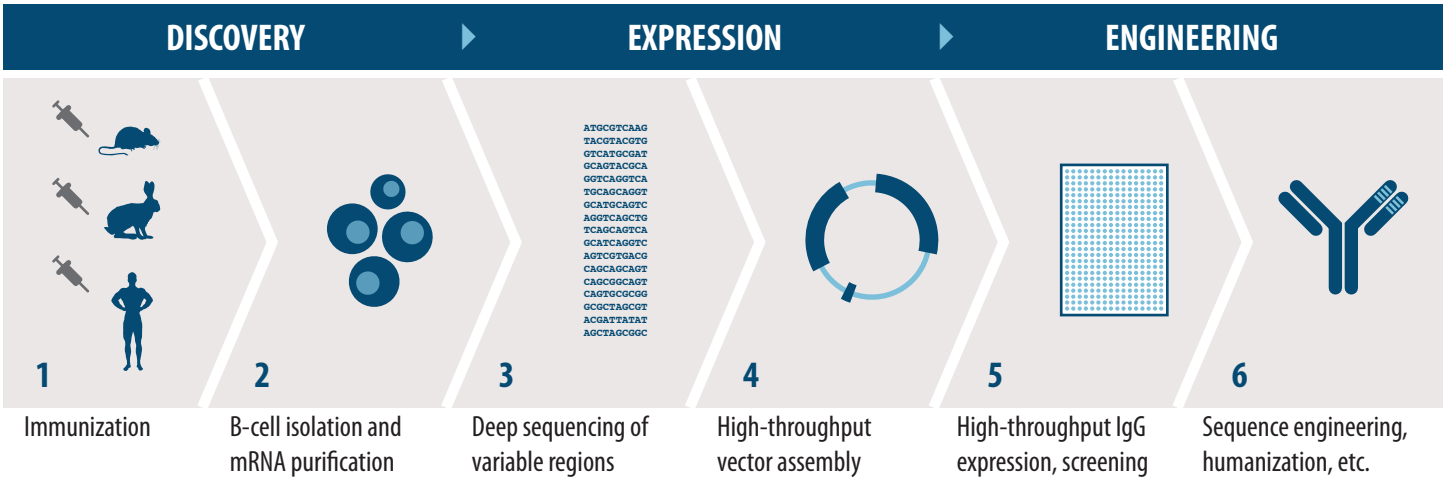
Recombinant Monoclonal Antibody Discovery:

Combining antibody repertoire profiling with high-thoughput IgG expression and screening

INTRODUCTION

New technologies and high-throughput approaches have created exciting opportunies at the intersection of genomics and immunology. We have developed a novel method using next-gen sequencing to profile the complete antibody repertoire of an organism. We have also created new methods using high-throughput gene synthesis and vector assembly to convert immune repertoire sequence information into large collections of full IgG antibodies that can be screened for antigen binding and other functional characteristics. Our novel pipeline enables fast and efficient discovery, expression, and screening of recombinant monoclonal antibodies from rabbit, mouse, or human.



Immunization	B-cell isolation and mRNA purification	Deep sequencing of variable regions	High-throughput vector assembly	High-throughput IgG expression, screening	Sequence engineering, humanization, etc.

METHODS

We have developed a unique workflow for immune repertoire sequencing for the analysis of human, mouse, rat and rabbit B-cells. Using rabbit as a model in this study, we profiled changes in expression levels of IgH and Igk sequences before and after immunization with a human recombinant protein. After B-cell isolation, we generated millions of sequences of the full variable region for IgG heavy and light chain mRNA transcripts. From the raw sequence data we performed clustering and lineage analysis to measure changes in frequency and somatic hypermutation of variable region sequences.

In parallel, we developed a novel pipeline for high-throughput variable region gene synthesis and vector assembly. Our immune repertoire sequence analysis software identifed the top 100 IgH and Igk sequences likely to be involved in the immune response and also provided a statistical likelihood of the pairing of IgH and Igk sequences. We then synthesized and cloned these variable region sequences into custom IgH and Igk expression vectors. Based on our pairing predictions for heavy and light chains, we expressed full IgG antibody proteins in a high-throughput plate-based format and screened for antigen binding with a custom bead-based ELISA assay. For the top binding antibodies identified, we validated binding specificity by western blot and successfully created humanized versions of the antibodies. **(see reverse for results)**

FOR MORE INFORMATION ON ANTIBODY DISCOVERY SERVICES, CONTACT:

Nathan Trinklein: email trinklein@panoplybio.com phone 650-323-6763 x139

PANOPLY bio

Recombinant Monoclonal Antibody Discovery

ANTIBODY REPERTOIRE SEQUENCE RESULTS

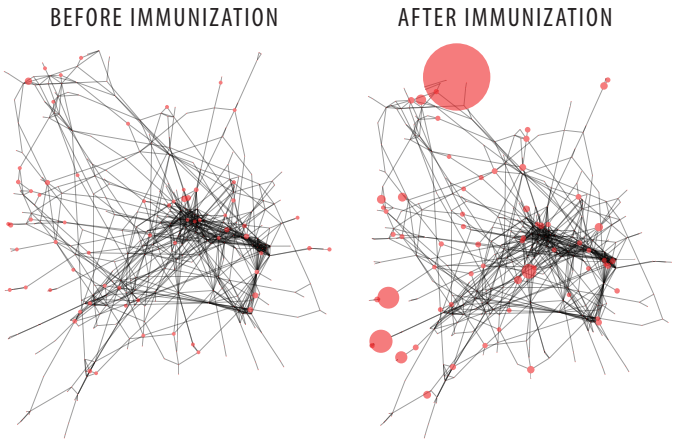


Figure 1: CDRH3 sequence similarity network

For each unique CDRH3 sequence before and after immunization, we used a force-directed layout to arrange the sequences as nodes for which the most similar sequences are located next to one other. The frequency of each sequence is calculated before and after immunization, and the area of the node is proportional to the frequency of that sequence at that time point. As seen in these network maps, some sequences expand significantly after immunization. The same analysis was done for CDRL3.

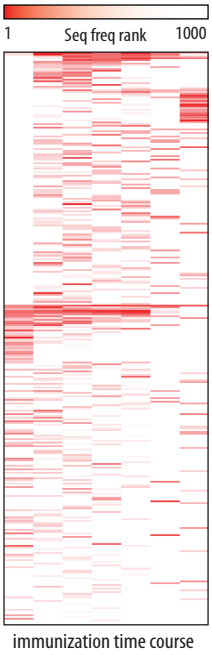


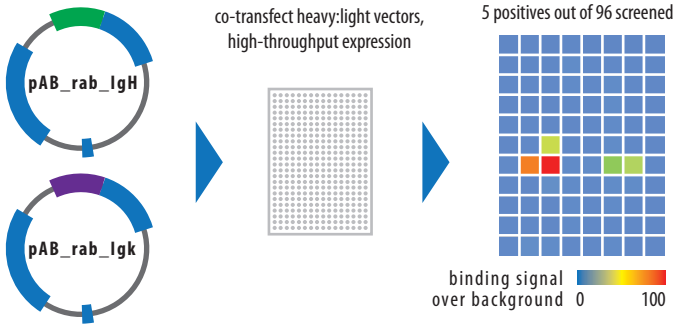
Figure 2: CDRH3 frequency changes during immune response

This heatmap shows a sample of CDRH3 sequences ranked by frequency in which each row is a unique CDR3 sequence. We compared the rank of each unique sequence across the immunization time course, and interesting classes of sequences are revealed by this rank analysis. Some sequences are ranked very low before immunization and rise to a high rank before decreasing at the end of the time course. A large group of sequences start high and decrease throughout the time course. Rank pattern analysis is one of the methods we use for priortizing sequences for subsequent functional testing.

RECOMBINANT IgG EXPRESSION & BINDING RESULTS

Figure 3: Multiple binding hits from antibody screen

Immune repertoire sequence analysis identified the top 96 heavy:light sequence pairs to express and test for binding. Using our high-throughput gene synthesis and assembly pipeline, we cloned each variable region sequence into the corresponding custom pAB_IgH and pAB_Igk expression vectors and expressed the full IgG antibodies in a plate-based format. We then harvested the supernatants containing the secreted antibody protein and measured antigen binding using a custom bead-based ELISA assay. Figure 3 shows that 5 of the 96 antibodies show significant binding ranging from 10 to 80-fold over background.



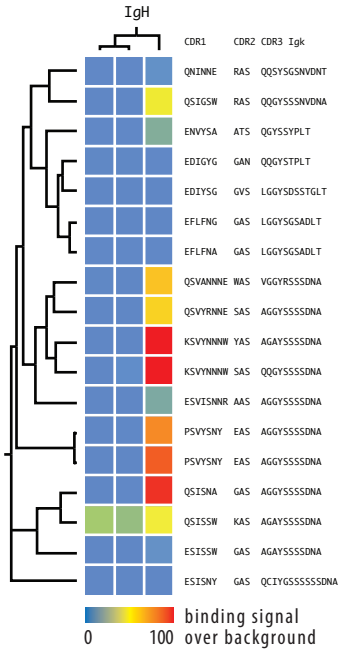


Figure 4: Binding data for related sequences

For the top hits, we selected additional related sequences from the same lineage clusters to express and test for antigen binding. We paired 3 heavy chain sequences with 18 light chain sequences (CDRs shown). The binding results shown here highlight interesting sequence-activity relationships. Using this combination of approaches we rapidly identified multiple monoclonal antibodies that strongly bind our antigen of interest.

Multiple high-affinity rabbit monoclonal antibodies were identified.

FOR MORE INFORMATION ON ANTIBODY DISCOVERY SERVICES, CONTACT:
Nathan Trinklein: email trinklein@panoplybio.com phone 650-323-6763 x139

PANOPLY bio