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Value from Innovation

IrvineScientific

Improvement of rAAV Production in HEK293 Fed-Batch Culture

Maintain Expi293 cell viability upon transfection with improved viral titer and quality of the viral particles, in a fed-batch process

Introduction

Recombinant adeno-associated virus (rAAV) vector is the leading vector of choice for gene delivery platform in gene therapy. The ability to elicit robust and long-term gene expression *in vivo*, together with minimal immunogenicity and little to no toxicity, make rAAV a perfect tool for this application. Availability of various serotypes with different tropisms makes rAAV adaptable to different gene therapy approaches as reflected by several approved rAAV-based medicines and numerous ongoing clinical trials.

On the other hand, current production yields often cannot satisfy the high demands of gene therapy applications. A typical phase III clinical trial requires 10^{16} to 10^{18} viral genomes (VG) with a therapeutic dosage of 10^{12} to 10^{14} VG per kg body mass, whereas current production yields are only about 10^{8} to 10^{11} VG/mL.

Interestingly, a majority of the produced recombinant capsids are genome-free, which is not useful for gene therapy and complicates downstream processing. These factors are part of the reason rAAV-based therapeutics can easily cost several thousands to millions of dollars for a course of treatment.

In this case study, the path taken by FUJIFILM Irvine Scientific to develop novel feed media for HEK293 cell culture is described to give useful insights into the methods used and parameters analyzed to boost rAAV production by increasing the virus titer and improving the viral packaging.

Material & methods

In this case study, we aimed to develop feed media for HEK293 cell culture to boost rAAV production by increasing the virus titer and improving the viral packaging. To this purpose, we adapted Expi293 cells (Thermo Fisher Scientific) to BalanCD HEK293 medium (FUJIFILM Irvine Scientific), subculturing them through several passages to ensure that growth was stabilized. We picked the cells from passage 3-25 and seeded them at 10^{6} cells/mL 24 h prior to transfection.

Cells were transfected using AAV2 triple plasmids (GOI, RC, and Helper plasmids) and polyethylenimine (Polyplus PEIpro) per industry standard. Cells were fed with either controls or one of the 13 different feed prototypes (A to M), 24 h post-transfection using 12% v/v of the culture. The cells were harvested 72 h post-transfection.

Cell health indicators were monitored before transfection and at the time of harvest **(Figure 1)**. The data showed that applying the feed in this panel did not negatively impact the cells, and all cell health indices including Viable Cell Density (VCD), Viability, and Cell Diameter are comparable to the “No feed” control.

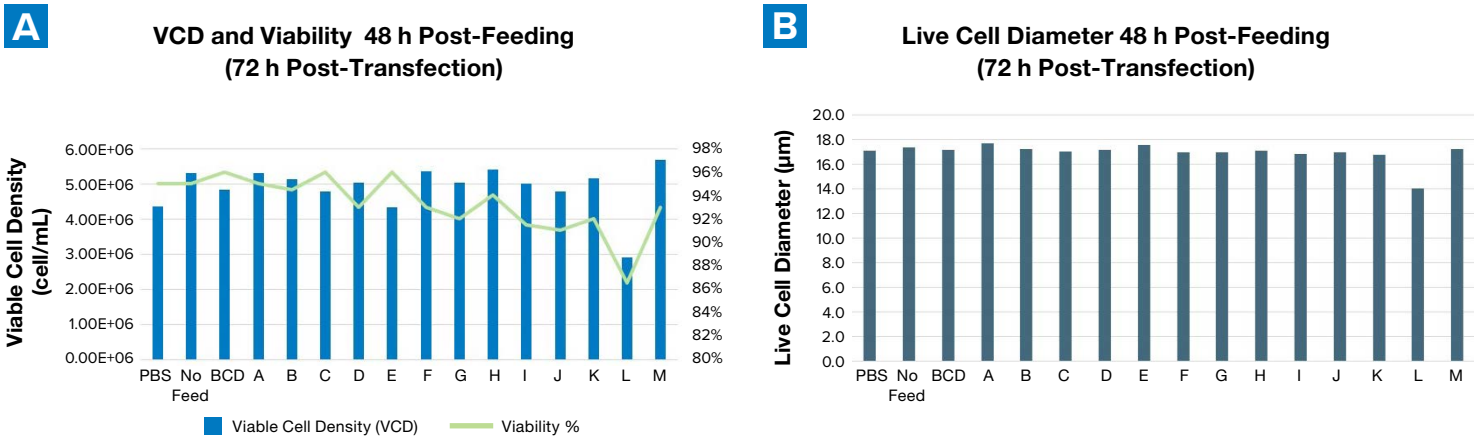

Figure 1. Expi293 cells were seeded at 10^6 cells per mL cell density in a fresh BalanCD culture. They were transfected 24 h later using the PEIpro method utilizing 1.5 µg DNA/mL and DNA:PEI 1:2. The cultures were split into 16 wells in a 24-deep-well plate and fed using either PBS, no feed, or BalanCD HEK293 (BCD, FUJIFILM Irvine Scientific) as controls or one of 13 prototypes (A-M) at 12% v/v at 24 h post transfection. **(A)** VCD and viability were recorded at time of harvest as well as **(B)** cell diameter, indicating that all of the feed media support cell health parameters for the duration of cell culture upon production of AAV2.

