Leveraging High-Throughput Platforms for the Discovery of Bispecific Antibodies

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Abstract

We present a high-throughput platform to accelerate antibody discovery by integrating gene synthesis, antibody production, and high-throughput automated workflows. This enables rapid identification and scalable production of thousands of antibody candidates, significantly reducing timelines and overcoming the bottlenecks of traditional methods. The platform has been successfully used on multiple formats including bispecific and multi-multispecific proteins.

This approach to accelerates antibody discovery by overcoming the limitations of traditional methods such as hybridoma technology and phage display, which are often low throughput. The solution integrates next-generation sequencing with both in vivo (e.g., B cells, PBMCs) and in vitro (e.g., phage display) discovery workflows to enable rapid and comprehensive antibody identification. NGS significantly enhances sequence diversity, uncovering 5–50x more novel candidates compared to conventional approaches such as random colony screening using Sanger sequencing. This increased depth enables more efficient identification of antibody candidates.

Using this integrated platform, we developed bispecific antibodies (BsAbs), which can simultaneously target two distinct antigens or epitopes. This capability supports the efficient generation of BsAbs for applications such as cancer immunotherapy. By combining NGS-based discovery, streamlined screening, and high-throughput workflows, this platform enables the rapid identification and scalable development of antibody candidates, including research tool antibodies and bispecific formats, providing an efficient and reliable pathway to high-quality therapeutic candidates.

Machine Learning Enabled Antibody Production

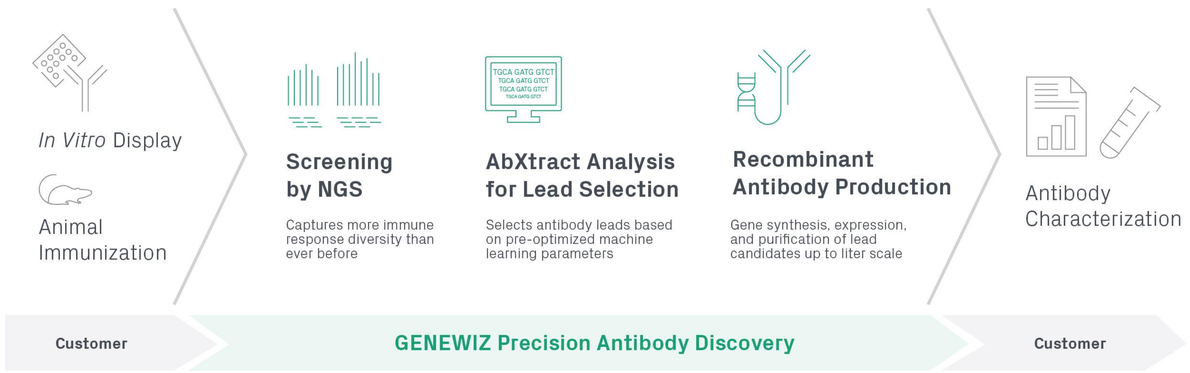
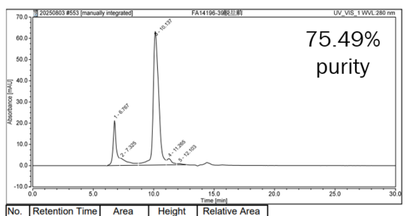


Figure 1. AbXtract Workflow- The bioinformatics pipeline utilizes machine learning for prioritization of optimal leads starts with quality filtering of input NGS data followed by annotating the sequences to identify regions of interest (ROI) and extract features. Relative Abundance and enrichment based 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: Purification of Bispecific Antibodies

Two-step purification of a CHO-expressed bispecific antibody using Protein A followed by CEX chromatography. Initial purification shows heterogeneous species with mispaired and truncated byproducts, while the second step removes impurities and yields a highly pure, homogeneous antibody, confirmed by chromatographic profiles and SDS-PAGE.

