IRB BARCELONA
INSTITUTE FOR RESEARCH IN BIOMEDICINE

IRB Barcelona
Functional Genomics Core Facility

SCENTINEL WORKSHOP I

September 2-6, 2024

Freddy Monteiro, PhD

IRB BARCELONA
INSTITUTE FOR RESEARCH IN BIOMEDICINE

Workshop Schedule with FGCF

Genomics	Monday	Tuesday	Wednesday	Thursday	Friday
Morning		fly prep (9-10) cell dissoc. (10-12) assay (~13h)	cDNA synthesis ? + purif. cDNA QC Library ampl. + purif.	Library QC? Conclusions Reporting	Free time + service details + quotations + questions
Lunch					
Afternoon	Intro session Freddy TD room 2 15-17:30h	buffer time	buffer time	Reporting	
		cDNA synthesis?	Library QC?	Questions	
	leave before 18h	*leave before 18h*	*leave before 18h*	*leave before 18h* *Dinner ?*	

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Location

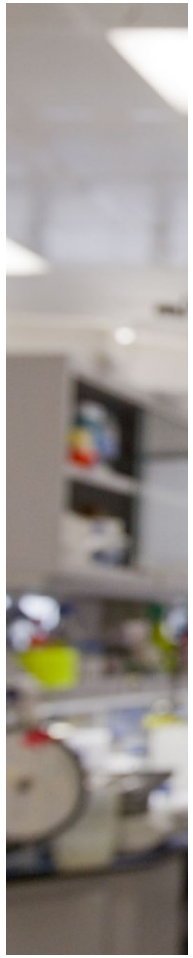
- PCB – Parc Científic de Barcelona

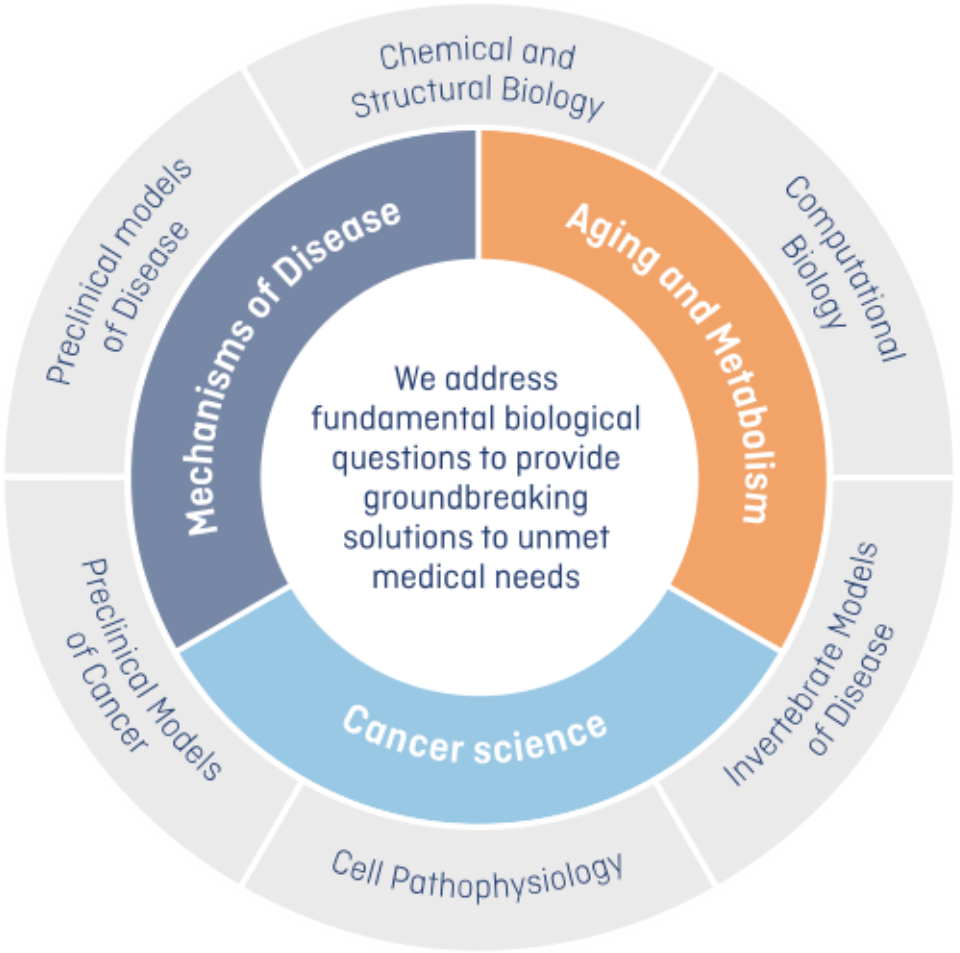
Location

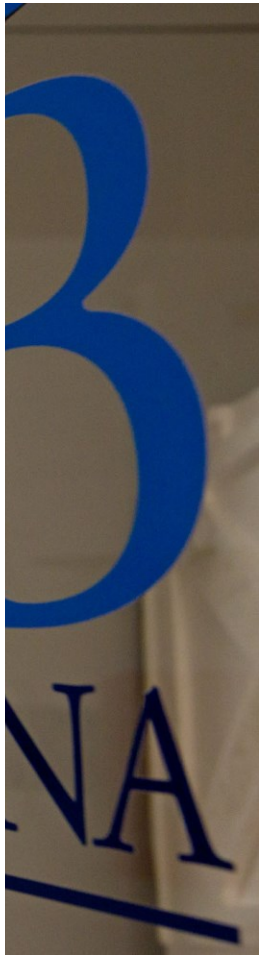
- PCB – Parc Científic de Barcelona

Institut de Recerca Biomèdica (IRB Barcelona)

Parc Científic de Barcelona. C/ Baldiri Reixac 10. 08028 Barcelona







523 Professionals

451 Scientific Staff

57% Women **43%** Men

3 Research Programmes

- Aging and Metabolism
- Mechanism of Disease
- Cancer Science

28 Research groups

9 Core facilities

6 Active spin-offs

https://www.irbbarcelona.org/annualreport2022/

Location

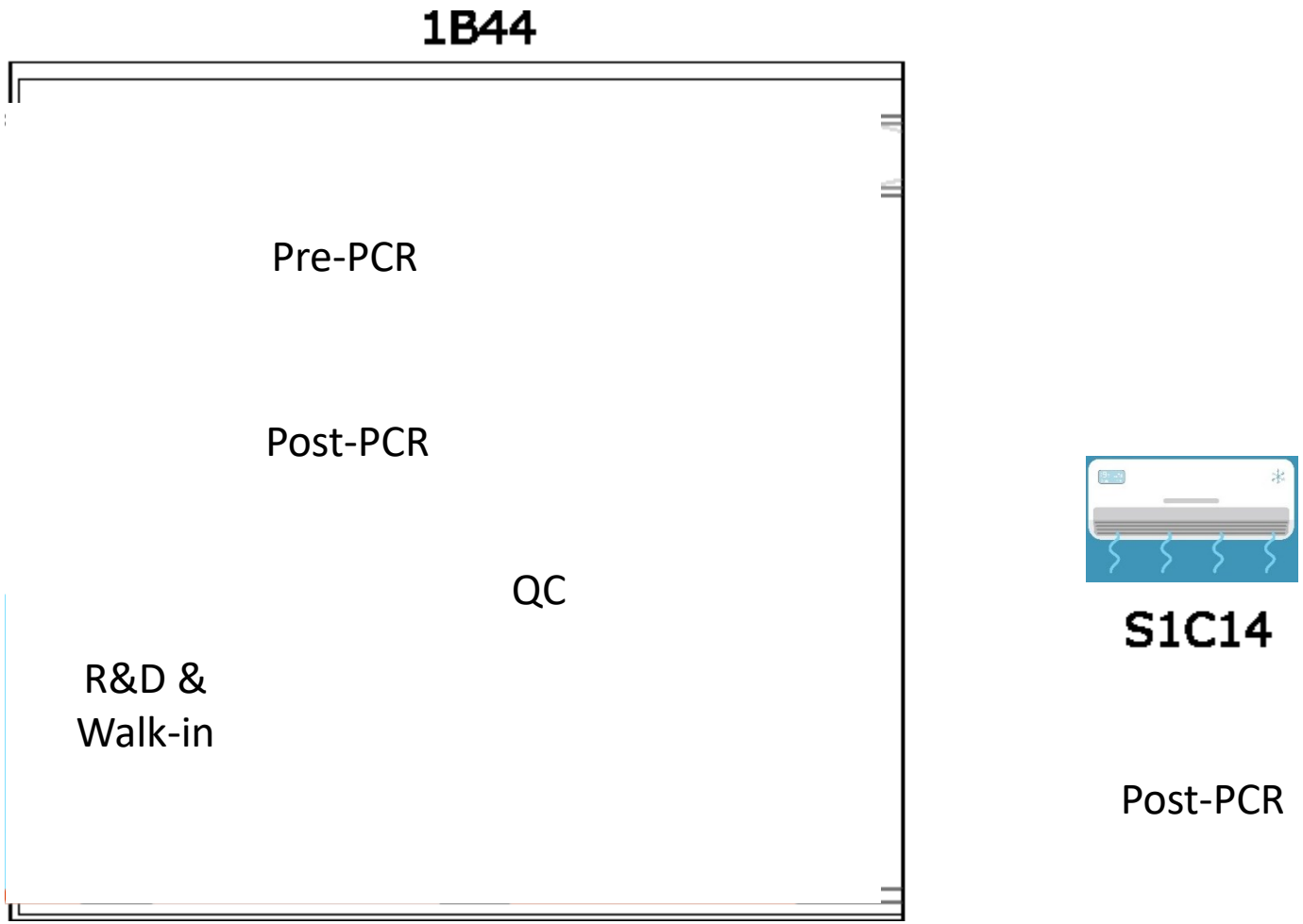
- Cluster I. Main Floor (1). Room 1B44.

Carrer de Josep Samitier

Carrer de Baldiri Reixac

Location

- Workflows distribution



S1C14

Post-PCR

Location

- Mercat del Peix 2026 (?)

Functional Genomics Core Facility (FGCF)

Team

Nacho Pons, Ph.D.
Senior Research officer

David Fernandez
Technical officer

Quim Perdices
Technical officer

Mariem Dris
Technical officer

Cecilia Garcia
Research Assistant
(Comp. Genomics Lab)

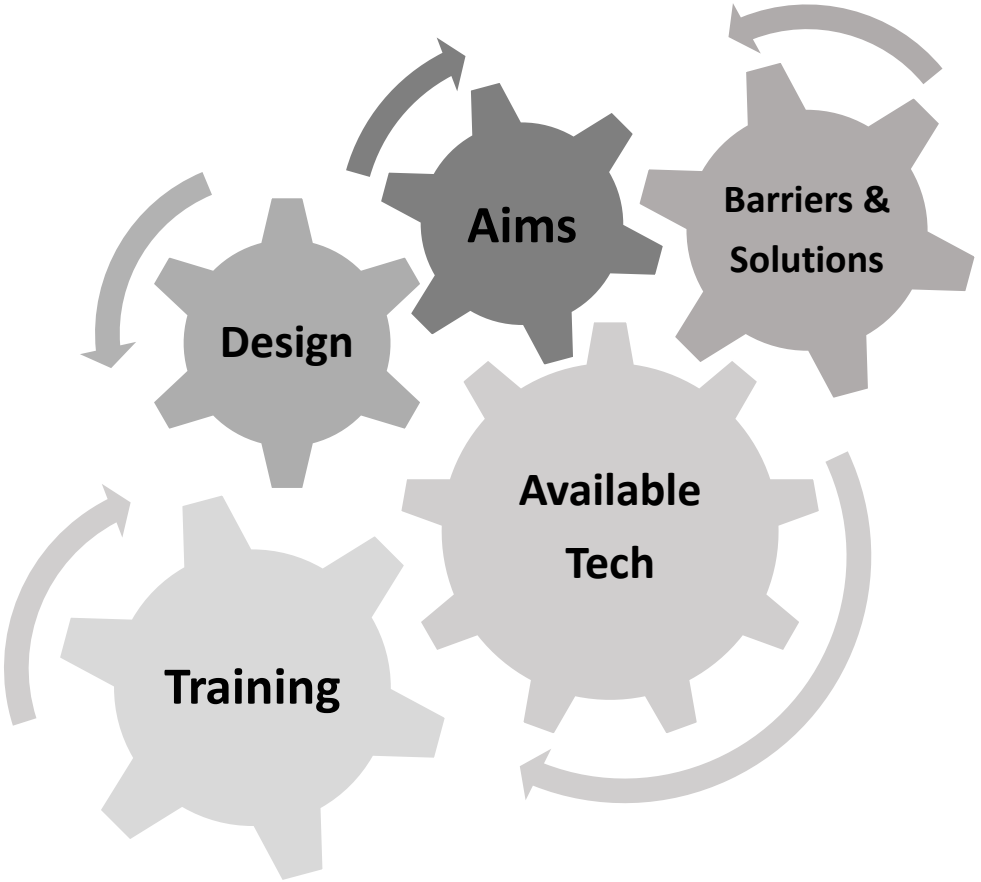
Freddy Monteiro, Ph.D.
FGCF Manager

genomics@irbbarcelona.org

https://www.irbbarcelona.org/en/research/functional-genomics

How to engage with us

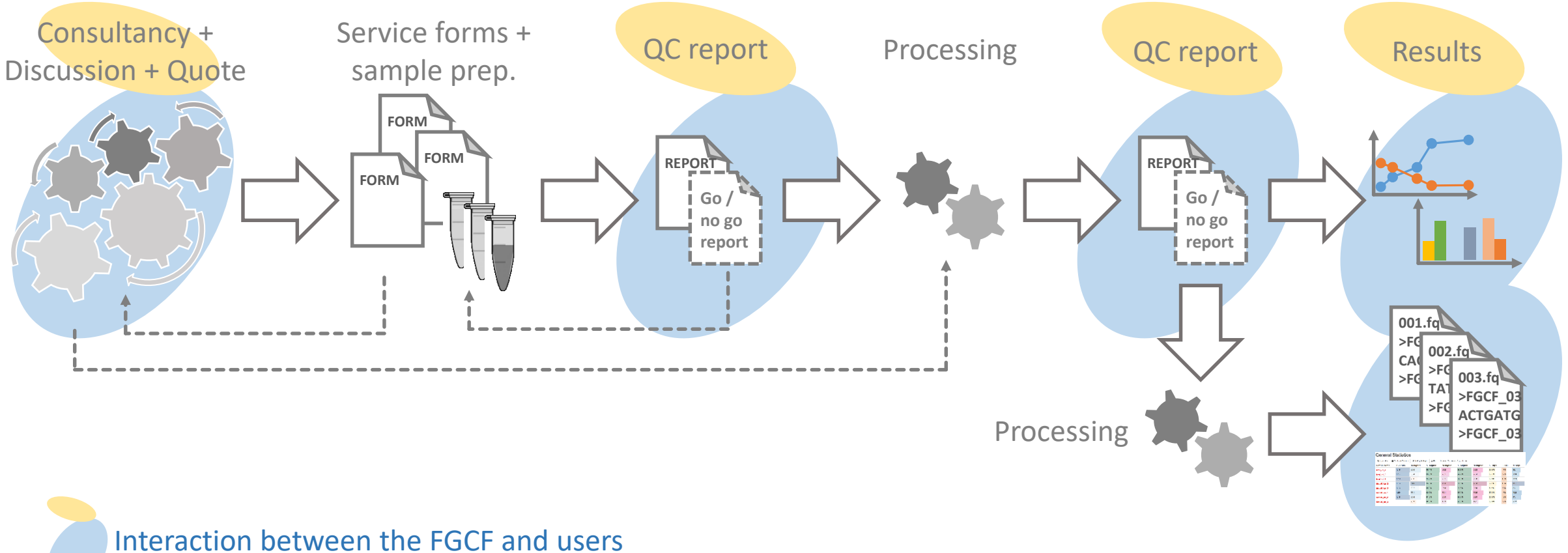
- *I am planning an experiment to genotype…?*
- *What are the sample requirements for…?*
- *How to remove clumps from my cell suspension?*
- *How to make sure cells are viable for the experiment?*
- *Should I use paired-end or single-end sequencing?*
- *Is this amount of cells enough for the experiment?*
- *What is the recommended sequencing depth?*
- *Quotes for service conditions with x samples and y conditions?*
- *Turn-around-time for results?*
- *“We did not expect this result… ”*
- *Can we try a different approach?*



