VHH discovery beyond B cells - antibody discovery from serum using machine learning models for in silico selection

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Abstract

Serum antibodies are a key component of the phenotype assessed when screening immunized animals or patients. Alicanto is a platform for identifying antibodies from serum that integrates B-cell receptor repertoire data with mass spectrometry measurements of serum antibodies to create a map of an individual's immune response to challenge.

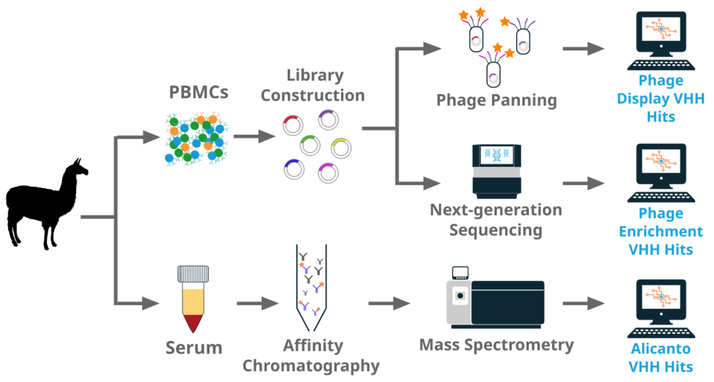
In this case study we compare different methods for capturing single domain antibodies, or VHHs, from the same immunized llama, including:

- B cell receptor repertoire
- phage library construction and panning
- serum antibodies discovered with Alicanto®

We identify strengths and limitations of each approach. We integrate structural modeling of VHHs to perform further selection and comparison of candidates.

Experimental set up

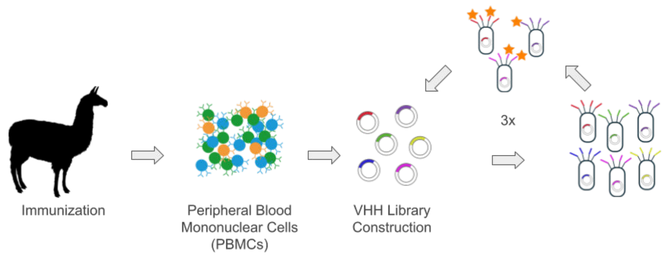
A llama was immunized with *Staphylococcus aureus* Cas9 (saCas9). Peripheral blood mononuclear cells (PBMCs) were collected along with serum.



Experimental overview of the VHH discovery project. From a single immunized animal, three analysis pathways identified candidate antibodies that bind saCas9.

VHH Phage Library Construction, Panning, and Sequencing

PBMCs were collected at week 9 and week 13 of the immunization. The PBMCs were lysed and RNA was extracted. The RNA was aliquoted for use either in phage library construction or repertoire sequencing



Workflow for VHH phage library construction and panning.

A phage library was constructed from the pooled week 9 and week 13 RNA. The library was panned over three rounds against the recombinant antigen, and colonies were selected as **Phage Display VHH Hits**. DNA from the pre-panned library, as well as after each panning round was analyzed by Abterra Biosciences' Reptor platform. The pre-panning library was compared to the direct B cell receptor repertoire (BCR) sequencing data. In addition, clone enrichment analysis was performed to identify **Phage Enrichment VHH Hits** that increase in prevalence across panning rounds.

About Abterra Biosciences

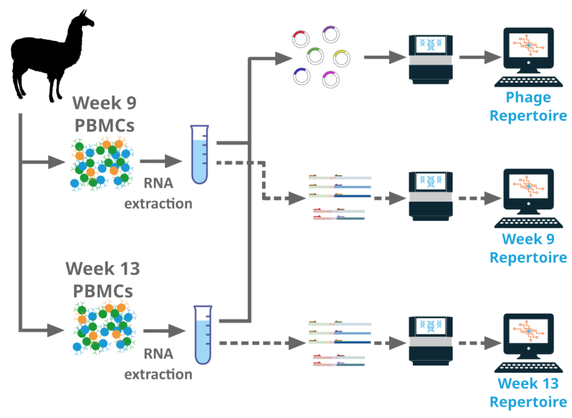
Check out our antibody related services:

- Alicanto®: antibody discovery directly from serum.
- Valens: monoclonal sequencing from protein service.
- Reptor: immune repertoire sequencing and analysis.
- Griffin: polyclonal antibody sequencing service.

