Revolutionizing Antibody Therapeutics: Harnessing Machine Learning for Bispecific Antibody Discovery

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Abstract

Machine learning is transforming antibody discovery by overcoming inefficiencies in traditional methods such as hybridoma technology and phage display. These conventional techniques are often low throughput. To address these limitations, we introduce a bioinformatics solution that accelerates antibody lead identification and production. This approach combines next-generation sequencing (NGS) with both in vivo (e.g., B-cells, PBMCs) and in vitro (e.g., phage display) discovery campaigns. NGS enhances the sequence diversity, uncovering 5–50 times more population diversity compared to traditional approaches like random colony screening using Sanger sequencing.

Herein, we applied a machine learning solution for the development of novel tool antibodies for microscopy and the rapid identification of bispecific antibodies (BsAbs), which simultaneously target two distinct antigens or epitopes. This enables the efficient development of BsAbs for applications such as cancer immunotherapy, where BsAbs can recruit immune cells (e.g., T cells) to tumor sites by targeting both a tumor-associated antigen and a T-cell receptor.

By integrating next-generation sequencing, machine learning, and advanced engineering, this streamlined solution accelerates antibody discovery, enabling the creation of both research tool kit antibodies and bispecific antibodies, offering an efficient and dependable pathway to high-quality therapeutic candidates.

Machine Learning Enabled Antibody Production

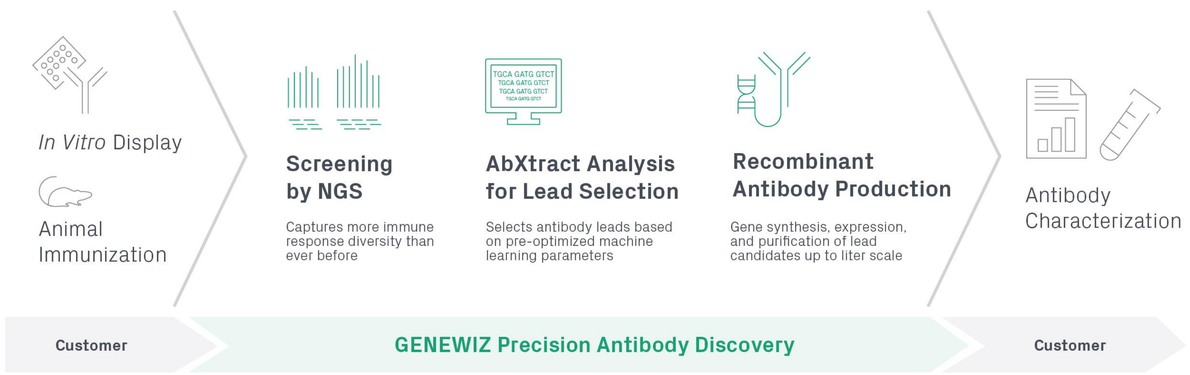


Figure 1. AbXtract Workflow- The bioinformatics pipeline utilizes machine learning for prioritization of optimal leads starts with quality filtering of input NGS data followed by annotating the sequences to identify regions of interest (ROI) and extract features. Relative Abundance and enrichment based on ROI can be calculated, the module then identifies abundant and rare clusters with unsupervised ML based on density-based clustering and quantifies sequence-based biophysical liabilities. The platform prioritizes the leads based on favorable NGS metrics to provide a gene synthesis ready output.

Application: Development of Antibodies for Microscopy

Antibodies are powerful tools in microscopy, enabling researchers to visualize and study specific proteins, molecules, or structures within cells and tissues. Their ability to bind with high specificity to target antigens makes them indispensable for various microscopy techniques. By utilizing the GENEWIZ generated antibodies in microscopy this highlights how these antibodies can be applied as tools in downstream workflows.

