DNA IMMUNIZATION

Custom Antibody Development

From Research to Discovery

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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
8 mice
Exclusive 63-day protocol

MONITORING
ELISA assay on each test bleed
Immunoreactivity comparison over time

3 HYBRIDOMA GENERATION

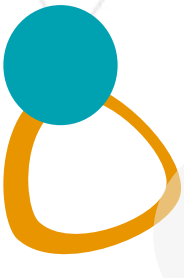
FUSION
Fusion of spleen cells with myeloma cell line
Culture in selective medium

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

4 HYBRIDOMA SELECTION

CLONING
Cell seeding by limiting dilution
ELISA tests to confirm positive clones (up to 6)

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



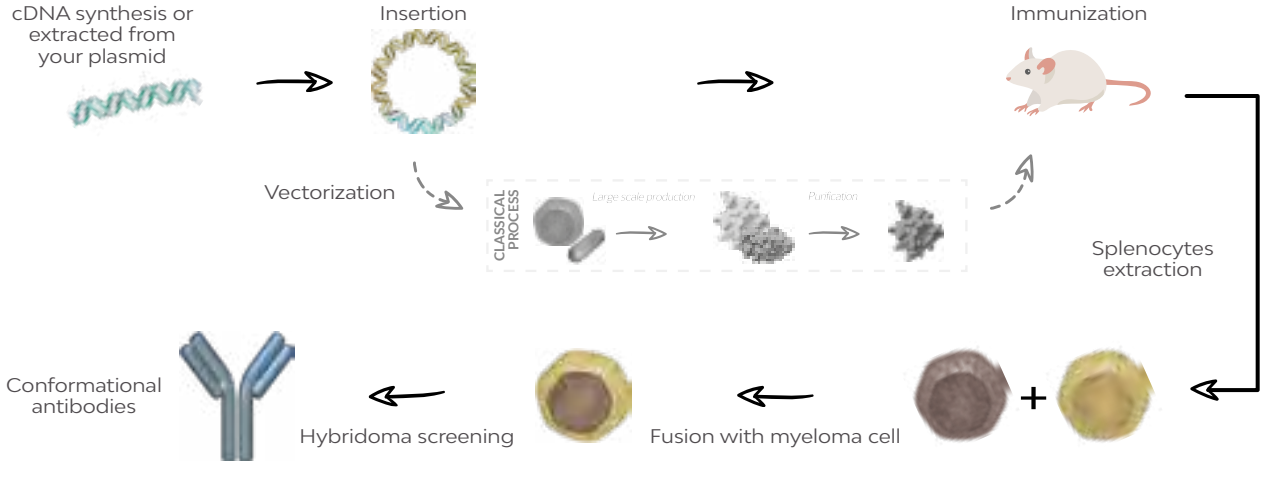
WHY PERFORM DNA IMMUNIZATION?

FROM THE GENE TO THE ANTIBODY

Classical immunization is based on biological samples inoculation (proteins, peptides...) which need 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 production. The DNA immunization is a powerfull alternative leading **decrease the project duration** and increase the project chance of success to obtain a **highly specific conformational antibody.**

OUR PROCESS



ADVANTAGES

NATURAL CONFORMATION

Conformational antibodies with all post translational modifications for mechanistic studies.

TIME-SAVING

Animals are immunized in shorter period of time and the immune response of the host animal is fast.

EFFICIENT

Slow, consistent presentation to immune system favours production of high-affinity antibodies.

SIMPLICITY

Simple start material cDNA or electronic sequence : no proteins / peptides 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 difficult antigens.

ROBUST

Optimized proprietary vectors to maximize the protein expression.

DETAILED PROTOCOLE

1. GENE CLONING

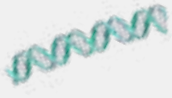
The first step of the development procedure consists in the plasmid preparation.

Extraction

Synthesis

--atgattcag--

You can either provide us with your plasmid if you already have it, or simply the coding sequence.



We extract or synthesize the ideal coding sequence...

Cloning

... which is then integrated into our proprietary vector optimized for *in vivo* DNA vectorization.

YOU RECEIVE

Sequencing results confirming DNA sequence insertion without any mutation.

