

1-28-2019

# Tangential flow filtration and scalability in viral vector purification

Eni Sterjanaj

Heather Mall

Rachel Legmann

Jacky Dang

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# Tangential Flow Filtration and Scalability for Gene Therapy Virus Purification (AAV)

**Continuously Improving Bioprocesses**

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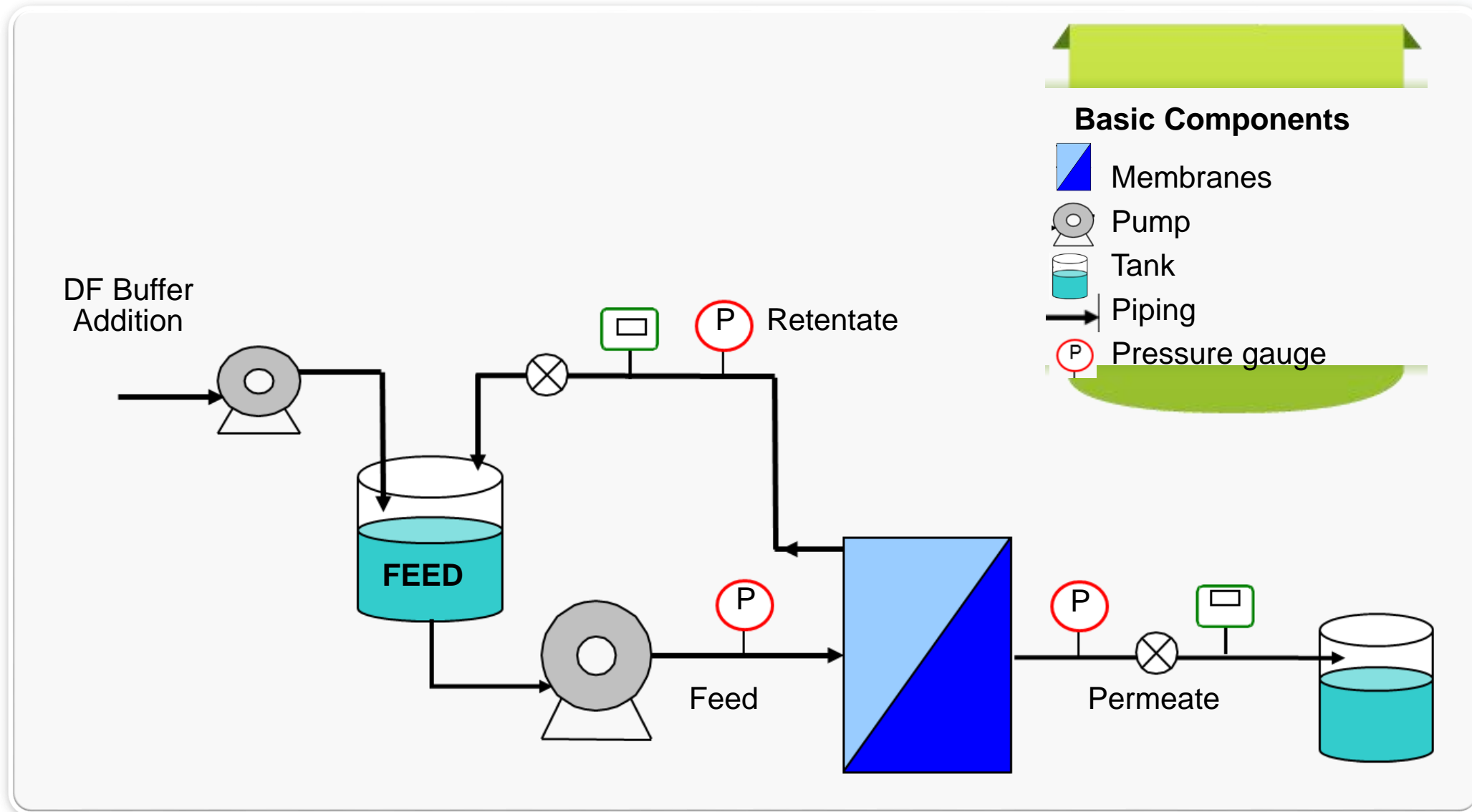
# Introduction

- Gene therapies are becoming more common, practical and effective
  - diabetes, hemophilia, cystic fibrosis, cancer, SMA, Huntington's,
- Advantages of AAV/ADV: easy to concentrate and high rates of infection
  - many cell types, dividing/non-dividing
- Downstream purification is a necessary tool in recovering final product
- Tangential flow filtration (TFF) clears low molecular weight impurities (i.e. less than filter retention rating) and buffer exchange is essential to stability

