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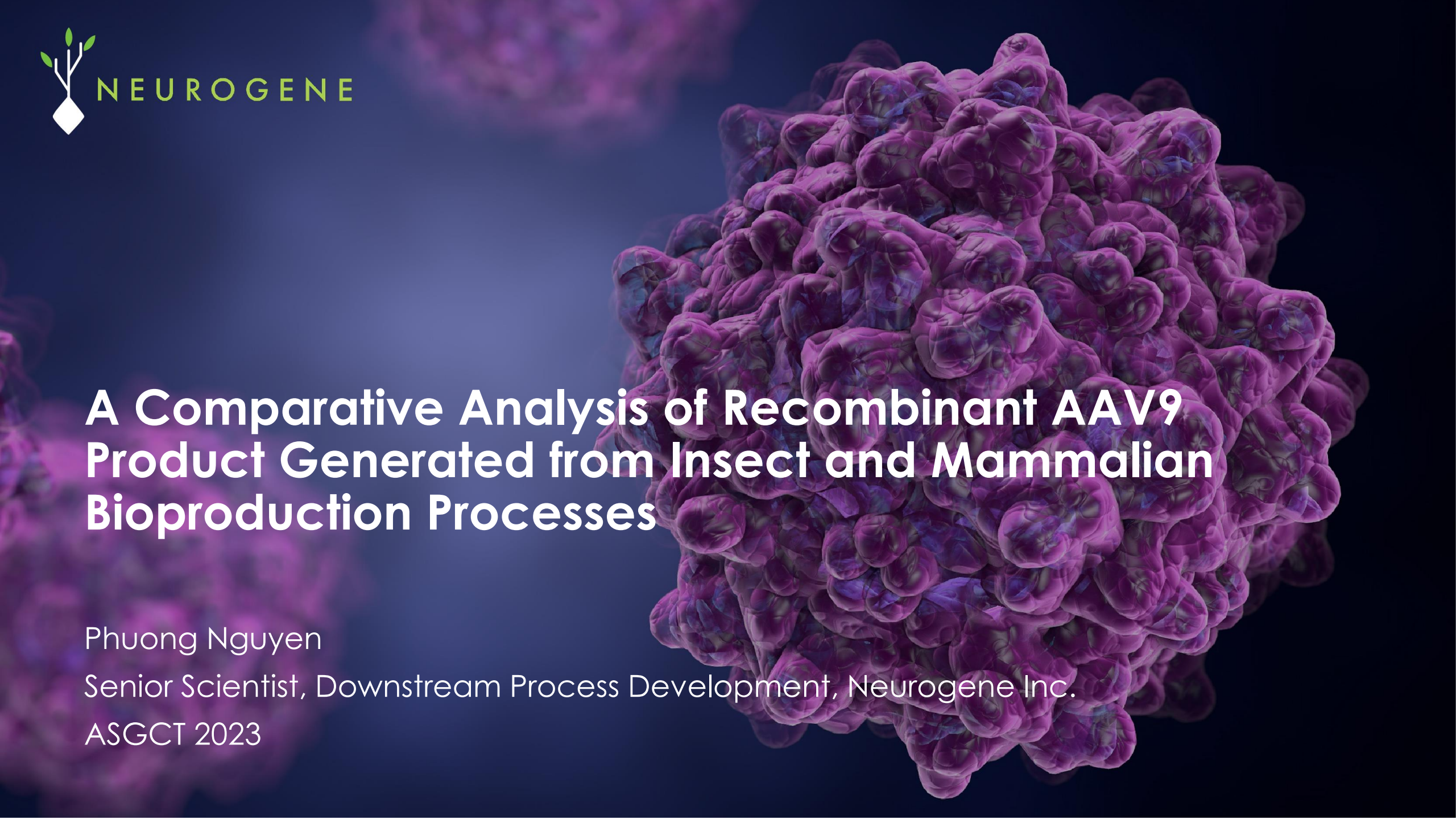

A Comparative Analysis of Recombinant AAV9 Product Generated from Insect and Mammalian Bioproduction Processes

Phuong Nguyen

Senior Scientist, Downstream Process Development, Neurogene Inc.

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Disclosures

Presenter: Phuong Nguyen

Phuong Nguyen is Senior Scientist, Process Development at Neurogene Inc.

