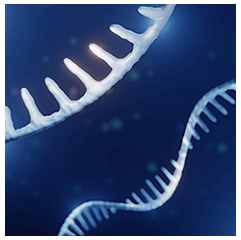
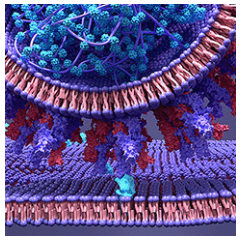
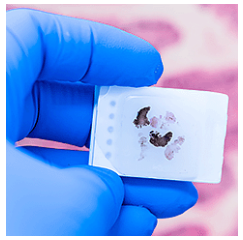
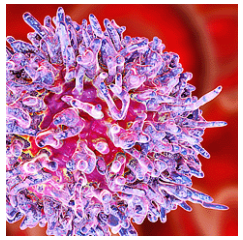
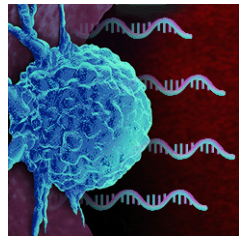






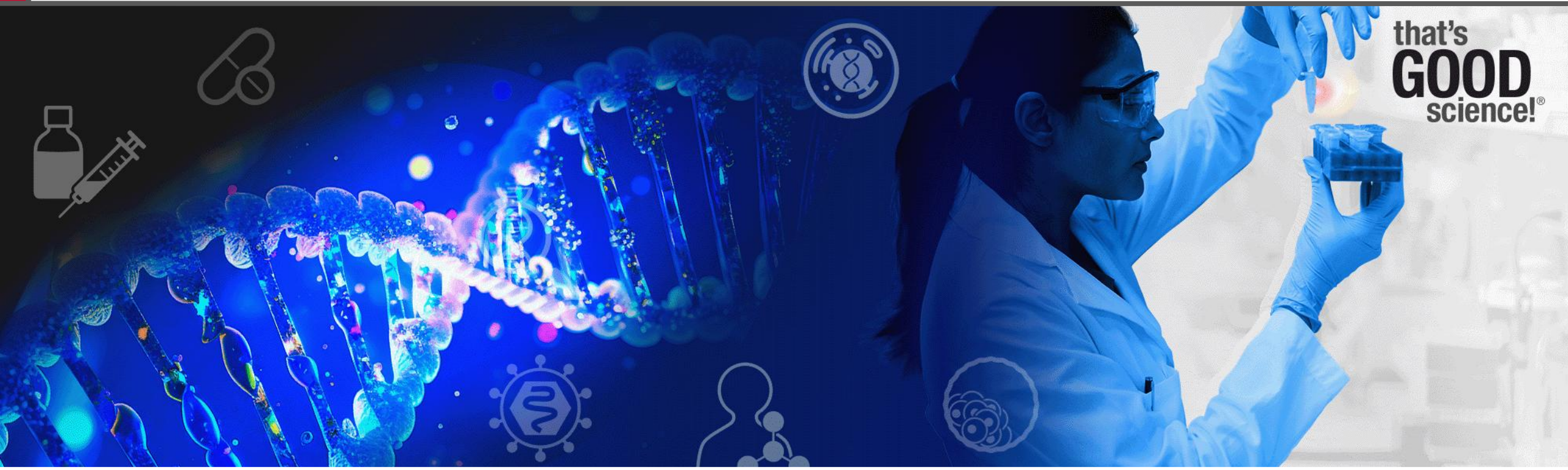
Unlocking precision in immune profiling: sensitivity, accuracy, and versatility across sample types

Yue Yun, PhD

Senior Director, NGS R&D

that's GOOD science!®

Takara Bio: core capabilities



NGS

Spatial genomics

PCR, qPCR, RT-PCR

Cloning

Nucleic acid purification

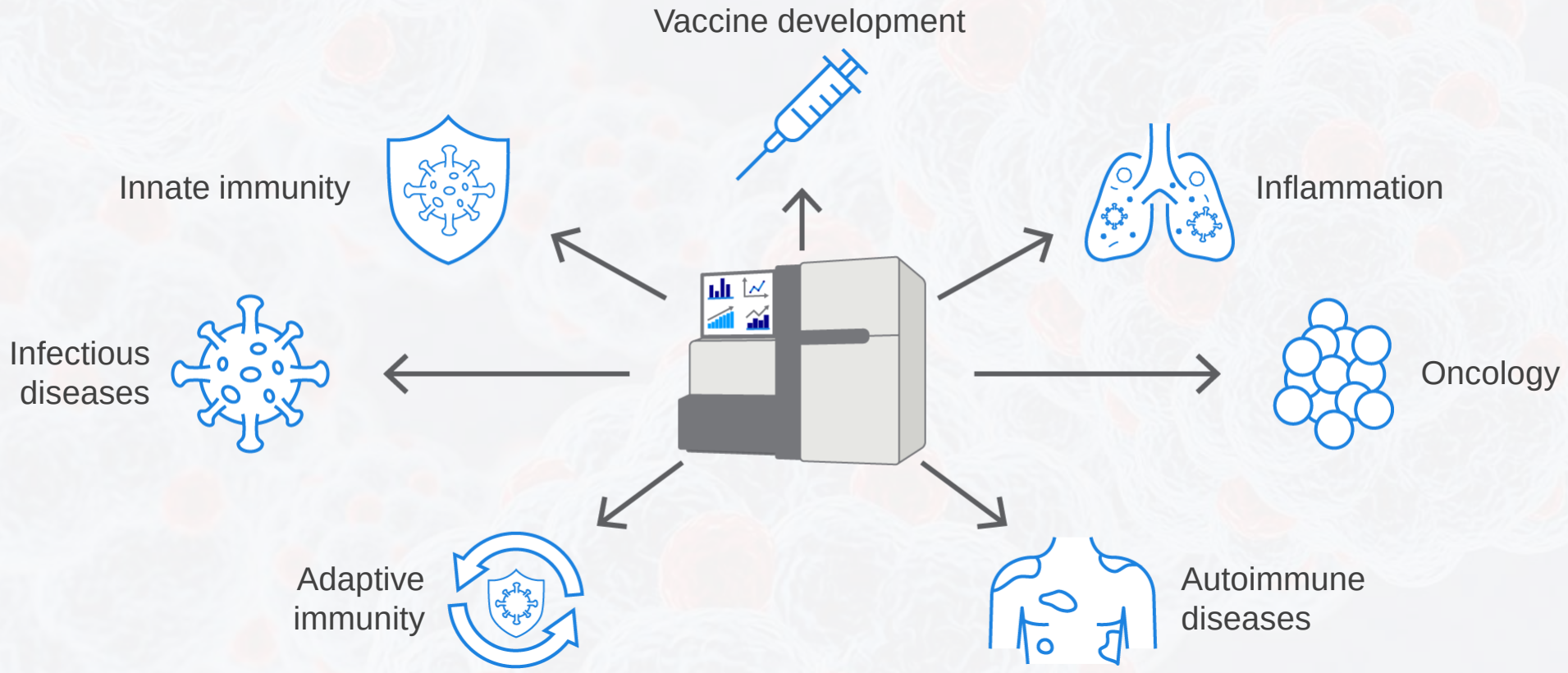
Gene delivery

Functional genomics

Protein expression & purification

OEM

Decoding the immune system through repertoire sequencing



Created with BioRender.com

Applications of TCR and BCR repertoire sequencing

T-cell receptors (TCRs)

- Study tumor-infiltrating T cells in cancer immunotherapy
- Monitor immune response to viral infection
- Investigate safety and clonal diversity of T cells used in T-cell/CAR-T/adoptive transfer therapies

B-cell receptors (BCRs)

- Understand antibody response to pathogens
- Identify B-cell clones associated with autoimmune disease symptoms or severity
- Develop new antibody therapies

Takara Bio offers diverse solutions for immune profiling

Multiplexing

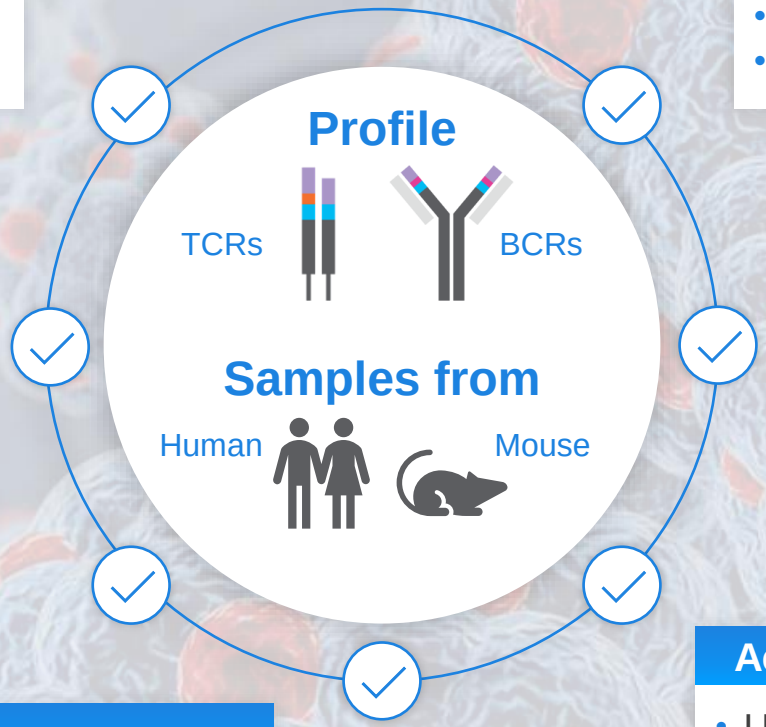
- Unique dual indexes (UDIs)
- Sequence 384 samples in a single run

Sequencing flexibility

- Full length sequencing (2 x 300 PE)
- CDR3 sequencing only (2 x 150 PE)

Captures isotypes

- **BCR:** IgA/D/E/G/M (heavy chain)
  IgK/L (light chain)
- **TCR:** TCRα, TCRβ



Sensitivity

- Low-abundant clonotypes
- Low-expressing TCRs and BCRs

Adaptable input

- 1 ng–1 µg RNA
- Direct cell input (TCR kits)

Various sample types

- RNA, direct cell inputs (e.g., T cells), PBMCs, whole blood, bone marrow, lymph nodes, FFPE & fragmented RNA

Accuracy

- Unique molecular identifiers (UMIs)
- Error correction
- Confident clonotype calling

Takara Bio’s advantages in NGS immune profiling

Unlocking precision in immune profiling

Accuracy
- Unbiased amplification
- Reproducibility
- Full-length reads

Versatility
- Bulk or single-cell
- Fresh/frozen or FFPE
- Clinical research samples

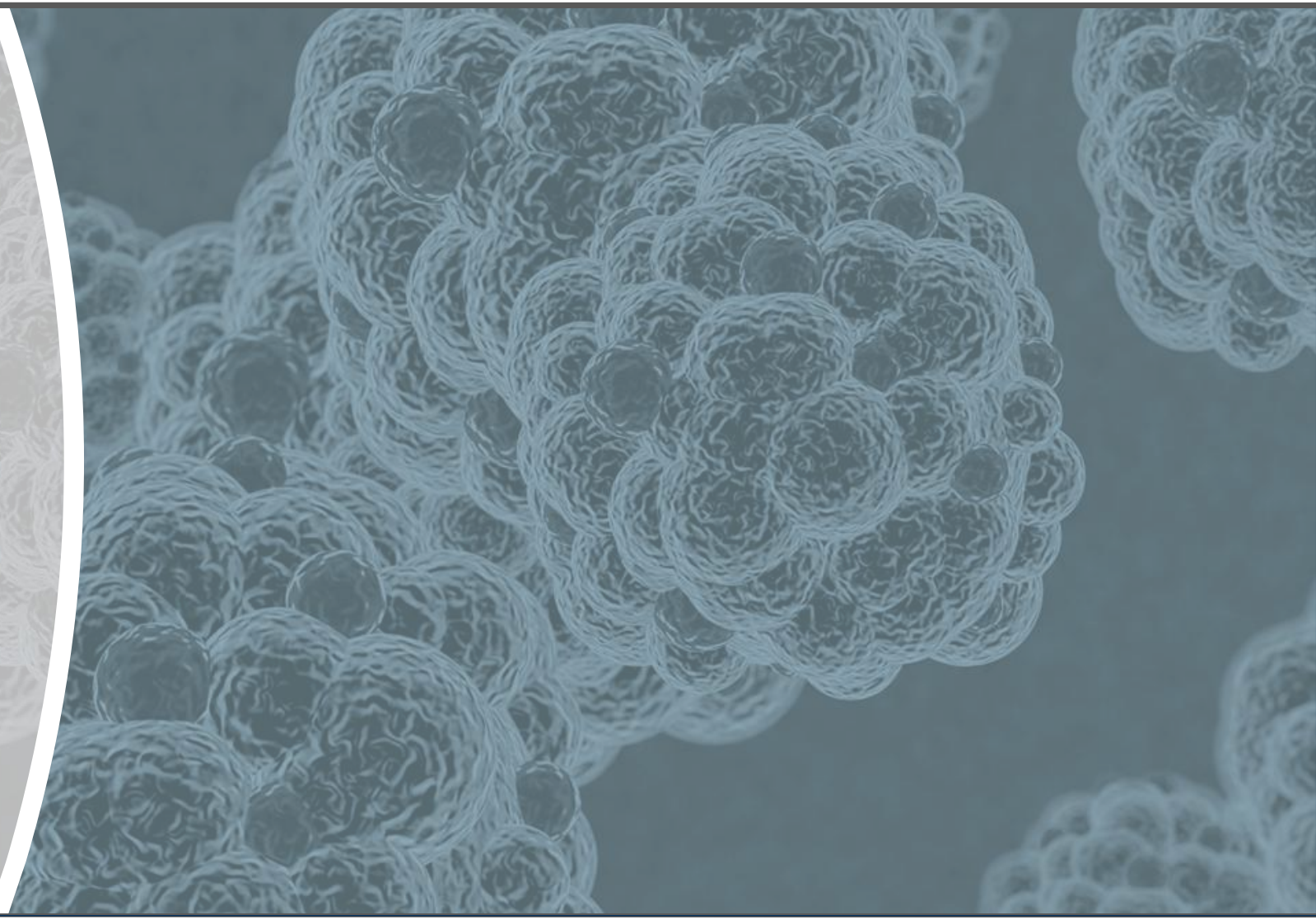
Scalability
- 100,000 cells per run
- 384 unique dual indexes

