

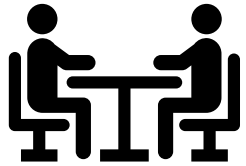
## **Abveris Antibody Discovery Case Study**

Accelerated Therapeutic Antibody Discovery  
Targeting a Cell Surface Receptor using a  
Beacon-Based Single B Cell Workflow



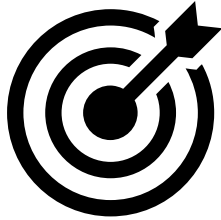
# DISCOVERY OF FUNCTIONAL ANTIBODIES TO CELL SURFACE TARGETS

## Case study background



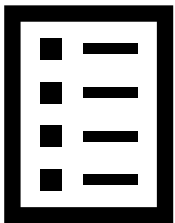
### Partner

- Well-established public biopharma
- Multiple successful campaigns completed in partnership with Abveris



### Target

- Cell surface receptor (FM1)
- Receptor family contains 9 highly conserved members (FM2-9)



### End Application

- Therapeutic antibody development for Partner's desired disease indication

### Goals

- 1 Specific on-cell binding to Family Member 1 (FM1) without off-target binding
- 2 Functional ligand blocking activity required
- 3 Sequence diversity desired
- 4 Tight timeline requirement

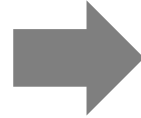


# LIMITED SUCCESS WITH ALTERNATIVE DISCOVERY PLATFORMS

Lack of functional candidate antibodies out of previous efforts necessitated de novo discovery

## Previous Discovery Attempts Prior to Abveris

40,000 Screened



6 Candidates



Additional Discovery  
Required

Hybridomas generated from wild-type mice were screened by ELISA then moved to flow validation

6 lead hybridomas were identified

- 1 FM1-specific binder, ligand blocker
- 2 non-specific binders, ligand blockers
- 3 FM1-specific binders, non-blocking

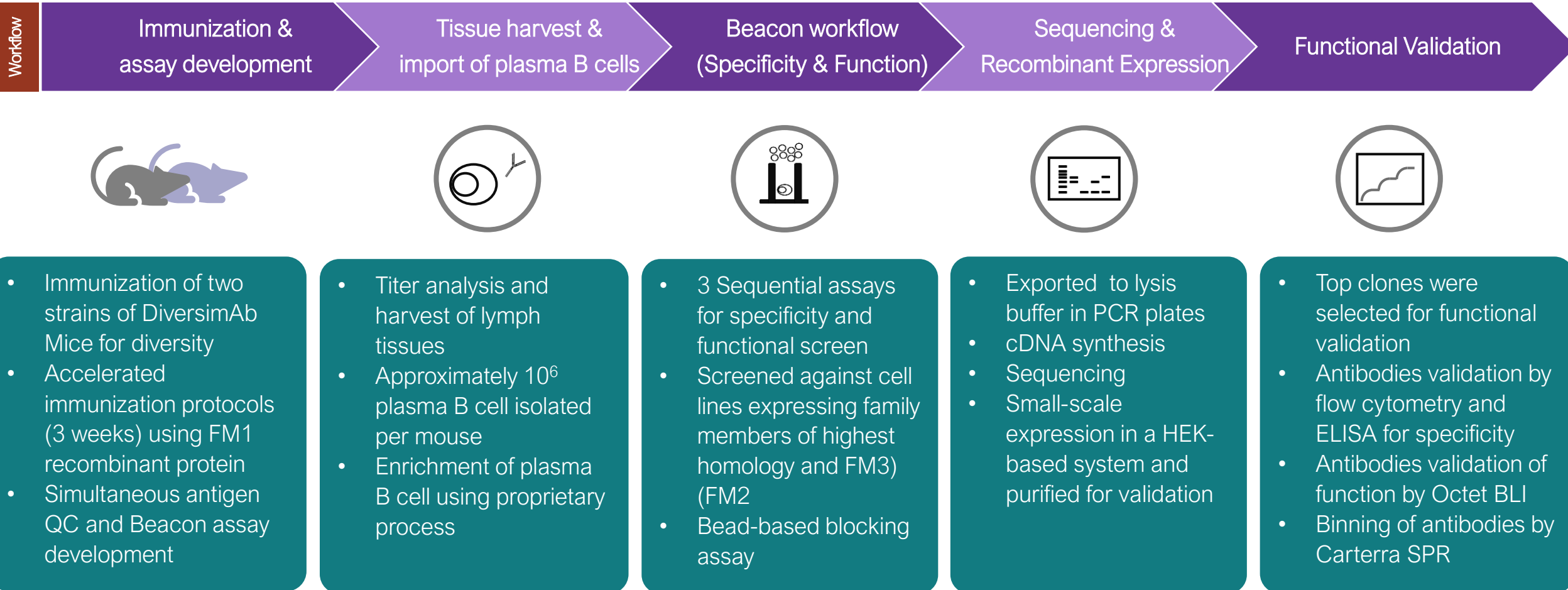
Primary Partner Goals

- Broader antibody diversity for discovery of rare candidates
- Function-forward, accelerated single B cell workflow for efficient lead identification



# OVERVIEW OF SINGLE B CELL WORKFLOW

Include parallel assay development

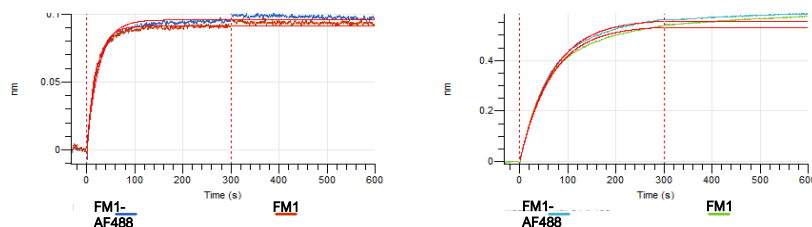




# ANTIGEN QC, ASSAY DEVELOPMENT, AND CELL LINE OPTIMIZATION

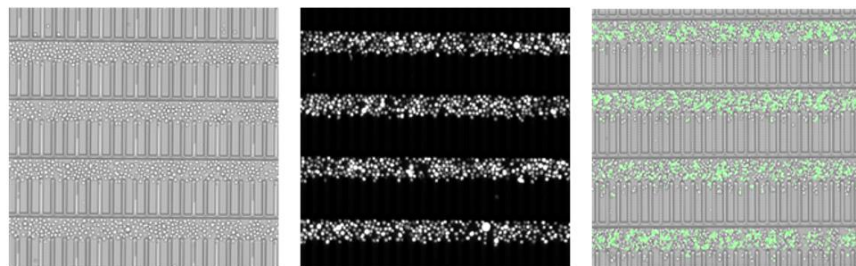
Labeled antigen QC both on and off Beacon enabled successful assay development

