Accelerating VHH lead candidate panel generation through high-throughput early triaging of optimal B cell clones

IMMUNOPRECISE ANTIBODIES

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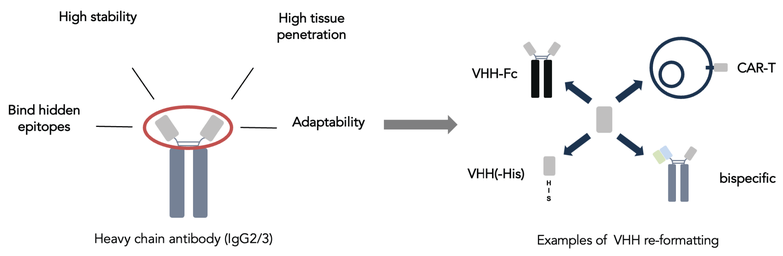
ImmunoPrecise Antibodies - Europe (Oss)

Introduction: VHHs, the variable domains of camelid heavy chain (IgG2/3) antibodies, have garnered substantial attention in recent years due to their unique advantages, including their versatility in CAR-T therapies and bi-/multispecific applications. While the interest in VHHs for clinical application is relatively recent, IPA brings over two decades of R&D expertise with these potent antigen-binding fragments.

Traditionally, VHHs are obtained from phage libraries generated from the B cells of (immunized) camelids. Building on this established method, IPA has introduced a high-throughput platform that accelerates the identification of VHHs with desired properties. This approach combines direct screening and automated hit selection of B cell clones with NGS-based VHH recovery. Following successful llama immunization, we here showcase how our platform screens up to 1 million nanowells per day, enabling early triaging of the most promising VHH-expressing clones and delivering a diverse panel of lead candidate VHHs.



Rapid, high-throughput VHH-discovery platform allowing early-stage triaging based on desired characteristics

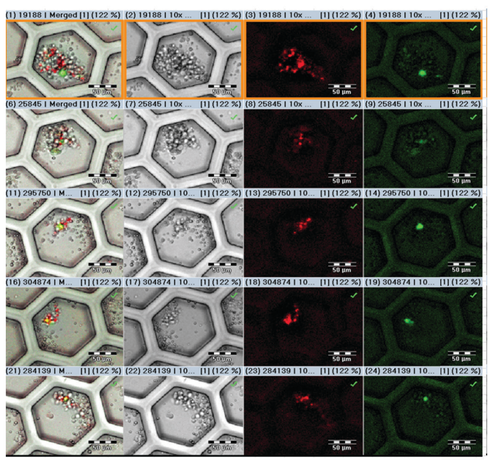
Plasma cell workflow

Seeding of PBMCs from immunized llama into nanowells containing target-expressing reporter cells and detection antibody

Screening and picking

- Fluorescence-based identification of target-binding (red) VHHs and receptor activating (green) hits
- Automated hit picking

High-throughput and rapid selection of affinity matured VHHs, including ones directed against difficult targets such as multi-pass membrane proteins



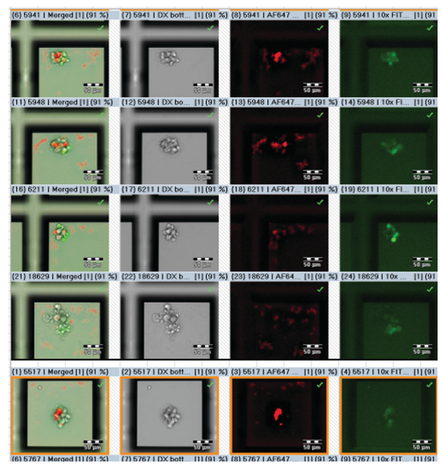
Memory B cell workflow

Selection of memory B cells from PBMCs of immunized llama on target, followed by seeding into nanowells for expansion and plasma cell differentiation

Screening and picking

- Addition of target-expressing reporter cells and detection antibody
- Fluorescence-based identification of target-binding (red) VHHs and receptor activating (green) hits
- Automated hit picking

Target-enrichment empowering efficiency and the throughput of nanowell-based screenings while maintaining natural clonal diversity