Superior ***sensitivity***

Obtain accuracy from your immune profiling studies

Accuracy

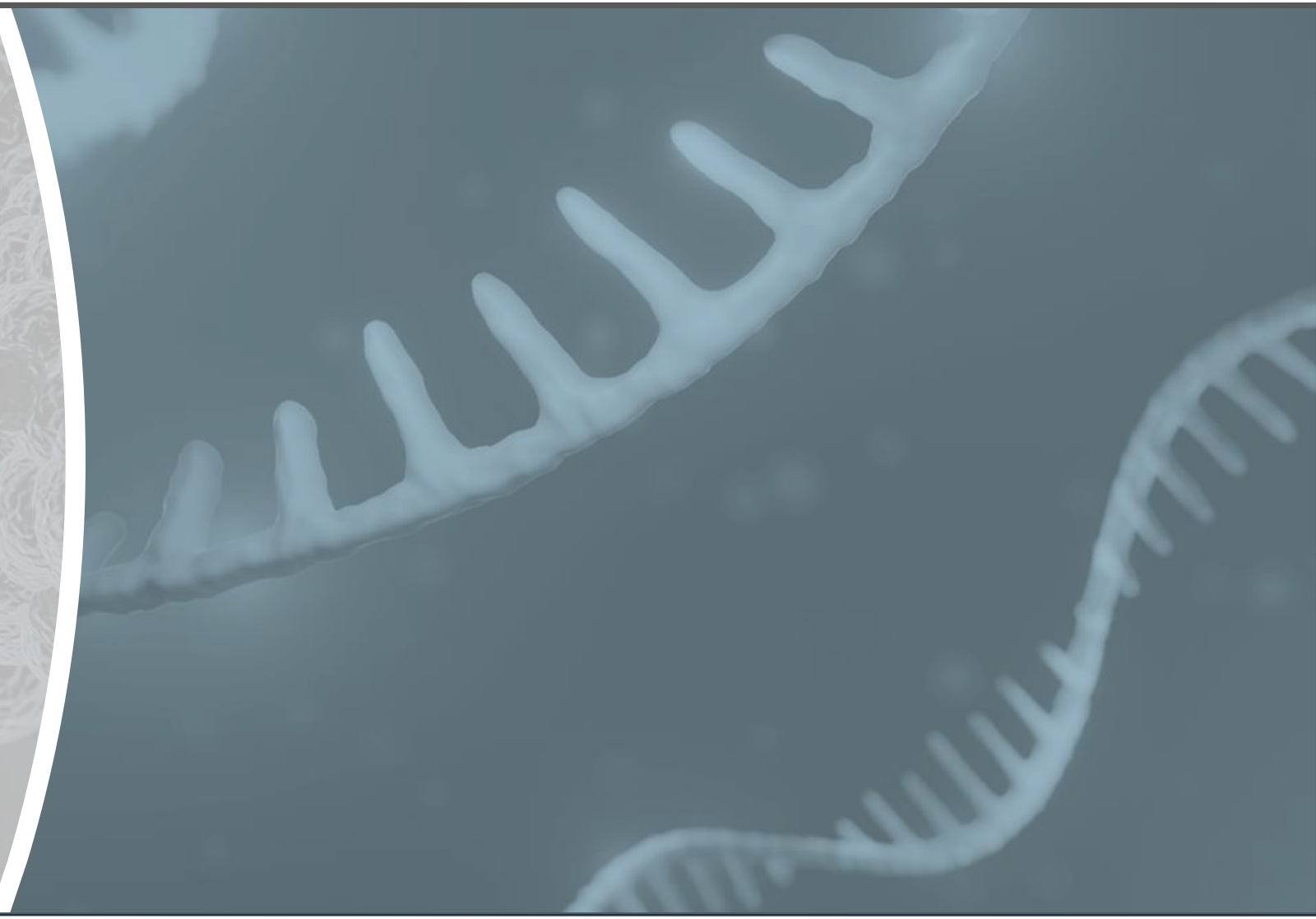
- Unbiased amplification
- Reproducibility
- Full-length reads
- Sensitivity



Spike-in control study (FDA)

Accuracy

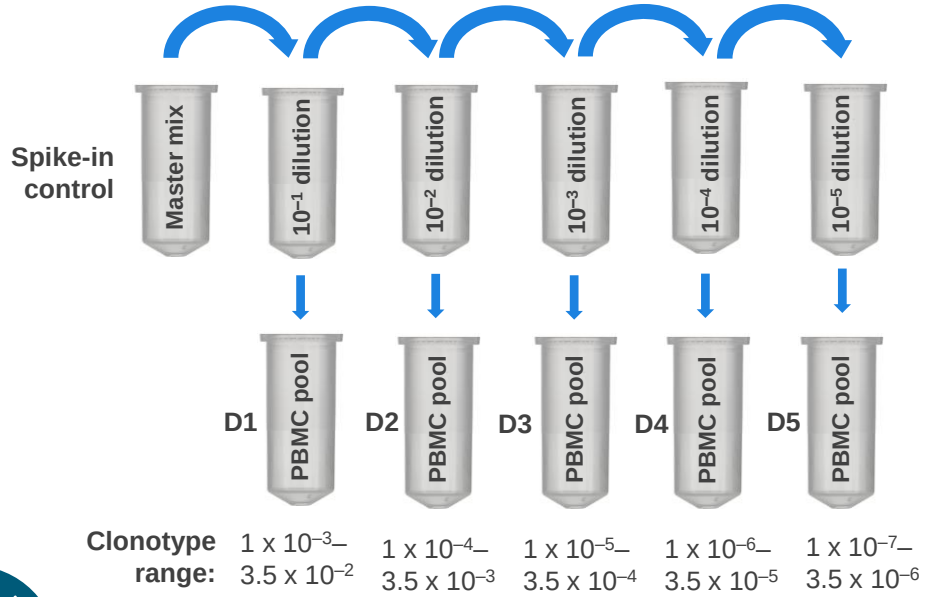
- **Unbiased amplification**
- **Reproducibility**
- Full-length reads
- **Sensitivity**



Evaluating bias in BCR repertoire profiling with SMART-Seq® Human BCR (with UMIs)

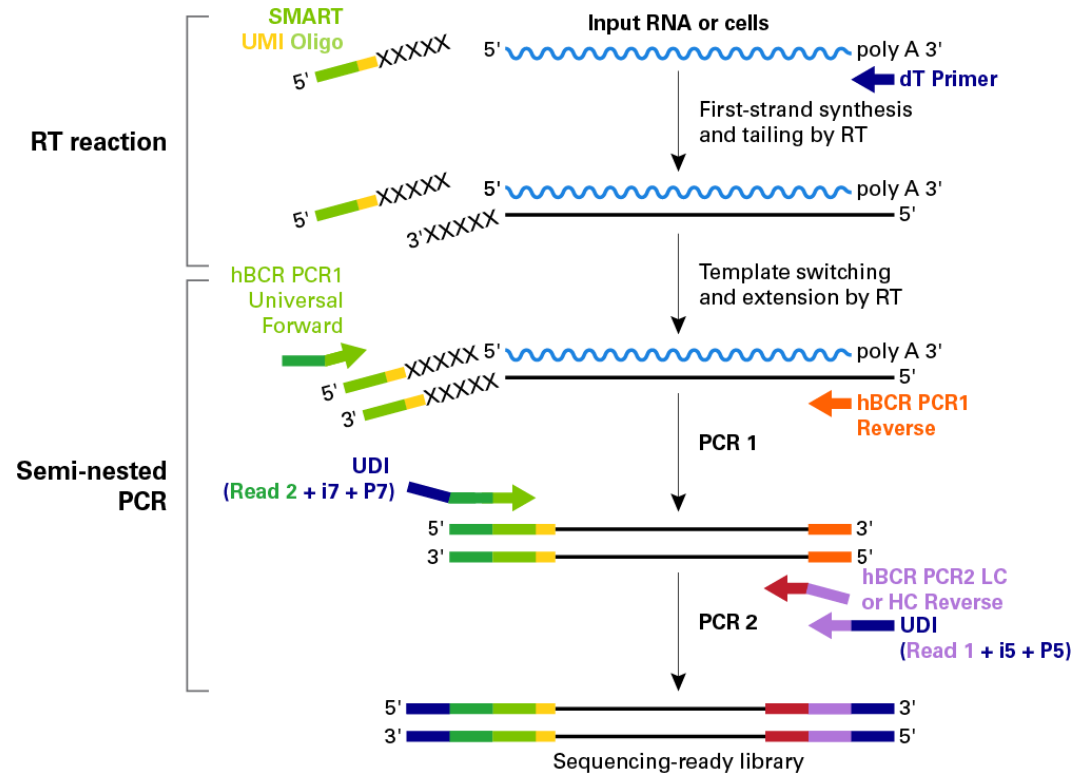
FDA consortium spike-in control study:

- Used mRNA from nine different cell lines as spike-in controls (each at a known concentration: 1–35% of the master mix)
- Spiked in control mRNA into a series of pooled PBMC dilutions



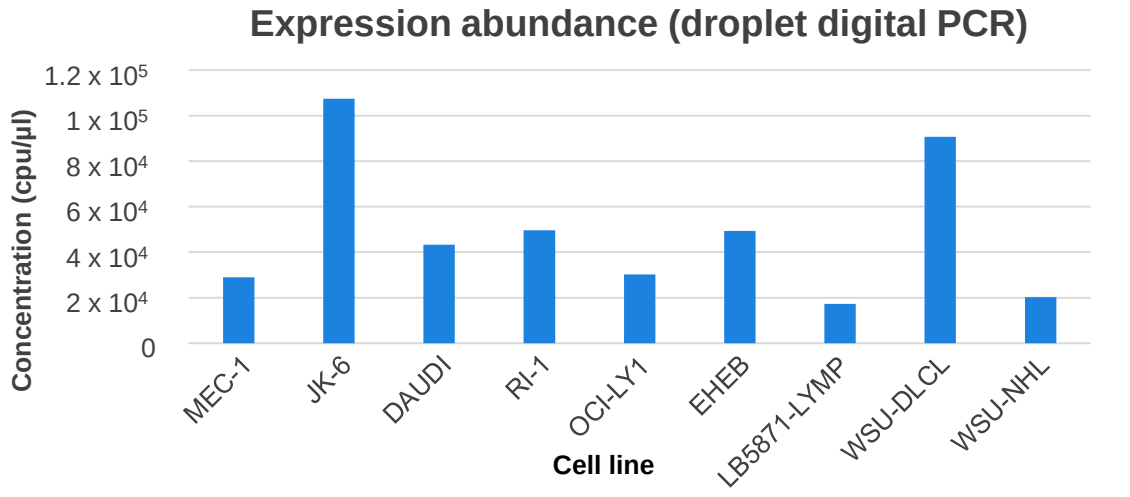
Unbiased amplification | **Reproducibility** | **Sensitivity**

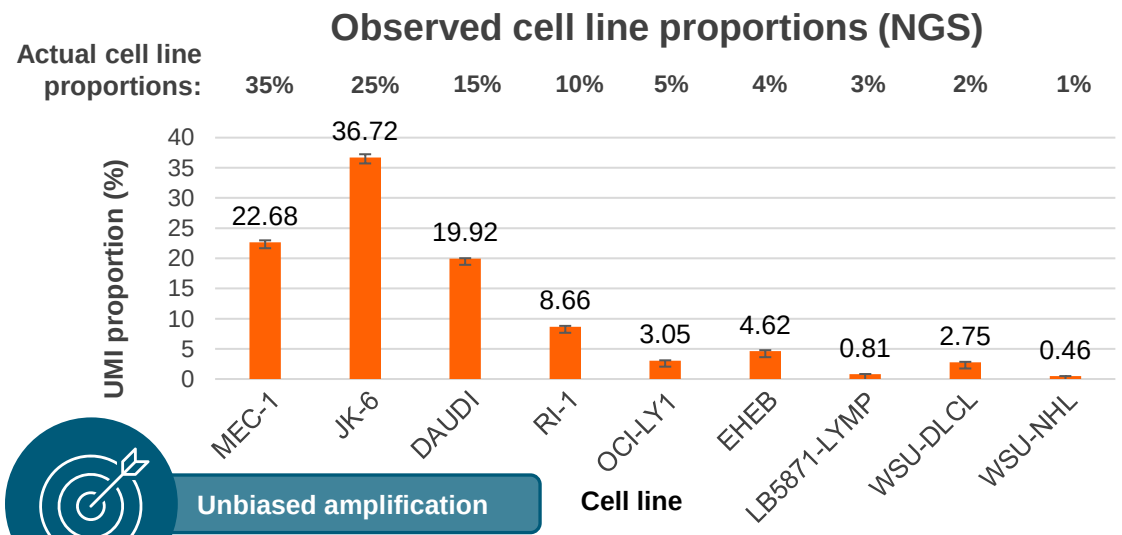
SMART-Seq Human BCR (with UMIs) workflow



Thanks to Dr. Wenming Xiao at the Food & Drug Administration for leading this study that brought together assay developers, sequencing platform providers, and bioinformatics solution providers.

