GRIFFIN: A Mass Spectrometry-Based Method for Polyclonal Antibody Sequencing Directly from Protein

Stefano Bonissone Thiago Lima Miin Lin
Richard Carson Brinley Moorman Natalie Castellana

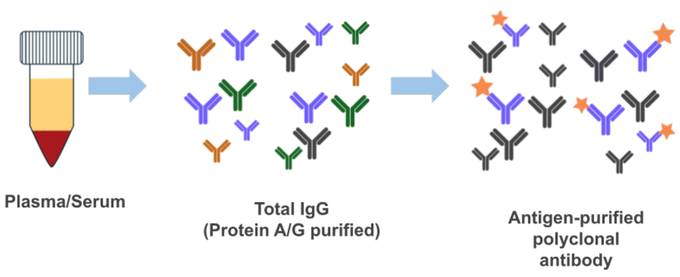
Abterra Biosciences, San Diego, CA

Abstract

The serum antibody repertoire represents a snapshot of the continually evolving humoral immune system, conferring protection from pathogens. Understanding and deconvoluting this mixture provides unique paths for novel therapeutic development when performed from human serum, and improves diagnostics and reagents when performed on animal serum.

Until recently, querying the polyclonal serum response without contemporaneous B-cell DNA/RNA information was not feasible. Advances in mass-spectrometry for long peptide generation, instrumentation, sequencing, and informatics have enabled the creation of GRIFFIN for deconvoluting serum polyclonal responses using only proteins. Here we present two case studies displaying the application of GRIFFIN for the analysis and recapitulation of both rabbit and human polyclonal antibodies.

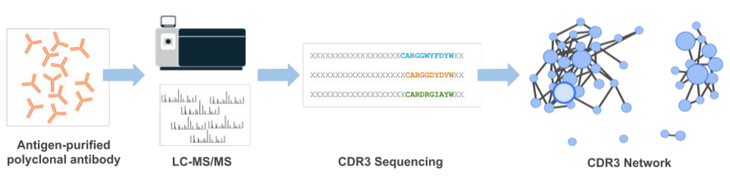
Griffin Stage 0: Antigen Purification



Antigen purification is required for sequencing with Griffin.

In most cases, total IgG (or other isotypes) purified from plasma or serum is too complex for sequencing with Griffin. A critical stage of the workflow is purification against the antigen to enrich for antibodies relevant to the intended purpose of the polyclonal antibody (pAb). In some cases, fractionation on different epitopes can be an excellent way to pursue diverse binding properties.

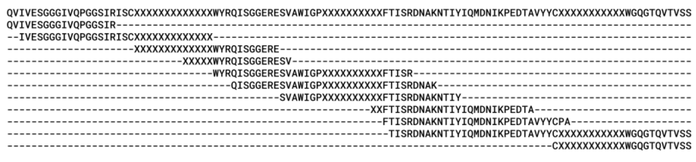
Griffin Stage 1: Clonality Assessment



Overview of the clonality assessment stage of Griffin.

A shallow first-past assessment of sequence diversity, particularly in the CDR3, reveals the level of complexity of the sample. The polyclonal antibody is digested with multiple enzymes and analyzed via mass spectrometry. The mass spectra are de novo sequenced, and CDR3 sequences are extracted. Too many CDR3s indicates that only the most abundant will be sequenceable. Simplification of the sample can be a good option to fractionate the polyclonal antibody into desired reactivity groups.

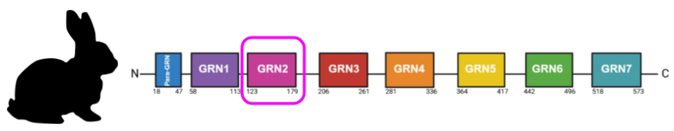
Griffin Stage 2: Sequencing & Assembly



An example assembly of a VHH from individual peptides

A deep second-pass analysis generates complete candidate sequences. Additional mass spectrometry data is generated, targeting longer peptides that are critical for full sequence assembly. Each sequence is assembled from hundreds of individual, overlapping peptides. The sequences candidates are reviewed in silico to eliminate unnatural sequences and structures, and tested recombinantly for binding to the target.

