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Case Study

An optimal upstream process development approach to increase the titers of the monoclonal antibodies (mAbs)

A comprehensive upstream development strategy must be devised at both the bench and pilot scales to develop the therapy in sufficient quantity and purity for regulatory approval and, eventually, commercialization. The decisions taken during upstream development, such as clone selection, DOE optimization, media and feed selection, and bioreactor scale, will ultimately determine the titers and purity of the biologics. This case study demonstrates how Aragen leveraged its almost two-decade of biologics knowledge to assist the client in developing an effective upstream process development strategy for a new mAb, resulting in a more than 100 percent increase in titers.

The project

The client was screening a specific Monoclonal Antibody (mAb) against a species of bacteria that causes several difficult-to-treat infections such as pneumonia and cystic fibrosis lung infection. This mAb candidate was being developed as a first-in-class adjunctive therapy. Aragen was approached to identify the high producing clone and to develop an upstream process development protocol for achieving greater therapeutic titers at different bioreactor levels, streamlining GMP manufacturing.

About the client

The client is a late-stage clinical development company leading the creation of transformative, first-in-class anti-infectives for life-threatening viral and bacterial respiratory infections. The company's pipeline of novel mechanism antibacterial and antivirals, sprung from its proprietary technology platforms, are designed to combat the growing public health threat of viral pandemics and antimicrobial resistant (AMR) bacteria.

Why Aragen?

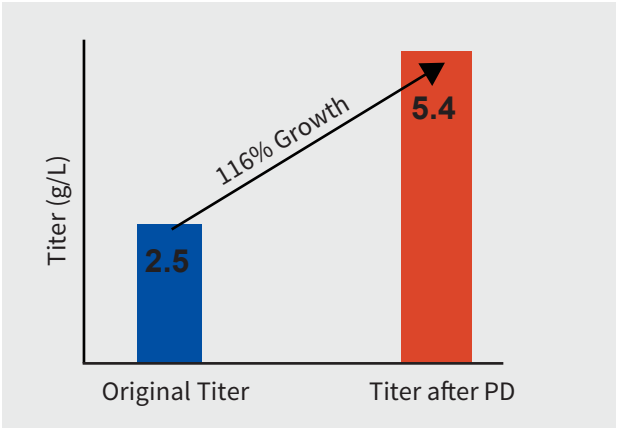
- Over 20 years of expertise in cell line engineering and upstream process development that yields higher results and supports regulatory filings.
- Experience of working on a variety of biologics and building their effective delivery roadmaps.
- World class infrastructure and high throughput analytical facilities at Morgan Hill, California spread across 47000 sq ft that allow us to upstream a variety of biologics in a timely manner.
- A robust IT infrastructure ensured complete data protection and IP security.

Aragen's approach

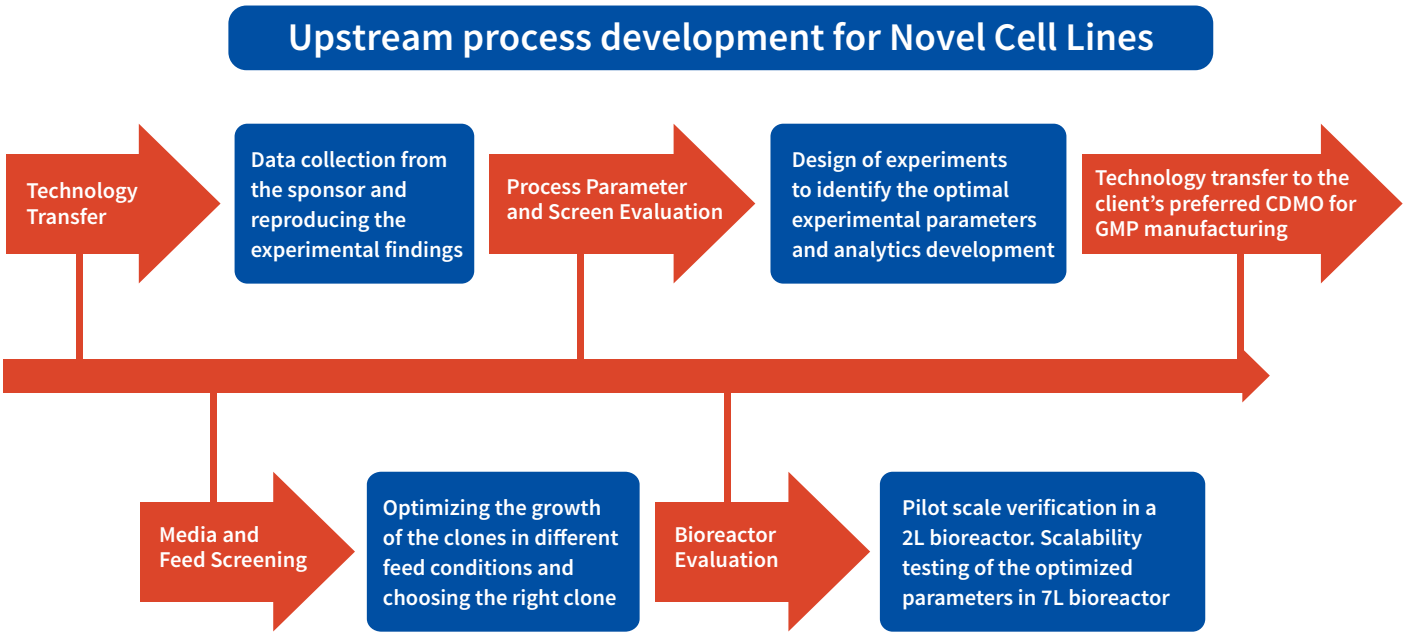
A multipronged approach was adopted to identify the high producing clone among the three developed by the client and to establish the optimum experimental parameters for upstream process development both at shaker flask and bioreactor levels. The cell line development team worked closely with the client's technical team for the tech transfer. Aragen's bioprocess technologists validated the optimum parameters at bench and pilot scale and all the analytics was performed by the developability team ensuring an unfailing and scalable upstream process. The process was developed in accordance with appropriate standards and was in accordance with industry best practices. All the complications and the associated risks in the developed process were consistently sought out and rectified to ensure the quality certified process to the client.

