Optimized Recombinant Antibody Production: A Comprehensive End-to-End Solution

P Rao, M Karney, S Kumar, C M Richardson
GENEWIZ From Azenta Life Sciences, South Plainfield, NJ 07080

Abstract

Recombinant antibody production is a cornerstone of modern biotechnology and research, offering precision and adaptability in the development of vital tools for diverse applications. Antibody engineering techniques, such as phage display, yeast display, and hybridoma technology, have enabled the isolation and optimization of antibodies with high specificity and affinity. Furthermore, the development of versatile recombinant expression systems has revolutionized the scalability and production of antibodies in an animal-free way. We present a pioneering end-to-end solution for high-quality antibody production to accelerate scientific research. Key features of GENEWIZ Multiomics & Synthesis Solutions from Azenta Life Sciences' expression platform include:

1.Mammalian Expression Platform: A robust platform optimized for both HEK293 and CHO cell lines.

2.Proprietary Expression Vectors: Our expression vectors have been meticulously designed and refined to enhance antibody production efficiency, ensuring reliable results from the small scale (1-5mL) to large-scale expression (1L).

3.Scalability: The fast and efficient expression achieved through GENEWIZ's vectors empowers researchers to scale up their projects with confidence.

4.Stringent Quality Control (QC) Measures: Rigorous QC measures, such as analytical SEC-HPLC and SDS-PAGE (both reducing and non-reducing) gel are in place to guarantee the delivery of high-quality antibodies that meet or exceed industry standards.

In conclusion, GENEWIZ's comprehensive solution facilitates expedited research and development, empowering scientists with the tools required to advance science and address pressing global challenges.

GENEWIZ Recombinant Antibody Production Service

Figure 1. Antibody workflow: an end-to-end solution for gene synthesis, antibody expression, and purification
GENEWIZ's streamlined recombinant antibody production workflow includes de novo synthesis of the variable heavy or light chain and cloning into the optimized vector backbones or custom vector. This is followed by an expression and purification step and a rigorous QC (A280 measurement, SDS-PAGE and SEC-HPLC). Purified antibodies can be delivered in as fast as 3 weeks.

Materials & Methods

GENEWIZ has streamlined the production of Recombinant Antibodies by optimizing the expression vectors to enhance the expression efficiency. To test the optimized expression vector, 12 antibody sequences (Human IgG1) were expressed in both HEK293 and CHO cell lines at a small-scale expression and purified using a one step Protein A-based purification and eluted in PBS pH7.4. Protein concentration was measured at A280 spectrometer reading and purity was tested using SDS-PAGE (reducing and non-reducing) gel. The Antibody titer is shown below (**Figure 2**). To further test the expression vector system, several different antibody formats, such as VHH, VHH-Fc, ScFv, ScFv-Fc were expressed

Results

Recombinant antibody production is a cornerstone of modern biotechnology and research, offering the development of vital tools for diverse applications. GENEWIZ has streamlined the production of Recombinant Antibodies by optimizing the expression vectors to enhance the expression efficiency. To test the optimized expression vector, 12 antibody sequences (Human IgG1) were expressed in both HEK293 and CHO cell lines at a small-scale expression and purified using a one step Protein A-based purification and eluted in PBS pH7.4. Protein concentration was measured at A280 spectrometer reading and purity was tested using SDS-PAGE (reducing and non-reducing) gel. The Antibody titer is shown below (**Figure 2**).

