Rapid Antibody Discovery on the Beacon® Platform

Keith Breinlinger[1], Minha Park[1], Ravi Ramenani[1], Adrienne Higa[1], Hariharasudhan Chirra[1], Kai Szeto, Yuxing Cheng[2], Jason Lavinder[2], Tao Sai[2], Jessica Yu[2], and Gabriel Cheung[2]

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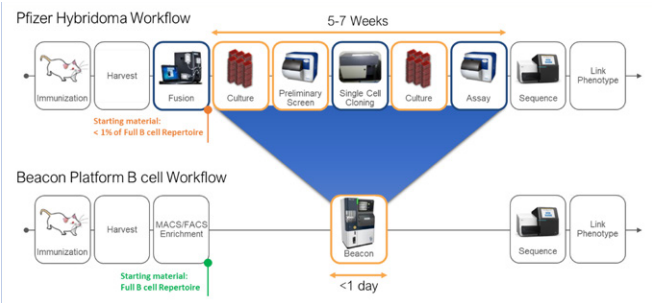
Introduction

Berkeley Lights, Inc. (BLI) has developed a platform that combines structured visible light technology and nanofluidic design to automate the screening and manipulation of thousands of primary antibody secreting cells (ASCs) in parallel, reducing antibody discovery timelines from months to a single day. The Beacon platform can screen thousands of primary ASCs for real-time secretion against multiple binding targets. For ASCs of interest, the Beacon platform can export live cells for downstream molecular biology, including direct cloning or sequencing.

Traditional methods of antibody discovery, including hybridoma, phage display, and EBV-transformed clones, are often labor-intensive, require months of work, and have inherent inefficiencies. The Beacon platform's one-day antibody discovery workflow enables scientists to bypass these traditional methods by directly screening primary ASCs.



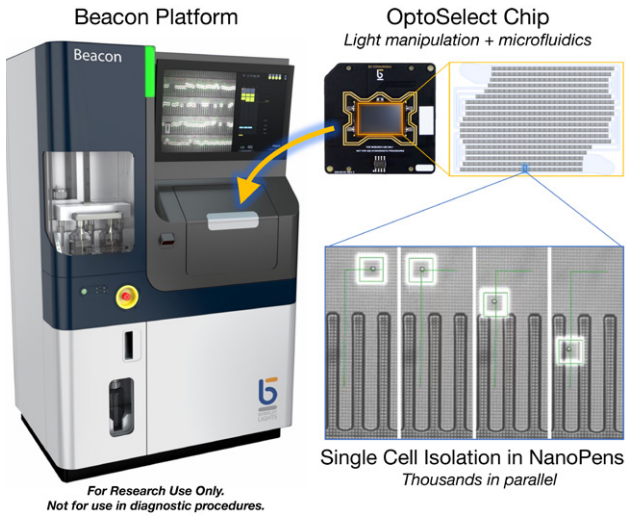
Beacon Platform

The Beacon platform blends novel nanofluidic design with semiconductor technology to enable thousands of single cell experiments in parallel.

BLI's OptoSelectTM chip overlays a perfusion-based nanofluidic system on top of light-activated phototransistor arrays. This enables the rapid and precise movement and positioning of single cells using controlled patterns of light.

The OptoSelect chip contains thousands of flow-isolated nanoliter pens or chambers, called NanoPensTM, which physically isolate cells from flow but enable diffusion-based exchange.

With the Beacon platform, thousands of single cells can be isolated, cultured, assayed, and exported for further analysis across multiple OptoSelect chips.



Materials and Methods

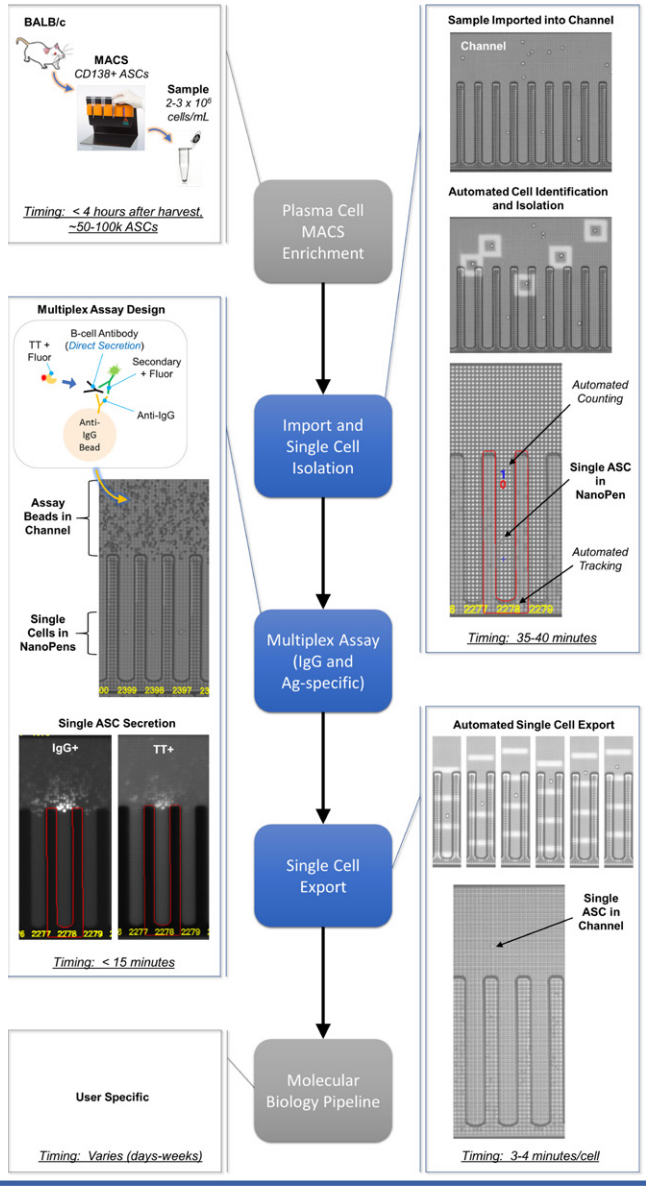
The Beacon platform antibody discovery workflow was demonstrated by screening mouse plasma cells.

