Accelerating antibody discovery and vaccine development

Chryssa Zografou

Technical Journal Club

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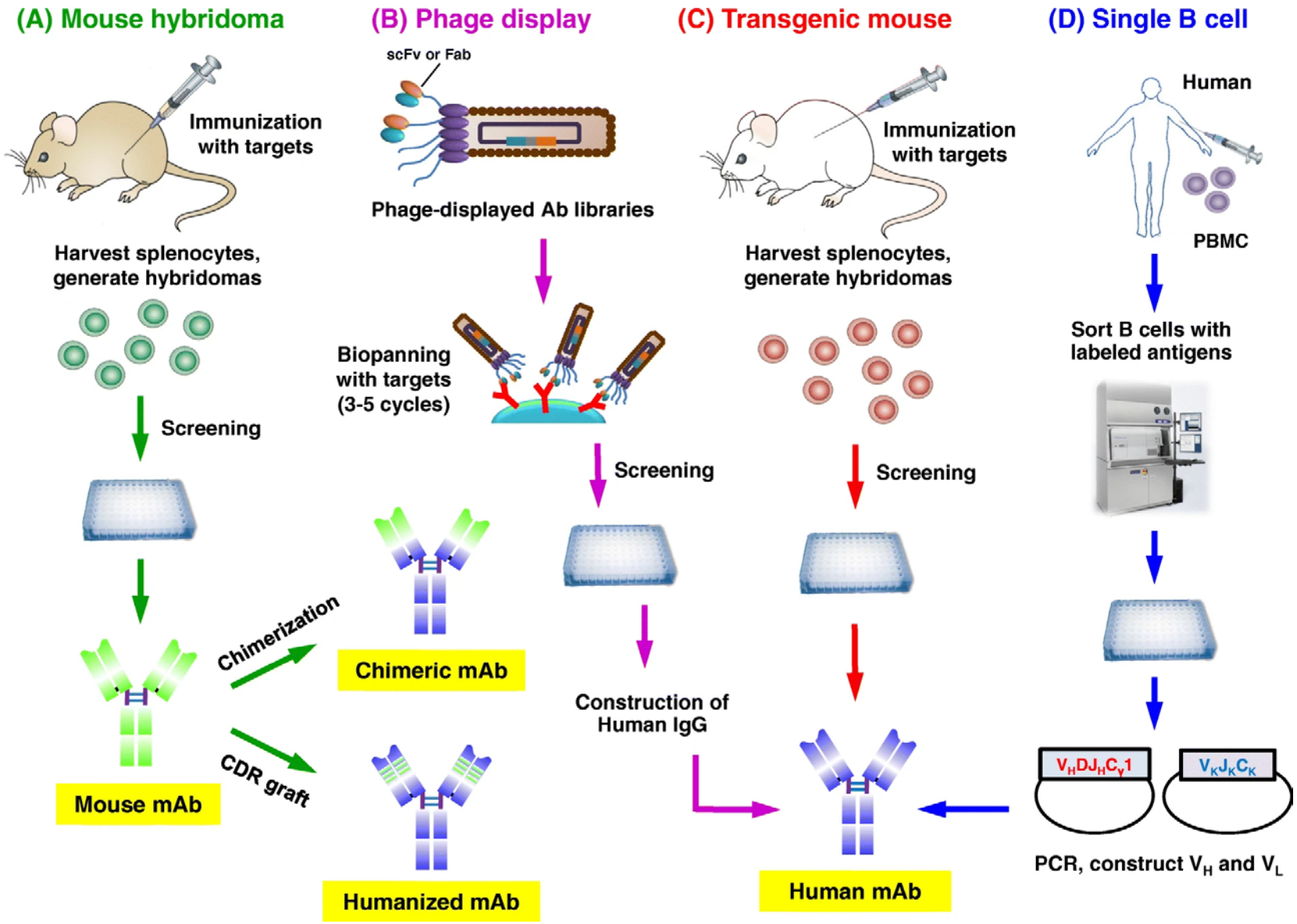
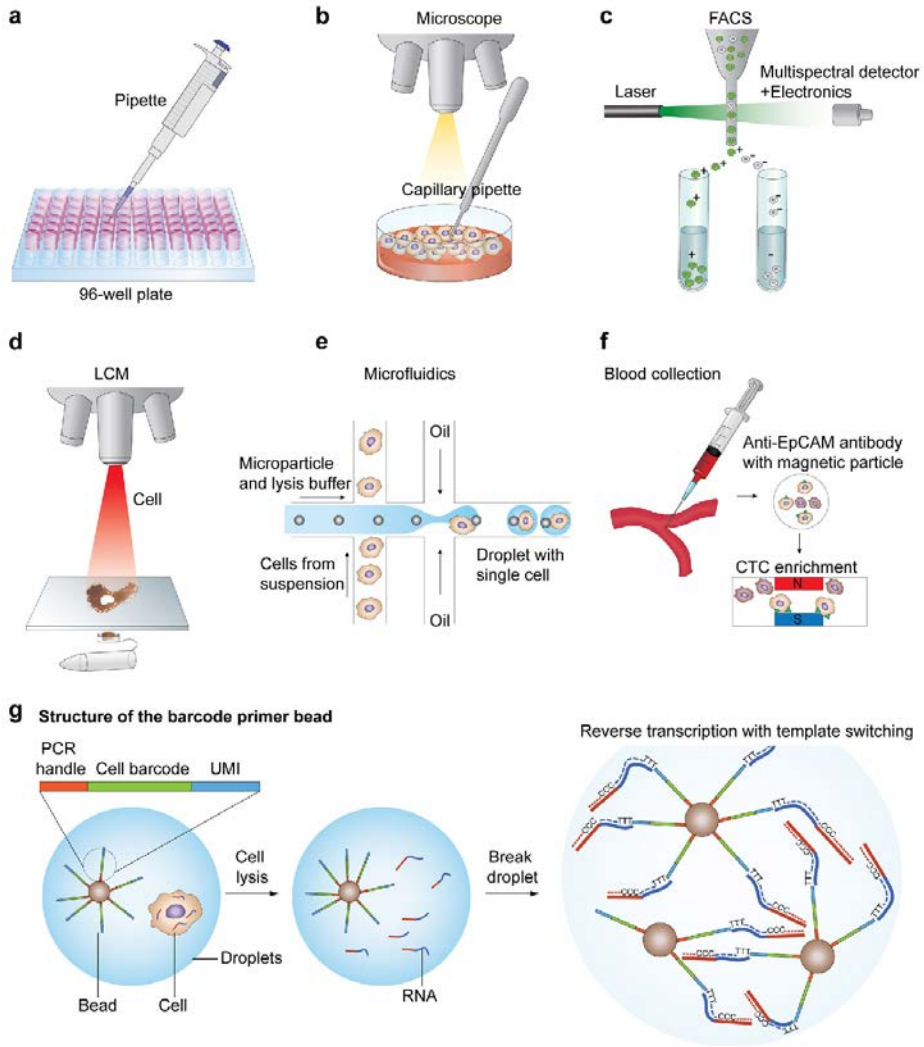
Traditional approaches to characterize antigen-specific B cells



Lu et al., J Biomed Sci. 2020 Jan 2;27(1):1

Advances in single-cell RNA sequencing have allowed for comprehensive analysis of the immune system



a The limiting dilution method.

b Micromanipulation involves collecting single cells using microscope-guided capillary pipettes.

c FACS isolates highly purified single cells by tagging cells with fluorescent marker proteins.

d Laser capture microdissection (LCM) utilizes a laser system aided by a computer system to isolate cells from solid samples.

e Microfluidic technology for single-cell isolation requires nanoliter-sized volumes.

f The CellSearch system enumerates CTCs from patient blood samples by using a magnet conjugated with CTC binding antibodies.

g A schematic example of droplet-based library generation.

Hwang et al., Exp Mol Med. 2018 Aug 7;50(8):96.

Resource

Cell

High-Throughput Mapping of B Cell Receptor Sequences to Antigen Specificity

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https://doi.org/10.1016/j.cell.2019.11.003

Introduction

The problem…

For antibody development and vaccine production we want to identify:

1. B cells with antigen specificity
2. Information on the B cell receptor

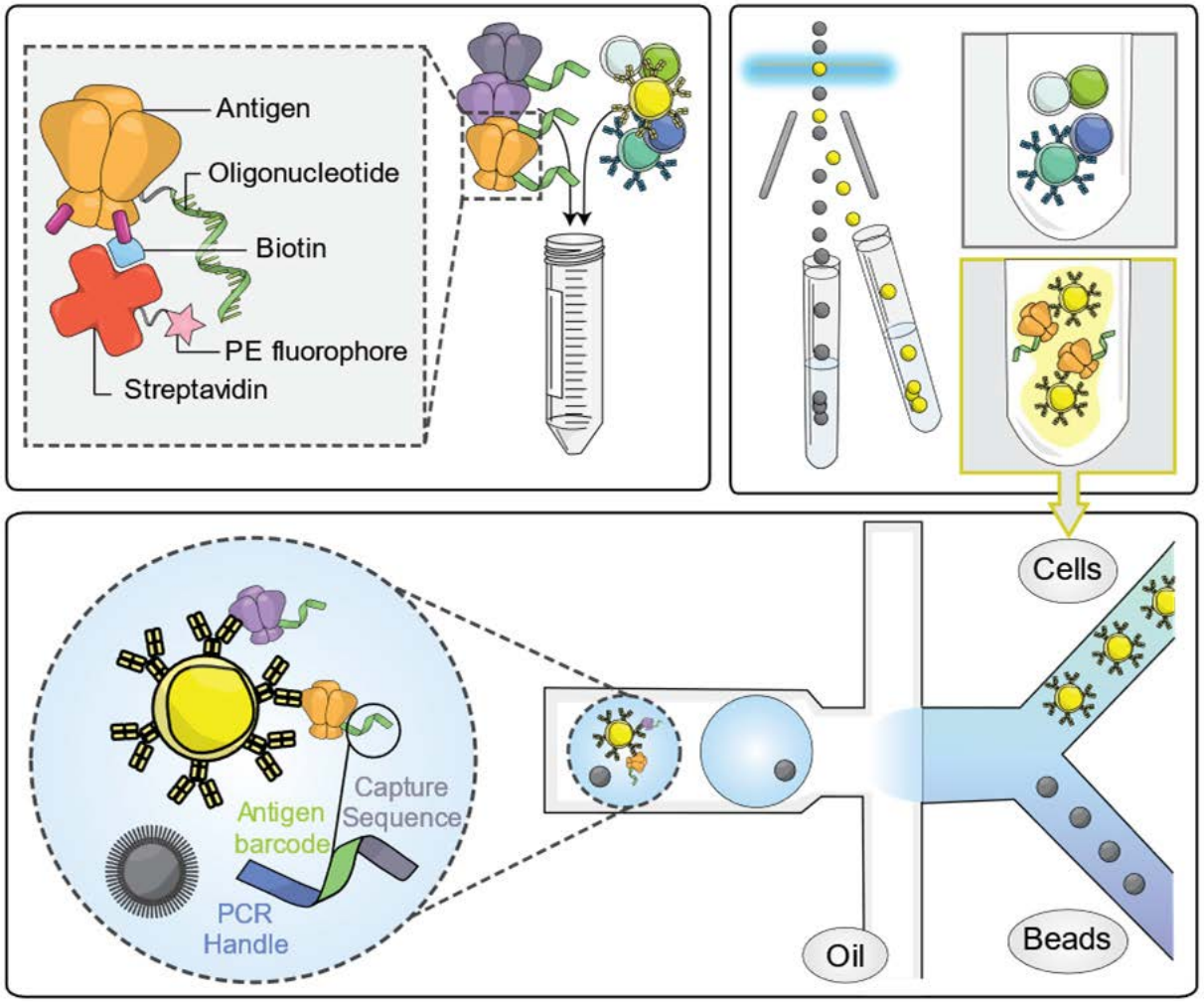
Current techniques on antigen-specific B cells/BCR sequencing have some limitations:

1. number of B cells
2. pairing of heavy and light
3. number of antigens that can be screened against
4. antigen specificity maps

The solution to the problem…

Antigen-specific BCR sequencing can be combined with LIBRA-Seq
((Linking B cell receptor to antigen specificity)

Linking BCR sequences to antigen specificity via next-generation sequencing



- LIBRA-seq transforms antibody-antigen interactions into sequencing-detectable events by conjugating barcoded DNA oligos to each antigen in a screening library.
- All antigens are labeled with the same fluorophore, which enables sorting of antigen-positive B cells by fluorescence activated cell sorting (FACS) before encapsulation of single B cells via droplet microfluidics.
- Antigen barcodes and BCR transcripts are tagged with a common cell barcode from bead-delivered oligos, enabling direct mapping of BCR sequence to antigen specificity.

