The BioContinuum™ Seed Train Platform

The gateway to next generation bioprocessing

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seed train intensification

The gateway to next generation bioprocessing

The seed train is a critical part of the bioprocess, generating sufficient biomass to inoculate the production bioreactor and begin protein production in an optimized manner. In a traditional seed train, this is achieved by passaging cells from a working cell bank vial through increasingly larger cultivation systems including shake flasks, rocking motion bioreactors, and stirred tank bioreactors.

The typical seed train process is time- and labor intensive, requiring operators to transfer and cultivate the cells manually. It also has an increased risk of contamination due to the open nature of the early stages of cell expansion, when cryovials are thawed and cells are manually transferred under a laminar-flow hood. The goals of seed train intensification are to lower manufacturing costs, achieve higher process throughput, increase flexibility, and reduce risk. This can be achieved by implementing perfusion operations in cell bank manufacturing to accelerate expansion and achieve greater cell density in the N-1 bioreactor and/or using high cell density cryopreservation methods.

The BioContinuum™ Seed Train Platform

With novel technologies, proven applications, and expert support, the BioContinuum™ Seed Train Platform empowers biomanufacturers to confidently move into next generation bioprocessing using seed train intensification.

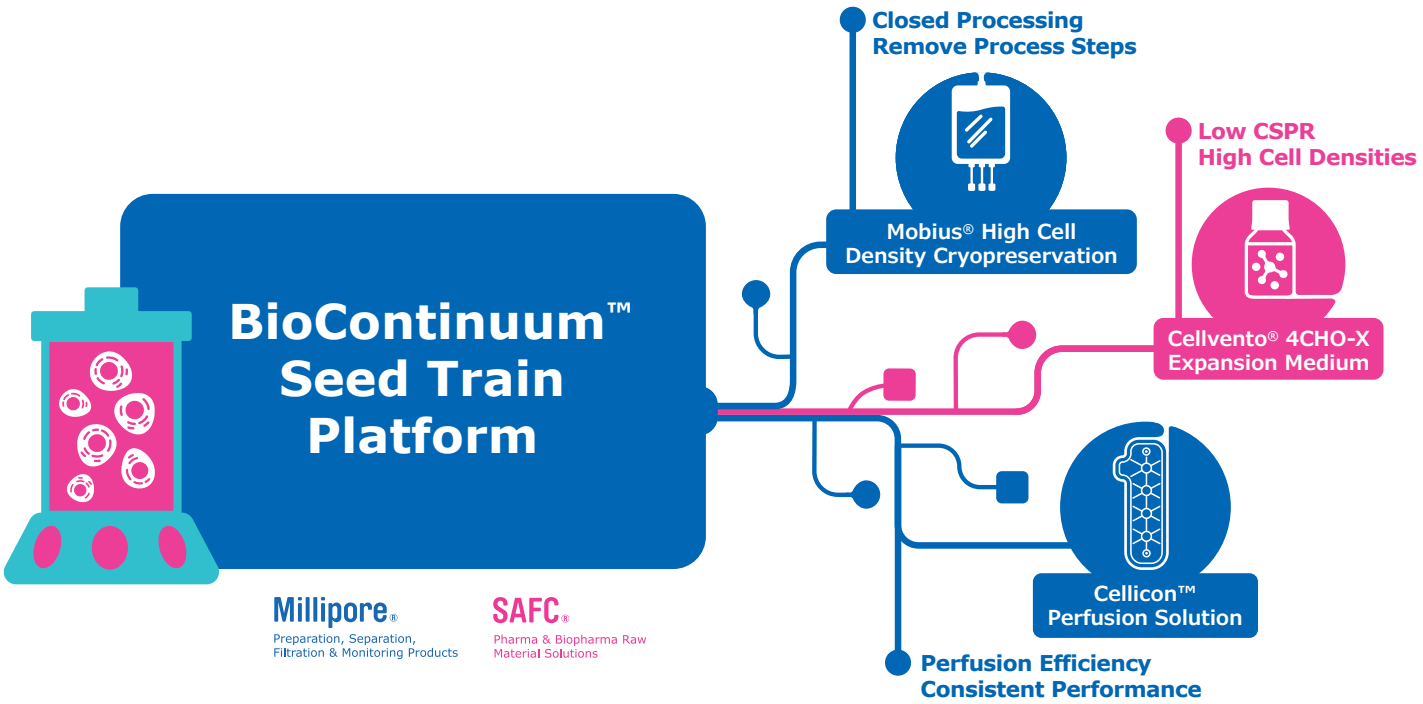
The BioContinuum™ Seed Train Platform provides an integrated and versatile solution to unleash the potential of perfusion, making the seed train faster, safer, and offering an unprecedented advantage for biomanufacturing. Each component of the platform is agnostic of the manufacturing template already in place and can be integrated into a fully compatible solution or used in a standalone approach. Using these technologies, we have developed workflows that can be transferred to accelerate process development and technical transfer.

The BioContinuum™ Seed Train Platform includes the following solutions for seed train intensification:

The **Cellicon™ Perfusion Solution** increases perfusion process efficiency and provides real-time monitoring and control for reliable and consistent performance.

Cellvento® 4CHO-X Expansion Medium is a chemically-defined cell culture medium specifically developed for N-1 perfusion for Chinese Hamster Ovary (CHO) cell lines. Using a balanced nutrient concentration, biomanufacturers can achieve high cell densities at low cell specific perfusion rates (CSPRs).

The **Mobius® HCDC R&D Assembly** is designed to facilitate the freeze and thaw of high cell density cell banks, eliminating the need for time-consuming manual scale-up steps and enabling risk reduction through closed banking and inoculation processes.



The Advantages of Perfusion in the Seed Train

In a perfusion process, fresh cell culture medium is introduced into the bioreactor at a constant rate while spent medium is removed, and cells are returned to the bioreactor using a cell retention device. With this approach, nutrient supply remains sufficient and waste products remain lower and stable over time, allowing increased cell mass in the bioreactor.

Perfusion has a long history in the biopharmaceutical industry and was initially used with adherent cultures for difficult-to-express proteins and those known to be sensitive to culture conditions. Industry moved away from perfusion as suspension cultures and the yields from batch processes improved. In the mid-1990s, attainable cell concentrations using fed-batch processes were about 5 × 10^6 cells/mL, with product concentrations of 1–2 g/L. Today, attainable concentrations are greater than 15 × 10^6 cells/mL, with product concentrations of up to 10 g/L. Although these numbers are impressive, pressure to reduce manufacturing costs and increase flexibility in the upstream setting continues to drive interest in perfusion and other intensified production processes.

Perfusion-based cell culture processes are well suited to new manufacturing environments and are enabled by the growing adoption of single-use technologies. Perfusion operating modes support high density cell cultures in both the seed train and production bioreactors (**Figure 1**).

By moving to a perfusion-based process, biomanufacturers can realize several key benefits over fed-batch cultures. These benefits includes higher volumetric productivity, increased flexibility, more consistent product quality, and lower capital investment. These benefits are achievable as the number and size of bioreactors can be reduced while maximizing their full potential.

While the biopharmaceutical industry has leveraged improvements in bioreactor operations, cell culture media formulations, and cell line engineering, the transition from fed-batch to perfusion production processes can be challenging due to the complexity of the process and the need to maintain a robust process over a long period of time. Introduction of perfusion technologies into the seed train is an efficient way to begin the transition toward next generation bioprocessing while maintaining compatibility with both fed-batch and perfusion production operations.

The seed train can be intensified in two critical ways: first, during the last phase of the seed train in the N-1 bioreactor to increase biomass production, or second, by utilizing high cell density cryopreservation (HCDC) to gain further flexibility and transition to a closed scale-up process.