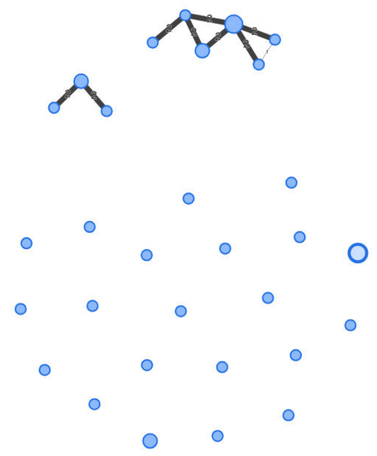
Stage 0: Rabbit pAb Sequencing



The progranulin protein is comprised of several granulin domains. In this project, we focused on sequencing a polyclonal antibody targeting the granulin 2 domain (GRN2).

Dr. Thomas Kukar from Emory University created a rabbit polyclonal reagent to GRN2, but supply was running low. The Kukar lab wanted to create an endless supply of the reagent to continue their research in neuro degeneration.

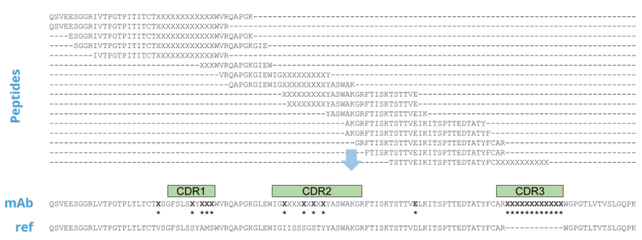
Stage 1: Rabbit pAb Sequencing



A CDR3 network generated for the heavy chain sequences. Each node is an amino acid CDR3 sequence, and edges are created between nodes if the CDR3s are within a similarity threshold.

CDR3s were identified and organized into a CDR3 network. The network revealed 7 heavy chain families and 3 light chain families

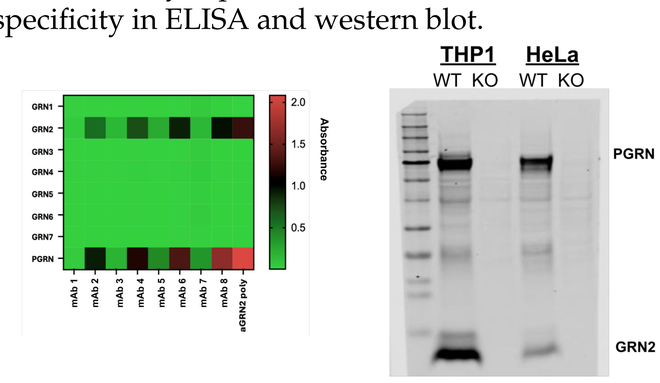
Stage 2: Rabbit pAb Sequencing



One heavy chain sequence assembly showing the peptides, the final mAb sequence with CDRs annotated, and the alignment with the closest germline reference V and J genes.

Validation

Ten antibodies from the seven main CDR3 families were recombinantly expression in CHO cells and tested for specificity in ELISA and western blot.



Above Left: ELISA validation against the GRN2 domain alone and the progranulin protein (containing GRN2) showed that several antibodies recapitulated the specificity of the starting polyclonal antibody. *Above Right:* One of the antibodies (mAb 8) showed specificity to both GRN2 and PGRN in western blot of lysate from two cell lines.

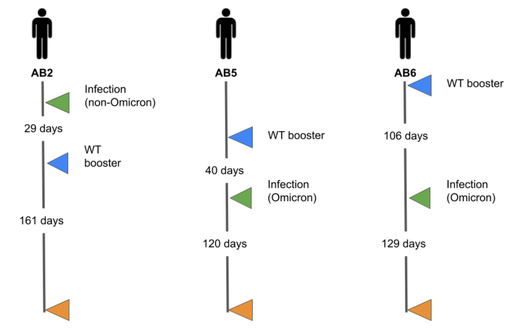
About Abterra Biosciences

Check out our antibody related services:

- Alicanto®: antibody discovery directly from serum.
- Valens: monoclonal sequencing from protein service.
- Reptor: immune repertoire sequencing and analysis.
- Griffin: polyclonal antibody sequencing service.

