

CASE STUDY

DEVELOPING A HIGH-AFFINITY ANTIBODY PAIR

that Powers Progranulin Detection



INSIDE YOU'LL FIND:

Aviva System Biology's proprietary workflow enabled the rapid identification of a high-affinity antibody pair for use in immunoassay detection of progranulin in clinical samples

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Developing a High-Affinity Antibody Pair

that Powers Progranulin Detection

Key Takeaways:

- Discovery and development of highly specific antibody pairs for immunoassay development can be challenging
- A solution that utilizes intelligent antigen design, recombinant technologies, and rapid high-throughput characterization can accelerate antibody discovery
- Aviva System Biology's proprietary workflow enabled the rapid identification of a high-affinity antibody pair for use in immunoassay detection of progranulin in clinical samples

THE CHALLENGE

Reliable monoclonal antibodies are an essential tool in research, drug development, and clinical diagnostics. The development of highly sensitive diagnostic assays hinges on having trustworthy antibodies with required sensitivity (in some cases sub-pg/mL detection), dynamic range, and target specificity across complex samples like cerebrospinal fluid (CSF), serum, and plasma. However, discovering and characterizing high-performing monoclonal antibody pairs for a chosen protein target can be difficult, especially in a high-throughput manner with quick turn-around-time.

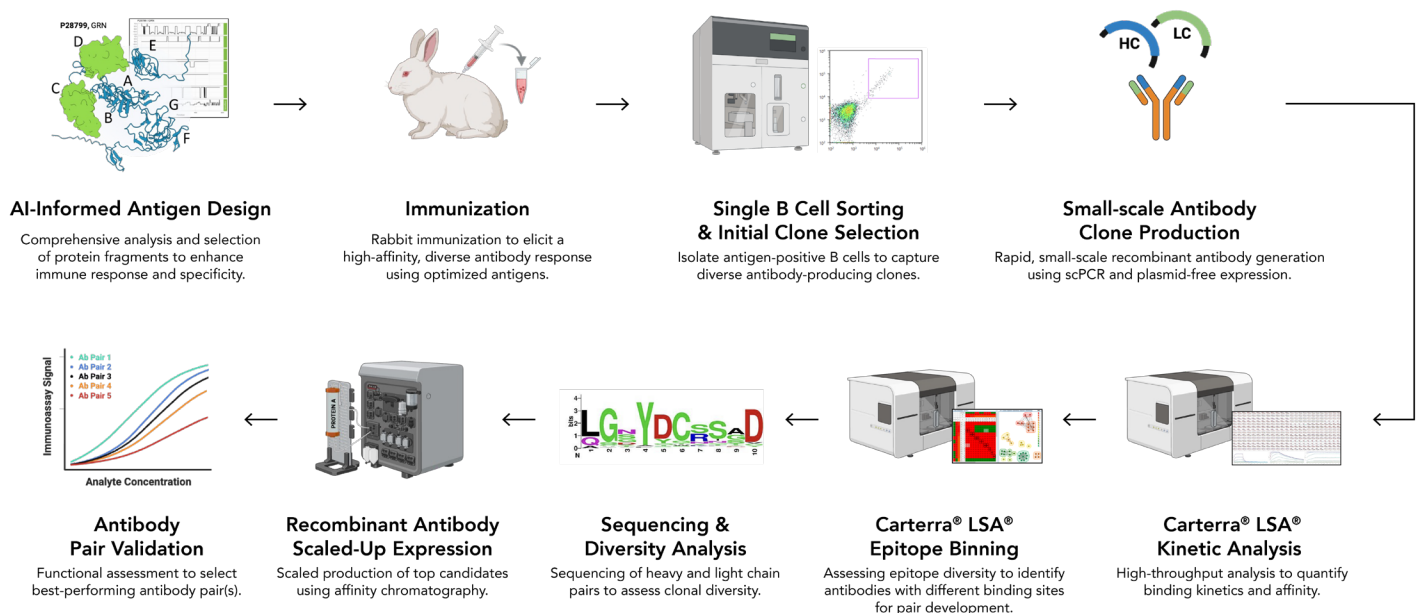
Given the critical role of antibodies in analyte detection, an urgent need exists for rapid discovery and reliable production of monoclonal antibody pairs that deliver high affinity and specificity. The use of data-informed design approaches, recombinant technologies, and high throughput characterization methods can accelerate discovery, development, and production of the optimized

monoclonal antibody pairs essential for advancing research and diagnostic assay development.