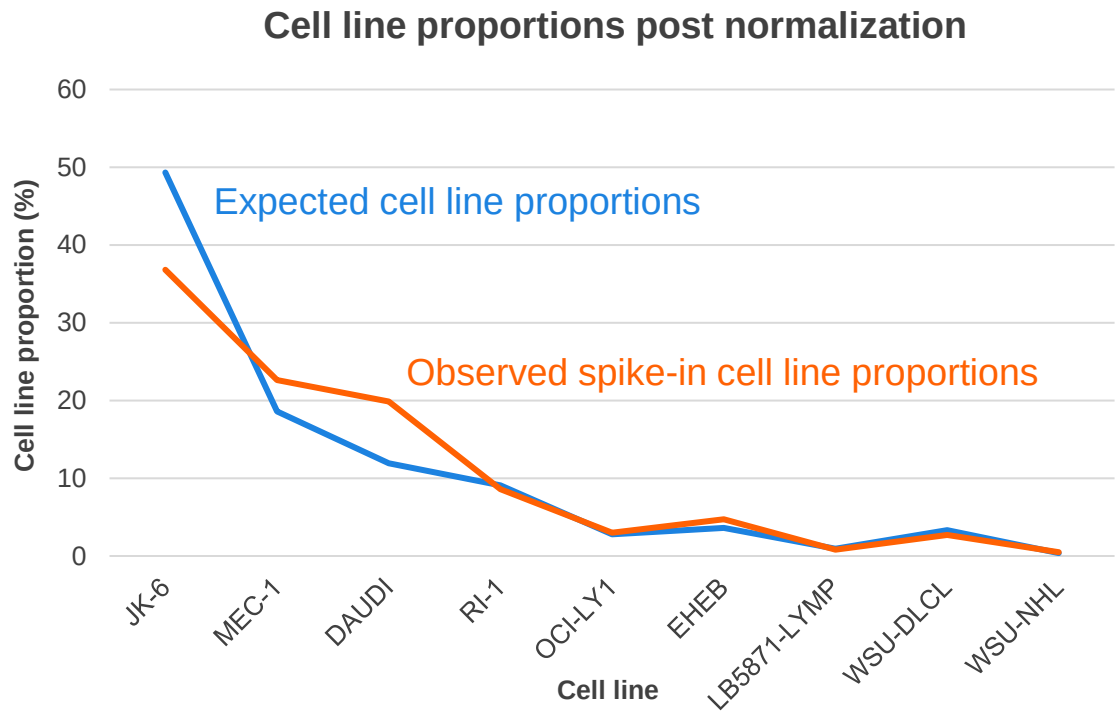
Unbiased amplification across spike-in cell lines





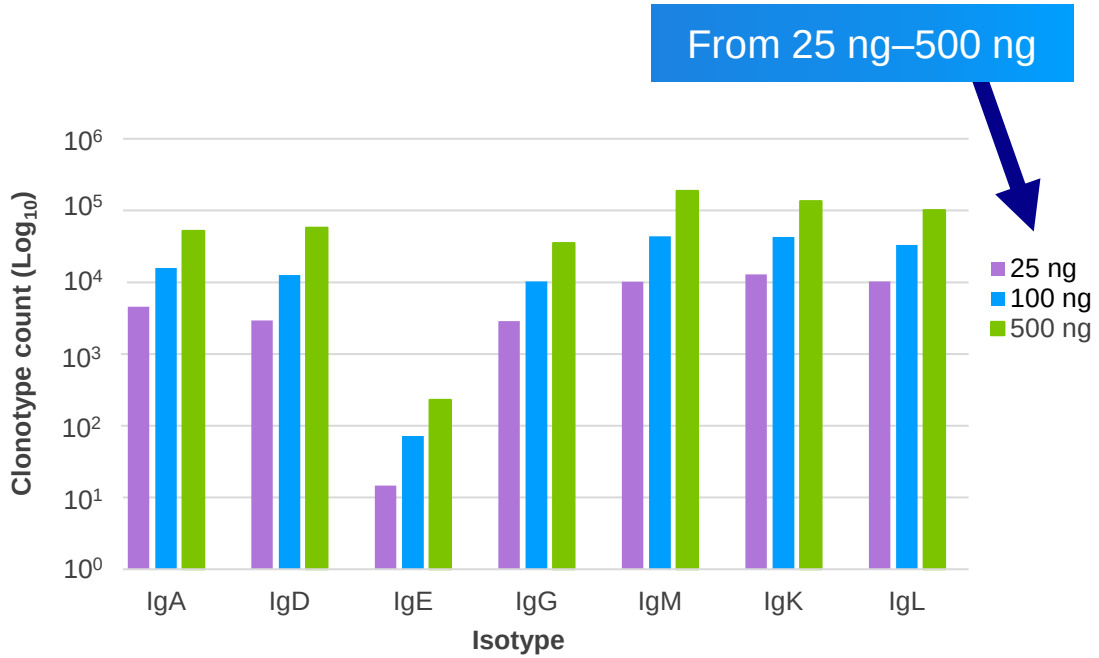
Unbiased amplification

Observed proportions are the same (by ranking) as expected, demonstrating **unbiased amplification**

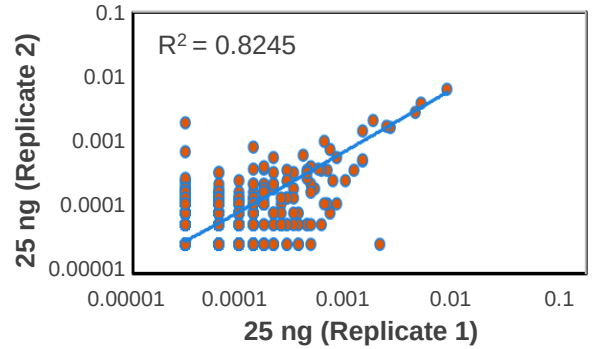


Reproducible performance across a wide mRNA input range

SMART-Seq Human BCR (with UMIs) performs consistently at low and high inputs

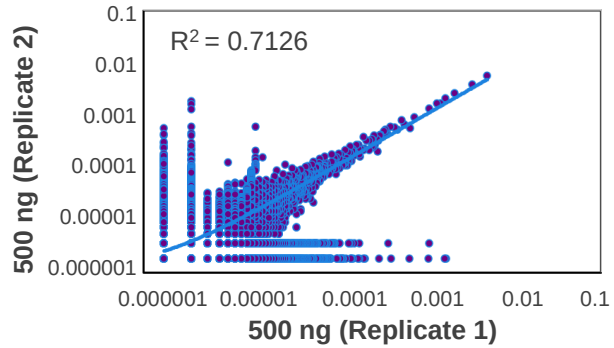


Clonotype frequencies correlate between technical replicates



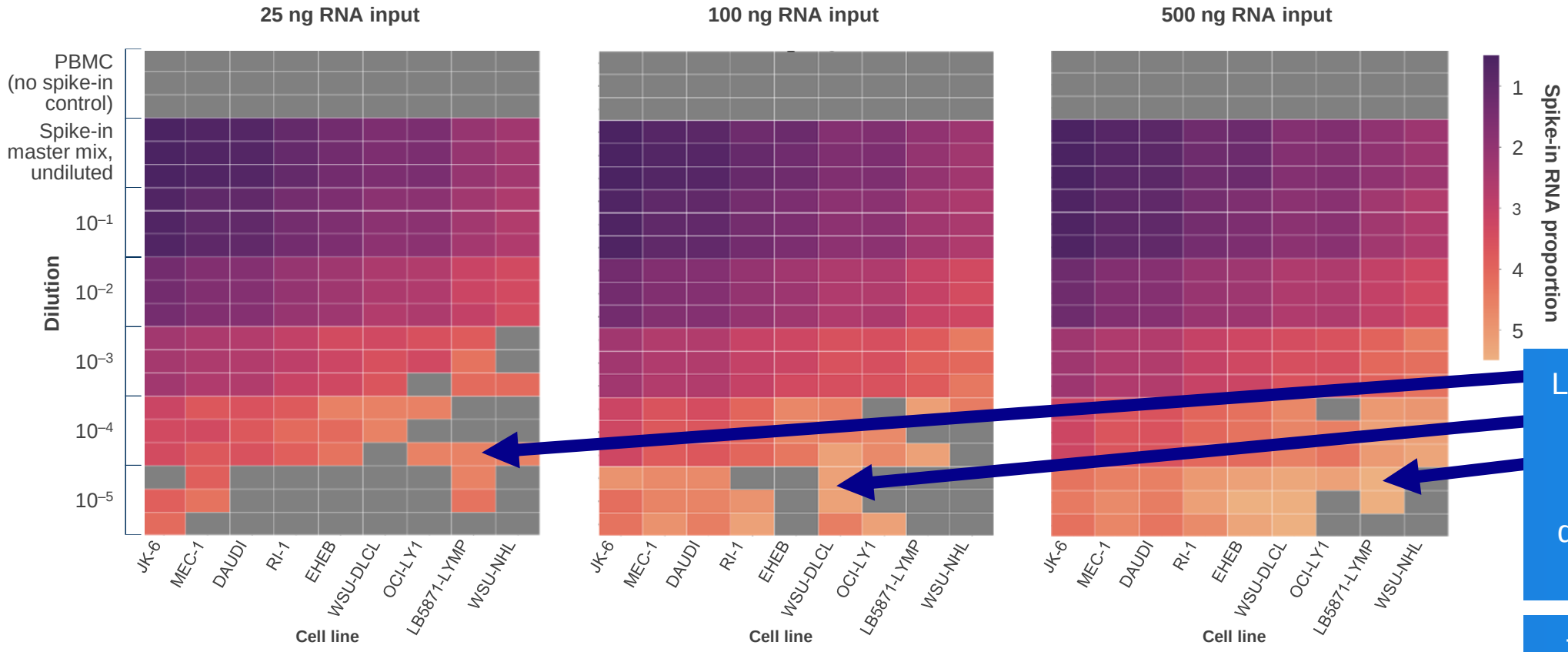
Reproducible at low inputs (25 ng)

Reproducible at high inputs (500 ng)



Reproducibility

Reproducible detection of low-abundance clonotypes

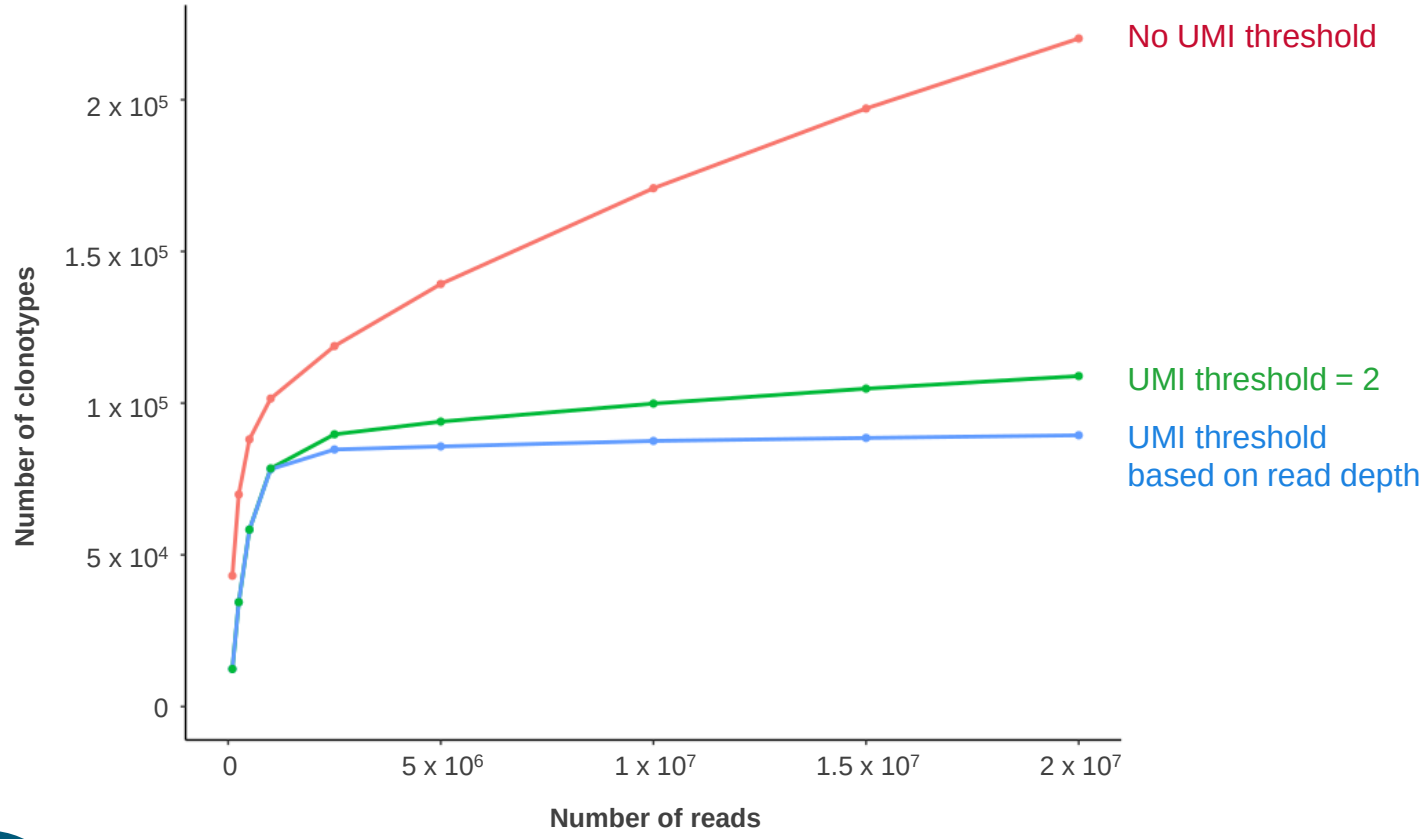


Libraries prepared with SMART-Seq Human BCR (with UMIs) are sensitive enough to detect low-abundance clonotypes

The limit of detection (LOD) was a clonotype frequency of 4 x 10^{-7} for an input of 500 ng

Reproducibility | **Sensitivity**

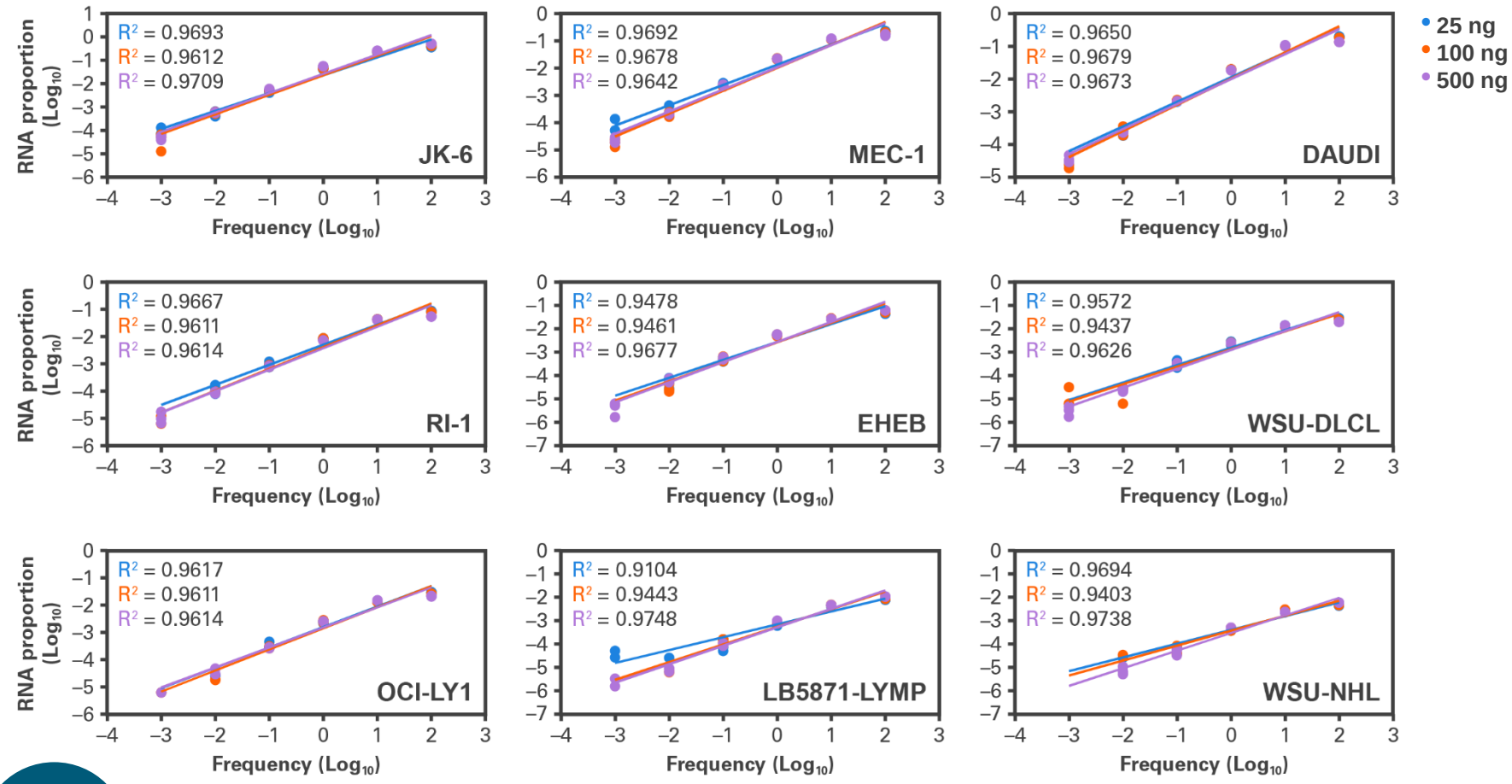
UMIs enhance immune profiling accuracy and quantification



