Abstract

The number of AAV gene therapy-based medicines for inherited genetic disorders is in constant growth, with a global 32% increase in new clinical trials in the last 4 years. AAVs 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 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. (D1) Suspension-adapted HEK-209 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 for the defined molecule is performed. (D4) rAAV2 are harvested after cell lysis by 3 thaw/freeze cycles. After incubation, cells are in exponential growth phase at around 2 million cells/mL. After cell counting, triple-transfection of rAAV2 coding for the defined molecule is performed. (D4) rAAV2 are harvested after cell lysis by 3 thaw/freeze cycles. After incubation, cells are in exponential growth phase at around 2 million cells/mL. After cell counting, triple-transfection of rAAV2 coding for the defined molecule is performed. (D4) rAAV2 are harvested after cell lysis by 3 thaw/freeze cycles. After incubation, cells are in exponential growth phase at around 2 million cells/mL. After cell counting, triple-transfection of rAAV2 coding for the defined molecule is performed.

Improved transfection protocol for large scale suspension bioreactors

By offering great flexibility in the transfection mixture, FectoVIR®-AAV allows to overcome the major limitations encountered in large-scale bioreactors transfection.

A. Description of the discovery process

B. Optimisation of titers and purity of viral capsids

C. Scalability and reproducibility

Conclusion

With 33% less DNA, an improvement in both viral genome production and packaging efficiency with FectoVIR®-AAV results in a 50-fold increase in functional AAV2 production in comparison with competitor.

Increased AAV titers using less DNA

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-209 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 volume of culture and incubated 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-209 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.

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