



INSTITUTE  
FOR RESEARCH  
IN BIOMEDICINE

# IRB Barcelona Functional Genomics Core Facility

## SCENTINEL WORKSHOP I

September 2-6, 2024

Freddy Monteiro, PhD

# 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	
	<i>leave before 18h</i>	<i>leave before 18h</i>	<i>leave before 18h</i>	<i>leave before 18h</i> Dinner ?	

# Location

- PCB - Parc Científic de Barcelona



# Location

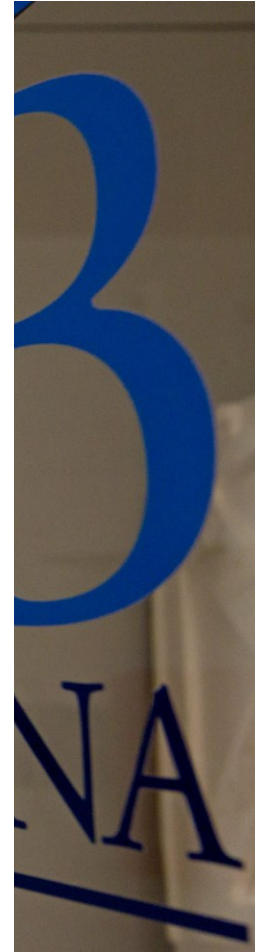
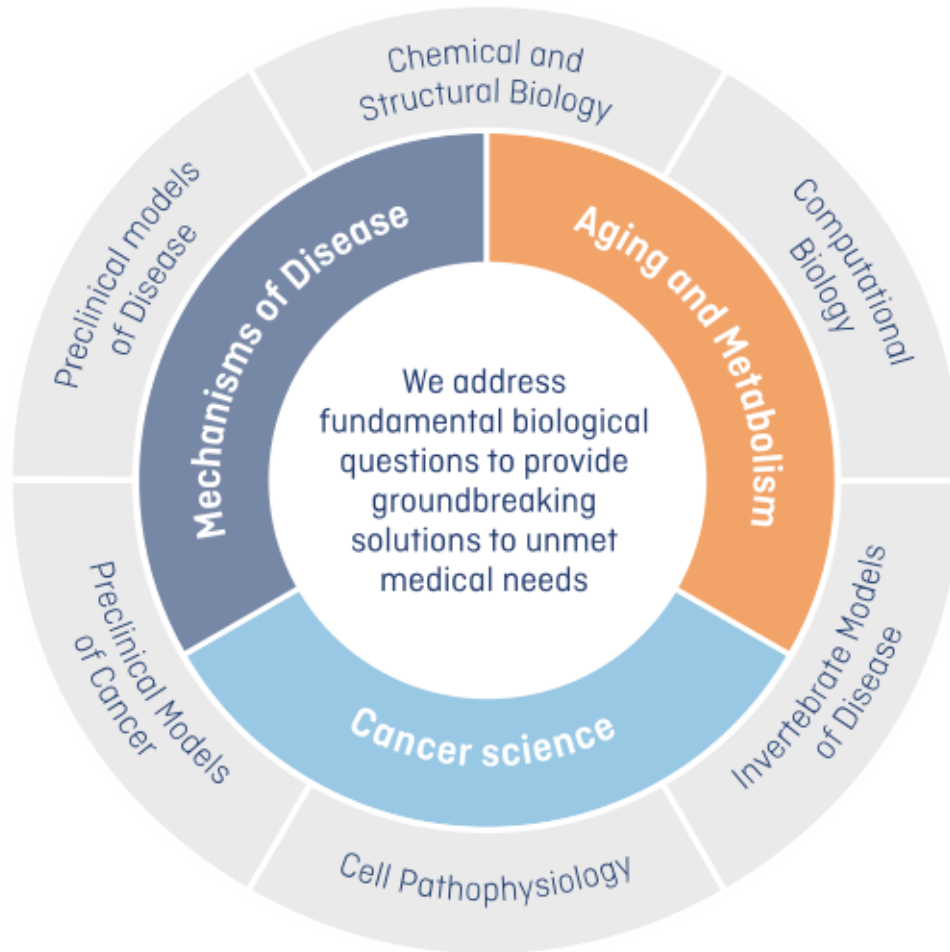
- PCB - Parc Científic de Barcelona





# Institut de Recerca Biomèdica (IRB Barcelona)

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



**523 Professionals**

**451 Scientific Staff**

**57% Women 43% Men**

**3 Research Programmes**

- Aging and Metabolism
- Mechanism of Disease
- Cancer Science

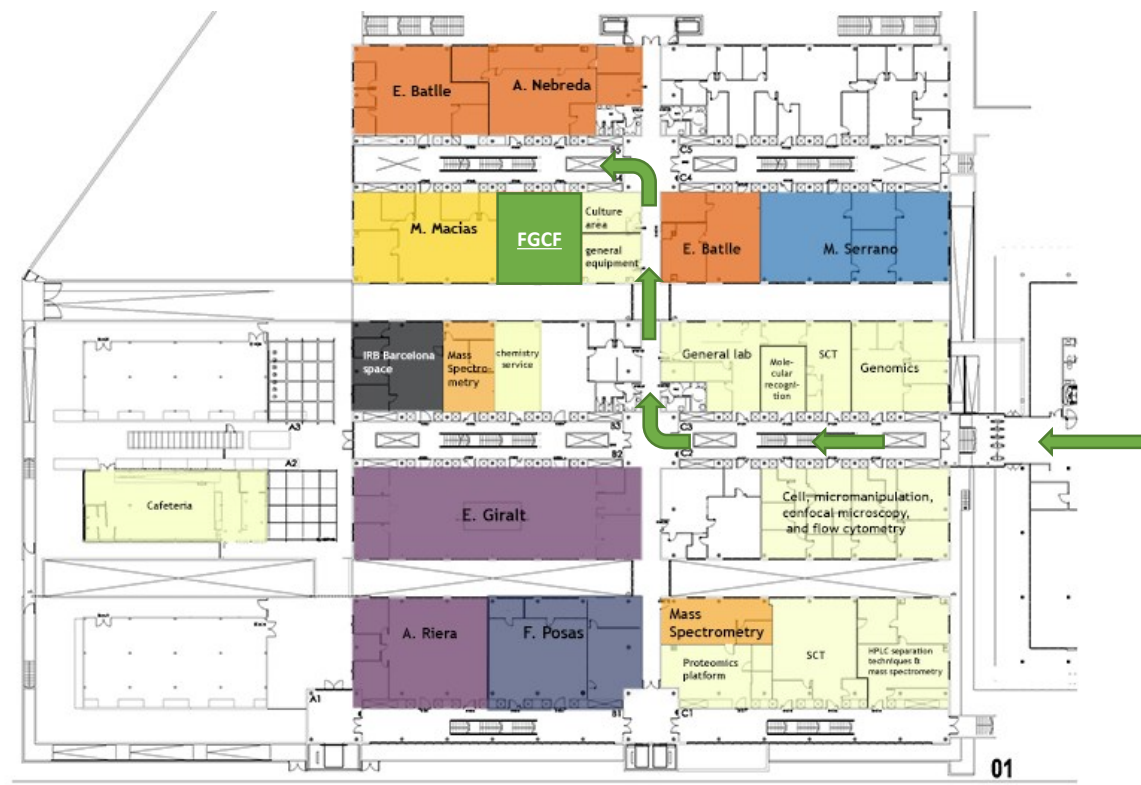
**28 Research groups**

**9 Core facilities**

**6 Active spin-offs**

# Location

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

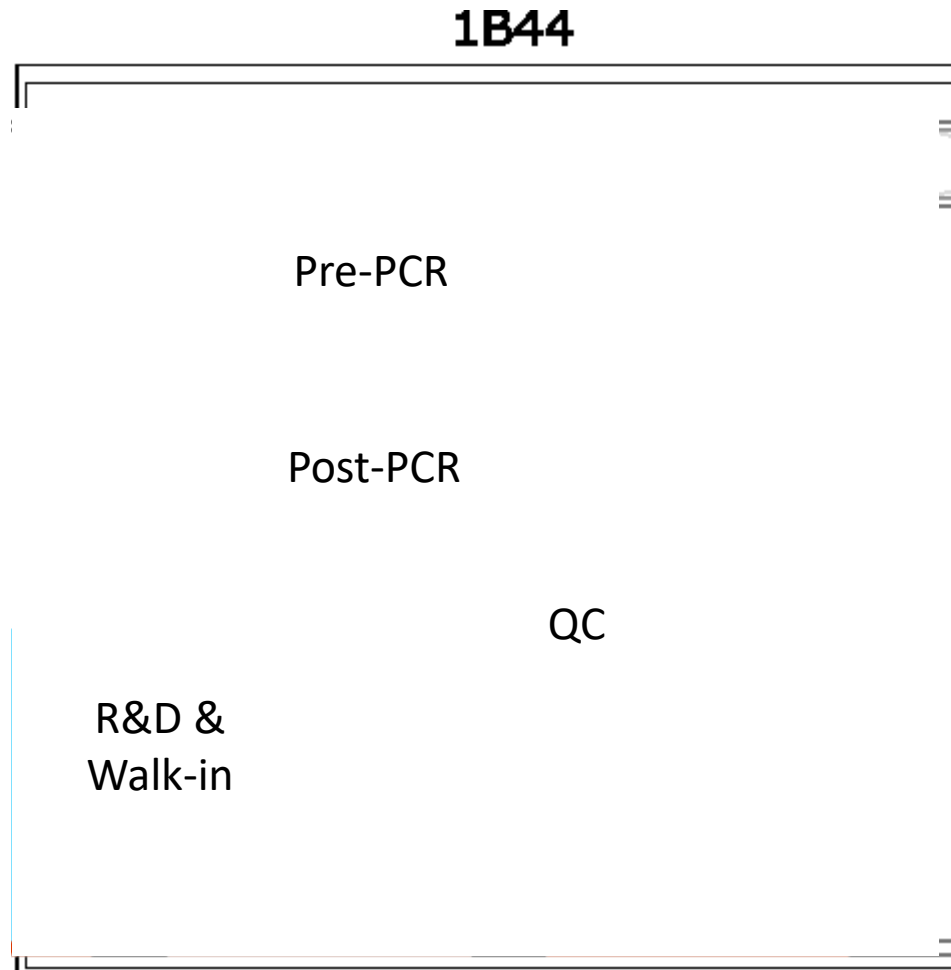


*Carrer de Baldiri Reixac*

*Carrer de Josep Samitier*

# 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)



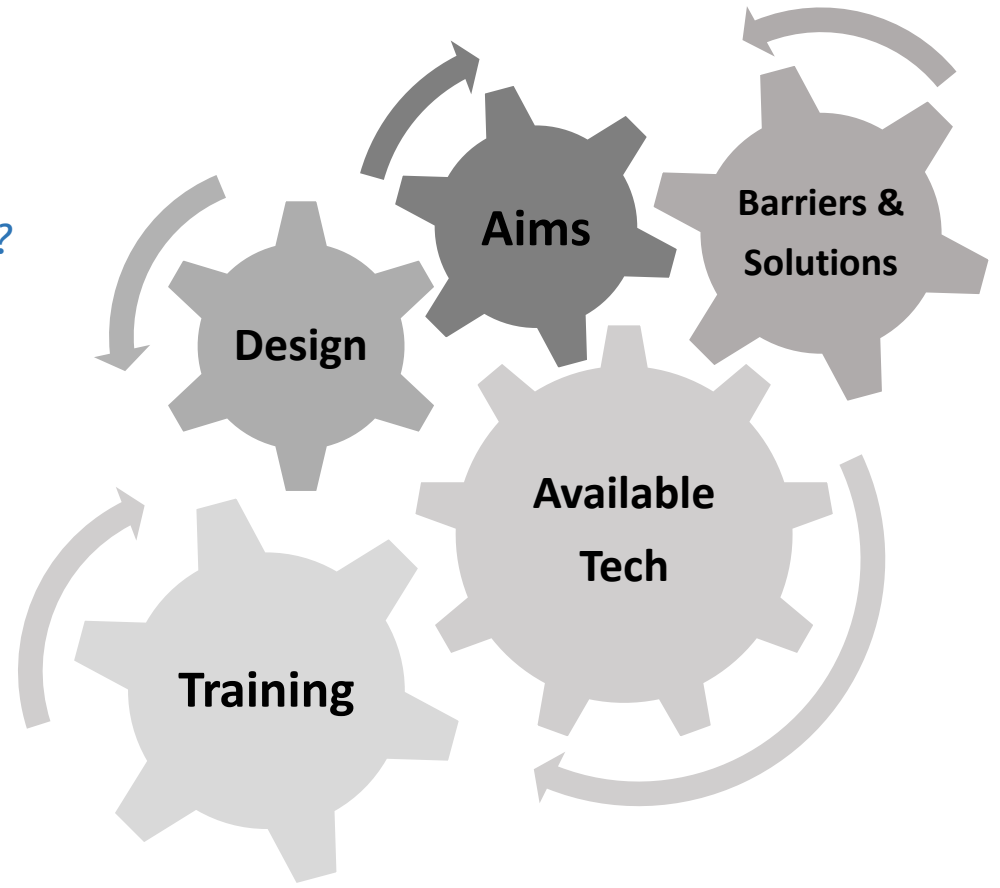
Freddy Monteiro, Ph.D.  
FGCF Manager



[genomics@irbbarcelona.org](mailto:genomics@irbbarcelona.org)

