Modular, scalable AAV purification process for safe vectors & recoveries of up to 50%

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P0031

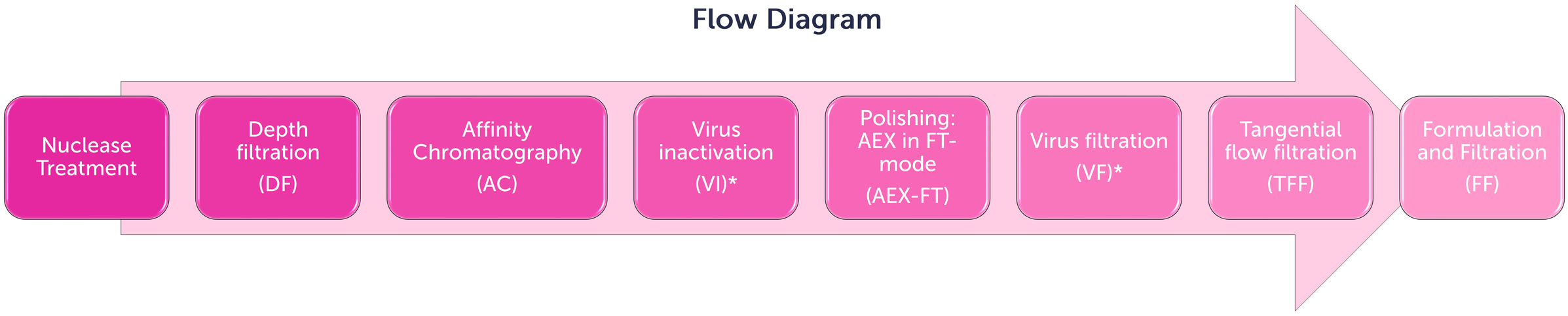
Abstract

The development of a fully scalable AAV purification process with high product yield and vector quality remains an important goal in the gene therapy field. Here we present the results of our modular and scalable AAV downstream process. The process was developed with AAV9 material produced with our proprietary suspension cell process.

Scalable options for DSP development are mainly chromatography and filtration steps while the scaling of preparative ultracentrifugation is more complex. We have therefore focused on the former. However, these steps also have their limitations, as columns and filters are only available in certain sizes or dimensions. In order to create a suitable scale-down model for future process robustness studies, the column and filter sizes were increased for production scale. This may have an impact on product yield, but our data show that changes performed during DSP scale-up development did not negatively impact high product yield and quality. To increase product safety, several downstream steps to remove adventitious viruses were tested for AAV9 regarding product stability and recovery. These steps can be implemented in a modular way to meet future regulatory requirements without impacting product quality and quantity.
Our 2-split plasmid system delivers AAV9 capsids with a %-full of up to 60 % (mass photometry) in upstream processing. We thereby implement high quality into the product from the beginning. A full-empty separation step, e.g. by AEX chromatography, might be dispensable for a number of applications which will be dependent on the intended dose, route of administration and overall risk/benefit profile of a given indication. Avoidance of full capsid enrichment results in higher DSP vector recovery. To further boost product quality, a high salt AEX chromatography in flow-through mode was developed as polishing step. By this step non-packaged process-derived impurities are further reduced, in many instances down to levels at or below the LOQ of our sensitive in-house analytical assays.

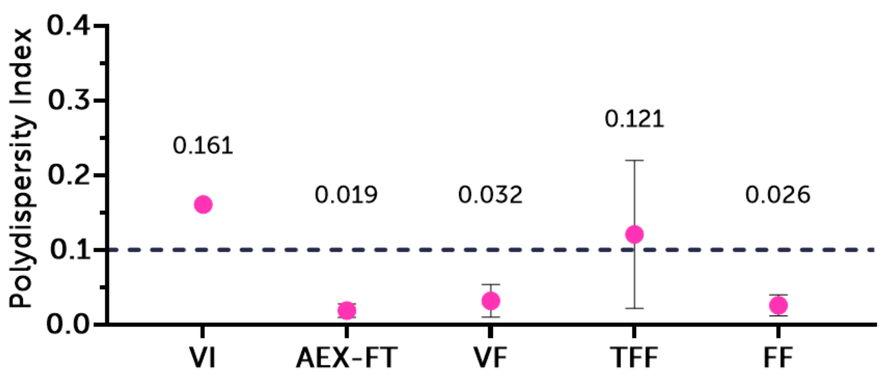
To sum it up, we successfully developed a fully scalable DSP platform enabling vector recoveries of ≥50 % at high vector quality and built-in adventitious virus removal steps to meet future regulatory requirements.

