Access Greater Antibody Sequence Diversity with Opto™ Plasma B Discovery 2.0 on the Beacon® System

APPLICATION SPOTLIGHT

- Access more B cell diversity to develop antibody therapeutics against difficult targets like GPCRs and ion channels
- Discover over 5,000 antigen-specific hits from a single plasma B cell sample
- Recover over 650 unique sequences in under 1 week

INTRODUCTION

Therapeutic monoclonal antibodies are proven and effective treatment options for a variety of diseases such as cancer, autoimmune disorders, and infectious disease. They act through multiple mechanisms, including blocking targeted molecule function, inducing apoptosis, or modulating signaling pathways. As the demand for monoclonal antibody therapies grows exponentially, so does the need for technologies that can shorten the development time for these life-saving drugs.

Antibody-producing B lymphocytes (B cells) are used to develop and produce therapeutic antibodies. Each B cell expresses a single B cell receptor (BCR), and the diverse range of BCRs expressed in the B cell population is referred to as the B cell repertoire. The B cell repertoire is highly diverse due to somatic recombination of the antibody genes and through somatic hypermutation and antibody heavy chain class-switching[1]. This highly diverse network of functional antibodies makes the B cell repertoire a powerful resource that can be leveraged to develop novel therapeutics. However, the therapeutics developed to date have primarily targeted simple targets because traditional technologies used to identify lead candidates, like hybridoma generation, do not efficiently sample the B cell repertoire. These methods can miss antibodies against difficult targets that elicit weak immune responses after immunization or targets with rare epitopes.

Other technologies, such as plasma B cell cloning using flow cytometry or droplet encapsulation, can access more antibody diversity because they enrich the plasma B cell population. These approaches, however, do not provide insights into antibody function and can thus increase the cost of development when nonfunctional or irrelevant hits progress further down the development process. Technologies that can functionally access more B cell diversity are required to realize the full potential of B cells and develop therapeutics against harder targets, like GPCRs and ion channels.

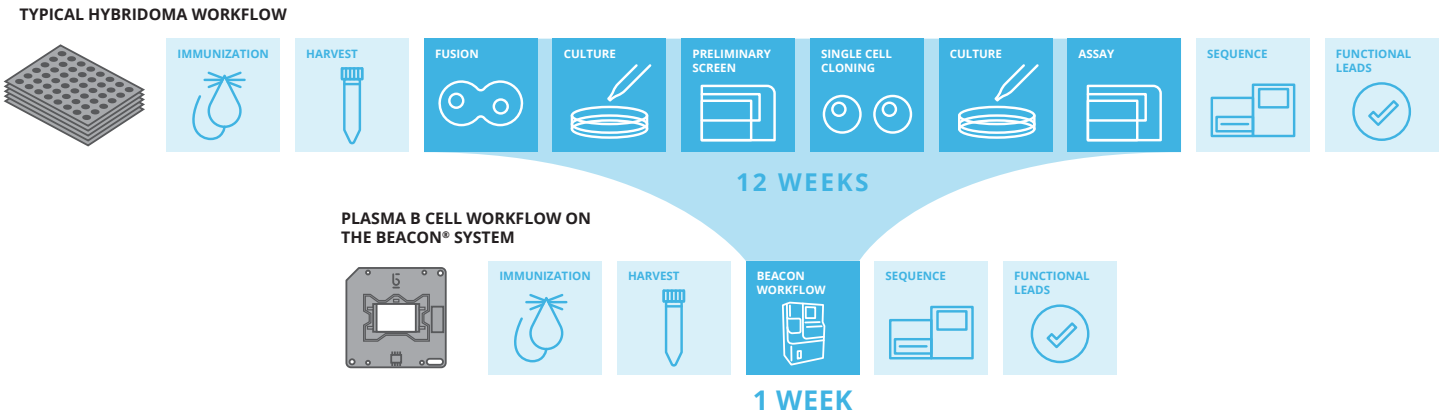


Figure 1. Opto Plasma B Discovery 2.0 on the Beacon system provides access to the plasma B cell repertoire to rapidly identify antigen-specific antibodies in less than one week. Plasma B cells are screened immediately after harvest and purification, bypassing the inefficient hybridoma fusion process.

The time it takes to screen B cells for lead candidates using a typical hybridoma workflow also significantly adds to the drug development timeline. After immunizing the animal and harvesting the B cells from the spleen, bone marrow, or lymph node, it can take at least 12 weeks to produce a hybridoma and screen through all of the potential hits, prolonging the development process.

