cytiva

A scalable AAV production process from cell culture to purified bulk

Webinar about AAV workflow and production

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Cytiva-Japan

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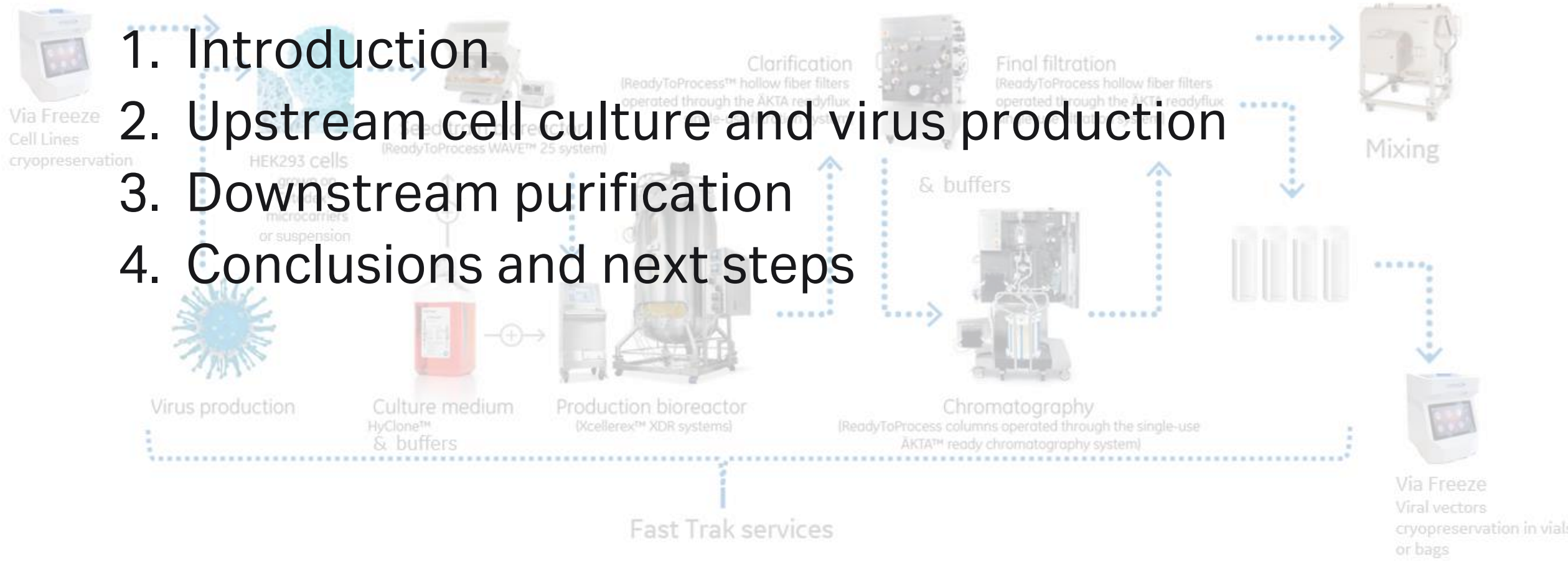
1. Introduction
2. Upstream cell culture and virus production
3. Downstream purification
4. Conclusions and next steps



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Introduction

Sizes of common viruses

Antibody
~ 5 nm

Preventive vaccines

JE
(40–60 nm)

Yellow Fever
(40–60 nm)

HAV
(30 nm)

VLP
(40–60 nm)

Polio
(30 nm)

Hep B
(42 nm)

Rota
(80 nm)

Corona
(125 nm)

Flu
(90–120 nm)

Mumps (200 nm)

Measles (100 × 300 nm)

Rabies (75 × 180 nm)

Recombinant virus vectors for cell and gene therapy

AAV
(25 nm)

Adeno
(70–90 nm)

Lenti
(80–120)

POX
(200–300)

Viral vector production and clinical use

Plasmid (AAV and lentivirus)

Plasmid design
Gene of interest
Plasmid backbone

Plasmid production
&
purification

Transfection

Viral vector production

Expansion of producer cells

Virus propagation

Virus purification

Virus fill & finish

Viral vector design
Gene of interest
Plasmid backbone

Virus seed bank

Infection

Virus (adenovirus and other)

Vaccines

Oncolytic therapy

Gene therapy

Cell therapy

Cytiva

Adeno-associated virus (AAV) production process

Upstream

- Triple plasmid transfection
- HEK293T suspension
- AAV2-GFP

WCB

Xcellerex™ XDR-10
Scaleable to 2000 L

Seed train

WCB = working cell bank

Virus production

Downstream

- **Cell lysis DNA fragmentation**
- **Clarification**
- **Concentration & buffer exchange**
- **Capture**
- **Polishing**
- **Concentration & buffer exchange**
- **Sterile filtration**

Analysis

Virus infectious titer
Transduction assay: flow cytometry

Virus titer
Viral genomes: qPCR
Viral capsids: ELISA, SPR (Biacore™)
Full-empty ratio: qPCR/ELISA, Analytic IEX, TEM

Host cell impurities
Total protein: BCA assay
Total DNA: Picogreen™ Assay
HC DNA: qPCR
HCP: ELISA

