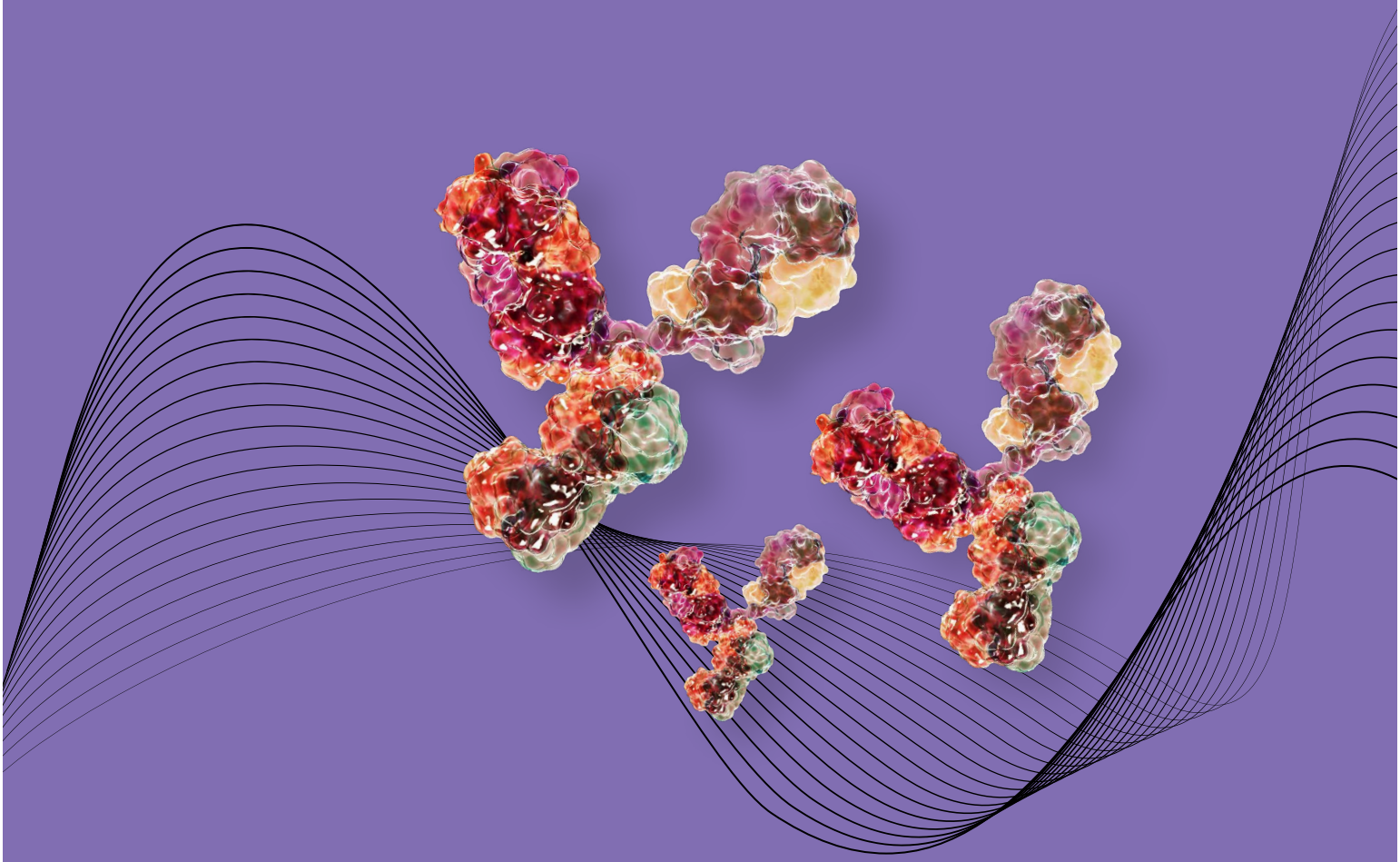
Genomics & Analytical Services

CASE STUDY

High-Throughput, Mid-Scale Production of Recombinant Antibodies



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Introduction

Recombinant antibody production is integral to most therapeutic antibody workflows. After screening *in vivo* or *in vitro*, leads undergo optimization and, if necessary, humanization. Engineering of antibody sequences to improve binding, specificity, stability, and function requires generation and testing of the purified protein. Transient antibody expression is typically used for research applications as it enables fast and parallel production without generation of stable cell lines[1]. Here, we discuss how recombinant antibody production at milligram scale by Azenta Life Sciences streamlined biologics research for a biopharmaceutical client.

