Improvement of Yield and Critical Quality Attributes through Process Development of a Novel Adeno-Associated Viral Vector Capsid, Anc80L65

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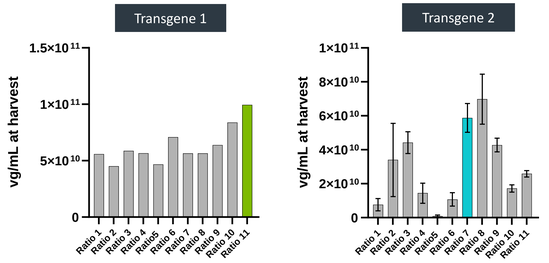
Abstract

Affinia Therapeutics is pioneering a shift to a new class of rationally designed gene therapies via our proprietary Affinia Rationally designed Therapies (ART) platform. One focus is developing novel capsids that enhance tissue specificity, immunological profile, and manufacturability and we have recently characterized multiple novel capsids with these attributes for skeletal muscle, heart and CNS diseases.

Here we wanted to demonstrate that our process improvement approaches can result in novel capsids having similar or better manufacturing and stability profiles vs. those of natural serotypes. For this purpose, we used Anc80L65, a capsid first identified by the Vandenberghe Lab, as an example. Multiple process development strategies were applied to improve total process yield and Critical Quality Attributes (CQA's). Our platform upstream process utilizes suspension HEK293 cells, so we started by testing different cell culture media, transfection reagents and transfection parameters utilizing Design of Experiment methodology. Through these efforts, we demonstrated over a log increase in yield at harvest in both shake flasks and bioreactors to >2e11 vg/mL. We then sought to develop a high yielding downstream purification method. We demonstrated that Anc80L65 can be purified with multiple commonly available affinity resins. We developed two polishing methods: with CsCl gradient, we achieved >90% full capsids and with anion exchange chromatography, we achieved >70% full capsids. We looked at other common critical quality attributes such as hcDNA, HCP, residual plasmid DNA and believe our process generates high quality vector. Stability is an important metric for AAV capsids, and we were able to show that Anc80L65 is stable at -80°C for at least 12 months. Overall, we have demonstrated, using Anc80L65 as an example, that process development can significantly improve yields and CQAs to commercially attractive ranges.

Upstream Process Development

Fig 1. DOE for optimal plasmid ratio



Upstream Process Development

Fig 2. Optimizing media and transfection parameters

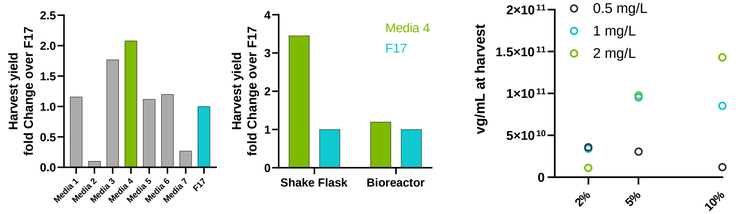
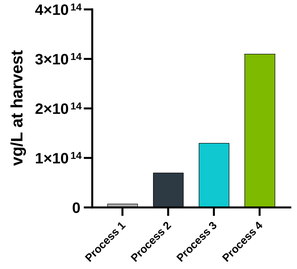


Fig 3. Output with best conditions



Downstream Process Development

Fig 4. Optimizing affinity chromatography resin

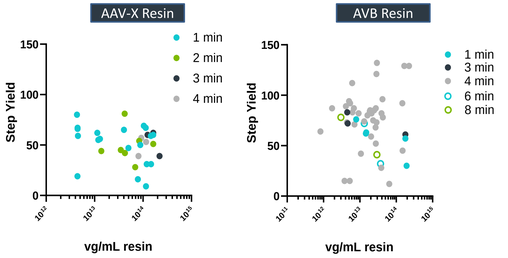


Fig 5. Optimizing CsCl for full capsid enrichment

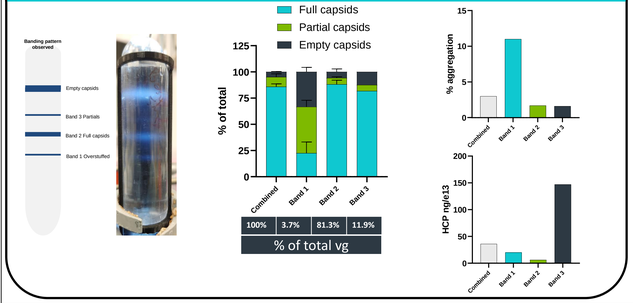
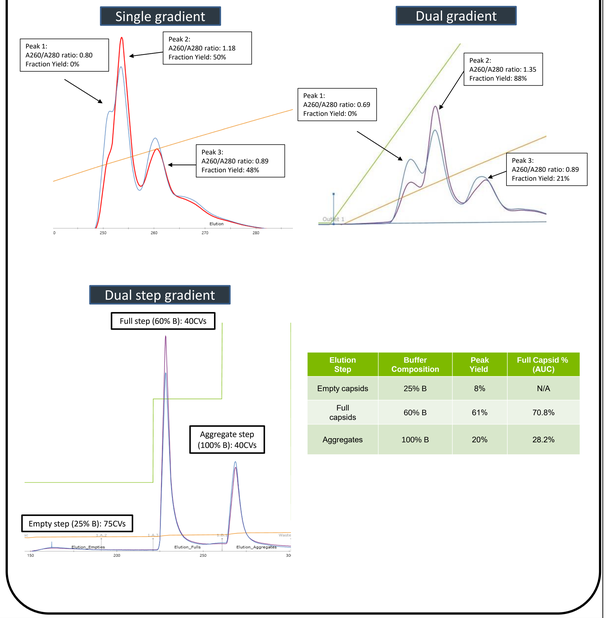


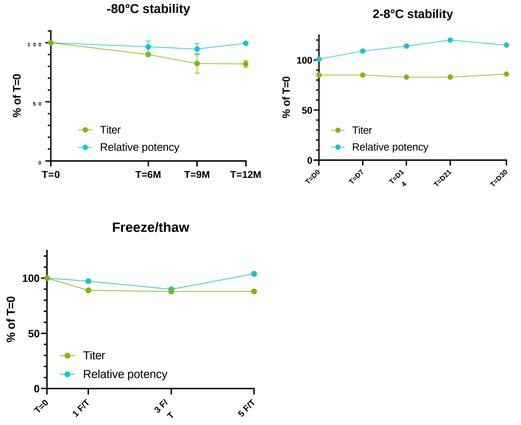
Fig 6. Optimizing AEX for full capsid enrichment



Elution Step	Buffer Composition	Peak Yield	Full Capsid % (AUC)
Empty capsids	25% B	8%	N/A
Full capsids	60% B	61%	70.8%
Aggregates	100% B	20%	28.2%

Stability Results

Fig 7. Anc80L65 shows good stability profile



Conclusions

Process development can significantly improve the manufacturability of novel capsids. With Anc80L65:

Harvest yield increased >40X to ~2e11 vg/mL

- High purity levels achieved
  - >90% full capsids with CsCl ultracentrifugation
  - >70% full capsids with AEX
- Resulting product showed stability at -80°C, 2-8°C and with multiple freeze thaws

This experience gives us confidence that other novel capsids, even if initially difficult to manufacture, can be improved into commercially attractive vectors through directed process development.