Evolution of Viral Vector Manufacturing

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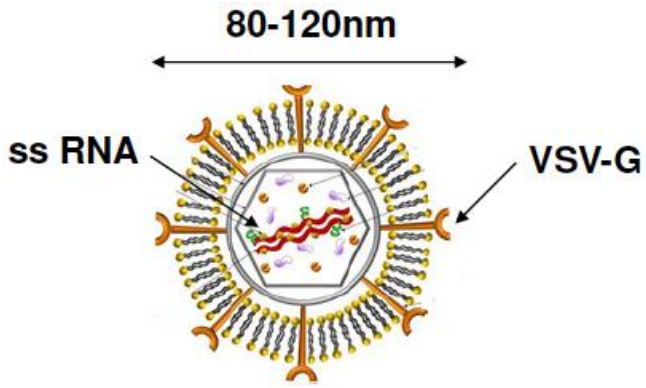
Viral vectors (LV) are used for cell and gene therapy

- Adeno Associated Viruses (AAV) and Lentiviral vectors (LV) are increasingly used for large patient populations such as Muscular Dystrophy (AAV) and Leukemia/Lymphoma (LVV).
- LVV is fragile, sensitive to pH and temperature and is used for stable gene integration into genome of dividing and non-dividing cells.



Lentiviral Vector (LVV)

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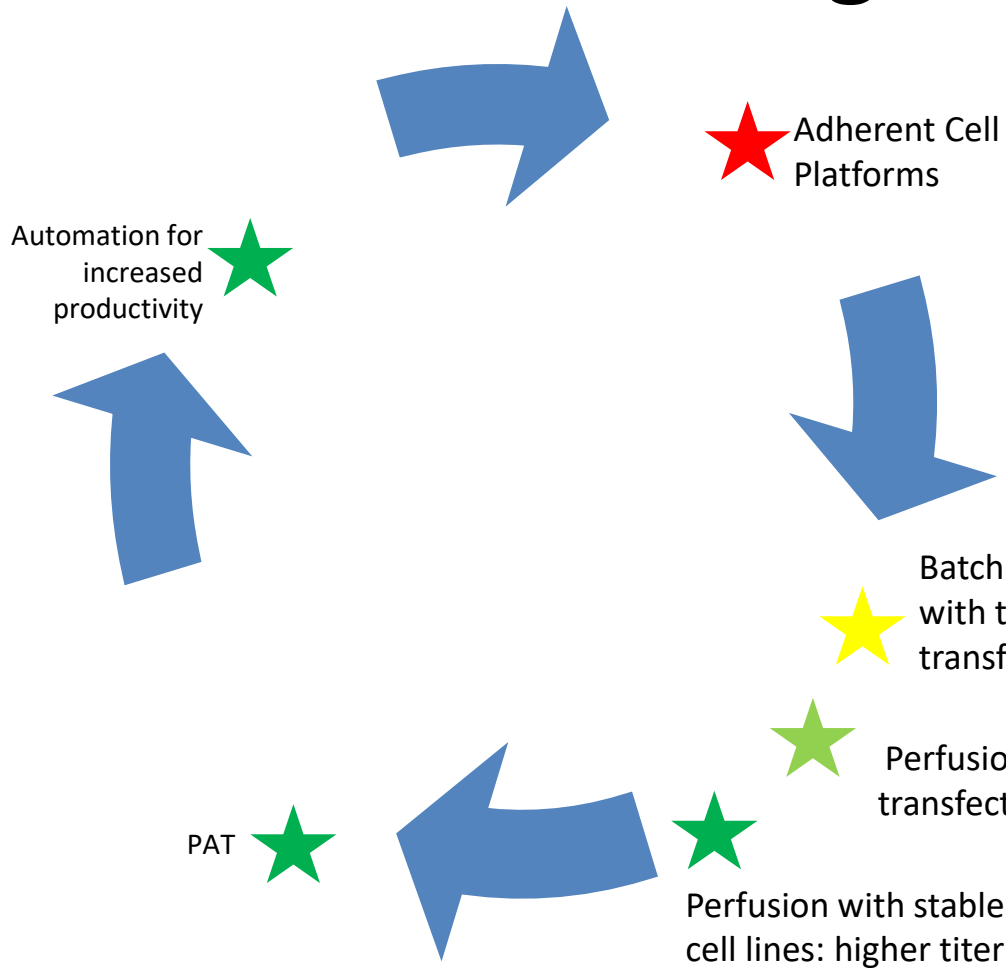
Platform Innovation for Viral Vector Manufacturing

AI and Machine Learning



Adherent Cell Platform: SCALE OUT. Eg. CF10, iCellis.

Suspension Cell Platform: SCALE UP. Aim for higher quality and higher yield

PAT aimed at characterizing vectors and achieving real time release

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Adherent cell Platform Processes

Adherent Cell Production Method:

- **Small scale for VV production**
- **T-flask for adherent cells (293T)**

i-Cellis 500L

?

- **The challenge with adherent cell systems is that tl . beyond 500L.**
- **When you scale-out COGS/dose stay high since you increase:**

  (1) the number of pieces of equipment,

  (2) the overall manufacturing footprint

  (3) the number of employees, i.e. no change in COGS

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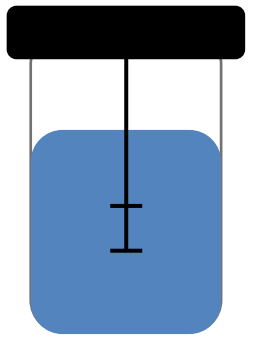
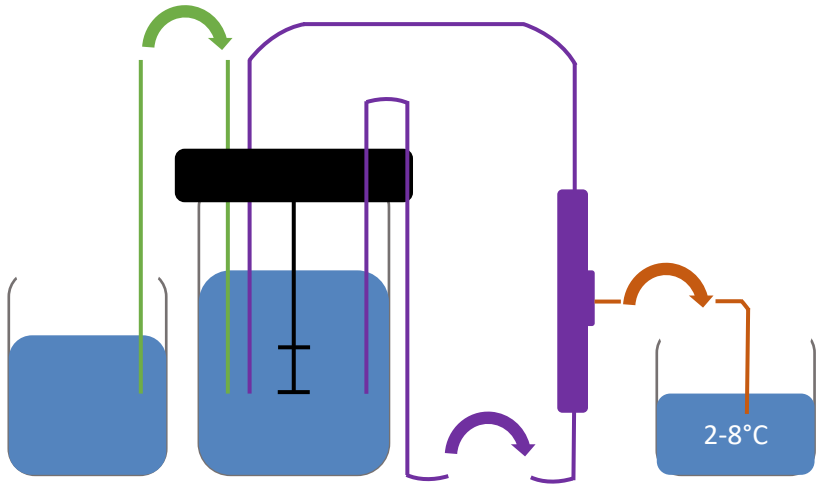
Suspension Cell Platform Processes

