UMGC Pilot Sequencing Program

Spring 2024

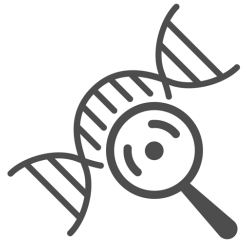
Aaron Becker, UMGC NGS Instrumentation Lead

Thursday, April 4

Today's Agenda

- UMGC – Aaron Becker
  - Pilot Sequencing Program overview
  - Subsidies
- Illumina – Logan Silber
  - Common NGS applications for a pilot
- 10X Genomics – Egon Ranghini
  - 3', 5' or Flex gene expression pilots
- Question and Answers

Program Overview - Goals



1. Enable researchers to generate pilot data to submit in support of future NIH/NSF grant proposals.

2. Promote the use of the genomics research solutions available at the UMGC.

To help PIs generate pilot data, the UMGC has partnered with:

- Illumina to subsidize sequencing pilot projects
- 10X Genomics to support single-cell pilot projects

Subsidies

- Illumina
  - Providing sequencing reagents of up to 1.2 billion reads for single-cell projects
  - 400 million reads for all other projects
  - Providing Illumina library prep reagents for applicable projects
- 10X Genomics
  - Providing 3’, 5’ gene expression or Flex reagents (limited qty)
- UMGC
  - Providing up to $500 to reduce labor costs per approved project

Subsidies

Note about pilots not using Illumina library prep reagents

- Pre-made libraries using non-Illumina library reagents are eligible for the sequencing subsidy
- Non-Illumina library methods (examples)

    1. Takara/Clontech low input/degraded RNA Pico reagents
    2. ATAC-seq: UMGC custom barcoding and amplification
    3. Cut and Run, Cut and Tag
    4. Your custom library here

Subsidies

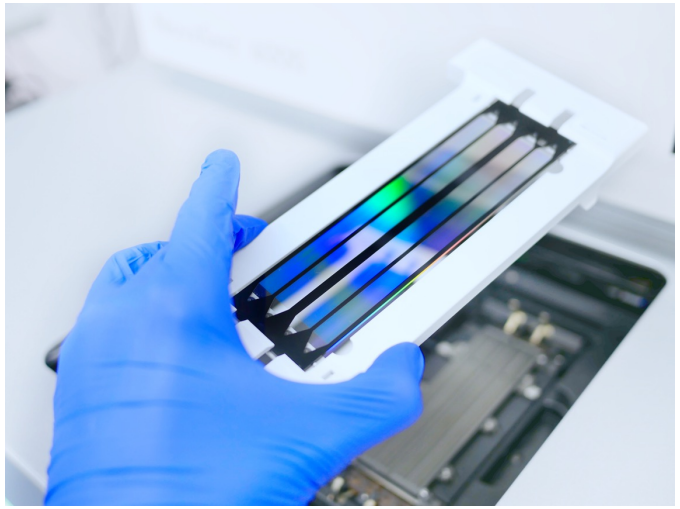
Note about 10X Genomics reagents

- Reagent subsidy is for the 3', 5' gene expression or Flex kits
- Limited quantity
  - Varying on the number of applications, not all may receive the library prep subsidy
- Spatial projects do qualify for sequencing subsidy and UMGC labor subsidy ($500) only
- Can be combined with the no-cost sequencing reagents

Pilot Project Examples

What type of pilot project qualifies?

- Any NGS application on our website can qualify for a sequencing subsidy
- Single-cell pilots should target ≤1.2 B reads
- All others must be for 400 million reads of sequencing or less



Pilot Project Examples

Bulk RNA-Seq

- 20 million reads/sample
- NextSeq P2, 150 PE run
- A pilot could support 20 samples

Subsidy

- Illumina library reagents
- Illumina sequencing reagents
- $500 UMGC labor costs

Pre-made libraries

- Illumina compatible
- Next-Seq, Mi-Seq

Subsidy

- Illumina sequencing reagents
- $500 UMGC labor costs

Pilot Project Examples

Visium Spatialomics Project

- Whole-transcriptome data for four samples, Cytassist
- 80% of 6.5 x 6.5 mm average capture area
- NextSeq P2, 2 x 50 PE run

Subsidy
- Illumina sequencing reagents
- $500 UMGC labor costs

Note:
The $500 doesn't apply towards UIC's or tissue labor

Pilot Project Examples

10X Genomics Single Cell Pilot

- Microfluidic capture of 16,000 cells across 2 captures, more captures for flexible project setup. Recipient pays for extra
- 3’, 5’ gene expression or Flex
- Illumina sequencing on a 28x90 bp NextSeq run
- Generating about 20,000 reads per cell

Subsidy

- 10X Genomics library reagents for two captures
- Illumina sequencing reagents
- $500 UMGC labor costs

Pilot Project Costs

Each project will have unique total costs due to:

- Number of samples
- Type of library prep
  - Illumina and 10X reagents vs. non-subsidized library prep reagents
- Different labor requirements

Consult a UMGC Service Manager to discuss your specific project details and to estimate your pilot project costs.

Pilot Project Costs (Savings Tier)

#	Description	QTY	Each	Total
1	Ribogreen Quant	20	$2.19	$43.80
2	Tapestation QC	20	$10.60	$212
3	Illumina mRNA Prep			
	3a Illumina reagents	*20*	*$76.8*	*subsidy*
	3b UMGC fees	*20*	*$42.57*	*$851.40*
4	NextSeq P2 150PE			
	4a Illumina reagents	*1*	*$3014.99*	*subsidy*
	4b UMGC fees	*1*	*lane*	*$687.71*
5	UMGC Labor Subsidy	1	*(-$500)*	*-$500.00*
			Grand Total	$1,294.91

Example 1
mRNA-Seq
20 samples

Subsidy in this example is ≈ $4,500

Pilot Project Costs (Savings Tier)

#	Description	QTY	Each	Total
1	Picogreen Quant	30	$2.16	$64.8
2	Nanodrop QC	30	$2.12	$63.60
3	DNA Prep			
	3a Illumina reagents	*30*	*$61.14*	*subsidy*
	3b UMGC fees	*30*	*$27.06*	*$811.8*
4	NextSeq P2 150PE			
	4a Illumina reagents	*1*	*$3,014.99*	*subsidy*
	4b UMGC fees	*1*	*lane*	*$687.71*
5	UMGC Labor Subsidy	1	*(-$500)*	*-$500.00*
			Grand Total	$1,127.91

Example 2
Metagenomics
30 samples

Subsidy in this example is ≈ $5,300

Who is eligible?

- UMN PIs
  - With a faculty position
  - Applying for a future grant as the PI
- UMN post-docs and doctoral researchers
  - With funding managed separately from their supervising PI
  - Applying for a future grant as the PI
- Applicants may not hold current awards directly related to the proposed research

3-Step Application Process

Step 1: Pilot Sequencing Proposal

- Sample and sequencing information
- Project summary - 1-page limit
  - How the pilot data would strengthen futu studies and grants
  - Plan for upcoming grant
    - Type of grant
    - Date of full proposal
    - Classification (new, renewal, resubmission)

Pilot Project Information

Sample Information	
1. *Number of samples*	
2. *Nature of your samples* *[Extraction method and QC measures]*	
3. *Type of pilot* *[RNA-Seq, exome, single-cell, 16S, amplicon, etc.]*	
4. *Library kit needed for pilot* *[Kits qualifying for a subsidy or write in if known]*	
5. *Anticipated sample submission date* *[Between June 3, 2024 - March 7, 2025]*	

Sequencing Information	
1. *Depth per sample*	
2. *Read length*	
3. *Total millions of reads for your pilot project* • *Must be ≤ 1.2 billion reads for single-cell pilots* • *All other pilots must be ≤ 400 million reads*	

Project Summary

1-page limit, 11 pt. font minimum
Use following page to provide a perspective for the proposed work, include:

1. How the pilot data would strengthen future studies/grants
2. Plan for upcoming grant proposal(s), include title, type (e.g. NIH R01, NSF, DOA, etc.), date for full proposal submission, and classification (New, Competing Renewal, or Resubmission)

3-Step Application Process

Step 2: Prepare an NIH-style biosketch

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME:

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE:

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY

A. Personal Statement

B. Positions, Scientific Appointments, and Honors

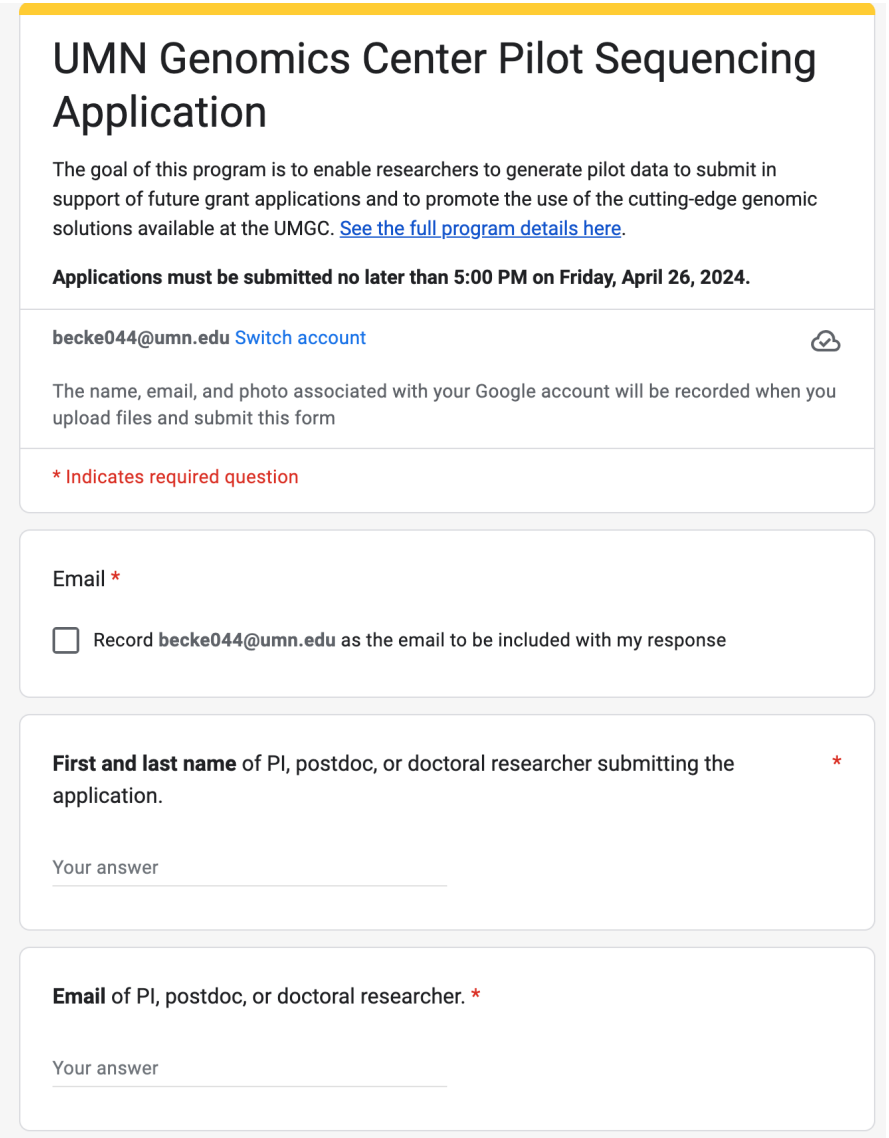
C. Contributions to Science

Templates can be found on the Pilot Sequencing web page

3-Step Application Process

Step 3: Pilot Sequencing Application

- Google Form
- Upload proposal
- Upload biosketch
- SUBMIT!



UMN Genomics Center Pilot Sequencing Application

The goal of this program is to enable researchers to generate pilot data to submit in support of future grant applications and to promote the use of the cutting-edge genomic solutions available at the UMGC. See the full program details here.

Applications must be submitted no later than 5:00 PM on Friday, April 26, 2024.

becke044@umn.edu Switch account

The name, email, and photo associated with your Google account will be recorded when you upload files and submit this form

* Indicates required question

Email *

Record becke044@umn.edu as the email to be included with my response

First and last name of PI, postdoc, or doctoral researcher submitting the application. *

Your answer

Email of PI, postdoc, or doctoral researcher. *

Your answer

Application Process

- One application may be submitted per PI
- Post-docs with funds managed separately from supervising PI are eligble
- Applicants will be informed by email on Friday, May 31
  - If application was approved
  - What components are subsidized (i.e. library prep reagents, sequencing)
- 40 applications are anticipated to be approved for a sequencing subsidy

Key Dates

Application open date	Monday, March 18, 2024
Application due	Friday, April 26, 2024
Decisions communicated to the applicants	Friday, May 31, 2024
Subsidy for approved projects begins	Monday, June 3, 2024
Sample submission deadline	Friday, March 7, 2025

Review Criteria

UMGC NGS experts will be evaluating eligible applications

10X Genomics will assist in reviewing applications utilizing 10X reagents

- Primary factor - technical feasibility of the proposed project
- Consideration will be given to new UMN PIs and PIs new to the UMGC
- Random drawing (if an overabundance of applications)
- No critiques will be provided to applicants

Approved Pilot Projects

Need to be able to support the balance of the pilot project:

- Projects exceeding $500 in UMGC labor costs will need a ChartField string (CFS) to cover the remaining balance.
- Projects not using Illumina library reagents, or not approved for 10X library reagents, will need a CFS to cover the library reagent costs and any UMGC costs beyond $500.