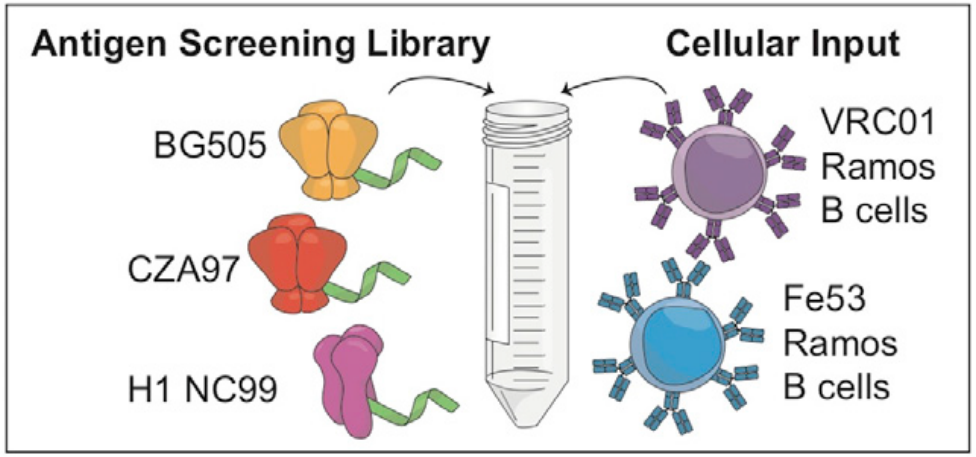
Assay design

Ramos B cell line – express IgM and as B cell mutate they knock out IgM
2 well characterized BCRs:

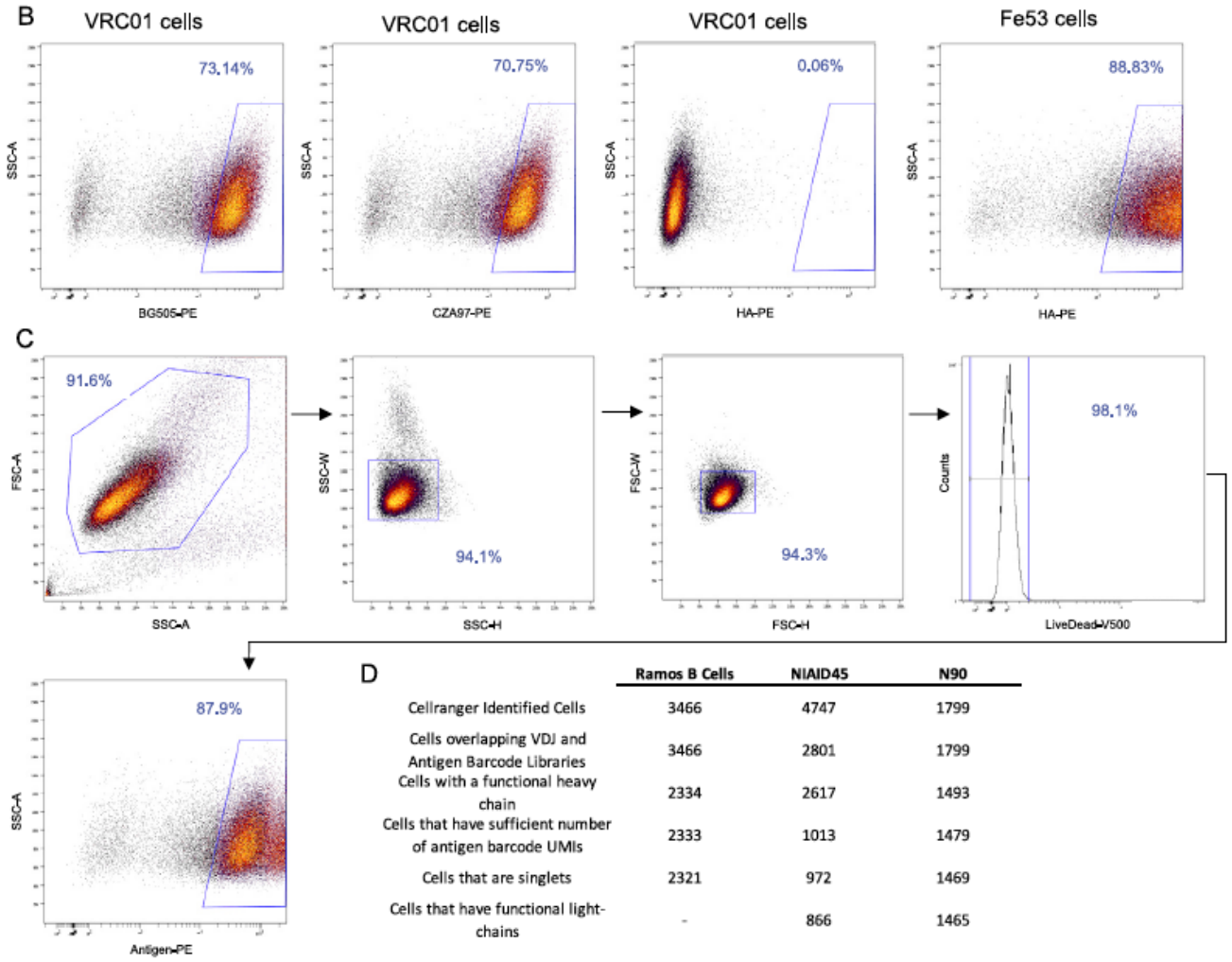
- VRC01, a CD4-binding-site-directed HIV-1 bNAb
- Fe53, a bNAb recognizing the stem of group1 influenza hemagglutinins (HAs)

B cell lines are mixed at a 1:1 ratio and incubated with three unique DNA-barcoded antigens:

1. two stabilized trimeric HIV-1 Env proteins (SOSIP) from strains **BG505** and **CZA97**
2. trimeric HA from strain H1 A/New Caledonia/20/1999, **H1 NC99**



Gating scheme for FACS sorting of Ramos B cell lines



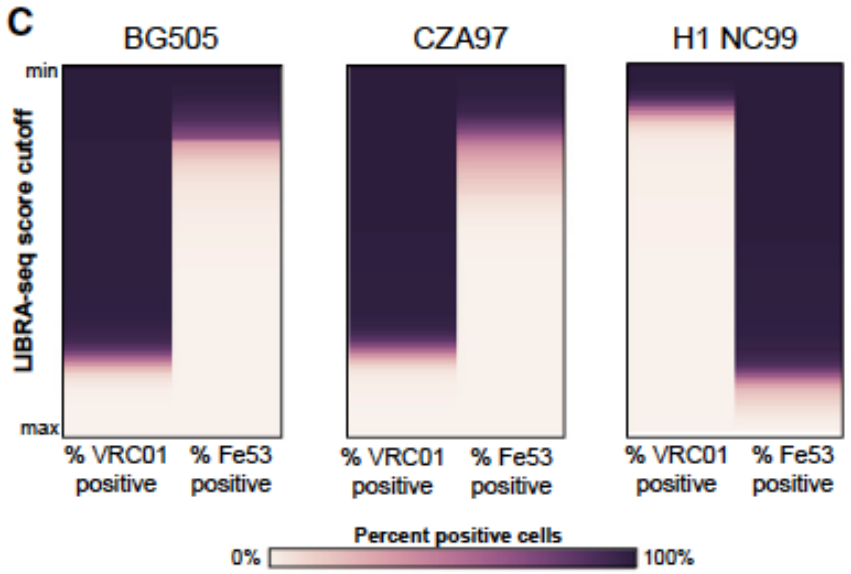
D

	Ramos B Cells	NIAID45	N90
Cellranger Identified Cells	3466	4747	1799
Cells overlapping VDJ and Antigen Barcode Libraries	3466	2801	1799
Cells with a functional heavy chain	2334	2617	1493
Cells that have sufficient number of antigen barcode UMIs	2333	1013	1479
Cells that are singlets	2321	972	1469
Cells that have functional light-chains	-	866	1465

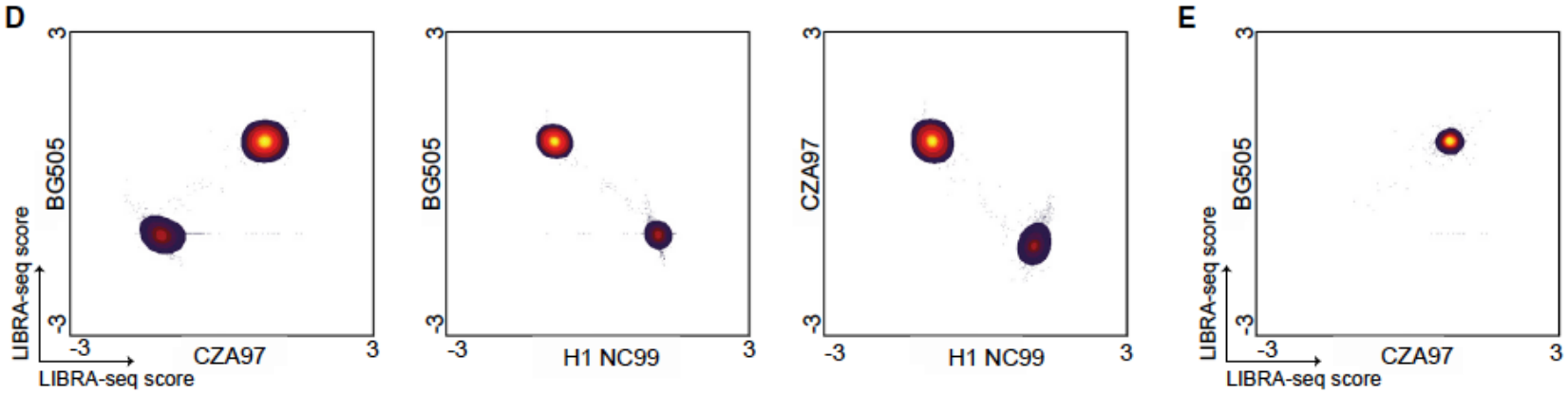
- 2,321 cells with BCR sequence and antigen mapping information were recovered
- VRC01 Ramos B cells bound both BG505 and CZA97 with a high correlation between the scores for these two antigens (Pearson's r = 0.84)

LIBRA-seq binding scores for each antigen

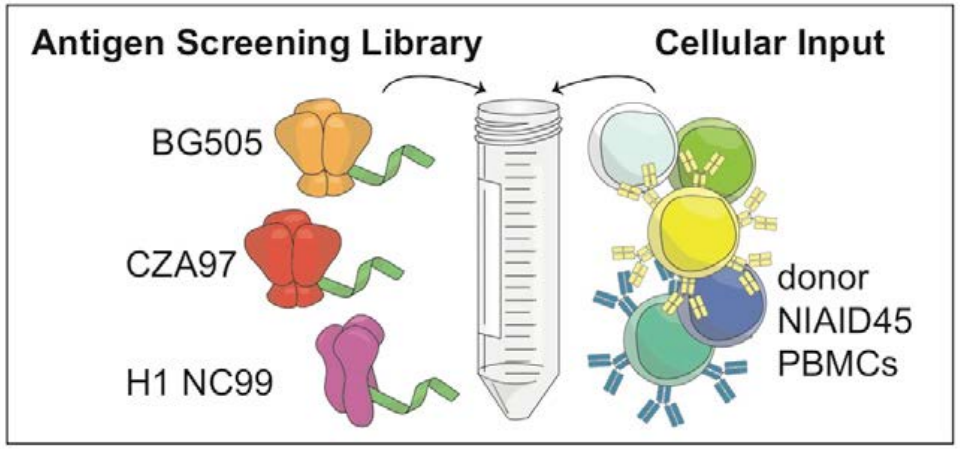
- Scores were computed as a function of the number of unique molecular identifiers (UMIs) for the respective antigen barcode
- All counts of 1, 2, or 3 UMIs were set to 0, and set to contain only cells with a count of at least 4 UMIs for at least 1 antigen.