Courtney Barlament is Associate Director, Upstream Process Development at Neurogene Inc.

Natasha Patel is a Scientist, Process Development at Neurogene Inc.

Adib Shafipour is an Associate Scientist, Process Development at Neurogene Inc.

Anandita Seth is VP of Process & Analytical Development at Neurogene Inc.

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Background

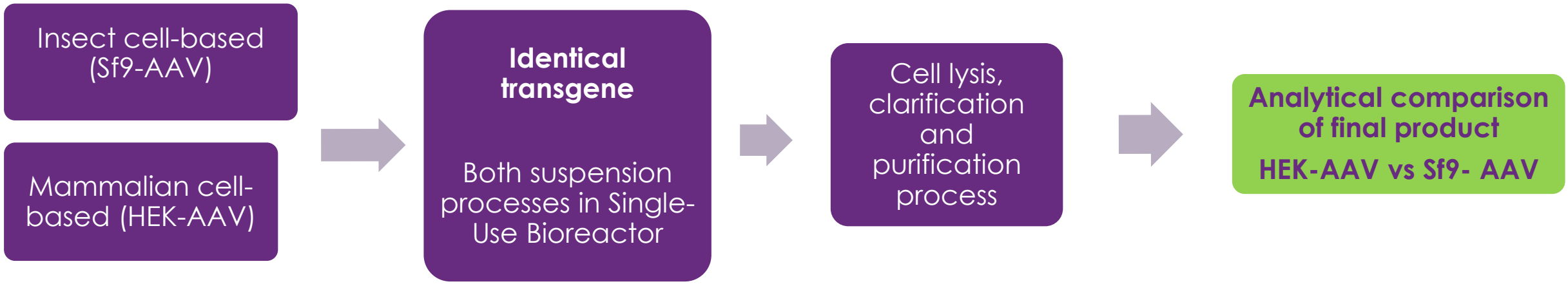
- Mammalian (HEK-AAV) and insect cell-based (Sf9-AAV) manufacturing systems are the two predominant AAV manufacturing platforms
- Neurogene has established both manufacturing platforms and have cleared INDs with each process

	Sf9-AAV	HEK-AAV
Advantages	• **Higher productivity** and lower COGS • Robust **scale-up** • Better **safety profile** (absence of proto-oncogene in production cells, less rcAAV) • Little or no expression of transgenes in insect cells	• **Flexibility** to switch from one serotype and/or transgene to another • **Speed** and established protocols to generate material
Challenges	• Requires master and working banks of both recombinant baculovirus clones (**upfront time and resource** utilization) • Might require viral clearance demonstration in early phases (even with Rhabdo-free cell line)	• **Lower productivity** and higher COGS • **Scale-up challenges**: Requires carefully controlled mixing at transfection step • Some transgene expression may affect performance of the cell culture system

AAV = adeno-associated virus; COGS = cost of goods sold; rcAAV = replication competent AAV; Sf9 = *Spodoptera frugiperda*; HEK = human embryonic kidney

Study Objective- Analytical Comparison of Mammalian and Insect Cell-based Manufacturing Systems

Two optimized, scalable platforms were utilized to generate AAV9 containing same **transgene of medical importance** (GMI)