Nano Talks Podcast

Excited about all things nanobody? We've collected questions from over 100 researchers and put them to the experts in a series of podcasts. Check out the videos with the QR code to the right.

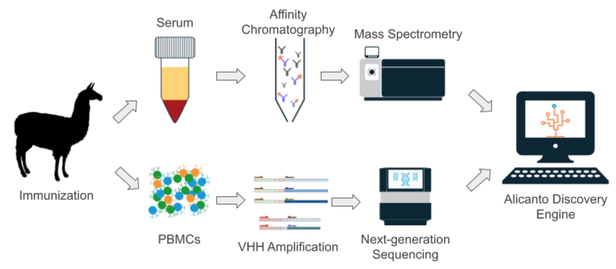
B Cell Receptor Repertoire Sequencing



Repertoires generated and compared using Reptor

From the same RNA pooled to create the phage library, the Reptor platform was used to generate BCR repertoires individually for the week 9 and week 13 PBMCs. Primers annealing to the leader peptide region and the heavy chain-only hinges (IgG2B and IgG2C) were used to amplify the target regions. The two isotypes that give rise to heavy chain-only antibodies were sequenced separately. Reptor generated a repertoire in AIRR-Seq format that was then used to compare the three repertoires: pre-panned phage, week 9, and week 13.

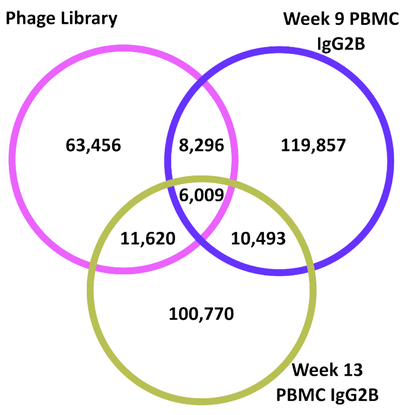
Alicanto



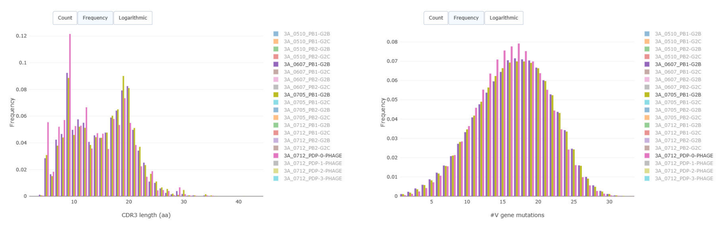
An overview of the proteogenomic process used by Alicanto.

Unlike B cell-focused antibody discovery approaches, Alicanto directly analyzes target-specific antibodies present in serum. Using mass spectrometry analysis of antigen-purified heavy chain-only antibodies from serum, Alicanto prioritizes high affinity antibodies that have been in vivo selected for secretion in serum. By combining the mass spectrometry data with a deeply sequenced BCR repertoire, Alicanto selects antibodies that are both diverse and high affinity (**Alicanto VHH Hits**).

BCR Repertoire vs Phage Library



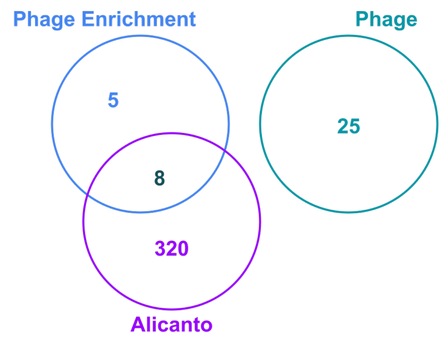
CDR3 overlap across the three repertoires (IgG2B only for the PBMC repertoires) was consistent with overlap across replicates from the same RNA extraction. The Jaccard similarity (CDR3s in the intersection over CDR3s in the union of the sets) ranged from 0.07 to 0.09)



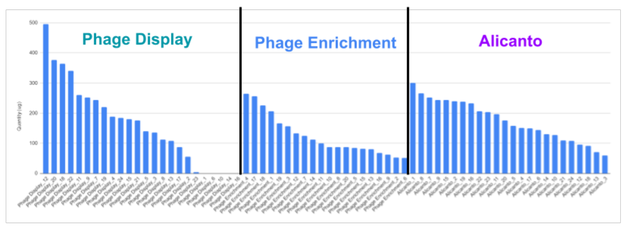
Above Left: The CDR3 length distribution for the week 9 IgG2B, week 13 IgG2B, and phage library repertoires. The phage library (pink) has slightly shorter CDR3s than the PBMC repertoires. *Above Right:* The histogram of V gene amino acid mutations for the phage library and the PBMC repertoires for IgG2B. The phage library repertoire (pink) was less mutated than the PBMC repertoires.

