DATASHEET

Opto™ Plasma B Discovery 2.0 Workflow

The Opto Plasma B Discovery 2.0 Workflow on the Beacon® optofluidic system accesses broad B cell diversity to select better antibody lead candidates in under 1 week. The workflow screens plasma B cells to discover antigen-specific antibodies and uses OptoSeq™ BCR to enable on-chip cDNA synthesis for efficient recovery of antibody heavy/light chain sequences. The workflow can enable discovery of thousands of antigen-specific antibodies and recovery of several hundreds of antibody sequences for sequencing and further downstream characterization.

WORKFLOW OVERVIEW

The Opto Plasma B Discovery 2.0 Workflow enables rapid screening of plasma B cells for discovery and recovery of diverse panels of antigen-specific antibodies ("hits") in under 1 week. The workflow's Opto™ Plasma B Discovery Sample Prep kit (Mouse/Human) enables preparation of plasma B cell samples for screening, and is compatible with plasma B cells from diverse organs, such as peripheral blood, spleen, bone marrow and lymph nodes (Figure 1A, B and C). This kit also includes optimized media for off-chip tissue culture to enable multiple on-chip workflows from single plasma B cell samples (Figure 1C). The Beacon system then automatically clones tens of thousands of single plasma B cells into NanoPen™ chambers on OptoSelect™ 11k chips in under 4 hours (Figure 1D). Antigen-binding and cross-reactivity assays are used to screen and select plasma

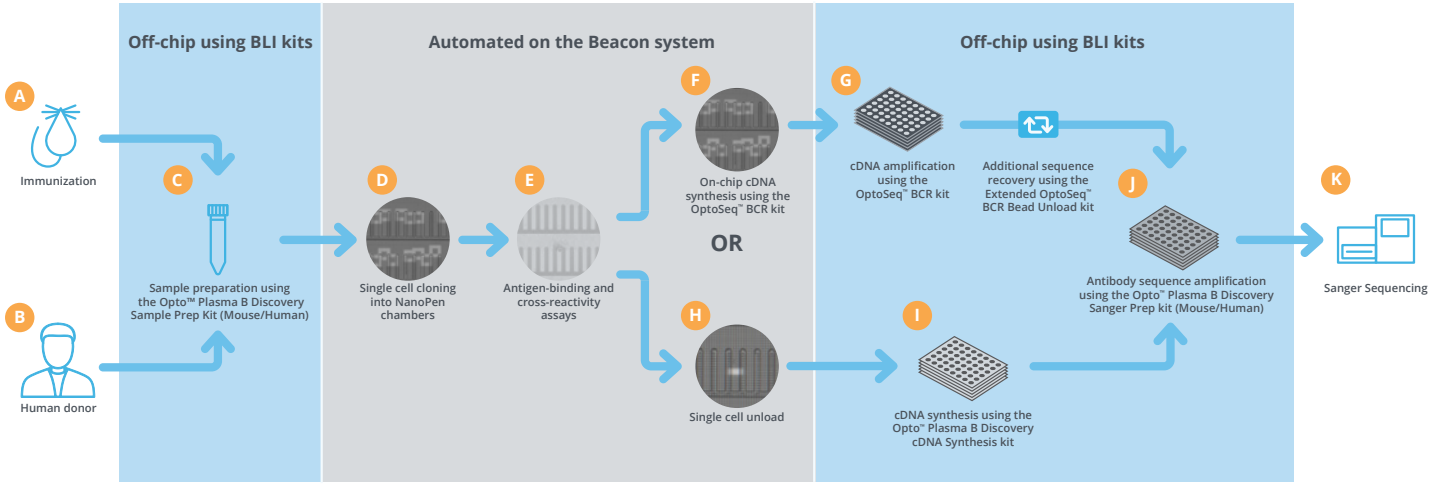

Figure 1. The Opto Plasma B Discovery 2.0 Workflow enables screening of plasma B cells for rapid discovery and recovery of antigen-specific antibodies.