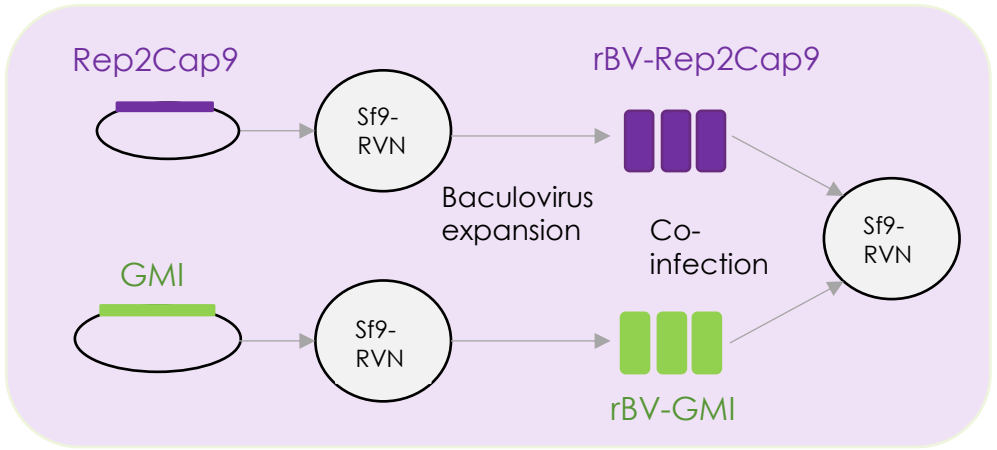


AAV = adeno-associated virus; GMI = transgene of medical importance

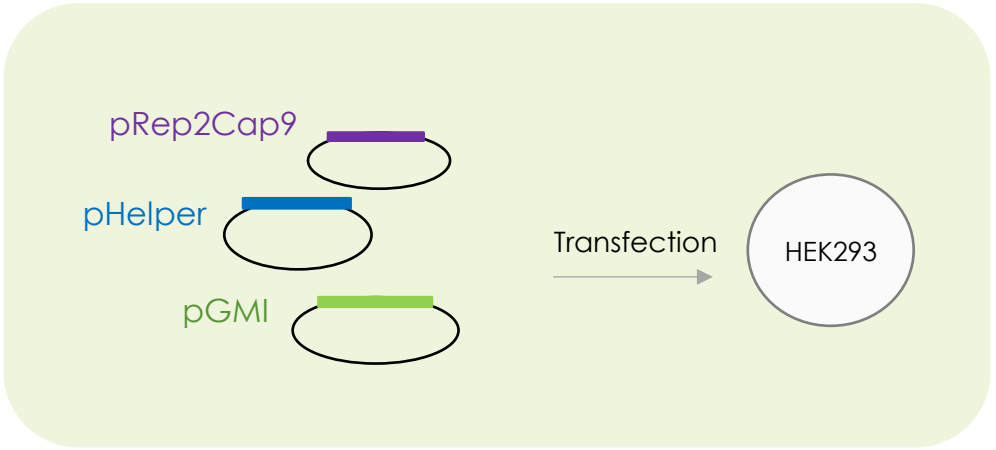
Process Overview of AAV9 Production Systems

