

# High-Throughput Mapping of Antibody Sequence to Antigen Specificity

**Ivelin Georgiev, Ph.D.**

Program in Computational Microbiology and Immunology

Vanderbilt Vaccine Center

Vanderbilt Institute for Infection, Immunology and Inflammation

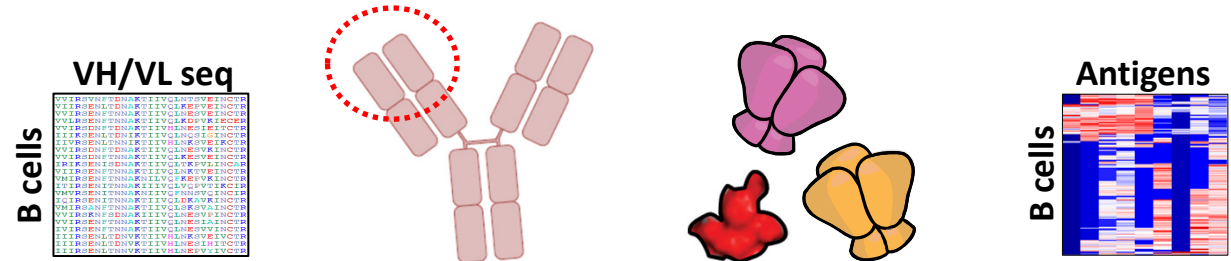
Department of Pathology, Microbiology, and Immunology, VUMC

Department of Electrical Engineering and Computer Science, Vanderbilt



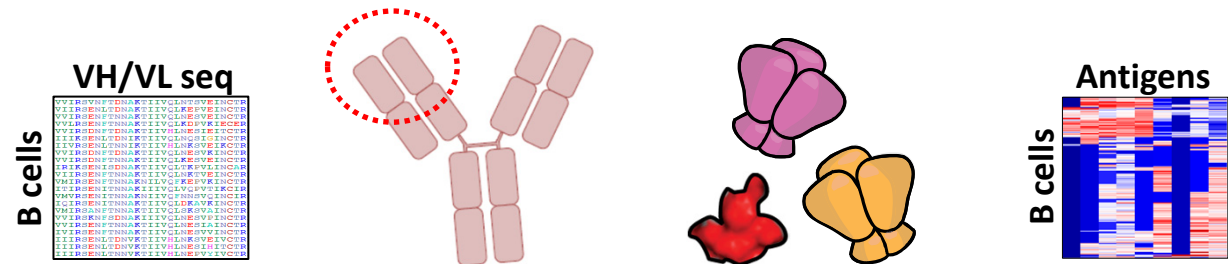
December 9<sup>th</sup>, 2020

# Current Approaches for Characterizing Antibody Repertoires Provide Limited Functional Information



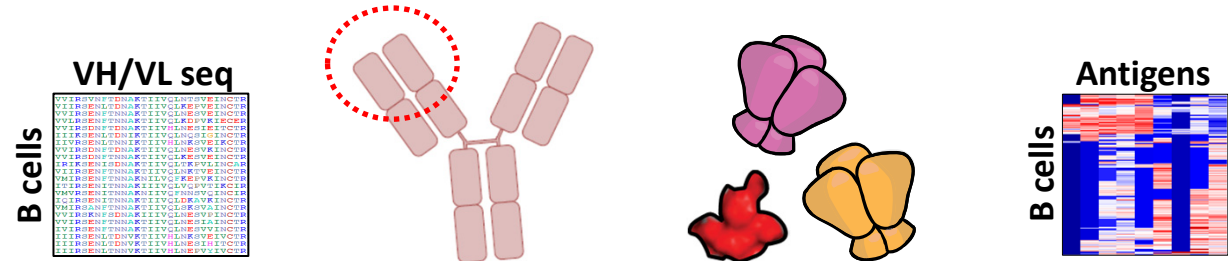
| Technique                | # B cells | Paired H-L sequences | Number of antigens | Antigen specificity map |
|--------------------------|-----------|----------------------|--------------------|-------------------------|
| Non-paired NGS           |           |                      |                    |                         |
| Paired NGS               |           |                      |                    |                         |
| Antigen-specific sorting |           |                      |                    |                         |
| Ag-sorting/NGS           |           |                      |                    |                         |
| Functional screening     |           |                      |                    |                         |
| Display technologies     |           |                      |                    |                         |

# Current Approaches for Characterizing Antibody Repertoires Provide Limited Functional Information



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| Non-paired NGS           | $10^8$ - $10^9$ | ✗                    | ✗                  | ✗                       |
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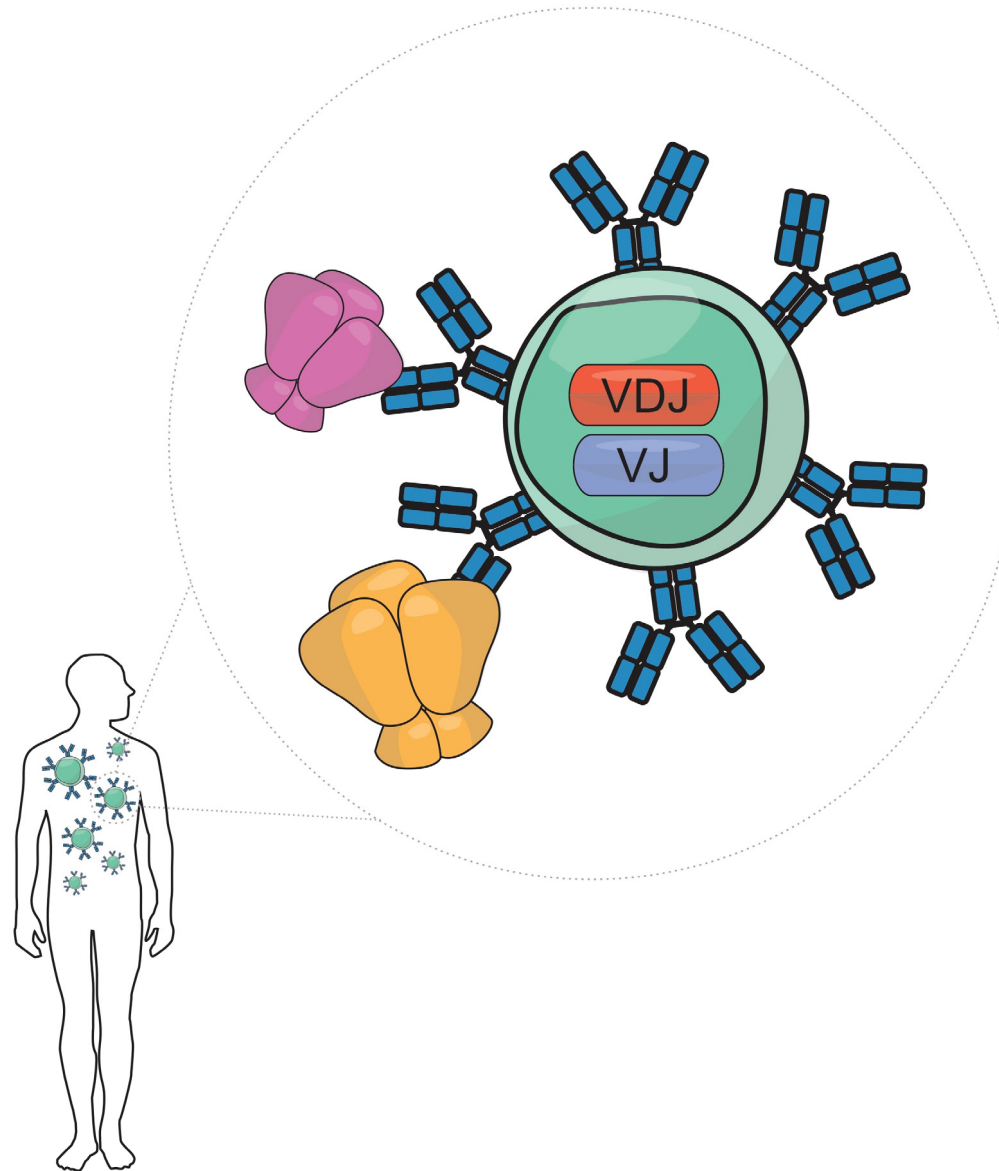
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# Is There a Way to Simultaneously Determine BCR Sequence and Antigen Specificity?

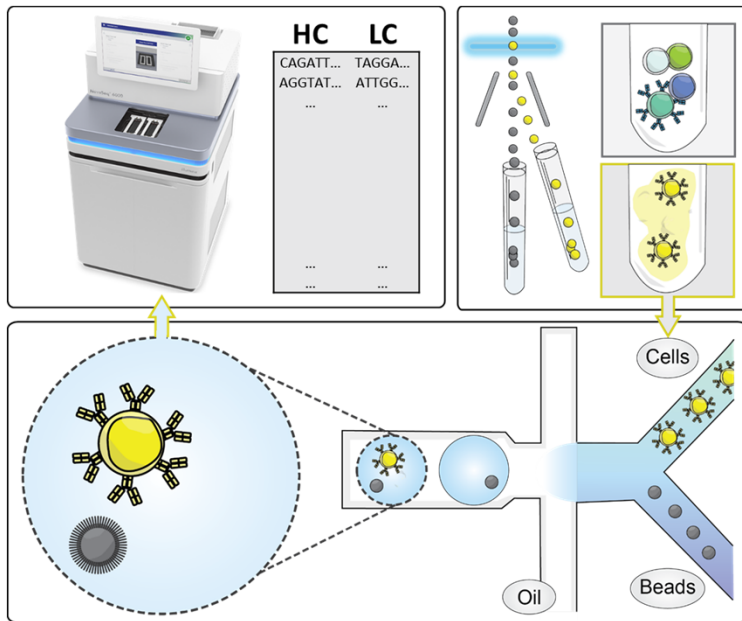
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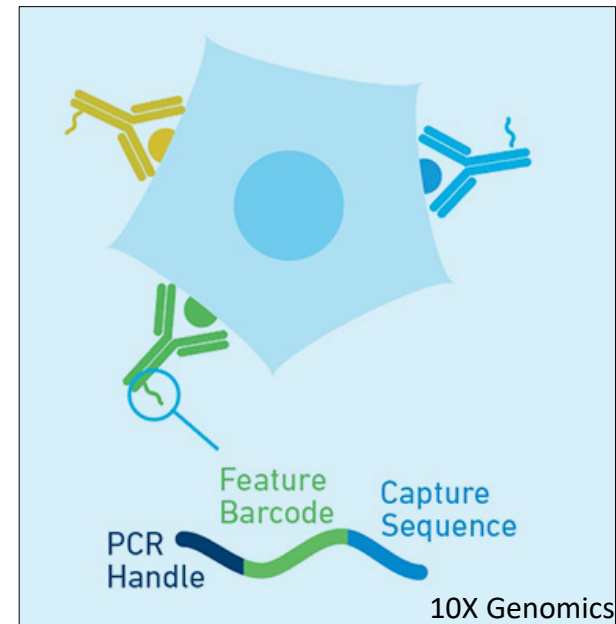
# Simultaneous Determination of BCR Sequence and Antigen Specificity: Backbone Technologies

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## Single-cell BCR sequencing

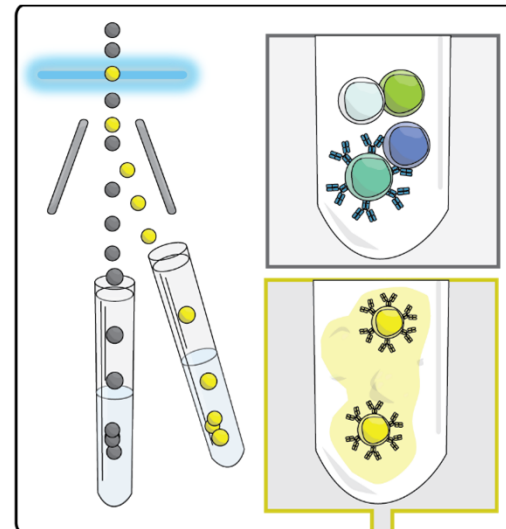
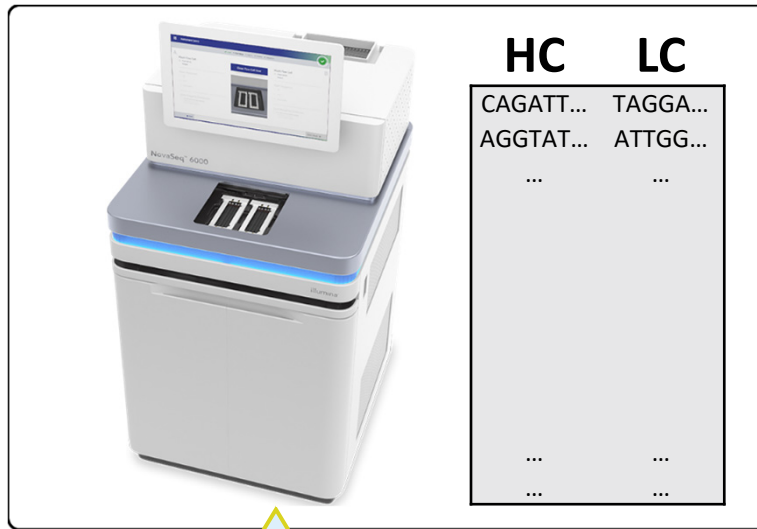


