Simplifying Progress

Keys to AAV Manufacturing Succes: Fast, Robust and High-yield Process Development with DoE, Automated Bioreactors and Selected Critical Raw Materials

Building a scalable, data-driven upstream platform for industrial AAV manufacturing

Hakima FLICI Scientific Support Specialist

SARTORIUS

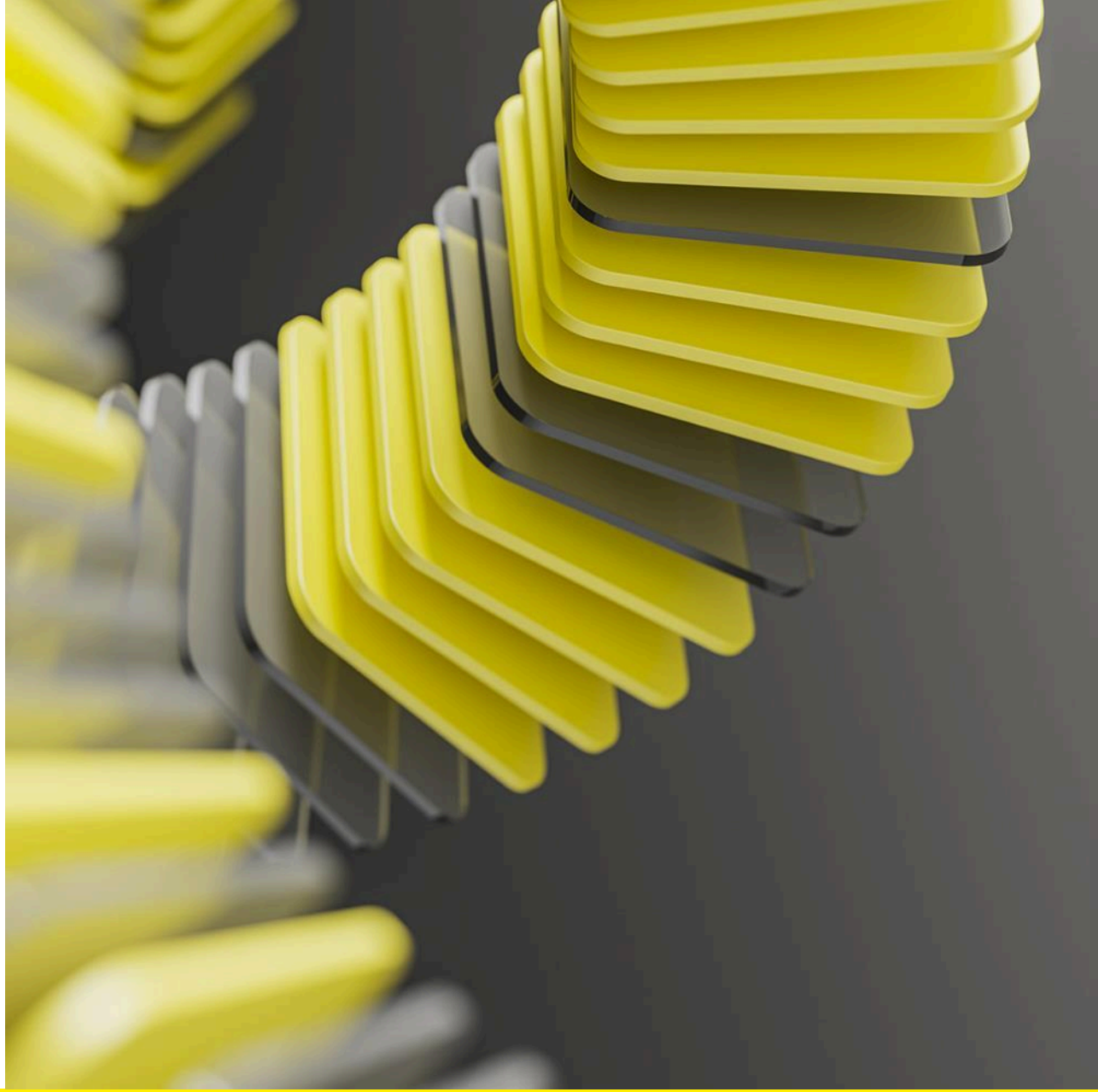
Agenda

1. rAAV gene therapy: background and current challenges
2. Sartorius solutions for AAV process development
3. Case Study: Development of a robust rAAV upstream platform

    Optimization of Bioreactor and transfection parameters (small scale)