THE SOLUTION

[Aviva Systems Biology](#) has built a high-throughput recombinant antibody discovery and development workflow designed to consistently generate high-quality antibodies, even against difficult targets (**Figure 1**). The workflow begins with an antigen design approach that layers a quantitative framework on top of AI-derived protein structure predictions for holistic antigen evaluation. The AlphaFold-derived models contribute an initial view of the predicted native-like protein structures, which are then further analyzed using a proprietary antigenicity scoring model. This model factors in additional biophysical details like amino acid enrichment, predicted disorder, and solvent accessibility. This multi-parameter lens enables deeper analysis of candidate protein fragments, ensuring structural

Figure 1: Overview of the Aviva recombinant antibody discovery workflow.



fidelity while systematically prioritizing those with the highest likelihood of functioning as effective antigens in antibody discovery.

To generate high yields of diverse antibodies to those selected antigens, Aviva utilizes a rabbit immunization model. This model is well-suited for diagnostic applications because the robust immune response and natural *in vivo* affinity maturation process ensures a diverse candidate repertoire and provides a more efficient and cost-effective method for antibody discovery than *in vitro* phage display methods. Multiple rabbits are immunized with a selected antigen, eliciting antibody generation and affinity maturation. Antigen-positive B cells are isolated via multi-color fluorescence-activated cell sorting (FACS), and those with robust antigen specificity are selected for highly-sensitive single-cell PCR. Variable region sequence pairs are transiently expressed in HEK293 cells as full-length IgGs, eliminating the need to generate and transfect hundreds of plasmids containing antibody candidates. This plasmid-free expression method allows us to conduct deeper candidate profiling more quickly, and earlier in the development process.

With this streamlined approach, we move quickly into antibody characterization using the Carterra® LSA®

platform, which employs Surface Plasmon Resonance (SPR) technology for rapid, high-throughput kinetic screening to identify antibodies with high affinity to the target, followed by epitope binning analysis to identify potential antibody pair combinations. Selected antibody candidates are sequenced and undergo production scale-up through recombinant cloning, large-scale expression and purification, followed by comprehensive, sandwich-ELISA functional testing of antibody pairs.

Here we describe how Aviva's recombinant antibody discovery and development platform successfully yielded high-affinity antibodies for pairing in an immunoassay targeting the progranulin protein, a potential biomarker and therapeutic target for cancer and neurodegenerative disease.

CASE STUDY: THE USE OF RAPID, HIGH-THROUGHPUT CHARACTERIZATION TO IDENTIFY PROGRANULIN IMMUNOASSAY ANTIBODY CANDIDATES

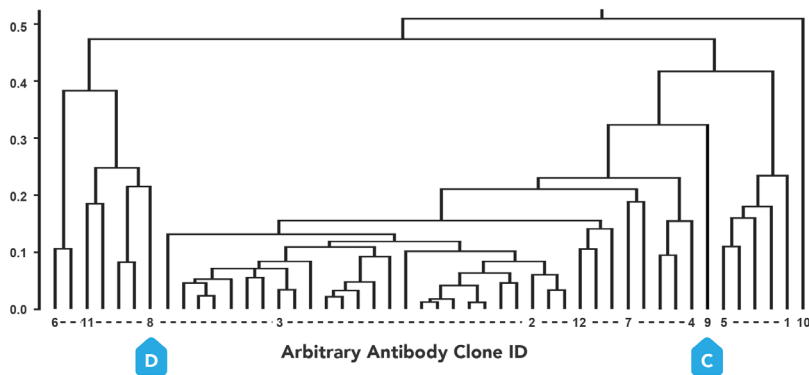
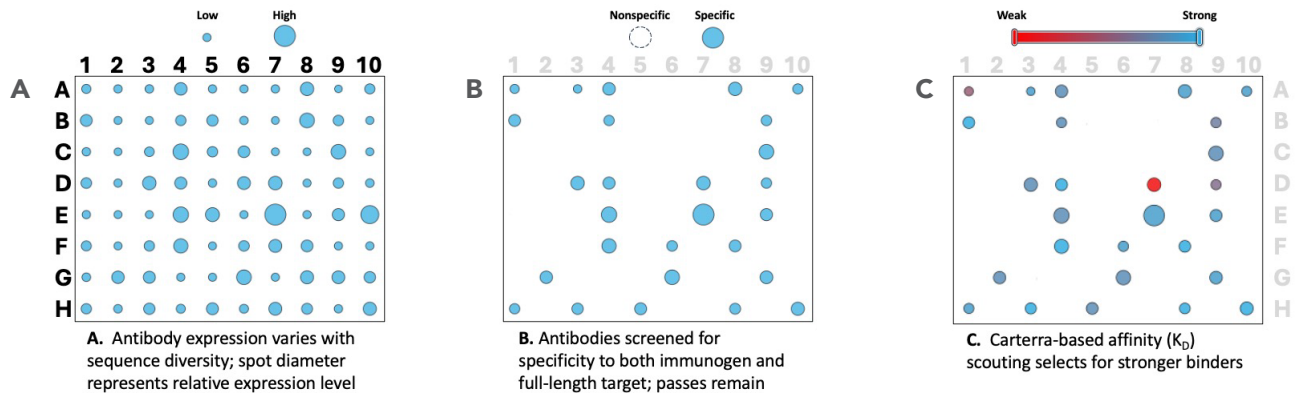
Progranulin is a glycoprotein with roles in tissue repair, inflammation, lysosomal function, and neuroprotection. Changes in progranulin levels are linked to neuropathology. Heterozygous loss of function mutations