We will align our pipelines to your needs and will be happy to troubleshoot new methodologies

genomics@irbbarcelona.org | (9340)-39803

Project life cycle



Interaction between the FGCF and users

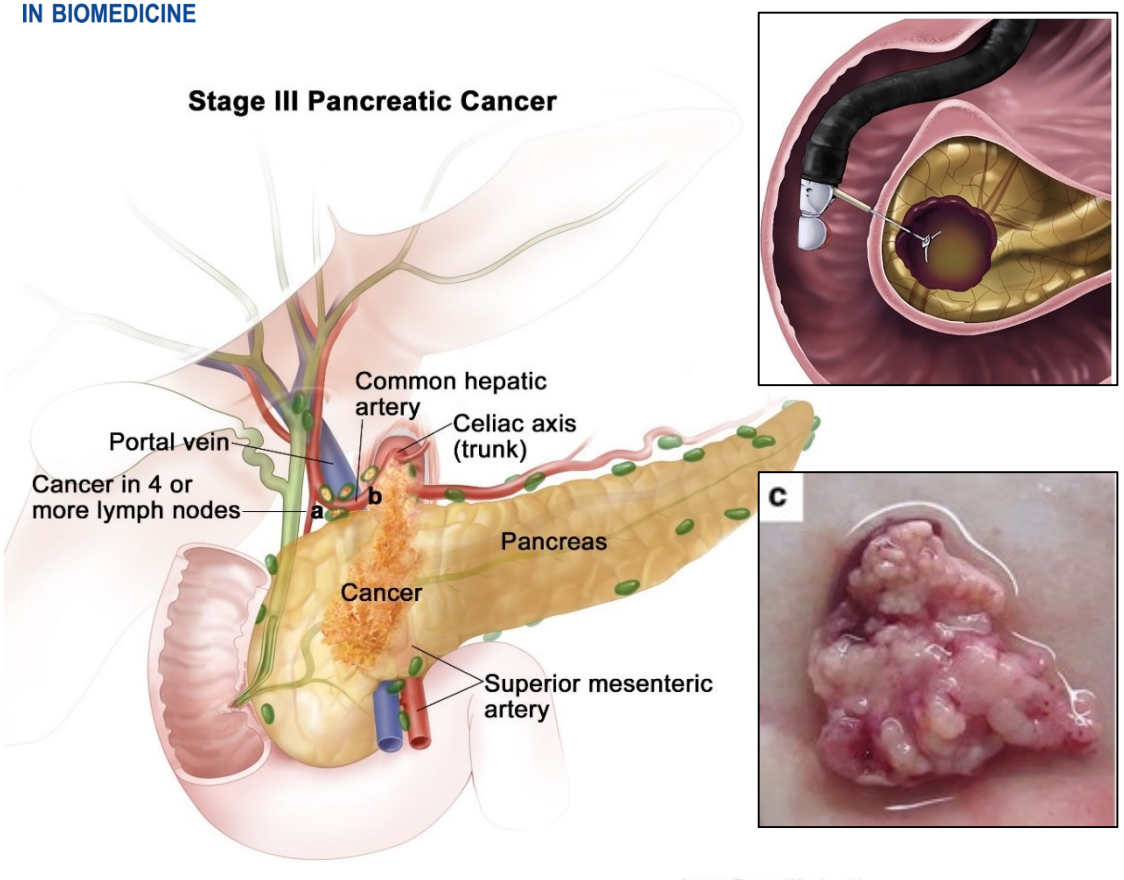
Why Single-Cell ?

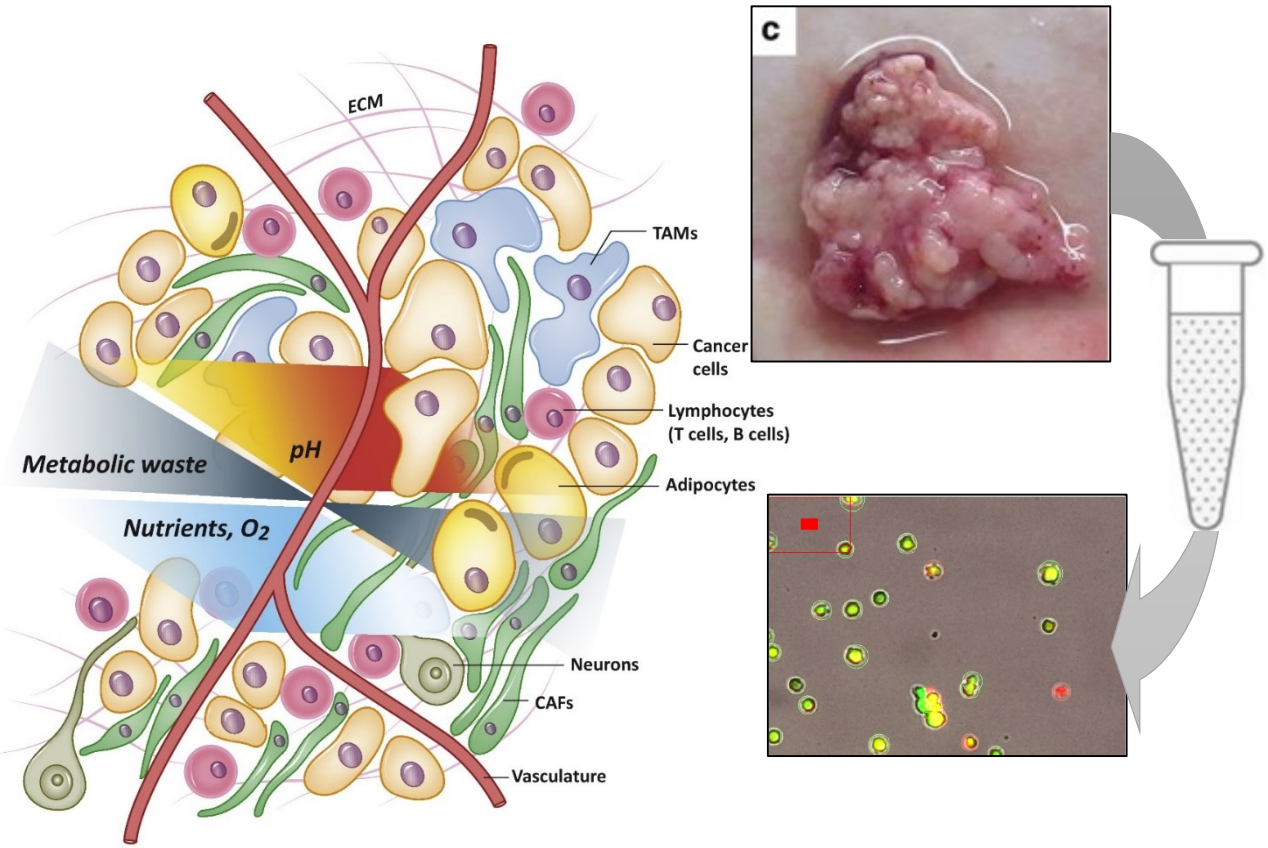
Bulk RNA-seq
=
Transcriptional Smoothie
=
Average gene expression of ALL cells

scRNA-seq
=
Transcriptional Fruit Salad
=
Gene expression of each cell
+
Absolute abundances

Image source: https://www.theblackpeppercorn.com/frozen-fruit-salad-smoothie/

Bulk RNA-seq or single-cell RNA-seq

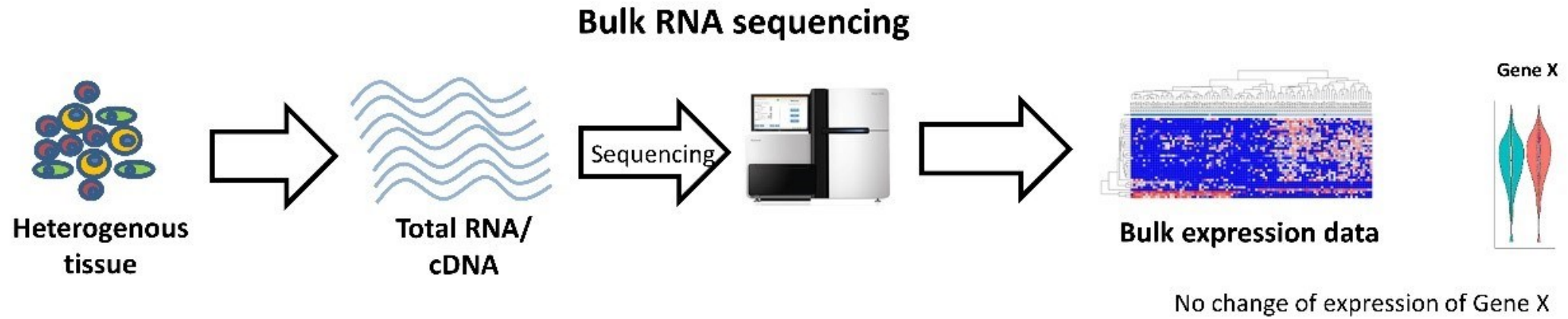


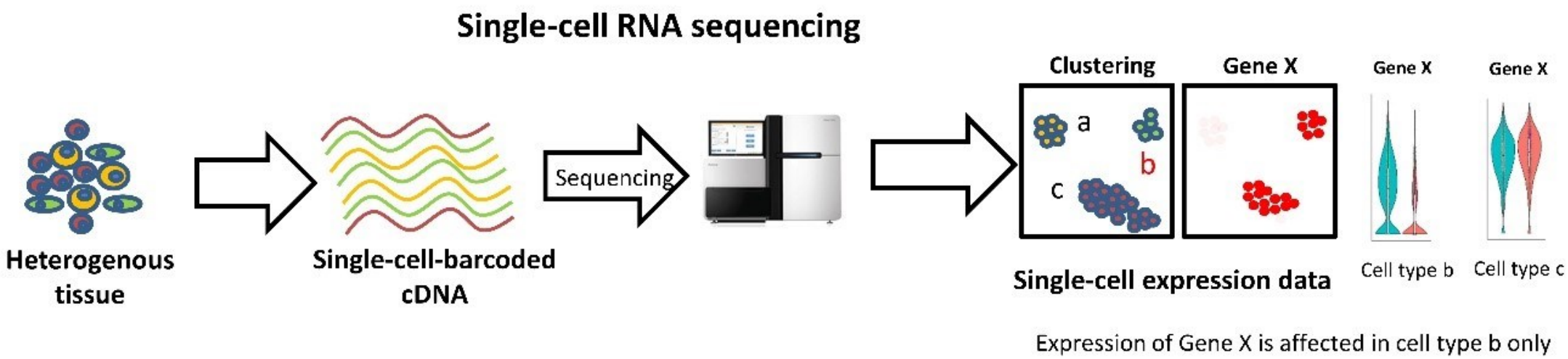


https://www.cancer.gov/types/pancreatic/patient/pancreatic-treatment-pdq
Cho et al. 2022
Payne et al. 2022

Lyssiotis and Kimmelman. 2017
Payne et al. 2022

Bulk *Vs* Single-Cell RNA-Seq





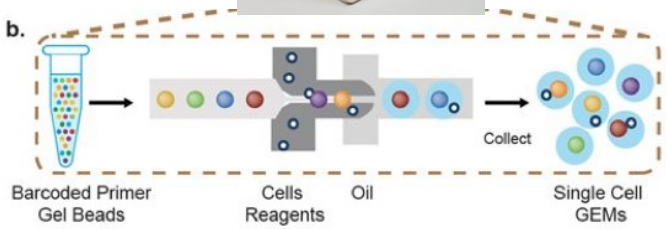
+INFO: https://www.singlecellcourse.org/

scRNAseq and multiomic possibilities @ the FGCF

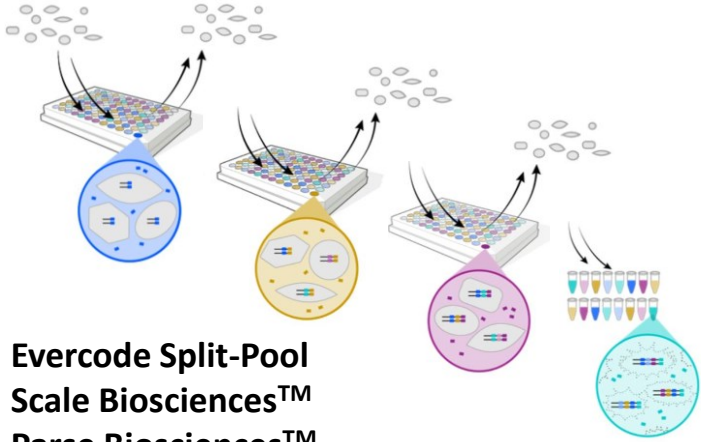
Single Cell Transcriptomics, Epigenomics and Multiomics