## Antigen QC



Octet QC using multiple control antibodies to labeled and unlabeled protein confirmed labeling did not impact protein integrity

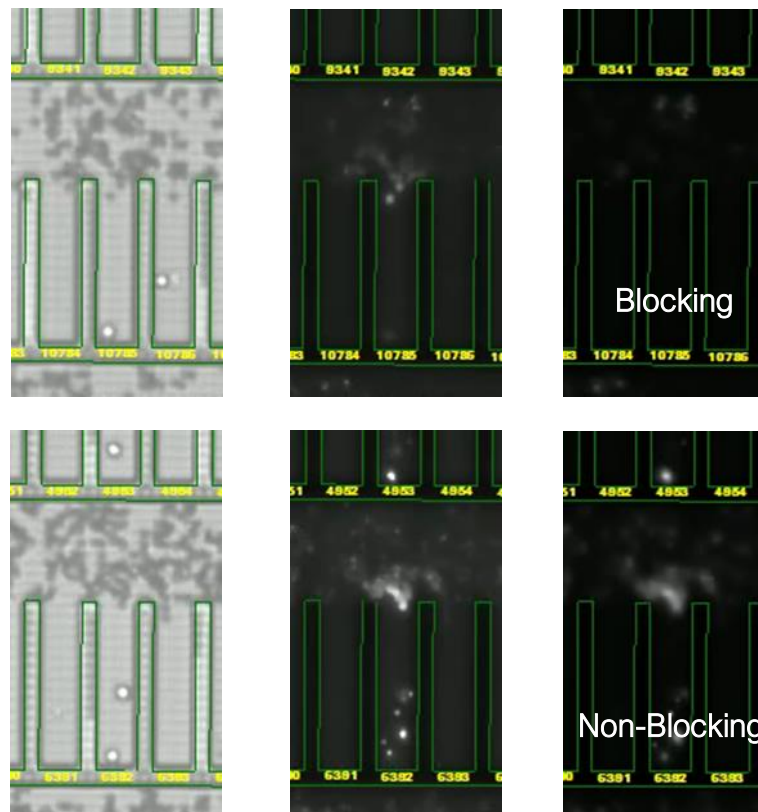
## Cell Line Optimization



FM1 Density Check    FM1 Expression Check    Cell Line Multiplex

Import cell density and cell line multiplex conditions were optimized for assay

FM1-AF488 + Ligand-AF647 in channel



Brightfield: Anti-Mouse IgG Capture Beads

FITC: FM1-AF488 Binding to Captured Control Antibody

CY5: Ligand-AF647 Binding to FM1-Control Antibody Complex

## Assay Development

Optimized on-Beacon ligand blocking assay using hybridomas expressing blocking and non-blocking control antibodies




# THREE SEQUENTIAL ASSAYS WERE EMPLOYED IN THE SCREENING WORKFLOW


Assay format overview

	Assay 1 Off-Target Binding		Assay 2 Blocking Assay		Assay 3 FM1 Specificity
Screening Reagent	FM2 cell line	FM3 cell line	FM1 protein (+)	Ligand (-)	FM1 cell line
Detection Channel	Cy5	Cy5	FITC	Cy5	Cy5
FM1 Specific, blocking	-	-	+	-	+
FM1 Specific, non-blocking	-	-	+	+	+


Legend




Secondary antibody-AF647




FM1-expressing cell line




B cell-secreted antibody




FM2-expressing cell line




FM1 recombinant protein-AF488



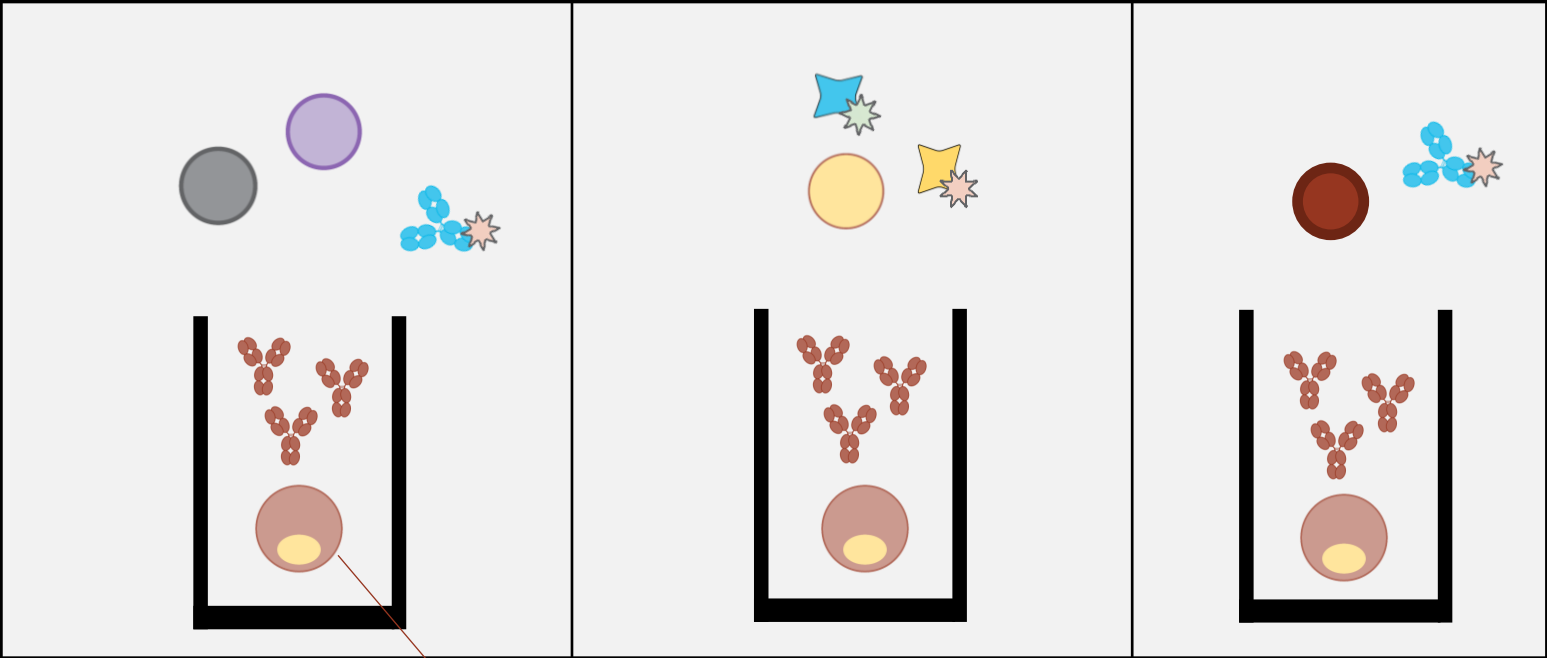
FM3-expressing cell line



Ligand-AF647



Anti-mouse IgG capture beads



Single IgG-secreting plasma B cell




# REPRESENTATIVE ASSAY RESULTS – SPECIFIC BLOCKING HITS


Assay results

	Assay 1 Off-Target Binding		Assay 2 Blocking Assay		Assay 3 FM1 Specificity
Screening Reagent	FM2 cell line	FM3 cell line	FM1 protein (+)	Ligand (-)	FM1 cell line
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FM1 Specific, blocking	-	-	+	-	+
FM1 Specific, non-blocking	-	-	+	+	+