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B cells secreting antigen-specific antibodies (Figure 1E). Antigen-specific antibody sequences can then be recovered using the OptoSeq™ BCR kit. Automated cell lysis and reverse transcription are performed on-chip (Figure 1F) to generate stable cDNA on OptoSeq BCR mRNA capture beads. Amplification of cDNA is performed after recovering OptoSeq BCR beads into 96-well plates by exporting each bead to a distinct well (Figure 1G). Alternatively, single antigen-specific plasma B cells can be recovered into 96-well plates (Figure 1H) followed by off-chip cDNA synthesis and amplification using the Opto™ Plasma B Discovery cDNA Synthesis kit (Figure 1I). Paired antibody heavy/light chain sequences are amplified from the cDNA of recovered beads or cells using the Opto™ Plasma B Discovery Sanger Prep kit (Mouse/Human), which includes a primer for conventional Sanger sequencing (Figure 1J and 1K).

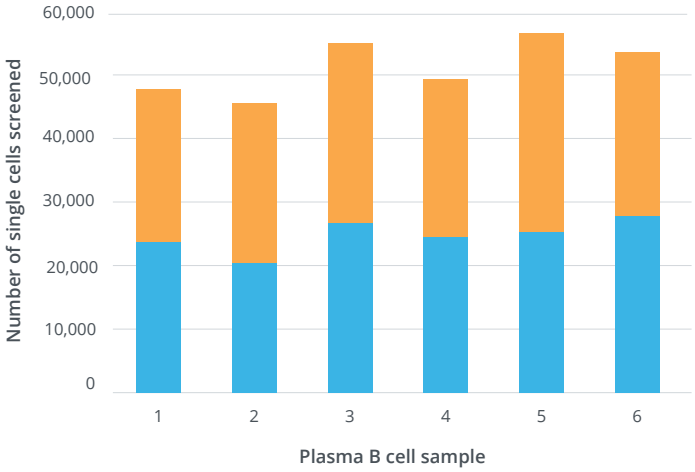

Figure 2. Screening throughput is increased by running two 4-chip Opto Plasma B Discovery 2.0 workflows from single plasma B cell samples. Plasma B cells were screened on the initial day of harvest and purification (Day 0 workflow ■) and the remaining sample was screened on the next day (Day 1 workflow ■) after overnight tissue culture using proprietary plasma B cell culture media developed by Berkeley Lights.

SCREENING THROUGHPUT

Approximately 50,000 plasma B cells can be screened by running multiple workflows from single plasma B cell samples (Figure 2). Plasma B cells are purified by MACS or FACS from diverse organs (spleen, bone marrow, lymph nodes, PBMCs) from immunized mice or humans. Purified plasma B cells are then processed using the Opto Plasma B Discovery Sample Prep kit (Mouse/Human) to prepare plasma B cell samples for on-chip workflows. Plasma B cells can be screened on the day of cell harvest and purification (Day 0 workflow) and after overnight tissue culture in proprietary plasma B cell culture media developed by Berkeley Lights (Day 1 workflow).

DETECTION OF ANTIGEN-SPECIFIC ANTIBODIES ("HITS")

Hits can be identified by screening plasma B cell samples for IgG secretion, antigen specificity and cross-reactivity using multiplexed, bead-based in-channel assays (Figure 3A). Greater than 5,000 hits were identified per plasma B cell sample by performing two workflows on each cell sample (Figure 3B). Antigen-positive hit rates were consistently equal or higher during Day 1 workflows as compared with Day 0 workflows, demonstrating that plasma B cells remain viable after overnight cell culture (Figure 3C). Screening of freeze/thawed plasma B cell samples yielded antigen-specific hits, though plasma B cells subjected to freeze/thaw cycles had reduced hit rates due to lower cell viability (Figure 3B and 3C). Hit rates may vary for different antigens based on antigenicity during animal immunization and purity of plasma B cells screened using the workflow.

AUTOMATED ASSAY SCORING

Cell Analysis Suite (CAS™) software uses machine learning to automatically score assays and identify NanoPen chambers that contain B cells secreting antigen-specific antibodies. The algorithm calls antigen-positive pens with an average accuracy of 90.3% for in-channel bead-based