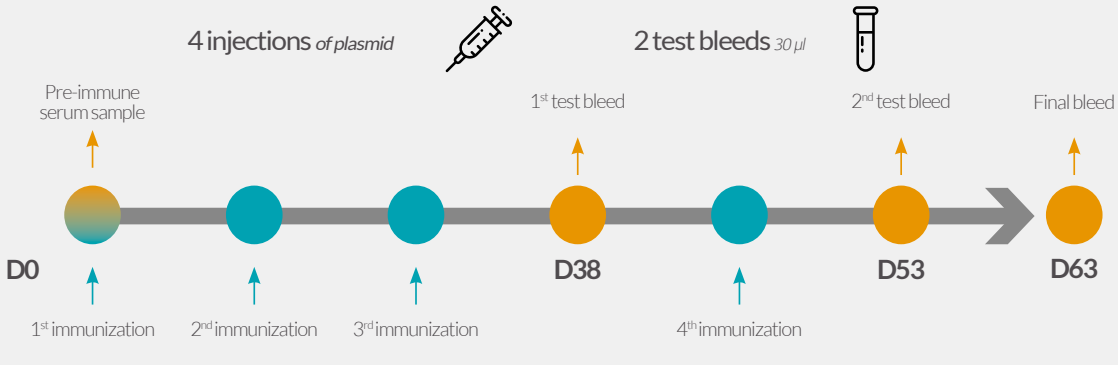
STORAGE & GUARANTEE

The gene expression is validated *in vitro* by cell line transfection before DNA immunization. Customer plasmid is stored at -20°C for 2 years.

2. ANIMAL IMMUNIZATION

The animals are transfected with the plasmid. The protein over-expression leads to an immune response. Test bleeds are assayed according to a detection method developed specifically for the project to adapt the protocol if necessary. In case of low immune response, additional injections and / or bleeds can be performed.

EXCLUSIVE 63-DAY PROTOCOL



All experiments are undertaken by experienced and authorised staff following H&S procedures, established according to the French legislation. Our animal house is registered under the reference C21 464 04 EA.

YOU RECEIVE

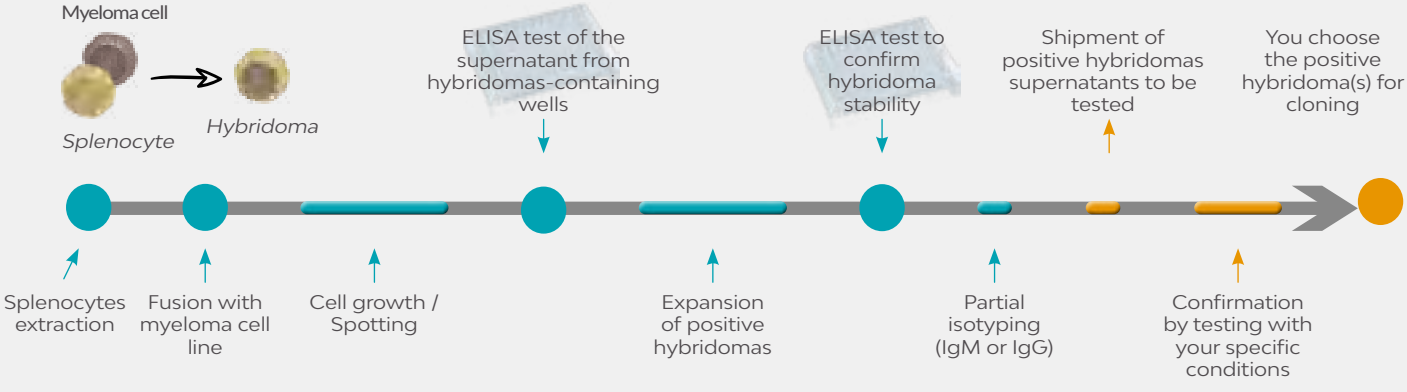
Test bleeds so that you can run tests in your specific condition in order to adapt the immunization protocol if the immunoreactivity is not as high as expected.

STORAGE & GUARANTEE

The Covalab plasmid with the customer insert is stored at -20°C for 2 years.

3. HYBRIDOMA GENERATION

After having selected the most suitable animal according to both our ELISA tests and your own results, 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