Characterization
SDS-PAGE, Western blotting
TEM SEC and IEX HPLC

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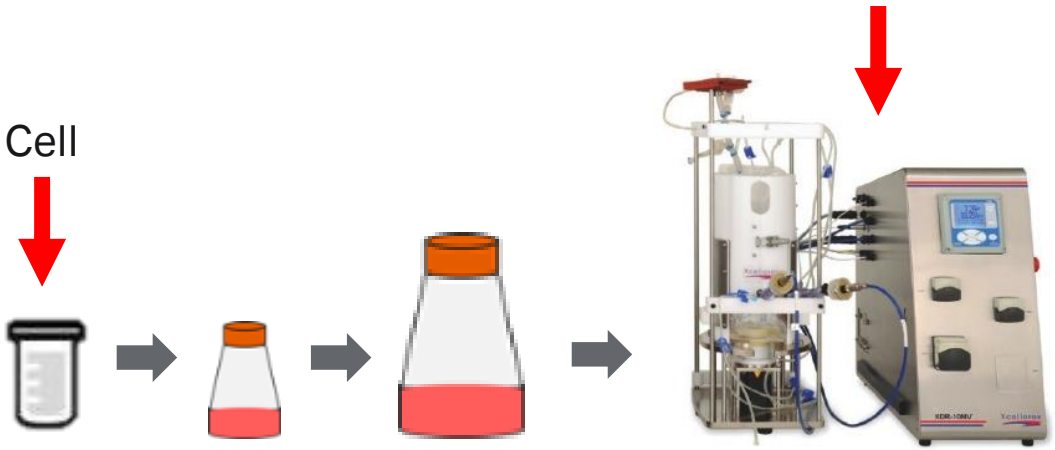
Upstream cell culture and virus propagation

AAV upstream strategy

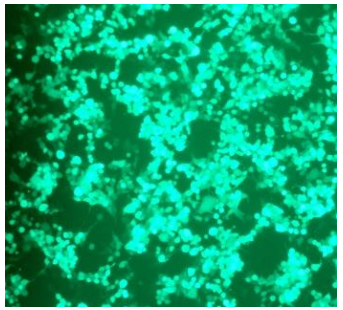
- Adaptation to serum free suspension cell culture
- Cell culture medium evaluation
- Cell density optimization
- Optimization of AAV transfection
- Optimization of culture conditions post transfection

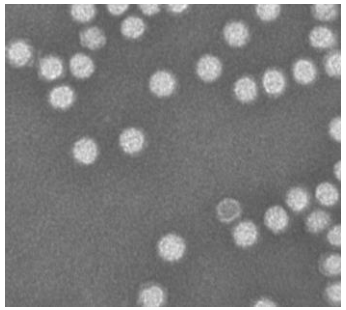
HyClone™
HyCell
TransFx-H

AAV plasmid production → Cell expansion → Transfection AAV plasmids → Cell lysis → Purification



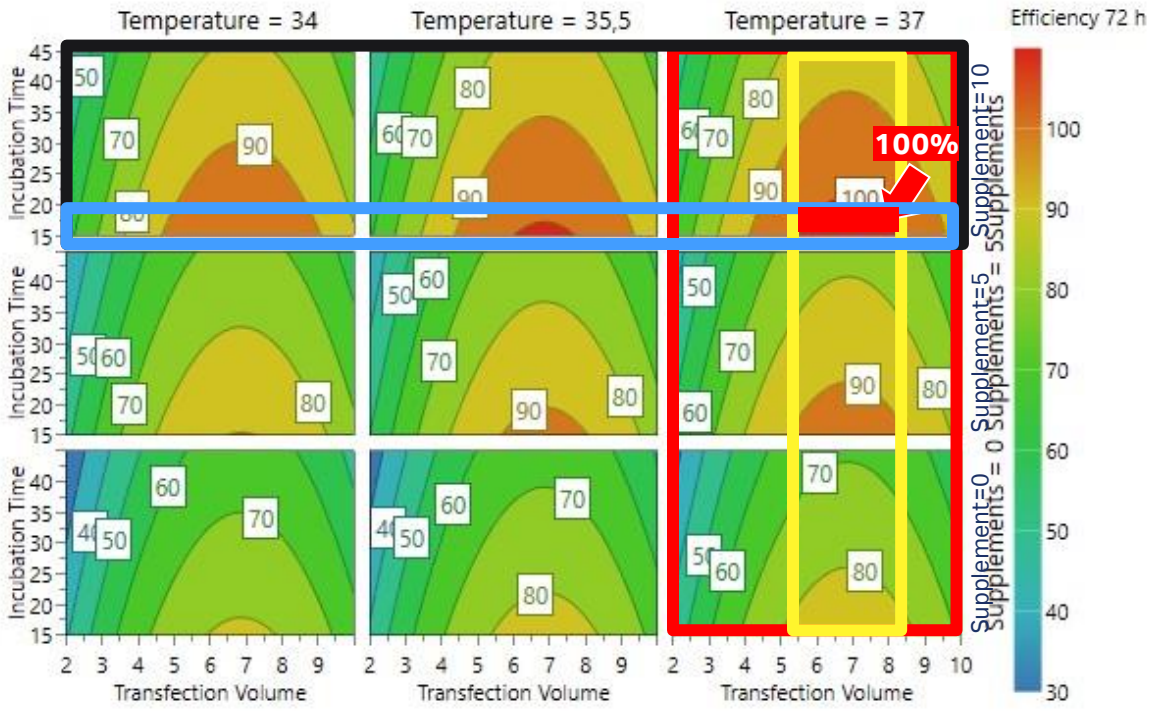
XDR bioreactor
(scalable up to 2000 L)