Access broad B cell diversity in days

In this application note, we demonstrate how Opto Plasma B Discovery 2.0 on the Beacon system can be used to access broad B cell diversity in a matter of days instead of months. The Opto Plasma B Discovery 2.0 workflow can access a critical part of the B cell repertoire, plasma B cells. Plasma B cells from the spleen, bone marrow, or lymph nodes can be screened on the Beacon system immediately after organ harvest and cell purification. This shortens the time required to screen the B cell repertoire to just 1 week since the hybridoma fusion process is bypassed (Figure 1). The Beacon system also enables automated, on-chip cDNA synthesis using the OptoSeq BCR Kit to rapidly recover broad antibody sequence diversity, increasing the chances of selecting better lead candidates.

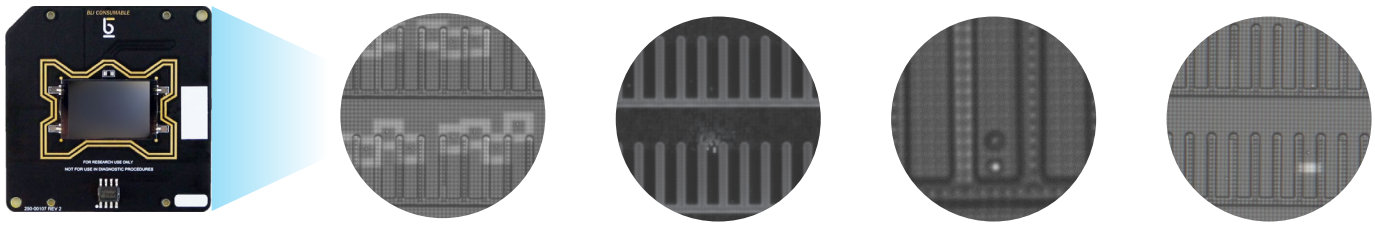
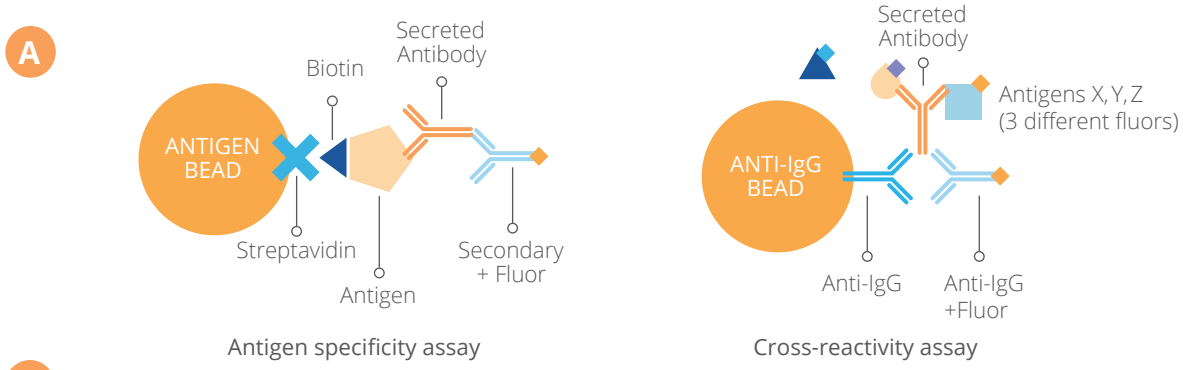


Figure 2. The Opto Plasma B Discovery 2.0 Workflow on the Beacon system. The Beacon system first loads plasma B cells into OptoSelect chips and performs single-cell cloning into NanoPen chambers using Berkeley Lights' proprietary technology. Antigen-specific hits are identified using binding assays followed by automated cell lysis, mRNA bead capture, and on-chip cDNA synthesis. Paired antibody heavy/light chain (VH/VL) sequences are amplified and sequenced.

