Production of AAV vector in HyPerforma S.U.B.s using the ExpiSf Expression System

Introduction

In this study, a 50 L Thermo Scientific™ HyPerforma™ DynaDrive™ Single-Use Bioreactor (S.U.B.), a 50 L HyPerforma™ 5:1 S.U.B., and a 3 L HyPerforma™ Glass Bioreactor were evaluated using the Gibco™ ExpiSf™ Expression System. A dual infection method was utilized to evaluate the production of recombinant adeno-associated virus type 2 (rAAV2). Gibco™ ExpiSf9™ Cells were infected with two baculoviruses. One of the baculoviruses carried the AAV RepCap2 plasmid, and the other carried a gene encoding Green Fluorescent Protein (GFP) that was flanked by inverted terminal repeats (ITRs). The RepCap2 vector supplied the viral genes needed for genome replication, genome packaging, and capsid assembly, while the ITR-GFP baculovirus contained the gene of interest. GFP expression was monitored to detect and quantify rAAV2 production. Two runs were performed with each reactor type. The production of rAAV2 particles was scalable from the 3 L benchtop scale to the 50 L bioreactor scale, and high viral genome (vg) titers of up to 1.5 x 10^{11} vg/mL of rAAV2 were attained.

Materials and methods

ExpiSf9 *Spodoptera frugiperda* cells were maintained in Gibco™ ExpiSf™ CD Medium throughout the seed train and in the bioreactor cultures. The key materials used in the study are listed in Table 1. The cell cultures were first grown in Fernbach shake flasks, then used to inoculate each of the bioreactors.

Table 1. Materials used for ExpiSf9 cell cultures and scale-up.

Component	Description
Cell line	ExpiSf9 Cells (Cat. No. A35243)
Cell culture medium and reagents	• ExpiSf CD Medium (Cat. No. A3767802) • ExpiSf Protein Production Kit (Cat. No. A3767808)
Baculovirus vectors	• RepCap2 • ITR-GFP
Benchtop bioreactor	HyPerforma Glass Bioreactor (120 V, heat only, Cat. No. F100-2680-002)
S.U.B. hardware	• HyPerforma 50 L DynaDrive S.U.B. (Cat. No. DDB0050.1011) • HyPerforma 50 L 5:1 S.U.B. (Cat. No. SUB0050.8100)
Bioprocess containers (BPCs) with Aegis 5-14 film	• HyPerforma 50 L DynaDrive BPC (Cat. No. SH31192.01) • HyPerforma 50 L 5:1 BPC (Cat. No. SH31073.01)

ExpiSf9 cell culture maintenance and P0 baculovirus production

ExpiSf9 cell growth and maintenance, baculovirus production, and quantification were performed as described in the ExpiSf Expression System User Guide [1]. A dual infection method was used to produce two unamplified P0 baculovirus stocks. The ExpiSf9 cells were transfected with the bacmid containing the gene that encodes RepCap2 and the ITR-GFP bacmid containing the gene of interest, GFP. The P0 baculoviruses were collected 4 days after transfection.

Bioreactor setup and rAAV production

The DynaDrive S.U.B., 5:1 S.U.B., and HyPerforma glass system were set up according to their respective user guides [2-4]. Thermo Scientific™ HyPerforma™ G3Pro™ Bioprocess Controllers were used to control the DynaDrive and 5:1 S.U.B.s, and the HyPerforma glass system was controlled with a HyPerforma™ G3Lab™ Bioprocess Controller. The operating parameters are listed in Table 2. Four days prior to infection, the ExpiSf9 cells were seeded in ExpiSf CD Medium to a density of 0.9 x 10^6 viable cells (vc) per mL in each bioreactor. The working volume of the glass vessel was 0.9 L, while the DynaDrive S.U.B. and 5:1 S.U.B. had working volumes of 15 L. The cell density one day prior to infection ranged from 6.0 x 10^6 to 7.5 x 10^6 cells/mL. The cultures were diluted to 3.0–3.5 x 10^6 cells/mL with fresh ExpiSf CD Medium, followed by 4 mL/L of Gibco™ ExpiSf™ Enhancer, in preparation for transfection the next day. The final volumes in the glass reactor and the S.U.B.s were 2 L and 30 L, respectively. The cultures were co-infected with the baculoviruses the next day. A multiplicity of infection (MOI) of 2 was applied for each baculovirus stock.

