Development and Optimization of a Novel Transfection Formulation for High Titer Recombinant Lentivirus and Adeno-associated Virus (AAV) Production

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Abstract
The tremendous success in clinical trials and FDA approval of several gene therapies in 2017 has led to an increased interest in the optimization and scale-up of virus manufacturing processes to deliver large quantities of high titer recombinant lentivirus and AAV. To address this need, we screened our lipid and polymer libraries using a functional titer read-out to identify a novel transfection formulation that provides robust titers for lentivirus and AAV production in both adherent, serum-containing cultures, as well as serum-free suspension 293-derived cell types.

Scale-up and reproducibility are key attributes in large-scale manufacturing. Transfection complex formation is a critical step for transient transfection; key parameters including buffer composition, incubation time and the volume of the complex formation were assessed and optimized. Proof-of-principle scaling experiments were performed in suspension 293 cells from multiwell formats to large shaker flasks. Reproducibility of the transfection process was also addressed through functional titer determination of multiple virus batches manufactured over an extended time period.

Our data demonstrate that transient transfection is a robust and reliable tool that can be harnessed for large-scale manufacturing of both recombinant lentivirus and AAV.

Development Goals
- Best-in-class generation of recombinant AAV and lentivirus
- Compatible with suspension and adherent 293-derived cell lines
- Robust, scalable protocol

Assays for Virus Detection

Transfection Complex Formation Buffer

Capsid Quantification

Transfection Efficiency During Virus Production

Functional Virus Titer

Virus Genome Number

Concentrated Transfection Complexes

Conclusions
- High Functional Titers: AAV and lentivirus produced in suspension and adherent 293-derived cells
- Scalable: Efficient across different formats
- Reliable: Consistent high titer production across independent experiments
- Robust: Compatible with multiple complex formation buffers and concentrations