DATASHEET
REV G | SEPTEMBER 2023

Antibody Discovery (AbD)

Opto® Plasma B Discovery 4.0 Workflow

The Opto® Plasma B Discovery 4.0 workflow on the Beacon® Optofluidic System enables functional profiling of the B cell repertoire to discover a broad diversity of antibody hits and rapidly down-select lead candidates. In just 1 week, the workflow allows:

- Recovery of 1,000s of hits from over 40,000 individual plasma B cells in a single 4-chip workflow
- Down-selection of lead candidates by functional profiling
- Sequencing and re-expression of >1,000 functionally-characterized antibodies

WORKFLOW OVERVIEW

The Opto Plasma B Discovery 4.0 workflow enables rapid screening of plasma B cells for discovery, recovery, sequencing, and re-expression of functionally characterized antibodies in just 1 week.

The Opto Plasma B Discovery 4.0 workflow begins with the preparation of plasma B cell samples for screening from diverse organs, such as spleen, bone marrow, and lymph nodes using the Opto® Plasma B Discovery Sample Prep kit (Mouse) [**FIGURE 1A and B**]. This kit also includes optimized media for off-chip tissue culture to enable multiple on-chip workflows from a single plasma B cell sample [**FIGURE 1B**]. The Beacon system then automatically clones tens of thousands of single plasma B cells into NanoPen® chambers on up to four OptoSelect® 11k or 20k chips in under 4 hours [**FIGURE 1C**]. Antigen-binding and cross-reactivity assays are used to screen and select plasma B cells secreting antigen-specific antibodies [**FIGURE 1D**]. Antigen-specific antibody sequences can then be recovered using the OptoSeq® Barcoded BCR kit. Automated cell lysis and reverse transcription are performed on-chip [**FIGURE 1E**] to generate stable cDNA on mRNA-capture beads with dual optical and genetic barcodes. Amplification of cDNA is performed after bulk recovery of up to 12 beads into each well for a maximum of 1,152 bead exports into a single 96-well plate [**FIGURE 1G**]. Additional beads can be recovered using the Extended OptoSeq® Bead Unload kit [**FIGURE 1F**]. NGS libraries are generated using the OptoSeq Barcoded BCR kit and Nextera XT DNA Library Preparation kit [**FIGURE 1I**] for accurate sequencing of the full-length variable region of paired heavy/light chain antibody genes on standard Illumina sequencing platforms [**FIGURE 1J**]. Bioinformatics software is then used to link the sequence and function of all recovered antibodies. Finally, the Opto® BCR Rapid Re-expression kit enables rapid production of antibodies for further downstream characterization [**FIGURE 1H**].