Upstream

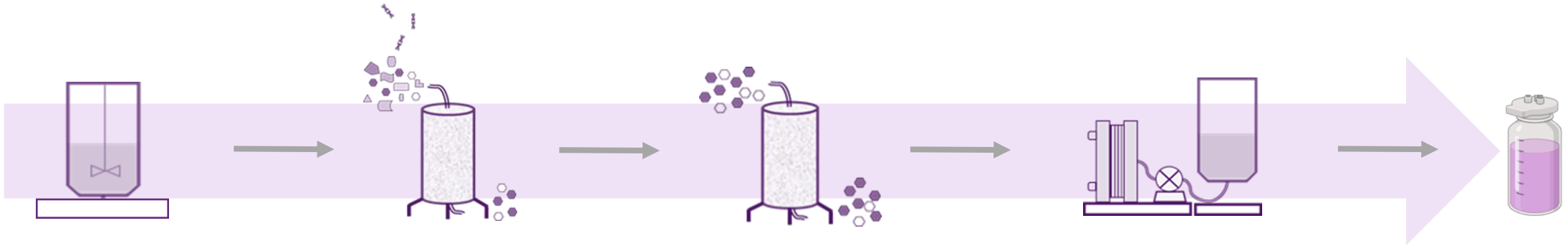
Sf9-AAV



HEK-AAV



Downstream



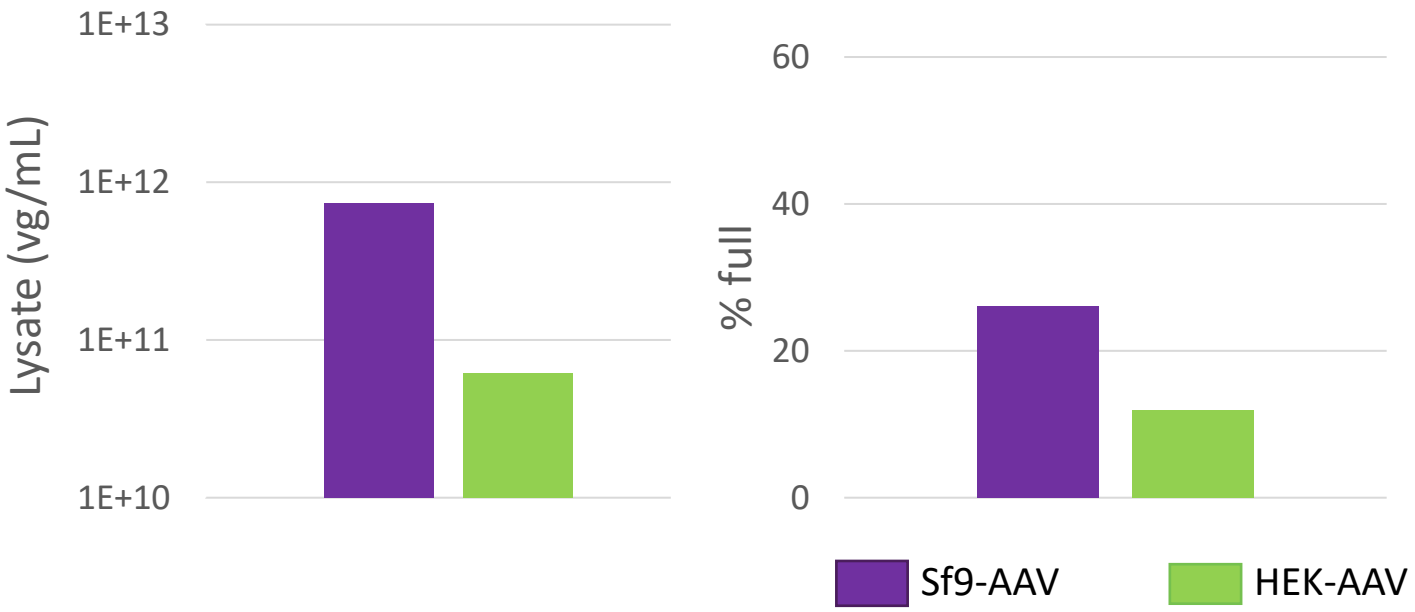
Same cell lysis, clarification, and purification processes

AAV = adeno-associated virus; GMI = transgene of medical importance

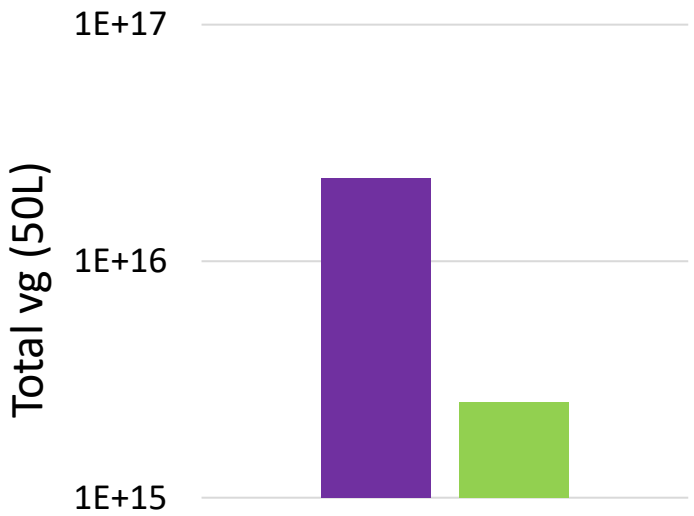
RVN= Rhabdovirus Negative

The Insect Cell-based System Yields Higher Productivity and Percent Full at Harvest

