Analysis of rAAV9 Expression Kinetics and Quality in Baculovirus Infected Sf9 Cells at Low and High MOI

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SUMMARY

- AAV9 produced from baculovirus infected Sf9 cells at low (0.002) and high (1) MOI are analyzed for productivity and product quality over different time intervals in order to select best process and harvest time for rAAV production.
- Low MOI production yielded overall lower titers than high MOI production.
- Low MOI infection resulted maximum titers 96-120 hours after infection whereas High MOI infection resulted maximum titers after 48 hours.
- %Full values were higher at high MOI infection. %Full increased over time in low MOI infection, and increased slightly over time in high MOI infection
- VP ratios and DNA integrity stayed stable over time in both low and high MOI infections.

INTRODUCTION

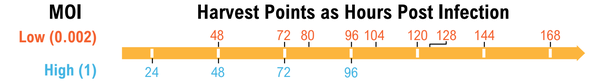
The Sf9/baculovirus expression system for rAAV production offers advantages such as higher yields and lower costs of raw materials, compared to other production systems. Generally, two baculovirus expression vectors – one containing the Rep/Cap elements and another with the gene of interest – are utilized to co-infect Sf9 cells. The infection may occur at low or high Multiplicity of Infection (MOI) and each process has its advantages and challenges. High MOI infection results in a high percentage of cells simultaneously co-infected so that cell division stops very rapidly. This synchronous infection could generate rAAV with more homogeneous product quality. However, high MOI infection can be costly due to large quantity of baculovirus (BV) needed, and may require BV production steps right as part of the AAV manufacturing process. Additionally, the risk of generating Defective Interfering Particles (DIPs) is also present.

In low MOI infection, a much lower quantity of virus is added to the culture and cells continue to divide until the baculovirus completely infects the culture, so that co-infection happens during secondary or consequent infection cycles. Small amounts of BVs added also reduce the probability of generating DIPs. However, low MOI infection yields rAAVs that are produced over a longer period in the culture, raising the question of whether the quality of the rAAV produced is homogenous throughout the culture.

In this study we evaluated the quality of the rAAV9 produced throughout the culture process in time intervals. 3.8 kb size Transgene encoding for SEAP-GFP fusion protein utilized as Gene of Interest.

rAAV productivity and product quality showed different patterns by hour and the MOI utilized, shedding light on the optimal harvest time for rAAV production.

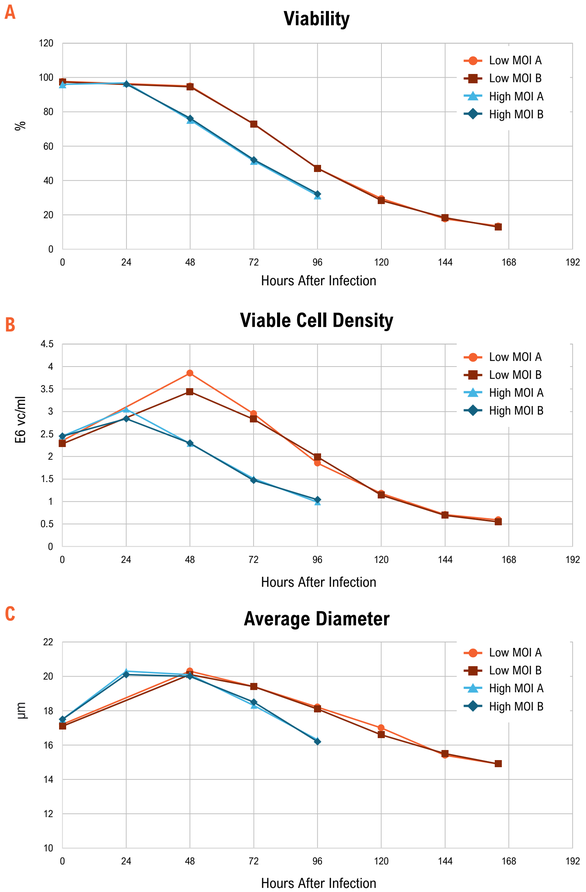
EXPERIMENTAL DESIGN



Cells are harvested at each harvest time point by detergent lysis. Lysate is clarified by 0.2 µm filter and analyzed by qPCR. Clarified lysate is affinity purified by an AAV Poros9 column. Eluates of affinity purification are utilized for product quality analysis.