# How to engage with us

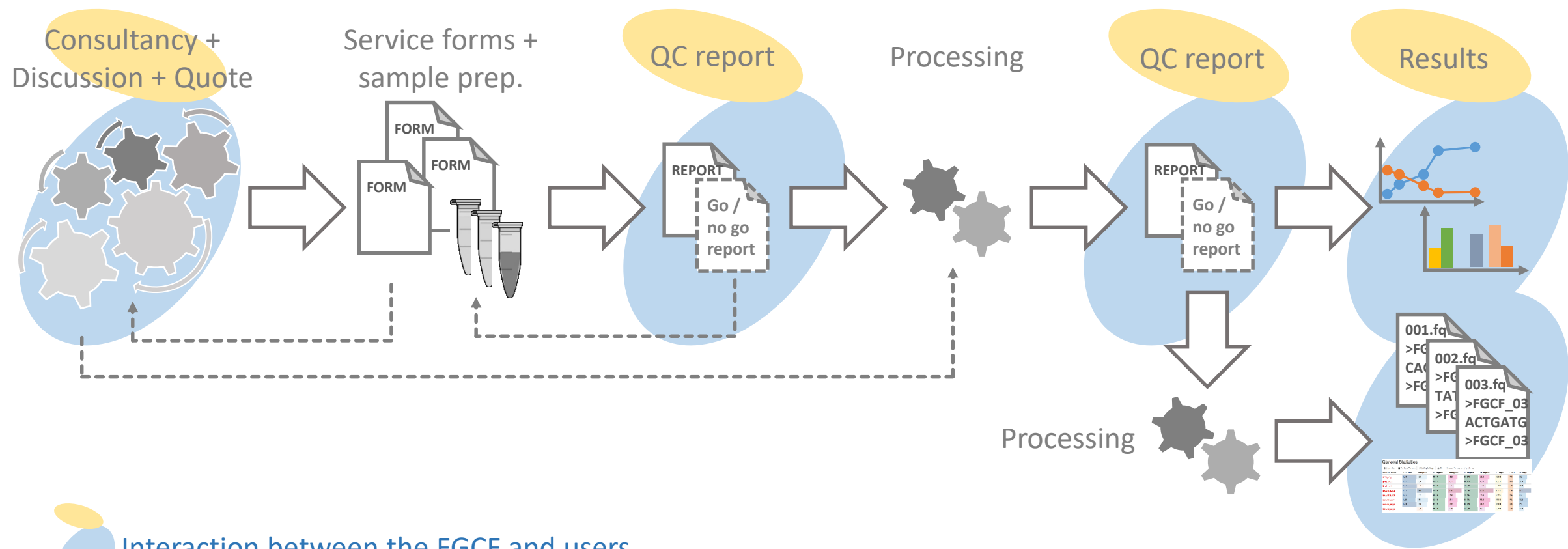
- *I am planning an experiment to genotype...?*
- *What are the sample requirements for...?*
- *How to remove clumps from my cell suspension?*
- *How to make sure cells are viable for the experiment?*
- *Should I use paired-end or single-end sequencing?*
- *Is this amount of cells enough for the experiment?*
- *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?*



genomics@irbbarcelona.org | (9340)-39803

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

# Project life cycle



# Why Single-Cell ?

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

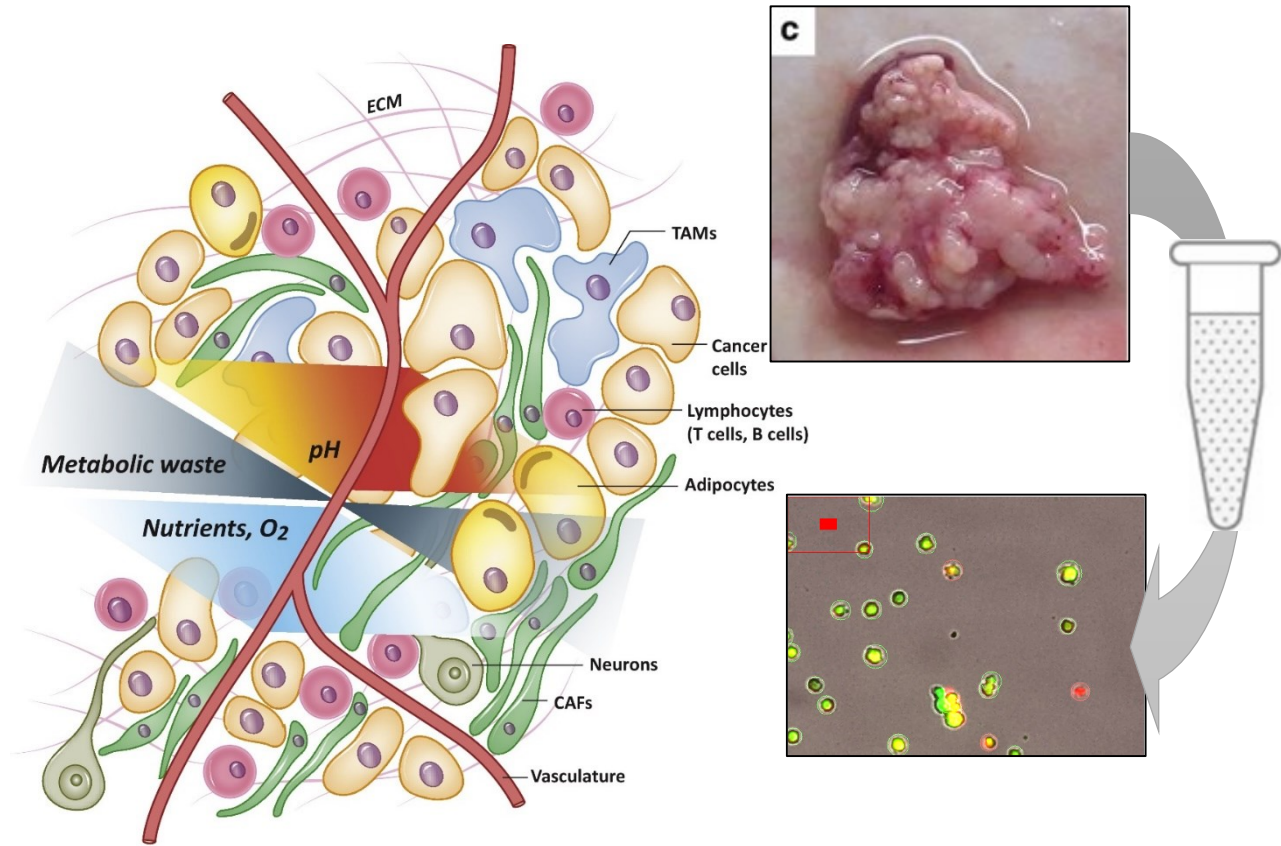
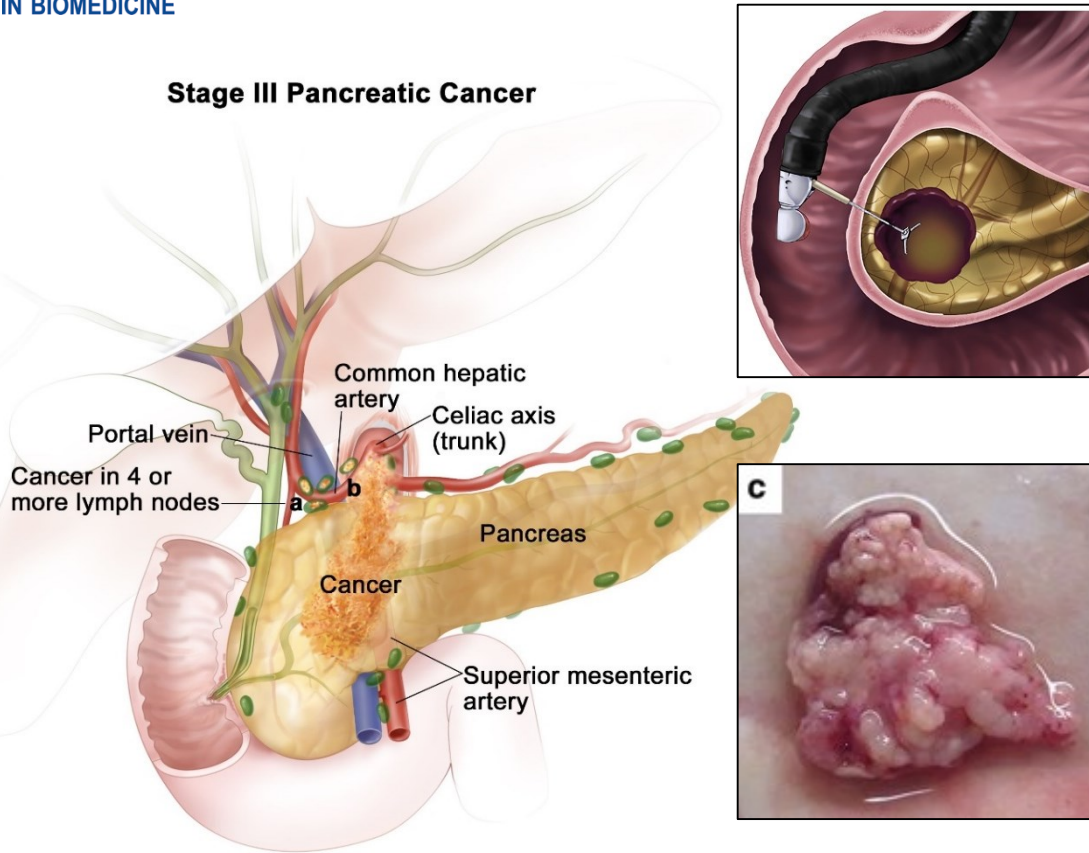


scRNA-seq  
=  
Transcriptional Fruit Salad  
=  
Gene expression of each cell  
+  
Absolute abundances