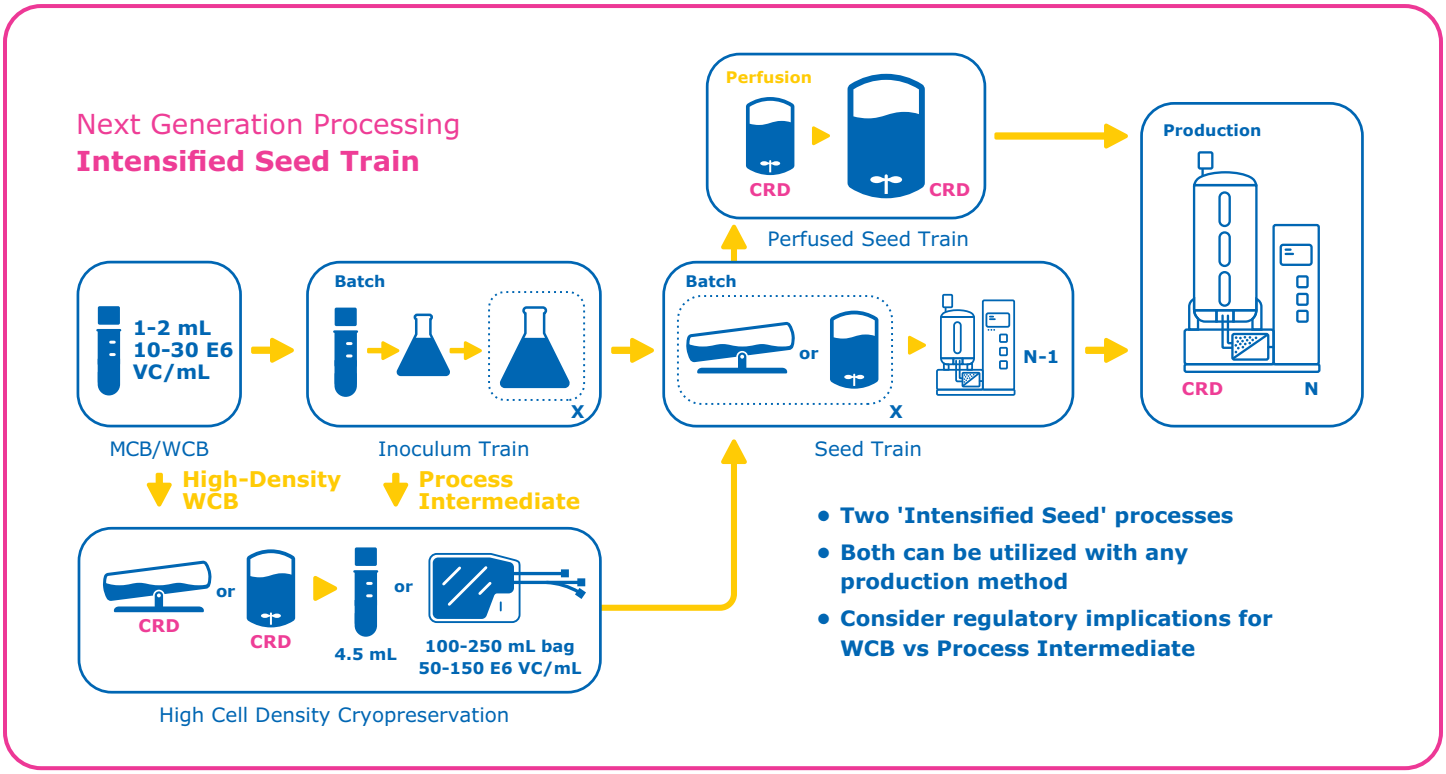


Figure 1. Perfusion can be used to increase viable cell density in the seed train to inoculate the production bioreactor, operating in either fed-batch or perfusion mode, with a higher cell density.

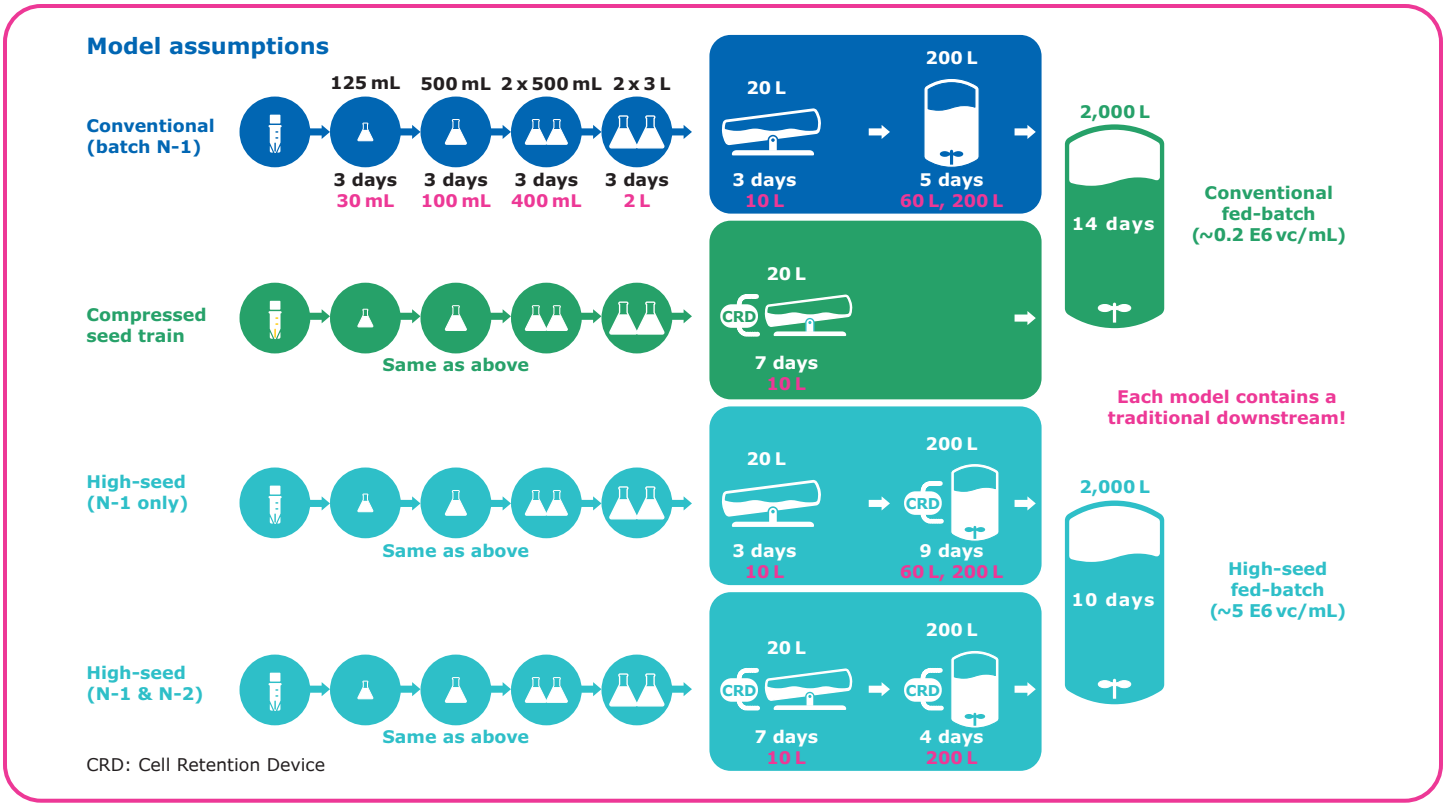


Figure 2: Description of representative seed train processes using N-1 perfusion.

