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Simplifying Progress

Enhancing AAV Productivity Through Helper Plasmid Design and Plasmid Ratio Optimization

Géraldine Guerin-Peyrou[1*], Mathieu Porte[1], Eric Mauro[2], Jonathan Havard[1], Laure Robert[1], Paul Giroud[1], Pauline Schorter[3], Quentin Bazot[3], Sylvain Julien[2], Patrick Erbacher[1]

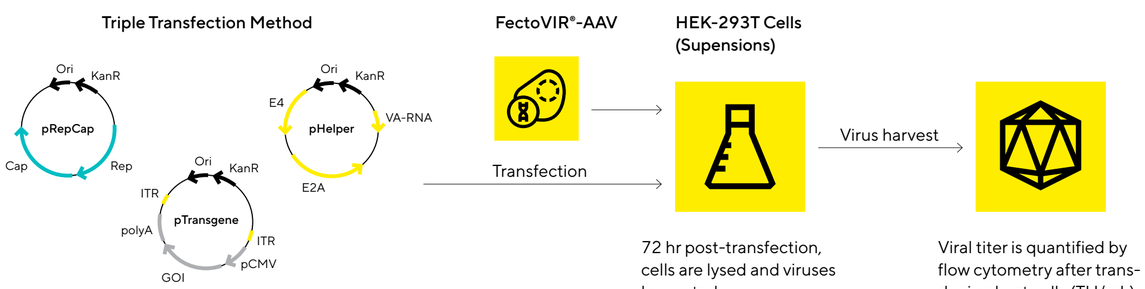
[1] Polyplus®, Illkirch, France
[2] Polyplus®, Loos, France
[3] ABL Europe, Lyon, France

* Corresponding author: g.guerin-peyrou@sartorius.com

1. Introduction

Harnessing rAAVs as viral vectors for therapeutic transgene delivery still requires improvements in yields and specificity to lower vector doses, and therefore manufacturing cost, as well as to improve patient safety. To this end, our research is focused on developing novel technologies to ensure manufacturing of high yielding rAAV particles using transient transfection, as well as enhancing viral particles quality and specificity. Here we present our state-of-the art approach to design new helper plasmids (pHelpers) with the aim of improving both the genomic titers and the infectivity (TU/mL) of the viral particle obtained from suspension cultures. We took the opportunity to exploit our proprietary DNA assembly method technology to explore the synergies of multiple genetic features modularly assembled in synthetic plasmids. We then explored how plasmid ratio optimization could further enhance AAV productivity through a Design of Experiment with a mixture design approach. This work led us to identifying plasmid ratios improving both VG titers and full | empty ratios.

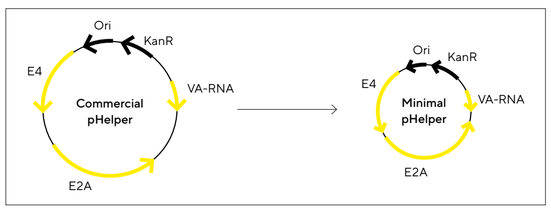
2. rAAV Production



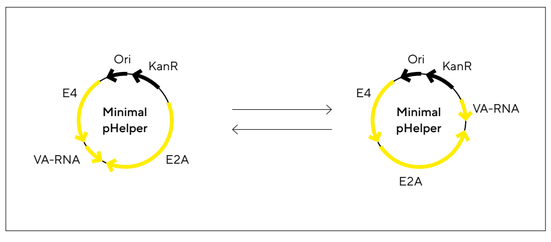
3. Plasmid Design Strategy

Various constructions of pHelper were made using e-Zyvec® plasmid assembly technology. Plasmids were transfected into HEK-293 cells and harvested 72 hr post-transfection. Infectious viral titers (TU/mL) of the produced rAAV were then compared to identify the best pHelper for optimal rAAV production.