Top-performing media enhances AAV2 titer and improves packaging efficiency

VG titer was measured using AAV real-time PCR titration kit (Takara Bio) and capsid titer was measured using Progen AAV2 titration ELISA kit **(Figure 2)**. The data showed different levels of improvement in VG and capsid titer across the prototypes. The highest VG and capsid titer were achieved by Feed J (4.72×10^9 VG/mL), enhancing the AAV2 titer approximately 10-fold compared to the "No feed" control. Feed I also effected an improvement, achieving 7.53×10^{10} capsid/mL.

Enhancing capsid production without improving the packaging produces only flimsy, empty capsids. Critically, a successful virus feed will help to improve viral packaging, or in other words, the VG/capsid ratio. **Figure 3** illustrates the packaging efficiency as measured by the VG/capsid ratio using prototypes A-J.

In this case, Feed A produced the best results, achieving a virus packaging efficiency of 32.77%, followed by Feed B (28.9%), and Feed J (21.81%).

Top feed performers were ascertained by ranking the prototypes based on highest to lowest VG titer, VG/capsid ratio, and adding the ranks. Then, the prototype media were sorted from lowest score to highest **(Figure 4)**. From a performance point of view, it is evident that Feed J not only improved VG titer by a log (10 times) but also improved the capsid production about 6-fold, and as a result improved the VG/capsid ratio 1.67-fold. Although Feed A resulted in higher packaging efficiency (2.51-fold improvement), VG titer was enhanced to a much lower level (only 1.55-fold) and capsid production was decreased.

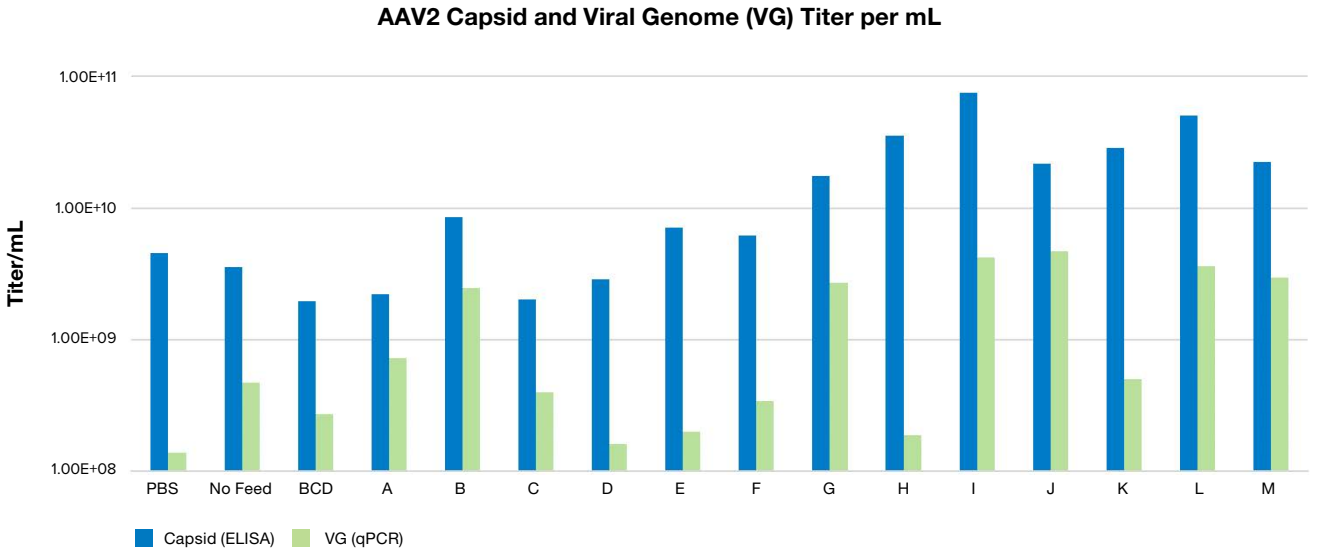

Figure 2. Cell extraction was carried out using three cycles of freeze-thaw. Extract was clarified by centrifugation and treated with DNase and lysis buffer according to Takara Bio AAV real-time PCR kit instruction prior to qPCR. Green bars indicate the VG titer per mL of the culture whereas the blue bars indicate the capsid titer per mL measured by Progen AAV2 titration ELISA kit. Feed J is the leading performer and enhances the AAV2 titer approx. 10-fold compared to the "No feed" control.

