APPLICATION NOTE No. 303 I March 2016

# Development of a Scale-Down Model for rAAV Viral Vector Production Using a Sf9/BEV System

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## Abstract

Single-gene disorders originate in the absence or loss of function of a protein due to a genetic mutation. Gene therapy is a promising therapeutic approach that delivers a normal version of the gene to affected cells to compensate for its missing or defective counterpart. It often employs viral vectors, such as recombinant Adeno Associated Viruses (rAAVs), to insert the genes. The insect cell line Sf9 provides a suitable host for virus production. Sf9 cells are cultured in suspension, and hence working volumes can be adapted to changing needs during process development and manufacturing much more easily than for adherent cell cultures. In this study, researchers at Généthon® developed a scale-down model for rAAV viral vector production in Sf9 cells using an Eppendorf DASbox® Mini Bioreactor System. Parallel experimentation in small working volumes allowed time- and cost-efficient evaluation of process performance.

## Introduction

Development and use of gene therapy for single-gene disorders requires optimization of the process for large-scale manufacturing of gene therapy vectors. Recombinant Adeno Associated Viruses (rAAV) are promising vector candidates. They are not known to be pathogenic, their DNA integrates into the human genome only very inefficiently, and the virus particles are very robust, to name only a few advantageous attributes. One big challenge on the road to therapeutic use is producing a sufficient amount of the virus for experimentation in large animal models, clinical trials, and actual treatment. A widely used host for rAAV production is the insect cell line Sf9. Cells are transfected with the genes needed to produce functional virus particles using baculovirus expression vector (BEV) systems. A major advantage of the Sf9/BEV system for large-scale virus production is the possibility of growing Sf9 cells in suspension culture. Higher cell densities can be reached than in adherent cell cultures, and the inherent scalability of the system allows for much easier adaption of production volumes during process development and manufacturing. Process development is usually carried out in small culture sizes, and subsequently scaled up to larger production volumes. It is highly desirable to also establish scale-down models that will mimic the production process in smaller volumes, and which can be used to optimize the process, troubleshoot, and implement changes.

Généthon is a non-profit biotherapy R&D organization created and funded by the Association Française contre les Myopathies, a French organization that supports patients and their families. Its mission is to design gene therapy products for rare diseases, to ensure their pre-clinical and clinical development, as well as the production in order to provide patients with access to these innovative treatments. Généthon is currently sponsoring two gene therapy projects that have reached clinical trial phase, and is involved in several other projects in preclinical or research stages.

This application note describes the successful development of a scale-down model for rAAV production using a Sf9/BEV system. The aim was to reproduce the production performance obtained in a 2 L bioreactor in an Eppendorf DASbox Mini Bioreactor System. It is optimized for parallel process development, meaning that

multiple experiments can be run, monitored, and controlled simultaneously by shared equipment. Processes can be carried out in working volumes as small as 60 mL, which helps to reduce the cost of media and supplements.

## Material and Methods

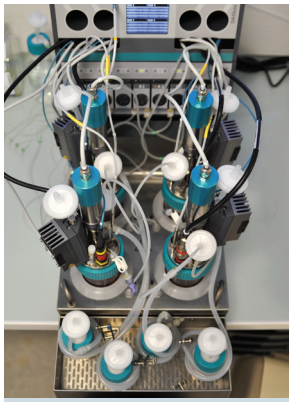

**Fig. 1:** The Eppendorf DASbox Mini Bioreactor System used at Généthon.

**Table 1:** Summary of experimental conditions. Three runs were performed in a DASbox Mini Bioreactor System. Several agitation speeds were compared within a given run. Cultures were analyzed for viable cell density, cell diameter, and cell viability (run 1) and viral genome titer per mL (VG/mL) and viral genome titer per cell (VG/cell), respectively (runs 2 and 3).