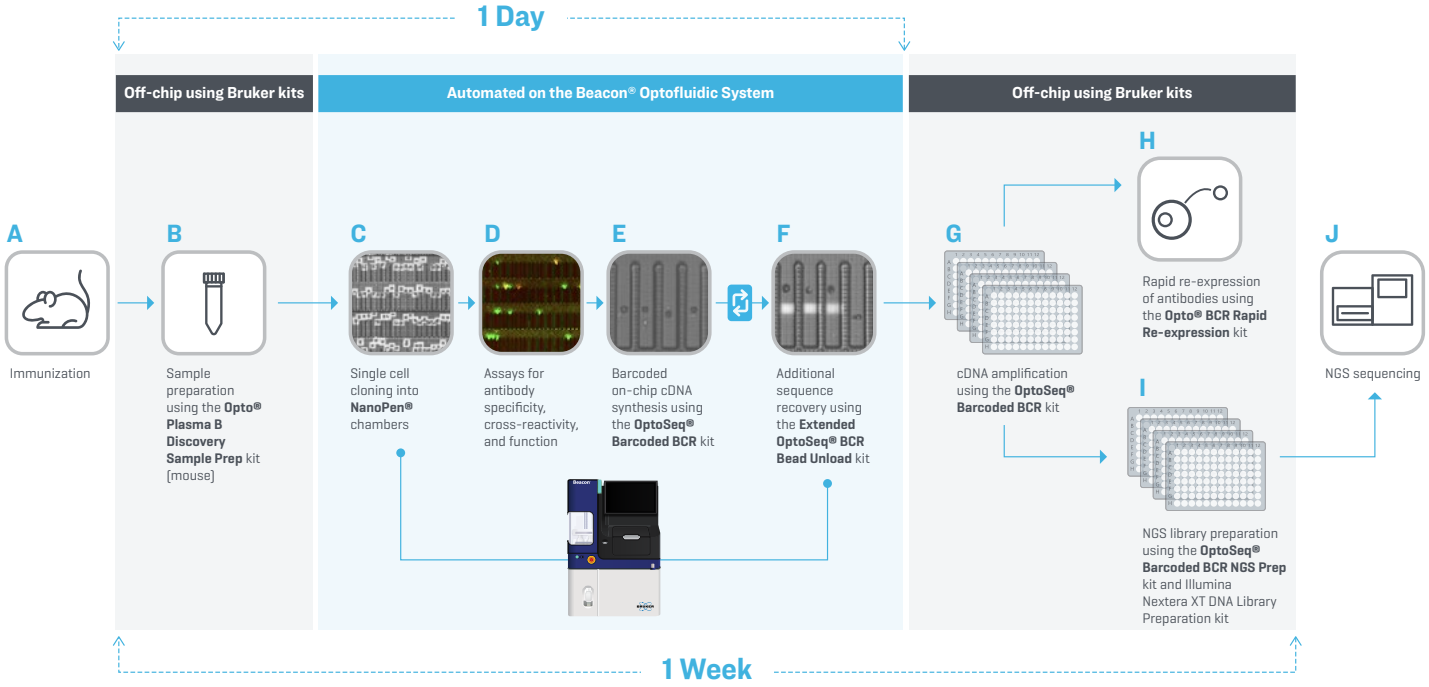


FIGURE 1: The Opto Plasma B Discovery 4.0 workflow. The Opto Plasma B Discovery 4.0 workflow enables screening of plasma B cells followed by sequencing and re-expression of lead molecules in just 1 week.

PLASMA B CELL FUNCTIONAL PROFILING

Plasma B cells are purified by MACS or FACS from diverse organs (spleen, bone marrow, lymph nodes, PBMCs) of immunized mice. Purified plasma B cells are then processed using the Opto Plasma B Discovery Sample Prep kit (Mouse) to prepare plasma B cell samples for on-chip workflows. Cells are then loaded onto either OptoSelect 11k or OptoSelect 20k chips that enable screening, on average, over 20,000 to 40,000 plasma B cells in a single automated 4-chip workflow (**FIGURE 2A**). Similar numbers of cells can be screened using workflows run with 4x OptoSelect 11k chips or workflows run with 2x OptoSelect 20k chips (**FIGURE 2A**). 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 Bruker (Day 1 workflow). This enables screening of over 80,000 plasma B cells from two workflows using 4 OptoSelect 20k chips each performed on a single sample.

Following loading, plasma B cells can be screened using multiple assays for antibody specificity, cross-reactivity, and function against soluble or cell membrane antigens. In a representative workflow in which mice were immunized with the KLH antigen, more than 5000 antigen-specific antibody hits were identified from a single 4x 20k chip workflow (**FIGURE 2B**). Plasma B cells can be assayed for multiple hours to enable multiple sequential assays (**FIGURE 2C**).