Approved Pilot Projects

- Use the subsidies solely to support the research described in the submitted application.
- Provide follow-up information regarding the long-term impact of the subsidy on your overall research and funding.
- Agree to use the UMGC for next-gen sequencing needs if/when the full proposal is funded.
- Acknowledge the "*University of Minnesota Genomics Center*" in publications.

Project Support

Office hours – Illumina & UMGC

illumina

Tuesday, April 16	Thursday, April 18	Thursday, April 18
9:00 AM – 2:00 PM	9:00 AM – 11:30 AM	1:00 PM – 4:00 PM
NHH 3-101	Cargill atrium (STP)	CCRB cafe

- *Logan Silber, Sequencing Specialist, and UMGC*
- Get support with latest technologies and methods in NGS
- Available for pilot project support
  - Library prep, sequencing, or data analysis
- Sign-up today or on the UMGC's website

Project Support

Office hours – 10X Genomics

Monday, April 15	Tuesday, April 16
9 AM – 12 PM	9 AM – 12 PM
Virtual	Virtual

- *Egon Ranghini PhD, Science and Technology Advisor, 10x Genomics*
- Get support with single-cell pilot projects and experimental design
- Available for Visium spatial support
  - Note: 400 M reads of sequencing can go towards a spatial project, but there isn't a reagent subsidy
- Sign-up today or on the UMGC's website

Project Support

Schedule a consult with the UMGC

Email us:

next-gen@umn.edu

single-cell@umn.edu

Email us with your technical questions or to arrange a consultation

FAQs

- Is this just for the Twin Cities campus?
  - No, applicants can be from any UMN system campus.
- I'm a graduate student, can I apply?
  - Grad students are not able to apply and should work with their PI on an application.
- Who will help interpret the results?
  - The UMGC will release data to PIs for analysis and interpretation. PIs should contact MSI for assistance.

FAQs

- What is the limit on number of samples for a pilot?
  - Up to 400 million reads worth of samples. Approved pilots will be for proposals with reads/sample that will generate meaningful preliminary data for a full proposal.
- For single cell pilot projects, would the PI prepare the library or just the cells?
  - For a 10X Genomics 3', 5' gene expression or Flex pilot, the PI would provide the UMGC a dissociated suspension of live cells or nuclei, or fixed cells and the UMGC would prepare the libraries.

FAQs

- Is whole genome sequencing a good fit for a pilot study?
  - If the WGS is for small genomes (i.e. bacteria, fungi etc.), then yes.
  - Unlikely to be enough coverage at 400 M reads for more than a single large genome.
- What is the best technique…, what method to study…, which technologies should I use to determine…?
  - Please contact a service manager to discuss your specific project goals and sign-up for Illumina or 10X's office hours.

Question & Answer Session

- Questions can be for UMGC, Illumina, or 10X
- UMGC, Illumina, and 10X will be available after the seminar for additional support

Applications are due by 5:00 PM on Friday, April 26

UMGC Pilot Program Kickoff 2024: Planning Your Pilot with Illumina

Logan Silber

Sequencing Specialist, Illumina

April 2024

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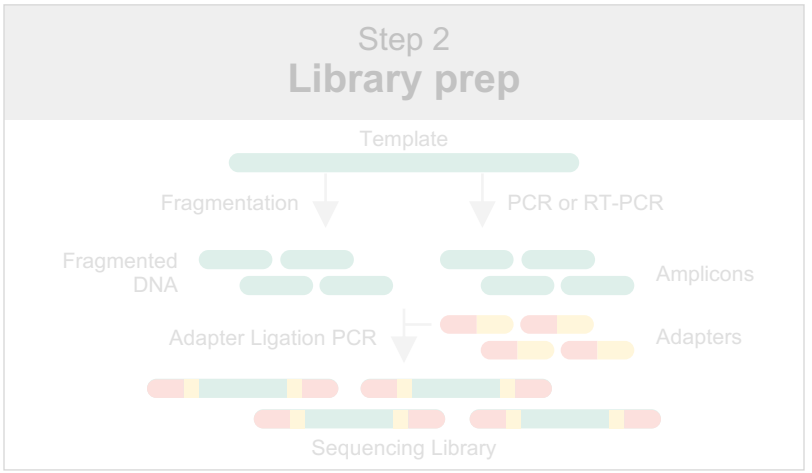
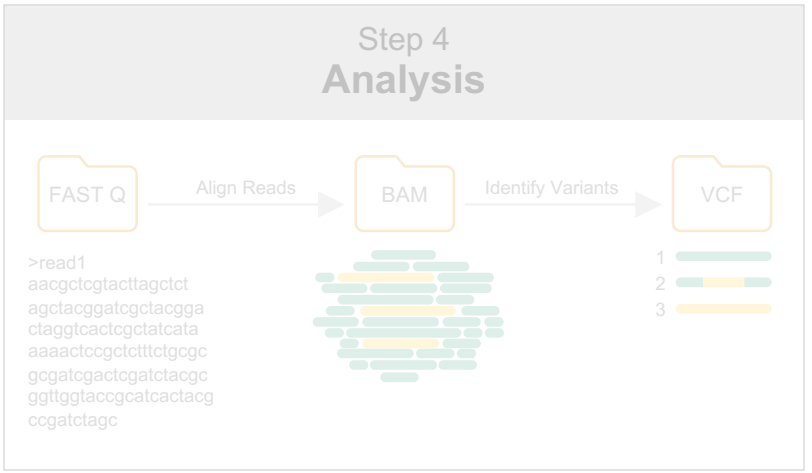
What Does an Illumina NGS Workflow Look Like?





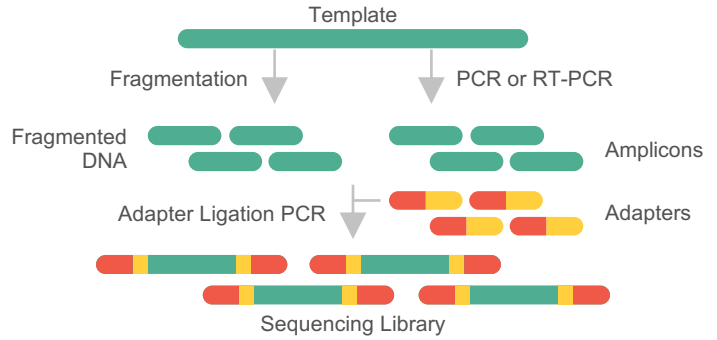
Step 1: Extraction

Nucleic acids are isolated from samples such as bulk tissue, individual cells, or biofluids

What Does a General NGS Workflow Look Like?

Step 1
Extraction

Step 2
Library prep



Step 2: Library prep

Nucleotides are prepared for Illumina sequencing. This may involve fragmenting DNA, adding adapters, and adding barcodes

Step 3
Sequencing

Step 4
Analysis

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What Does a General NGS Workflow Look Like?

Step 1
Extraction

Step 2
Library prep

Step 3
Sequencing

Step 4
Analysis

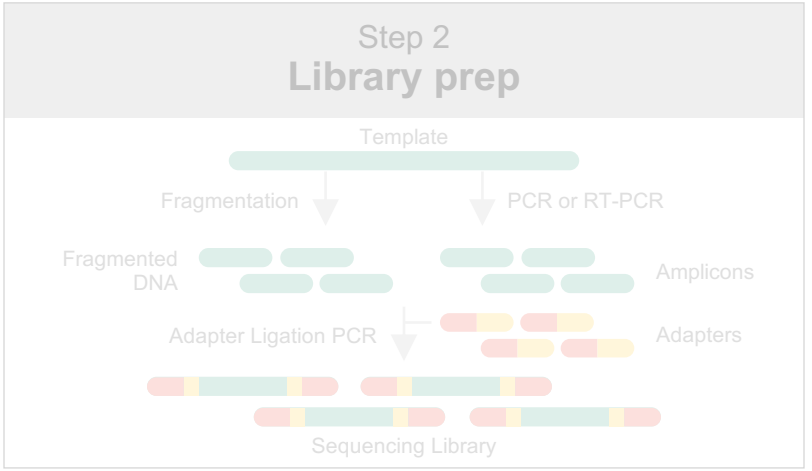
Step 3: Sequencing

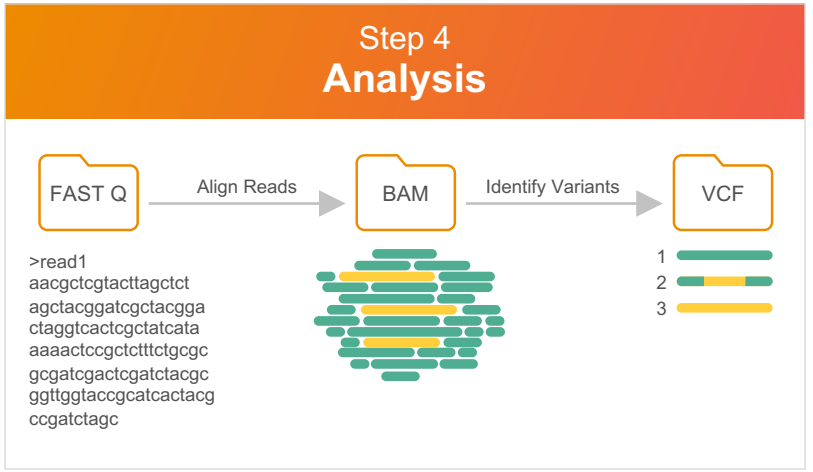
Nucleotides are read on an Illumina sequencer at a read length and depth that's recommended for a particular use-case

Key:

- **Read length** – the length of a DNA fragment that is read on a sequencer
- **Read depth** – the number of "reads" that are obtained per sample

What Does a General NGS Workflow Look Like?





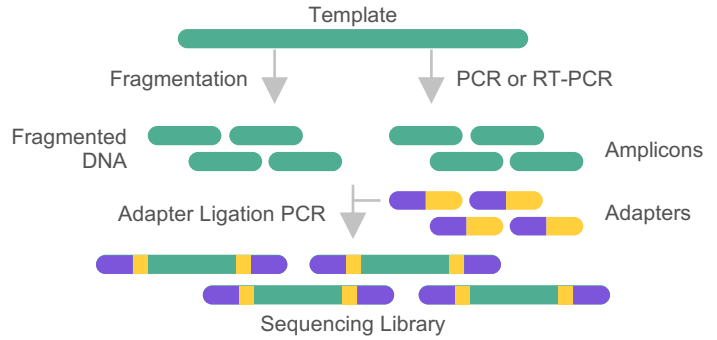
Step 4: Analysis

Bioinformatics tools are used to make sense of the series of A's, T's, G's and C's, or reads, that leave an Illumina sequencer

What Does a General NGS Workflow Look Like?

Step 1
Extraction

Step 2
Library prep



Step 3
Sequencing

Step 4
Analysis

Today we will be focusing on these considerations for DNA or RNA based applications:

- **Library Preparation**
- **Sequencing**

Library Prep: Choosing the Right One

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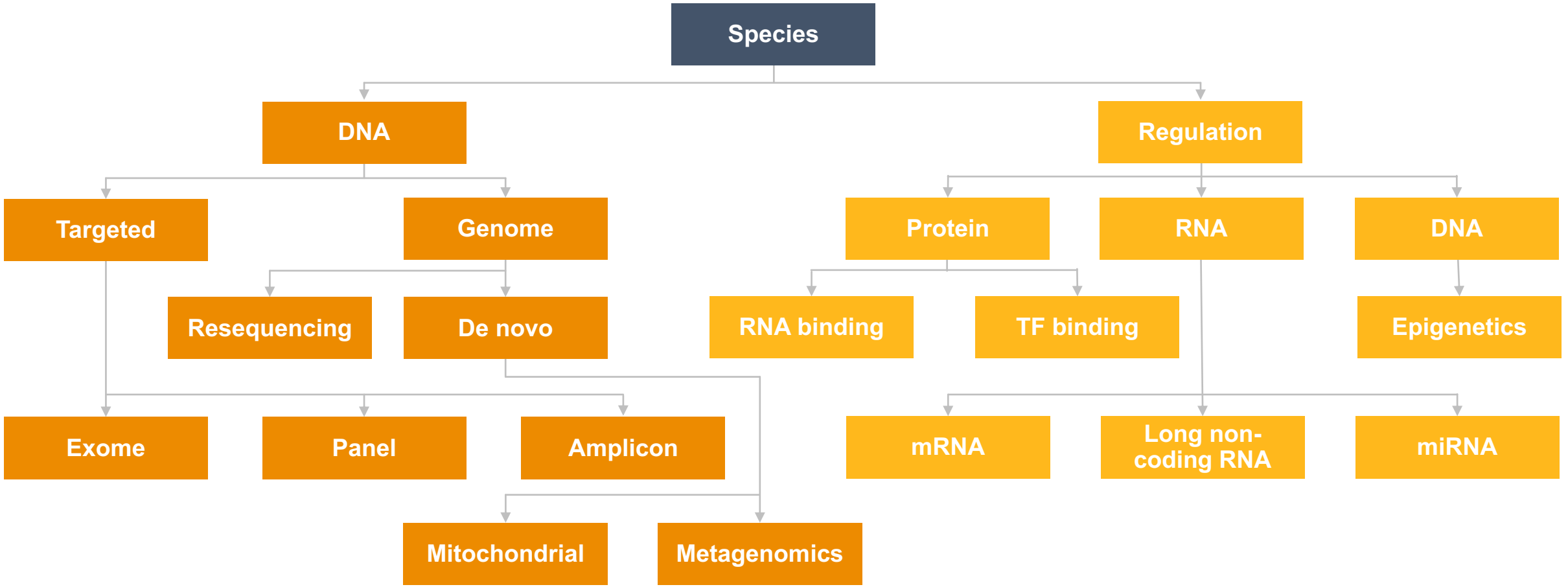
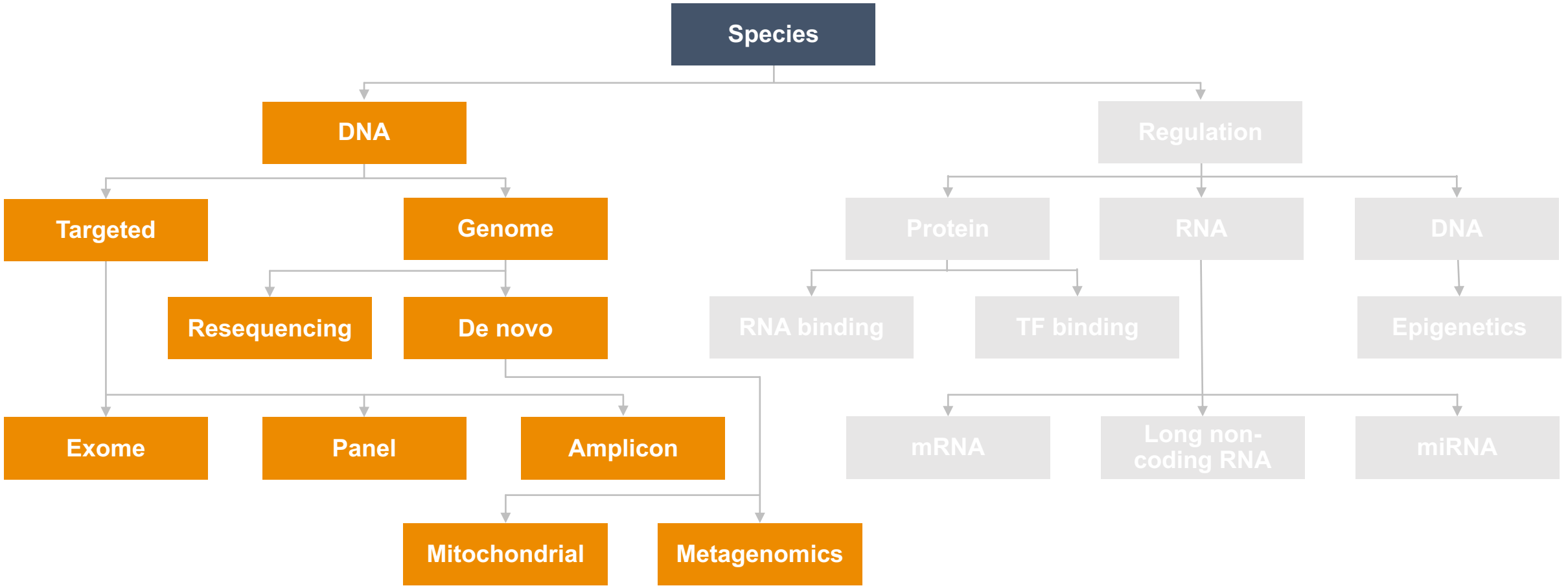
Your application will determine your library prep kit!



TF = Transcription Factor

Your application will determine your library prep kit!



TF = Transcription Factor

What Methods Are Commonly Used to Study the Genome with NGS?

Whole-Genome-Sequencing

Sequencing region: Entire genome

Coverage: ~30X*

Use-case: Characterization of **non-coding** and **coding** genome, genome-wide **structural** variants, regulatory region identification

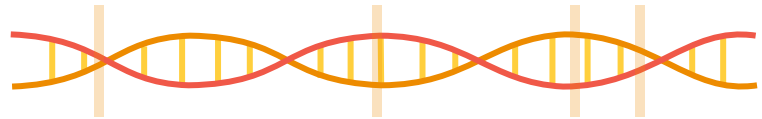
Whole-Exome-Sequencing

Sequencing region: 1% of the genome that encodes proteins

Coverage: ~50X–100X*

Use-case: Deeper analysis of the regions of the genome most-associated with cancer mutations. Often used to ID SNPs, INDELs, SVs (in coding regions).

Targeted DNA Sequencing



Sequencing region: Specific genes or regions-of-interest. Often customized.

Coverage: >500X*

Use-case: Deep sequencing of specific regions to identify rare variants. If less reads are used and less depth is required, this method can benefit from decreased cost per sample.

Coverage

* Coverage requirements vary depending on use-case, such as rarity of factor being measured

What Library Preps Are Commonly Used to Study the Genome with NGS?

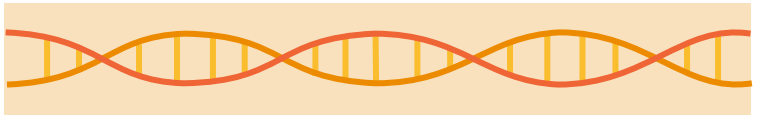
Whole-Genome-Sequencing	Whole-Exome-Sequencing	Targeted DNA Sequencing
Illumina DNA PCR-Free Prep	Illumina Exome v2.5	Illumina Targeted Panels
Illumina DNA Prep	Illumina DNA/ctDNA Prep with Enrichment	

For Research Use Only. Not for use in diagnostic procedures.

* Coverage requirements vary depending on use-case, such as rarity of factor being measured

When to choose Illumina DNA PCR Free Prep vs Illumina DNA Prep

Whole-Genome-Sequencing



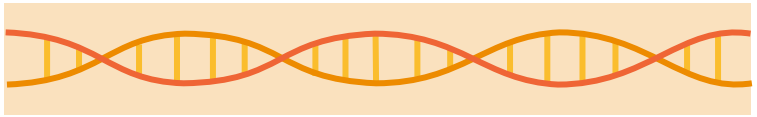
Illumina DNA PCR-Free Prep → Recommended if input concentration is at least 25ng, and if PCR-induced errors are a concern. A common choice for translational research and human WGS

Illumina DNA Prep

* Coverage requirements vary depending on use-case, such as rarity of factor being measured

When to choose Illumina DNA PCR Free Prep vs Illumina DNA Prep

Whole-Genome-Sequencing

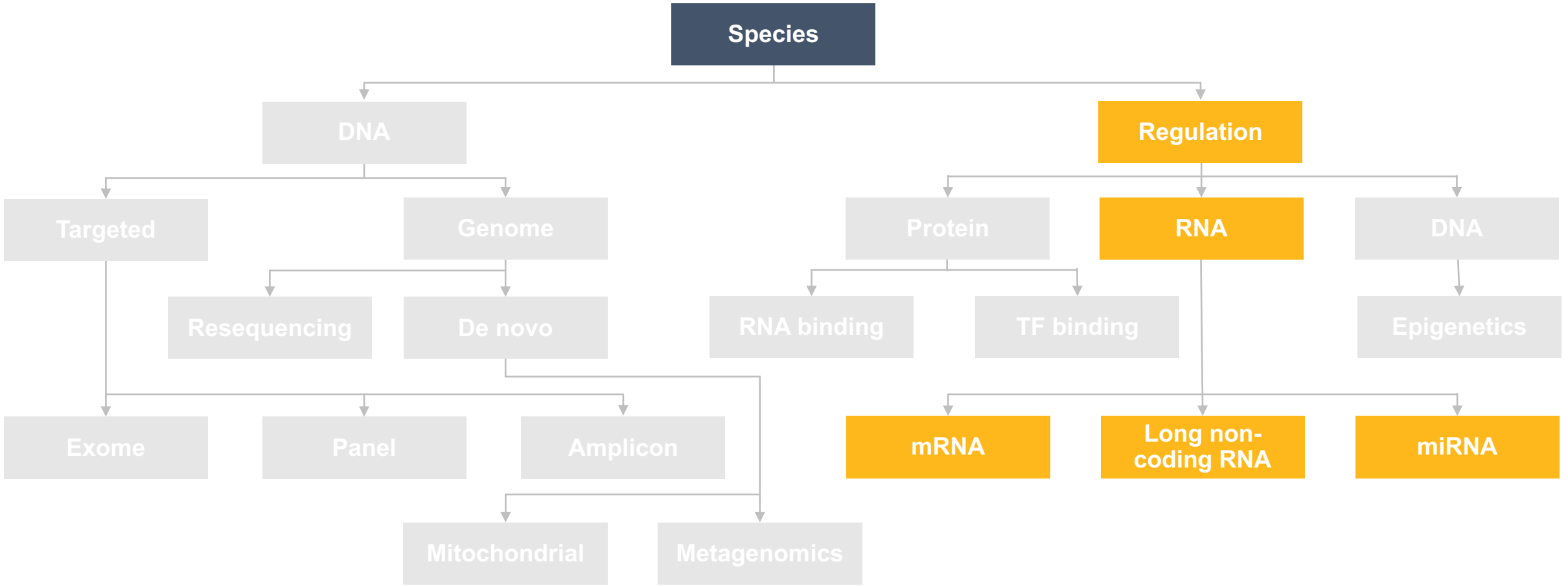


Illumina DNA PCR-Free Prep

Illumina DNA Prep → Compatible with as low as 1ng input. Recommended for small and large genomes from a variety of species, including microorganisms like bacteria

* Coverage requirements vary depending on use-case, such as rarity of factor being measured

Your application will determine your library prep kit!



TF = Transcription Factor

Bulk RNA Sequencing Comprehensively Profiles the Average Gene Expression of Many Cells

Bulk Sequencing

- Workflows involve lysing cells, extracting nucleic acids, and sequencing.
- No resolution of the specific cells where these changes occur
- Can be combined with flow cytometry to analyze populations based on biomarker expression

Benefits of Bulk Sequencing:

- No specialized equipment required
- Workflows have been validated in thousands of publications
- Less NGS experience required prior to experiments
- Lower cost

Illumina RNA Library Preparation Suite

16 & 96
Sample kit sizes

384
Unique Dual Indexes[3]

	1	2	3
	Illumina Stranded Total RNA Ligation with Ribo-Zero Plus	**Illumina Stranded mRNA** Ligation	**Illumina RNA Prep with Enrichment** (L) Tagmentation
Detection	Coding & Non-coding regions	Coding transcriptome w/ Poly A tail	Targeted coding region
FFPE Compatibility	✓	-	✓
Input	1-1,000 ng[1] 10ng for optimal quality & FFPE	25-1,000 ng	10ng non-FFPE 20ng FFPE
Total Time (hours)[2]	7	6.5	< 9
Hands-on time (hours)[2]	< 3	< 3	< 2
Automation Friendly	✓	✓	✓
	• Multi-species rRNA depletion • Includes cDNA synthesis reagents	• Includes Illumina Poly A capture kit • Includes cDNA synthesis reagents	• Illumina Tested with Illumina Exome & Illumina Respiratory Viral Panel v2

1. Minimum input for high-quality RNA shown, 10ng minimum recommended for optimal quality and FFPE for Total RNA workflow
2. Hands-on and total time based on manual processing of up to 24 samples for Total & mRNA workflows and 1 sample on Enrichment workflow
3. Up to 192 UDIs available now, 384 coming soon

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For Research Use Only. Not for use in diagnostic procedures.

What About Microbes?

4 Core Methods in Microbiology

Whole-Genome Sequencing

Generate accurate reference genomes, for microbial identification

Ex. **Illumina DNA Prep**

Shotgun Metagenomics and Metatranscriptomics

Detect very low abundance members of the microbial community.