| Run | Bioreactor 1 | Bioreactor 2 | Bioreactor 3 | Bioreactor 4 | Analysis |
|---|---|---|---|---|---|
| 1 | 300 rpm | 375 rpm | 450 rpm | - | Viable cell density; Cell diameter; Viability |
| 2 | 300 rpm | 300 rpm | 400 rpm | 400 rpm | VG/mL; VG/cell |
| 3 | 300 rpm | 400 rpm | - | - | VG/mL; VG/cell |

**Cell culture**

The Généthon research team cultivated Sf9 cells in Sf-900™ III SFM culture medium (Thermo Fisher Scientific® Inc., USA) at 27°C and dissolved oxygen set to 50 %. The reference production system used a glass bioreactor with a working volume of 2 L, and scale-down experiments used an Eppendorf DASbox Mini Bioreactor System (Eppendorf AG, Germany, Fig. 1), with a working volume of 250 mL. Both systems used marine impellers. The agitation speed of the 2 L production system was 180 rpm. Proper culture mixing and oxygen transfer have to be ensured during development of scale-down models. Agitation speeds of 300 rpm, 375 rpm, and 450 rpm were tested in three vessels simultaneously (Table 1).

Cell density, cell viability, and cell diameter were determined offline, using the Cell Viability Analyzer Vi-CELL® XR (Beckman Coulter®, Inc., USA).

**Virus production and quantification of production yield**

The researchers at Généthon used Sf9 cells as hosts for the production of recombinant Adeno Associated Virus serotype 8 (rAAV8). 72 hours after inoculation they co-infected the Sf9 cells with two distinct baculovirus clones, one carrying the genes for the production of the virus capsid and the other carrying the gene of interest. Through their lytic life cycle, the baculoviruses replicate using the Sf9 cell machinery, infect other cells in the culture, and produce the rAAV8 vectors. The culture was harvested 96 hours after infection, and the vectors were released using a detergent treatment. The team determined the rAAV8 vector titer by measuring viral DNA using real-time quantitative PCR. This analysis leads to a viral genome titer (VG), which is interpreted as the number of viral vector particles carrying the gene of interest. Specific productivity (VG/cell) was calculated by normalizing the viral genome titer to the viable cell density at the time of infection.

In parallel processes production yields were compared for cultures agitated at 300 rpm and 400 rpm, respectively. To test for reproducibility, three bioreactor runs were performed for each agitation speed (Table 1).

## Results

The study aimed to scale-down rAAV production from a 2 L working volume to a 250 mL working volume, while reproducing production performance. The Généthon research team first compared growth of non-virus infected Sf9 cells in the 2 L glass vessel and in the 250 mL working volume vessels of the DASbox Mini Bioreactor System.

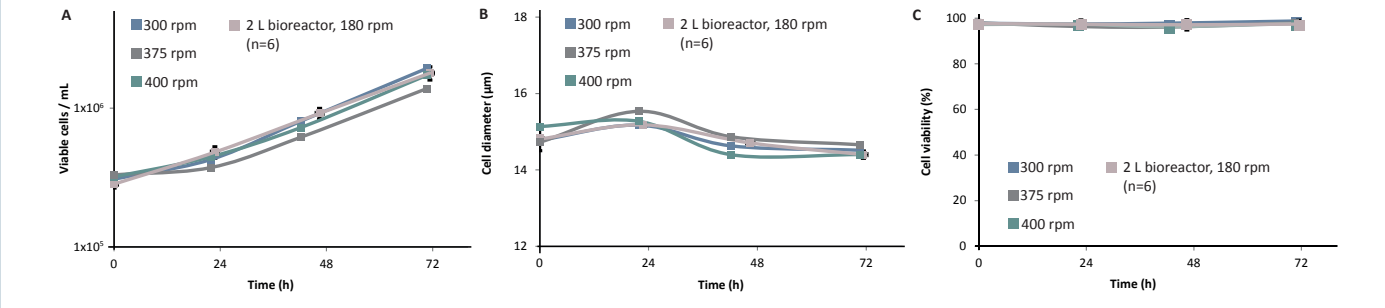


**Fig. 2:** Growth performance of Sf9 cells. Sf9 cells were cultivated in a DASbox Mini Bioreactor System and a 2 L bioreactor. Several agitation speeds were tested. Viable cell density **(A)**, cell diameter **(B)**, and cell viability **(C)** were determined offline.

