Antibody development

# Unleashing the power of automation for high-throughput antibody synthesis

## Introduction

In the rapidly evolving field of antibody therapeutics, the efficiency and efficacy of discovery and development workflows are paramount. To mitigate the risk of late-stage failure, manufacturers screen hundreds of antibodies by conducting *in vivo*, *in vitro*, and *in silico* campaigns. The antibodies are expressed in mammalian cells and tested early in development to identify the most promising candidates for specific targets. Since developability assays require only microgram quantities, there is no need for large-scale antibody expression. Small-scale expression enables higher throughput, so it is a more economical way to screen hundreds or even thousands of antibody candidates.

The Invitrogen™ GeneArt™ HTP Antibody Expression Service can reliably perform small-scale expression and purification of hundreds of antibodies in parallel. We have applied Six Sigma™ principles to develop a semi-automated platform that requires minimal operator intervention for high lot-to-lot consistency.

Full workflow integration with our Manufacturing Execution System (MES) and seamless barcode tracking from gene synthesis to the final product also provide end-to-end traceability.

The high-throughput (HTP) platform utilizes Invitrogen™ GeneArt™ GeneOptimizer™ software for sequence optimization. The GeneOptimizer algorithm evaluates more than 20 parameters that can affect gene expression, including transcription, splicing, translation, and mRNA degradation. Sequence optimization by the algorithm frequently leads to an increase in antibody expression [1], and an electronic DNA or amino acid sequence is the only input required. The workflow includes gene synthesis, cloning, and antibody production with the Gibco™ Expi293™ Expression System or ExpiCHO™ Expression System (Figure 1). Depending on the downstream application, the customer can choose to have antibodies expressed in the supernatant or use our antibody purification service, which includes buffer exchange and sterile filtration.

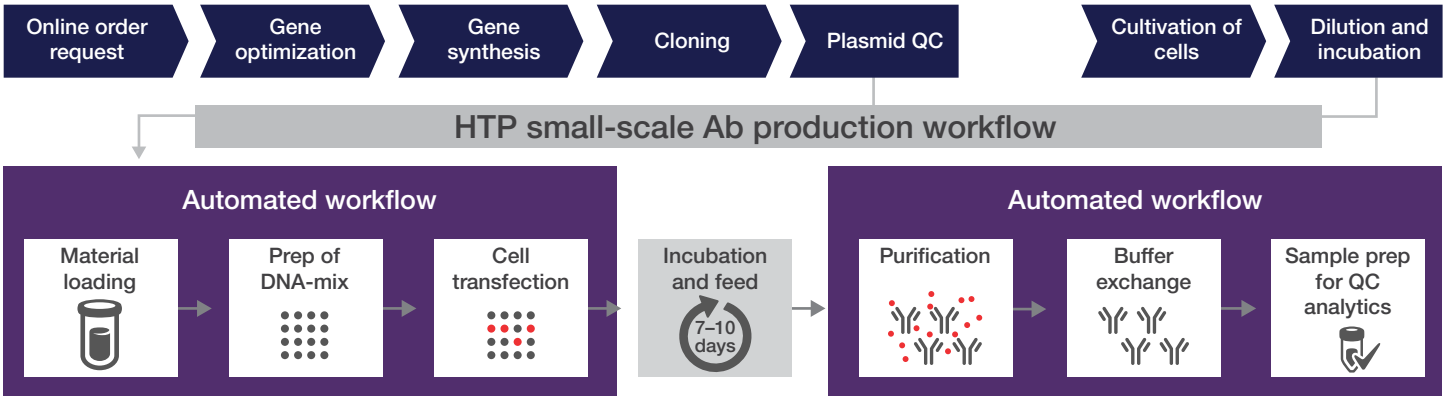


**Figure 1. Schematic overview of the GeneArt HTP Antibody Expression Service workflow.** Antibodies can be ordered as amino acid or DNA sequences. Sequences encoding the heavy chain (HC) and light chain (LC) are optimized to enhance expression in the ExpiCHO or Expi293 Expression System. The sequences are then synthesized and cloned into the Invitrogen™ pcDNA™ 3.4 TOPO™ vector or an expression vector provided by the customer. Transfection, protein purification, buffer exchange, and filtration are all automated for high reproducibility and robustness. The use of 2D barcoded tubes and workflow integration with our MES help ensure full traceability through the entire workflow.