Ex. **Illumina DNA Prep** for Metagenomics

Ex. **Illumina Stranded Total RNA** for Metatranscriptomics

16S rRNA Sequencing

Identify and compare bacteria present within a given sample. Going out of style in favor of low pass shotgun

Ex. Amplicon Based 2-Step PCR Approach

Virology

Determine the sources of infection, route of transmission, and molecular pathways. May start with DNA or RNA

Ex. **Illumina DNA/RNA Prep with Enrichment**

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Sequencing: Working within the Optimal Parameters

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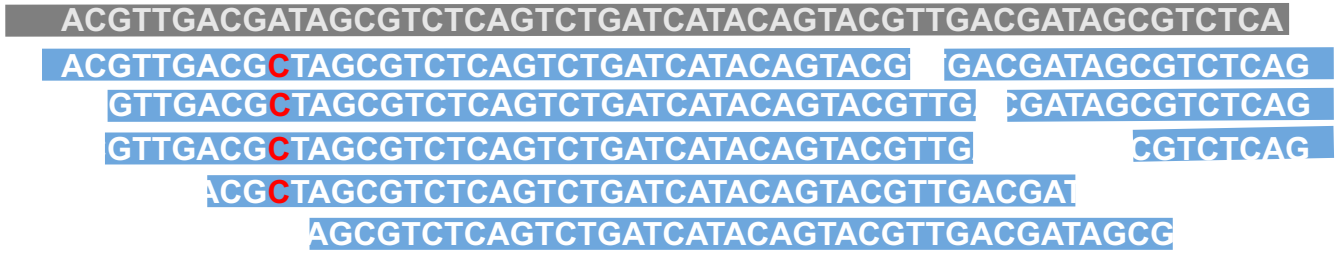
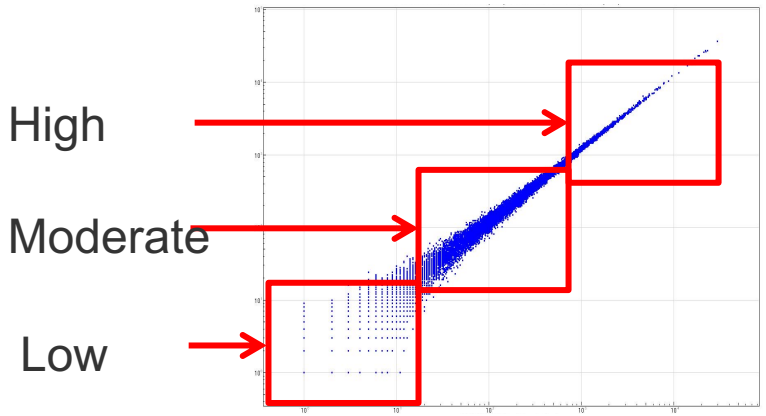
“How much sequencing do I need for my samples?”

Coverage Depth vs Number of Reads

- **More coverage = greater confidence about the sample sequence**



- **RNA-Seq: use number of reads**



Contact Illumina and the Core for help with calculating how many samples you can sequence!

Examples for when we need coverage

Application	Mean coverage depth	Cost/Sample
Exome **Germline** Variant Detection	50x – 120X	$
Exome **Somatic** Variant Detection	200x – 600X	$$
Whole Genome **Germline** Variant Detection	30X – 40X	$$
Whole Genome **Somatic** Variant Detection	300X	$$$$$

Germline
A variant that will be present in all cells

Somatic
A *de novo* variant, like in cancer, that will be rare in bulk tissue.
You must sequence deeply to reliable detect it

Whole Exome Project Example

Understanding the coding regions of the human genome

Study Goals

- Focus on coding regions to get a deeper analysis of the regions of the genome most-associated with cancer mutations.
- Often used to ID coding single nucleotide polymorphisms (SNPs), insertion/deletions (INDELs), Copy Number Variants (CNVs), etc.

Method

Targeted Enrichment

- Liquid Biopsy samples
- **50X Coverage (12 samples) or 120X Coverage (8 samples)**
- Read length 2x100 bp

*Excludes post-prep quantification and normalization time

Powered by the Illumina DNA Prep with Enrichment, NextSeq™ 2000 and DRAGEN™

Prepare Library	 Illumina DNA Prep with Exome 2.5 Enrichment	8-19 Samples	Total Time* ~6.5 Hours
Sequence	 NextSeq™ 2000	Up to 8-19 Samples	Run Time 19 Hours
Analyze	DRAGEN DRAGEN™	8-19 Samples	Run Time 30 Minutes

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RNA-Seq Coverage

How Many Reads Are Needed?

Application	Recommendation (# reads)		
	mRNA	Total RNA	RNA Exome/Enrichment
RNA Profiling	12-25M	50M	25M
Deep transcriptome Analysis or Assembly	50M	100M	50M

- This applies to mammals
- Check Encode guidelines and literature: encodeproject.org

illumina®

Total RNA Project Example

Maximize discovery power

Study Goals

- Study coding and multiple forms of noncoding RNA
- Analyze abundance values for every transcript isoform from each gene
- Identify novel transcript isoforms, gene fusions, and/or identify allele-specific expression

Example Method

Total RNA-Seq

- As low as 1ng input (10ng for low-quality/FFPE)
- **50 million reads/sample**
- Read length: 2x75 bp / 2x100bp

Powered by the Illumina Stranded Total RNA Prep, NextSeq™ 2000 and DRAGEN™

Prepare Library	 Illumina Stranded Total RNA prep	8 Samples	Total time 7 Hours
Sequence	 NextSeq™ 2000	Up to 8 Samples	Run Time 21 Hours
Analyze	DRAGEN DRAGEN™	8 Samples	Run Time 30 Minutes

Community characteristics of the gut microbiomes of competitive cyclists

Lauren M. Petersen ✉, Eddy J. Bautista, Hoan Nguyen, Blake M. Hanson, Lei Chen, Sai H. Lek, Erica Sodergren & George M. Weinstock

Microbiome **5**, Article number: 98 (2017) | Cite this article

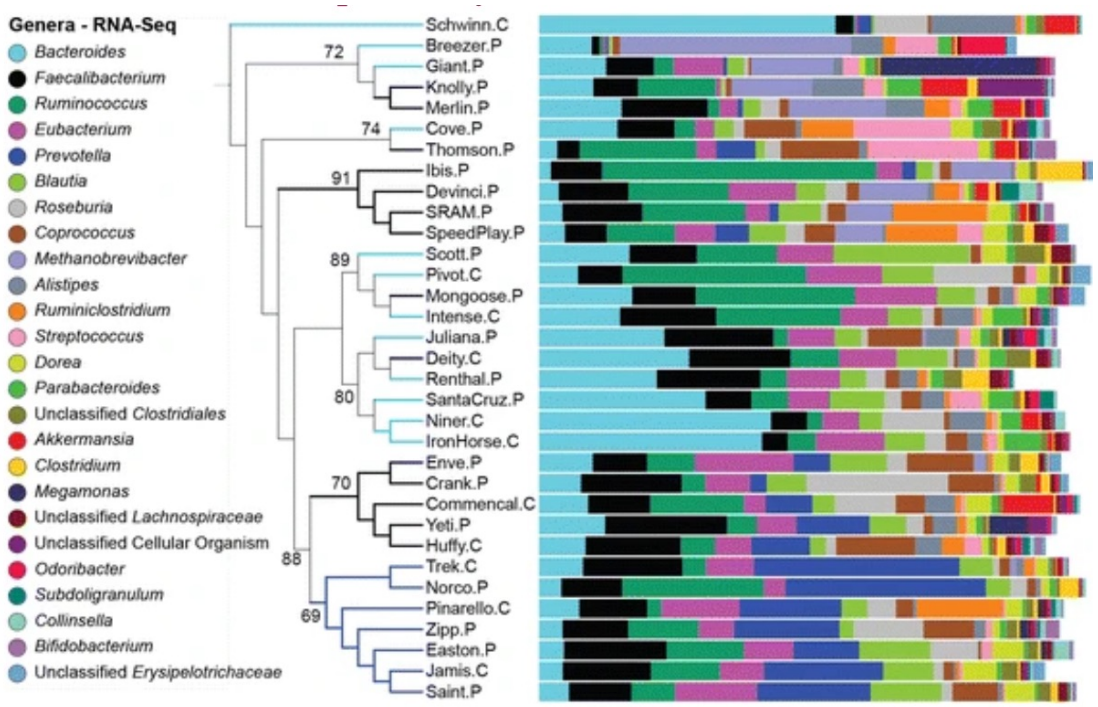
12k Accesses | **139** Citations | **96** Altmetric | Metrics

Do athletes have gut microbes that make them better athletes?

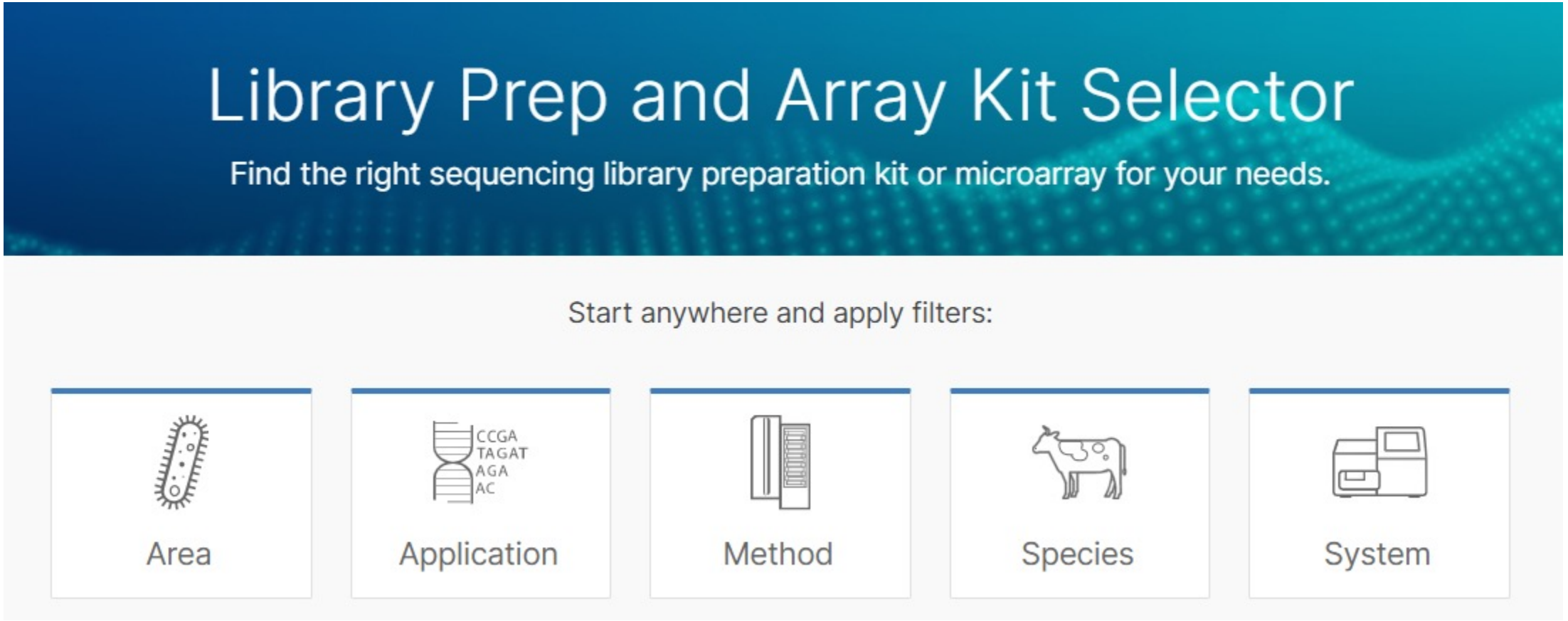
Methods: 16S profiling and RNA-Seq to extend our understanding of healthy microbiomes and to see if an athletic or 'golden' microbiome exists.

Findings:

- *Prevotella sp* was more common in the athletes.
- Gene expression showed that *Methanbrevibacter smithii* was a high expresser (relative to its abundance) in the most active participants.
- Methane production pathways were elevated as were ancillary carbohydrate metabolism and energy utilization pathways in these individuals.



Not Sure Which Kit to Use?

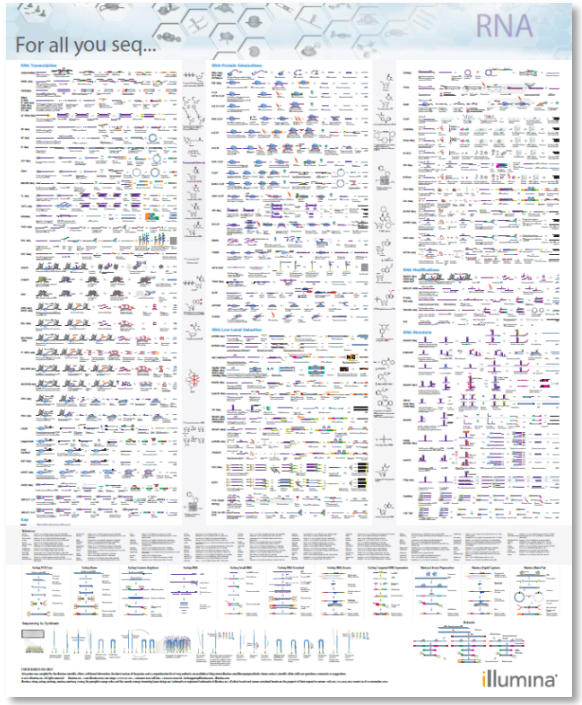


https://www.illumina.com/library-prep-array-kit-selector.html

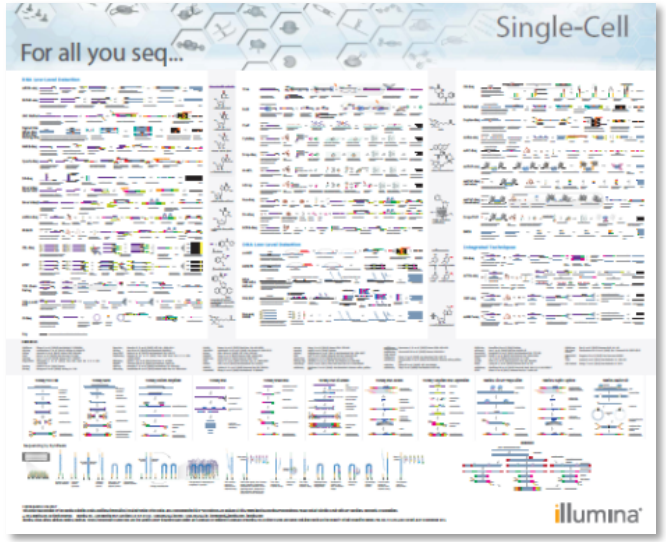
You're not limited by off-the-shelf options… There are many methods out there!