    Process scale-up

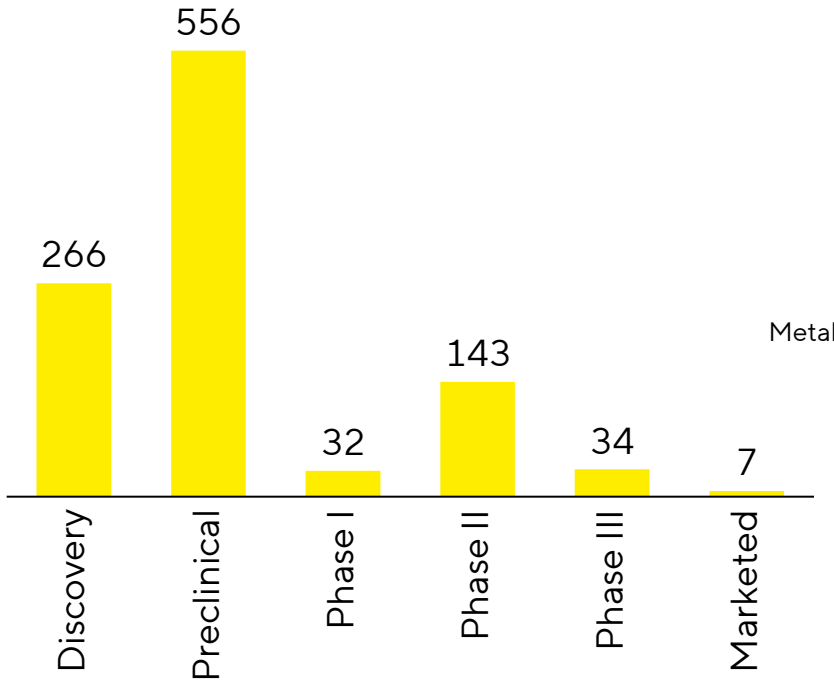
4. Key results and takeaways



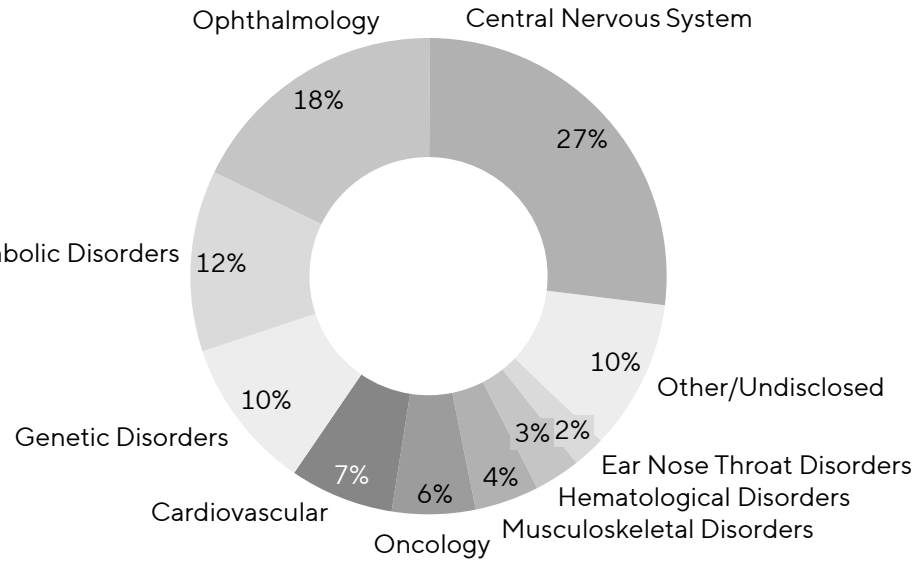
rAAV gene therapy: background and current challenges

Expanding Demand Highlights Urgency in AAV Gene Therapy Advancement

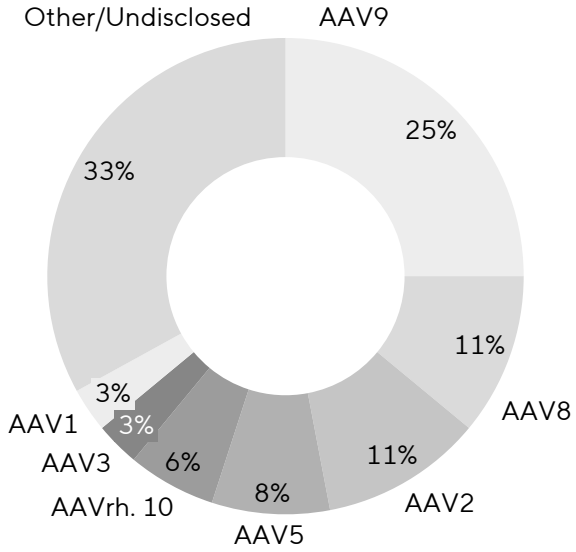
Unique AAV-based Drugs by Highest Development Stage



Unique AAV-based Drugs by Therapy Area



Proportion of different serotypes used



Global Data Drug Pipeline Database, as of January 2026

Cell & Gene Therapy Insights 2024; 10(6), 821–840 DOI: 10.18609/CGTI.2024.095

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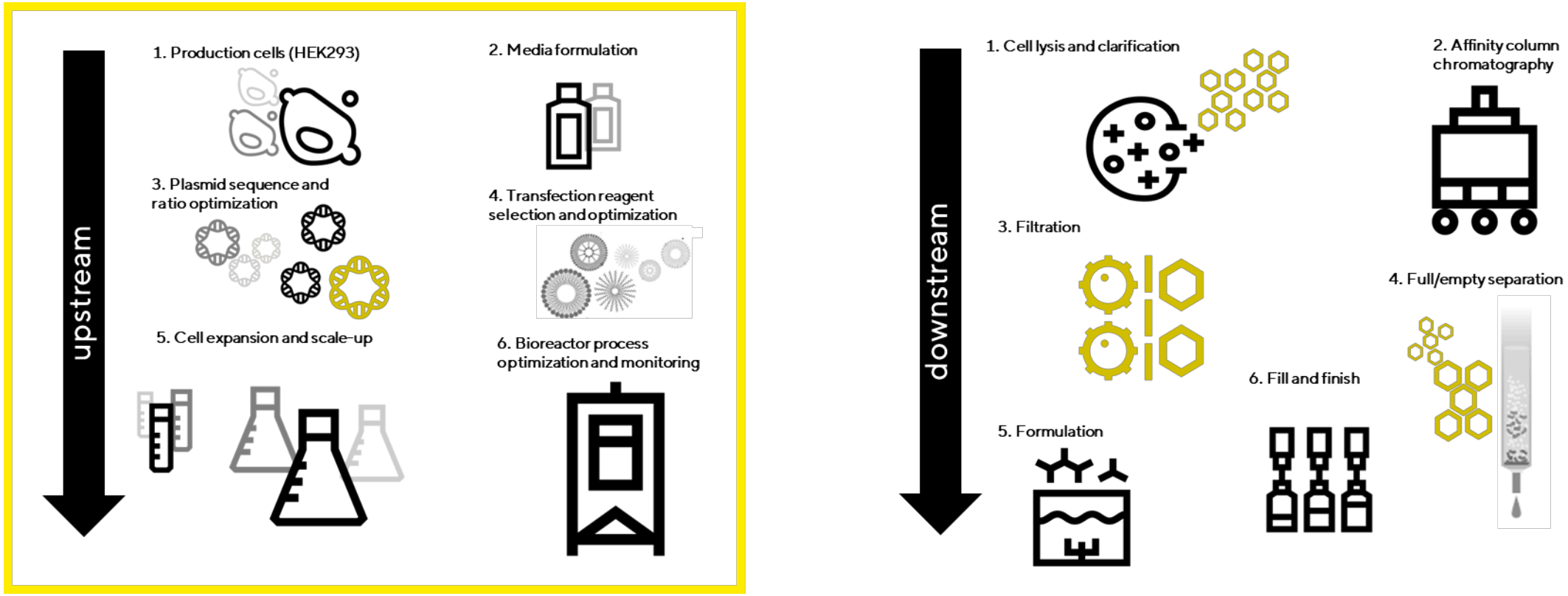
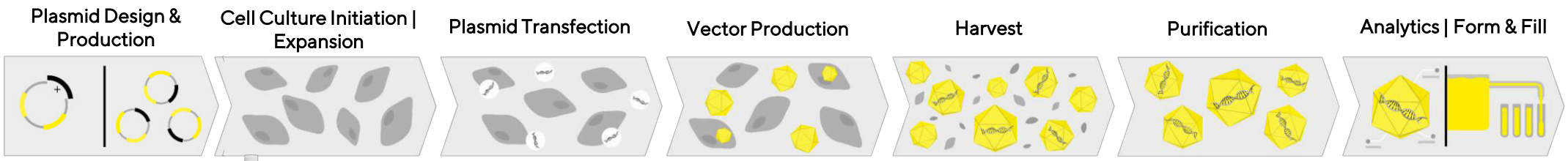
Overview of AAV Production Using Plasmid Transfection