Legend




Secondary antibody-AF647




B cell-secreted antibody




FM1 recombinant protein-AF488




Ligand-AF647




FM1-expressing cell line



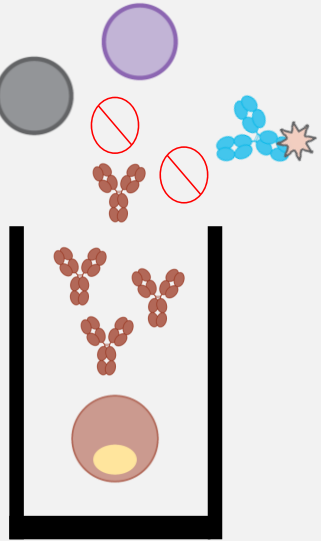
FM2-expressing cell line

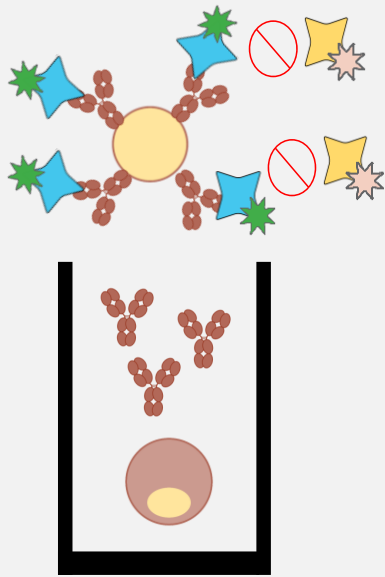


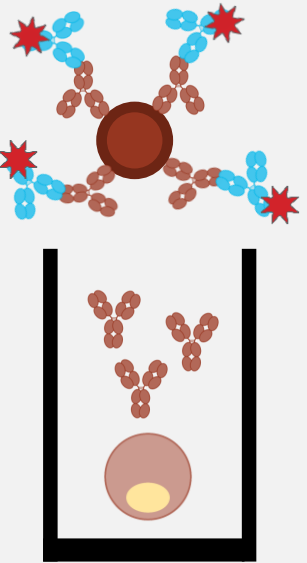
FM3-expressing cell line



Anti-mouse IgG capture beads













## REPRESENTATIVE ASSAY RESULTS – SPECIFIC BLOCKING HITS



## Assay results



		Assay 1 Off-Target Binding		Assay 2 Blocking Assay		Assay 3 FM1 Specificity
Screening Reagent	Detection Channel	FM2 cell line	FM3 cell line	FM1 protein (+)	Ligand (-)	FM1 call line
FM1 Specific, blocking		-	-	+	-	+
FM1 Specific, non-blocking		-	-	+	+	+

**Legend**

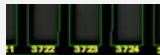
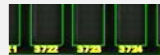
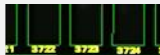
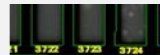
 Secondary antibody-AF647
  FM1-expressing cell line

 B cell-secreted antibody
  FM2-expressing cell line

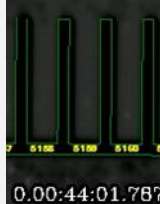
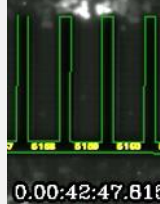

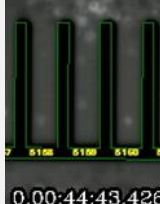
 FM1 recombinant protein-AF488
  FM3-expressing cell line

 Ligand-AF647
  Anti-mouse IgG capture beads

T<sub>0</sub>

T<sub>detection</sub>





# REPRESENTATIVE ASSAY RESULTS – SPECIFIC NON-BLOCKING HITS


Assay results


	Assay 1 Off-Target Binding		Assay 2 Blocking Assay		Assay 3 FM1 Specificity
Screening Reagent	FM2 cell line	FM3 cell line	FM1 protein (+)	Ligand (-)	FM1 call line
Detection Channel	Cy5	Cy5	FITC	Cy5	Cy5
FM1 Specific, blocking	-	-	+	-	+
FM1 Specific, non-blocking	-	-	+	+	+


Legend


Secondary antibody-AF647


B cell-secreted antibody


FM1 recombinant protein-AF488

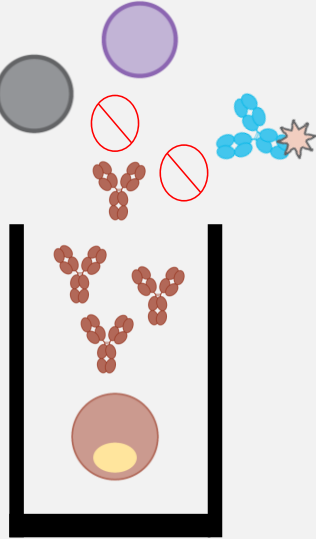
Ligand-AF647

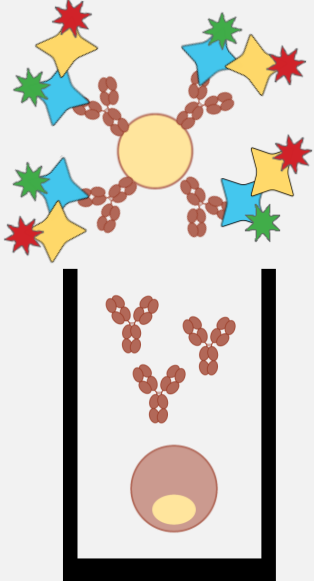
FM1-expressing cell line

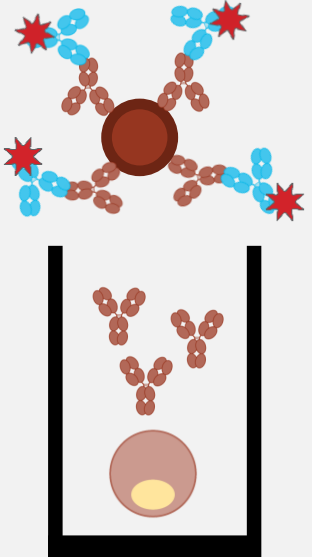
FM2-expressing cell line

FM3-expressing cell line

Anti-mouse IgG capture beads













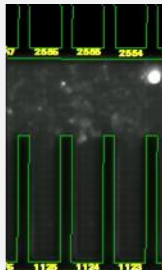
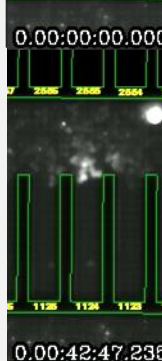
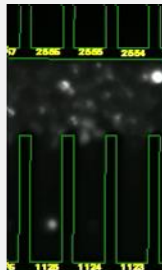
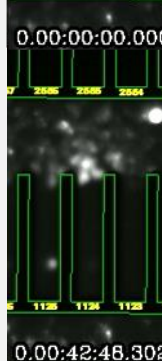