For all you seq... DNA

For all you seq... RNA



For all you seq... Single Cell



https://emea.illumina.com/content/dam/illumina-marketing/documents/applications/ngs-library-prep/for-all-you-seq-dna.pdf
https://emea.illumina.com/content/dam/illumina-marketing/documents/applications/ngs-library-prep/for-all-you-seq-rna.pdf
https://emea.illumina.com/content/dam/illumina-marketing/documents/applications/ngs-library-prep/for-all-you-seq-single-cell.pdf

Have questions about designing your pilot? Contact us!

Email your local Illumina team!

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Sr. Territory Account Manger

Dylan Cole

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Field Service Engineer

Can't make the office hours on the 16th and 18th? Scan here!

Thank You!

Logan Silber

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illumina®

Illumina Targeted Panels

Cancer

- BRCA — A
- Cancer Hotspot Panel v2 — A
- Comprehensive Cancer Panel — A
- TruSight™ Cancer — E
- TruSight Hereditary Cancer — E
- TruSight Oncology 500 ctDNA — E
- TruSight RNA Fusion — E
- TruSight RNA Pan Cancer — E
- Childhood Cancer Panel — A
- Comprehensive Panel v3 — A
- Focus Panel — A
- Myeloid Panel — A
- TruSight Oncology 500 — E

Exome & Inherited

- Illumina Exome 2.5 — E
- Exome (Access) — E
- Transcriptome - Human — A
- TruSight One & Expanded — E

Immunology

- Immune Repertoire Plus, TCR beta — A
- TCR beta-SR (CDR3) — A
- Immune Response — A

Infectious disease

- Viral Surveillance Panel — E
- Urinary Pathogen ID/AMR Panel — E
- COVID Seq
- Respiratory Virus Oligo Panel v2 — E
- Respiratory Pathogen ID/AMR — E
- Pan Coronavirus Panel — E

Cardio

- TruSight Cardio — E

Custom

- Custom panels — E A
- Community Panels — A

DNA

RNA

DNA /RNA

A AmpliSeq for Illumina

E hyb enrichment

illumina®

Prices Effective March 2023

For Research Use Only. Not for use in diagnostic procedures.

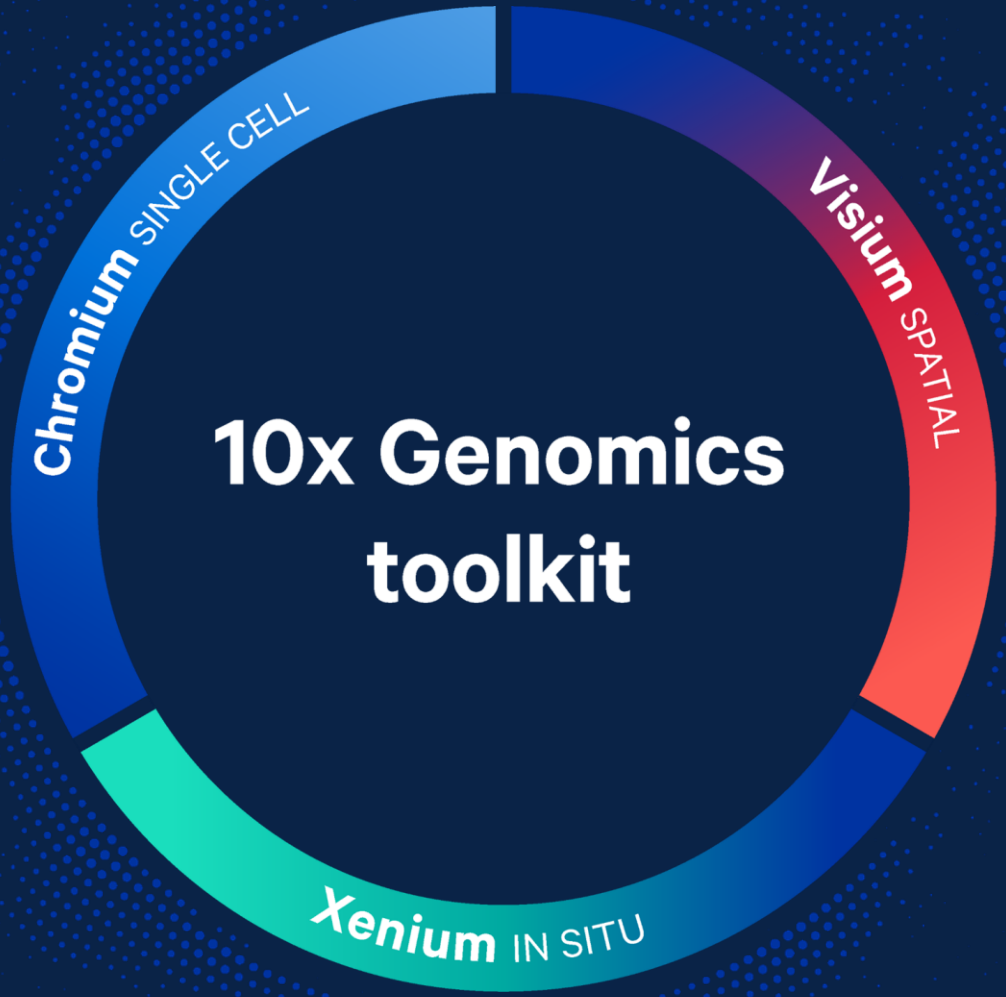
2024 Pilot Sequencing Program

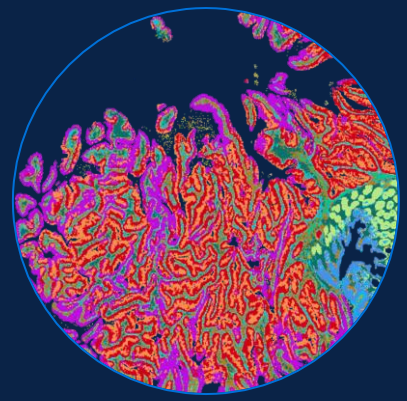
University of Minnesota – April 4th, 2024

Egon J. Ranghini, PhD
Sr. Science & Technology Advisor
egon.ranghini@10xgenomics.com

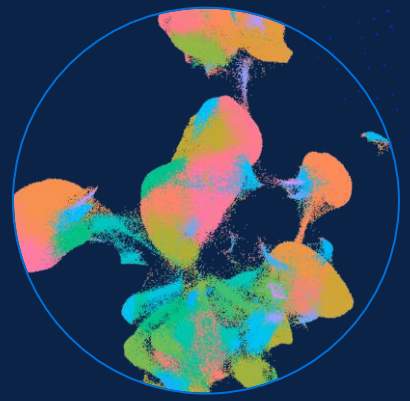
10X GENOMICS

Three platforms to **resolve biology's complexity**

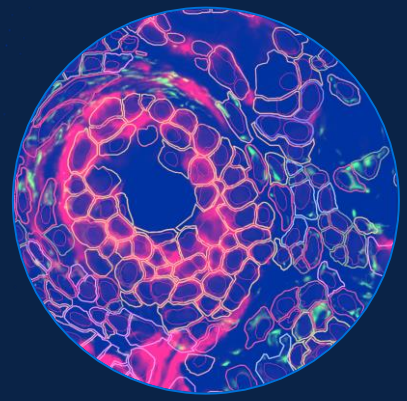




Visium Spatial



Chromium Single Cell



Xenium In Situ

10x Products are Empowering Impactful Science

7,100+

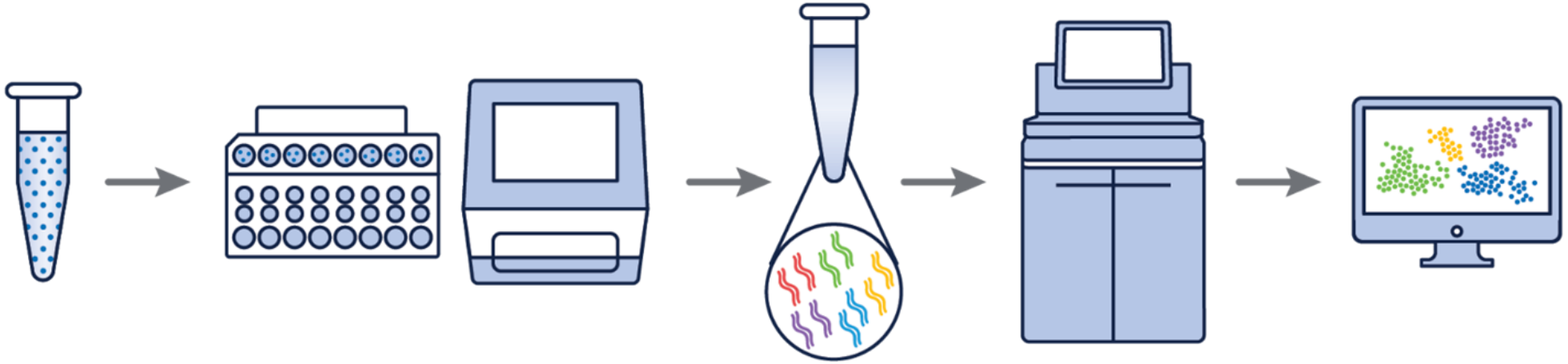
Streamlined and robust workflow for your experiment

Sample Input
(Cells or Nuclei)

GEM Generation & Library Construction

Library Sequencing

Data Analysis & Visualization



Sample Input
User-supplied Cell or Nuclei Suspension*
(labeled or unlabeled)

Cell Capture, Library Construction

UMGC

Sequencing

Analysis
10x Genomics Cell Ranger
10x Genomics Loupe Browser

**Nuclei are not supported with Cell Surface Protein analysis*

10X GENOMICS

Introducing the Next Generation Single Cell Solutions

New versions of 3’ and 5’

- **Efficient partitioning**: tens of thousands of cells in 6 minutes
- **Built to scale**: 500–20K cells per channel, for up to 160K cells per run, run up to 8 samples in parallel
- **Cell size flexibility**: no lower limits
- **High cell capture rates**: up to 80% cell recovery
- **Low doublet rates**: 0.4% per 1,000 cells
- **Instrument compatibility**: Chromium X series

- Powered by **GEM-X Technology**

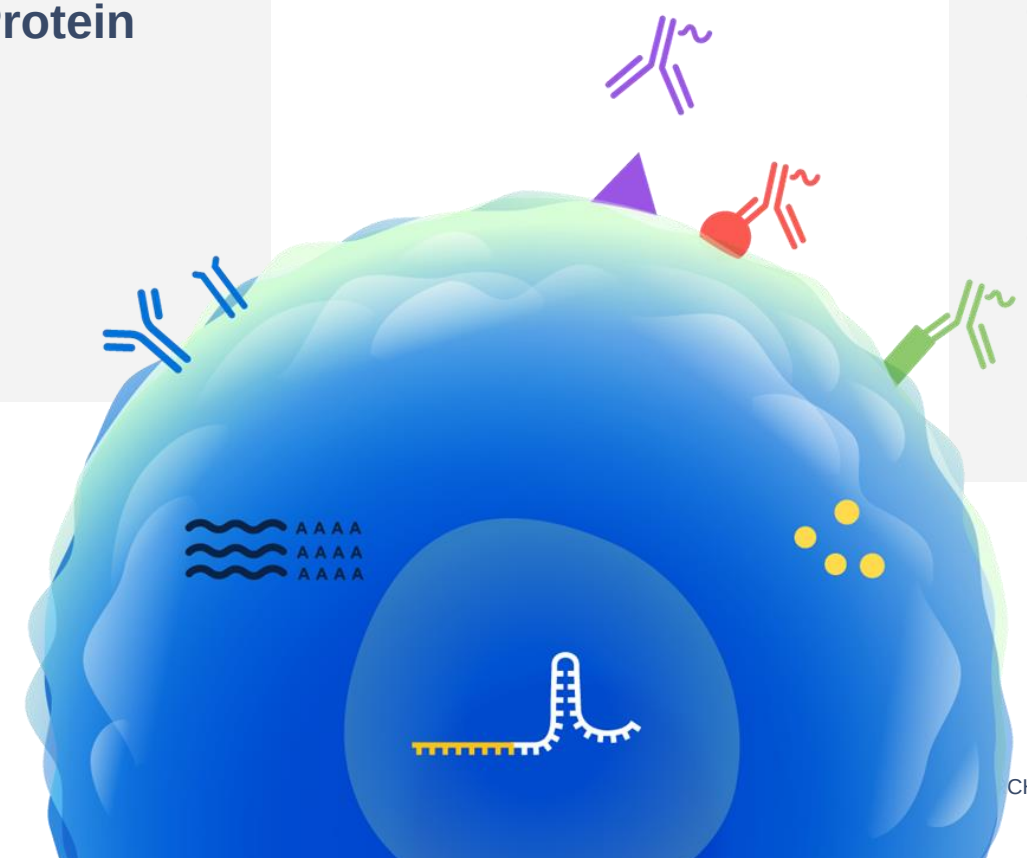
Multiomics are enabled on 3’ v4 and 5’ v3 assays