Adapted from Cell & Gene Therapy Insights 2024; 10(6), 821–840 DOI: 10.18609/CGTI.2024.095

Sartorius solutions for AAV process development

Comprehensive solutions for CGT development & manufacturing



Starting & Raw Materials

Preparative & Analytical Chromatography

Supporting Services

Foundational Bioprocessing Solutions

4Cell® HEK TF Medium

Ready-to -Use pPLUS® Plasmids

Transfection Reagents

Recombumin®

PATfix® Analytical Platform

CIM plates & monoliths

pDNA Engineering | pDNA Manufacturing

HEK Cell Banking

Viral Testing Services

Media Optimization & Development

Process Development Services

Bioreactor Technologies

Fluid Management

Data Analytics

Filtration Solutions

Case Study: Development of a robust rAAV upstream platform

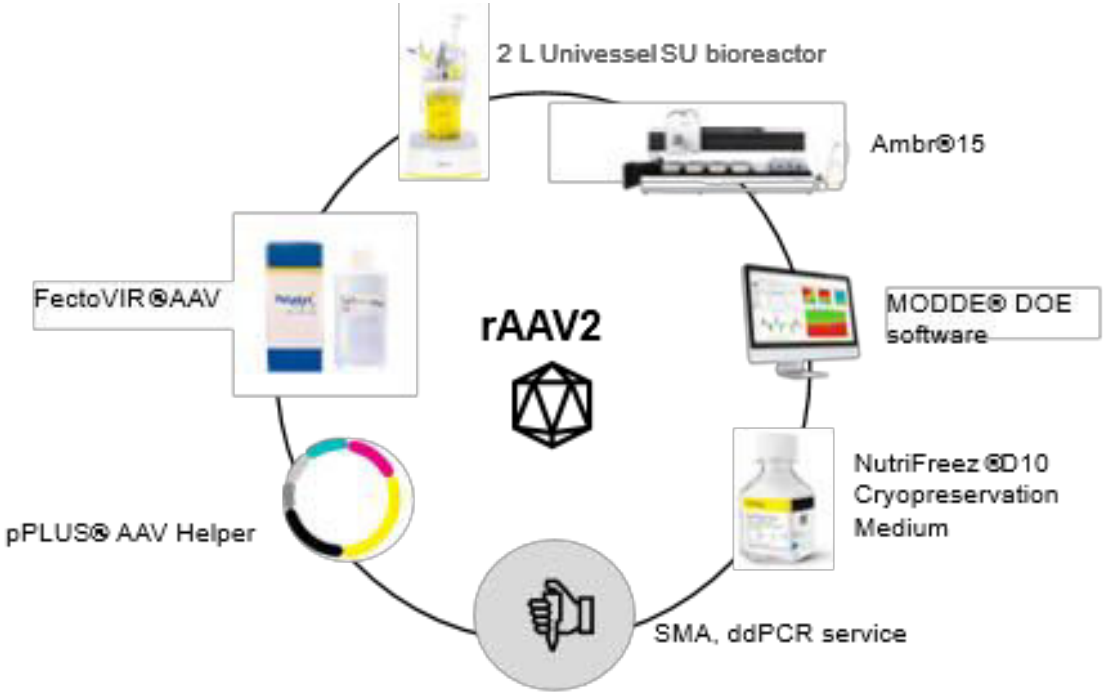
Case study

Building a Robust AAV Upstream Platform

Objectives

Increase yield & Develop a Robust AAV Production Platform by Leveraging Comprehensive Design-of-Experiment (DoE) & High-Throughput Automated Bioreactors Powered by Next Gen Raw Materials

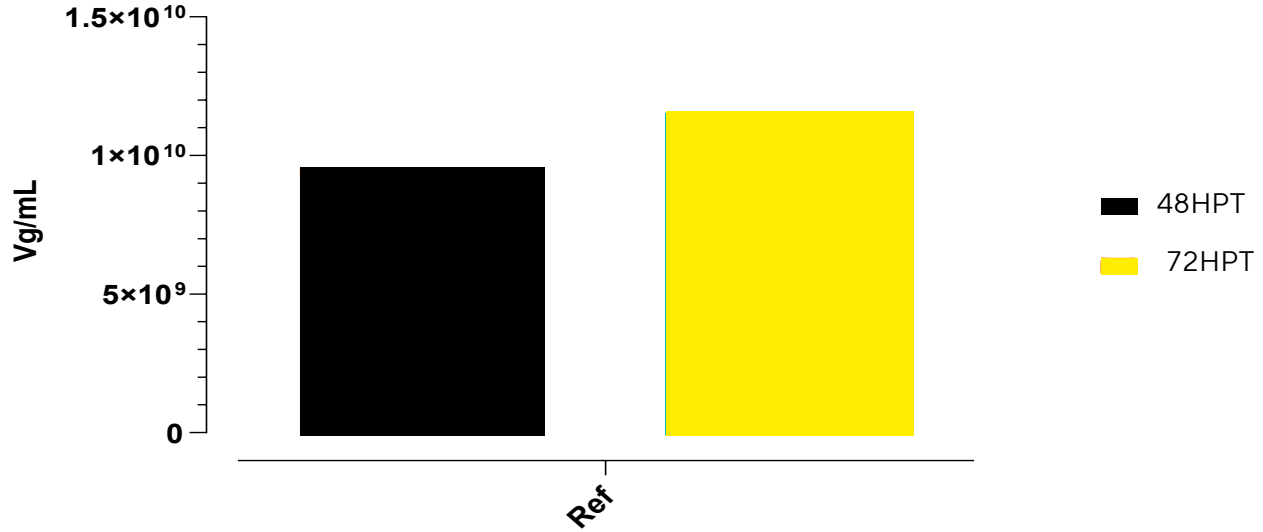
Raw Materials Used in This Study



Case study

AAV Production, Where We Started

rAAV2-GFP : Productivity of **1.1E+10 vg/mL** (Bulk harvest) with the standard culture medium in shake flask.