REPRESENTATIVE ASSAY RESULTS – SPECIFIC NON-BLOCKING HITS

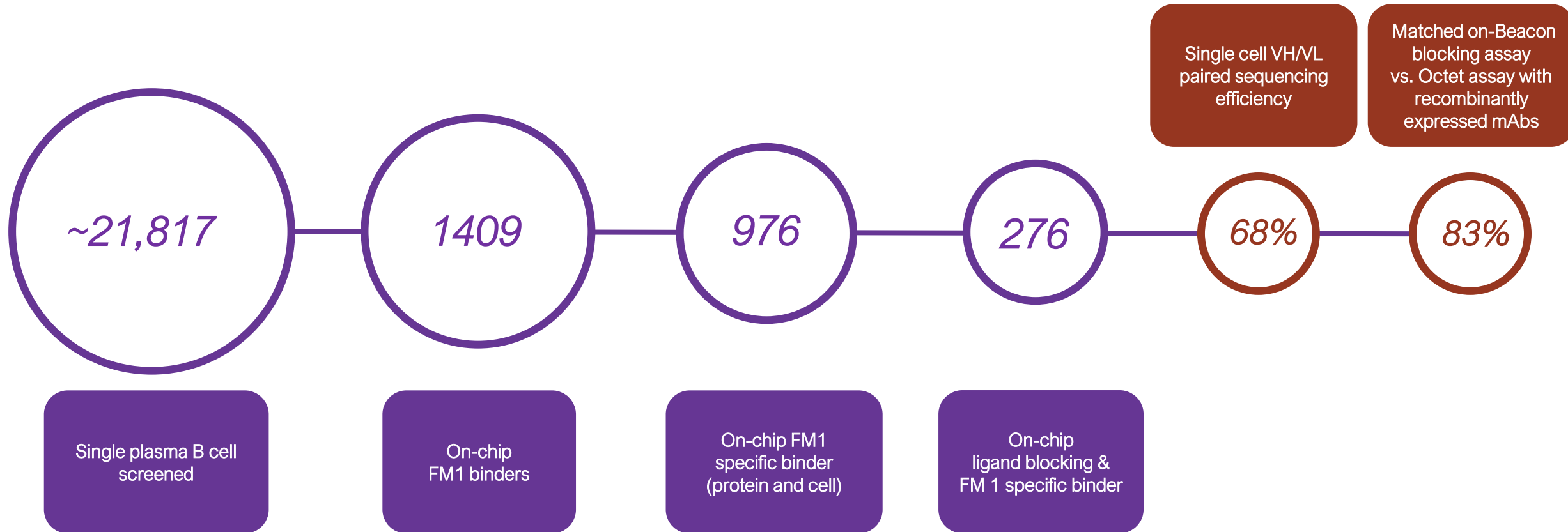
Assay results

		Assay 1 Off-Target Binding		Assay 2 Blocking Assay		Assay 3 FM1 Specificity
Screening Reagent		FM2 cell line	FM3 cell line	FM1 protein (+)	Ligand (-)	FM1 cell line
Detection Channel		Cy5	Cy5	FITC	Cy5	Cy5
FM1 Specific, blocking		-	-	+	-	+
FM1 Specific, non-blocking		-	-	+	+	+
<div><div><div><div><div></div><div>Secondary antibody-AF647</div></div><div><div></div><div>B cell-secreted antibody</div></div><div><div></div><div>FM1 recombinant protein-AF488</div></div><div><div></div><div>Ligand-AF647</div></div></div><div><div><div><div></div><div>FM1-expressing cell line</div></div><div><div></div><div>FM2-expressing cell line</div></div><div><div></div><div>FM3-expressing cell line</div></div><div><div></div><div>Anti-mouse IgG capture beads</div></div></div></div><div><div><div><div><div><math>T_0</math></div><div></div></div><div><div><math>T_{\text{detection}}</math></div><div></div></div></div><div><div><div><div></div><div></div></div><div><div><div><div></div><div></div></div></div></div></div></div></div><div data-bbox="147 1322 170 1322" data-label="Page-Footer"><p>TWIST BIOSCIENCE</p></div><div data-bbox="2023 1322 2425 1322" data-label="Page-Footer"><p>Twist Bioscience 2022 Non-confidential   10</p></div></div></div></div>						