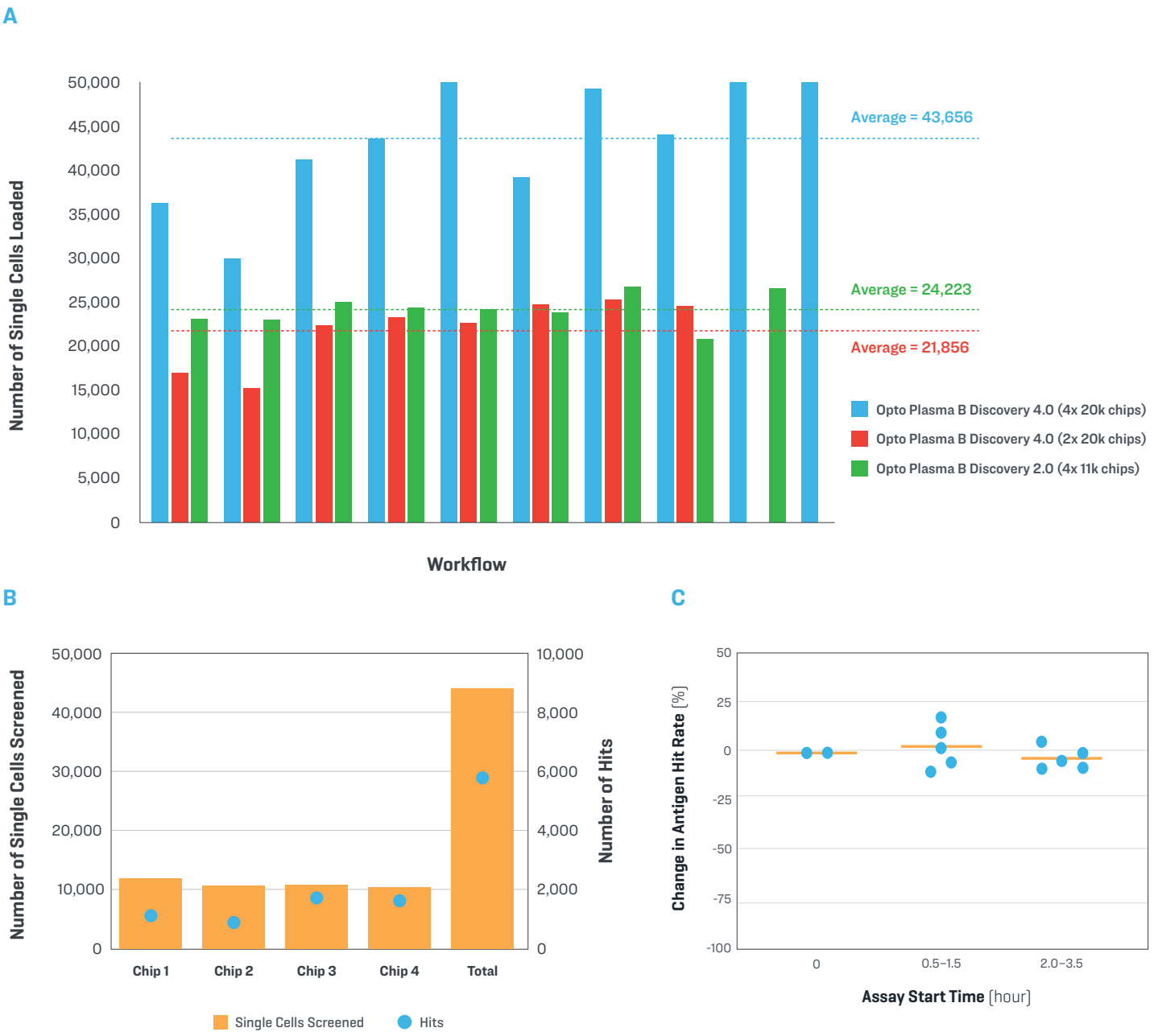


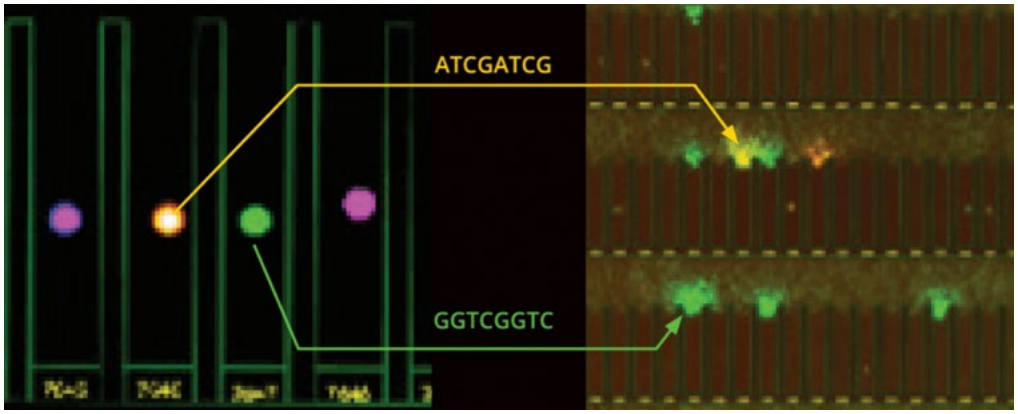
FIGURE 2: A) The Opto Plasma B Discovery 4.0 workflow enables screening of over 20,000 to 40,000 single plasma B cells in a single Beacon workflow using four OptoSelect 11k or 20k chips. **B)** Binding assays can be performed to identify thousands of hits. **C)** Plasma B cells can be assayed for multiple hours to characterize antibody specificity, cross- reactivity, and function.

SEQUENCE RECOVERY

Accurate sequencing of thousands of full-length antibody genes is prohibitively expensive and slow by conventional Sanger methods. The OptoSeq Barcoded BCR method enables rapid, efficient sequencing of thousands of antibodies in a single run. Antibody sequences are recovered in bulk by using a mixture of beads containing 12 distinct optical and genetic barcodes [FIGURE 3A].