GEM-X Single Cell Gene Expression v4

- **Gene Expression**
- **Cell Surface Protein** (TotalSeq™-B)

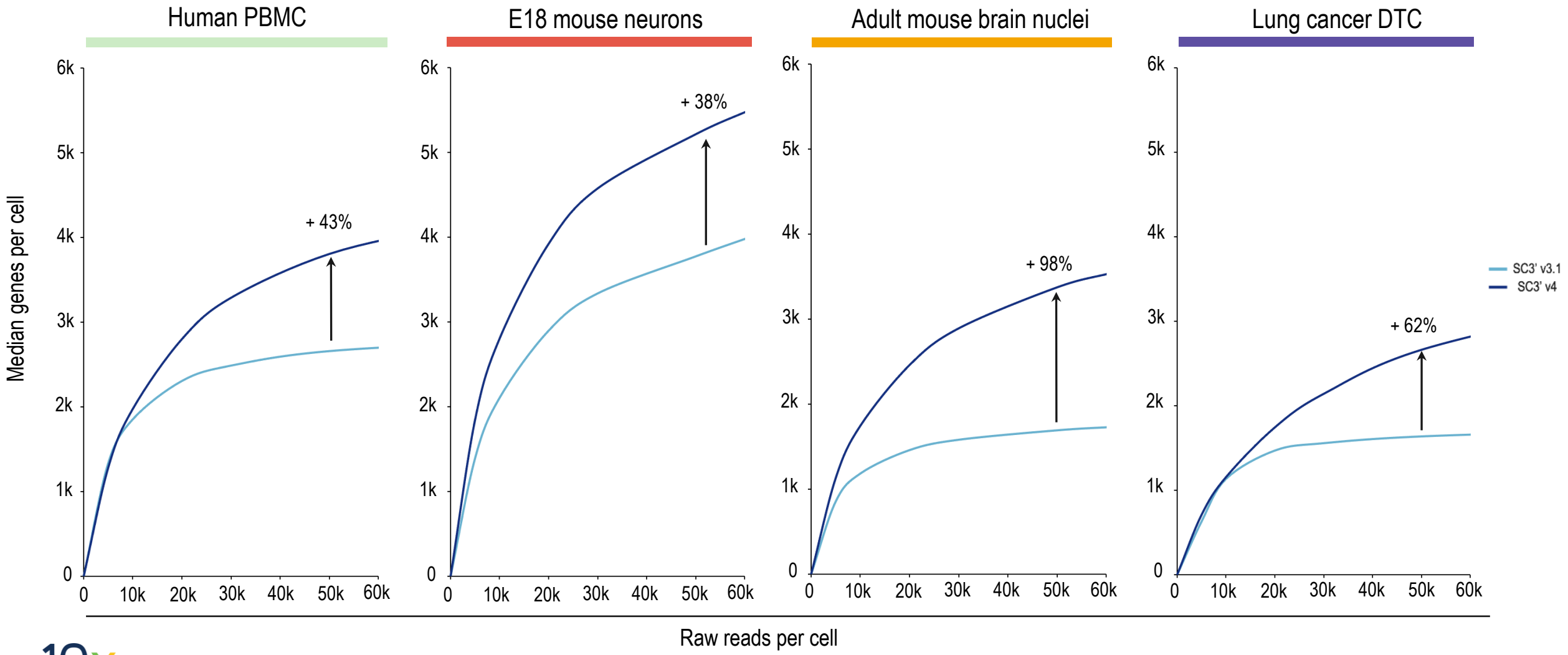
GEM-X Single Cell Immune Profiling v3

- **Gene Expression**
- **V(D)J**
- **Cell Surface Protein** (TotalSeq™-C)
- **CRISPR/gRNA**