Adding UMIs allows you to:

- **Eliminate duplicates created during PCR and sequencing**
- **Correct errors**
- **Achieve quantitative accuracy**
- **Avoid over-sequencing**

Precise quantification of low-abundance clonotypes



Ratio of detected spiked-in BCR clonotypes versus total reads:

- Showed strong correlation with the corresponding spike-in concentration
- Indicates **unbiased detection**

25 ng RNA input: R^2 = 0.910–0.969

100 ng RNA input: R^2 = 0.940–0.967

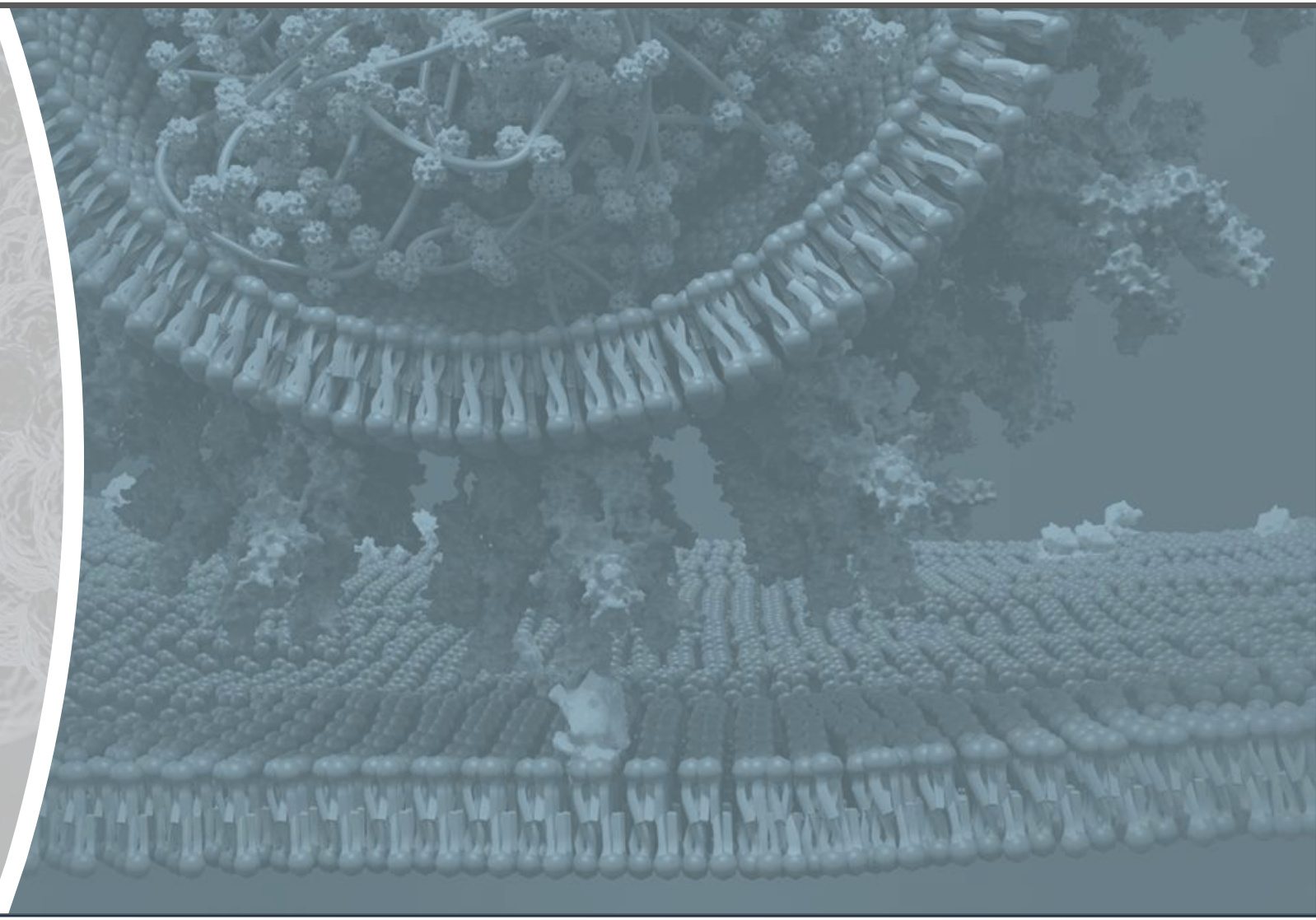
500 ng RNA input: R^2 = 0.961–9.974

Sensitivity

The power of full-length V(D)J sequencing (SARS-CoV-2 study)

Accuracy

- Unbiased amplification
- Reproducibility
- **Full-length reads**
- Sensitivity

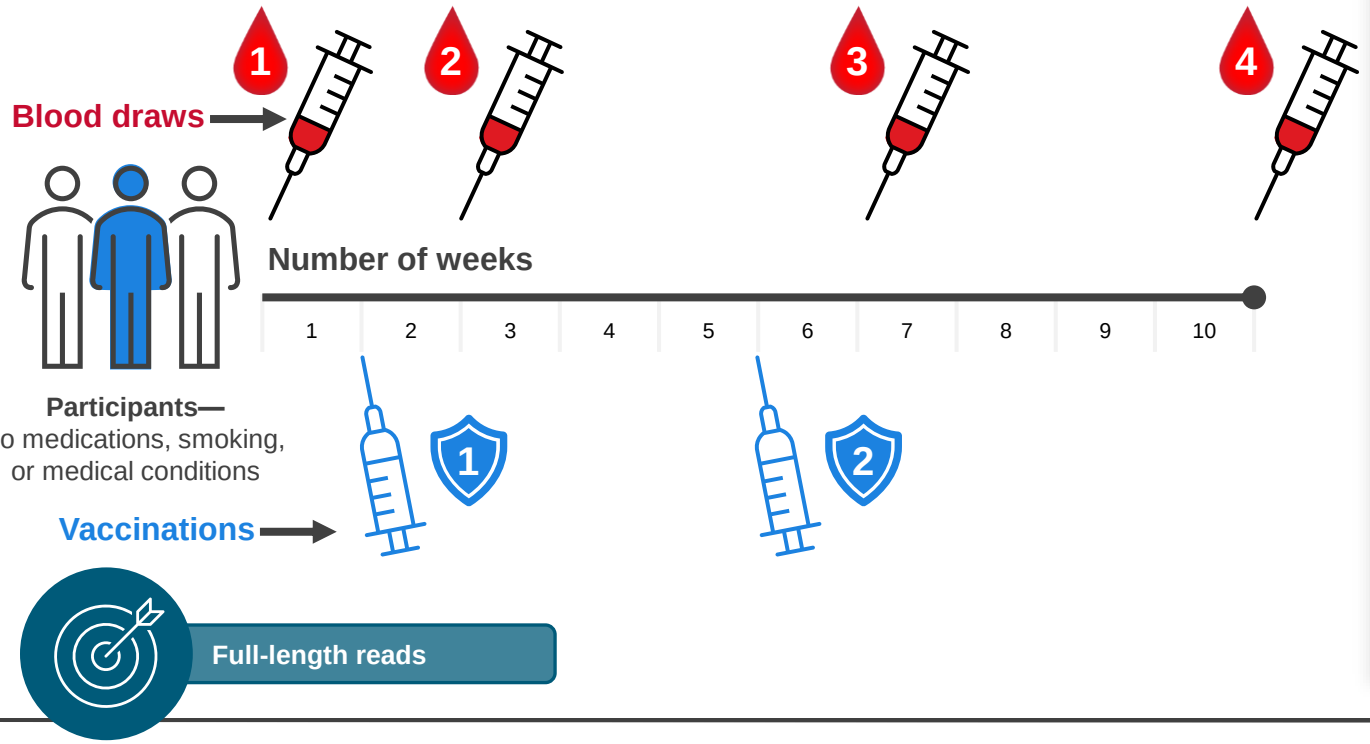


Tracking immune expansion after Covid-19 vaccination

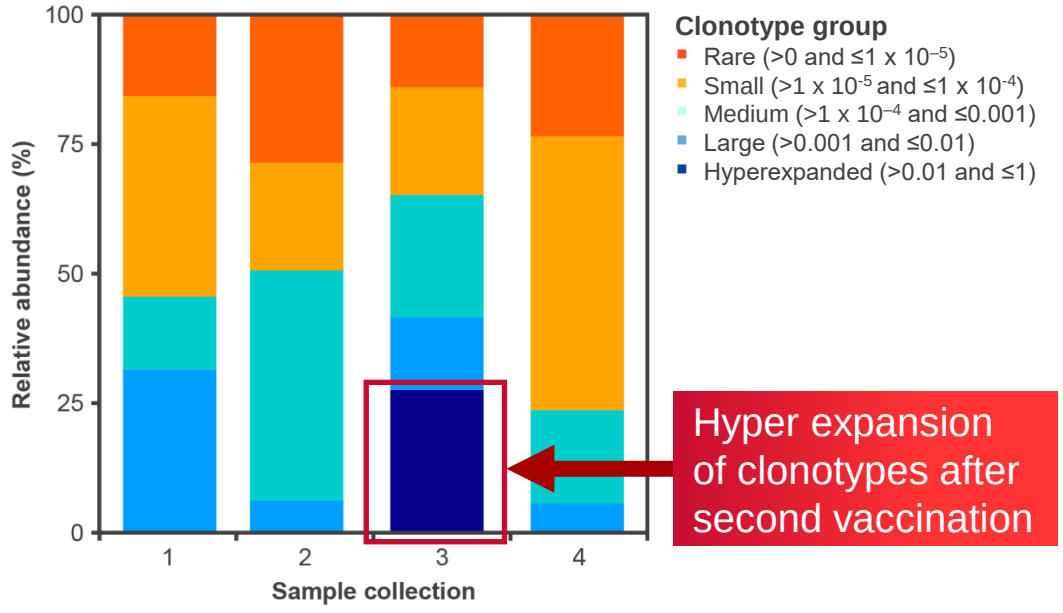
Project workflow

1. PBMC samples collected at four time points
2. RNA extraction with NucleoSpin RNA kit
3. Library preparation with SMART-Seq Human BCR (with UMIs) kit
4. Sequencing with Illumina® NextSeq® 2000
5. Data analysis with Cogent™ NGS Immune Profiler

Sample collection



A closer look at individual #1—clonotype abundance at four time points



Critical sequences are missed with CDR3 only libraries

A closer look at the hyper expansion of clonotypes in individual #1 after second vaccination

Species	Gene	Allele	AccNum	Domain label	Functionality	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)
Homsap	IGHV3-33	IGHV3-33*01	AB019439	VH	F	QVQLVESGG.GVVQP	GRSLRLSCAAS	GFTF....SSYG	MHWVRQAP	GKGLEWVAV	IWYD..GSNK YYADSVK.G RFTISRDNSK NTLYLQMNSLRA EDTAVYYC AR
Homsap	IGHV3-48	IGHV3-48*01	M99675	VH	F	EVQLVESGG.GLVQP	GGSLRLSCAAS	GFTF....SSYS	MNWVRQAP	GKGLEWVSY	ISSS..SSTI YYADSVK.G RFTISRDNAK NSLYLQMNSLRA EDTAVYYC AR

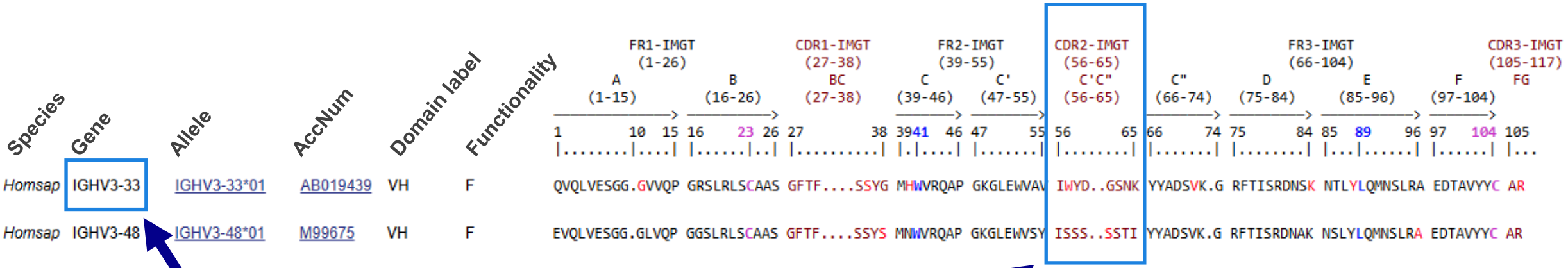
With a CDR3-only BCR library, **IGHV3-48** appeared to be most abundant

Most kits on the market only sequence the **CDR3 region**

Full-length reads

Full-length V(D)J libraries uncover missed clonotypes

A closer look at the hyper expansion of clonotypes in individual #1 after second vaccination



Species	Gene	Allele	AccNum	Domain label	Functionality	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)
Homsap	IGHV3-33	IGHV3-33*01	AB019439	VH	F	QVQLVESGG.GVVQP GRSLRLSCAAS	GFTF....SSYG	MHWVRQAP GKGLEWVAV	IWYD..GSNK	YYADSVK.G RFTISRDNSK NTLYLQMNSLRA EDTAVYYC AR
Homsap	IGHV3-48	IGHV3-48*01	M99675	VH	F	EVQLVESGG.GLVQP GGSLRLSCAAS	GFTF....SSYS	MNWVRQAP GKGLEWVSY	ISSS..SSTI	YYADSVK.G RFTISRDNAK NSLYLQMNSLRA EDTAVYYC AR