Significant productivity gains are needed to meet today's rAAV clinical requirement

(with a dosing ranging from 10^{10} to 10^{16} vg per patient, depending on the therapeutic area)

Overview of the DoE Runs

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1 RUN ~ Timelines ~ 25 days

~15 days

Seed in Shake Flasks (day -11) → Inoculation (day 0) → Transfection (day +1) → Production rAAV2 → Harvest (day +3) → Analysis (12 days)

RUN1: Optimizing bioreactor operating parameters	**RUN2:** Optimizing cell culture parametesr	**RUN3:** Optimizing the transfection conditions	**RUN4:** Optimizing the transfection conditions	**Responses**
DoE factors: **pH** ▪ Before TFX (Low, Middle, High ▪ After TFX (Low, Middle, High **Stirring** ▪ At seeding (High) ▪ Post-transfection (Low, high) **Media** ▪ Medium 1, 2 and 3	**DoE factors:** **Fresh Medium addition before transfection** ▪ YES ▪ NO **Media** ▪ Medium 1 ▪ Medium 2 ▪ Medium 3	**DoE factors:** **Seeding VCD (10^6 cells/mL)** ▪ Low, Middle, High **DNA amount (µg/106 cells)** ▪ Low, Middle, High **FectoVIR®-AAV volume (µL/106 cells)** ▪ Low, Middle, High	**DoE factors:** Plasmid ratio optimization	**Output / Method:** **Genome Titer (vg/mL)** ▪ qPCR ▪ ddPCR **Total Capsid Titer (VP/mL)** ▪ ELISA ▪ mass-photometry **Full capsids %** ▪ Genome titer/Total Capsid titer ▪ mass-photometry **Transfection efficiency** efficiency (Flow cytometry)

Transfection reagent: FectoVIR®-AAV

Helper plasmid: pALD-X80 | **Helper plasmid:** pPLUS®-AAV Helper

Control medium: Expi293$_{TM}$ Expression Medium

Helper plasmid control: pALD-X80

Metabolites and ddPCR/ELISA services

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Case study

Optimizing Bioreactor Operating & Cell Culture Parameters

RUN1: Optimizing bioreactor operating parameters	**RUN2:** Optimizing cell culture parametesr
DoE factors: **pH** ▪ Before TFX (Low, Middle, High ▪ After TFX (Low, Middle, High **Stirring** ▪ At seeding (High) ▪ Post-transfection (Low, high) **Media** ▪ Medium 1, 2 and 3	**DoE factors:** **Fresh Medium addition before transfection** ▪ YES ▪ NO **Media** ▪ Medium 1 ▪ Medium 2 ▪ Medium 3
Transfection reagent: FectoVIR®-AAV	
Helper plasmid: pALD-X80	**Helper plasmid:** pPLUS®-AAV Helper
Control medium: Expi293TM Expression Medium	
	Helper plasmid control: pALD-X80

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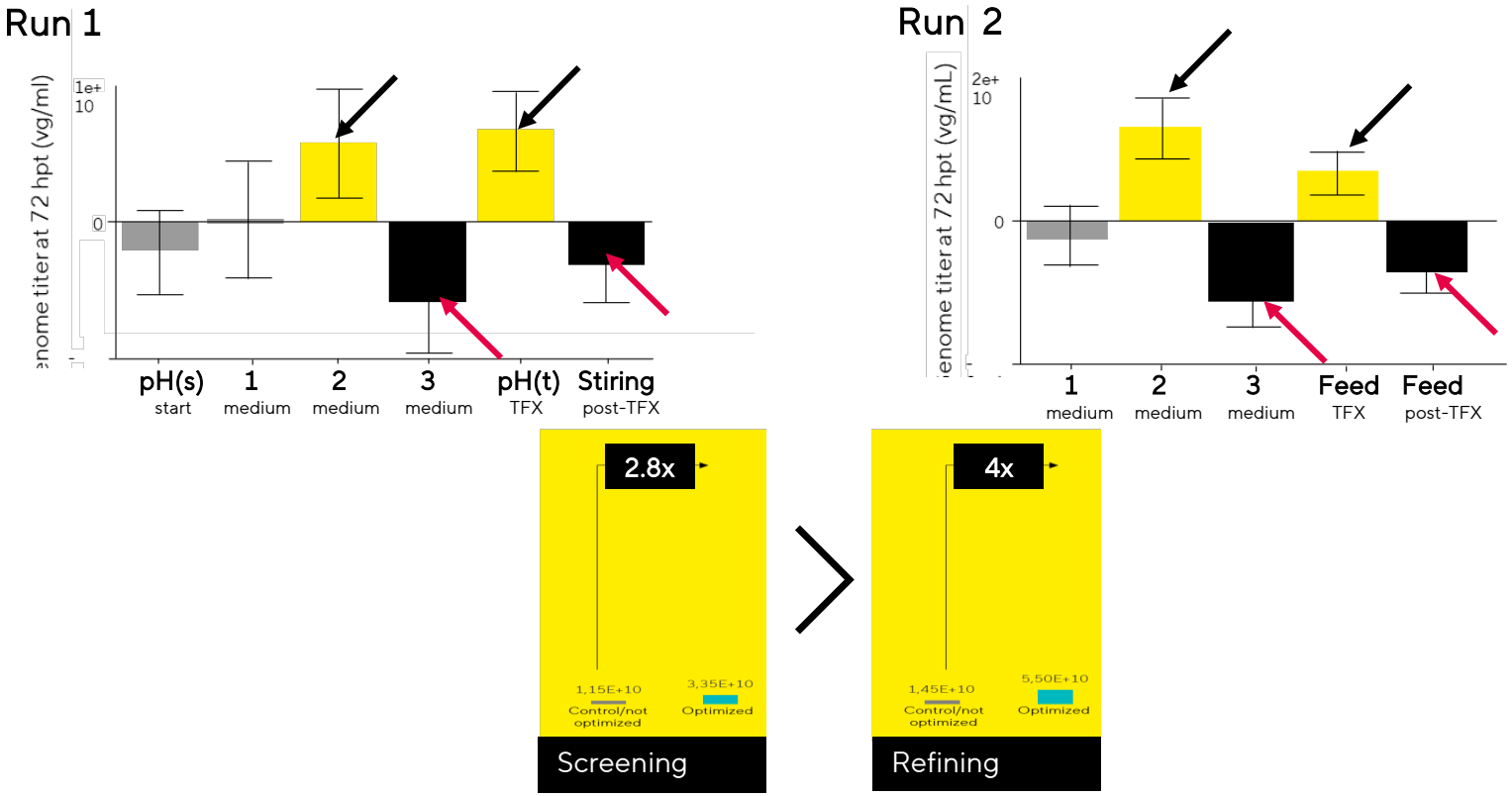
Advanced Therapies 2026 | Do not replicate

Case study

Optimizing Bioreactor Operating Parameters & Cell Culture Conditions



To Increase Genome Titers:

- **Medium 2** is the most performing medium.
- **Increase pH** during transfection
- **Decrease stirring speed** at the time of transfection