DoE results

Transfection efficiency



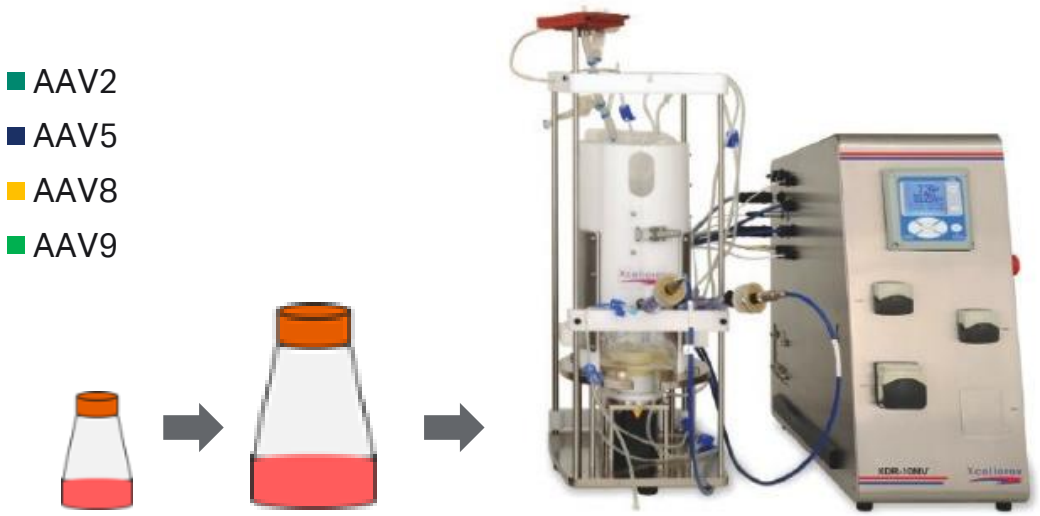
Transfection efficiency: % GFP expressing cells

Criteria:
Titer (ELISA) > 10^9 capsids/mL

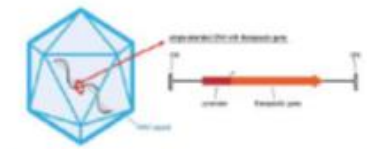
Optimized transfection protocol

VCD: 1×10^6/mL
DNA (µg/µL): 1
PEI/DNA ratio: 2
Transfection volume (% of total): 5
Incubation time: 20 minutes
Temperature: 37°C
DNA ratio: 1:1:2
(Rep/cap: helper: transgene plasmid)
Sodium Butyrate: 5 mM

AAV productivity in different scales



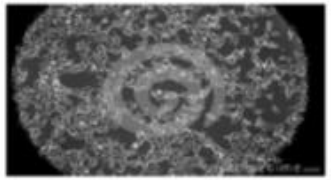
Homology's Upstream HEK293 Transfection Production Platform Shows Linear Scalability Up to 500 L



