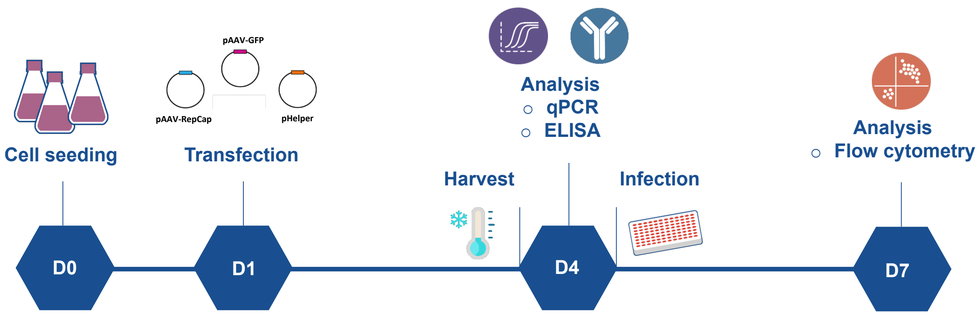
Next-Generation Transfection Reagent for Large Scale AAV Manufacturing

Mathieu Porte, Mégane Denu, Marine Ricordel, Jonathan Havard, Coralie Stritt, Yann Philipson, Malik Hellal, Patrick Erbacher
Polyplus-transfection, Bioparc, 850 Boulevard S. Brant, 67400 Illkirch, France

Abstract

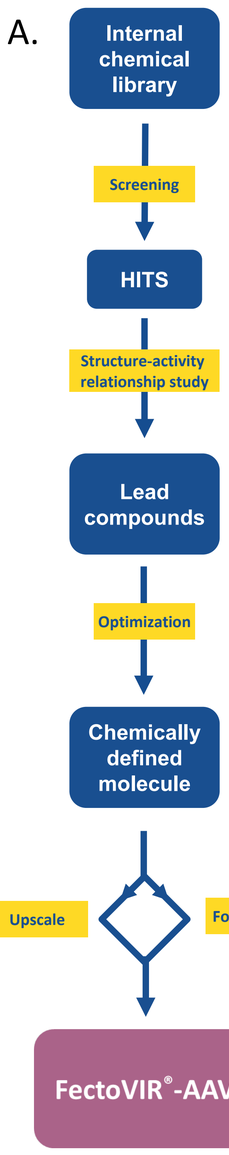
The number of ATMP therapeutic-based medicines for inherited genetic disorders is in constant growth, with a global 32% increase in new clinical trials in the last 4 years. ATMPs have demonstrated their success with already more than ten approved for commercialization. The success of AAV as the most promising viral vector for gene therapy is due to low immunogenicity, broad tropism and non-integrating properties. One major challenge for translation of promising research to clinical development is the manufacture of sufficient quantities of AAV. Transient transfection of suspension cells is the most commonly used production platform, as it offers significant flexibility for cell and gene therapy development. However, this method presents some limitations in large scale bioreactors: inadequate transfection protocol, reduced transfection efficiency and lower productivity. To address this concern, we present data on a novel transfection reagent showing: *i)* increased AAV titers, *ii)* improved transfection protocol for large scale bioreactors and *iii)* reproducibility of viral titers at different production scale. The aforementioned optimized parameters make this novel transfection reagent ideal for cell and gene therapy developers by combining the flexibility of transient transfection with scalability and speed to market.

Materials & Methods



Workflow chart of the rAAV2 production and analysis. (D0) Suspension-adapted HEK-293T cells are seeded at 1x10^6 cells/mL in 30 mL Freestyle™ F17 culture medium in a 125 ml shaker flask. The cells are incubated at 37°C and 8% CO2 at 130 rpm. (D1) After incubation, cells are in exponential growth phase at around 2 million cells/mL. After cell counting, triple-transfection of rAAV2 coding plasmids with GFP as gene of interest is performed. (D4) rAAV2 are harvested after cell lysis by 3 thaw/freeze cycles. After centrifugation, the virus physical titer is assayed in the supernatant either by capsid ELISA (Viral Particles or VP) or by qPCR (Viral Genomes or VG) analysis. The functional titer (Transduction Units or TU) is measured with an infectivity assay : adherent HEK-293T cells are infected with serial dilutions of harvested rAAV2. (D7) three days post-infection, cells are analyzed by flow cytometry to determine the number of GFP-positive transduced cells.

A.



