Simplifying Progress

pPLUS® AAV-Helper, Novel Engineered pHelper Plasmid to Improve Yield and Quality of Several AAV Serotypes in Suspension Cell Culture Systems

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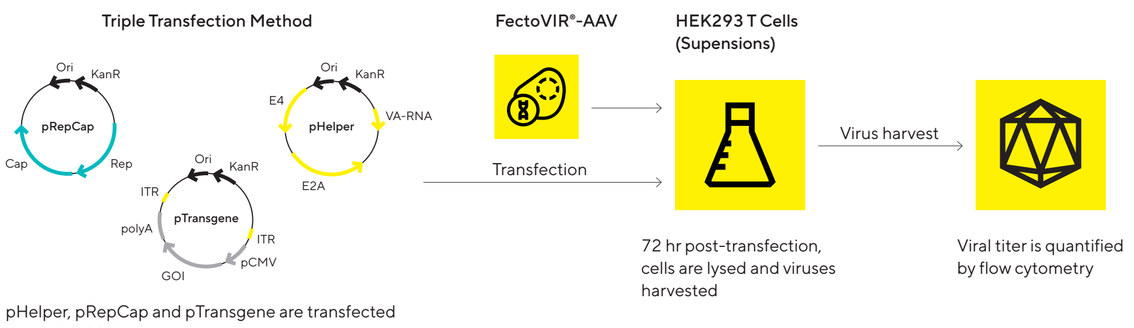
1. Introduction

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 features of rAAV vectors that act on the overall size of packaged material and specificity of delivery. Here we present our state-of-the art approach to design new helper plasmids (phelpers) with the aim of improving both the infectiosity (TU/mL) and the quality (full | empty ratio) 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. Comparison of the biological activity of several versions of rationally designed pHelpers led us to identify the optimal configuration able to outperform existing helper plasmids in every tested bioproduction conditions. Our expertise in DNA plasmid design and assembly together with our scalable transfection solutions for rAAV manufacturing gives us the potential to improve both productivity and specificity of gene therapy products.

2. rAAV Production



pHelper, pRepCap and pTransgene are transfected into HEK293 T cells

Strategy

- Various constructions of pHelper were made using e-Zyvec® plasmid assembly technology
- Plasmids were transfected into HEK293 T cells and harvested 72 hr post-transfection
- Viral titers (TU/mL) of the produced rAAV were then compared to identify the best pHelper for optimal rAAV production

3. Removing Non Essential Elements

Construction of a Minimal pHelper (mpH)

- 19 constructions tested, 3 series

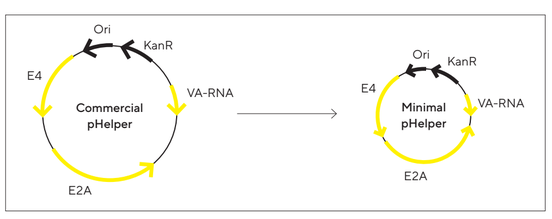
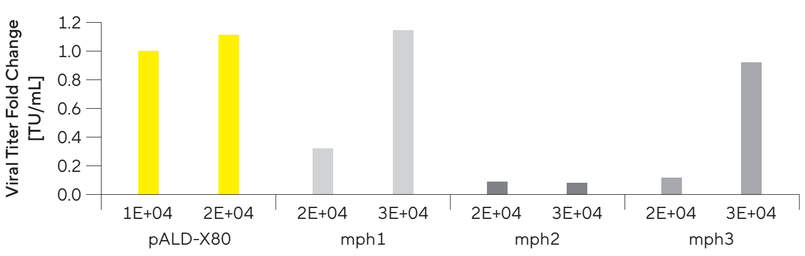


Figure 1: *Viral Titers of rAAV2 (Normalized to pALD-X80)*



Note. Copy | cell of the pHelpers are shown above plasmids name.

