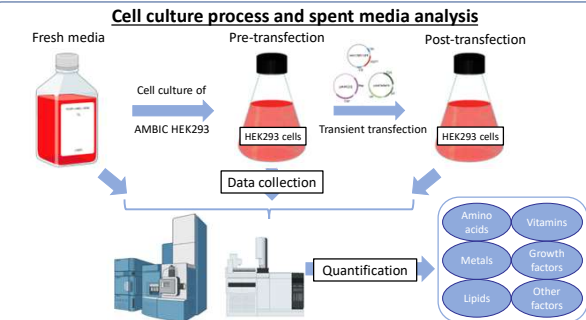# Maximizing Yields of rAAV and Elucidating the Role of Media Components Through Design and Optimization of AMBIC.293 Media
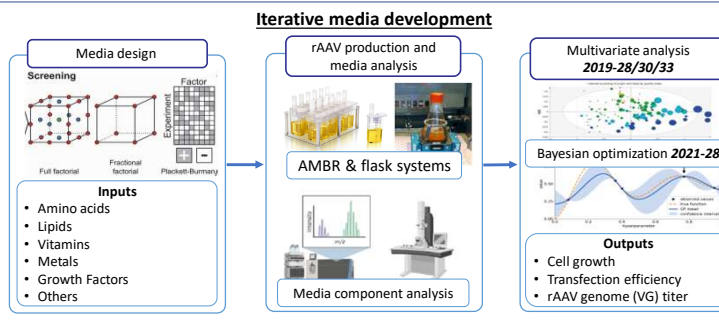
PIs: David McNally (UML), Seongkyu Yoon (UML), Kelvin Lee (UDel), Michael Betenbaugh (JHU)
Students: Yongdan Wang (UML), Houria Ahi (UML), Nelson Ndahiro (JHU), Justin Sargunas (JHU), Erica Green (UDel), Thomas Leibiger (UDel)

AMBIC
Johns Hopkins University
University of Massachusetts Lowell
University of Delaware
Clemson University
University of Maryland

Research Approach

Cell culture process and spent media analysis

Fresh media → Cell culture of AMBIC HEK293 → Pre-transfection (HEK293 cells) → Transient transfection → Post-transfection (HEK293 cells)

Data collection → Quantification

Amino acids, Vitamins, Metals, Growth factors, Lipids, Other factors

- Deleted media will be designed to modify levels of different nutrient classes.
- Concentration of amino acids, metal, lipids, vitamins will be measured via LC/MS, ICP-MS, and GC-MS.
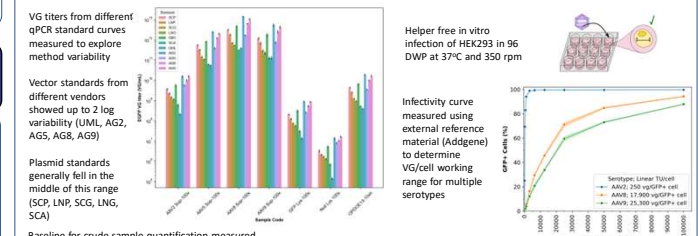
Iterative media development

Media design — Screening (Full factorial, Fractional factorial, Plackett-Burman), Factor Experiment

Inputs:
- Amino acids
- Lipids
- Vitamins
- Metals
- Growth Factors
- Others

rAAV production and media analysis — AMBR & flask systems, Media component analysis

Multivariate analysis **2019-28/30/33** → Bayesian optimization **2021-28**

Outputs:
- Cell growth
- Transfection efficiency
- rAAV genome (VG) titer

- Impact of different media component concentrations on **productivity** and **quality** will be defined by DOE studies
- **Predict** the media component concentration for **maximal VG titer** and **optimal quality**

