcode
16 nt 10x Genomics Barcode
~3.6 M defined barcode sequences

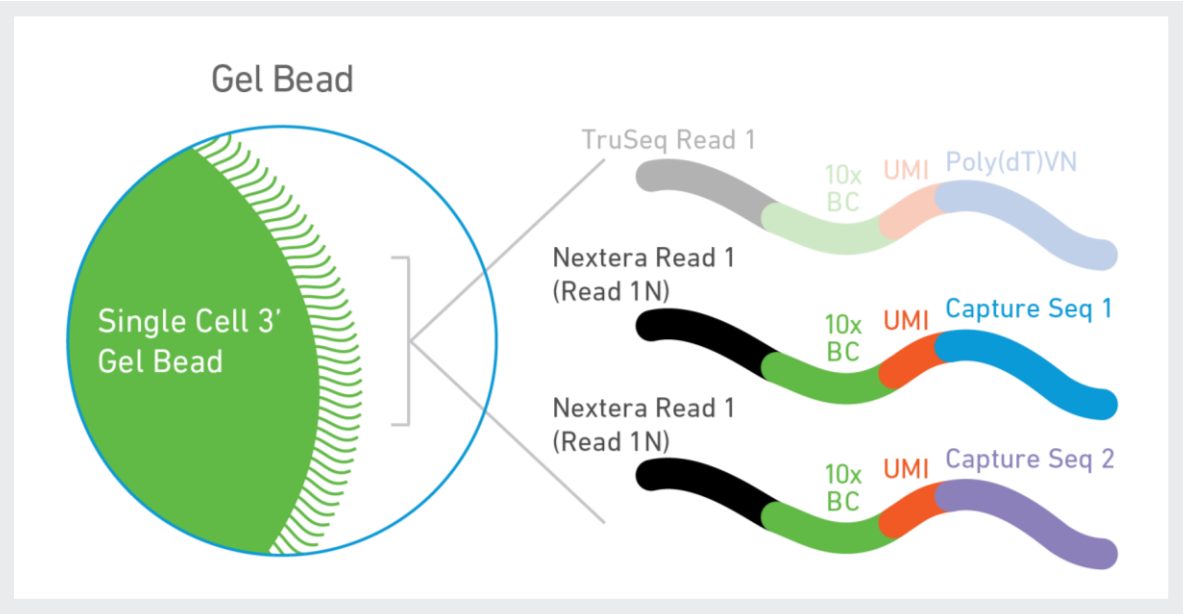
iii. UMI
12 nt Unique Molecular Identifier

iv. Poly(dT)VN
30 nt Poly(dT) sequence (Enables capture of poly-adenylated mRNA molecules)

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Feature Barcode technology enabled via Single Cell 3' Gel Beads



i. Nextera Read 1 (Read 1N)
22 nt Partial Illumina Nextera Read 1 sequence (Enables selective enrichment of the Feature Barcode construct)

ii. 10x Genomics Barcode
16 nt 10x Genomics Barcode
~3.6 M defined barcode sequences

iii. UMI
12 nt Unique Molecular Identifier

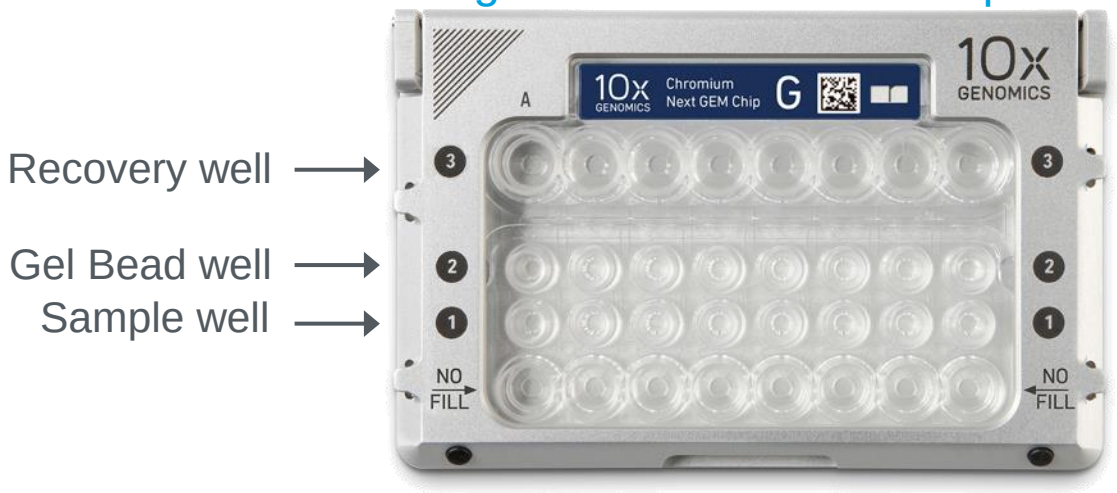
iv. Capture Sequence 1 or 2
22 nt sequence that is the reverse complement of the sequence inserted into the DNA (Antibody) or RNA (sgRNA) based sequence

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Chromium Single Cell Gene Expression Solution with Feature Barcode technology

Single-use microfluidics chip



- Up to 8 channels processed in parallel
- 500 to 10,000 cells per channel
- 18 minute run time (per chip)
- Up to 30 µm cell diameter tested
- Up to 65 % cell processing efficiency
- Temperature Range 18-28°C

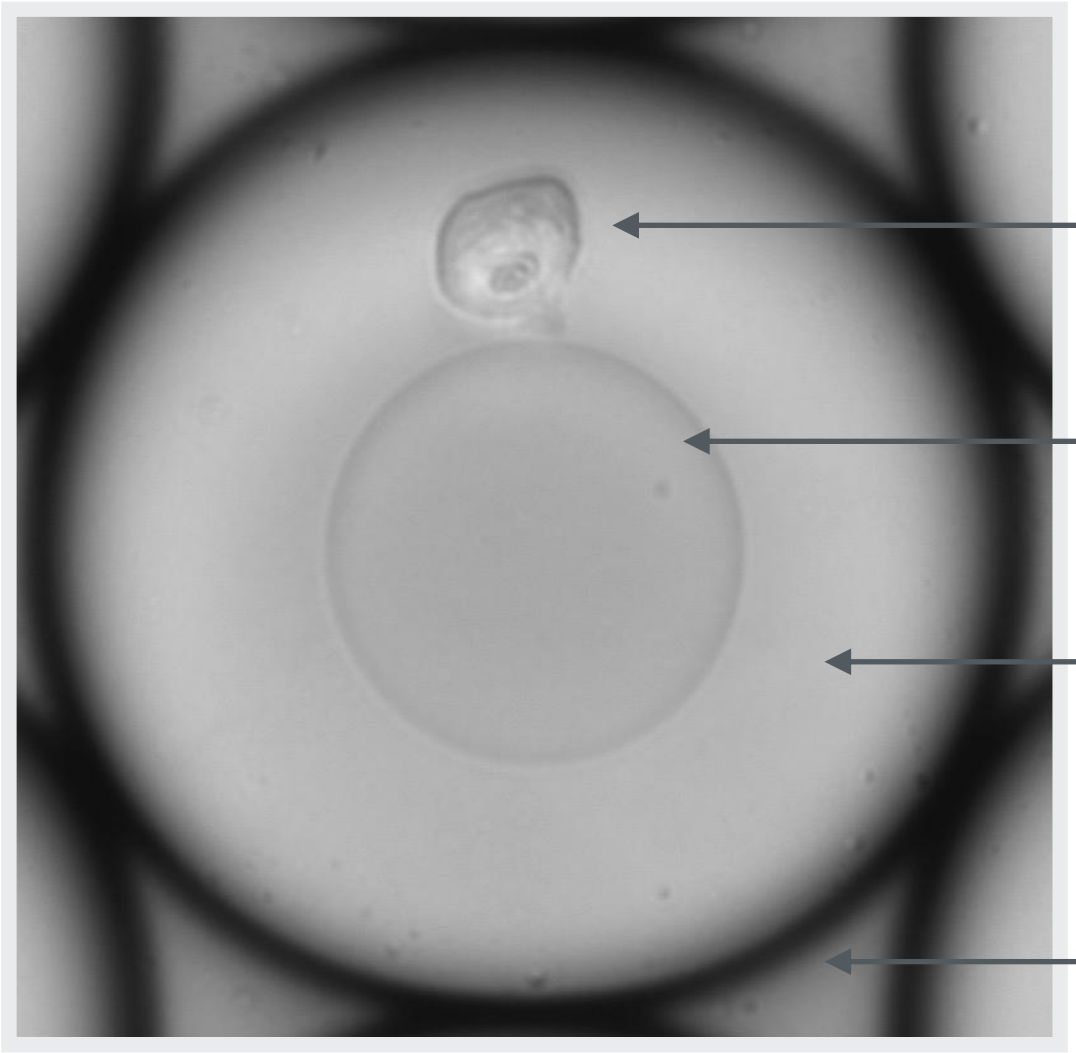
User controlled trade-off between cell numbers and doublet rate

Number of cells loaded	Number of cells	Expected multiplet rate (%)*
~800	~500	~0.4
~1,600	~1,000	~0.8
~8,000	~5,000	~3.9
~16,000	~10,000	~7.6

Firmware 4.00 or higher is required for running this assay

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Gel Bead-in-Emulsion (GEM)



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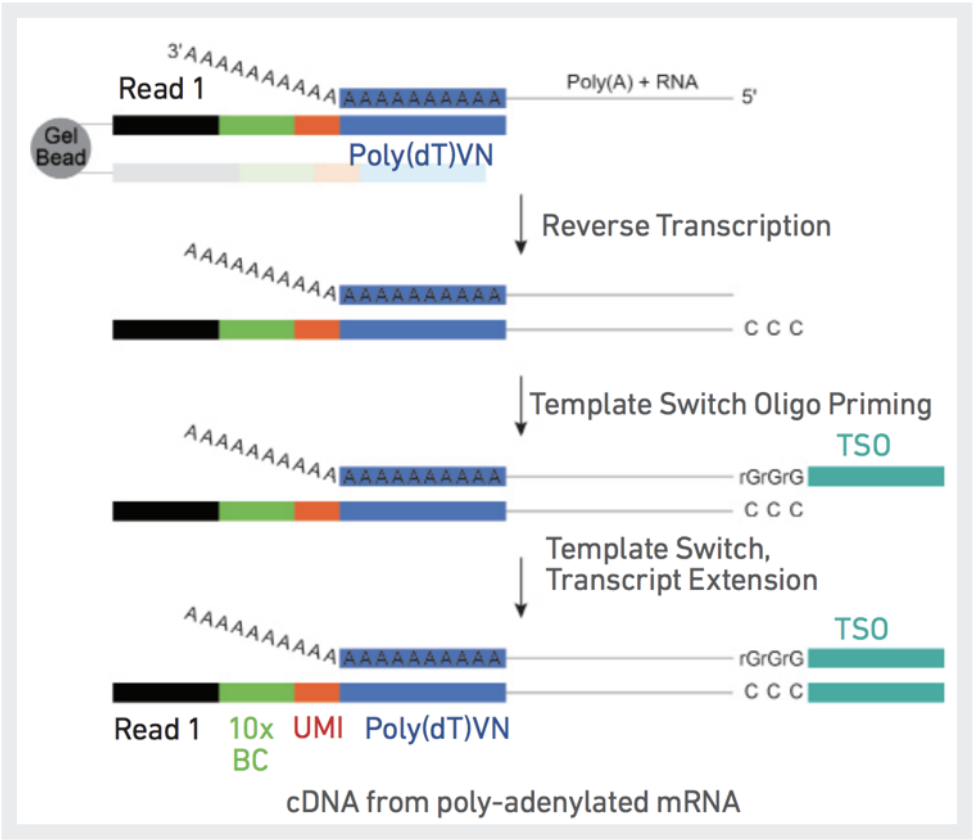
The biochemistry behind the Single Cell Gene Expression Solution

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Single Cell Gene Expression workflow: target capture in GEMs