N-1 perfusion

Introduction of N-1 perfusion can be considered in the context of four different scenarios (representative examples described in **Figure 2**). A conventional seed train, using a combination of flasks and batch-based N-2, N-1, and N bioreactors, is described in the first scenario.

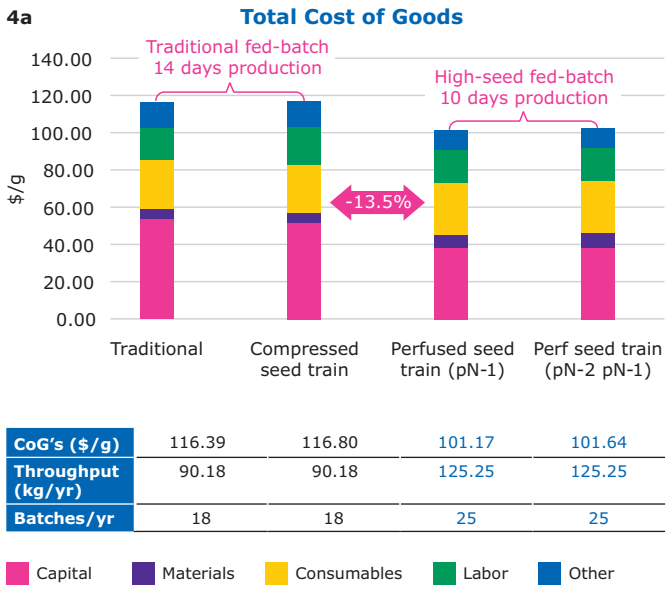
In the second scenario, demonstrating a compressed seed train, N-1 perfusion is used to used to inoculate a standard fed-batch process. In this case, a single, smaller bioreactor can be used to seed the same production bioreactor without requiring significant changes in the production process.

In the third scenario, N-1 perfusion is used to seed the production bioreactor with a 25-fold higher initial cell density, referred to as a high-seed fed-batch process. In the fourth scenario, an alternative bioreactor configuration using perfusion for both the N-1 and N-2 bioreactors is demonstrated, showing similar results. This is a highly impactful change that requires use of N-1 perfusion. Production time is significantly shortened by reducing the requirement for cell expansion within the production bioreactor. Using this high seed fed-batch approach, biopharmaceutical manufacturers have consistently reported 20-50% increases in titer, which are largely dependent upon the cell culture media formulation. This increase is mainly due to a larger cell population secreting the protein of interest rather than an increase in cell specific productivity.

In both cases, clear efficiencies emerge with respect to equipment footprint and CAPEX needs. However, in the second scenario, where the seed train is compressed but the production process remains unchanged, these savings are offset by the increased cell culture medium demands. In contrast, by using N-1 perfusion to enable transition into a high-seed fed-batch production process in the third and fourth scenarios, a nearly **14% reduction in CoGS** is obtained (**Figure 3**), and **facility throughput increases more than 35%** due to the shorter production run length.

Our white paper on cost modeling of the perfused train has many more details to show you how your manufacturing costs can be reduced through seed train intensification. Find it here: https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/159/870/cost-analysis-of-perfused-seed-train-scenarios-wp5879Een-ms.pdf

Constant production titer 4 g/L



CoG's ($/g)	116.39	116.80	101.17	101.64
Throughput (kg/yr)	90.18	90.18	125.25	125.25
Batches/yr	18	18	25	25

Figure 3: Total cost of goods process modeling for a perfused seed train. Process models were developed using BioSolve® (Biopharm Services).

High Cell Density Cryopreservation (HCDC)

The traditional process of thawing a single vial of cells to initiate cell expansion for a commercial manufacturing batch is time- consuming and requires open cell culture operations, increasing the risk of contamination (**Figure 4**). Use of cells banked at high density and high volume, which can be fed into the first seed train bioreactor, can streamline the overall process.

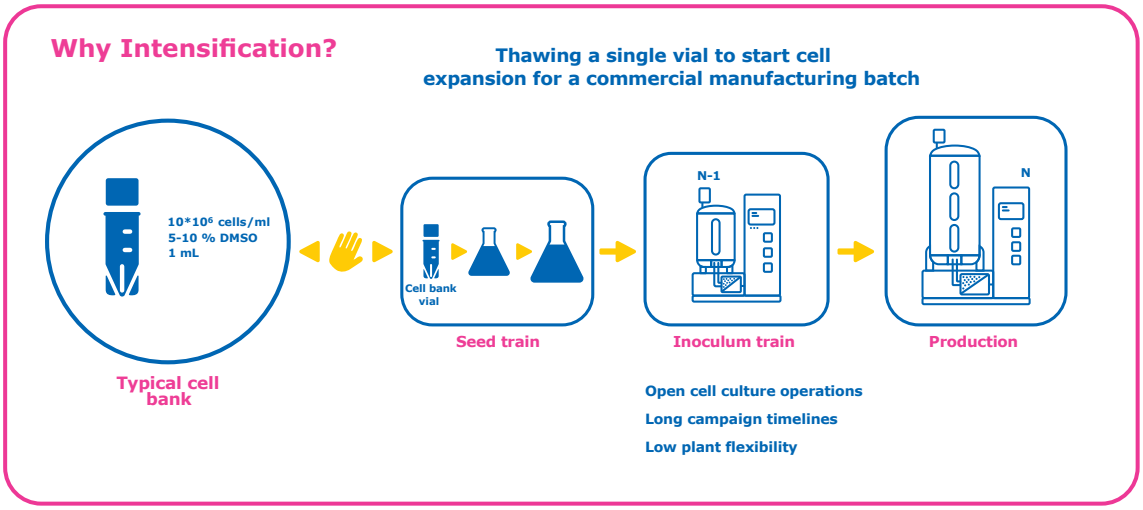
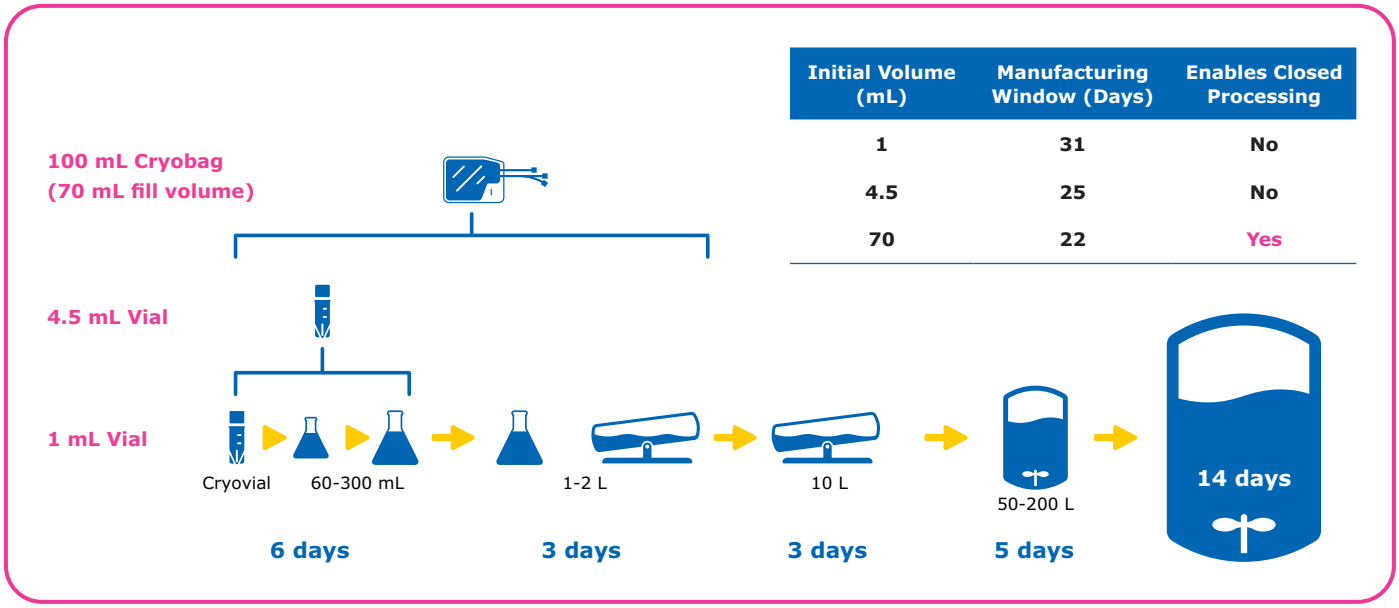


Figure 4. Thawing a single vial of cells to start expansion is a time-consuming process.

