Simplifying Progress

A High-Throughput Potency Assay Enables rAAV Process Optimization

Y. Huang[1]*, S. Matthias[1], S. Ali[1], N. Wagner[2], K. Teschner[3], S. Boi[1], U. Jung[1], K. Thiele[1]

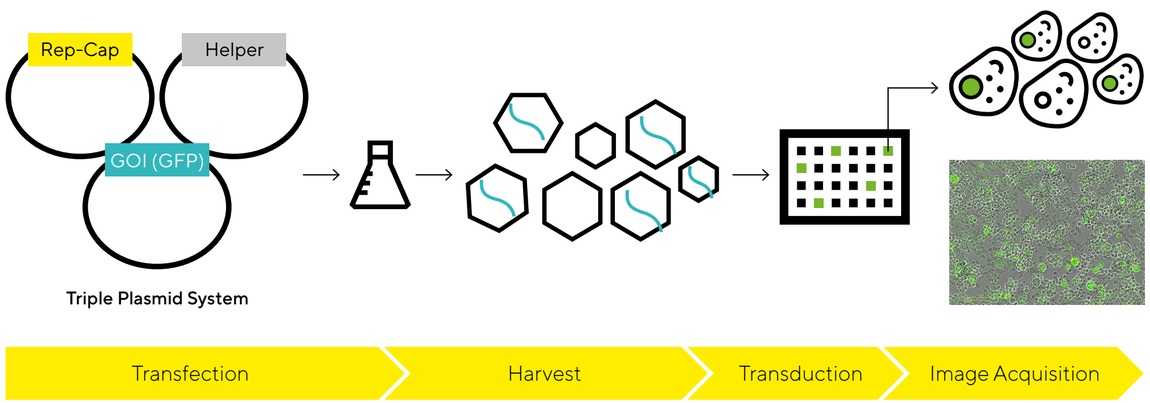
[1]Sartorius Stedim Cellca GmbH, 89081 Ulm, Germany
[2]Sartorius Stedim Biotech GmbH, 37079 Göttingen, Germany
[3]Sartorius Xell GmbH, 33689 Bielefeld, Germany
*Corresponding author: yi-hsuan.huang@sartorius.com

Introduction

Recombinant adeno-associated viruses (rAAVs) have emerged as favorable gene therapy tools due to their safety profiles in humans. Among the quality criteria, the infectious titer is critical as it represents vectors that can transduce cells and is, therefore, relevant to vector efficacy. In this study, we produced AAV2-GFP by transient transfection as a model system. We developed a cell-based assay by quantifying GFP expression using Incucyte® to capture the fluorescence and conduct high-throughput analyses. This assay allowed us to optimize process parameters such as cell density and transfection time points. We then compared transduction efficiency and transgene expression of two engineered vectors with commercial vectors in the Ambr® 15 bioreactor system.

Methods

HEK293 suspension cells were transfected with dual or triple plasmids using polyethylenimine (PEI, PEIpro®, PolyPlus). Post-transfection (72 hours), the bulk culture was collected and stored at -80 °C. Subsequently, the suspension was thawed and lyzed in lysis buffer. The crude lysate supernatant (diluted 2-fold) was used to transduce HEK293 suspension cells in 96-well plates. Post-transduction (72 hours), the green fluorescence signal was acquired and analyzed by the Incucyte® image system and presented as GFP+[%] of total cells (Figure 1).



