



VHH Discovery via Single B Cell Screening

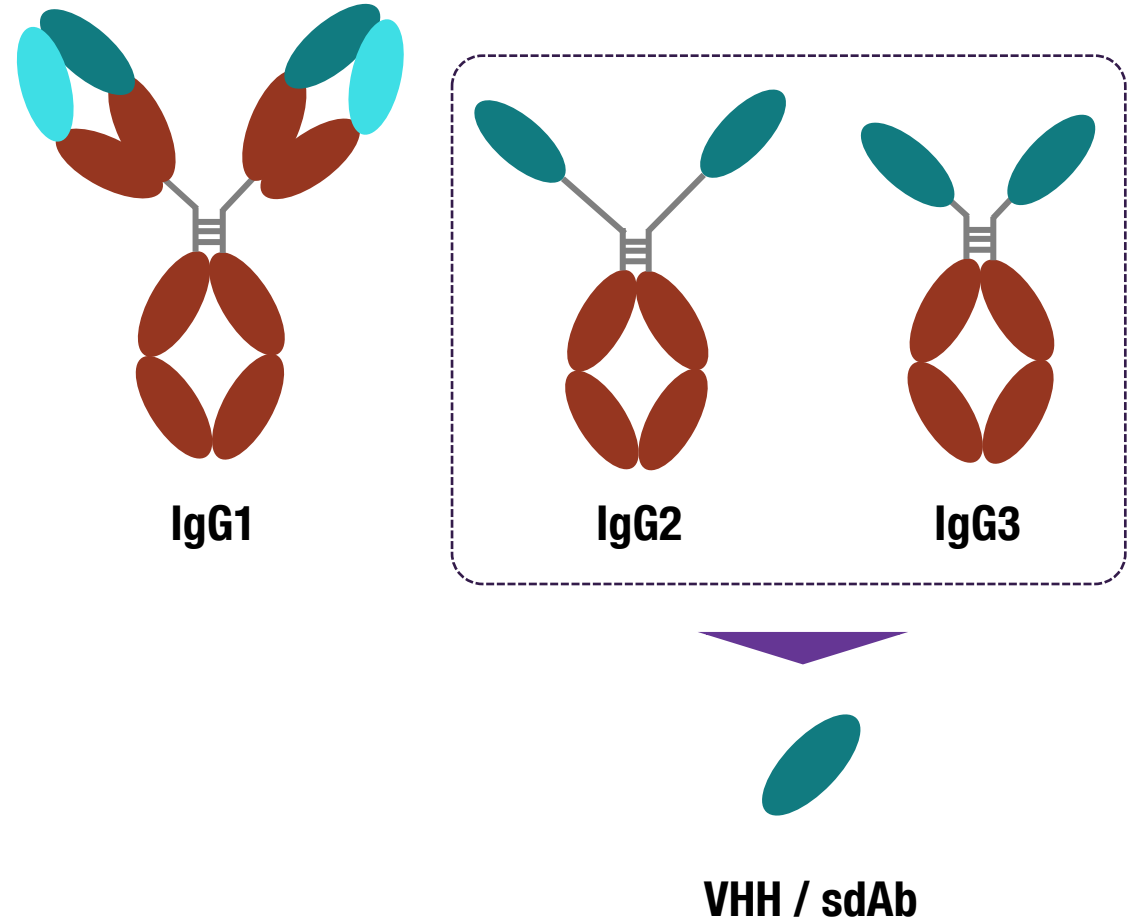
Industry Leading Timelines with Function & On-Cell
Screening Abilities