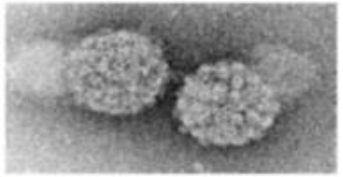
1. Construct Selection

2. Plasmid Engineering & fine tuning

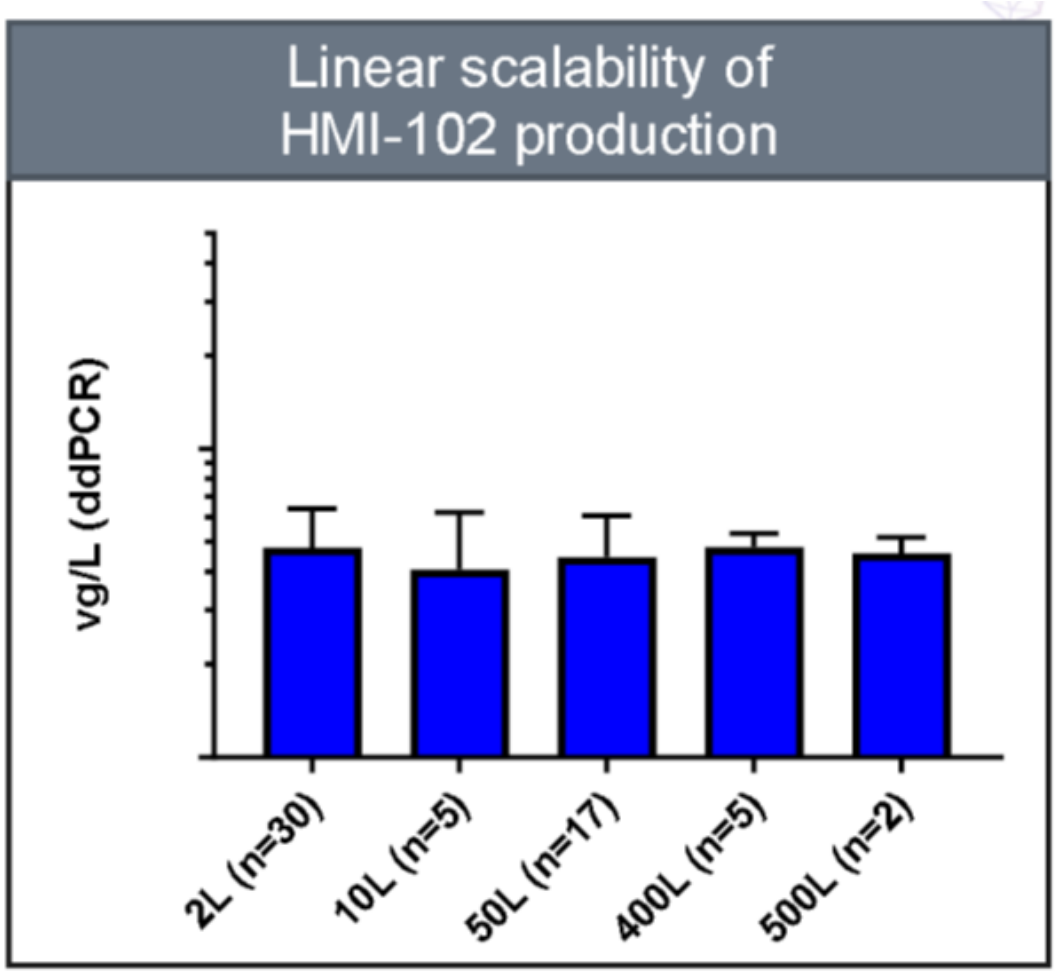
3. Cell growth & propagation



4. Transfection



5. Harvest product frozen & stored



HOMOLOGY Medicines, Inc.

Courtesy of Homology Medicines Inc

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Clarification
Downstream purification

Harvest: Cell lysis, DNA fragmentation and Clarification

Cell lysis
DNA fragmentation

Clarification

Conc. and buffer exchange

Capture

Polishing

Conc. and buffer exchange

Sterile filtration

Harvest:
0.5 % Tween™ 20
300 mM NaCl
1 mM $MgCl_2$
40 U/mL Denarase™ (DNA nuclease)
Incubation in Bioreactor at 37°C with mixing for 4 hours

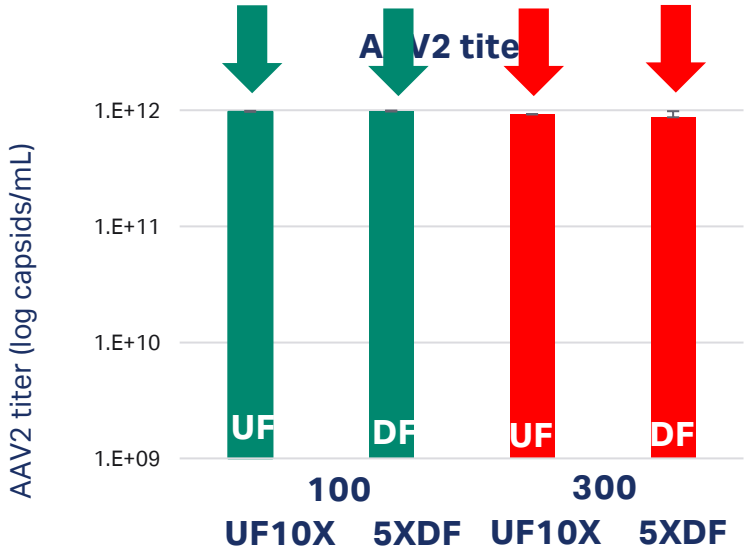
Normal flow filtration:
ULTA™ capsules 5 µm + 2 µm + 0.6/0.2 µm HC
Flow 30 to 50 LMH
Recovery 74 to 80 %

Concentration and buffer exchange: tangential flow filtration hollow fiber with 300 kDa NMWCO

Downstream

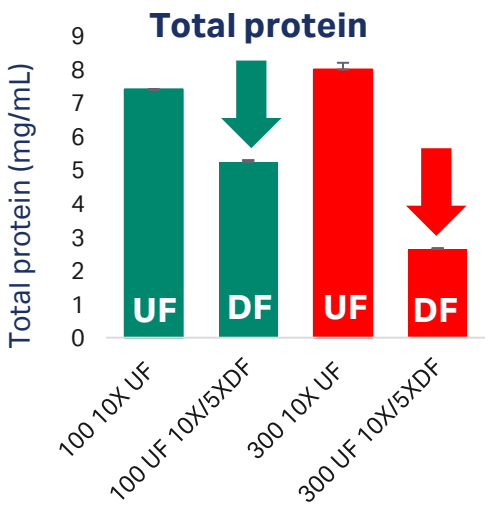
- **Cell lysis DNA fragmentation**
- **Clarification**
- **Conc. and buffer exchange**
- **Capture**
- **Polishing**
- **Conc. and buffer exchange**
- **Sterile filtration**

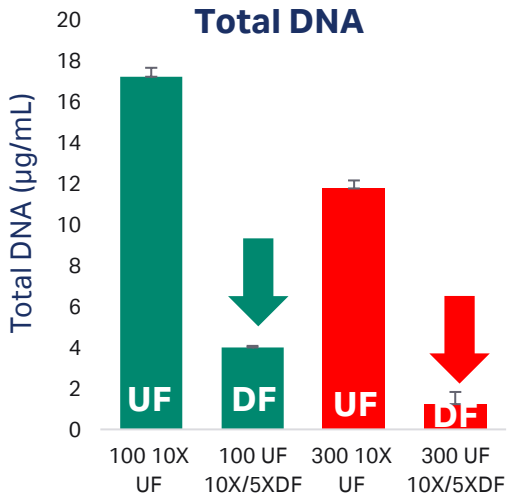
UF= Ultrafiltration
DF= Diafiltration
AIEX = Anion exchange chromatography