## Materials and methods

In this study, we evaluated a panel of 38 antibodies in three formats with different target antigens. The panel consisted of 8 chimeric, 18 human, and 12 humanized antibodies with different expression levels. This provided a comprehensive and balanced representation of antibody types, which was crucial to obtain generalizable results (Figure 2).

Automated transfection of Gibco™ Expi293™ and ExpiCHO™ cells was performed in triplicate at the 2.5 mL scale. After 7 days of antibody expression in the Expi293 system and 10 days of expression in the ExpiCHO system, the plates were transferred back to the liquid handling platform for automated antibody harvest, purification, buffer exchange, and filtration.

To assess antibody expression rates and final recoveries, the antibody titers in the supernatants were measured via biolayer interferometry (BLI). Affinity purification with Thermo Scientific™ Pierce™ Protein A/G Agarose beads was performed using the Thermo Scientific™ KingFisher™ Presto Purification System. After the antibodies were eluted in Tris-glycine buffer, Thermo Scientific™ Zeba™ size exclusion resins were used for buffer exchange. This approach was faster and more efficient than traditional dialysis, enabling nearly 100% buffer exchange and 92% to 95% antibody recoveries regardless of protein concentration. The concentrations of the purified antibodies were measured on a spectrophotometer after sterile filtration, and purity was assessed by capillary electrophoresis. All instruments were validated via measurement system analysis prior to use.

To evaluate precision within runs with each cell line, automated production of each antibody in the test panel was performed in triplicate with replicates distributed randomly across plates. Experiments were repeated on different days by different operators to assess variability between runs. The coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean of the values considered and reported as a percentage.

## Results

The data showed remarkably low variation within runs for each antibody expressed in Expi293 cells. The average CVs of antibody titers in the supernatants and the purified antibody concentrations were 3.7% and 4.3%, respectively (Figure 3). There was slightly more variation in runs performed with ExpiCHO cells. The average CVs of the antibody titers in supernatants and the purified antibody concentrations were 9.0% and 9.5%, respectively. When we analyzed the precision between runs, variation was slightly higher for most antibodies with average CVs of 11.2% in Expi293 cells and 9.8% in ExpiCHO cells. This could be attributed to biological variation between the two cell lines.

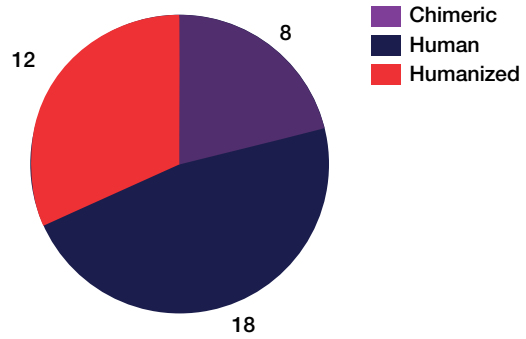


**Figure 2. Composition of the antibody test panel.** The test panel included 38 antibodies with different target antigens and expression levels in three formats: chimeric antibodies (purple, n = 8), human antibodies (navy, n = 18), and humanized antibodies (red, n = 12).

