

Single B Cell Technology for Antibody Discovery

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WuXi Biologics
Global Solution Provider

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Agenda

01

Single B cell Technology

- Introduction of major technologies for antibody discovery
- Beacon[®] Optofludic System for Single B Cell Screening
- Assays on Beacon

02

Antibody Discovery by Single B Screening

- Case 1: Isolation of Blocking Antibodies
- Case 2: Identify Antibodies with Diverse Epitopes and Affinities
- Case 3: Antibody Discovery on a Multi-pass Transmembrane Target
- Case 4: Develop Rabbit Antibody with Super High Affinity
- Case 5: Develop Phospho-Specific Rabbit Antibody

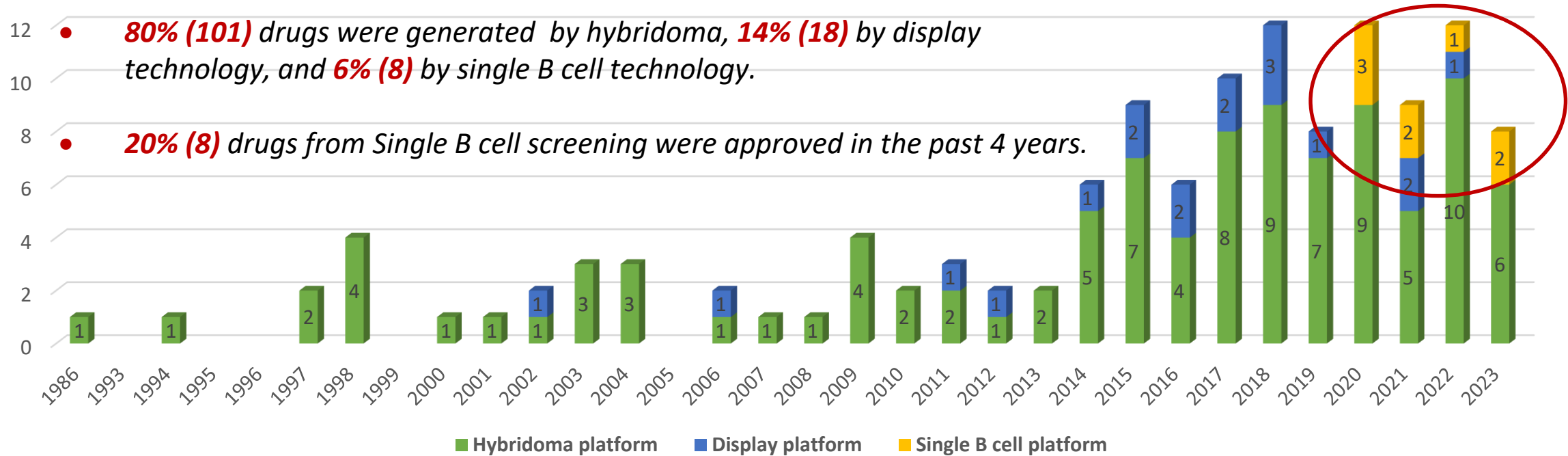
03

Summary/Q&A

Single B Cell Technology

01

FDA Approved Therapeutic Antibodies by Technology Platform



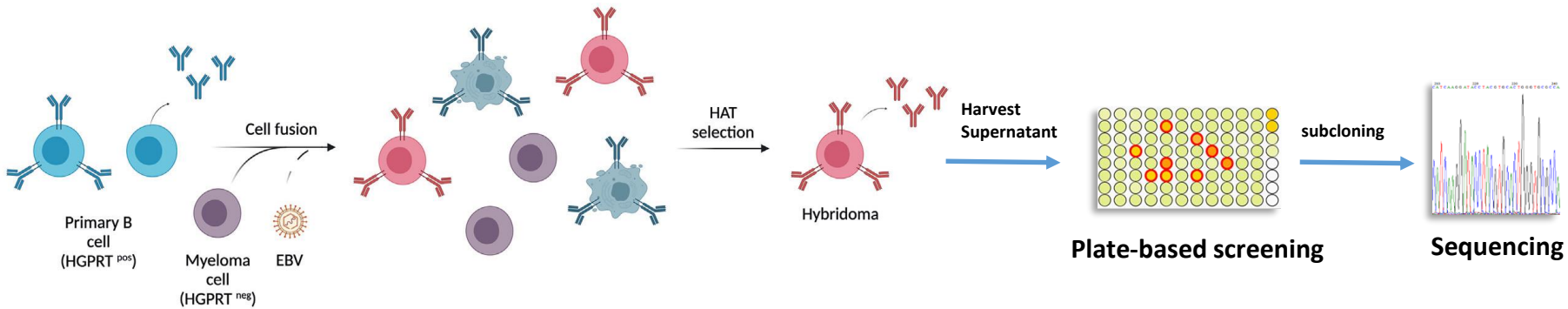
Drugs from B cell technology	Brand name	Target; Format
Aducanumab, aducanumab-avwa	Aduhelm	Amyloid beta; Human IgG1
Evinacumab	Evkeeza	Angiopoietin-like 3; Human IgG4
Ansuvimab	Ebanga	Ebola virus; Human IgG1
Atoltivimab, Maftivimab, and Odesivimab-ebgn	Inmazeb	Ebola virus; mixture of 3 human IgG1
Eptinezumab	Vyepti	CGRP; Humanized IgG1
Tixagevimab, cilgavimab	Evusheld	SARS-CoV-2; Human IgG1
Nirsevimab	Beyfortus	RSV; Human IgG1
Rozanolixizumab	RYSTIGGO®	FcRn; Humanized IgG4

<https://www.antibodysociety.org/resources/approved-antibodies/>

Lu et al. Journal of Biomedical Science (2020) 27:1

Methods for Antibody Generation and Screening (1/2)

1. B Cell Immortalization Technologies (Hybridoma, Viral immortalization of the B cell)

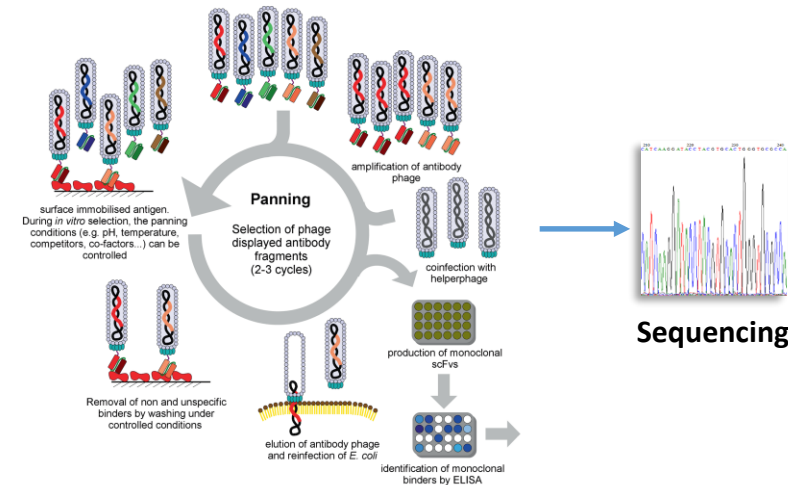


- Pros**
- High affinity
 - Developability
 - Function screen
- Cons**
- Partial B cells
 - Lack of proper fusion partner

2. Phage/Yeast Display Technologies

Mouse/human/rabbit
B cell
(immunized or naïve)

Library construction
(Ab fragment)



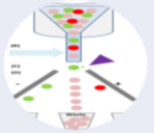
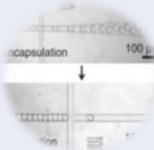
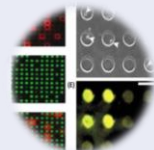
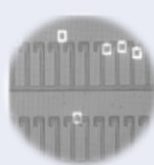
Sequencing