No virus losses using
300 kDa NMWCO
Recovery was 75 to 80 %

100 NMWCO UF10X/5XDF
300 NMWCO UF10X/5XDF

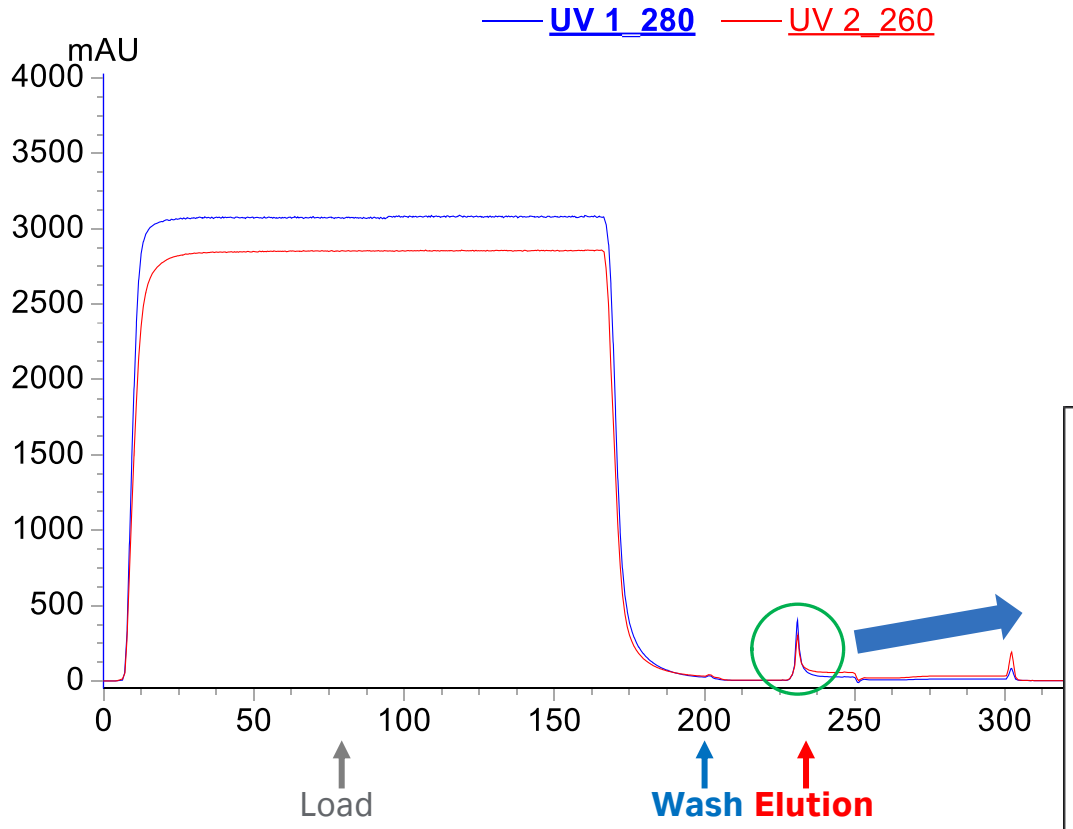




Better impurity removal
using 300 kDa NMWCO

Capture: Affinity chromatography with **Capto™ AVB**

- Cell lysis
  DNA fragmentation
- Clarification
- Conc. and buffer exchange
- **Capture**
- Polishing
- Conc. and buffer exchange
- Sterile filtration

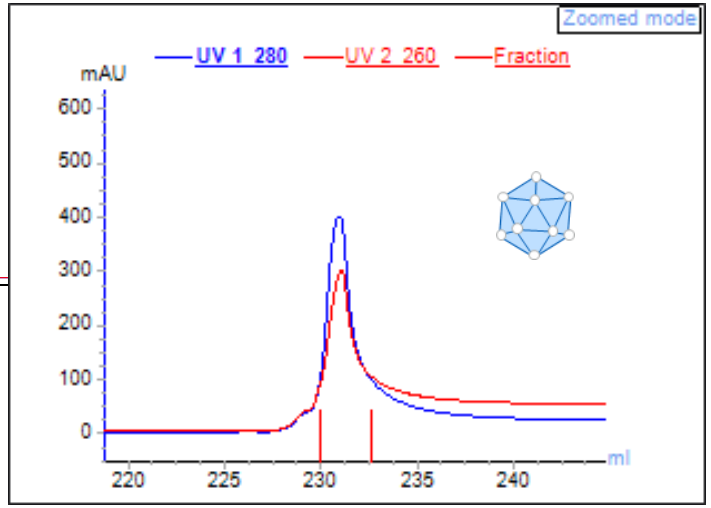


ÄKTA™ pure 25
HiTrap™ Capto AVB 5mL

Load flow: 5 mL/ min (1 min RT)
Eluate flow: 2.5 mL/min (2 min RT)

Eq buffer: 20 Mm Tris, pH 8.0 + 500 mmM NaCl
Washing buf. ①50 Mm Tris, pH 8.0 + 300 mmM NaC
②20 Mm Tris pH 8.0
Elution buf: 50 mM Citrate pH 3.5 500mM NaCl
500 mM Arginine

Binding capacity: ~ 1 x 10^{14} capsids/mL resin
Concentration factor: ~ 100×
Recovery range: 60 to 80 %