# SCREENING RESULTS IN NUMBERS

Number of clones screened and hits in each stage of the single B cell workflow

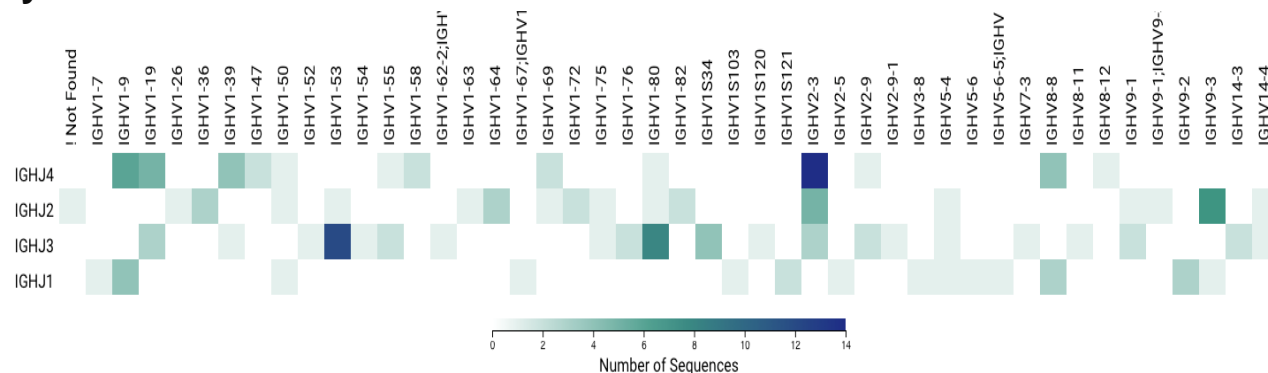




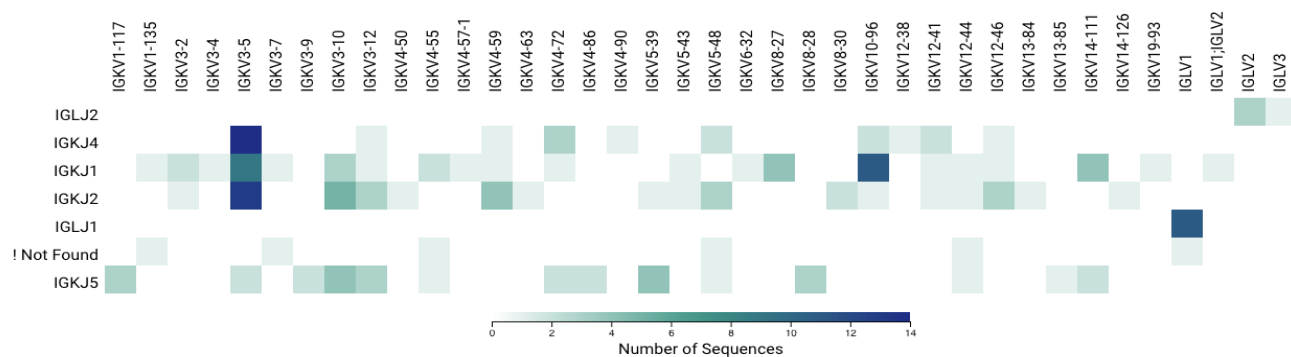
# VALIDATION RESULTS: SEQUENCING OF B CELLS FROM BEACON

78% of paired HC/LC sequences were unique

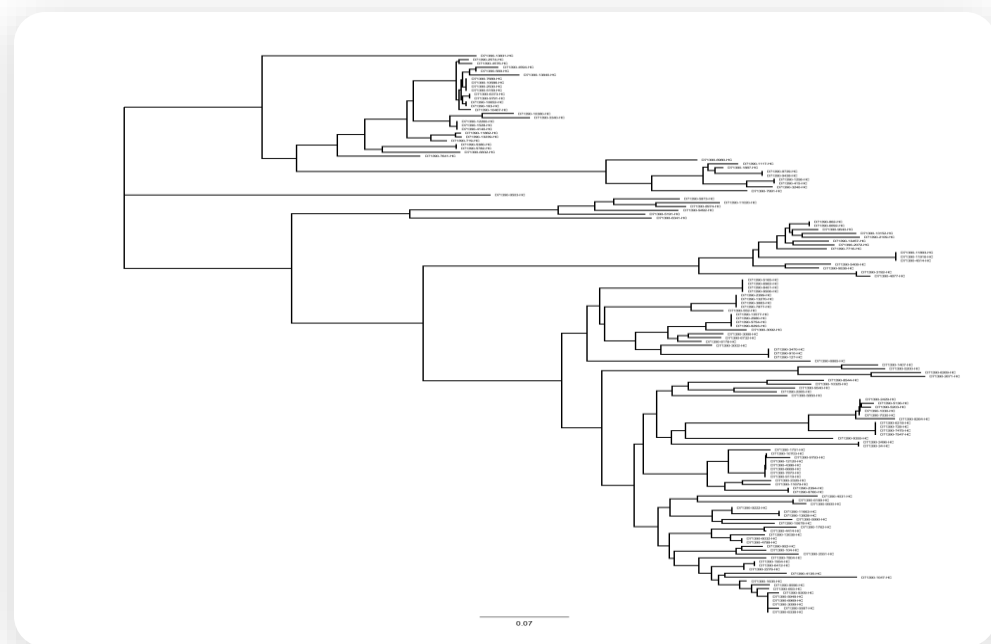
## Heavy VJ Gene



## Light VJ Gene



## Phylogenetic Diagram



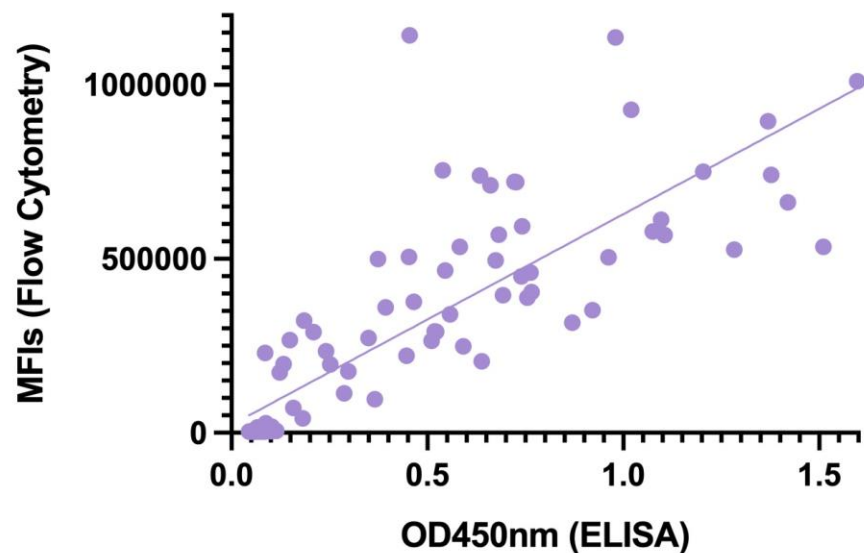
- 129 paired HC/LC sequences recovered from 191 B cells
- 100 out of 129 unique sequences identified
- Broad V-gene usage across both HC and LC
- Diverse clonal lineages identified



# VALIDATION RESULTS: SINGLE-POINT FACS, ELISA FOR SPECIFICITY

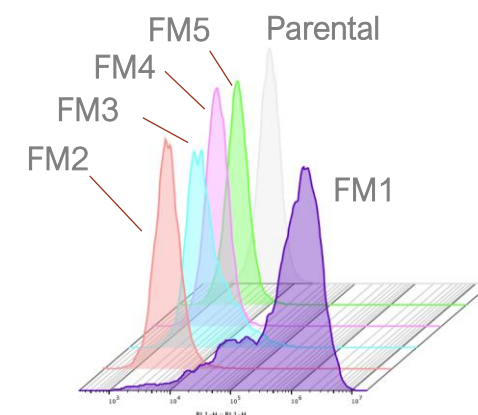
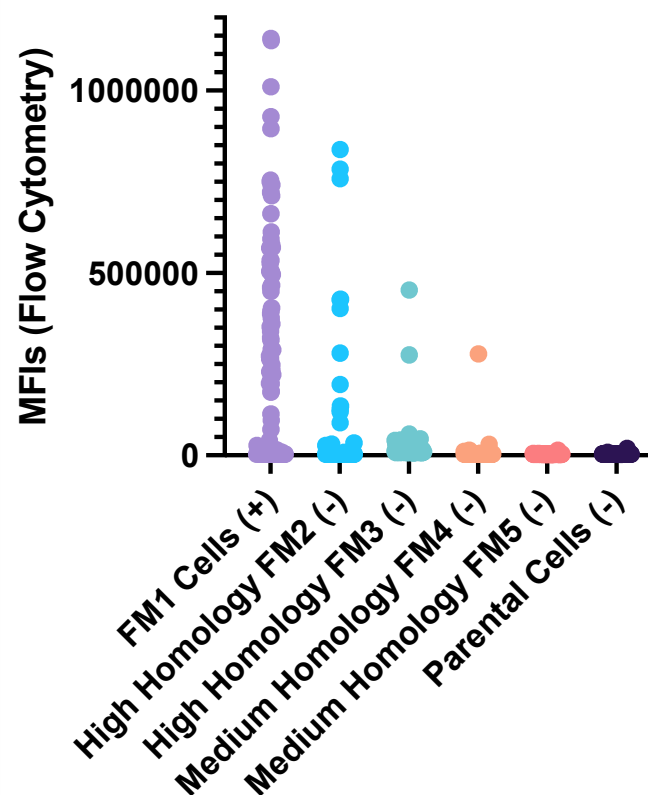
Candidates recapitulated Beacon binding profiles as recombinantly expressed antibodies