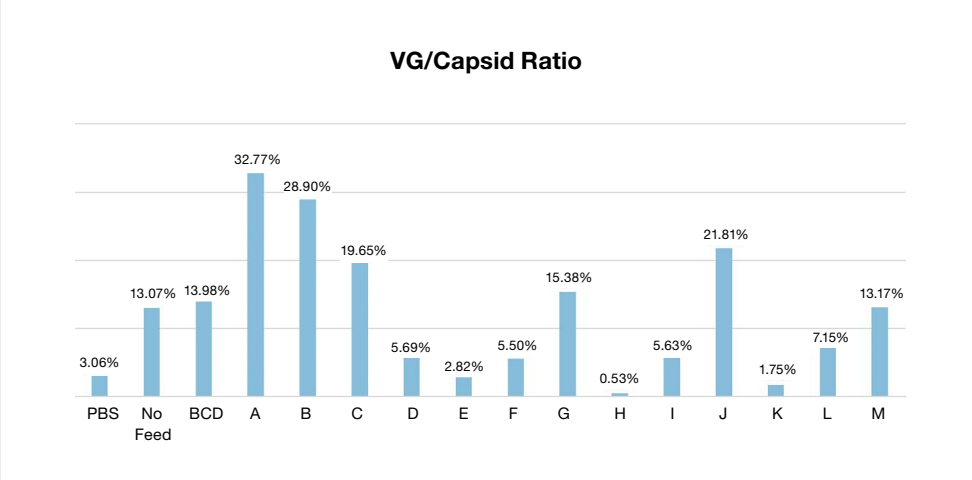

Figure 3. Viral genome per capsid ratio; an indication of viral packaging efficiency. Feed A packaging efficiency is about 33% followed by Feed B (approx. 29%) and Feed J (approx. 22%).

Selection of the Top-Performing Media Prototypes

Rank			
Feed Type	Titer	VG/capsid	Total
J	1	3	4
B	6	2	8
A	7	1	8
G	5	5	10
M	4	7	11
L	3	9	12
I	2	11	13
C	10	4	14
No Feed	9	8	17
BCD	12	6	18
K	8	15	23
F	11	12	23
D	16	10	26
E	13	14	27
PBS	16	13	29
H	14	16	30

X Times Improvement of Each Prototype Over No Feed Control			
Feed Type	VG	Capsid	Vg/capsid
A	1.55	0.62	2.51
B	5.29	2.39	2.21
J	10.05	6.02	1.67

Fold Improvement Over No Feed Control

Lower Titer — Titer remains the same — Higher Titer

Figure 4. Top-performing media were identified factoring the VG titer and VG per capsid ratio. Feed J enhances AAV production by approximately one log (10X) while improving viral packaging (1.67X). Feed A achieved the most efficient packaging compared to the other prototypes.

Lead candidate optimization to increase stability in liquid form

Verification of the VG titer improvement was accomplished in 30 mL shake flask scale **(Figure 5)**. An approximate 10-fold increase of AAV2 titer was observed for Feed J compared to the "No feed" control.

Notwithstanding the promising results with regard to VG titer and capsid production, Feed J had an unfortunate tendency to precipitate at 4-5 weeks post-hydration of the powder. In order to investigate the root cause of this issue, we analyzed the precipitate using HPLC and identified the compounds present in the pellet. About 73% of Compound 1 precipitated whereas the other components such as 2, 3, and 4 were negligible **(<0.1%, Figure 6).** Compound 1 was titrated and analyzed in a media optimization experiment. This experiment showed that removal of compound 1 from Feed J does not impair cell growth (data not shown) and improves the VG titer **(Figure 7)**.