LUNA-FL
Cell counter

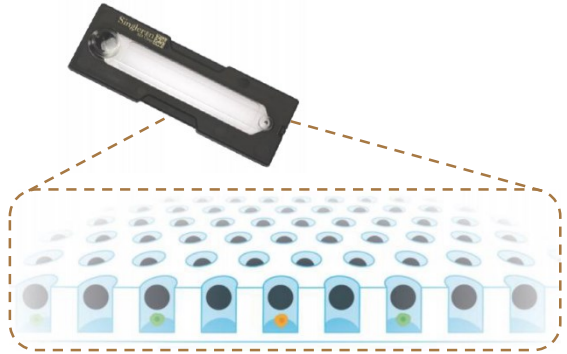
Chromium Controller
10x Genomics™



Matrix
Singleron Biotechnologies™



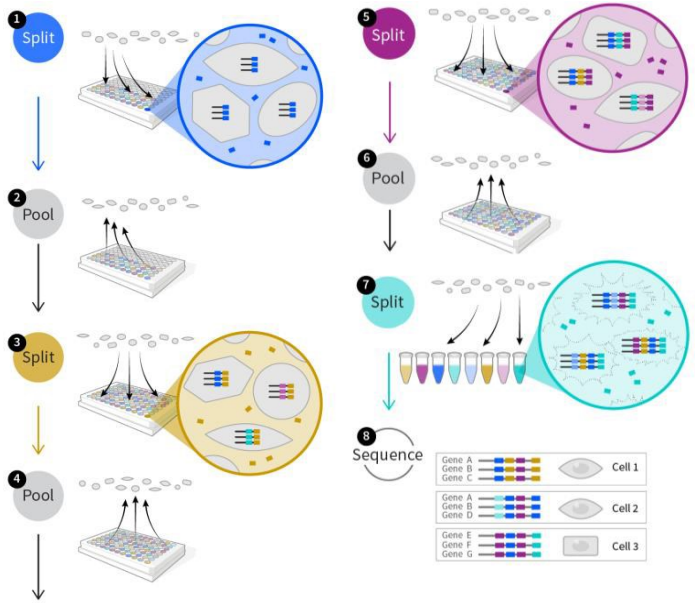
Evercode Split-Pool
Scale Biosciences™
Parse Biosciences™



Split-pool

Single-cell transcriptomics and epigenomics

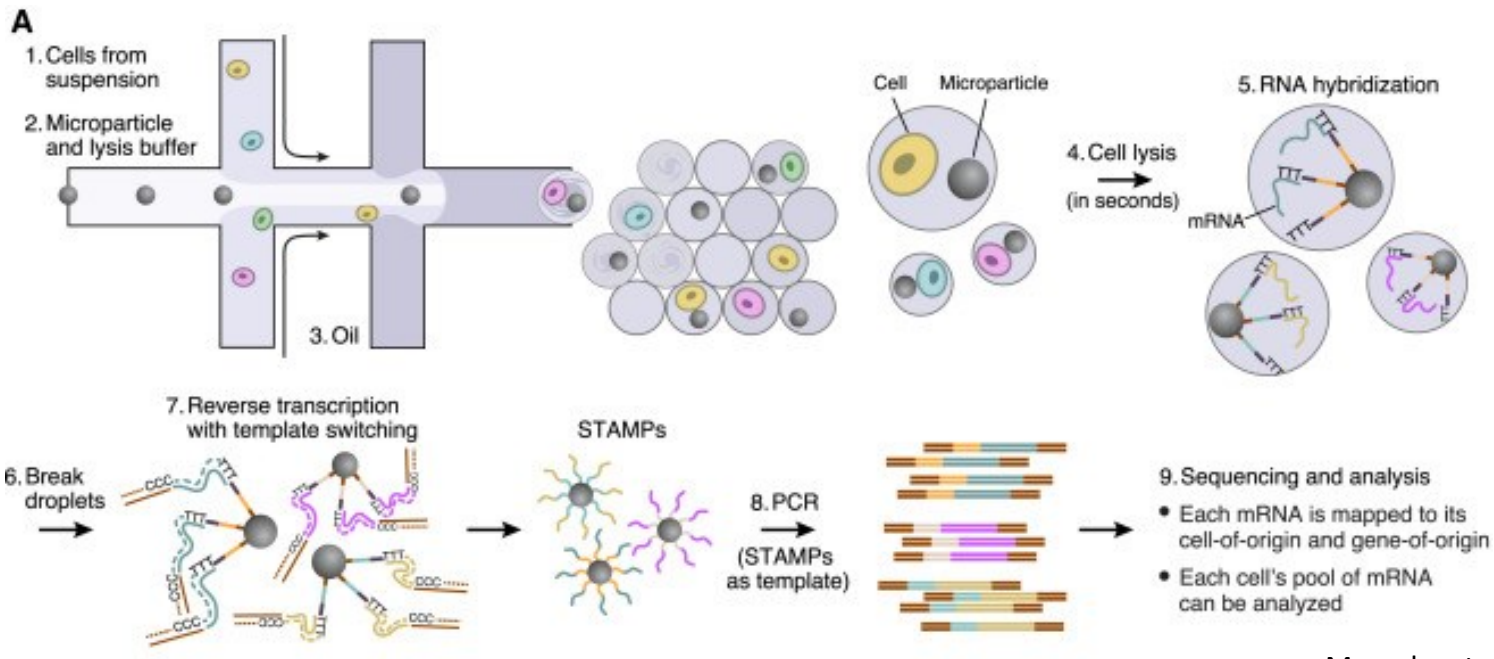
- Combinatorial indexing solution for large-scale projects that aim to profile up to 100,000 cells/nuclei, across 1 to 48 samples
- Fixation solution for sample storage that enables pooling of multiple samples from different time points into a single experiment.
- Lower multiplets than microfluidics-based methodologies



Droplets

Single-cell transcriptomics and epigenomics

- Microfluidics technology from 10x Genomics for sc whole/targeted transcriptome interrogation, immune profiling, assay for transposase accessible chromatin, and Multiomics.
- Fast (18 minutes encapsulation + 9 hours library preparation)
- High-throughput (100-10.000 cells)

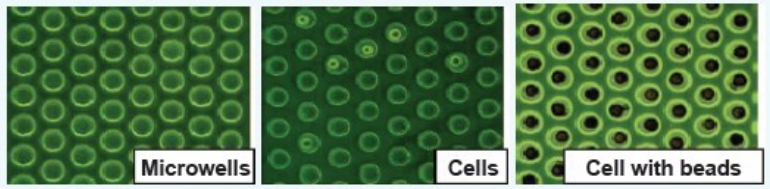


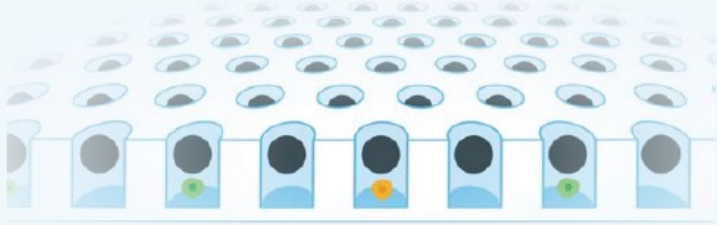
Macosko et al. Cell 2015.

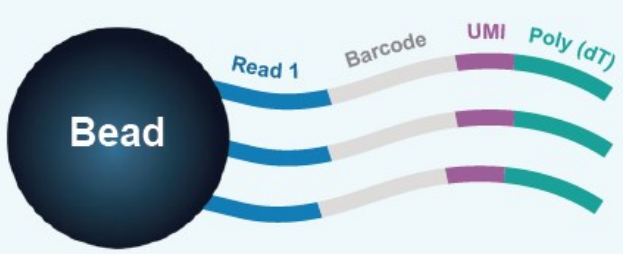
Microwells

Single-cell transcriptomics and epigenomics

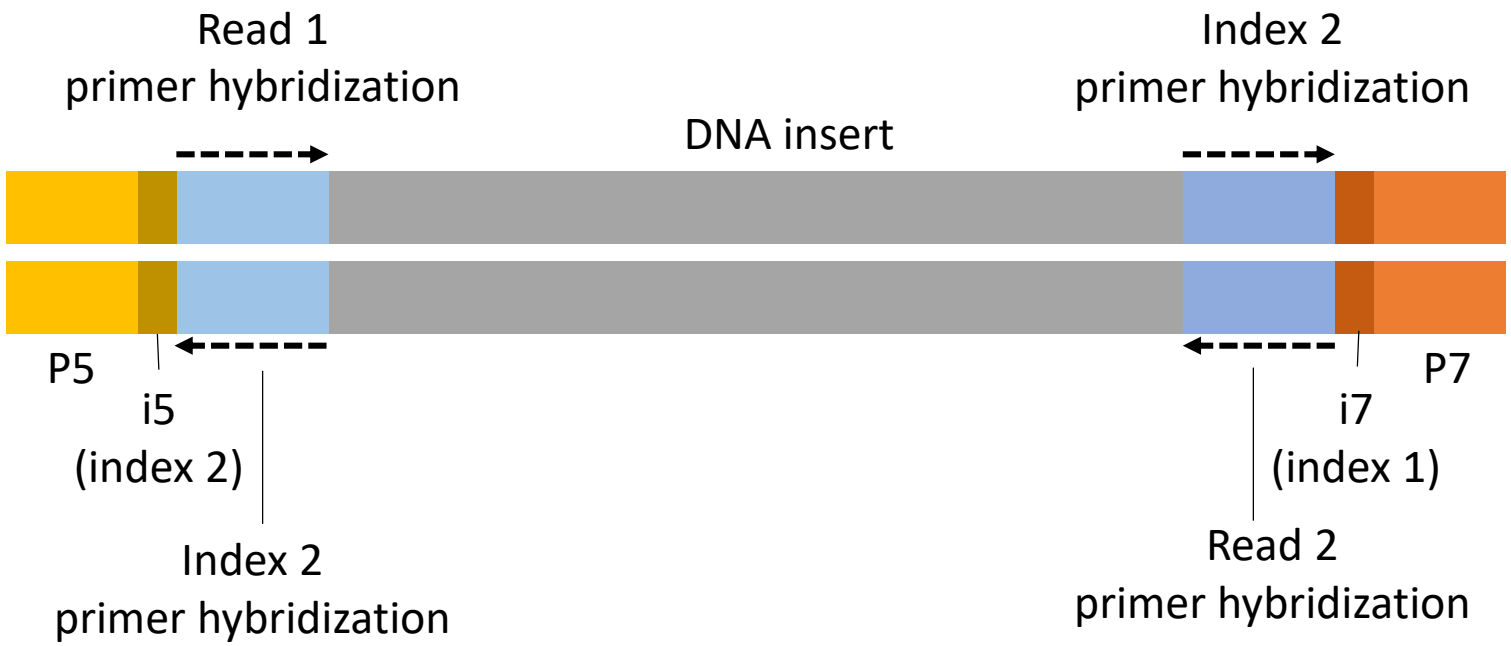
- SCOPE-chip from Singleron captures single cells by partitioning them into hundreds of thousands of microwells
- Standard chip: 500-10,000 single cells; High-density chip up to 30,000 cells per sample, or up to 120,000 cells on one HD chip when multiplexing samples with CLindex
- Large-well chips ensure analysis of cell sizes up to 100 µm
- Manual or Automated workflow.







Archetypal Illumina library structure



P5 & P7 are required for *clustering*

Primer-binding regions are required for sequencing

Unique i7/i5 indexes are required for combining multiple samples (multiplexing)

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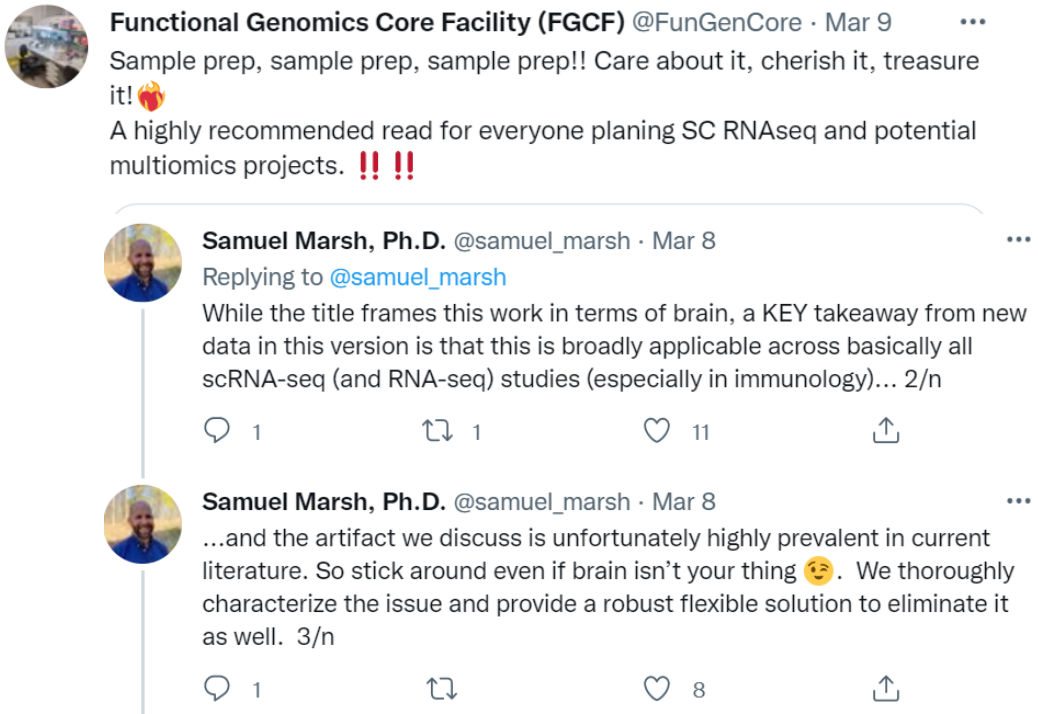
Sample preparation is (by far) *THE MOST* important factor for the success of your scRNA-seq and scMultiomics experiment

ARTICLES
https://doi.org/10.1038/s41593-022-01022-8
nature neuroscience
Check for updates

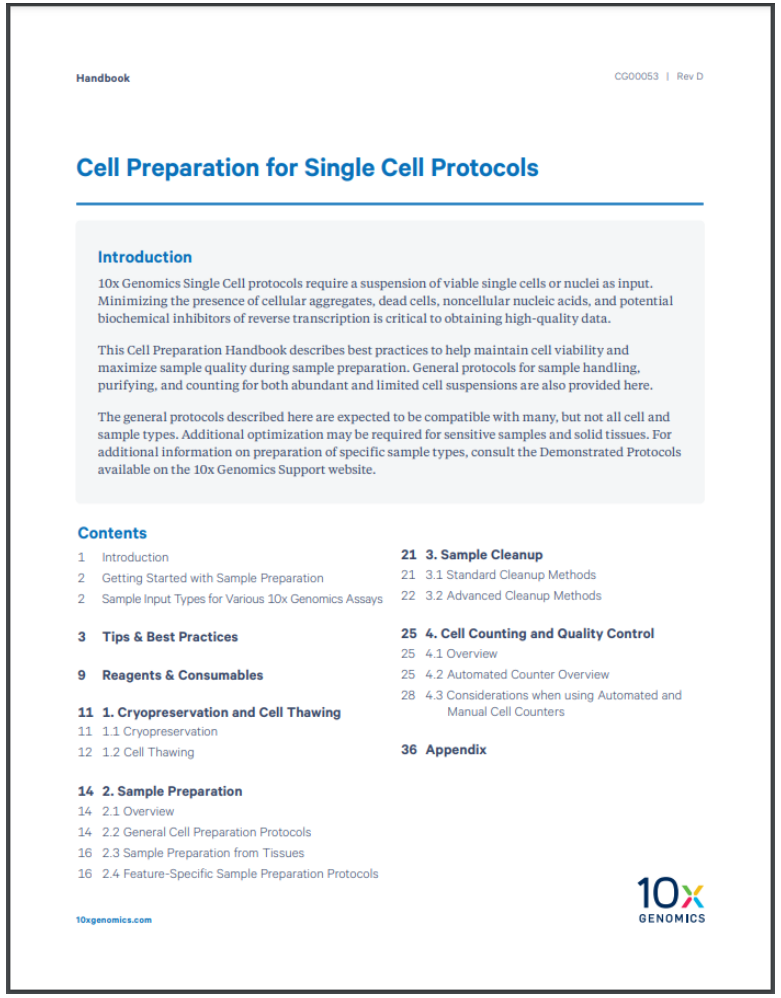
Dissection of artifactual and confounding glial signatures by single-cell sequencing of mouse and human brain

Samuel E. Marsh[1,2,3], Alec J. Walker[1,2,3], Tushar Kamath[2,3], Lasse Dissing-Olesen[1,2,3], Timothy R. Hammond[1,2,3], T. Yvanka de Soysa[1,2,3], Adam M. H. Young[4], Sarah Murphy[1], Abdulraouf Abdulraouf[3], Naeem Nadaf[3], Connor Dufort[1], Alicia C. Walker[1], Liliana E. Lucca[5], Velina Kozareva[3], Charles Vanderburg[3], Soyon Hong[6], Harry Bulstrode[4], Peter J. Hutchinson[7], Daniel J. Gaffney[8], David A. Hafler[5,9], Robin J. M. Franklin[4], Evan Z. Macosko[3,10] and Beth Stevens[1,2,3,11]

Marsh et al. 2022. Nature Neuroscience



Resources for sample preparation



Handbook CG00053 | Rev D

Cell Preparation for Single Cell Protocols

Introduction

10x Genomics Single Cell protocols require a suspension of viable single cells or nuclei as input. Minimizing the presence of cellular aggregates, dead cells, noncellular nucleic acids, and potential biochemical inhibitors of reverse transcription is critical to obtaining high-quality data.