4. Shuffling

Re-Organization of Plasmid Configuration to Optimize Its Efficiency to Produce rAAV

- 9 constructions tested, 2 series

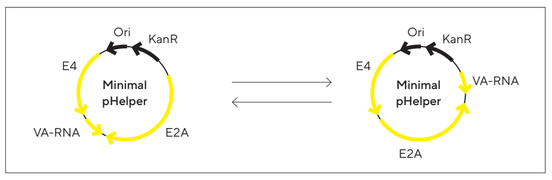
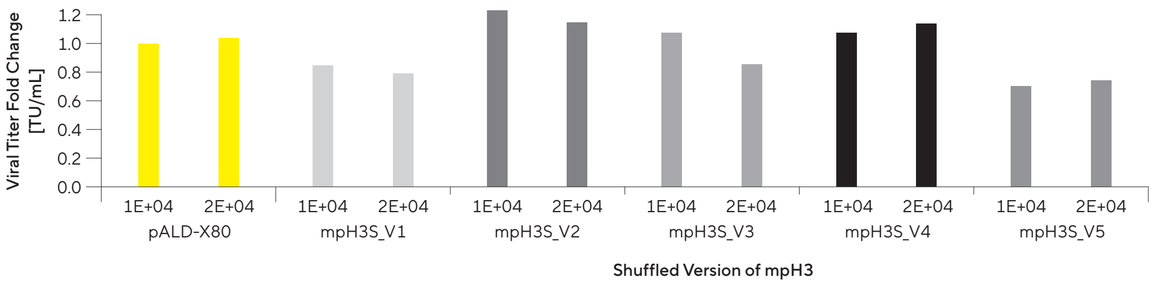


Figure 2: *Viral Titers of rAAV2 (Normalized to pALD-X80)*



Note. Copy | cell of the pHelpers are shown above plasmids name.

5. Addition of New Helper Elements

Identification of New Sequences to Add to the pHelper to Boost rAAV Production

- 29 constructions tested, 5 series

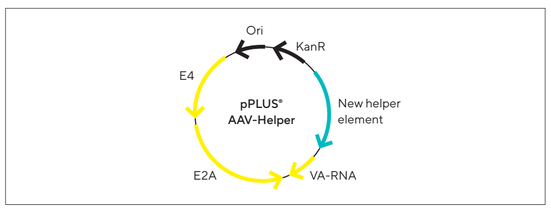
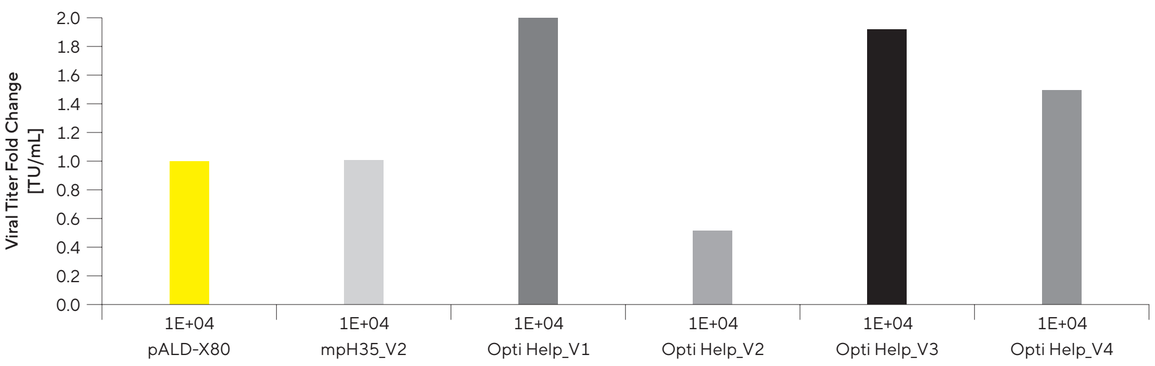


