High-throughput mapping of B cell receptor sequences to antigen specificity

Researchers from Vanderbilt University Medical Center developed LIBRA-seq, a method to rapidly map paired heavy- and light-chain B-cell receptor (BCR) sequences to their corresponding antigens. They studied blood samples from two subjects with HIV, identifying the antigen specificity of thousands of single B cells, and confirming these specificities with both known and novel broadly neutralizing HIV- and influenza-specific antibodies (1). These findings position LIBRA-seq as an integral tool for not only antibody discovery, but also vaccine and immunotherapy development.

Snapshot	10x Genomics product
Research area: Vaccines & Immunotherapies – Antibody Discovery, Infectious Disease **Organism:** Human **Sample type:** Peripheral blood mononuclear cells from two subjects with HIV, and Ramos B-cell lines **Research question:** How can single cell immune sequencing methods be leveraged to link B-cell receptors with their corresponding antigens? How can these methods accelerate antibody discovery?	**Chromium Single Cell Immune Profiling Solution** • Chromium Single Cell 5′ Library & Gel Bead Kit • Chromium Single Cell V(D)J Enrichment Kit, Human B Cell • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines

Experiment overview

LIBRA-seq method and validation

- Validated LIBRA-seq on two engineered B-cell lines expressing BCR sequences of the known neutralizing antibodies VRC01 (targeting HIV-1) and Fe53 (targeting influenza)
- Conjugated barcode oligos to fluorophore-tagged antigens, mixed antigens and B cells, then performed flow cytometry to isolate antigen-positive B cells
- Performed single cell immune sequencing, recovering 2,321 cells with paired, full-length BCR sequences from both IgH/L and antigen mapping information

Isolation of antibodies from a known HIV bNAb lineage from donor NIAID45

- Used validation scheme to perform experiments with samples from human donor
- Analyzed antibody repertoire of donor against oligo-barcoded antigen screening library, recovering 866 cells with paired, full-length BCR sequences from both IgH/L and antigen mapping information, then calculated LIBRA-seq scores
- Confirmed ability of a subset chosen based on phylogenetic analysis of identified antibodies to bind antigen probes and verified binding with ELISA
- Tested neutralization potency of antibody lineage members on tier 1, tier 2, and control viruses

Identification of additional anti-HIV and anti-influenza antibodies from donor NIAID45

- Produced an additional seven putative HIV-specific monoclonal antibodies and two influenza-specific monoclonal antibodies from donor
- Compared patterns of LIBRA-seq scores and ELISA area under the curve (AUC) values to confirm antigen binding

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

- Screened B cells from donor against nine antigens—five HIV-1 Env trimers and four diverse HA trimers
- Recovered paired, full-length BCR sequences from both IgH/L and antigen mapping information for 1,465 cells, including 18 B cells of the known VRC38 lineage
- Focused analysis on one anti-HIV member, 3602-870, from a novel antibody lineage that had a high LIBRA-seq score and was heavily mutated in both the heavy- and light-chain V genes
- Confirmed antigen binding with ELISA and neutralization potency against tier 2 viruses

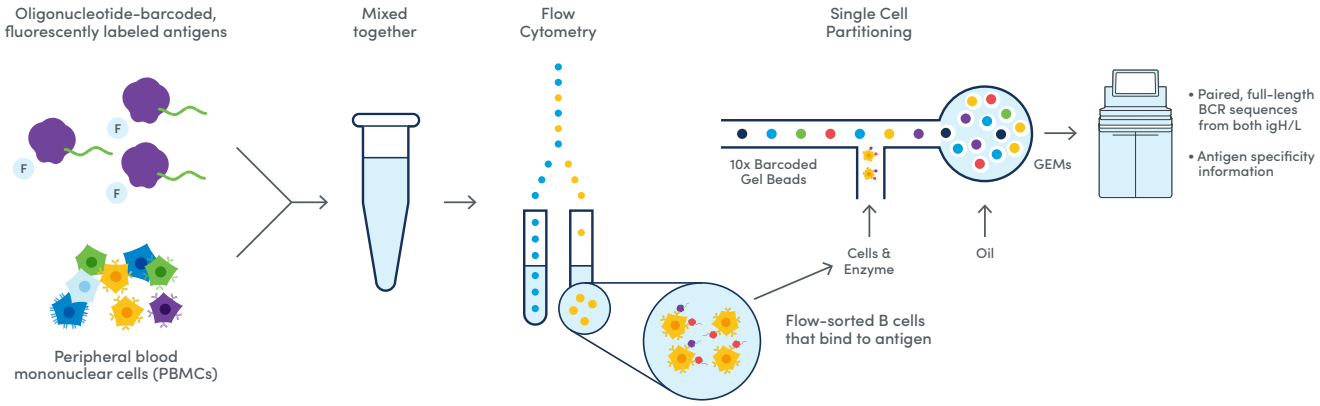

Figure 1. LIBRA-seq Workflow. The antigen screening library is mixed with donor PBMCs. Antigen-binding B cells are sorted via flow cytometry, then partitioned into oil droplets with oligonucleotide-barcoded gel beads and enzymes that drive a reverse transcription reaction. Both cellular BCR transcripts and antigen barcodes are captured by bead-delivered oligos, enabling direct mapping of BCR sequence to antigen specificity following sequencing.

Why single cell?

There are billions of B cells in the human body, each of which produces a single species of antibody with a unique epitope specificity. Many traditional methods to characterize antigen-specific B cells use fluorescence to determine binding of antibody to antigen and are low throughput, limiting the number of antigens against which B cells can be simultaneously screened due to spectral overlap. The LIBRA-seq method overcomes this limitation, enabling analysis of antibody–antigen interactions via a sequencing-based readout (instead of a fluoresce readout) in single cells, and giving researchers broad access to study and leverage the human body's antibody diversity. Importantly, this method pairs full-length heavy- and light-chain antibody sequences at the single cell level, enabling production of recombinant antibodies.

