DNA IMMUNIZATION

Expert in Antibody Engineering

From Research to Discovery

ISO 9001 : 2015 certified

covalab
R&D in Biotechnology

PROTOCOL OVERVIEW

1 GENE CLONING

CODING SEQUENCE GENERATION
Extraction from custom plasmid or
de novo synthesis from numeric
sequence

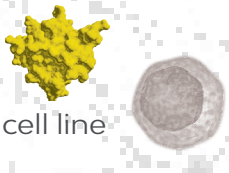
EXPRESSION PLASMID CLONING
Proprietary plasmid
Optimized for *in vivo* DNA vectorization
Plasmid validation

2 IMMUNIZATION

IMMUNIZATION
Mice or rabbit
Exclusive 63-day protocol

MONITORING
ELISA assay on each serum
Immunoreactivity comparison over time

3 HYBRIDOMA GENERATION (MOUSE ONLY)



FUSION
Fusion of spleen cells with myeloma cell line
Culture in selective medium

SCREENING
ELISA tests on growing cell hybridomas to
confirm positive hybridomas stability

4 HYBRIDOMA SELECTION (MOUSE ONLY)

CLONING
Cell seeding using limiting dilution method
ELISA tests to confirm positive clones

SELECTION
ELISA tests to confirm antibody production
stability. Complete isotyping *(isotype, class, subclass and light chain isotype)* and freezing

ABOUT DNA IMMUNIZATION

Classical immunization is based on biological samples inoculation (proteins, peptides...) which needs to be produced and purified prior to the immunization. The protein production is a critical step which is **time consuming** and requires **several optimisation steps.** Even after a long optimisation process, many proteins such as transmembrane proteins failed to give consistent results. **The low successful rate** of these proteins production and immunization are mainly due to their large hydrophobic domains which are leading to the protein precipitation even at low concentration.

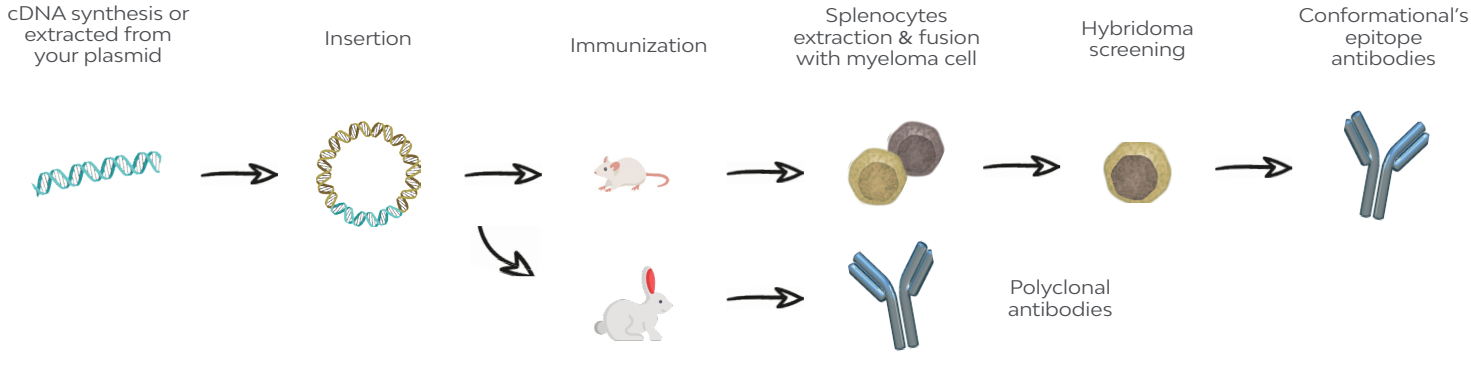
DNA immunization permits *in vivo* antigen production by avoiding all protein production steps and offer **better results with hydrophobic proteins.** The antigen is displayed on its natural conformation with **all post translational modifications** and the quality is significantly improved compared to bacterial or yeast productions. The DNA immunization is a powerfull alternative that leads to **decreased project duration** and increase project chance to obtain **highly specific antibodies against conformational epitope.**

COVALAB, YOUR PARTNER IN ANTIBODY ENGINEERING SINCE 1995

MADE IN FRANCE - ISO 9001 : 2015 CERTIFIED

OUR PROCESS

FROM THE GENE TO THE ANTIBODY



ADVANTAGES

NATURAL CONFORMATION

Conformational protein antibodies with all post translational modifications for mechanistic studies.