Culture monitoring

Samples were collected daily to assess the growth rate and health of the cells. Approximately 10 mL was pulled from each reactor and discarded to clear the sample line. A fresh 10 mL sample was then collected to measure nutrient and metabolite levels using a Nova Biomedical™ BioProfile™ FLEX2™ Automated Cell Culture Analyzer. A Beckman Coulter™ Vi-CELL™ XR Cell Viability Analyzer was used to evaluate the concentration and viability of the cells, and an Oakton™ pH 150 Meter was used for offline pH measurements.

rAAV genome titer measurement

An Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System was used to perform quantitative PCR (qPCR) to measure the rAAV viral genome titers. GFP-specific primers and a GFP-specific probe were employed to target the gene of interest. Unencapsulated DNA was removed from the crude lysates prior to qPCR by treating them with DNase I. Proteinase K treatment released the encapsulated AAV genomes from the viral particles. The linearized GFP-containing plasmid was used to construct a standard curve to determine the rAAV titers.

Table 2. Bioreactor parameters.

Parameter	3 L Glass Bioreactor	50 L DynaDrive S.U.B.	50 L 5:1 S.U.B.
Working volume (L)	0.9/2	15/30	15/30
Temperature (°C)	27	27	27
pH*	6	6	6
Agitation (rpm)	200/220	110/120	160/186
Dissolved oxygen (DO) set point (%)	30	30	30
DO cascade	O_2 through drilled pipe sparger, 7 x 0.8 mm holes	O_2 through enhanced drilled-hole sparger (DHS), 1,448 x 0.08 mm holes	O_2 through standard DHS, 360 x 0.178 mm holes
Headspace aeration (slpm)	0.05 (air)	0.75 (air)	0.75 (air)
Target seeding density	0.9 x 10^6 cells/mL	0.9 x 10^6 cells/mL	0.9 x 10^6 cells/mL

* Not controlled

Results

Figure 1 shows the growth behavior of the ExpiSf9 cells during the two bioreactor runs. The cultures were similar in terms of viable cell numbers, viability, and morphology across the reactors. They reached the expected cell densities of 6.0–7.5 x 10^6 cells/mL prior to infection. This corresponded to a specific growth rate of 0.657 per day with an average doubling time of 25.3 hours, which was consistent with the results of previous experiments (data not shown). Cell viability was similar in each of the bioreactors and remained above 95% until the cells were infected. After infection, cell viability declined rapidly due to the lytic nature of the baculoviruses.

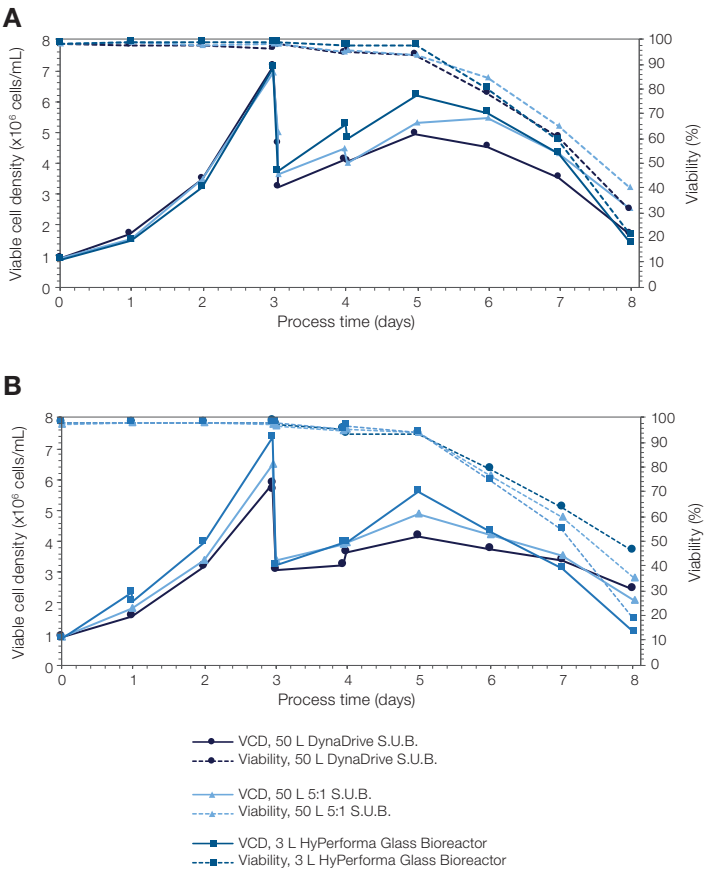

Figure 1. Viable cell density (VCD) and viability of two separate cultivations (A, B) during ExpiSf rAAV production in stirred-tank bioreactors.

Four days post-infection, the AAV titers were similar in all vessels tested (Figure 2), and differences between the large and small bioreactors were less than 15%. This clearly demonstrated the scalability of the ExpiSf Expression System for recombinant AAV production. The highest viral titers of 1.2–1.6 x 10^{11} vg/mL were observed three days post-infection.