Abstract

The process development began with tech-transfer of three RCB clones from the client's laboratory to Aragen's. To identify the superior clone first step was to increase the quantity of all the three clones i.e., to generate the development cell banks (DCBs). Initial shaker flask studies were conducted with all three DCBs using media, feed, and experimental conditions like those of the client and based on metrics such as protein titer and other physicochemical characteristics one clone was selected for master cell bank (MCB) development. Media and feed screening were performed on the selected clone using a shaker flask, and eight different media were tested.



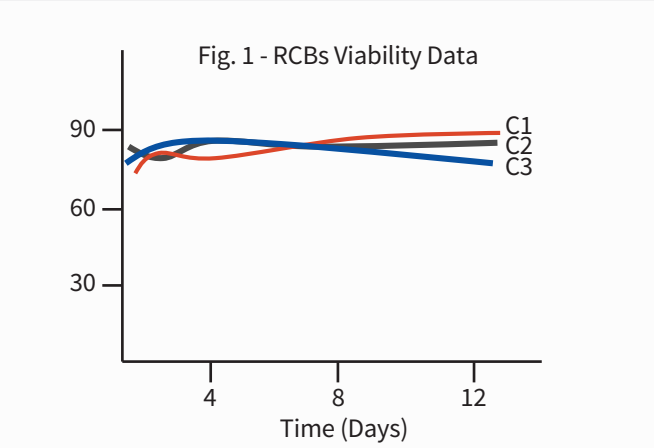
After extensive DOE in shaker flask, screen assessment, and parameter optimization, the process was translated to pilot scale bioreactors (2L). Paraments were further optimized in pilot scale and the product was analyzed for its physicochemical characters using high throughput analytical techniques. Optimised parameters were tested for scalability in 7L bioreactor and finally the optimised upstream process was transferred to client's preferred CDMO. The total increase in the titer after the bioreactor evaluation was equivalent to 116% with titer improving at every stage of the process development.