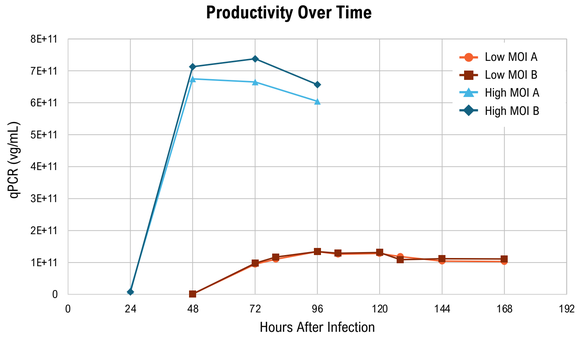
RESULTS

Figure 1. Culture parameters over course of infection



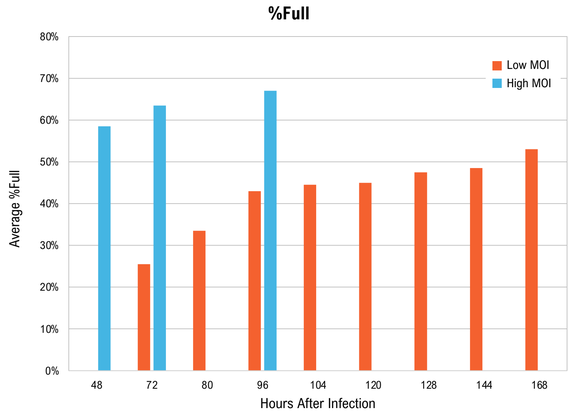
Sf9 cells are either infected with Low or High MOI. Conditions are run in duplicate flasks. Cell parameters are measured with ViCell counter every 24 hours.

Figure 2. Low MOI production yielded overall lower titers than high MOI production



Cells are lysed with Triton and clarified. Clarified lysates are analyzed with qPCR to obtained vector genome/mL productivity.

Figure 3. %Full is higher at high MOI infection and increases over time in low MOI infections



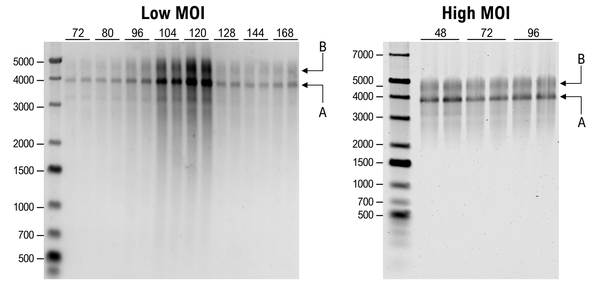
Affinity column purified material is analyzed for % Full using size exclusion chromatography (SEC). Online multi angle light scattering (MALS) combined with refractive index (RI) is used to determine the molar mass, geometry, and protein fraction of the aggregates. This assay measures %Full by deconvoluting the molar mass and determining the contribution from the DNA component of the monomer peak. Duplicates flask measurements are averaged since the variation was very low.

Table 1. VP ratios do not change dramatically over time

VP Ratios (VP3:VP2:VP1)				
	Low MOI		High MOI	
Hours After Infection	A	B	A	B
48			8:01:01	7:01:01
72	7:01:02	7:01:02	7:01:01	7:01:01
80	7:01:02	7:01:02		
96	6:01:02	6:01:02	8:01:01	8:01:01
104	7:01:02	8:01:02		
120	10:01:03	7:01:02		
128	9:01:02	6:01:02		
144	6:01:02	7:01:02		
168	7:01:02	7:01:02		

VP ratios are measured using sodium dodecyl sulfate capillary gel electrophoresis (CE-SDS) in conjunction with laser induced fluorescence (LIF) to separate and quantify the major structural proteins: VP1 (82 kDa), VP2 (66 kDa), and VP3 (60 kDa), along with any other protein-based impurities present in significant amounts. Affinity column purified material is used for the analysis.

Figure 4. Vector genome integrity remains stable over time



1% denaturing agarose gel is utilized to do electrophoretic analysis of AAV vector genome size. Affinity Column Purified Material is utilized for the analysis. A – Full Length SEAP-GFP Transgene, ~3.8kb ; B – Presumed packaging limit for AAV ~4.7kb.

CONCLUSIONS

- These comparisons show a different profile of productivity and product quality in low and high MOI infection over time.
- High MOI infection is more favorable for productivity and time spent in production, and results in similar, if not better, PQ profiles to low MOI infection.
- Performance analyses from both processes - including impurities, potency and purification performance - are planned for selecting the final optimal MOI and harvest time.

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