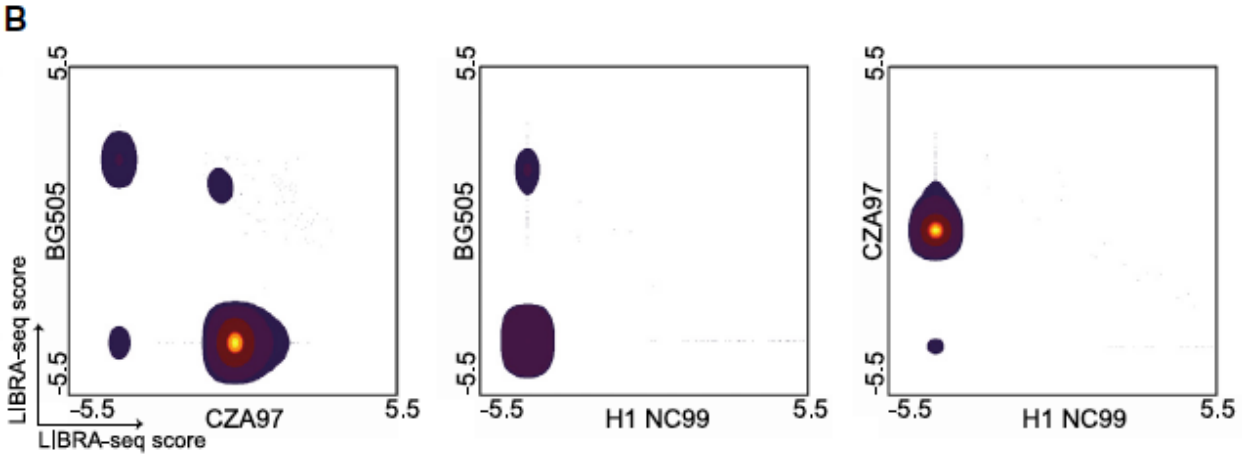
Scores for each pair of antigens were plotted



Antibodies from an HIV bNAb donor NIAID45



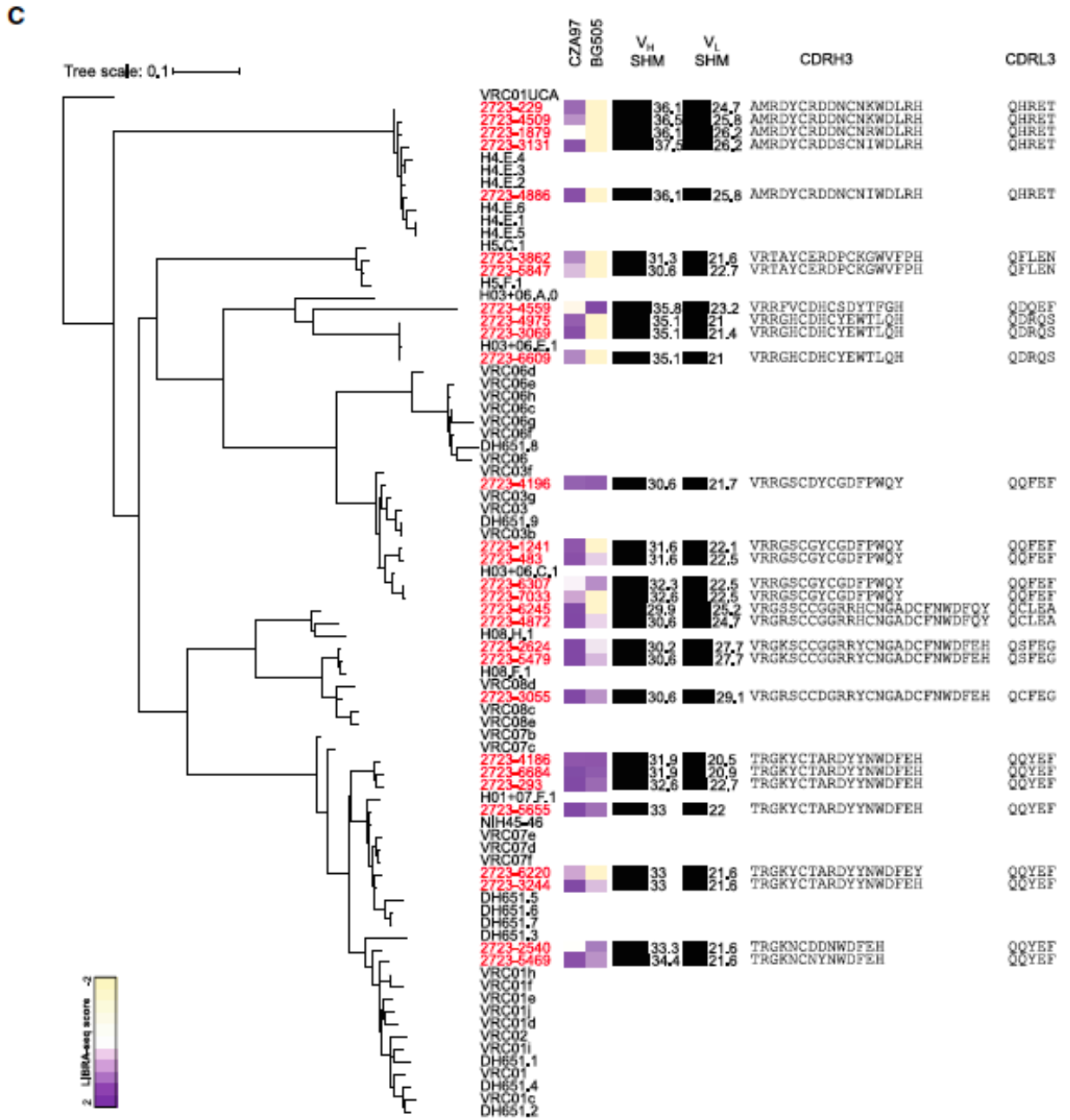
Same approach:
BG505, CZA97, and H1 NC99 antigen screening library
Recovered paired VH:VLantibody sequences with antigen mapping for 866 cells



Different patterns of LIBRA-Seq scores: cross reactive B cells to BG505 and CZA97

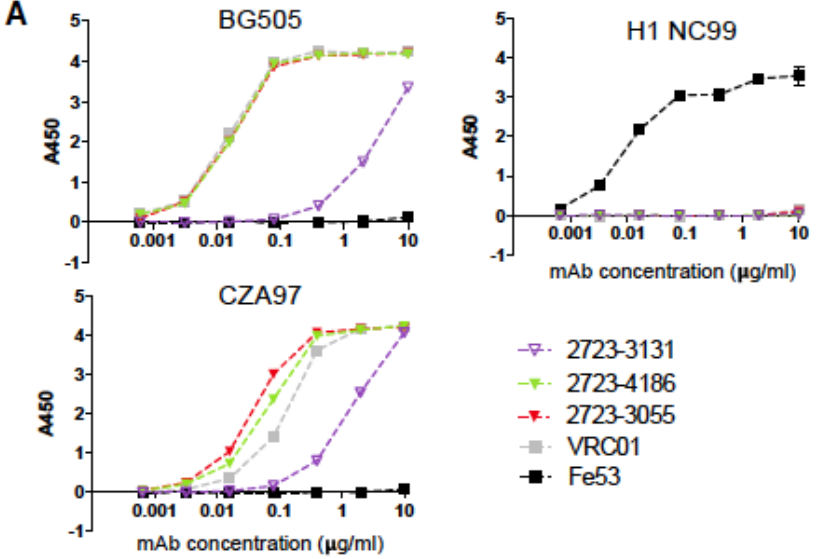
Validating the utility of LIBRA-seq in monoclonal antibody isolation

- A large lineage of HIV-1 bNAbs had been identified previously from donor NIAID45
- This lineage consists of the prototypical bNAb VRC01 as well as multiple clades of clonally related antibodies with diverse neutralization phenotypes
- Compared LIBRA-seq members to the ones from the VRC01 antibody lineage
- Found **29 BCRs** that were clonally related to previously identified members of the VRC01 lineage



Characterization of LIBRA-seq-identified antibodies from donor NIAID45

A



B

Virus phenotype	Env pseudoviruses	IC50 (µg/ml) 2723-3131	2723-4186	2723-3055	VRC01
Tier 1	MW965.26	2.99	0.044	0.043	0.025
	MN.3	16.13	0.013	0.323	0.044
	6644.V2.C33	>50	0.134	0.062	0.139
Tier 2 (global panel)	TRO.11	>50	0.271	0.059	0.226
	CH119.10	>50	0.168	0.309	0.71
	CNE55	>50	0.144	0.113	0.156
	25710.2.43	>50	0.216	0.051	0.47
	CE0217	>50	0.108	0.042	0.136
	X1632.S2.B10	>50	0.045	1.136	0.15
	X2278.C2.B6	>50	0.035	0.024	0.091
	246.F3.C10.2	>50	0.23	0.088	0.321
	398.F1.F6.20	>50	0.066	0.064	0.13
	CE1176.A3	>50	0.692	0.109	1.17
	BJOX002000.03.2	>50	>25	0.099	>50
Tier 2	BG505.W6M.C2.T332N	>50	0.073	0.037	0.06
Control	MLV	>50	>25	>25	>50

