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Five Intelligent Ways to Accelerate Cell Line Development

Biologics, also known as biopharmaceuticals, are manufactured using biotechnological methods. These products originate from biological sources usually involving live organisms or their active cellular components.[1] Biologics account for almost 50% of recent new drug approvals and include products such as vaccines, gene therapies, and recombinant proteins. Over the past four years, 84% of the biologics approved for therapeutic use were produced using mammalian cells.[2,3]

Cell Line Development: Challenges and Solutions

High-performing, stable and robust cell lines form the foundation of biologics production. However, as outlined in Figure 1, cell line development is logistically and technically demanding. Costly errors can occur and impede the process of drug development.

Gene Cloning and Initial Clone Selection

Expression vectors are transfected into a host cell line, creating a mixed population. Cells carrying the recombinant gene are isolated and proliferated.

Clone Selection and Confirmatory Analytics

Monocultures from the previous stage are screened and the most stable cells producing the highest titer of product selected.

Cultivation and Media Optimization

Growth conditions such as media, gaseous input, temperature and pH are optimized to maximize viability and output.

Cell Line Evaluation and Characterization

The best performing clones are fully characterized and critical quality attributes are evaluated.

Cell Banking

Selected clones are proliferated to a high density and frozen down in cryovials to create high quality cell banks.

Figure 1. The five stages of cell line development.[4]

Cell-based systems produce biologics that are heterogenous and difficult to purify when compared to chemically synthesized drugs. They are also more sensitive to manufacturing or environmental changes, and to microbial contamination. Hence, protocols must be carefully designed to minimize cell culture disruption and maintain optimal cultivation conditions.[1,5]

Despite the popularity of mammalian cell lines, they are complex to develop; the process can take 12-18 months on average and often has multiple bottlenecks along the way.[4] Making the right decisions early is key to avoiding costly delays, however this can be difficult with traditional manual techniques.

Automation can significantly reduce the time spent on intricate protocols, such as single-cell isolation, and also increase productivity, efficiency, multiplexing and reliability of processes. High-throughput capabilities allow multiple clones and growth conditions to be examined simultaneously, allowing key decisions to be made earlier. Automated filling of cryovials can increase batch size and ensure high-quality cell banks.

Here are five intelligent ways automated solutions can streamline cell line development, by minimizing bottlenecks, improving yield and reducing costs.

1. Rapidly screen and select clones

Transfecting an expression vector into a cell line results in a heterogenous population as not all cells will successfully express the gene of interest. Therefore, bulk analysis only provides averaged data and results in the loss of valuable information. This is not conducive to the production of biologics, which requires monocultures to achieve maximum yield.

Single-cell isolation is used to achieve homogenous populations, however current methods are challenging and have negative implications for cell health. Manual techniques, such as limiting dilution, are time consuming and have low rates of success paired with extensive use of consumables.[6] Other traditional methods, such as fluorescence activated cell sorting (FACS), offers a faster workflow for isolating subpopulations, but the process has been shown to cause cell damage and reduce viability.[7]

A high-throughput single-cell isolation platform such as the CellCelector Flex (Figure 2) increases the efficiency and speed of clone selection, while reducing the need for consumables. The automated screening, selection and isolation process allows for one-round cloning from liquid and semi-solid media. Plates containing up to 130,000 nanowells in a single plate allow seeded cells to share the same media and support each other's growth, without compromising monoclonality. The CellCelector's ability to screen, select and isolate ideal clones in a single, one-day workflow, with 100% proof of monoclonality, makes it the ideal alternative to traditional methods.

Figure 2. The CellCelector single cell and colony picking platform.

2. Identify high-productivity clones early on

The goal of cell line development is to produce a monoculture expressing desired characteristics, such as the production of a specific antibody. For biologics, these characteristics, known as critical quality attributes (CQAs), must fall within a specific range to ensure the efficacy and safety of the product.[8] CQAs should be defined and validated early on in the cell line development process to reduce time lost pursuing poorly performing clones. This also gives confidence that an observed effect is reproducible in later stages. Often, the methods for identifying optimal clones are time consuming, single endpoint assays, such as enzyme-linked immunosorbent assays (ELISAs). The process required to examine CQAs and measure protein output is therefore a significant bottleneck with multiple, lengthy assays.

The iQue® Advanced Flow Cytometry Platform (Figure 3), combined with the iQue® Human IgG Titer and Viability Kit is a high-throughput analysis platform that can use as little as 10 µL to simultaneously report on antibody output, specific productivity and cell viability. The no-wash, no-dilution workflow reduces variability of results, while the walkaway automation with rapid protocols reduces time cost.

For non-destructive analysis of clones, the Octet® BLI Label-Free Detection System (Figure 4) provides real-time, label-free protein quantitation in as little as two minutes. As an alternative to ELISAs and high-performance liquid chromatography, the Octet® can examine a variety of parameters, including antibody titer and binding, and glycosylation. Using both purified and unpurified samples, this biolayer interferometry platform can select optimal clones with the desired critical attributes of products.