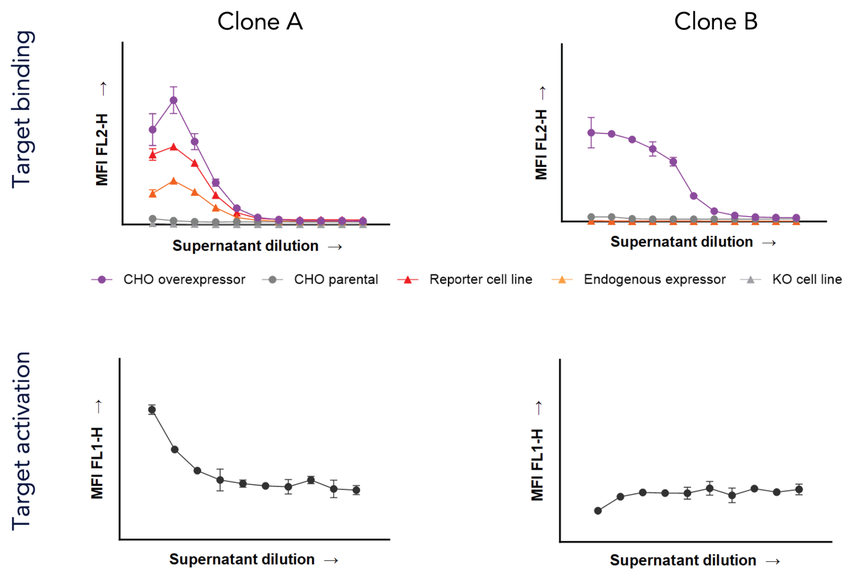
NGS-based sequence recovery and validation of recombinant antibodies

NGS of hits - RNA isolation, VHH amplification, barcoding, MiSeq, NGS data processing and **consensus sequence** determination

Phylogenetic tree of the VHH sequences of picked hits

Interestingly, 3 sequences obtained from our dual B cell strategy overlapped with output obtained from a parallel phage display approach validating the platform but also supporting the uniqueness of each discovery approach

Recombinant expression of VHHs and **reactivity and activity** verification



In parallel to the dual B cell approach, PBMCs from the same llama were used for **phage display-based** discovery, yielding **~50 sequences with unique CDR3s.** In short, following RNA isolation from PBMCs and generation of a phage library from amplified VHH repertoire, the VHH-library was subjected to different selection strategies including cell-based pannings, followed by screening of single clones and sequencing of target-reactive VHHs.

A subset of VHH sequences was reformatted to VHH-Fc and recombinantly expressed. Culture supernatant was used for a binding study (upper graphs) towards a target-overexpressing CHO cell line, CHO parental, a target reporter cell line, an endogenous expressor cell line and a KO of that endogenous cell line. Furthermore, the culture supernatants were also tested for their ability to activate the target using the target reporter cell line (bottom graphs).

VHH hit discovery flexibility: Rapid, sensitive and high-throughput with diverse outputs

Single plasma - Speed

Direct screening of single plasma cells

Multi-pass membrane proteins

Memory B Cell - Diversity

Culturing and screening of target-enriched memory B cell maintaining natural diversity

Direct functional read-out (reporter cell)

Phage - Versatility

Versatile phage display-enrichment of VHHs matching multiple lead requirements

Multi-pass membrane proteins/low immunogenic targets

Specific cross-reactivity and specificity requirements

The solution for every VHH discovery need

Conclusion: Our recently implemented camelid B cell workflow allows for rapid identification of VHHs with desired characteristics shortly after peripheral blood withdrawal. A streamlined NGS-based workflow for VHH sequence recovery of automatically picked hits and subsequent validation of recombinantly expressed VHHs further speeds up the process of lead candidate selection. Our approach presents a rapid way forward towards therapeutic VHH discovery. As target type and critical lead characteristics are key to designing the most suitable antibody discovery strategy, IPA is at the forefront of VHH discovery leveraging its broad expertise in camelid immunizations, phage display and single B cell technologies, offering highly customizable and robust discovery technologies to generate diversified VHH panels.