in GRN, the gene encoding progranulin, have been shown to cause a highly penetrant form of Frontotemporal Dementia (FTD-GRN). Progranulin deficiency has been implicated in other diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), and limbic-predominant age-related transactivation response DNA-binding protein 43 encephalopathy (LATE). Aviva leveraged its recombinant antibody discovery and development platform to identify optimal monoclonal antibody pairs targeting progranulin, generating antibodies suitable for immunoassay development for discovery research, drug development, and diagnostic applications.

Rapid Identification of Viable Antibodies

Hundreds of recombinant antibodies were screened for target specificity and binding affinity using the high-throughput Carterra® LSA®. Carterra-derived data was used to estimate the relative antibody expression levels of individual clones (Figure 2A). In this study, antibodies were screened for specificity to both the antigen and full-length target protein (Figure 2B), and the affinity of each antibody is reported as its dissociation constant, or K_D , as measured by SPR kinetic characterization (Figure 2C). Epitope binning was performed and a dendrogram (Figure 2, bottom) was assembled from this data to visualize epitope clades and pair candidates. Antibody clones from several different clades, suggestive of different binding sites, were selected for expression scale-up and subsequent testing by sandwich ELISA.

Figure 2: Carterra LSA screening of antibody candidates. (A) Estimated relative antibody expression levels of individual clones (B) antibodies were screened for specificity to both the antigen and full-length target (C) Affinity assessment of antibody candidates, reported as a dissociation constant (K_D) (Bottom) Pairwise testing of competitive antibody binding to antigen is used to build community plots of mutually exclusive signals and thus epitope bins; a dendrogram is assembled from this data to enable selection of best performing antibodies to move forward for functional characterization.



The streamlined characterization of antibody candidates using the high-throughput Carterra LSA system enables rapid down-selection and identification of top antibody candidates early in the discovery process, saving time and directing resources toward further development of antibody candidates with a higher potential for success.

Functional Validation of Progranulin Antibody Pairs

With antibody pair candidates identified, we performed functional testing under a variety of analytical conditions (Figure 3). We analyzed seven combinations of detection and capture antibodies via sandwich ELISA across multiple progranulin concentrations, identifying a pair of antibodies that produced the highest signals for progranulin detection at all concentrations (Figure 3A, in green).