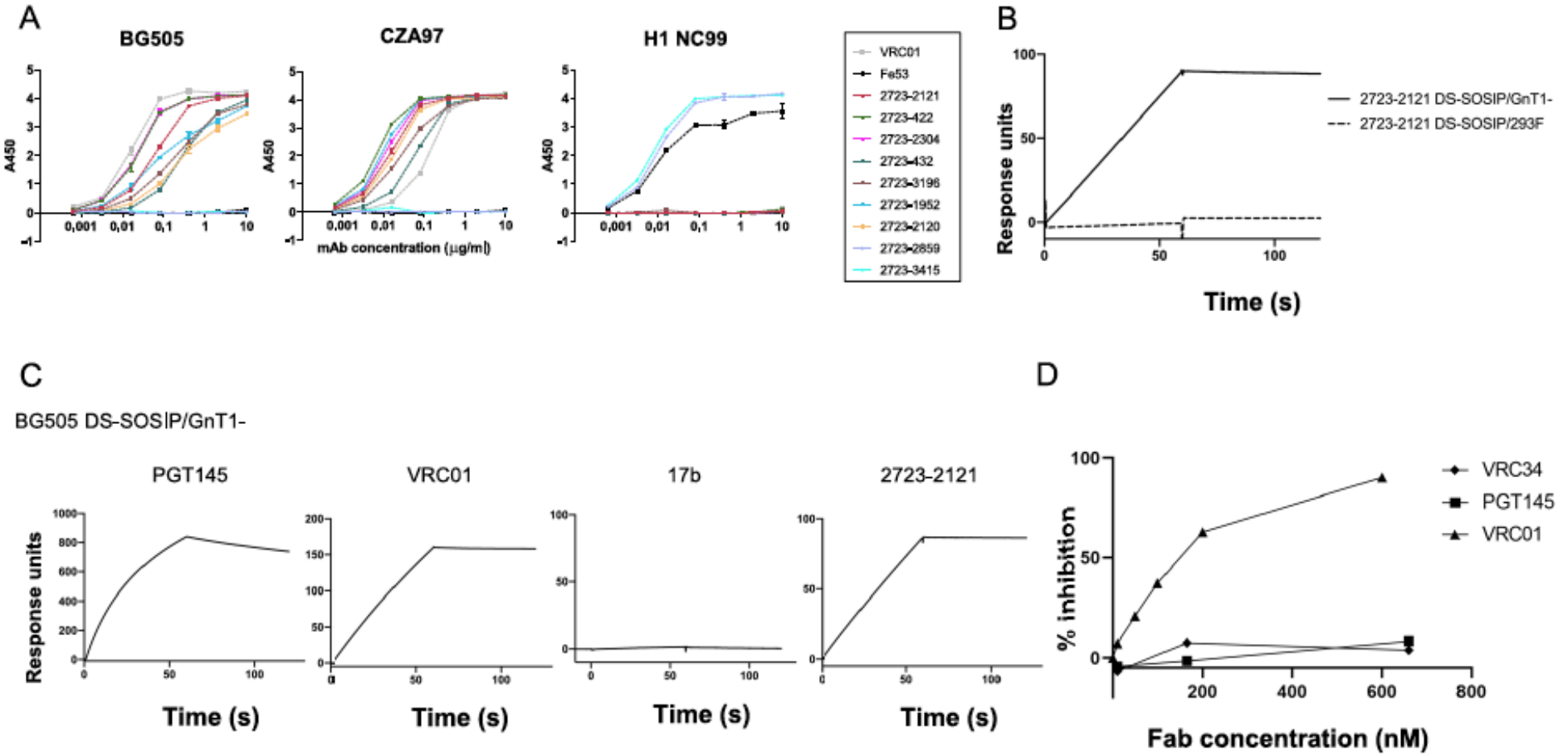
IC50 (µg/ml)
0.0001 1 50

C

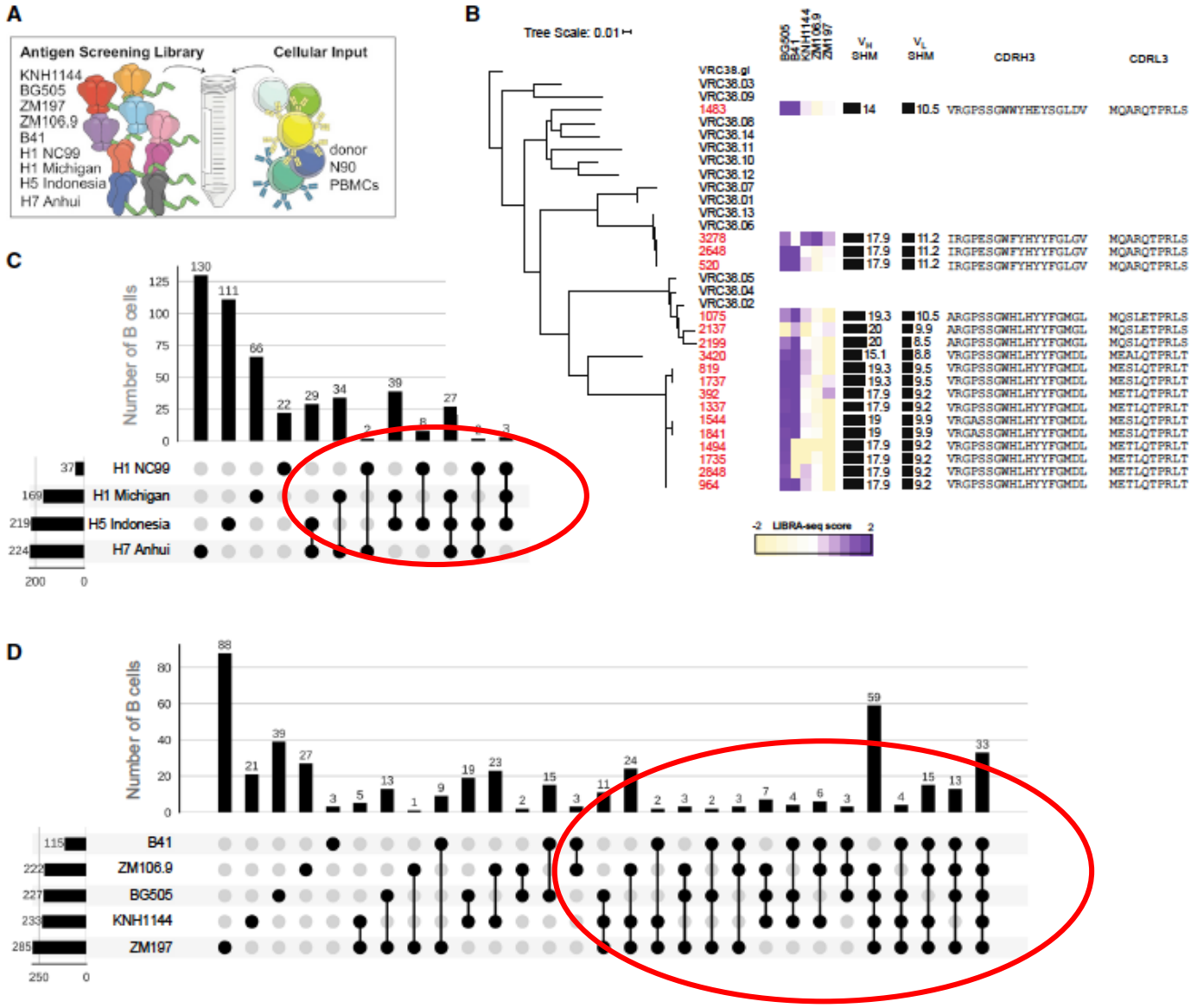
Name	VH gene	VH % identity	CDRH3 length	VDJ junction	VL gene	VL % identity	CDRL3 length	VJ junction	LIBRA-seq score BG505	CZA97	HA	ELISA AUC BG505	CZA97	HA
2723-2121	IGHV4-39	86.55	21	ARHRADYDFWNGNNLRGYFDP	IGKV3-20	94.33	9	QQYGSSPTT						
2723-422	IGHV4-39	85.91	21	ARHRANYDFWGGSNLRGYFDP	IGKV3-20	88.65	9	QQYGTSPTT						
2723-2304	IGHV4-39	85.22	21	ARHRADYDFWGGSNLRGYFDP	IGKV3-20	90.07	9	QQYGTSPTT						
2723-432	IGHV3-23	83.68	26	ARDEVLRGSASWFLGPNEVRHYGMDV	IGKV2-29	88.1	8	MQSLQLRS						
2723-3196	IGHV1-69	79.17	16	VGRQKYISGNVGDFDF	IGKV1-33	89.96	11	QQYTNLPPALN						
2723-1952	IGHV4-38	83.33	14	ATRGIAASGFYFQH	IGKV1-5	89.61	9	HHYNSFSHT						
2723-2120	IGHV4-61	84.54	18	AREHTMIFGVAEGFWFDP	IGLV3-19	89.25	11	SSRDTDDISVI						
2723-2859	IGHV1-69	90.97	15	VTMSGYHVSNTYLDA	IGKV3-20	94.68	9	QQYANSPLT						
2723-3415	IGHV1-18	91.32	9	ARGRVYSDY	IGKV3-20	95.74	10	QQSGTSPPWT						

-2 LIBRA-seq score 2
min ELISA AUC max

Characterization of LIBRA-seq-identified antibodies from donor NIAID45



Discovery of an HIV bNAb from donor N90 using an antigen screening library



- Increased the number of antigen to 9:
- Recovered paired VH:VL antibody sequences with antigen mapping for 1,465 cells
- Antigen specificity maps:

Number of B cells with high scores

- 17 had high LIBRA-seq scores for at least one HIV antigen, and one had no high LIBRA-seq scores but had a mid-range score for two SOSIPs
- 32 cells that had high LIBRA-seq scores for three of the four influenza antigens

Discovery of an HIV bNAb from donor N90 using an antigen screening library

- 3602-870 is highly mutated 29% deviation from the germline in the heavy chain and 17% deviation from the light chain
- 3602-1707 mAb has broad influenza recognition, with high correlation between LIBRA-seq scores and ELISA

A

Name	VH gene	VH % identity	CDRH3 length	VDJ junction	VL gene	VL % identity	CDRL3 length	VJ junction		BG505	B41	KNH1144	ZM106.9	ZM197	H1 NC99	H5 Indonesia	H1 Michigan	H7 Anhui
3602-870	IGHV1-46	71.53	19	ARDAGERGLRGYSVGFFDS	IGKV3-20	82.98	9	HQYGTTPYT	LIBRA-seq score									
									ELISA AUC									
3602-1707	IGHV3-23	87.15	24	AKVVAGGQLRYFDWQEGHYYGMDV	IGKV2-28	96.60	9	MQSLQTPHS	LIBRA-seq score									
									ELISA AUC									

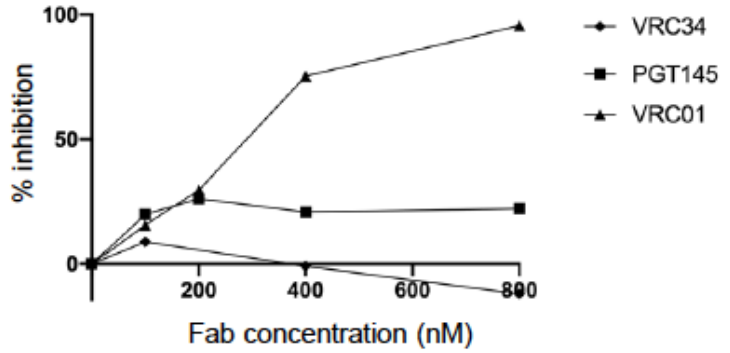
-2 LIBRA-seq score 2
min ELISA AUC max

B

Virus phenotype	Env-pseudoviruses	IC_{50} (µg/ml) 3602-870
Tier 2 (global panel)	TRO.11	0.18
	CH119.10	0.58
	CNE55	>10
	25710.2.43	0.33
	CE0217	0.19
	X1632.S2.B10	>10
	X2278.C2.B6	0.06
	246_F3.C10.2	0.78
	398.F1.F6.20	>10
	CE1176.A3	0.76
	BJOX002000.03.2	0.16
Tier 2	CZA97	1.41
	ZM197	4.94
	BG505.W6M.C2.T332N	0.13
Control	MLV	>10