# Bulk RNA-seq or single-cell RNA-seq

Stage III Pancreatic Cancer



<https://www.cancer.gov/types/pancreatic/patient/pancreatic-treatment-pdq>

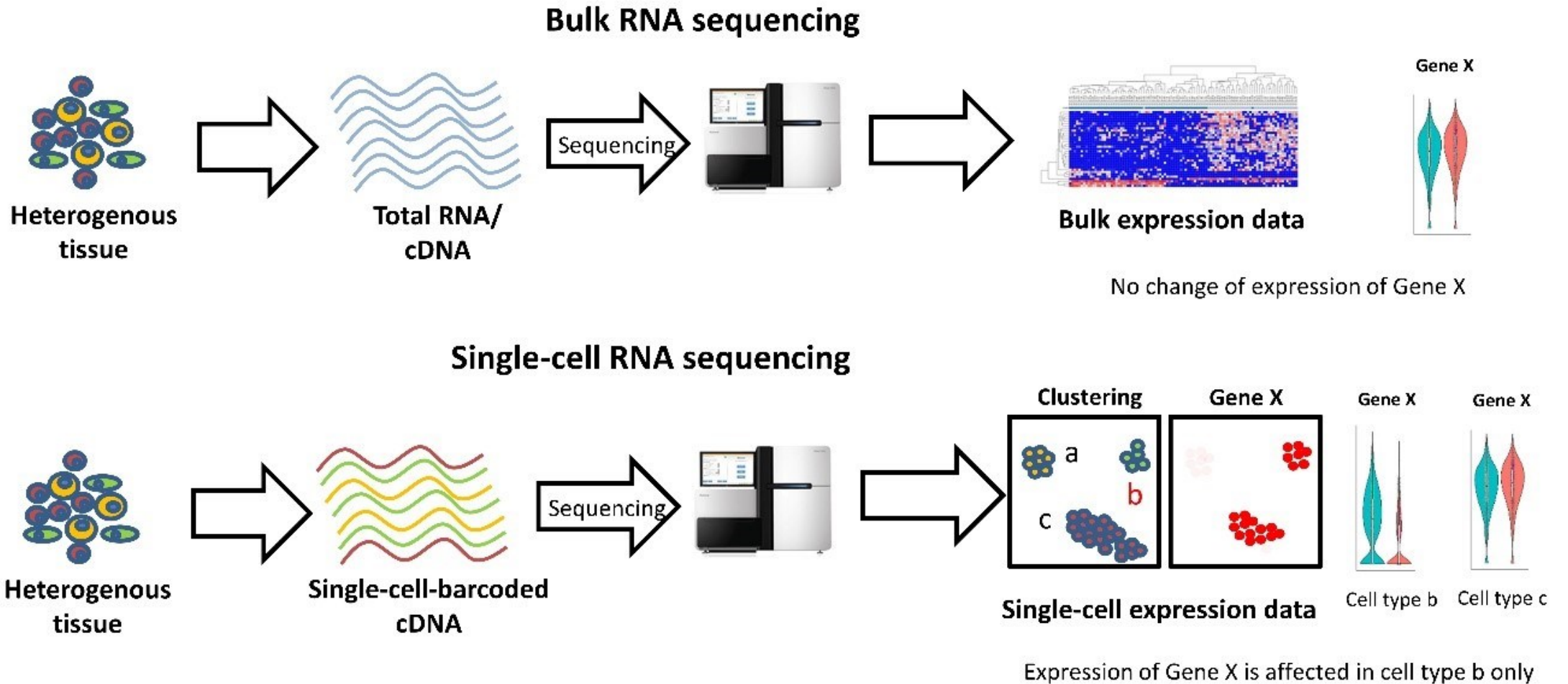
Cho et al. 2022

Payne et al. 2022

Lyssiotis and Kimmelman. 2017

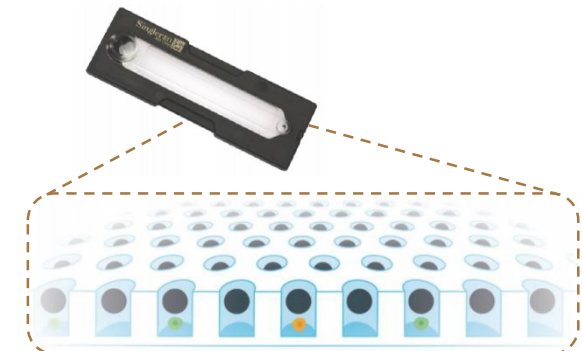
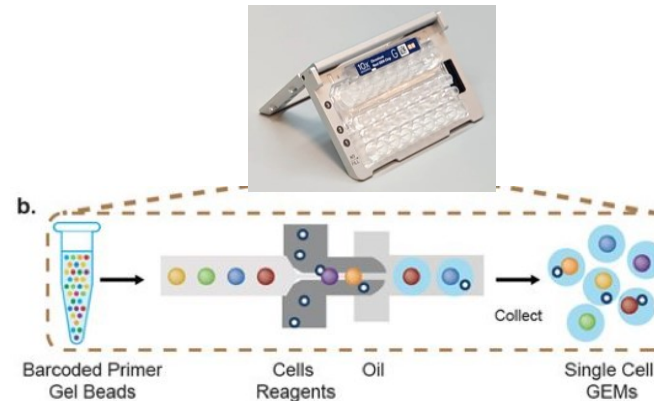
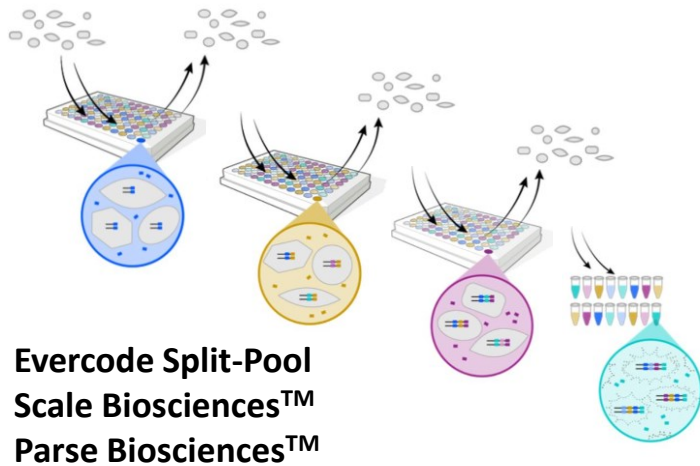
Payne et al. 2022

# Bulk Vs Single-Cell RNA-Seq



# scRNAseq and multiomic possibilities @ the FGCF

## Single Cell Transcriptomics, Epigenomics and Multiomics

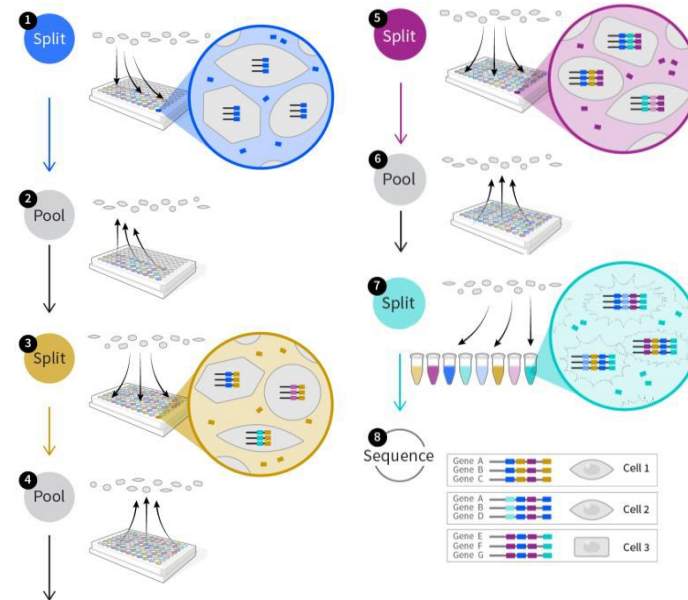




# Split-pool

## 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

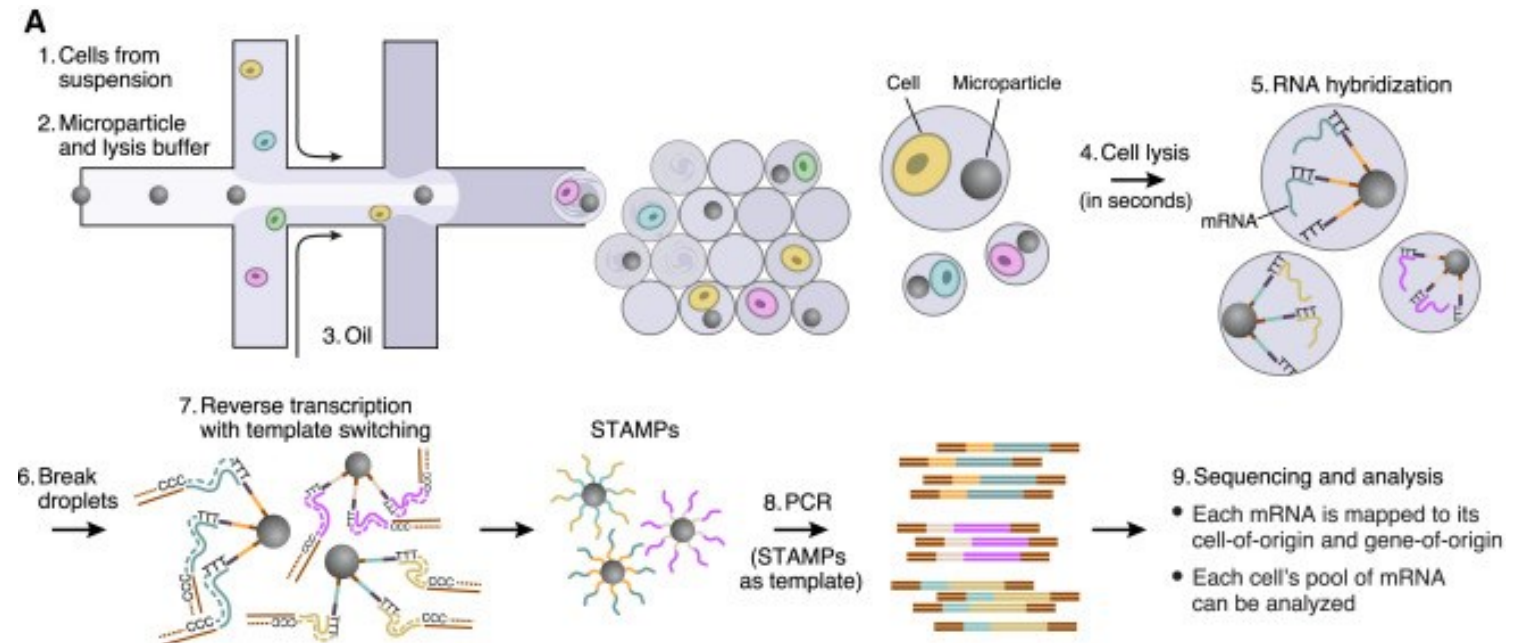




# Droplets

## Single-cell transcriptomics and epigenomics

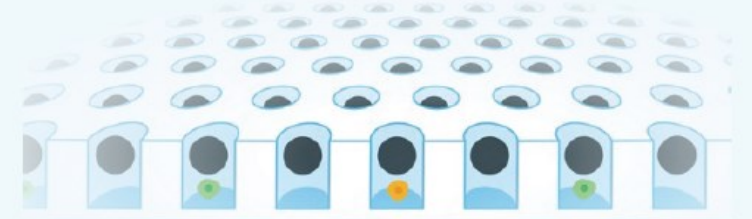
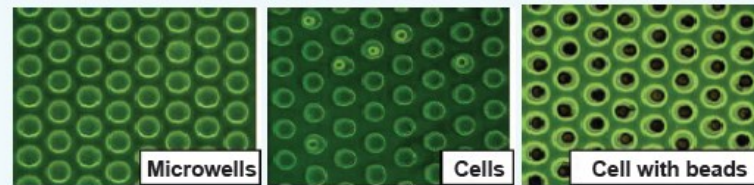
- Microfluidics technology from 10x Genomics for sc whole/targeted transcriptome interrogation, immune profiling, assay for transposase accessible chromatin, and Multiomics.
- Fast (18 minutes encapsulation + 9 hours library preparation)
- High-throughput (100-10.000 cells)