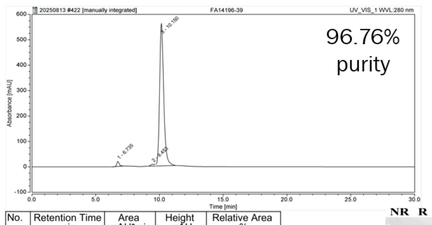
Expression cell	Expression volume	1st step purification	1st step yield	1st step purity	2st step purification	2st step yield	2st step purity
CHO-K1	1000 mL	Protein A	265 mg	75.49%	CEX	61.7 mg	96.76%



No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	6.767	6.477	21.093	14.58
2	7.325	2.667	3.309	6.00
3	10.137	33.531	62.951	75.49
4	11.265	1.454	2.967	3.27
5	12.103	0.286	0.570	0.64

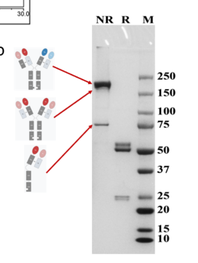
1st step Protein A purification:
Main impurity: Hole-Hole homodimer/Hole half antibody

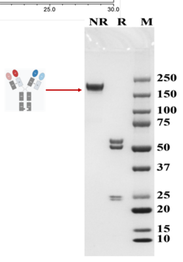
2st step CEX purification:
By-products removal



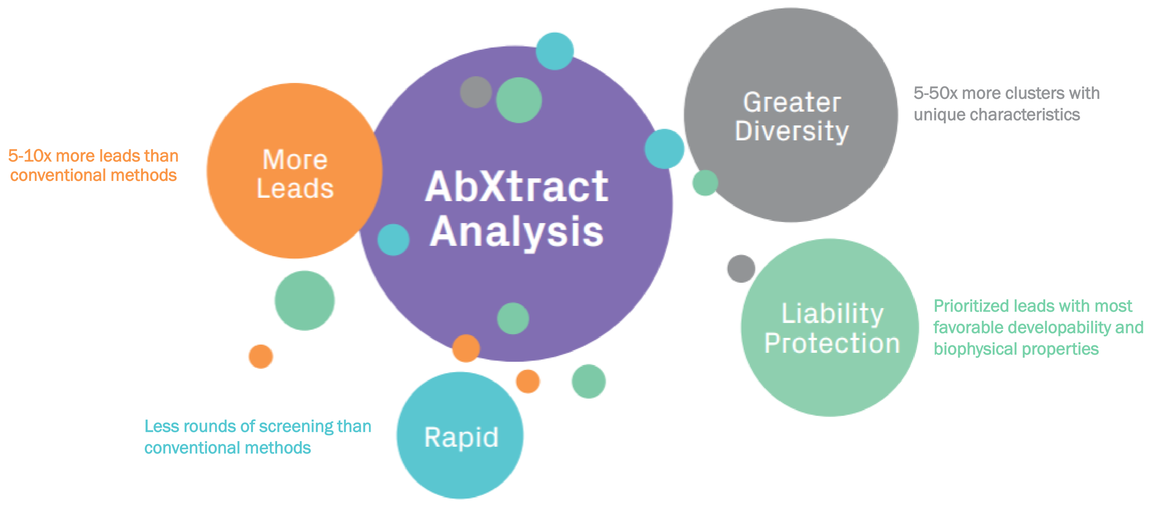
No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	6.735	4.375	20.036	2.02
2	9.453	2.663	6.942	1.23
3	10.150	209.918	560.440	96.76

Crossmab BsAb





Advantages



Key Technical Contacts

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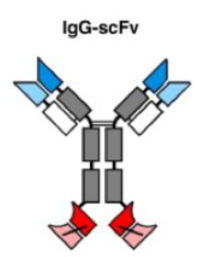
Optimizing Expression of Bispecific Antibodies

Asymmetric bispecific antibodies are a specialized class of engineered antibodies designed to simultaneously bind two different antigens or epitopes. 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.



Production Metrics and Quality Profile	
Final amount	968.76 mg
Concentration	7.02 mg/L
Purity of SEC-HPLC	96.39%
Purity of LC-MS	100.00 %
Endotoxin	<0.015 EU/mg

Table 1. Production Metrics and Quality Profile for IgG-scFv. Protein yield, concentration and purity evaluation. This approach enabled the production of a highly purified bispecific antibody with strong yield and low endotoxin levels, making it suitable for in vivo applications. Structural integrity and purity were further confirmed using orthogonal analytical methods, including LC-MS and SEC.

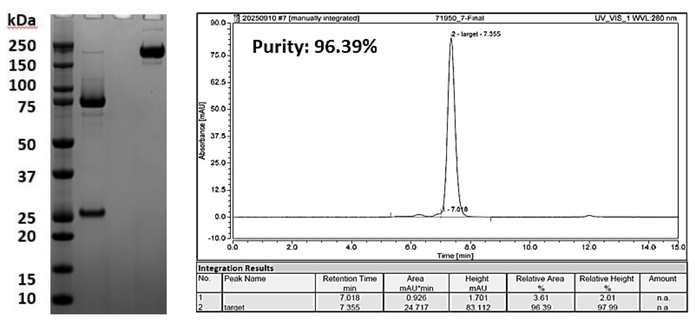


Figure 2. SDS-PAGE and SEC-HPLC results for IgG-scFv.