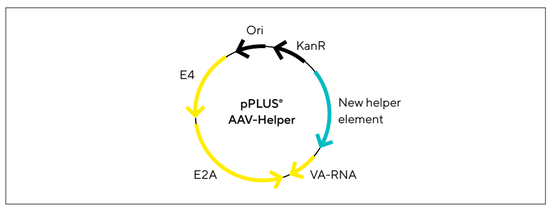
1. Removing Non-Essential Elements
- Construction of a minimal pHelper (mpH)
- 19 constructions tested, 3 series



2. Shuffling
- Re-organization of plasmid configuration to optimize its efficiency for rAAV production
- 9 constructions tested, 2 series



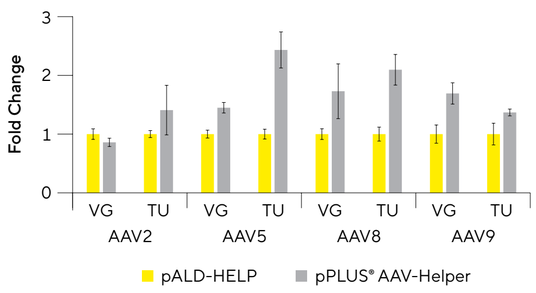
3. Addition of New Helper Elements
- Identification of new sequences to add to the pHelper to boost rAAV production
- 29 constructions tested, 5 series



4. Performance of pPLUS® AAV-Helper

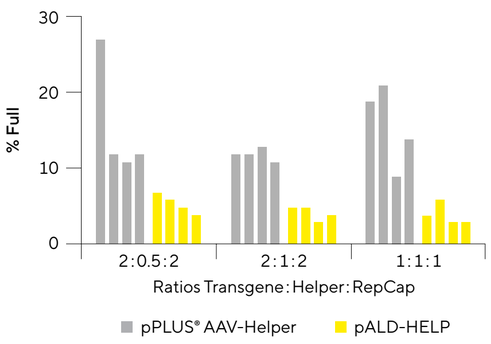
The best performing plasmid (named pPLUS® AAV-Helper) was then selected and further evaluated internally and through third-parties to confirm its performance as shown in the below experiments.

Figure 1: *Improved Titers With pPLUS® AAV-Helper for Different Serotypes*



Note. AAV2, AAV5, AAV8 and AAV9 were produced in HEK293T cells adapted in suspension in F17 medium and transfected with FectoVIR®-AAV. Infectivity assay was performed on HT-1080 cells. Improvement of titers are shown as fold increase in reference to pALD-HELP.

Figure 2: *Improved Full | Empty Particle Ratio With pPLUS® AAV-Helper*

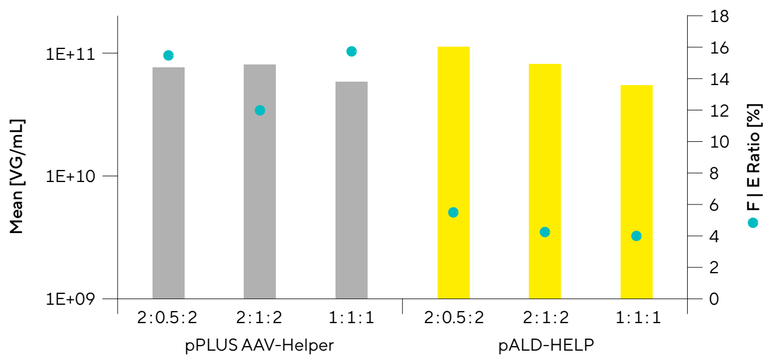


Note. AAV2 was produced in HEK293 suspension cells and transfected with FectoVIR®-AAV. Two independent experiments were conducted where each transfection was performed in duplicate. Three different plasmids ratio were evaluated (Transgene:Helper:RepCap). The % of full capsids showed is the results of qPCR:ELISA ratio (data kindly provided by ABL).

5. Enhancing Productivity Through Plasmid Ratio Optimization

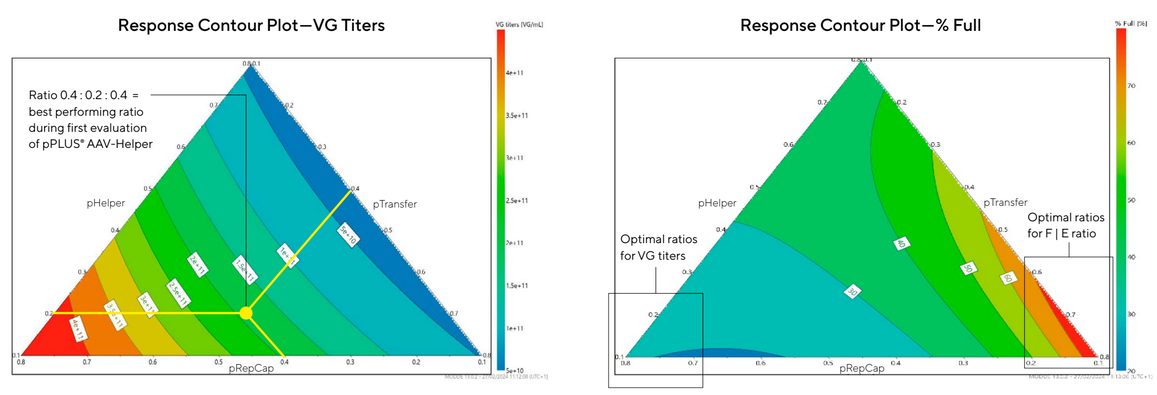
In this case study, pPLUS® AAV-Helper was evaluated by ABL Europe, a GMP CMO, completely dedicated to the provision of viral vector GMP contract manufacturing services with full scientific support provided by Polyplus.