THE OPTO PLASMA B DISCOVERY 2.0 WORKFLOW

The Beacon platform completely automates the Opto Plasma B Discovery 2.0 Workflow. Plasma B cells harvested from the spleen, bone marrow, or lymph nodes of immunized host animals are enriched using magnetic-activated cell sorting (MACS). The Beacon system then loads the plasma B sample onto multiple OptoSelect chips and performs single-cell cloning into NanoPen™ chambers using proprietary technology for on-chip manipulation of cells using light (Figure 2). Cells are cloned into NanoPen chambers in parallel so that several tens of thousands of cells can be cloned across 4 chips in just under 2 hours. Antigen-binding and cross-reactivity assays are then used to screen antibodies secreted by the B cells to select hits (Figure 3). Multiple assays are performed for thorough characterization of promising lead candidates. Antigen-specific antibody sequences are then recovered using Berkeley Lights' OptoSeq BCR Kit. Automated cell lysis and reverse transcription are performed on-chip to generate stable cDNA, which are subsequently recovered for paired heavy/light chain amplification and sequencing.



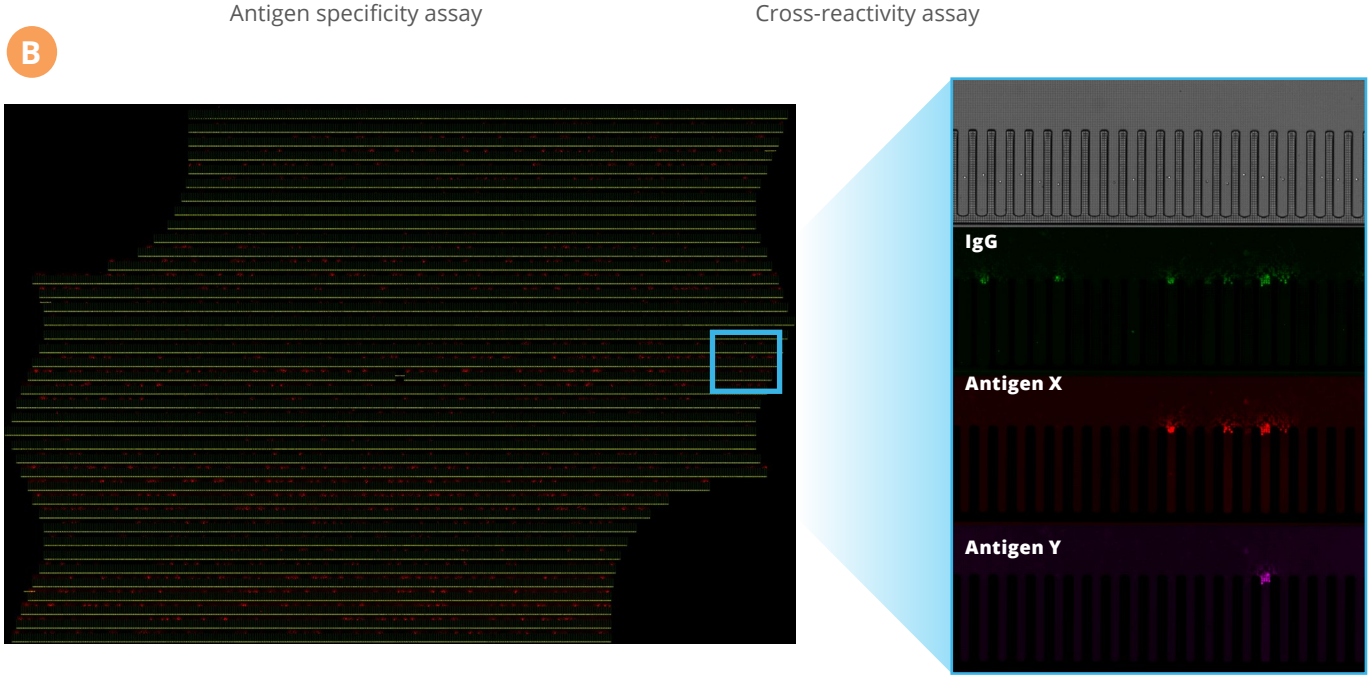


Figure 3. Assays for antigen specificity and cross-reactivity. A. The Beacon system enables down-selection of lead candidates through multiple assays for antigen specificity (left), cross-reactivity (right), and function that can be performed either in serial or in parallel. **B.** Demonstration of the simultaneous identification of antibodies that are specific to antigen X, antibodies that are cross-reactive to both antigens X and Y, and antibodies that do not bind antigens X or Y.

MATERIAL AND METHODS

Generating plasma B cells

We harvested bone marrow and spleen of wild-type mice immunized with a model antigen. Plasma B cells were enriched by magnetic-activated cell sorting (MACS) using the CD138+ Plasma Cell Isolation Kit, Mouse Kit (Miltenyi Biotec).

