A Recombinant Antibody Platform for Discovery and Development of High Affinity Antibody Pairs for Diagnostic Applications

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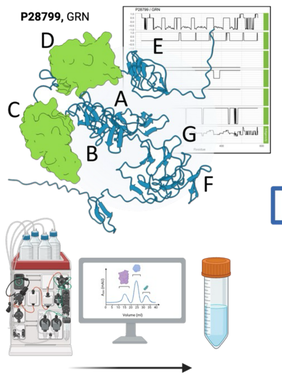
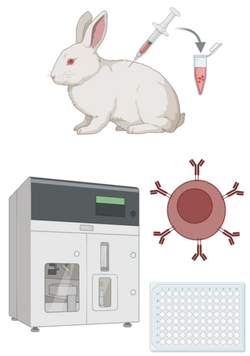
Poster #968

Aviva's Immunoassay Development Workflow Begins with High Throughput Antibody Discovery For Optimized Outcomes

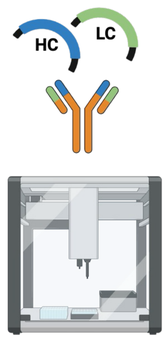
Proprietary Antigen Design & High Throughput Protein Expression (immunogen)



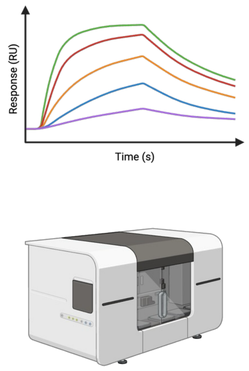
Immunization, Antigen-Positive B Cell Sorting, & Initial Clone Selection



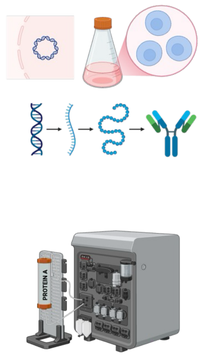
Immunoglobulin DNA Amplification & Plasmid-Free Antibody Expression



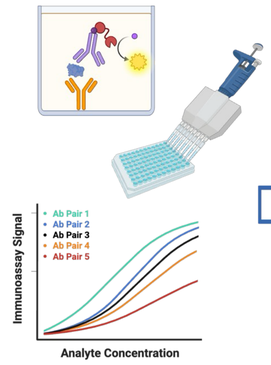
Carterra® LSA™ High-Throughput Screening & Epitope Binning



Transient Mammalian Cell Expression of Recombinant Ab & Affinity-based Purification



Functional ELISA-based Antibody Pair Screening & Assay Optimization



Analytical Validation

Antibody Robustness
Sensitivity
Specificity
Accuracy
Precision
Dynamic Range
LOD & LLOQ
Parallelism
Spike-and-Recovery
Interfering Substances
Reagent Stability

Operational Transfer
(SOPs, Specs, CoAs)

Introduction

The demand for antibodies to neurodegenerative disease-associated biomarkers is clear and present. Quantitative proteomic analysis of disease-associated biomarkers has driven the demand for more powerful immunoassay platforms. To this day these best-in-class detection platforms still depend upon high affinity monoclonal antibodies. Immunoassay developers must rapidly generate and characterize robust affinity reagents across a variety of matrices (CSF, serum, plasma, etc.) with the goal of sub-pg/mL sensitivity.

We have developed a streamlined approach to developing immunoassay antibody pairs for neurological targets that begins with efficient, high-throughput antibody discovery followed by rapid affinity assessment, and functional testing. **A case study of this approach is shown here to pursue high affinity immunoassays to Progranulin (UniProt P28799).**

Progranulin exists as a glycosylated 593 amino acid precursor protein which is cleaved into a variety of granulin peptides. The multifunctional nature of progranulin has growing significance in neuroscience and immunology. As a secreted glycoprotein, it plays central roles in inflammation, tissue repair, lysosomal function, and neuroprotection, making it a valuable target for researchers studying complex diseases such as frontotemporal dementia, Alzheimer's, and Parkinson's.

The work presented here establishes the foundation for innovative immunoassay development work that is set to span a wide range of biomarkers and detection modalities.