Case study

Optimizing Transfection conditions

RUN1: Optimizing bioreactor operating parameters	**RUN2:** Optimizing cell culture parametesr	**RUN3:** Optimizing the transfection conditions
DoE factors: **pH** ▪ Before TFX (Low, Middle, High ▪ After TFX (Low, Middle, High **Stirring** ▪ At seeding (High) ▪ Post-transfection (Low, high) **Media** ▪ Medium 1, 2 and 3	**DoE factors:** **Fresh Medium addition before transfection** ▪ YES ▪ NO **Media** ▪ Medium 1 ▪ Medium 2 ▪ Medium 3	**DoE factors:** **Seeding VCD (10^6 cells/mL)** ▪ Low, Middle, High **DNA amount (µg/106 cells)** ▪ Low, Middle, High **FectoVIR®-AAV volume (µL/106 cells)** ▪ Low, Middle, High
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Control medium: Expi293TM Expression Medium		
	Helper plasmid control: pALD-X80	

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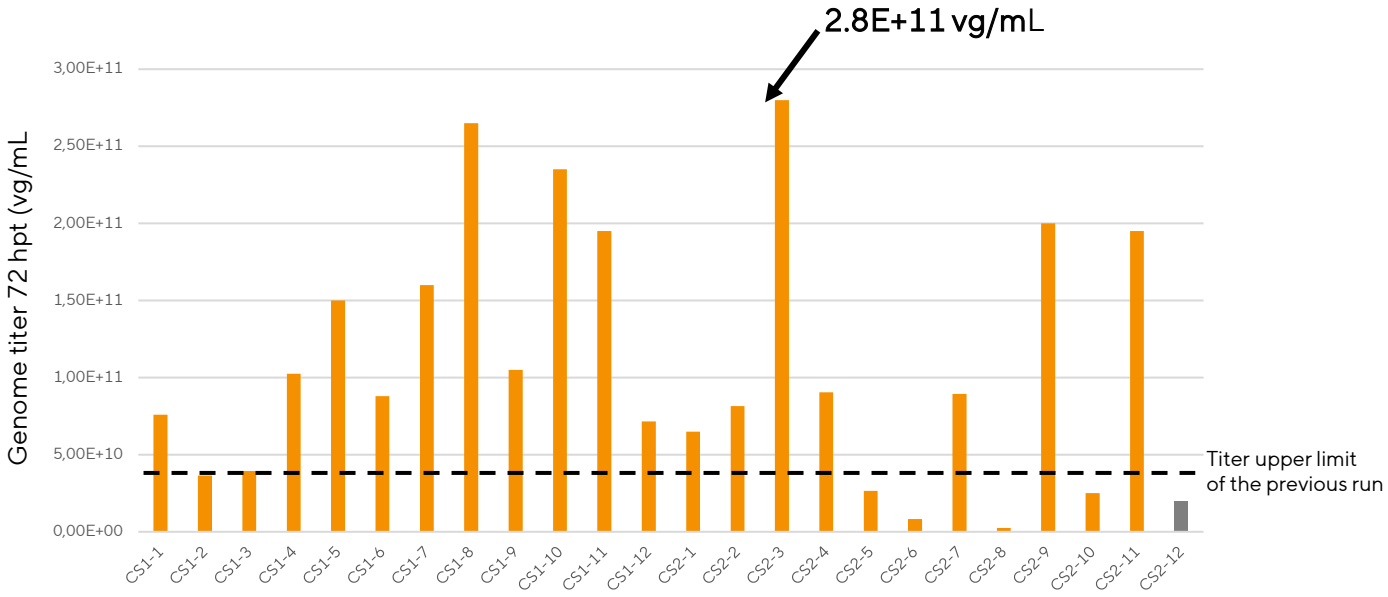
Case study

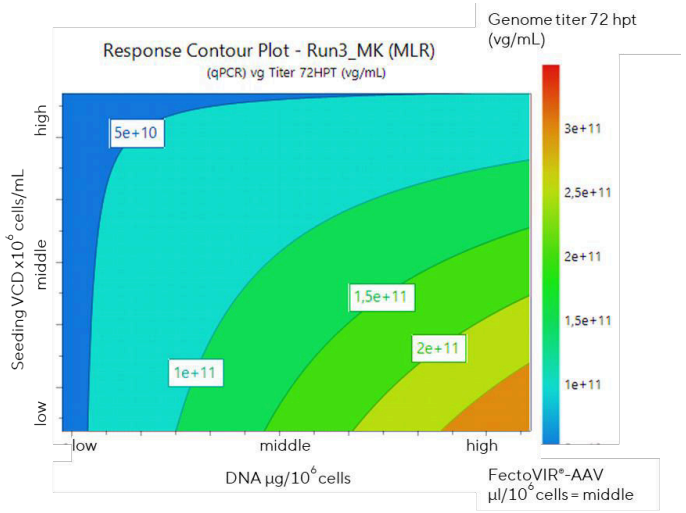
Optimizing Transfection Conditions

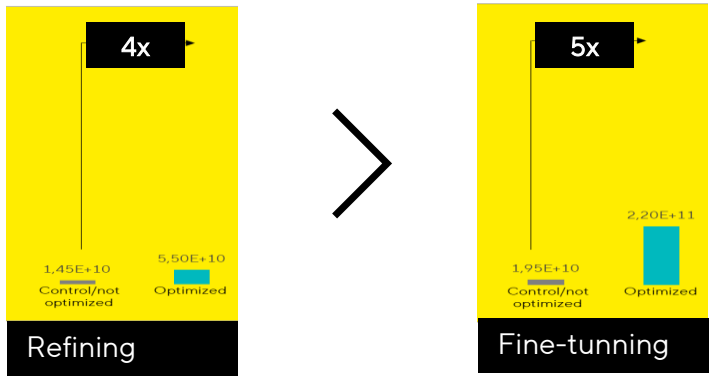
Run 3



To improve productivity:

- **Decrease** cell seeding density
- **Increase** DNA amount





Case study

Optimizing Transfection Conditions

RUN1: Optimizing bioreactor operating parameters	**RUN2:** Optimizing cell culture parametesr	**RUN3:** Optimizing the transfection conditions	**RUN4:** Optimizing the transfection conditions
DoE factors: **pH** ▪ Before TFX (Low, Middle, High ▪ After TFX (Low, Middle, High **Stirring** ▪ At seeding (High) ▪ Post-transfection (Low, high) **Media** ▪ Medium 1, 2 and 3	**DoE factors:** **Fresh Medium addition before transfection** ▪ YES ▪ NO **Media** ▪ Medium 1 ▪ Medium 2 ▪ Medium 3	**DoE factors:** **Seeding VCD (10^6 cells/mL)** ▪ Low, Middle, High **DNA amount (µg/106 cells)** ▪ Low, Middle, High **FectoVIR®-AAV volume (µL/106 cells)** ▪ Low, Middle, High	**DoE factors:** Plasmid ratio optimization

- **Transfection reagent:** FectoVIR®-AAV
- **Helper plasmid:** pALD-X80
- **Helper plasmid:** pPLUS®-AAV Helper
- **Control medium:** Expi293TM Expression Medium
- **Helper plasmid control:** pALD-X80