A single barcoded bead is loaded into each NanoPen chamber containing an antigen-specific plasma B cell and cells are lysed to capture mRNA. After on-chip cDNA synthesis, the optical barcodes of every bead are decoded by fluorescent imaging [FIGURE 3A]. Up to 1,152 beads can then be recovered into a single 96-well plate by bulk recovery of up to 12 beads with distinct barcodes into each well. From just two 4-chip workflows, nearly 12,000 beads can be recovered with high (>90%) efficiency [FIGURE 3B].

A



B

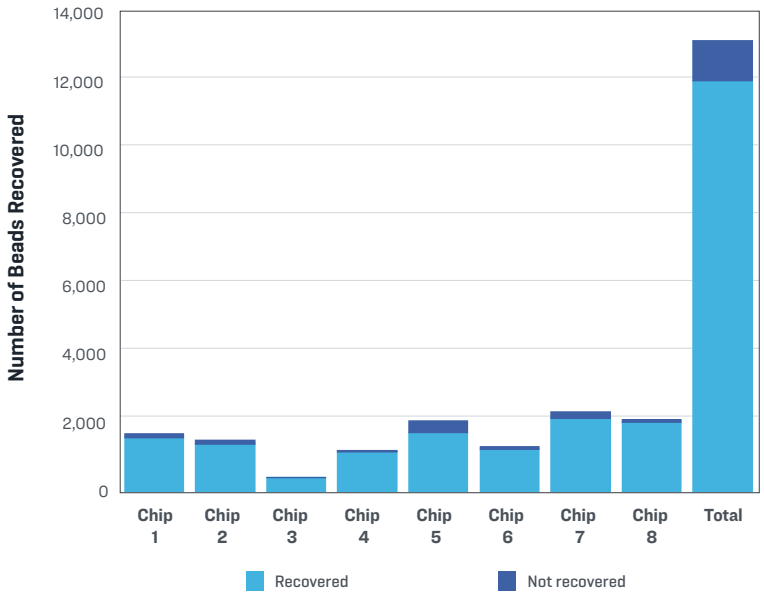


FIGURE 3: OptoSeq Barcoded BCR enables linkage of sequence to function of thousands of antibodies. A) Dual genetic and optical barcodes enable bulk bead recovery while preserving the linkage of sequence to function. B) Nearly 12,000 beads can be recovered from 2x 4-chip Opto Plasma B Discovery workflows with high efficiency (>90%).