IC_{50} (µg/ml)
0.0001 1 50

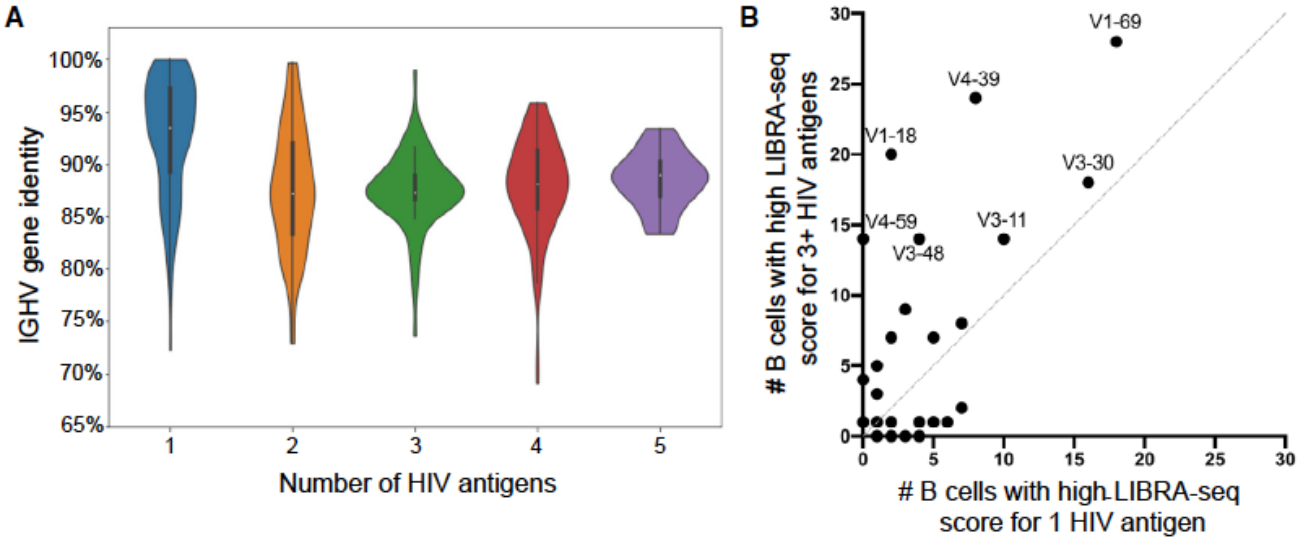
C



LIBRA-Seq method
Conclusions

- Detection of increased SHM in B cells with high scores for a single antigen compared to others with >1 antigen
- type-specific antibodies have lower levels of SHM

- Use of specific germline genes to be more frequent in B cells that exhibited broad as opposed to strain-specific HIV-1 antigen reactivity



- Novel method for interrogating antibody-antigen interactions via sequencing-based readouts:
  1. Antibody sequence
  2. Antigen reactivity
- Platform for simultaneously screening B cell repertoires against multiple diverse antigen targets
- Coupling of antibody sequence and specificity can enable high-resolution immune profiling; highly important for vaccine development

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PMID: 30457979

Spec-seq unveils transcriptional subpopulations of antibody-secreting cells following influenza vaccination

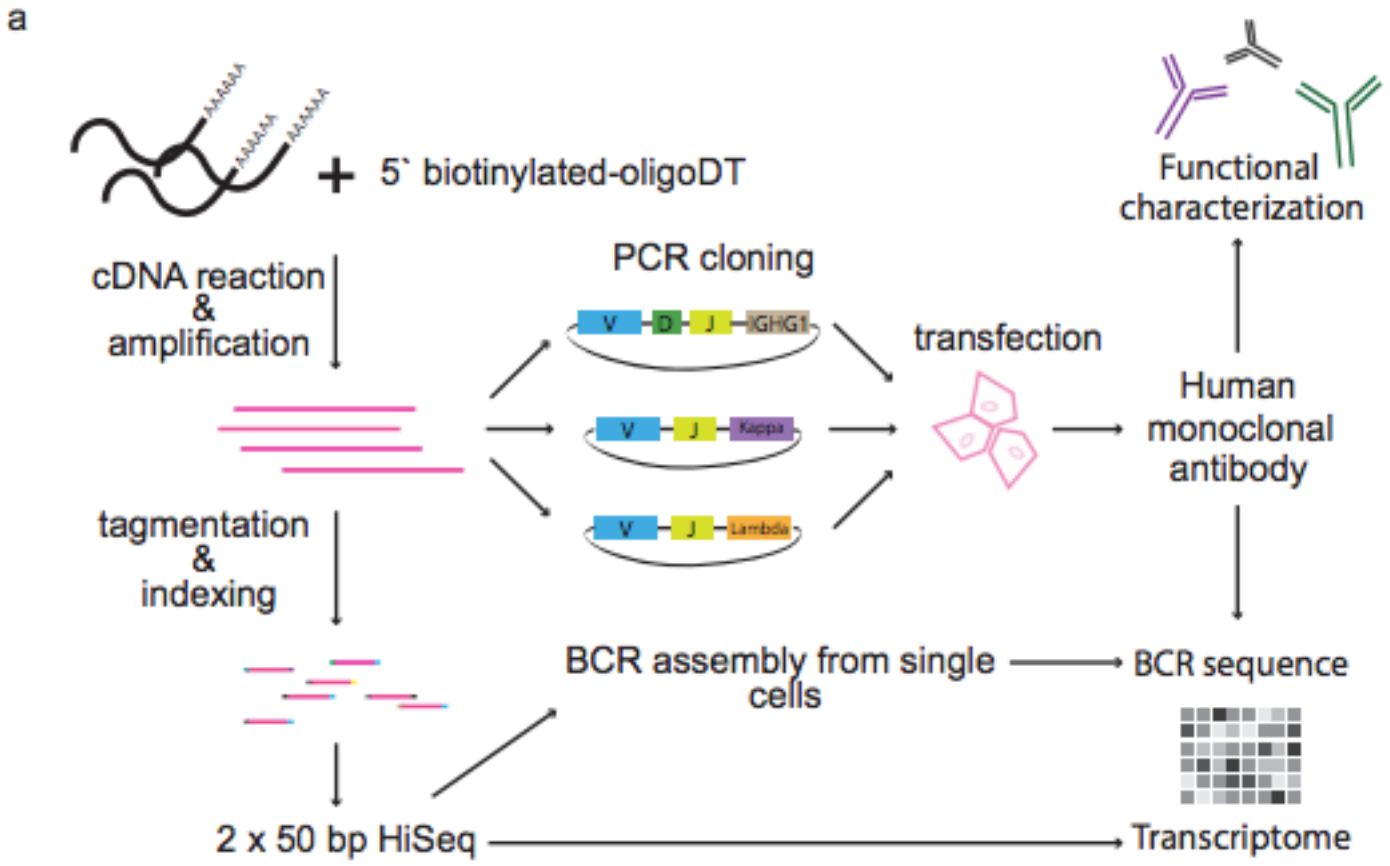
Karlynn E. Neu,[1,2] Jenna J. Guthmiller,[2] Min Huang,[2] Jennifer La,[3] Marcos C. Vieira,[4] Kangchon Kim,[4] Nai-Ying Zheng,[2] Mario Cortese,[5] Micah E. Tepora,[1] Natalie J. Hamel,[1] Karla Thatcher Rojas,[2] Carole Henry,[2] Dustin Shaw,[1,2] Charles L. Dulberger,[6] Bali Pulendran,[5] Sarah Cobey,[4] Aly A. Khan,[7] and Patrick C. Wilson[1,2]

The Spec-Seq framework

mAb characterization and
transcriptional profiling from the same single B cell

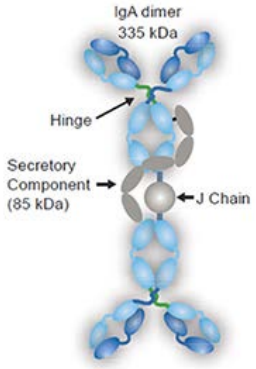
Spec-seq allows for simultaneous analysis of B cell receptor sequence, receptor binding properties and transcriptional profile from a single cell.

- RT-PCR & cDNA reaction
- Amplification with PCR and SmartSeq2 protocol
- The amplified V(D)J sequence is cloned into an expression vector, matched heavy and light chains are transfected into HEK293A cells and a fully human monoclonal antibody is generated
- Indexed libraries are generated from 0.5 ng of the remaining cDNA prior to next generation sequencing.



Introduction

- Plasmablasts are the main ASCs in the peripheral blood and are thought to be terminally differentiated and short-lived
- Present at a low level in the peripheral blood of healthy humans and undergo significant expansion 7 days after immunization or infection
- The vaccine-induced population is predominantly antigen-reactive
- The majority of plasmablasts induced by vaccination express the IgG isotype, although produce IgA
- Steady-state plasmablast population comprises mainly of IgA
- IgA antibodies have enhanced neutralization capacity and antigen sequestration
- Shared IgA antibody sequences found in both the serum and the gut



an ideal target population for vaccines aimed to induce localized mucosal protection

Exploring the immune transcriptome through Spec-Seq

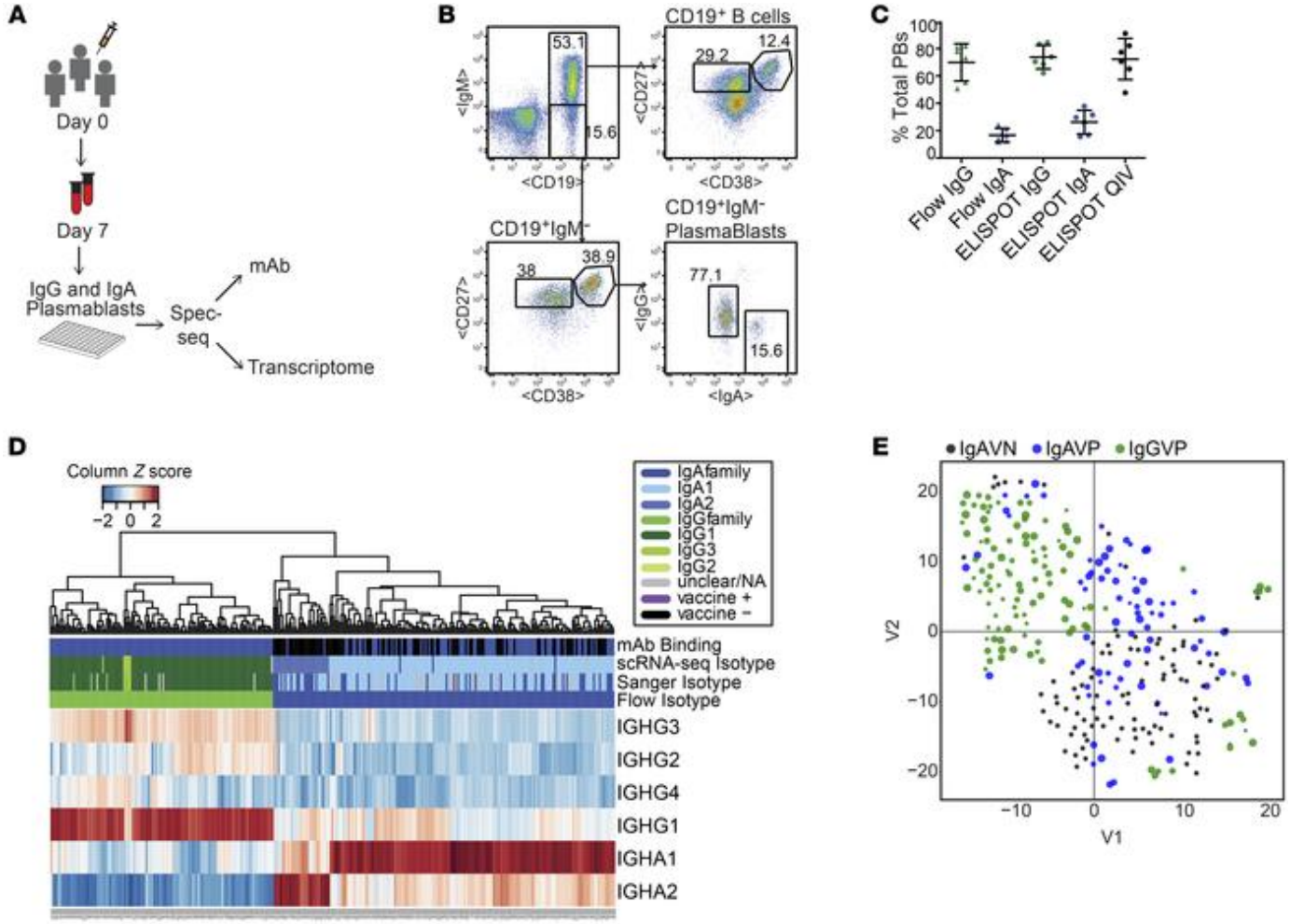
Developed to explore plasmablast transcriptional diversity in the context of BCR repertoire and antigen specificity