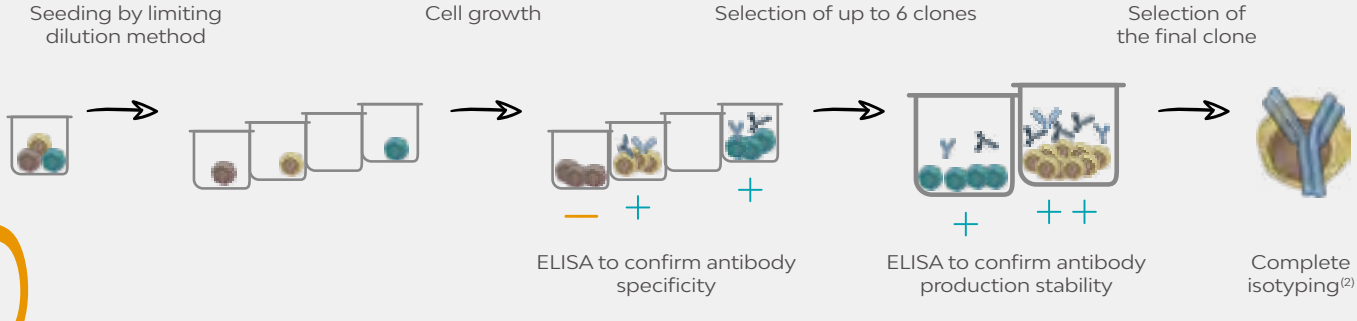
Supernatant from each positive hybridoma to run tests in your specific conditions so that you can choose one or several hybridomas to be cloned.

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

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 clone you choose.



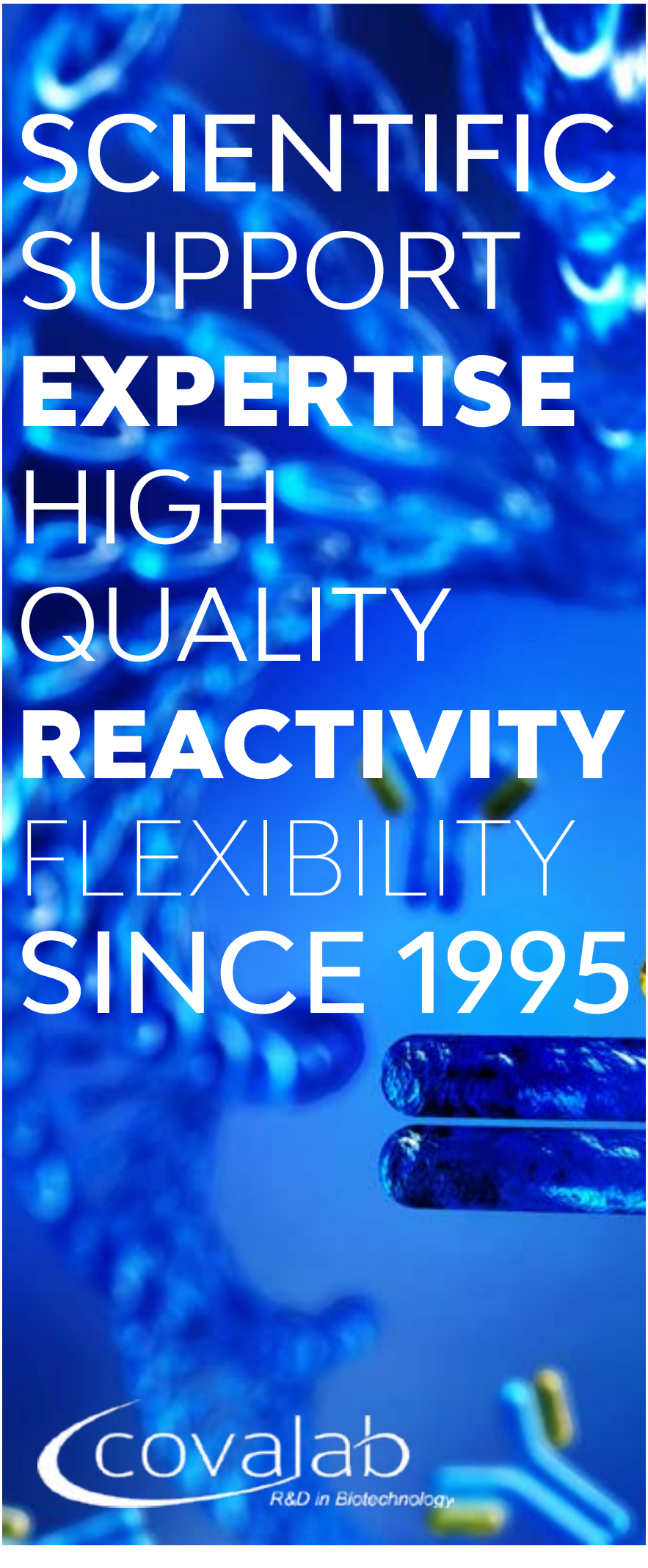
YOU RECEIVE

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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