- Pros**
- No species limitation
 - Epitope guided
- Cons**
- Low affinity
 - Developability

Trends in Immunology, December 2021, Vol. 42, No. 12
F. Tomszak et al. Advances in Experimental Medicine and Biology, 2016

Methods for Antibody Generation and Screening (2/2)

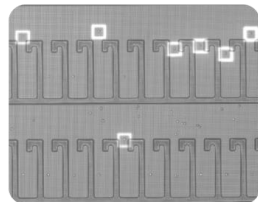
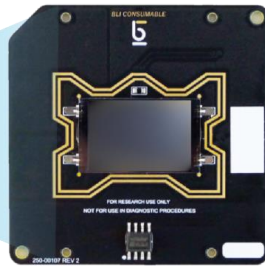
3. Single B Cell Screening Technologies

Technology	Features	Limitations	Providers
 FACS Sorting	A well established and validated single cell screening method	<ul style="list-style-type: none"> • Only soluble antigens screening • Cell-surface binding only (memory B cell only) 	
 Nanodroplet (Microdroplet)	Encapsulate individual cells by capturing them in two-phase droplets. Droplets are then sorted by Fluorescence activated droplet sorting	<ul style="list-style-type: none"> • Can screen secreted antibody, but single assay w/o wash • Generation and manipulation of the droplets requires very precise conditions • Low rate of single B in droplet 	<ul style="list-style-type: none"> • HiFi Bio • Sphere Fluidics
 Nanowells (Microwells)	Seeding of individual cells into a chip containing microengraved wells and analyzing directly in the well or by capturing secreted proteins on a slide sealed	<ul style="list-style-type: none"> • Random distribution of B cells • Single or single multiplexed assay in nanowells w/o wash 	<ul style="list-style-type: none"> • Enumeral Biomedical • ISAAC
 Microfluidic Chamber	<p>These devices employ a microfluidic chip like chambers where cells are captured and cultured. Run micro-assays in each micro-chamber.</p> <ul style="list-style-type: none"> • High rate of single B cell distribution • Flexibility in number and format of assays 	<ul style="list-style-type: none"> • Dedicated expensive equipment • Experts for assay development and machine running 	<ul style="list-style-type: none"> • Abcellera • Berkeley Lights

Beacon Optofluidic Technology



OptoSelect™ chips use light to automatically move individual cells.
[CHIP SHOWN ACTUAL SIZE]



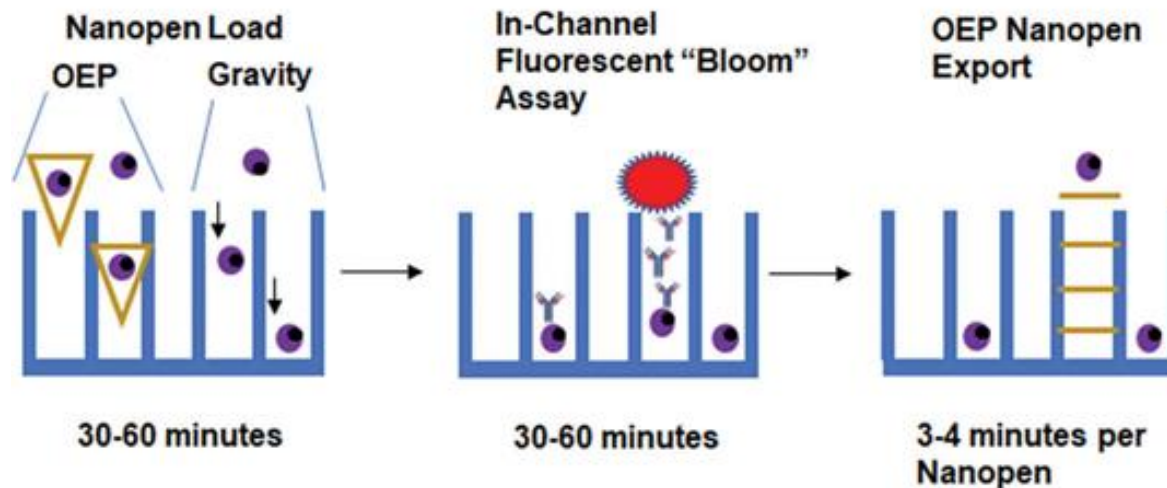
Cells are cloned and assayed in individual 500 pL or 1 nL NanoPens™. Each pen is ~100,000 times smaller than a microwell.

- Automatically identifies single cells and directs them into NanoPen chambers all at once. Light patterns move them into position for export to a well plate.
- The OptoSelect chips replace typical well plates. Each OptoSelect chip contains thousands of NanoPen chambers, which are like wells on a microplate. **It enables high resolution screening of antibody secreting B-cells with High-throughput in one day.**
- Days after tissue collection, the Beacon provides a rich data set about each antibody screened to identify the rare, active, cross-reactive mAbs. Those antibodies are then sequenced and expressed rapidly to advance them to downstream assays.
- Assays in the nanopen or in the channel. 5 fluorescence channels to support multiplex assays. Cells can be cultured in the nanaopen.

General Workflow for Mab Discovery on Beacon

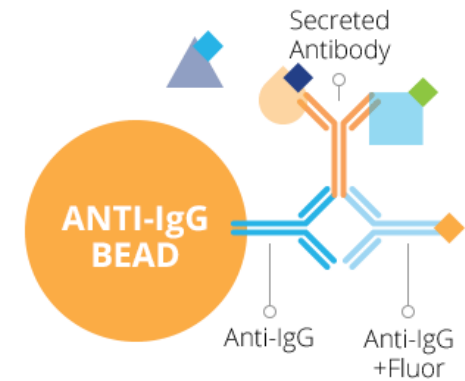
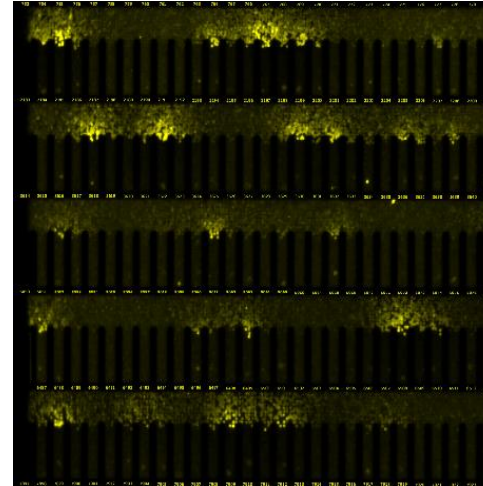
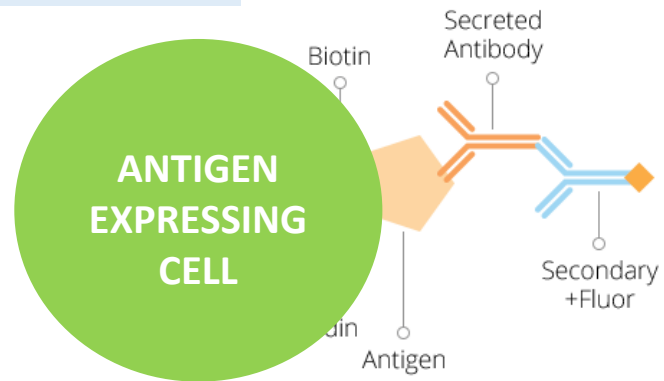