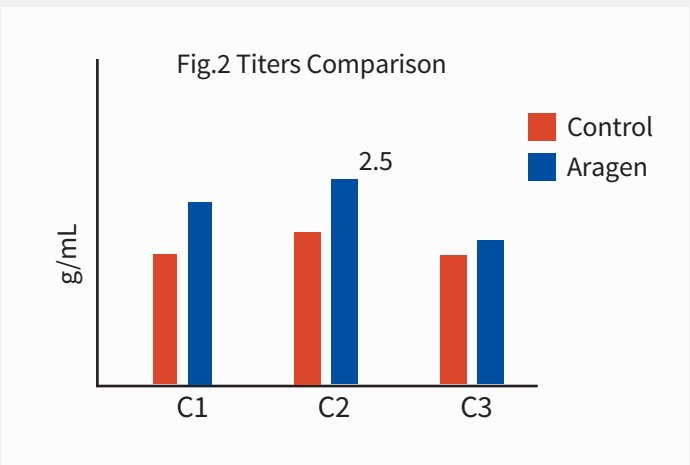


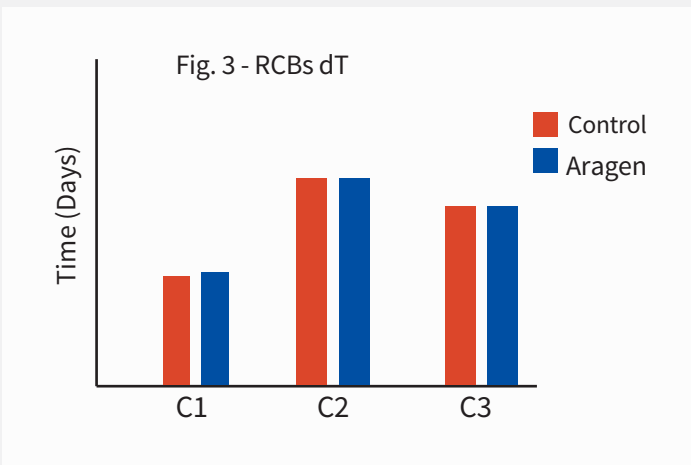
Process Development

Step 1: Technology transfer

- Tech-transfer of three clones (C1, C2, C3) from the client's laboratory to Aragen's facility at Morgan Hill, California and expanding them into DCBs.
- Seeding the cells at 0.5 cells/mL every third day for 12 days and evaluating the cell density, proliferation, and viability data.
- Result: All three clones showed a cell viability of 90% to 95% (Fig.1). Titers for all three clones (Fig.2) were comparable to those at client's lab. Highest titer of C2 ~2.5g/mL. Doubling time (Fig. 3) of all the clones was equivalent in both labs.
- Duration: 5-6 weeks





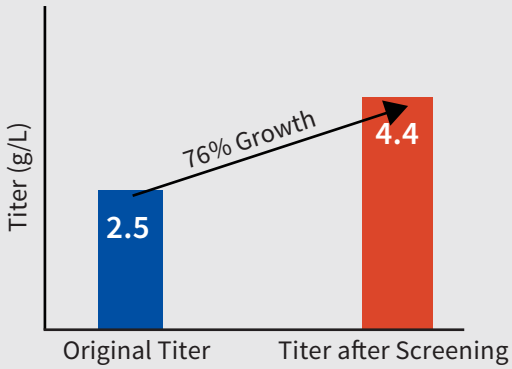


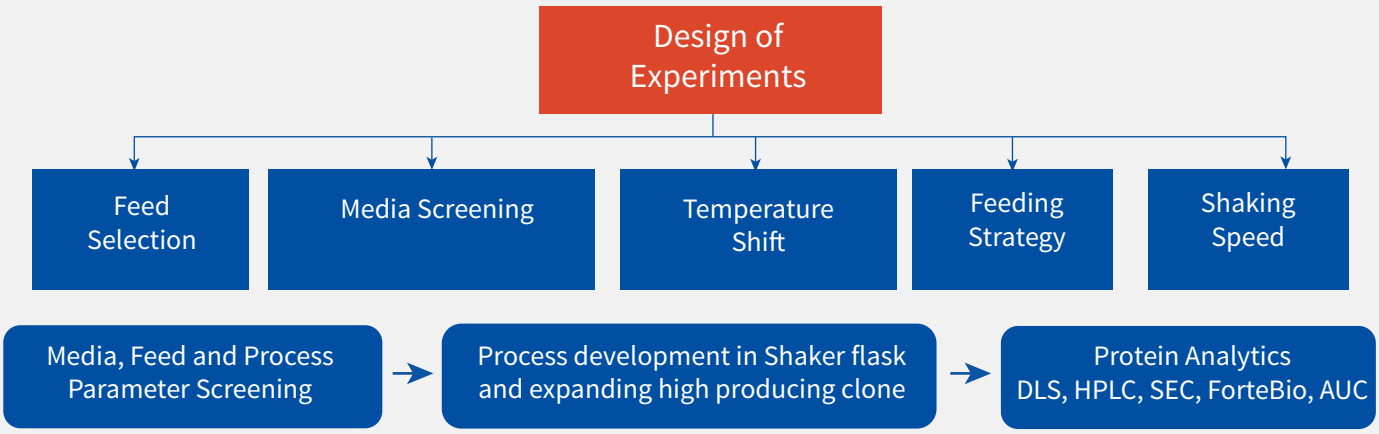
Step 2: Feed screening and selection of a clone