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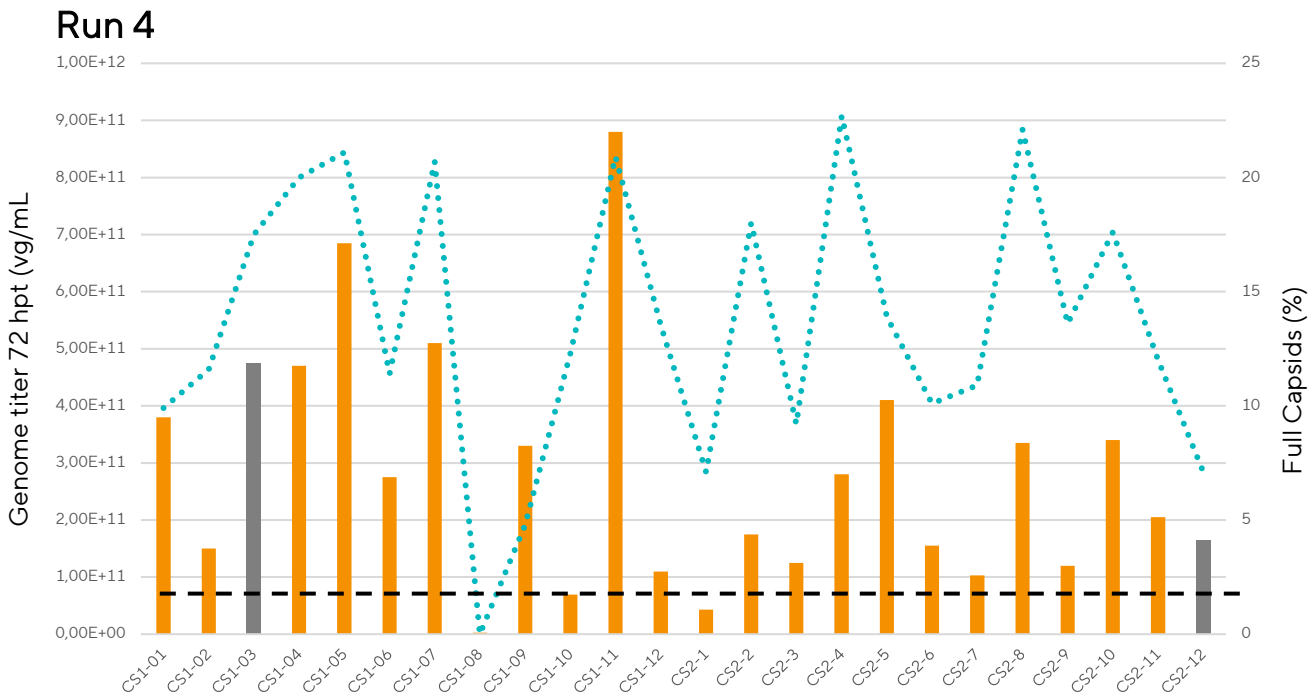
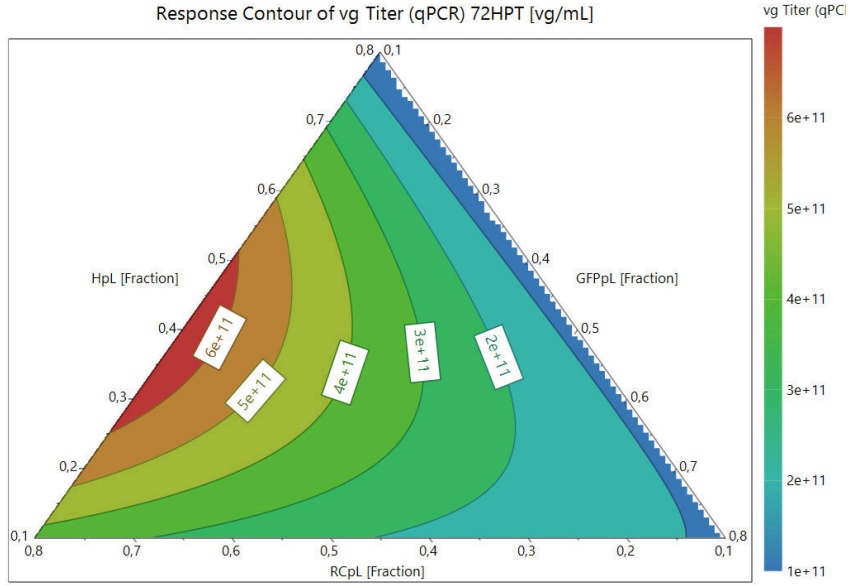
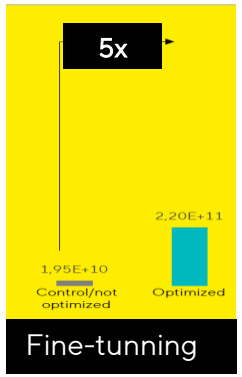
Optimizing Plasmid Ratio

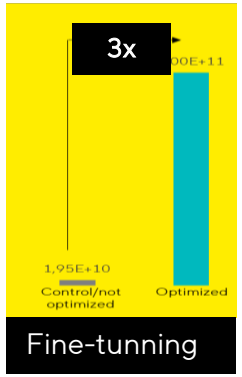




Optimal plasmid ratio for improved productivity:

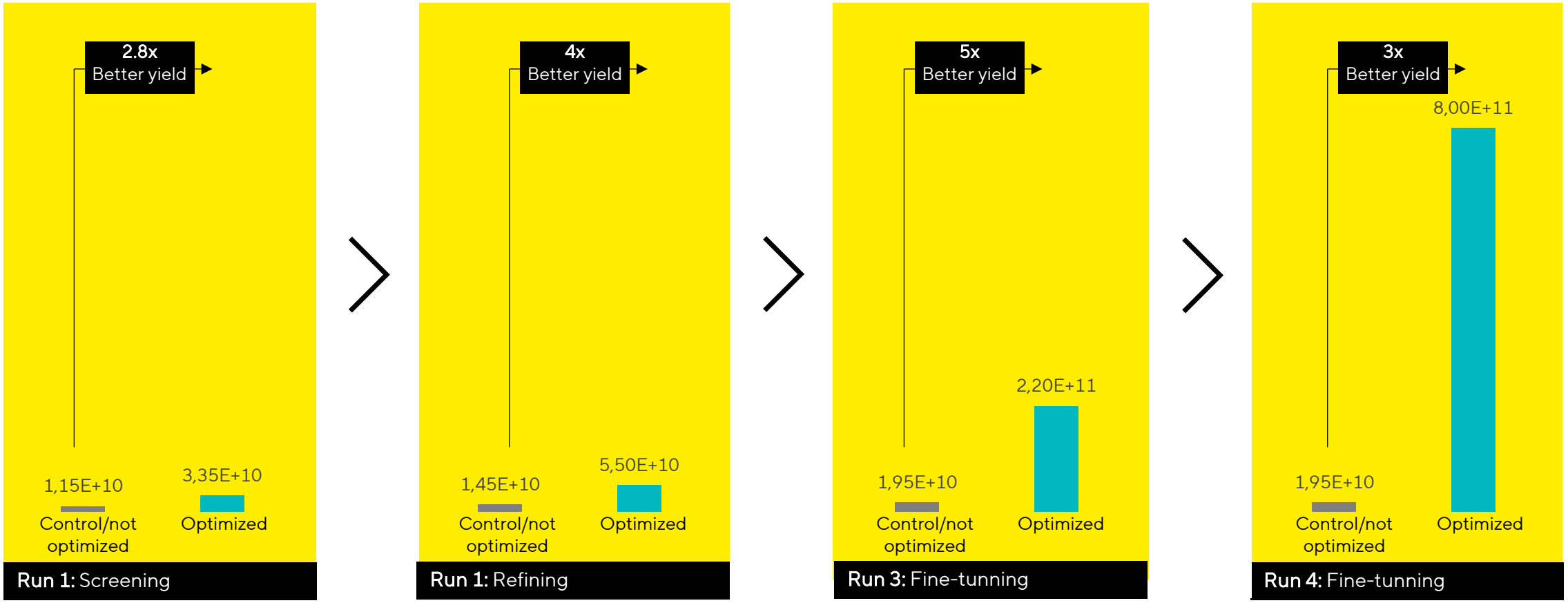
- pPLUS®AAV-Helper: 45%
- pAAV-RC2: 45%
- pAAV-GFP: 10%