Productivity and % full at harvest



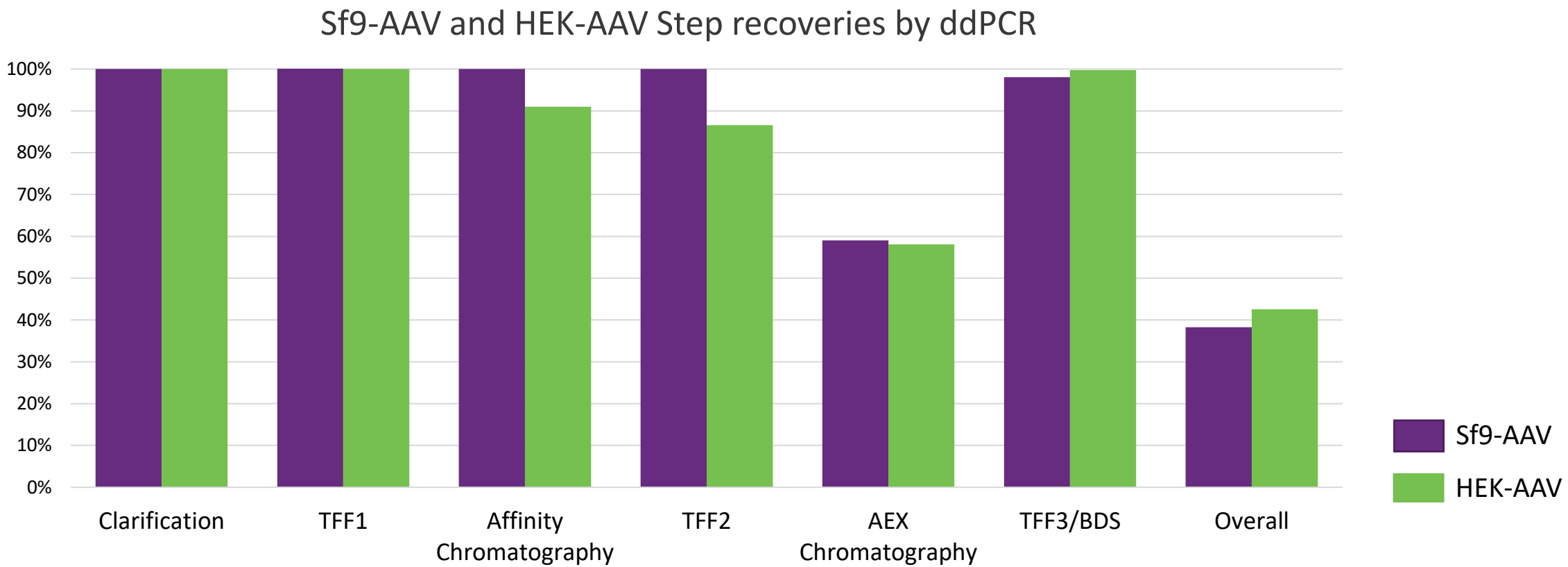
Volumetric yield



Total yield from the same scale runs is ~10-fold higher using the Sf9-AAV system

AAV = adeno-associated virus; vg = vector genome

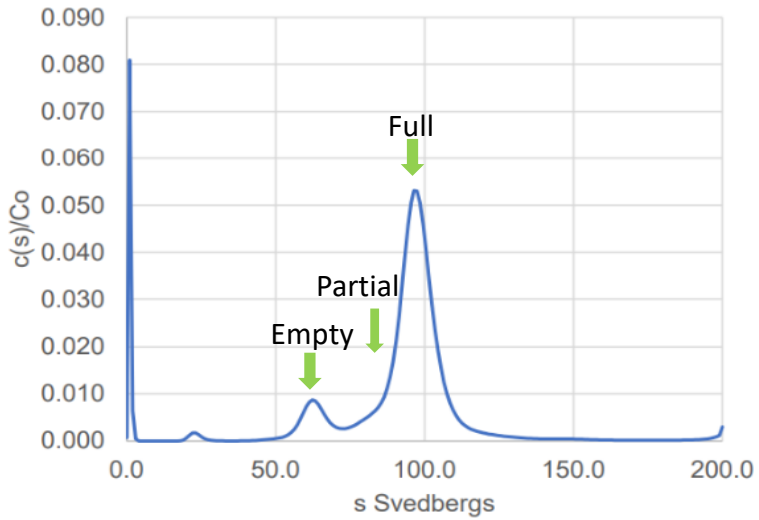
Recoveries From Each Unit Operation are Similar Between the Two Processes



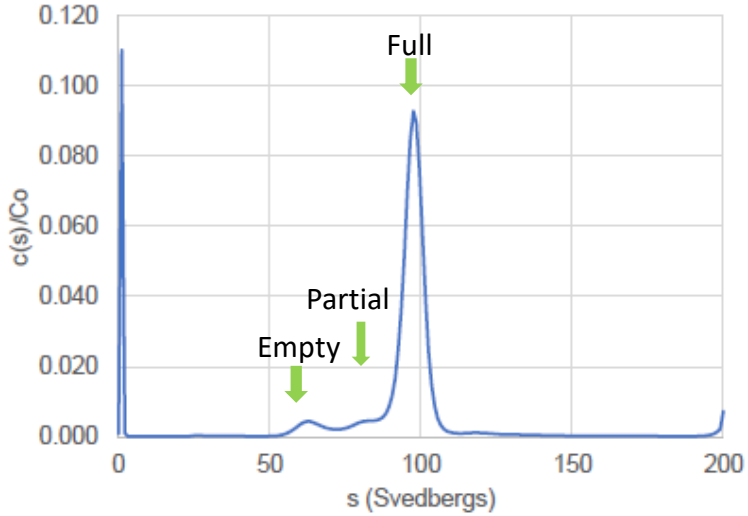
TFF = tangential flow filtration; AEX = anion exchange; BDS = bulk drug substance; ddPCR = droplet digital polymerase chain reaction