Capture: Affinity chromatography with **Capto™ AVB**

Downstream

- **Cell lysis DNA fragmentation**
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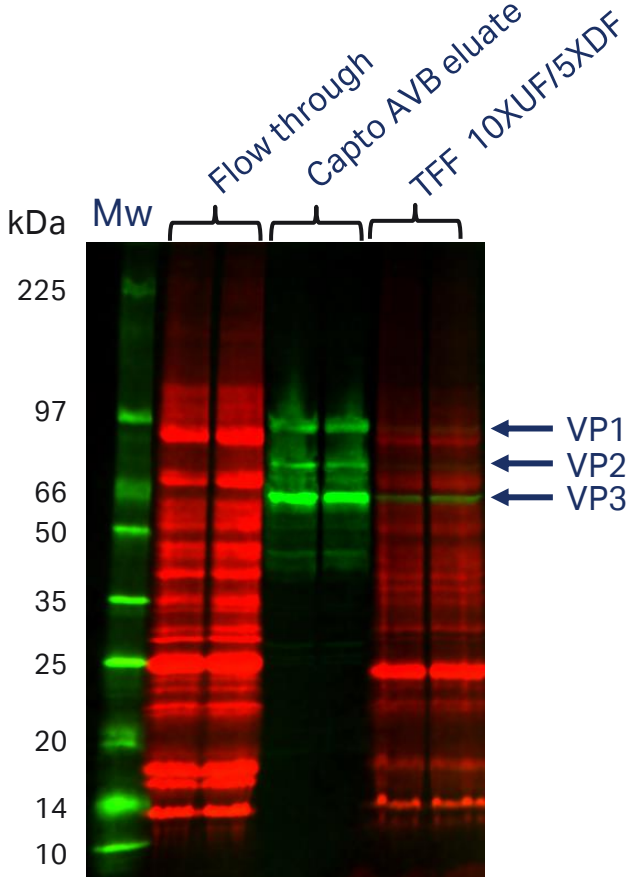
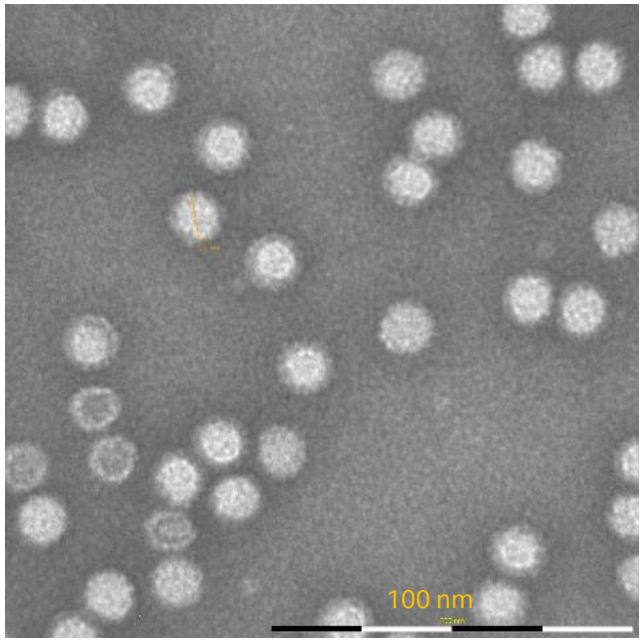
Membrane image
Cy™3/**Cy5** overlay