Direct capture of poly-adenylated mRNA inside individual GEMs

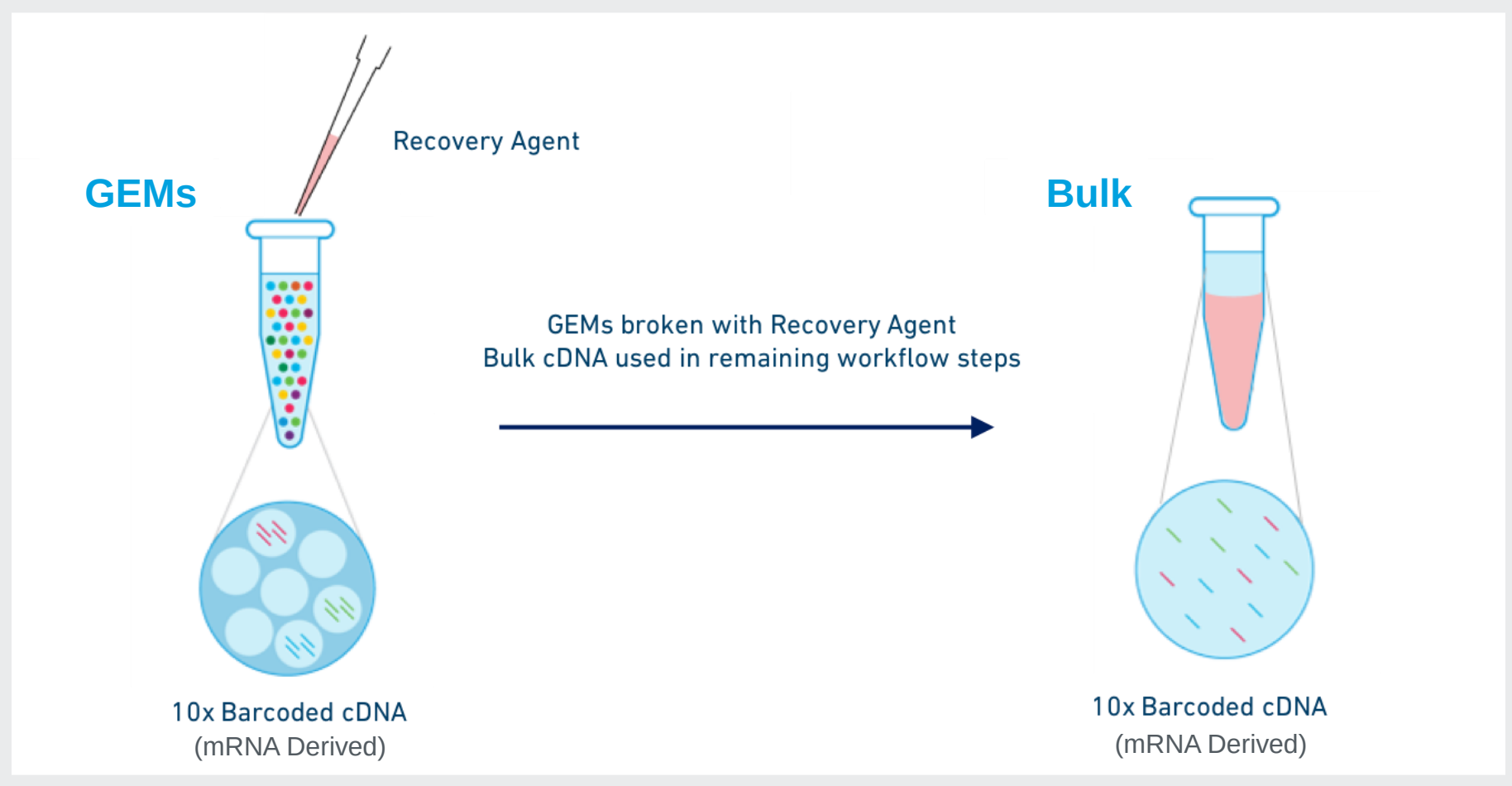
Barcoded, full-length cDNA from poly-adenylated mRNA



Note: gene expression poly-adenylated mRNA is directly captured by the corresponding gel bead oligo by reverse transcription and template switching

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Single Cell Gene Expression workflow: Breaking GEMs

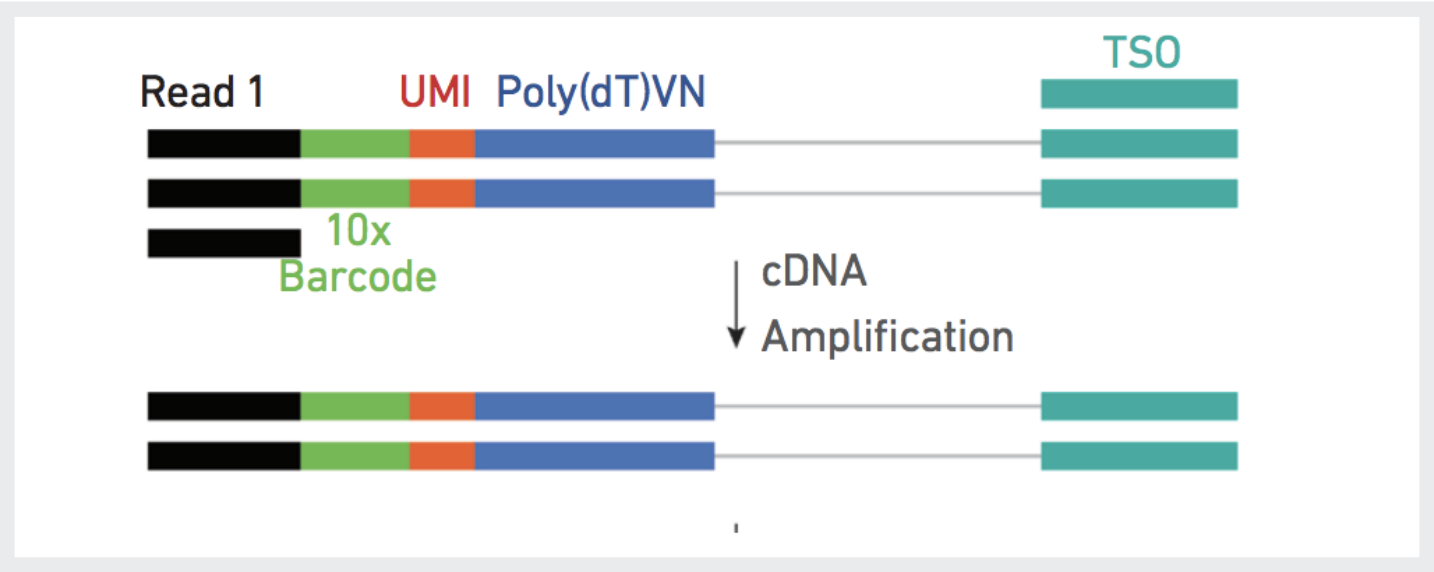


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Single Cell Gene Expression workflow: cDNA amplification

In bulk amplification of cDNA

cDNA Amplification of poly-adenylated mRNA



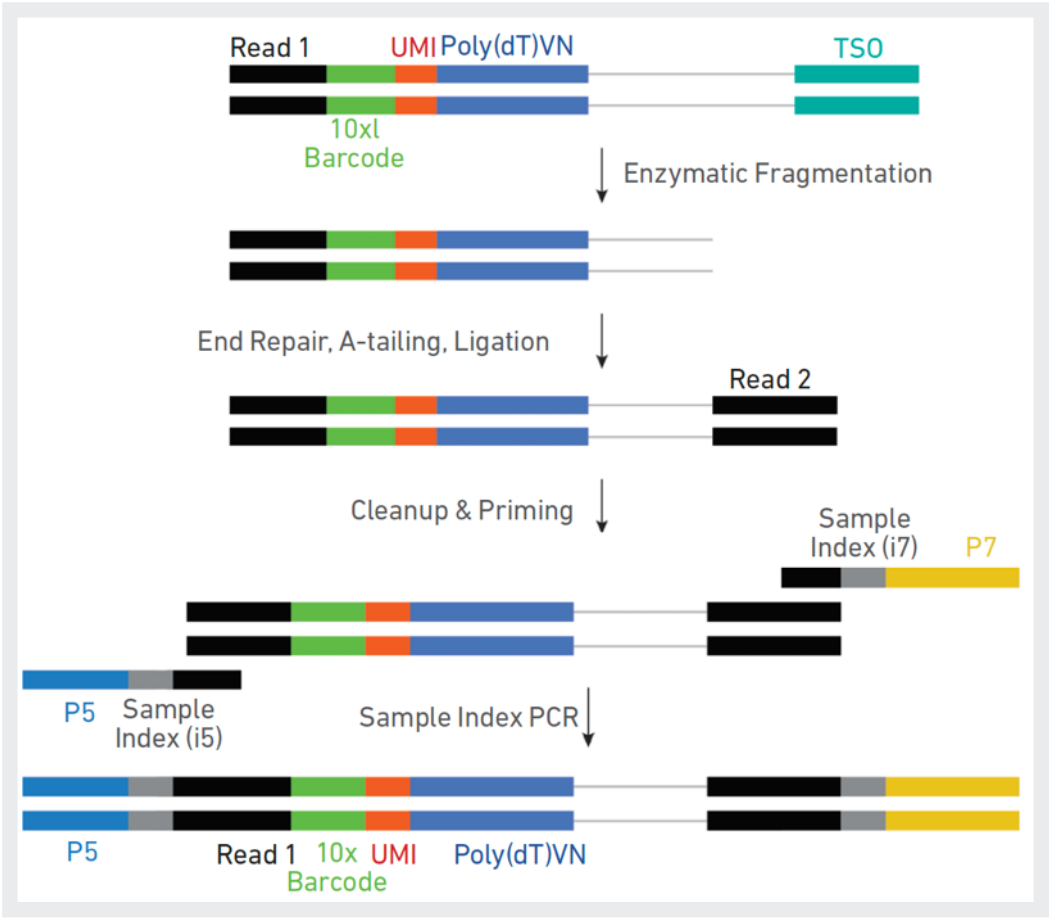
Note: For cDNA amplification of poly-adenylated mRNA please use: cDNA Primers (PN 2000089)

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Single Cell Gene Expression workflow: Library construction

In bulk library construction

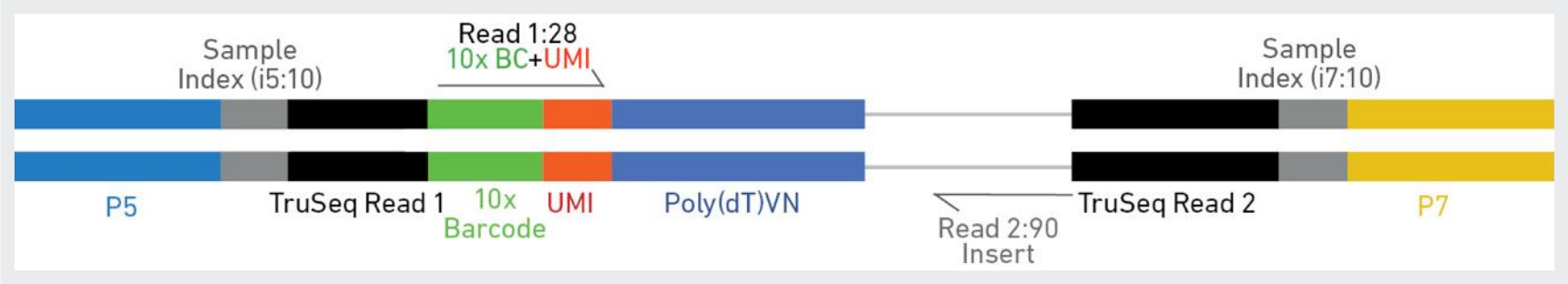
Library construction of poly-adenylated mRNA



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Recommended read length

Single Cell 3’ Gene Expression Library

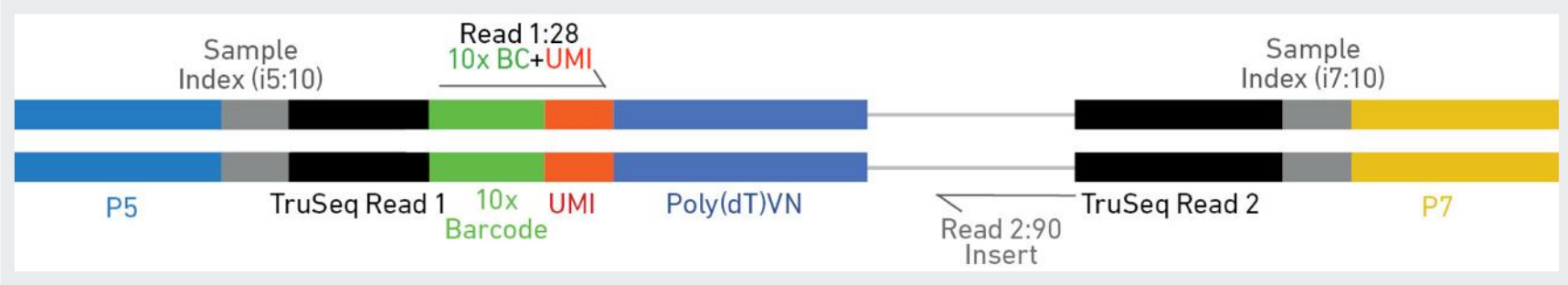


	Read 1	i7 Index	i5 Index	Read 2
Purpose	10x Barcode & UMI	Sample Index	Sample Index	Transcript
Length	28	10	10	90