OptoSeq Barcoded BCR enables rapid, accurate sequencing of paired heavy/light chain antibody genes by DNA fragmentation, NGS sequencing, and bioinformatics analysis using proprietary PrimeSeq software (**FIGURE 4A and B**). PrimeSeq assembles full-length antibody variable regions from short reads (150 x 150), enabling accurate sequencing of thousands of antibodies from a single run on standard Illumina sequencing platforms. PrimeSeq uses a machine learning model (the BLIModel) to select the top heavy and light chain sequences recovered from each bead. From Opto Plasma B Discovery 4.0 workflows performed on 46 chips, the average paired heavy/light chain sequence recovery rate was 65% (ranging from 43%-79%) (**FIGURE 6A and B**). Sequence recovery rates vary based on cell preparation, viability of cells at time of cDNA generation, and library preparation. Primers are optimized for mouse heavy and kappa light chains.

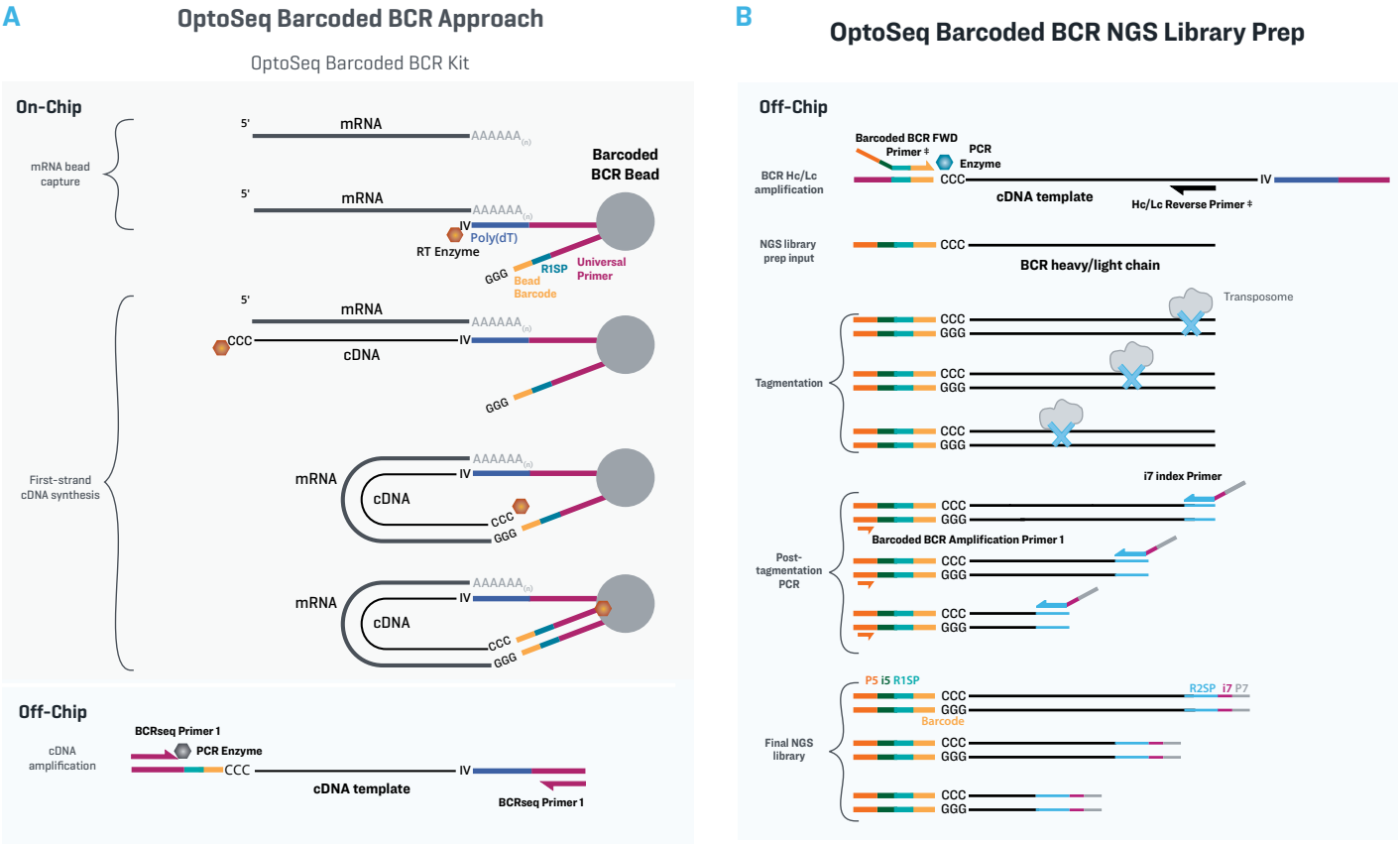


FIGURE 4: OptoSeq Barcoded BCR enables accurate sequencing of full-length, paired heavy/light chains (Hc/Lc) from >1,000 antibodies from a single run on standard Illumina NGS sequencing platforms. A) The OptoSeq Barcoded BCR kit is used for on-chip cell lysis, on-chip cDNA synthesis and off-chip cDNA amplification from recovered beads. **B)** The OptoSeq Barcoded BCR NGS Prep kit and Nextera XT DNA Library Prep kit use amplified cDNA to prepare NGS libraries for 150x150 sequencing compatible with standard Illumina sequencing platforms.

