Abterra bio

Alicanto®

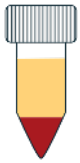
Polyclonal Antibody Sequencing

Convert your polyclonal antibody into monoclonals via sequencing

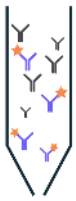
BENEFITS

- **Eliminate batch-to-batch variability**
- **Protect your IP by protecting your sequences**
- **Engineer, conjugate, and reformat your products into new tools**

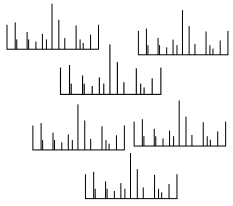
The Sequencing Workflow



Polyclonal antibody or serum/plasma



Affinity chromatography



Mass spectrometry

Alicanto Discovery Engine

STAGE 1

Clonality/Complexity Assessment

STAGE 2

Assembly and Sequencing

STAGE 3

Expression and Validation

Deliverables:

- **Full-length sequences** of heavy and light chain
- **Clone clustering** of families of related sequences
- **Interactive** report of candidates with proteomic evidence
- **Recombinant expression** and binding validation

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All work performed in the USA!

www.abterrabio.com

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Case Study

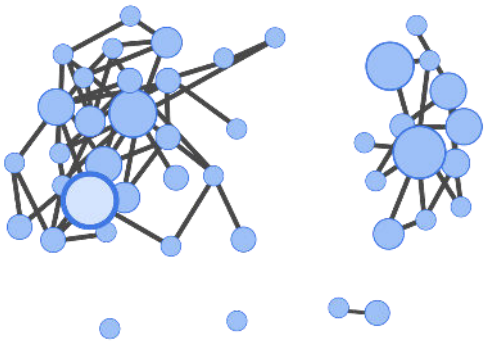
A llama was immunized against a protein antigen for the purpose of generating a polyclonal VHH antibody reagent. The animal died, preventing any further production of the polyclonal antibody (pAb). The goal of the project was to identify monoclonal antibodies that recapitulate the pAb activity.

Mass spectrometry data generation

The heavy chain-only antibodies were purified from llama serum, and subsequently purified against the antigen using affinity chromatography. The pAb was digested with multiple enzymes to obtain peptide sequences that covered every region of the antibody. Using proprietary methods, long peptides were generated to enable assembly and phasing of CDRs.

STAGE 1 Clonality/Complexity Assessment

A shallow first-pass assessment of sequence diversity reveals the level of complexity of the sample. Too many CDR3s indicates that only the most abundant will be sequenced. The CDR3 network to the right shows two major families of CDR3s present in the pAb.



STAGE 2 Assembly and Sequencing

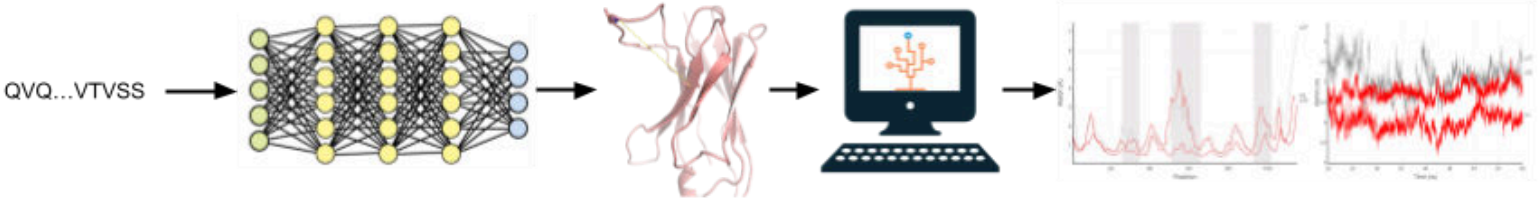
A deep second-pass analysis generates candidate assemblies. Each assembly is reviewed in silico to eliminate unnatural sequences and structures. The figure shows the peptide support for one assembled VHH

STAGE 3 Expression and Validation

CDR3 clustering at 80% similarity, single linkage, revealed 8 clone families. Representatives from each family were recombinantly expressed and tested. ELISA binding showed that 44% of all VHHs bound the target, and 5 of the 8 families showed activity. The 5 largest families all contained at least 1 binder, while the 3 singleton families all failed to show binding

Family ID	# VHHs	# Binders
A	13	6
B	2	1
C	2	1
D	3	1
E	2	2
F	1	0
G	1	0
H	1	0
Total	25	11

A post-hoc analysis using molecular dynamics (MD) simulations of clones with similar sequences was predictive of failure to bind.



Predict Structure **Simulate Movement** **Analyze Instability**

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