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Supported sequencers

Single Cell 3’ Gene Expression Library



	Single Cell 3’ Gene Expression
Raw Read Pairs per Cell	Minimum 20,000



MiSeq



NextSeq 500/550



HiSeq2500



HiSeq 3000/4000

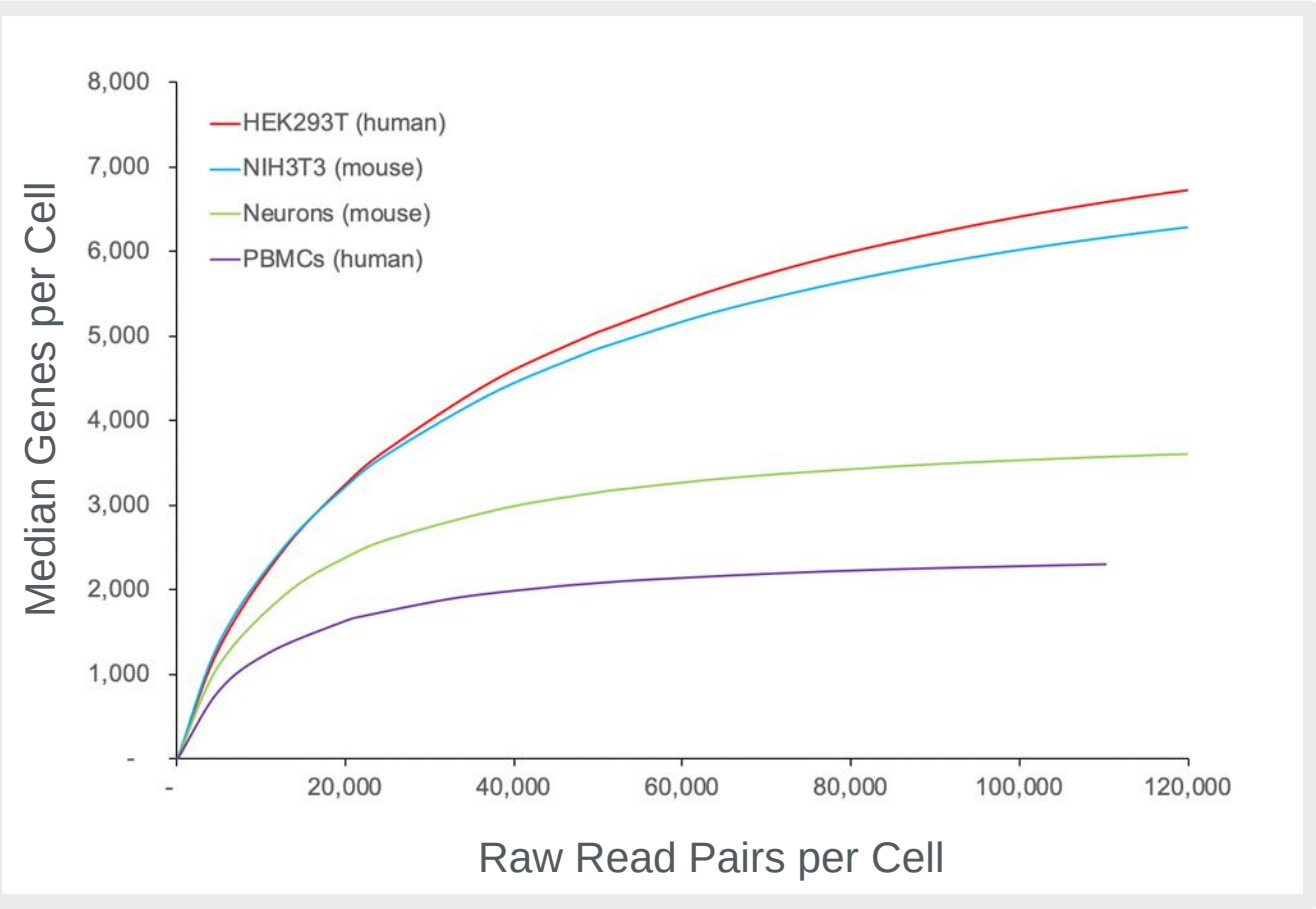


NovaSeq

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Sequencing depth for typical samples

Gene Expression Libraries



- 20,000 raw read pairs per cell is the recommended minimum sequencing depth for typical samples
- Given variability in cell counting and loading, extra sequencing may be required if the cell count is higher than anticipated

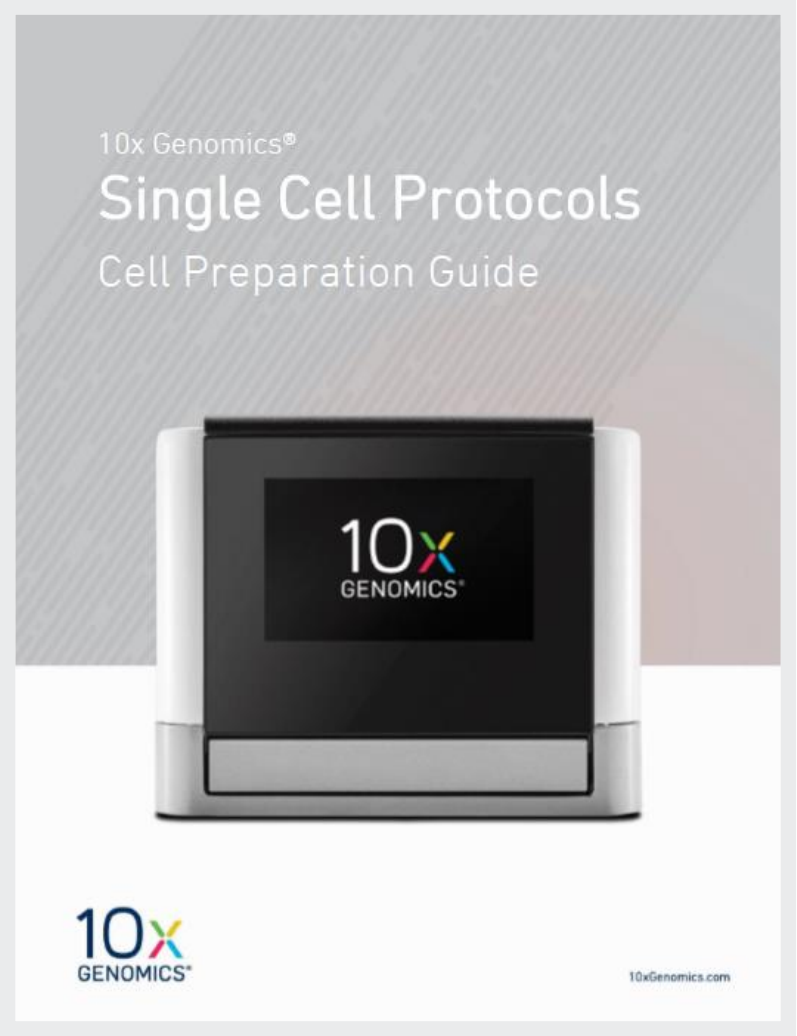
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Sample preparation recommendations

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Cell Preparation Guide

- Available on the 10x Genomics Support website
- Cell Preparation Guide includes best practices and general protocols for:
  - Washing and resuspension
  - Straining
  - Counting
  - Cell concentration
  - Working with limited cell suspensions (less than 100,000 total cells)
- Flow-sorted and primary white blood cells can generally be treated like suspension cell lines



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Single Cell Gene Expression with Feature Barcode technology

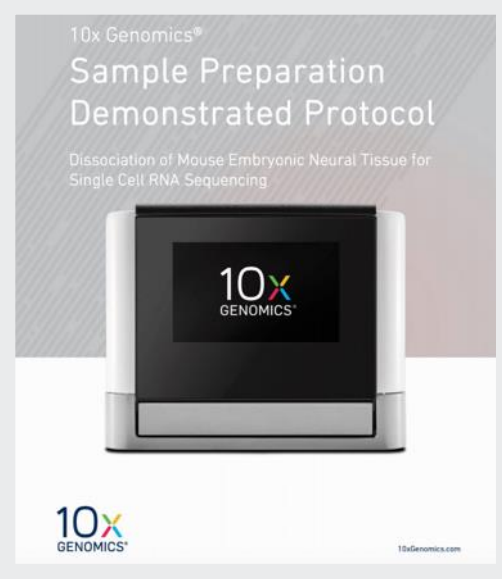
Routine use of demonstrated protocols – optimization may be required

CG00014
Fresh Frozen Human-Mouse Cell Line Mixtures for Single Cell RNA Sequencing





CG00055
Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing





CG00039
Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing





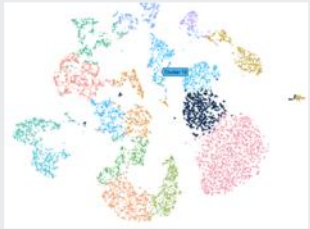
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General cell handling recommendations

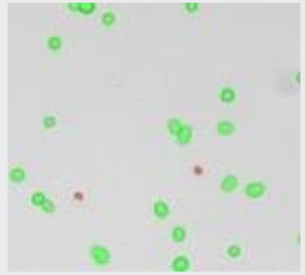
Analysis of single cell transcriptomes

- 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



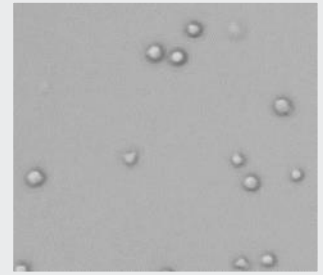
Importance of input cell quality

- 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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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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Single Cell Gene Expression workflow

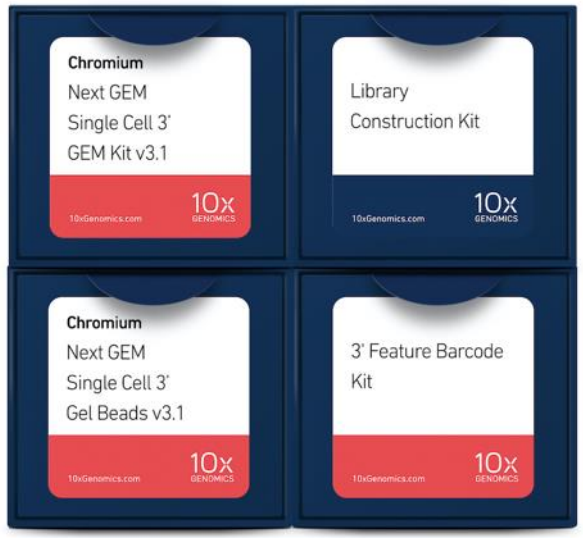
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Protocol steps & timing

Day	Steps		Timing	Stop & Store
2 h	**Cell Preparation**			
	Dependent on Cell Type		~1-1.5 h	
4 h	**Step 1 – GEM Generation & Barcoding**			
	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip G	10 min	
	1.3	Run the Chromium Controller	18 min	
	1.4	Transfer GEMs	3 min	
	1.5	GEM-RT Incubation	55 min	STOP 4°C ≤72 h or –20°C ≤1 week
6 h	**Step 2 – Post GEM-RT Cleanup & cDNA Amplification**			
	2.1	Post GEM RT-Cleanup – Dynabead	45 min	
	2.2	cDNA Amplification	40 min	STOP 4°C ≤72 h or –20°C ≤1 week
	2.3	cDNA Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h –20°C ≤4 weeks
	2.4	cDNA QC & Quantification	50 min	
8 h	**Step 3 – 3' Gene Expression Library Construction**			
	3.1	Fragmentation, End Repair & A-tailing	50 min	
	3.2	Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	3.3	Adaptor Ligation	25 min	
	3.4	Post Ligation Cleanup- SPRIselect	20 min	
	3.5	Sample Index PCR	40 min	STOP 4°C ≤72 h
	3.6	Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	STOP 4°C ≤72 h or -20°C long term
	3.7	Post Library Construction QC	50 min	

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Getting started: Equilibrate Reagents



From -80°C storage – Place at room temperature:



Remove the:

- **Chromium Next GEM Single Cell 3' v3.1 Gel Beads** from -80°C storage and equilibrate to room temperature for **30 min.**

Failure to equilibrate Gel Beads for 30 minutes will lead to run failure

From -20°C storage – Place at room temperature:



Remove the:

- **RT Reagent B (blue cap)**
- **Reducing Agent B (white cap)**
- **Template Switch Oligo (blue cap)**

provided as lyophilized oligos; after resuspension allow to sit at room temperature 30 min, store unused primers at −80°C

from the **Chromium Next GEM Single Cell 3' GEM Kit v3.1** stored at -20°C and equilibrate to room temperature for at least **10 min.**

From -20°C storage – Place on ice:

Remove the:

- **RT Enzyme C (blue cap)**

from the **Chromium Next GEM Single Cell 3' GEM Kit v3.1** stored at -20°C and place on ice

From room temperature storage:

Remove the:

- **Partitioning Oil (clear cap)**
- **Next GEM Chip G, Gasket, Chip Holder** from room temperature storage

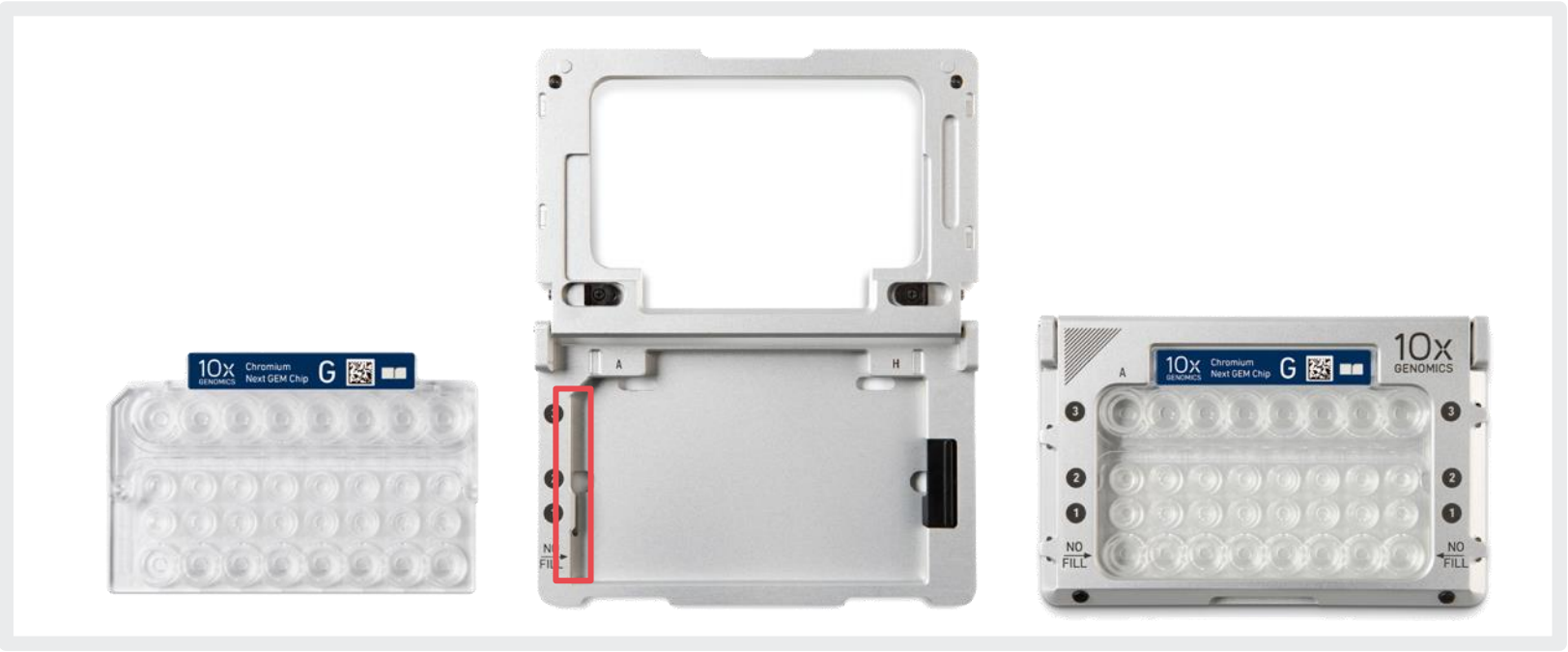
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Single Cell Gene Expression workflow

Assemble a Chromium Next GEM Chip G in a Next GEM Chip Holder

- Chips come packaged in mylar bags containing desiccant packet
  - Chip should be run within 24 hrs of opening the mylar bag
  - Shelf life if unopened: one year from date of packaging
- Insert a Chromium Next GEM Chip G into a Next GEM Chip Holder



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Single Cell Gene Expression workflow

Unused channels must be loaded with 50% glycerol

- Assemble a Chromium Next GEM Chip G in a Chromium Next GEM Chip Holder
- If running less than 8 samples per chip, dispense 50% glycerol into the unused wells
  - 70 µl to unused wells in Row 1
  - 50 µl to unused wells in Row 2
  - 45 µl to unused wells in Row 3
  - This should be done prior to adding cells to the aliquoted Master Mix
- Failure to add glycerol to unused wells can lead to sample loss and may damage the Controller
- Do NOT add glycerol to the bottom row of NO FILL wells
- Do NOT use any substitute for 50% glycerol solution

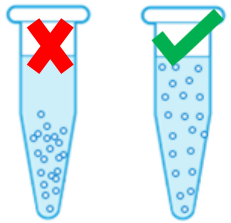


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Single Cell Gene Expression workflow

Determine targeted number of cells

- Cell Suspension Volume Calculator Table is provided in the User Guide
- Targeted Cell Recovery numbers take into account a 65% cell processing efficiency
- For example: Targeting 5000 cells with a cell concentration of 1000 cells/µl:
  - Add 35.0 µl water to aliquoted Master Mix
  - Add 8.3 µl cell suspension to Master Mix containing water
- Remember to resuspend the cells gently whenever taking aliquots to count or add to Master Mix



Cell Suspension Volume Calculator Table
(for step 1.2 of Chromium Next GEM Single Cell 3' v3.1 protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of Nuclease-free Water per reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 35.0	16.5 26.7	33.0 10.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 39.1	8.3 35.0	16.5 26.7	24.8 18.5	33.0 10.2	41.3 2.0	n/a	n/a	n/a	n/a	n/a
300	2.8 40.5	5.5 37.7	11.0 32.2	16.5 26.7	22.0 21.2	27.5 15.7	33.0 10.2	38.5 4.7	44.0 -0.8	n/a	n/a
400	2.1 41.1	4.1 39.1	8.3 35.0	12.4 30.8	16.5 26.7	20.6 22.6	24.8 18.5	28.9 14.3	33.0 10.2	37.1 6.1	41.3 2.0
500	1.7 41.6	3.3 39.9	6.6 36.6	9.9 33.3	13.2 30.0	16.5 26.7	19.8 23.4	23.1 20.1	26.4 16.8	29.7 13.5	33.0 10.2
600	1.4 41.8	2.8 40.5	5.5 37.7	8.3 35.0	11.0 32.2	13.8 29.5	16.5 26.7	19.3 24.0	22.0 21.2	24.8 18.5	27.5 15.7
700	1.2 42.0	2.4 40.8	4.7 38.5	7.1 36.1	9.4 33.8	11.8 31.4	14.1 29.1	16.5 26.7	18.9 24.3	21.2 22.0	23.6 19.6
800	1.0 42.2	2.1 41.1	4.1 39.1	6.2 37.0	8.3 35.0	10.3 32.9	12.4 30.8	14.4 28.8	16.5 26.7	18.6 24.6	20.6 22.6
900	0.9 42.3	1.8 41.4	3.7 39.5	5.5 37.7	7.3 35.9	9.2 34.0	11.0 32.2	12.8 30.4	14.7 28.5	16.5 26.7	18.3 24.9
1000	0.8 42.4	1.7 41.6	3.3 39.9	5.0 38.3	6.6 36.6	8.3 35.0	9.9 33.3	11.6 31.7	13.2 30.0	14.9 28.4	16.5 26.7
1100	0.8 42.5	1.5 41.7	3.0 40.2	4.5 38.7	6.0 37.2	7.5 35.7	9.0 34.2	10.5 32.7	12.0 31.2	13.5 29.7	15.0 28.2
1200	0.7 42.5	1.4 41.8	2.8 40.5	4.1 39.1	5.5 37.7	6.9 36.3	8.3 35.0	9.6 33.6	11.0 32.2	12.4 30.8	13.8 29.5
1300	0.6 42.6	1.3 41.9	2.5 40.7	3.8 39.4	5.1 38.1	6.3 36.9	7.6 35.6	8.9 34.3	10.2 33.0	11.4 31.8	12.7 30.5
1400	0.6 42.6	1.2 42.0	2.4 40.8	3.5 39.7	4.7 38.5	5.9 37.3	7.1 36.1	8.3 35.0	9.4 33.8	10.6 32.6	11.8 31.4
1500	0.6 42.7	1.1 42.1	2.2 41.0	3.3 39.9	4.4 38.8	5.5 37.7	6.6 36.6	7.7 35.5	8.8 34.4	9.9 33.3	11.0 32.2
1600	0.5 42.7	1.0 42.2	2.1 41.1	3.1 40.1	4.1 39.1	5.2 38.0	6.2 37.0	7.2 36.0	8.3 35.0	9.3 33.9	10.3 32.9
1700	0.5 42.7	1.0 42.2	1.9 41.3	2.9 40.3	3.9 39.3	4.9 38.3	5.8 37.4	6.8 36.4	7.8 35.4	8.7 34.5	9.7 33.5
1800	0.5 42.7	0.9 42.3	1.8 41.4	2.8 40.5	3.7 39.5	4.6 38.6	5.5 37.7	6.4 36.8	7.3 35.9	8.3 35.0	9.2 34.0
1900	0.4 42.8	0.9 42.3	1.7 41.5	2.6 40.6	3.5 39.7	4.3 38.9	5.2 38.0	6.1 37.1	6.9 36.3	7.8 35.4	8.7 34.5
2000	0.4 42.8	0.8 42.4	1.7 41.6	2.5 40.7	3.3 39.9	4.1 39.1	5.0 38.3	5.8 37.4	6.6 36.6	7.4 35.8	8.3 35.0

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Single Cell Gene Expression workflow: Loading Chip G

Load sample into row 1

- Prepare Master Mix and aliquot into strip tube
- Add water and cell suspension for each sample to Master Mix aliquot(s)
- Gently pipette mix Master Mix + water + Cell Suspension and load 70 µl into row labeled 1
- Proceed to prepare gel beads

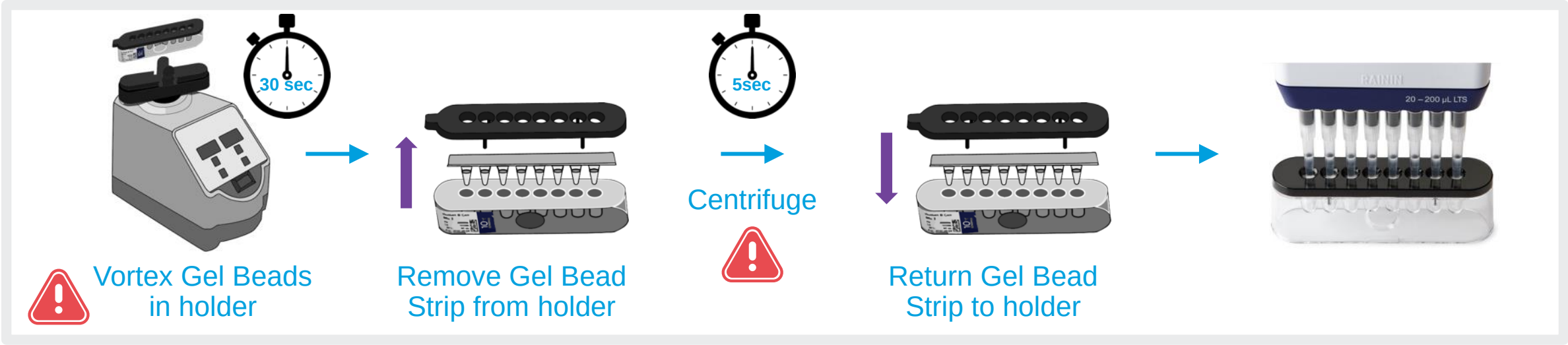


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Single Cell Gene Expression workflow: Loading Chip G

