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Scalable protein and AAV production in the ExpiSf™ Expression System

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Introduction

The Baculovirus Expression Vector System (BEVS) is a versatile platform for expression of individual recombinant proteins, multimeric protein complexes, virus-like particles (VLPs), and membrane proteins. Recently, BEVS has demonstrated particular utility in the large scale production of vaccines and viral vectors. Here, we describe the ExpiSf™ Expression System, the first chemically defined insect expression system that enables high-yield production of proteins and viral particles with consistent performance using a fast, streamlined workflow. The system demonstrates scalability for production of proteins and viral vectors in stirred tank, rocking motion, and microbioreactors. We discuss optimal bioprocessing parameters and protocols to enable high quality process development for production of targets of interest.

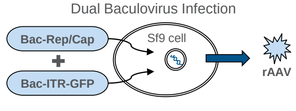
Materials and Methods

The following experiments were performed with:

- Gibco™ ExpiSf Expression System Starter Kit:
  - ExpiSf9™ Cells
  - ExpiSf™ CD Medium
  - ExpiSf™ Enhancer
  - ExpiFectamine™ Sf Transfection Reagent
  - Opti-MEM™ Reduced Serum Medium
  - MAX Efficiency DH10Bac™ Competent Cells
  - Bac-to-Bac™ Vector Kit
- POROS™ CaptureSelect™ AAVX Affinity Resin
- Thermo Scientific™ HyPerforma™ G3Lab Controller
- Thermo Scientific™ HyPerforma™ 3L Glass Bioreactor
- Cytiva ReadyToProcess™ WAVE 25 bioreactor system
- Cytiva 22 L Cellbag™ Disposable Bioreactor
- Sartorius ambr® 15
- ÄKTA Pure™ purification system

Protein Expression

Protein expression was optimized for scale down (ambr 15) and scale up (rocking motion and stirred tank bioreactor) production in the ExpiSf Expression system. Bioreactors were inoculated at 0.5 x 10^6 – 1.0 x 10^6 viable cells/mL three to four days before infection. The day before infection, when cell density should have reached 6.0 x 10^6 – 8.0 x 10^6 viable cells/mL, the culture was diluted with fresh medium to a specified density, and ExpiSf Enhancer was added. After culturing overnight, the cells were infected using a multiplicity of infection (MOI) of 5 with a baculovirus containing the gene of interest. Product was harvested 3 to 5 days post infection. The figures presented here describe optimized parameters in each production format.



AAV Production

A dual-infection method was adopted to evaluate the production of two different recombinant adeno-associated virus (rAAV) serotypes, AAV2 and AAV6, in the ExpiSf system [1]. For this purpose, ExpiSf9 cells were infected with two baculoviruses: RepCapX, where X denotes serotype 2 or serotype 6, and inverted terminal repeat (ITR)–green fluorescent protein (GFP). The RepCap baculovirus supplies the necessary viral genes for genome replication, genome packaging, and capsid assembly. The ITR–GFP baculovirus contains the gene of interest (encoding GFP) flanked by AAV ITR sequences. rAAV production runs were similar to the protein expression protocol, except that the starting cell density was 3.0 x 10^6 cells/mL and an MOI of 2 was used for each baculovirus stock (RepCapX and ITR-GFP).

Results

ExpiSf system scale down into ambr 15 microbioreactors

Optimized ambr15 Conditions						
Final Volume	DO	Temperature	RPM	Vessel Type	Stirring Direction	N_2 Flow Rate
15 mL	40 %	27ºC	1000	Low-Temp Spargeless	Up	0.2 mL/min.

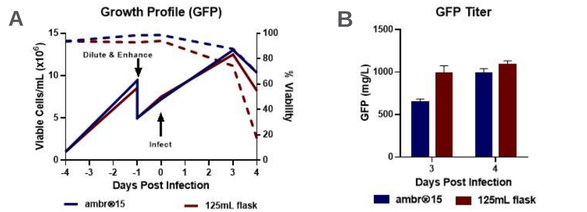


Figure 1. Protein expression in the ambr15 microbioreactor system. (A) Viable cell densities (VCD) and cell viabilities of ExpiSf9 cells in GFP expression runs in 125 mL shake flasks and ambr 15 microbioreactors. Solid lines represent viable cell density; dotted lines represent percent viability. (B) GFP titers of ExpiSf9 cultures from 125 mL shake flasks and ambr 15 microbioreactors. Samples were collected 3 and 4 days post infection. Data represents optimized microbioreactor conditions after several rounds of testing.