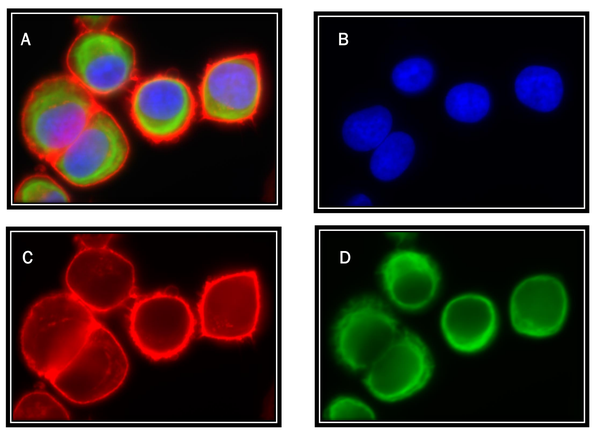
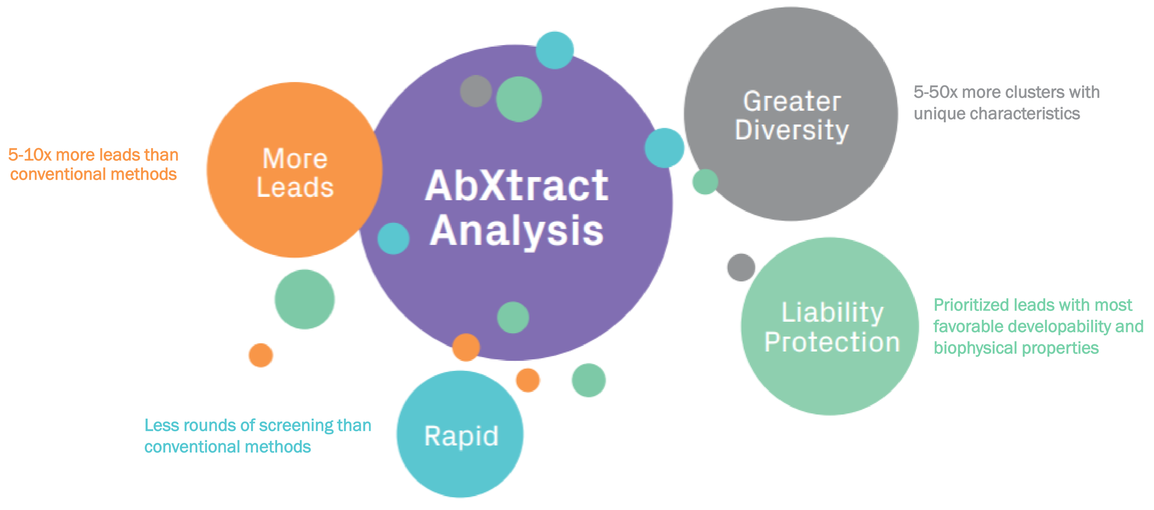


Figure 2. Robust recombinant antibody production for microscopy. Fixed SKBR3 Cells, 63x widefield microscopy
A. Overlay
B. F-actin stained using Phalloidin-647
C. DNA stained using Hoechst 33342
D. Antibody made by GENEWIZ- Anti pan Cytokeratin, at 3 ug/ml with an anti-Human-488 Secondary 1:1000

Advantages



Key Technical Contacts

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Optimizing Antibody Discovery

AbXtract identified ≥1000 antibodies candidates selected against SARS-CoV-2 Spike trimer protein, its monomer S1 and the receptor binding domain (RBD). Utilizing PacBio NGS followed by *in silico* AbXtract analysis and recombinant antibody production, 200 Ab were produced as IgG and recognized receptor binding domain (RBD). The RBD binding affinities of 143 Abs ranged from 34 pM to 1µM, with 30 Ab better than 100 pM, as measured by surface plasmon resonance (Figure 3). The Ab selected with the traditional colony screening method (pink dots) had overall worse affinity when compared to clones identified by AbXtract (blue dots).

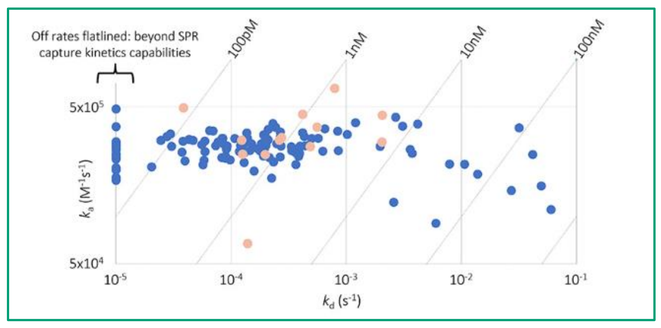

Figure 3. Clones and their affinities by traditional screening method versus NGS. Isoaffinity plot of 13 picked clones (Pink Dots), or 143 clones identified by AbXtract synthesized, expressed, and purified (Blue Dots). Affinities are indicated by the diagonal lines.

Machine learning (ML) is transforming the design and development of bispecific antibodies (BsAbs) by addressing the complexities involved in their engineering. Bispecific antibodies, which can bind to two different antigens or epitopes, require precise design to ensure proper pairing, stability, and functionality. ML offers innovative solutions to optimize these processes. Through combining the AbXtract platform for robust antibody discovery with high throughput antibody production, hundreds to thousands of unique antibodies can be evaluated for tool applications like microscopy or therapeutic applications like Bispecific antibody generation.

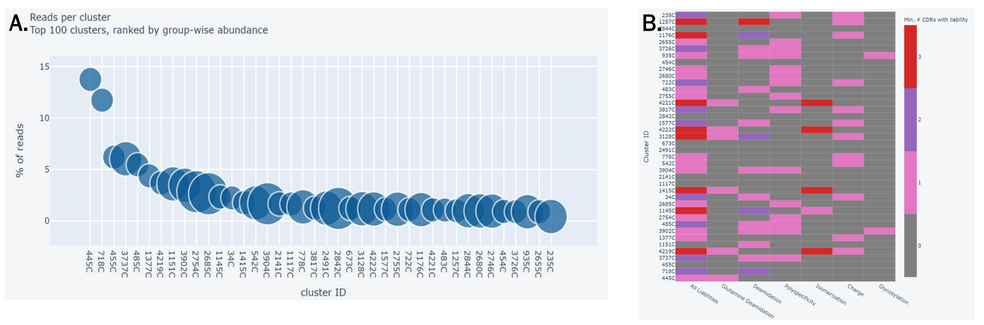

Figure 4. AbXtract Report. (A) Percentage of NGS Reads versus Cluster. The figure highlights the reads per cluster for the top 100 clusters that have been ranked by group-wise abundance. The size of blue circle indicates the number of reads associated with each cluster. (B) Cluster IDs versus CDR Liabilities. This chart shows the minimum number of CDRs with sequence liabilities across each clustered sequences. The top 100 clusters are ranked by group-wise abundance. Furthermore, the clusters with less liabilities are predicted to provide a better candidate for antibody development and production.

