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.

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### Antigen Requirements

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Requirement</th>
</tr>
</thead>
<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>

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### The Alicanto® workflow

![Diagram of 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!

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<table>
<thead>
<tr>
<th>14 weeks</th>
<th>4 weeks</th>
<th>1 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunization</td>
<td>Mass Spectrometry</td>
<td>Selection</td>
</tr>
<tr>
<td>Next Gen Sequencing</td>
<td></td>
<td></td>
</tr>
</tbody>
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**Deliverables:**

- **Sequences** for candidates selected by Alicanto.
- **Interactive repertoire report** with access to entire measured repertoire.
- **Expressed monoclonal antibodies** with binding validation.

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Case Study

Two juvenile New Zealand white rabbits were immunized with a recombinant beta-galactosidase antigen over 14 weeks. Boosts were administered every 3-4 weeks after initial immunization.

B cells and next-generation sequencing
Peripheral blood mononuclear cells (PBMCs) from 9 time points were sampled, as well as spleen and bone marrow at 14 weeks. No enrichment was performed on the cells prior to RNA-extraction, variable region amplification, and next-generation sequencing. In total 1.4M heavy chain reads and 1.9M light chain reads were obtained.

Serum antibodies and mass spectrometry
At week 14, serum was collected from the rabbits. Target-specific antibodies were enriched by affinity purification against beta-galactosidase. The polyclonal antibodies were then separated by chain and digested with multiple proteases prior to analysis by mass spectrometry.

Discovery and expression
Candidate antibody sequences were selected based on quality of proteomic evidence. In total 11 heavy chain and 9 light chain clonal lineages were transiently expressed in HEK293, allowing all pairs of heavy and light chain. Expression was performed by Absolute Antibody, Ltd. A dilution ELISA (see right) was performed with a commercially available antibody. Kinetic analysis of two antibodies demonstrated sub-nanomolar binding, and increased affinity over the control antibody.

Data access on the Alicanto web portal
The entire measured immune response is accessible through the Alicanto web portal.