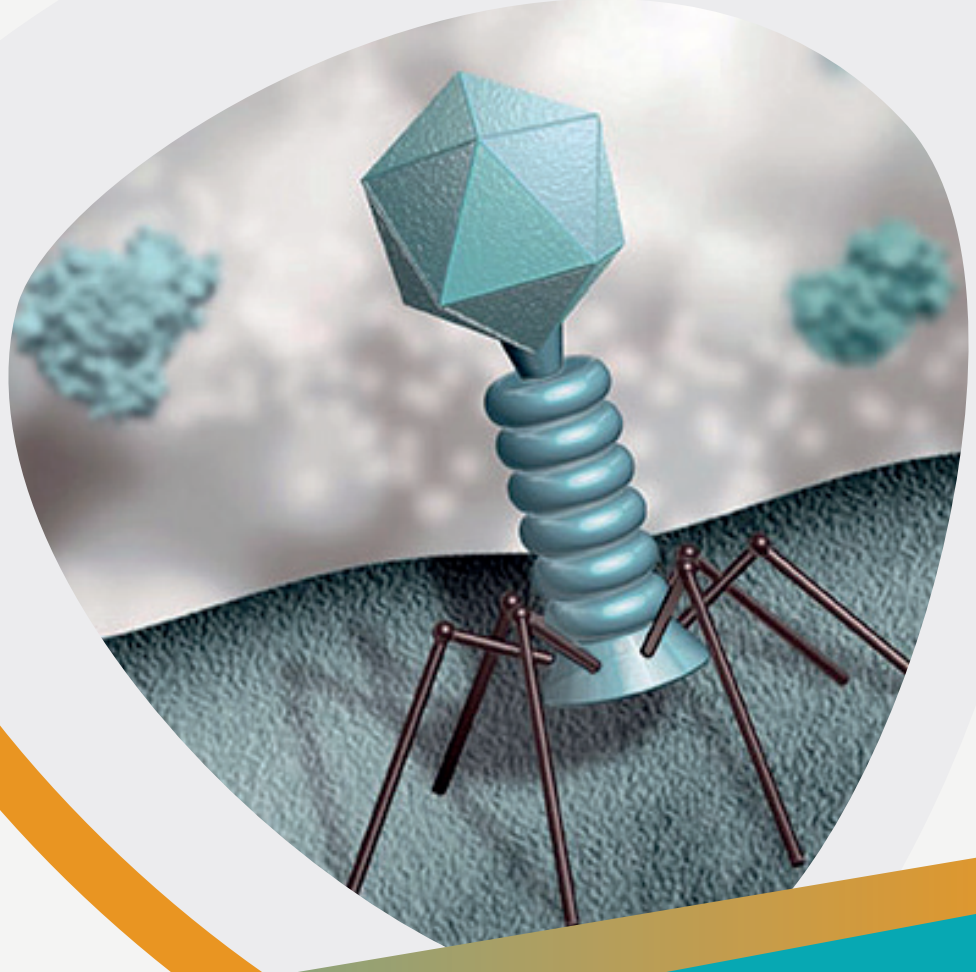
CAMELID VHH FRAGMENT



Expert in Antibody Engineering

From Research to Discovery

ISO 9001 : 2015 certified

covalab
R&D in Biotechnology

PROTOCOL OVERVIEW

1

IMMUNOGEN SELECTION

Protein
Microorganism
Peptide & PTM
Hapten
Other

2

LLAMA IMMUNIZATION

88-day protocol
4 injections & 4 test sera

ELISA tests on each serum
Follow-up of serum immunoreactivity over time

3

PHAGE LIBRARY CONSTRUCTION

PBMCs isolation and RNA extraction

cDNA synthesis and VHH amplification

Phage vector cloning

4

PANNING

Target interaction antigen-phages

Phages amplification

Selection of cDNAs coding for specific VHH

5

HIGH SCALE PRODUCTION

Subcloning in high yield expression vector

Culture, lysis and affinity chromatography purification

ABOUT VHH

Camelids produce conventional antibodies made of 2 heavy chains and 2 light chains bound together with disulphide bonds in a Y shape. However, they also produce a unique subclasse of immunoglobulin G, also known as heavy chain antibody (HC-Ab).