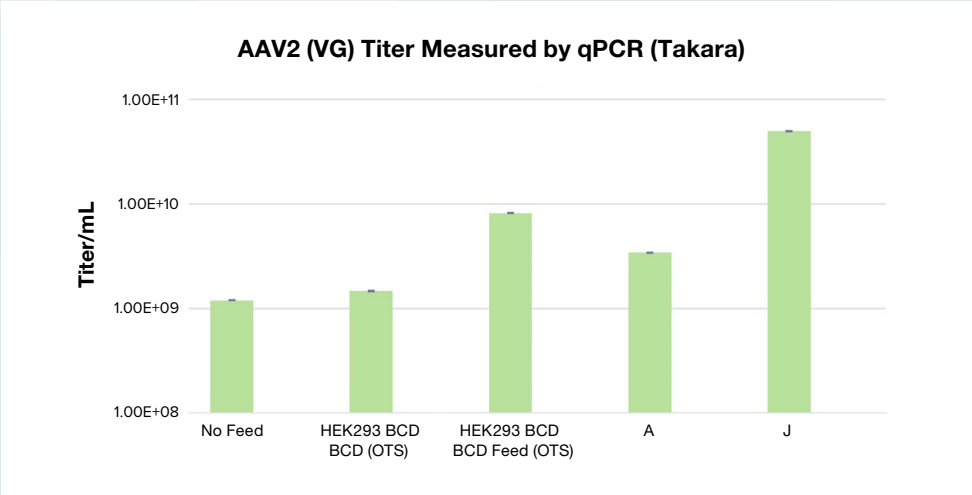

Figure 5. Verification experiment in 30 mL shake flask; AAV2 (VG) titer improved about 10-fold upon applying Feed J comparing to "No feed" control.

Component	% in the Pellet Compared to the Fresh Media
Component 1	73.46
Component 2	0.03
Component 3	0.03
Component 4	0.02

Figure 6. HPLC analysis revealed that the majority of the precipitate at 4-5 weeks post-hydration is composed of one component (73.46%).

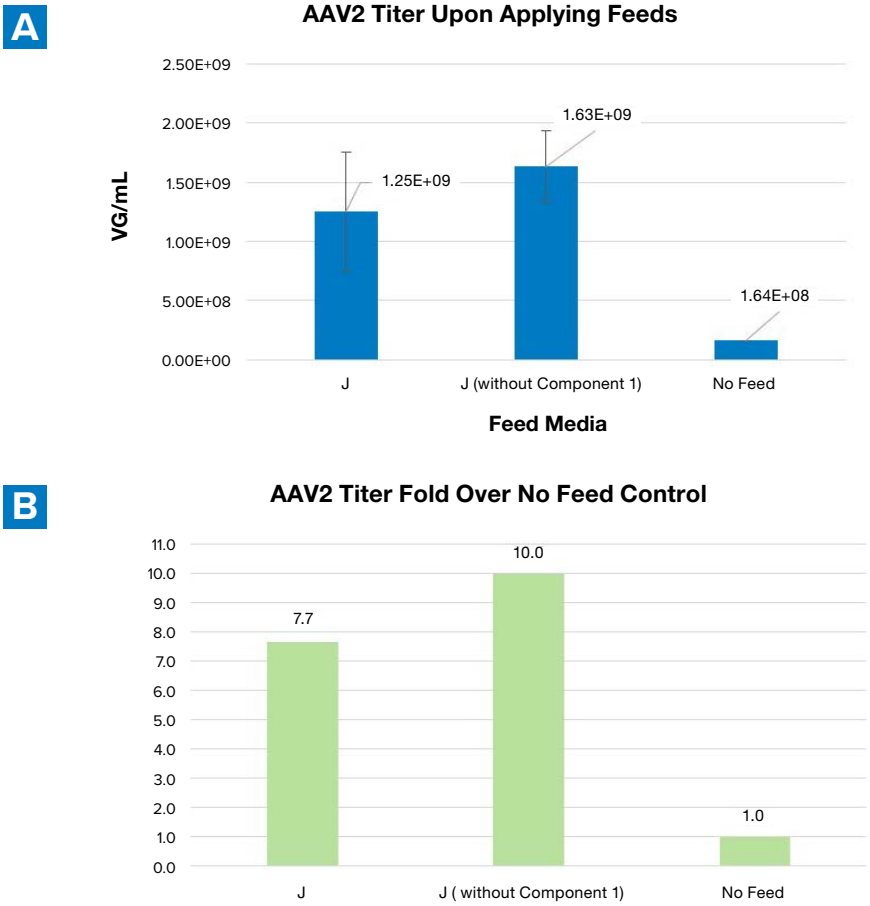

Figure 7. Removing Component 1 from the Feed J formula increases AAV2 titer. The experiment was carried out in 30 mL scale shake flask.

Summary & outlook

FUJIFILM Irvine Scientific has developed a feed medium that can boost AAV2 viral genome titer by about 10-fold and improves the packaging efficiency 1.67-fold, without compromising cell growth and health. Stability studies and reconfiguration of the formula yielded a feed that is stable in liquid form.

The novel feed media are being assayed with other AAV serotypes such as AAV5 and AAV9 at shake flask scale, and trials in 10 L bioreactors are in progress.