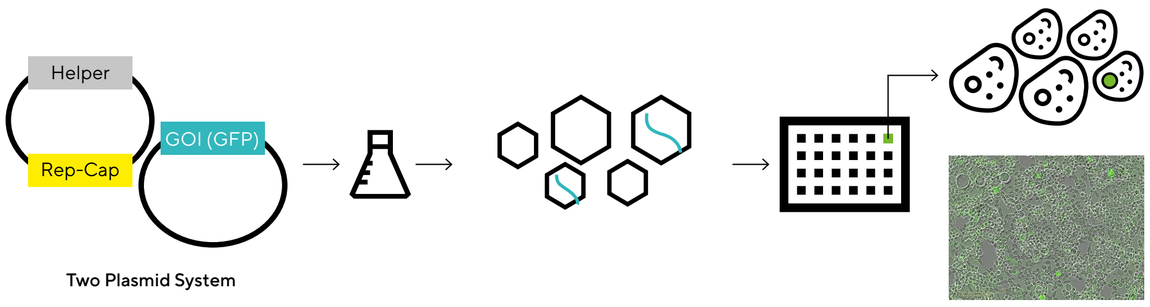


Figure 1: *Diagram of the Workflow*

Results

Analysis Setup and Validation

We chose HEK293 suspension cells for transduction for the cell-based assay due to their high susceptibility to AAV2. The analysis mask on the Incucyte® was optimized by adapting the parameters, like diameter, eccentricity, and cell edge smoothness, to the cell morphology and green fluorescence intensity (Figure 2).

The cells' autofluorescence and the free GFP molecules in the lysate were the major sources of background signals, which may hinder the recognition of weak signals from samples with lower titers. Therefore, we set criteria—linear range, precision, and robustness—for the quantitative detection of GFP expression and, calculated the transducing titer with different dilutions from six replicates. We used 2-20% as a cut-off for titer calculation as the average GFP+[%] from the mock transduction was close to 2%; the value above 20% would underestimate the titer due to the possibility of multiple viral particles transducing one cell.

The coefficient of variation (% CV) was calculated with six replicates for technical replicates, independent runs, and individual operators (Table 1). For assay robustness, we tested the samples from small-scale production processes, such as Ambr® 15 and shake flasks, and the methods remained unaffected.

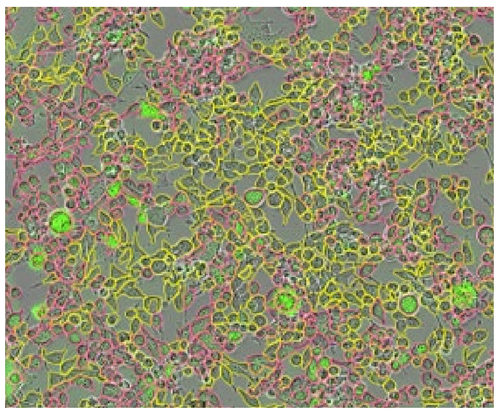


Figure 2: *Image View of Cell and Fluorescence Recognition Within the Analysis Mask*
Yellow: Cell Boundary
Pink: Cells With Green Mean Intensity Above the GFP-Positive Threshold

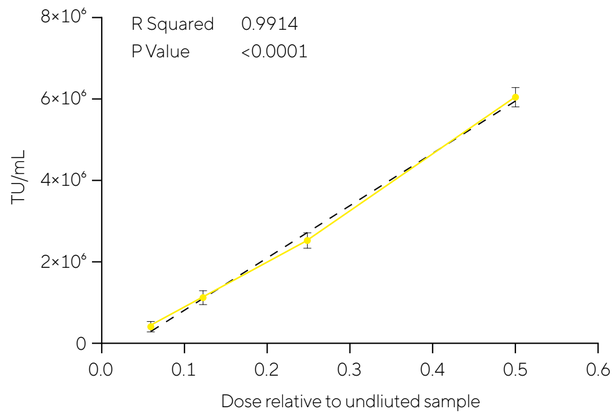


Figure 3: *Linear Range of AAV2 Transducing Titer to Virus Inoculum*

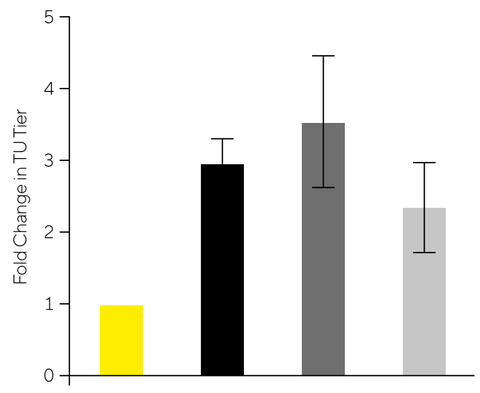
Coefficient of Variation (CV)	[%]
Intra-Plate	9.95
Inter-Plate	10.88
Inter-Operator	8.39

Table 1: *Coefficient of Variation (CV) For Intra-Plate, Inter-Plate, and Inter-Operator Precision*

Process Optimization of rAAV Production

During AAV process optimization, we first evaluated the effects of seeding density and the interval between seeding and transfection on production in shake flasks. The titers were normalized to our existing procedure (Figure 4). The results show improved potency (over 2-fold) when we transfected 2×10^6 cells/mL instead of 4×10^6 cells/mL and that we could shorten the production by one day with seeding and transfection on the same day.