April 2022

Industry-leading platform for generating VHHs against cell surface receptors

Abveris *In Vivo* VHH Discovery Differentiators

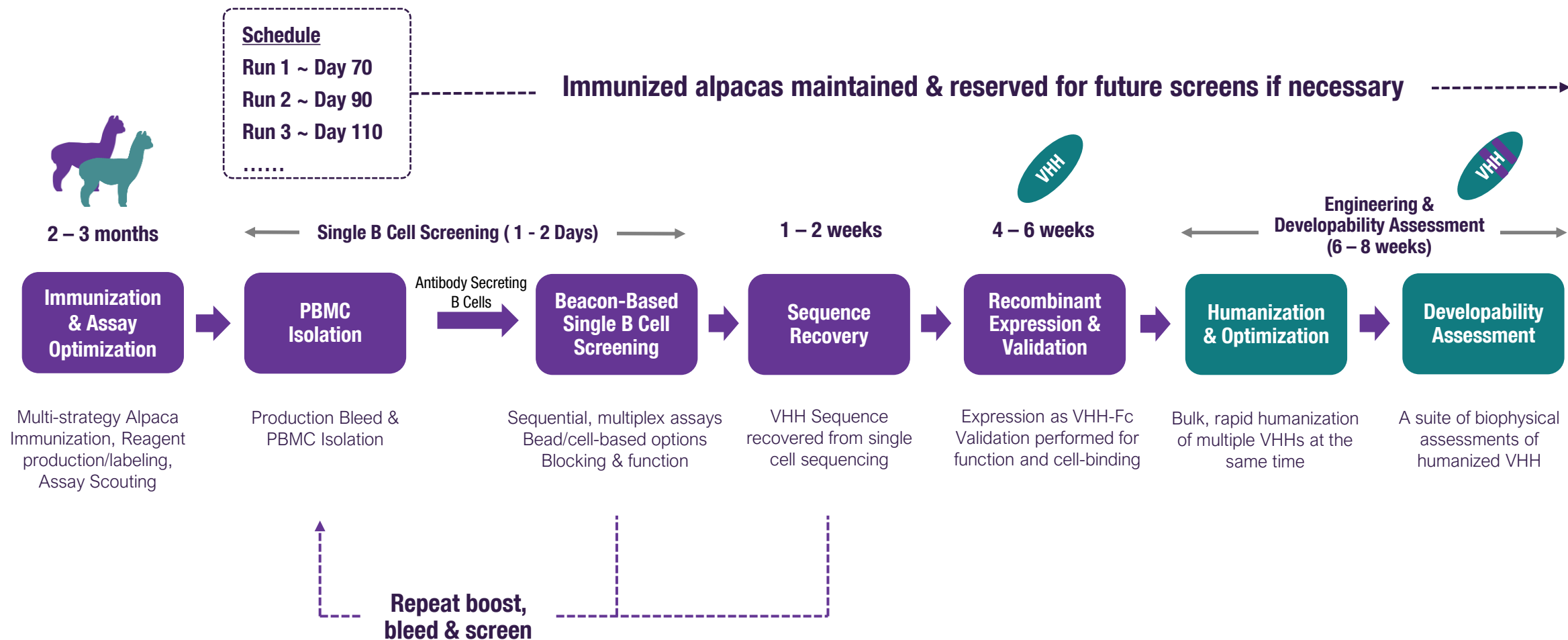
- Superior VHH discovery against cell surface targets
 - Upfront on-cell screens for specificity/function
 - Overcoming known limits of display technologies for addressing cell surface targets
- High-affinity antibodies
 - Natural *in vivo* affinity maturation
 - Beacon-based affinity screening
- Rapid timeline
 - Direct screening on IgG2/3 secreting camelid B cells
 - No need to build immune-specific libraries
- Royalty free for campaigns initiated in 2022



In camelids, VHHs are derived from IgG subclasses of IgG2 and IgG3.

OVERVIEW OF ABVERIS VHH VIA SINGLE B CELL SCREENING

Industry-leading platform for generating VHHs against cell surface receptors



ABVERIS SINGLE B CELL SCREENING PLATFORM

Optimized, Beacon-based screening for the rapid identification of ideal, rare antibodies



THROUGHPUT

- Tens of thousands of mAbs simultaneously screened
- ~100K of B cells screened in a day
- 1,000+ specific hits assayed in many cases



RESOLUTION

- Multiple sequential screens including on-cell, multiplexed, and functional screens
- Industry-leading resolution among all single B cell platforms



CUSTOMIZATION

- Highly flexible assay setups with assay development services offered



SPEED

- Project start to paired HC/LC sequence delivery in as few as 29 days



Photo credit: www.berkeleylights.com

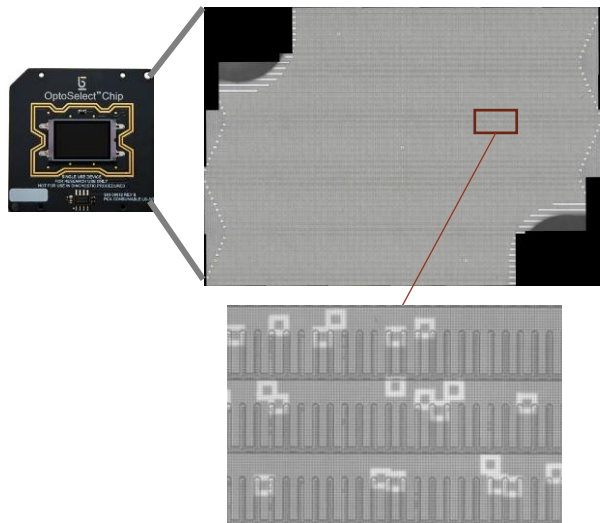
SINGLE B CELL DISCOVERY WORKFLOW IN ONE DAY

