CAPABILITIES
POLYCLONAL ANTIBODIES AGAINST CYTOPLASMIC PROTEIN HUMAN RAS

THE CHALLENGE

Genovac was approached by a client who wanted polyclonal antibodies against the human protooncogene RAS GTPase in a rat host animal. However, this was a challenge since this intracellular target shares an extremely high amino acid sequence similarity with its rat orthologue, at 98%.

THE SOLUTION

In order to generate the polyclonal antibodies, genetic immunization was used. Here, the protein was routed to the cell surface to stimulate an immune response.

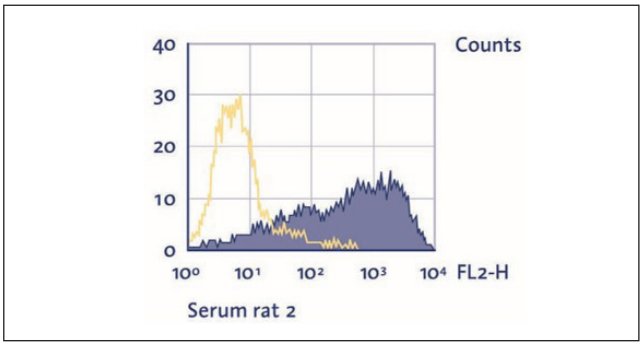

Fig. 1: Exhibits the RAS recognition on transiently transfected cells. Flow cytometry was performed to detect the binding of the specific antibodies to the RAS protein.

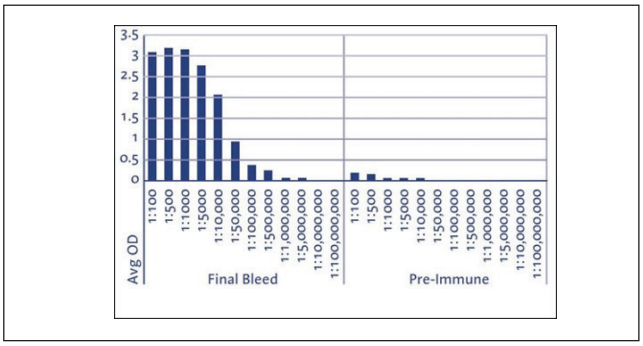

Fig. 2: Depicts the detection of RAS protein levels pre-immunization and after the final bleed. Despite the high sequence similarity, a highly sensitive polyclonal antiserum was generated that could still differentiate human RAS at a 1:500,000 dilution.