Both Processes Resulted in Similar AAV Particle Content by AUC

Sf9-AAV



HEK-AAV

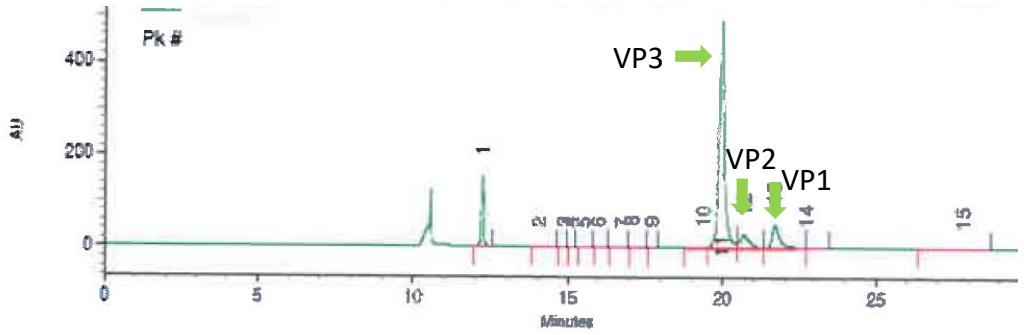


Particle Content (%)	Sf9-AAV	HEK-AAV
Empty	10	6
Partial	8	7
Full	82	87

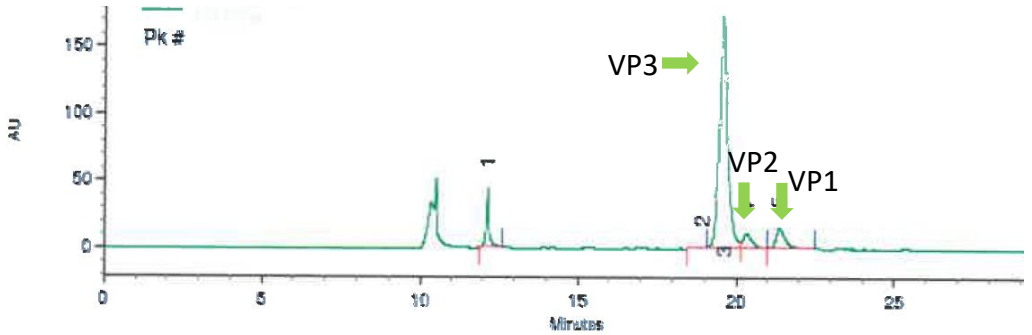
AUC = analytical ultracentrifugation

Similar Capsid Composition (Viral Protein Ratio) Observed in Both Products by CE-SDS