To reduce the duration of cell expansion, higher density cell banking approaches in larger vials (5 mL) have been used. This approach results in some improvements but does not eliminate the contamination risk due to manual transfer of cells. The shortcomings of this approach are overcome with use of high-cell density cryopreservation (HCDC) single-use assemblies, in which single-use bags containing larger volumes of high cell density cell banks (50-150 x10^6 cells/ mL) are produced using a perfusion process.

Traditional seed train expansion can require up to 20–30 days before inoculation of the production bioreactor. In contrast, incorporation of HCDC into the process enables a significant reduction in the time required to inoculate the production bioreactor.

In the example shown in **Figure 5**, a 70 mL bag of 50 x 10^6 cells/ mL reduced the standard process by ten days as compared to starting with a 1 mL vial of 10 x 10^6 cells/mL.



Initial Volume (mL)	Manufacturing Window (Days)	Enables Closed Processing
1	31	No
4.5	25	No
70	22	Yes

Figure 5. Use of single-use bags with high cell densities generated by perfusion processes reduces the seed train length and enables closed processing.

In addition to time savings, specially designed cryobags eliminate open cell culture operation steps, lead to better reproducibility in seed train expansion, and decouple cell expansion and batch production. Unlike vials requiring expansion during each campaign at the production site, cryobags can be distributed globally from a central expansion facility to global production facilities, improving the consistency of the inoculum of the production bioreactor (**Figure 6**).

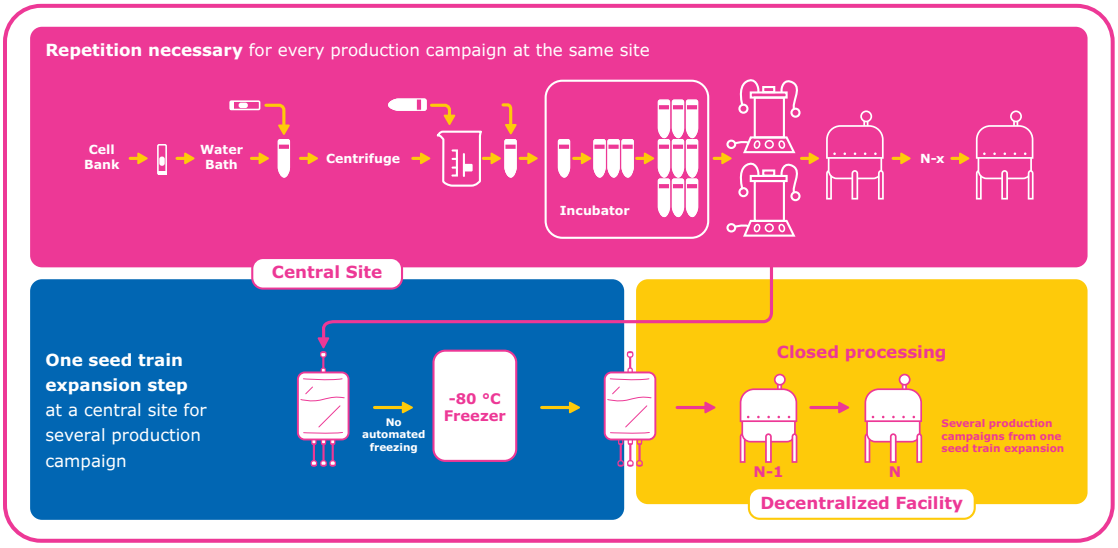


Figure 6. HCDC enables one seed train expansion step at a central site to support several production campaigns, including decentralized manufacturing facilities.

Cost Modeling: Enabling streamlined seed train production

It is essential to consider how adoption of new technologies like HCDC can benefit the ongoing risk mitigation efforts in your manufacturing process. By moving away from traditional open scale-up processes, HCDC supports your transition into a closed process that reduces risk of contamination. Process development is less constrained by the requirements for rigorous characterization of cell banks, and introduction of HCDC may be easier to implement in these processes. However, the regulatory requirements for testing of master and working cell banks are well-described and can be applied to a variety of cell banking scenarios. Careful and proactive consideration of the cell bank characterization requirements will enable adoption of HCDC processes in the development of highly-characterized cell banks for manufacturing processes.

In comparison to a traditional seed train process, HCDC also provides clear cost and resource advantages (**Figure 7**), especially when paired with N-1 perfusion.

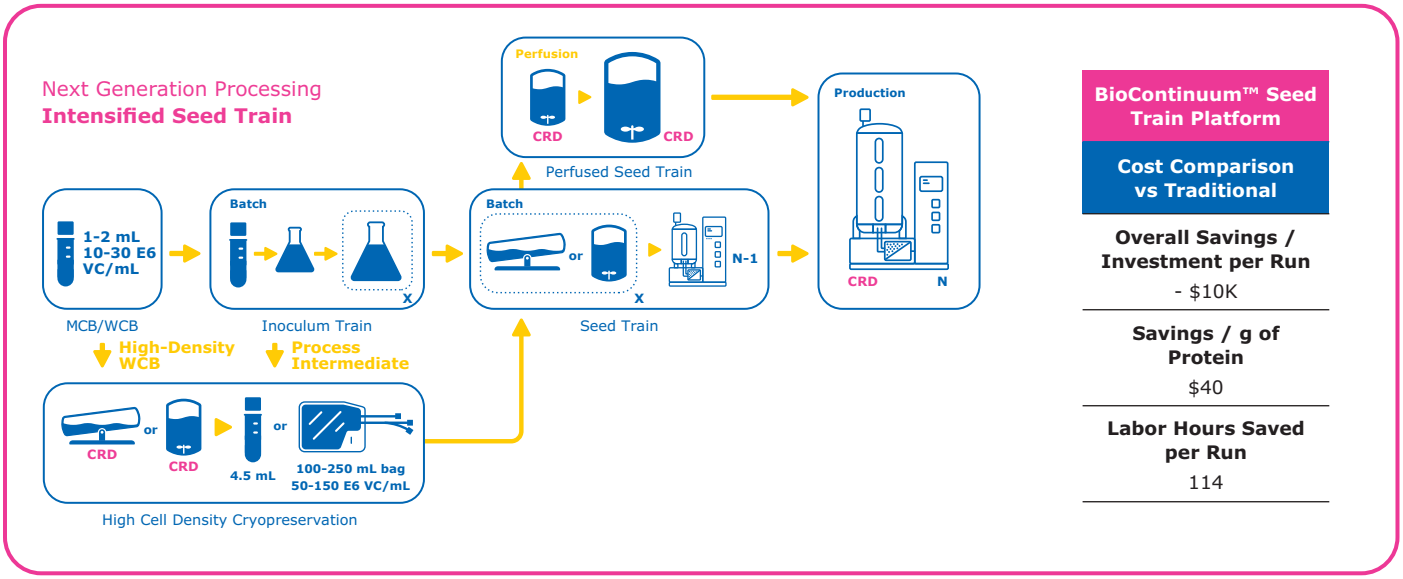


Figure 7. N-1 perfusion and high cell density cryopreservation enable significant cost reductions and reduce labor requirements.