BALB/c mice were immunized with 10 µg of Tetanus Toxoid (TT) on Days 1, 14, and 28, with a final boost three days before harvest. On the day of experiments, bone marrow from high-titer mice (EC50: > 1:50,000 serum titration) were isolated and enriched for CD138+ B cells with magnetic-activated cell sorting, or MACS.

Following enrichment, the cell population was imported into the Beacon platform to start the workflow. On the Beacon platform, thousands of single cells were loaded, using BLI's proprietary light technology, into NanoPens with volumes less than 1 nL. After loading, the cells were screened for TT antigen-binding and IgG secretion using a multiplexed bead-based assay with single-cell sensitivity.

Beacon Platform One-day Antibody Discovery Workflow

Beacon platform steps in blue, off-chip steps in gray



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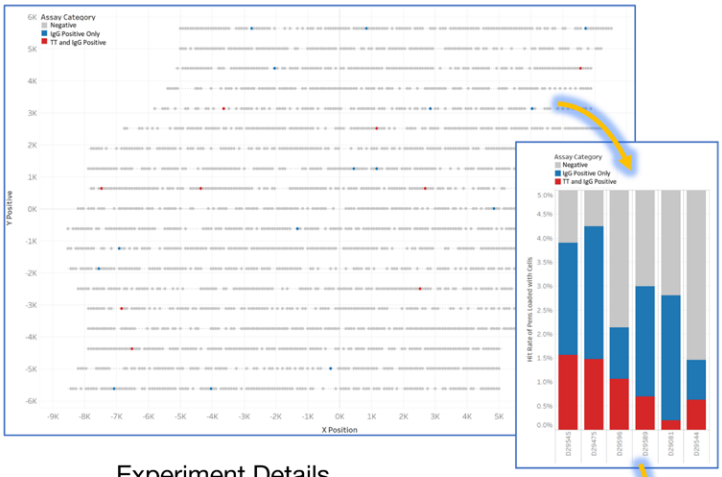
Results

The multiplex assay identified TT-binders as early as 10 minutes, with a hit rate ranging from 0.25-1.5%. Total IgG hit rates ranged from 1.5-4.2%. On-chip assay results matched TT-binding hit rates from an ELISpot off-chip control. TT-binders were exported into 96 well plates to recover paired heavy chain (HC) and light chain (LC) sequences. Up to 90% of the exported individual cells yielded paired HC and LC sequences with more than 80% having TT-binding confirmed through ELISA.

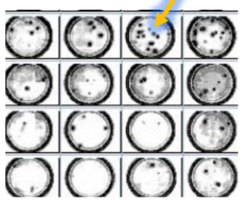
Chip-level and NanoPen-level Tracking



Experiment Details

	Experiment 1	Experiment 2
Total Bone Marrow Cells	~200,000,000	~188,000,000
CD138+ Cells	~350,000	~20,000
Cells Analyzed	13,721	3,041
IgG+ Hit Rate (%)	1.0-1.8	1.5-4.2
TT+ Hit Rate (%)	0.2-0.8	0.2-1.5
Cells Exported	25 (7 IgG+, TT+, clonal)	61 (16 IgG+, TT+, clonal)
Cells Cloned	20	
Cells with Confirmed TT-binding	16	

Beacon Platform Assay Hit Rates Match Off-chip ELISpot Controls



Conclusions

- The Beacon platform can rapidly isolate and screen thousands of primary ASCs in a single-day workflow.
- Primary ASCs that demonstrate antigen-specific binding can be exported from the Beacon platform for the recovery of paired heavy and light chain sequences.
- The paired heavy and light chain sequences recovered from the exported primary ASCs have shown antigen-specific binding upon re-expression, demonstrating are relevance of Beacon platform's assays to the antibody discovery process.