These antibodies are made of only two heavy chains without the CH1 region, and an antigen binding domain at their N-terminus called VHH.

The unique feature of hcIgG is the capacity of their monomeric antigen binding regions (VHH) to bind antigens with specificity, affinity and especially diversity that are comparable to conventional antibodies without the need of pairing with another region. Using molecular engeenering VHH fragments can be generated.

COVALAB, YOUR PARTNER IN ANTIBODY ENGINEERING SINCE 1995

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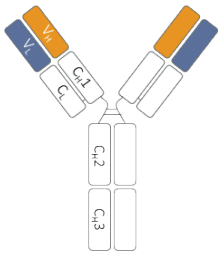
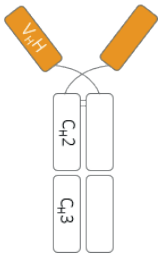


Conventional antibody IgG
(150 kDa)



Camelid IgG
(80 kDa)

VHH fragment
(15 kDa)

WHY USE VHH FRAGMENTS RATHER THAN CONVENTIONAL ANTIBODIES?

Small & accessible
From 12 to 15 kDa - Bind the hidden epitope - Reach hardly accessible antigens

Penetration
Quicker - Target intracellular proteins - Precise staining of tissue sections

Soluble & stable
Thermostable up to 70°C / 158°F - Resist to extreme pH

Easy to manufacture
Easy to manufacture in yeast or microbial systems

Specificity & affinity
Nano to picomolar affinity

Clearance
Rapid blood elimination - Able to pass renal filter

DETAILED PROTOCOLE

1. IMMUNOGEN SELECTION

Protein

Microorganism
Inactivated virus
Inactivated yeast
Inactivated bacteria

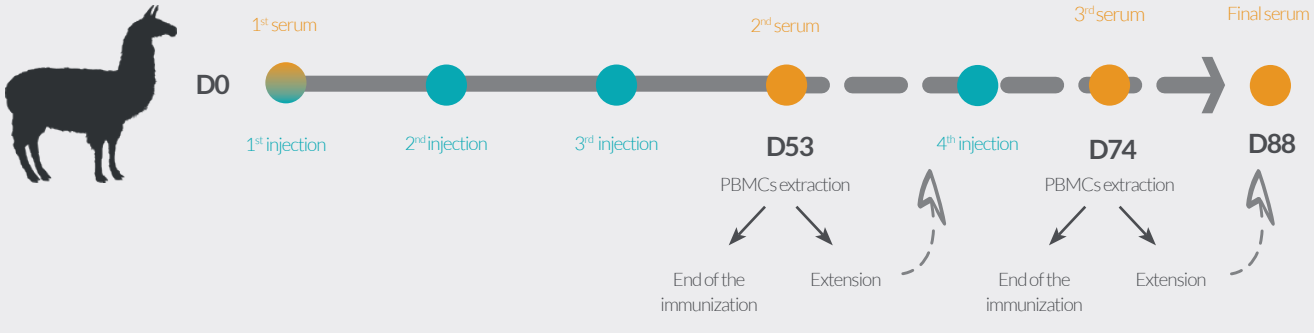
Peptide
PTM

Hapten
Carbohydrates
Chemicals & Toxins
Natural or modified nucleotides

Other
Cell extracts
Tissus extracts
Others *(please contact us)*

2. LLAMA IMMUNIZATION

According to our exclusive protocol, the immunization last from 53 to 88 days, according to the results, with up to 4 injections and 2 test sera. The immunoreactivity is monitored by the detection method developed for the project.



All the experiments are carried out by experienced and authorised personnel according to H&S procedures, established in accordance to the French legislation.