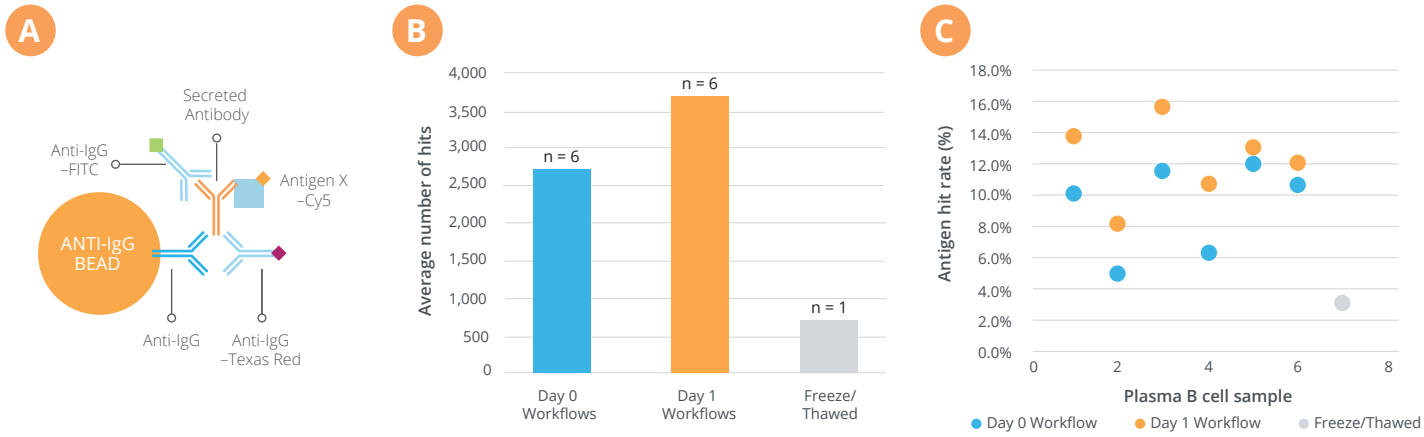

Figure 3. >5000 hits can be identified by running two 4-chip Opto Plasma B Discovery 2.0 Workflows per plasma B cell sample. A. Hits were identified using a triplex assay in which secreted antibodies were captured on anti-IgG beads and detection was performed using antigen labeled with Cy5, and two secondary anti-IgG antibodies labeled with Texas Red and FITC fluorophores, respectively. Hits were classified as antigen-specific antibodies that scored positive in all 3 assays. **B.** and **C.** The percentage of single cells secreting antigen-specific antibodies (antigen hit rate) for Day 1 workflows was equivalent or higher than hit rates for Day 0 workflows. Plasma B cells subjected to freeze/thaw cycles had reduced hit rates due to lower cell viability. Hit rates may vary for different antigens based on antigenicity during animal immunization and purity of plasma B cells screened using the workflow.

assays (Figure 4). Following automated hit scoring, the software automatically initiates OptoSeq BCR by loading OptoSeq BCR beads into antigen-positive pens for on-chip cDNA synthesis.

ANTIGEN-SPECIFIC ANTIBODY SEQUENCE RECOVERY

The Opto Plasma B Discovery 2.0 Workflow provides two approaches for recovery of paired heavy/light chain sequences for antigen-specific antibodies (Figure 5):

1. OptoSeq BCR Bead Approach: on-chip cell lysis, mRNA bead capture, and cDNA synthesis followed by bead recovery into 96-well plates for off-chip amplification of cDNA and antibody heavy/light chain sequences (Figure 5A and 5C); or,
2. Single Cell Approach: recovery of single cells into 96-well plates to perform off-chip cell lysis, cDNA synthesis and amplification, and antibody heavy/light chain amplification (Figure 5B and 5C).

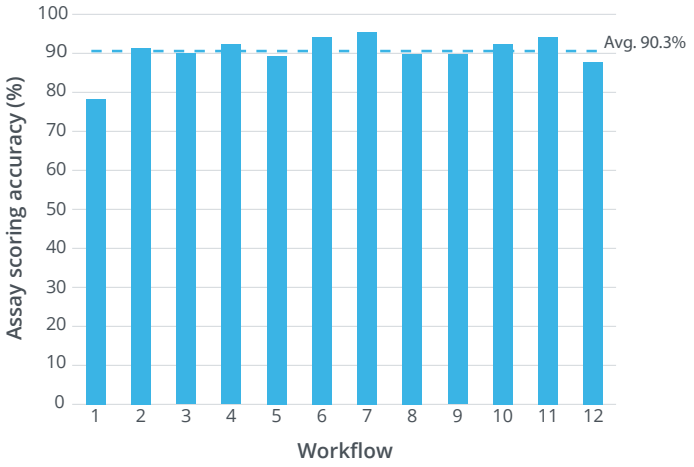

Figure 4. Accurate automated assay scoring in Cell Analysis Suite (CAS) software minimizes manual data analysis. Accuracy of the automated assay scoring algorithm was measured by manual verification of in-channel, bead-based assay images for multiple workflows (n = 12). Accurate software scoring minimizes manual intervention to enable walk-away automation.