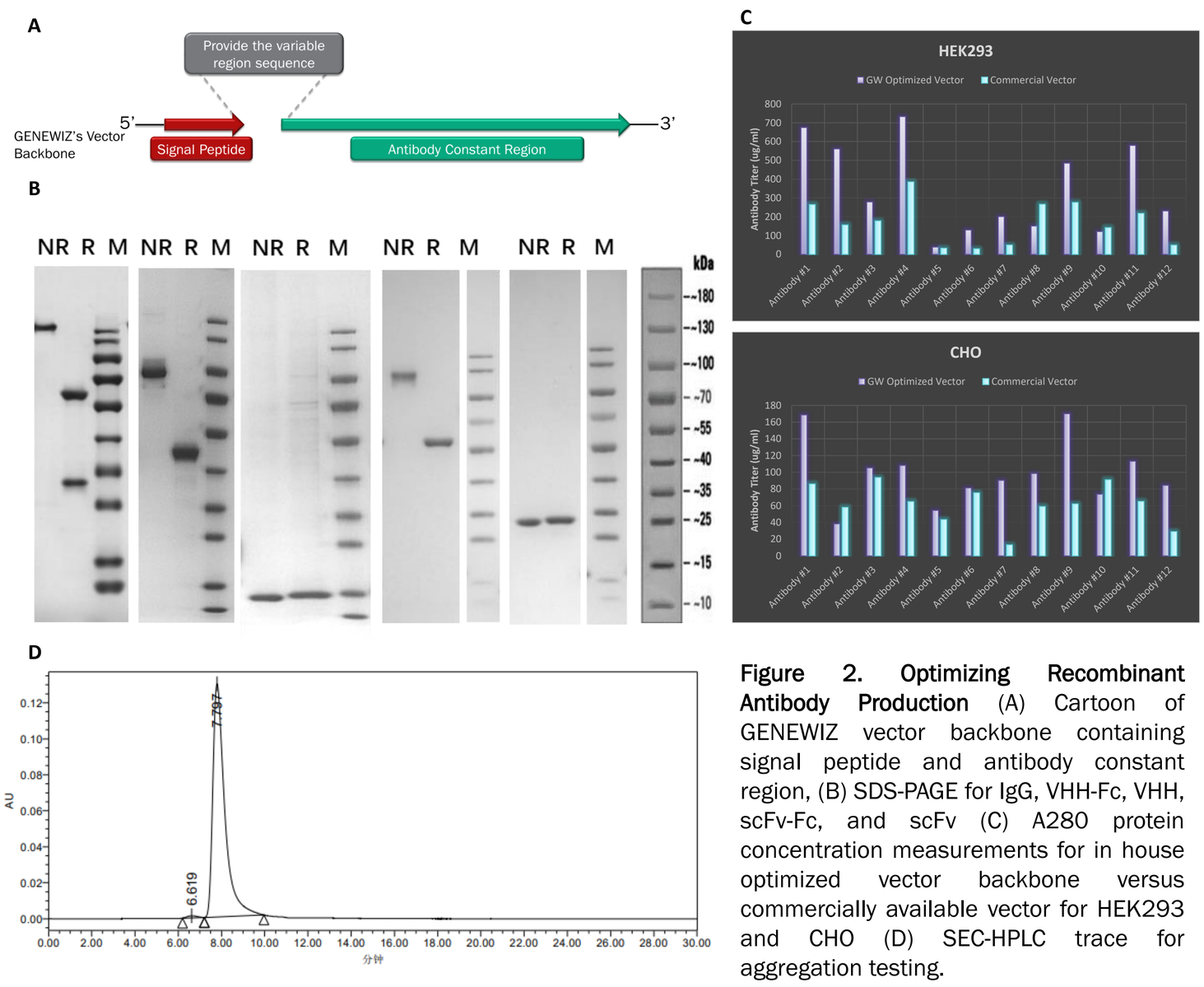

Figure 2. Optimizing Recombinant Antibody Production (A) Cartoon of GENEWIZ vector backbone containing signal peptide and antibody constant region, (B) SDS-PAGE for IgG, VHH-Fc, VHH, scFv-Fc, and scFv (C) A280 protein concentration measurements for in house optimized vector backbone versus commercially available vector for HEK293 and CHO (D) SEC-HPLC trace for aggregation testing.

Customer Case Study

A customer provided an antibody sequence that was challenging to express. GENEWIZ scientists optimized the DNA sequence and designed a cloning strategy into a high expressing vector. Both Heavy and Light chains constructs were QC verified prior to co-transfection in a 100ml HEK293 cell line. Supernatants were harvested after 5 days of expression and the resulting antibody was purified using a Protein A resin. High quality purified antibody delivered by GENEWIZ was used for microscopy on fixed SKBR3 Cells with 63x widefield microscopy (**Figure 3**).

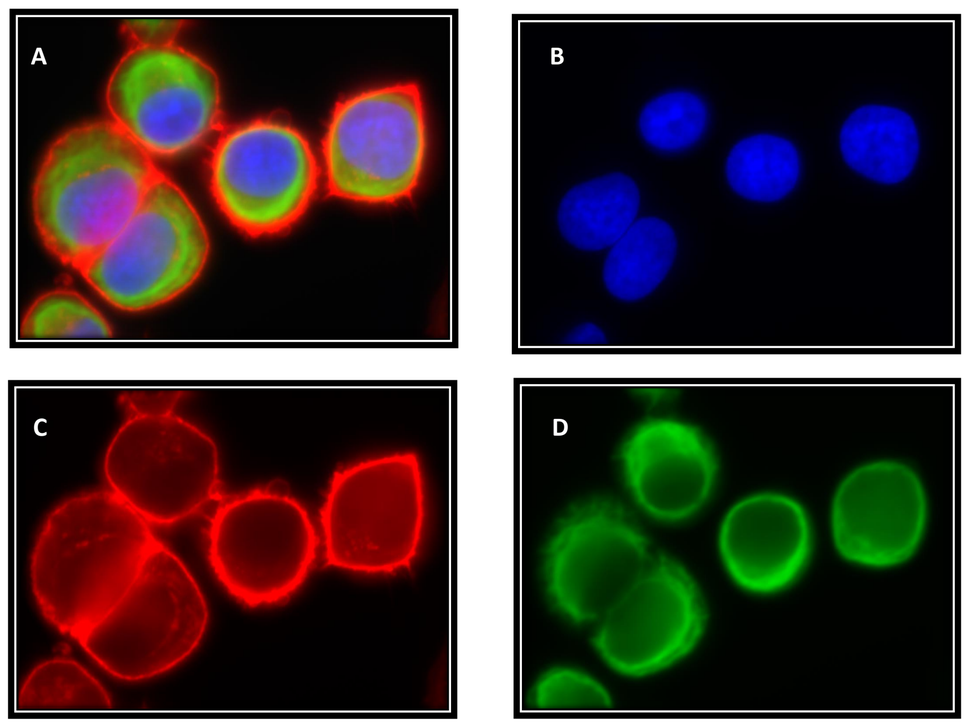

Figure 3. Robust recombinant antibody production for microscopy (Customer case study)
Fixed SKBR3 Cells, 63x widefield microscopy
A. Overlay
B. F-actin stained using Phalloidin-647
C. DNA stained using Hoechst 33342
D. Antibody for the customer - Anti panCytokeratin, at 3ug/ml with an anti-Human-488 Secondary 1:1000

Conclusions

- GENEWIZ from Azenta Life Sciences provides a robust, end-to-end service for antibody expression and purification
- GENEWIZ has proprietary optimized expression vectors for expression in both HEK293 and CHO platforms
- Multiple Antibody formats can be expressed using expression vectors

Key Contact

GENEWIZ PROJECT MANAGEMENT: GS@Azenta.com