- Evaluation of different feeds for effects on productivity of the different clones.
- Titer of all three clones nearly doubled with cell boost feed.
- However, Efficient Feed C (EFC) feed had a titer that was almost equal to the control feed.
- Based on affinity data, potency testing and Aragen's experimental finding, clone C2 was selected for developing the upstreaming process.
- Duration: 4-5 weeks

Step 3 - Media and feed screening for the selected clone

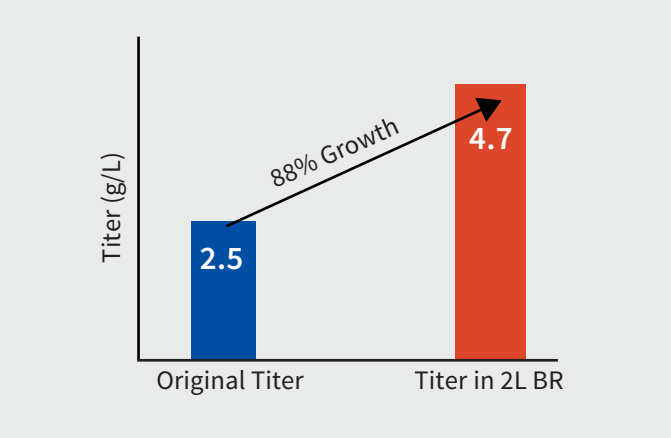
- Performed design of experiments and shaker flask studies to identify suitable media, control feed, feeding period, temperature shift, and shaking speed.
- Estimation of titers was done using two different methods i.e., HPLC and ForteBio.
- Duration: 3-4 weeks

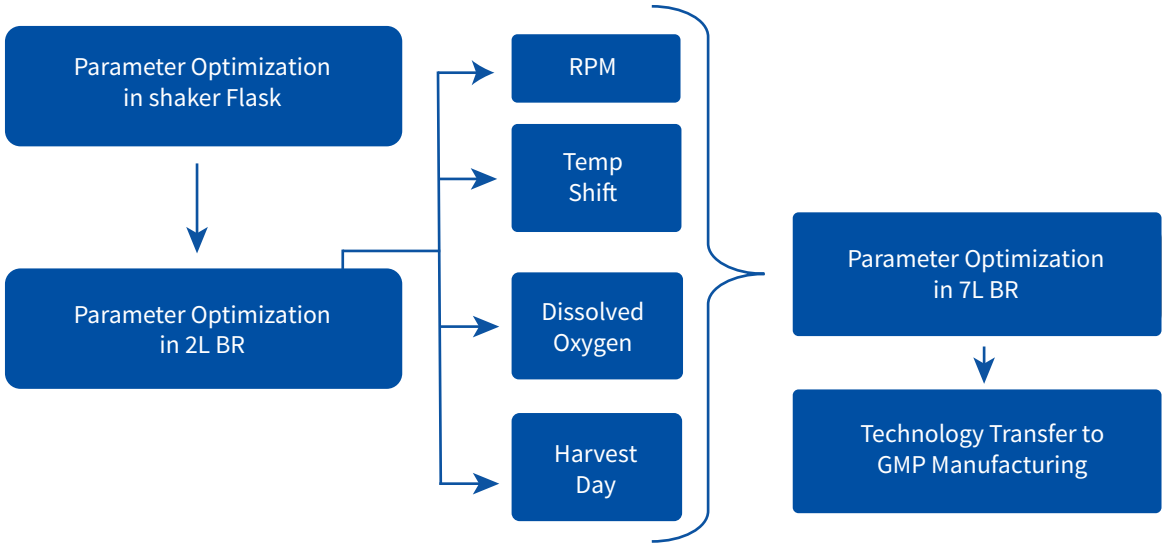




Step 4 – Process parameter and screen evaluation in 2L and 5L bioreactor

- Using ActiPRO and BalanCD medium, eight investigations carried out in 2L bioreactors using cell boost feed
- Observations: Acti-prio medium produced greater titers, sodium butyrate did not appear to promote expression, and lower RPMs were linked to higher titers
- Further evaluation in 7L bioreactor to ensure further scalability of the process.
- Duration:
  - process parameter screening in 2L bioreactor: 3 weeks
  - optimization in 7L bioreactor: 2 weeks





Project outcome

The total increase in the titer of the harvest was equivalent to 116% with titer improving at every step of the process development.

The efficient process development developed by Aragen was submitted to the client for scaling up to GMP manufacturing. Subsequently, the client successfully conducted Phase I trials.