Reference Material & Analytics

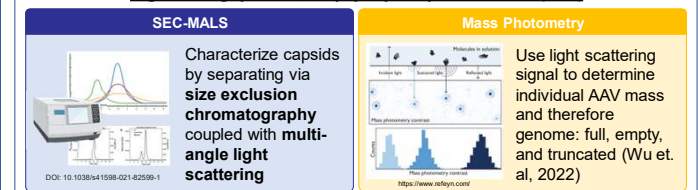### AAV9-EGFP Reference Material Generation (UDel)
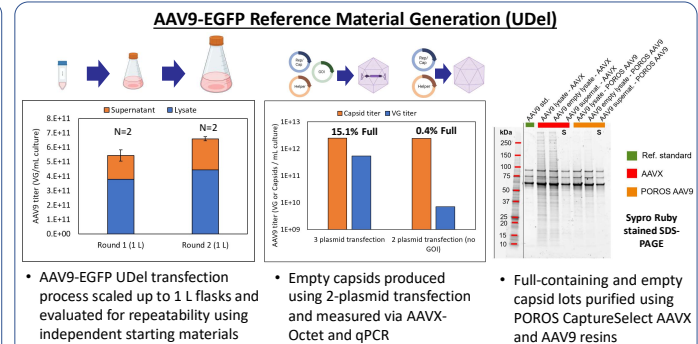
- AAV9-EGFP UDel transfection process scaled up to 1 L flasks and evaluated for repeatability using independent starting materials
- Empty capsids produced using 2-plasmid transfection and measured via AAVX-Octet and qPCR
- Full-containing and empty capsid lots purified using POROS CaptureSelect AAVX and AAV9 resins
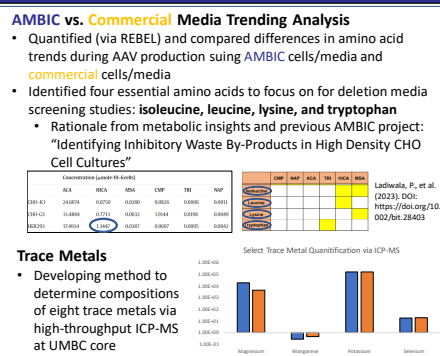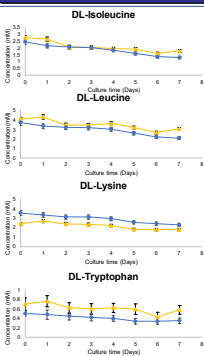
15.1% Full   0.4% Full

Sypro Ruby stained SDS-PAGE — Ref. standard, AAVX, POROS AAV9

Titer Methods: Quantitative PCR and Infectivity (UDel)

VG titers from different qPCR standard curves measured to explore method variability

Vector standards from different vendors showed up to 2 log variability (UML, AG2, AG5, AG8, AG9)

Plasmid standards generally fell in the middle of this range (SCP, LNP, SCG, LNG, SCA)

Baseline for crude sample quantification measured with GFP and Null lysates

Helper free in vitro infection of HEK293 in 96 DWP at 37°C and 350 rpm

Infectivity curve measured using external reference material (Addgene) to determine VG/cell working range for multiple serotypes

Serotype: Linear TU/cell — AAV2: 250 vg/GFP+ cell; AAV8: 17,900 vg/GFP+ cell; AAV9: 25,300 vg/GFP+ cell

High Throughput Full/empty capsid quantification (JHU)

SEC-MALS — Characterize capsids by separating via **size exclusion chromatography** coupled with **multi-angle light scattering**
DOI: 10.1038/s41598-021-82599-1

Mass Photometry — Use light scattering signal to determine individual AAV mass and therefore genome: full, empty, and truncated (Wu et. al, 2022)
https://www.refeyn.com

Recent Results (JHU)

DL-Isoleucine, DL-Leucine, DL-Lysine, DL-Tryptophan (Concentration (mM) vs Culture time (Days))