- How is BCR isotype or specificity affecting the transcriptome?
- Do these features affect other specializations than the BCR?
- How IgA vaccine-positive compare to IgG positive and IgA negative populations?
- Can transcriptional subpopulations correlated with BCR binding be identified?

Exploring the immune transcriptome through Spec-Seq

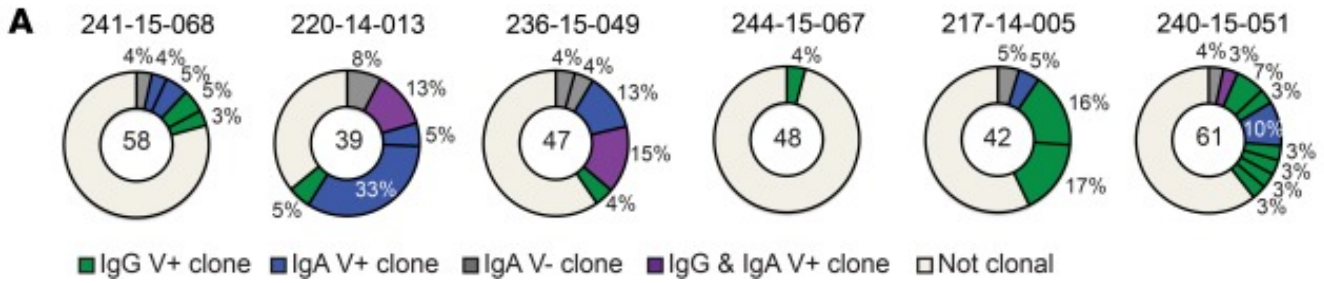
- IgA and IgG peripheral blood plasmablasts were single-cell sorted from 6 donors 7 days post- influenza vaccination

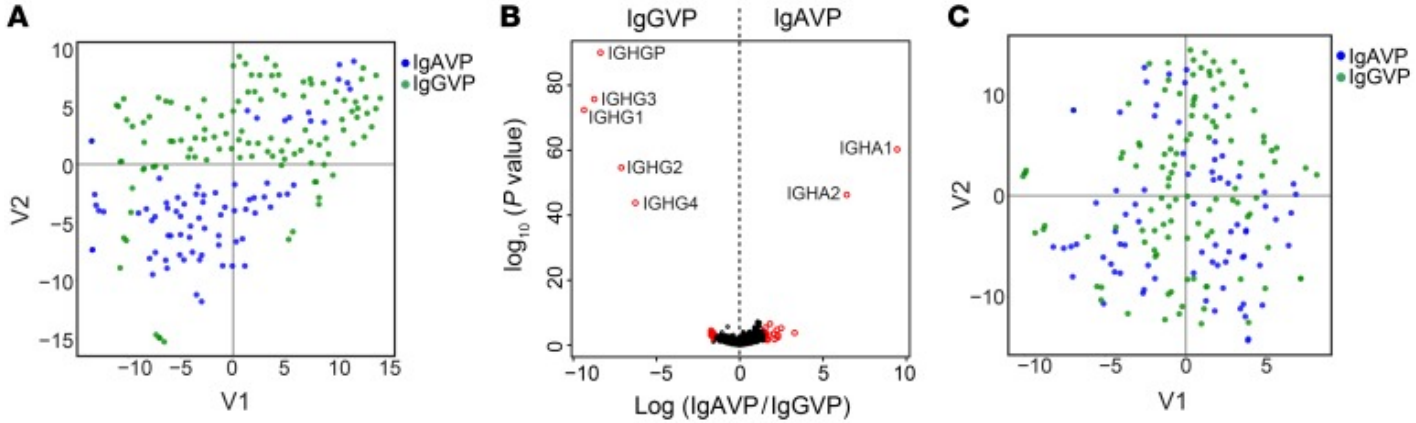
- Gating strategy from IgM- to IgG and IgA positive
- Plasmablast expansion post-vaccination
- Heatmap of 291 single-cells clustered by the expression of IgA/G subtype genes
- The IgAVP and IgAVN cells cluster together and away from the IgGVP population

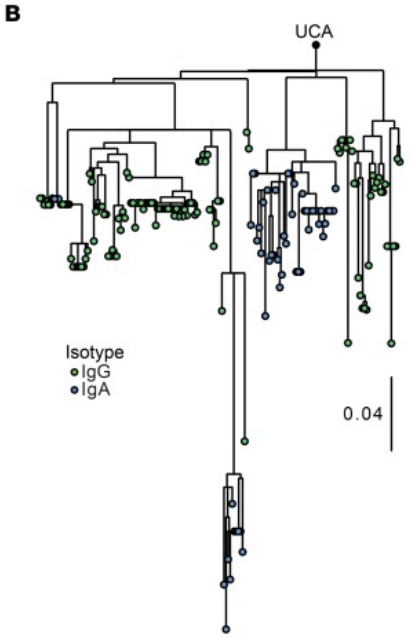


Exploring the immune transcriptome through Spec-Seq

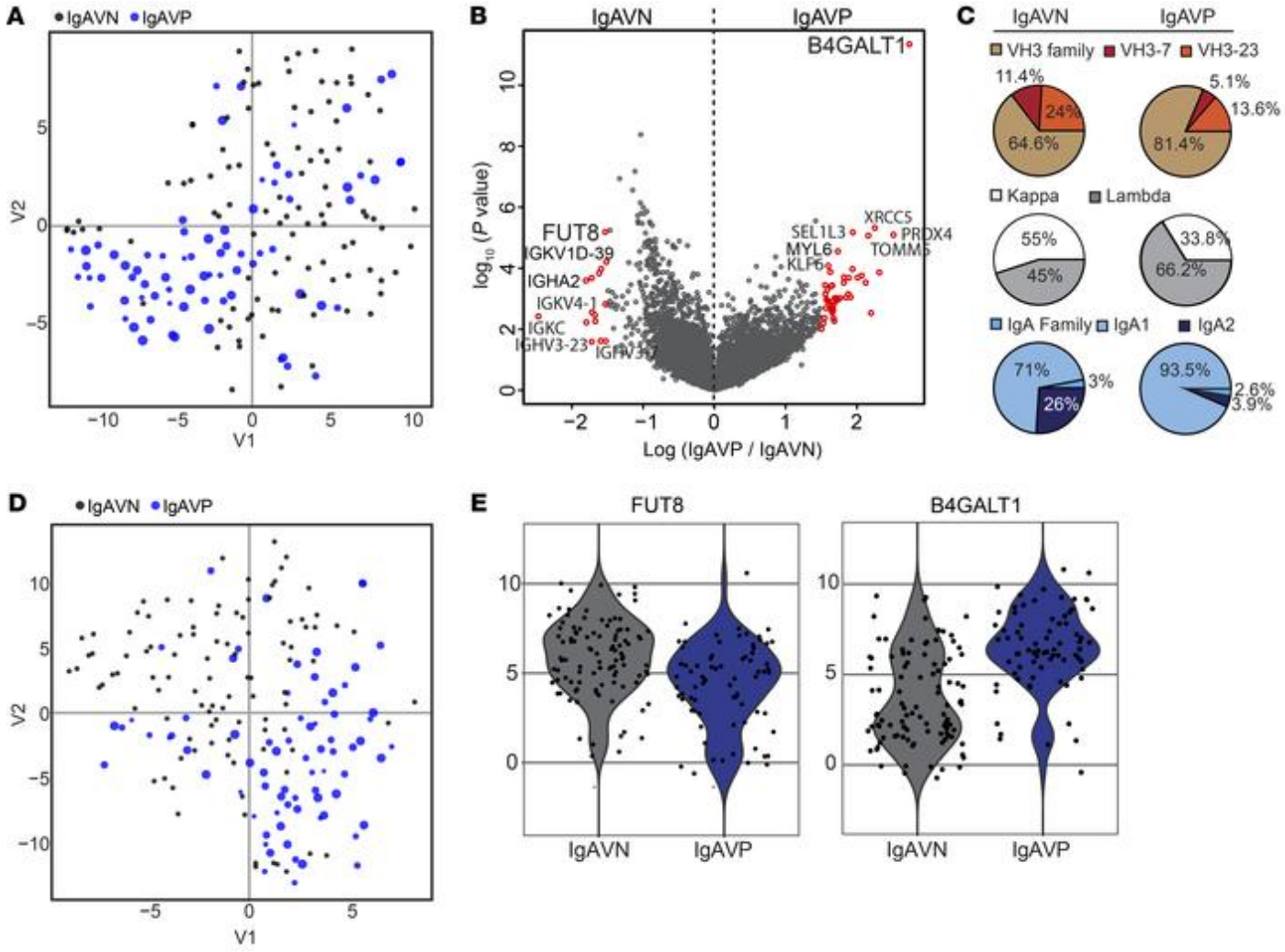
- Clonal expansions by donor
- Lineage tree reveals increased transcriptional similarities
- Frequency of clones containing both IgG and IgA members for the 3 donors