Preparing Gel Beads

- Snap the tube strip holder with the gel bead strip into a 10x Genomics Vortex Adapter
- After loading row 1 with cells and Master Mix, simultaneously prime row 1 microfluidic channels, while vortexing Gel Beads for 30 sec at highest speed
- Remove the Gel Bead Strip from the holder and centrifuge for ~5 sec. Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead Strip back in the holder. Secure the holder lid.
- Pierce foil and very slowly aspirate Gel Beads and dispense into row 2, avoiding bubbles

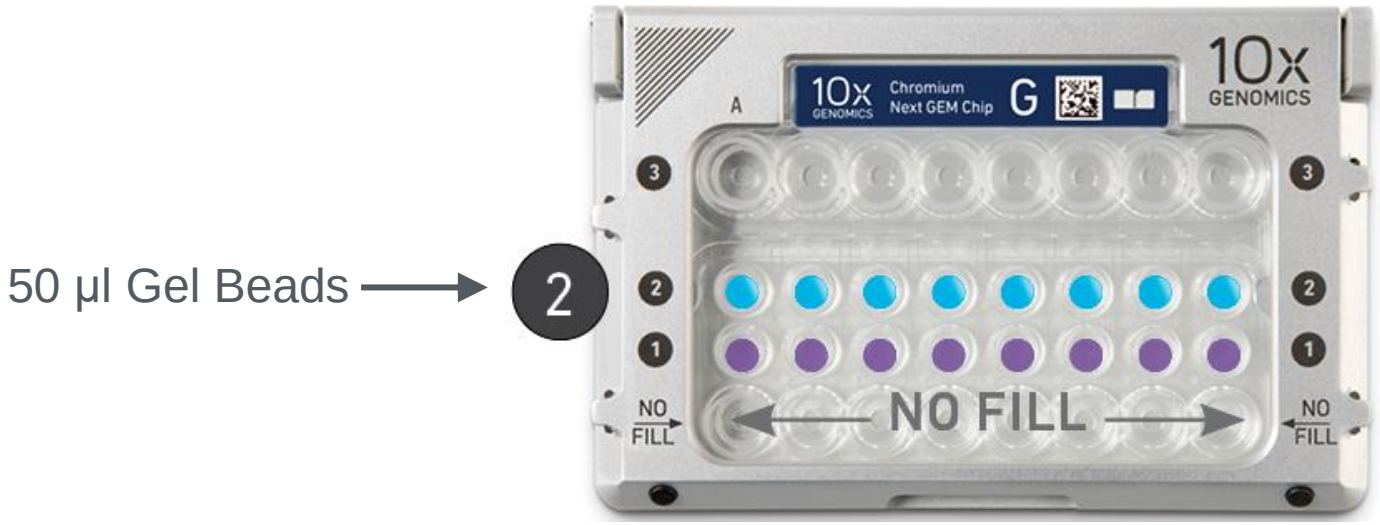


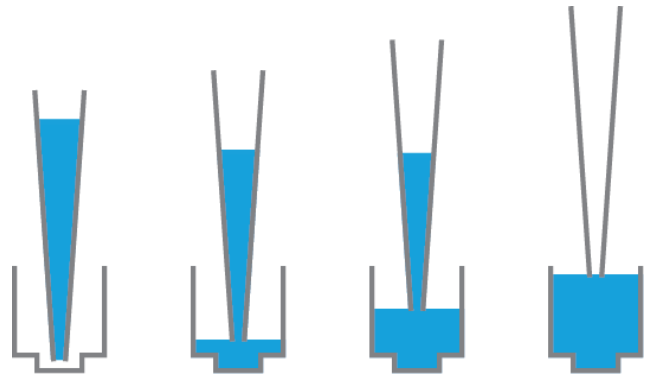
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Single Cell Gene Expression workflow: Loading Chip G

Load Gel Beads into row 2

- Pierce foil seal on the required number of Gel Bead Tubes
- Slowly aspirate 50 µl and dispense into row labeled 2
- **Wait 30 seconds** before proceeding to loading partitioning Oil





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Single Cell Gene Expression workflow: Loading Chip G

Load partitioning oil into recovery wells (row 3)

- Load 45 µl partitioning oil from a reservoir into row labeled 3



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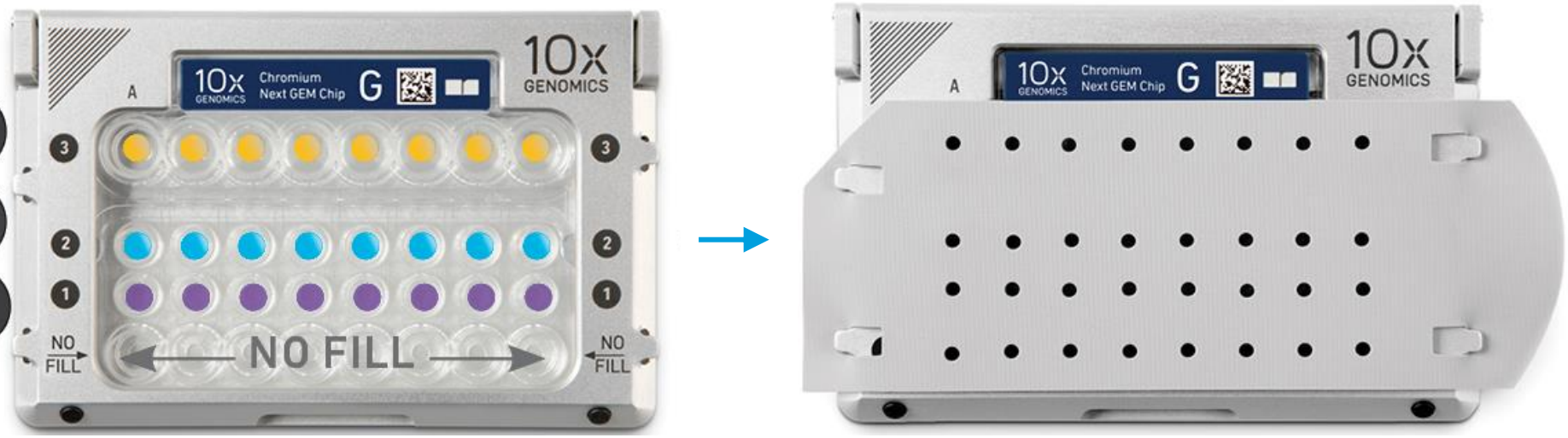
Single Cell Gene Expression workflow: Loading Chip G

Attach gasket

Partitioning oil 3

Gel Beads 2

Master Mix + sample 1

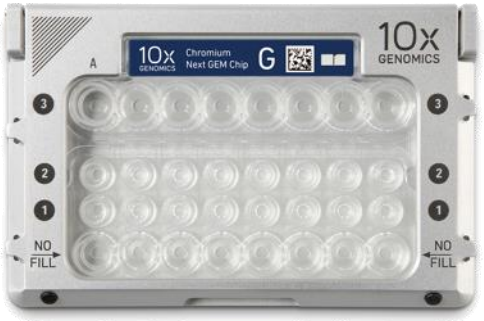


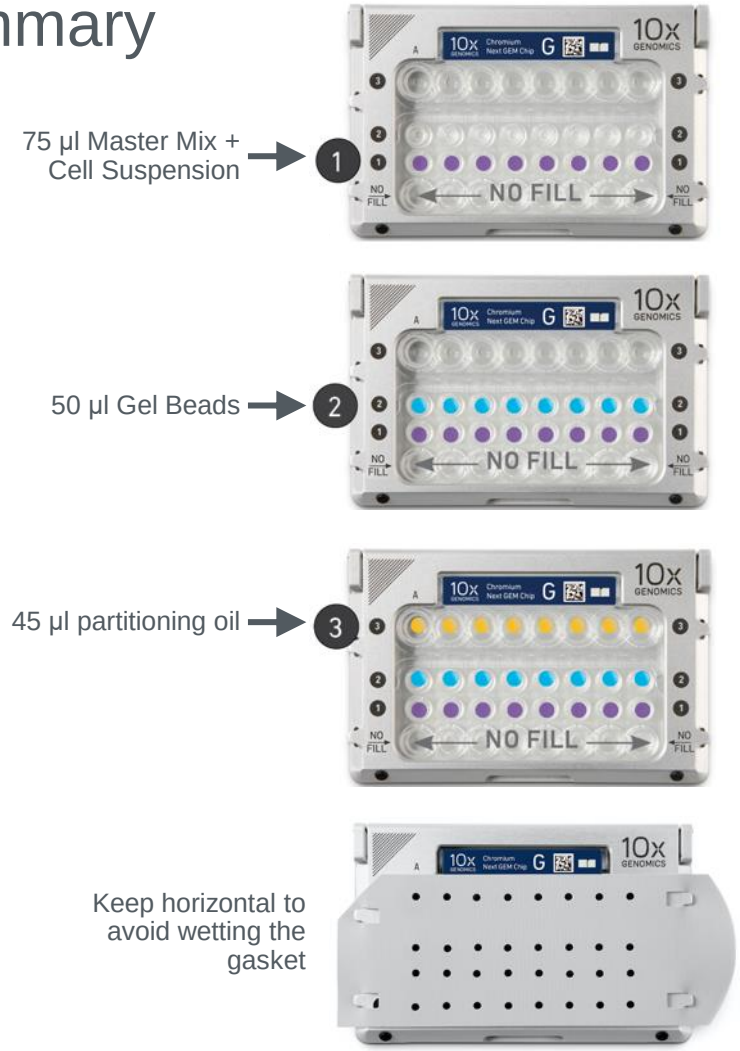
Keep horizontal to avoid wetting the gasket. **DO NOT** press down on the gasket

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Single Cell Gene Expression workflow

Chip G loading summary





Firmware 4.00 or higher is required for running this assay

- Assemble a chip into a chip holder and load all unused wells in rows 1, 2 & 3 with 50% glycerol
- Prepare and aliquot Master Mix
  - Add water to Master Mix
  - Add cells to Master Mix
    - **Gently resuspend each cell sample immediately before adding to Master Mix
- Load Chromium Chip G:
  - Load row 1: 70 µl cells and Master Mix
  - Prime chip and vortex Gel Beads for 30 seconds
  - Load row 2: 50 µl Gel Beads. Wait 30 seconds
  - Load row 3: 45 µl partitioning oil
  - DO NOT load reagents in the bottom row labeled NO FILL
- Attach 10x Genomics Gasket
- Run on Chromium Controller immediately after loading the partitioning oil (Run time ~18 mins)

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Single Cell Gene Expression workflow

Generating GEMs

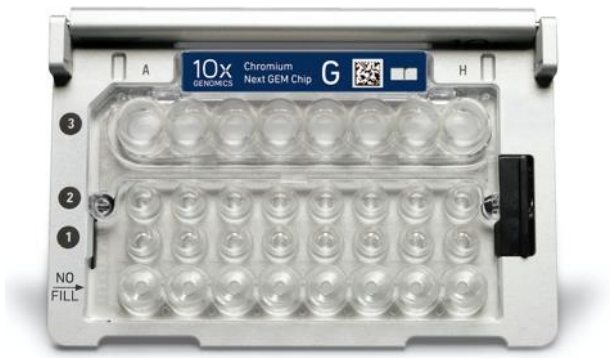
- Chips should be run immediately after loading
- Chip runtime is ~18 minutes
- Recover emulsions immediately after run is complete
- After transferring to a strip tube on ice, GEMs may be stored on ice for up to an hour before beginning the GEM-RT reaction



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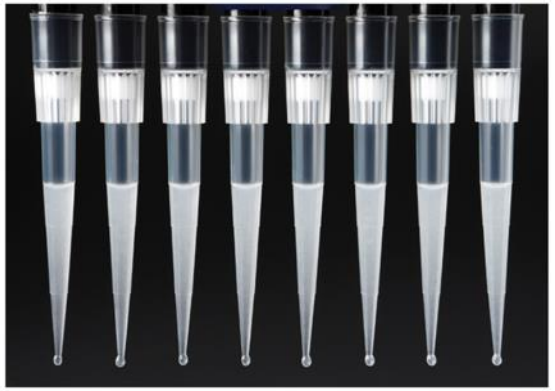
Single Cell Gene Expression workflow