## DNA barcoding

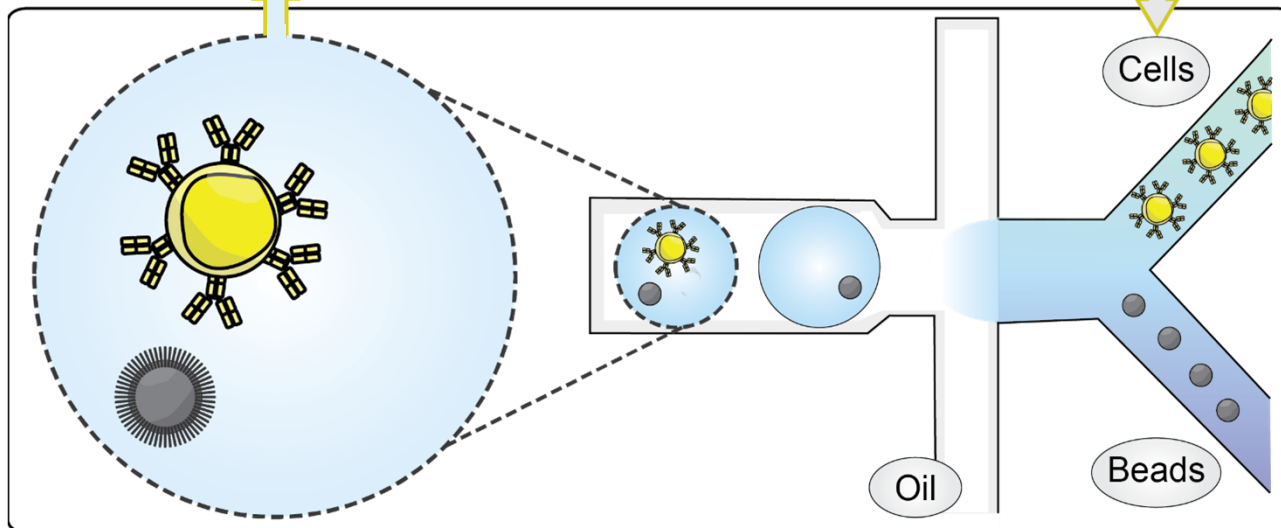


# Antigen-Specific BCR Sequencing Helps Select for B cells with Target Specificities

**3**  
NGS  
(NovaSeq)



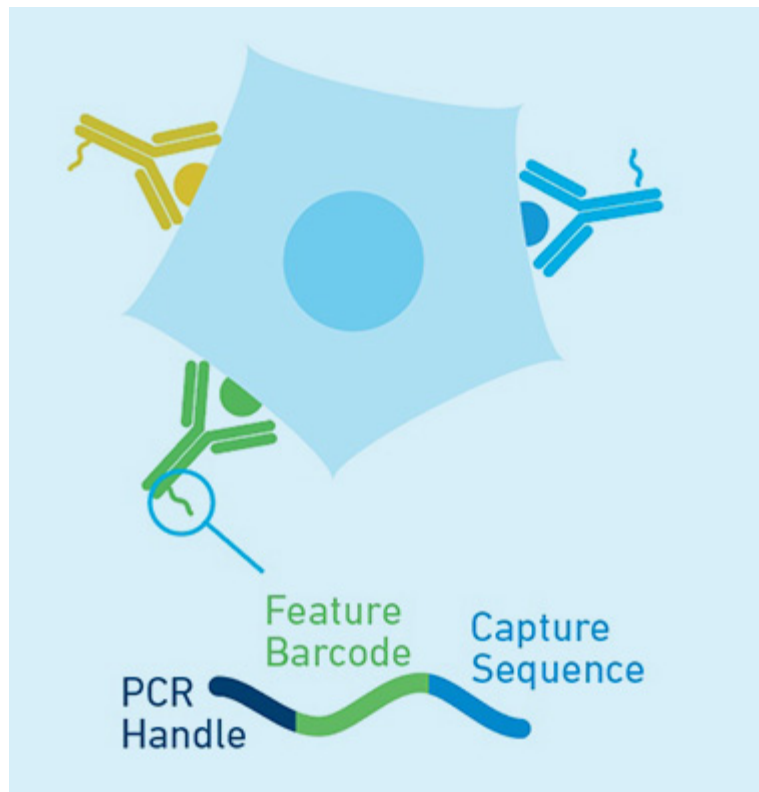
Antigen-specific B cell sorting **1**



Single cell solution (10X) **2**

# DNA Barcoding: An Established Approach for Single-Cell Protein Surface Marker Screening

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Stoeckius et al., Nature Methods, 2017

Peterson et al., Nature Biotechnology, 2017

Shahi et al., Scientific Reports, 2017

Stoeckius et al., Genome Biology, 2018

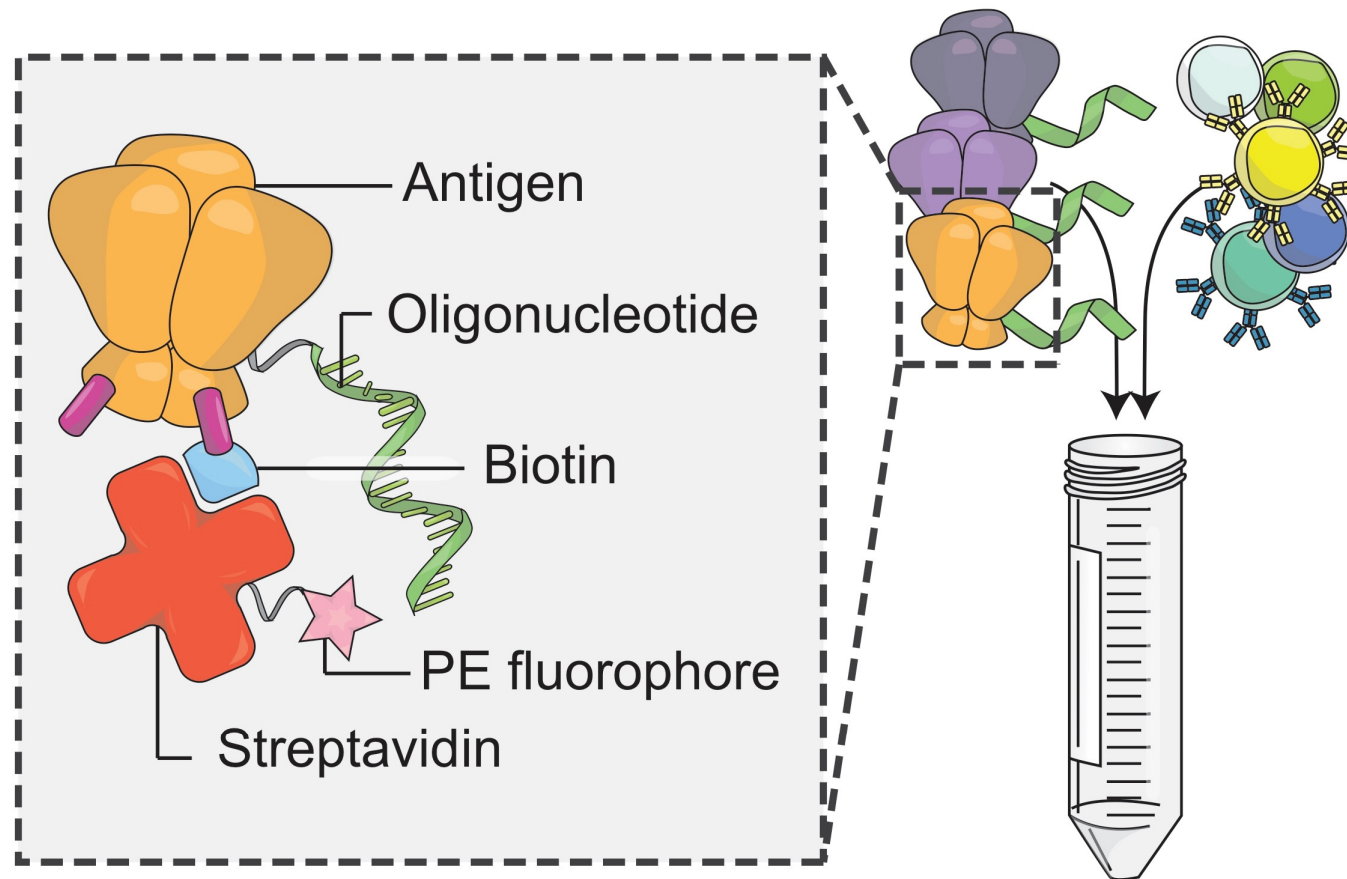
Zhang et al., Nature Biotechnology, 2018

Mimitou et al., Nature Methods, 2019

Stuart et al., Cell, 2019



# LIBRA-seq: Linking B-cell Receptor to Antigen Specificity through Sequencing



# Overview of LIBRA-seq Workflow

Step 1

Mix **B cells** with **barcoded antigens**

Step 2

Antigen-specific B cell **sorting**

Step 3

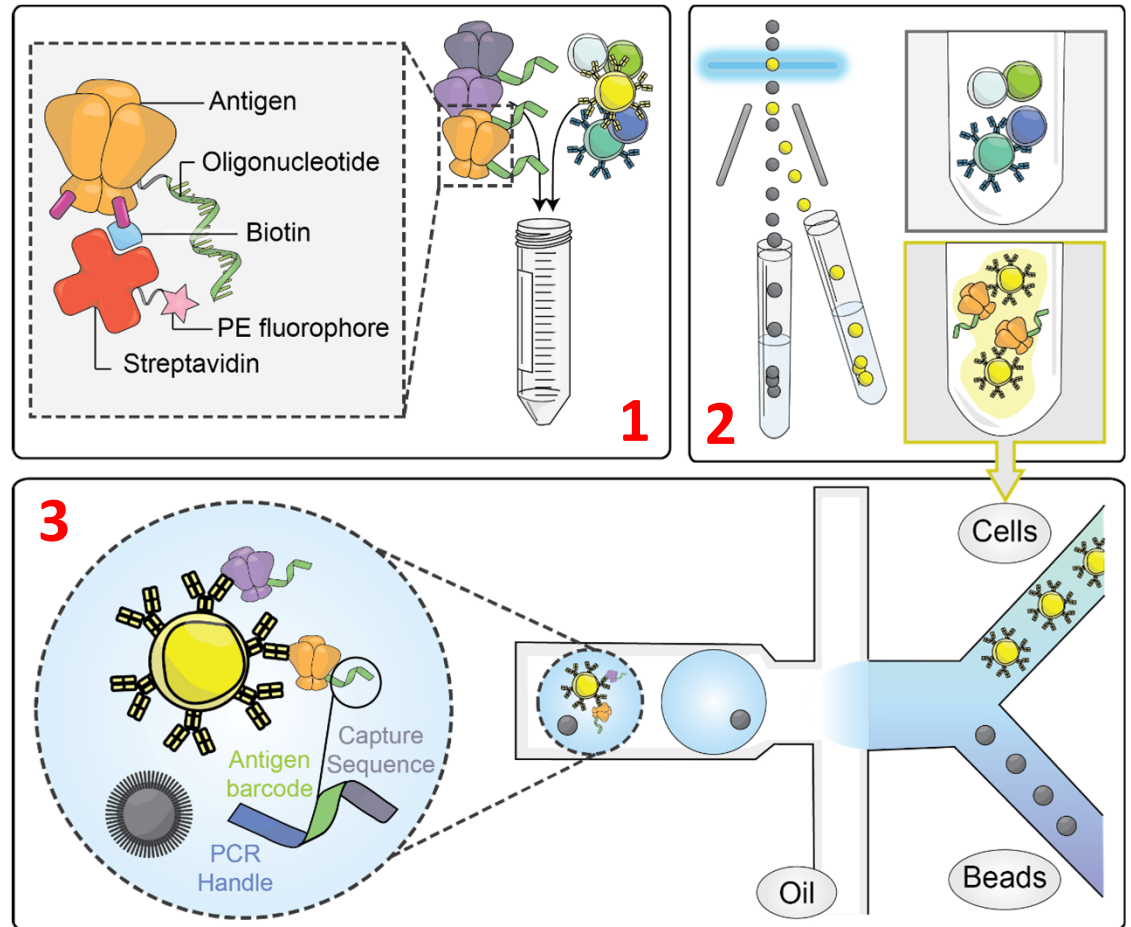
**Single cell** solution (10X)

Step 4

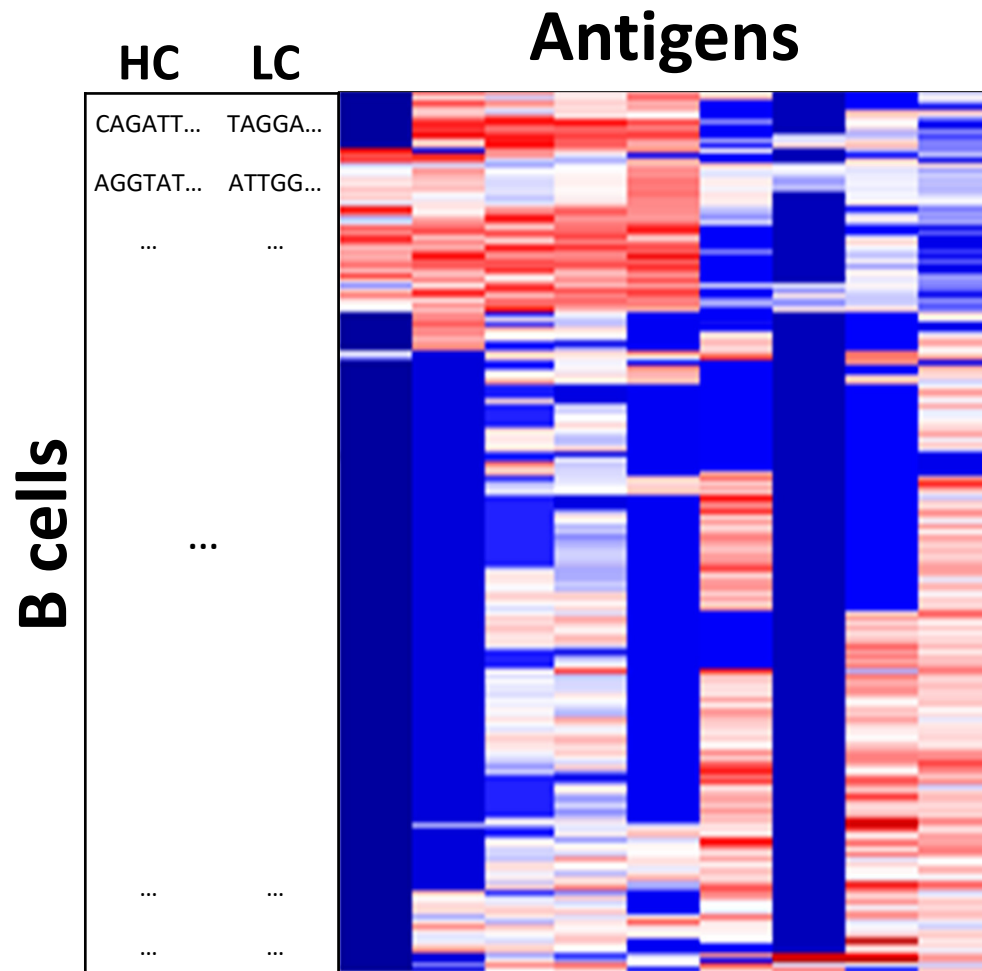
**NGS** (NovaSeq)