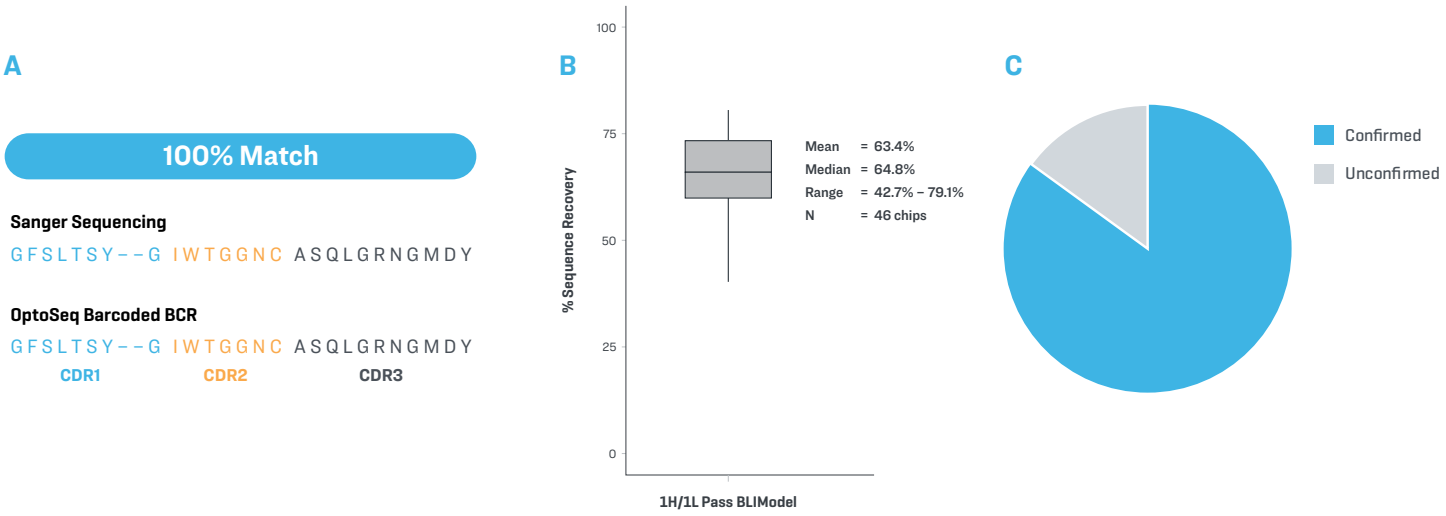


FIGURE 5: OptoSeq Barcoded BCR enables accurate sequencing and re-expression of >1,000 antibodies in just 1 week. **A)** Accurate, full length variable region sequences are obtained using a combination of fragmentation, NGS sequencing, and bioinformatics analysis. The ability to reconstruct full-length antibody genes from short reads enables rapid sequencing of hundreds of antibodies from a single run on standard Illumina sequencing platforms. **B)** From Opto Plasma B Discovery 4.0 workflows performed on 46 chips, the average paired heavy/light chain sequence recovery rate was 65% (ranging from 43%-79%). **C)** Over 85% of re-expressed antibodies (n = 271) demonstrated confirmed function in plate-based ELISA assays.

RAPID RE-EXPRESSION

Production of lead molecules for downstream characterization typically includes costly gene synthesis and laborious bacterial cloning.

The Opto BCR Rapid Re-expression kit enables re-expression of antibodies in just 1 week to confirm function in plate-based assays [**FIGURE 6A**]. Amplified cDNA is cloned into expression constructs in one step to enable generation of >1,000 antibodies for functional confirmation. Over 85% of re-expressed antibodies (n = 271) demonstrated confirmed function in plate-based ELISA assays [**FIGURE 5C**].