A quick look at the Beacon-based screening process

Loading of B Cells

- 14K pens per chip
- 1 chip = 145 x 96-well plates
- 10k+ IgG expressing single B cells loaded into individual nanopens per chip

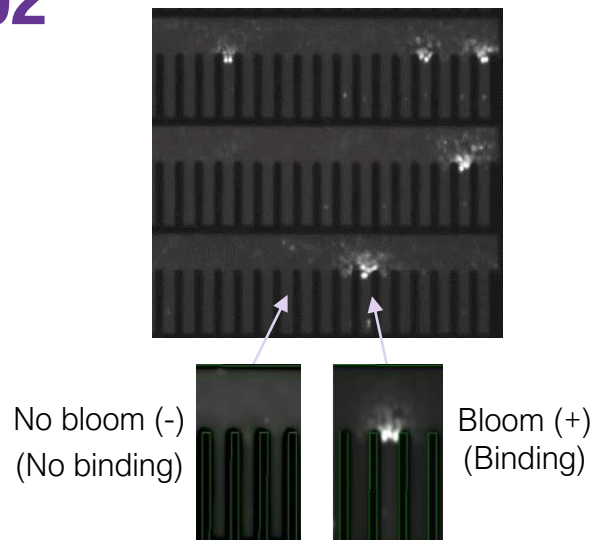
01



Screening

- Sequential, multiplex assays performed to identify hits
- Fluorescent readout detected as blooms in the channels

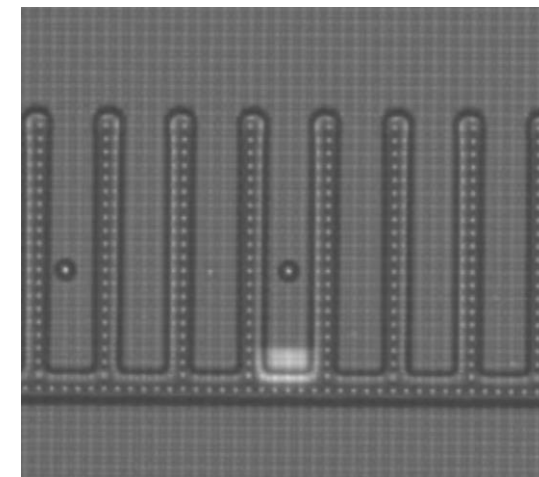
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Export

- Export hits for single cell sequencing
- Downstream expression and validation off-Beacon

03

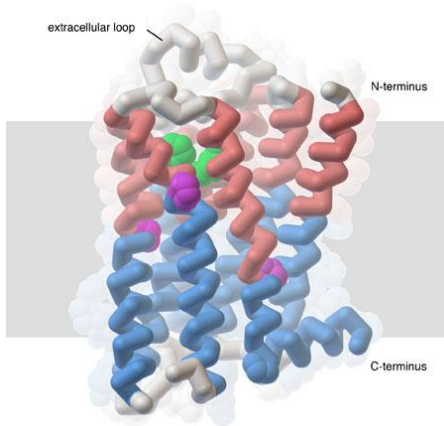


SINGLE B CELL ON-CHIP SCREENING CAPABILITIES

Unique techniques constructed for traditionally difficult antigens

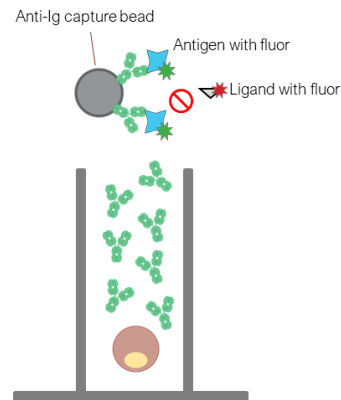
Cell Binding

- Multi-pass transmembrane proteins – GPCRs, ion channels
- Use of adherent cell lines



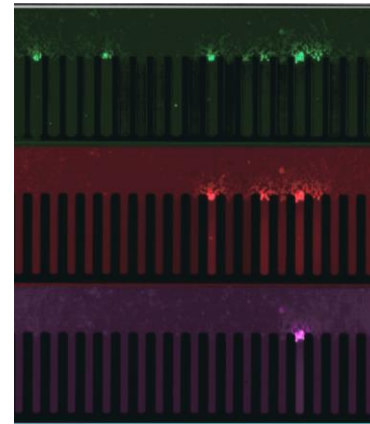
Ligand blocking

- Receptor blockers
- Competition
- Neutralization



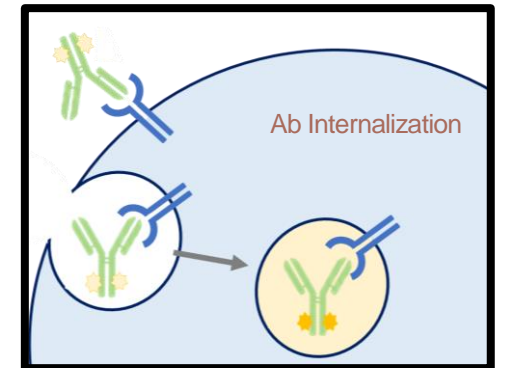
Multiplexed Assay

- Up to 4 sequential 3-color multiplexed assays
- Species cross-reactivity
- High homology specificity



