Advanced Antibody Discovery Workflow to Capture Maximum Repertoire Diversity

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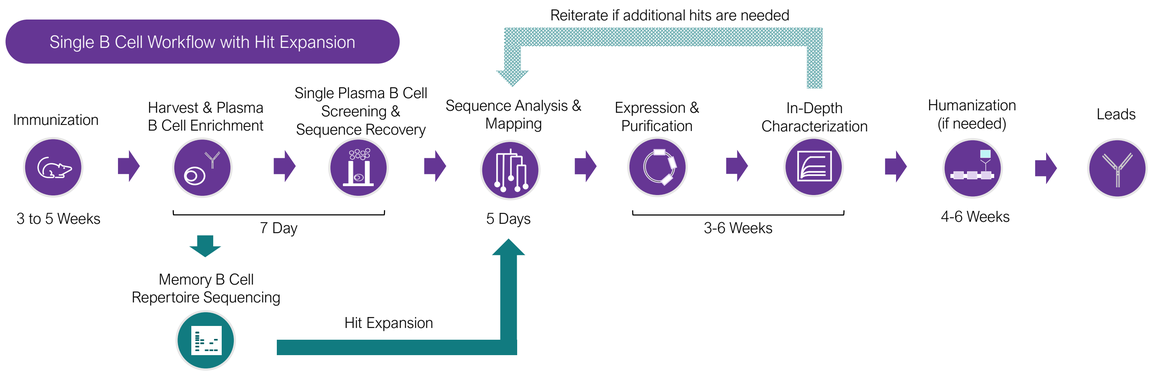
ABSTRACT

The complexity of antibody therapeutic targets continues to evolve, which in turn necessitates the evolution of integrated discovery methodologies. Incorporating state-of-the-art, high-resolution techniques enables reliable candidate triage more efficiently than traditional techniques. Major advancements in critical tools have improved antibody discovery by providing robust and thorough analysis of target specificity, function, and developability earlier in the drug discovery process. Effective integration of these technologies bolsters the antibody discovery process and facilitates lead candidate selection within as few as two months.

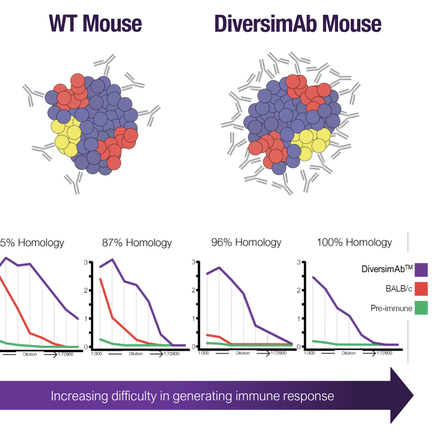
ANTIBODY DISCOVERY APPROACH

Overview of the process for upstream discovery workflow employed for rapid, high-content antibody discovery is presented below (purple). Repertoire sequencing of memory B cells (green) is incorporated into the workflow to capture the full repertoire for maximum antibody diversity, thus driving the discovery of more rare functional hits and providing additional insight into necessary engineering (such as humanization) for expanded therapeutic leads selection.

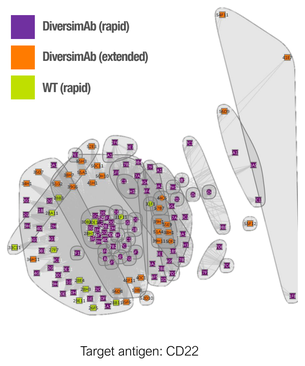


REPERTOIRE GENERATION AND CAPTURE

Immunization and Animal Model



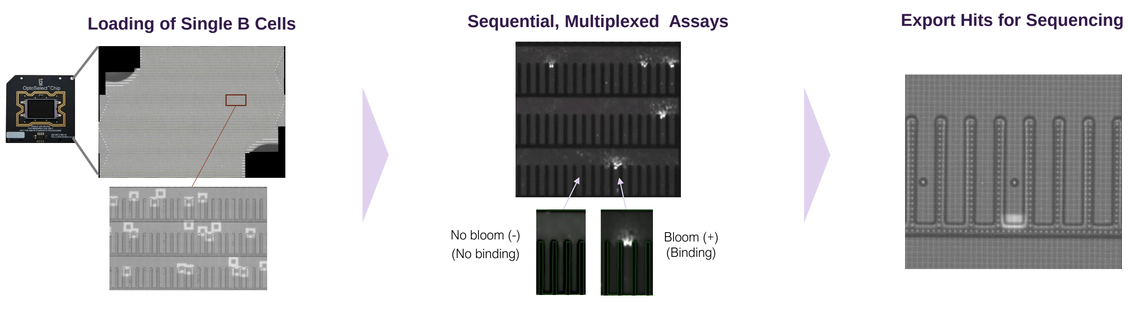
The DiversimAb hyperimmune mouse model was designed specifically to overcome the problem of limited repertoire diversity and epitopic access of the antibodies produced in WT or other genetically-engineered strains. This translates to an efficient immune response to high homology targets (left), conserved epitopes and expanded coverage of multiple epitopes for large proteins (right).