Batch Process



- ✓ Fixed volume
- ✓ Limited number of cells
- ✓ Low vector stability at 37°C

Perfusion Process



- ✓ Provides fresh nutrients and removes toxins
- ✓ Higher cell density – increased vector production
- ✓ Continuous transfer of unstable vectors to 2-8°C – improved stability
- ✓ Potential for higher yields and improved vector quality

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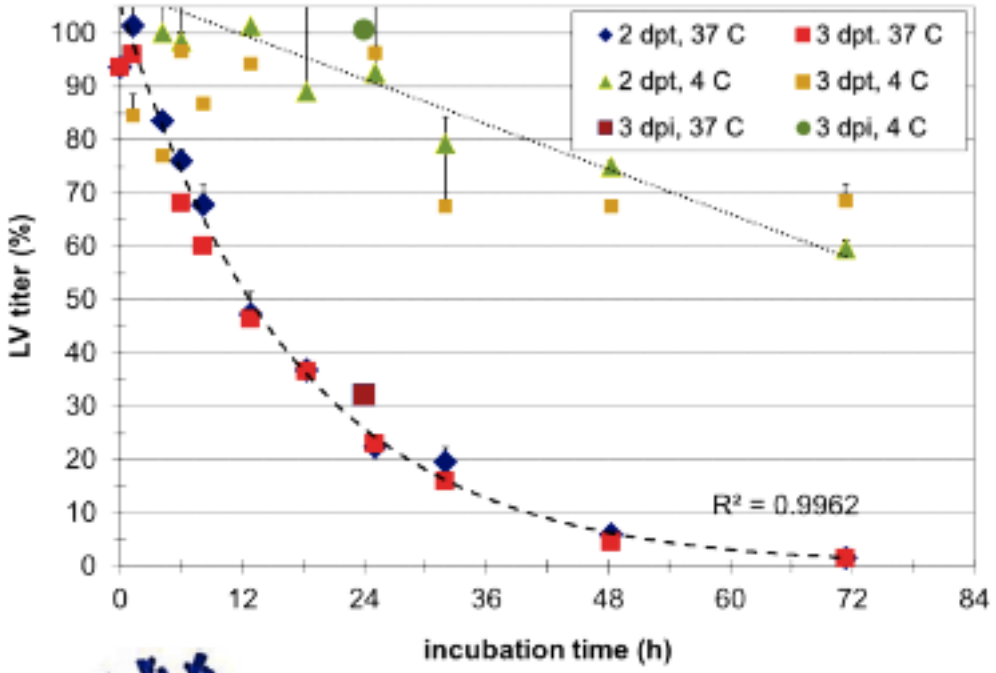
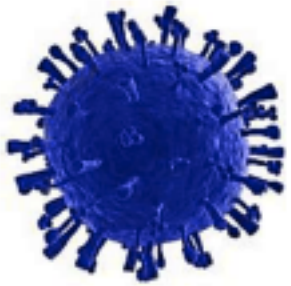
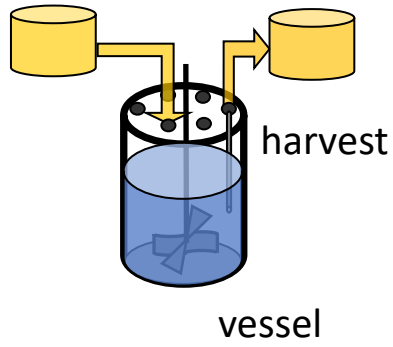
LV Stability



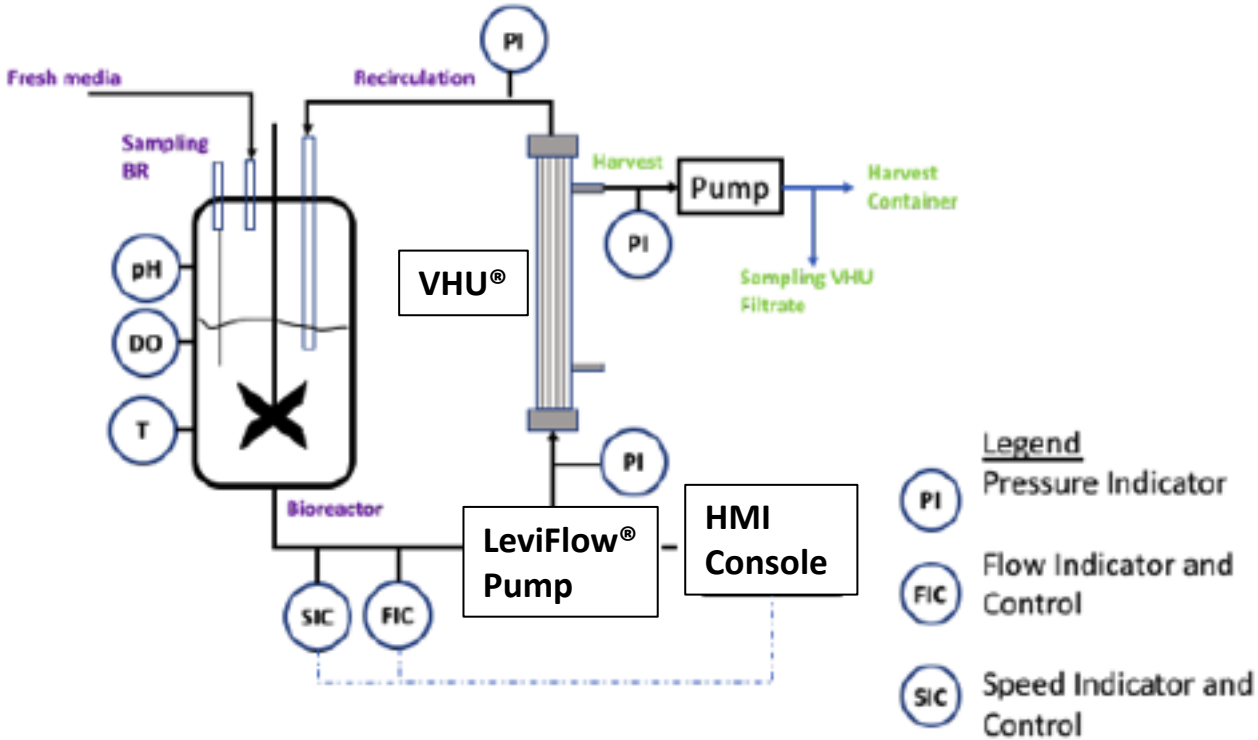


- What operating mode is ideal for stable product recovery?