Scalable protein production in a rocking motion bioreactor

Optimized 22 Liter Rocking Motion Bioreactor Conditions						
Final Volume	DO	Temperature	RPM	Rocking Angle	Gas Flow Rate	Headspace Overlay
8 - 10 L	30 %	27ºC	18 - 20	8˚	0.3 L/min.	N_2

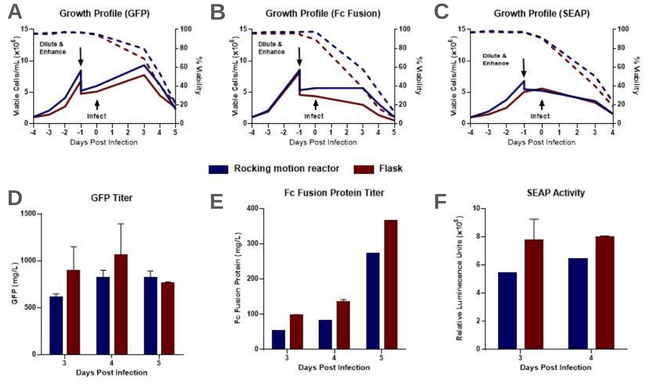


Figure 2. Protein expression in a rocking motion bioreactor. Viable cell densities (VCD) and cell viabilities of ExpiSf protein expression runs for (A) GFP, (B) Fc Fusion protein, and (C) Secreted Alkaline Phosphatase (SEAP) show similar growth kinetics in 125 mL shake flasks and 22L rocking motion bioreactors. Solid lines represent viable cell density; dotted lines represent percent viability. (D, E, F) GFP, Fc Fusion protein, and SEAP titers of ExpiSf9 cultures from 125 mL shake flasks and rocking motion bioreactors. Samples were collected 3, 4, and 5 days post infection. At least 80% protein yield was achieved in rocking motion bioreactors when compared to shake flasks.

Scalable protein production in the HyPerforma stirred tank bioreactors

Optimized 3 Liter Stirred Tank Conditions						
Final Volume	DO	Temperature	RPM	P/V	Sparge	Headspace Overlay
2 L	40 %	27ºC	180	4.5 – 10 W/m^3	Macro	0.5 L/min. air

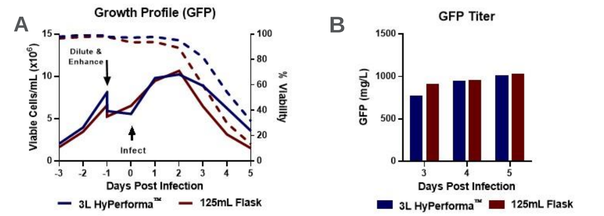


Figure 3. Protein expression in the HyPerforma stirred tank bioreactors. (A) Viable cell densities (VCD) and cell viabilities of ExpiSf GFP expression runs in 125 mL shake flasks and 3L HyPerforma stirred tank bioreactors showed comparable growth kinetics. Solid lines represent viable cell density; dotted lines represent percent viability. (B) GFP titers of ExpiSf9 cultures from 125 mL shake flasks and 3L HyPerforma stirred tank bioreactors. Samples were collected 3, 4, and 5 days post infection. Data represents optimized stirred tank bioreactor conditions after several rounds of testing.