Both of these automated, high-throughput solutions can relieve bottlenecks, and give more in-depth insights for clone ranking, to obtain rapid, actionable results early in the cell line development process.

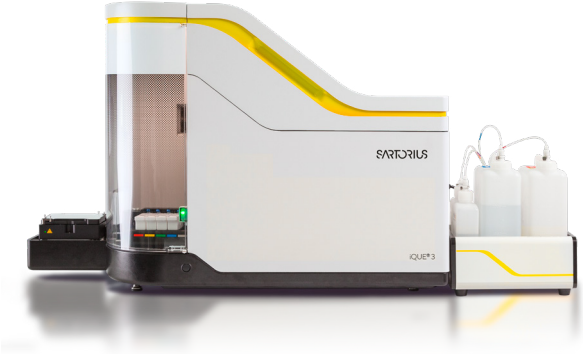

Figure 3. iQue®Advanced Flowcytometry Platform.

3. Screen cultures in parallel

Sub-optimal feeding and growth conditions can negatively impact protein yields and even cause the loss of desirable clones. Using design of experiment (DOE) studies to identify, characterize and validate the cell culture conditions, allows cell lines to function at peak capacity.[9] Traditionally, this process has been performed in individual bioreactors or shake flasks, a time-consuming process requiring large amounts of space. To comply with good manufacturing practice (GMP) guidelines, it is important to identify optimal media composition early on. Any changes made during later stages of clinical development may be considered a significant amendment to the manufacturing process and require extensive validation.[10]

The Ambr® 15 Cell Culture (Figure 5) is an advanced, automated microbioreactor system for up to 48 parallel cultures under aseptic conditions. Each individual bioreactor has its own atmospheric controls, including gas composition, pH, temperature, and stirring setpoints, vastly accelerating media and condition optimization. Additionally, the Ambr® 15 Cell Culture control software allows for the creation of multiple media blends directly in the microbioreactors. This automated, fine-tune control over culture and environmental conditions can increase viable cell counts by up to 20% compared to standard shake flasks,[11] while also reducing human error. The option for integrating accessories, such as cell counters and metabolite analyzers, further streamlines the process.

The Ambr® 15 Cell Culture bioreactor system enables rapid selection of optimal environmental settings, media and feeds under small scale bioreactor conditions in preparation for scale up to a manufacturing environment. The bioreactor system significantly reduces labor, laboratory space, consumables and time compared to traditional shake flask protocols.

Figure 4. Octet®BLI Label-Free Detection System.

Figure 5. Ambr® 15 Cell Culture microbioreactor.

4. Quickly evaluate and characterize the best clones

In order to determine the ideal media and environmental conditions, CQAs must be examined during the bioreactor stage. This can also help to select the best clones for the next stage of development. Performance is usually based on cell growth and viability, metabolite analysis and product titer. Assays for these analyses can often be lengthy and samples may need to be submitted to other laboratory groups.

The Ambr® 15 Cell Culture bioreactor system and the Octet® BLI Label-Free Detection System can be combined to reduce analysis turnaround and streamline CQA evaluation. The automated liquid handler of the Ambr® 15 can take daily samples from all the reaction vessels, ready for direct transfer to the Octet®. This label-free detection system can then perform high-throughput product analytics without the need for sample processing. One measurement can give information on binding kinetics and specificity, glycosylation and product titer in real time. This allows for identification of the top-performing clones, and the best growth conditions while significantly reducing hands-on time compared to traditional sampling and analysis methods.[12]

5. Automate cryovial cell banking

Once the highest performing clones have been selected and cultured to high cell densities, the final stage in cell line development is cell banking. Rapid, aseptic filling of cryovials is essential to comply with GMP standards, however this can be difficult using manual methods, which often create a bottleneck. There are many challenges associated with manual cell banking including, variations in volume, batch size constraints, process times and injury due to repetitive strain. Introducing a simple, automated cryovial processing system like the Fill-It (Figure 6) easily overcomes these issues and prepares consistent, high-quality cell banks. The Fill-It shows greater accuracy and precision than manual dispensing, and the viability of revived cells is comparable with manually prepared vials.[13] The Fill-It is a compact bench-top device, able to fit inside a standard biological safety cabinet. The automated filling of up to 96 cryovials allows cell banks to be prepared in a single batch, reducing quality control costs. The rapid, precise filling of large numbers of cryovials at once makes it the ideal solution to cell banking bottlenecks.

Figure 6. The Fill-It automated cryovial filling system.

Conclusion

Cell line development is one of the most time consuming processes in biomanufacturing; it often takes biobanks up to 18 months to produce high-performing, GMP-compliant cell lines.[2] As the demand for biologics increases, biomanufacturing methods must also improve to meet it. Sartorius offers a range of high-throughput, automated solutions for the rapid development of cell lines that produce high yields of recombinant proteins. These solutions can also remove bottlenecks, reduce costs and ensure early selection of the best-performing clones, ensuring a more streamlined cell line development process.

Learn more about accelerating cell line development

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