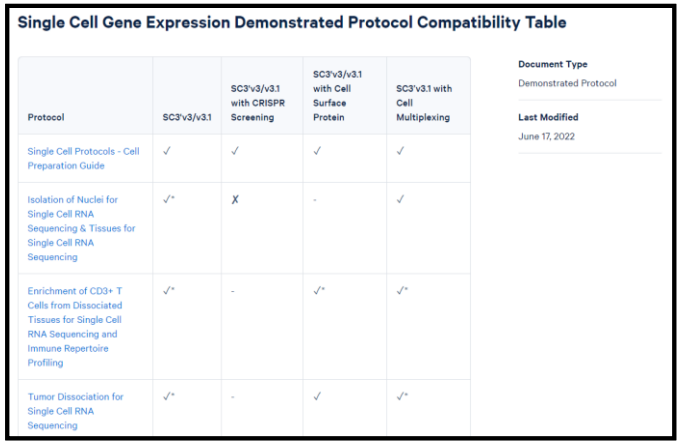
This Cell Preparation Handbook describes best practices to help maintain cell viability and maximize sample quality during sample preparation. General protocols for sample handling, purifying, and counting for both abundant and limited cell suspensions are also provided here.

The general protocols described here are expected to be compatible with many, but not all cell and sample types. Additional optimization may be required for sensitive samples and solid tissues. For additional information on preparation of specific sample types, consult the Demonstrated Protocols available on the 10x Genomics Support website.

Contents

10xgenomics.com

https://cdn.10xgenomics.com/image/upload/v1686678481/support-documents/CG00053_Handbook_CellPreparation_SingleCellProtocols_Rev_D.pdf



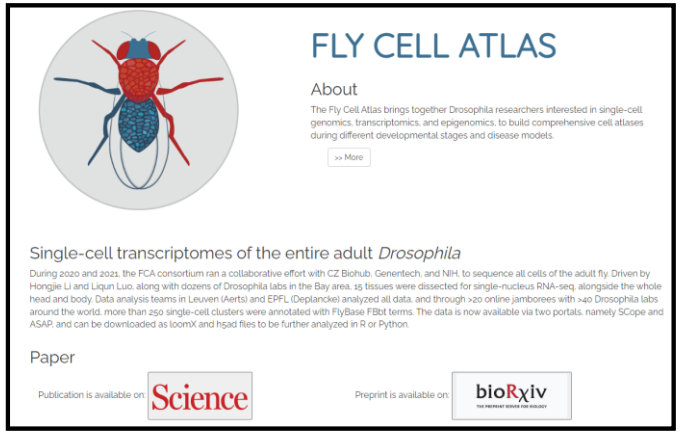
Single Cell Gene Expression Demonstrated Protocol Compatibility Table

Protocol	SC3'v3/v3.1	SC3'v3/v3.1 with CRISPR Screening	SC3'v3/v3.1 with Cell Surface Protein	SC3'v3.1 with Cell Multiplexing
Single Cell Protocols - Cell Preparation Guide	✓	✓	✓	✓
Isolation of Nuclei for Single Cell RNA Sequencing & Tissues for Single Cell RNA Sequencing	✓*	✗	-	✓
Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling	✓*	-	✓*	✓*
Tumor Dissociation for Single Cell RNA Sequencing	✓*	-	✓	✓*

Document Type
Demonstrated Protocol

Last Modified
June 17, 2022

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/single-cell-gene-expression-demonstrated-protocol-compatibility-table



FLY CELL ATLAS

About

The Fly Cell Atlas brings together Drosophila researchers interested in single-cell genomics, transcriptomics, and epigenomics, to build comprehensive cell atlases during different developmental stages and disease models.

>> More

Single-cell transcriptomes of the entire adult *Drosophila*

During 2020 and 2021, the FCA consortium ran a collaborative effort with CZ Biohub, Genentech, and NIH, to sequence all cells of the adult fly. Driven by Hongjie Li and Liqun Luo, along with dozens of Drosophila labs in the Bay area, 15 tissues were dissected for single-nucleus RNA-seq, alongside the whole head and body. Data analysis teams in Leuven (Aerts) and EPFL (Deplancke) analyzed all data, and through >20 online jamborees with >40 Drosophila labs around the world, more than 250 single-cell clusters were annotated with FlyBase FBbt terms. The data is now available via two portals, namely SCope and ASAP, and can be downloaded as loomX and h5ad files to be further analyzed in R or Python.

Paper

Publication is available on: Science

Preprint is available on: bioRxiv

https://flycellatlas.org/

Workflow

Qualification: Meeting to discuss objectives and experimental design

Quotation: Cost estimation

Scheduling: Day and approximate time of the experiment

Experiment day:

- 30 minutes before the experiment
- Sample submission and cell QC (demo submission form)
- Chip loading and Chromium run

Reporting: Document processing (demo report)

Workflow

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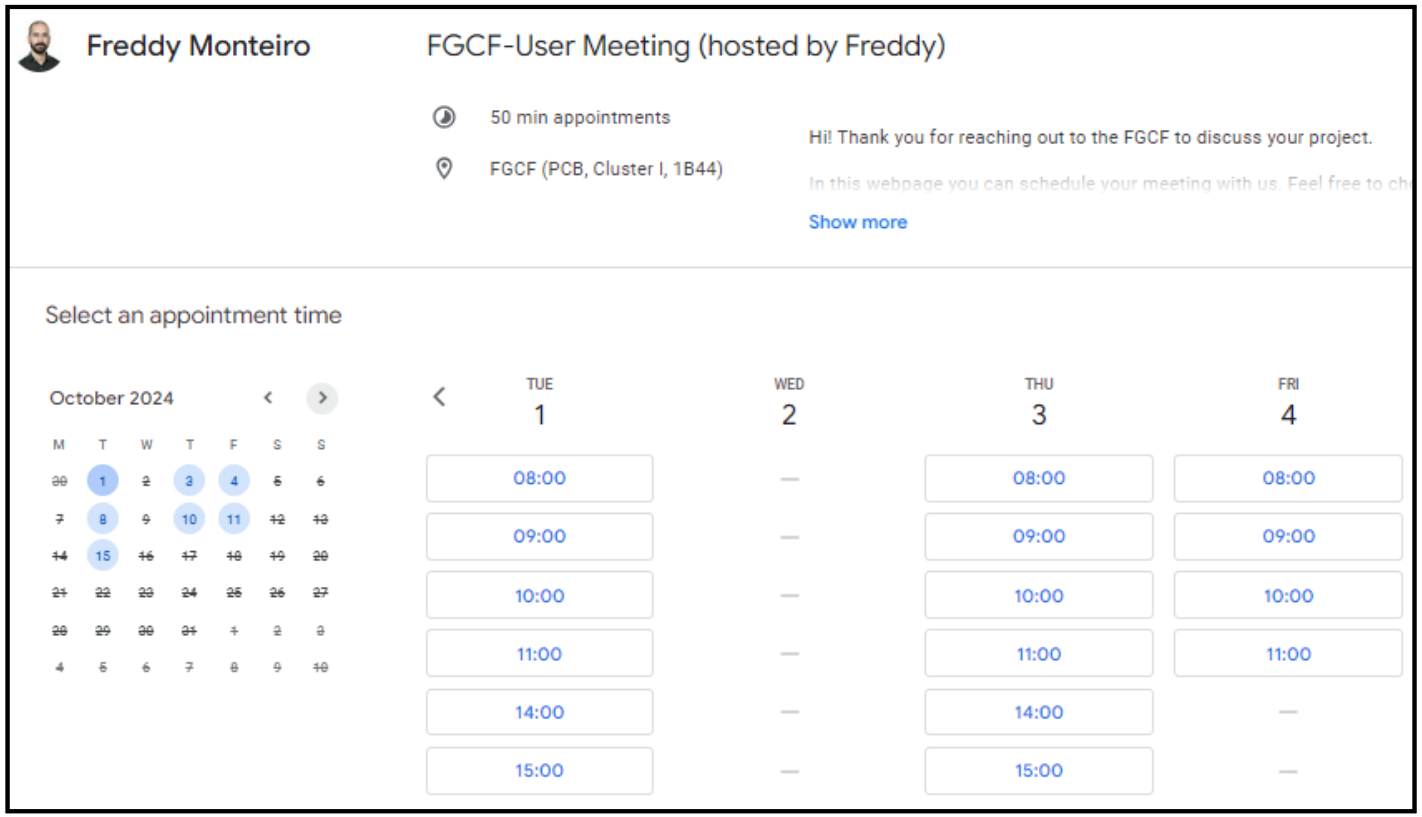
Workflow: Qualification



https://calendar.app.google/dszBnvYmr6ox5xYeA

Objectives. Feasibility and Qualify 3' or 5' application.

Organism & Tissue. Experienced/First timer, Dissociation pilots, FACS, bead-based enrichment, straining, etc.

Cell yield, viability and Single Cell Purity. Expectations/Objectives and sample requirements to obtain those objectives (conc. and volume).

Multiplexing and Cell Hashingestrategy . Experienced/First timer, Dissociation pilots.

Controls and Replictes. Experienced/First timer, Dissociation pilots.

Reagents and consumables purchase. Stock management.

Deliverables. Libraries, fastq, analysis*,

Quotation. Ror revision and PI approval.

Workflow

✓ Qualification: Meeting to discuss objectives and experimental design

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Scheduling: Day and approximate time of the experiment

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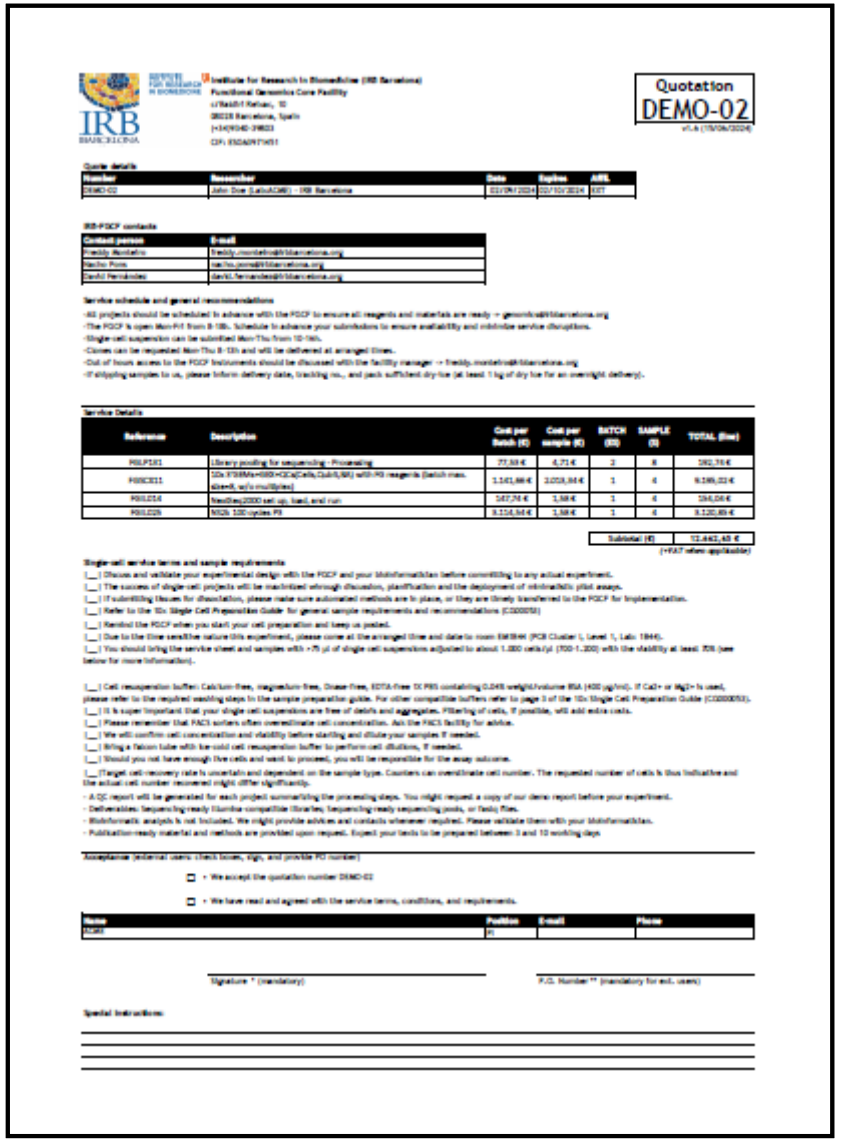
Reporting: Document processing (demo report)