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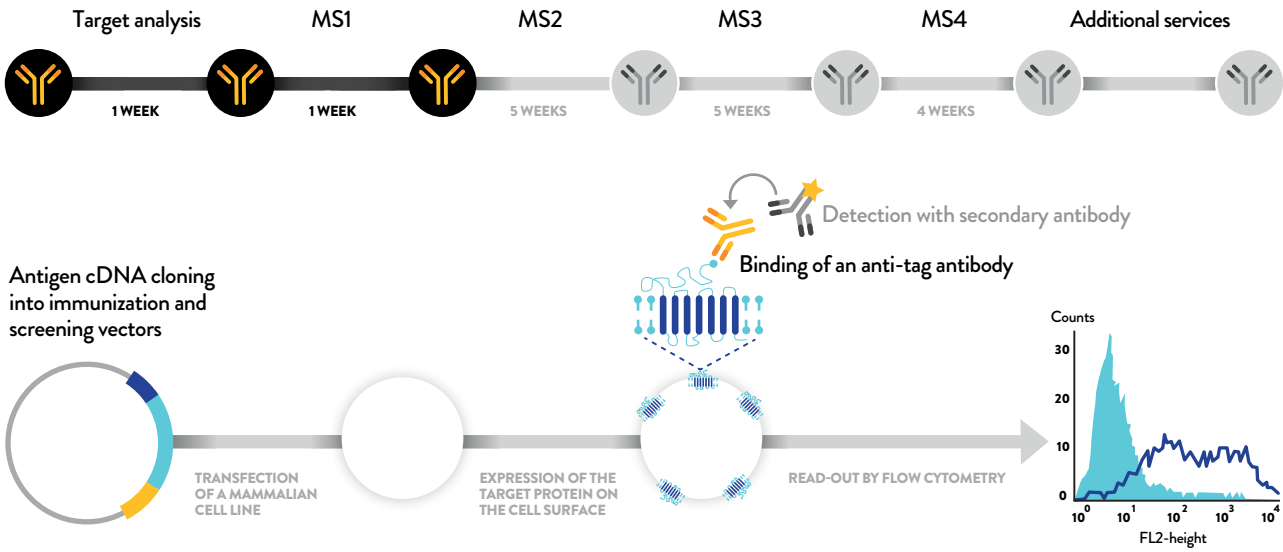
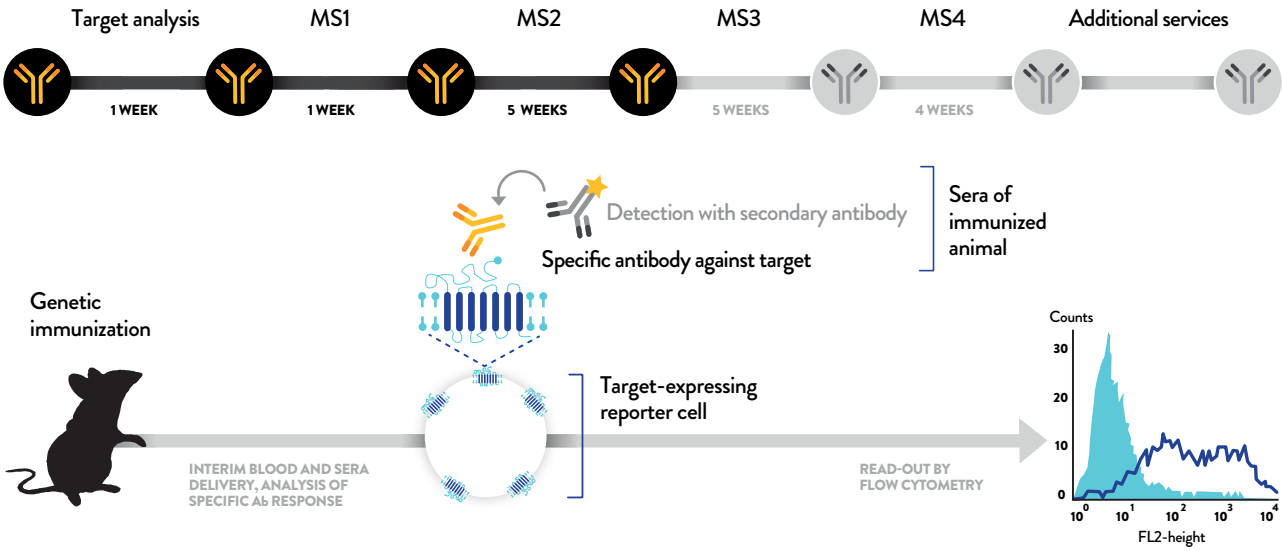
Milestone 1: Molecular cloning and expression test



Milestone 2: Genetic immunization and sera test



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THE RESULT

The methods that were used in this project and described in this case study demonstrate the possibility to generate antibodies against a highly conserved, intracellular protein by genetic immunization. Due to immune tolerance, some proteins that are highly conserved between mice and humans are not very immunogenic in mice, making it difficult to generate antibodies using a conventional approach. Our genetic immunization proved to be an effective approach for the generation of antibodies against such a “difficult antigen”.

The client’s request was to generate polyclonals against the human RAS, thus the project terminated at Milestone 2 (generation of polyclonal serum) instead of going all the way through to Milestone 4 (generation of monoclonal antibodies).

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