The Challenge

Researchers at a top pharmaceutical company needed to produce over twenty monoclonal antibodies as part of their antibody discovery process. DNA constructs had to be synthesized and expressed to generate five to six mg of human IgG protein at high concentration while maintaining low endotoxin levels for downstream applications. The antibodies had not been previously produced, so expected expression levels were unknown. With resource constraints to complete the work internally, the pharmaceutical company approached Azenta Life Sciences to provide a high-throughput solution.

The Solution

Azenta Life Sciences used its gene-to-antibody workflow for recombinant antibody production (Figure 1). The variable regions of the antibody DNA sequences were synthesized and cloned into an expression vector provided by the customer. The constructs were verified by Sanger sequencing, and maxi-scale endotoxin-free preparations of the plasmid DNA were generated. Each antibody was transiently expressed in 250 mL of ExpiCHO™ cells, following internal protocols. Protein levels in the supernatant were evaluated by biolayer interferometry (BLI) using the Octet® platform, enabling the customer to make a "go" or "no-go" decision to proceed with purification. BLI analysis allowed the customer to identify and remove low or non-expressing samples from their project, saving both time and money during the subsequent purification. Single-step affinity chromatography purification was performed on the ProteinMaker™ system, and the final product was dialyzed into a customer-specified formulation buffer and sterile-filtered. Antibody concentration was adjusted to >13 mg/mL, and purity was assayed by SDS-PAGE. Endotoxin levels were measured, and subset of antibodies was tested for aggregation by analytical size exclusion chromatography (aSEC).

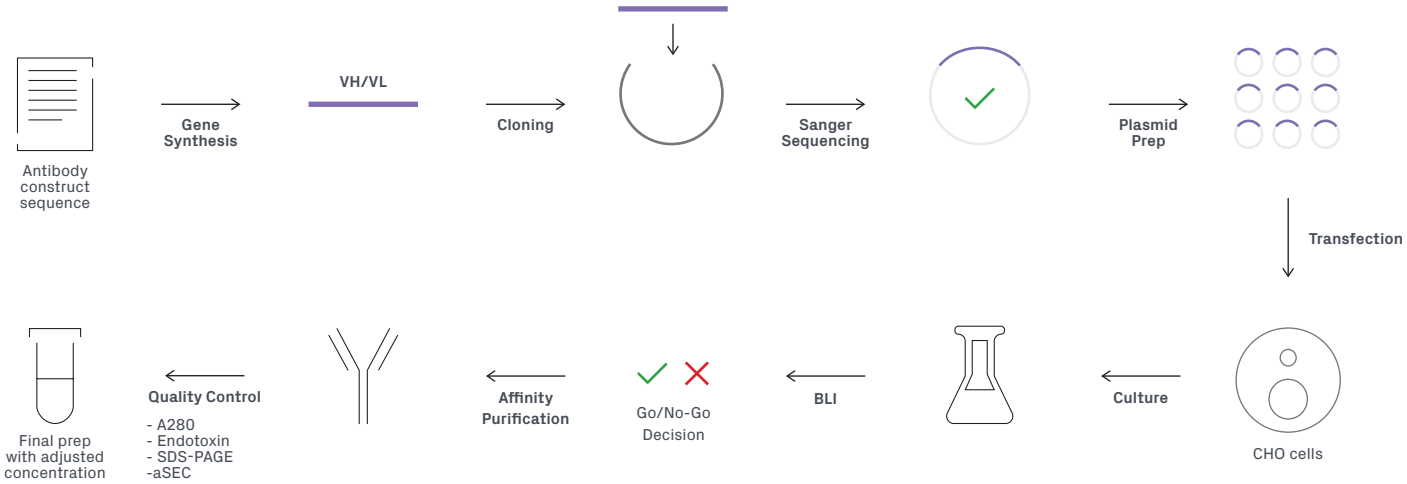


Figure 1: Recombinant antibody production workflow. The process starts with a nucleotide sequence provided by the customer. The DNA construct is synthesized, cloned, and verified by Sanger sequencing. The plasmid DNA is prepped and transfected into mammalian cells for expression. BLI analysis enables a checkpoint step to evaluate expression levels for each construct. Antibody protein is then purified and assayed for concentration and purity.