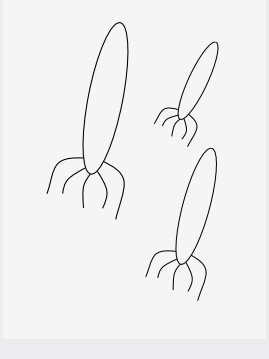
YOU RECEIVE

- Serua to run tests in your specific conditions and adapt the protocol according to the immunoreactivity.

GUARANTEE

- The immunized animal is kept in the animal facility to perform additional total RNA extraction if necessary.

3. PHAGE LIBRARY CONSTRUCTION



PBMCs isolation and RNA extraction
Peripheral blood mononuclear cells (PBMCs) are isolated from the final serum and total RNA is extracted.

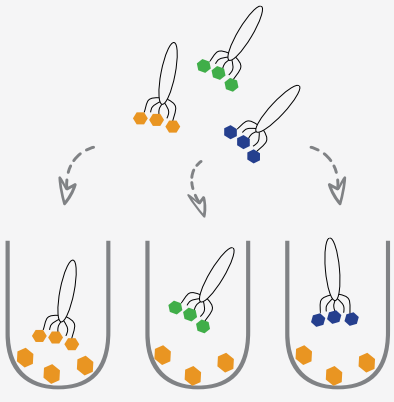
cDNA synthesis and VHH amplification
RT-PCR and VHH antibody fragment amplification
Specific primers are design to perform retro-transcription and amplification of VHH antibody fragments.

Phage vector cloning
VHH coding genes are integrated into a phage vector which is then used to transform competent bacteria for phage library production.

4. PANNING

Several selection rounds are required for target specific recombinant phage enrichment.

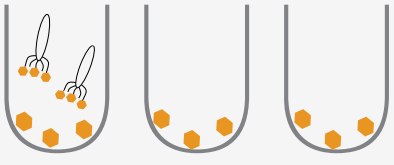
Target interaction



The antigen is immobilized in the bottom of microtiter plate wells.

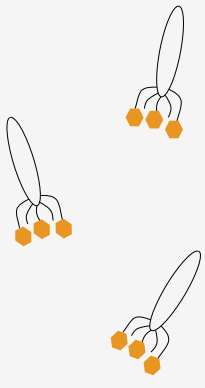
Phages preparations are loaded into the wells to allow them to bind the antigen through the VHH expressed at their surface.

Amplification



After washing off unbound phages, remaining phages are transformed into competent bacteria for enrichment and amplification.

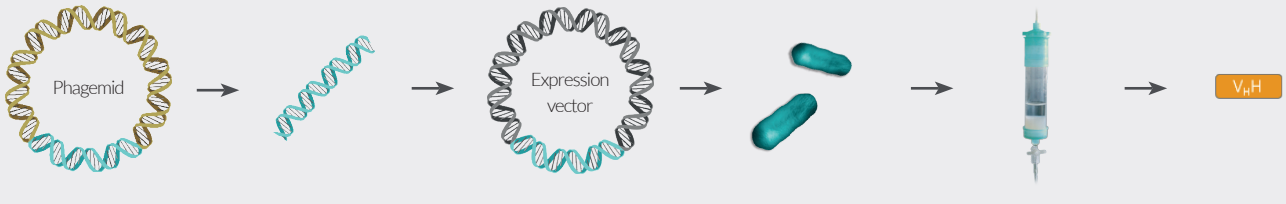
Selection



At the end of panning stage, monoclonal ELISA screening is performed for individual clones and cDNAs coding for VHH specific to the antigen are isolated and sequenced

5. HIGH SCALE PRODUCTION

The cDNAs coding for VHH of interest can then be used for high-scale production. The VHH gene are subcloned into our expression vector. VHH are then produced after transformation and growth of competent bacteria followed by affinity chromatography purification.



PCR amplification of the selected VHH gene.

Inserts are then integrated into a high yield expression vector which is used to transform competent bacteria.

Specific VHH are purified from cells lysis supernatants by affinity chromatography.

YOU RECEIVE

- VHH preparations purified from bacteria cell culture medium by antigen-affinity chromatography

GUARANTEE

- All cDNAs coding for specific VHH are kept frozen at -80°C in our laboratory

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