Recovering GEMs



Expose wells at
45 degrees



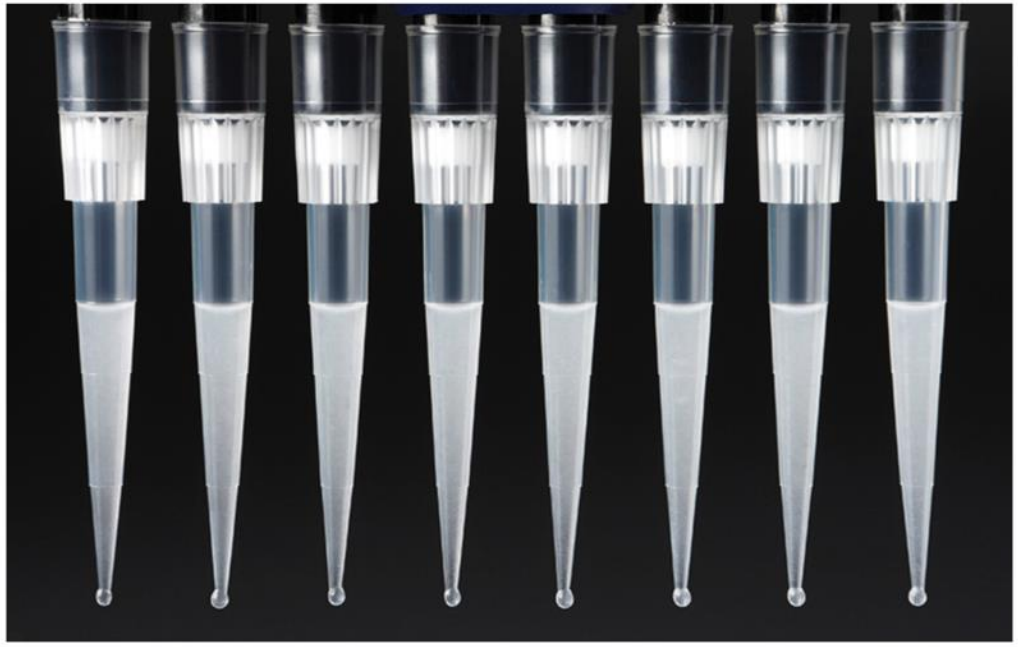


GEMs should appear
opaque and uniform across
all channels

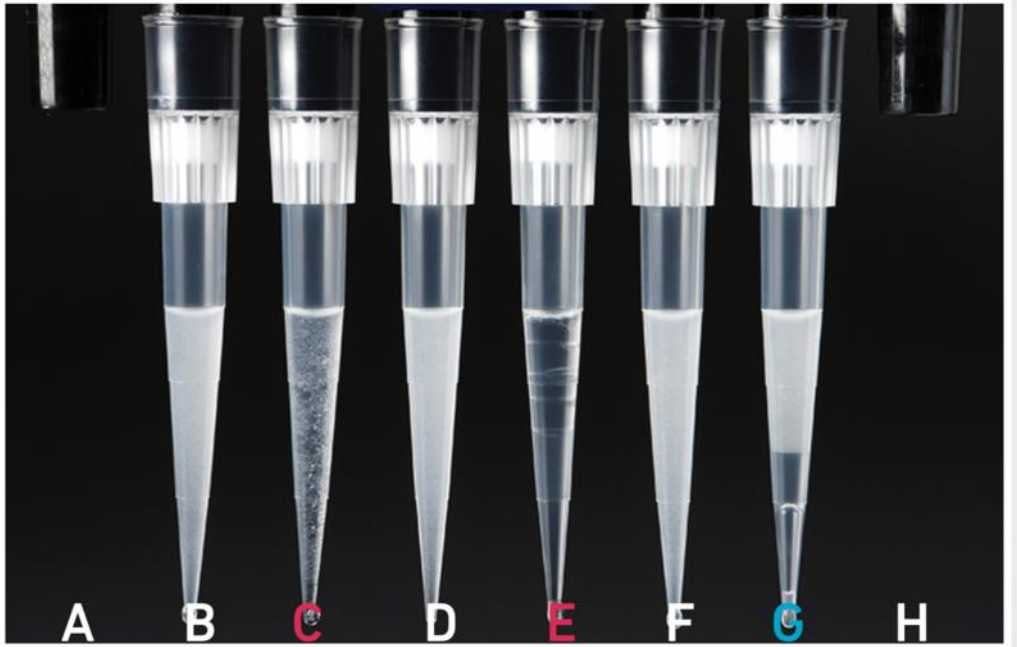
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Single Cell Gene Expression workflow

Visually inspect GEMS to confirm the presence of a uniform emulsion



- All liquid levels are similar in volume and opacity without air trapped in the pipette tips



- Pipette tips C and E indicate a wetting failure
- Pipette tip C contains partially emulsified GEMs
- Emulsion is absent in pipette tip E
- Pipette tip G indicates a reagent clog

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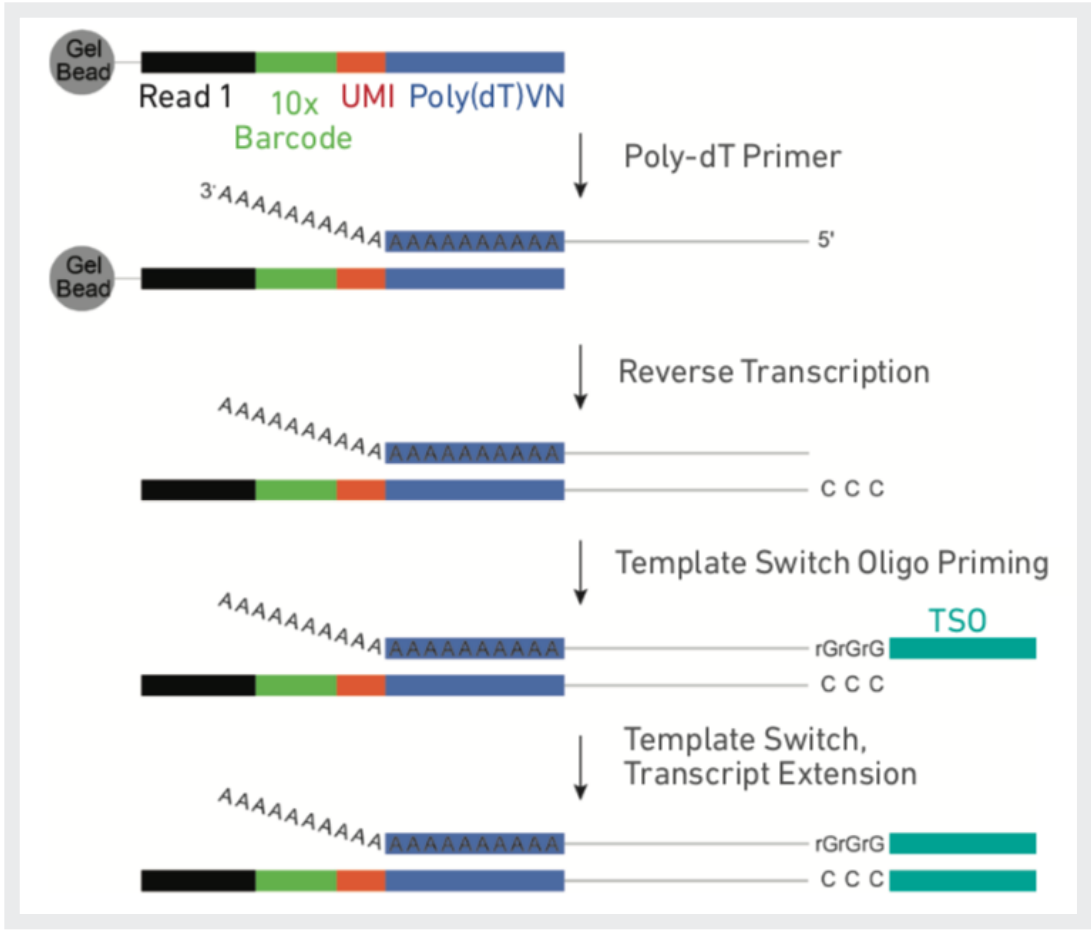
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Single Cell Gene Expression workflow

GEM RT incubation

Lid temperature	Reaction volume	Runtime
53°C	125 µl	~55 min
Step	**Temperature**	**Time**
1	53°C	0:45:00
2	85°C	0:05:00
3	4°C	Hold

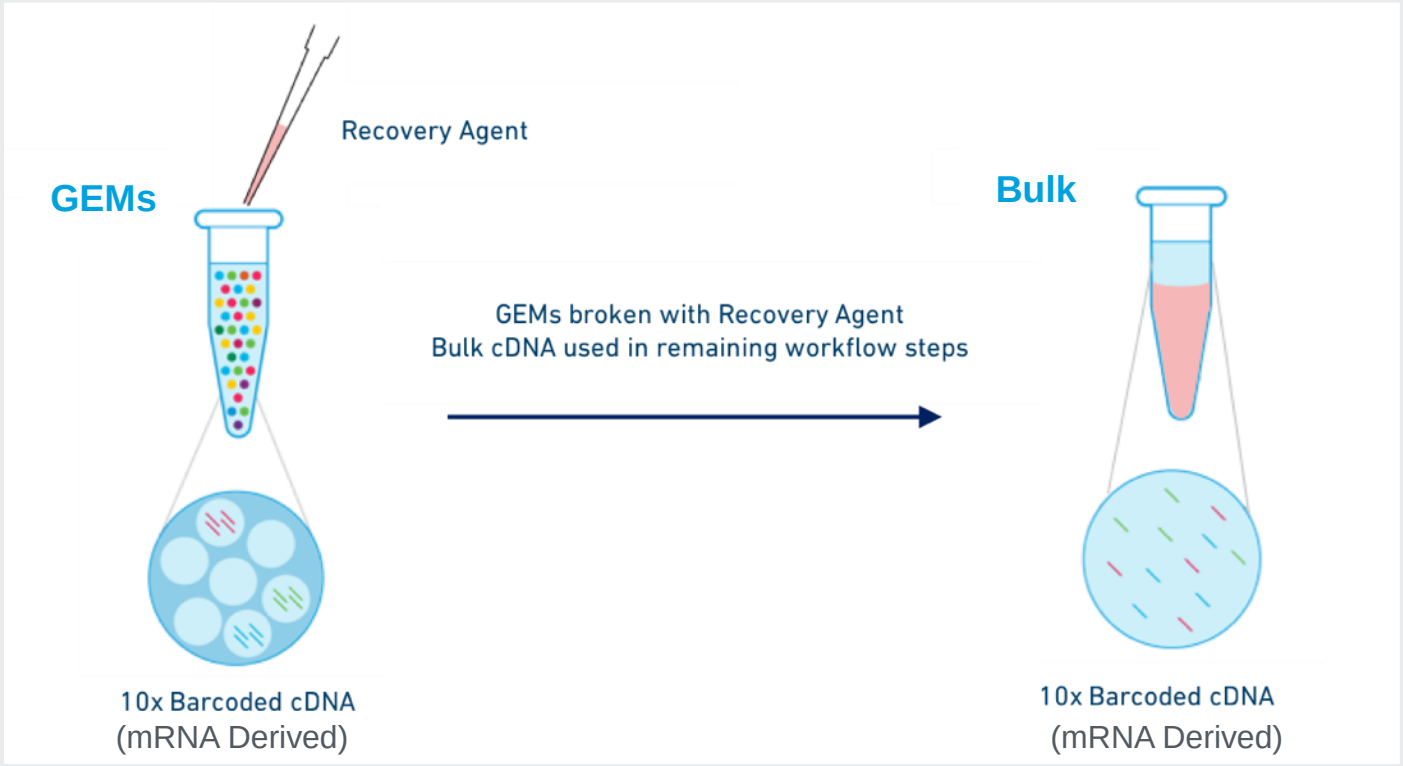
Inside individual GEMs



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Single Cell Gene Expression workflow

Breaking GEMs



Biphasic mixture



- Wait 2 minutes after adding recovery agent to each sample
- If biphasic separation is incomplete: firmly cap strip tubes, gently invert 5x, and centrifuge briefly

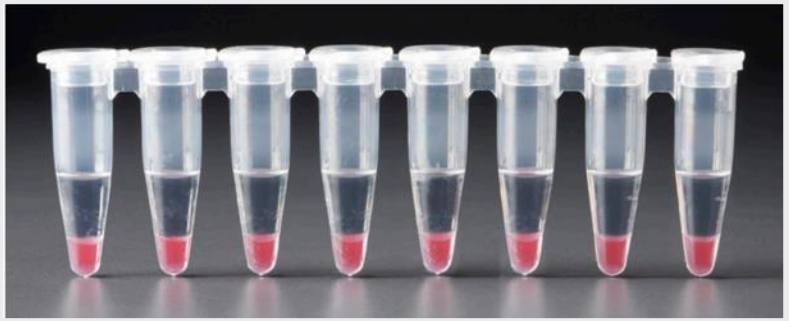
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Single Cell Gene Expression workflow