Step 5

**Bioinformatics**



# LIBRA-seq Output



LIBRA-seq scores:

high

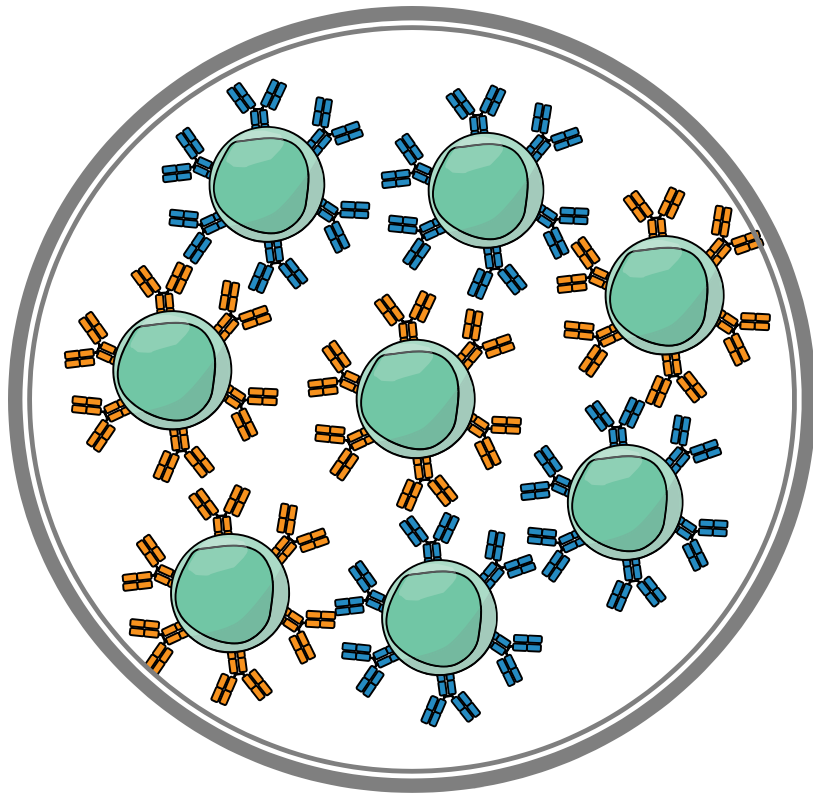
low

- High-resolution **antigen specificity map** for a large number of B cells

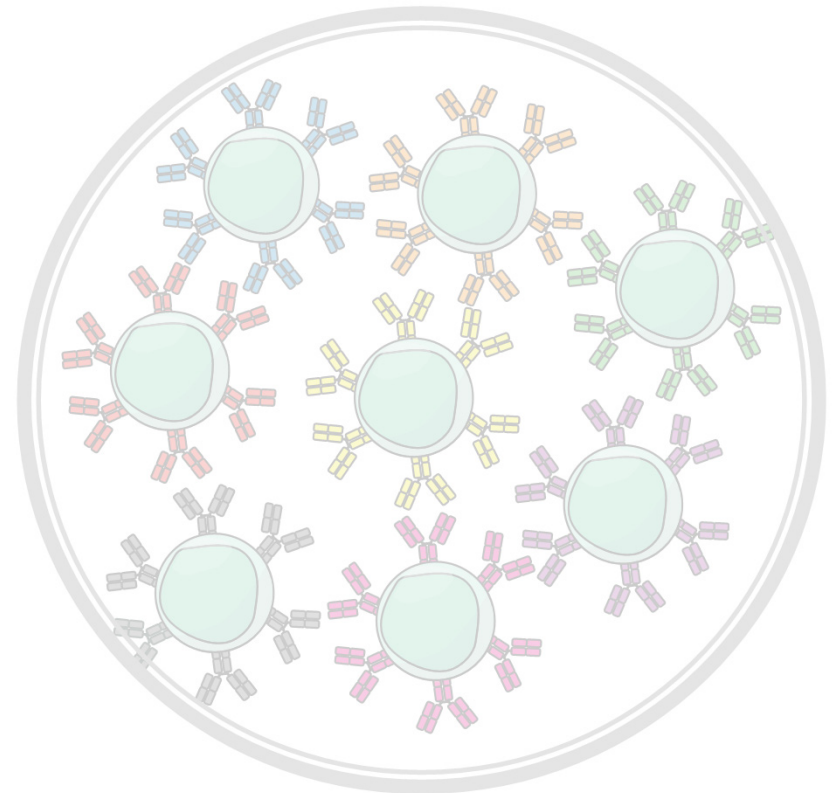
# Does LIBRA-seq Actually Work in Practice?

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**B cell lines with known  
BCR sequence and antigen specificity**

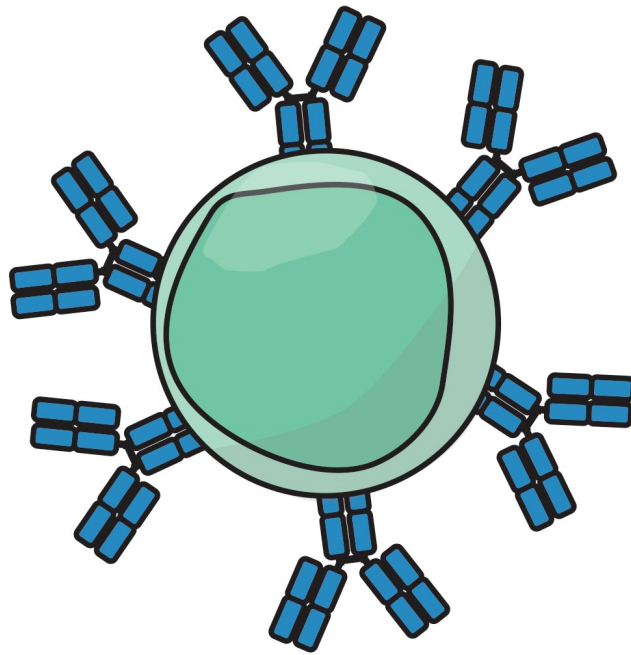


Human infection samples

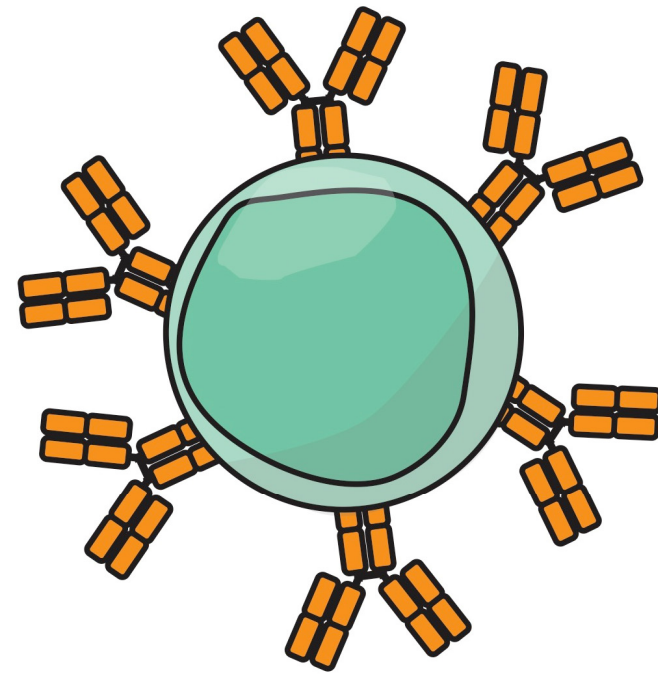


# LIBRA-seq Validation: B Cell Lines with Known BCR and Antigen Specificity

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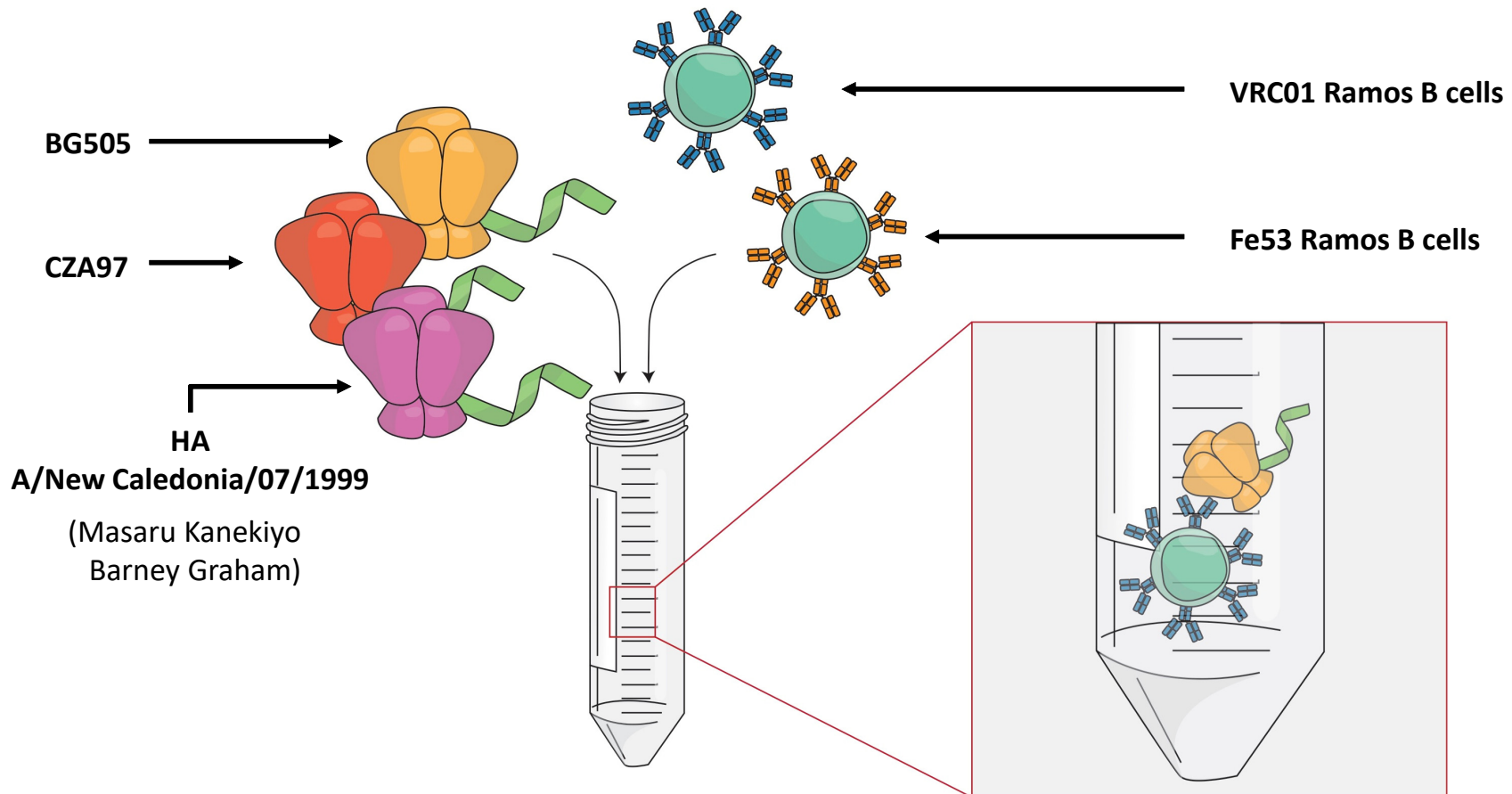


VRC01 Ramos B cells  
(HIV)