With a full-length BCR library, **IGHV3-33** was found to be most abundant

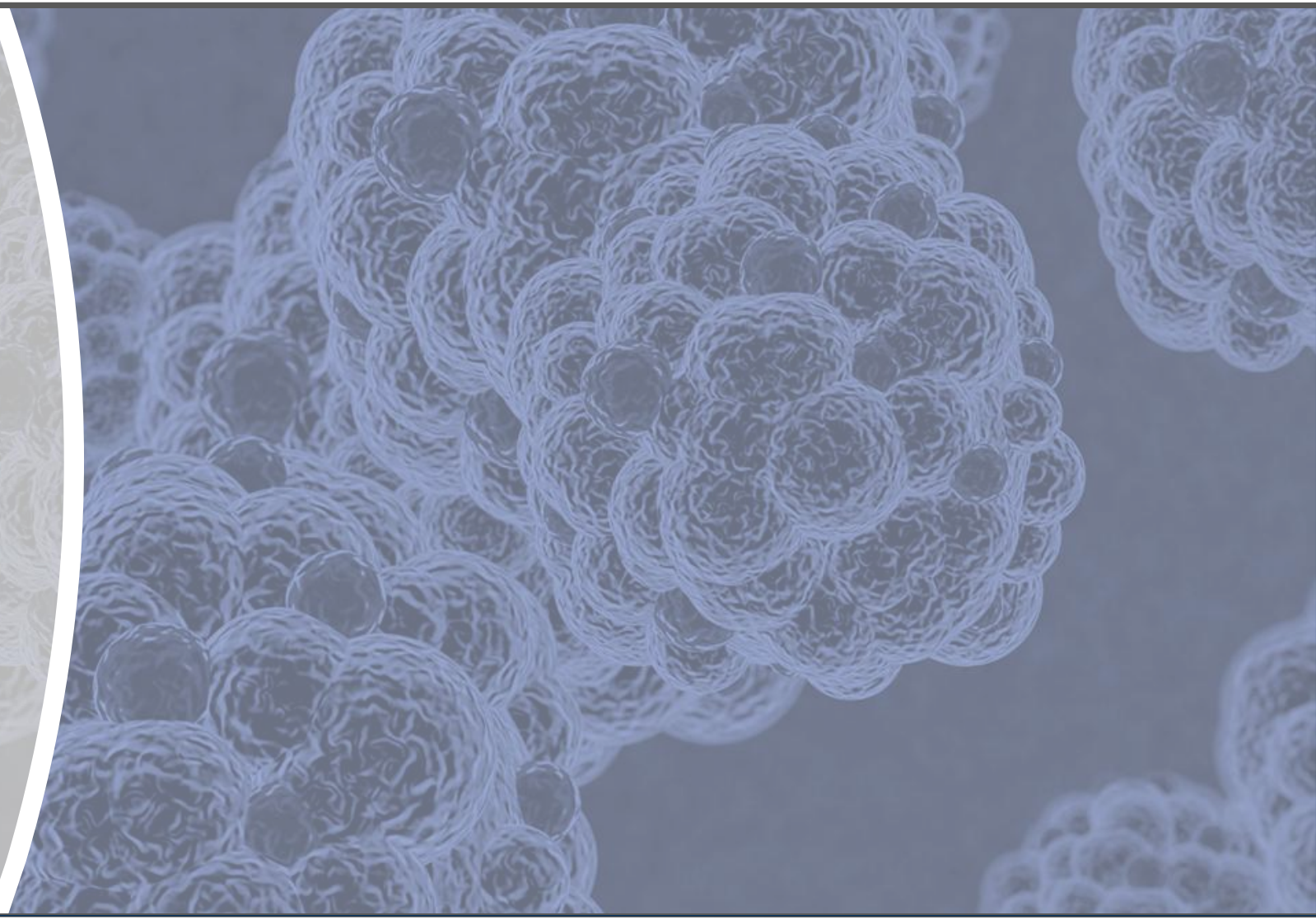
Takara Bio's full-length V(D)J sequencing captures the difference between the IGHV3-33 and IGHV3-48 genes in the CDR2 region

Full-length reads

Obtaining versatility across different sample types

Versatility

- Bulk or single-cell
- Fresh/frozen or FFPE
- Clinical research samples
- Sensitivity



Technology—Coming soon!

MaxiSeq™ Human FFPE TCR+BCR (with UMIs) kit

Versatility

- Bulk or single-cell
- **Fresh/frozen or FFPE**
- Clinical research samples
- Sensitivity

TaKaRa

Unlocking immune biomarkers from vital archived samples

FFPE samples are challenging

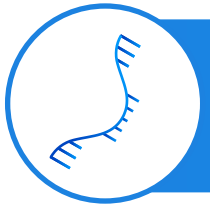
Starting with DNA

Advantages:

- Captures full repertoire—whether actively expressed or not
- More stable—archived FFPE samples may be used

Disadvantages:

- No isotype information
- Single copy per cell—high input required



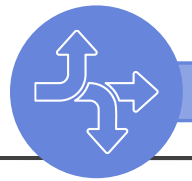
Starting with RNA

Advantages:

- Reflect active expression
- Identify different isoforms
- Multiple copies per cell—lower inputs can be used

Disadvantages:

- Less stable—degraded and fragmented RNA



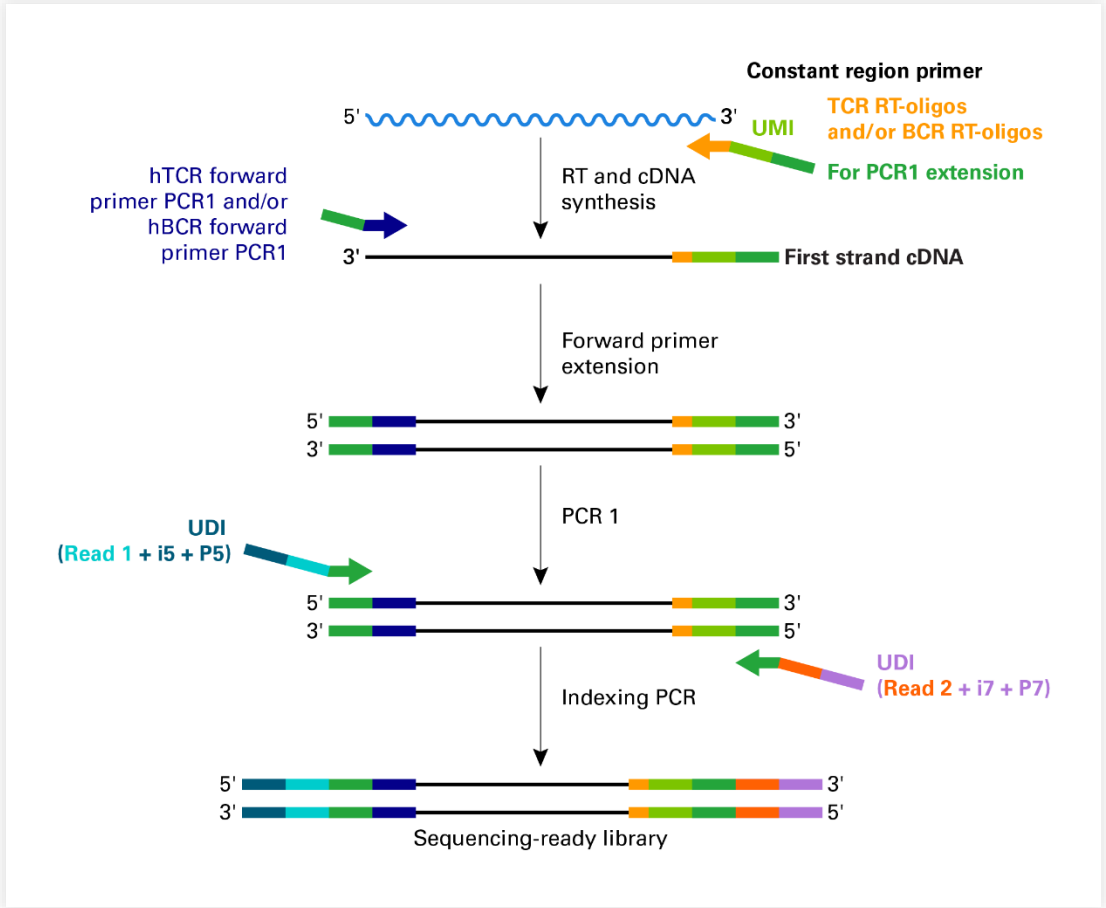
Fresh/frozen or FFPE

TaKaRa

Coming soon!
MaxiSeq Human FFPE TCR+BCR (with UMIs)

FFPE RNA immune profiling with UMI-based accuracy

- Supports input range of 200 ng–1 µg
- Works with low RIN and DV200 values
- Detects all human
  - TCR chains (TCRα, β, γ, δ)
  - BCR chains (IgA, D, E, G, M, K, L)
- Sequences CDR3 with UMI-powered accuracy
- Includes free, user-friendly analytical tools

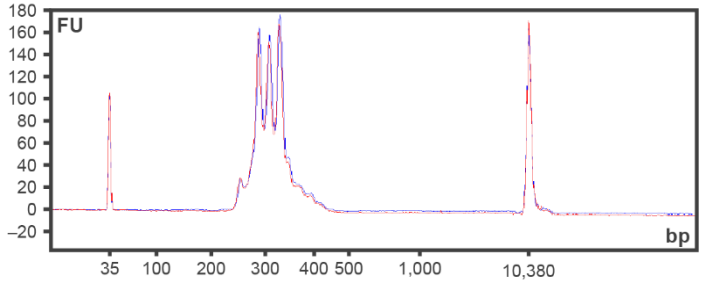


Fresh/frozen or FFPE

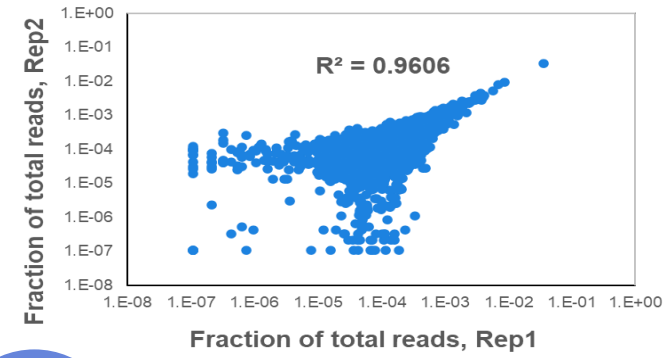
MaxiSeq—reliable solution for immune profiling from highly degraded FFPE RNA samples

Degraded RNA sample A
RIN: 2.8; DV200: 54%; TILs: medium

Sensitive detection

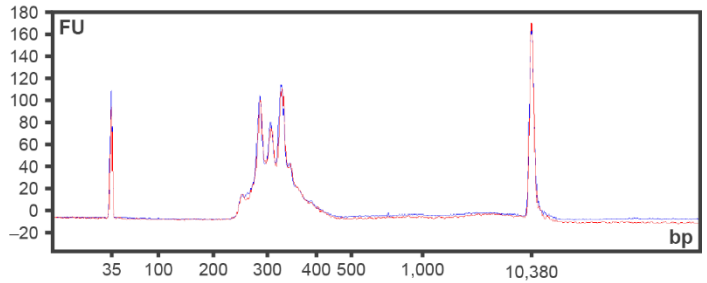


Reproducible detection

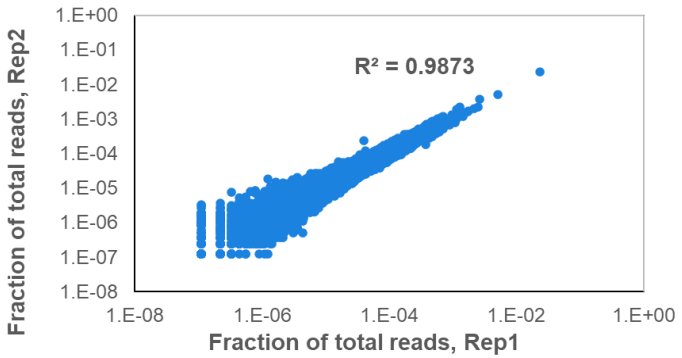


Degraded RNA sample B
RIN: 3.0; DV200: 50%; TILs: high

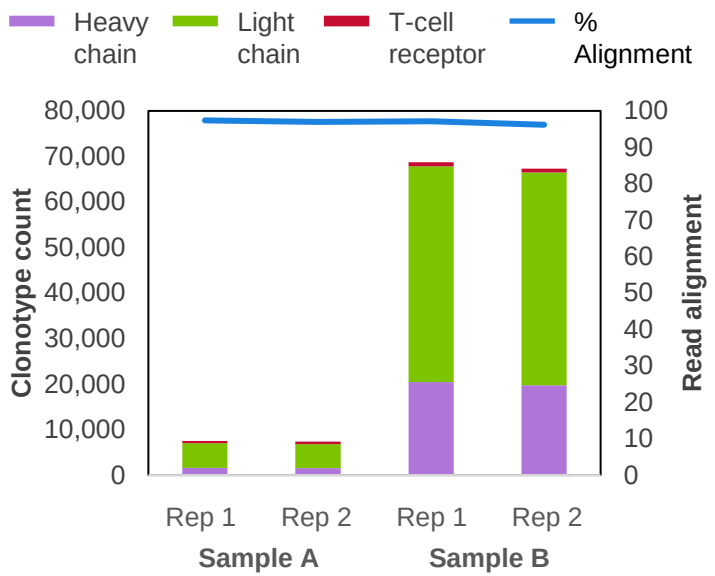
Sensitive detection



Reproducible detection



Degraded RNA samples with varying TIL content: successfully processed with high reproducibility using MaxiSeq chemistry

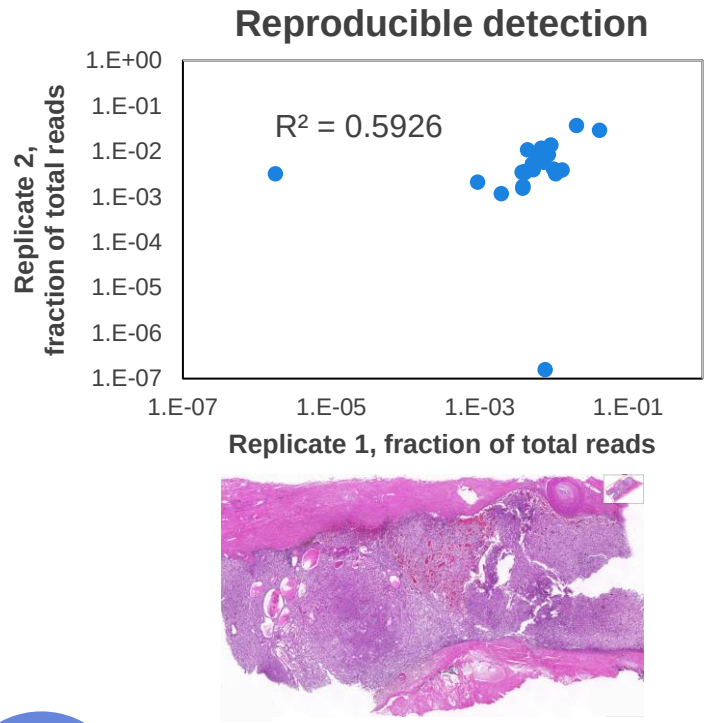


Fresh/frozen or FFPE

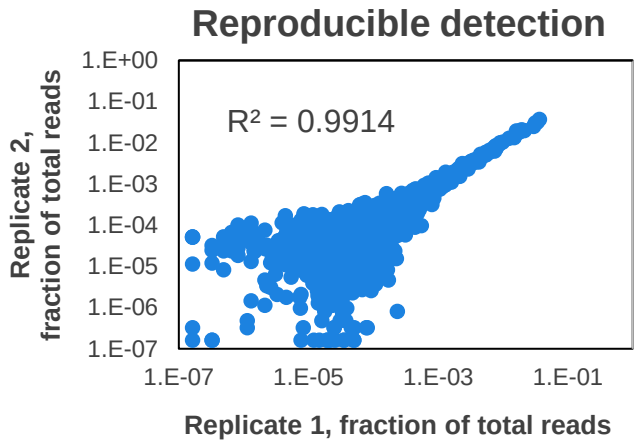
TIL = tumor infiltrating lymphocytes

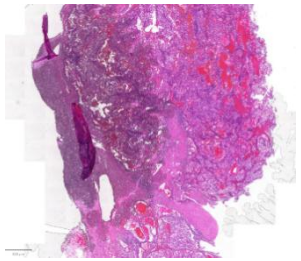
MaxiSeq—reliable detection of TCRs in highly degraded RNA isolated from renal cell carcinoma FFPE samples