Overview
A bispecific antibody required for in vivo studies could not be successfully produced using standard approaches.

Synthesis and Cloning
The DNA sequence was re-engineered and optimized for CHO expression using advanced codon optimization tool to improve expression efficiency and overall construct performance.

Expression and Purification
Transient expression was performed in CHO-K1 cells at large scale volume, followed by a custom multi-step purification workflow.

Despite their potential, the structural complexity of BsAbs introduces significant challenges in expression, including chain mispairing, formation of undesired byproducts, and lower overall yields compared to monoclonal antibodies. Optimizing expression is therefore critical for successful development. Our team uses strategic approaches such as codon optimization, rational sequence engineering, and the use of robust expression systems like CHO cells improve protein folding, assembly, and expression efficiency. In parallel, we combined tailored purification strategies that were essential to remove impurities such as homodimers and half-antibody species, ensuring high purity and product consistency. By integrating optimized design, efficient expression platforms, and multi-step purification workflows, we were able to overcome these challenges and achieve reliable production of high-quality bispecific antibodies.

Production 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.

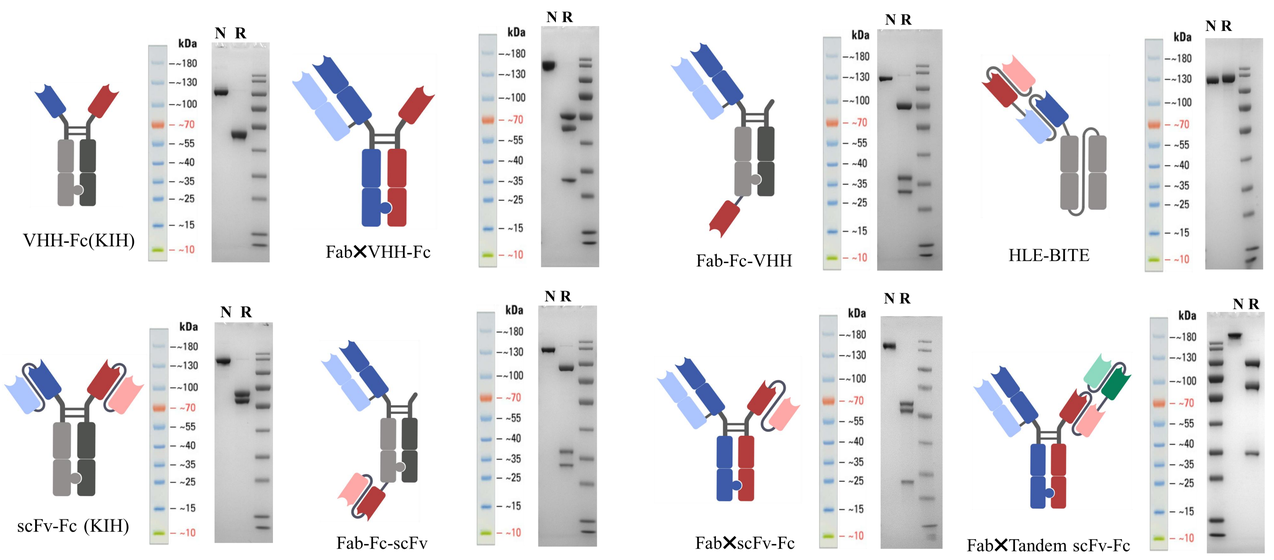


Figure 2 Optimizing Recombinant Formats. A variety of asymmetric IgG-like Bispecific KIH antibodies are shown here with the corresponding representative SDS-PAGE.

Summary

Bispecific antibodies have become highly valuable in areas such as oncology, immunotherapy, and targeted drug delivery. However, their structural complexity introduces challenges in expression, including chain mispairing and formation of unwanted byproducts, making optimized design, expression, and purification strategies essential for achieving consistent, high-quality production suitable for research and clinical development. By integrating machine learning, optimized sequence design, CHO-based expression, and tailored purification strategies, this approach overcomes key bispecific production challenges and enables reliable generation of high-quality candidates for downstream applications. This integrated solution accelerates the identification and production of high-quality therapeutic candidates.

References

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Acknowledgements: Suhani Nagpal[1], Amy Migliori[1], M. Frank Erasmus[2], Laura Spector[2], Katheryn Perea-Schmittle[2]
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