Specialized B cell isolation methods for mouse/ rat/ alpaca /llama/ rabbit

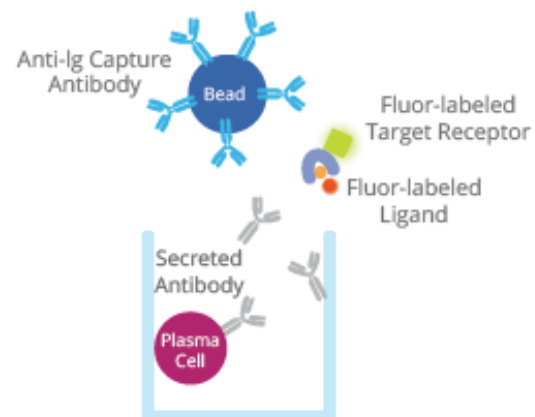
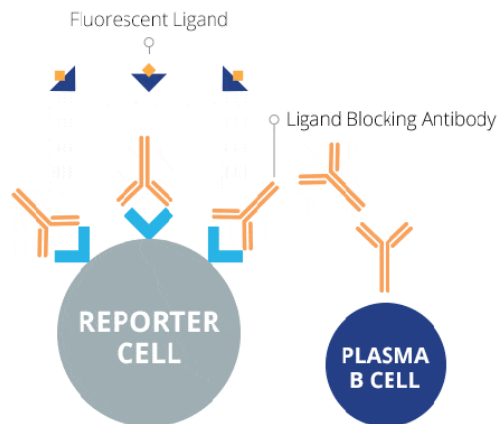


Assays on Beacon

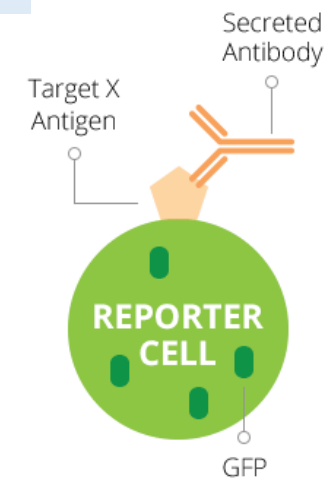
Antigen Binding Assay



Receptor/Ligand Blocking Assay



RGA Assay



<https://www.berkeleylights.com>

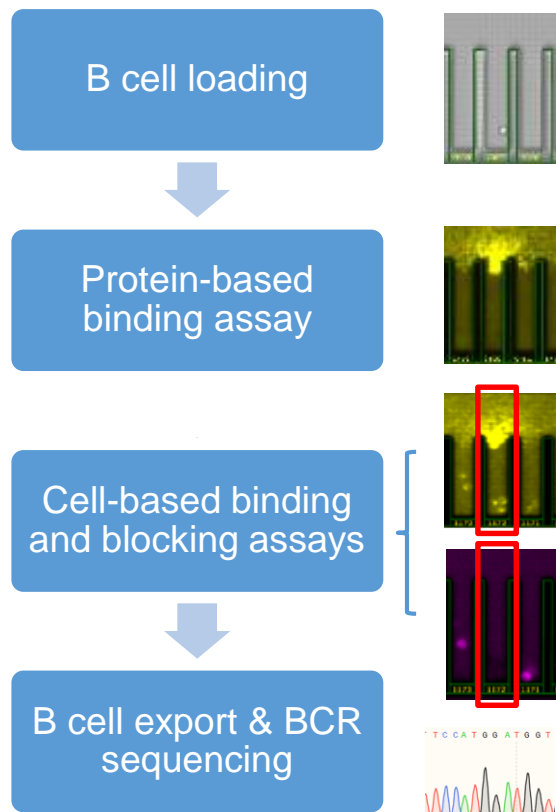


**Antibody Discovery by
Single B Screening**

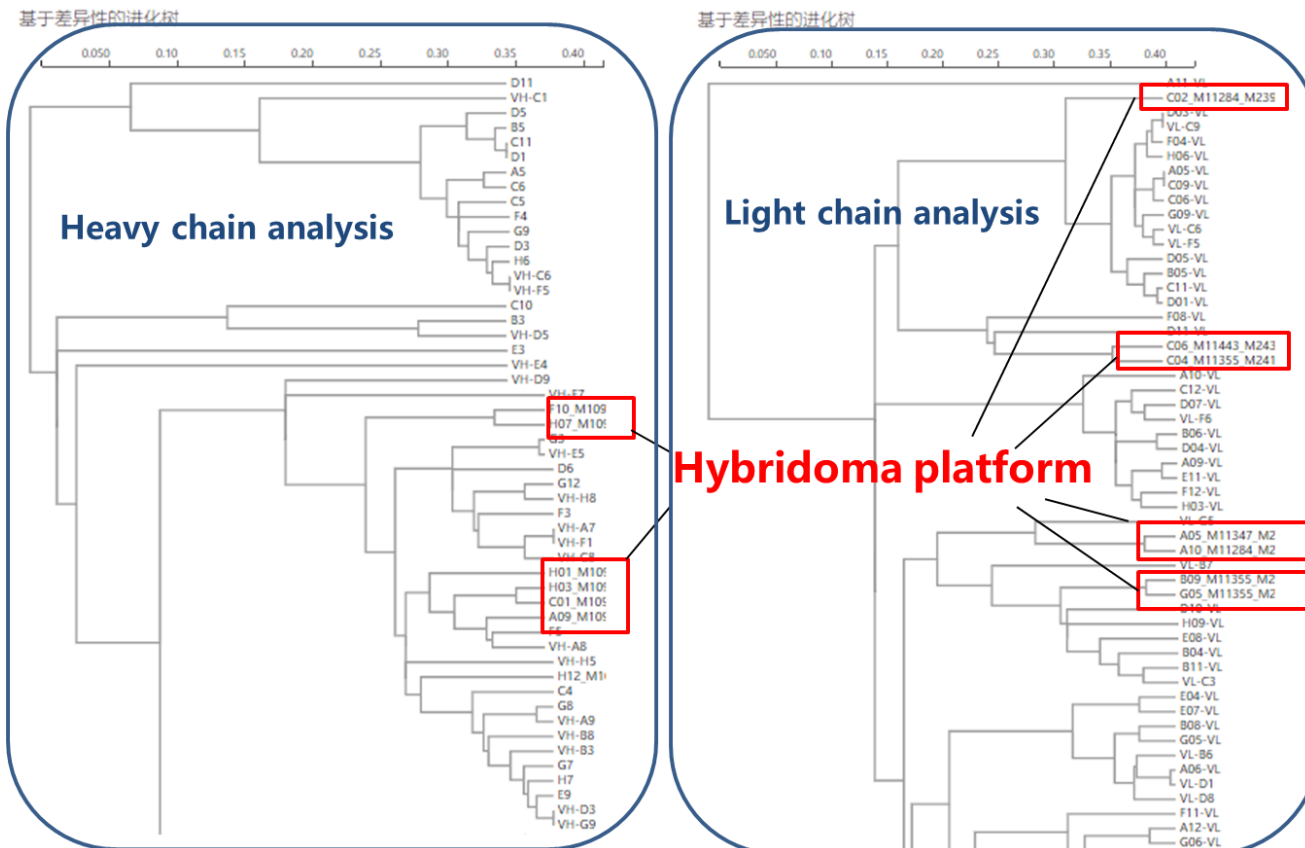
02

Case 1: Isolation of Blocking Antibodies with Diverse Sequences (1/2)

Workflow on Beacon®



Antibody sequences clustering analysis



- 40+ antibodies with blocking activity were identified from one chip.
- Antibodies selected via single B cell screening are highly diverse in both heavy chain and light chain.
- Many sequences were exclusively found from single B screening.