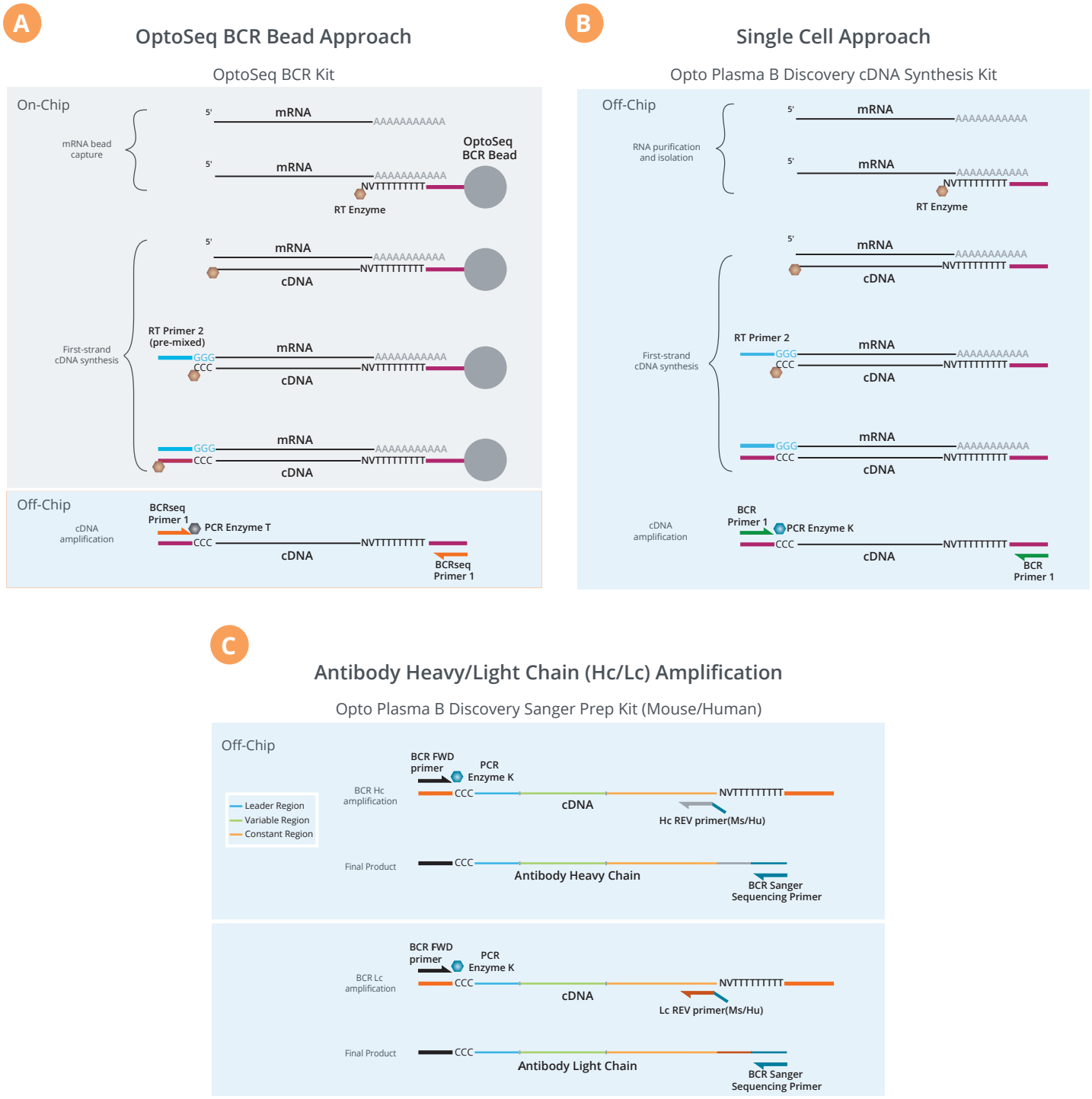

Figure 5. Antigen-specific antibody heavy/light chain (Hc/Lc) sequences can be obtained using either the OptoSeq BCR Bead Approach or the Single Cell Approach. A. Using the OptoSeq BCR bead approach, the OptoSeq BCR kit is used for on-chip cell lysis, on-chip cDNA synthesis and off-chip cDNA amplification from recovered beads. **B.** Using the Single Cell Approach, all molecular biology steps (lysis, reverse transcription, cDNA synthesis and amplification) are performed after recovery of cells in 96-well plates. **C.** The amplified cDNA from either the OptoSeq BCR Bead Approach or the Single Cell Approach is used for amplification of the antibody heavy/light chain genes by the Opto Plasma B Discovery Sanger Prep Kit (Mouse/Human). This figure depicts cDNA generated by OptoSeq BCR.

Antigen-specific antibody heavy/light chain (Hc/Lc) sequences can be recovered with consistently high efficiency (average = 86%) using the OptoSeq BCR Bead Approach (Figure 6). Using this approach, sequence recovery efficiency was comparable for both fresh and frozen plasma B cell samples. By comparison, the Single Cell Approach yielded an average paired sequence recovery of 75%.

SEQUENCE DIVERSITY