Workflow: Quotation



Quotation
DEMO-02

Specific to each service

IRB Barcelona - Functional Genomics 2024

Service	Public and CERCA Institutions (€)	
qPCR (FGQP)	**Batch**	**Sample**
qPCR Consultancy (per hour)	77,89	0,00
Analysis per plate (absolute/relative quantification; Variant detection by High Resolution Melting)	259,52	0,00
QuantStudio 6 Pro self-service (per hour)	15,00	0,00
Illumina library quantification using SYBR qPCR (1-12 libraries per 96-well plate)	120,77	9,22
RT reaction (first strand)	65,12	9,98
Primer design and optimization (unit: transcript of interest)	218,08	5,68
PCR Concentrations and Annealing Optimization (unit: pair of primers)	122,59	25,00
96-well qPCR set up and run (unit: pair of primers; batch max size=96)	124,95	12,69
Single-cell (FGSC)	**Batch**	**Sample**
Single Cell Consultancy (per hour)	77,89	0,00
Cell Counting & Viability Quality Control (batch max. size=8)	26,28	6,47
10x GEMs and cDNA with user's reagents (batch max. size=8 w/o multiplex)	231,13	9,83
10x GEX Library with user's reagents (batch max. size=8, w/o multiplex)	313,68	13,12
10x GEMs+GEX+QCs(Cells,Qubit,BA) with user's reagents (batch max. size=11, w/o multiplex)	810,34	36,86
10x 3'GEMs and cDNA with FG reagents (batch max. size=8, w/o multiplex)	637,58	2.090,83
10x GEX Library with FG reagents	339,63	25,82
10x 3'GEMs+GEX+QCs(Cells,Qubit,BA) with FG reagents (batch max. size=8, w/o multiplex)	1.242,74	2.130,57
10x FB Library with user's reagents (batch max. size=8)	121,38	13,18
10x 3'FB Library with FG reagents (batch max. size=8)	147,32	78,84
10x 3'GEMs+GEX+FB+QCs(Cells,Qubit,BA) with FG reagents (batch max. size=8)	1.502,06	2.212,46
10x 5'GEMs and cDNA with FG reagents (batch max. size=8, w/o multiplex)	651,58	2.299,58
10x BCR/TCR Amplification with user's reagents	105,54	8,21
10x BCR/TCR Amplification with FG reagents	131,49	136,77
10x Chromium 5'-Gene Expression v2 BCR/TCR - Full Workflow (batch max. size=8, w/o multiplex)	1.727,86	2.500,87
10x ATAC Transposition (batch max. size=8, w/o multiplex)	61,27	2,64
10x ATAC GEMs + cleanup with user's reagents (batch max. size=8, w/o multiplex)	228,92	9,89
10x ATAC GEMs + cleanup with FG reagents	636,40	2.191,39
10x ATAC Library v2 with user's reagents (batch max. size=8, w/o multiplex)	312,76	9,26
10x ATAC Library v2 with FG reagents	325,74	21,97
10x ATAC v2 GEMs+Library+QCs with FG reagents (batch max. size=8, w/o multiplex)	1.334,97	2.231,24
10x Multiome pre-split amplification (batch max. size=8, w/o multiplex)	131,59	9,45
10x Multiome GEMS+GEX+ATAC+QCs(BA) with user's reagents (batch max. size=8, w/o multiplex)	1.525,30	61,72

https://www.irbbarcelona.org/en/research/functional-genomics

Workflow

✓ Qualification: Meeting to discuss objectives and experimental design

✓ Quotation: Cost estimation

Scheduling: Day and approximate time of the experiment

Experiment day:

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- Chip loading and Chromium run

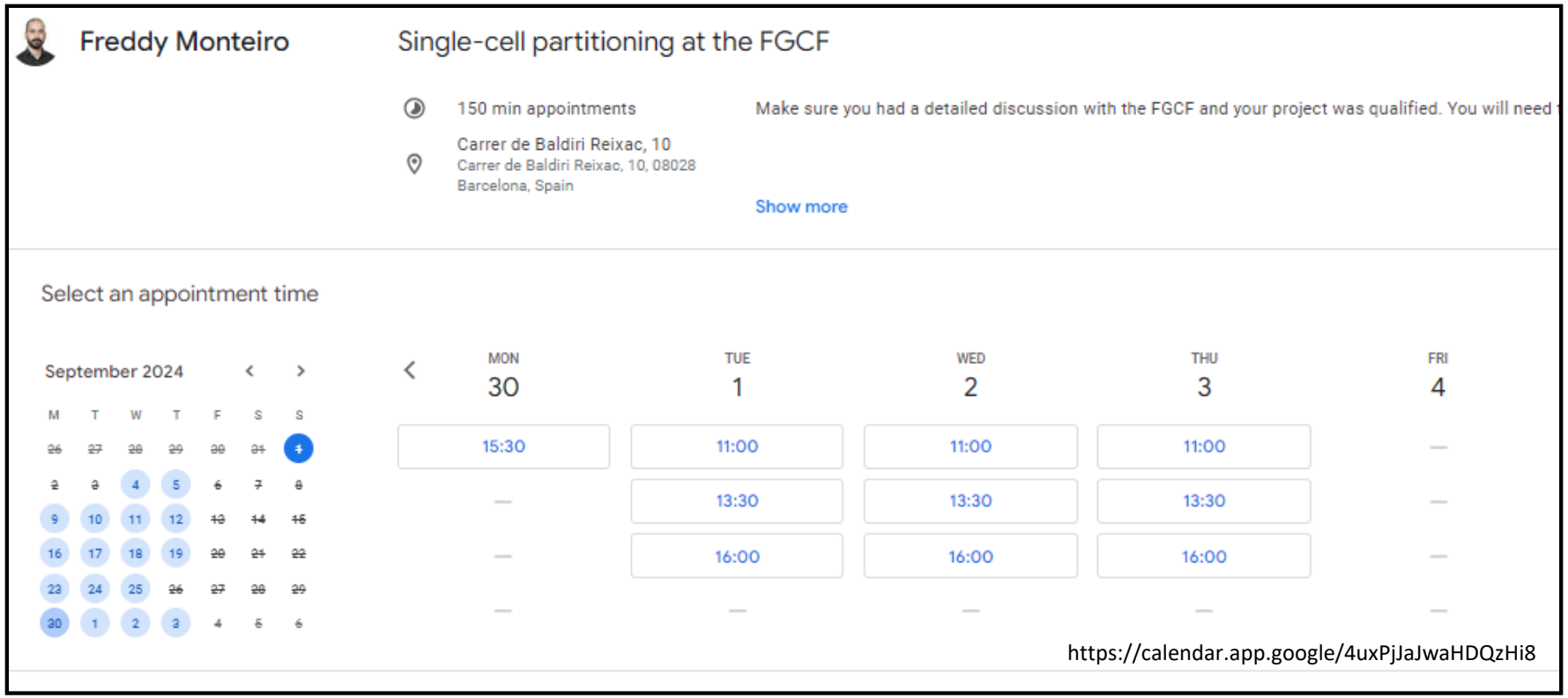
Reporting: Document processing (demo report)

Workflow: Scheduling

15 days in advance from quotation acceptance for reagents not in stock

72 hours in advance for qualified projects with reagents in sock **AND** confirmed staff availability

NEXT DAY scheduling for patient-derived samples of qualified projects **AND** reagents in stock **AND** staff availability



Workflow

✓ Qualification: Meeting to discuss objectives and experimental design

✓ Quotation: Cost estimation

✓ Scheduling: Day and approximate time of the experiment

Experiment day:

30 minutes before the experiment
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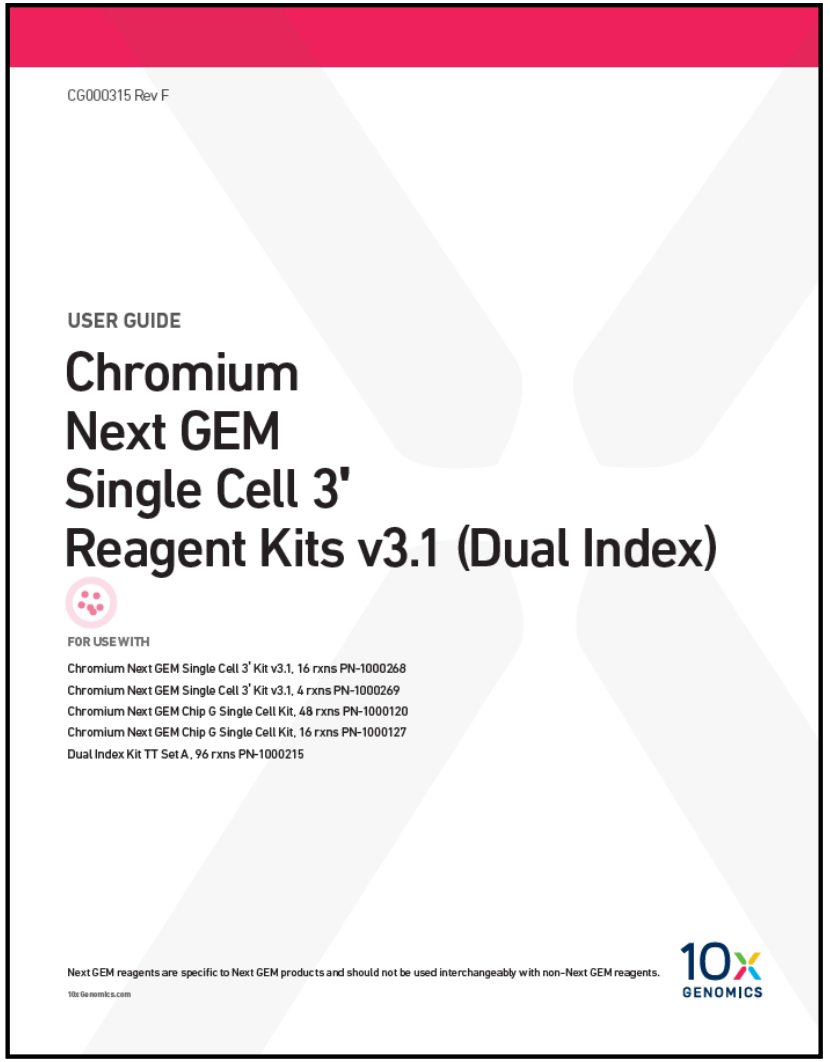
Chip loading and Chromium run

Reporting: Document processing (demo report)

Workflow: Set up


CG000315 Rev F

USER GUIDE

Chromium
Next GEM
Single Cell 3'
Reagent Kits v3.1 (Dual Index)

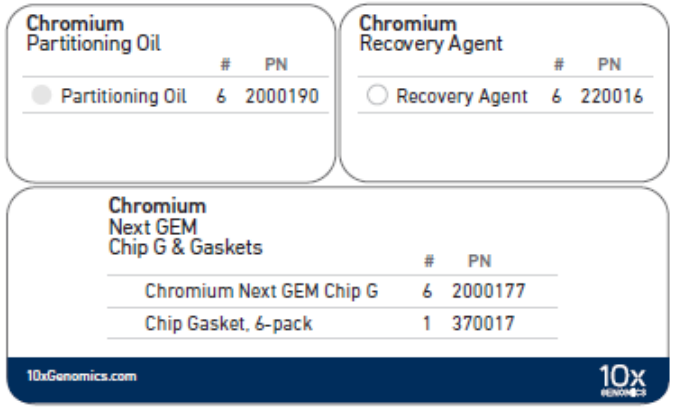
FOR USE WITH

Chromium Next GEM Single Cell 3' Kit v3.1, 16 rxns PN-1000268
Chromium Next GEM Single Cell 3' Kit v3.1, 4 rxns PN-1000269
Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120
Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127
Dual Index Kit TT Set A, 96 rxns PN-1000215

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

10xGenomics.com

10x GENOMICS


Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	6	2000177
Chip Gasket, 6-pack	1	370017

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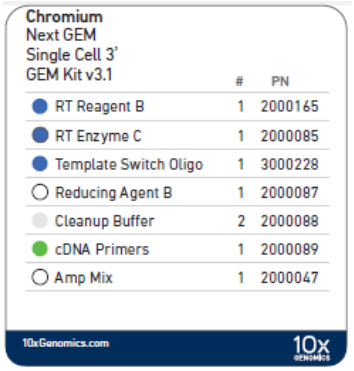
Chromium
Next GEM
Single Cell 3'
GEM Kit v3.1

	#	PN
RT Reagent B	1	2000165
RT Enzyme C	1	2000085
Template Switch Oligo	1	3000228
Reducing Agent B	1	2000087
Cleanup Buffer	2	2000088
cDNA Primers	1	2000089
Amp Mix	1	2000047

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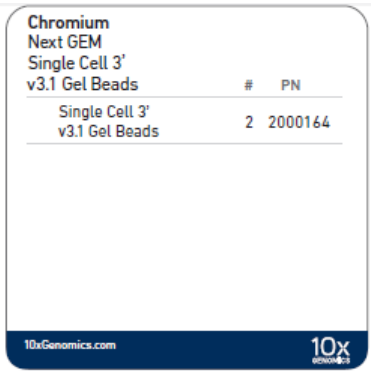
Chromium
Next GEM
Single Cell 3'
v3.1 Gel Beads

	#	PN
Single Cell 3' v3.1 Gel Beads	2	2000164

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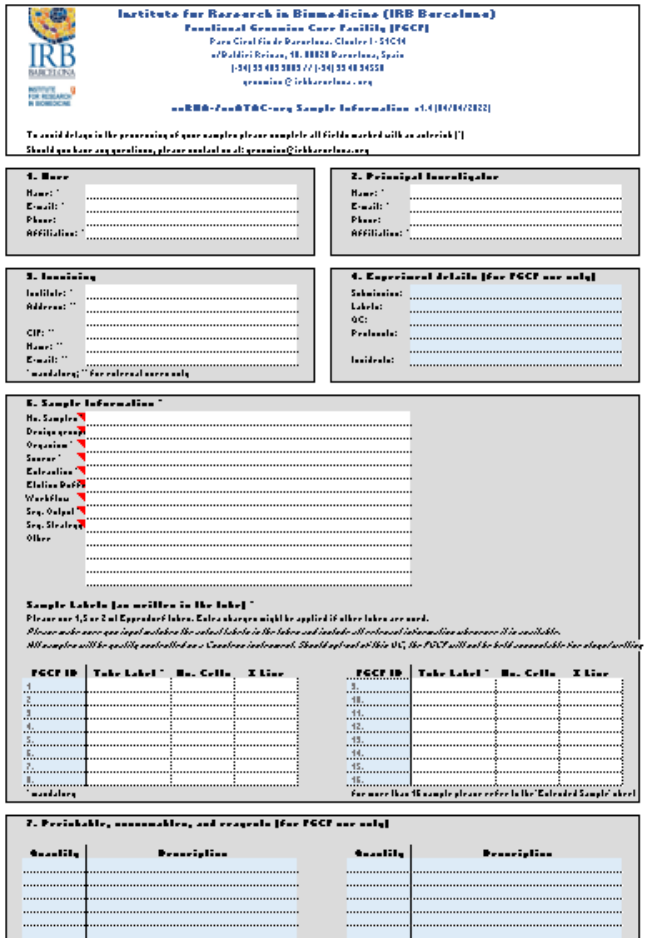
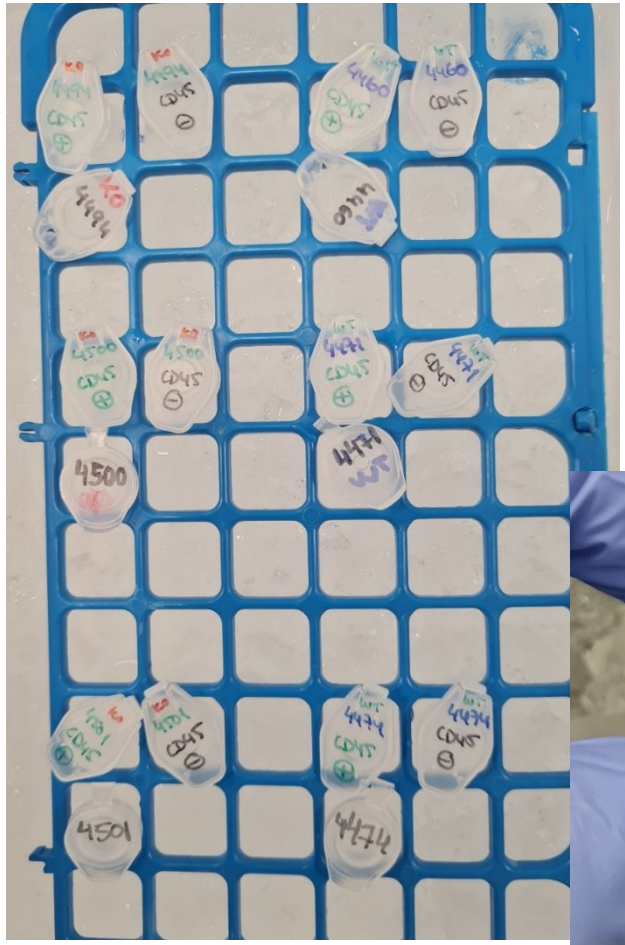
https://cdn.10xgenomics.com/image/upload/v1722285481/support-documents/CG000315_ChromiumNextGEMSingleCell3__GeneExpression_v3.1_DualIndex__RevF.pdf