Case 1: Isolation of Blocking Antibodies with Diverse Sequences (2/2)

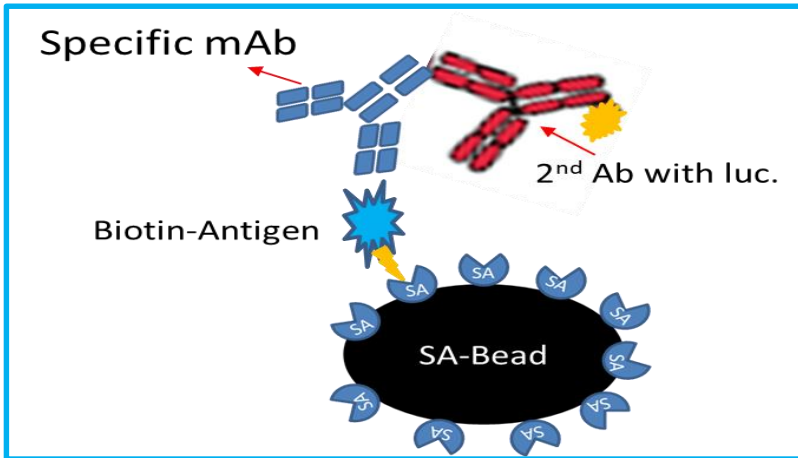


Figure 1. Antigen-beads binding in channel

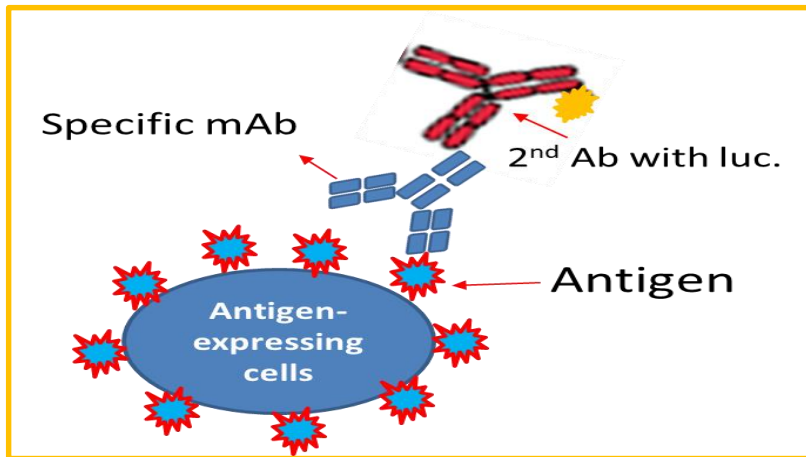
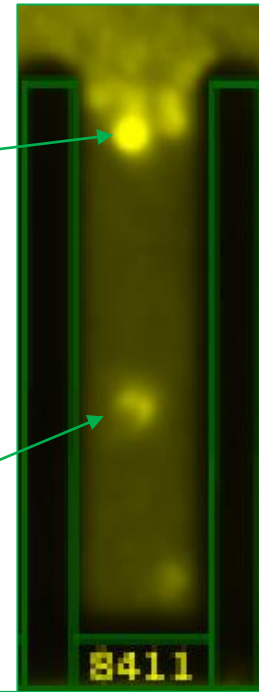
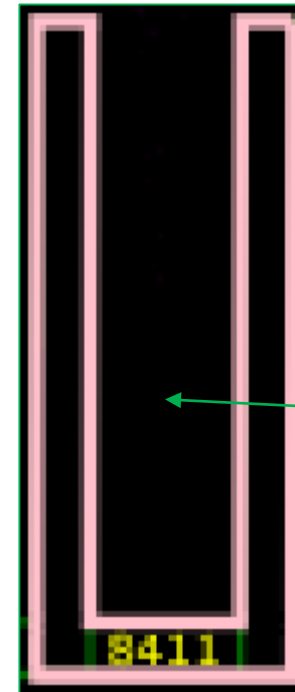


Figure 2. Antigen-cell binding in pen

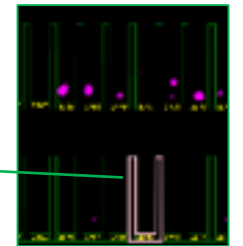
Antigen Binding Positive + Ligand Binding Negative = Blocker



Antigen binding (+)



Ligand binding (-)



Case 2: Discovery of mAbs against a Multi-Pass Transmembrane Target (1/2)

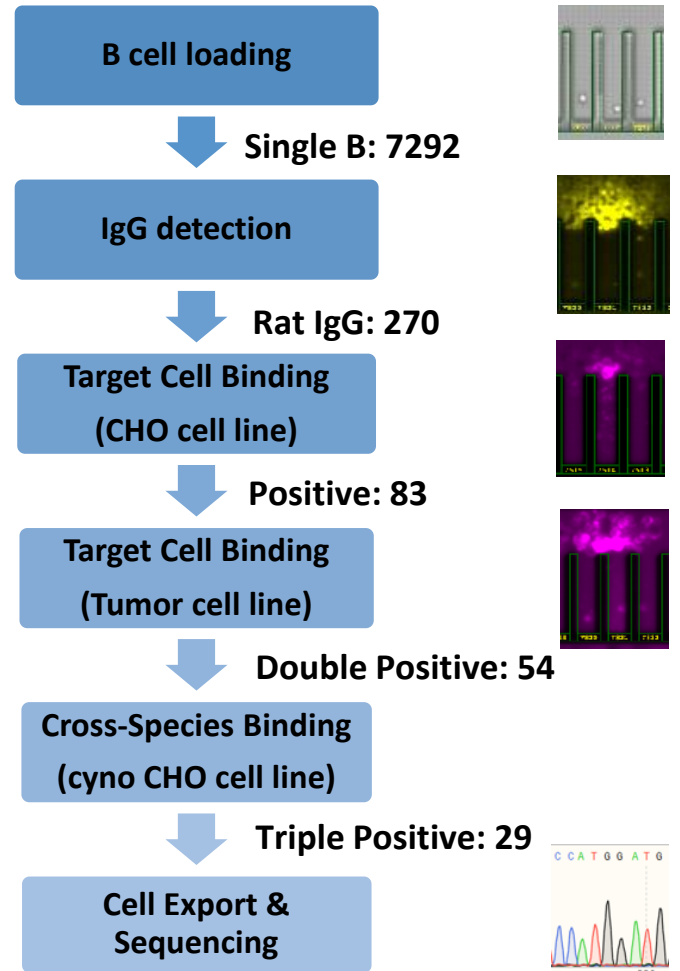
Rat Immunization

Serum Titer	Group 1 (Cell Immunization)		
Animal No.	#1	#2	#3
2 nd bleed	72900/24300	218700/24300	72900/8100
1 st bleed	218700	218700	72900
Pre-bleed	<100	<100	<100