TIME-SAVING

Short immunization period and fast host's immune response.

EFFICIENT

Slow and good presentation to immune system favours the production of high-affinity antibodies.

SIMPLICITY

Simple start material cDNA or electronic sequence : no protein / peptide required which make us skip the difficult and time consuming steps of protein production and purification. No risk of antigen contamination.

HIGH SUCCESS RATE

Compared to classical immunization for hard-to-produce antigens.

ROBUST

Optimized proprietary vectors that maximize the proteins' expression.

DETAILED PROTOCOLE

1. GENE CLONING

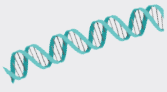
The first step of the development procedure consists in plasmid preparation.

Extraction

Synthesis

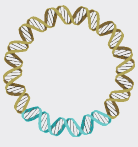
--atgattcag--

You can either provide us with your plasmid or simply the coding sequence.



We extract or synthesize the optimized coding sequence...

Cloning



... which is then integrated into our proprietary vector optimized for *in vivo* gene expression.

YOU RECEIVE

Sequencing results confirming DNA sequence insertion without mutation.

STORAGE & GUARANTEE

Customer plasmid is stored at -20°C for 2 years.

2. ANIMAL IMMUNIZATION

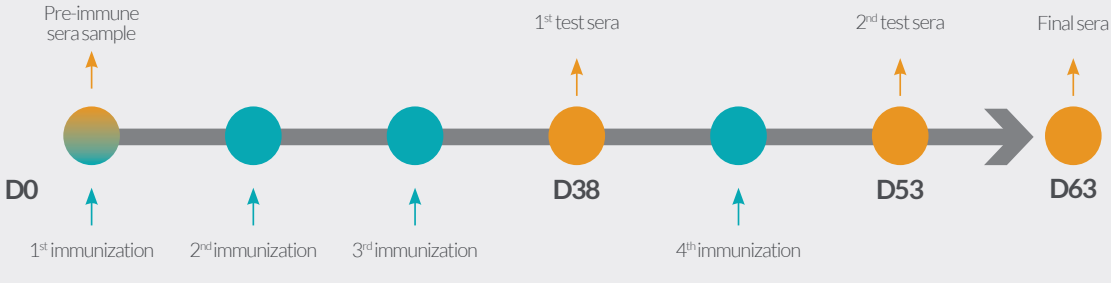
The animals are transfected with the plasmid. The protein expression leads to an immune response. Test sera are assayed according to a detection method developed and adapted specifically for the project. In case of low immune response, additional injections and / or test sera can be performed.

EXCLUSIVE 63-DAY PROTOCOL

2 species

4 injections *of plasmid*

2 test sera *30 µl*



All the experiments are carried out by experienced and authorised personnel according to HSE procedures, established in accordance to the French legislation. Our animal house is registered under the reference C21 464 04 EA.