We then evaluated the productivity of two host cell lines with triple-plasmid transfection in the Ambr® 15 bioreactor system. The titers were normalized to that of one cell line transfected with a commercial two-plasmid system (Figure 5). Comparing the two cell lines showed that the AAV productivity was not associated with a specific plasmid combination and may instead lie in the intrinsic property of the cells.



Seeding density [×10⁶/mL]	2	2	1	2
Density at transfection [×10⁶/mL]	4	2	2	2
Time between seeding and transfection [h]	24	1	24	24

Figure 4: *Effects of Cell Density and the Time Between Seeding and Transfection on the Fold Change of Transducing Titer*

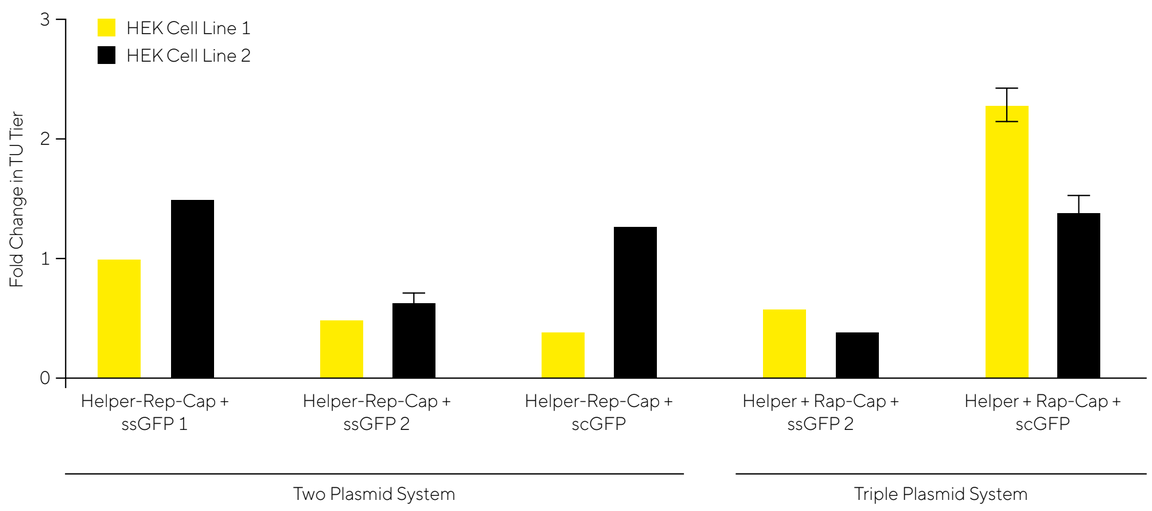


Figure 5: *Evaluation of Two Cell Lines With a Commercial Two-Plasmid System and a Triple-Plasmid System. ss: Single Strand; sc: Self Complementary*

Conclusion

In this study, we developed a high-throughput potency assay to titrate rAAV2 encoding GFP in a 96-well format. Our results show a dose-response relationship between the GFP-positive cell population and virus inoculum with intra-, inter-plate, and inter-operator reproducibility. We evaluated the transfection conditions (viable cell density, plasmid combinations) in shake flasks and the Ambr®15 to produce AAV with a higher transduction capability. As the transducing titer is critical for vector efficacy, together with capsid titer and genome titer, we can get a better picture of the AAV characteristics and, therefore, better production conditions. Despite the promising results for AAV2-GFP, further development is required for other serotypes and transgenes.

References

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- A. Westhaus, High-Throughput in vitrom ex vivo, and in vivo screen of adeno-associated virus vector base on physical and functional transduction. 2020
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