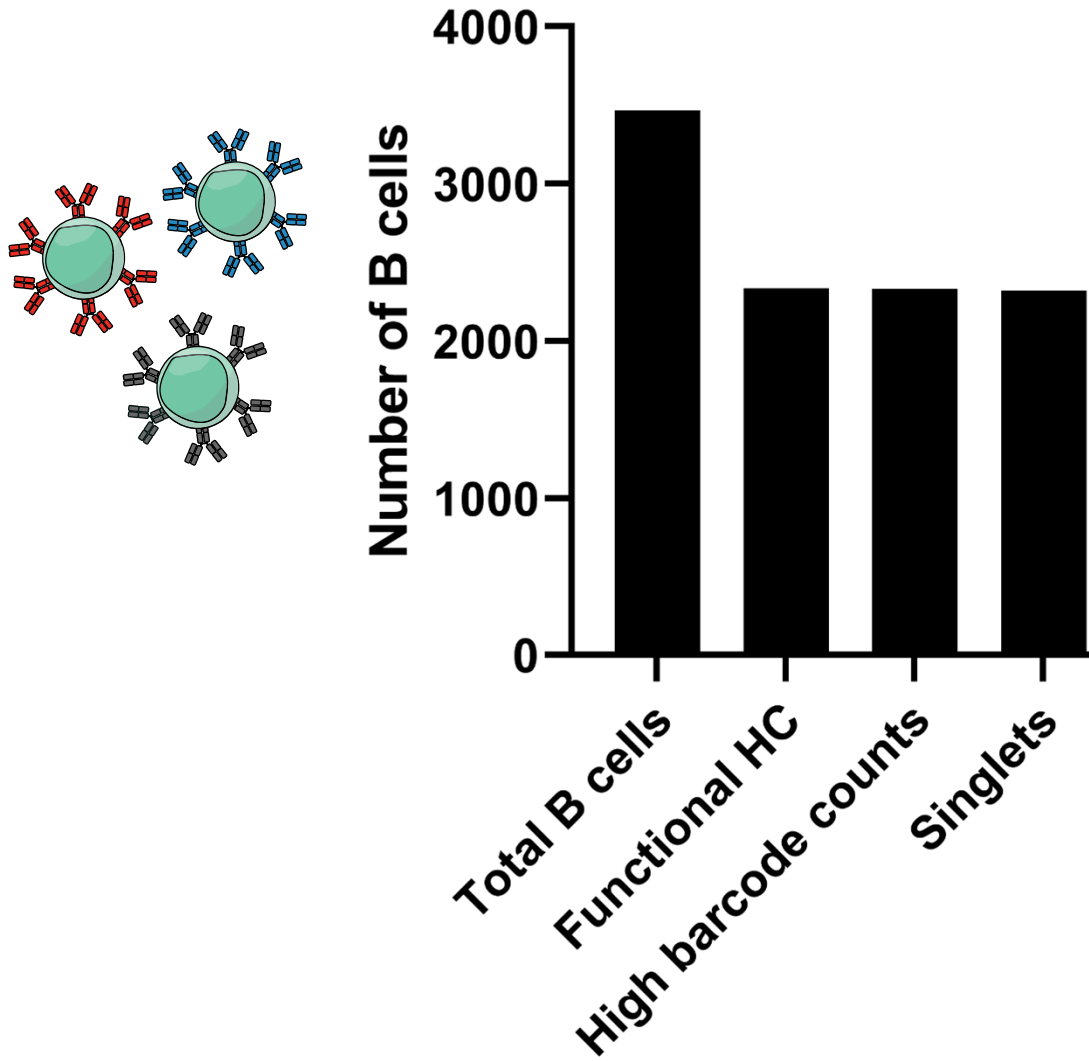
Fe53 Ramos B cells  
(Influenza)

# LIBRA-seq Validation: B Cell Lines with Known BCR and Antigen Specificity



# LIBRA-seq Successfully Recovered Thousands of Antigen-Specific B Cells

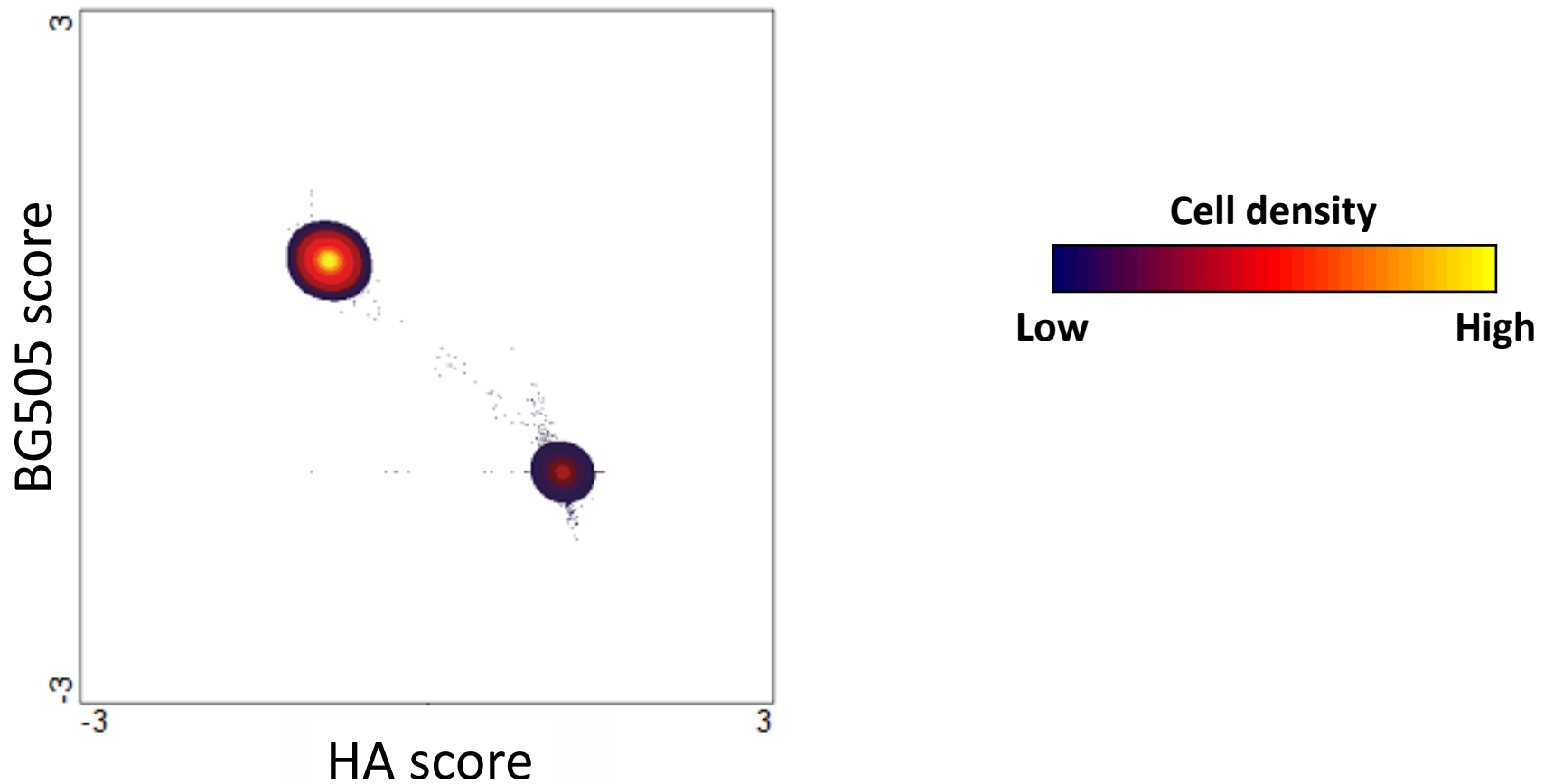
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# LIBRA-seq Can Successfully Discriminate between Different Types of Antigen Specificity

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## Antigen-specific LIBRA-seq scores

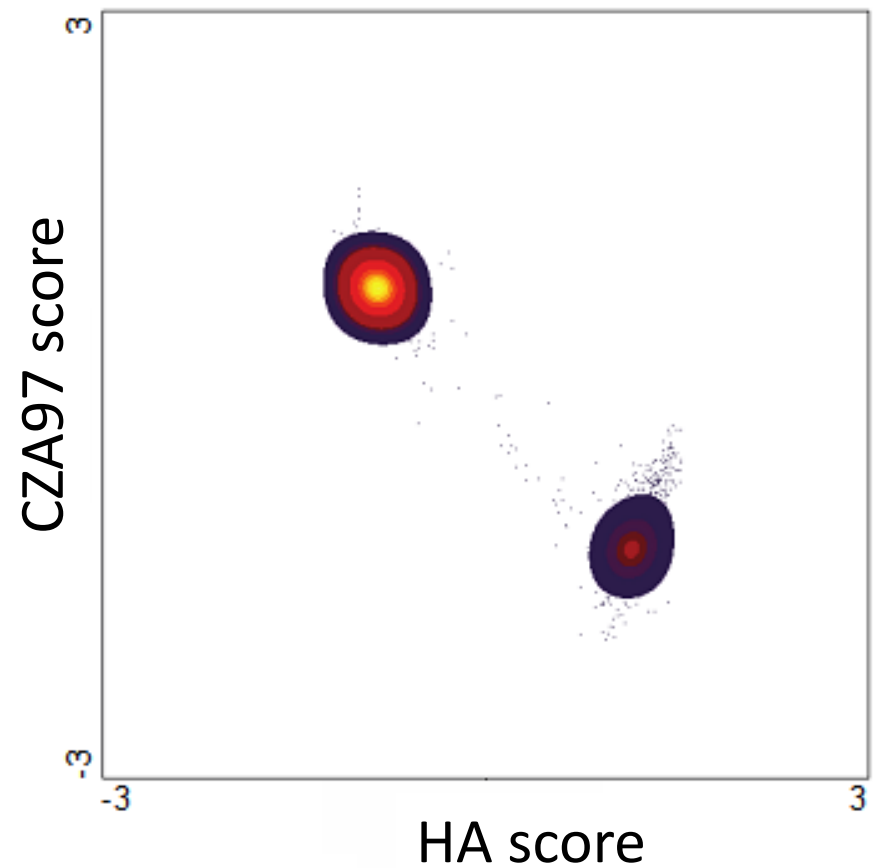
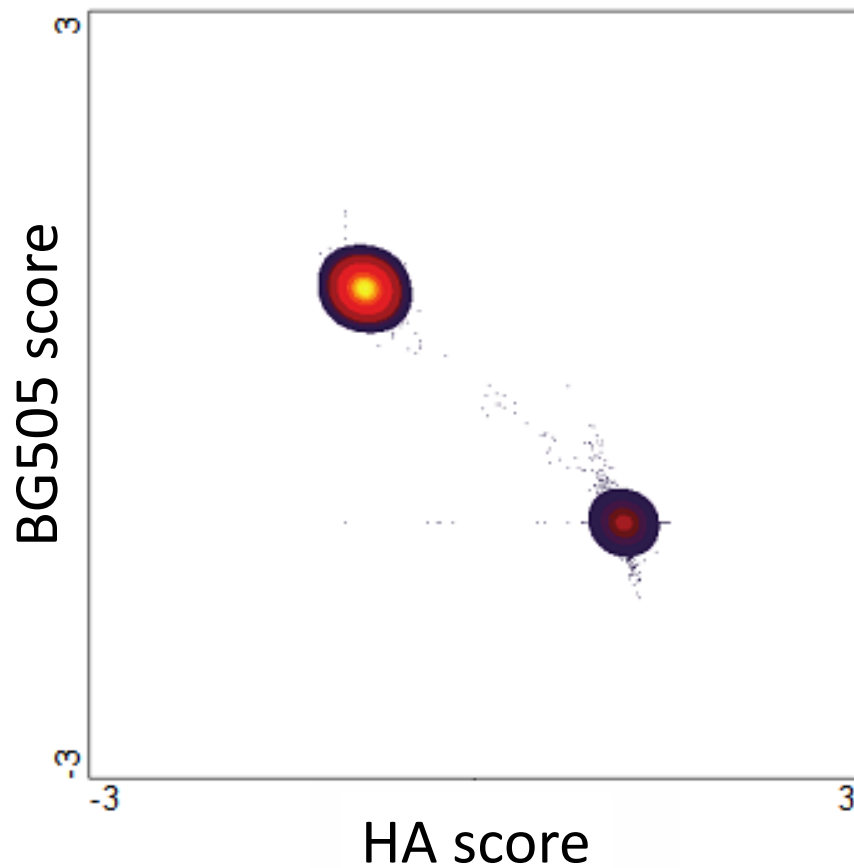




# LIBRA-seq Can Successfully Discriminate between Different Types of Antigen Specificity

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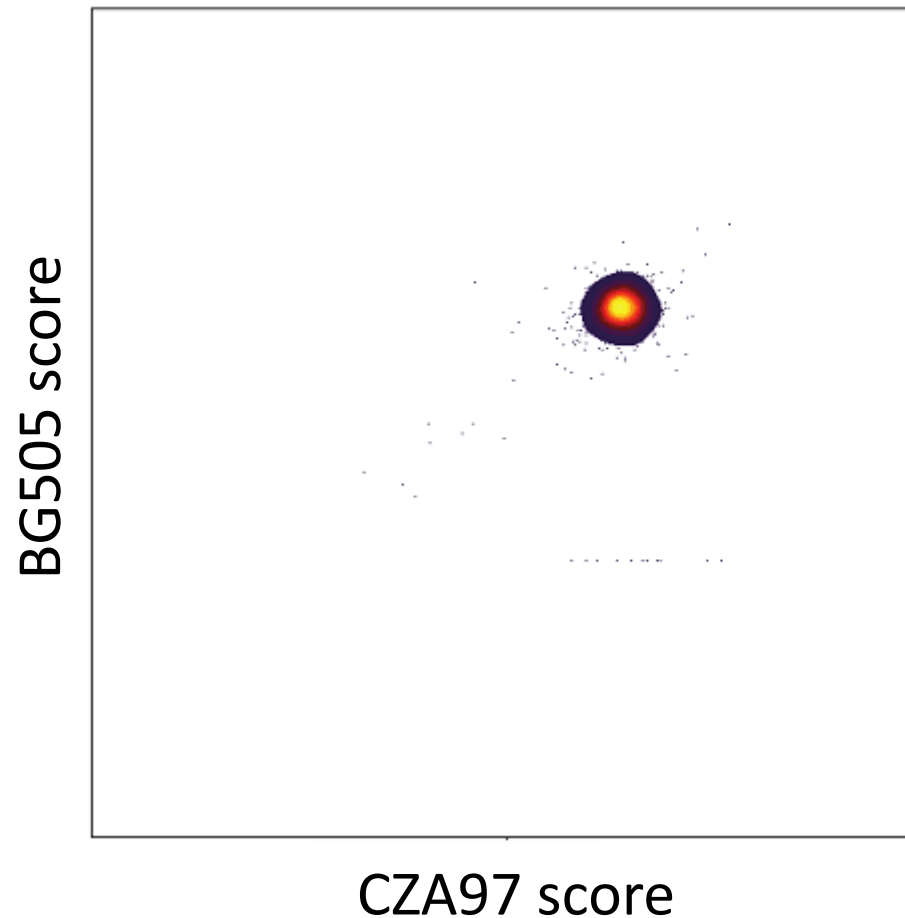
## Antigen-specific LIBRA-seq scores