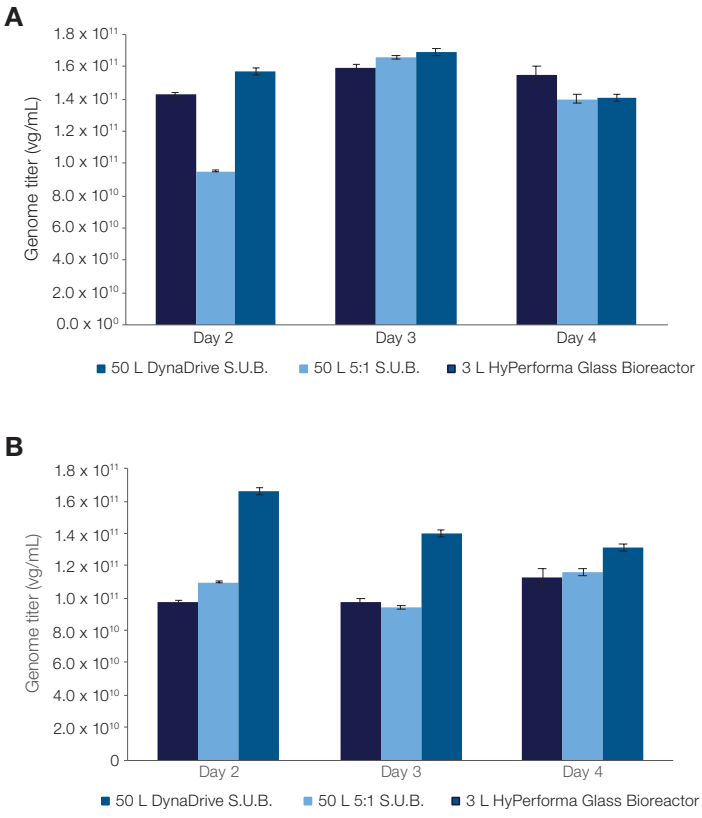

Figure 2. rAAV genome titers of ExpiSf cell lysates from two separate cultivations (A, B) in stirred-tank bioreactors. Samples were collected 4 days post-infection.

Volumetric oxygen consumption, which is the ratio of total oxygen gas and the working volume, is plotted in Figure 3. Less oxygen was consumed in the DynaDrive S.U.B. and 5:1 S.U.B. than in the 3 L HyPerforma Glass Bioreactor. The DynaDrive S.U.B. had an enhanced DHS system, and oxygen mass transfer was better at comparable agitation and aeration rates (data not shown). The residence time of gas bubbles in the DynaDrive BPC was longer due to its higher height-to-diameter ratio, which allowed more efficient oxygen transfer.

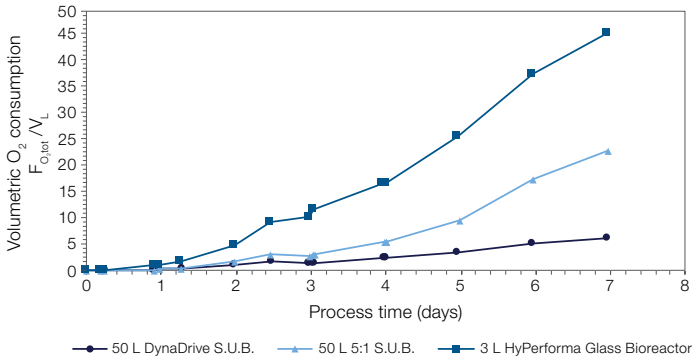

Figure 3. Volumetric oxygen consumption in stirred-tank bioreactors.

Conclusions

The ExpiSf Expression System can be paired with both the HyPerforma DynaDrive S.U.B. and the HyPerforma 5:1 S.U.B. for larger-scale production processes. A key advantage of using these reactor systems for production is the ability to utilize the turndown capability to scale the cell concentration as needed. Another advantage is the ability to optimize gas sparging by utilizing the DHS of each system. Oxygen mass transfer was better in both of these systems than it was in the 3 L glass reactor, and the DynaDrive S.U.B.s did an extraordinary job of minimizing gassing in the system.

The ExpiSf Expression System is a scalable option for baculovirus processes for research use; it allows for high titers that are comparable to those typically seen in glass reactors. It is a versatile system that can be used for research applications other than protein expression. In this study, we demonstrated the scalable production of recombinant AAV for gene therapy research.

References

1. ExpiSf Expression System User Guide. Carlsbad, CA, Thermo Fisher Scientific, April 2018.
2. HyPerforma Glass Bioreactor Quick Start Guide. Santa Clara, CA, Thermo Fisher Scientific, November 2019.
3. 50 and 500 L HyPerforma DynaDrive S.U.B. User's Guide. Logan, UT, Thermo Fisher Scientific, December 2020.
4. HyPerforma 5.1 Single-Use Bioreactor (S.U.B.) User's Guide. Logan, UT, Thermo Fisher Scientific, July 2019.

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