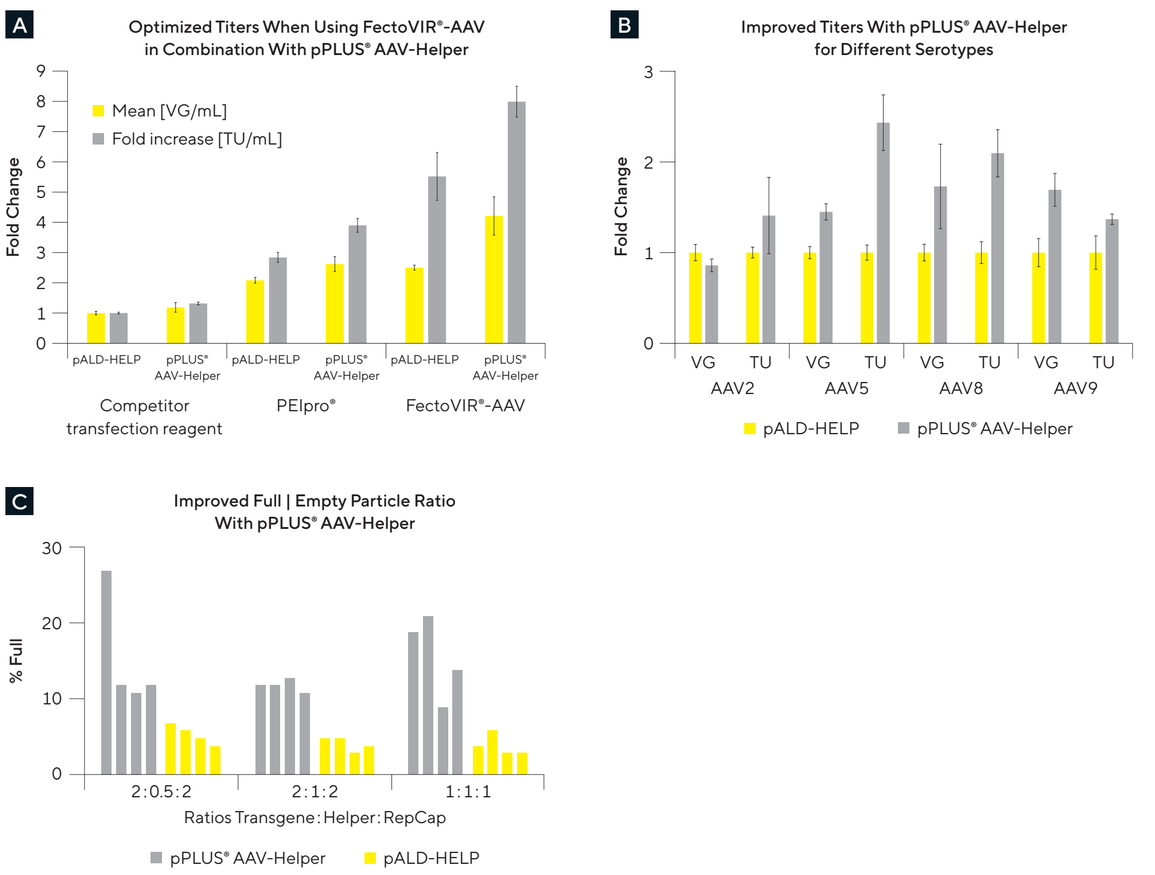
Figure 3: *Viral Titers of rAAV2 (Normalized to pALD-X80)*



Note. Copy | cell of the pHelpers are shown above plasmids name.

6. pPLUS® AAV-Helper to Improve Yield and Quality of Different AAV Serotypes

Figure 4: *pPLUS® AAV-Helper Is a Novel Helper Plasmid for AAV Production in Suspension HEK293 Cell Lines to Improve Production Yields of All AAV Serotypes Tested (AAV2, AAV5, AAV8, AAV9)*



Note. A) Improved productivity and infectivity with an optimized efficiency when used in synergy with FectoVIR®-AAV transfection reagent. AAV9 were produced in HEK293 T cells adapted in suspension in F17 medium and transfected with FectoVIR®-AAV. Transduction assay was performed on HEK293 T cells. B) Improved titers with pPLUS® AAV-Helper for different serotypes. AAV2, AAV5, AAV8 and AAV9 were produced in HEK293 T cells adapted in suspension in F17 medium and transfected with FectoVIR®-AAV. Transduction assay was performed on HT-1080 cells. Improvement of titers are shown as fold increase in reference to pALD-HELP. C) Improved full | empty particle ratio with pPLUS® AAV-Helper. AAV2 was produced in HEK293 suspension cells and transfected with FectoVIR®-AAV. Two idependents 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 provided by ABL).