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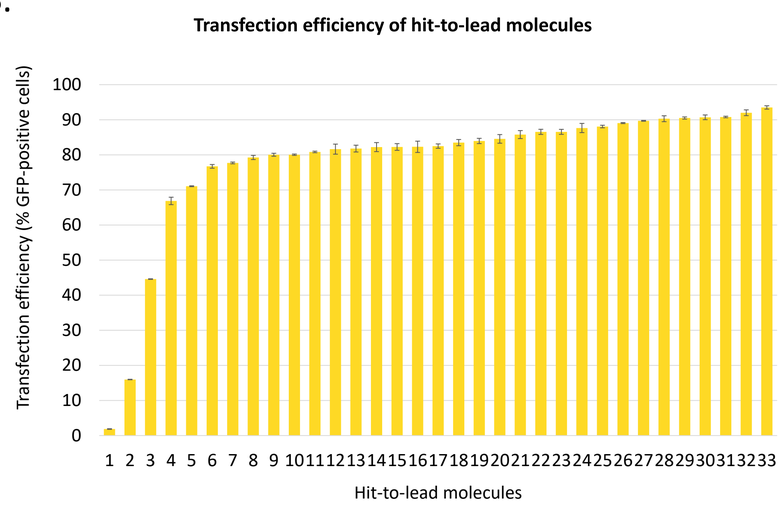

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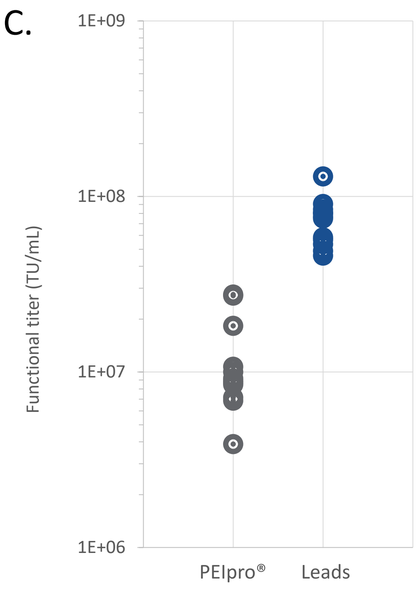
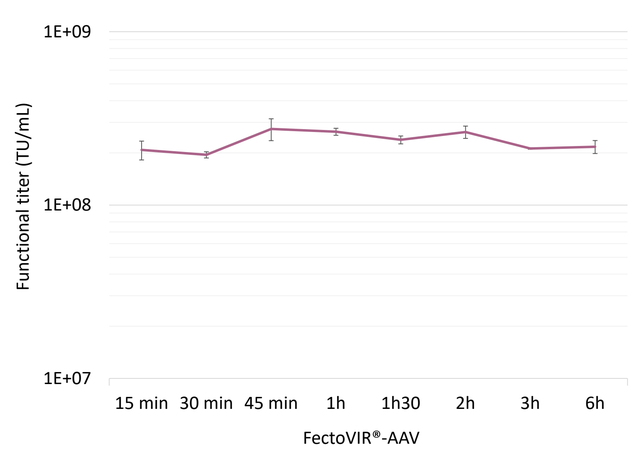


Discovery process and validation of a breakthrough transfection reagent for suspension rAAV production.
A. Description of the discovery process followed to identify and optimize the perfect reagent for suspension rAAV production.
B. Suspension HEK-293T cells were transfected with a GFP plasmid using molecules from the hit-to-lead screening. Transfection efficiency was assayed at 48h post-transfection using flow cytometry.
C. rAAV were produced in suspension HEK-293T cells either with PEIpro® or with the lead compounds. The graph shows the results of 12 different molecules in 12 different experiments. Functional titer was measured with an infectivity assay

Improved transfection protocol for large scale suspension bioreactors

A.



