

From idea to injection: Flexible modular manufacturing options

Andreas Castan, PhD Director Strategic Technologies

Agenda

- Enterprise Solutions introductions
- Plasmid DNA a critical raw material and therapeutic
- Scalable AAV process
- mRNA how to deal with the unknown
- Drug product innovation –closing the loop from idea to injection

The process is central to biomanufacturing



 Focus should be on understanding the product and its manufacturing process.

Enterprise Solutions – Adapting and evolving in a more diverse industry



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Plasmid DNA – a critical raw material and therapeutic

Plasmid applications – the start of many therapies



Plasmid GMP manufacturing



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Modular biomanufacturing solutions for pDNA



50 L process example

- *E. coli* low optical density (OD) fermentation,
 0.2 g/L titer
- 40% total recovery

Day 1 to 2

• 60 batches/yr with 90% facility utilization

•••

Day 3 to 4

~ 4 g of plasmid/batch;
 ~ 0.25 kg/yr



FlexFactory[™] manufacturing line

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables support
- Enabling services and training speed to engineering runs



KUBio[™] box facility solution

- Designed for the 50 to 200 L plasmid FlexFactory™ manufacturing line
- Biosafety level 1
- Expandable design for capacity increase



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Day 4 to 6



A scalable AAV manufacturing process

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Adeno-associated virus (AAV) process



TEM = Transmission electron microscopy AUC = Analytical ultracentrifugation

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Capture: Affinity chromatography Optimized protocols

Downstream





Affinity capture AAV2 Capto™ AVB resin HiTrap™ column, 1 mL

Step	Volume	Buffer/sample
Equilibration	5 CV	20 mM Tris, pH 7.8 + 200 mM NaCl
Sample load	170-215 CV	TFF retentate Load 1–3 x10 ¹⁴ VP/mL resin
Wash	10 CV	20 mM Tris, pH 7.8 + 200 mM NaCl
Elution AAV2	4 CV	50 mM citrate pH 3.5, 500 mM NaCl, 500 mM arginine
Elution AAV5	5 CV	50 mM glycine pH 2.7

AAV5

Lower pH, glycine buffer No salt or arginine Good recovery 60% to 90% Binding capacity ~ 2–5 ×10¹⁴ VP/mL Concentration ~ 100-fold << 1% aggregation in eluate

AAV2 similar performance (different protocol)

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Polishing: Anion exchange to reduce empty capsids AAV5 final protocol and scale up to 10 L

A 280



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Conductivity

Column volume 51 mL, bed height 10 cm. Capto[™] Q ImpRes resin, HiScale[™] column

Step	Volume	Buffer/material
Equilibration	3 CV	20 mM Tris pH 9.0 + additives*
Load	~5 CV	Capto™ AVB eluate diluted 10 times in
		equilibration buffer (~2 ×10 ¹³ VP/mL resin)
Wash 1	4 CV	20 mM Tris pH 9.0 + additives*
Elution hold at	5 CV	A: 20 mM Tris pH 9.0 + 18 mM MgCl ₂ +
0% B		additives*
		B: Buffer A +1 M NaCl
Elution	15 CV	Linear gradient 0%-20% B
Elution hold at	5 CV	
20% B		

*1% sucrose + 0.1% poloxamer-188 Confidential - Company Proprietary

Capto[™] Q ImpRes resin Capacity: 1–3 x 10¹³ VP/mL resin Recovery: 60% to 80% VG Full capsids: 40% to 65%

Analytics: critical for success but time-consuming

Detection range - compatibility with samples of different concentrations (LOD/LOQ)

Assay variation - intra- and inter-assay reproducibility

Accuracy - include controls and references

Matrix or sample buffer interference - detergents, salt, additives, HCP, and hcDNA

Orthogonal methods - for full and empty capsid analysis

Throughput - manual vs automation, simplify to reduce time

Assay validation is critical

Analysis

Virus infectious titer

Transduction assay: flow cytometry

Virus titer

Viral genomes: qPCR Viral capsids: ELISA, SPR (Biacore[™] assay) Full-empty ratio: qPCR/ELISA, analytic IEX, AUC, TEM

Host cell impurities

Total protein: BCA assay Total DNA: PicoGreen™ assay hcDNA: qPCR HCP: ELISA

Characterization

SDS-PAGE, Western blotting TEM SEC and IEX HPLC

LOD = Limit of detection, LOQ = Limit of quantitation, HCP = Host cell protein, hcDNA = Host cell DNA, SPR = Surface plasmon resonance, AUC = Analytical ultracentrifugation, TEM = Transmission electron microscopy, SEC = size exclusion chromatography, IEX = ion exchange

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Modular biomanufacturing solutions for AAV



200 L Fed batch process example

- Triple transfection of HEK293 producer cell line
- 1.0E+14 vp/L titer
- 36% total recovery
- 28 batches per year with 80% facility utilization
- 7.3E+15 purified vp /batch and 2.0E+17 purified vp /year



FlexFactory[™] manufacturing line

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables
- Enabling services and training- speed to engineering runs

KUBio[™] box facility solution

- Designed for the 50-200L AAV workflow*
- Biosafety level 2
- Expandable design for capacity increase



* Larger scales supported by other designs





mRNA – how to deal with the unknown

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Plasmid and mRNA manufacturing process



1. Plasmid manufacturing



How to go from mL to L



Never too early to think big – develop process with manufacturing in mind

No mRNA process is the same – how to deal with process diversity



LNP formulation technology, from discovery to commercialization



Scalable solutions for mRNA-LNP formation can shorten time to commercialization.

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Modular biomanufacturing solutions for mRNA

Example mRNA process

- IVT at 2-5 g/L titer
- 40-60% total recovery
- 55 batches/yr with 80% facility utilization
- 40-60 g/batch

2.2-3.3 kg/yr

*

FlexFactory[™] manufacturing line

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables support
- Enabling services and training for faster time to engineering runs

KUBio[™] box facility solution

- Designed for the 50 L mRNA FlexFactory[™] manufacturing line, with optional prep and filling space
- Biosafety level 1
- Expandable design for capacity increase



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Aseptic filling for advanced therapies

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Maximizing yield for multiple clients

- Goals:
 - Reduce risk
 - Maximize dosage yields
 - Serve multiple clients
- Cytiva SA25 Aseptic Filling Workcells can fill all molecules and dosage types
- Scale out with standardized systems





Reduced risk via closed robotic workcells

- Remove human operator
- Remove quality risks
- Reduce product hazard pathway / single-use flowpath
- Container / closure capable of -80°C



Customer examples



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Summary

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Give your manufacturing room to grow with fast, integrated, and flexible solutions at any scale.



- Enterprise Solution
- Plasmid DNA
- Scalable AAV process
- mRNA
- Aseptic filling

Never too early to think about manufacturing.

Stay flexible with platforms and standardization.

Gain time with solutions for optimized manufacturing.

Thank you

Andreas Castan

andreas.castan@cytiva.com

linkedin.com/in/andreas-castan-91570b1/

cytiva.com



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