Workflow: Sample submission

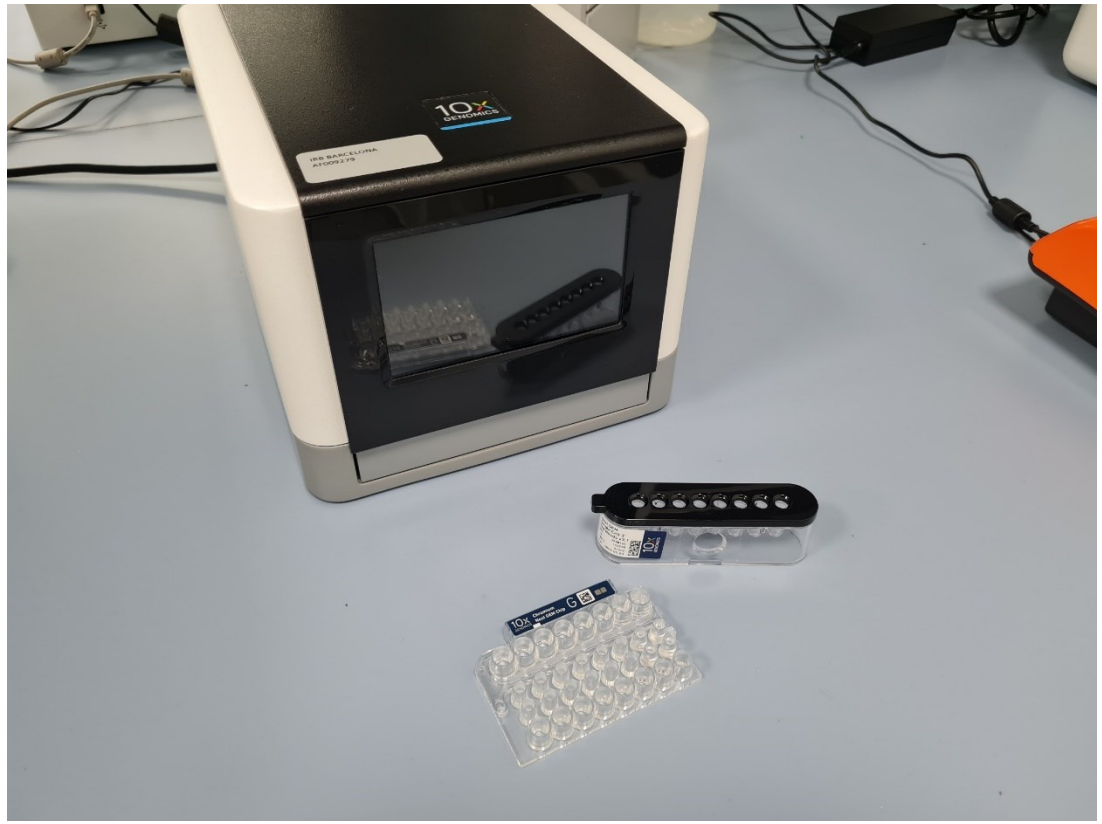


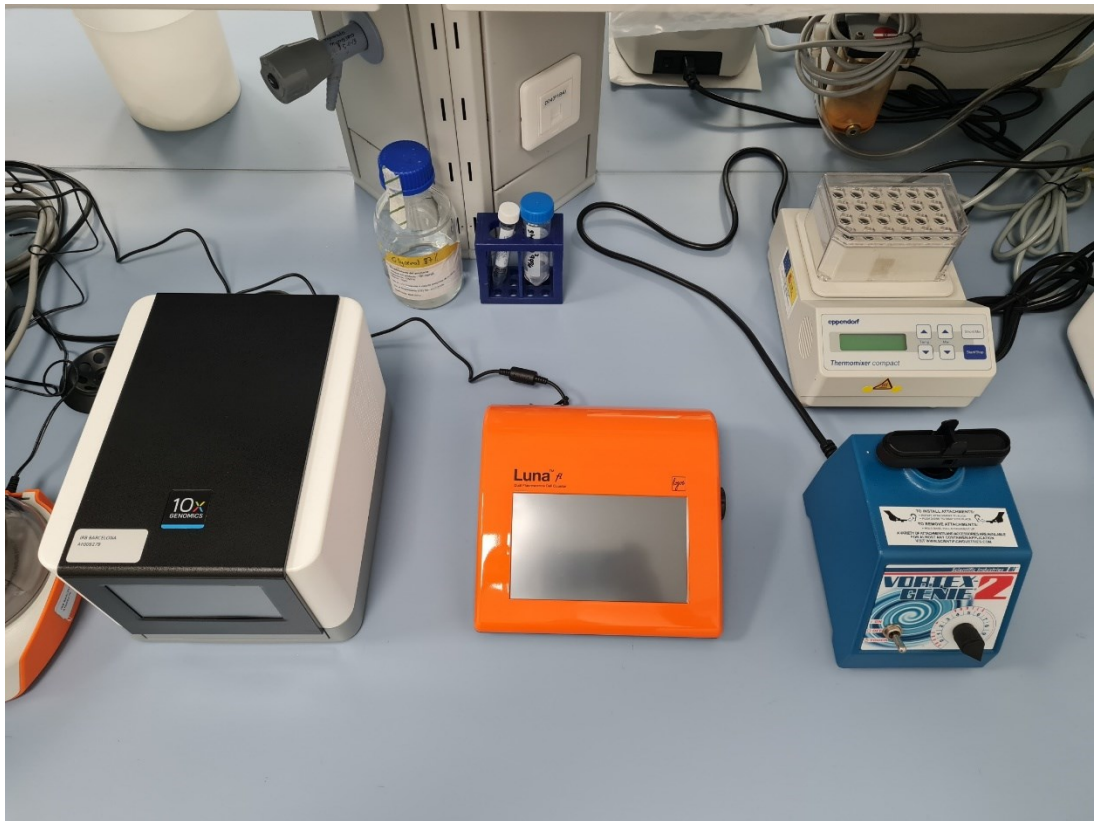
Institute for Research in Biomedicine (IRB Barcelona)
Functional Genomics Core Facility (FGCF)

1. User
2. Principal Investigator
3. Invoicing
4. Experiment details (for FGCF use only)
5. Sample Information *
6. Perishable, consumables, and reagents (for FGCF use only)



Workflow: Workbench and reagents preparation





Workflow: Cell QC

Cell Count Report 1/2

File name

fg-21032023_003350_3-1

Date

21 Mar., 2023, 00:34

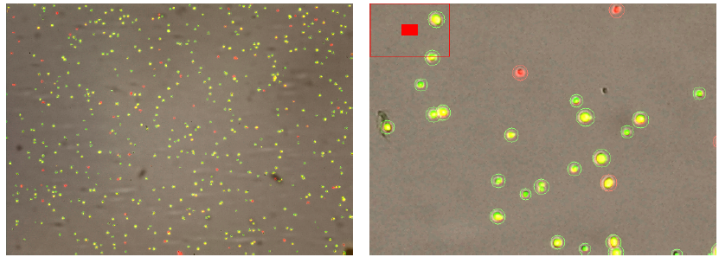
Cell count results

Total cell concentration: 1.46 x 10^6 cells/mL
Live cell concentration: 1.23 x 10^6 cells/mL
Dead cell concentration: 2.34 x 10^5 cells/mL
Viabillity: 84.0 %
Average cell size: 15.4 µm
Total cell number: 595
Live cell number: 500
Dead cell number: 95

Protocol

Protocol name: DEFAULT
Dilution factor: 1.11
Min. cell size: 3 µm
Max. cell size: 60 µm
Size gating: 3 ~ 60 µm
Green fluorescence threshold: 5
Red fluorescence threshold: 5
Green exposure: 7
Red exposure: 10
Green calibrated value: 0x3000
Red calibrated value: 0x5000

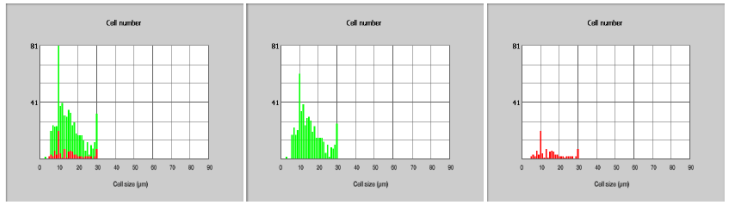
Cell Image (Average intensity: 97)



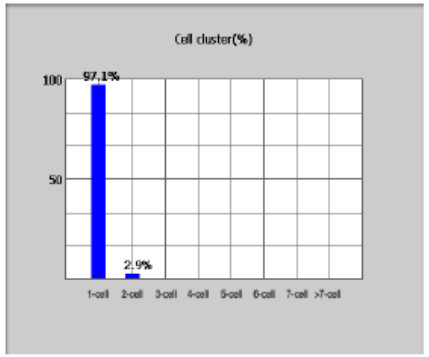
Cell Count Report 2/2

Cell size histogram expressed by cell number

Total cells | Live cells | Dead cells



Cell cluster graph



qc

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Workflow: Loading volume

10x genomics: **65 %** recovery (NextGEM 3' v3.1)

distributor: **50 %** recovery (NextGEM 3' v3.1)

reality: **35 %** recovery (NextGEM 3' v3.1)

Next GEM

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)

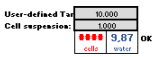
DO NOT add nuclease-free water directly to single cell suspension. Add nuclease-free water to the Master Mix. Refer to step 1.2b.

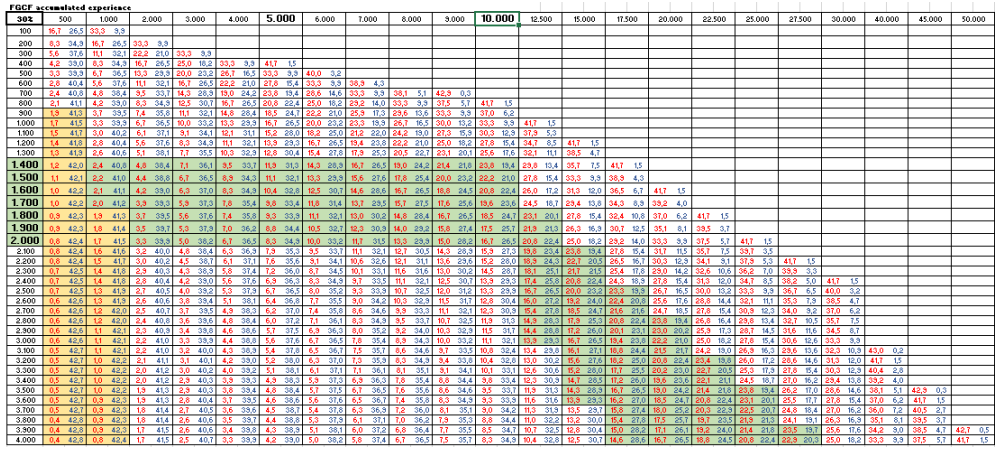
Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 35.0	16.5 26.7	33.0 10.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 39.1	8.3 35.0	16.5 26.7	24.8 18.5	33.0 10.2	41.3 2.0	n/a	n/a	n/a	n/a	n/a
300	2.8 40.5	5.5 37.7	11.0 32.2	16.5 26.7	22.0 21.2	27.5 15.7	33.0 10.2	38.5 4.7	n/a	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

Grey boxes: Volumes that would exceed the allowable water volume in each reaction

Yellow boxes: Indicate a low transfer volume that may result in higher cell load variability

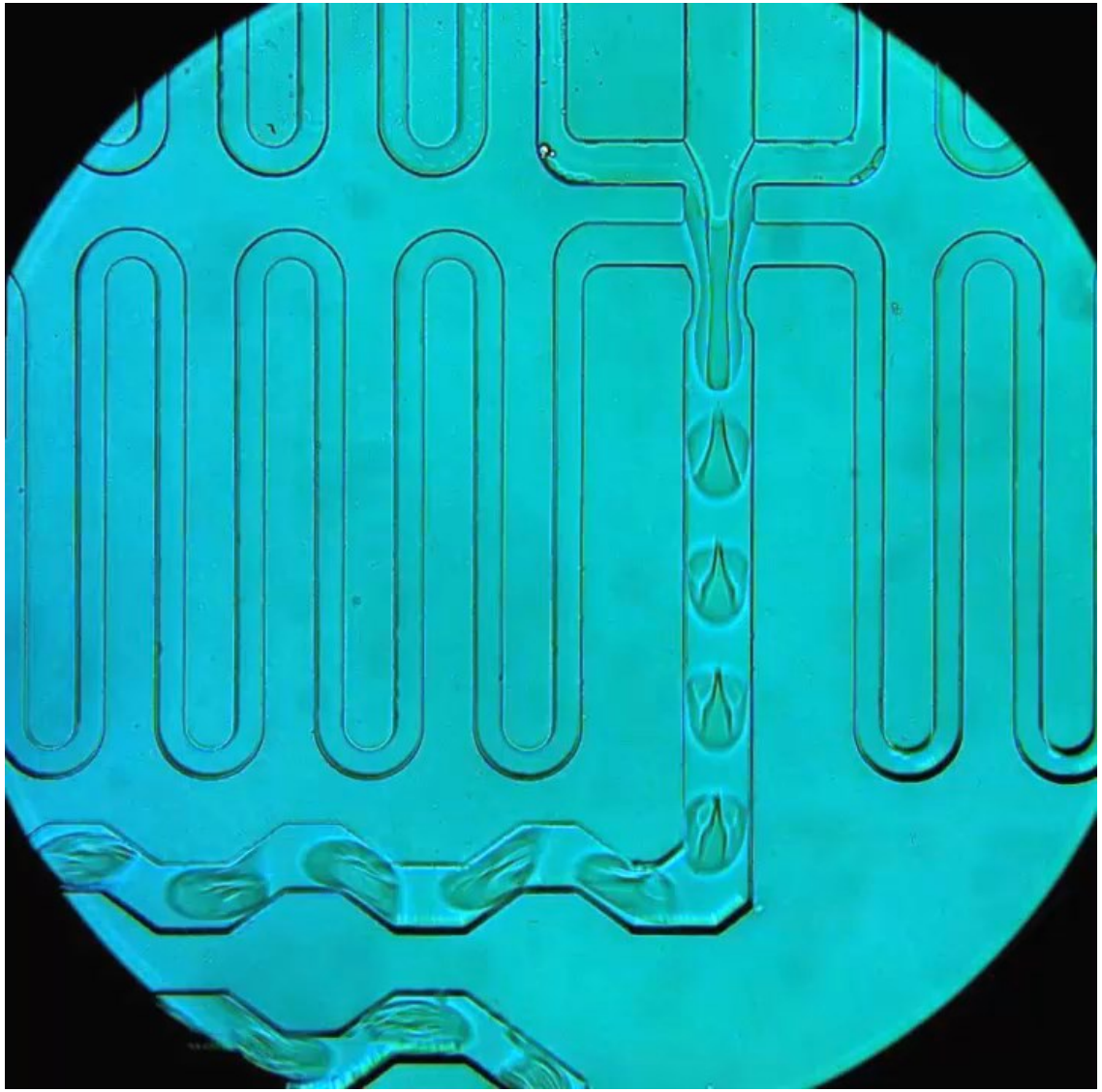
Blue boxes: Optimal range of cell stock concentration to maximize the likelihood of achieving the desired cell recovery target