Renal cell carcinoma sample 1
RIN: 1.9; DV200: 42%; TILs: low

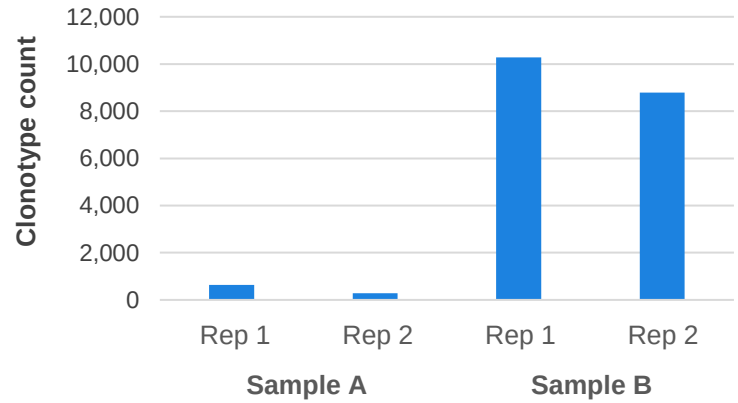


Renal cell carcinoma sample 2
RIN: 1.2; DV200: 34%; TILs: high





Libraries successfully prepared with FFPE samples with varying degrees of RNA degradation



The clonotypes identified depends on the tumor-infiltrating lymphocyte content in the sample

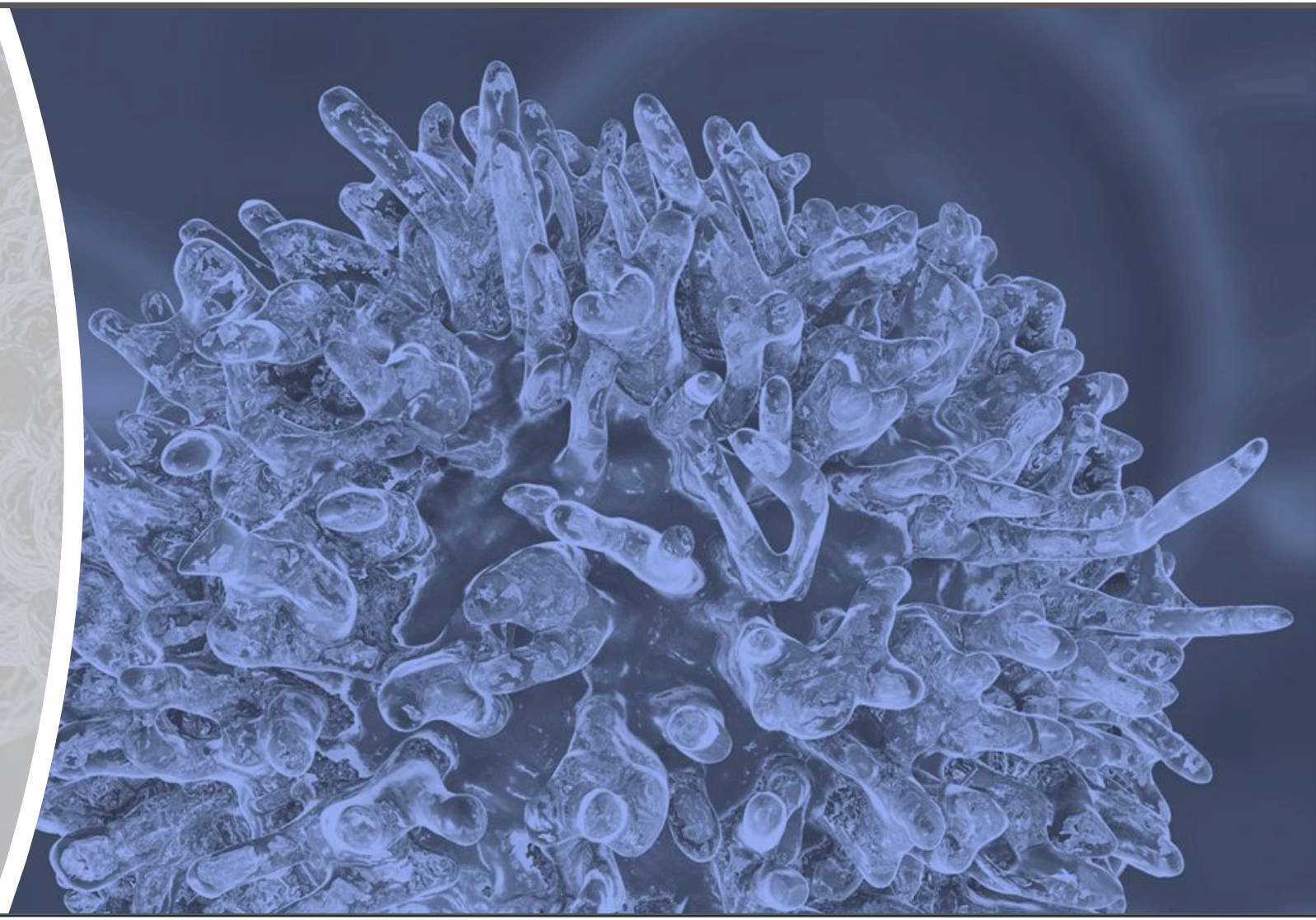
Fresh/frozen or FFPE

TIL = tumor infiltrating lymphocytes

Leukemia study *(in collaboration with FDA, NIH, and Illumina)*

Versatility

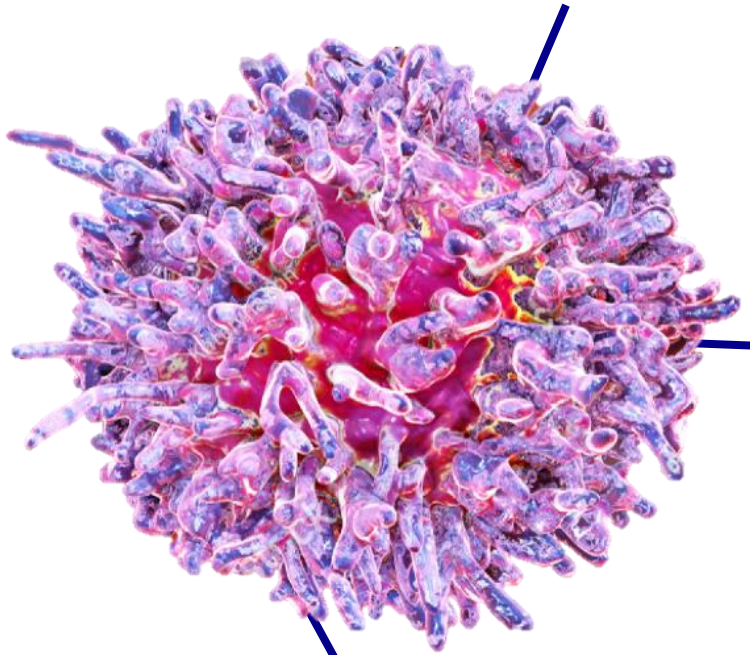
- Bulk or single-cell
- Fresh/frozen or FFPE
- **Clinical research samples**
- Sensitivity



TaKaRa

Hairy cell leukemia (HCL) arises from B cells

HCL is a rare B-cell malignancy found in 2% of leukemia cases



HCL arises from mature B cells with a restricted BCR repertoire

Hairy cells have hairy cytoplasmic projections

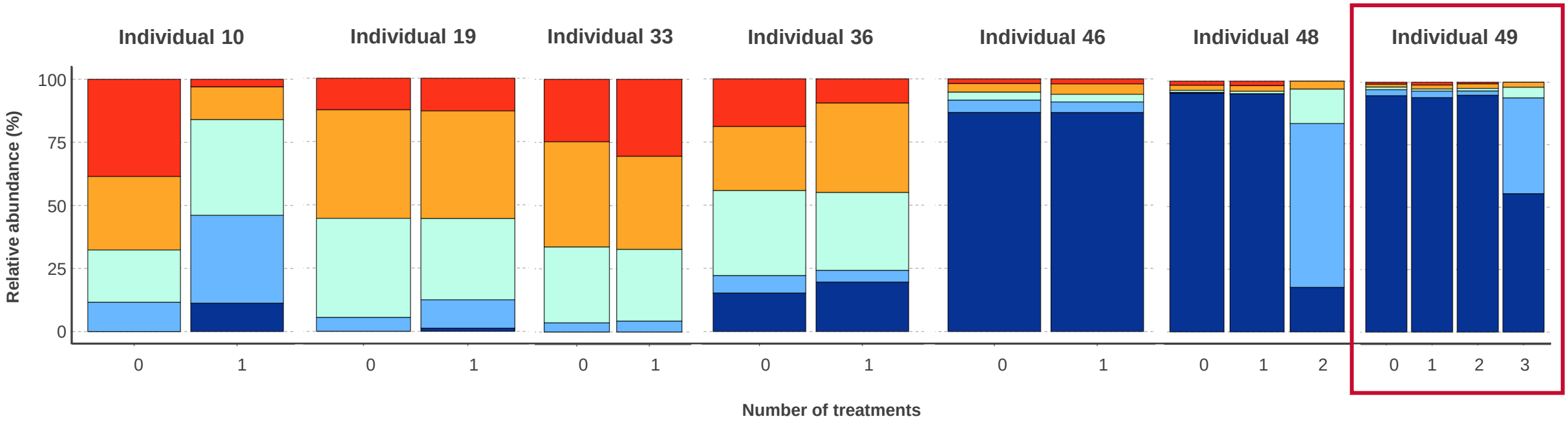
Goal: Identify biomarkers in a cohort of HCL samples to understand how the BCR repertoire changes over the course of treatment

Immunoglobulin heavy chain (V) gene sequences identify and track residual leukemic cells

This study is ongoing, so we will focus on one individual here

Clinical research samples

Tracking clonal proportions before and after treatment



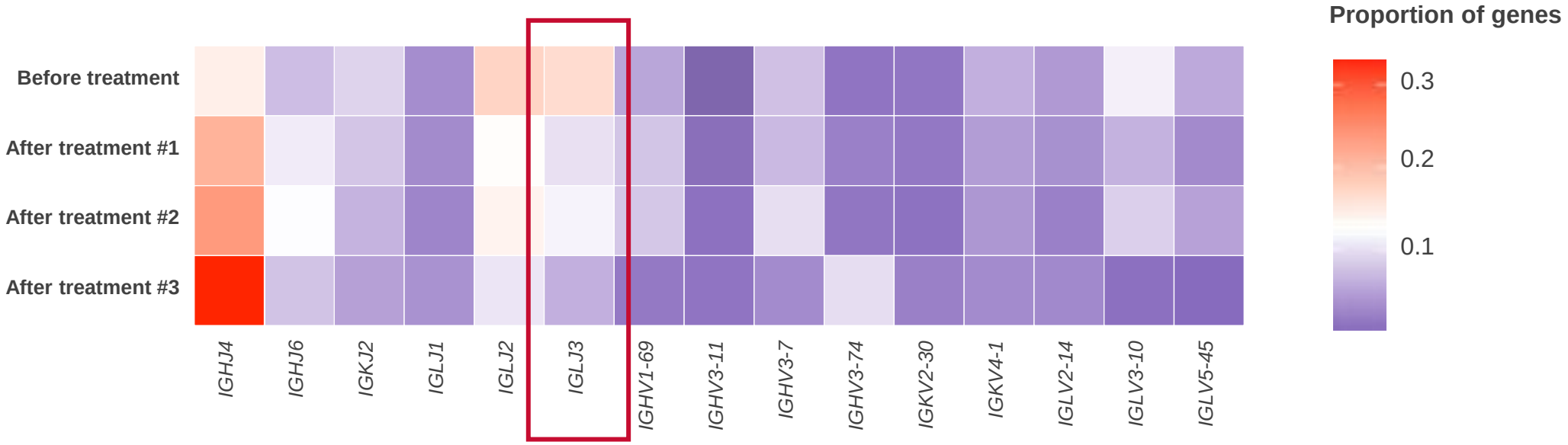
Treatment alters the distribution of clonotype proportions in a subset of samples, highlighting **dynamic changes in the BCR immune repertoire**

Clonotype group
- Rare (>0 and ≤1 x 10^{-5})
- Small (>1 x 10^{-5} and ≤1 x 10^{-4})
- Medium (>1 x 10^{-4} and ≤0.001)
- Large (>0.001 and ≤0.01)
- Hyperexpanded (>0.01 and ≤1)