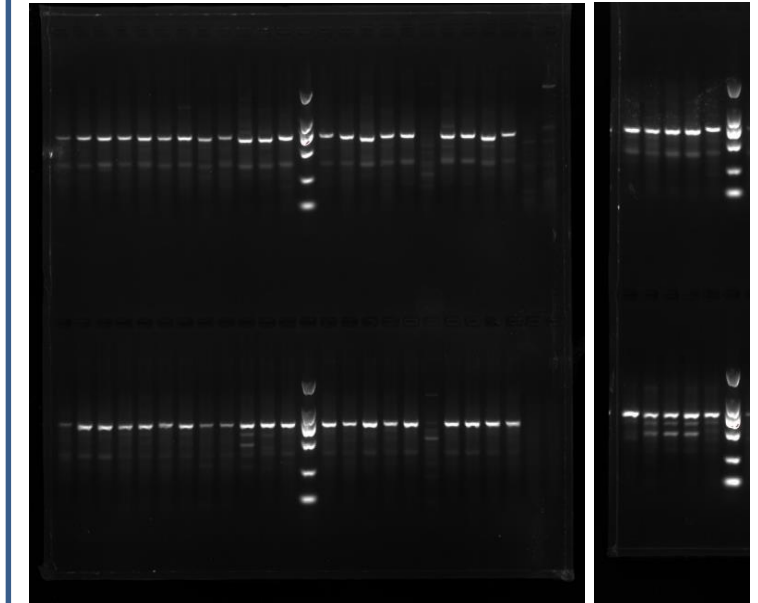
Serum Titer	Group 2 (DNA Immunization)		
Animal No.	#1	#2	#3
2 nd bleed	72900/300	72900/<100	218700/<100
1 st bleed	300	900	8100
Pre-bleed	<100	<100	100

- ADC candidates against an eight-pass transmembrane protein.
- SD Rats were immunized with cell or DNA using WuXi Bio's immunization protocols

Single B Screening on Beacon[®]

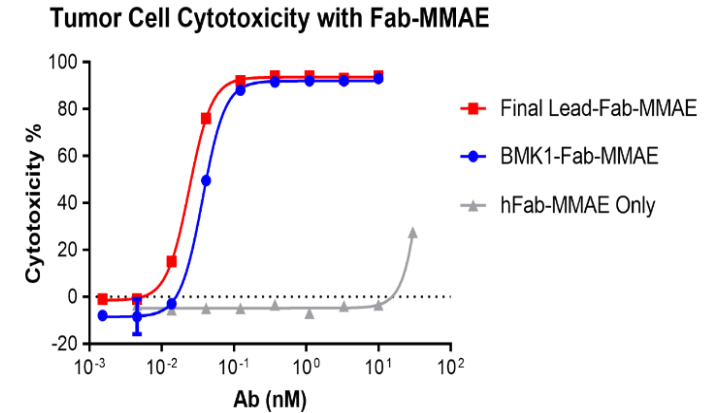
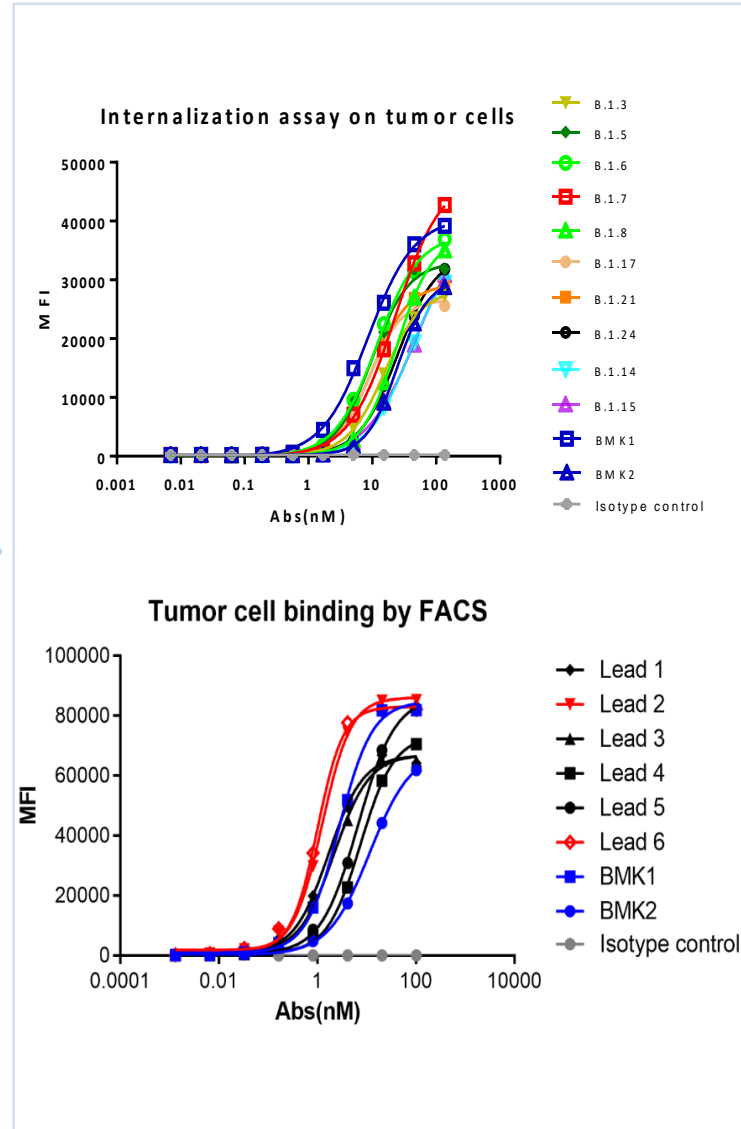
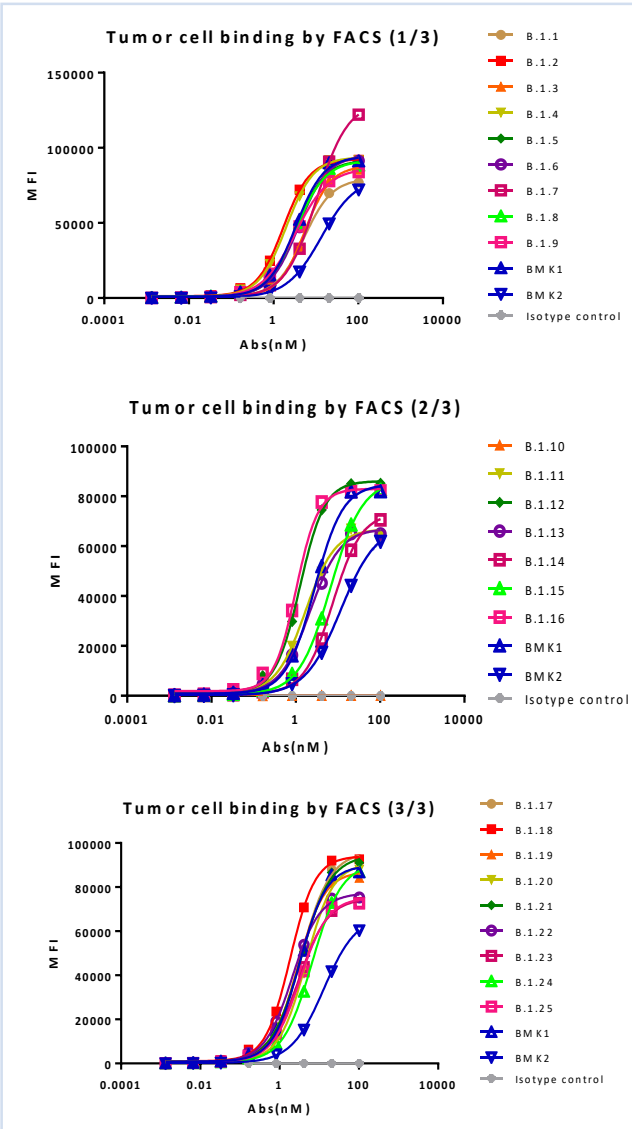


VH/VL Sequencing



- A total of 29 single B cells were sequenced and 25 pairs of VH/VL sequences were obtained (86% recovery).
- 24 out of 25 (96%) recombinantly expressed mAbs maintained binding activities against the target cell line.