Post GEM incubation cleanup – Dynabeads

- Prepare Dynabeads Cleanup Mix
  - Vortex Dynabeads thoroughly for 30 sec
    - Confirm that Dynabeads have been fully resuspended by aspirating the full liquid volume with a pipette tip. If clumps are present, pipette mix to resuspend completely
  - Use only Reducing Agent B and Cleanup Buffer from Chromium Next GEM Single Cell Gene Expression Reagent Kits
- Always use fresh preparations of 80% ethanol
- Elute in 35 µl of Elution Solution I

After removing recovery agent (pink) from aqueous phase



After adding Dynabeads Cleanup Mix to aqueous phase



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Single Cell Gene Expression workflow

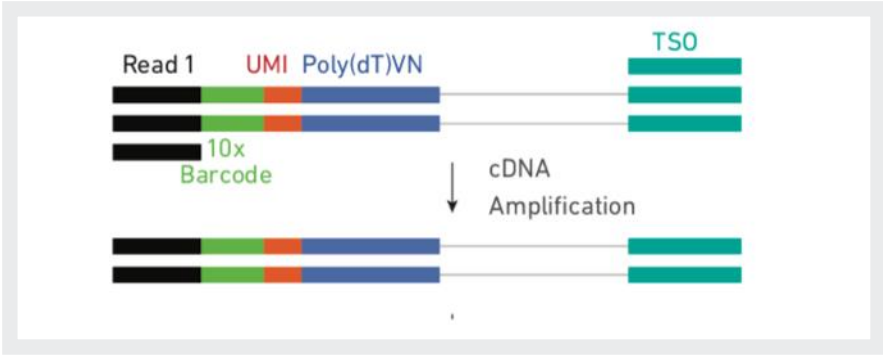
cDNA Amplification

- Prepare cDNA Amplification Reaction Mix
  - Primers must be selected based on whether generating only Gene Expression Libraries, or Gene Expression and Cell Surface Protein or CRISPR Libraries
- Add 65 µl cDNA Amplification Reaction Mix to 35 µl of sample

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30-45 min
Step	**Temperature**	**Time**
1	98°C	00:03:00
2	98°C	00:00:15
3	63°C Version Specific Updated Temperature	00:00:20
4	72°C	00:01:00
5	Go to Step 2, see table below for total # of cycles	
6	72°C	00:01:00
7	4°C	Hold

- Use cell load to determine number of total cycles for cDNA amplification

Pooled cDNA amplification



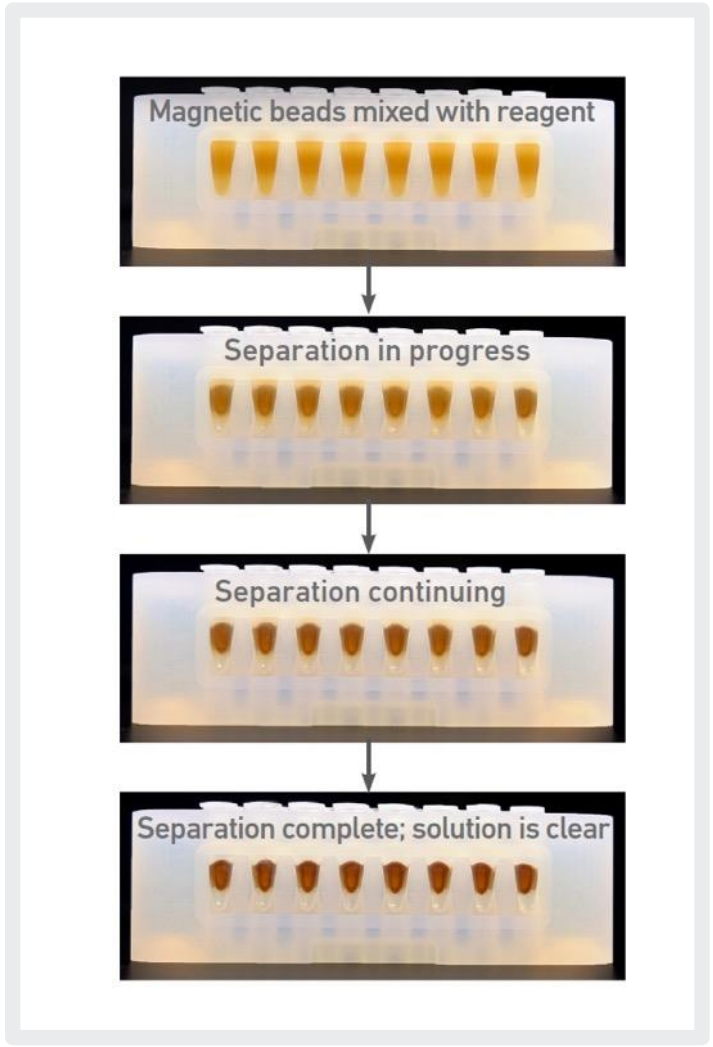
Cell load	Total cycles
<500	13
500–6,000	12
>6,000	11

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Single Cell Gene Expression workflow

SPRIselect cDNA cleanup

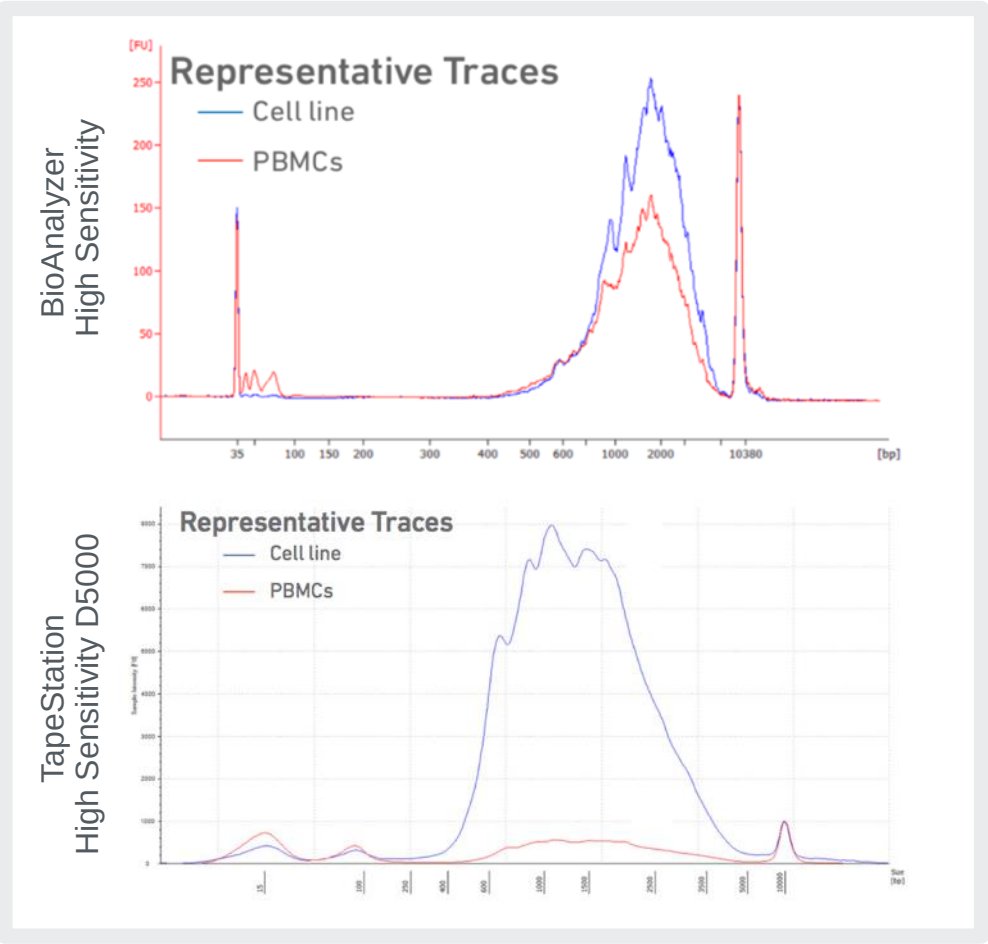
- Vortex SPRIselect Reagent thoroughly before adding to samples and ensure samples are fully mixed with beads
- If generating Cell Surface Protein or CRISPR screening libraries, **DO NOT** discard supernatant after the first bead incubation. See appropriate User Guide for application-specific guidance
- Always use fresh preparations of 80% ethanol
- Perform a 0.6x single-sided SPRI cleanup, eluting in 40 µl of Buffer EB



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Single Cell Gene Expression workflow

cDNA QC and quantification



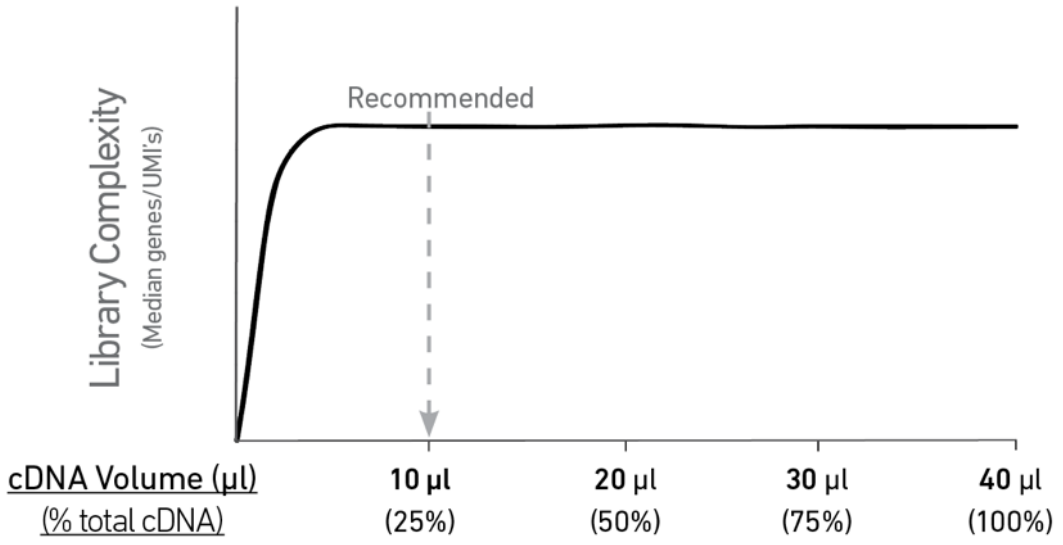
- For input cells with low RNA content (<1 pg total RNA/cell), 1 µl undiluted product may be run
- Lower molecular weight product (35-150 bp) may be present. This is normal and does not affect sequencing or application performance
- Carry forward **only 25%** of total cDNA yield into 3' Gene Expression Library construction
  - i.e. use only 10 µl of total 40 µl elution volume
  - Quantification (based on BioAnalyzer estimate) will be used to determine number of Sample Index PCR cycles
  - You can save remaining cDNA for future uses if desired

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Single Cell Gene Expression workflow

Carrying forward only 25% of total cDNA is sufficient to capture full library complexity

Correlation: cDNA input vs library complexity



Library construction input mass and SI PCR cycle examples

Cell Type	Targeted Cell Recovery	Total cDNA Yield (ng)	cDNA Input into Fragmentation		SI PCR Cycle Number
			Volume (µl)	Mass (ng)	
High RNA Content	Low	250 ng	10 µl	62.5 ng	13
	High	1900 ng	10 µl	475 ng	10
Low RNA Content	Low	1 ng	10 µl	0.25 ng	16
	High	200 ng	10 µl	50 ng	12