Figure 3: *Performance of pPLUS® AAV-Helper in First Evaluation*



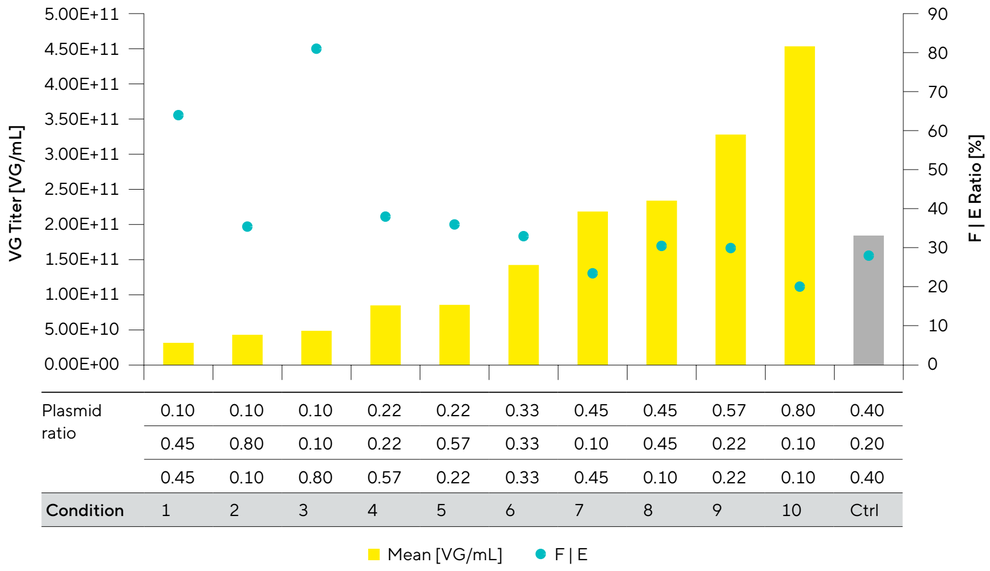
Note. AAV2, were produced in VPC2.0 cells grown in VPM medium (ThermoFisher) and transfected with FectoVIR®-AAV. Three standard plasmid ratios expressed as mass ratios (Transgene:Helper:RepCap) were evaluated.

In an attempt to further optimize the obtained titers with pPLUS® AAV-Helper, a DOE was conducted using MODDE® Pro Software. Through a mixture design approach, 10 different plasmid molar ratios were evaluated as shown in the opposite design space. Both VG titers and VP titers were measured for each data point.



6. Conclusion

Figure 4: *VG Titers and F | E Ratios Obtained Through DOE*



Note. AAV2, were produced in VPC2.0 cells grown in VPM medium (ThermoFisher) and transfected with FectoVIR®-AAV following a mixture design. VG titers were measured using qPCR and F | E ratios are expressed based on qPCR:ELISA ratios.

- Combining the obtained results for VG titers and full | empty ratio shows the tradeoff relationship between the two analytical parameters. Performing the DOE enabled to identify conditions leading to an increase in VG titers compared to the initial ratio tested in the first evaluation (grey bar in the graph)
- Condition 10 shows the best improvement in the VG titer (2.5-fold improvement compared to the initial ratio tested) but led to a slight decrease in the full | empty ratio, while condition 9 led to a lower increase in the VG titers (1.8-fold improvement) but maintaining the full | empty ratio at the same level
- This highlights the importance of monitoring various analytical parameters when optimizing a process, and the need to find a compromise between VG titers and full | empty ratio when choosing optimized conditions for production. Leveraging mixture designs in DOE allowed to identify an optimal plasmid ratio to maintain a high F | E ratio while improving VG titers
- Design of a novel Helper plasmid combined to plasmid ratio optimization through DOE led to a significant enhancement of the AAV production process