Red = HCP
Green = Viral proteins

Capto AVB eluate

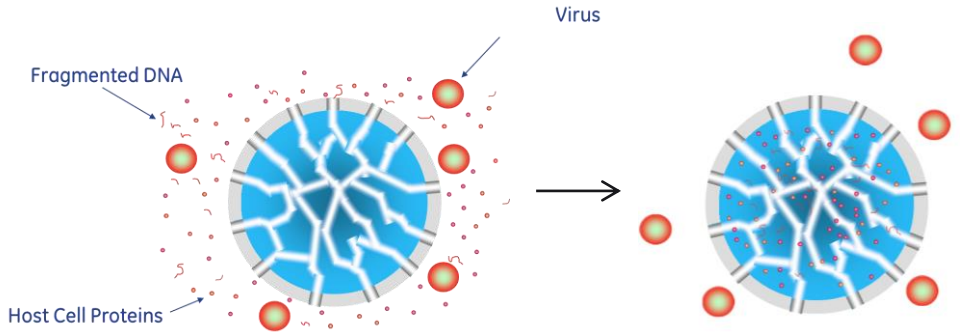


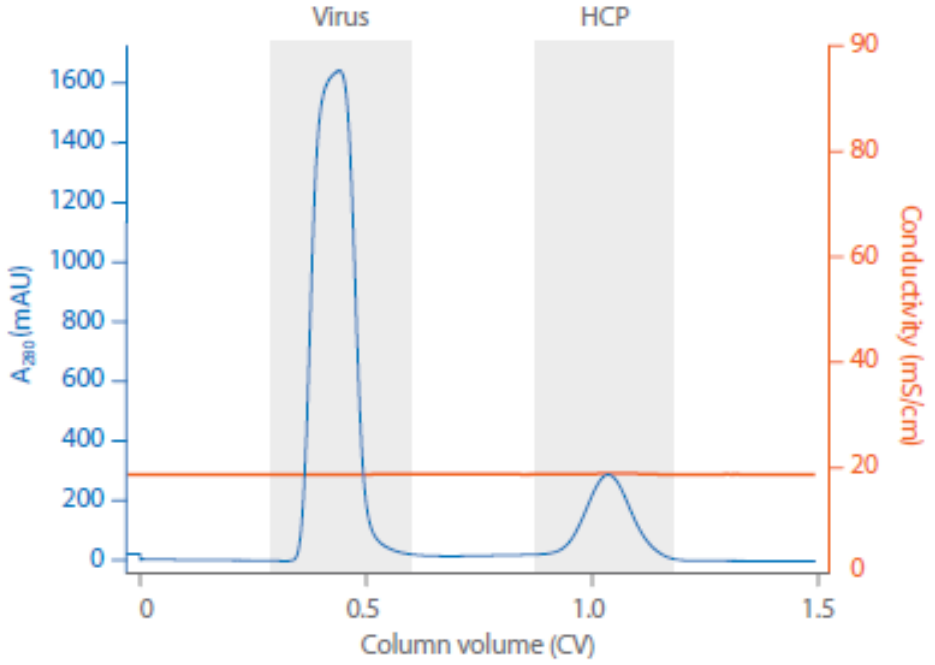
Transmission electron microscopy in collaboration with Vironova AB using MiniTEM™ system, Stockholm, Sweden

Polishing: Using **Core Technology** for reduction of impurities.

AAV

	Capto Core 400	Capto Core 700
Matrix	Highly cross-linked agarose	
Average particle size (d_{50v})	90 µm	85 µm
Ligand	Octylamine	
Binding capacity*	22 mg ovalbumin/mL resin	13 mg ovalbumin/mL resin
Average molecular weight cutoff	M_r 400 000	M_r 700 000
Maximum flow velocity	700 cm/h in column with 20 cm bed height at < 2 bar (0.2 MPa)	500 cm/h in column with 20 cm bed height at < 2 bar (0.2 MPa)
pH stability Operational† CIP‡	 3 to 13 3 to 14	
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide (NaOH)§, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol.	
Avoid	Oxidizing agents, citrate buffers	
Storage	20% ethanol at 4°C to 30°C	





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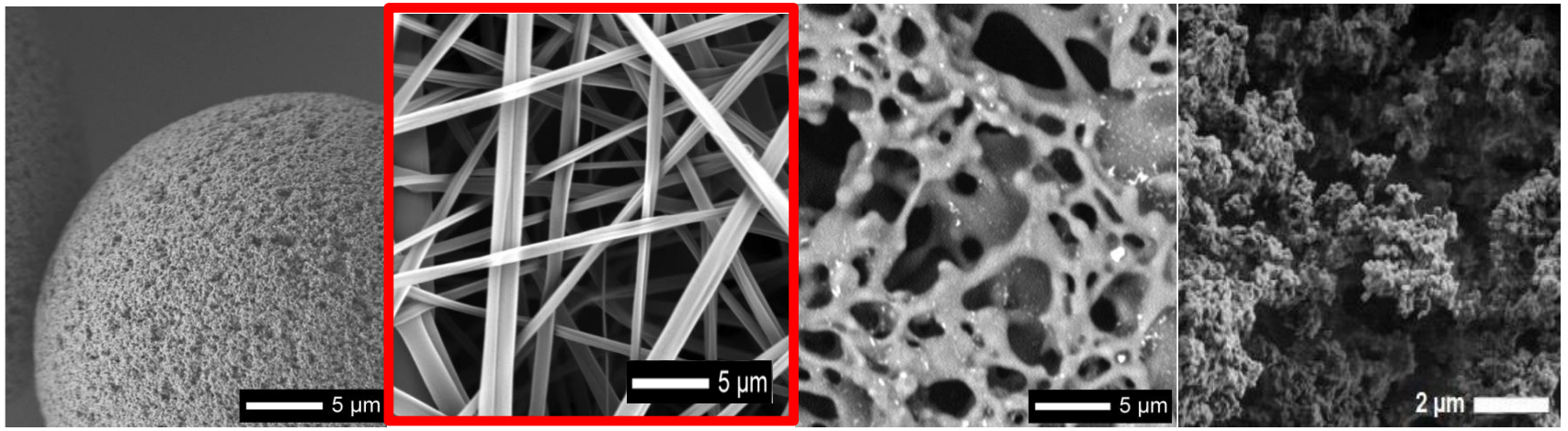
Technology formats

Evolution in formats :
Fibro – well-suited for viral vector chromatography



Bead resin | Fibro | Membrane adsorber | Monolith

Parameter	Bead resin	Fibro	Membrane	Monolith
Pore size	15–40 nm	0.2–2.0 µm	3–5 µm	2 µm
Surface area	~ 40 m^2/g	~ 10 m^2/g	< 2 m^2/g	< 7 m^2/g