Target antigen: CD22

B Cell Screening and Sequence Analysis

Primary B Cell Selection

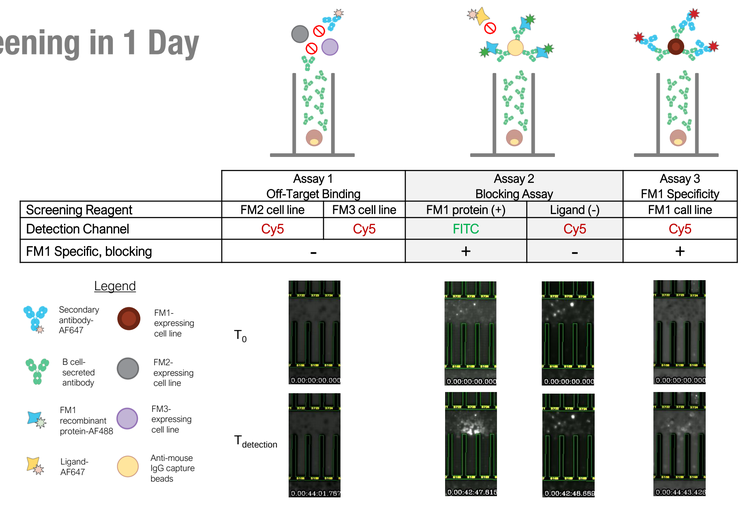


Overview of a typical single B cell screening workflow. Using the Berkeley Lights Beacon, customized loading results in 8,000 to 12,000 viable plasma single B cells into one Beacon chip (multiple chips per workflow is supported). Typically, 3 to 4 sequential assays are run with multiple reagents detected in each assay in a multiplexed manner. Lead candidates are selected for export to sequence heavy and light chain V regions.

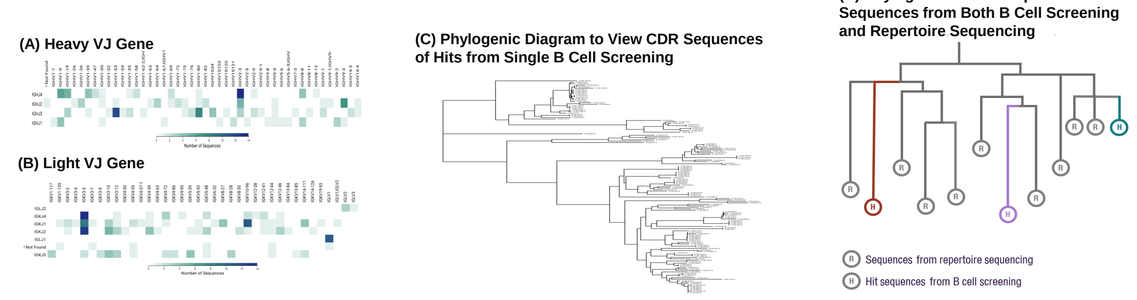
Case Study: Functional Screening in 1 Day

Screening results from a project interrogating three different cell lines and a ligand blocking assay in one workflow

- Three sequential assays were performed on-Beacon for specificity and functional screens
- Assay 1: Multiplexed screen against cell lines expressing family members of highest homology (FM2 and FM3)
- Assay 2: Multiplexed bead-based assay for blocking function
- Assay 3: Confirmatory specificity screen for on-cell binding to FM1-expressing cell line
- 191 hits of FM1 specific, ligand blockers were identified and exported for sequence recovery



