# Process and Platform Development for Production and Purification of CNS-Tropic Engineered AAV Capsids

Abstract #1460

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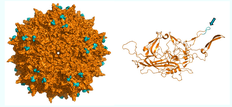
## Introduction

- Conventional AAV serotypes exhibit limited delivery to the central nervous system (CNS) when employing less invasive routes of administration.
- Modification of the AAV capsid can improve delivery efficiency and tissue specificity, however, these changes may also necessitate optimization of the manufacturing process and purification conditions.
- Design and optimization of manufacturing processes that enable large scale production and purification of AAV vectors is challenging, but critical to bridge preclinical and clinical studies.
- In this study, we report the development of a scalable process for production and purification of engineered AAV capsids that show significantly enhanced CNS delivery relative to AAV9, the most widely used vector for CNS gene therapy[1,2]. The optimized process demonstrates the manufacturing feasibility as well as product quality attributes that are critical to advancing novel genomic medicines to treat CNS disorders.

### Engineered AAV Capsids

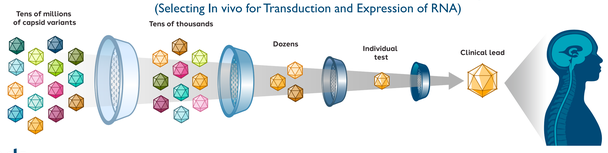
Full icosahedral capsid | Viral protein monomer



Peptide insertion in Variable Region VIII influences cell and tissue tropism

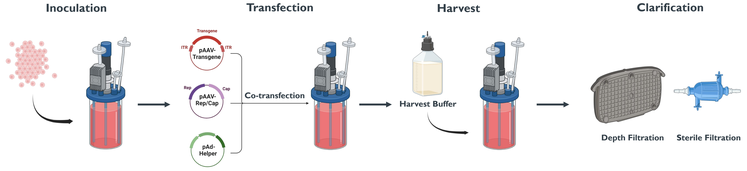
### SIFTER Platform for Identification of Novel AAV Capsids

(Selecting In vivo for Transduction and Expression of RNA)



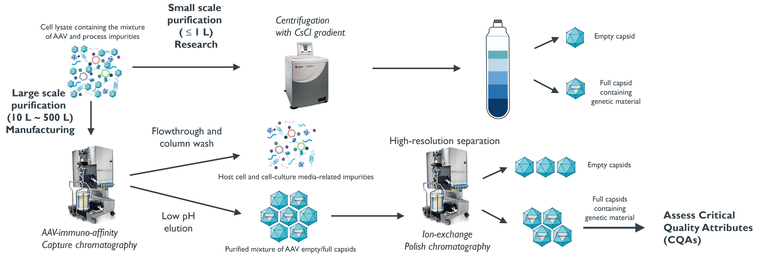

## Methods

### AAV Production in HEK293 Cell Suspension System



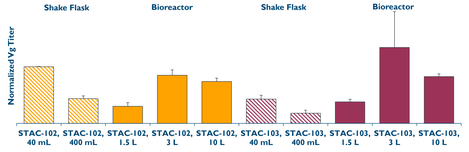

*Created with BioRender.com*

### AAV Downstream Purification




## Results

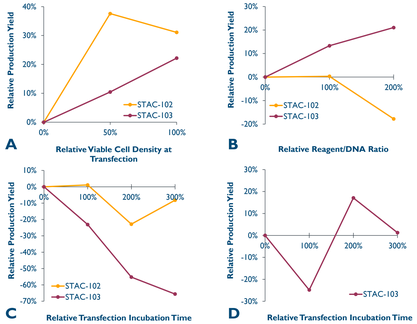
### Productions at Various Scales




Comparison of clarified harvest vg titer from productions at various scales: 40mL, 400mL, 1.5L, 3L, and 10L for both engineered AAV capsids: STAC-102 and STAC-103.

- AAV production in shake flask vs. bioreactor have different productivity performance.
- We demonstrated scalable production processes in bioreactors for both engineered AAV capsids STAC-102 and STAC-103 by showing comparable yields during scale-up.

### Upstream Process Optimization




The impact on production yield by varying in-process parameters **(A)** viable cell density at transfection **(B)** ratio of transfection reagent over total DNA plasmids amount and **(C)** incubation time of transfection complexation media at 40mL scale in shake flask production; **(D)** verification of the impact of transfection complexation medium incubation time in 1L bioreactor.