Commercial HEK293 Cells and Media; AMBIC HEK293 Cells and Media

AMBIC vs. Commercial Media Trending Analysis

- Quantified (via REBEL) and compared differences in amino acid trends during AAV production suing AMBIC cells/media and commercial cells/media
- Identified four essential amino acids to focus on for deletion media screening studies: **isoleucine, leucine, lysine, and tryptophan**
  - Rationale from metabolic insights and previous AMBIC project: "Identifying Inhibitory Waste By-Products in High Density CHO Cell Cultures"

Concentration (μmole-16-4cells)	ACA	HICA	MSA	CMP	TRI	HICA	NAP
CHO-K1	2.65074	0.2750	0.0280	0.0026	0.0006		0.0011
CHO-GS	31.4884	0.7711	0.0632	1.0644	0.4198		0.0006
HEK293	17.4934	1.3647	0.0187	0.0607	0.0005		0.0063

Ladiwala, P., et al. (2023). DOI: https://doi.org/10.1002/bit.28403

Select Trace Metal Quantification via ICP-MS — CMP, NAP, ACA, TRI, HICA, AAV

Trace Metals

- Developing method to determine compositions of eight trace metals via high-throughput ICP-MS at UMBC core

Magnesium, Manganese, Potassium, Selenium — Formulation, Measured

---

Recent Results (UML)

Preliminary transfection

Transfection Variables	AMBIC Media verification Condition	
1. PEI:DNA (-)	1:1 (Fixed)	
2. Cell density (+)	4.35*10^6 Cells/mL	
3. pHelper: pGol (-)	1:2	
4. pHelper: pRepCap (+)	1:2	
5. DNA amount (+)	1.0pg/cell (Fixed)	
6. Cocktail volume (+)	3mL/10% (Fixed)	
7. Incubation time (+)	7min(Fixed)	
	Simulated	Measured
Transfection efficiency	53.25%	58.95%
	Simulated	Measured
Titer	1.86E13 vg/L	1.43E13vg/L

Data from AMBIC manufacturing project:
- Establish transfection baseline (control)
- Initial evaluation of rAAV2 and rAAV9

Preliminary LCMS essay runs for amino acids concentration

NEAA — Formulation, Fresh media batch 1, Fresh media batch 2

Observation:
- Six out of eight nonessential amino acids showed <50% error between formulation and two fresh media batches
- Overall consistency between two fresh media batches (batch 1 and batch 2)
- Overall consistency of triple injection for each sample

Identified five non-essential amino acid to focus on for deletion media screening: **Arginine, Serine, Tyrosine, Aspartic acid, and Asparagine**
- Rationale from existing CHO media optimization and HEK293 metabolism on production of viral vector and vaccine
https://doi.org/10.1016/j.vaccine.2015.05.097
https://doi.org/10.1002/btpr.2706

Milestones and Timelines

Analytical
- Establish analytical methods for selected media components, including LCMS, ICPMS, GCMS
- Preliminary testing with fresh media and validate the analytical consistency between sites

Spent media study
- Conduct initial spent media study and design DOE based on results
- Conduct multiple DOEs and study the impact of media components impacts on productivity and quality
- Predict the media component concentration for maximal titer and quality via MVDA and modeling

Reference Material
- Establish purification methods for full and empty capsid isolation
- Share AAV9-EGFP between sites for analytical method validation

Vector Characterization
- Comparison of full/empty capsid method results within and between sites
- Share qPCR standard material between sites

Next Steps

UML
- Conduct spent media analysis for preliminary transfection samples
- Design depleted media and DOE experiment for essential amino acid screening
- Establish analytical methods for other media components, including ICPMS and GCMS

JHU
- Design and run deleted media to analyze role of essential amino acids in AAV titer and quality
- Establish analytical method to study role of trace metals in AAV production
- Develop Multi-angle Light Scattering method for assaying empty vs full viral particle content

UDel
- Develop AAV9 LC-MS peptide mapping workflow for assessment of capsid quality attributes
- Evaluate methods for full capsid enrichment and apply method for reference material generation
- Establish high-throughput HPLC-AEX method for full vs. empty capsid measurement