YOU RECEIVE

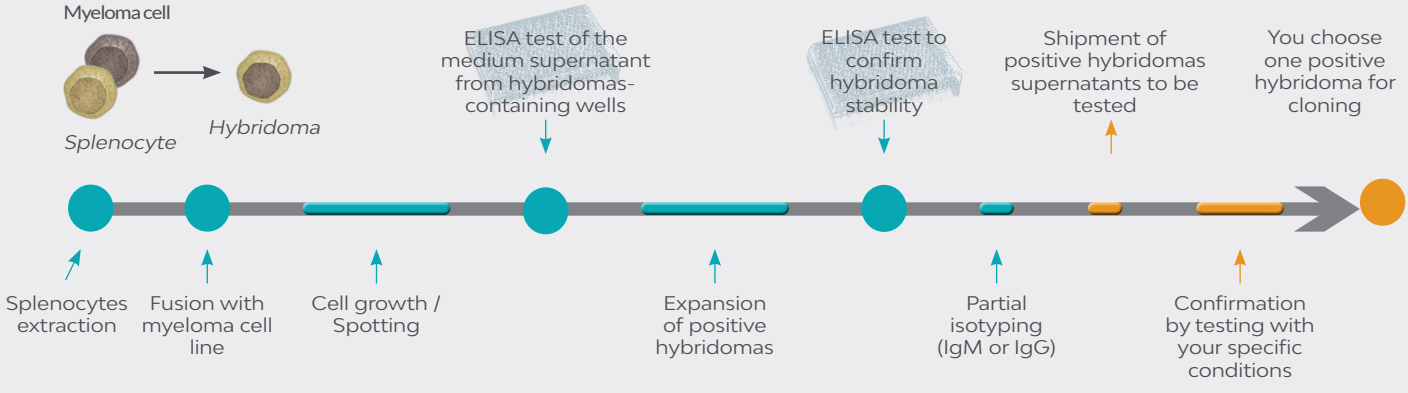
Test sera are provided to run tests in your specific conditions.

STORAGE & GUARANTEE

The Covalab plasmid containing your insert is stored at -20°C for 2 years.

3. HYBRIDOMA GENERATION
(MOUSE ONLY)

Based on the immune response, the best animal is selected according to both our ELISA tests and your own results. Then, we proceed to the extraction of its immunoglobulin-secreting lymphocytes. The cells are subsequently fused with immortalized myeloma cells to generate hybridomas which are subsequently screened to isolate single cells secreting monoclonal antibodies.



YOU RECEIVE

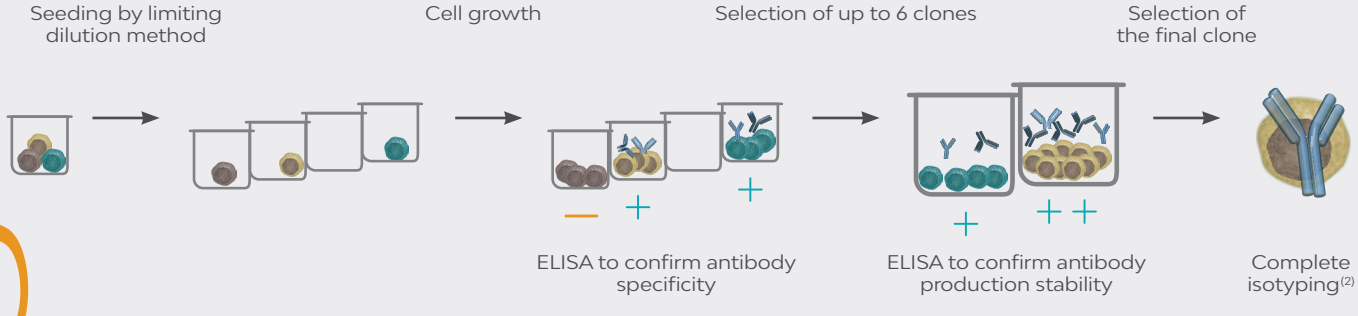
Supernatant from each positive hybridoma to run tests in your specific conditions and you can choose one or several hybridomas to be cloned.

STORAGE & GUARANTEE

Up to 10 positive hybridomas (at polyclonal stage) are kept frozen in liquid nitrogen to perform additional cloning steps if necessary.

4. HYBRIDOMA SELECTION
(MOUSE ONLY)

The final step of the development procedure consists in seeding the previously selected hybridomas using the limiting dilution method, in order to isolate the clones from each other. ELISA tests are then performed to identify the clones which produce the expected antibodies in a stable manner, before a complete characterization of the chosen clone.