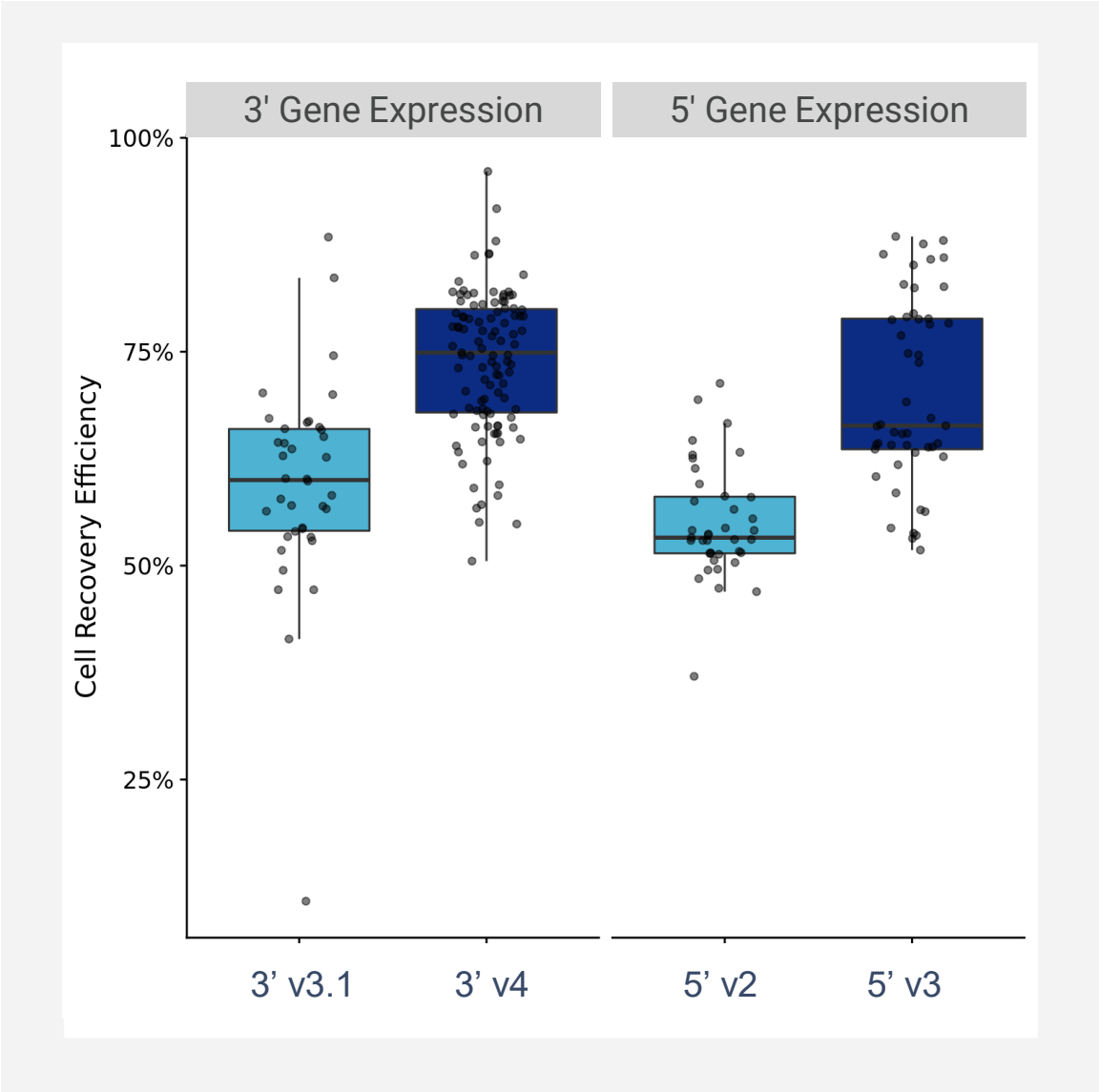


Substantially improved gene sensitivity

GEM-X Single Cell Gene Expression v4

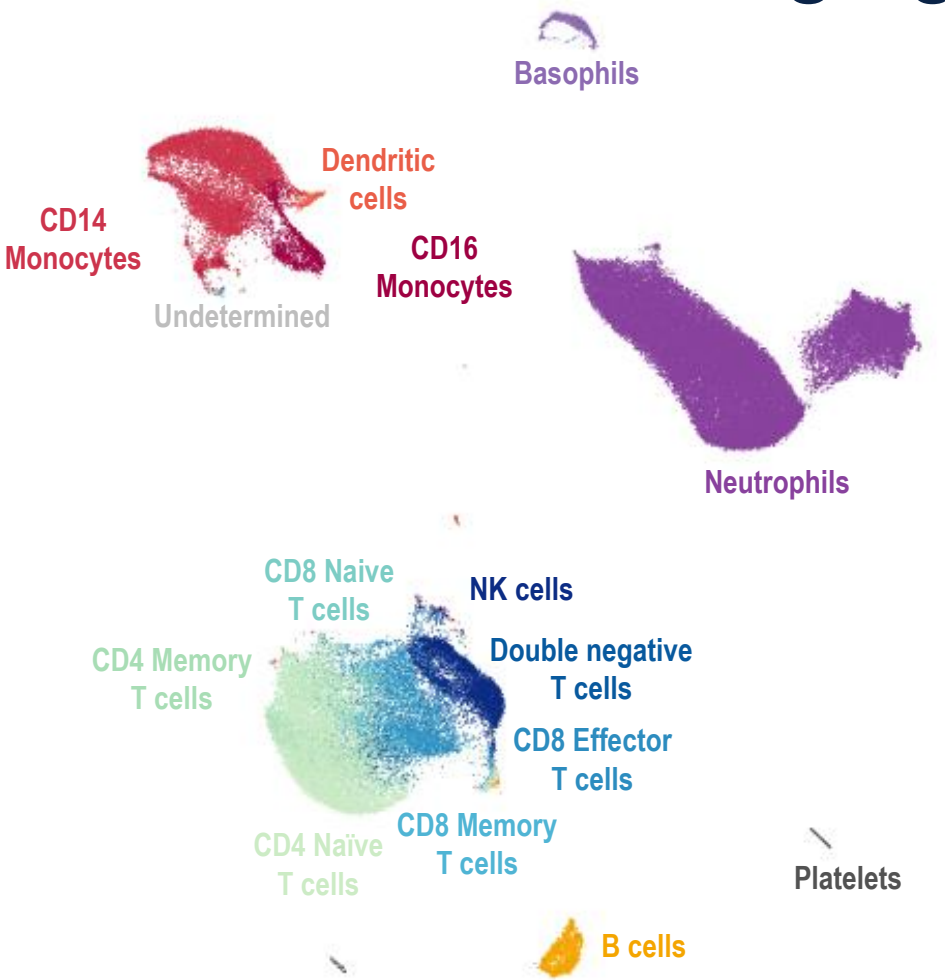


Up to 80% cell recovery

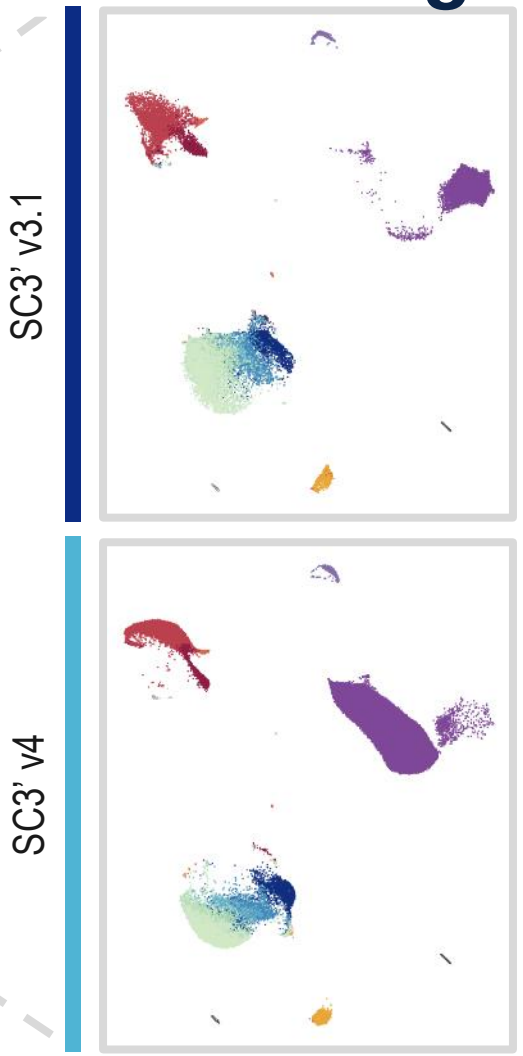


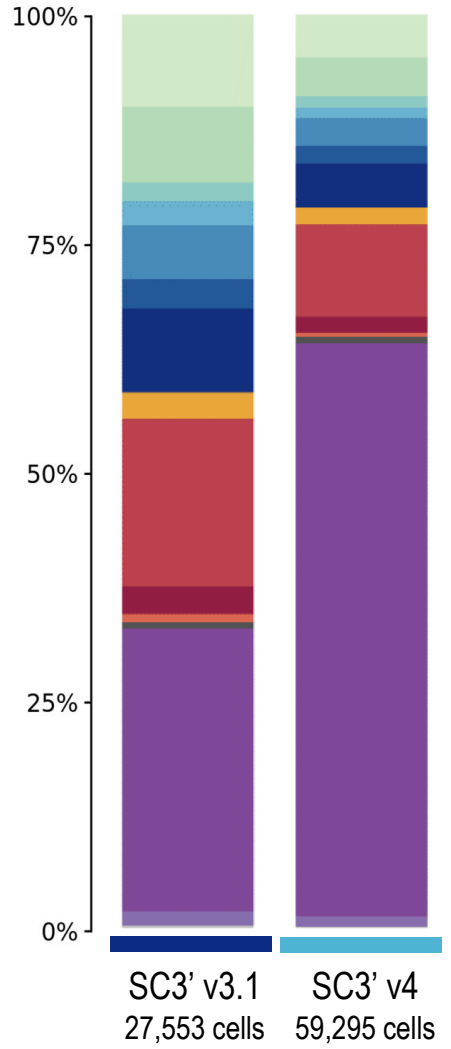
- Make every run, every cell count with improved robustness and cell recovery efficiency enabled by a new chip design
- Optimized for samples typically yielding few cells such as tissue biopsies or previously flow sorted cells
- GEM-X boosts cell recovery from up to 65% to up to 80%

GEM-X Single Cell Gene Expression v4 enables enhanced detection of challenging cell types including neutrophils

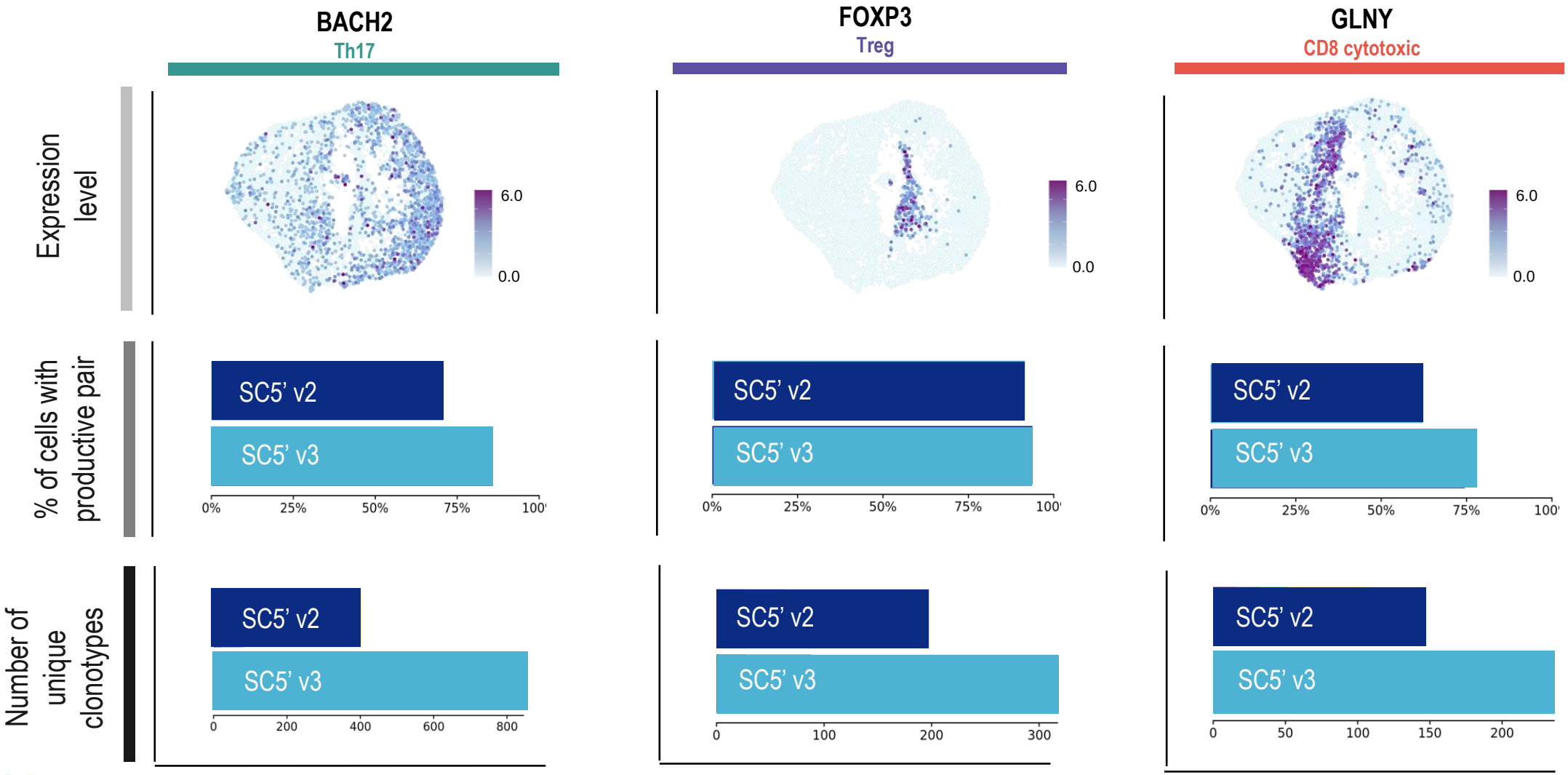


Fresh human leukocytes





GEM-X Single Cell Immune Profiling v3 reveals more unique clonotypes with productive VDJ transcripts

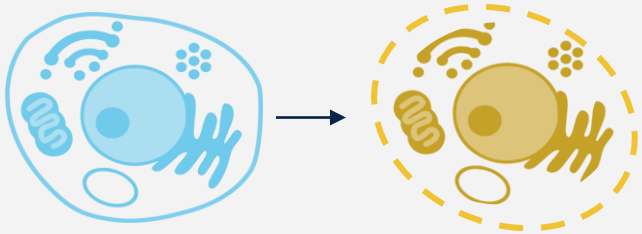


Working with living fresh cells can remain challenging

Limited flexibility

- Hard to collect at different times
- May want to decide which samples to run later

Sample degradation



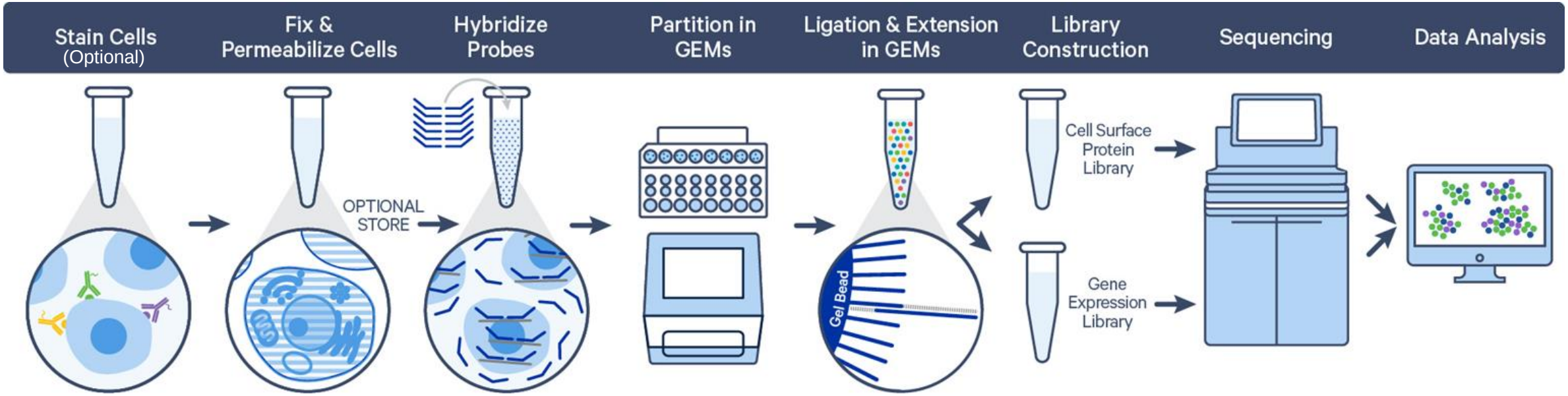
- Fast sample prep is needed
- Some cells cryopreserve better than others

Logistic constraints

- Unavailability of cryopreservation
- Transport can compromise quality

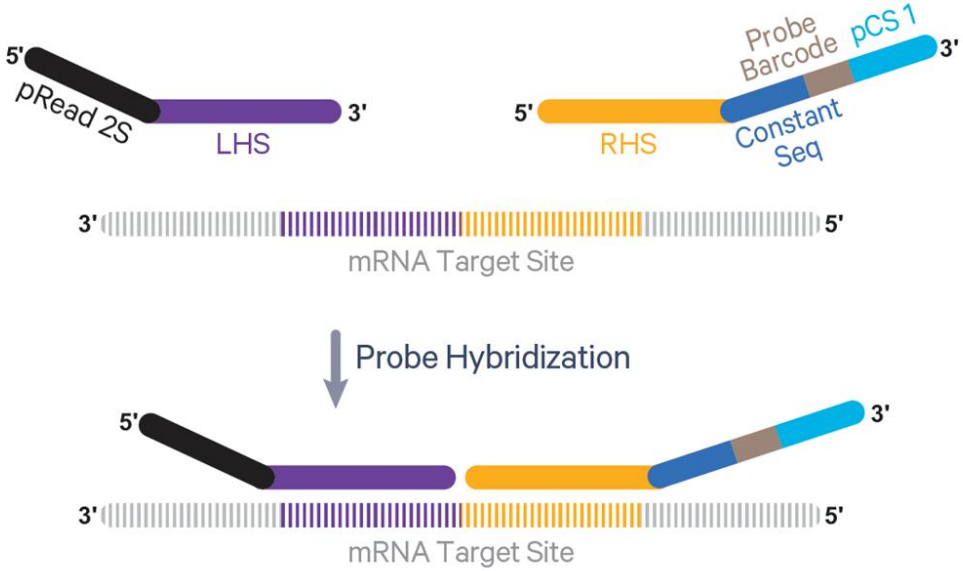
Sample stabilization is crucial!

Single Cell Gene Expression Flex – A Streamlined Workflow



- Sensitive, probe-based **whole transcriptome assay**
- Assay compatible with Feature Barcode technology for **profiling cell surface proteins** (with TotalSeq™-C antibodies)
- Does not depend on polyA capture; covers more than 18,000 human or mouse genes

How it Works – Design and Hybridization of Probe Set



Summary of human & mouse probe set:

- >18,000 coding genes specifically detected
- >55,000 probe pairs
- Design strategy maximizes specificity, sensitivity, and sequencing efficiency
- Minimum of just **10,000 rrpc** is recommended depth for typical samples

Tiling

- **3-fold coverage for most genes** to maximize sensitivity and robustness on fixed samples
- 1-fold coverage for top high-expressing genes
  - Includes mitochondrial protein genes
  - Dampens reads/cell on highest expressors

Excluded Genes

- TCR, Ig joining and variable regions
- Ribosomal proteins
- HLA, KIR, readthrough genes, lncRNA

Design priorities

- Probes designed to account for **all annotated isoforms** for a given gene
- Probes **avoid common sequence variants**
- Empirically determined Tm and sequence preferences
- Most probes target exons