Summary

- Hundreds of recombinant antibody clones to progranulin were characterized on Carterra LSA for expression, affinity, specificity, and epitope diversity.
- Top antibody candidates from distinct epitope bins were assessed for immunoassay sensitivity, dynamic range, and functionality in human samples.
- Progranulin levels were measured in neurodegenerative disease patient samples utilizing an ELISA constructed with top performing antibodies.

Conclusion

- Aviva Systems Biology has established a robust recombinant antibody development workflow to identify high-affinity binders that display diverse epitope repertoires.
- This high-throughput workflow allows for rapid orthogonal functional binding assessments with multiple backup antibodies mitigating the risk of failure in downstream validation activities.
- This pipeline accelerates development of diagnostic antibody reagents for clinically relevant targets that may be used in a variety of assay formats and platforms.

For More Information

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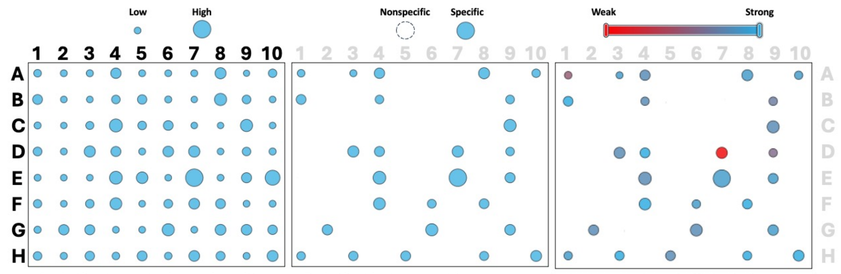
2 For a copy of this poster please scan with your smartphone

Results

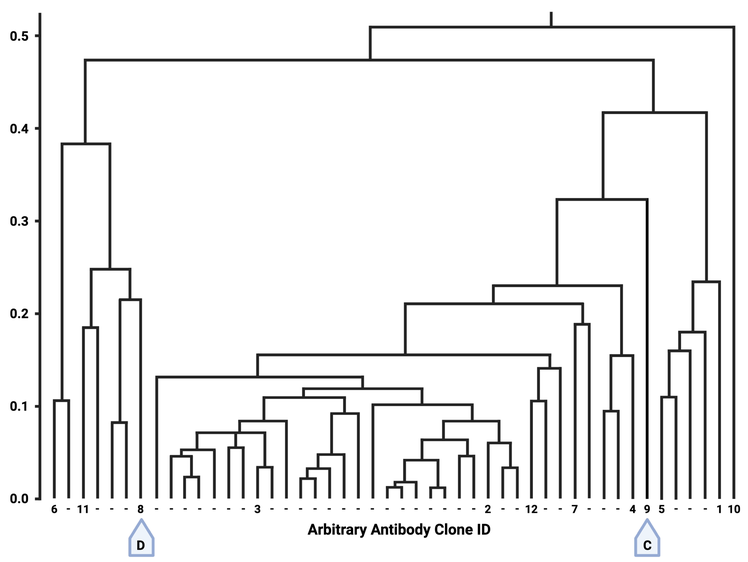


A. Antibody expression varies with sequence diversity; spot diameter represents relative expression level

B. Antibodies screened for specificity to both immunogen and full-length target; passes remain

C. Carterra-based affinity (K_D) scouting selects for stronger binders

Figure 1. Representative data from expression, specificity, and sensitivity testing on the Carterra high-throughput SPR platform. Hundreds of antibodies were down-selected based on key assay performance properties to focus the development efforts on smaller groups of high-impact clones. Final clones are then subjected to more advanced binding characterization.