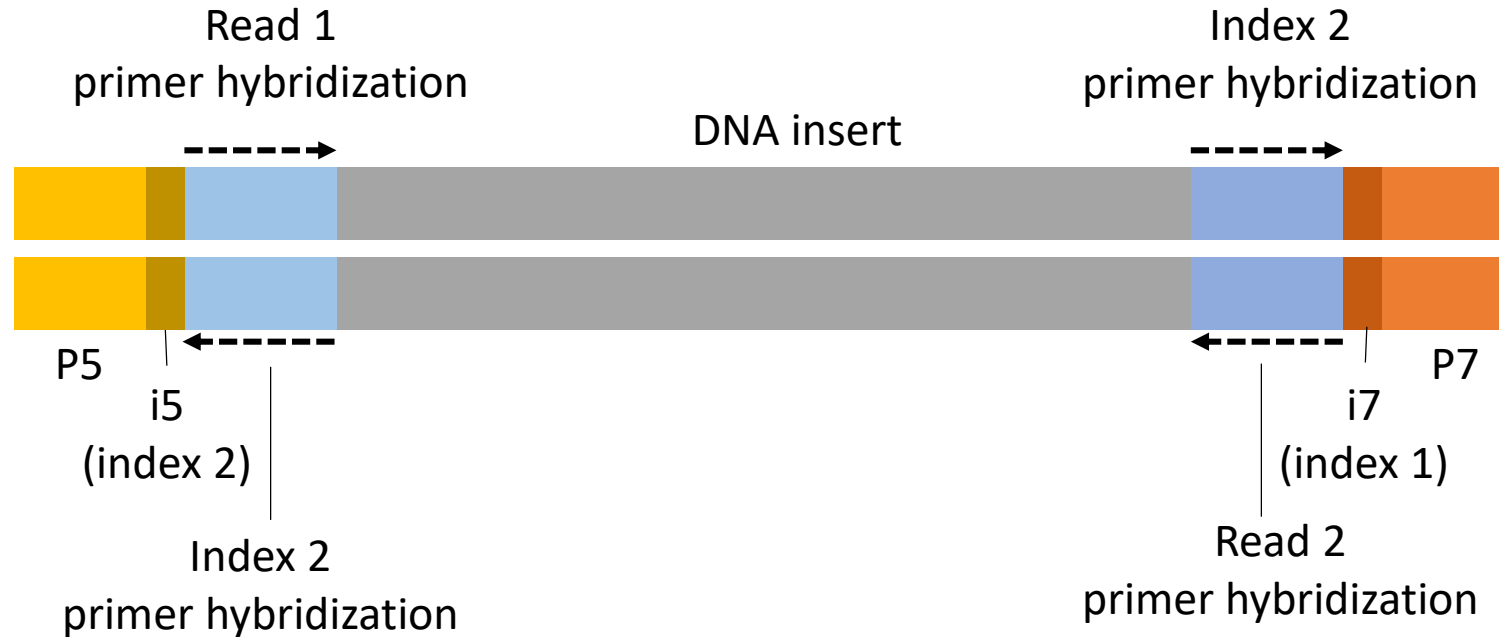
# Microwells

## Single-cell transcriptomics and epigenomics

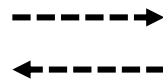
- SCOPE-chip from Singleron captures single cells by partitioning them into hundreds of thousands of microwells
- Standard chip: 500-10,000 single cells; High-density chip up to 30,000 cells per sample, or up to 120,000 cells on one HD chip when multiplexing samples with CLIndex
- Large-well chips ensure analysis of cell sizes up to 100  $\mu\text{m}$
- 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)

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



Marsh et al. 2022. Nature Neuroscience



**Functional Genomics Core Facility (FGCF)** @FunGenCore · Mar 9

Sample prep, sample prep, sample prep!! Care about it, cherish it, treasure it! 🧡

A highly recommended read for everyone planing SC RNAseq and potential multiomics projects. !!!



**Samuel Marsh, Ph.D.** @samuel\_marsh · Mar 8

Replying to @samuel\_marsh

While the title frames this work in terms of brain, a KEY takeaway from new data in this version is that this is broadly applicable across basically all scRNA-seq (and RNA-seq) studies (especially in immunology)... 2/n



1



1



11



**Samuel Marsh, Ph.D.** @samuel\_marsh · Mar 8

...and the artifact we discuss is unfortunately highly prevalent in current literature. So stick around even if brain isn't your thing 😊. We thoroughly characterize the issue and provide a robust flexible solution to eliminate it as well. 3/n



1



1



8





# 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**

- 1 Introduction
- 2 Getting Started with Sample Preparation
- 2 Sample Input Types for Various 10x Genomics Assays
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  - 12 1.2 Cell Thawing
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  - 14 2.2 General Cell Preparation Protocols
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  - 16 2.4 Feature-Specific Sample Preparation Protocols
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  - 21 3.1 Standard Cleanup Methods
  - 22 3.2 Advanced Cleanup Methods
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**10x GENOMICS**


10xgenomics.com

[https://cdn.10xgenomics.com/image/upload/v1686678481/support-documents/CG00053\\_Handbook\\_CellPreparation\\_SingleCellProtocols\\_Rev\\_D.pdf](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	SC3v3/v3.1	SC3v3/v3.1 with CRISPR Screening	SC3v3/v3.1 with Cell Surface Protein	SC3v3.1 with Cell Multiplexing	Document Type
Single Cell Protocols - Cell Preparation Guide	✓	✓	✓	✓	Demonstrated Protocol
Isolation of Nuclei for Single Cell RNA Sequencing & Tissues for Single Cell RNA Sequencing	✓*	X	-	✓	Last Modified June 17, 2022
Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling	✓*	-	✓*	✓*	
Tumor Dissociation for Single Cell RNA Sequencing	✓*	-	✓	✓*	

<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 asloomX and h5ad files to be further analyzed in R or Python.

Paper

Publication is available on  Preprint is available on 

<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)

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# Workflow: Qualification



**Freddy Monteiro**

FGCF-User Meeting (hosted by Freddy)

50 min appointments

FGCF (PCB, Cluster I, 1B44)

Hi! Thank you for reaching out to the FGCF to discuss your project.

In this webpage you can schedule your meeting with us. Feel free to ch

[Show more](#)

Select an appointment time

October 2024

M	T	W	T	F	S	S
28	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			
4	5	6	7	8	9	10

< >

TUE  
1

WED  
2

THU  
3

FRI  
4

	08:00	—	08:00	08:00
	09:00	—	09:00	09:00
	10:00	—	10:00	10:00
	11:00	—	11:00	11:00
	14:00	—	14:00	—
	15:00	—	15:00	—

<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 Hashing strategy.**

Experienced/First timer, Dissociation pilots.

**Controls and Replicates.** Experienced/First timer, Dissociation pilots.

**Reagents and consumables purchase.** Stock management.

**Deliverables.** Libraries, fastq, analysis\*,

**Quotation.** Ror revision and PI approval.



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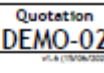
INSTITUTE FOR RESEARCH IN BIOMEDICINE

# Workflow: Quotation



**Quotation DEMO-02**

H/A 15/04/2024



**Service Details**

Number	Researcher	Date	Expire	URL
DEMO-02	John Doe (LABORANT) - IRB Barcelona	03/04/2024	22/10/2024	PDF

**IRB-FCQP contact**

Contact person	E-mail
Trinity Montolio	trinity.montolio@irbbarcelona.org
Trinity Pons	trinity.pons@irbbarcelona.org
Trinity Ferrández	trinity.ferrandez@irbbarcelona.org

**Service schedule and general recommendations**

- All projects should be scheduled in advance with the FCQP to ensure all reagents and materials are ready - [genomics@irbbarcelona.org](mailto:genomics@irbbarcelona.org)
- The FCQP is open Mon-Fri from 9:00. Schedule in advance your submissions to ensure availability and minimize service disruptions.
- Single cell suspension can be submitted Mon-Thu from 10:00.
- Clones can be requested Mon-Thu 9-10h and will be delivered at arranged times.
- Out of hours access to the FCQP instruments should be discussed with the facility manager - [trinity.montolio@irbbarcelona.org](mailto:trinity.montolio@irbbarcelona.org)
- If shipping samples to us, please inform delivery date, tracking no., and pack sufficient dry ice (at least 1 kg of dry ice for an overnight delivery).

**Service Details**

Reference	Description	Cost per Batch (€)	Cost per sample (€)	BATCH (€)	SAMPLE (€)	TOTAL (€)
FCQP01	Library pooling for sequencing - Processing	73,88 €	6,71 €	2	€	150,76 €
FCQP02	10x 3' GEMs+GEX+QC+Cells, Qubit, BA) with user reagents (batch max. size=8, w/o multiplex)	1.013,84 €	2.028,84 €	1	€	6.286,22 €
FCQP03	NextSeq 2020 cell up. NextSeq run	147,74 €	2,98 €	1	€	156,20 €
FCQP04	NextSeq 2020 lanes PR	3.154,84 €	2,98 €	1	€	6.120,80 €
<b>Subtotal (€)</b>						<b>12.462,82 €</b>

**Single-cell service terms and sample requirements**

- Discuss and validate your experimental design with the FCQP and your submission before committing to any actual experiment.
- The success of single cell projects will be maximized through discussion, clarification and the deployment of robustness pilot assays.
- If submitting clones for distribution, please make sure automated methods are in place, or they are closely transferred to the FCQP for implementation.
- Refer to the 10x Single Cell Application Guide for general sample requirements and recommendations (C000003).
- Refresh the FCQP when you start your cell preparation and keep us posted.
- Due to the time sensitive nature of this experiment, please come at the arranged time and date to room B3B34 (PCR Cluster), Level 3, Lab 3B46.
- You should bring the service sheet and samples with +75 µl of single cell suspensions adjusted to about 1.200 cells/µl (500-1.200) with the viability at least 70% (see below for more information).
- Cell suspension buffer: Calcium-free, magnesium-free, DMSO-free, EDTA-free 10x PBS containing 0.24% weight/volume BSA (400 µg/ml), 0.1%  $\beta$ -2-microglobulin, please refer to the required washing steps in the sample preparation guide. For other compatible buffers refer to page 3 of the 10x Single Cell Preparation Guide (C000003).
- It is super important that your single cell suspensions are free of debris and aggregates. Filtering of cells, if possible, will add extra costs.
- Please remember that FACS sorting often concentrate cell concentration. Ask the FACS facility for advice.
- We will confirm cell concentration and viability before starting and dilute your samples if needed.
- Bring a clean tube with ice-cold cell resuspension buffer to perform cell dilutions, if needed.
- Should you not have enough live cells and want to proceed, you will be responsible for the assay volume.
- Target cell recovery rate is variable and dependent on the sample type. Counters can overestimate cell number. The requested number of cells is thus indicative and the actual cell number recovered might differ significantly.
- A QC report will be generated for each project summarizing the processing steps. You might request a copy of our data report before your experiment.
- Deliverables: sequencing-ready libraries compatible libraries, sequencing-ready sequencing pools, or data files.
- Bioinformatics analysis is not included, we might provide advice and contacts whenever required. Please contact them with your submission.
- Publications-ready material and methods are provided upon request. Expect your data to be prepared between 3 and 10 working days.

**Acceptance (please check boxes, sign, and provide PID number)**

We accept the quotation number DEMO-02

We have read and agreed with the service terms, conditions, and requirements.

Name	Position	Initial	Phone
_____	_____	_____	_____


Signature \* (mandatory) P.I.D. Number \*\* (mandatory for external users)

**Special instructions:**

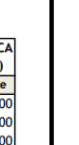
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**IRB Barcelona - Functional Genomics 2024**



Service	Public and CERCA Institutions (€)	
	Batch	Sample
<b>qPCR (FGQP)</b>		
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
<b>Single-cell (FGSC)</b>	<b>Batch</b>	<b>Sample</b>
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 3GEMs 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 3GEMs+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 3FB Library with FG reagents (batch max. size=8)	147,32	78,84
10x 3GEMs+GEX+FB+QCs(Cells,Qubit,BA) with FG reagents (batch max. size=8)	1.502,06	2.212,46
10x 5GEMs 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>

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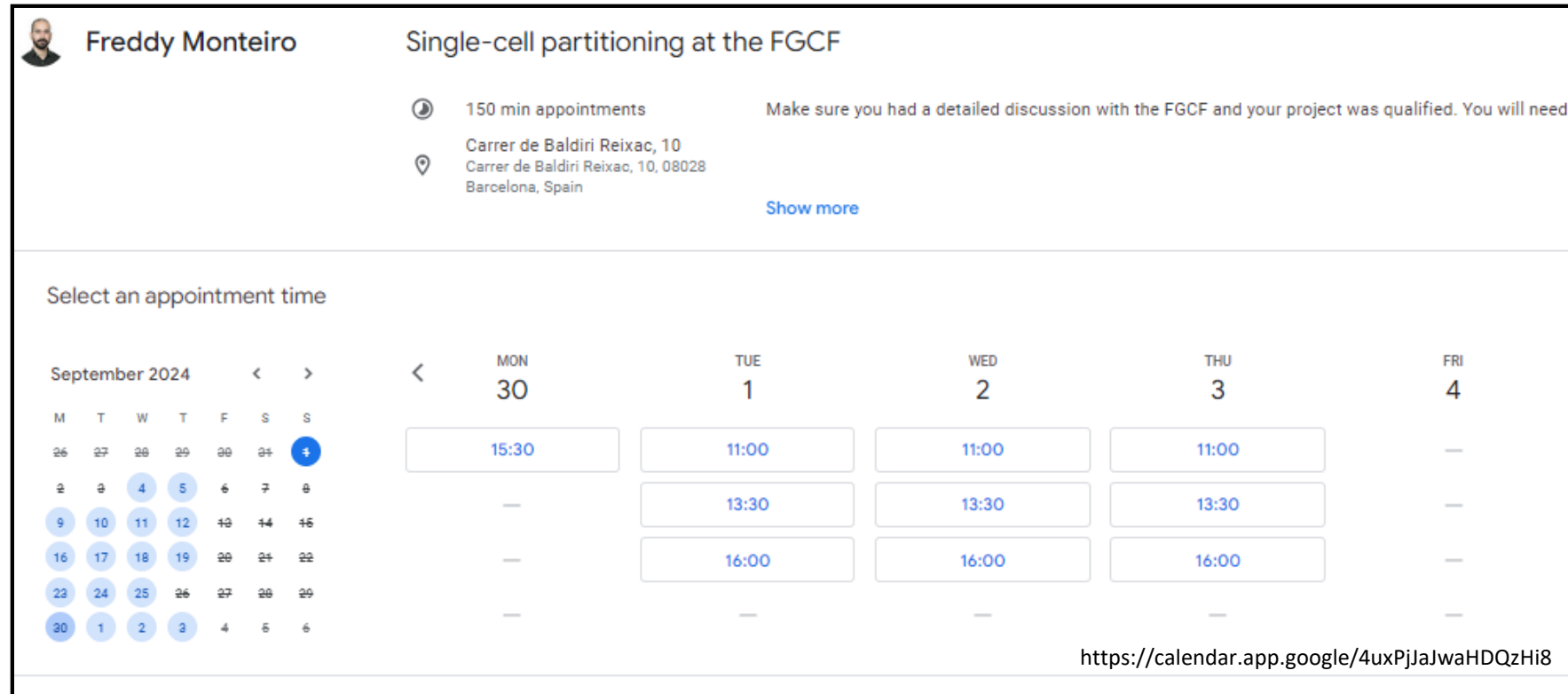
Reporting: Document processing (demo report)

