Optimization of AAV Vector Production using Statistical Design of Experiment

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Xingcheng Chen, Nida S. Zubairy, Timothy Ryan, Dogan Ornek, Tao Xiang, Chi-Ming Yu, W. Roy Lin, Chih-Cheng Chen
Boston Institute of Biotechnology, Southborough, MA 01772

Boston Institute of Biotechnology

Abstract

Applications of adeno-associated virus (AAV) in gene therapy has increased significantly in the last two decades due to its remarkable safety profiles and efficiency of gene delivery into various tissues. Demand on recombinant AAV vectors has also increased to fulfill pre-clinical and clinical requirements. Helper-virus-free mediated transient transfection has been widely adopted. Low titer and process scalability remain two key issues in AAV manufacturing. The productivity of AAV vectors is often affected by the AAV serotype, cell culture type, the size of Gene of Interest (GOI), cell density, transfection method, plasmid DNA concentration and other factors.

Based on the risk assessment of these factors that may impact AAV production with helper-virus-free mediated transient transfection, we have evaluated 6 process parameters using JMP's Definitive Screening Design. In this study, critical process parameters (CPP) were identified as plasmid DNA to cell density ratio, cell density per surface area and incubation time post transfection. Their operating ranges were established for optimum and robust AAV production, which can be applied while scaling up to manufacturing. AAV expression level was improved to 2E11 vg/mL.

Introduction

Over the past twenty years, three types of AAV expression systems have been developed and used for gene therapy related efforts. These include tri-plasmid transient transfection in HEK293 cells, helper virus mediated expression system using HSV or baculovirus in HEK293 or Sf9 cells respectively, and stable HEK293 cell lines integrated with Rep-Cap or Rep-Cap and ITR flanked transgene.

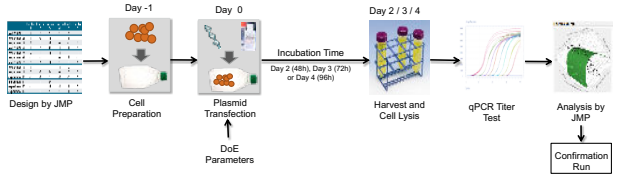
Of these expression systems, plasmid mediated transient transfection in HEK293 cell line has the advantage of being the quickest to develop and easy to scale up. However, its AAV expression level is usually low at 10^9 to 10^{10} vg/mL in titer, making it less suitable for large scale production.

Therefore it is of great interest to understand, develop and optimize this expression system to achieve higher titer in order to support the fast-paced gene therapy development and manufacturing, especially for rare diseases with urgent unmet medical demand.

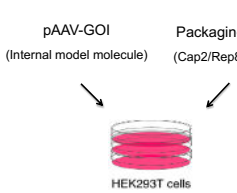
Material & Methods

- HEK 293T adherent cell line
- PEI
- AAV packaging plasmids (Cap2/Rep8/pHelper)
- AAV-GOI plasmid
- Experimental design and analysis by JMP for 6 parameters in 17 conditions
- Thaw and establish cell culture
- Transfect cells at designed conditions
- Extract crude AAVs from transfected cell culture
- Measure AAV titers using qPCR with GOI specific primers/probe

Workflow



AAV Packaging System & Analytical



AAV quantification by TaqMan qPCR

- AAV extraction (0.5% Triton)
- Residual plasmid cleanup: (DNase I)
- qPCR assay
  - Primer/probe: GOI specific
  - Standard: plasmid DNA containing GOI

Design of Experiment & Results

1. Parameters in the Study:

No.	Parameters	−	0	+
1	PEI:Total DNA	1:1	2:1	3:1
2	GOI:Packaging	1:1	2.5:1	4:1
3	DNA (ug/E6 cells)	1.43	1.71	2.00
4	Cell seeding number/ Surface area (E6/cm^2)	0.0667	0.0933	0.1200
5	Culture time (hour)	48	72	96
6	FBS (%)	5%	10%	15%