Multiple workflows on the same plasma B cell samples yielded unique antibodies, thus increasing overall hit diversity. Comparison of the CDR3 sequences from antigen-specific antibody sequences recovered from Day 0 and Day 1 workflows confirmed that the majority of sequences identified in each workflow are unique (Figure 7).

OptoSeq BCR also provides access to greater sequence diversity by enabling the efficient recovery of hundreds of unique antigen-specific antibody sequences from a single workflow. By stabilizing cDNA on beads, antibody sequences can be recovered for several days without sequence degradation due to death of plasma B cells. Each round of recovery provides access to an additional 192 OptoSeq BCR beads containing antigen-specific antibody sequence information. In a single experiment,

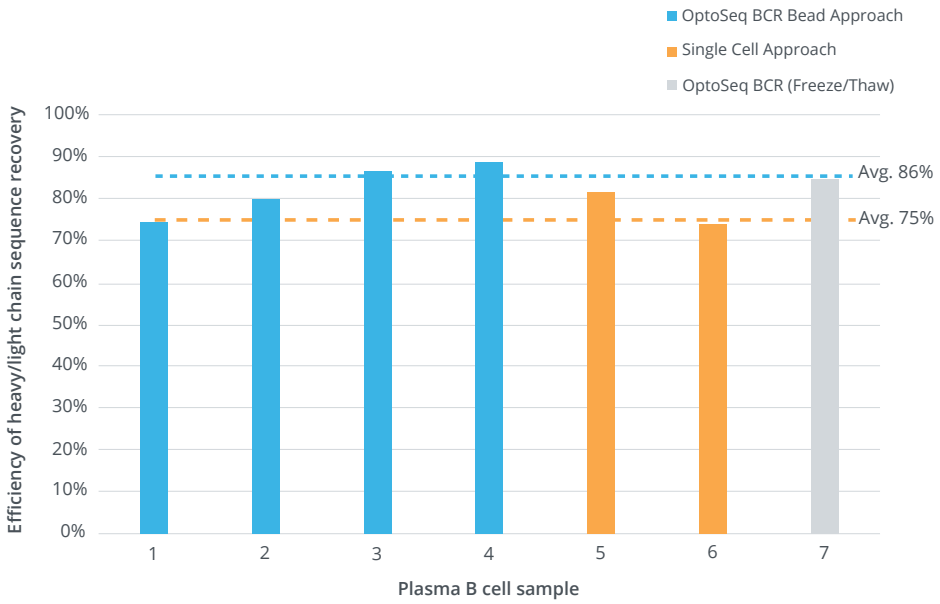

Figure 6. OptoSeq BCR enables recovery of antigen-specific antibody sequences from plasma B cells with high efficiency. OptoSeq BCR recovered paired heavy/light chain antibody sequences from an average of 86% of plasma B cells across multiple workflows and multiple plasma B cell samples. This high sequence recovery efficiency was observed for both fresh and frozen plasma B cell samples. By comparison, recovery of single cells without on-chip cDNA synthesis yielded an average paired sequence recovery of 75%.