Mobius® HCDC R&D Assembly

Filling and inoculation using a Mobius® HCDC R&D assembly is straightforward and safe. These single-use assemblies are compatible with storage at -80°C, supporting user needs throughout process development and in other process settings where storage at -80°C is desired. In the example shown in **Figure 8**, the HCDC process begins by filling a 250 mL cryobag with 50 mL of the cryopreservation medium (including DMSO) followed by 100 mL of the cell suspension, which is transferred via a direct connection from the bioreactor. Once a cryobag is filled with the prepared freezing medium containing the cells and mixed thoroughly, it is easily disconnected via the NovaSeptum® crimping tool, placed in a protective case and stored in the freezer at -80 °C. The protective case preserves the integrity of the cryobag throughout the freezing and thawing process. For inoculation, the cryobag is removed from the freezer, thawed under temperature-controlled conditions, and connected to the seed train bioreactor for inoculation.

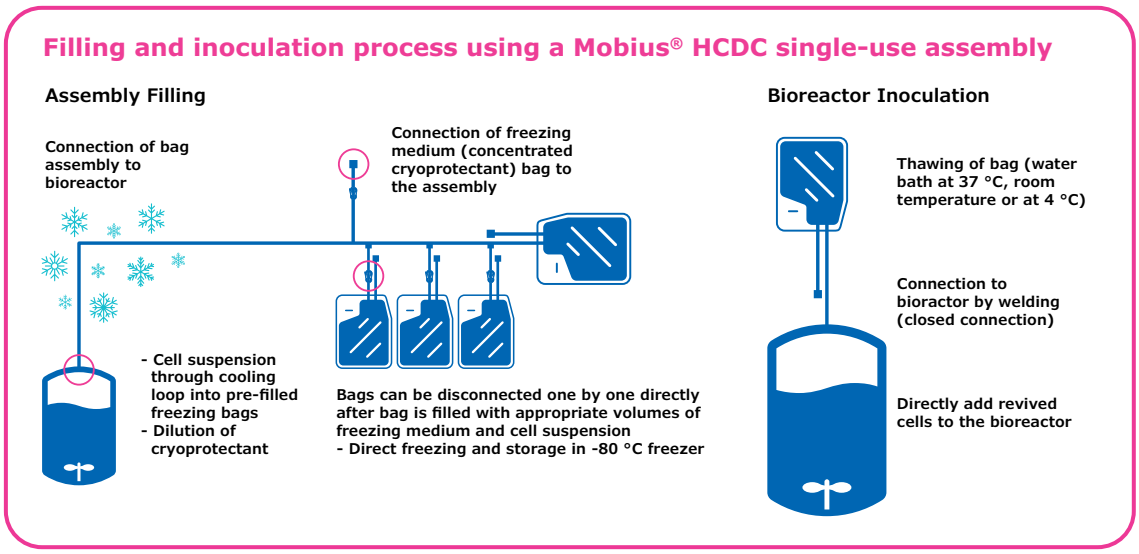


Figure 8. Filling and inoculation process using a Mobius® HCDC R&D single-use assembly.

Figure 9 shows the cell growth and titer from an N-1 bioreactor inoculated from a Mobius® HCDC R&D Assembly and another bioreactor seeded with inoculum originated from a standard expansion from a vial; both bioreactors were run in perfusion. Subsequently, two additional bioreactors were inoculated with cells from the first two bioreactors, run in perfusion to generate sufficient biomass, and transitioned into a steady-state perfusion production process at 50 x 10^6 viable cells/mL. Results were comparable, confirming that the HCDC application can be implemented while preserving the expected cell performance from a traditional scale-up process.

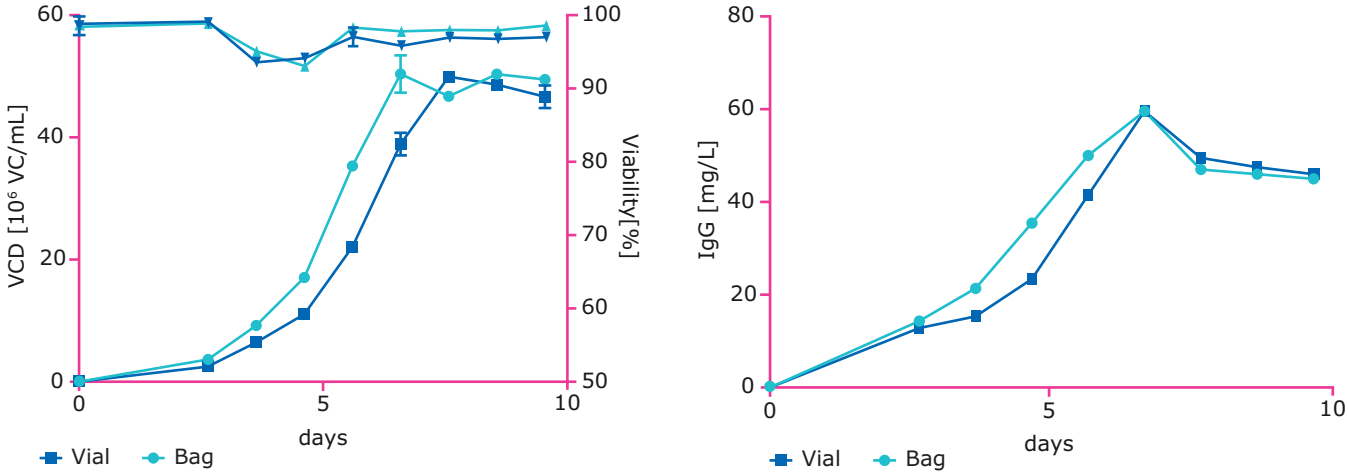


Figure 9. Cell growth and productivity comparing processes inoculated from a traditional vial-based cell bank and a Mobius® HCDC R&D Assembly; N-1 bioreactors were cultivated under identical conditions (data not shown) prior to inoculation of the N production bioreactor, displayed here.

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Lower Risk of Contamination and Better Reproducibility

Using HCDC assemblies in a seed train process removes the requirement for manual transfer of cells between the vial and the culture vessels, reducing the risk of accidental contamination early in the seed train by supporting a closed process. Furthermore, by including HCDC methods alongside other seed train intensification methods like N-1 perfusion, the value of closed processing is even greater, as fewer culture vessels are required to achieve a similar biomass.

The HCDC method also improves batch reproducibility compared to the traditional seed train method. HCDC enables one seed train expansion step to be stored for use in more than one manufacturing campaign, allowing each bioreactor run to start at an equivalent starting point.

Our Mobius® HCDC R&D assembly is a single-use assembly specially designed to facilitate the freeze and thaw of cryopreserved cells at -80 °C. Available in two assembly bag sizes of 100 mL and 250 mL, Mobius® HCDC R&D assemblies enable seed train inoculation with cells at a higher cell density and larger volume compared to the traditional seed train process using vials, which significantly accelerates the cell expansion process. Our Mobius® single-use assembly also provides a closed processing environment which prevents contamination and reduces process risk.