# LIBRA-seq Can Be Used to Identify Cross-Reactive B Cells

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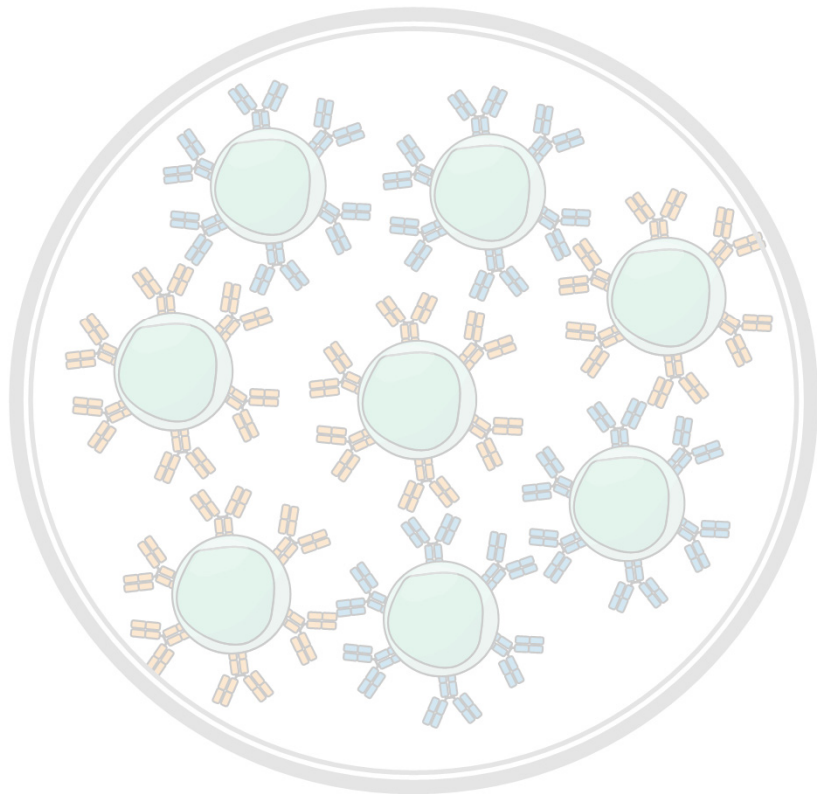
VRC01 cells



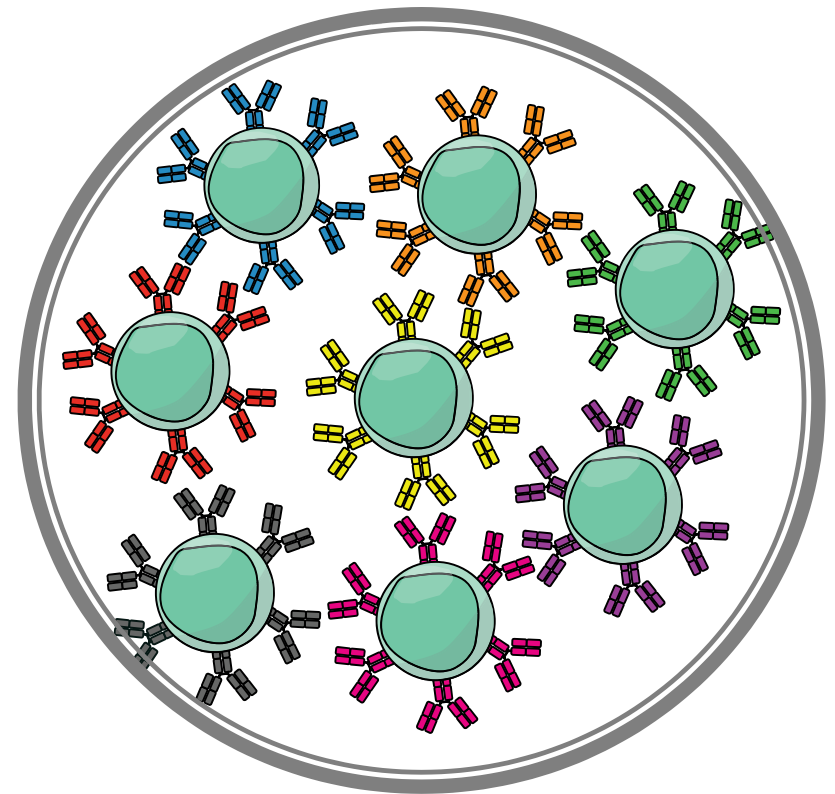
# Does LIBRA-seq Actually Work in Practice?

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B cell lines with known  
BCR sequence and antigen specificity



Human infection samples



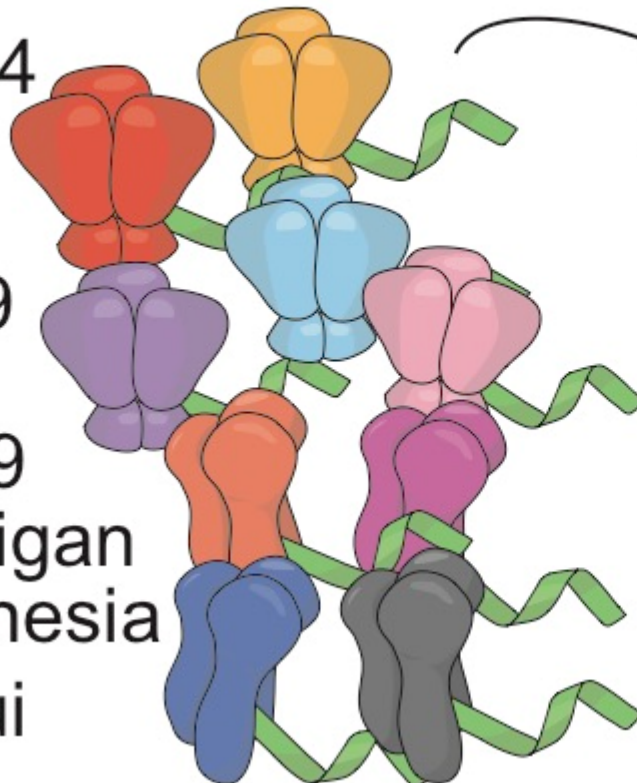
# HIV-1 Broadly Neutralizing Antibody Discovery Using LIBRA-seq

9 antigens

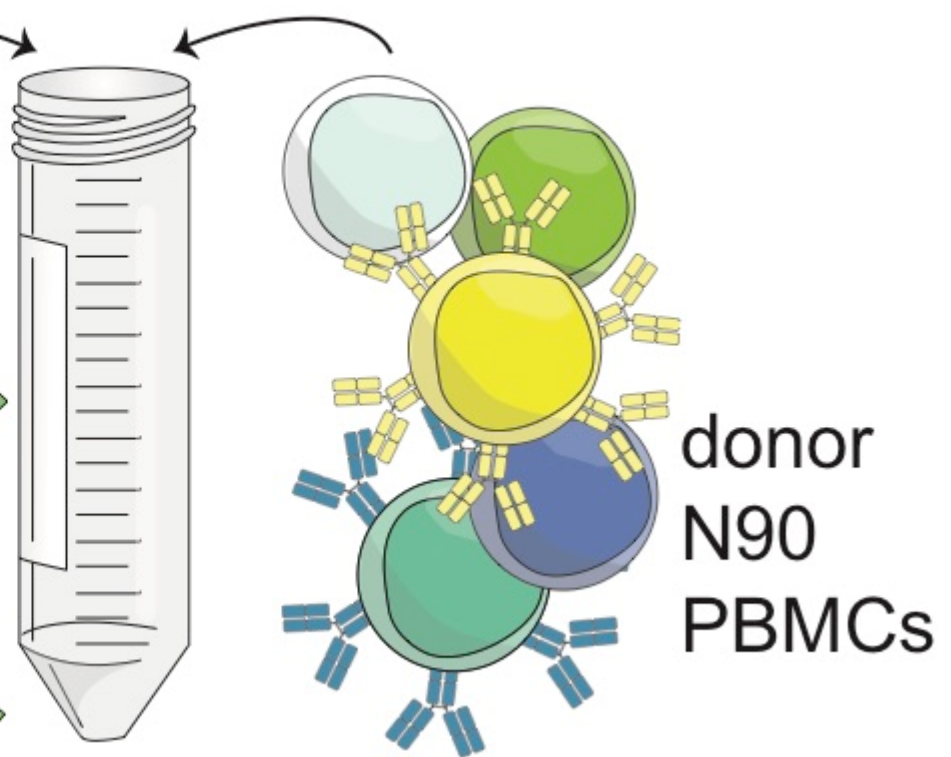
Chronic HIV Infection

Antigen Screening Library

KNH1144  
BG505  
ZM197  
ZM106.9  
B41  
H1 NC99  
H1 Michigan  
H5 Indonesia  
H7 Anhui

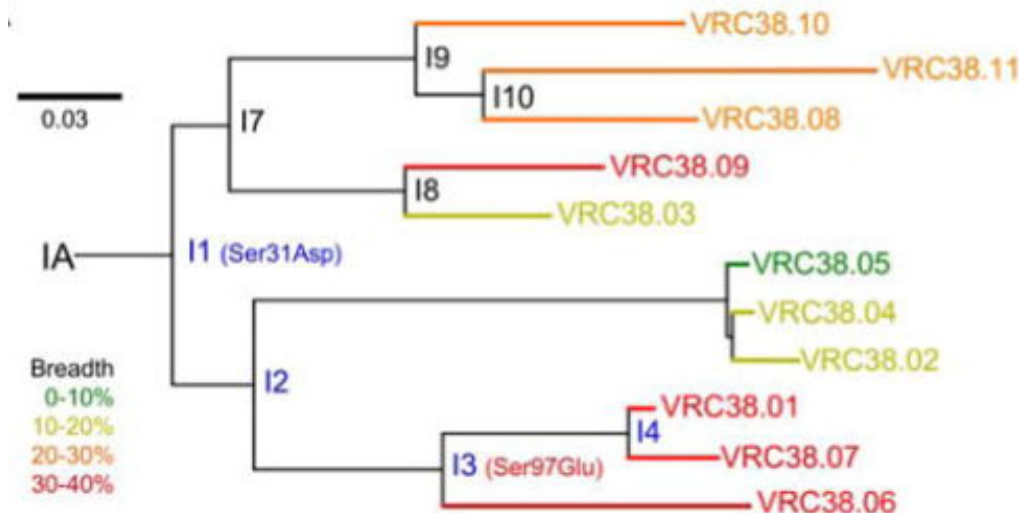


Cellular Input



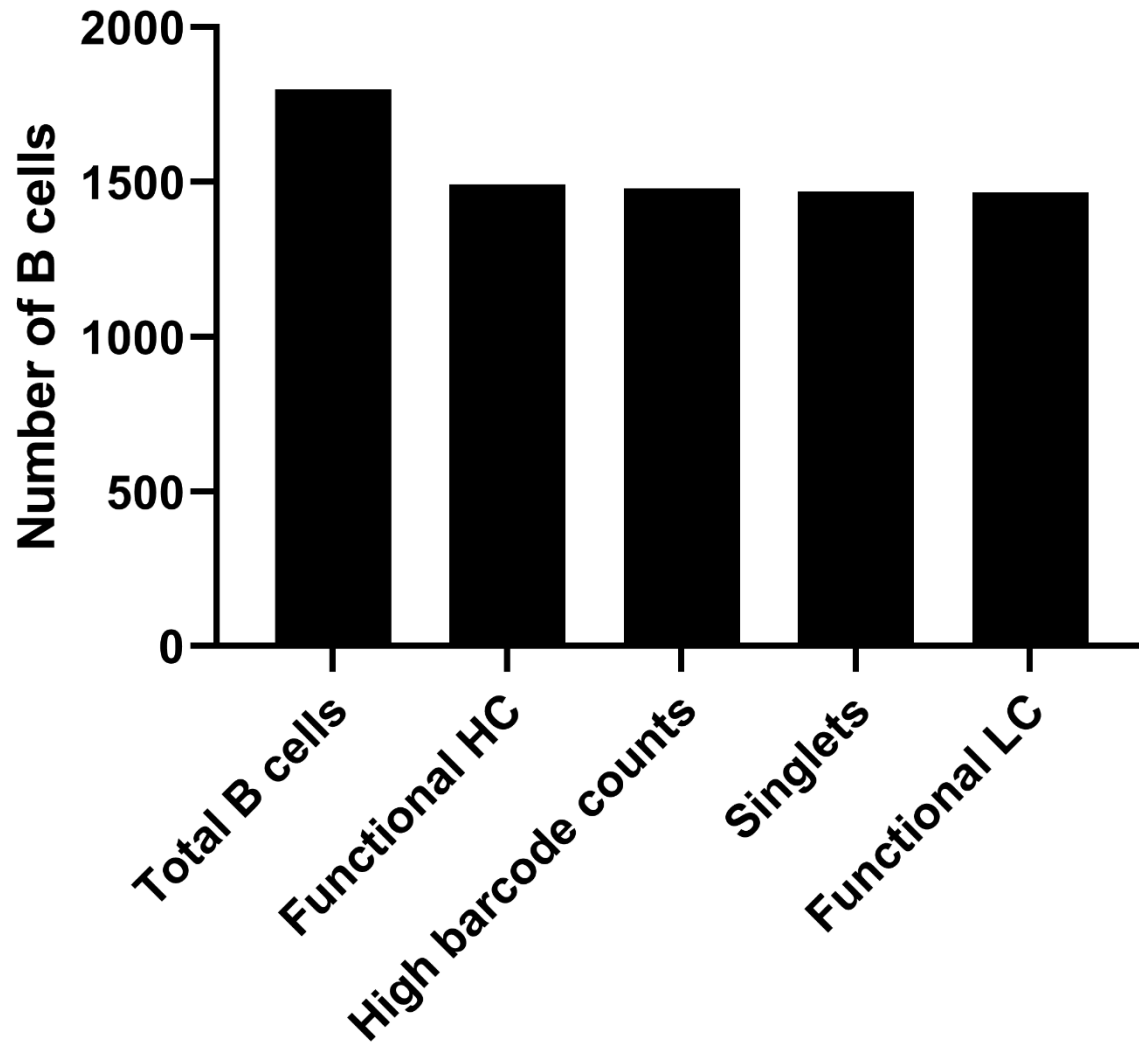
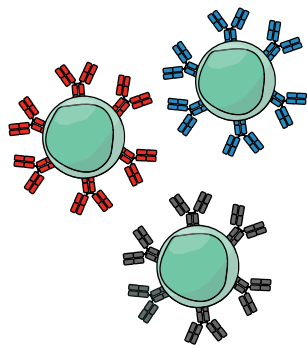
# LIBRA-seq Analysis of Donor N90

- Chronic infection
- bNAb lineage with limited neutralization breadth (30%)
- Can we identify antibodies from the VRC38 lineage with LIBRA-seq?
- Can we identify new – and broader – bNAbs?