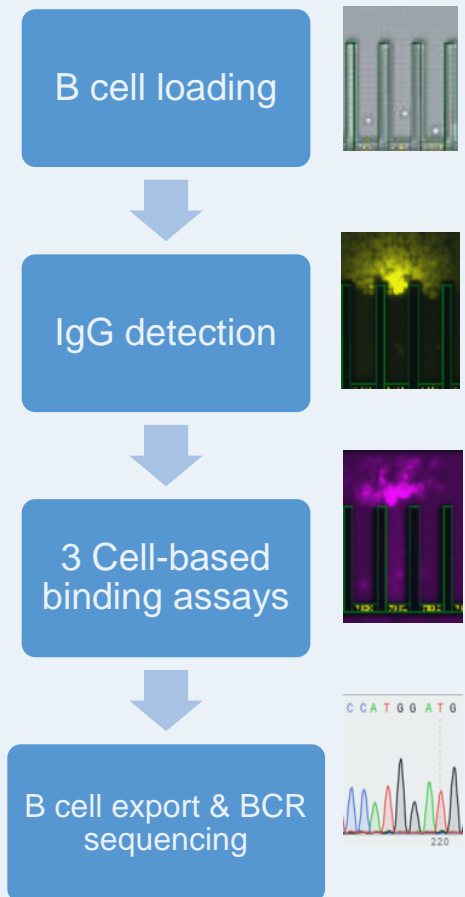
Case 2: Discovery of mAbs against a Multi-Pass Transmembrane Target (2/2)



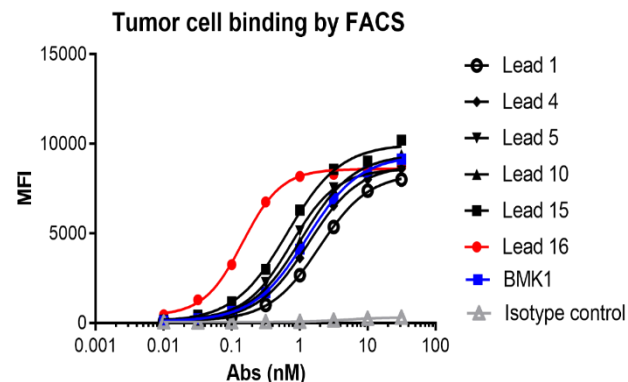
- All the Single B derived antibodies showed nanomolar binding EC_{50} to target-expressing cell line.
- Some candidates showed better binding activity than benchmark antibodies and strong internalization activity.
- The final lead antibody showed better cytotoxicity activity than BMK.

Case 3: Identify Antibodies with Diverse Epitopes and Affinities

Workflow on Beacon

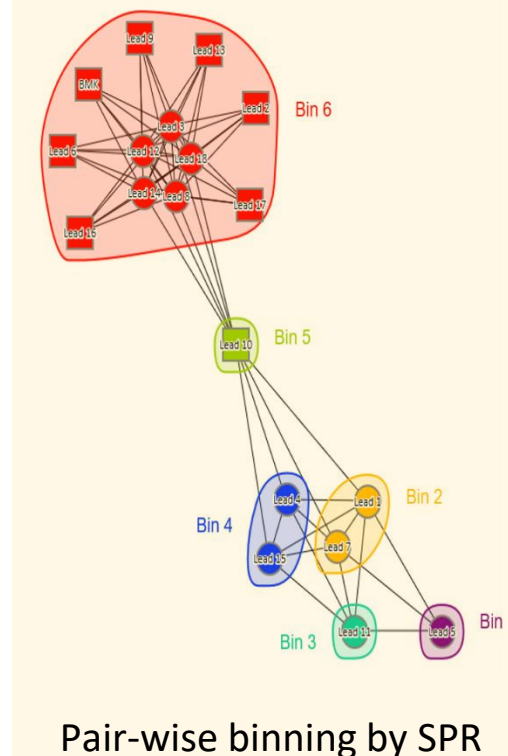


In-vitro Lead Characterization



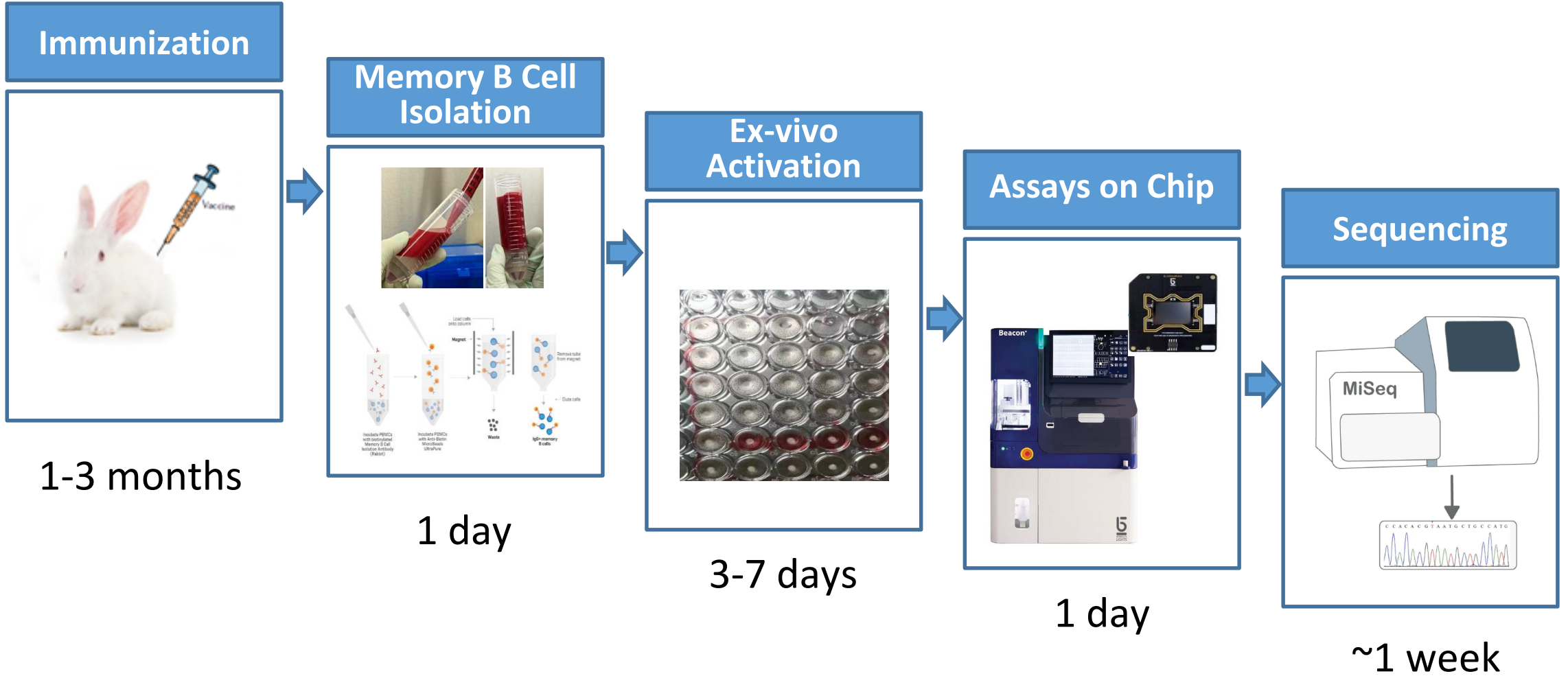
Ligand	K_D by SPR (M)
Lead 1	1E-10
Lead 4	1E-08
Lead 5	4E-10
Lead 10	7E-09
Lead 15	2E-09
Lead 16	5E-10
BMK1	3E-08