# Workflow: Scheduling

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

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

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



**Freddy Monteiro** Single-cell partitioning at the FGCF

150 min appointments Make sure you had a detailed discussion with the FGCF and your project was qualified. You will need

Carrer de Baldiri Reixac, 10  
Carrer de Baldiri Reixac, 10, 08028  
Barcelona, Spain

Show more

Select an appointment time

September 2024 < >

	MON	TUE	WED	THU	FRI
30	15:30	11:00	11:00	11:00	—
1	—	13:30	13:30	13:30	—
2	—	16:00	16:00	16:00	—
3	—	—	—	—	—

<https://calendar.app.google/4uxPjJaJwaHDQzHi8>



# 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: 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.

10x GENOMICS

Chromium Next GEM Single Cell 3' GEM Kit v3.1		
	#	PN
<input checked="" type="radio"/> RT Reagent B	1	2000165
<input checked="" type="radio"/> RT Enzyme C	1	2000085
<input checked="" type="radio"/> Template Switch Oligo	1	3000228
<input type="radio"/> Reducing Agent B	1	2000087
<input type="radio"/> Cleanup Buffer	2	2000088
<input checked="" type="radio"/> cDNA Primers	1	2000089
<input type="radio"/> Amp Mix	1	2000047

10x GENOMICS

Chromium Partitioning Oil			Chromium Recovery Agent		
	#	PN		#	PN
<input checked="" type="radio"/> Partitioning Oil	6	2000190	<input type="radio"/> Recovery Agent	6	220016

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

10x GENOMICS

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

10x GENOMICS



# Workflow: Sample submission

**IRB**  
**BARCELONA**  
 INSTITUTE FOR RESEARCH IN BIOMEDICINE

**Instituto for Research in Biomedicine (IRB Barcelona)**  
**Functional Genomics Core Facility (FGCF)**  
 Parc Científic de Barcelona, Clustor 1-51C14  
 s/Naldíri Rovira, 18, 08028 Barcelona, Spain  
 t: +34 93 403 3083 / f: +34 93 403 3458  
 g: genoma@irbbarcelona.org

**IRB-GenTAC-req Sample Information v1.4 (14/04/2022)**

To avoid delays in the processing of your sample please complete all fields marked with an asterisk [\*]  
 Should you have any questions, please contact us at: genoma@irbbarcelona.org

**1. Name**  
 Name: .....  
 E-mail: .....  
 Phone: .....  
 Affiliation: .....

**2. Principal Investigator**  
 Name: .....  
 E-mail: .....  
 Phone: .....  
 Affiliation: .....

**3. Shipping**  
 Institute: .....  
 Address: .....  
 City: .....  
 Name: .....  
 E-mail: .....  
 \*codetara: \*for external use only

**4. Experimental details [for FGCF use only]**  
 Submission: .....  
 Labels: .....  
 QC: .....  
 Protocols: .....  
 Incident: .....

**5. Sample Information \***  
 No. Samples: .....  
 Design group: .....  
 Operation: .....  
 Source: .....  
 Extraction: .....  
 Elution Date: .....  
 Workflow: .....  
 Seq. Output: .....  
 Seq. Strains: .....  
 Other: .....

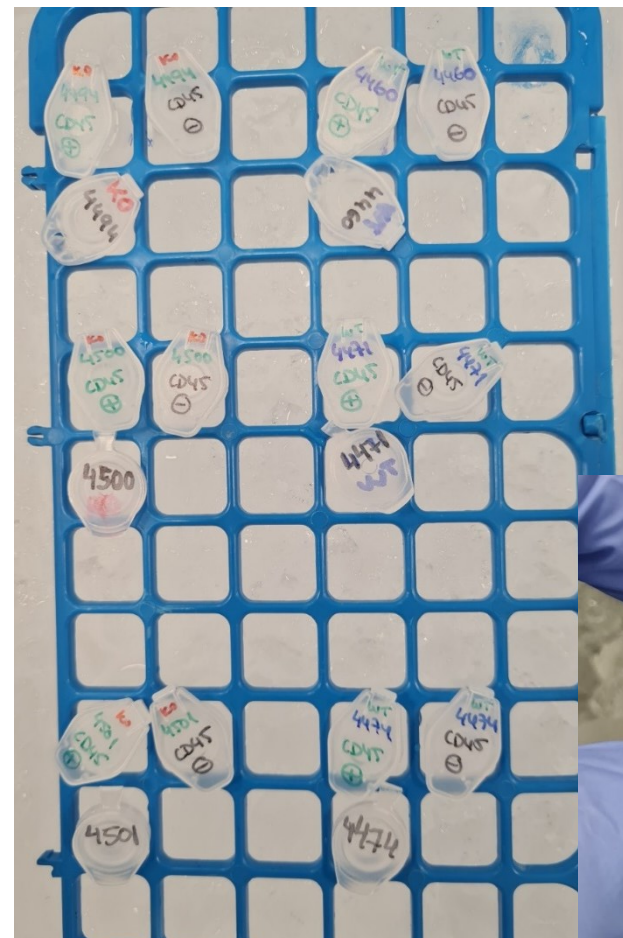
**Sample Labels [as written in the lab] \***  
 Please use 1, 5 or 2 ml Eppendorf tubes. Extra charges might be applied if other tubes are used.  
 Please make sure you label multiple the correct labels in the tubes and include all relevant information whenever it is available.  
 All samples will be quality controlled as a standard instrument. Should you opt out this step, the FGCF will not be held responsible for steps written

FGCF ID	Tube Label	No. Cells	X Line	FGCF ID	Tube Label	No. Cells	X Line
1				16			
2				17			
3				18			
4				19			
5				20			
6				21			
7				22			
8				23			
9				24			
10				25			
11				26			
12				27			
13				28			
14				29			
15				30			

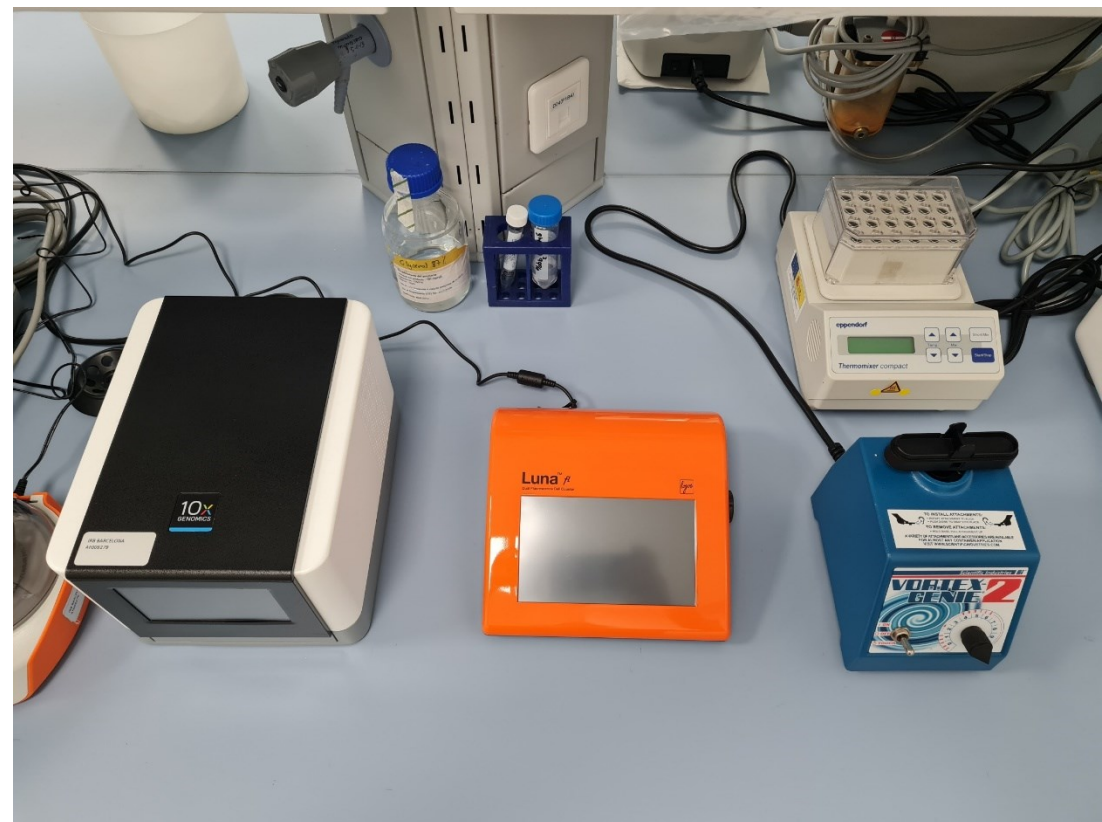
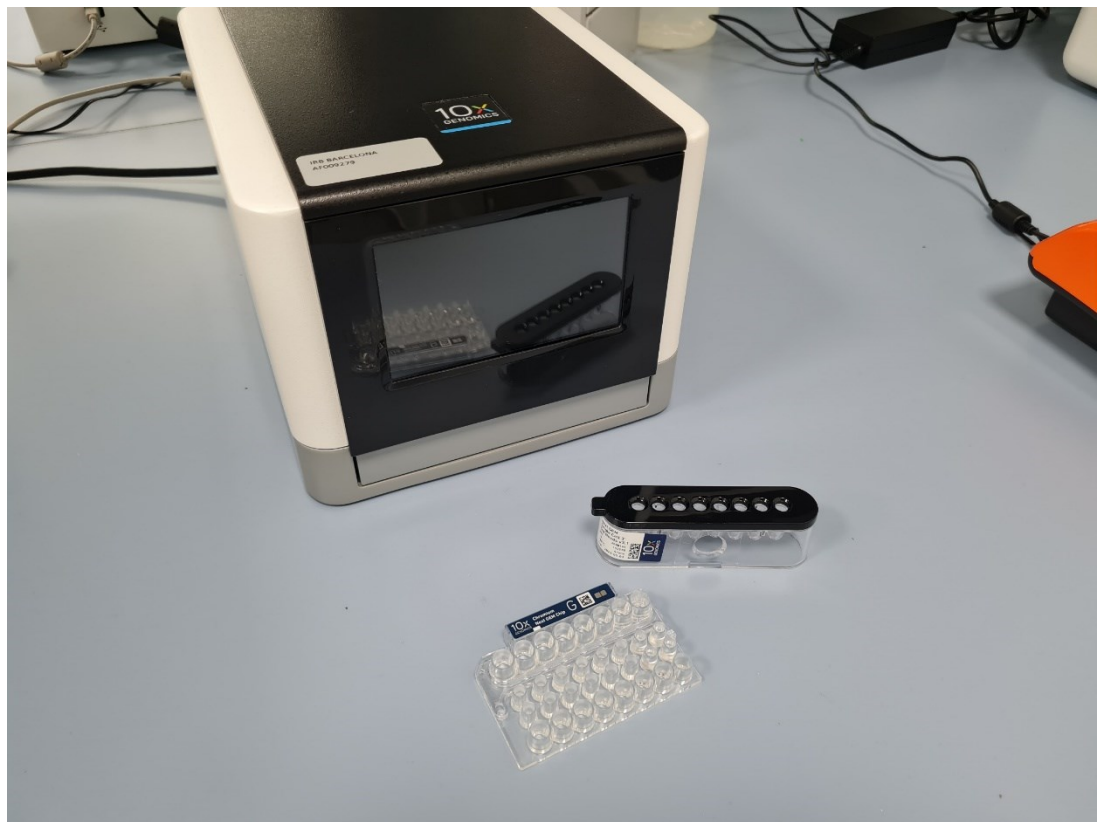
\*codetara: \*for external use only

**7. Protable, unmeasurable, and vials [for FGCF use only]**

Quantity	Description	Quantity	Description
.....	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....



