

# CAPABILITIES

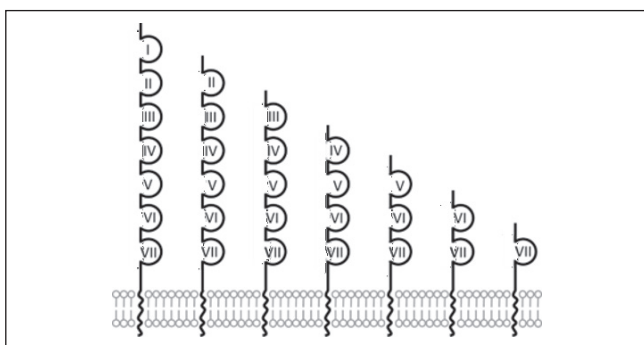
## EXCELLENT EPITOPE COVERAGE: ANTIBODIES RECOGNIZE SPECIFIC PROTEIN DOMAINS OF HUMAN VEGFR2

### THE CHALLENGE

Genovac was approached by a client who had a unique technological challenge with respect to the lifespan of synthetic arterial stents. The shortened lifespan of the stent added patient costs by increasing the number of replacement surgeries and stent monitoring. Synthetic arterial stents are required in patients where their own arteries are narrowed or blocked. One major problem of using synthetic stents is that they become blocked through restenosis, the formation of neointimal tissue inside the stent, after a relatively short time. In order to tackle this challenge, Genovac developed a novel way to develop a panel of antibodies that enabled the tethering of endothelial cells to the stents by utilizing VEGFR2. A secondary goal was to develop antibodies that would be specific to different domains.

### THE SOLUTION

The idea is to disguise the foreign material by coating it with the patients' endothelial cells to prolong the life span. However, endothelial stem cells circulate in low concentrations. They carry a VEGFR2 receptor protein on their cell surface, so antibodies have been developed to capture these stem cells and stimulate them to build the endothelial cell layer to line the inner wall of the stent. There are several issues that arise with using this strategy. The antibodies must have high affinity to overcome shear forces present in the coronary arteries to be treated. Furthermore, a battery of antibodies that will bind different epitopes should strengthen this binding.



**Fig. 1:** Describes the subtractive screening strategy utilizing truncated VEGFR2 screening constructs of various lengths. The idea was that all the antibodies tested would bind to the full length, but for each domain removed, fewer antibodies would bind. For example, all 40 antibodies bound the full-length construct. Twelve binders remained through domains IV-VII and only two binders remained when using the most truncated domain VII.

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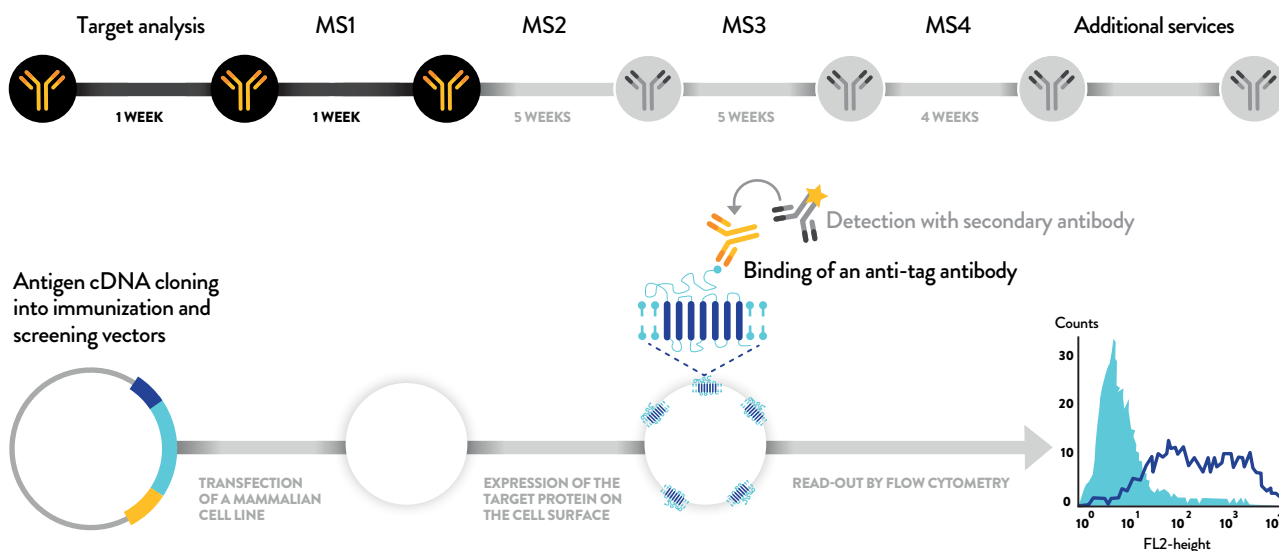
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## PROJECT SCOPE