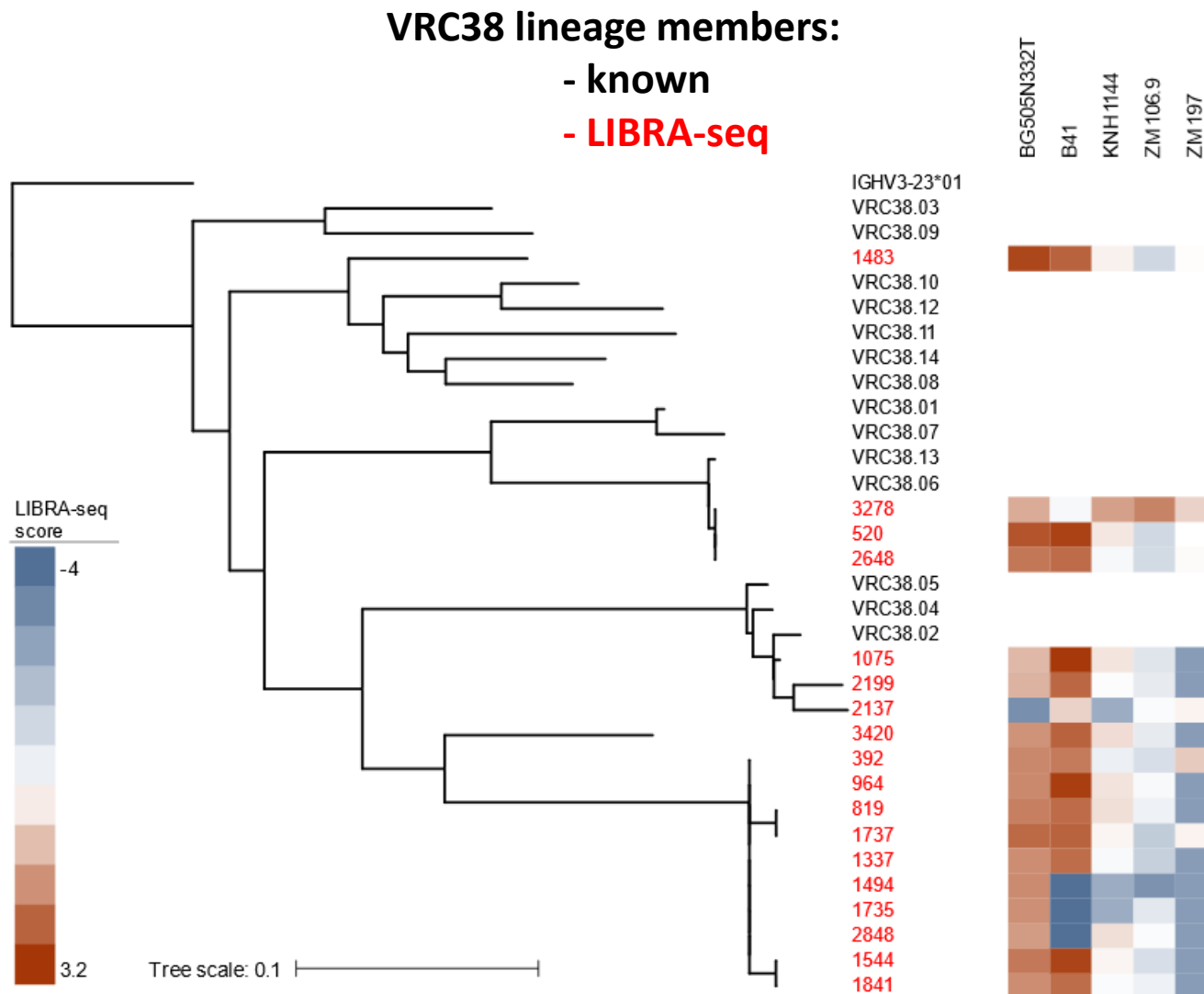


# LIBRA-seq Recovered ~1,500 Antigen-Specific B cells from a Donor N90 Sample

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# LIBRA-seq Identified 18 VRC38 Lineage Members

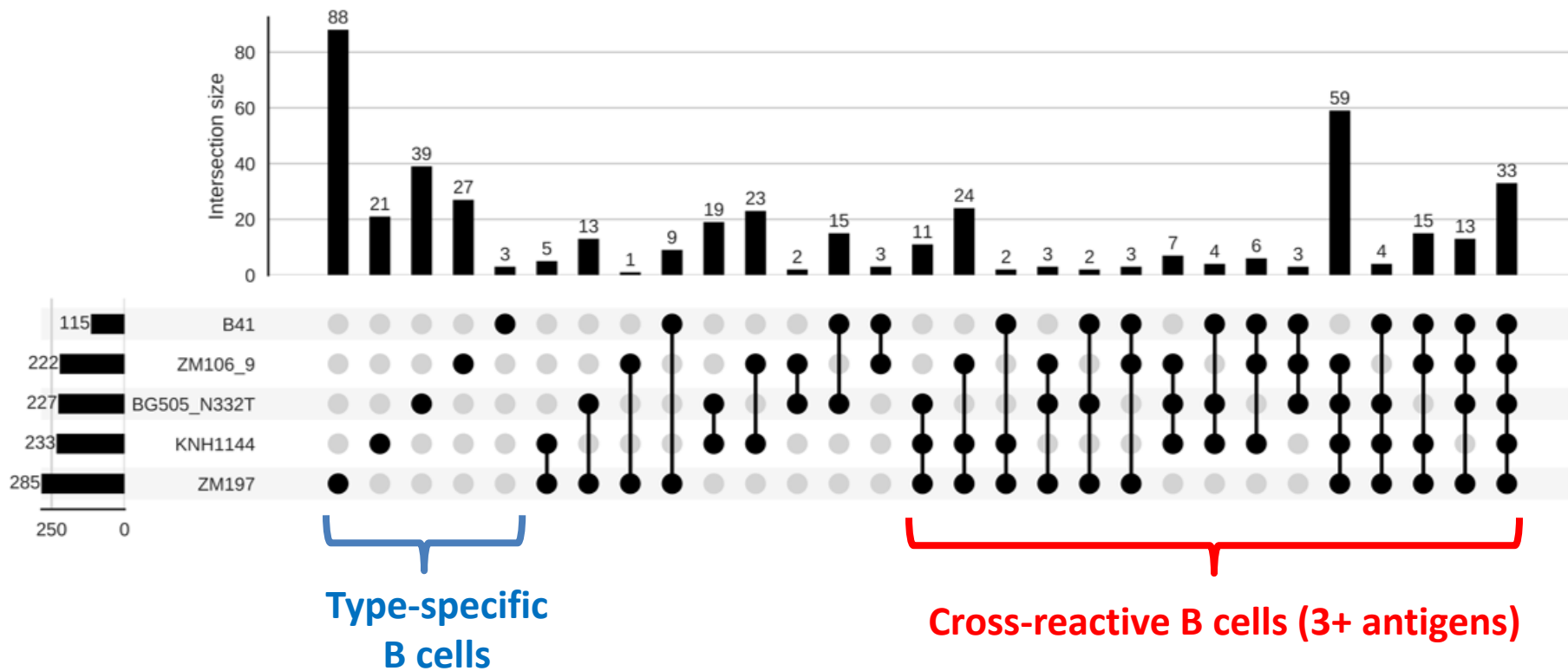


# **Can We Identify Antibodies with Greater Neutralization Breadth?**

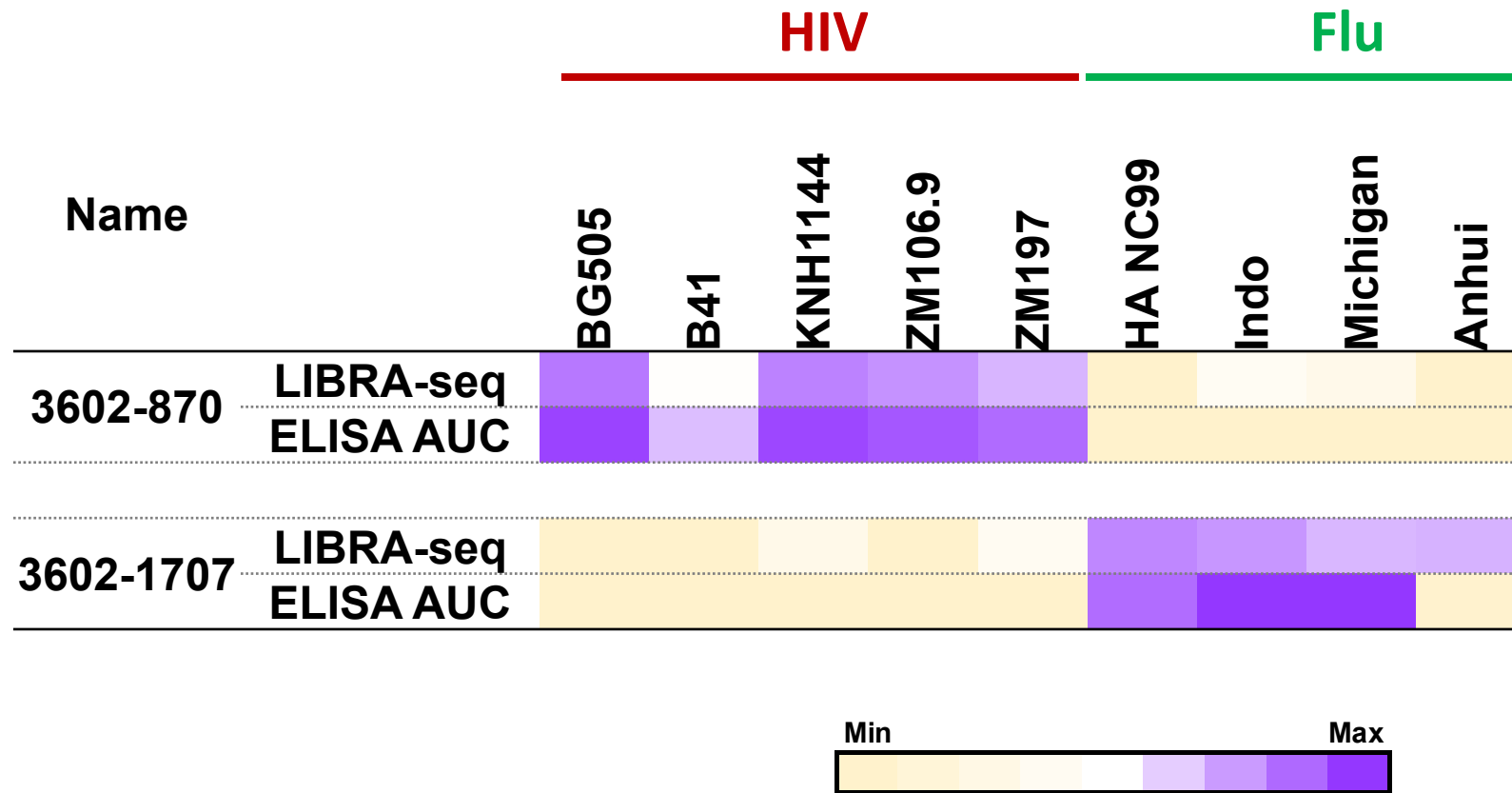
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# HIV-specific B Cells Indicate Different Levels of Cross-Reactivity



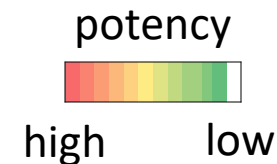
# LIBRA-seq Scores Correlate with mAb Antigenicity