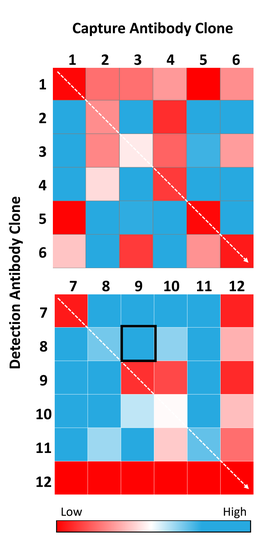


Figure 2 (above). Carterra LSA-based epitope bin analysis of highest-performing GRN antibody clones. Antibody clones selected by target specificity and K_D were analyzed by epitope binning on Carterra LSA. A community dendrogram was assembled that represents antibodies clustered by binding modality. Antibody clones from several different clades, suggestive of different binding sites, were selected for expression scale-up and subsequent testing by antibody sandwich ELISA. The chosen antibody pair for eventual characterization is marked as "D" (progranulin detection antibody) and "C" (progranulin capture antibody).

Figure 3 (left). Heat maps representing GRN protein binding efficacy (blue, high binding signal; red, low binding signal) across 12 recombinant antibodies tested via ELISA based screening. Scenarios in which a clone was used in the capture or detection step are indicated. Upper panel, antibody clones 1-6. Lower panel, antibody clones 7-12.

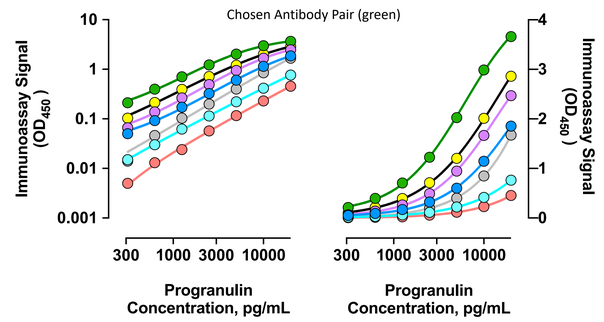


Figure 4. Rank-ordered assessment of progranulin antibody pairs via chromogenic ELISA. 14 antibody pair combinations (capture or detect; only 7 shown here; utilizing the predicted highest performing antibodies) were run under sandwich ELISA conditions using full-length progranulin analyte. Both linear and log scales of signal shown for clarity.

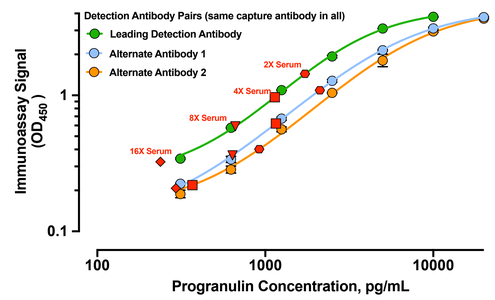


Figure 5. Functional testing of progranulin antibody pairs in human serum. Following optimization of the leading ELISA pair, it was tested along side alternative detection antibodies (using same optimized capture antibody). These results reaffirmed the leading choice moving forward.

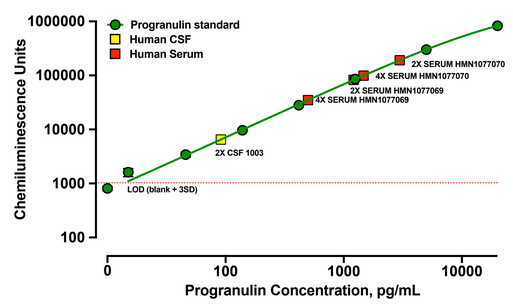


Figure 6. Optimized Aviva progranulin ELISA detects progranulin in human CSF and serum. Locked ELISA conditions include conversion from chromogenic to chemiluminescent readout, and a limit of detection (LOD) of 14 pg/mL.

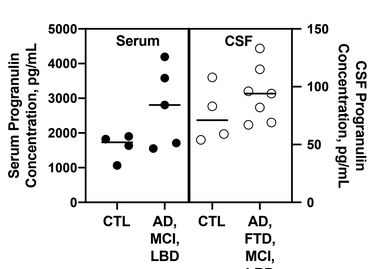


Figure 7. Progranulin detected in human subjects suffering from neurodegenerative diseases. Sera or CSF was obtained from normal age-matched control donors (CTL) or individuals diagnosed with Alzheimer's disease (AD), Frontotemporal Dementia (FTD), Mild Cognitive Impairment (MCI), or Lewy Body Dementia (LBD). Each data point is the average of replicate measurements per subject, with group median values indicated by the horizonal line.