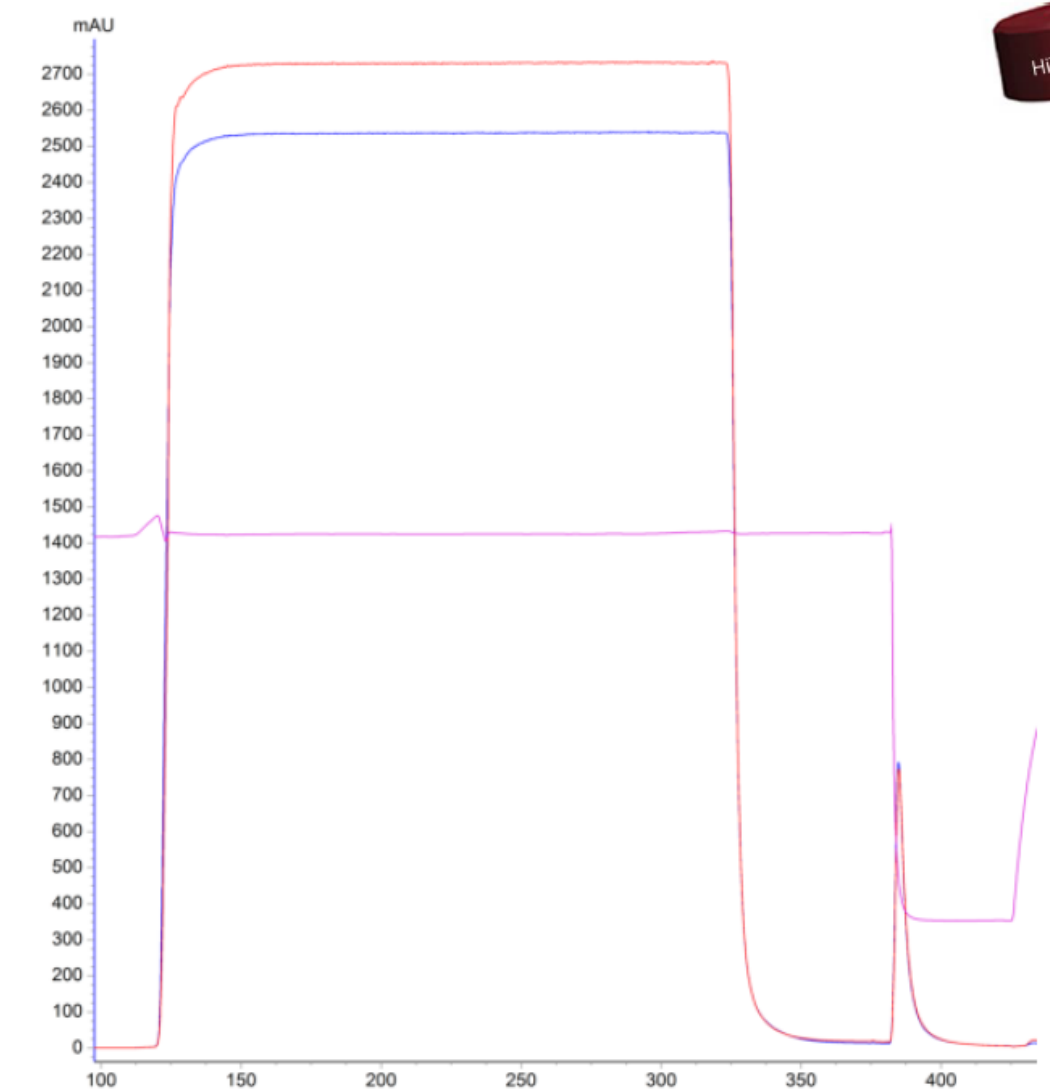
Fibro has high surface area for high binding capacity and macroporosity needed for viruses

Fibro prototype for AAV5 purification

Biomanufacturer case study: clarified sample

- Residence time in seconds rather than minutes
- Loading time reduced from hours to minutes
- Recovery > 80% (based on vg)
- Purification performance similar to affinity resins
- Simple elution

Clarification:	NFF + uncharged DF
Load:	9×10^{13} vg clarified AAV5
Load volume:	500 Fibro volumes (500 × 400 µL = 200 mL)
Load rate:	25 Fibro volumes/min
Residence time:	**2.4 s**
Load time:	**20 min**
Elution:	pH 2.5



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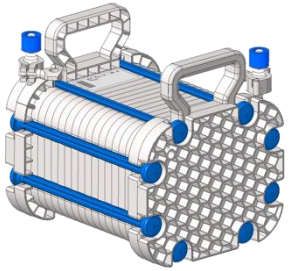
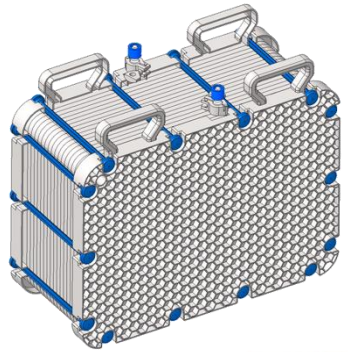
Data from collaboration with Belief BioMed

Fibro formats and ÄKTA™ systems





Lab-scale Fibro unit

- Screening and HTPD tool
- 0.4 mL adsorbent volume
- ~ 1 L AAV feed

Small Fibro unit

- PD tool – scale-down mimic
- 4 mL adsorbent volume
- ~ 10 L AAV feed

Medium Fibro unit

- GMP compatible
- Up to 160 mL adsorbent volume
- Up to 500 L AAV feed

Large Fibro process unit

- GMP compatible
- Up to 2400 mL adsorbent volume

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Conclusions

Conclusions

- ✓ Scalable supension cell culture process with chemically defined medium
- ✓ High AAV titers with optimized transfection protocol (~ 10^{11} capsids/mL)
- ✓ TFF with 300 NMWCO hollow fiber
- ✓ Efficient affinity capture chromatography with Capto™ AVB
- ✓ Fibro technology improves AAV capture

Thank you

www.cytivalifesciences.co.jp

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