FUNCTIONAL ASSAY

- Antibody internalization
- Apoptosis
- Custom assays with fluorescent readouts

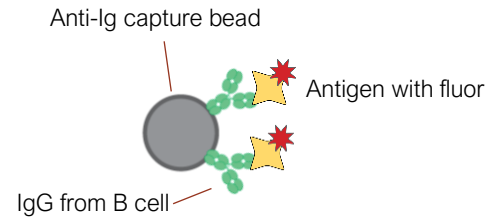


Ultimate flexibility to run up to 4 sequential, multiplexed assays of choice in the same run

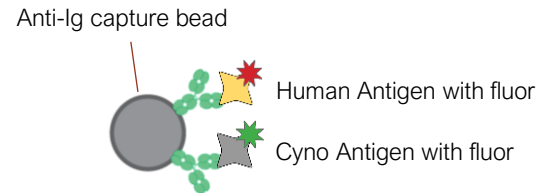
Examples of Beacon-based on-chip assay deployed at Abveris

Bead-Based Assays

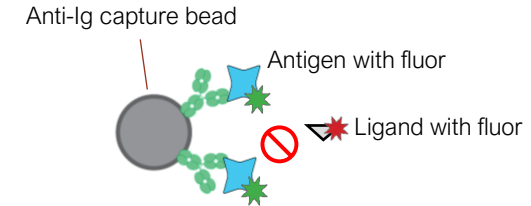
Protein binding



Multiplexed, Cross-Reactivity

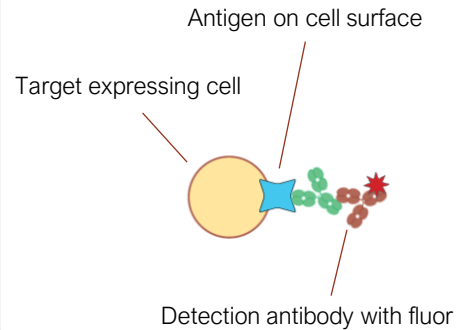


Multiplexed, Blocking Assay

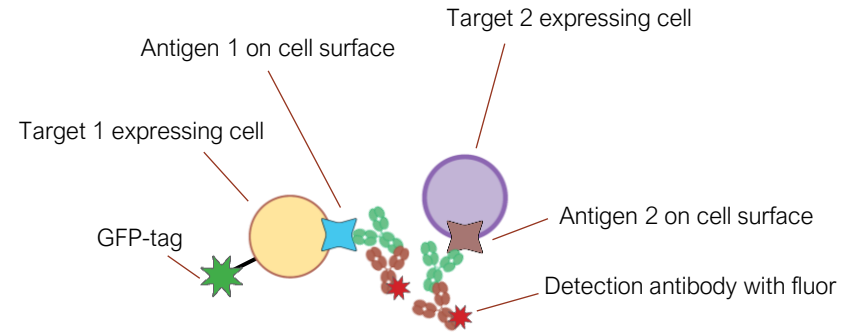


Cell-Based Assays

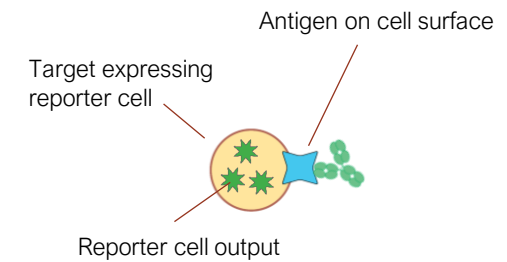
On-Cell Binding



Multiplexed, Cross-Reactivity



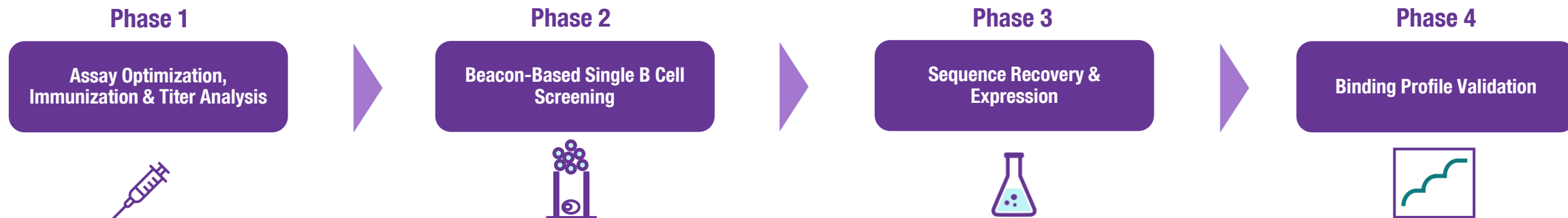
Functional Assay



CASE STUDY: ANTI-PSMA VHH DISCOVERY WITH IMMUNIZED ALPACA

Validating Beacon-based VHH discovery workflow targeting prostate-specific membrane antigen (PSMA)