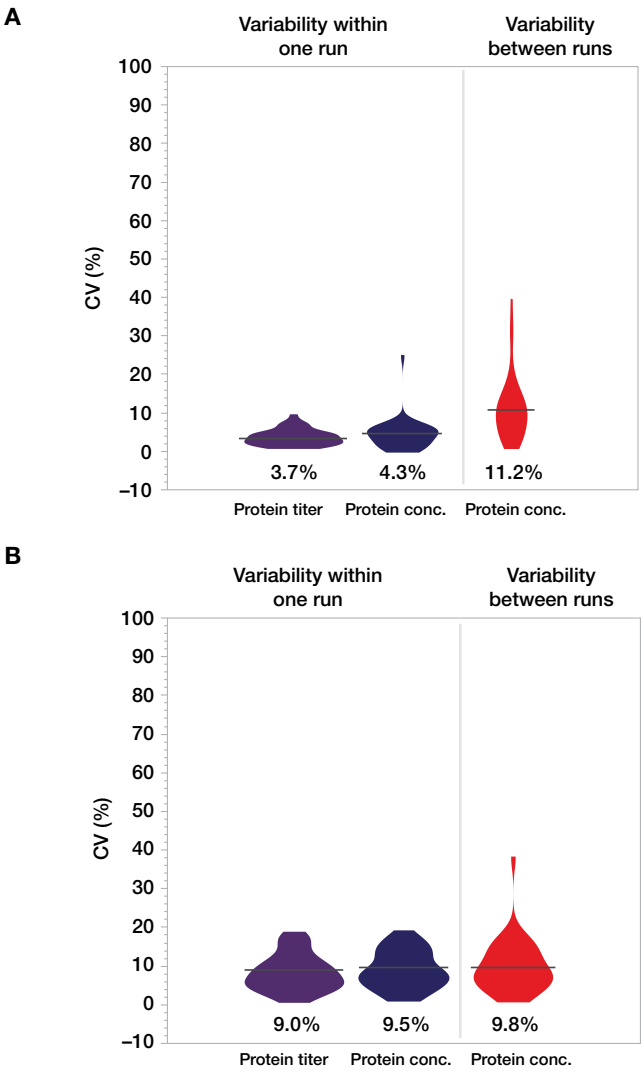


**Figure 3. Variability of antibody expression with (A) the Expi293 Expression System and (B) the ExpiCHO Expression System.** Left: CVs reflecting the intra-run precision in supernatant titers (purple) and purified antibody concentrations (navy). Right: CVs reflecting the inter-run precision in purified antibody concentrations (red).

All of the antibodies were individually expressed in two separate runs, and the correlation between each set of runs was assessed. $R^2$ values above 0.9 indicated a high level of consistency between runs (Figure 4). As expected, the variability between runs was slightly higher after purification, buffer exchange, and sterile filtration. Overall, the automated workflow delivered consistent results across both titer and purified protein measurement ranges and produced antibodies of high purity in quantities that would be sufficient for many developability assays.

We also examined the expression profiles of three antibodies in the test panel at three different scales in each cell line. Insights like these are essential for academic research and industrial applications, because they provide a roadmap for scaling up antibody production efficiently and reliably. Antibody expression was evaluated at culture volumes of 2.5 mL, 200 mL, and 1 L (Figure 5). These scales reflected the different stages of research and production from initial small-scale testing to production on a larger scale.

The antibody expression patterns remained constant across scales, which was a key observation. This level of stability is critical for comparative studies, as it allows researchers to reliably compare the performance and characteristics of different antibodies under varying conditions. However, absolute expression levels will likely vary with production scale, the type of cell line used, and the specific antibody candidate. These variations must be considered when planning large-scale production or switching to a different cell line.