Scalable production of infectious recombinant Adeno-associated virus

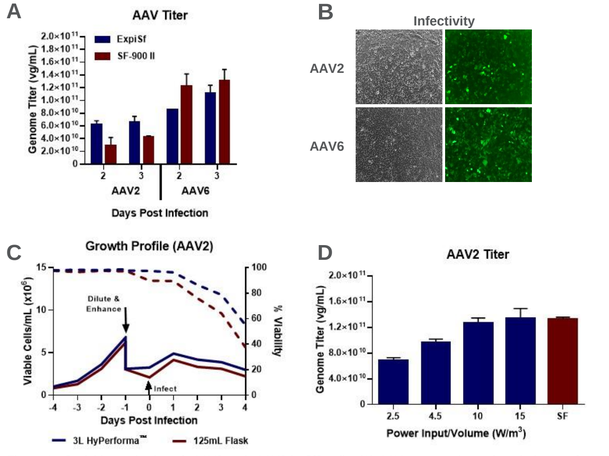


Figure 4. Scalable production of rAAV2 and rAAV6 in Sf9 cells. (A) AAV genome titers of crude lysates from ExpiSf9 cells and Sf9 cells in Sf-900™ II SFM. Sf9 cells in Sf-900 II SFM medium were seeded at 2 x 10^6 viable cells/mL and infected using an MOI of 2 for each baculovirus. Production runs were performed at 25 mL scale in 125 mL shake flasks and crude lysates were collected two and three days post infection. (B) Light and fluorescent images of HT1080 cells inoculated with rAAV2 and rAAV6 ExpiSf crude lysates; rAAV from ExpiSf crude lysates are infectious. (C) Viable cell densities (VCD) and cell viabilities of ExpiSf rAAV2 production runs in 125 mL shake flasks and 3L HyPerforma stirred tank bioreactors showed comparable growth kinetics. Solid lines represent viable cell density; dotted lines represent percent viability. (D) AAV2 genome titers of crude lysates 3 days post infection from production runs in 3L HyPerforma stirred tank bioreactors with Power Input per Volume values of 2.5, 4.5, 10, and 15 W/m^3. Higher Power Input per Volumes (10 and 15 W/m^3) showed equivalent rAAV2 titers to 125 mL shake flask (SF) control.

Purification of recombinant Adeno-Associated Virus (rAAV6)

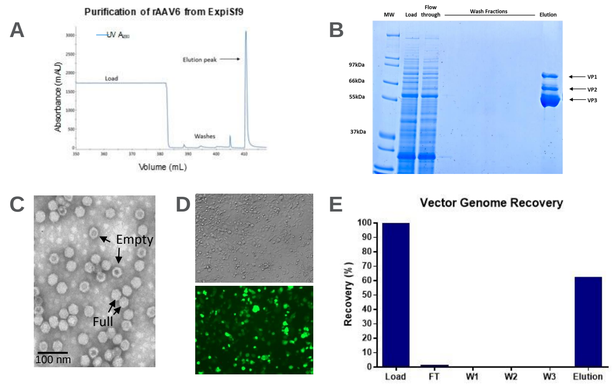


Figure 5. Purification of rAAV6 from ExpiSf production run. (A) ÄKTA chromatogram showing elution peak of rAAV6 purified on POROS CaptureSelect AAVX affinity resin. (B) Fractions from AAV6 purification run on a Coomassie stained gel. The capsid proteins VP1, VP2, and VP3 are indicated. (C) Transmission electron microscope image of purified rAAV6 particles. Empty and full particles are indicated in the image. (D) Light and fluorescence microscopy images of HT1080 cells infected with purified rAAV6 virus. Crude lysates were heated prior to infection to inactivate baculoviruses. (E) Vector genome recovery, relative to the load, in flow-through (FT), wash (W1–W3), and elution fractions of rAAV6 purification run.

Conclusions

Here, we demonstrate the adaptability of the ExpiSf Expression System to a number of formats and scales for the production of recombinant proteins. Optimization of bioprocess parameters resulted in similar protein titers in scale down (microbioreactor) and scale up (rocking motion and stir tank bioreactor) formats compared to shake flask controls. Finally, the production and purification of infectious rAAV particles emphasizes the versatility of the ExpiSf Expression System.

References

1. Smith, RH, Levy, JR, and Kotin, RM (2009). A simplified baculovirus-AAV expression vector system coupled with one-step affinity purification yields high-titer rAAV stocks from insect cells. Mol Ther. 11: 1888-1896.2

Resources

1. ExpiSf™ Expression System
2. Thermo Fisher Bioprocessing Solutions
3. Scalable production and purification of adeno-associated virus (AAV) vector using the ExpiSf Expression System, 2020. Application note
4. Adaptation of the ExpiSf™ Expression System for high-level protein production with the WAVE™ Bioreactor System, 2019. Application note

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