- We have evaluated viable cell density at transfection, the ratio of transfection reagent over total DNA plasmid amount, and transfection complexation media incubation time.
- Data shown here denotes the percentage of value change for each parameter vs. the reference process parameter (0, 0).
- The transfection complexation media incubation time was deemed as a critical process parameter at 40 mL for STAC-103 production in shake flask. However, after verification in 1L bioreactor, this parameter was less impactful than expected and the standard parameter value was already optimal for the process.

### Capture Chromatography Optimization

| Clarified Harvest | Affinity Resin | Wash | Elution |
|---|---|---|---|
| STAC-102 | POROS CaptureSelect | 20 mM Tris, 1 M NaCl, pH 8 | Buffer A (pH 3) |
| STAC-103 | | | Buffer B (pH 3 + additives) |

| Serotypes | Elution Buffer | Vg Recovery (by ddPCR) | |
|---|---|---|---|
| | | FT | Elution |
| STAC-102 | Buffer A | 0% | 74% |
| | Buffer B | 0% | 43% |
| STAC-103 | Buffer A | 1% | 49% |
| | Buffer B | 0% | 21% |

### Polish Chromatography Optimization

| Columns | STAC-102 | STAC-103 |
|---|---|---|
| POROS 50 HQ | ✓(E) | ✓(E) |
| CIMmultus QA* | ✓(E) | ✓(E) |
| POROS XS | ? (FT & E) | ? (FT & E) |

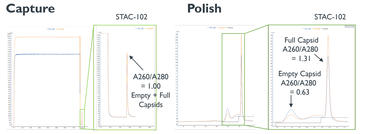
* Desirable if empty capsid removal is required

FT: Flow Through
E: Elution

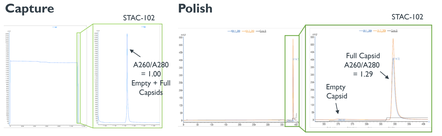
#### Empty capsids in capture eluate?


- The A260 / A280 values for Capture eluates were approx. 1.0, which represents approx. 70% of full capsids
- **To separate the Empty and Full capsids, CIMmultus QA is used for the Polish Step**

### Small Scale Purification: Optimization

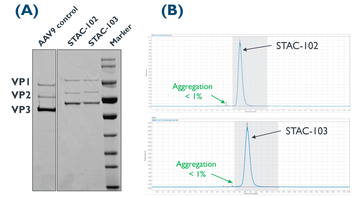



### Large Scale Purification: Scale Up




### Critical Quality Attributes

| Critical Quality Attributes | Assay | STAC-102 | STAC-103 |
|---|---|---|---|
| Vg Recovery | ddPCR | Capture: > 70%<br>Polish: > 90% | Capture: > 50%<br>Polish: > 90% |
| Purity | SDS-PAGE (A) | VP1:VP2:VP3<br>1: 0.88: 7.72 | VP1:VP2:VP3<br>1: 1.29: 8.81 |
| | SEC (B) | < 1% aggregation | < 1% aggregation |
| | DLS | No aggregation | No aggregation |
| Infectivity | TCID50 | P/I ratio : 22 | P/I ratio : 9 |




Sample purity results assessed by various assays **(A)** SDS-PAGE for inter-viral protein ratio; **(B)** SEC for sample aggregation indication.

## Conclusion

- We have successfully developed robust and scalable production processes (up to 10L) for CNS-tropic engineered AAV capsids STAC-102 and STAC-103.
- Production process parameters were evaluated and optimized for high product yield.
- Produced AAV capsids (STAC-102 and STAC-103) were purified using an affinity capture chromatography column (POROS CaptureSelect) and an anion-exchange chromatography column (CIMmultus QA).
- The column chromatography purification method is easily scalable to the target manufacturing scale.
- The purified AAV capsids showed acceptable yield, purity, and infectivity.

## References

1. Au H.K.E., et al. Gene Therapy Advances: A Meta-Analysis of AAV Usage in Clinical Settings. *Front. Med.* (2022) 8:809118. https://doi.org/10.3389/fmed.2021.809118
2. Lykken, E.A., et al. Recent progress and considerations for AAV gene therapies targeting the central nervous system. *J Neurodevelop Disord* 10, 16 (2018). https://doi.org/10.1186/s11689-018-9234-0

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## Disclosures

*These authors contributed equally

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