Cellvento® 4CHO-X Expansion Medium

Media optimization can have a significant impact on growth and productivity in upstream process steps, especially for intensified processes. Cell culture media formulations can be optimized for specific phases, such as clone selection, cell expansion, cryopreservation, and production (**Figure 10**).

Specialized media formulations based on a foundational platform can effectively direct cell metabolism toward growth or production. Using the Cellvento® cell culture media platform, Cellvento® 4CHO-X delivers key advantages for seed train intensification:

- Prevent cell damage during freezing and thawing
- Ensure fast growth with minimal or no lag phase after thawing
- Support a constant growth rate and productivity over thaw, expansion, and production
- Minimize cell stress during transitions to different media formulations between thaw, expansion, and production so as not to slow timelines
- Achieve high productivity at low perfusion rates

Cellvento® 4CHO-X Expansion Medium has been specifically formulated to control the metabolic profile of the cells, both to minimize toxic byproducts and to ensure adequate nutrient levels for high cell densities. Preventing nutrient depletion can increase robustness and predictability of seed train operations, setting the stage for optimized performance in the production bioreactor. By deliberately designing this formulation for compatibility with both fed-batch and perfusion processes, the requirements for lengthy adaptation into a different medium can also be avoided.

This medium supports high growth and viability including in N-1 perfusion, thus increasing cell biomass for cryopreservation or inoculation of another reactor. Furthermore, the medium improves IgG productivity in the N bioreactor when used in a production process with EX-CELL® Advanced HD Perfusion medium (N perfusion) and in fed-batch production with Cellvento® 4CHO medium and Cellvento® 4Feed. Cellvento® 4CHO-X Expansion Medium can be used for all seed train expansion steps including N-1 perfusion, reaching robust growth rates at low cell-specific perfusion rates (CSPR).

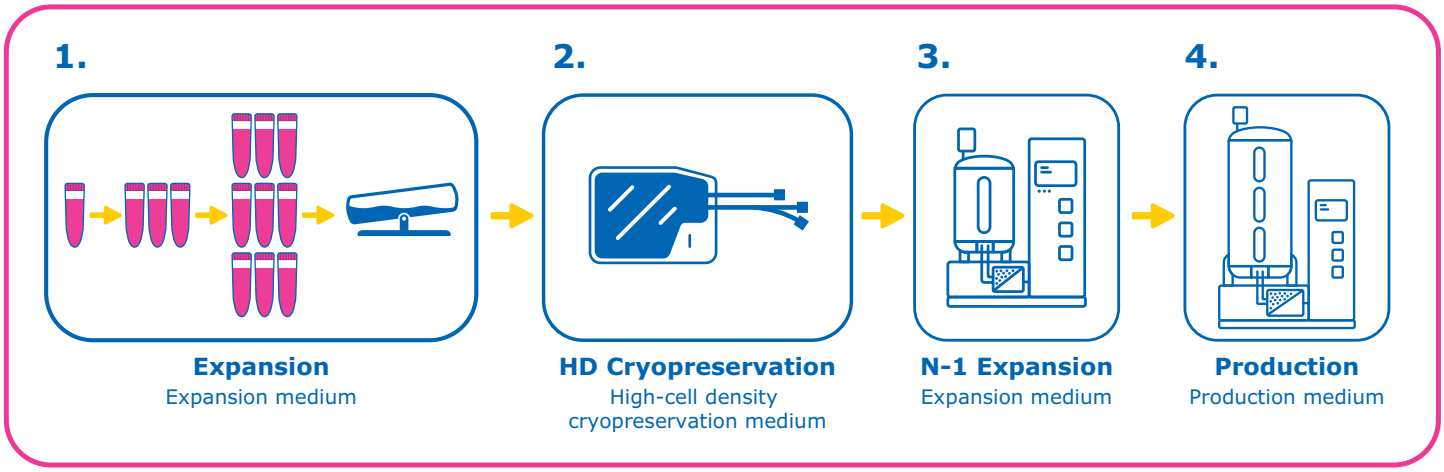

Figure 10. To optimize the upstream process, specialized media is used for expansion, freezing, and perfusion.

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Case Study

In the following case study, the Cellicon™ perfusion filter and controller were utilized in an N-1 perfusion process with Cellvento® 4CHO-X Expansion Medium prior to a fed-batch production bioreactor. The results were compared to a control process with a conventional seed train. Cell growth, titer, nutrients, metabolites and product quality were assessed throughout the production process. Additionally, the ability of N-1 perfusion to support upstream process intensification was evaluated using a high-seed fed-batch process (**Figure 11**).

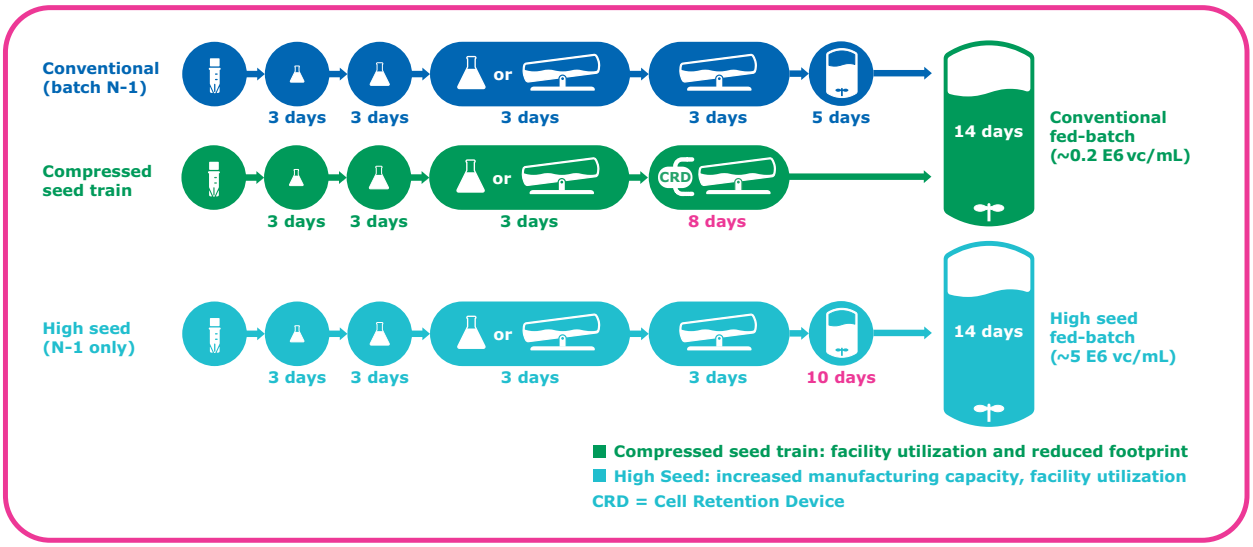

Figure 11. Description of conventional processes relative to production processes utilizing a perfused seed train.