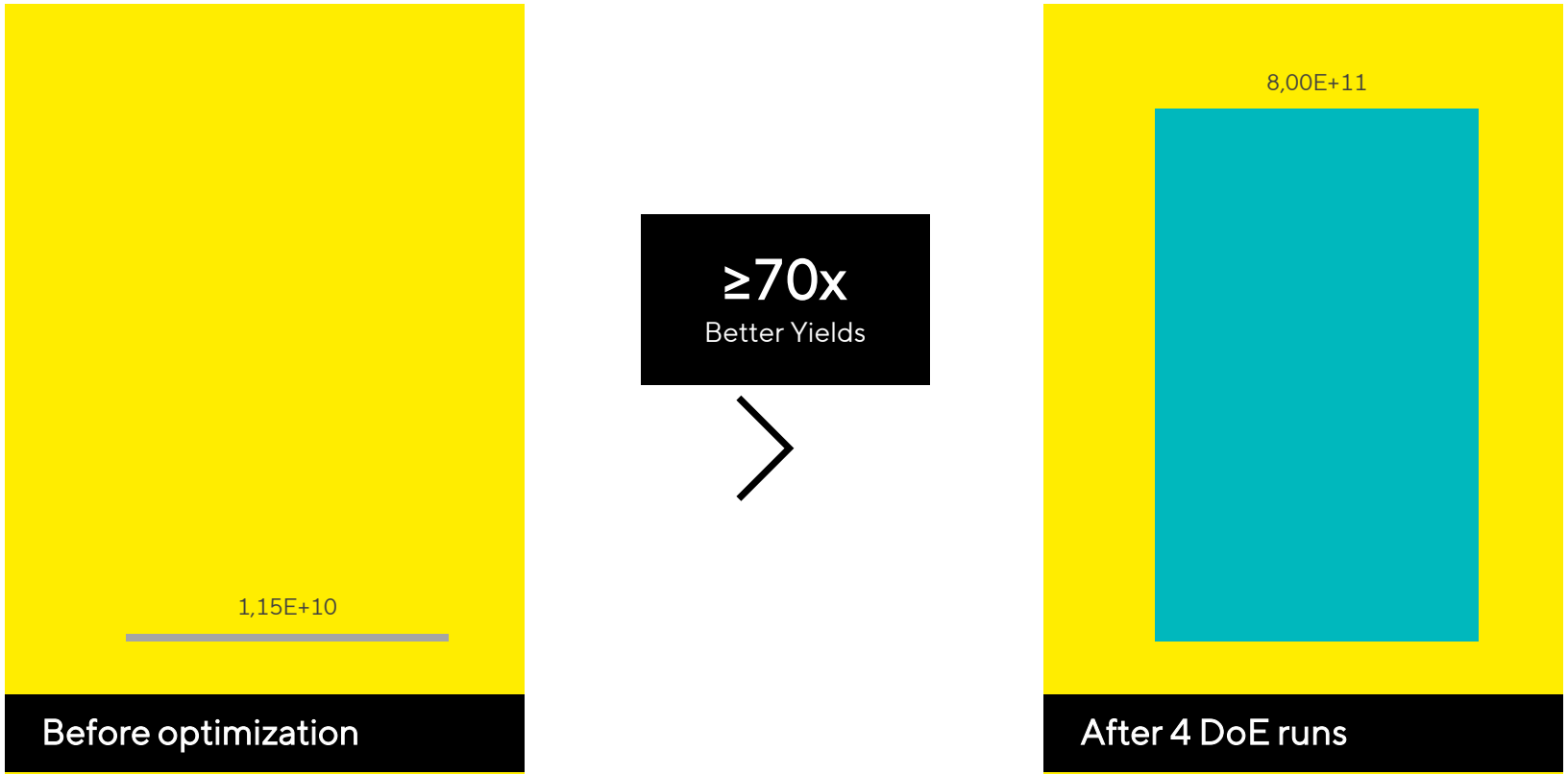
Case study

Four Experimental Designs to Achieve Significant Increase in VG



Case study

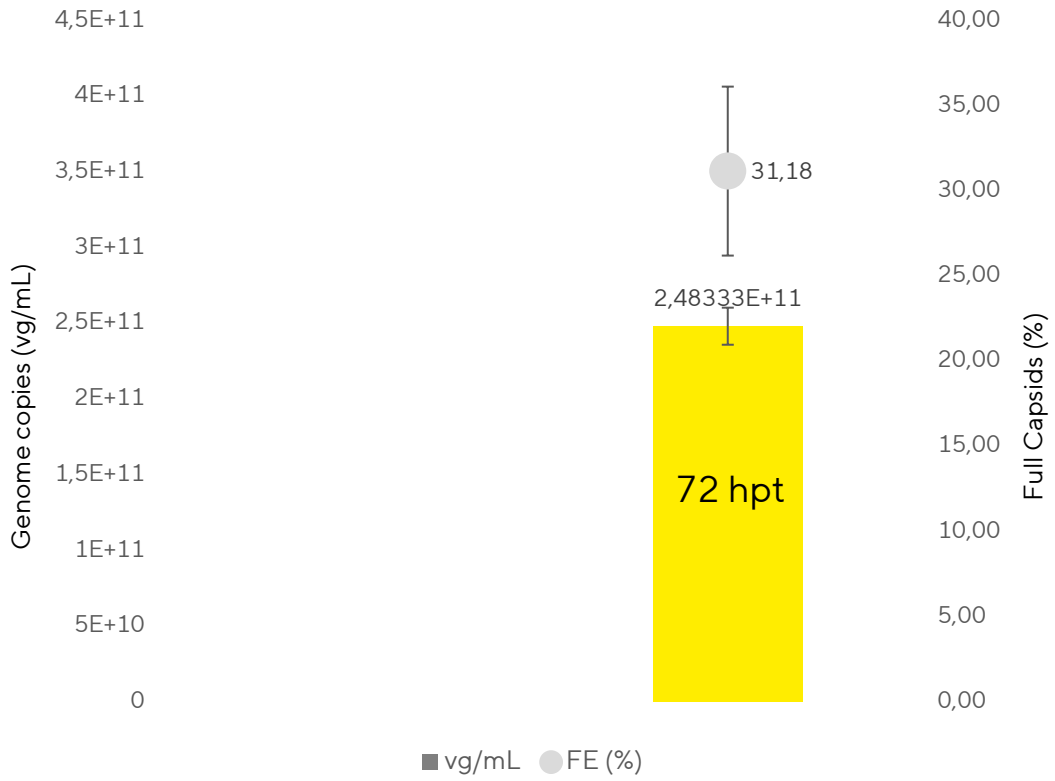
Four Experimental Designs to reach a 70-fold Increase in VG

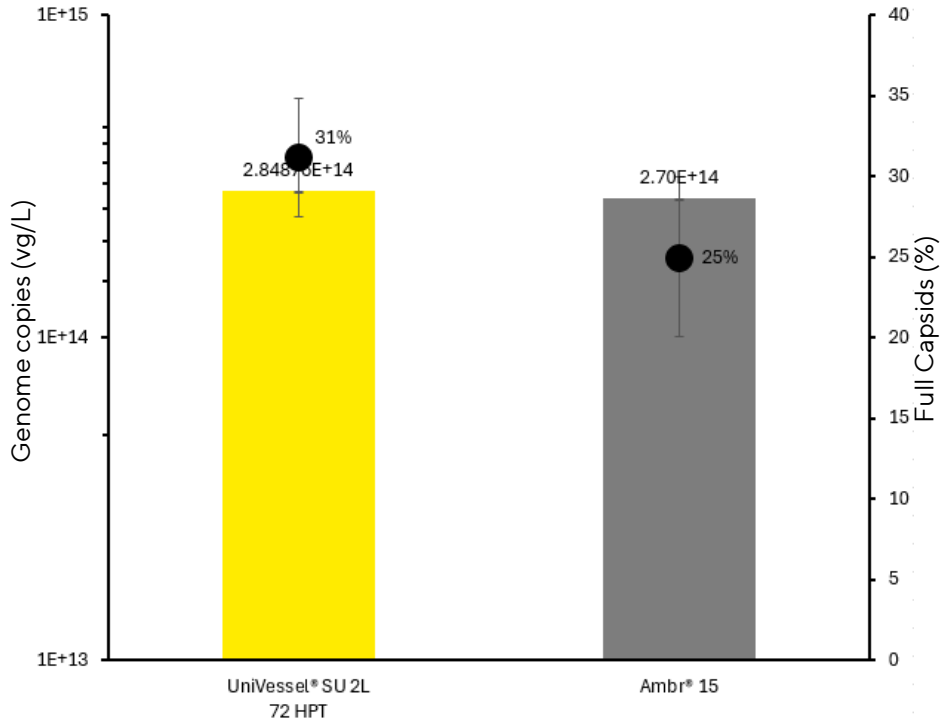


Case study

Scalable Success: Consistent Productivity at 2 L with Optimized conditions

2 L Univessel SU bioreactor





Key results and take aways

Summary and Key Takeaways

- **70-fold increase in rAAV2 productivity**

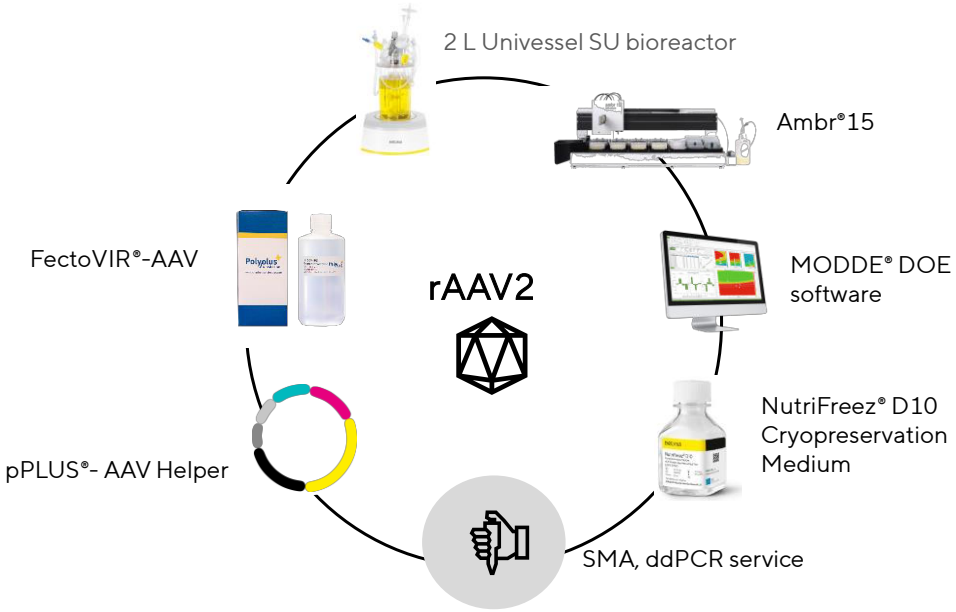
1.2E+10 → 8.8E+11 vg/mL in 4 data-driven iterations

- **Structured DoE in Ambr® 15 revealed critical multi-factor interactions**

Enabled definition of a robust operating window and deeper process understanding

- **Optimized conditions successfully translated to 2 L scale**

Demonstrating scalability and accelerated rAAV process development



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Booth number 79

Poster number 33

Decentralized Manufacturing of ATMPs: Navigating Regulatory Innovations and Strategic Partnerships for Enhanced Patient Care
Speaker : Ravid Grimberg
Date / Time : 18th March 2026 at 11.30-11.50 GMT

Thank you.

Specifications subject to change without notice.