Custom antibody discovery from serum

The natural immune response is a rich source of antibody candidates for research use, diagnostics, and therapeutics. Our Alicanto platform comprehensively mines the immune system using next-generation sequencing together with mass spectrometry analysis and produces a diverse collection of antibody candidates derived from nature. Our service builds on recent technology development across immunogenomics and proteomics (1-4).

**Key Features:**

**Specificity:** Focus on specificity in the earliest stages instead of during downstream screening.

**Diversity:** Directly analyze the diverse, natural immune response.

**Transparency:** Full access to every sequenced antibody.

### Antigen Requirements

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Requirement</th>
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<tbody>
<tr>
<td>Rabbit</td>
<td>4 mg</td>
</tr>
<tr>
<td>Llama/Alpaca</td>
<td>7 mg</td>
</tr>
<tr>
<td>Goat</td>
<td>7 mg</td>
</tr>
<tr>
<td>Human</td>
<td>Varies</td>
</tr>
</tbody>
</table>

### The Alicanto® workflow

**Expedited**

Save time by starting with material from an existing immunization!

**End-to-End**

We can express the selected candidates at any scale!

14 weeks

Immunization

4 weeks

Mass Spectrometry

Next Gen Sequencing

1 week

Selection

Candidates

Absolute antibody

Deliverables:

**Sequences** for candidates selected by Alicanto.

**Interactive repertoire report** with access to entire measured repertoire.

**Expressed monoclonal antibodies** with binding validation.

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.