References

1) Fitzgerald et al, **Methods** (2017) 116:34-42

2) Starkie et al, **PLoS One** (2016) 11(3)

3) Miltenyi Biotec. (2017) "CD138 MicroBeads, mouse." Auburn, CA.

4) Nduati et al, **PLoS One** (2010) 5(11)

5) Halliley et al, **Immunity** (2015) 43(1):132-45

Sampling of Recovered HC/LC Sequences

from Single ASCs Exported from Beacon platform

Chip ID	IGHV	IGHD	IGHJ	Hc-CDR3	IGKV/IGLV	IGKJ/IGLJ	Kc-CDR3/Lc-CDR3
D29596	IGHV6-3*03	IGHD1-1*01	IGHJ2*01	TGGYYYGTSFYFDY	IGKV19-93*01	IGKJ1*01	LQYDNLLRT
D29596	IGHV1S135*01	IGHD2-1*01,IGHD2-10*01,IGHD2-10*02	IGHJ3*01	TRSGNFAY	IGKV9-124*01	IGKJ1*01	LQYASYPWT
D29544	IGHV8-12*01	IGHD1-1*01,IGHD1-2*01	IGHJ3*01	ARIYYGWFAY	IGKV4-58*01	IGKJ2*01	QQWSGYPYT
D29544	IGHV5-9-4*01	IGHD1-1*02	IGHJ4*01	ARRAHGSYEDYAMDY	IGKV8-24*01	IGKJ2*01	QQHYSIPYT
D29589	IGHV8-8*01	IGHD1-1*01	IGHJ2*01	ARTITTVGHFDY	IGKV8-21*01	IGKJ1*01	KQSYSLPT
D29589	IGHV5-9-3*01	IGHD2-10*02,IGHD2-11*01	IGHJ4*01	ARHRYGNGGFYAMDY	IGKV1-110*01	IGKJ2*01	SQSTHVPYT
D29545	IGHV1-69*02	IGHD2-3*01	IGHJ2*01	ARDGRYYYYFDY	IGKV4-55*01	IGKJ1*01	QQWSSYPRT
D29545	IGHV5-9-3*01	IGHD2-10*02,IGHD2-11*01	IGHJ4*01	ARHRYGNGGFYAMDY	IGKV1-110*01	IGKJ2*01	SQSTHVPYT
D29596	IGHV1-14*01	IGHD2-3*01	IGHJ4*01	TRNLYNGYYVKYYAMDY	IGKV6-17*01	IGKJ1*01	QQHYTTPWT
D29545	IGHV8-8*01	IGHD1-1*01	IGHJ2*01	ARTITTVGHFDY	IGKV13-84*01	IGKJ2*01	QQYWNTPYT
D29545	IGHV1-9*01	IGHD1-1*01	IGHJ3*01	ARHYYGSSYFAY	IGKV4-91*01	IGKJ4*01	QQGINIFT
D29081	IGHV2-2*02	IGHD2-14*01	IGHJ2*01	VRGNYRYFDY	IGKV12-41*01	IGKJ1*01	QHFWSIPWT
D29475	IGHV1-26*01	IGHD2-2*01,IGHD2-7*01,IGHD2-9*01	IGHJ2*01	ARRKAYGYDGDWDY	IGKV3-4*01	IGKJ1*01	QQSNEDPWT
D29475	IGHV1-14*01	IGHD1-1*02	IGHJ4*01	ARCLYGGSRYYAMDY	IGKV4-80*01	IGKJ4*01	HQWSSFT
D29475	IGHV1-39*01,IGHV1S135*01	IGHD1-1*01	IGHJ3*01	ARAYGSSSWFAY	IGKV4-61*01	IGKJ1*01	QQYHSYPWT
D29475	IGHV2-6*02	IGHD1-2*01	IGHJ4*01	ARNNGPYYTMLY	IGKV12-44*01	IGKJ1*01	QHHYGAPWT
D27708	IGHV2-9*02	IGHD2-1*01,IGHD2-10*01,IGHD2-11*02	IGHJ2*01	ARDFSYGNYFDY	IGKV4-91*01	IGKJ2*01	QQGSSIPRT
D27708	IGHV2-6-1*01	IGHD2-4*01,IGHD2-9*02	IGHJ4*01	ARHGGGLRRFLYSMDY	IGKV6-13*01	IGKJ5*01	QQYSSYPLT
D27708	IGHV5-9-4*01	IGHD2-14*01	IGHJ4*01	ARGNYRYDGAMDY	IGKV12-44*01	IGKJ1*01	QHHYGIPRT
D27700	IGHV1-14*01	IGHD2-3*01	IGHJ4*01	ARHYDGYYGFPYALDY	IGKV10-96*01,IGKV10-96*01_Mus_spretus	IGKJ4*01	QQVNTLPFT