Genovac developed a strategy based off a comprehensive target analysis utilizing its proprietary genetic immunization technology and advanced hybridoma platform. Immunization and screening constructs were generated in less than 10 days and rats were immunized the following week. A serum test was performed on day 35 of the immunization and cell fusion occurred a week later. 28 days post fusion, stable hybridoma clones were established that produced antibodies specific for VEGFR2. After subcloning, we were able to generate 40 stable hybridoma clones that secreted antibodies recognizing different epitopes of the VEGFR2.

### Milestone 1: Molecular cloning and expression test



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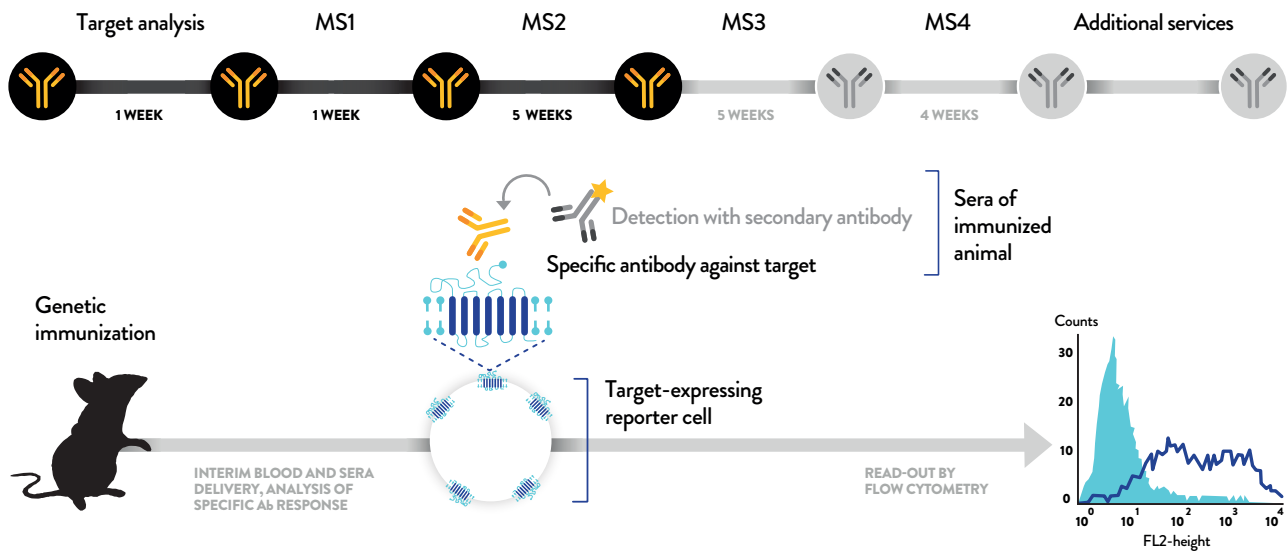
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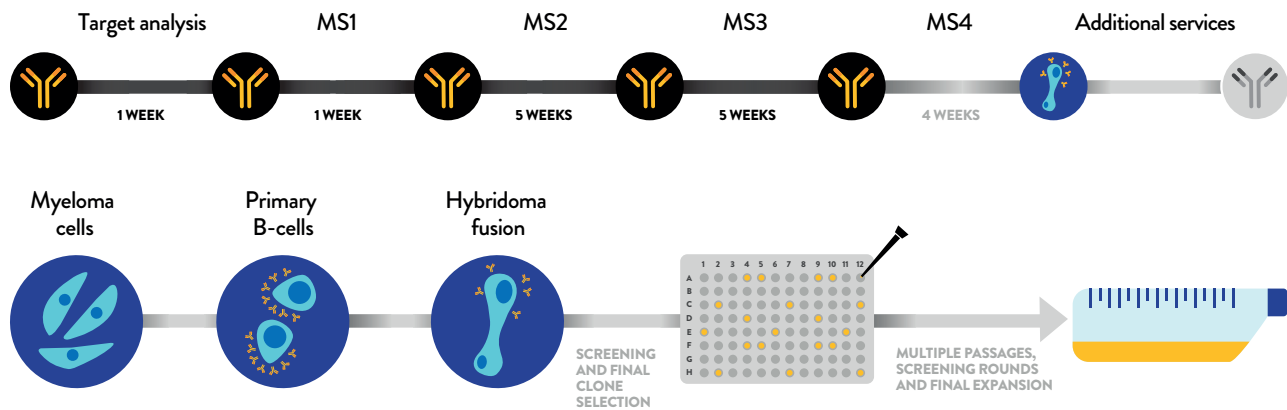
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## Milestone 2: Genetic immunization and sera test