How it Works – Design and Hybridization of Probe Set

Species compatibility

Human

Mouse

Sample type compatibility

Cell lines

Primary cells

Nuclei

Fresh/Frozen tissue

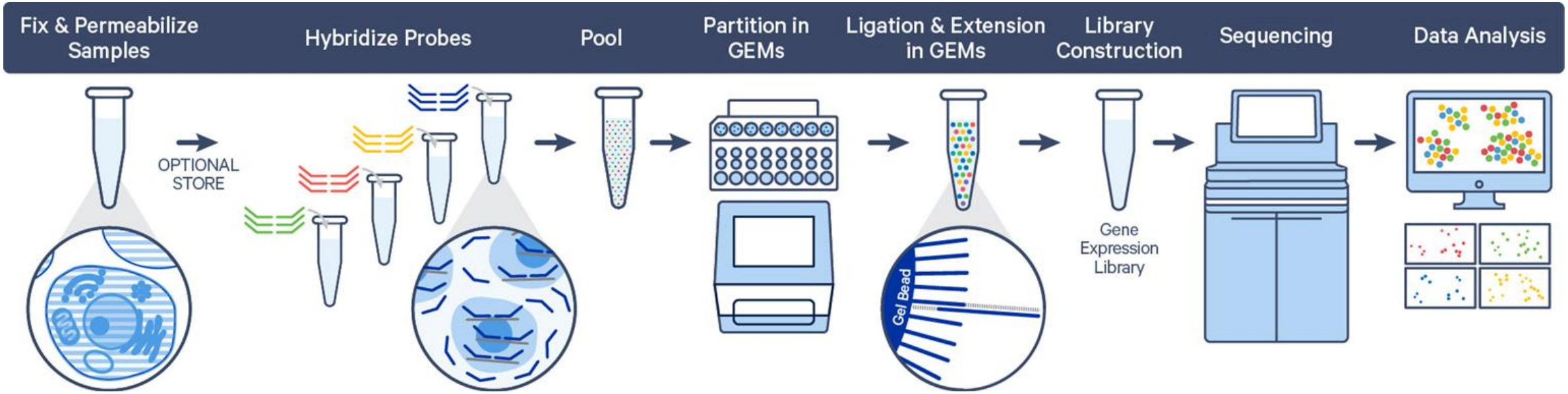
Formaldehyde fixed tissue

FFPE tissue

Use of custom probes not supported or validated

Built-in Sample Multiplexing

Streamlined multiplexing workflow, with no additional steps



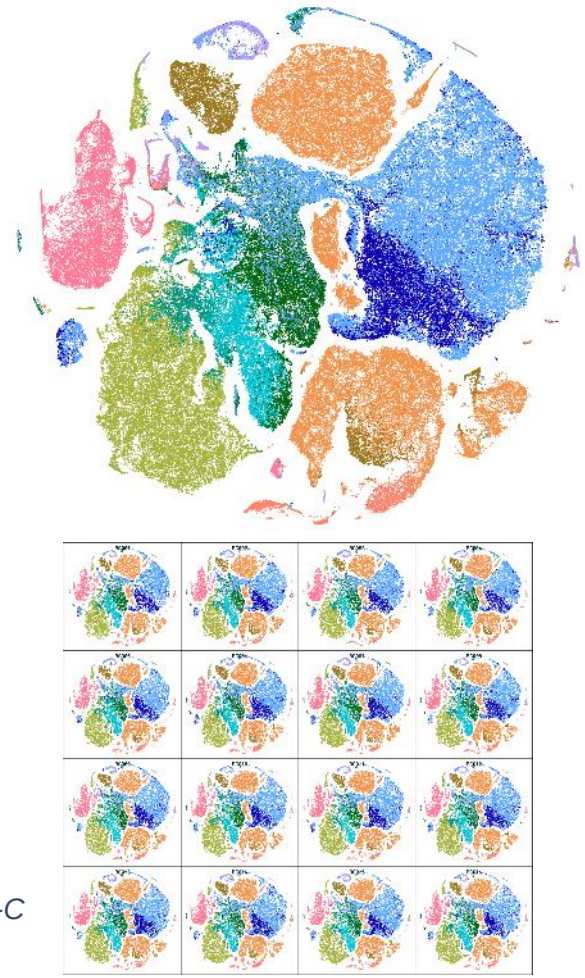
- Each set of probes contains a **unique barcode**; each individual molecule can be assigned back to its sample of origin
- Enables pooling of **up to 16 samples** and **up to 128,000 cells** in one channel
- Reduces cost per sample, and allows for higher throughput and batching
- No need to decide up front which samples to pool together

Multiomic characterization of multiplexed samples

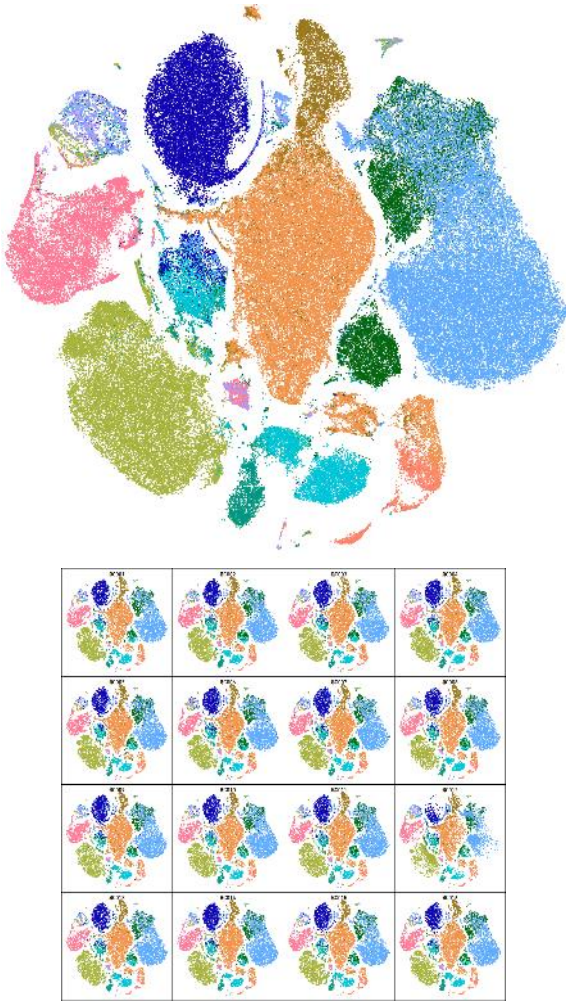
- Feature Barcode Multiplexing kit supports up to 16 multiplexed samples targeting 128,000 cells
- Human PBMCs profiled for gene expression and cell surface protein in a multiplexed format
- Inclusion of cell surface protein data enabled increased resolution of T-cell subpopulations

Multiplexing configuration only supports TotalSeq™-C

Gene Expression



Cell Surface Protein



Human PBMCs

- B cells
- Dendritic cells
- CD14 monocytes
- CD16 monocytes
- NK cells
- Platelets
- Double negative T cells
- Effector cytotoxic T cells
- Memory cytotoxic T cells
- Naïve cytotoxic T cells
- Memory helper T cells
- Naïve helper T cells
- Undetermined

Gene Expression Flex supports FFPE tissues

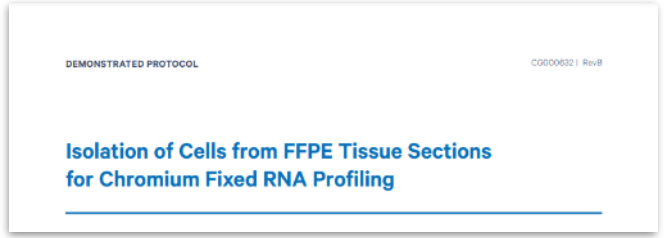
Two robust methods for FFPE dissociation

FFPE Block

FFPE Curls

Dissociated tissue

- We are providing **two methods** for FFPE tissue dissociation, as described in Demonstrated Protocol (CG000632)
  - Instrument-based workflow with the gentleMACS™ Octo Dissociator
  - Manual workflow with a pestle
- Appendix provides additional guidance regarding:
  - Cell counting recommendations
  - Cell yields derived from >20 samples with section thickness and cross-section size
- Each method has been extensively tested with the tissues listed below



HUMAN

Brain (healthy, Alzheimer's, glioblastoma)
Breast cancer
Colorectal cancer
Heart
Kidney
Lung (cancer, healthy)
Ovarian cancer
Lymph Node (reactive, healthy)
Liver (cancer, healthy)
Pancreas
Prostate cancer
Skin melanoma
Testis
Thymus
Tonsil
Spleen

MOUSE

Brain (cerebellum, cortex, hippocampus)
Heart
Kidney
Liver
Spleen
Thymus

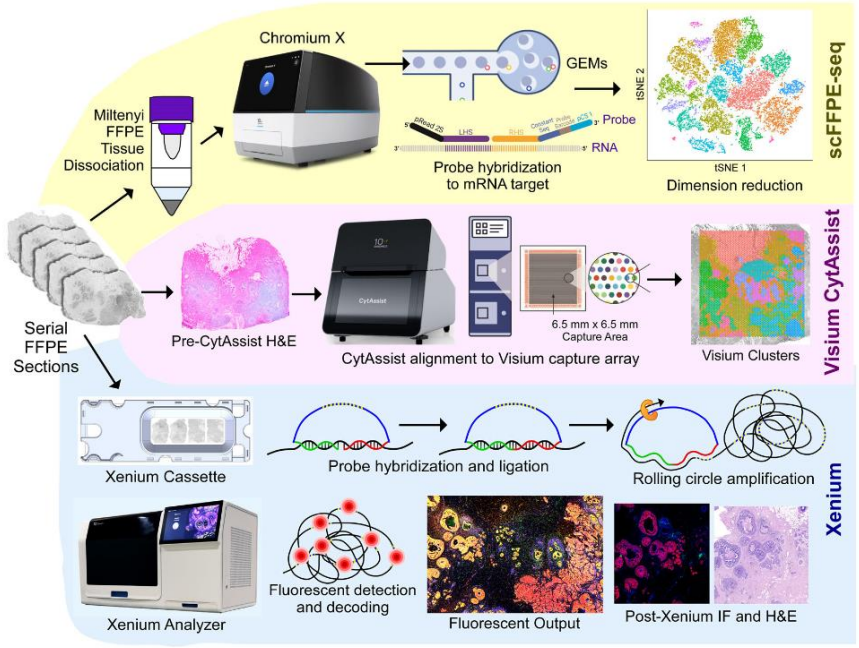
Recent Single Cell FFPE publications

nature communications

Article

https://doi.org/10.1038/s41467-023-43458-x

High resolution mapping of the tumor microenvironment using integrated single-cell, spatial and in situ analysis



https://doi.org/10.1038/s41467-023-43458-x

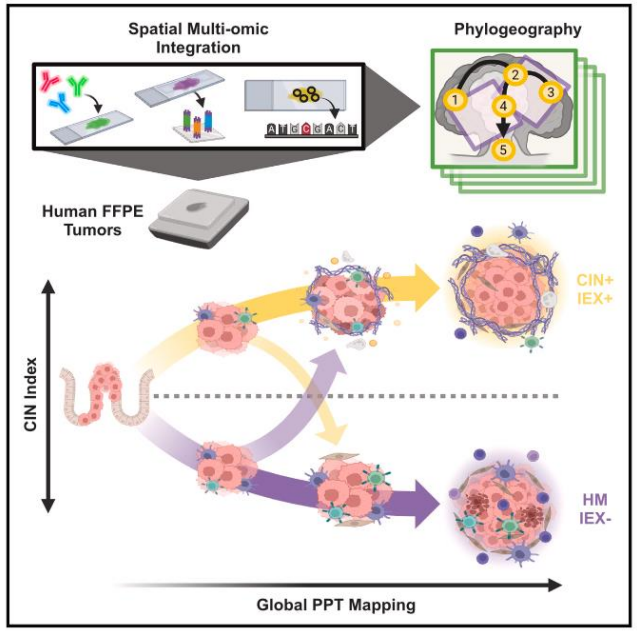
CellPress OPEN ACCESS

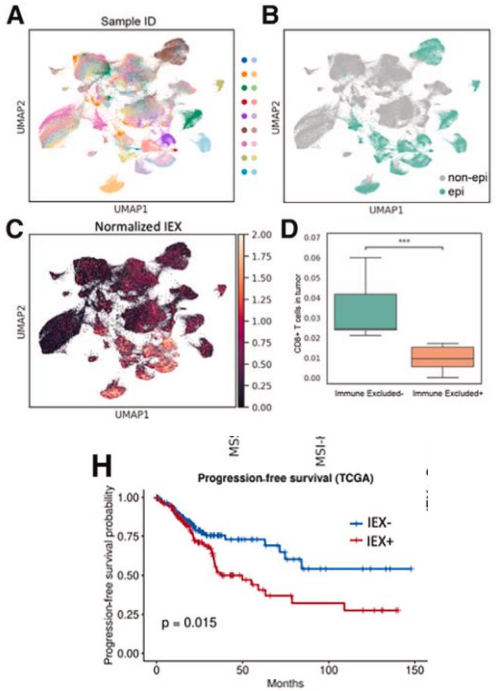
Cell

Resource

Molecular cartography uncovers evolutionary and microenvironmental dynamics in sporadic colorectal tumors

Graphical abstract





https://doi.org/10.1016/j.cell.2023.11.006

Single Cell Pilot Projects – Typical Cases

Single Cell 3’ or 5’ GEM-X

- 2-4 samples; GEX (+ VDJ) only; 10,000 cells; 20,000 read pairs/cell

Single Cell Gene Expression Flex

- 2-4 samples; GEX only; 10,000 cells; 10,000 read pairs/cell

The reagent subsidies will cover a limited amount of the following 10x solutions at no cost:

- Single Cell Gene Expression 3’ v4, or
- Single Cell Immune Profiling 5’ v3, or
- Single Cell Gene Expression Flex

Thank you! Questions?

Your local 10x Team

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egon.ranghini@10xgenomics.com

10x Office Hours!

April 15th and April 16th, 9am - 12pm CDT

Zoom meetings