## Recombinant Expression Binding



- 96 clones from Beacon were expressed and validated off-Beacon for binding to FM1
- 92% of sequences produced appreciable IgG quantities
- Strong correlation between FM1 recombinant protein binding and FM1 on-cell binding

## Flow Cytometry Binding



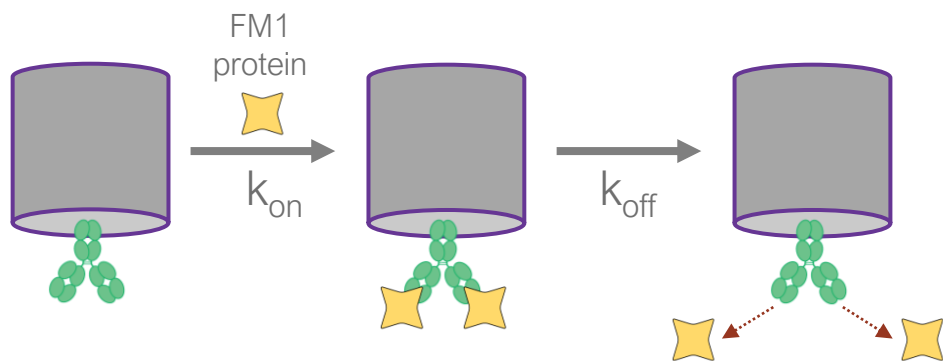
- 70% of clones exhibited binding to FM1 cells
- 61% of FM1 binders maintained stringent specificity off-Beacon



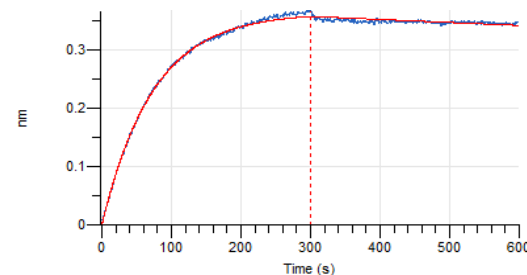
# VALIDATION RESULTS: OCTET BLI – AFFINITY ANALYSIS

A diverse range of affinities was obtained with clones in nanomolar and picomolar range