Opto Plasma B Discovery 2.0 experiments on the Beacon System

Fresh plasma B cells were cloned and screened in OptoSelect 11k chips using the Opto Plasma B Discovery 2.0 Workflow in Cell Analysis Suite (CAS™) 2.0 software on the Beacon system.

We were able to perform multiple workflows from individual plasma B cell samples by standard overnight tissue culture of cells using proprietary media in the Opto™ Plasma B Discovery Sample Prep Kit, Mouse (Berkeley Lights). Antigen specificity and cross-reactivity assays were performed using Assay Beads (Berkeley Lights) conjugated to target antigen and fluorescently-labeled secondary anti-mouse antibodies. We then performed automated cell lysis, mRNA bead capture, and on-chip cDNA synthesis by using the OptoSeq BCR Kit (Berkeley Lights). Amplification of heavy and light chain sequences was performed using the Opto™ Plasma B Discovery Sanger Prep Kit, Mouse (Berkeley Lights).

Sequencing, analysis and re-expression

We then used next-generation sequencing (NGS) to sequence antibodies and analyzed the resulting sequences using custom bioinformatics software. We confirmed antigen binding by integration of the heavy/light chain cDNA into expression constructs and transfecting these constructs into HEK293 cells using standard methods. Supernatants were collected 4 days post-transfection, and the presence of IgG immunoglobulin and antigen-binding activity in these supernatants was measured by ELISA.

RESULTS AND DISCUSSION

Thousands of hits discovered from a single plasma B cell sample

Plasma B cell samples were loaded onto four OptoSelect 11k chips on the Beacon system. Each chip contains over 11,000 NanoPen chambers, each <1 nanoliter in volume. This small volume enables secreted antibodies to reach detectable concentrations within minutes. We performed antigen specificity and cross-reactivity assays using bead-based fluorescent sandwich assays (Figure 3) and identified more than 5,000 antigen-specific antibodies (hits) in two workflow runs from the same sample (Figure 4). This approach was repeated for >10 different plasma B cell samples.

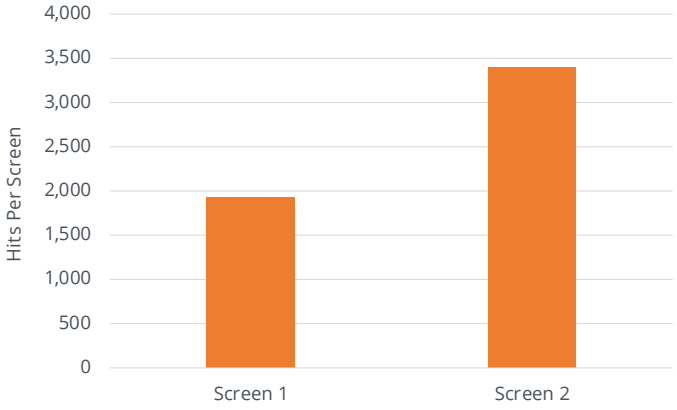


Figure 4. Over 5,000 antigen-specific hits discovered from single plasma B cell samples. Berkeley Lights' proprietary media extends the survival of plasma B cells in tissue culture, making it possible to perform multiple screening workflows on a single plasma B cell sample. Screen 1 was performed on the same day as plasma cell isolation, while Screen 2 was performed after 1 day of overnight tissue culture.

More than 650 unique, functional BCR sequences in <1 week

Following assay characterization, mRNA capture beads were delivered to targeted NanoPen chambers containing plasma B cells secreting antibodies with desired antibody-antigen specificity and cross-reactivity. We then performed automated cell lysis, mRNA bead capture and on-chip cDNA synthesis using the OptoSeq BCR Kit (Figure 5A). By stabilizing the cDNA sequences on beads within the NanoPen chambers, we could recover sequences over multiple days without any observed sequence degradation due to apoptosis of the B cell. This sequential sequence recovery made it possible to recover over 650 unique, VH/VL antigen-specific antibody sequences from a single workflow in just 4 days (Figure 5B).