# Workflow: Workbench and reagents preparation





# Workflow: Cell QC



## Cell Count Report

1/2

File name	Date
fg-21032023_003350_3-1	21 Mar., 2023, 00:34

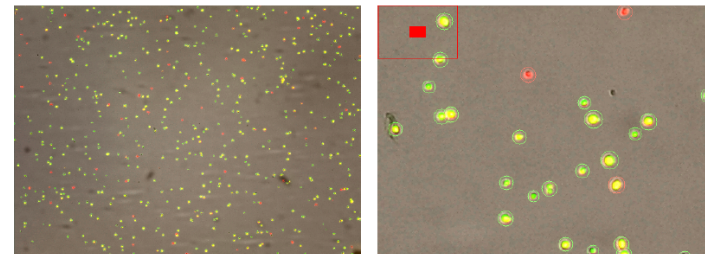
### Cell count results

Total cell concentration:  $1.46 \times 10^6$  cells/mL  
 Live cell concentration:  $1.23 \times 10^6$  cells/mL  
 Dead cell concentration:  $2.34 \times 10^5$  cells/mL  
 Viability: 84.0 %  
 Average cell size: 15.4  $\mu$ m  
 Total cell number: 595  
 Live cell number: 500  
 Dead cell number: 95

### Protocol

Protocol name: DEFAULT  
 Dilution factor: 1.11  
 Min. cell size: 3  $\mu$ m  
 Max. cell size: 60  $\mu$ m  
 Size gating: 3 ~ 60  $\mu$ m  
 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)

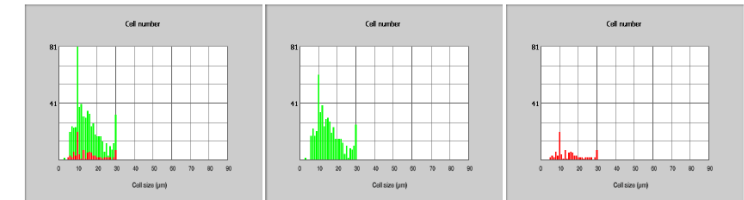


## Cell Count Report

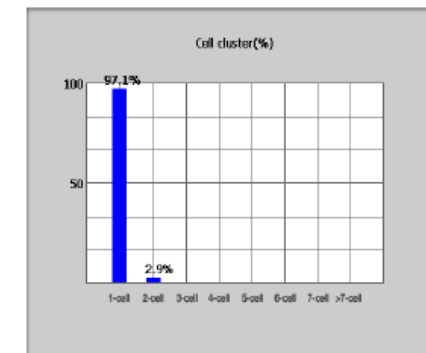
2/2

### Cell size histogram expressed by cell number

Total cells      Live cells      Dead cells



### Cell cluster graph





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# Workflow: Loading volume



## 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	20.1 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	23.4 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 32.2	11.0 30.8	12.4 29.5	13.8 29.5
1300	0.6 42.6	1.3 41.7	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	4.5 38.9	5.4 38.0	6.3 37.1	7.2 36.3	8.1 35.4	9.1 34.5
2000	0.4 42.8	0.8 42.4	1.7 41.6	2.5 40.7	3.4 39.9	4.4 39.1	5.3 38.3	6.2 37.4	7.1 36.6	8.0 35.8	8.9 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

10x genomics: **65 %** recovery (NextGEM 3' v3.1)  
 distributor: **50 %** recovery (NextGEM 3' v3.1)  
 reality: **35 %** recovery (NextGEM 3' v3.1)

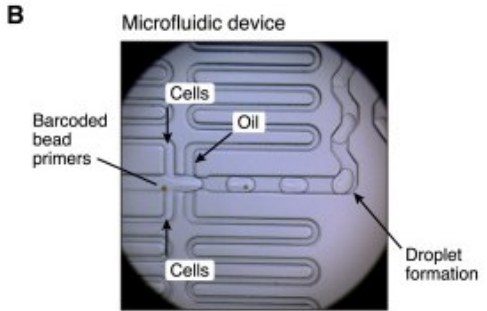
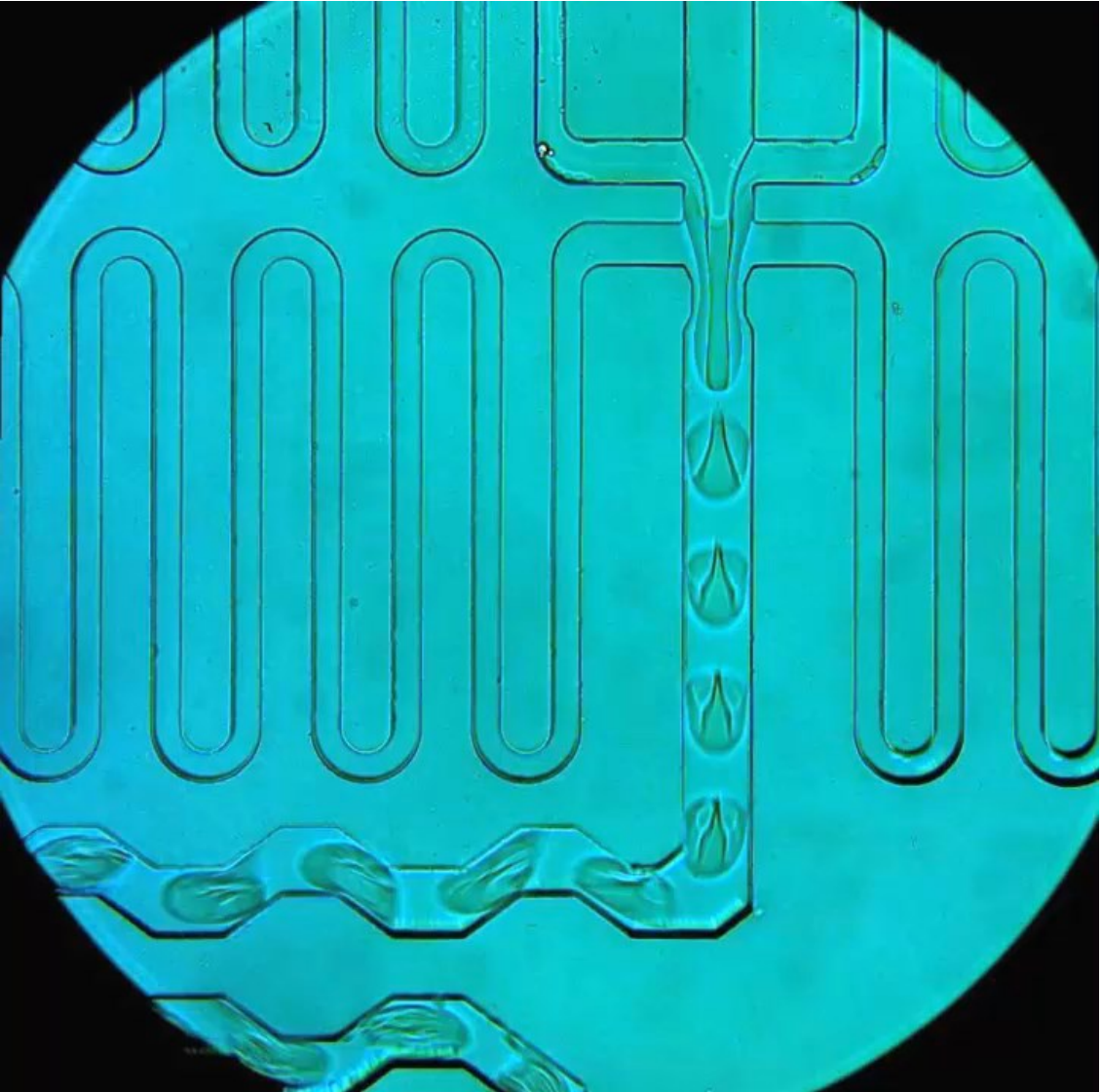
User-defined Target  
 Cell responsive  
 10,000  
 5,000  
 5,000 cells

FCSE accumulated experience

500	1000	2000	5000	4000	5000	6000	7000	8000	9000	10000	12500	15000	17500	20000	22500	25000	27500	30000	40000	45000	50000	
900	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
800	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
700	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
600	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
500	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
400	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
300	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
200	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												
100	8.3	16.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0												

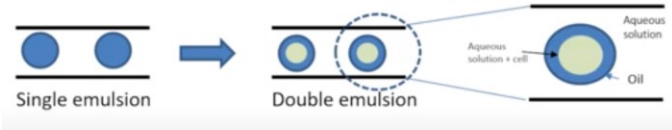
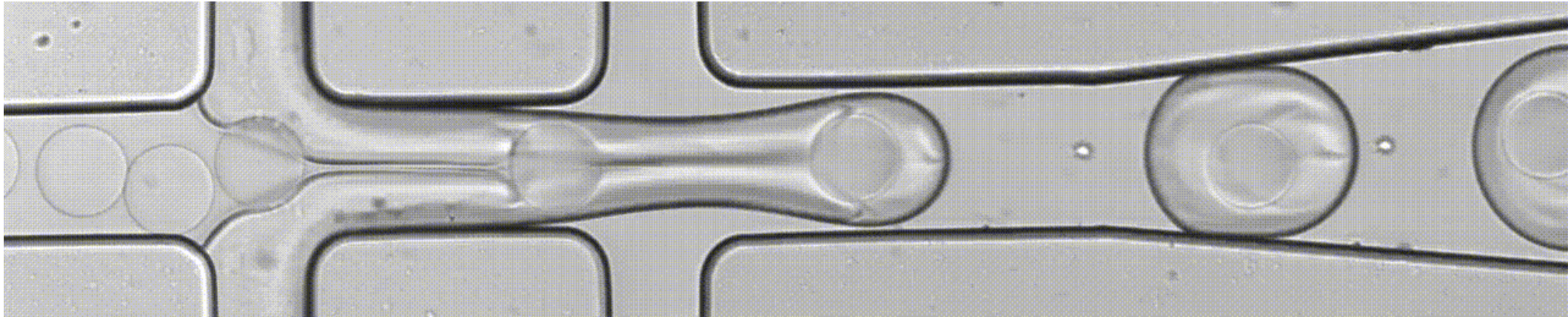
In house table for 30% recovery and overloading targets