The Results

While achieving low endotoxin levels at such high concentration is difficult in recombinant antibody expression, Azenta Life Sciences delivered this with high-quality antibodies at the required concentration and yield (Table 1 and Figure 2). Protein purity was 89% or higher for all antibodies.

Antibody #	Concentration (mg/mL)	Yield (mg)	Endotoxin (EU/mg)	SDS-PAGE Purity (min)	aSEC Aggregation
1	14.1	29.2	0.09	90%	ND
2	14.8	65.8	0.06	90%	1.0%
3	14.0	62.3	0.08	90%	ND
4	14.6	32.9	0.13	90%	7.0%
5	14.8	75.2	0.10	90%	ND
6	14.8	56.0	0.08	90%	ND
7	14.0	31.7	0.14	90%	ND
8	14.0	32.3	0.10	95%	ND
9	14.6	7.8	2.2	98%	ND
10	14.7	22.7	0.16	95%	ND
11	14.3	35.4	0.14	90%	ND
12	14.8	42.2	0.10	95%	ND
13	14.1	24.3	0.13	90%	ND
14	13.9	25.6	0.13	89%	ND
15	14.5	18.1	0.28	95%	ND
16	14.2	7.8	1.1	98%	ND
17	14.1	26.7	0.16	90%	ND
18	13.8	8.7	1.8	98%	ND
19	14.0	20.3	0.15	90%	ND
20	13.7	16.2	0.04	90%	14.1%
21	14.0	29.7	0.15	95%	0.6%

ND: not determined

Table 1: Yield and quality data for recombinantly expressed human IgG1 and IgG3 antibodies.

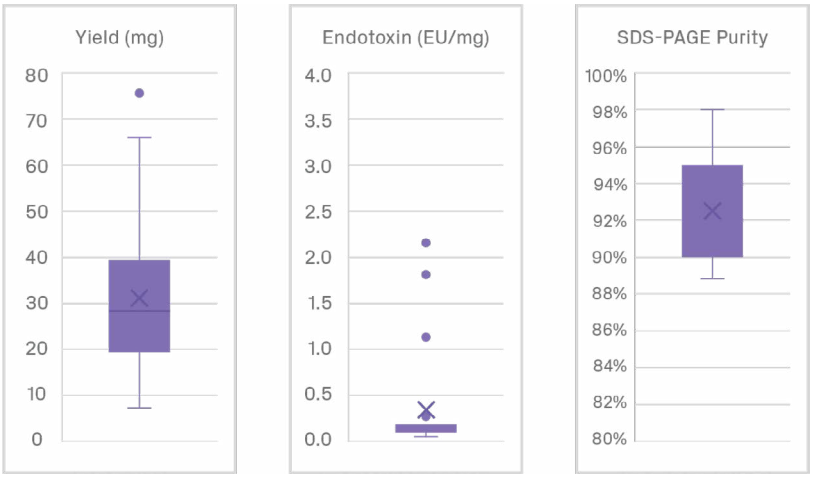


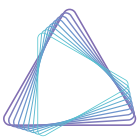
Table 2: Summarized data from Table 1.

Conclusion

A high-throughput recombinant expression workflow enables fast and efficient production of antibodies. BLI analysis was a critical step to ensure quality of the final recombinant product while also saving the customer project time and reducing project cost. Azenta Life Sciences has the capability to generate high-quality antibodies from DNA sequences to free up time and resources for pharmaceutical organizations.

Reference

1. Frenzel, A., Hust, M. & Schirrmann, T. Expression of Recombinant Antibodies. Frontiers in Immunology vol. 4 (2013).



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