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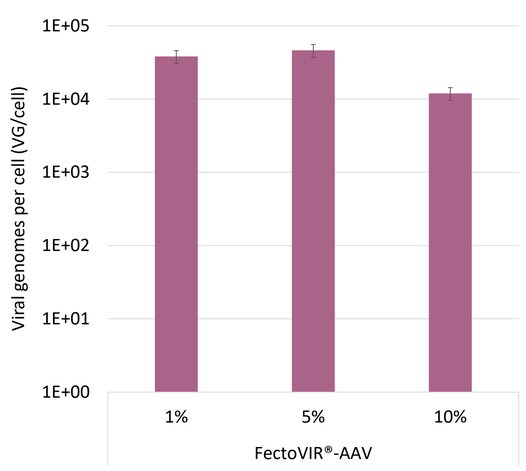
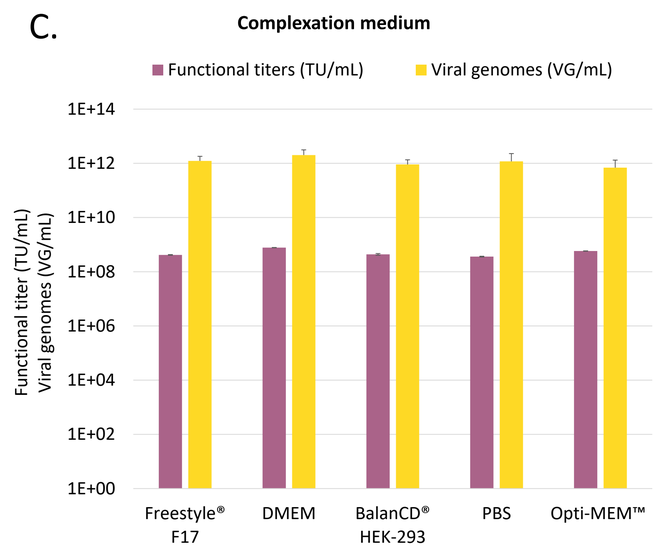
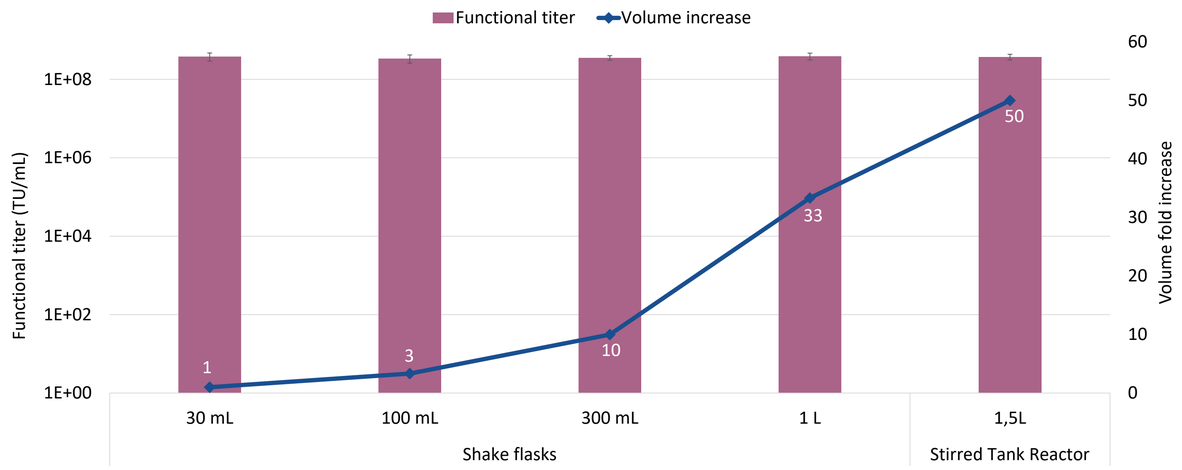


By offering great flexibility in the transfection mix preparation, FectoVIR®-AAV allows to overcome the major limitations encountered in large-scale bioreactors transfection.
For each experiment, HEK-293T cells in suspension were transfected with FectoVIR®-AAV using 1 µg DNA/million cells and a DNA : reagent ratio of 1 µg : 1 µL. If not mentioned otherwise, transfection complexes were prepared in DMEM in 5% of the final volume of culture and incubated 30 min before being added to the cells.
A. After preparation, the transfection mix were incubated at room temperature at rest from 15 min to 6h. Functional titer was measured with an infectivity assay.
B. Transfection complexes were prepared in a volume ranging from 1 to 10% of the final volume of culture (30 mL). Viral genomes were measured using qPCR analysis.
C. Transfection complexes were prepared in different complexation media. Viral genomes were assayed using qPCR analysis and functional titer was determined in an infectivity assay.

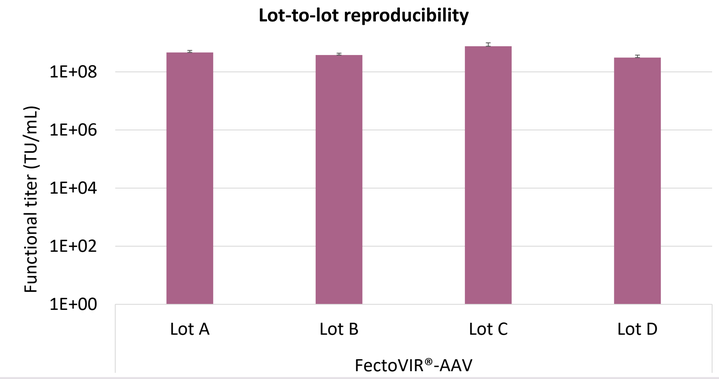
C.