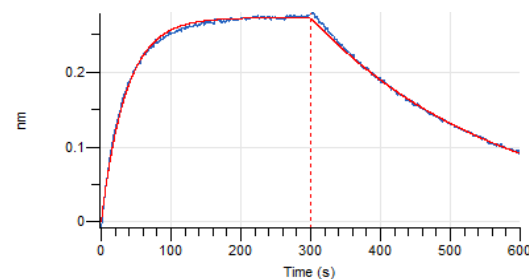
$K_D$  measurement



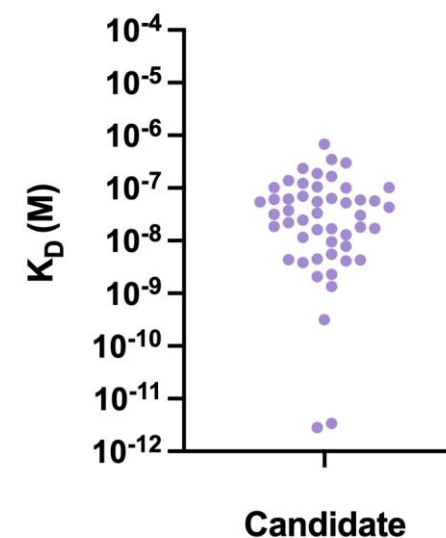
Example clone 1



Example clone 2



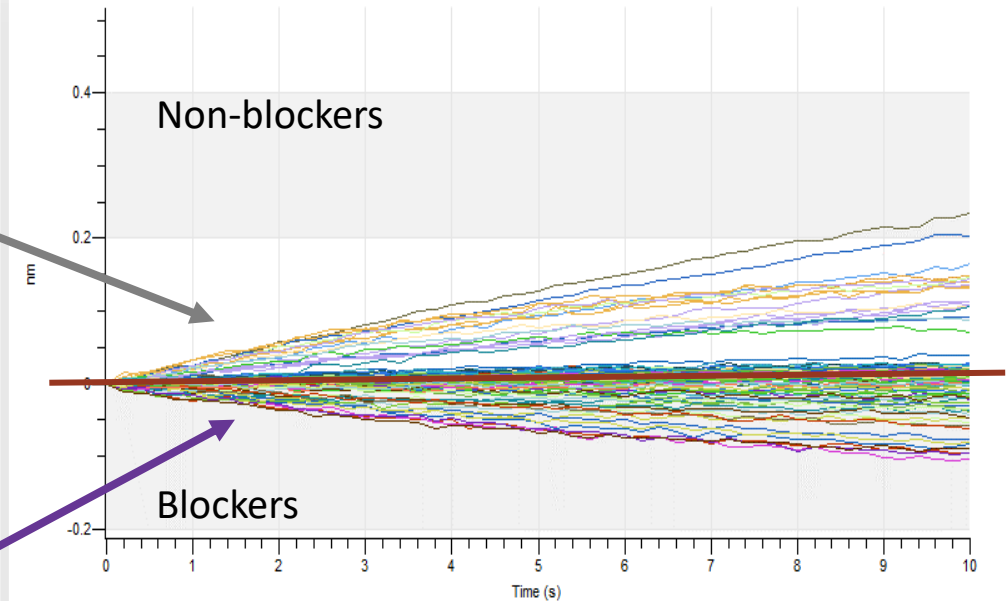
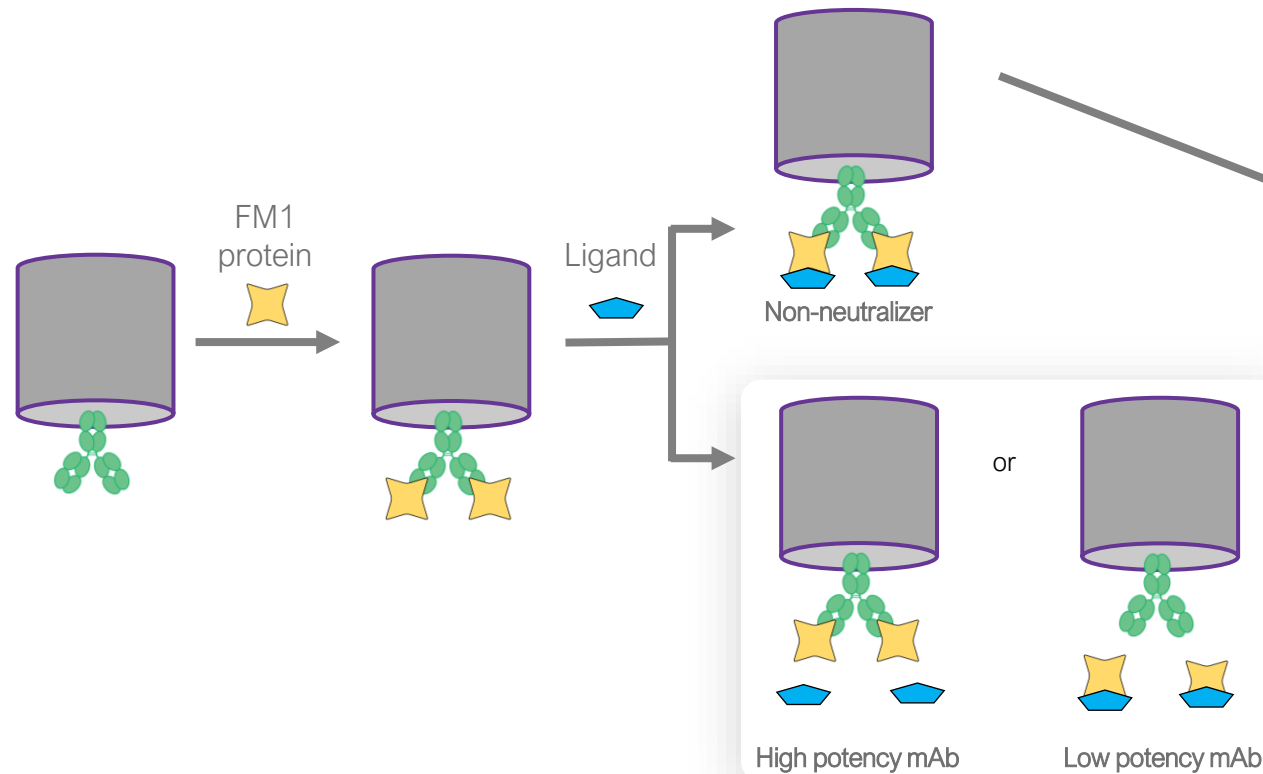
## Single-Point $K_D$ Analysis





# VALIDATION RESULTS: OCTET BLI – SEQUENTIAL FORMAT FOR BLOCKING ANALYSIS

Sequential format allows definitive non-blocking clone determination

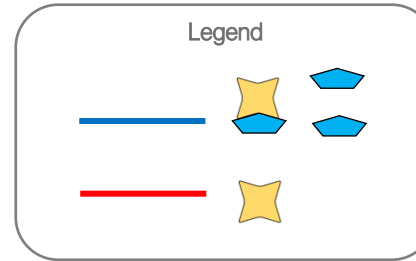
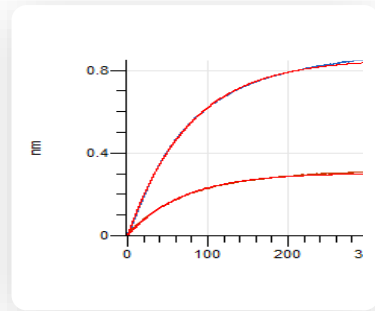
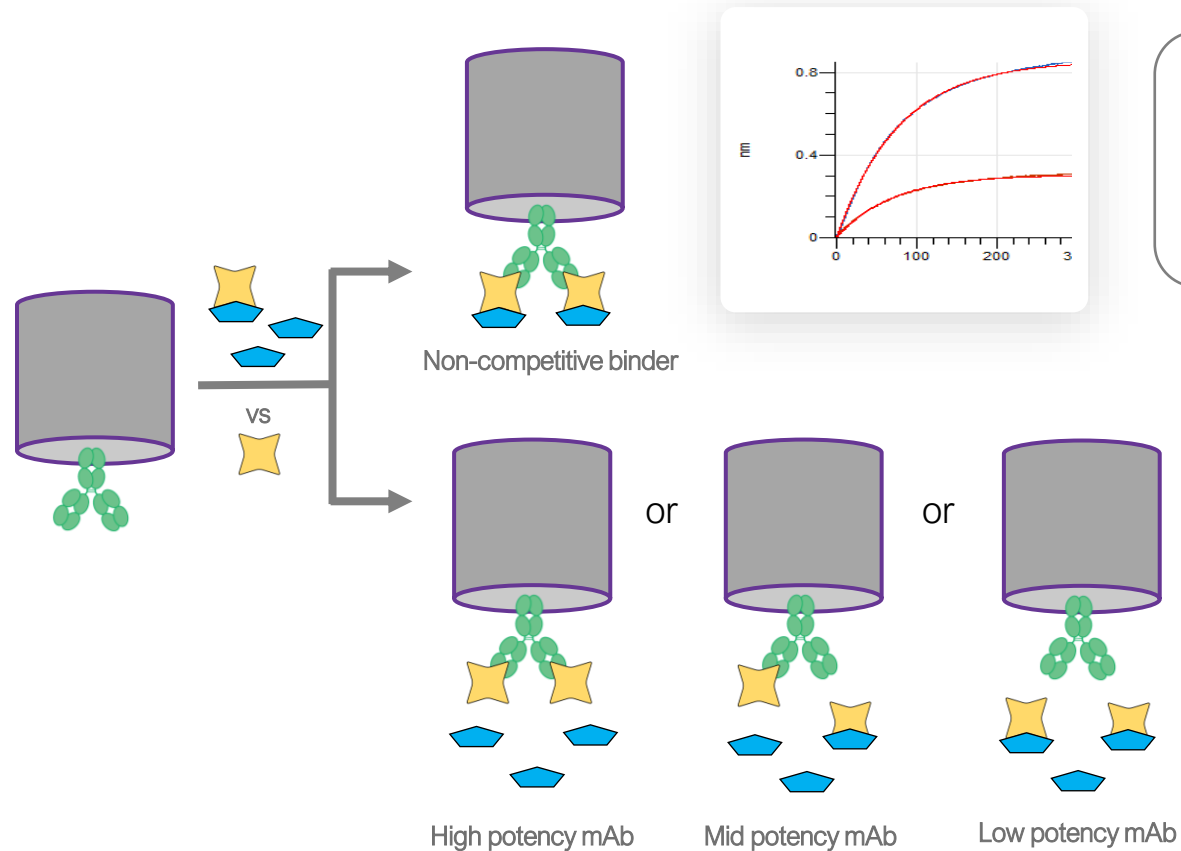