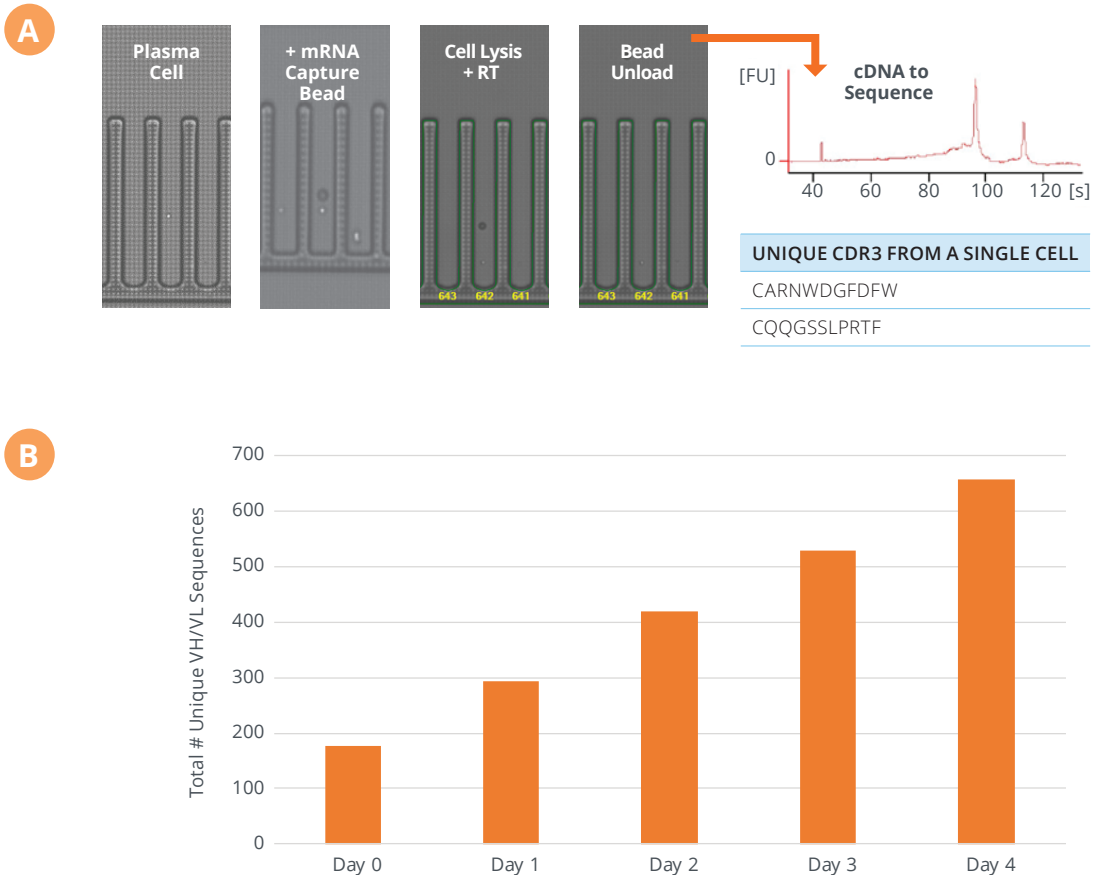


Figure 5. OptoSeq™ BCR Kit enables recovery of >650 antigen-specific antibody sequences from a single 4-chip workflow. A. The OptoSeq BCR workflow. mRNA capture beads are loaded into specific NanoPen chambers and plasma B cells are lysed to capture their mRNA. Reverse transcription is then performed on-chip to generate stable cDNA. The captured beads are then recovered for downstream sequencing. **B.** The stable cDNA can be recovered over several days, increasing the number and diversity of unique VH/VL antigen-specific sequences.

Every antibody sequence can then be mapped directly to a known antibody function. Sequence relationships can identify individual mutations that confer antigen specificity and cross-reactivity. We found the antigen-specific sequences recovered over multiple days from the identified plasma B cells to be rich in CDR3 diversity (Figure 6). 192 antibodies were selected for cloning and re-expression of which 85% demonstrated confirmed antigen-binding using standard ELISA.