Clinical research samples

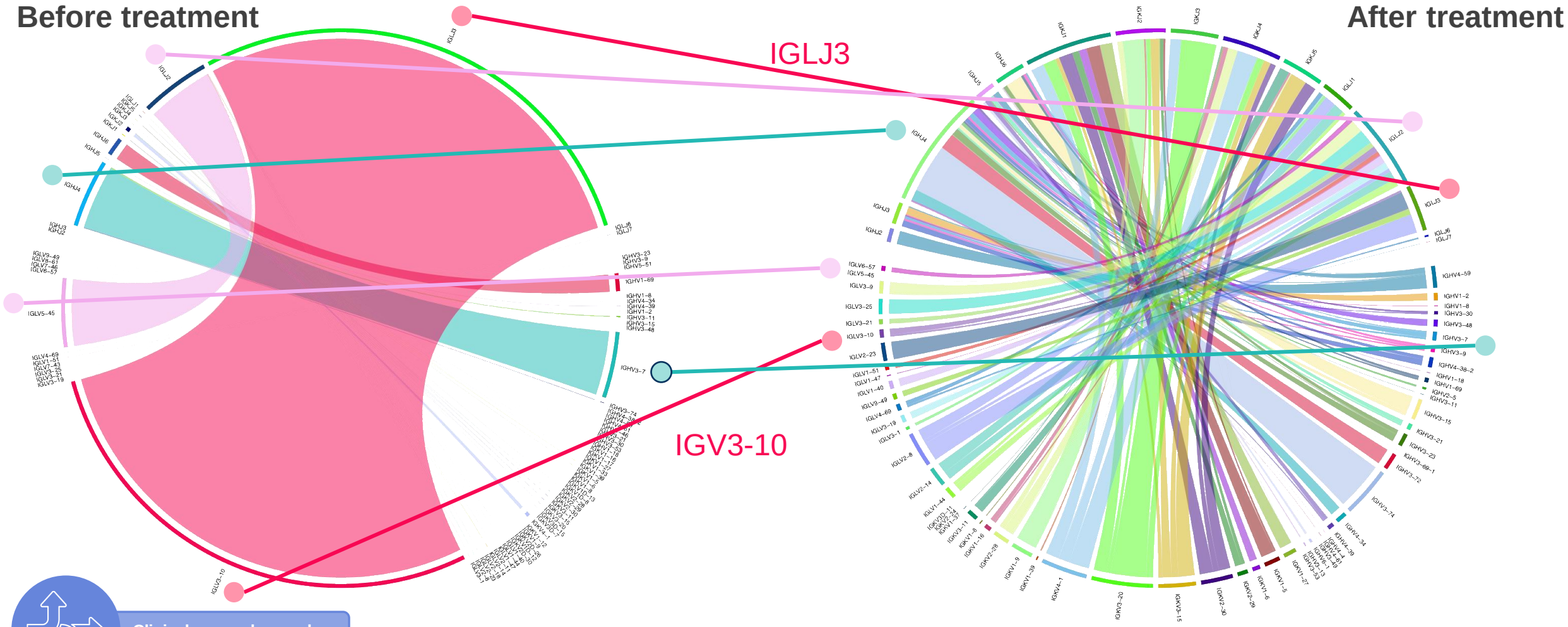
Dynamic shift in V and J gene usage over the course of treatment in individual 49

Selected genes



Clinical research samples

BCR diversity increased after treatment in individual 49

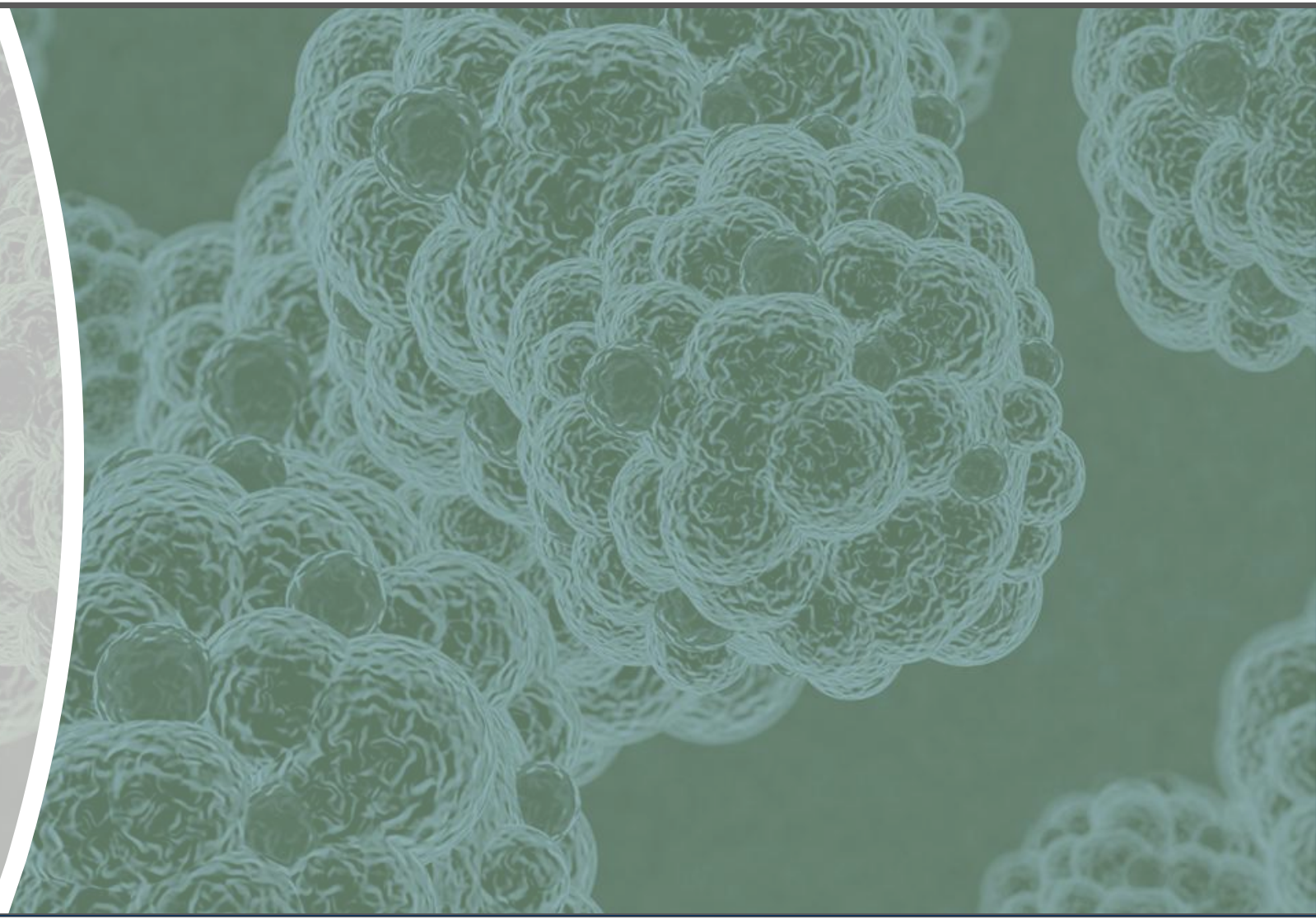


Clinical research samples

Single-cell workflows can be scaled up to 100,000 cells per run

Scalability

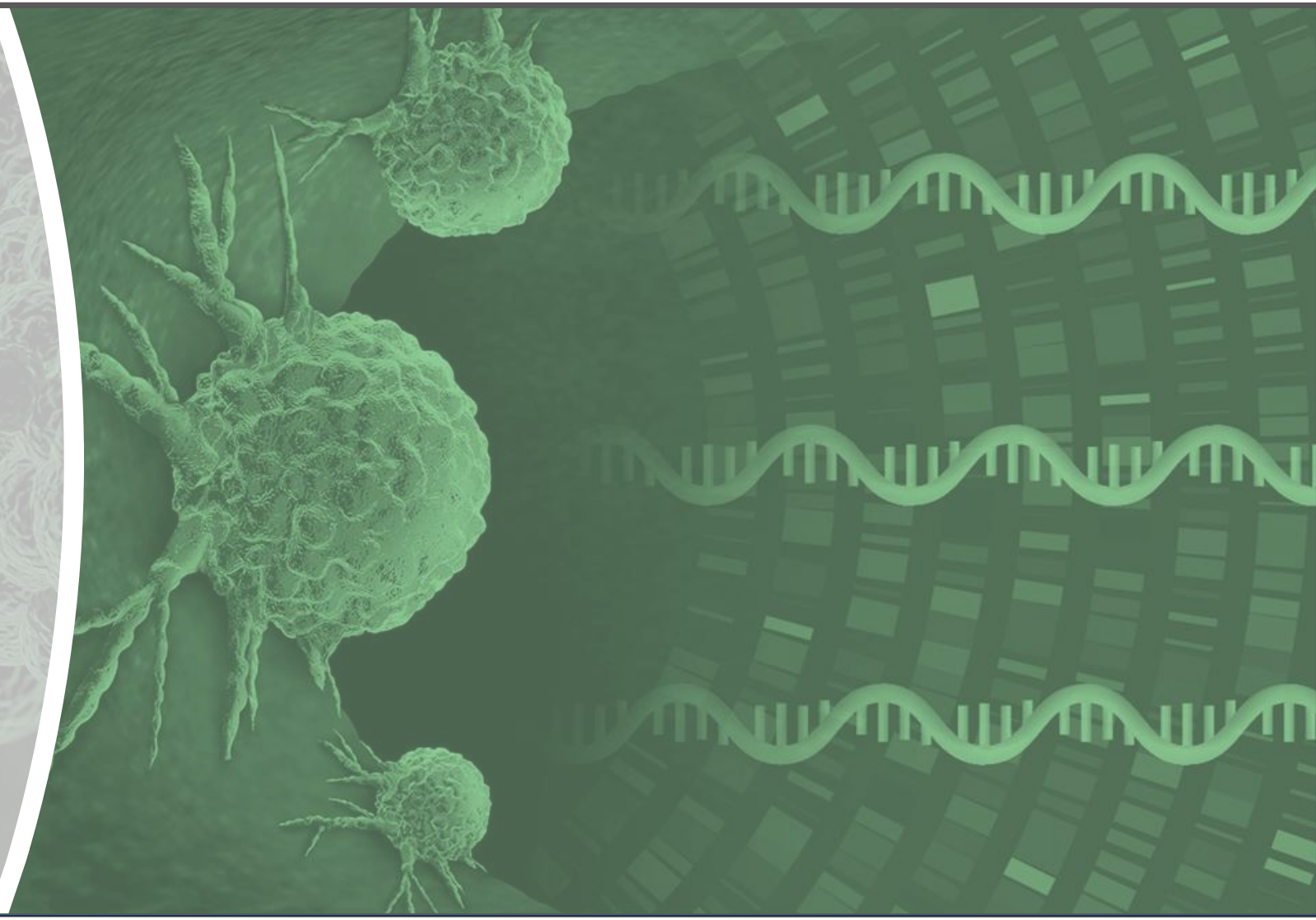
- 100,000 cells per run
- 384 unique dual indexes
- Sensitivity