Computational analysis

The research team used Cell Ranger to process BCR sequence and antigen barcode reads. They developed a pipeline that uses FASTQ files of oligo libraries to generate a cell barcode—antigen barcode UMI count matrix. This matrix allowed them to count how much of a unique oligo-barcoded antigen bound to the B-cell receptors of a particular B cell, and derive a LIBRA-seq score after transforming and scaling the matrix data. They leveraged this LIBRA-seq score to predict antigen specificities of each cell and prioritize further study on promising monoclonal antibody leads.

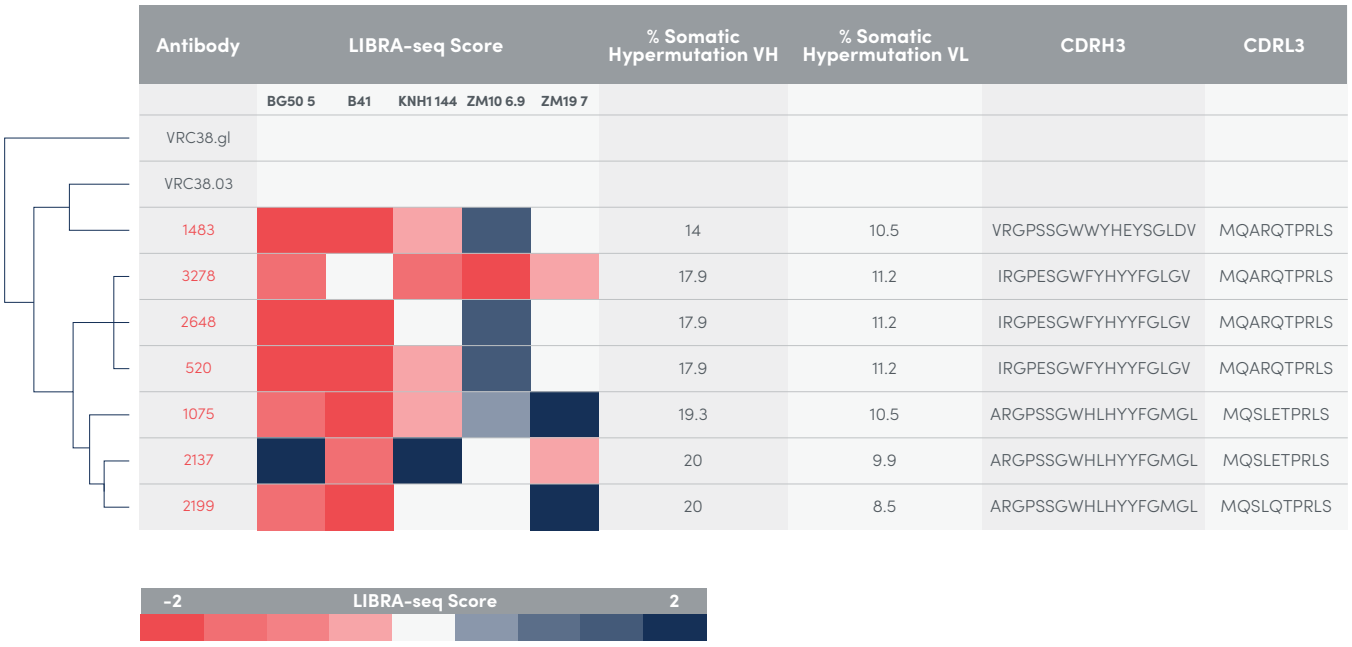

Figure 2. Aligning LIBRA-seq-identified BCR sequences with known B-cell lineages. A simplified phylogenetic tree that visualizes how similar BCR sequences from known B-cell lineages are to those identified via LIBRA-seq and the Cell Ranger V(D)J assembler (in red). VRC38.gl indicates the inferred germline BCR sequence. Heatmap shows predicted antigen specificity for five HIV antigens.

Results

Setliff et al. sought to validate LIBRA-seq on two previously characterized human HIV samples. Past studies using samples from Donor NIAID45 identified a lineage of HIV-1 bNAbs, including VRC01. The team revisited this polyclonal sample using LIBRA-seq, and found 29 BCRs clonally related to members of the bNAb VRC01 lineage. Further testing for three of these antibodies via ELISA confirmed they were cross-reactive with the two HIV-1 antigens used in the LIBRA-seq library. Additionally, two of these three antibodies neutralized 12 of 12 and 11 of 12 viruses, respectively, on a global panel of HIV-1 variants. These findings demonstrate that LIBRA-seq can be used to identify cross-reactive bNAbs in prospective antibody discovery efforts.

LIBRA-seq was further validated on another HIV sample. Past studies on Donor N90 have also revealed a set of anti-HIV antibodies in the VRC38 lineage. The team identified 18 B cells from this lineage but focused their study on one anti-HIV member, 3602-870, from a novel antibody lineage that had a high LIBRA-seq score and was heavily mutated in both the heavy- and light-chain V genes. Further testing showed that this antibody neutralized over 70% of viruses on a panel of diverse HIV pseudoviruses, including several viruses that were not neutralized by antibodies from the VRC38 lineage. ELISA revealed that this antibody competed with VRC01 for binding to the HIV envelope trimer, suggesting it represents a newly discovered anti-HIV bNAb lineage in N90 that previous methods missed.

References

1. I Setliff et al., High-Throughput Mapping of B cell Receptor Sequences to Antigen Specificity. *Cell*. 179, 1–11 (2019).

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