Diverse Sequence Recovery with Hit Expansion Workflow

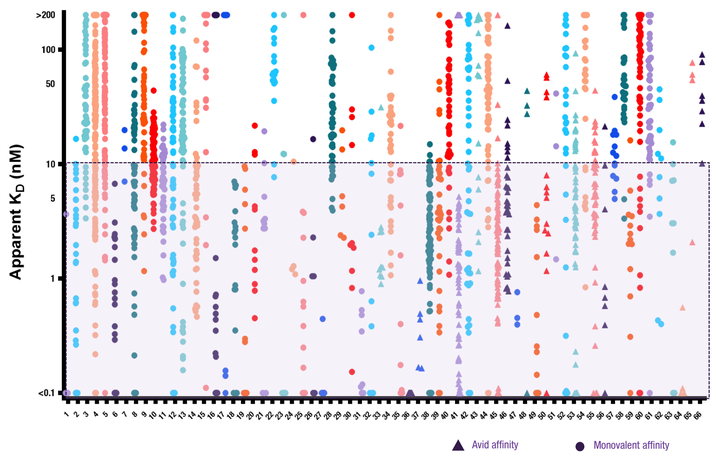


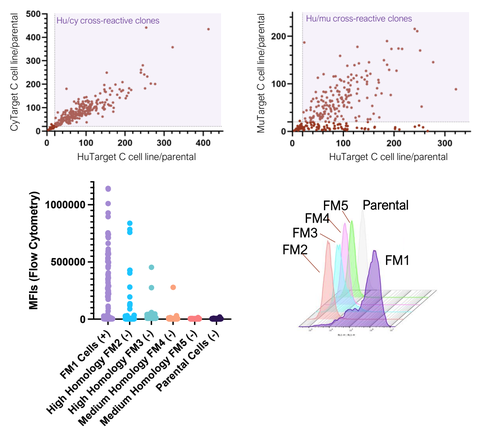
Hits from screening of plasma B cell through single B cell workflows were exported for sequence recovery to yield a diverse set of VH/VL paired sequences (A, B, and C).
Concurrently, repertoire sequences from memory B cells were analyzed by NGS as a complementary Hit Expansion Workflow. All sequences are mapped to visualize lineage relationship between single B cell screening hits and repertoire sequences (D). Related sequences are utilized to provide further opportunities in expansion of functional hit selection, removal of severe sequence liabilities, insight for engineering, and additional IP protection.

CHARACTERIZATION AND LEAD SELECTION

Antibody Characterization

Antibody affinity is determined by BLI (ForteBio Octet) or SPR (Carterra LSA) for candidate antibodies. For select projects, apparent on-cell affinity could be determined via flow cytometry. The DiversimAb mouse platform consistently generates mAbs with a wide range of affinities as demonstrated in 66 discovery campaigns over various types of target antigens.

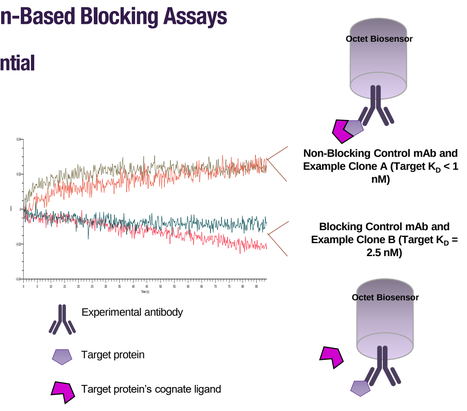




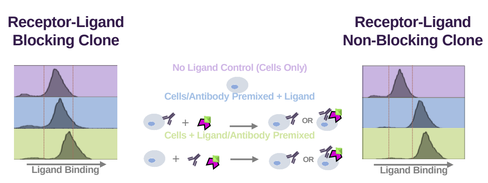
Binding specificity and cross-reactivity are determined by ELISA and/or flow cytometry depending on the target type and screening reagent availability. Cross-reactivity to human, cyno, and murine orthologs are common requirements for ideal candidates. Specificity to one member within a family of closely related proteins is typically necessary to minimize off-target activity of a candidate. Both species cross-reactivity and target specificity require strict epitope targeting and DiversimAb mice enable discovery of candidates to these regions.

Functional Analysis and Deep Characterization

Protein-Based Blocking Assays



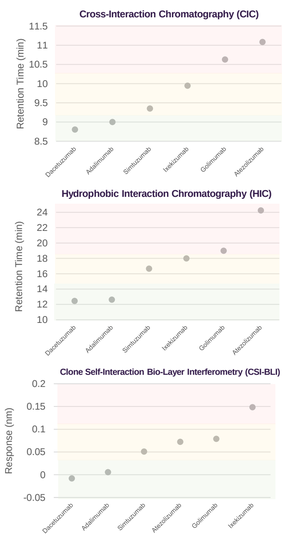
Cell-Based Blocking Assays



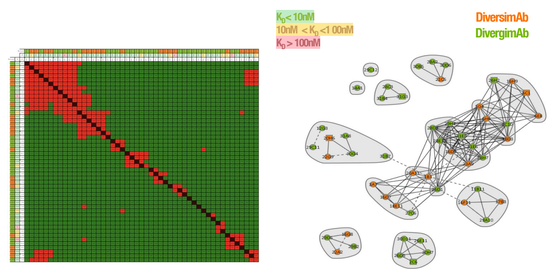
Candidate antibodies are assessed for functional activity in blocking assays to identify antibodies capable of disrupting protein-protein interactions. Both sequential and premix formats are utilized to determine activity and relative potency, respectively, for BLI and SPR-based assays. On-cell assays via flow cytometry (below) confirm blocking activity and potency with the receptor in its native state.

Physical Stability Assessment

Lead candidates are compared in molecular assessment assays to identify those with developability concerns. Reference antibodies with known properties are used to determine intra-assay cutoffs to determine regions of high (red), medium (yellow) and low (green) risk. High throughput assays such as CIC, HIC and CSI-BLI enable rapid assessment of the entire candidate pool.



Epitope Binning



Antibody binning analysis provides detailed information regarding epitope targeting. When combined with other characterization data, ideal leads from relevant epitopes are selected to nominate a development-ready candidate.

DISCUSSION

Antibody discovery requires both compressed timelines and high-resolution screening to effectively address modern targets. The combination of diverse starting repertoire, deep screening and high content validation produces lead candidates to complex targets. Scaling and integration of technologies and workflows that enable this process is a cornerstone to the next generation of antibody discovery.

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