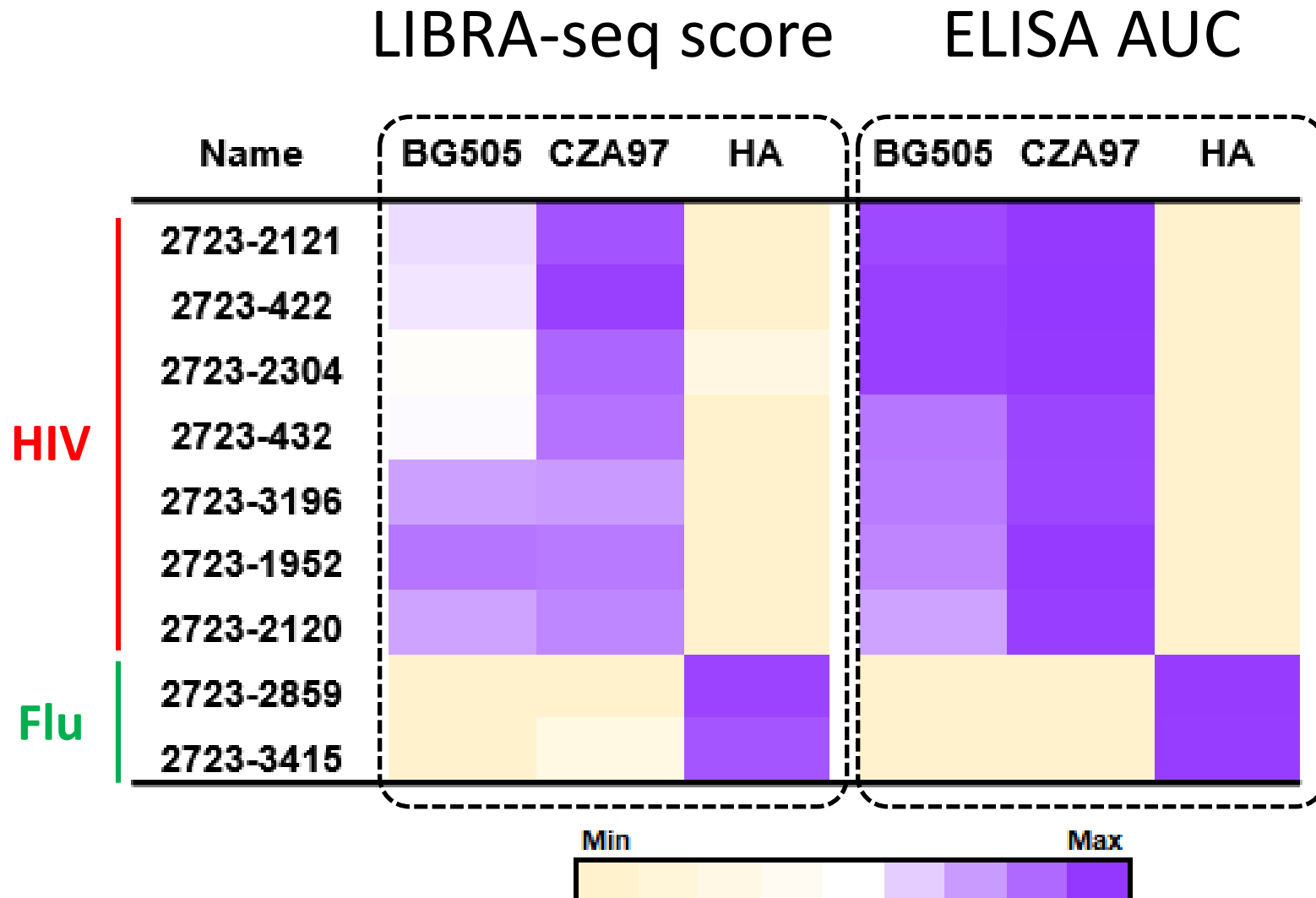
LIBRA-seq scores and ELISA AUC were highly correlated for these mAbs

# Antibody 3602-870 Has Greater Neutralization Breadth than the VRC38 Lineage

| Virus Phenotype          | Strain name     | Virus clade | 3602-870 | VRC38.01 |
|--------------------------|-----------------|-------------|----------|----------|
| Tier 2<br>(Global Panel) | TRO_11          | B           | 0.176    | >50      |
|                          | CH119_10        | BC          | 0.506    | >50      |
|                          | CNE55           | AE          | >25      | >50      |
|                          | 25710_2_43      | C           | 0.321    | >50      |
|                          | CE0217          | C           | 0.177    | 0.073    |
|                          | X1632_S2_B10    | G           | >25      | >50      |
|                          | X2278_C2_B6     | B           | 0.066    | 1.88     |
|                          | 246_F3_C10_2    | AC          | 0.704    | 19       |
|                          | 398_F1_F6_20    | A           | >25      | >50      |
|                          | CE1176_A3       | C           | 1.033    | >50      |
|                          | BJOX002000_03_2 | BC          | 0.360    | 1.43     |



# LIBRA-seq Scores Correlate with mAb Antigenicity: a Second HIV Infection Sample



# LIBRA-seq: Linking B-cell Receptor to Antigen Specificity through Sequencing

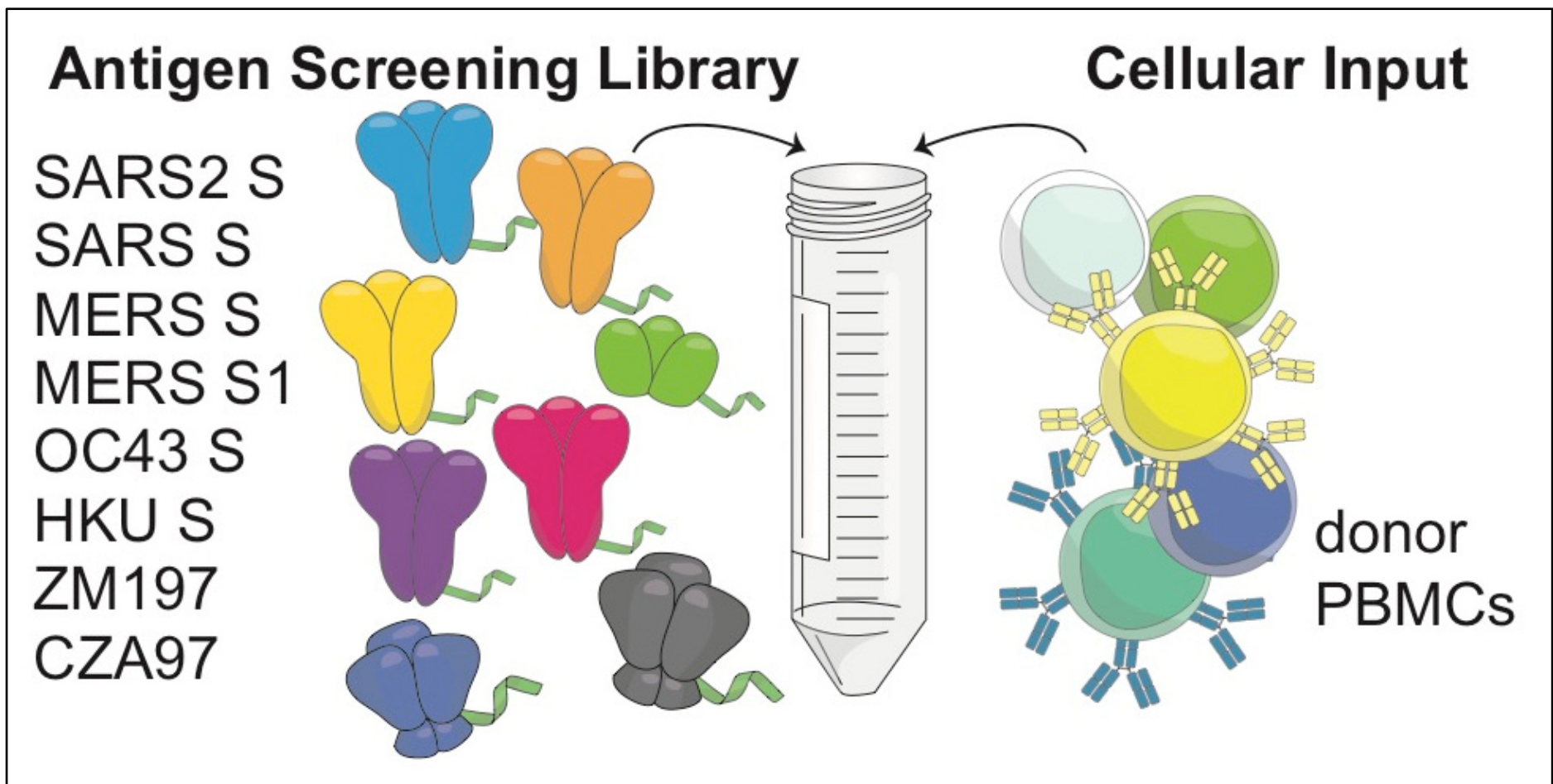
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| Technique                | Sequencing depth | Paired H-L sequences | Number of antigens | Antigen specificity map |
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| Display technologies     | $10^2$ - $10^3$  | ✓                    | sequential sorting |                         |
| <b>LIBRA-seq</b>         | $10^4$ - $10^5$  | ✓                    | many               | ✓                       |

# LIBRA-seq Development

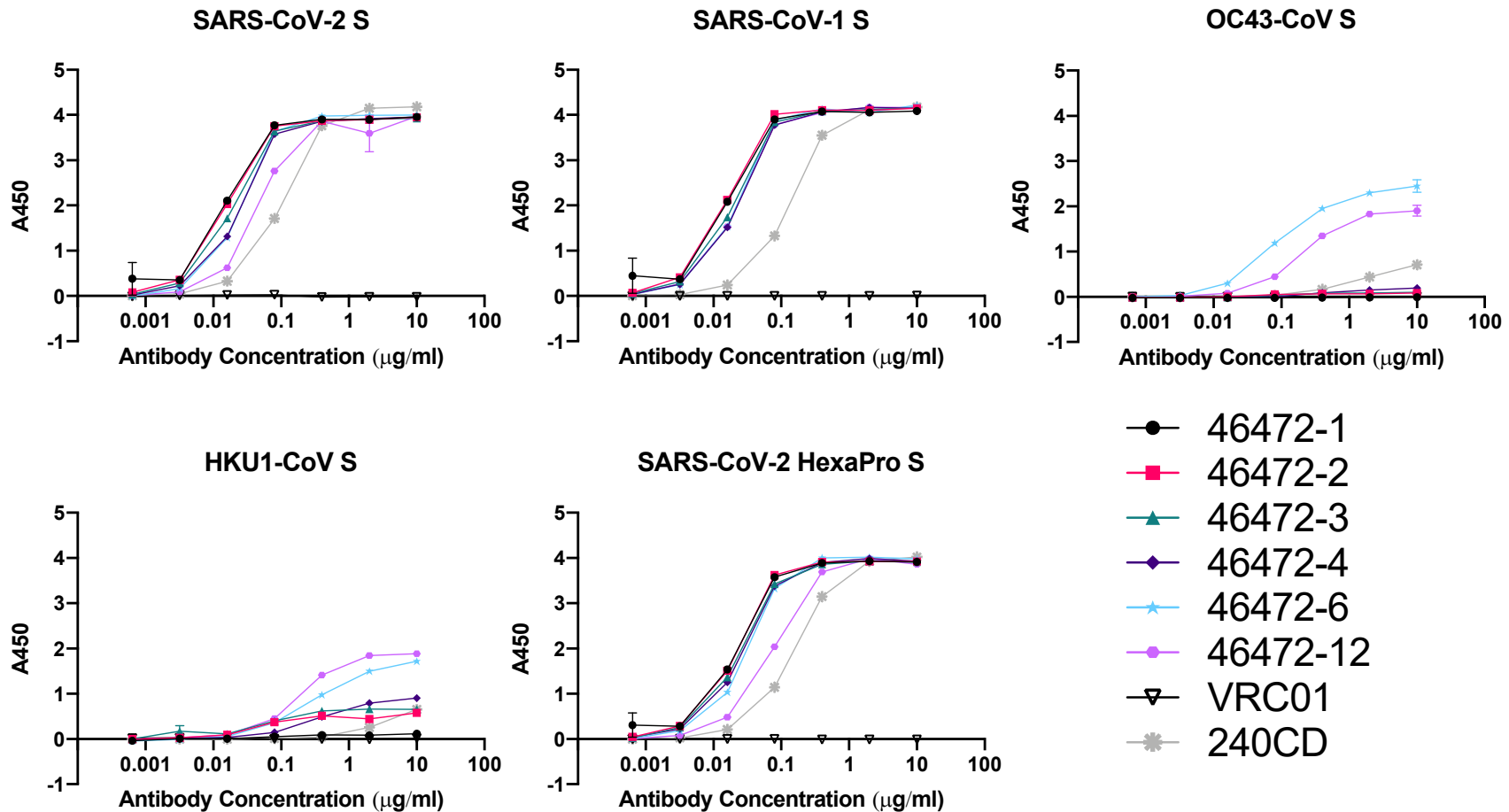


# LIBRA-seq and SARS-CoV-2 Antibody Discovery



- SARS-CoV-1 convalescent sample
- > 10 years post infection
- Diverse CoV antigens from McLellan Lab