Diverse Epitope

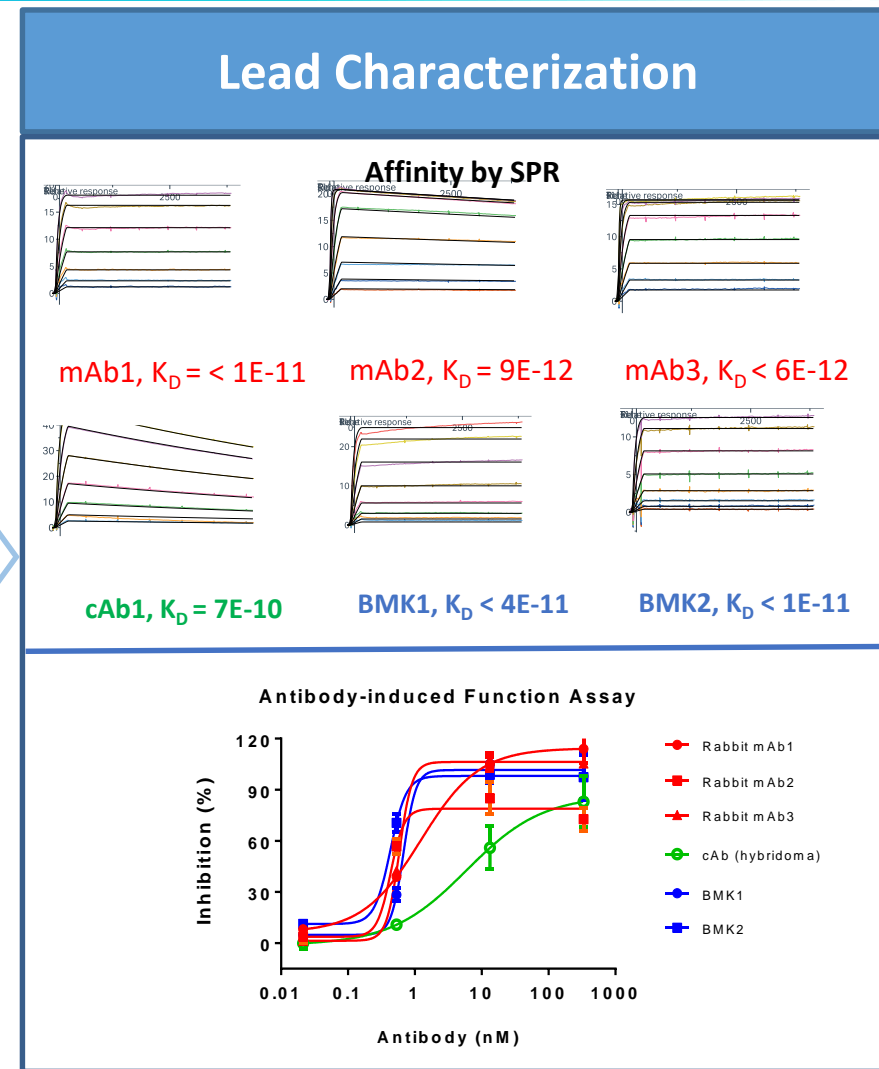
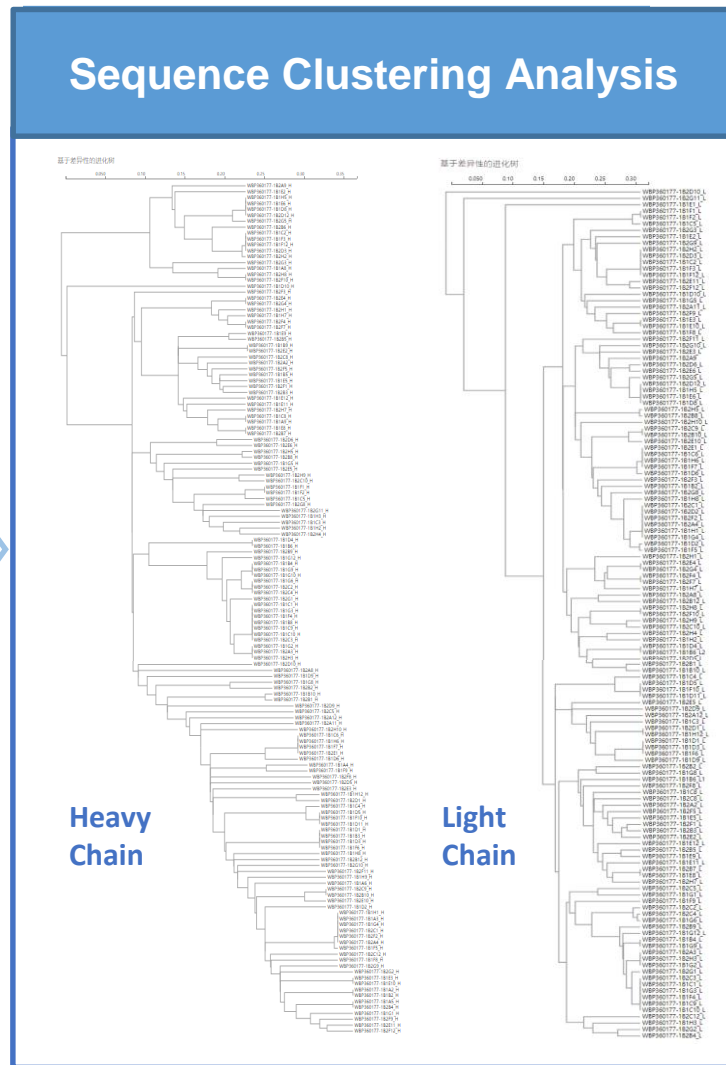
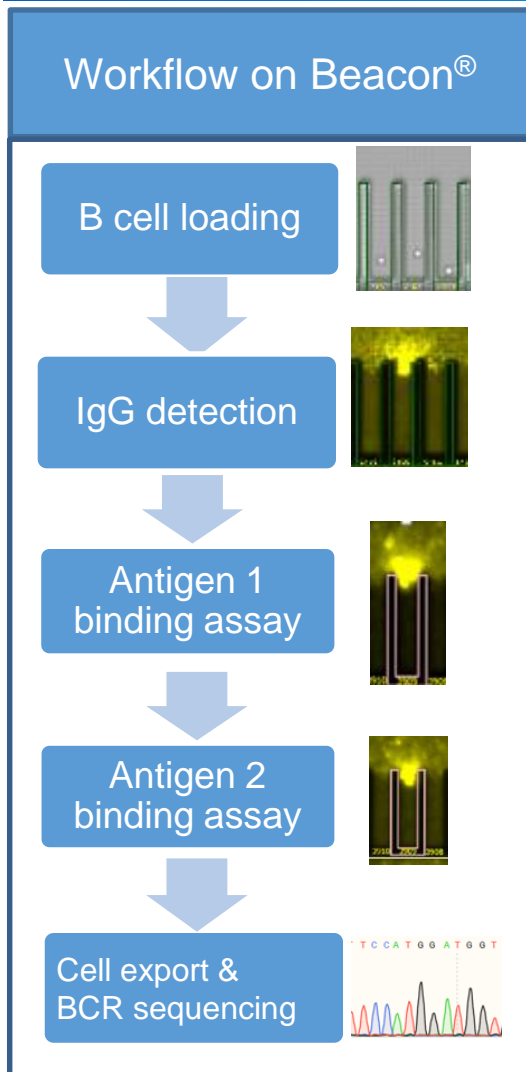


- A series of antibodies against a tumor associated antigen (TAA) target for the construction of T cell engagers (TCE).
- Selected lead antibodies showed wide-range affinity and diverse epitopes