POC Study



Goals of POC Study

- 1 Validate the immunization protocol and confirm class switch of alpaca B cells to IgG2/3 isotype
- 2 Optimize Beacon screening to identify IgG2/3 antigen-specific binders secreted by alpaca B cells
- 3 Establish the sequencing protocol and subsequent expression productivity of VHH for downstream characterization

Results

- Successfully elicited robust antigen-specific immune responses in alpaca with class switch to IgG2/3 confirmed in as few as 70 days
- Successfully screened and identified antigen-specific IgG2/3 on-Beacon to guide hit identification and export
- Successfully demonstrated the recovery of VHH sequences and recombinant expression of VHH (as VHH-Fc)
- Binding profiles of VHH to the target were validated off-Beacon by ELISA, Octet BLI and Catterra SPR to show single-digit and subnanomolar affinities

POC STUDY: ABVERIS SINGLE B CELL VHH DISCOVERY

Successful class switch was observed at Day 70 of immunization

POC Study

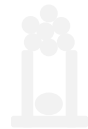
Phase 1

Assay Optimization,
Immunization & Titer Analysis



Phase 2

Beacon-based Single B Cell
Screening



Phase 3

Sequence Recovery &
Expression



Phase 4

Binding Profile Validation



Immunization Results & Discussion:

- An alpaca was immunized by recombinant protein of the PSMA ECD
- Titer analyses from alpaca sera collected on Day 41, 87, and 97 showed robust immune responses (Figure 1A)
- Subsequent Beacon-based single B cell screens confirmed detection of IgG2/3 (Figure 1B)

Figure 1A. Anti-human PSMA-His Titer of Alpaca Sera

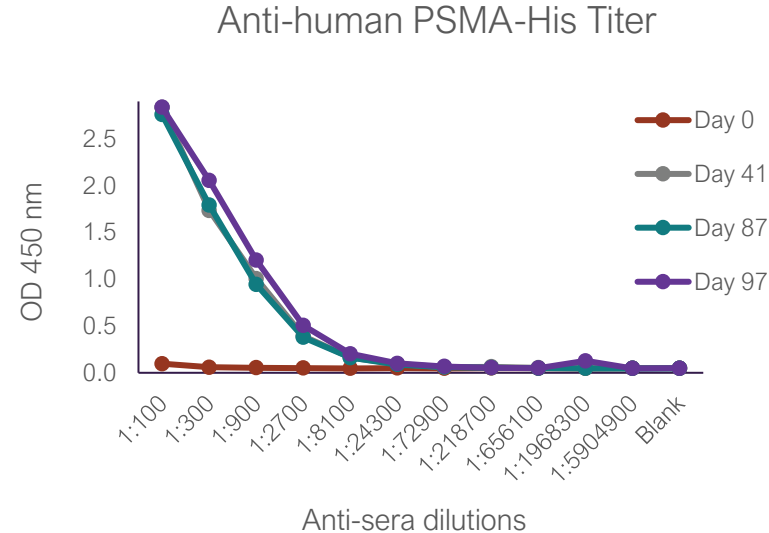
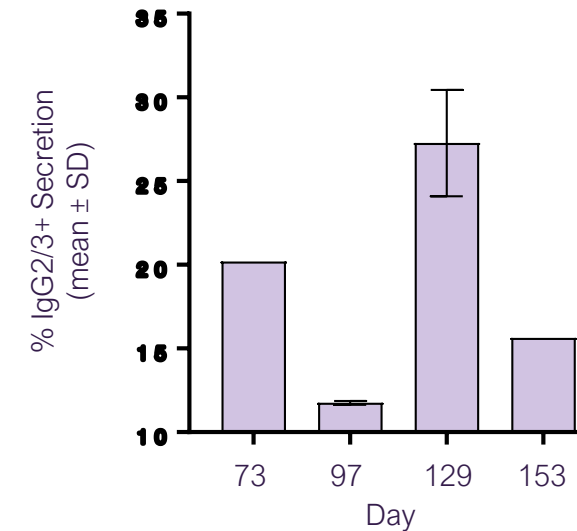