# Antibodies with Diverse Antigen Cross-Reactivity Patterns

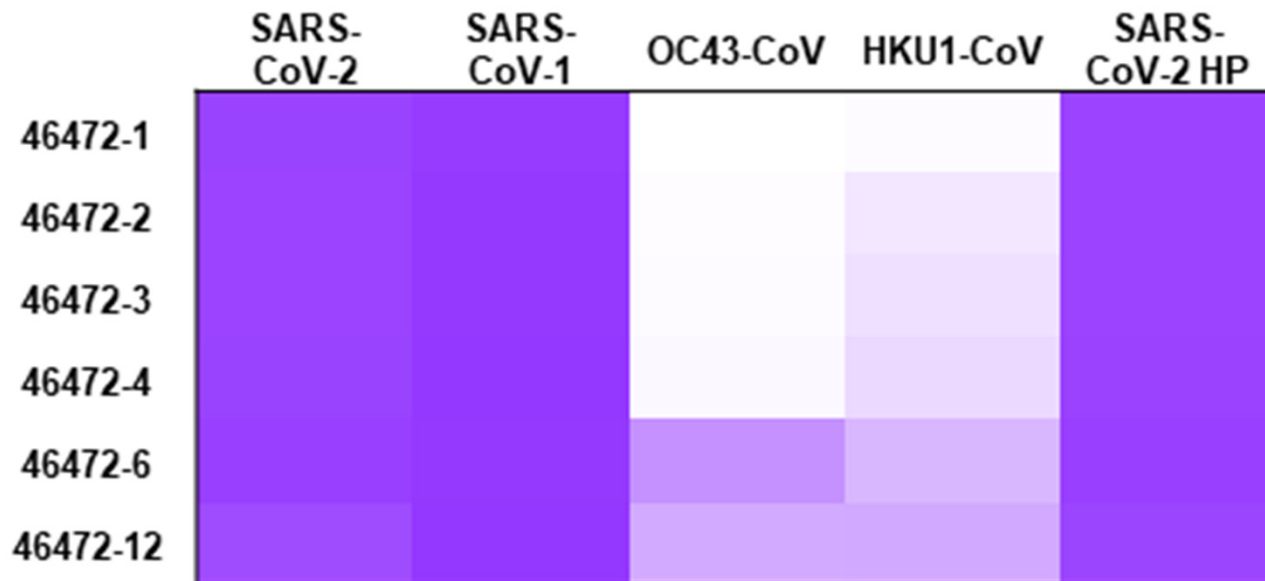




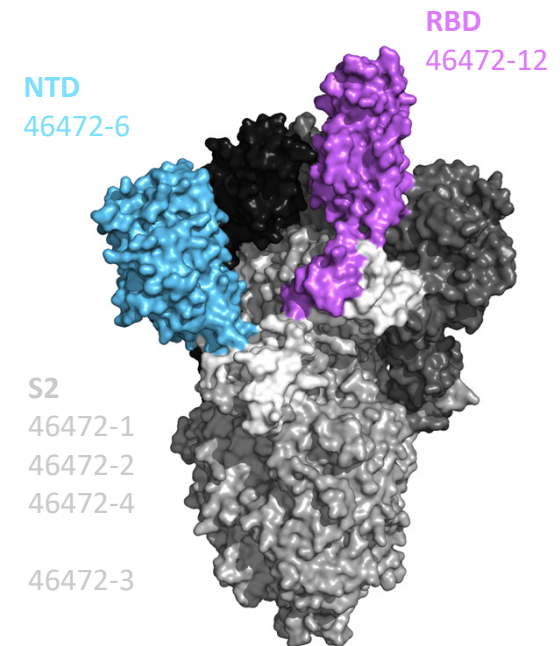
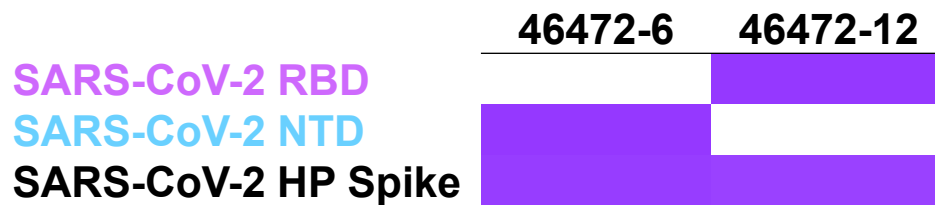
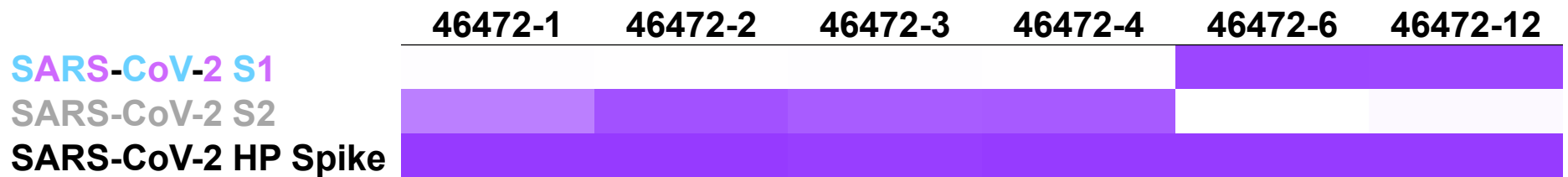
# Antibodies with Diverse Antigen Cross-Reactivity Patterns

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AUC (ELISA Binding)

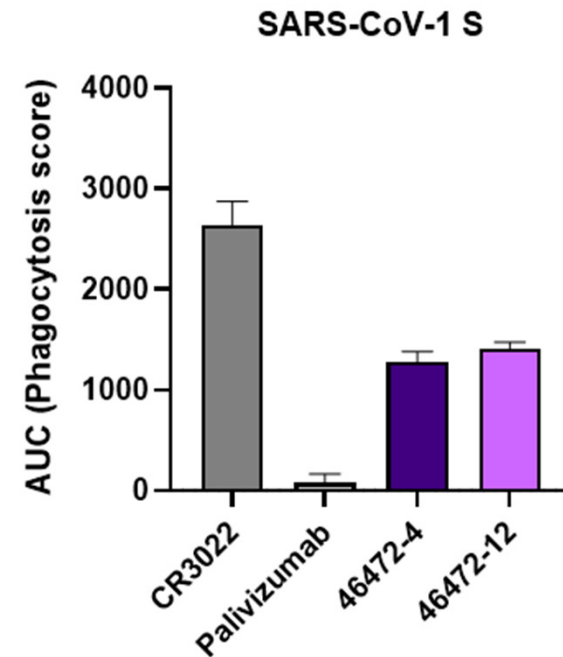
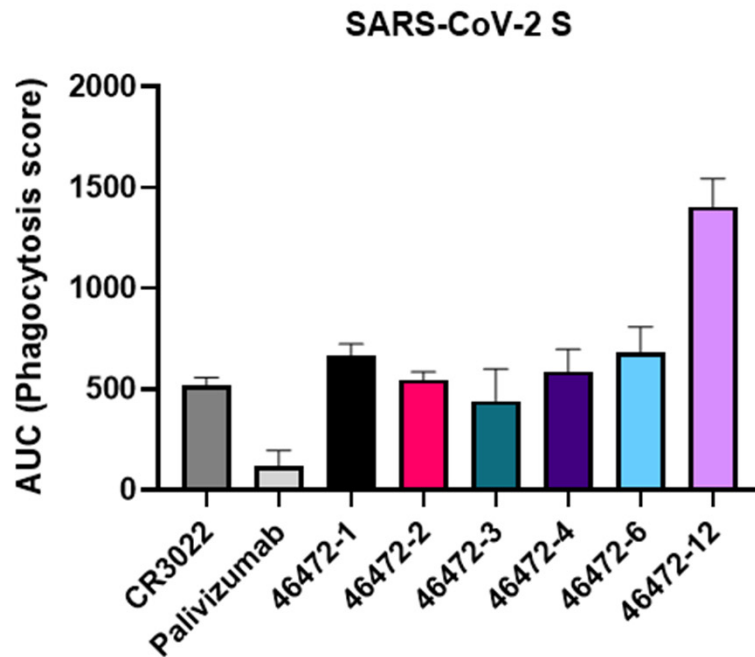


# Cross-Reactive Antibodies Target Diverse Epitopes on SARS-CoV-2 S



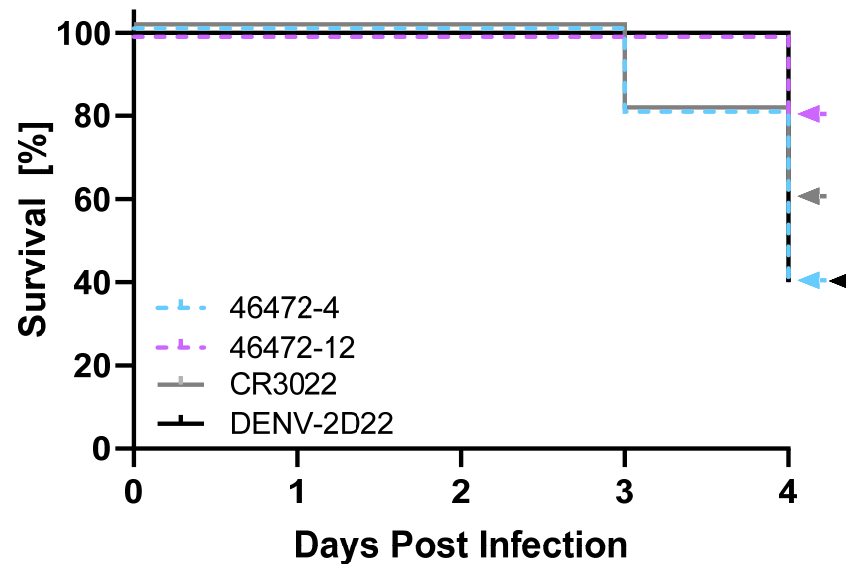
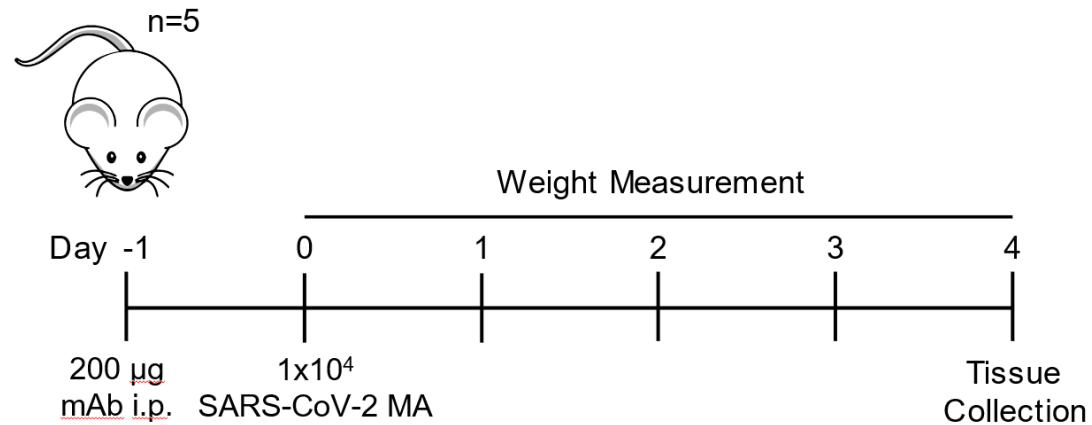
# Cross-Reactive Antibodies Exhibit a Diversity of Fc Effector Functions

## ADCP

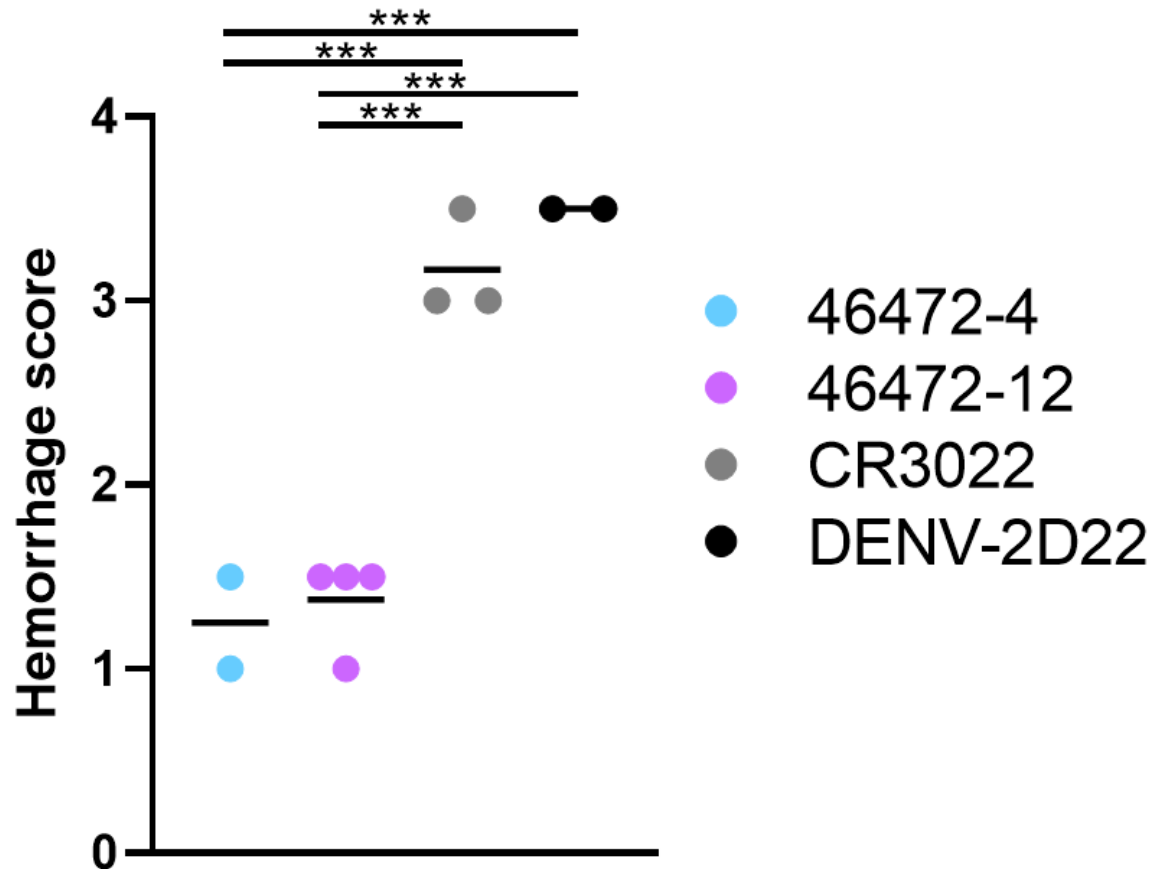


- Also, ADCT for cell-surface expressed antigen

# Cross-Reactive Antibodies Mitigate Lung Hemorrhage in a Prophylaxis Model



# Cross-Reactive Antibodies Mitigate Lung Hemorrhage in a Prophylaxis Model



# Summary of Cross-Reactive CoV Antibodies

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- Identified a set of **cross-reactive** SARS-1/SARS-2 antibodies
- **Diverse** specificities indicate multiple cross-reactive **epitopes** on S
- Non-neut, but **Fc functions**
- Potential *in vivo* effects

# CoV Antibodies



# Acknowledgements



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Rachel Sutton

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Michael Diamond  
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***COVID-19 Fund***