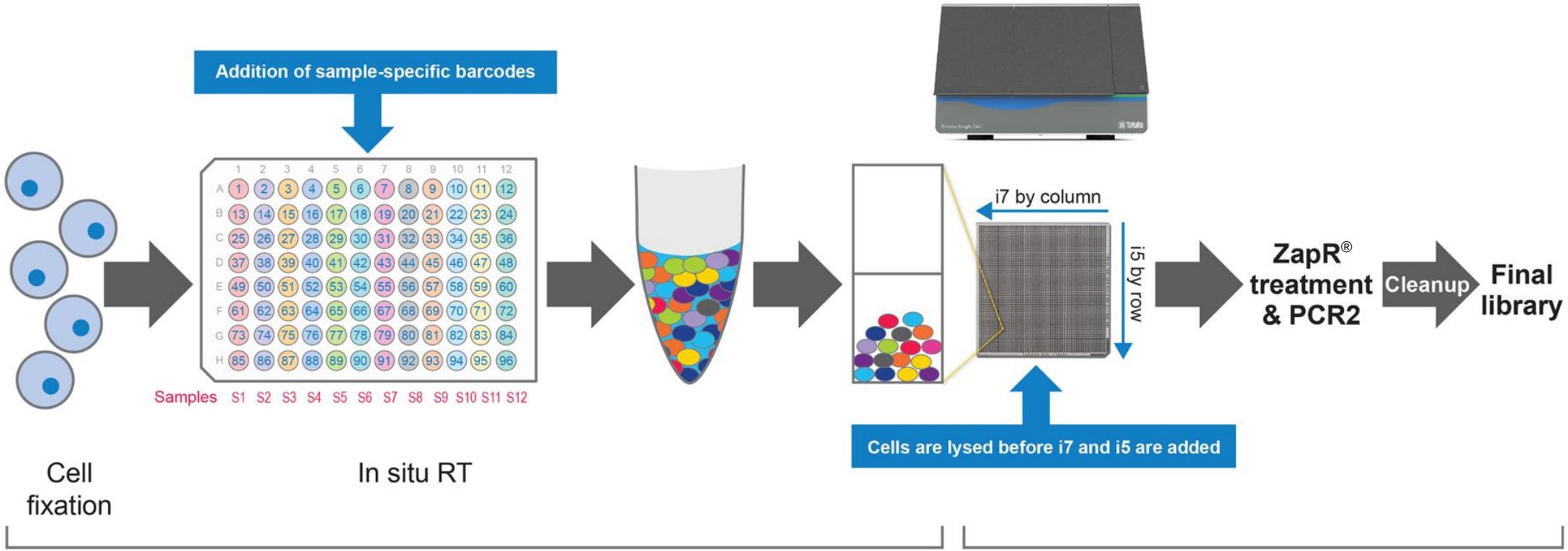
Technology
Shasta™ Total RNA-Seq Kit

Scalability

- **100,000 cells per run**
- 384 unique dual indexes
- Sensitivity



Shasta Total RNA-Seq Kit: a high-throughput, full gene-body coverage workflow



100,000 cells per run

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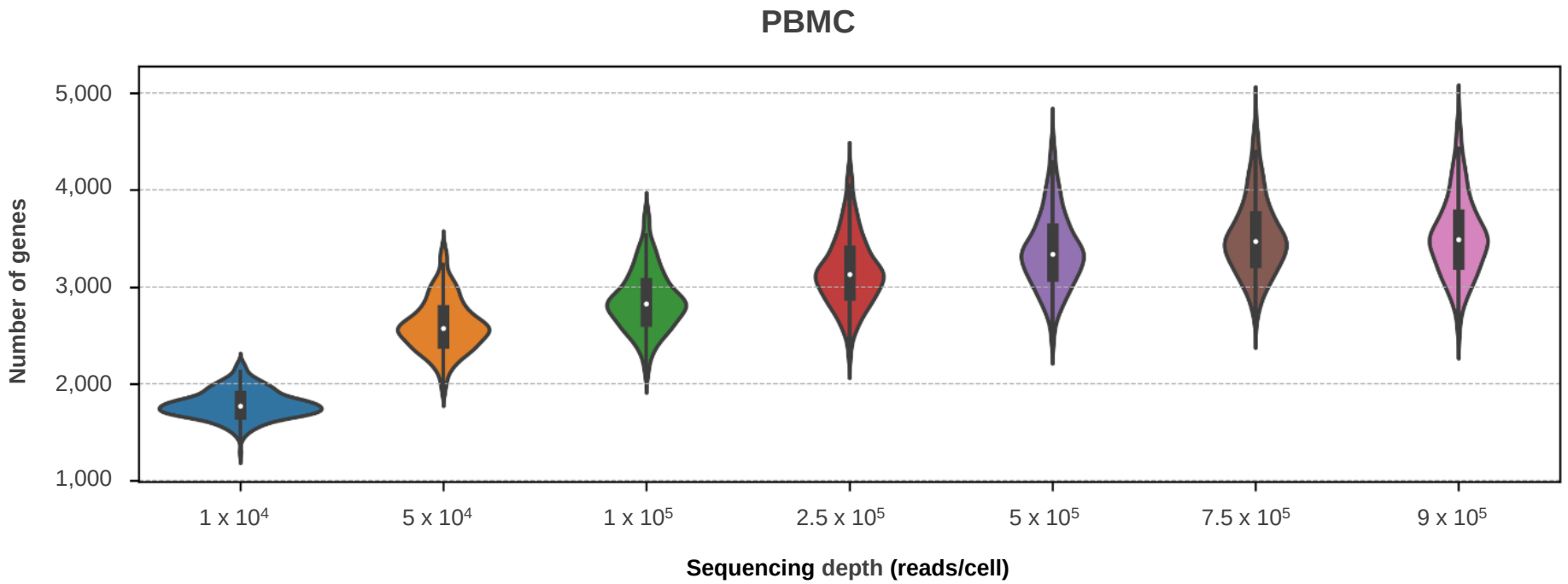
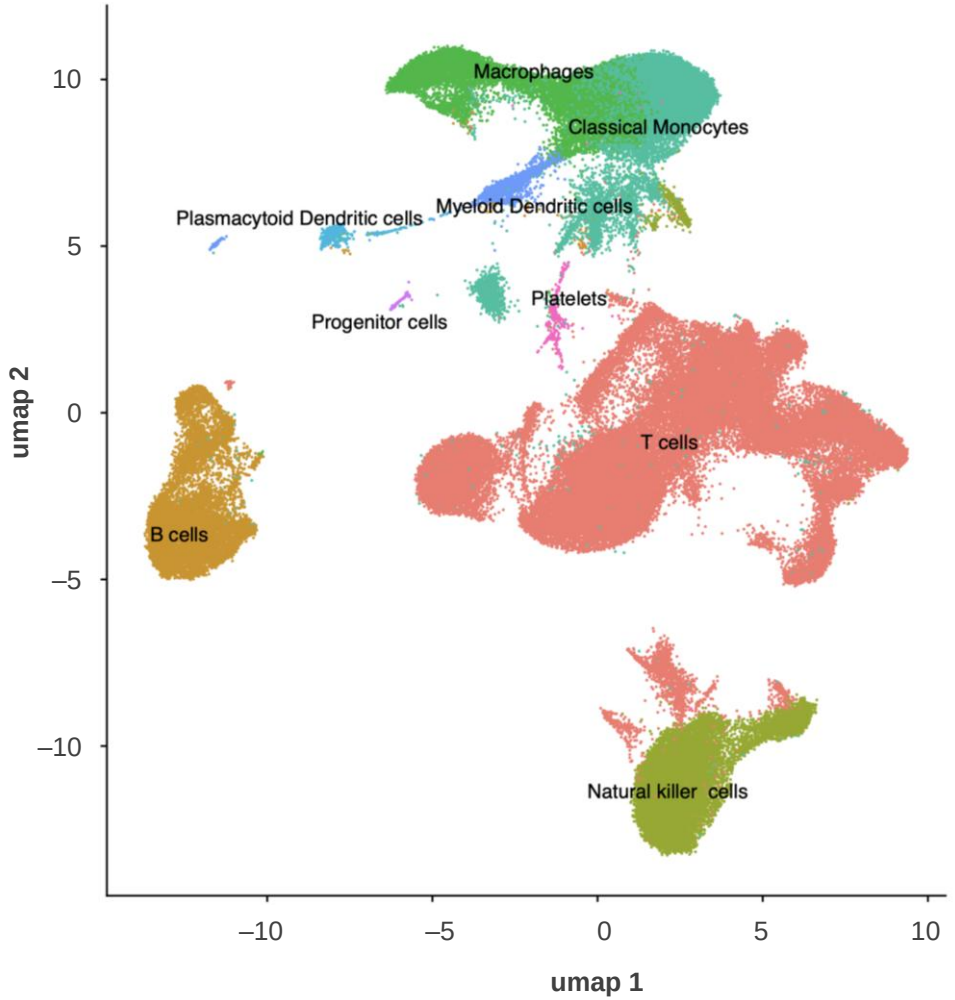
Detect more genes by increasing sequencing depth using the Shasta Total RNA-Seq Kit



100,000 cells per run

Sequencing saturation not observed until read depth exceeds 5 x 10^5 reads per cell

UMAP analysis allows detection of cell types in the Shasta Total RNA-Seq library

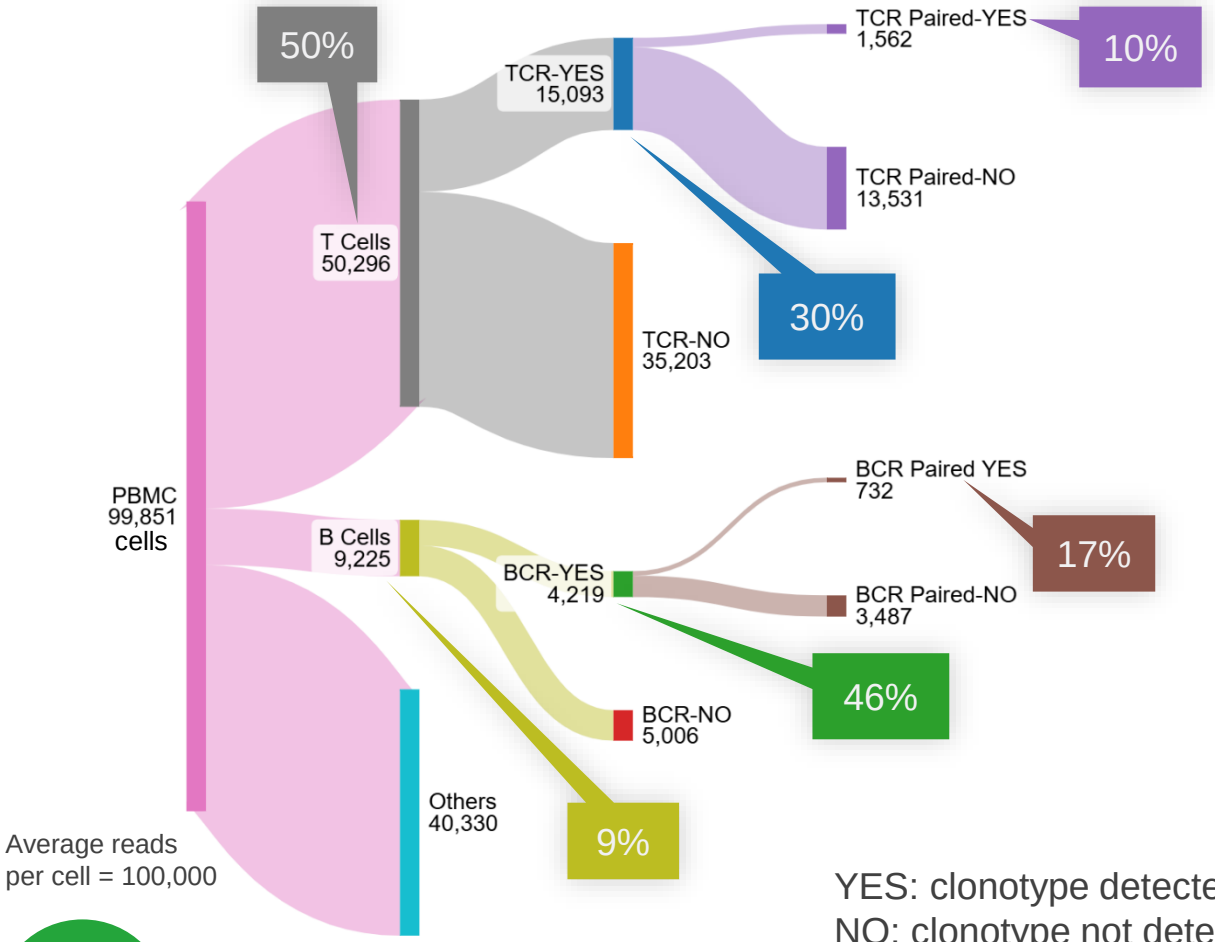


Total cells in this run: 99,851

- T cells
- B cells
- Natural killer cells
- Macrophages
- Classical monocytes
- Plasmacytoid dendritic cells
- Myeloid dendritic cells
- Progenitor cells
- Platelets

100,000 cells per run

TCR and BCR clonotypes detected in the Shasta Total RNA-Seq library



100,000 cells per run

YES: clonotype detected
NO: clonotype not detected

Made at SankeyMATIC.com

Shasta Total RNA-Seq Kit provides a wealth of data:

- High-quality scRNA-seq library
- Full gene-body coverage
- Detailed UMAP to identify cell types
- Valuable TCR and BCR clonotype detection

Takara Bio's high-quality portfolio provides accurate, versatile, and scalable solutions for immune profiling

Accuracy

- Unbiased amplification
- Reproducibility
- Full-length reads
- Sensitivity

Versatility

- Bulk or single-cell
- Fresh/frozen or FFPE
- Clinical research samples
- Sensitivity

Scalability

- 100,000 cells per run
- 384 unique dual indexes
- Sensitivity

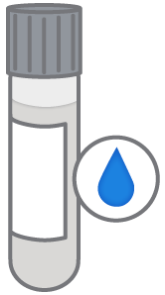
Takara Bio's immune profiling NGS product portfolio—an end-to-end solution

Sample

Direct cell input

RNA from tissues, cells, or FFPE

Single cells

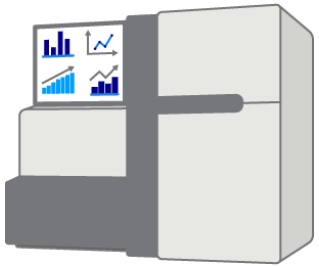


Cell lysis
Nucleic acid extraction
Quantification
RNA quality

Library prep

Bulk TCR (human & mouse)
Bulk BCR (human & mouse)
FFPE TCR+BCR (human)*
Single-cell TCR (plate-based)
Shasta Single Cell System*

Sequencing



Illumina
Element
BGI

Data analysis

Free bioinformatics tools
Cogent NGS Immune Profiler
Cogent NGS Immune Viewer

**New offerings*

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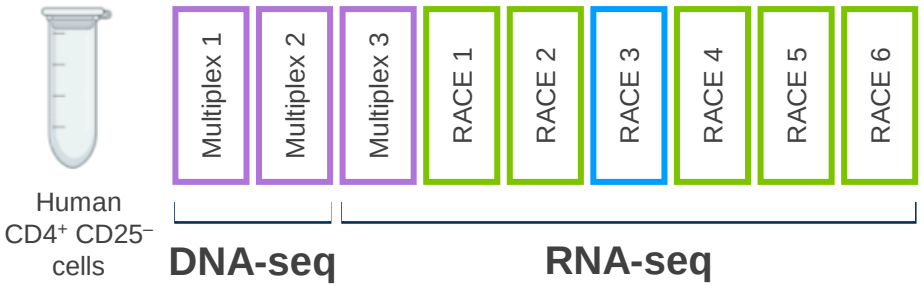
SMART® technology outperforms in immune profiling

A study systematically compared the results of nine commercial and academic TCR-seq methods, including:

- Takara Bio's **S**witching **M**echanism **a**t 5' end of **R**NA **T**emplate (SMART) technology
- Rapid amplification of cDNA ends (RACE)

Takara Bio's SMART TCR technology outperformed in both **sensitivity and reliability**

Rank values are between 2 (best) and 7 (worst)



None of the multiplex PCR methods can detect TCR α chains

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TR chain	Method	Replicability	Reliability	Sensitivity	Cost per sample ($)	Controls and standards	Format type	FASTQ data availability
TRA	RACE-1	7	4	4	~230	—	Lab protocol	Yes
	RACE-1_U	4	5	4	~230	UMI	Lab protocol	Yes
	RACE-2	5	4	5	230–280	—	Service or kit	Yes
	RACE-2_U	4	5	5	230–280	UMI	Service or kit	Yes
	RACE-3	3	2	3	~150	—	Kit	Yes
	RACE-4	5	6	4	~150	—	Lab protocol	Yes
	RACE-5	2	3	3	~300	—	Lab protocol	Yes
TRB	mPCR-1	3	3	3	~300–550	Synthetic TCRs	Service or kit	No
	mPCR-2	6	7	7	~25	—	Lab protocol	Yes
	mPCR-3	5	5	3	~300–550	—	Service or kit	Yes
	RACE-1	6	5	4	~230	—	Lab protocol	Yes
	RACE-1_U	4	6	5	~230	UMI	Lab protocol	Yes
	RACE-2	6	6	6	230–280	—	Service or kit	Yes
	RACE-2_U	6	6	7	230–280	UMI	Service or kit	Yes
	RACE-3	2	2	3	~150	—	Kit	Yes
	RACE-4	3	5	4	~150	—	Lab protocol	Yes

Takara Bio SMART TCR

Multiplex PCR with gDNA

Takara Bio SMART TCR

Barennes, P. et al. Benchmarking of T cell receptor repertoire profiling methods reveals large systematic biases. *Nature Biotechnology* 39(2), (2021)

Over 1,000 publications in high-impact journals

Sample publications

- Barennes, P. et al. Benchmarking of T cell receptor repertoire profiling methods reveals large systematic biases. *Nat. Biotechnol.* **39,** 236–245 (2020).
- Caduff, N. et al. KSHV infection of B cells primes protective T cell responses in humanized mice. *Nat. Commun.* **15,** 1–13 (2024) under a CC BY 4.0 license.
- Chen, R. et al. NUDCD3 deficiency disrupts V(D)J recombination to cause SCID and Omenn syndrome. *Sci. Immunol.* **9,** (2024).
- Lopez, J. et al. Autogene cevumeran with or without atezolizumab in advanced solid tumors: a phase 1 trial. *Nat. Med.* **31,** 152–164 (2025) under a CC BY 4.0 license.
- Ogega, C. O. et al. Convergent evolution and targeting of diverse E2 epitopes by human broadly neutralizing antibodies are associated with HCV clearance. *Immunity* **57,** 890-903.e6 (2024).

Generating more meaningful immunological insights

Takara Bio immune profiling solutions

- **Highest sensitivity for low-input samples**
- **Versatility across sample types**
- **Accuracy powered by UMIs with easy and free analytical tools**
- **Discovery of rare TCR/BCR biomarkers**

Acknowledgements

We would like to thank the following collaborators for their vital contributions

- Wenming Xiao at the Food & Drug Administration (FDA) for leading the spike-in control study that brought together assay developers, sequencing platform providers, and bioinformatics solution providers
- Wenming Xiao at the FDA, Evgeny Arons at the National Institutes of Health, and Robin Bombardi at Illumina for helping with the leukemia study

TaKaRa

that's
GOOD
science!®