Application: Development of Asymmetric IgG-like Bispecific Antibodies

Asymmetric bispecific antibodies are a specialized class of engineered antibodies designed to simultaneously bind two different antigens or epitopes. Unlike symmetric bispecific antibodies, which have identical heavy and light chains, asymmetric bispecific antibodies are constructed with distinct heavy and light chains, allowing for greater flexibility and functionality in targeting diverse biological pathways. These antibodies are particularly valuable in therapeutic applications, as they can engage multiple targets with high specificity. For example, they can simultaneously bind a tumor-associated antigen and a T-cell receptor, effectively recruiting immune cells to attack cancer cells. This dual-targeting capability enhances therapeutic efficacy while minimizing off-target effects.

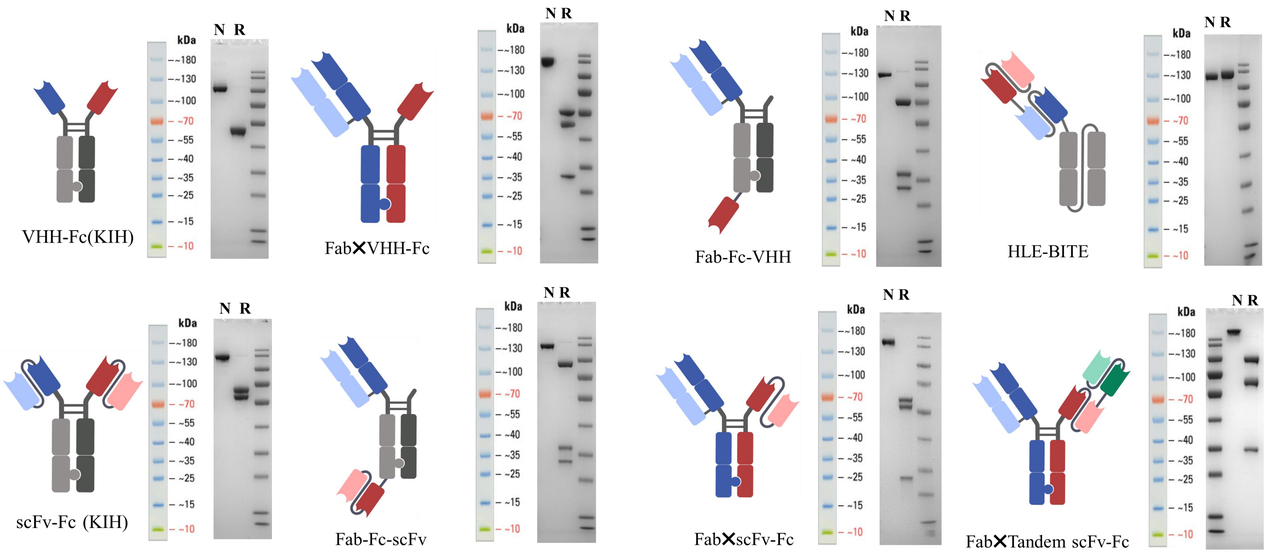

Figure 5 Optimizing Recombinant Formats. A variety of asymmetric IgG-like Bispecific KIH antibodies are shown here with the corresponding representative SDS-PAGE.

Summary

Machine learning and next-generation sequencing (NGS) are revolutionizing antibody discovery by addressing the inefficiencies of traditional methods. By combining NGS with *in vivo* and *in vitro* campaigns, this approach uncovers up to 50 times more sequence diversity. It also enables the development of novel tool antibodies for microscopy and bispecific antibodies (BsAbs) for applications like cancer immunotherapy. This integrated solution accelerates the identification and production of high-quality therapeutic candidates.

References

1. Erasmus, M.F., Ferrara, F., D'Angelo, S. et al. Insights into next-generation sequencing guided antibody selection strategies. Sci Rep, 13: 18370 (2023).
2. Ferrara, F, Erasmus, M.F., et al. A pandemic-enabled comparison of antibody discovery platforms: a naïve library directly delivering antibodies as potent as immune sources. Nature Comm. 13:462 (2022).
3. Song, E., Zhu, P., Lee, SK. et al. Antibody-mediated in vivo delivery of small interfering RNAs via cell-surface receptors. Nat Biotechnol, 23: 709 (2005).
4. Zhang X, Roth MJ. Antibody-directed lentiviral gene transduction in early immature hematopoietic progenitor cells. J Gene Med, 12: 945 (2010).

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