Figure 1B. IgG2/3 Secretion Assay by Beacon



POC STUDY: ABVERIS SINGLE B CELL VHH DISCOVERY

Custom assays by Abveris enabled the identification of antigen-specific IgG2/3 on-Beacon

POC Study

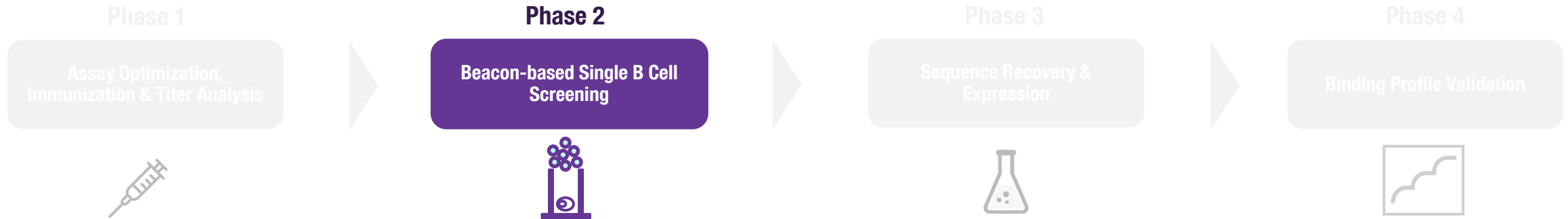


Figure 2A. Assay #1: IgG2/3 Secretion Confirmation

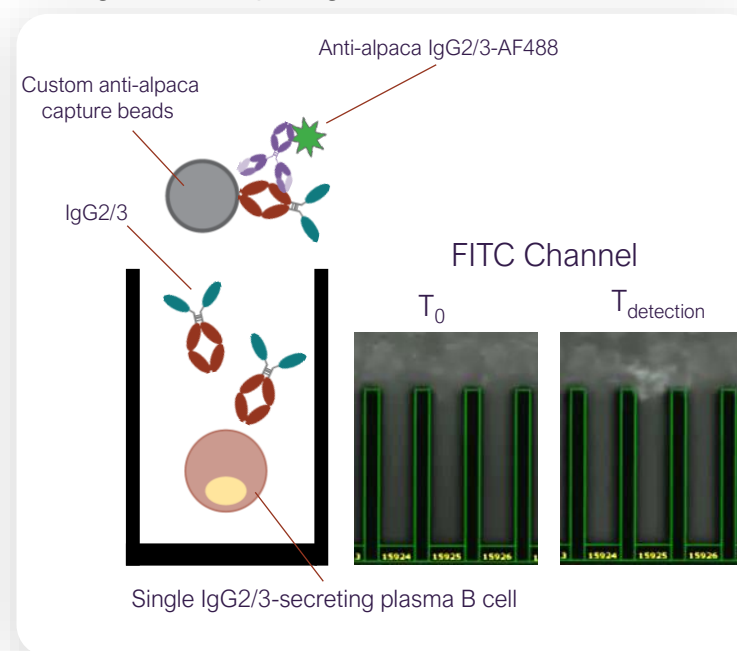
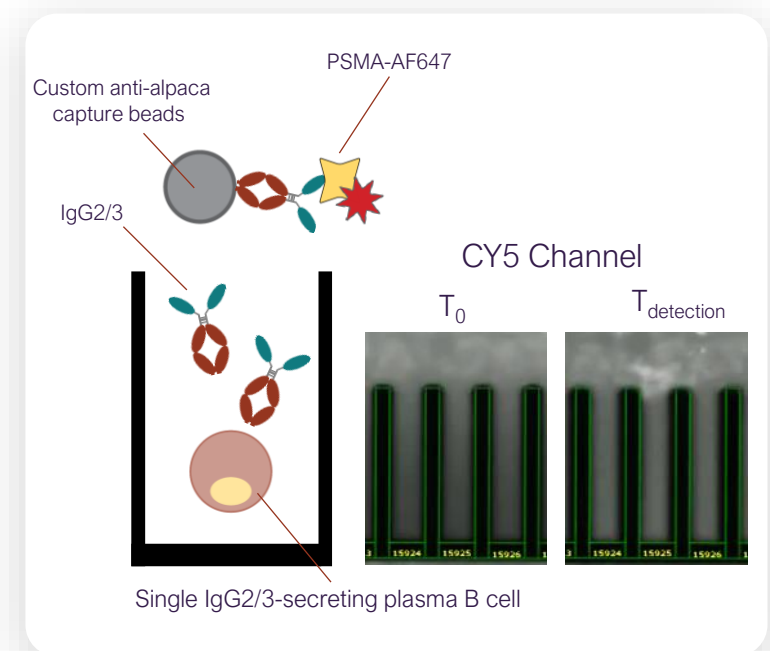


Figure 2B. Assay #2: PSMA Protein Binding Assay



Sequential single B cell assay results:

Assay #1 – IgG2/3 secretion assay using a custom bead that was developed at Abveris (Figure 2A).