→ Perfusion



TFF Perfusion: VHU® filter module and PuraLev® Pump (Patent # US10,358,626 & US10,988,725)



TFF Perfusion: VHU® filter module and PuraLev® low-shear pump.

VHU1 1L

VHU2 10L

VHU3 100L

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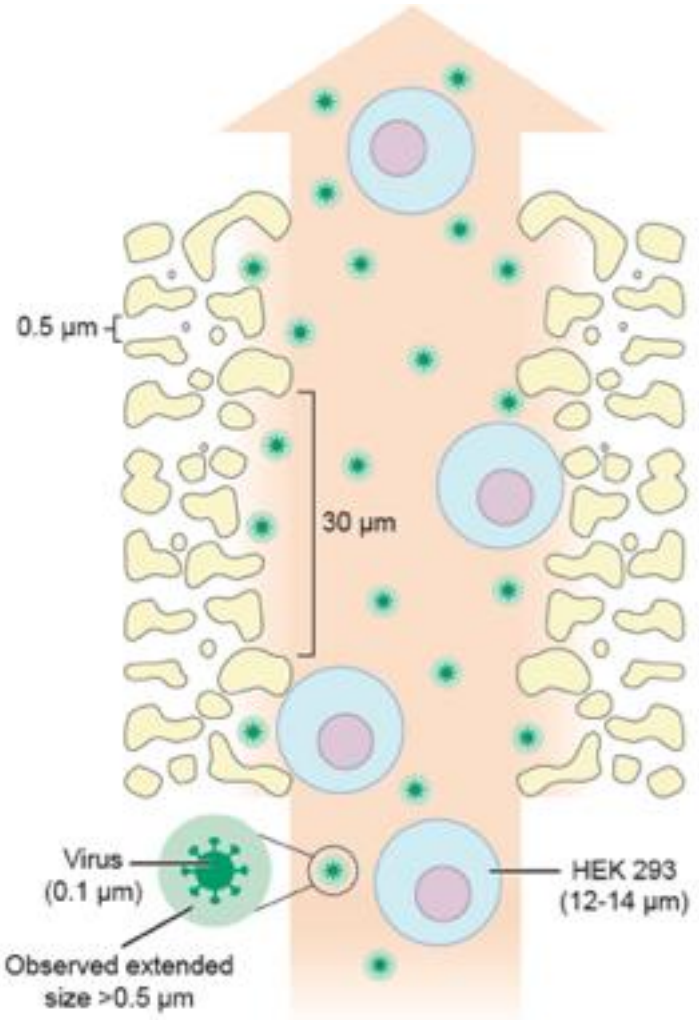
Current ATF Perfusion Technology:

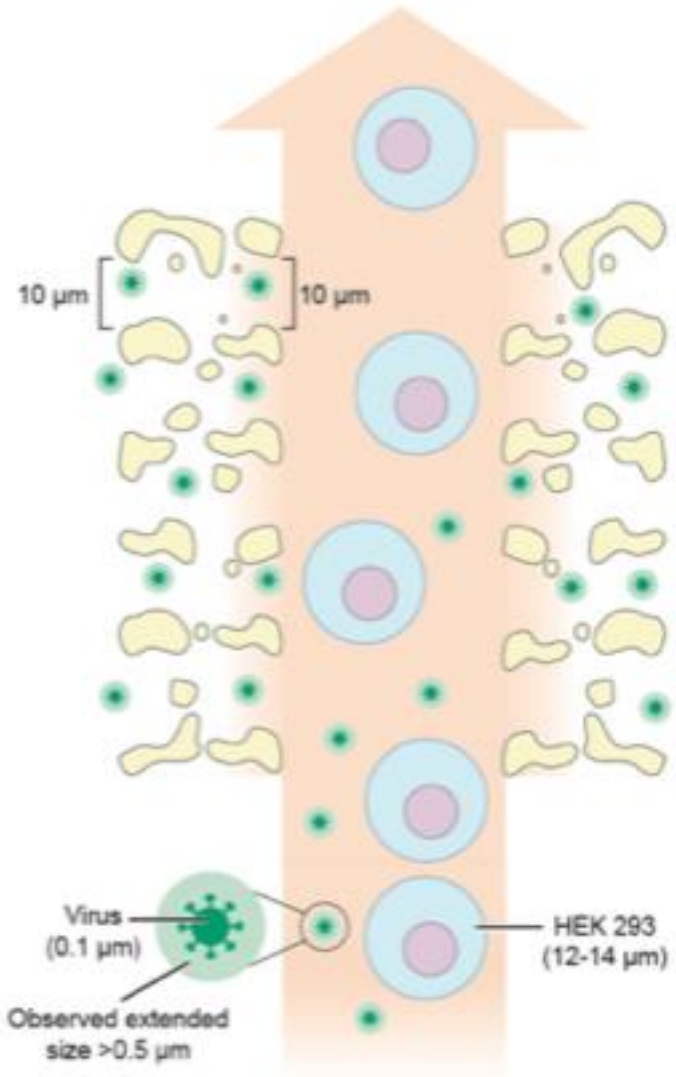
Hollow Fiber Membrane

Virus does not flow through the pores of the membrane



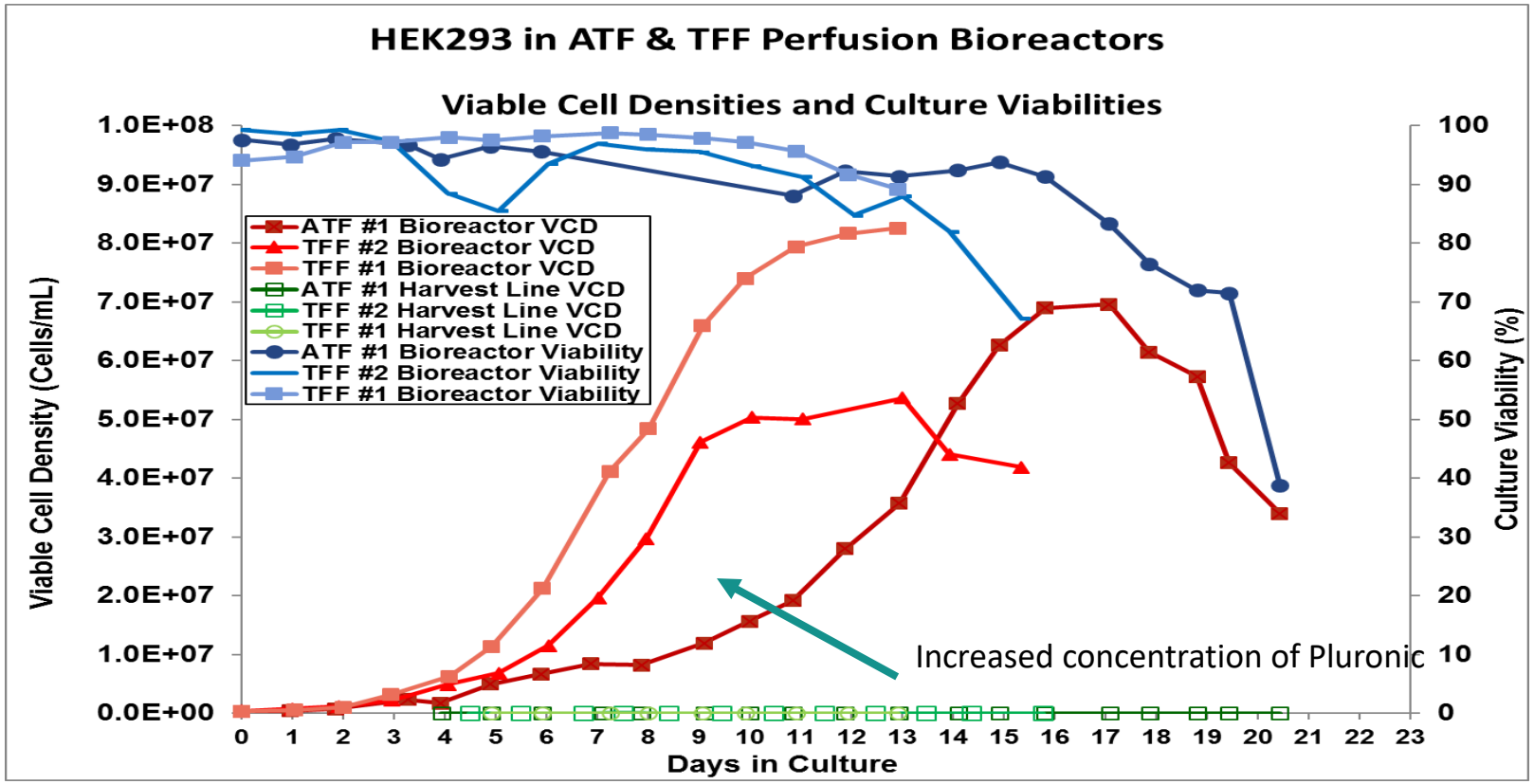
Our TFF Perfusion Technology:

Tubular Membrane



Cells are retained and virus flows through the pores of the membrane

Process Intensification: VHU-TFF vs. HFM-ATF



- Experiment was run for 21 days
- Tubular Membrane Filter in TFF mode (VHU-TFF) reached a maximum cell density of 80 million viable cells/mL
- Hollow Fiber Membrane (HFM) in ATF mode (HFM-ATF) reached maximum viable cell density of 70 million viable cells/mL
- The HFM-ATF showed Slower growth compared to VHU-TFF runs likely due to greater shear forces with the HFM.

*Note: Cell counts in harvest line are less than 4e4 c/mL, as is expected (perfusion should maintain cells within bioreactor)

Case Study 1: Artemis VHU® for LV Production and Harvesting Published Operating Parameters

	Batch Run	Perfusion Run
Day -1	Set up bioreactor, add media	Set up bioreactor, add media
Day 0	Seed at 0.3E6	Seed at 0.3E6
Day 1	~0.5E6	~0.5E6
Day 2	~1E6, Transfection	~1E6, Transfection After 2h start recirculation
Day 3		Crossflow: 333 mL/min, (shear rate equivalent to VHU2 @ 1L/min crossflow, ~620s^{-1}). Start Perfusion at 0.5vvd
Day 4		Increase crossflow to 540mL/min (~1000s^{-1}) Increase to 1vvd
Day 5-7	Day 5 Harvest	Harvest into bag stored at 2-8°C. Monitor nutrient levels and supplements as needed. Pull Sterile samples for TU analysis. Terminate when viability ≤50% or at 120h post transfection.

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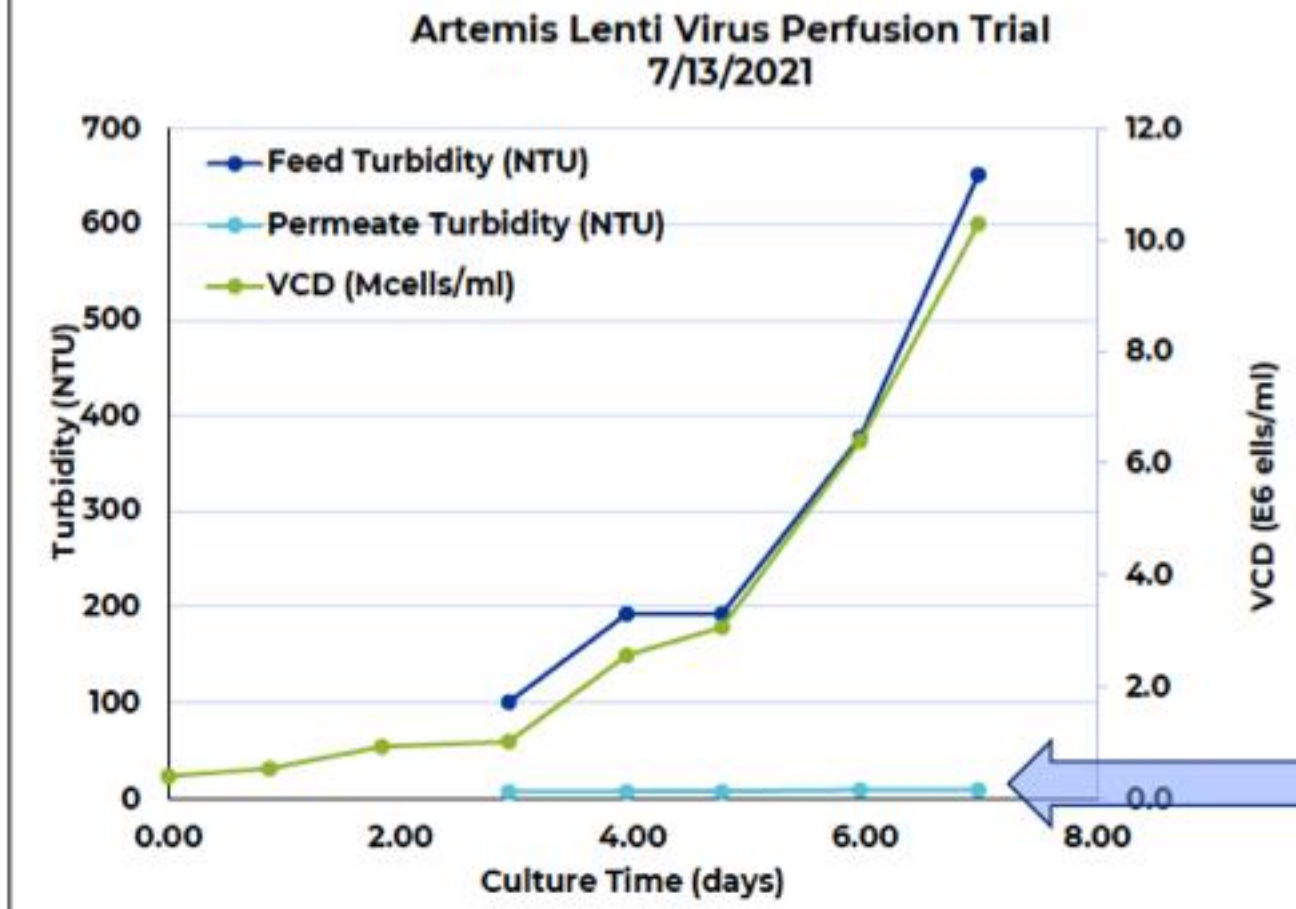
Artemis TFF Perfusion Bioreactor: Pressure and Turbidity Results