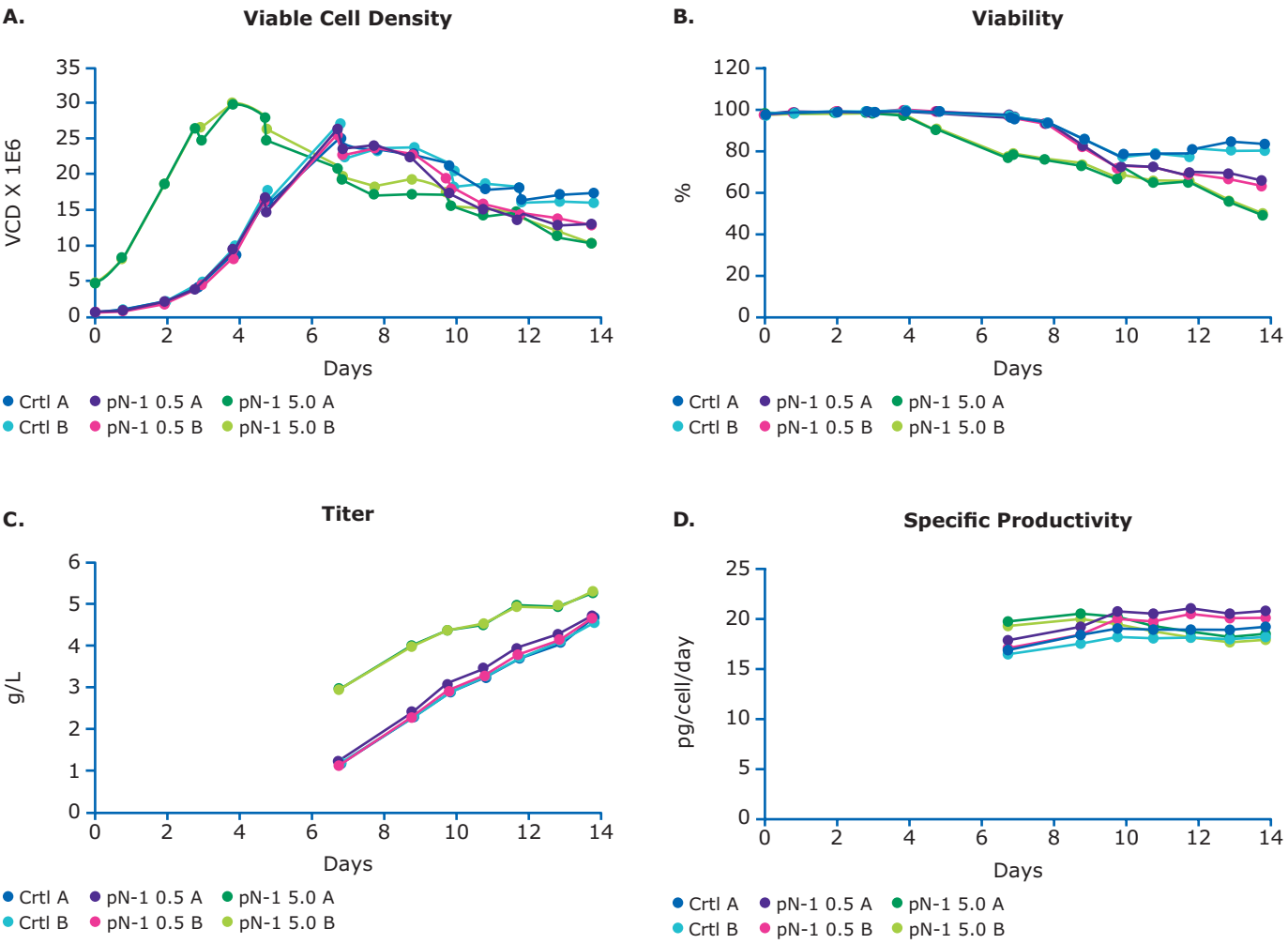

Figure 12. Comparability of traditional and perfused seed trains cultivated in Cellvento® 4CHO-X Expansion Medium. Control samples were expanded in a traditional, shake flask-based seed train, while pN-1 samples were expanded in N-1 perfusion. All production bioreactors were run in EX-CELL® Advanced™ Fed-Batch basal medium with a 50:50 mixture of EX-CELL® Advanced™ CHO Feed 1 and Cellvento® 4Feed. VCD (**A**), Viability (**B**), productivity (**C**), and cell-specific productivity (**D**) are represented for duplicate runs of each condition.

In this case study, the traditional seed train and perfused seed train generated highly similar results in the production bioreactor utilizing the lower seeding density (**Figure 12**), demonstrating the easy transition to an intensified seed train using Cellvento® 4CHO-X Expansion medium. However, more significant benefits can be realized by transitioning to a higher seed fed-batch production process, which is enabled by the high biomass generated in N-1 perfusion. The high-seed bioreactors experienced a greater peak viable cell density and integral viable cell count overall. This led to increased titer since the specific productivity was in the same range as the control conditions. An increased titer was highly beneficial since greater productivity was achieved within the same time period. Alternatively, the N production process could be shortened by 4 days with the same productivity, enabling manufacturers to use their production facilities more efficiently. There were no significant differences in the nutrient/metabolite concentrations or the product quality between the high-seed bioreactors or the standard seed bioreactors.

The Cellvento® 4CHO-X Expansion Medium provided an optimized formulation of components for the seed train steps by maintaining an exponential cell growth rate, high viability, and low media turnover requirement during the process. The Expansion Medium also showed seamless adaptation to the basal media of the fed-batch production bioreactor.

For additional details from this case study, please refer to the application note here:

https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/pharmaceutical-and-biopharmaceutical-manufacturing/monoclonal-antibody-manufacturing/higher-productivity-cho-cell

Cellicon™ Perfusion Solution

The cell retention device is essential for optimizing perfusion-based processes. The device maintains cells within the bioreactor while removing other components such as spent media, cell debris, and protein products. Many conventional cell retention technologies utilize membrane filtration to achieve this separation and have demonstrated success in intensifying upstream processes. However, these cell retention technologies often come with mechanical or performance challenges related to throughput, efficiency, product retention, or process scalability.

The Cellicon™ Perfusion Solution is designed to overcome these challenges and enable true workflow intensification. The solution consists of a controller and a flat sheet cell retention filter with a single-use assembly running in tangential flow filtration mode. This easy-to-use solution increases perfusion process efficiency and provides real-time monitoring and control for reliable and consistent performance.

Cellicon™ Perfusion Filter

Cellicon™ Perfusion Controller

Low crossflow and gentle on cells

In a perfused seed train, high cell densities must be achieved while preserving cell viability. This can be accomplished by supporting optimal growth conditions in the bioreactor and by reducing physical stress on the cells, which can occur as the culture is circulated through the cell retention device. The Cellicon™ Perfusion Filter allows perfusion to be run at crossflow rates ten times lower than traditional perfusion solutions. Under these conditions, a smaller pump can be used, which is especially beneficial at larger scales. The low-shear levitating pump and unique filter design enable higher cell density and reduced residence time of cells outside of the bioreactor, supporting high viability cultures of > 100 x 10^6 cells/mL (**Figure 13**).

N-1 process cell growth with Cellicon™ Perfusion Solution

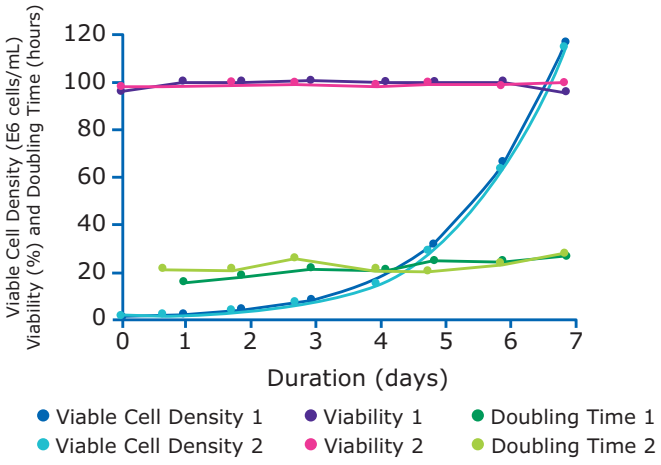

Figure 13. Viable cell density, viability and doubling time profiles of Cellicon™ filter.