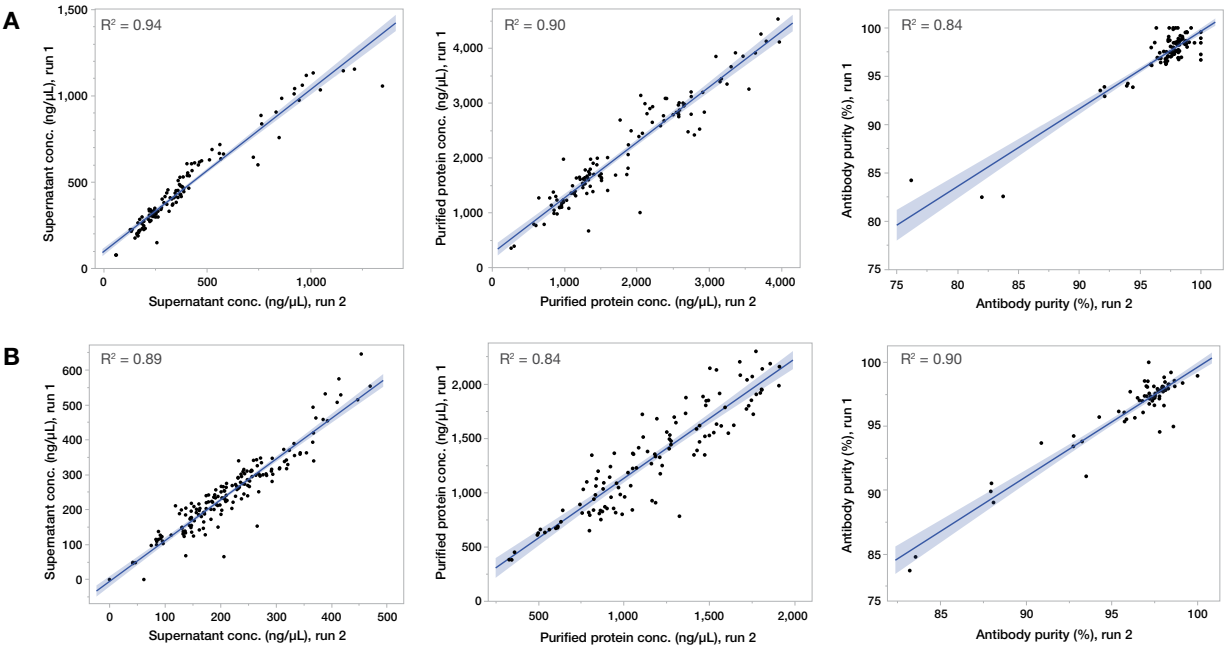


**Figure 4. Small-scale antibody production in independent runs performed with (A) the Expi293 Expression System and (B) the ExpiCHO Expression System.** Each antibody was expressed in two independent runs, and each transfection was performed in triplicate. Antibody titers in the supernatants (left panels), purified antibody concentrations (center panels), and percent purity (right panels) were compared, and the correlation between each pair of runs was calculated.

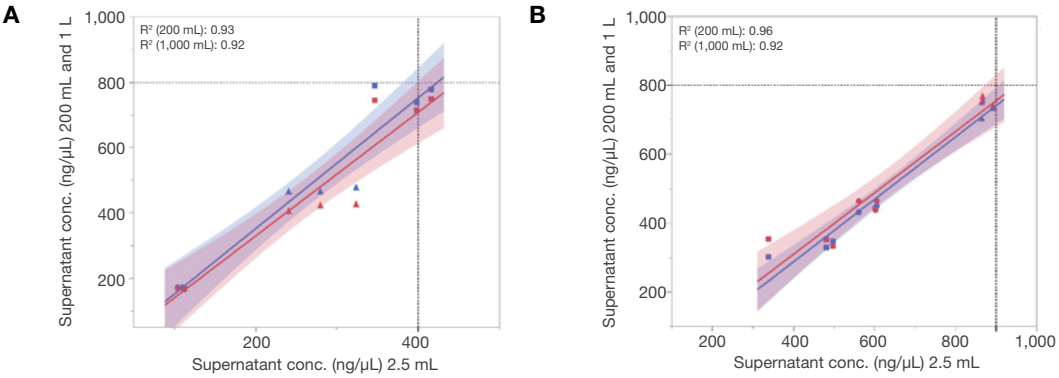


**Figure 5. Scalability of antibody production with (A) the Expi293 Expression System and (B) the ExpiCHO Expression System.** Three antibodies were expressed in individual experiments at three different scales. Each shape represents a different antibody. Antibody titers in the supernatants were measured after seven days of expression with the Expi293 system and ten days of expression with the ExpiCHO system. Antibody titers measured at 200 mL (blue) and 1 L (red) are plotted along the y-axes. The x-axes represent titers measured at the 2.5 mL scale, which is commonly used in initial research and development phases.

## Conclusion

In this study, the GeneArt HTP Antibody Expression Service consistently demonstrated robust performance with two cell lines at different scales. The platform can efficiently produce ready-to-screen antibodies with exceptional speed and reproducibility. This capability can help users efficiently screen antibodies and scale up production as needed. It also addresses the challenge of generating large volumes of high-quality data, which is necessary for training machine learning algorithms designed to predict the developability attributes of antibodies. By offering customizable options, providing reliable data, and supporting a wide range of applications, we help empower customers to achieve high-quality outcomes more quickly and consistently.

### References

1. Fath S, Bauer AP, Liss M et al. (2011) Multiparameter RNA and codon optimization: a standardized tool to assess and enhance autologous mammalian gene expression. *PloS One* 6(3):e17596.

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