Rabbit Antibody Discovery by Single B Cell Screening

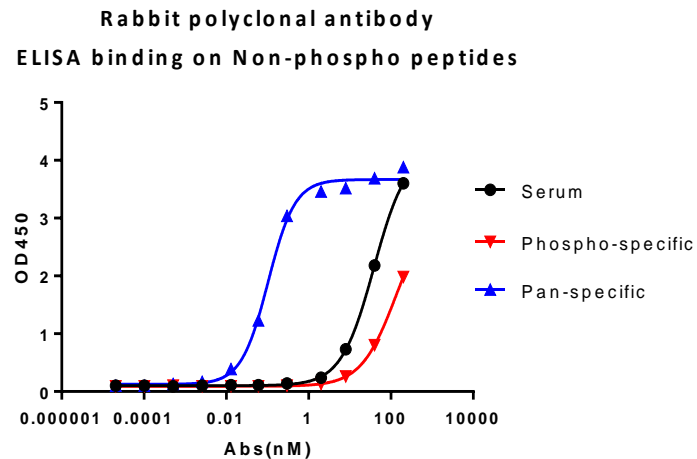
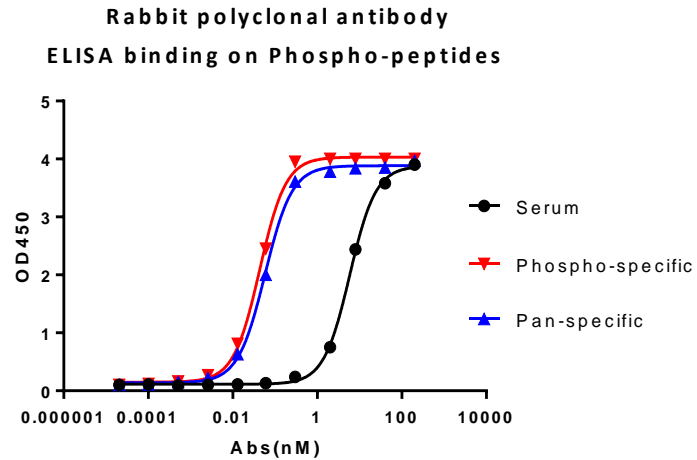


Case 4: Develop Rabbit Antibody with Super High Affinity



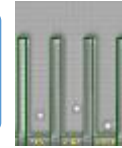
Case 5: Develop Phospho-Specific Rabbit Antibody

Polyclonal Rabbit Antibody

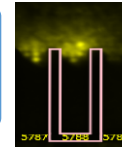


Monoclonal Rabbit Antibody

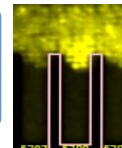
B cell loading



IgG detection



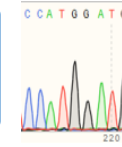
Phospho peptide binding



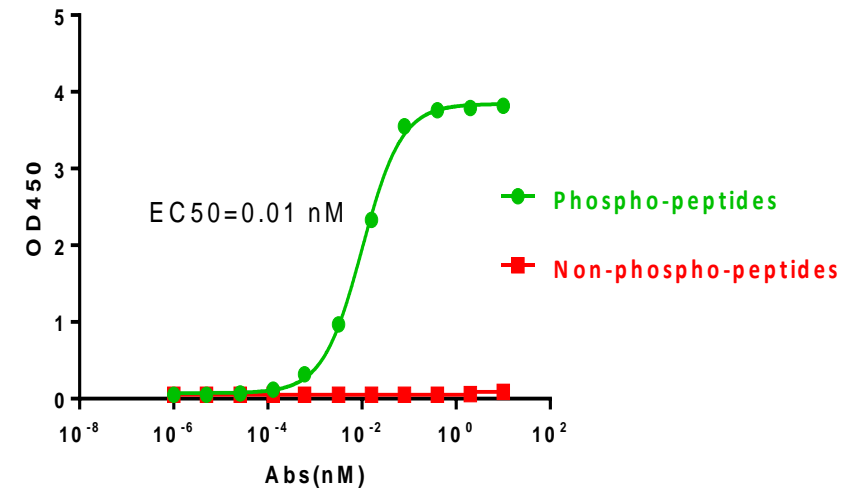
Non-phospho peptide binding



Cell export & BCR sequencing



ELISA Binding on Phospho & Non-phospho Peptides



Summary

03

Summary 1

Reference Data

Workflow #	# of Cells Screened	# Antigen-Specific Hits	Hit Rate	# VH/VL Sequences Analyzed	# Unique VH/VL Sequences	% Unique VH/VL Sequences
1	19,065	219	1.15%	125	112	90%
2	22,430	66	0.29%	33	23	70%
3	23,119	36	0.16%	24	24	100%
4	34,006	236	0.69%	127	123	97%
5	36,375	4	0.01%	1	1	100%
Total	134,895	561	0.42%	310	283	91%

<https://www.berkeleylights.com>

- Rodent IgG secreting B cells can reach 10-20% through WuXi Bio's B cell isolation methods.
- Hit rate varies a lot and highly depends on the target.
- Multiple assays can further narrow down the hit# and limit the # of antibodies to be produced.
- Single B cell platform can improve sequence diversity.

WuXi Bio Data (Data from one chip screening)

Project #	# of Single B Cell*	# IgG+ secreting B cell	IgG+ B cell ratio	Target binding positive B cell	Hit Rate	# Exported B cell	# Unique VH/VL sequence
1	7450	633	8.6%	93	1.3%	37	23
2	8002	643	8.0%	19	0.24%	19	16
3	8713	1150	13%	69	0.79%	18	12
4	8363	2250	27%	139	1.66%	94	44
5	15106	1470	9.7%	74	0.49%	30	25
6	7158	1580	22%	484	6.8%	68	54
7	7485	923	12%	24	0.32%	24	18
8	9071	1822	20%	321	3.54%	109	62
9	8076	1034	13%	282	3.49%	95	83

Summary 2

Pros and Cons of Single B Technology

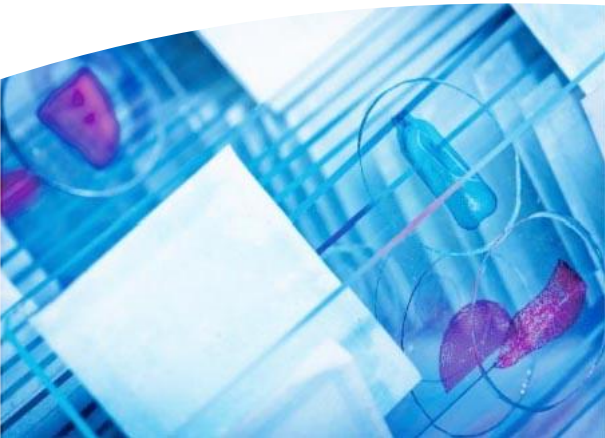
Advantages

- Fast screening and delivery of antibody sequences early
- **Multiple cell based binding assays simultaneously or sequentially**
- Super high screening throughput
- Discover novel antibodies from species without traditional fusion partners (e.g. rabbit, llama, human)
- **Ability to identify rare, therapeutically relevant antibodies directly from B-cells.** No selection on B cells. Even with optimized electrofusion protocols, only 1 of 5000 input B cells survives fusion, becomes immortalized, and secretes antibody.

Drawbacks

- Highly specialized equipment and relatively expensive
- Need experts to develop assays and run the experiments.
- Need special reagents (fluorescently-labeled reagents) for some assays
- Not all the plate-based function assays can be adopted to Beacon® system.

Thank You





WuXi Biologics Vision

“Every drug can be made and every disease can be treated” by building an open-access platform with the most comprehensive capabilities and technologies in the global biologics industry.

Learn More



Contact Us

Email: info@wuxibiologics.com

Website: wuxibiologics.com/discovery