Assay #2 – PSMA binding was confirmed by a custom bead-based assay (Figure 2B). Combining the results of both assays guides the identification of PSMA-binding, IgG2/3+ candidates to export the hits for single cell sequencing, downstream expression and validation.

POC STUDY: ABVERIS SINGLE B CELL VHH DISCOVERY

Sequencing protocol by Abveris led to successful VHH sequence recovery from clones exported from Beacon

POC Study

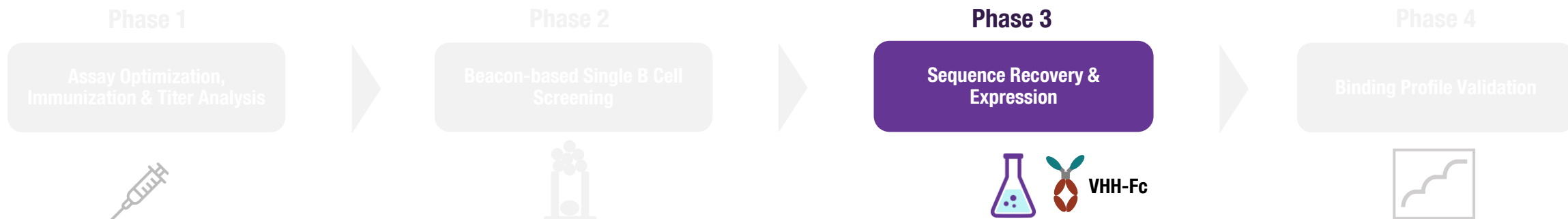
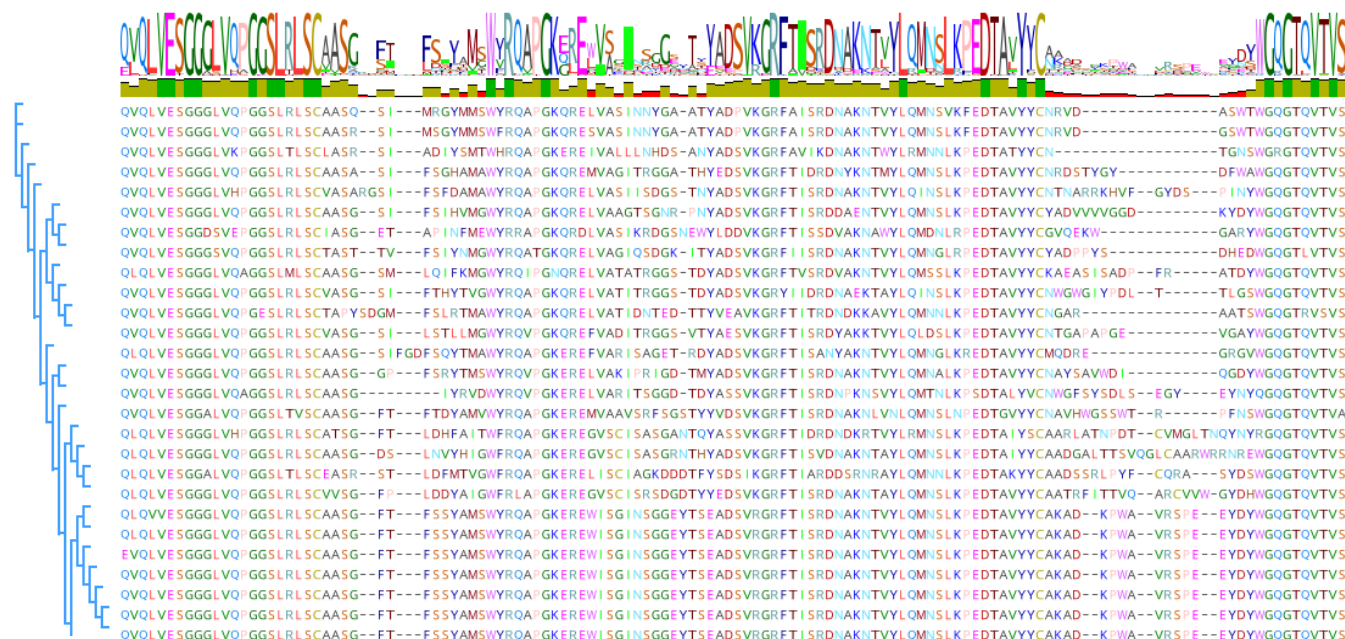