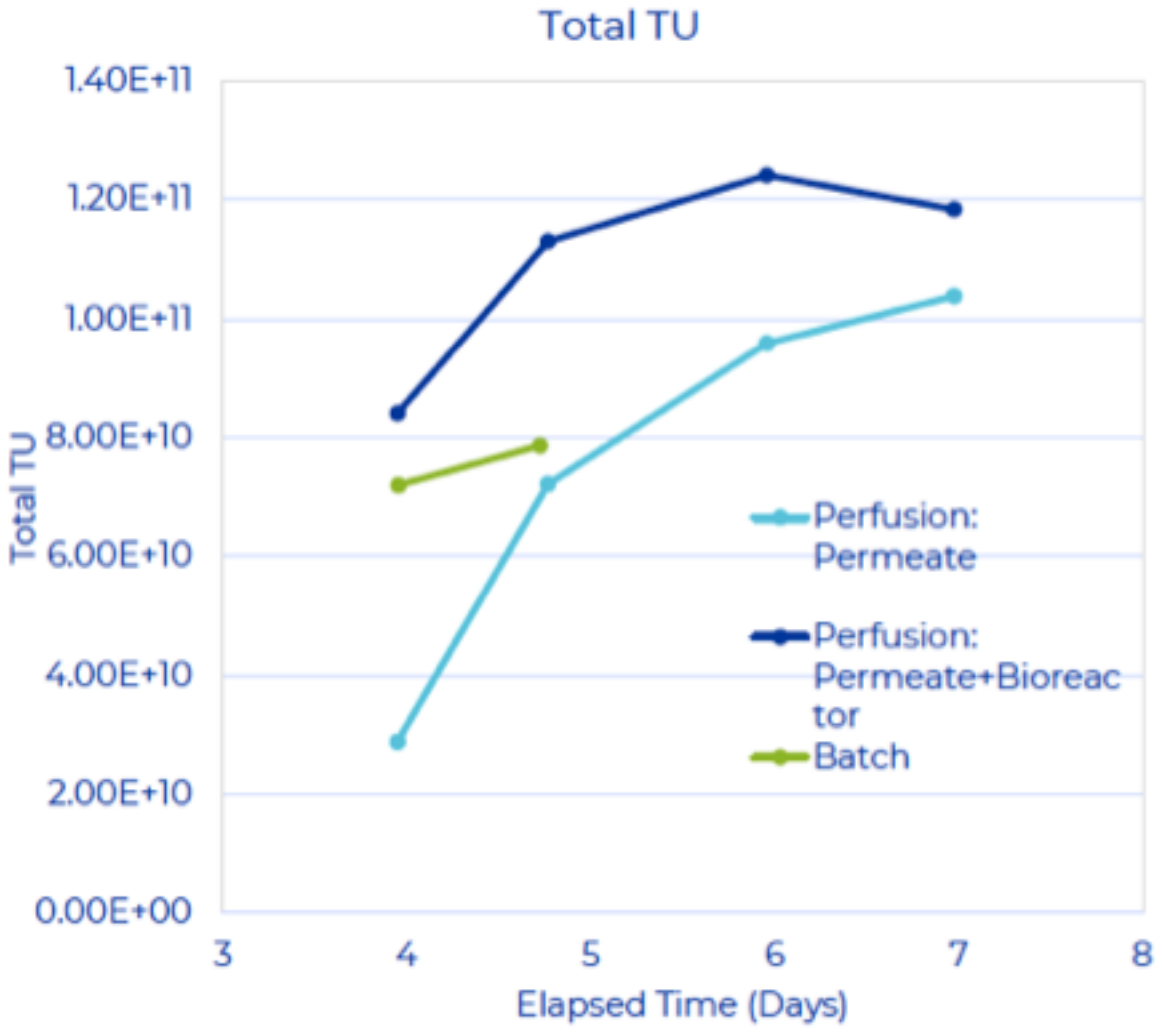
Perfusion Rate (vv/day)	Process Day	Permeate Flow Rate (ml/min)	Feed Flow Rate (ml/min)	Feed Pressure (psi)	Retentate Pressure (psi)	Permeate Pressure (psi)
0.50	3	0.8	333	0.34	0.00	0.11
0.50	4	0.8	333	0.34	0.00	0.14
1.00	4	1.7	540	0.44	0.00	0.18
1.00	5	1.7	540	0.44	0.02	0.22
1.00	6	1.7	540	0.44	0.00	0.22