Flow Diagram



*dedicated "viral clearance" steps to comply with new Q5A(R2) guideline

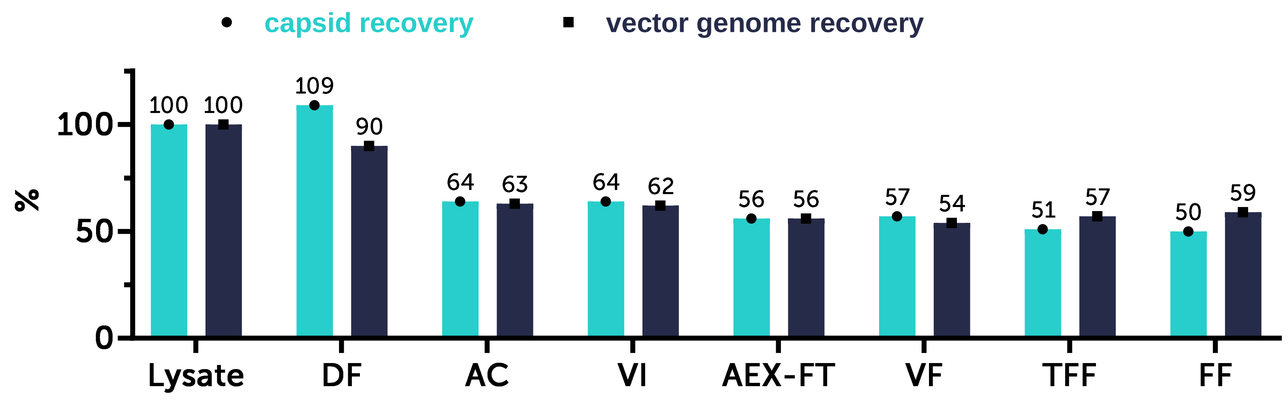
Aggregation*



- Until 0.1 particles are defined as "monodisperse". Therefore, a dotted line is included to demonstrate the limit.
- Due to elevated conductivity levels, aggregation level of AAV9 capsids is low during most of the DSP steps.

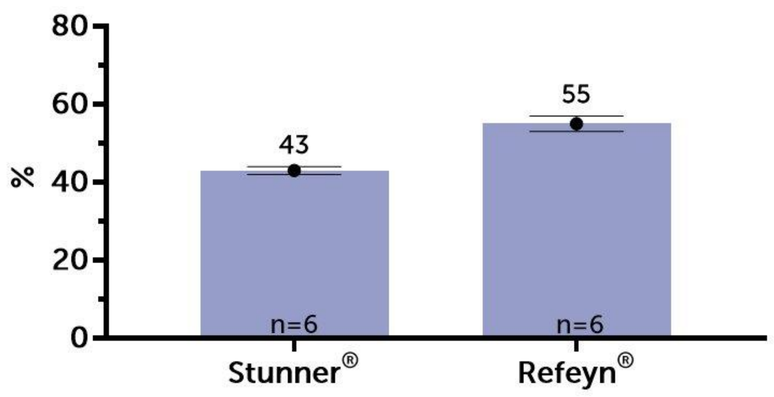
*Aggregation level determined by DLS (Stunner®).

Overall Recovery



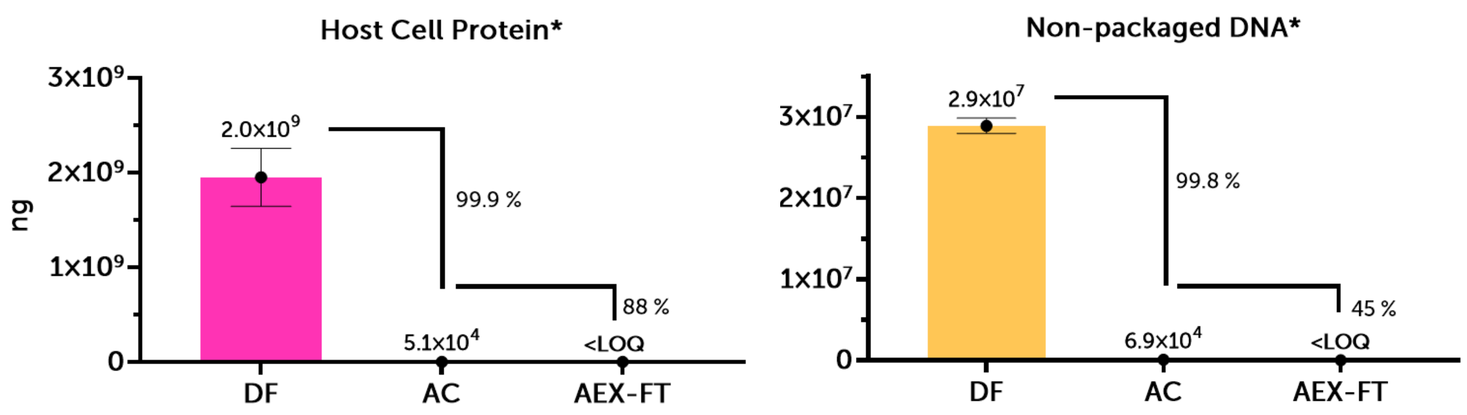
- Affinity chromatography is the only step where a clear vector loss happens. Therefore, the focus of future studies is to improve the step yield of the affinity chromatography.
- Difference between capsid and vg titer is not considered significant. Hence % full remains constant during the process.
- **50%** overall recovery was achieved with the whole purification process!

%Full data



- Full-empty ratio is up to 60% by mass photometry (Refeyn®) without enrichment step!

Host cell protein and non-packaged DNA removal



- 99.9 % Host cell protein and 99.8 % non-packaged DNA removal by affinity chromatography!
- Post AEX in FT-mode HCPs and non-packaged DNA are below the limit of quantification!

*Host cell proteins were determined by Gyrolab® ELISA and non-packaged DNA by Qubit® fluorometer,

Summary

Our new high-salt DSP can control virus aggregation at any process step and achieves overall recoveries of ≥ 50% with built in viral clearance steps to meet current and future regulatory requirements.
Our fully-scalable DSP reduces process derived impurities, specifically non-packaged HCD, HCP, nuclease (data not shown) and affinity ligand (data not shown) to levels below LOQ of highly sensitive analytical methods.
The already achieved high % full ratios can be further increased by an optional AEX chromatography based full enrichment step.

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