VHH Candidate Comparison

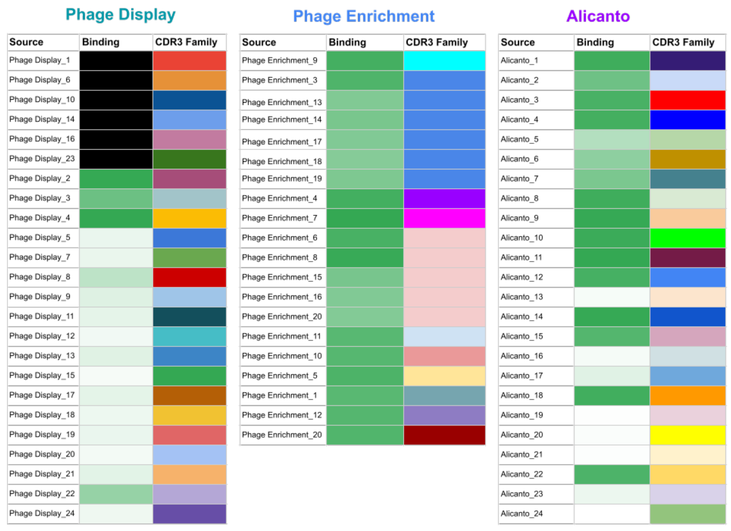
Each method for antibody discovery delivered VHH candidates. The candidates were compared based on CDR3 amino acid sequence in the Venn diagram below



Interestingly, the final set of phage candidates had no overlap with the Alicanto candidates. Some candidates discovered by phage library enrichment analysis were also discovered by Alicanto



Up to 24 candidates from each method were recombinantly expressed in CHO cells with a human Fc. The expression yield is shown for each candidate. Yield varied considerably, however, 6/24 phage display candidates fail to express in mammalian cells.

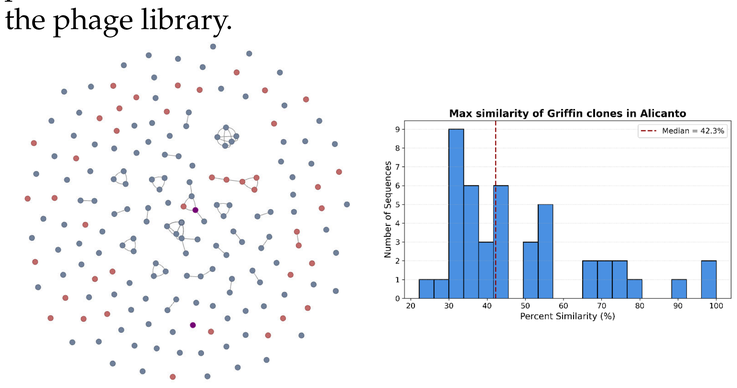


Expressed candidates were tested for binding in ELISA. The binding strength and CDR3 family label are shown for each candidates. The CDR3 family was determined based on CDR3 amino acid similarity.

Overall both Alicanto and phage display delivered at least 24 candidates with diverse sequences, though Alicanto delivered significantly more candidates than were tested in this study. Alicanto candidates were more likely to express recombinantly in a mammalian expression system, and bind the target in ELISA. The phage enrichment candidates were also easy to recombinantly express, and showed strong binding to the target but had lower sequence diversity than Alicanto.

VHHs found only in serum with Griffin

Griffin is a mass spectrometry-based platform that sequences antibodies directly from serum, without using B cell information at all. Griffin was used to profile the clones present in the serum that were not detected in the BCR nor the phage library.



Above Left: A CDR3 network where each node is a CDR3 identified by Alicanto (red) or Griffin (blue) or both (purple) and edges are drawn between nodes if the CDR3s are 80% similar. *Above Right:* the edit distance between CDR3s found by Griffin and the closest sequence in the BCR repertoire. Most Griffin CDR3s were less than 43% similar to the closest repertoire sequence.