Sf9-AAV



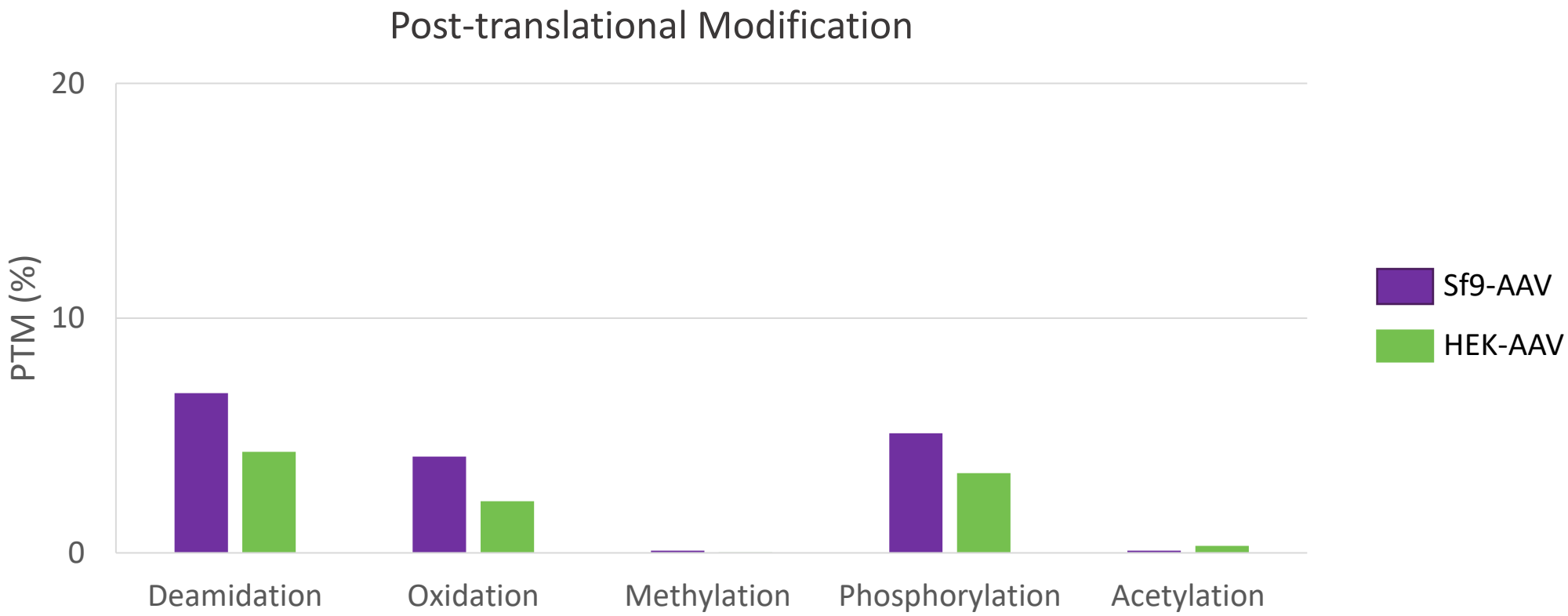
HEK-AAV



Species	VP1	VP2	VP3	AAV Purity (%)
Sf9-AAV	1.5	1.0	10	90
HEK-AAV	1.1	0.8	10	93

CE-SDS = capillary electrophoresis-sodium dodecyl sulfate

Overall Low Levels of PTM on the Capsid Surface, and the Difference between Products is within Assay Variability



AAV = adeno-associated virus; PTM = post-translational modifications

MiSeq Data Analysis Showed Similar Genome Integrity for Both Processes

Regions	Sf9-AAV (reads aligning to map %)	HEK-AAV (reads aligning to map %)
NGN Construct (GMI)	86	91
Starting Plasmid Backbone	0.02	1.30
Baculo RepCap/Plasmid RepCap	0.18	0.48
Shuttle Vector	0.010	N/A
Helper Plasmid	N/A	0.21
Host Cell DNA	1.10	0.57

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AAV = adeno-associated virus; GMI = transgene of medical importance

Residual (Impurity) Analysis and Safety Testing Showed Comparable Profiles

Assay	Sf9-AAV	HEK-AAV
Endotoxin (EU/mL)	< 0.05	< 0.05
SEC (%)	Monomer = 97.6 HMWS = 2.4	Monomer = 99.2 HMWS = 0.8
Replication competent AAV (in 1E+11 vg)	<10 rcAAV	<10 rcAAV
Residual Host Cell Protein (ng/mL)	8.1	<2.0
Residual Host Cell DNA (ng DNA/E+13 vg)	< 0.1	2.5
Residual baculovirus DNA/plasmid (copies/E+13 vg)	2.0E+6	2.0E+11