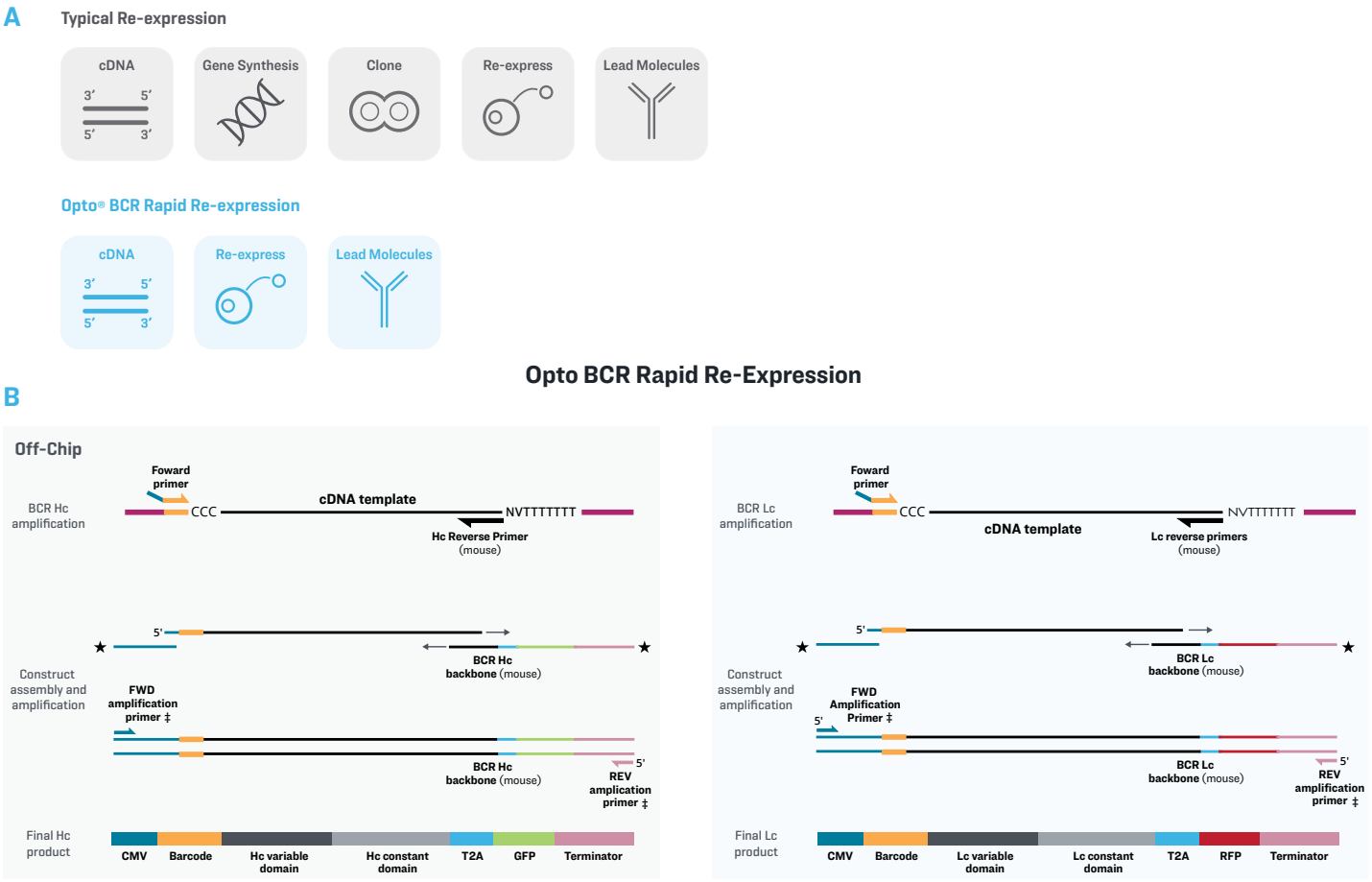


FIGURE 6: The Opto BCR Rapid Re-expression kit converts antibody sequences into molecules in just 1 week for plate-based confirmation assays. A) Opto BCR Rapid Re-expression bypasses costly gene synthesis and laborious bacterial cloning to re-express antibodies for plate-based confirmation assays. **B)** cDNA from the OptoSeq Barcoded BCR kit is amplified and directly cloned into heavy and light chain expression constructs for rapid re-expression of mouse IgG2a antibodies.

CONCLUSION

In just 1 week, the Opto Plasma B Discovery 4.0 workflow enables recovery of 1,000s of hits by screening 20,000 to 40,000 plasma B cells, down-selecting lead candidates by functional profiling, and sequencing and re-expressing >1,000 functionally-characterized antibodies. By integrating functional profiling of the B cell repertoire with high-throughput sequencing and rapid re-expression, the workflow enables users to rapidly profile broad B cell diversity.

ORDERING INFORMATION

PART NO.	PRODUCT NAME	QUANTITY
750-02004	**Opto Plasma B Discovery 4.0 kit, Mouse (4-chip)** • 4 OptoSelect 20k chips • 1 Opto Plasma B Discovery Sample Prep kit Mouse • Assay Beads • OptoSeq Barcoded BCR kit • OptoSeq Barcoded BCR NGS Prep kit, Mouse • Opto BCR Rapid Re-expression kit, Mouse	Suitable for a single 4-chip workflow with up to 1152 OptoSeq Barcoded BCR exports in 1x 96-well plate, and re-expression of up to 192 antibodies