Permeate turbidity was 7-9 NTU while bioreactor turbidity climbed to 652 NTU

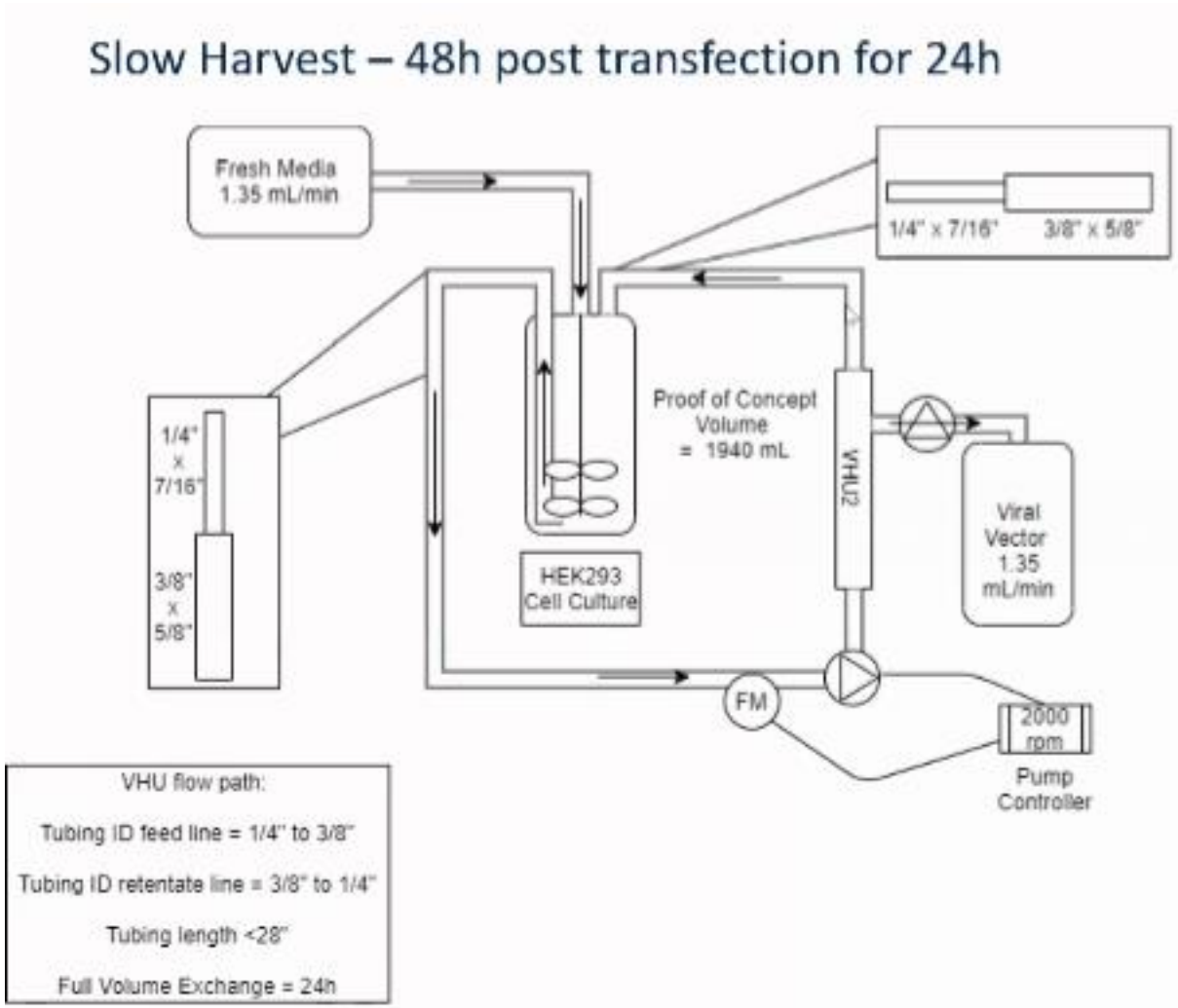
Total TU and specific productivity results

	Batch	Artemis Perfusion
Total harvested (TU)	Pre-clarification: 7.86E10 Post-clarification: 2.05E10*	9.59E10**
Clarified Pool titer (TU/L)	8.55E9	1.60E10**
Pool Volume (L)	2.4	6
Bioreactor time	5 days	6 days
Turbidity (NTU)	Pre-clarification: 407 Post-clarification: 3.98	9.08

*V100P + EKV, Virus transmission 26±6%
** Calculated



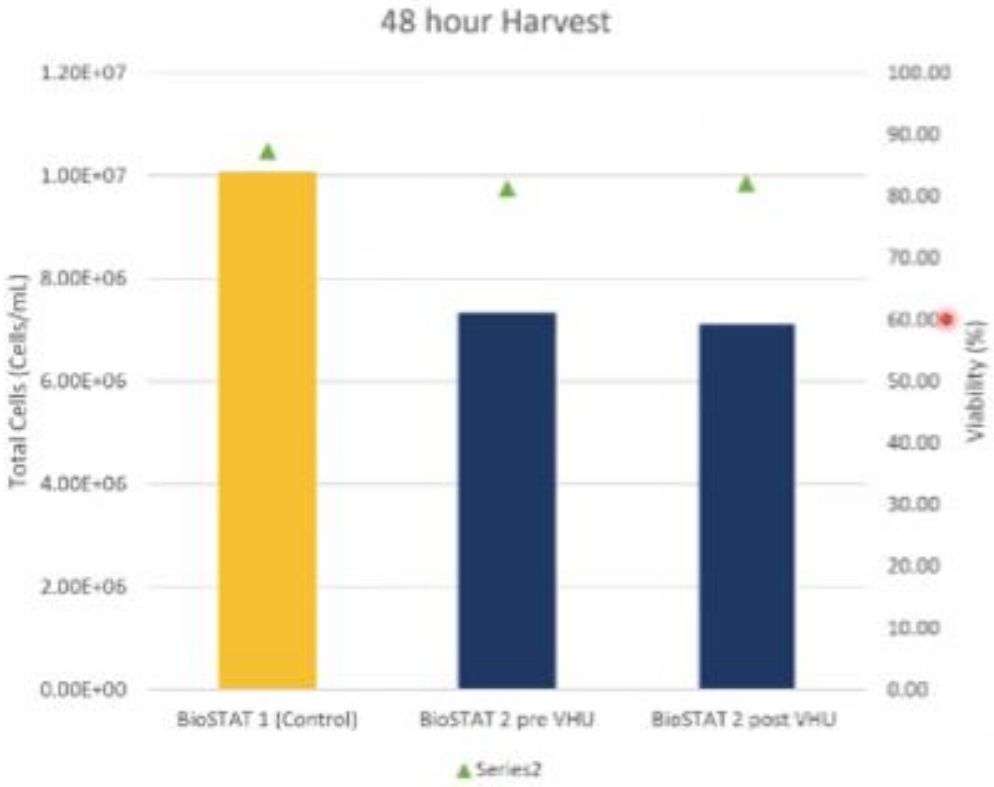
Case Study 2: TFF Perfusion with Transient Transfection