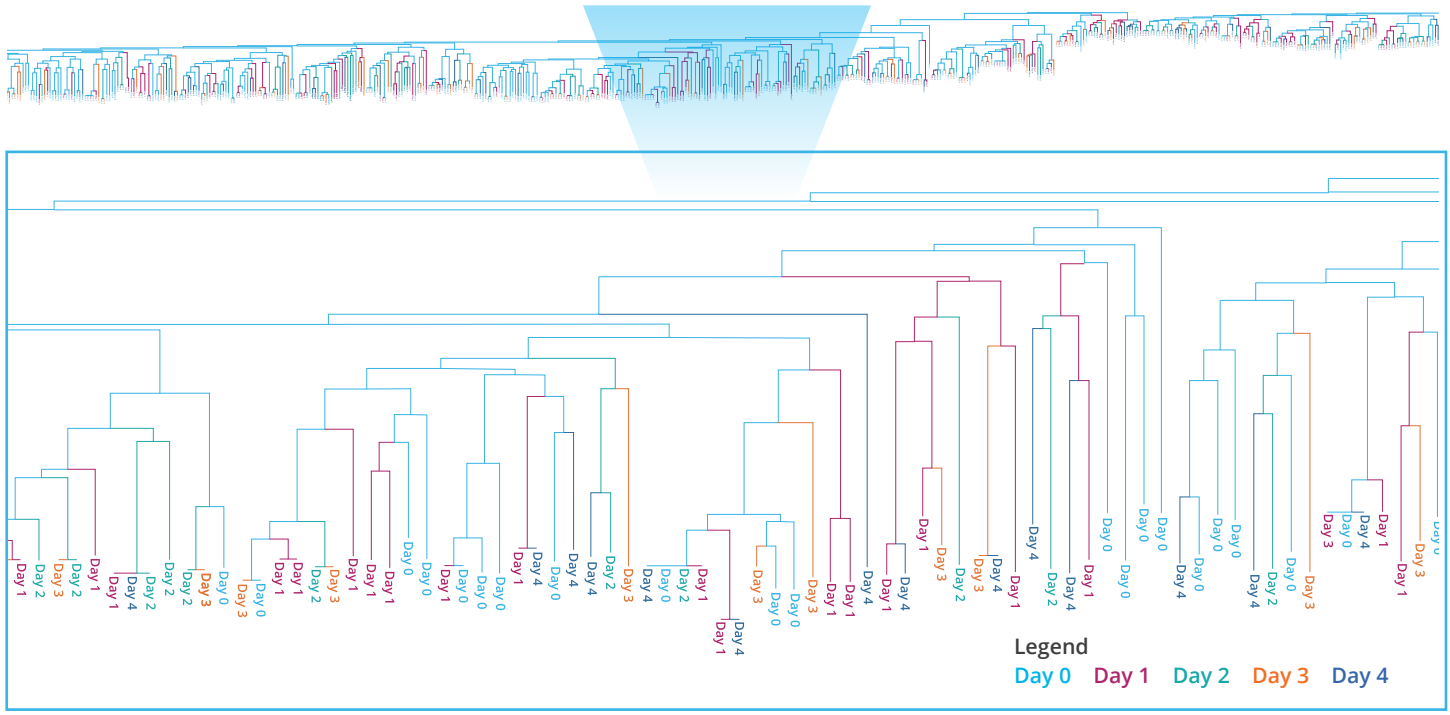


Figure 6. Recovery of antibody sequences over multiple days using OptoSeq BCR delivers rich CDR3 diversity. Over 650 antigen-specific sequences were recovered in just 5 days, increasing the chances of identifying better lead candidates.

CONCLUSION

Methods that rapidly screen the B cell repertoire are a requirement to keep up with the demand for fast-paced therapeutic antibody development against difficult targets. Opto Plasma B Discovery 2.0 on the Beacon system provides an automated workflow that decreases the time to obtain antigen-specific antibody sequences to less than 1 week. On-chip cDNA generation stabilizes the antibody sequences to enable recovery over multiple days, delivering antigen-specific sequence diversity that increases the chances of discovering better lead candidates to difficult targets.

We demonstrate how accessing the B cell repertoire with Opto Plasma B Discovery using the Beacon system connects antibody sequence to function in less than 1 week compared to months using conventional hybridoma methods. More than 5,000 antigen-specific hits from a single plasma B cell sample and automated cDNA synthesis enabled recovery of over 650 unique antigen-specific antibody sequences in 4 days. This deep characterization of a B cell sample meets the market's demand for better and faster methodologies to streamline antibody therapeutic development in order to bring novel drugs against hard targets to the clinic sooner.

REFERENCES

1. Wu, Y.C., Kipling, D., Dunn-Walters, D. Assessment of B Cell Repertoire in Humans. *Immunosenescence* (2015).

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