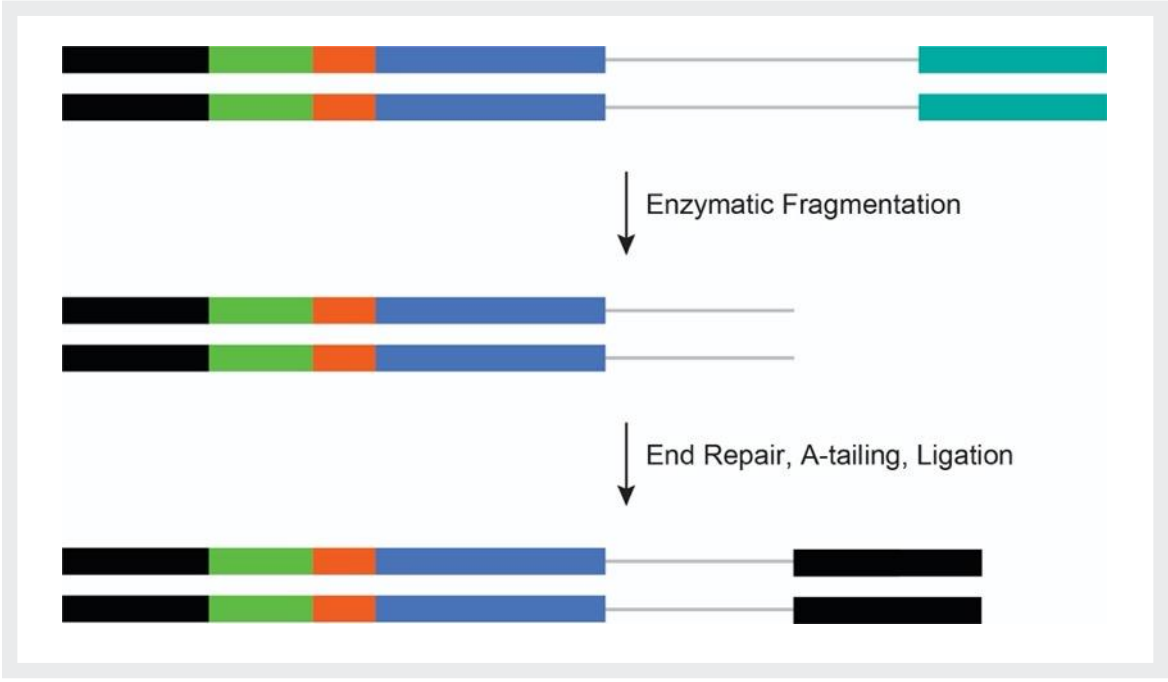
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Single Cell Gene Expression workflow

Library construction

- Fragmentation, end repair and A-tailing
  - It is critical to pre-chill the thermal cycler block to 4°C prior to assembling the reaction
- Double-sided SPRIselect cleanup
- Adaptor ligation
- Single-sided SPRIselect cleanup
- Proceed to Sample Index PCR

Pooled library construction



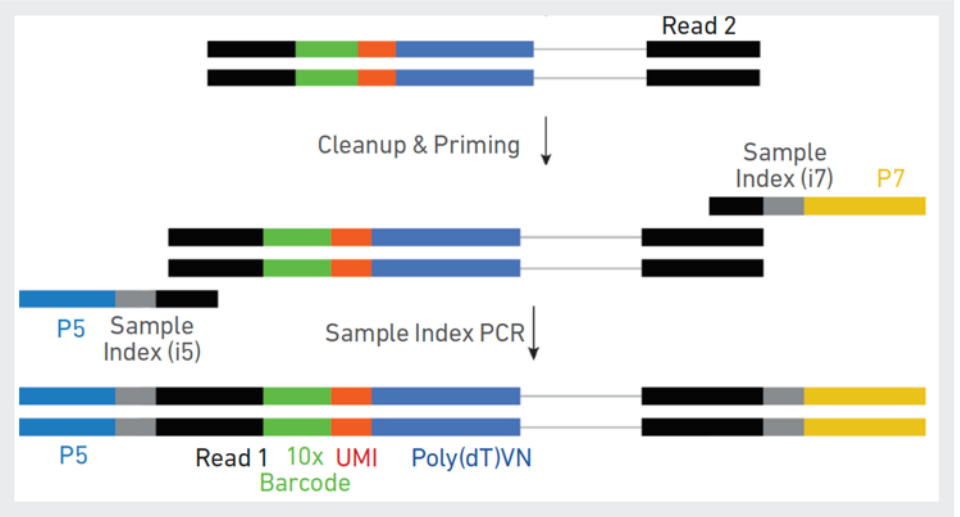
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Single Cell Gene Expression workflow

Sample index PCR

- Uses Dual Index Kit TT Set A
- Record the Well ID used for each sample. This is needed for de-multiplexing the sequencing data
- Incubate on the thermal cycler using the appropriate number of cycles
  - cDNA input informs total cycles
  - **Should be optimized based on cDNA mass (ng) carried forward** (25% of total cDNA yield)
- Double-sided size selection with SPRIselect (0.6X, 0.8X) to remove excess primers and optimize final library size distribution

Pooled library construction



Recommended cycle numbers

cDNA input	Total cycles
1–25 ng	14–16
25–150 ng	12–14
150–500 ng	10–12
500–1,000 ng	8–10
1,000–1,500 ng	6–8

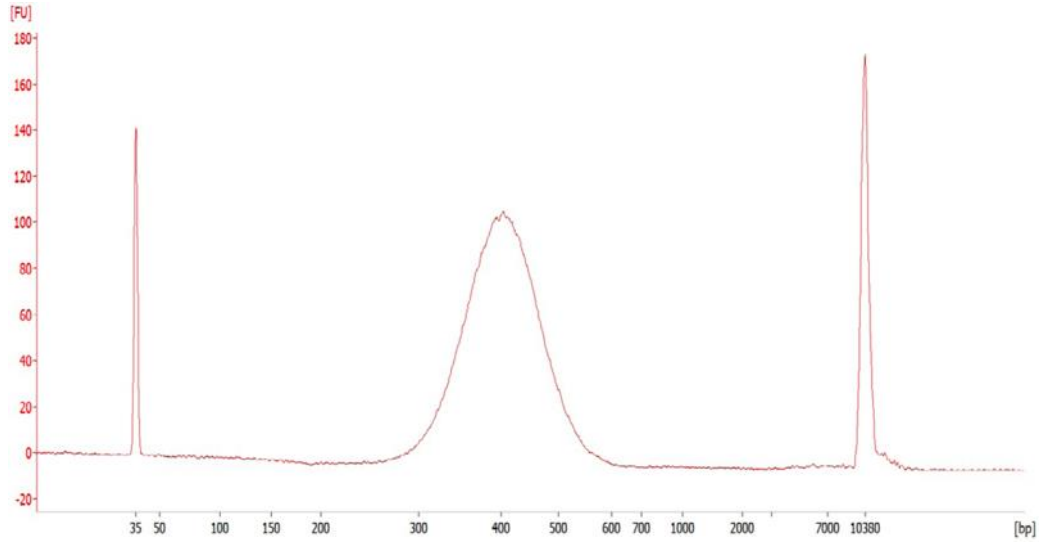
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Single Cell Gene Expression workflow

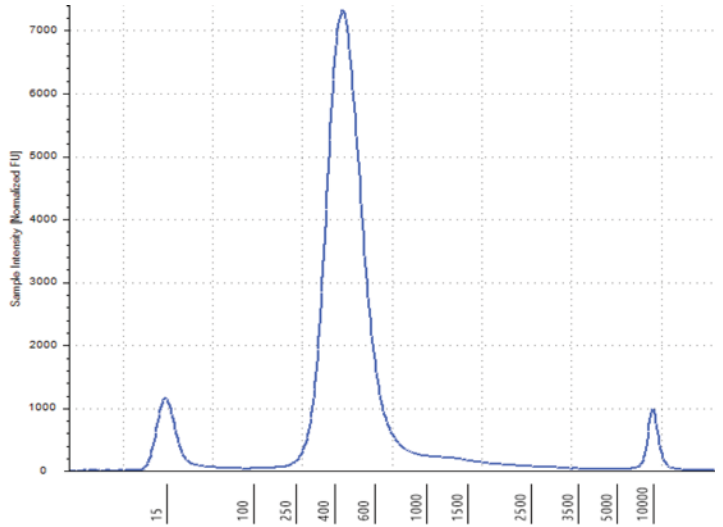
Post library construction QC and quantification

- Final library shows size distribution between ~300–600 bp
- Libraries should be quantified by qPCR, using the KAPA Library Quantification Kit for Illumina Platforms

Representative Trace (Bioanalyzer)



Representative Trace (TapeStation)



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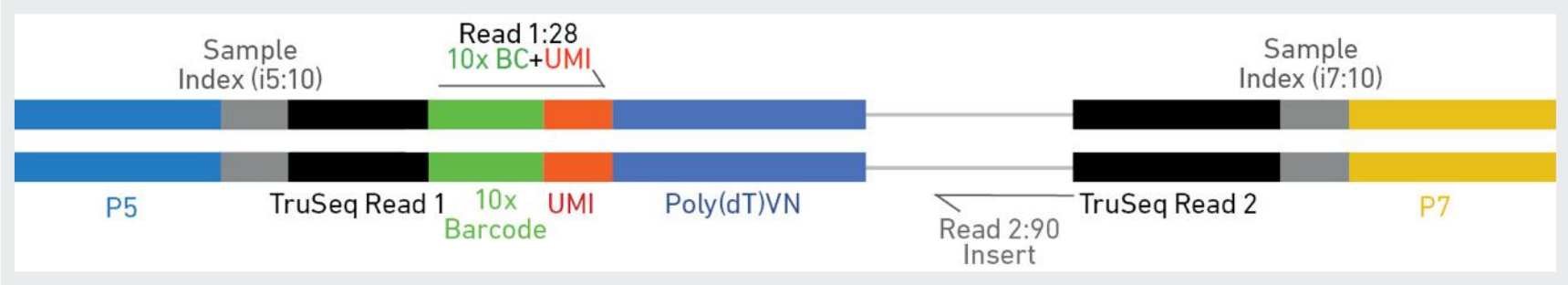
Sequencing specifications

Single Cell 3’ Gene Expression Libraries

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Recommended read length

Single Cell 3’ Gene Expression Library

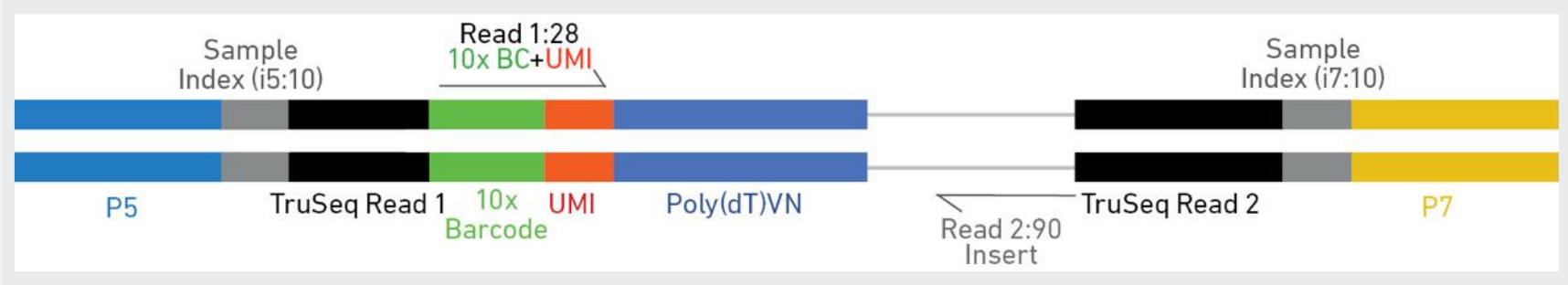


	Read 1	i7 Index	i5 Index	Read 2
Purpose	10x Barcode & UMI	Sample Index	Sample Index	Transcript
Length	28	10	10	90

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Supported sequencers

Single Cell 3’ Gene Expression Library



	Single Cell 3’ Gene Expression
Raw Read Pairs per Cell	Minimum 20,000



MiSeq



NextSeq 500/550



HiSeq2500



HiSeq 3000/4000



NovaSeq

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Sequencing recommendations: Pooling guidelines

Single Cell 3’ Gene Expression Libraries

The following **dual index** libraries can be pooled for Illumina sequencing:

- Single Cell 3’ v3.1 Dual Index Gene 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 Libraries*
- Spatial Gene Expression Libraries

* If run with 28 cycles for Read 1

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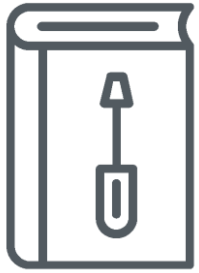
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Reference materials and resources

Available at support.10xgenomics.com



Specifications



User guides



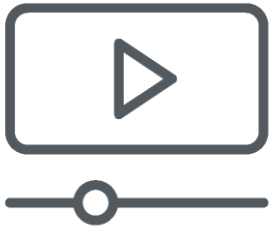
Product sheet



Technical notes



Public data sets



How-to-videos



Demonstrated protocols

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10x Genomics Support contact information

Support Overview:
http://support.10xgenomics.com

Q&A Knowledgebase:
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