Figure 3. Representative VHH recovered from Beacon Screening



Hit Identification and Sequence Recovery:

- Selected hits identified on-Beacon were exported for single cell sequencing
- VHH sequences were successfully recovered from IgG2/3 secreting B cells
- VHHs were expressed in the format of VHH-Fc with an average yield of 0.6mg at a high throughput scale using a transient HEK expression system

POC STUDY: ABVERIS SINGLE B CELL VHH DISCOVERY

Validation of recombinantly expressed VHH-Fc warrants the quality of Beacon workflow to generate high-quality hits

POC Study



Figure 4A. Validation #1: Recombinantly expressed VHH-Fc were validated to bind recombinant PSMA ECD protein

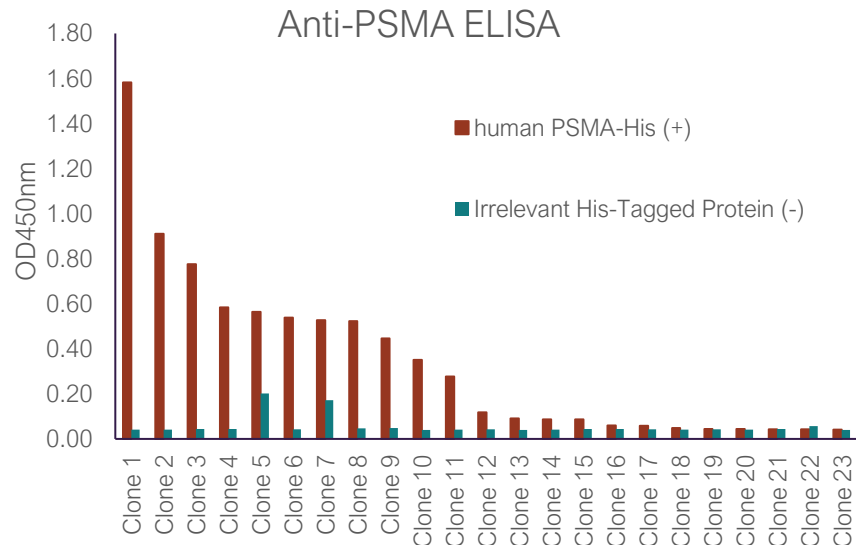
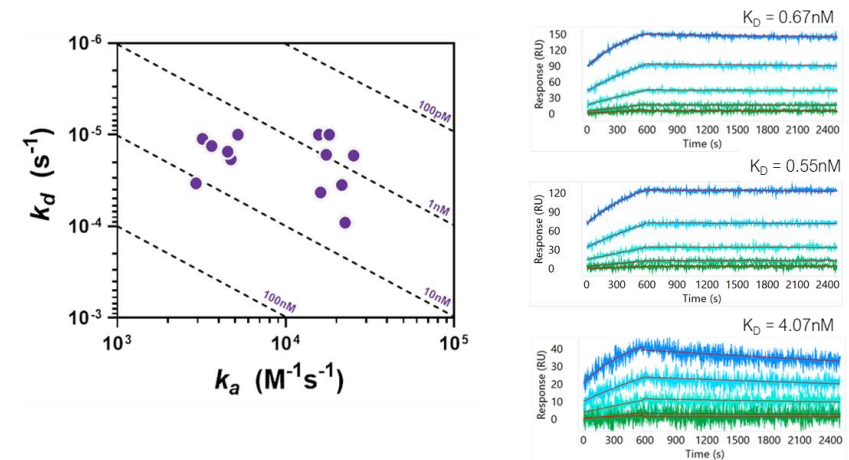


Figure 4B. Validation #2: Recombinantly expressed VHH-Fc validated by Carterra SPR show single-digit nanomolar and subnanomolar affinities to recombinant PSMA ECD protein

Carterra SPR Validation Results:

- Clones of single-digit and subnanomolar affinities were identified (left)
- Representative kinetics sensograms showing mAb binding to PSMA recombinant protein are shown (right)
- Validated route for high-affinity VHH discovery



ABVERIS

IN

NUMBERS

100+

successful mAb discovery campaigns
against surface receptors

80%

of our partners return for
additional campaigns

7+

mAb candidates discovered by Abveris
have proceeded to clinical trials

29

days required from immunization start to
antibody sequence for a single B cell campaign

10

weeks required from immunization start to
antibody sequence for a hybridoma campaign

PARTNERSHIP MODELS DESIGNED TO EMPOWER EVERY TEAM

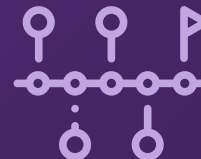
Flexible and dynamic partnership structures for diverse antibody discovery teams



FEE-FOR-SERVICE



SUCCESS-BASED



MILESTONE



EQUITY-BEARING