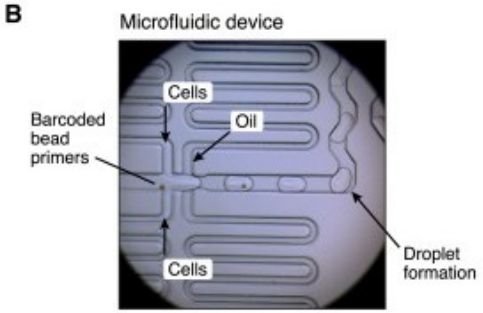




In house table for 30% recovery and overloading targets

Workflow: Cell Partitioning





Macosko et al. Cell 2015.

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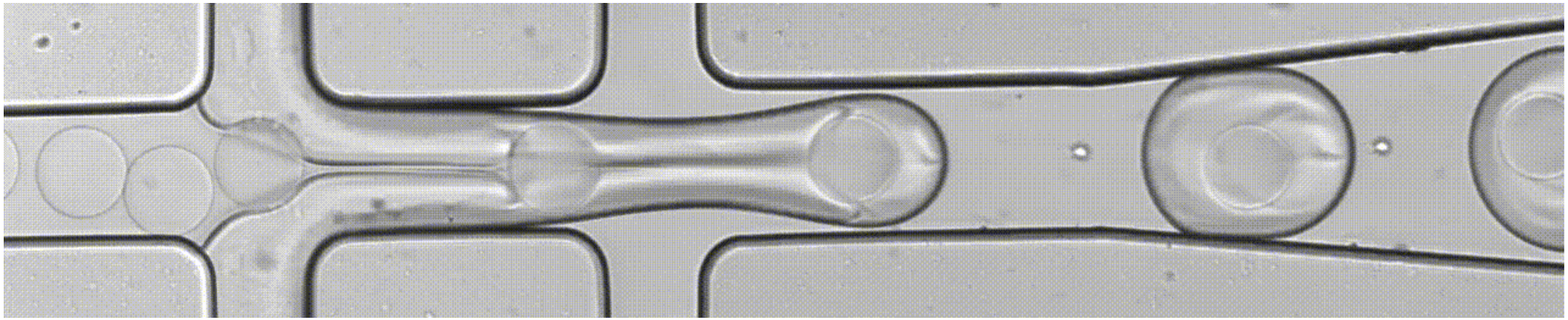
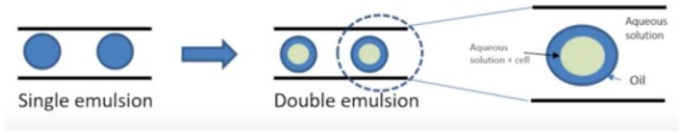
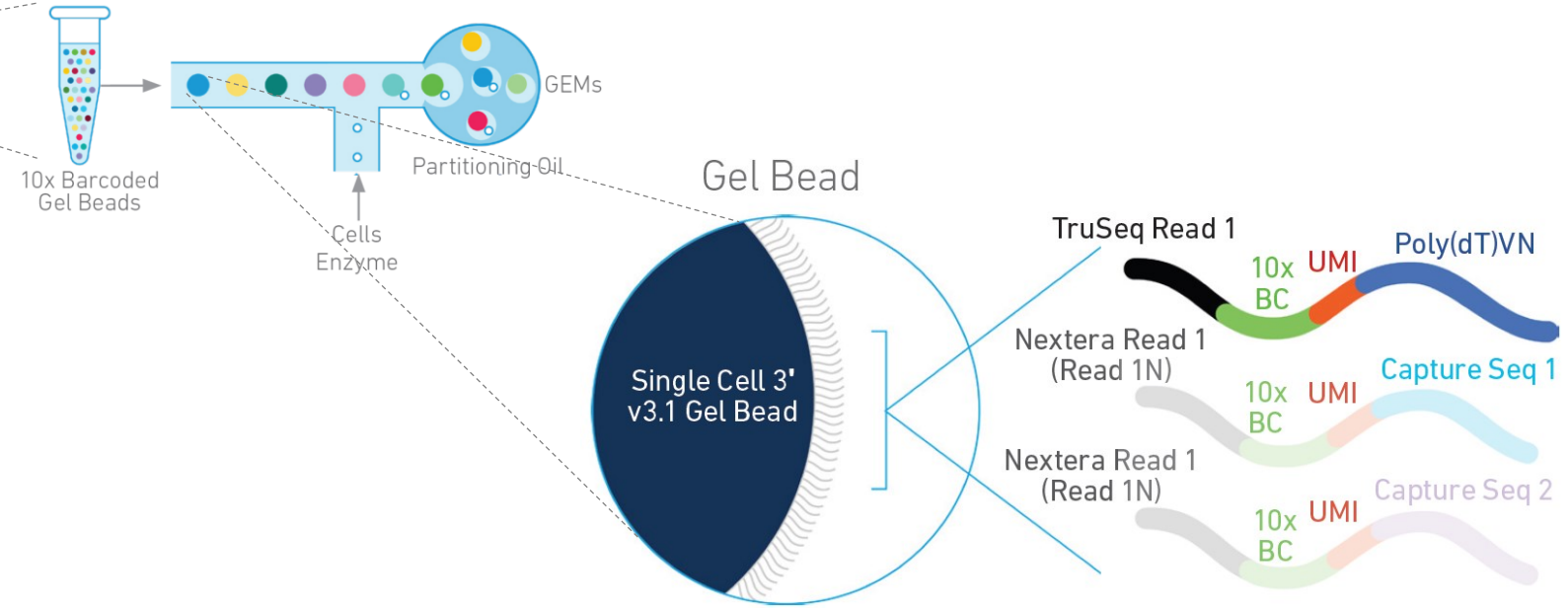
Workflow: Cell Partitioning





10x genomics 3’ v3.1 Gel Beads



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

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

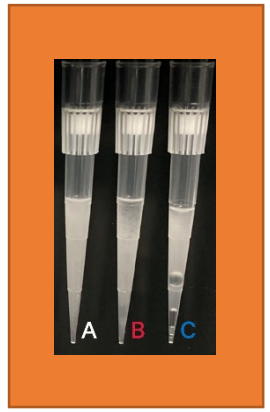
ii. 10x Barcode
16 nt 10x Barcode
~3.6 M defined barcode sequences

iii. UMI
12 nt Unique Molecular Identifier

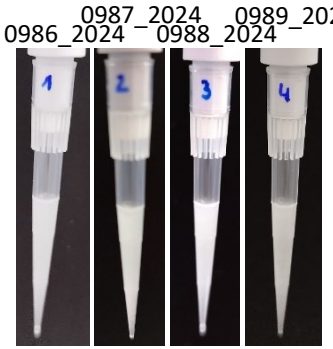
iv. Poly(dT)VN
30 nt Poly(dT) sequence
Enables capture of poly-adenylated mRNA molecules

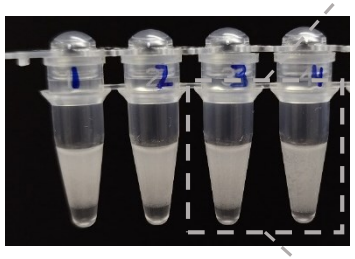
iv. Capture Sequence 1 or 2
22 nt sequence that is the reverse complement of the sequence inserted into the DNA (Antibody) or RNA (sgRNA) based sequence

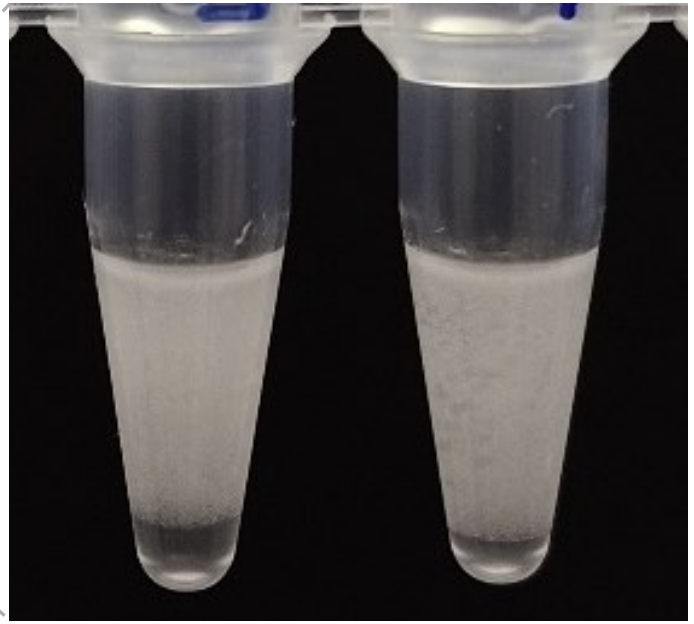
GEMs recovery and visual QC



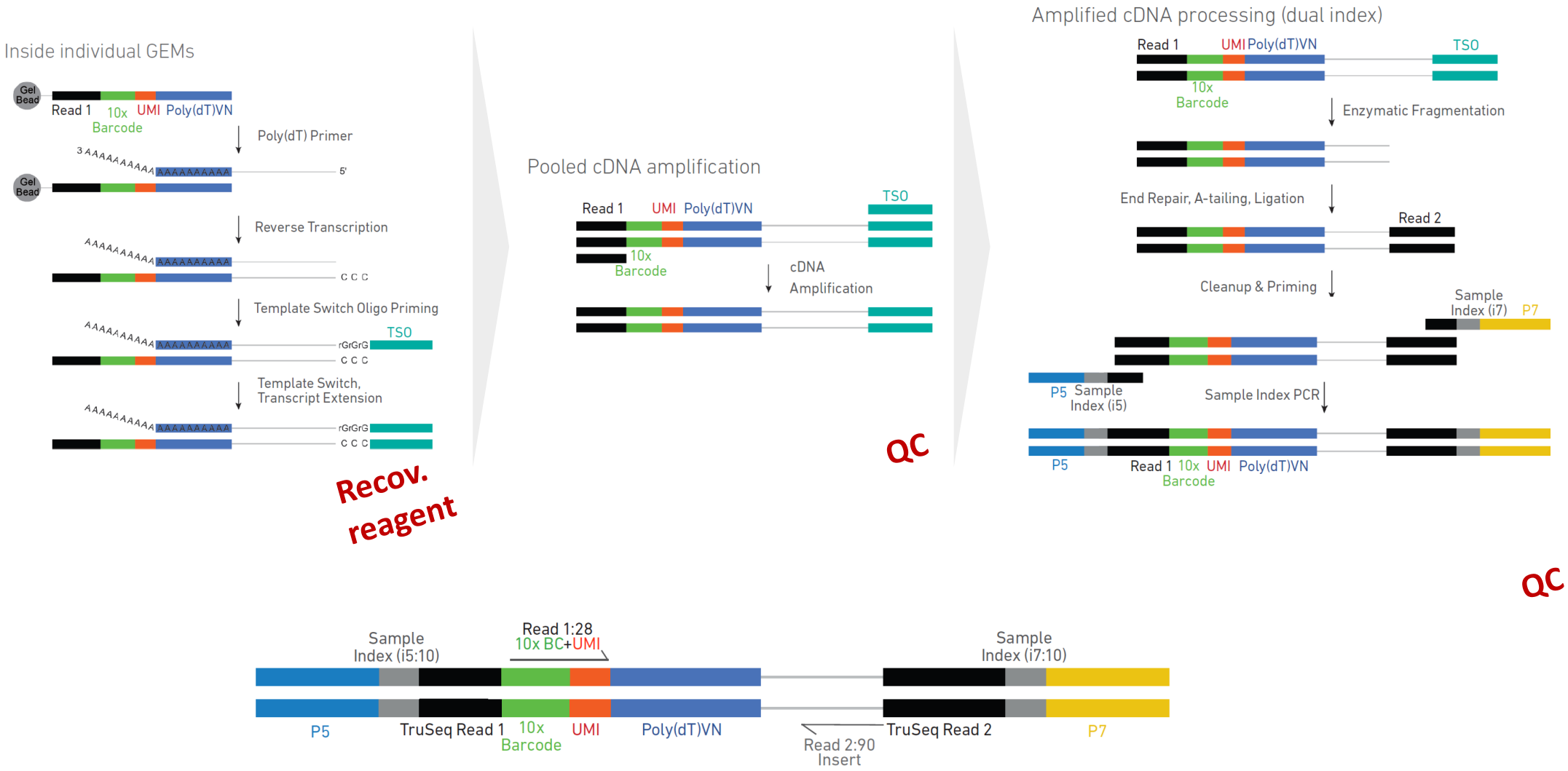
https://kb.10xgenomics.com/hc/en-us/articles/218135863-What-is-a-wetting-failure-and-how-can-they-be-recognized