# Workflow: Cell Partitioning

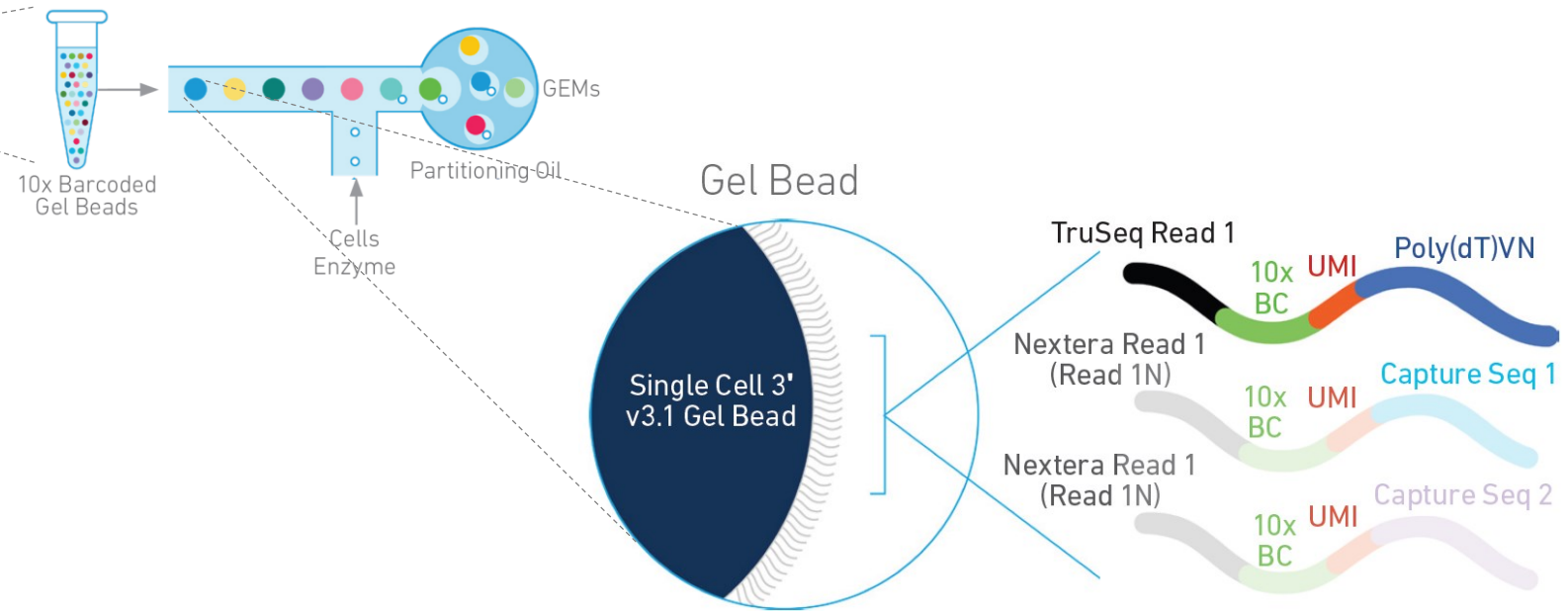




# Workflow: Cell Partitioning



# 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

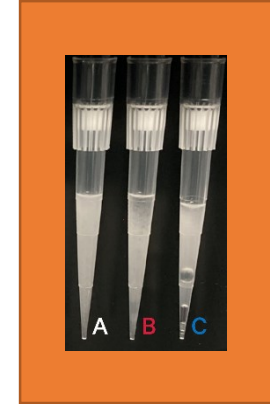
iv. Capture Sequence 1 or 2

22 nt sequence that is the reverse complement of the sequence inserted into the DNA (Antibody) or RNA (sgRNA) based sequence



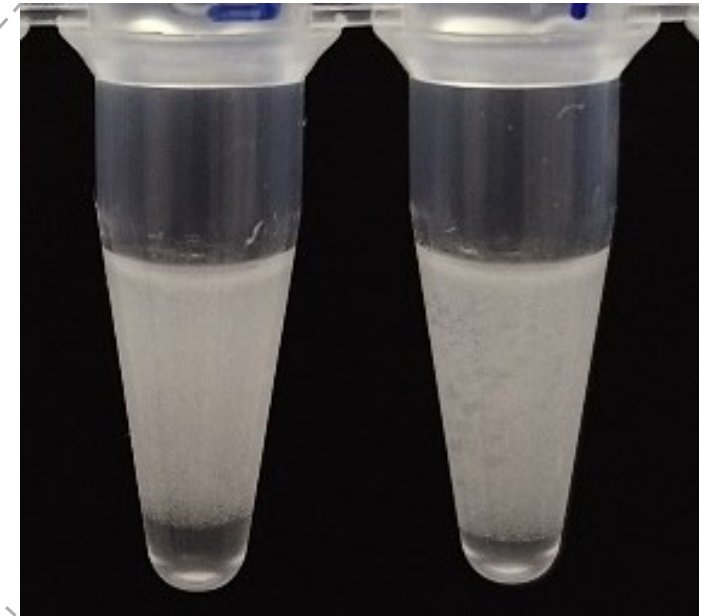
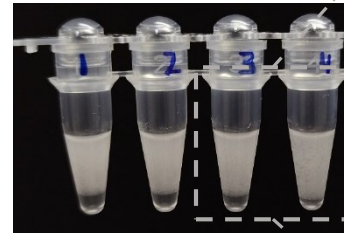
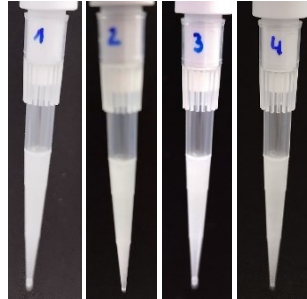
# GEMs recovery and visual QC

Transfer GEMs



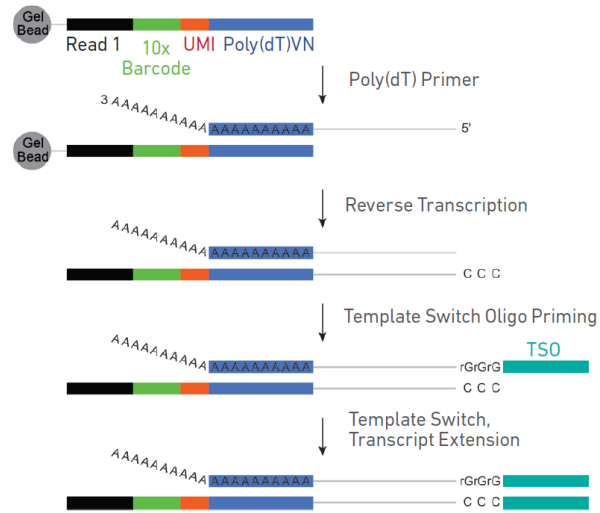
<https://kb.10xgenomics.com/hc/en-us/articles/218135863-What-is-a-wetting-failure-and-how-can-they-be-recognized>

0987\_2024\_0989\_2024  
0986\_2024\_0988\_2024



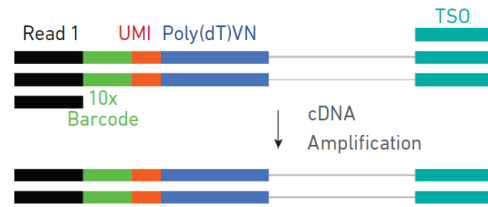
# 10x genomics library preparation procedures

Inside individual GEMs



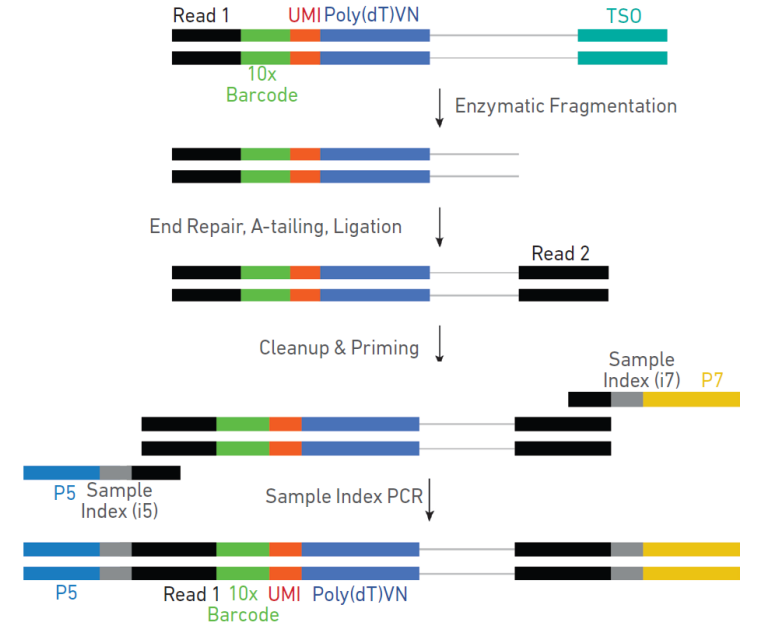
**Recov.  
reagent**

Pooled cDNA amplification

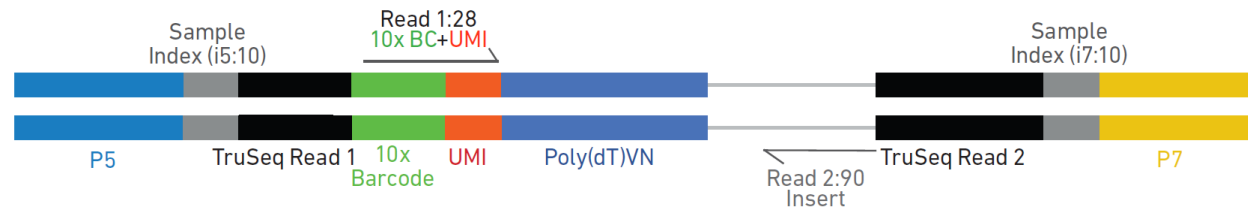


**QC**

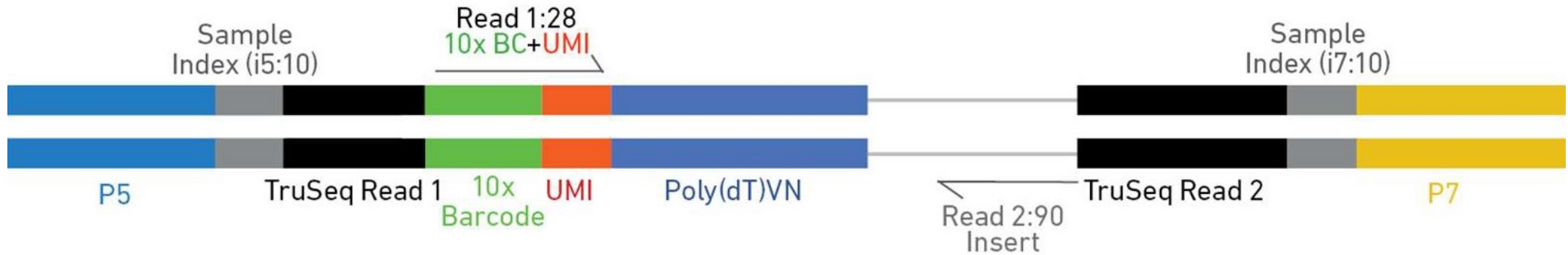
Amplified cDNA processing (dual index)



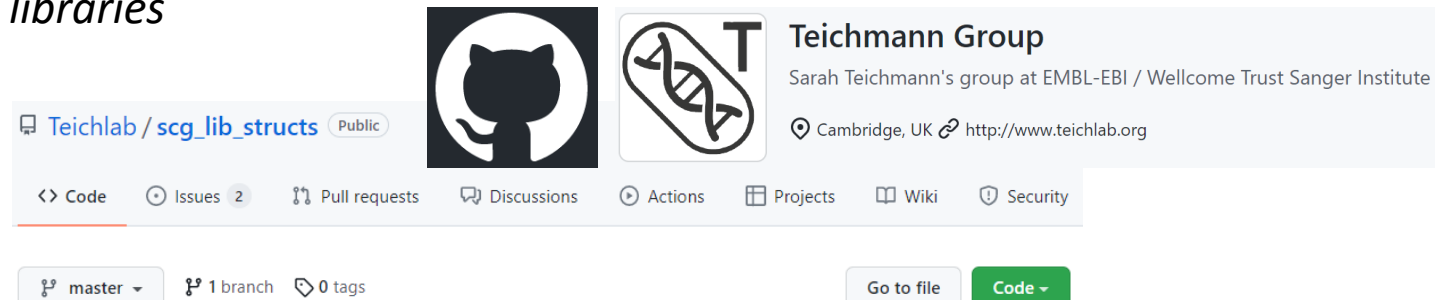
**QC**



# 3' v3.1 GEX library structure and resources



*Actual sequences and other sc libraries*



Teichlab / [scg\\_lib\\_structs](#) Public

**Teichmann Group**  
Sarah Teichmann's group at EMBL-EBI / Wellcome Trust Sanger Institute  
Cambridge, UK <http://www.teichlab.org>

<> Code Issues 2 Pull requests Discussions Actions Projects Wiki Security

master 1 branch 0 tags Go to file Code

# 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)

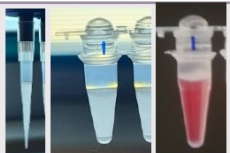
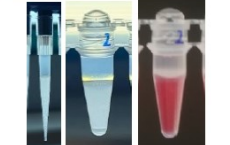
# Workflow: Reporting