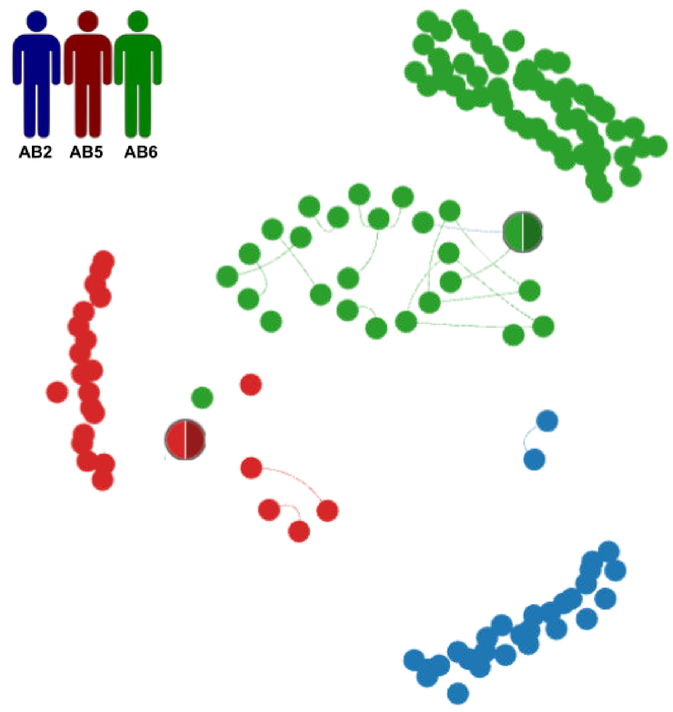
Stage 0: Human pAb Sequencing

Blood was collected 3+ months from the most recent infection/vaccination for three human donors. Donor AB2 was only exposed to wild-type vaccines and a non-omicron natural infection. Donors AB5 and AB6 received wild-type vaccines and suspected omicron natural infections.



Timeline of exposure to SARS-CoV-2 antigens of three donors.

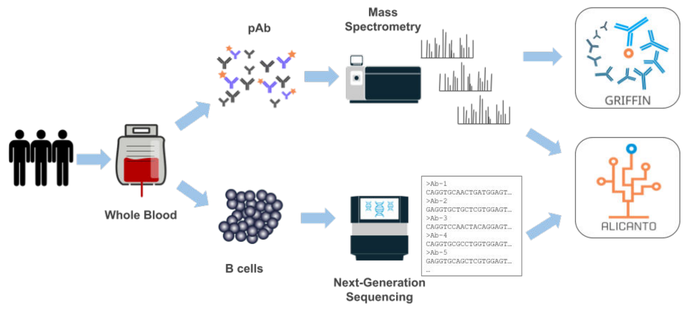
Stage 1: Human pAb Sequencing



Color coded CDR3 network across the individuals. Two CDR3s (noted by split dark/light circles), one from AB5 and one from AB5 were also found in parallel B cell sequencing efforts.

The CDR3 networks showed no clonal convergence across donors, and limited clustering of identified clones except for AB5. AB6 had the largest number of CDR3s identified by Griffin, as well as the largest connected component of similar CDR3s.

B Cells versus Serum Antibodies



Griffin and Alicanto are two technologies for identifying antibodies in serum. Griffin prioritizes antibodies that are abundant in serum, while Alicanto looks for corroborating evidence between serum antibodies and B-cell receptor sequences.

Concurrently with Griffin analysis, Alicanto was used to analyze both the B-cell receptor repertoire of the donors as well as the serum antibodies that match B-cell receptor sequences. We compared the CDR3 sequences found by Alicanto to those found by Griffin.

Donor	Griffin	Alicanto	Shared
AB2	29	17	0
AB5	25	188	1
AB6	80	49	1