IgA plasmablasts cluster by their vaccine-binding properties

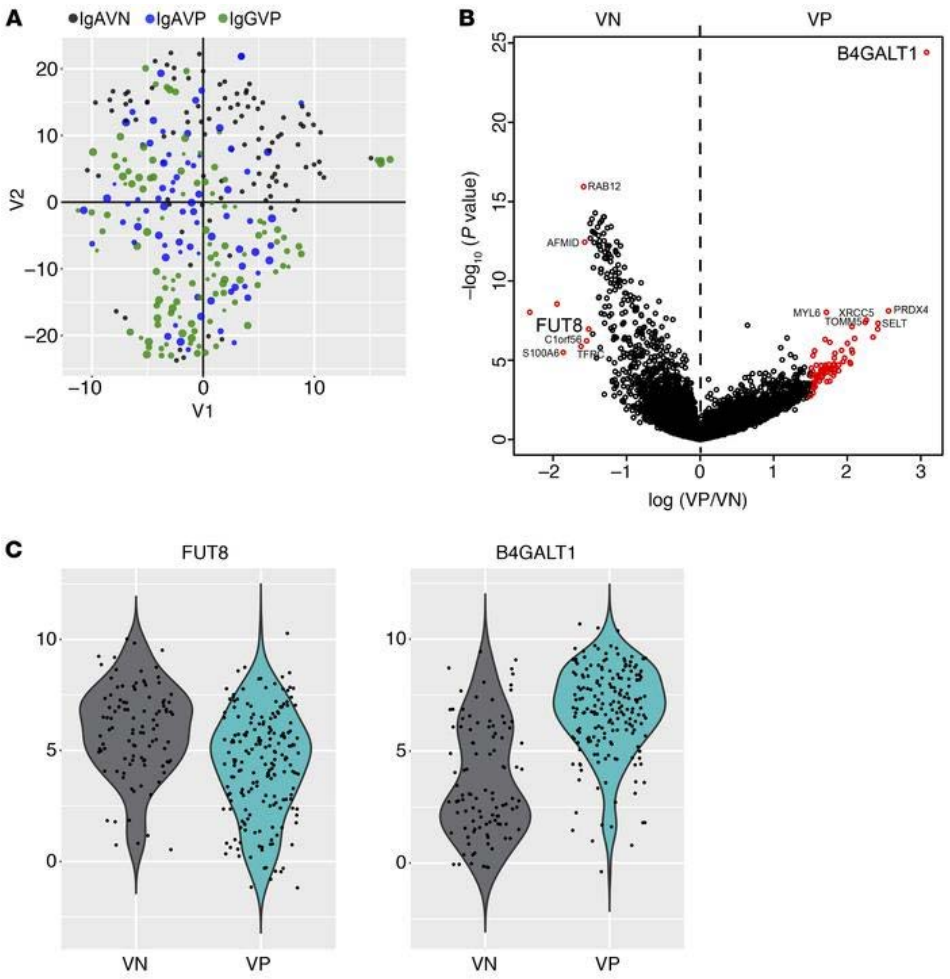


tSNE projection of the IgA cells suggests transcriptional segregation between these 2 populations -- could this indicate different functional properties or specificity for epitopes?

tSNE repeated without Ig genes and similar clustering indicate that transcriptional differences exist beyond repertoire usage

Differentially expressed genes identified are involved in glycosylation, affecting binding properties of BCR and altering immunogenicity

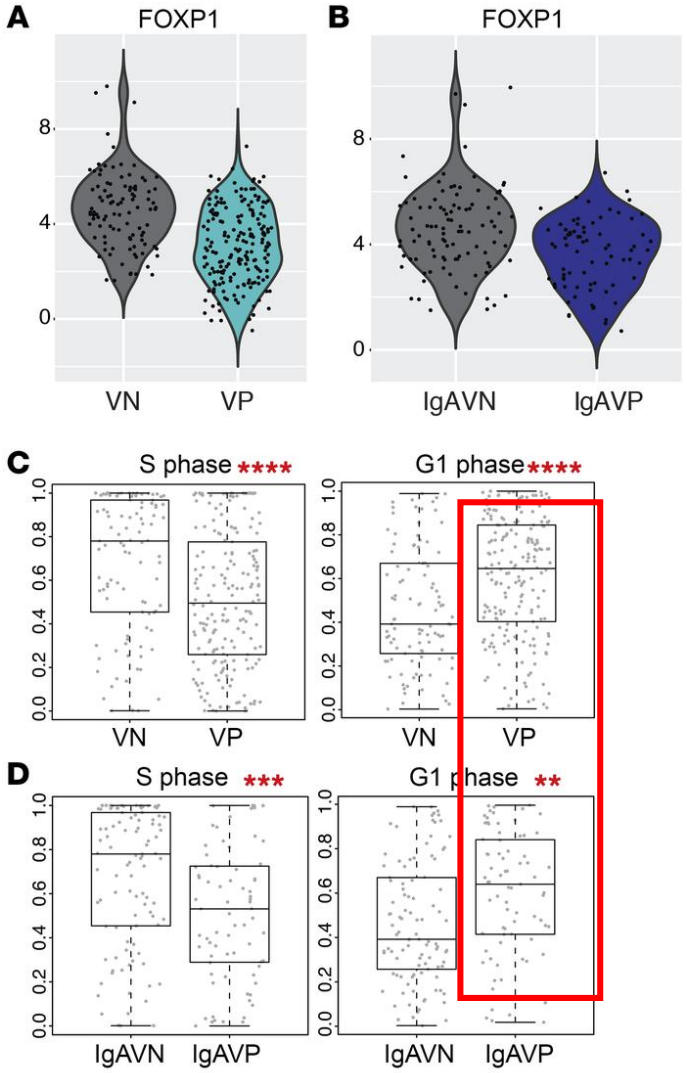
Total plasmablasts cluster similarly



- IgGVP and IgAVP cells cluster together and away from the IgAVN cells
- Glycosylation genes are identified in large numbers between VN and VP cells

plasmablast BCR specificity directs transcriptional differences that can ascribe key effector properties to the antibodies secreted by that cell

FOXP1 is differentially expressed between VN and VP cells



The increased expression of *FOXP1* within the vaccine-negative plasmablast population may suggest upregulation of pathways designed to prevent terminal differentiation into traditional antigen-induced plasmablasts

G1 phase increase in VP cells indicates terminal differentiation into antibody-secreting cells

Spec-Seq method
Conclusions

- B cell biology is linked to BCR specificity; somatically rearranged receptors provide distinct antigen specificities and effector functions
- Vaccine-induced plasmablasts are transcriptionally distinct from the vaccine-negative IgA population
- Identification of dominant BCR transcripts is not surprising
- No significant changes in the gene expression profiles of cells expressing IgG versus IgA BCRs were found
- IgAVN steady-state B cell clones are maintained over time in the peripheral blood and have a homeostatic role. what is their specificity and how are they connected to the local mucosa?

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Simultaneous epitope and transcriptome measurement in single cells

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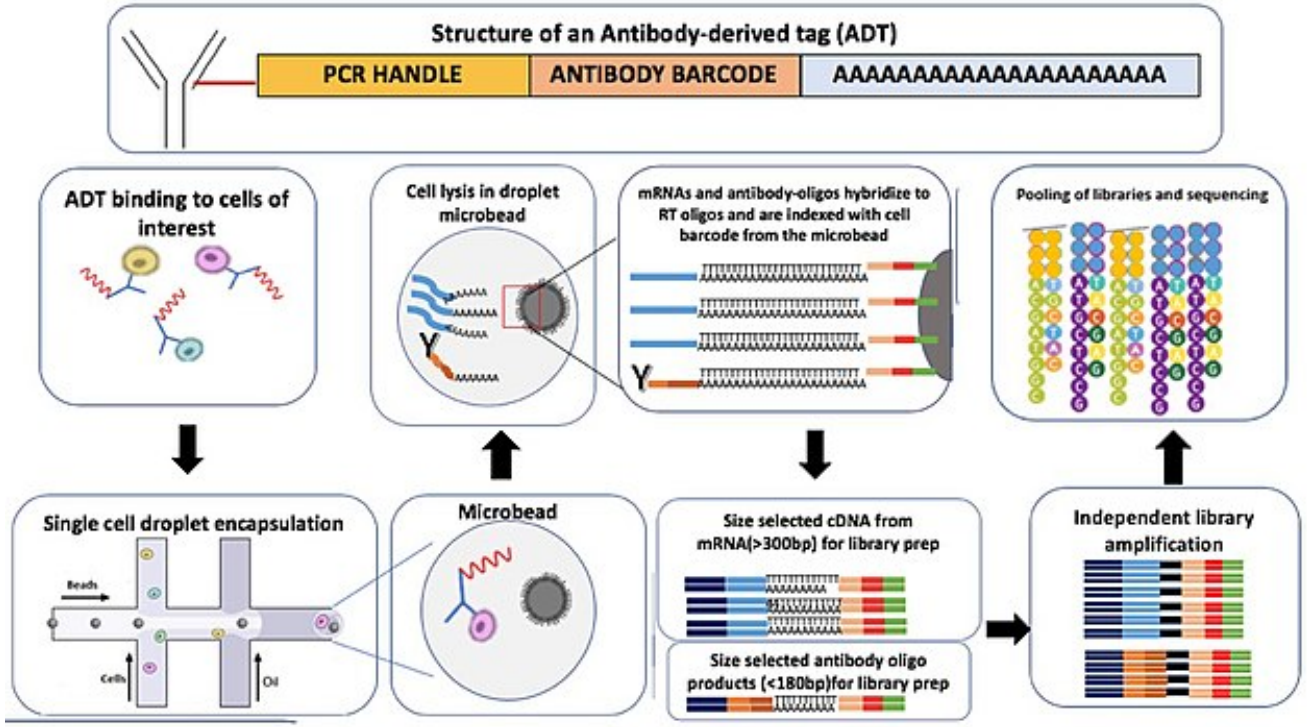
Nature Methods **14**, 865–868(2017) | Cite this article

Detecting transcriptomes and protein markers with CITE-Seq
(Cellular Indexing of Transcriptomes and Epitopes by Sequencing)



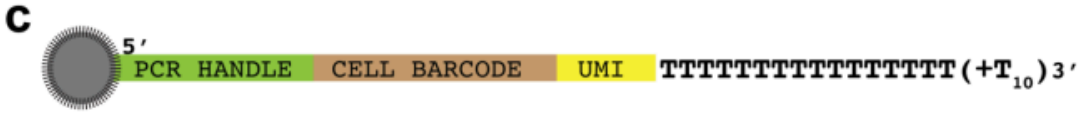
- CITE-Seq allows the simultaneous measurement of transcripts and proteins in single-cells so to get a sequenceable readout from an antibody
- Multiplexing protein marker detection with transcriptome profiling
- Cell phenotyping is made possible by conjugating DNA oligos to antibodies that contain a barcode for identification, can be captured by oligo-dT primers, and include a handle for PCR amplification

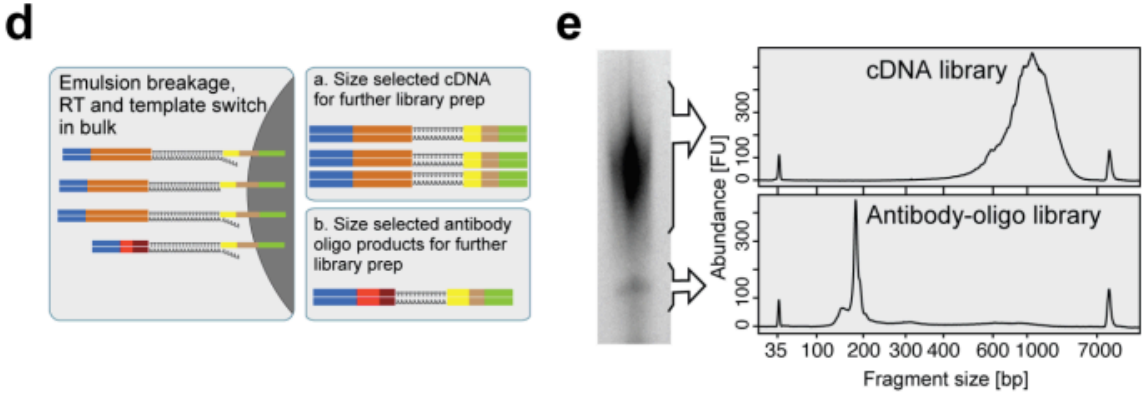
CITE-Seq workflow

Preparation of CITE-seq oligos &
Antibody-Derived Tags (ADTs)

- biotinylation of oligos
- streptavidin labeling of antibodies
- conjugation of streptavidin-antibodies with biotinylated-oligos
- cells stained
- cDNA synthesized
- libraries are prepared

Antibody-oligonucleotide complexes appear as a high-molecular-weight smear when run on an agarose gel.
Cleavage of the oligo from the antibody by reduction of the disulfide bond collapses the smear to oligo length.