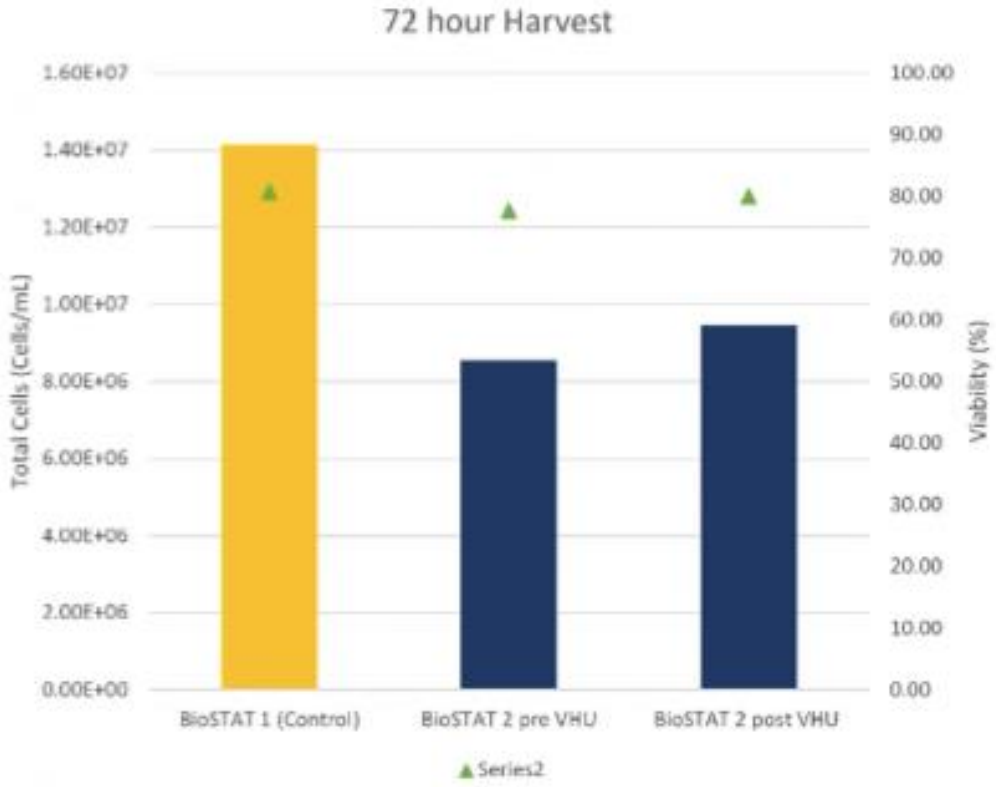


FM = Flow Meter

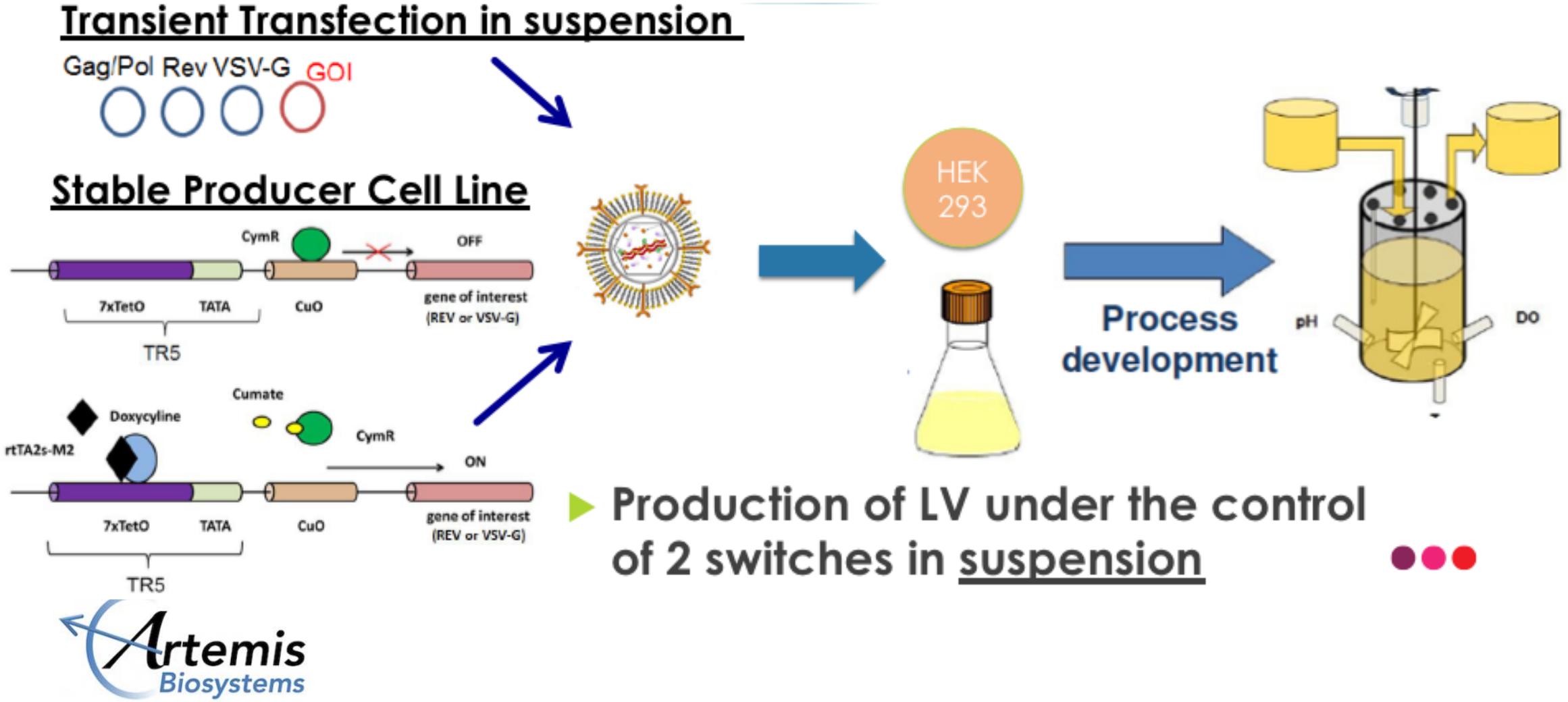
LV Perfusion with multiple LV harvesting

- 77.7% media exchange pre-transfection (1.5 x Bioreactor Volume).
- Harvest 1 x bioreactor volume 48h post TFX.
- Harvest 1 x bioreactor volume 72h post TFX.