Scalability and reproducibility



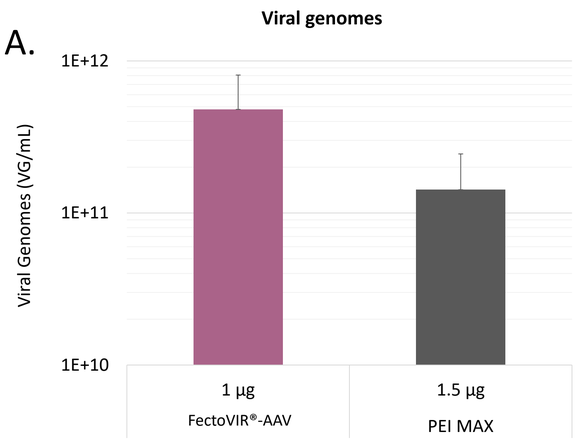
FectoVIR®-AAV shows a great scalability with perfectly consistent results between shake flasks and stirred tank reactor in a 50-fold volume increase study. HEK-293T cells in suspension were transfected with FectoVIR®-AAV with 1 µg DNA/million cells and a DNA : reagent ratio of 1 µg : 1 µL. Complexes were prepared in DMEM in 1% of the final culture volume and incubated for 30 min before being added to the cells. Functional titer was measured with an infectivity assay



As a robust and reliable product, FectoVIR®-AAV demonstrates an excellent lot-to-lot reproducibility. HEK-293T cells in suspension were transfected with FectoVIR®-AAV with 1 µg DNA/million cells and a DNA : reagent ratio of 1 µg : 1 µL. Complexes were prepared in DMEM in 5% of the final culture volume and incubated for 30 min before being added to the cells. Functional titer was measured with an infectivity assay.

Increased AAV titers using less DNA

A.



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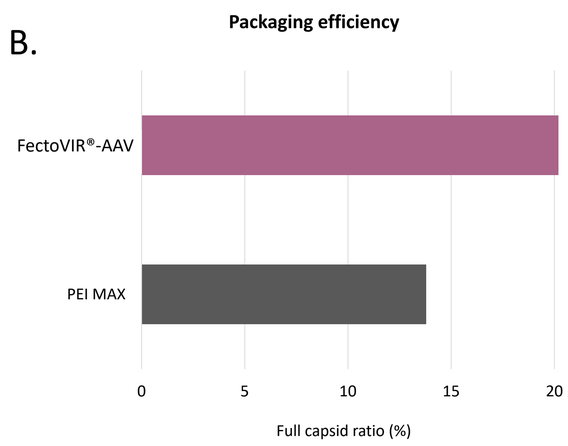

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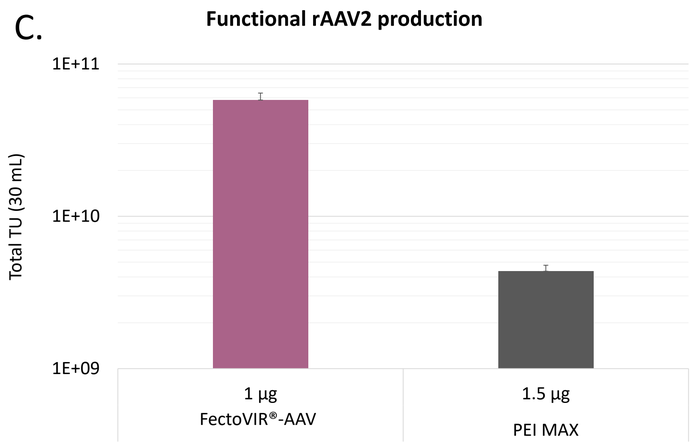

With 33% less DNA, an improvement in both viral genome production and packaging efficiency with FectoVIR®-AAV results in a 10-fold increase in functional rAAV2 production in comparison with competitor.
Suspension-HEK-293T cells were transfected with the optimal conditions for PEI MAX (1.5 µg/million cells, ratio DNA : PEI of 1 µg : 4 µL) and FectoVIR®-AAV (1 µg/million cells, ratio DNA : reagent of 1 µg : 1 µL) following the recommended protocol for each reagent.
A. The viral genome production was measured by qPCR analysis.
B. Capsid titer was determined by ELISA. The full capsid ratio was calculated by dividing the VG titer (VG/mL) by the capsid titer (VP/mL).
C. Functional titer was measured with an infectivity assay

Conclusion

FectoVIR®-AAV

- High performance : superior yields for rAAV production in suspension
- Ease of use : reduces complexation volume, stable transfection mix
- Cost-saver : reduces DNA amount
- Scalable : from small to large scale industrial production
- Flexible : compatible with different culture medium
- GMP compliance : GMP grade coming soon

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