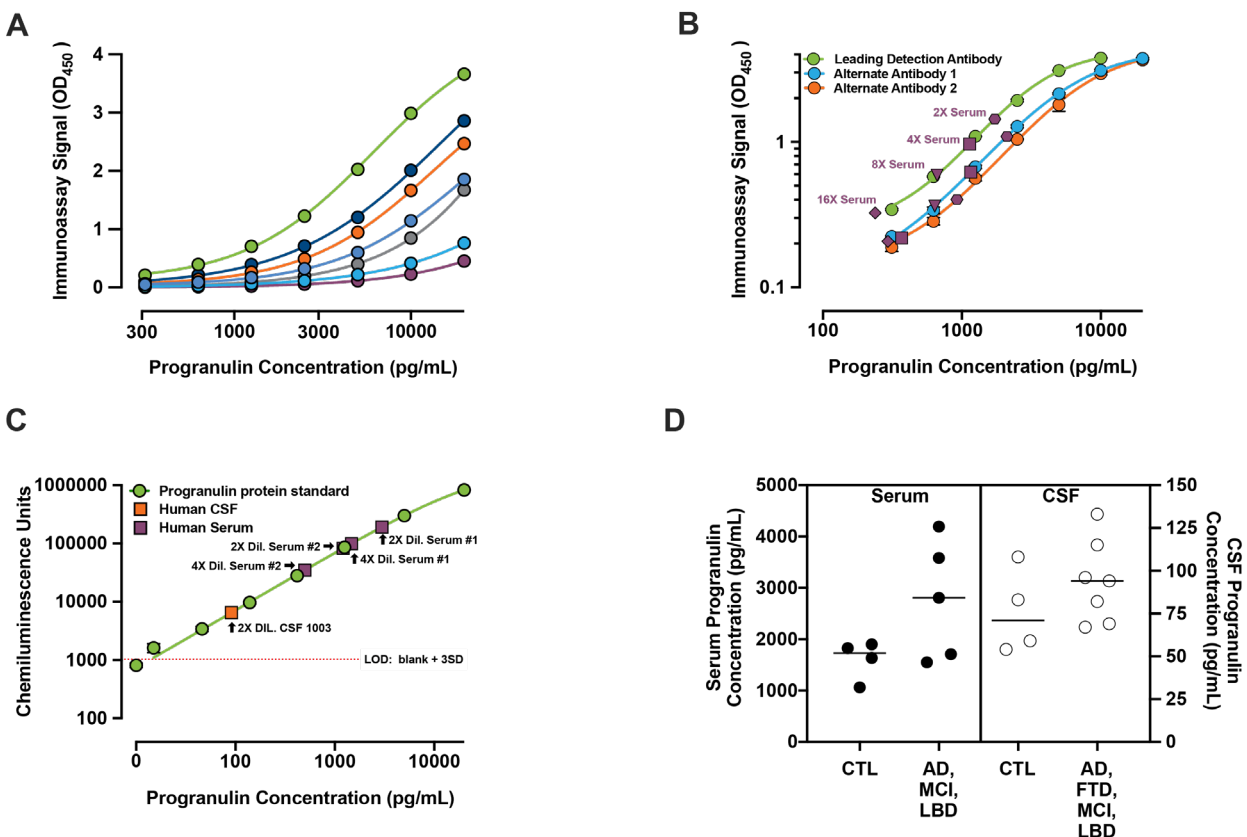
Additional functional testing of the leading progranulin capture antibody with alternate detection antibodies was conducted in human serum to evaluate the functionality of multiple pairings in clinical samples. Results of this experiment reaffirmed the selection of the leading

antibody pair prior to further development (Figure 3B, in green).

The highest performing antibody pair was then developed into an optimized chemiluminescent ELISA, using a wide range of progranulin protein standard concentrations, within which progranulin was successfully detected in both human CSF and serum samples. The analytical limit of detection (LOD) of the assay was determined at 14 pg/mL, demonstrating the high sensitivity of the chosen antibody pair (Figure 3C).

Finally, progranulin levels were measured in both CSF and serum samples (samples were collected from patients with a neurodegenerative condition and from normal age-matched control donors) using the optimized chemiluminescent ELISA. Results show the successful detection of progranulin in both CSF and serum clinical samples with notable differences in the average signal between patients and controls in serum samples (Figure 3D).

Figure 3: Results of the functional validation of progranulin antibody pairs.



CONCLUSION

Aviva's recombinant antibody discovery and development platform allows for rapid, high-throughput SPR-based assessment of hundreds of antibody candidates to quickly narrow the field to those with the greatest potential for downstream success. This proven approach enables faster discovery of high-performance antibodies for challenging targets.

Your target, our technology

Partner with Aviva to [develop high-affinity recombinant antibodies](#) for your research and diagnostics projects. [Contact us](#) to discuss your recombinant antibody needs—we offer flexible, modular solutions for all stages of discovery and development.

References

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