520-00053	**Assay Beads**	Suitable for up to 16 binding assays
750-02050	**Opto Plasma B Discovery Sample Prep kit, Mouse** • Plasma B Cell Media, Mouse • Loading Reagent • Wetting Additive • DNA Clean Up	Suitable for a single 4-chip workflow
750-08097	**Extended OptoSeq BCR Bead Unload kit**	Suitable for recovery of beads into 2x 96-well plates from a single workflow
750-00019	**OptoSelect 20k chip**	1
750-01005	**OptoSeq Barcoded BCR kit**	Suitable for a single 4-chip workflow with up to 1152 OptoSeq Barcoded BCR exports in 1x 96-well plate
750-02052	**OptoSeq Barcoded BCR NGS Prep kit, Mouse**	Suitable for up to 1152 mouse B cells in 1x 96-well plate
750-02031	**Opto BCR Rapid Re-expression kit, Mouse***	Suitable for up to 192 antibodies

* Compatible with Opto Plasma B Discovery 1.0, 2.0, and 4.0 workflows.

OTHER CONSUMABLES AND REAGENTS

PART NO.	PRODUCT NAME	QUANTITY
520-70000	**DNA Clean Up**	1
750-08029	**Loading Reagent**	1
750-08091	**Wetting Additive kit**	1
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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