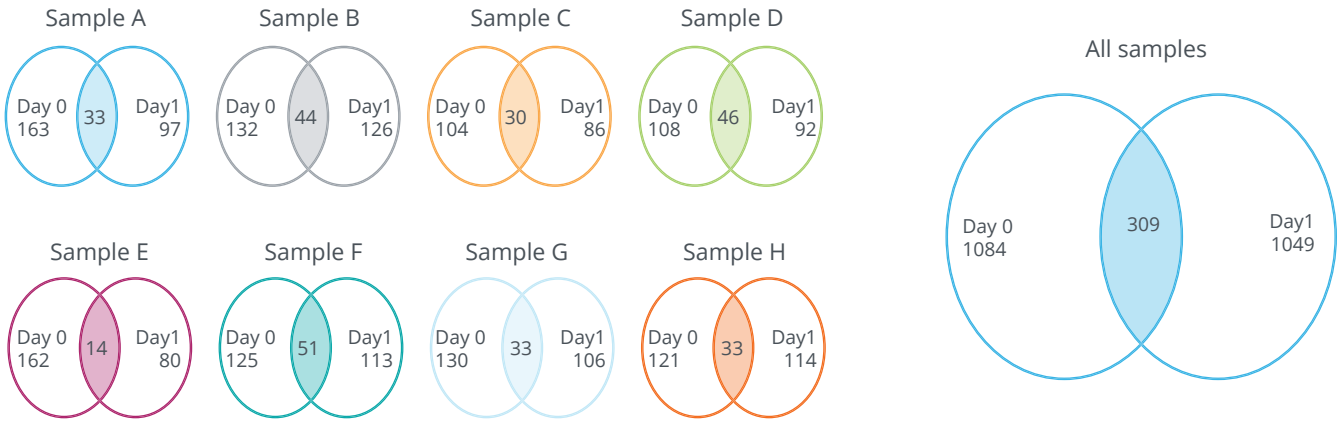

Figure 7. Greater sequence diversity can be accessed by performing multiple workflows from the same plasma B cell sample. Comparison of CDR3 sequences of antigen-specific antibodies from Day 0 and Day 1 workflows confirmed that the majority of antibodies found in each workflow are unique. Across all samples, fewer than 15% of antibodies had CDR3 sequences that were found in both Day 0 and Day 1 workflows.

greater than 650 unique antigen-specific antibody sequences were obtained from a single 4-chip workflow by using the OptoSeq BCR kit, and an Extended OptoSeq BCR Bead Unload kit for each additional round of bead recovery (Figure 8).

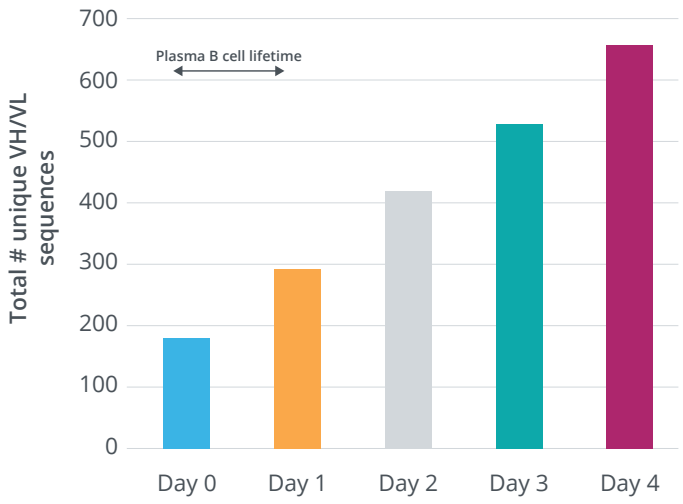

Figure 8. OptoSeq BCR enables efficient recovery of >650 unique antigen-specific antibody sequences from a single 4-chip workflow. On Day 0, plasma B cells were screened to identify hits. On-chip cDNA synthesis was performed using the OptoSeq BCR kit. Beads were then unloaded for multiple days using Extended OptoSeq BCR Bead Unload kits. Antibody heavy/light chain sequences were amplified for sequencing using the Opto Plasma B Discovery Sanger kit.

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 antibodies and antigen-binding activity in these supernatants was measured by ELISA. From 192 randomly selected antibodies, we confirmed that 85% of antibodies bound the target antigen (data not shown).