## Milestone 3: Well-based fusion, screening and selection



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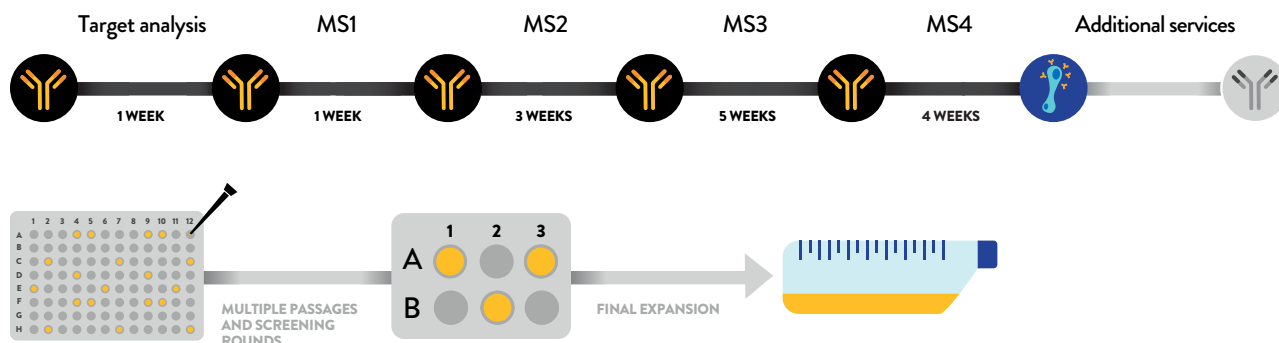
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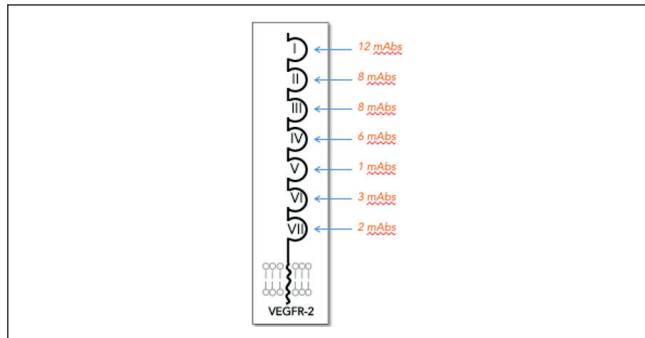
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## Milestone 4: Subcloning, screening, and selection



## THE RESULT

By utilizing the subtractive screening strategy described above, Genovac was able to generate an antibody panel with antibodies binding to all of the seven different domains on the VEGFR2 protein. Several of these antibodies cross-react with the pig orthologue, which our partner has successfully been used for in vivo testing in a pig model.



**Fig. 2:** Exhibits the generation of antibodies specific to each of 7 individual extracellular domains of VEGFR2. This result supports the success of the subtractive screening strategy described in Figure 1.

This subtractive construct method can be applied to various applications. The strategy provides an alternative technique to approaching unique project requirements. A recent example of this is using this strategy to aid in the fight against the Sars-Cov-2 pandemic. Here, we focused on the Sars-Cov-2 Spike protein, where the full-length Spike, the S1 domain, and the receptor-binding domain (RBD) were utilized for genetic immunization. We successfully generated different sets of antibodies against these antigens.

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