Growth performance results were comparable for Sf9 cells in the 2 L glass vessel and the vessels of the DASbox system, at agitation speeds of 300 rpm, 375 rpm, and 450 rpm. At 72 hours post-inoculation, for all experimental conditions tested, the viable cell density had increased from $3x10^5$ cells/mL to around $1.5x10^6$ cells/mL (Fig. 2A). The average cell diameter depends on the cell cycle phase, and correlates with infection by baculoviruses. It is routinely determined in Sf9/BEV systems and was around 15 µM for non-infected cells (Fig. 2B). Close to 100 % of the cells were viable (Fig. 2C). Next, the research team monitored virus production performance, comparing processes carried out in the DASbox system at agitation speeds of 300 rpm and 400 rpm, and in the 2 L glass vessel. Virus genome titers and specific productivities were comparable for all experimental conditions tested. Results from three experimental runs were highly reproducible (Fig. 3).

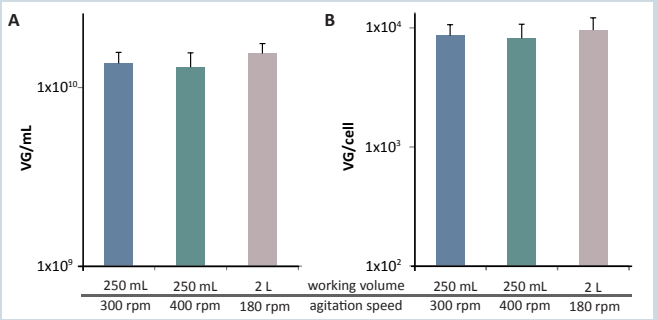


**Fig. 3:** Virus production performance. rAAV was produced using a Sf9/BEV expression system in a DASbox Mini Bioreactor System at an agitation speed of 300 rpm and 400 rpm, respectively, and in a 2 L bioreactor at an agitation speed of 180 rpm. Experiments in the DASbox system were carried out in triplicate, and experiments in the 2 L bioreactor were carried out in duplicate. Means and standard deviations are shown. Virus genome titer per mL (VG/mL) **(A)** and specific productivity (VG/cell) **(B)** were determined.

## Conclusion

The results demonstrate the successful scale-down of rAAV production using a Sf9/BEV system. The cell growth and production performance obtained in a 2 L glass vessel were reproduced in an Eppendorf DASbox Mini Bioreactor System with a working volume of 250 mL. This study exemplifies the value of the Eppendorf DASbox Mini Bioreactor System for process scale-down. In the course of scale-down model development, multiple experiments must be performed to test a variety of experimental conditions and to ensure the reproducibility of process performance. The use of a parallel bioreactor system saves time, and ensures maximum comparability of experimental runs. By using the DASbox, researchers at Généthon shortened development timelines. They state: "The biological timing is impossible to shorten due to the rAAV production kinetics. But the DASbox system shortens the development timelines, by being able to run the same amount of bioreactors in one week whereas it takes two weeks in 2 L glass bioreactors." Using the DASbox system also helped reduce costs, by facilitating the performance of studies in small-scale bioreactors.

**Ordering information**

| Description | Order no. |
|---|---|
| **DASbox® Mini Bioreactor System for Cell Culture Applications**, max. 5 sL/h gassing | |
| 4-fold system | 76DX04CC |
| 8-fold system | 76DX08CC |
| 16-fold system | 76DX16CC |
| 24-fold system | 76DX24CC |
| **DASware® control**, incl. PC, OS, and licenses | |
| for 4-fold DASbox® system | 76DXCS4 |
| for 8-fold DASbox® system | 76DXCS8 |
| for 16-fold DASbox® system | 76DXCS16 |
| for 24-fold DASbox® system | 76DXCS24 |
| **DASware® control professional**, incl. PC, OS, and licenses | |
| for 4-fold DASbox® system | 76DXCSP4 |
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