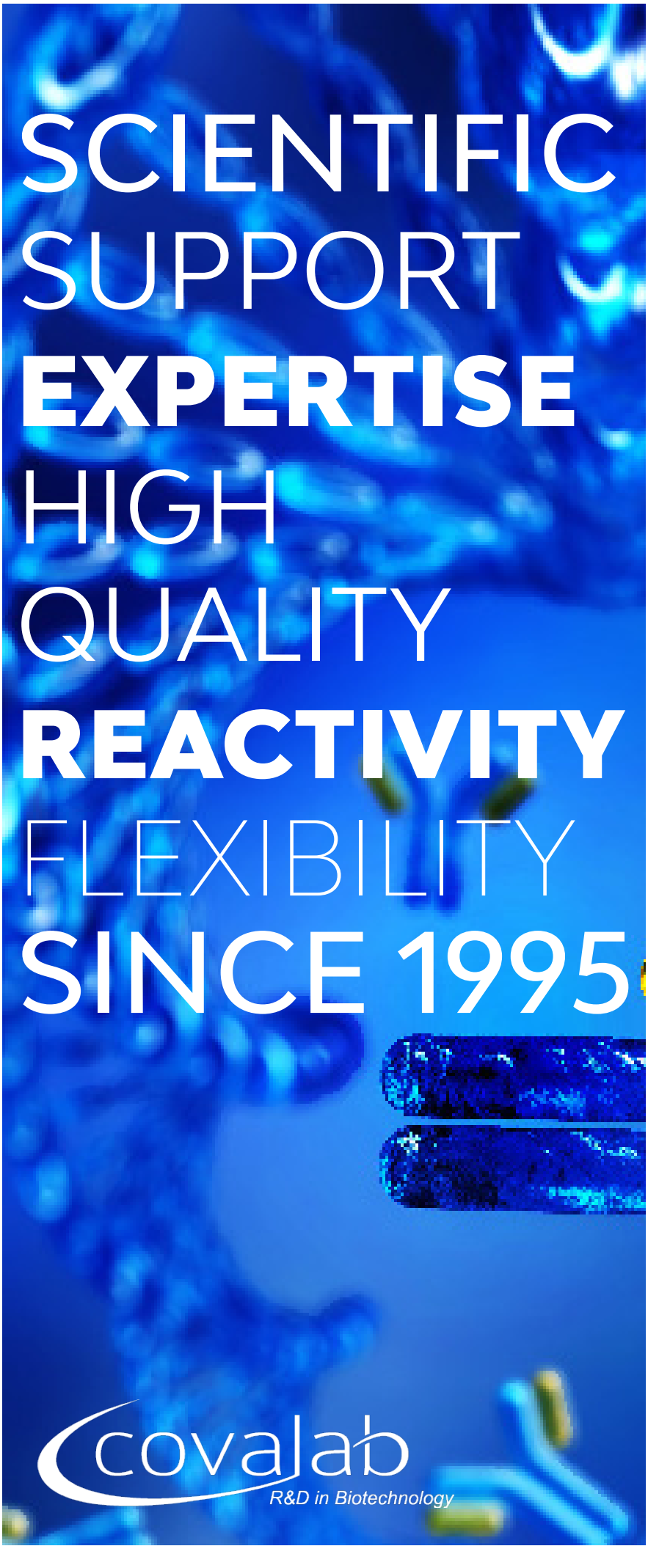
YOU RECEIVE

Supernatant from each confirmed stable positive clone to run tests in your specific conditions.
The final clone frozen in a cryotube vial, based on both our results and yours.

STORAGE & GUARANTEE

Cryotubes vials of the final clone you select are kept frozen in liquid nitrogen.

(2) Complete isotyping includes the determination of the class and subclass of the heavy chains as well as the isotype of the light chain.



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