## Single-Cell Transcriptomics service report

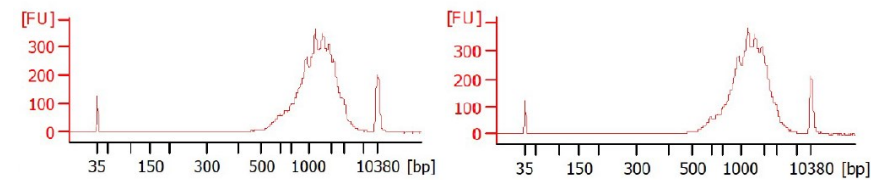
User: John Doe  
 PI: Jane Doe  
 Project: JDoeJan22\_scRNAseq  
 Date: 01/01/2022



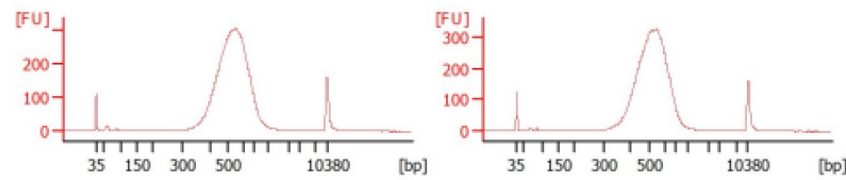
INSTITUTE FOR RESEARCH IN BIOMEDICINE

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



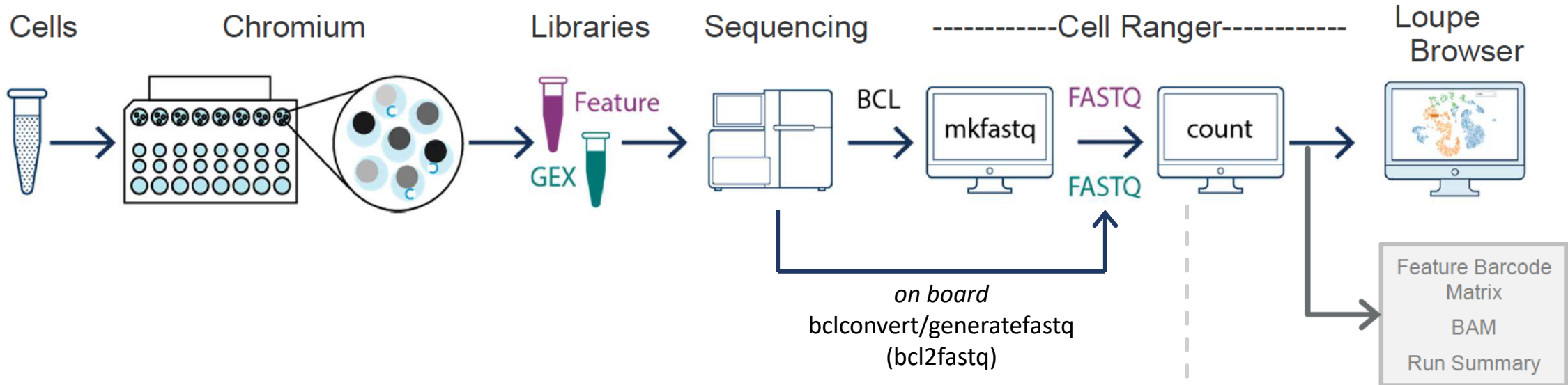
FGCF ID	cDNA used amount (ng) in 10 μl	Dual Index TT Set A	Cycles	Library concentration (ng/μl)	Volume (μl)	Yield (ng)	Average size (bp)
00X1_2022	85.1	SI-TT-X1	12	19.7	35	689.5	526
00X2_2022	121	SI-TT-X2	12	30.3	35	1060.5	538



Reads	Valid reads	Cells	Median UMI per Cell	Median Genes per Cell	Saturation	Reads/Cell	Cell recovery
259.775.175	97,90%	<b>13.311</b>	<b>3.039</b>	902	53,40%	<b>19.516</b>	53%
251.695.980	97,50%	<b>13.211</b>	<b>2.520</b>	917	53,00%	<b>19.052</b>	56%
126.980.438	97,70%	<b>6.170</b>	<b>2.649</b>	794	48,70%	<b>20.580</b>	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="$ID" \
  --transcriptome=/scratch/offg/fdeoliveiramonteiro/ref/mouse/refdata-gex-GRCm39-2024-A \
  --fastqs=~/.HN00220094_10X_RawData_Out/"$ID"/HH77JDSXC,~/.HN00222740_10X_RawData_Out/"$ID"/HN1CFDSXC \
  --sample="$ID" \
  --localcores=16 \
  --localmem=280
```

# 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

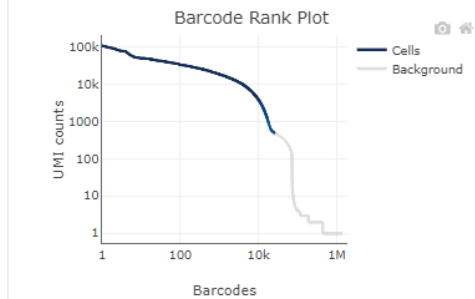
1,124

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%

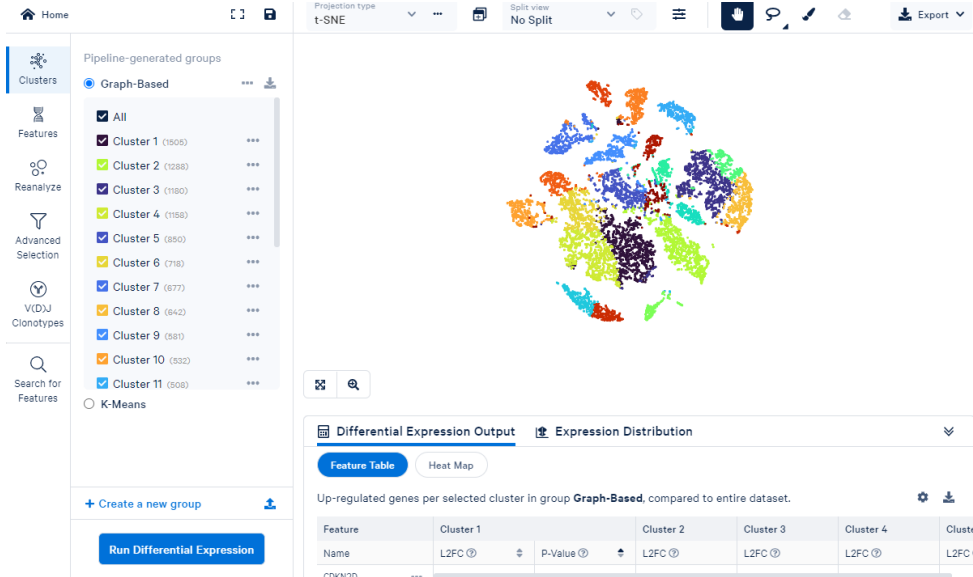
## Cells



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



Projection type: t-SNE

Split view: No Split

Export

Home

Pipeline-generated groups

Graph-Based

- All (1905)
- Cluster 1 (1905)
- Cluster 2 (1288)
- Cluster 3 (1180)
- Cluster 4 (1158)
- Cluster 5 (850)
- Cluster 6 (718)
- Cluster 7 (677)
- Cluster 8 (642)
- Cluster 9 (581)
- Cluster 10 (532)
- Cluster 11 (508)

K-Means

Run Differential Expression

Differential Expression Output

Feature Table | Heat Map

Up-regulated genes per selected cluster in group Graph-Based, compared to entire dataset.

Feature	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Name	L2FC	L2FC	L2FC	L2FC	L2FC
P-Value					

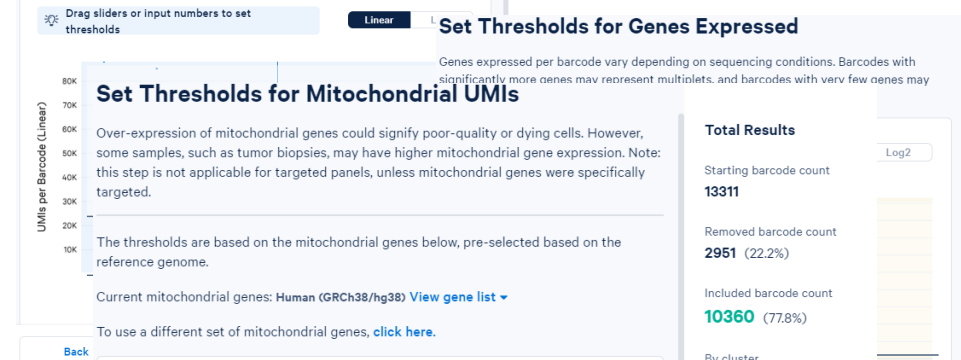
## Set Thresholds for UMIs

UMIs per barcode vary depending on sequencing conditions. Barcodes with unexpectedly high counts of UMIs (Unique Molecular Identifiers) may represent multipllets, and barcodes with very few UMIs may represent low-quality cells or empty droplets—those with fewer than 3 are unavailable for Reanalyze.

## Total Results

Starting barcode count  
**13311**

Removed barcode count  
**232 (1.7%)**



Drag sliders or input numbers to set thresholds

Linear

### Set Thresholds for Mitochondrial UMIs

Over-expression of mitochondrial genes could signify poor-quality or dying cells. However, some samples, such as tumor biopsies, may have higher mitochondrial gene expression. Note: this step is not applicable for targeted panels, unless mitochondrial genes were specifically targeted.

The thresholds are based on the mitochondrial genes below, pre-selected based on the reference genome.

Current mitochondrial genes: Human (GRCh38/hg38) [View gene list](#)

To use a different set of mitochondrial genes, [click here](#).

Back

## Set Thresholds for Genes Expressed

Genes expressed per barcode vary depending on sequencing conditions. Barcodes with significantly more genes may represent multipllets, and barcodes with very few genes may represent low-quality cells or empty droplets—those with fewer than 3 are unavailable for Reanalyze.

## Total Results

Starting barcode count  
**13311**

Removed barcode count  
**2365 (17.8%)**

Included barcode count  
**10946 (82.2%)**



Drag sliders or input numbers to set thresholds

Log2

### Set Thresholds for Genes Expressed

Genes expressed per barcode vary depending on sequencing conditions. Barcodes with significantly more genes may represent multipllets, and barcodes with very few genes may represent low-quality cells or empty droplets—those with fewer than 3 are unavailable for Reanalyze.

The thresholds are based on the mitochondrial genes below, pre-selected based on the reference genome.

Current mitochondrial genes: Human (GRCh38/hg38) [View gene list](#)

To use a different set of mitochondrial genes, [click here](#).

Back

Total Results

Starting barcode count  
**13311**

Removed barcode count  
**2951 (22.2%)**

Included barcode count  
**10360 (77.8%)**

By cluster

Graph-based

Cluster	Count	Removed
Cluster 1	1505	69 (4.6%)
Cluster 2	1288	104 (8.1%)
Cluster 3	1180	20 (1.7%)
Cluster 4	1158	166 (14.3%)
Cluster 5	850	343 (40.4%)
Cluster 6	718	344 (47.9%)
Cluster 7	677	446 (65.9%)
Cluster 8	642	5 (0.8%)
Cluster 9	581	81 (13.9%)
Cluster 10	532	105 (19.7%)
Cluster 11	508	41 (8.1%)
Cluster 12	470	162 (34.5%)
Cluster 13	437	25 (5.7%)
Cluster 14	389	379 (97.4%)
Cluster 15	361	115 (31.9%)
Cluster 16	318	184 (57.9%)
Cluster 17	318	48 (15.1%)
Cluster 18	317	1 (0.3%)
Cluster 19	296	0
Cluster 20	289	51 (17.6%)
Cluster 21	266	225 (84.6%)
Cluster 22	211	37 (17.5%)

By cluster

Cluster Count Removed

- Cluster 1 1505 28 (1.9%)
- Cluster 2 1288 2 (0.2%)
- Cluster 3 1180 2 (0.2%)
- Cluster 4 1158 134 (11.6%)
- Cluster 5 850 315 (37.1%)
- Cluster 6 718 330 (46.0%)
- Cluster 7 677 403 (59.5%)
- Cluster 8 642 0
- Cluster 9 581 5 (0.9%)
- Cluster 10 532 56 (10.5%)
- Cluster 11 508 1 (0.2%)
- Cluster 12 470 132 (28.1%)
- Cluster 13 437 0
- Cluster 14 389 379 (97.4%)
- Cluster 15 361 98 (27.1%)
- Cluster 16 318 177 (55.7%)

Barcodes

Next

Reset Min 0% Max 10.702% Removed barcodes

Back Skip to final step Next