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Comprehensive monitoring and precise control

The controller's touchscreen interface is easy to use. The P&ID screen monitors all active parameters, and the data display provides real-time processing at a glance (**Figure 14**).

Monitoring of feed, retentate and perfusate pressure allows users to quickly adjust conditions in real time. A consistent crossflow is maintained via a proportional-integral (PI) control loop, enabling high reproducibility from run to run. For individualized process control, visible and audible alarms can be enabled and configured to alert you to any changes in conditions. The solution is easily integrated into a distributed control system (DCS) for remote monitoring.

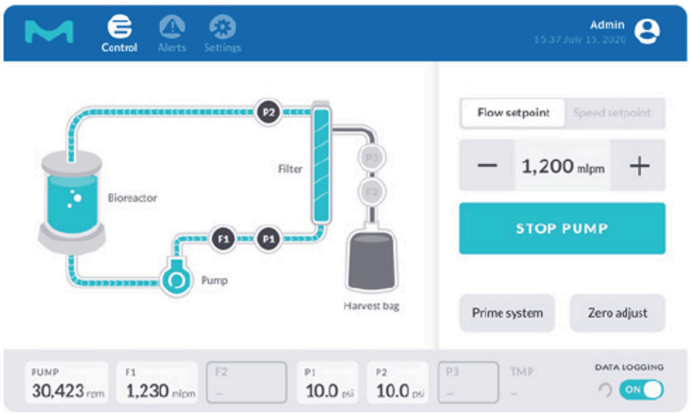

Figure 14. Main touchscreen of the Cellicon™ controller.

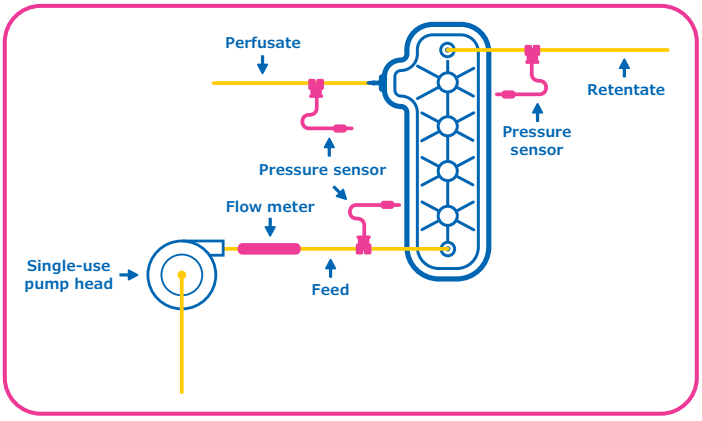

Figure 15. Diagram of Cellicon™ perfusion filter assembly, with components labeled.

Ready to process in minutes

This intuitive single-use system can be up and running in minutes. The filter assembly is supplied gamma-irradiated and dry (preservative-free), eliminating the need for flushing. Detailed assembly components are shown in **Figure 15**.

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Case Study

In the same case study described previously, the Cellicon™ Perfusion Solution was implemented during N-1 perfusion. Here, the cell retention device demonstrates robust performance through the entire duration of the seed train, supported by consistent doubling times and high viability (**Figure 16a**), stable pressures in the cell retention device (CRD) (**Figure 16b**), and low accumulation of lactate and ammonium, which can inhibit growth and reduce viability.

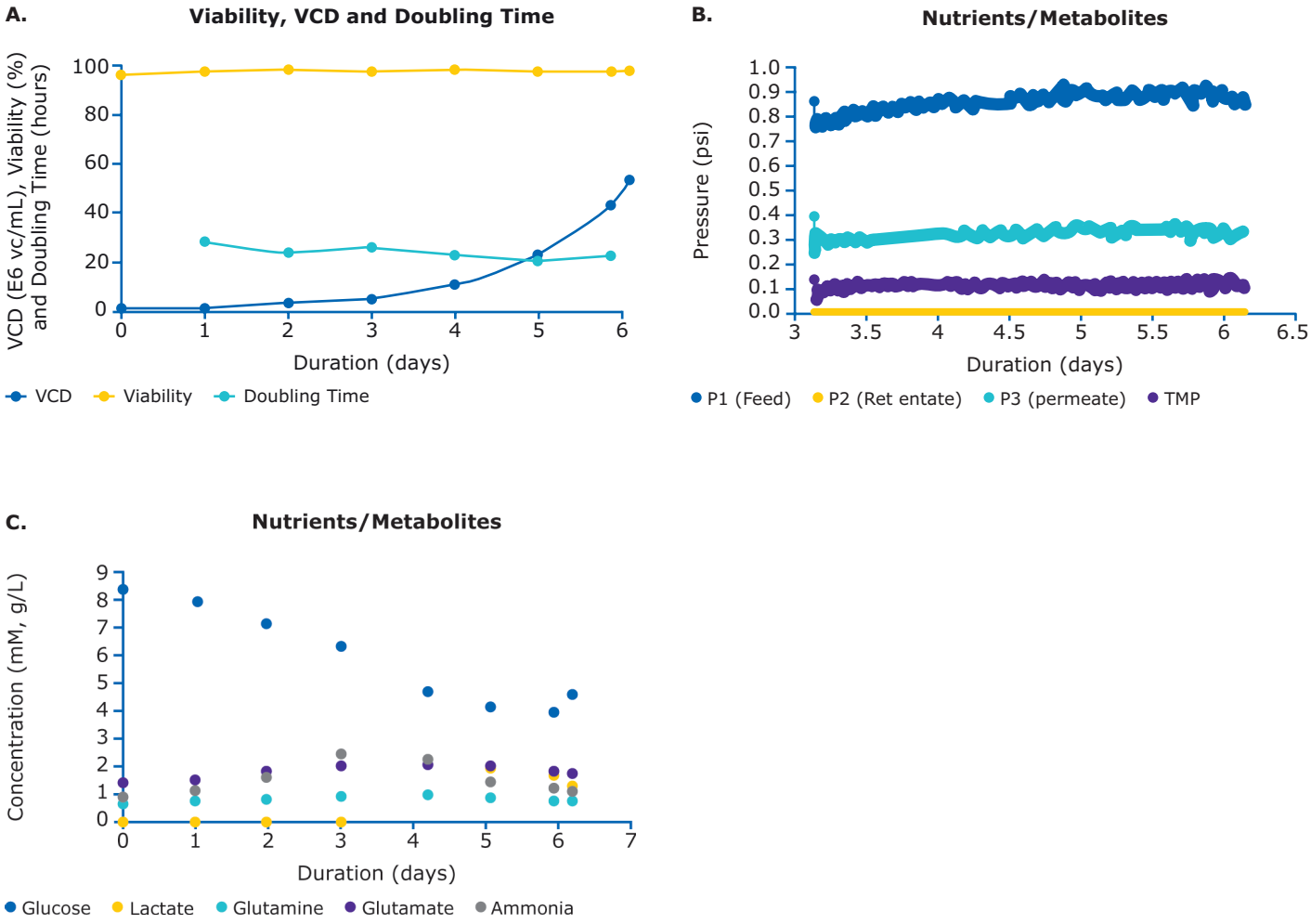

Figure 16 a/b/c. (**A**) Viability, viable cell density, and doubling times of cells in an N-1 perfusion process using the Cellicon™ Perfusion Solution. (**B**) Cellicon™ filter performance during the N-1 perfusion process. (**C**) Nutrient and metabolite concentrations during the N-1 perfusion process.

For additional information on this case study and performance of the Cellicon™ Perfusion Solution, please refer to the application note here.

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To place an order or receive technical assistance, please visit
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