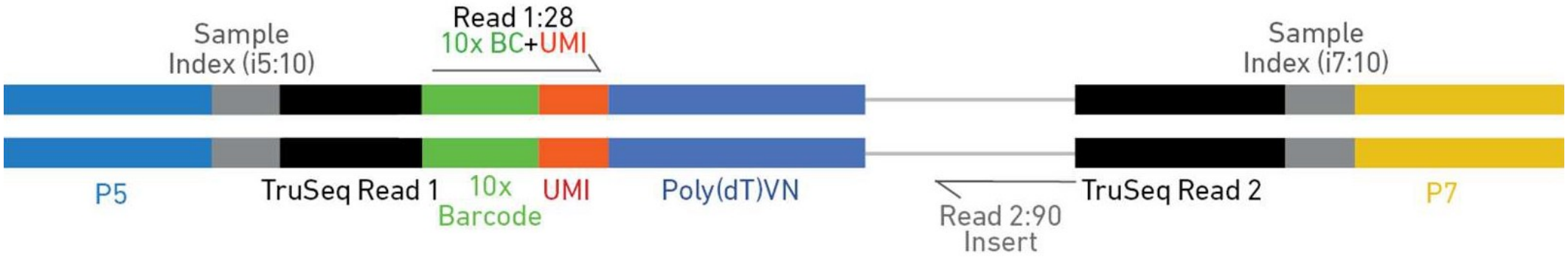


10x genomics library preparation procedures

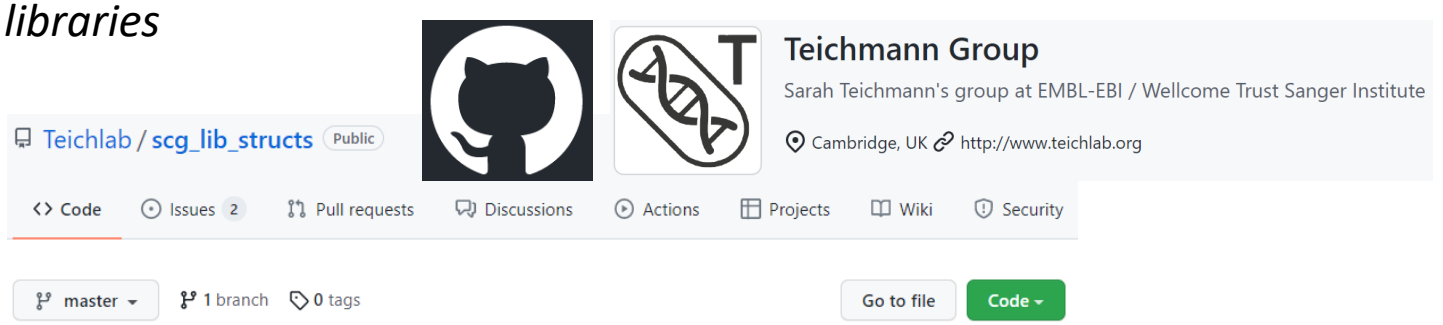


https://cdn.10xgenomics.com/image/upload/v1722285481/support-documents/CG000315_ChromiumNextGEMSingleCell3__GeneExpression_v3.1_DualIndex__RevF.pdf

3’ v3.1 GEX library structure and resources



Actual sequences and other sc libraries



Workflow

✓ Qualification: Meeting to discuss objectives and experimental design

✓ Quotation: Cost estimation

✓ Scheduling: Day and approximate time of the experiment

✓ Experiment day:

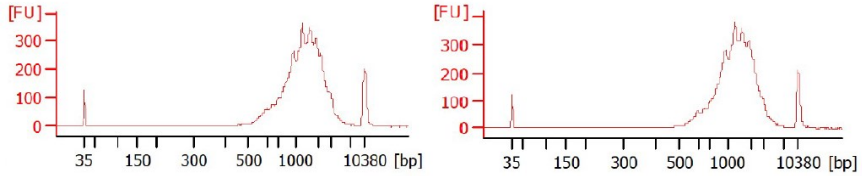
30 minutes before the experiment
Sample submission and cell QC (demo submission form)
Chip loading and Chromium run

Reporting: Document processing (demo report)

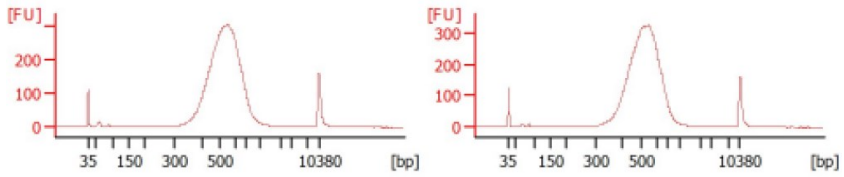
Workflow: Reporting

FGC ID	Sample Name	Pictures
00X1_2022	Sample #1	
00X2_2022	Sample #2	

FGCF ID	Cycles	cDNA concentration (ng/µl)	Total yield in 40 µl (ng)
00X1_2022	11	8.51	340.4
00X2_2022	11	12.1	484

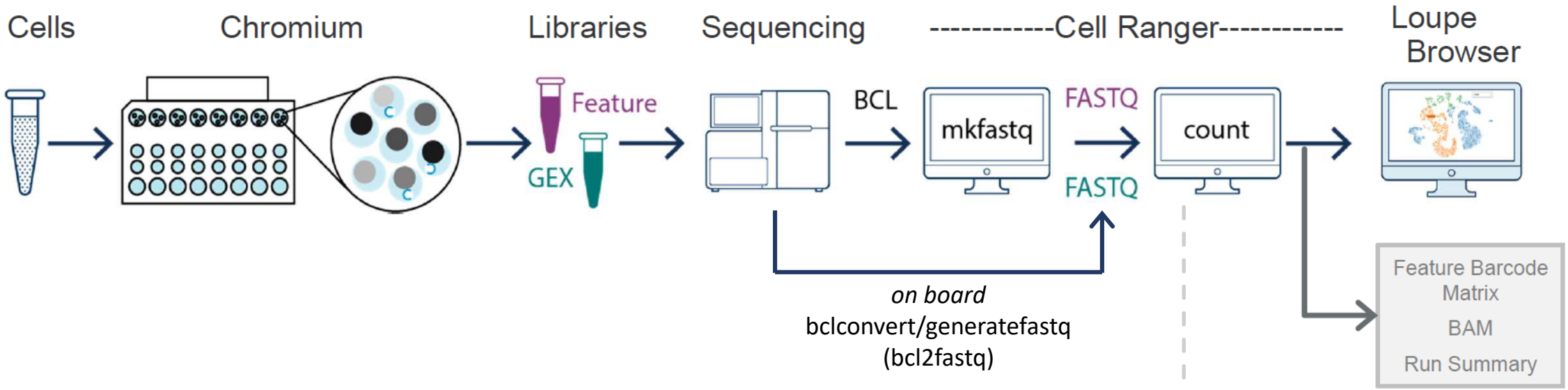


FGCF ID	cDNA used amount (ng) in 10 µl	Dual Index TT Set A	Cycles	Library concentration (ng/µl)	Volume (µl)	Yield (ng)	Average size (bp)
00X1_2022	85.1	SI-TT-X1	12	19.7	35	689.5	526
00X2_2022	121	SI-TT-X2	12	30.3	35	1060.5	538



Reads	Valid reads	Cells	Median UMI per Cell	Median Genes per Cell	Saturation	Reads/Cell	Cell recovery
259.775.175	97,90%	**13.311**	**3.039**	902	53,40%	**19.516**	53%
251.695.980	97,50%	**13.211**	**2.520**	917	53,00%	**19.052**	56%
126.980.438	97,70%	**6.170**	**2.649**	794	48,70%	**20.580**	33%

From cells to data: CellRanger *count*




```
#!/bin/bash
#SBATCH -J cnt-1134
#SBATCH -c 16
#SBATCH --qos=medium
#SBATCH --partition=irb_cpu_iclk
#SBATCH --ntasks=1
#SBATCH --export=ALL
#SBATCH --time=0-12:00
#SBATCH --mem=300G
#SBATCH --mail-type=begin        # send email when job begins
#SBATCH --mail-type=end          # send email when job ends
#SBATCH --mail-type=fail         # send email if job fails
#SBATCH --mail-user=freddy.monteiro@irbbarcelona.org

ID="1134_2024";
cellranger count --id="$ID" \
                 --transcriptome=/scratch/cffg/fdeoliveiramonteiro/ref/mouse/refdata-gex-GRCm39-2024-A \
                 --fastqs=~/HN00220094_10X_RawData_Outs/"$ID"/HH77JDSXC,~/HN00222740_10X_RawData_Outs/"$ID"/HNTCFDSXC \
                 --sample="$ID" \
                 --localcores=16 \
                 --localmem=280
```

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count outputs: QC

10x GENOMICS Cell Ranger • count

1458_2024

Alerts

The analysis detected 1 informational notice.

Alert	Value	Detail
Intron mode used		This data has been analyzed with intronic reads included in the count matrix. This behavior is different from previous Cell Ranger versions. If you would not like to count intronic reads, please rerun with the "include-introns" option set to "false". Please contact support@10xgenomics.com for any further questions.

Summary | Gene Expression | Antibody

22,595
Estimated Number of Cells

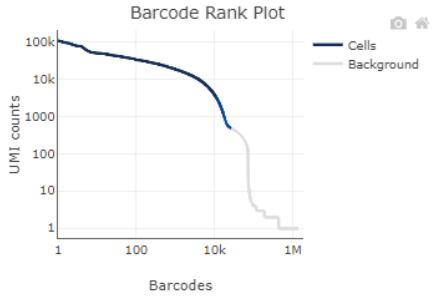
13,530
Mean Reads per Cell

1,124
Median Genes per Cell

Sequencing

Number of Reads	305,715,909
Number of Short Reads Skipped	0
Valid Barcodes	97.3%
Valid UMIs	100.0%
Sequencing Saturation	42.7%
Q30 Bases in Barcode	94.3%
Q30 Bases in RNA Read	92.4%
Q30 Bases in UMI	93.1%

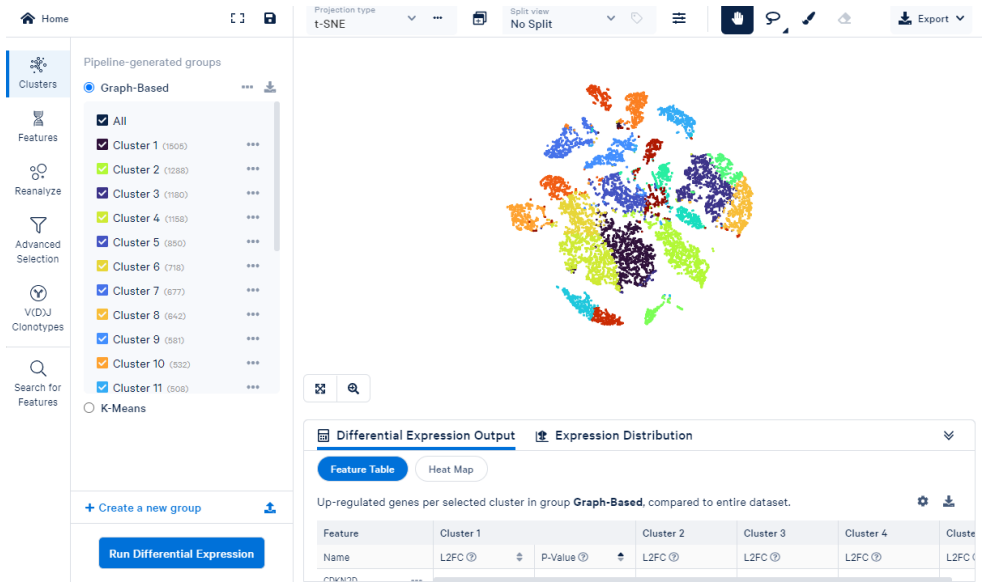
Cells

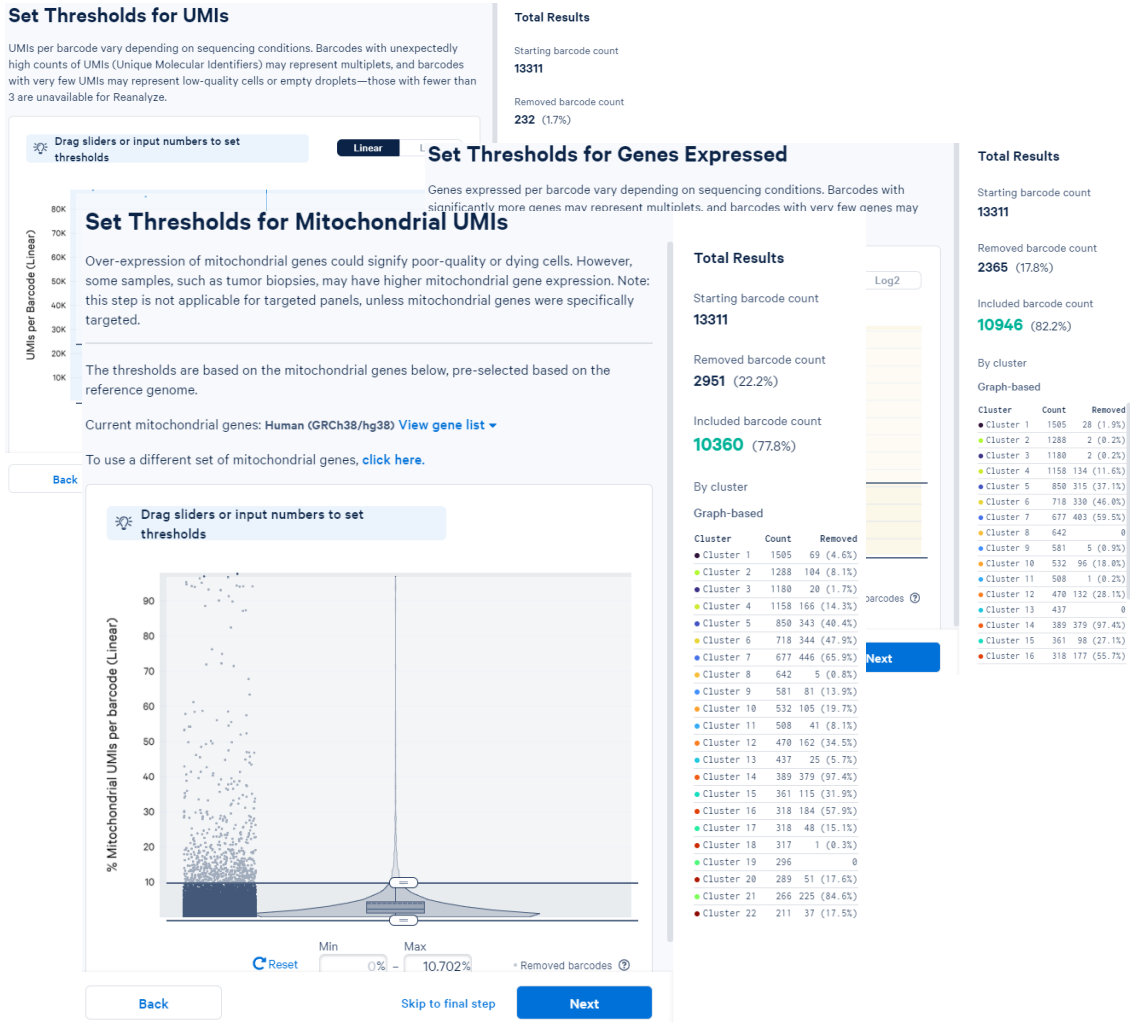


Estimated Number of Cells	22,595
Fraction Reads in Cells	86.3%
Mean Reads per Cell	13,530
Median UMI Counts per Cell	3,166
Median Genes per Cell	1,124
Total Genes Detected	24,194

.html web summary

count outputs: Loupe Browser





.cloupe