- 70% of clones exhibited binding to recombinant FM1, of which 83% matched Beacon-predicted ligand blocking profiles
- 100% of non-blocking candidates were correctly identified as non-blocking on Beacon, indicating successful elimination of non-ideal candidates

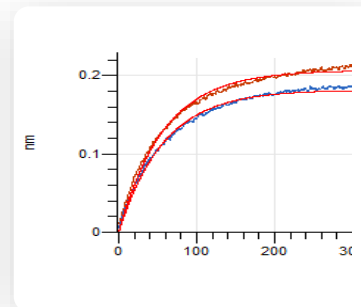


# VALIDATION RESULTS: OCTET BLI – PRE-MIXED FORMAT FOR BLOCKING ANALYSIS

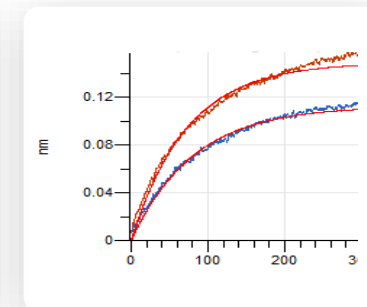
Pre-mixed format allows differentiation in potency among blocking clones



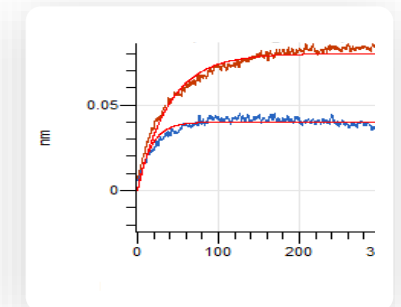
Antibodies with different potencies were identified



High potency mAb



Mid potency mAb

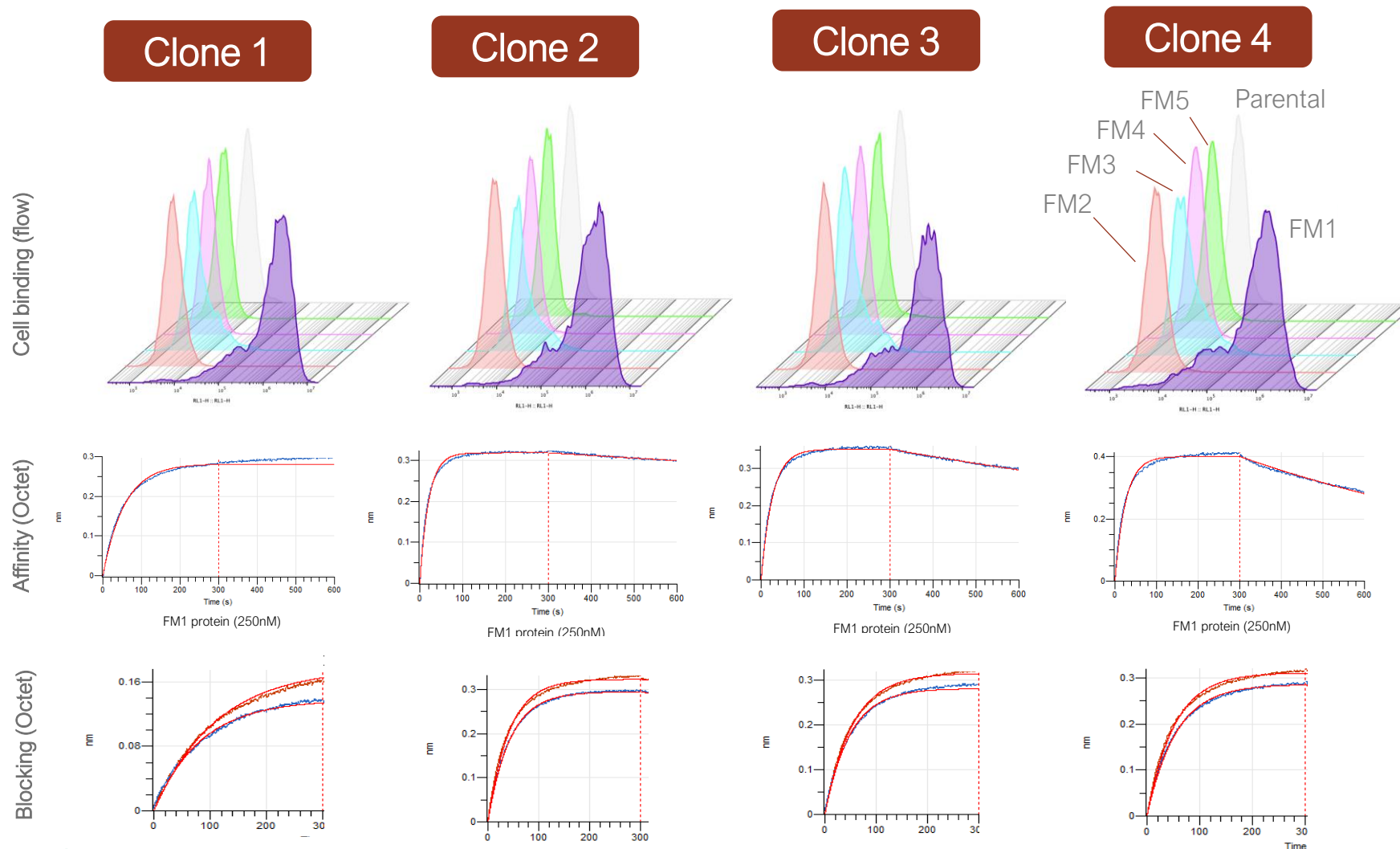


Low potency mAb



# LEAD IDENTIFICATION FOR THERAPEUTIC CANDIDATE NOMINATION

Representative lead candidates from the workflow



Successful  
Additional Discovery

## Primary Partner Goals

- ✓ Broader antibody diversity for discovery of rare candidates
- ✓ Function-forward, accelerated single B cell workflow for efficient lead identification



# CASE STUDY SUMMARY

Successful delivery rare hits of desired specificity and function within a tight timeline

1 Specific on-cell binding to Family Member 1 (FM1)  
without off-target binding

2 Functional ligand blocking activity required

3 Sequence diversity desired

4 Tight timeline requirement

- 62 FM 1-specific binders identified and validated off-Beacon by flow cytometry
- 51 ligand blocking antibodies identified and validation off-Beacon by Octet
- Expression recovery of approximately 90% from unique sequences

- 100 unique sequences or 80% unique sequence recovery
- Diverse affinities down to nanomolar range

- Delivery of validated, purified antibodies in 2.5 months





## **PREMIER ANTIBODY DISCOVERY PARTNER**

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Contact: Garren Hilow  
[ghilow@abverisantibody.com](mailto:ghilow@abverisantibody.com)