Case Study 3: Stable Producer Cell Lines



- Production of LV under the control of 2 switches in suspension

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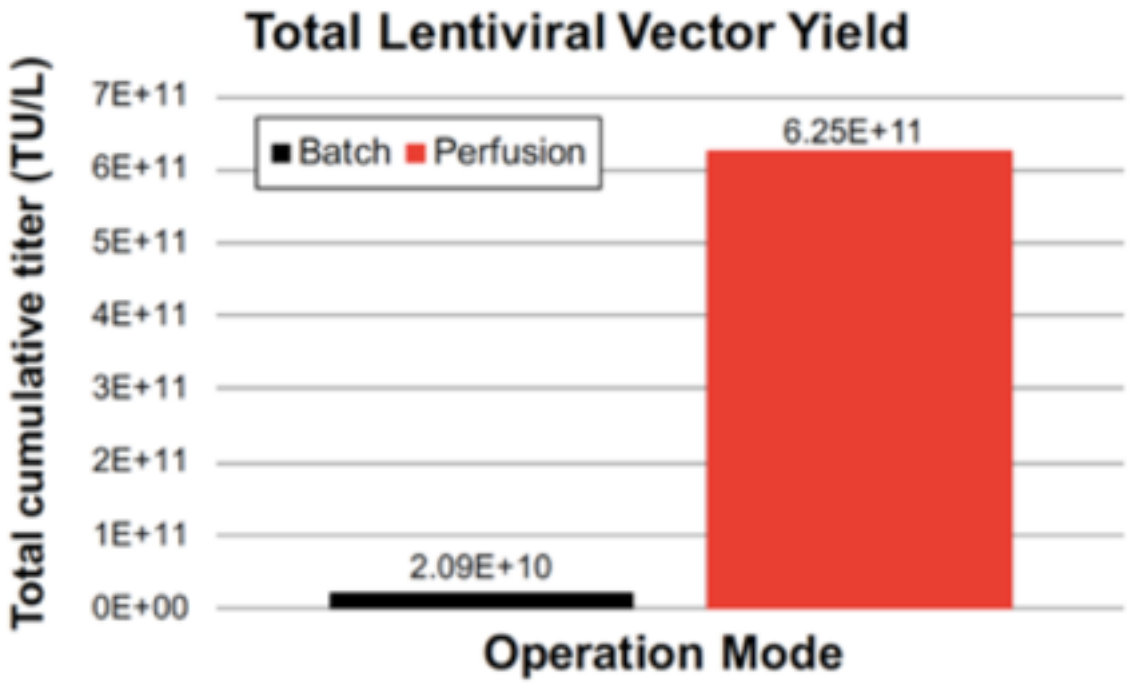
Increase capacity within existing facility

- The TFF-VHU acts as a yield multiplier, eg. a 50L perfusion is equivalent to a 500L batch
- Add capacity by simply adding a VHU perfusion module to your existing bioreactor
- Reduce the equipment footprint for the same total yield



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Lower Cost of Goods with VHU® Perfusion

Assumptions:

Annual supply of 10,000 doses of LV viral vector

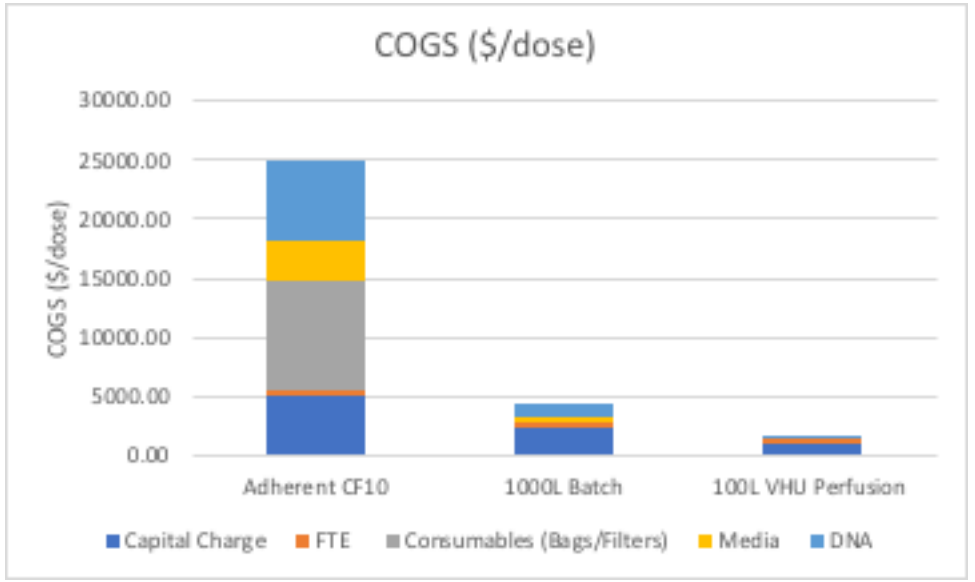
Outcome for VHU

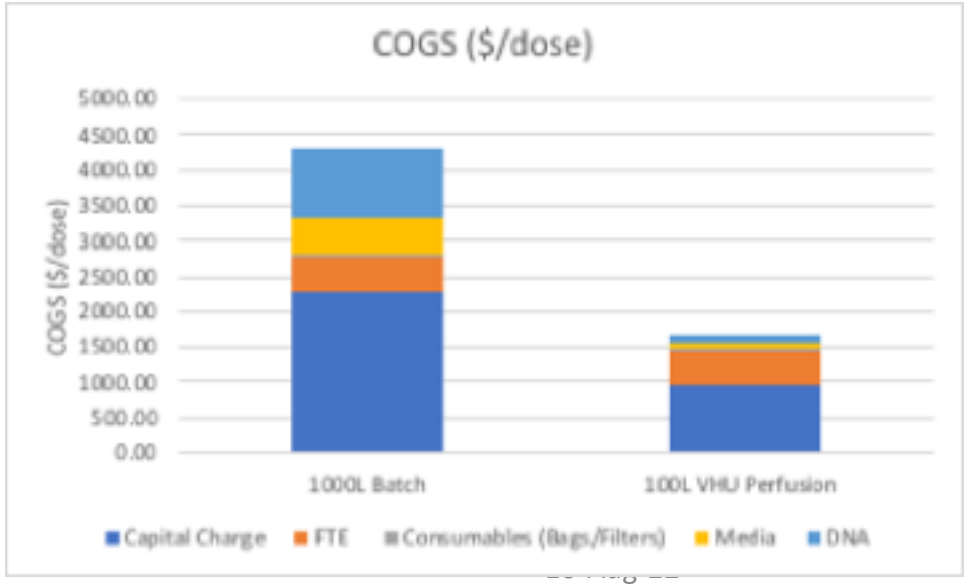
90% reduction in COGS versus adherent process and 50% reduction compared to suspension

90% reduction in consumables versus adherent plates which translates in a significant reduction of plastic disposal.

90% lower capital charge compared to adherent and 50% lower compared to suspension

>50% reduction in DNA costs





Take home message

- **VHU® Perfusion Process consisting of a macropore filter module and a low-shear Levitronix® pump to yield high titers of functional lentiviral vectors (6E11 TU/L)**

- **Scale up production to 1000L**

  -Robust Process

  -Productivity maintained throughout the scale-up

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Thank you!

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