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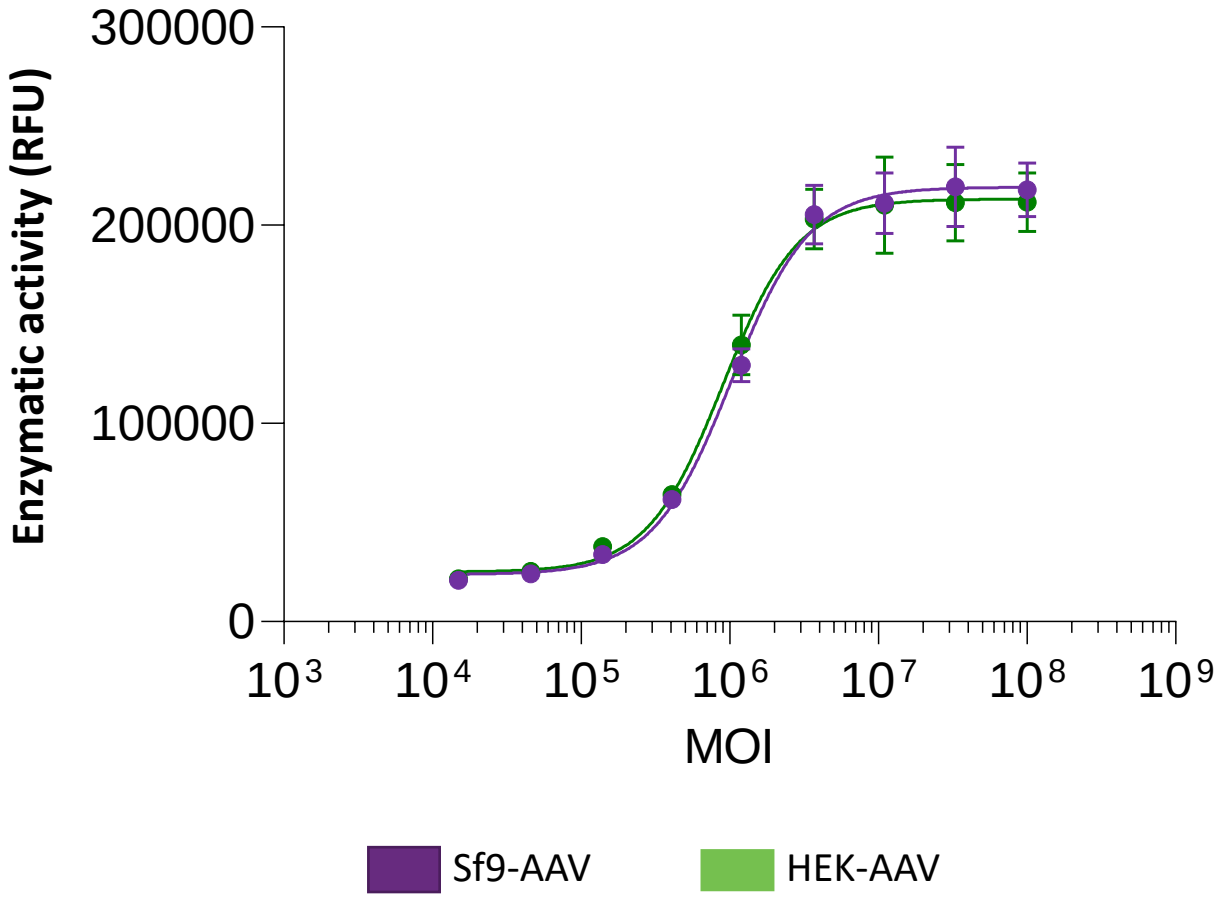
SEC = size exclusion chromatography; HMWS = high molecular weight species

vg = vector genome

AAV Products from Both Processes Show Similar Activity Using a Functional (Enzymatic) Potency Assay



Process	Relative Potency (%)
Sf9-AAV	100
HEK-AAV	87
Assay variability is +/- 25%	

AAV = adeno-associated virus; GMI = transgene of medical importance; MOI = multiplicity of infection

Conclusions

- We thoroughly characterized and compared the final products (containing the same GMI) generated using an Sf9 and a HEK process in order to address the question of which is a better process
- Using developed processes, both methods yielded high quality vector with low amounts of impurities, a high % of full capsids, and low levels of post translational modifications
- Considerations/Caveats
  - Design of RepCap construct plays a significant role in high quality product from Sf9 system, and we have a used an optimized design in this study.
  - Downstream process has some differences in buffer pH for the anion-exchange chromatography step.
  - Does not include long-read sequencing data
  - No in-vivo studies performed
- While there were minor differences in the product quality, the biological function was comparable for Sf9 and HEK derived products
- Sf9 had consistently higher yields and is our platform of choice, while we use HEK for indications requiring less drug product

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GMI = transgene of medical importance

Acknowledgements

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