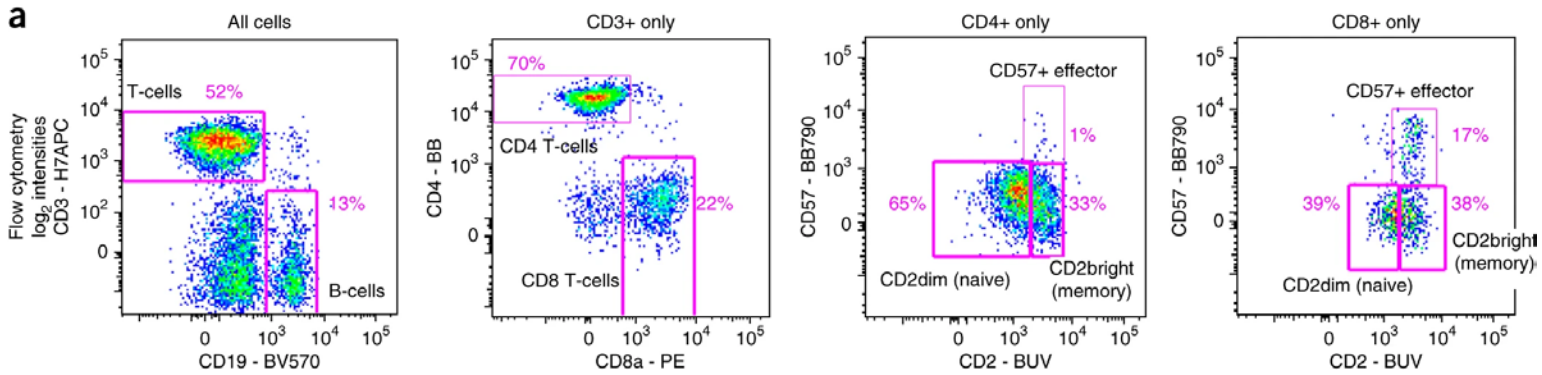




Drop-seq beads with conjugated oligonucleotides comprise of a common PCR handle, a cell barcode, followed by a unique molecular identifier (UMI) and a polyT-tail

RT and template switch is performed in bulk after emulsion breakage. After amplification, full length cDNA and antibody-oligo products are separated by size and amplified independently

CITE-seq is merging RNA-Seq and flow cytometry technologies



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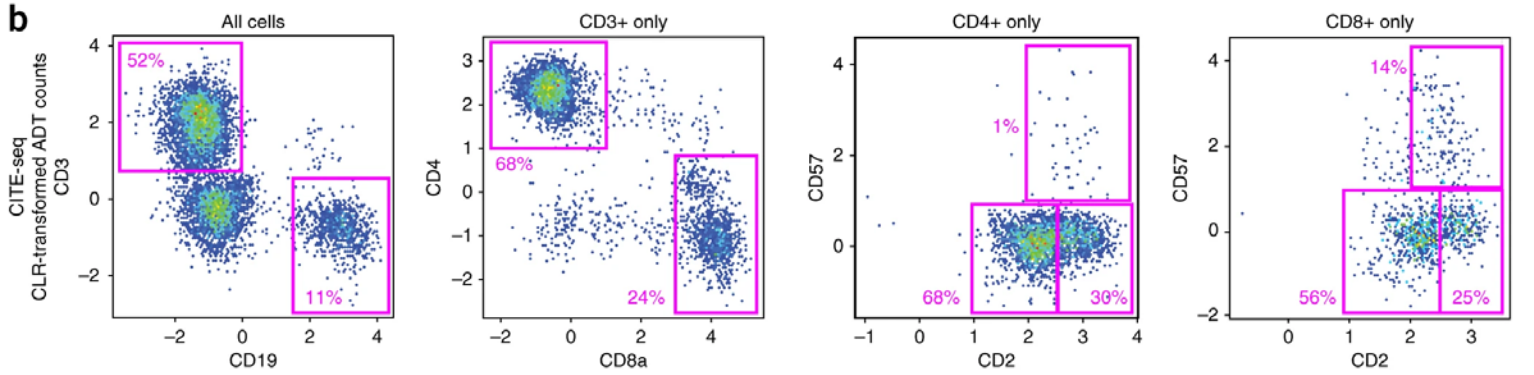
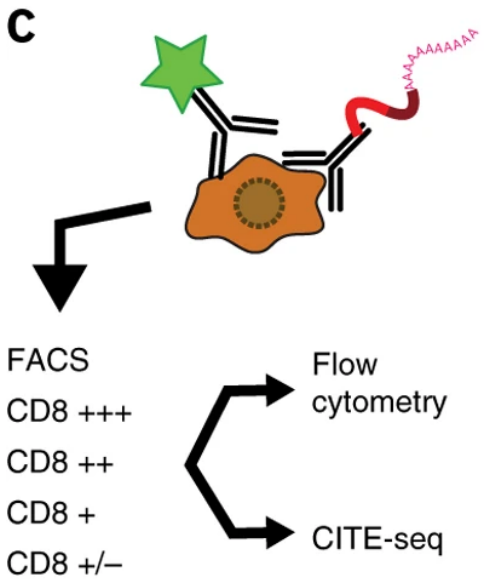
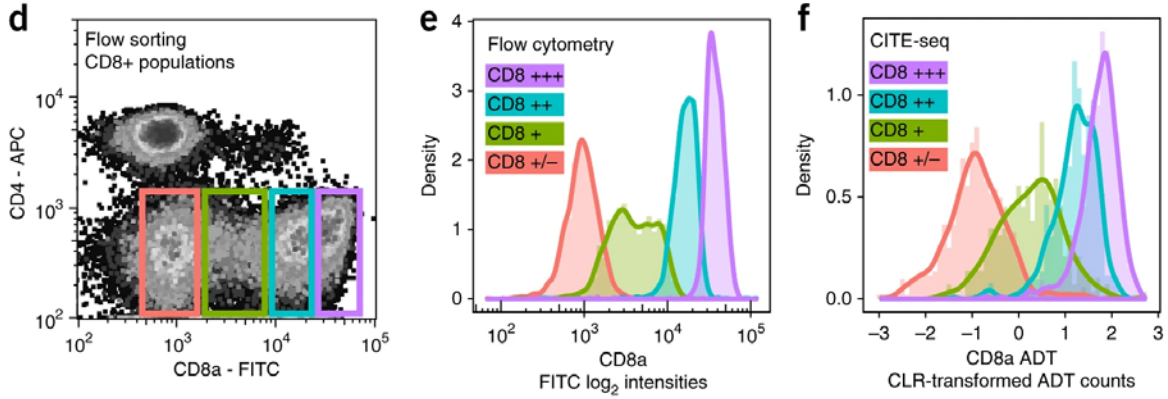
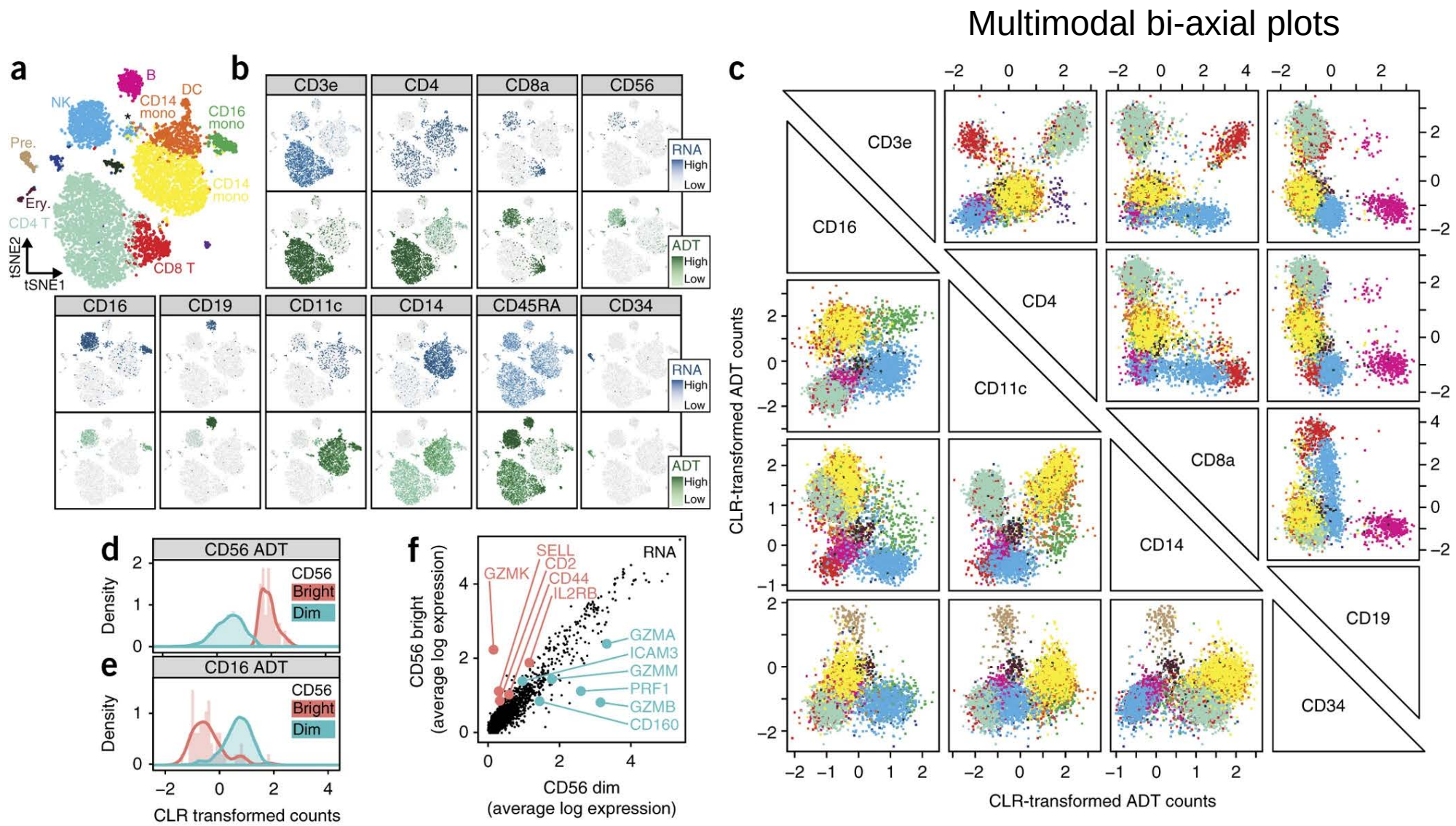


CITE-Seq





CITE-seq allows for detailed multimodal characterization of cord blood mononuclear cells



CITE-Seq method
Conclusions

- CITE-seq enables simultaneous analysis of the transcriptome as well as the proteome of single cells
- It enables large-scale immunophenotyping with panels of tens to hundreds of antibodies, in contrast to flow and mass cytometry
- Can be adapted to detect small molecules, RNA interference, CRISPR, and other gene editing techniques
- Could be adapted for intracellular markers
- Can be modified so that only ADTs are analyzed on a massively parallel scale without capturing cellular mRNAs (cytometry by sequencing)

Thank you for your attention!