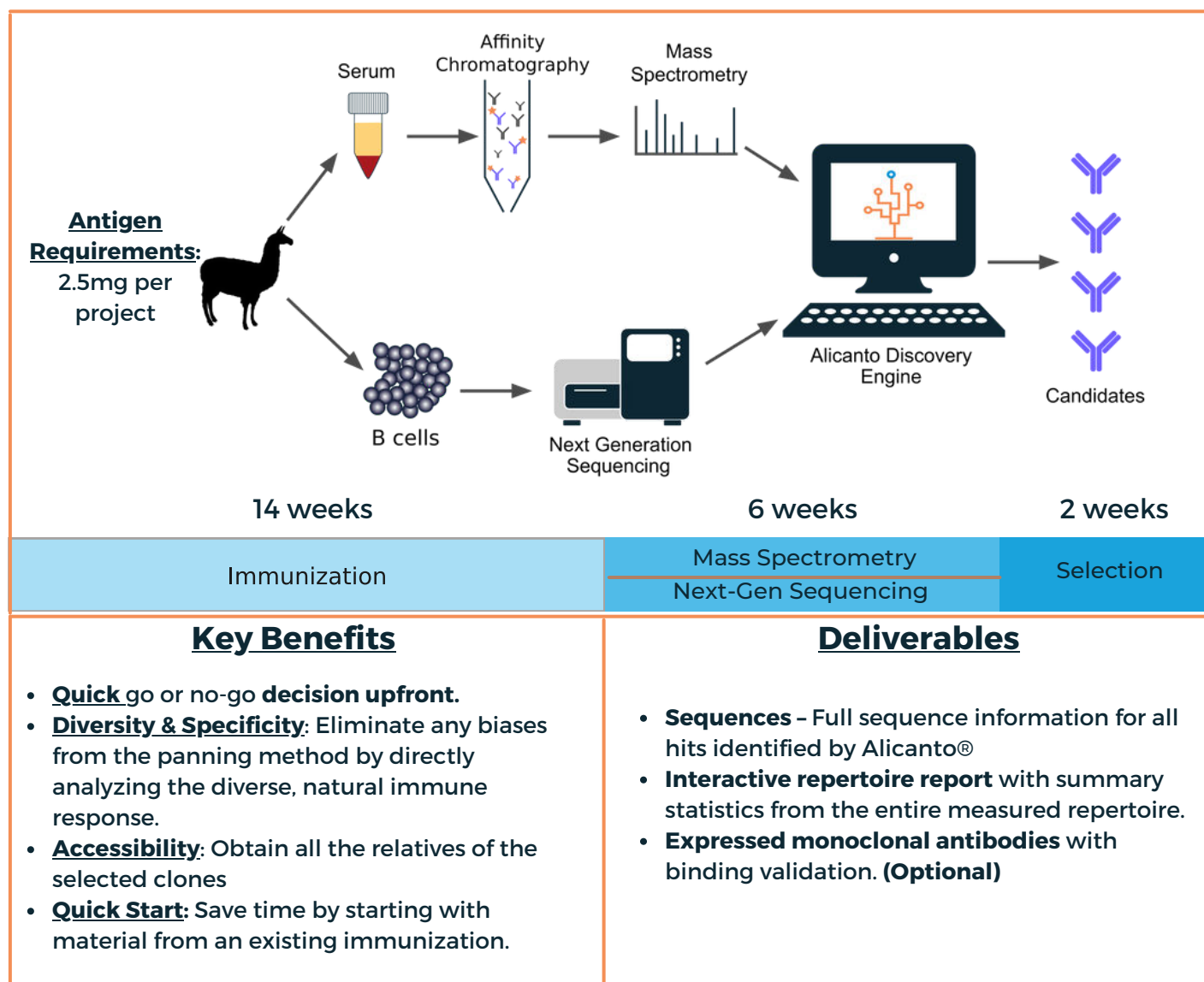


# Custom Antibody Discovery From Serum

Diverse Single Domain (VHH) Antibody Discovery by Mapping the Entire Antibody Response

## The Alicanto® Workflow



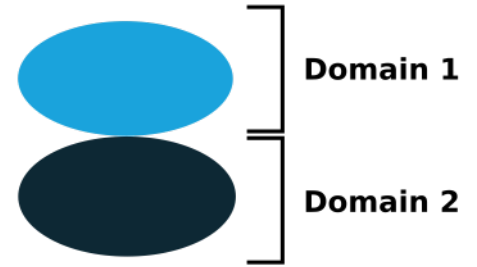
No more panning!

Royalty Free!

Customizable workflow!

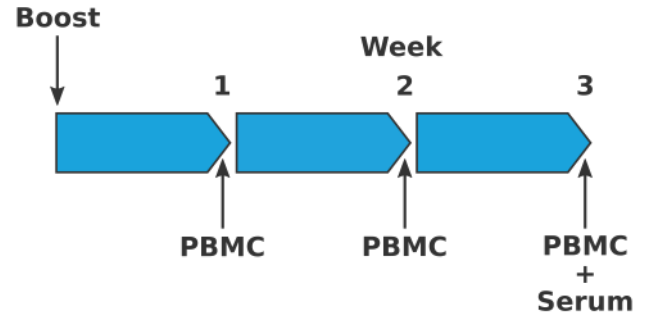
## Case Study

Two alpacas were immunized with a multi-domain protein target. The goal of the project was to recover single chain antibodies targeting two different domains; domain 1 and domain 2. After the final boost, peripheral blood mononuclear cells (PBMCs) were collected 1 week, 2 weeks, and 3 weeks after the boost (Schedule shown to right).



## B cells and next-generation sequencing

We performed next-generation sequencing of the single chain IgG isotypes. In total, over 115,000 distinct antibody sequences were recovered from alpaca 1 and 146,000 distinct antibody sequences were recovered from alpaca 2.



## Serum antibodies and mass spectrometry

3 weeks after the final boost, serum from both alpacas was affinity purified using either the entire antigen (Ag), only domain 1 (Ag-d1), or only domain 2 (Ag-d2). Mass spectra were generated for each fraction, and mapped to the B-cell repertoires described above.

## CDR3 discovery

The collection of CDR3s observed across the different enrichment fractions is shown in the plot to the right. Each row represents a distinct CDR3 amino acid sequence (465 total). Each column represents a fraction of affinity purified antibodies. These results showed that:

- **Alpaca 1 and Alpaca 2 produced completely different CDR3s.**
- **The Ag-d1 and Ag-d2-enriched fractions shared CDR3s with the Ag-enriched fraction.**
- **Both the Ag-d1 and Ag-d2-enriched fractions enabled deeper sampling of those specificities, beyond what was observed in the Ag-enriched fraction.**