- Symbols:
  - −: -1 level value
  - 0: Center point level value
  - +: 1 level value
- Fixed parameters:
  - Surface area to total transfection volume (cm^2/ mL): 6.25
  - Percentage of transfection mixture in total transfection volume: 5%

2. Definitive Screening Design with 6 Parameters:

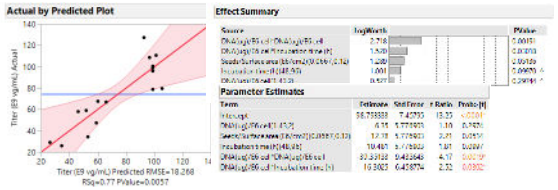
Exp No.	PEI: Total DNA (ug:ug)	GOI : Packaging	DNA (ug/E6 cells)	Seeding (E6/cm^2)	Culture time (hr)	FBS (%)	Titer (Vg/mL)
1	3	4.0	1.430	0.0933	48	15	4.73E+10
2	3	1.0	2.000	0.1200	48	10	5.90E+10
3	2	1.0	1.430	0.0667	48	5	5.79E+10
4	3	2.5	1.430	0.1200	96	5	6.76E+10
5	3	4.0	2.000	0.0667	72	5	3.42E+10
6	1	1.0	1.430	0.1200	72	15	6.68E+10
7	1	4.0	1.430	0.0667	96	10	2.59E+10
8	1	2.5	2.000	0.0667	48	15	2.91E+10
9	3	1.0	1.715	0.0667	96	15	1.09E+11
10	1	1.0	2.000	0.0933	96	5	1.27E+11
11	2	4.0	2.000	0.1200	96	15	7.95E+10
12	2	2.5	1.715	0.0933	72	10	1.00E+11
13	1	4.0	1.715	0.1200	48	5	1.11E+11
14	2	2.5	1.715	0.0933	72	10	9.59E+10
15	2	2.5	1.715	0.0933	72	10	1.70E+11
16	2	2.5	1.715	0.0933	72	10	7.86E+10
17	2	2.5	1.715	0.0933	72	10	9.88E+10

Note: Experiment No. 12/14/15/16/17 are 5 center point experiments.

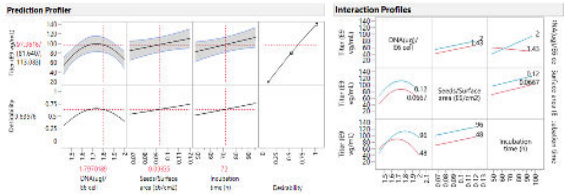
Statistical Analysis by JMP

- Model chosen describes the process behavior well as RSq=0.77
- It estimates three significant parameters:

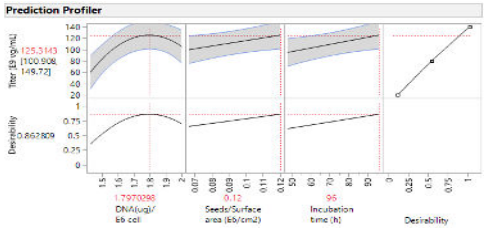
  a. DNA/E6 cells, b. Cell Seeding Number/Surface Area, c. Incubation Time



- Correlation between significant parameters and titer are shown in these figures.



- Under optimized operating conditions the predicted titer is 1.25E11±0.25E11 vg/mL



- **2E11 vg/mL was achieved at optimized operating conditions.**

Conclusion

1. Definitive screening design was used to evaluate the impacts of 6 parameters on AAV titer using 17-run experiment. AAV titer has:
   - Quadratic interaction with "Total DNA transfected to host cells".
   - Linear correlation with "Cell seeding number/Surface area"
   - Linear correlation with "Incubation time"
2. Significantly higher titer at 2E11 vg/mL was achieved at optimum conditions.
3. CPP were identified as plasmid DNA to cell density ratio, cell density per surface area, and incubation time post transfection.