CONCLUSION

In under 1 week, the Opto Plasma B Discovery 2.0 Workflow provides access to broad B cell diversity through direct screening of the plasma B cell repertoire and automated recovery of antigen-specific antibody sequences. Access to greater B cell diversity can enable selection of better antibody lead candidates.

ORDERING INFORMATION

Mouse Plasma B Cells: OptoSeq BCR

PART NUMBER	PRODUCT NAME	QUANTITY (4-CHIP / 8-CHIP WORKFLOWS)
750-08090	OptoSelect™ Chip 11k	4/8
750-02002	Opto™ Plasma B Discovery 2.0 Kit, Mouse Contains: • Opto™ Plasma B Discovery Sample Prep Kit, Mouse (Part Number 750-02050) • OptoSeq™ BCR Kit (Part Number 750-01003) • Opto™ Plasma B Discovery Sanger Prep Kit, Mouse (Part Number 750-01004)	1/2
750-08096	Import Wells	1 (suitable for up to 25 x 4-chip workflows)
520-00053	Assay Beads	1 (suitable for up to 16 assays)
750-01004	Opto™ Plasma B Discovery Sanger Prep Kit, Mouse	1 for each 192 OptoSeq BCR beads
750-08097 (optional)	Extended OptoSeq™ BCR Bead Unload Kit	1 for each 192 additional OptoSeq BCR beads

Human Plasma B Cells: OptoSeq BCR

PART NUMBER	PRODUCT NAME	QUANTITY (4-CHIP / 8-CHIP WORKFLOWS)
750-08090	OptoSelect™ Chip 11k	4/8
750-02003	Opto™ Plasma B Discovery 2.0 Kit, Human Contains: • Opto™ Plasma B Discovery Sample Prep Kit, Human (Part Number 750-02051) • OptoSeq™ BCR Kit (Part Number 750-01003) • Opto™ Plasma B Discovery Sanger Prep Kit, Human (Part Number 750-02041)	1/2
750-08096	Import Wells	1 (suitable for up to 25 x 4-chip workflows)
520-00053	Assay Beads	1 (suitable for up to 16 assays)
750-02041	Opto™ Plasma B Discovery Sanger Prep Kit, Human	1 for each 192 OptoSeq BCR beads
750-08097 (optional)	Extended OptoSeq™ BCR Bead Unload Kit	1 for each 192 additional OptoSeq BCR beads

Hardware and Software

PART NUMBER	PRODUCT NAME	DESCRIPTION
870-08027	Opto™ Plasma Discovery 2.0 Upgrade Package	Upgrade package includes Beacon hardware upgrades, on-site FSE, training kit, and up to 4 days of on-site FAS training. Opto Plasma B Discovery 2.0 requires CAS 2.0.

Opto Plasma B Discovery 2.0 Workflow

Other Consumables and Reagents

PART NUMBER	PRODUCT NAME	QUANTITY
750-02050	Opto™ Plasma B Discovery Sample Prep Kit, Mouse Includes: • DNA Clean Up (Part Number 520-70000) • Loading Reagent (Part Number 750-08029) • Plasma B Cell Media (for mouse plasma B cells) • Wetting Additive	1
750-02051	Opto™ Plasma B Discovery Sample Prep Kit, Human Includes: • DNA Clean Up (Part Number 520-70000) • Loading Reagent (Part Number 750-08029) • Plasma B Cell Media (for human plasma B cells) • Wetting Additive	1
520-70000	DNA Clean Up	1
750-08029	Loading Reagent	1
750-08091	Wetting Additive Kit	1
750-02030	Opto™ Plasma B Discovery cDNA Synthesis Kit	1
750-01003	OptoSeq™ BCR Kit	1
750-08097	Extended OptoSeq ™ BCR Bead Unload Kit	1 for each 192 additional OptoSeq BCR beads
750-01004	Opto™ Plasma B Discovery Sanger Prep Kit, Mouse	1 for each 192 OptoSeq beads or mouse B cells
750-02041	Opto™ Plasma B Discovery Sanger Prep Kit, Human	1 for each 192 OptoSeq beads or human B cells
750-08096	Import Wells	1 (suitable for up to 25 x 4-chip workflows)
520-00053	Assay Beads	1 (suitable for up to 16 assays)

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BERKELEY LIGHTS®

5858 Horton Street | Suite 320 | Emeryville, CA 94608

info@berkeleylights.com
+1-510-858-2855
berkeleylights.com

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