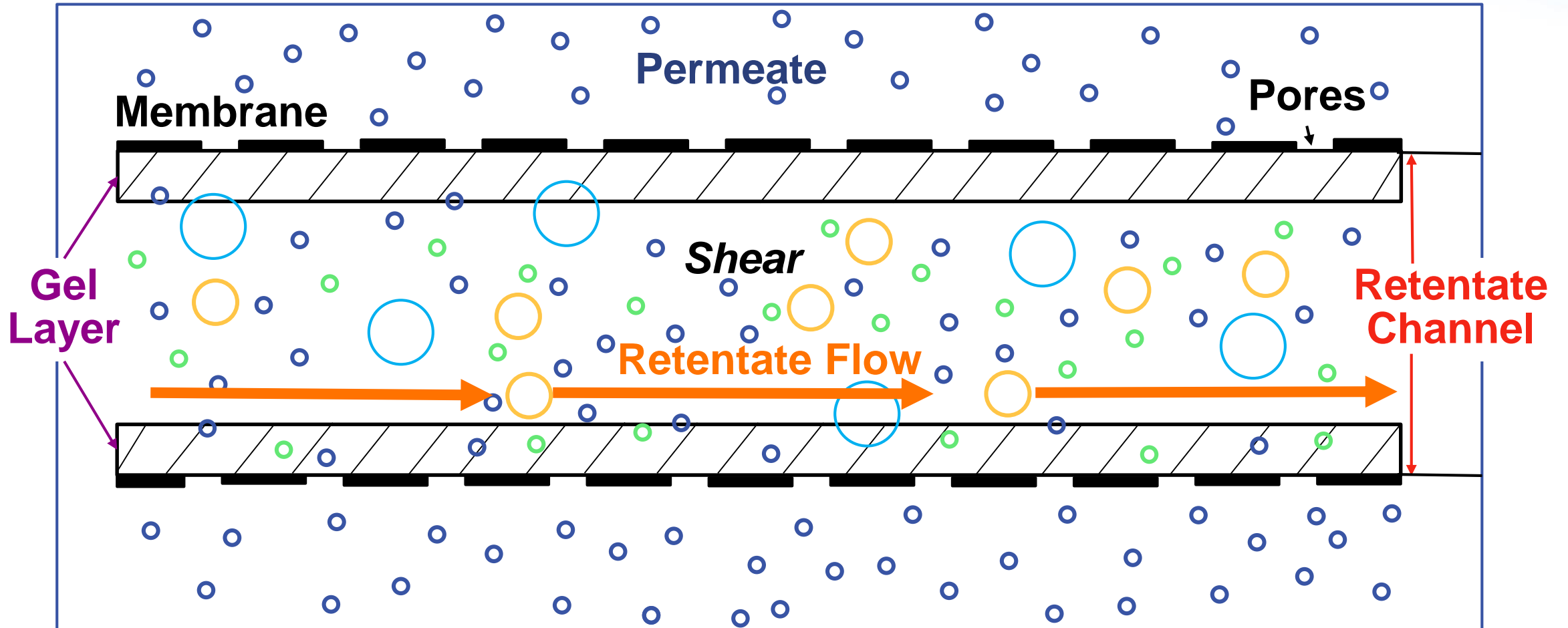
## Abstract

- Pall Biotech has linearly scalable tangential flow filtration (TFF) technology that can process volume ranges from less than 1 L to over 2000 L
- Programmable skids are available for industrial scale volumes
- In this presentation, TFF linear scalability of up to 200 L is covered starting from a 9 L initial volume and maintaining similar pressures, processing times and yields at all scales. Yield was upwards of 90% for both runs
- Using this scalable technology, Pall Biotech enables biologics companies to serve their target markets by going to clinical trials and to commercial scale manufacturing successfully

# Basic TFF System Components



# Close Up of TFF Flow Path



# Materials

- Cell culture harvest of AAV
- SupraCap100 V100P depth filter (2-4 microns)
- Stax with V100P media for large scale
- Manual TFF assembly (small scale TFF)
- 0.1 m<sup>2</sup> surface area, 300 kDa Pall Centramate™ TFF cassette
- 2.5 m<sup>2</sup> surface area, 300 kDa Pall Centrasette™ TFF cassette
- Allegro single-use TFF system (CS1000)
- Allegro single-use biocontainer

## Methods

- Cell culture material was clarified through a 2-4 micron pore size depth filter for large and small scale using V100P depth media. Depth filter was flushed with 25 mM Tris, 100 mM NaCl, pH 7.5 to maximize recovery
- Filtrate from the previous step was concentrated 10x in small (0.1m<sup>2</sup>) and large (2.5m<sup>2</sup>) scale. TFF set up and buffer exchanged 5x into 25 mM Tris, 100 mM NaCl, 0.005% Tween 20, pH 7.5
- Supor<sup>®</sup> EKV membrane Kleenpak<sup>™</sup> capsule was used for sterilizing grade filtration at the end of the TFF step
- SDS-Page was used to examine impurity clearance per process step
- qPCR was used to determine titer



# Large Scale Single-Use TFF

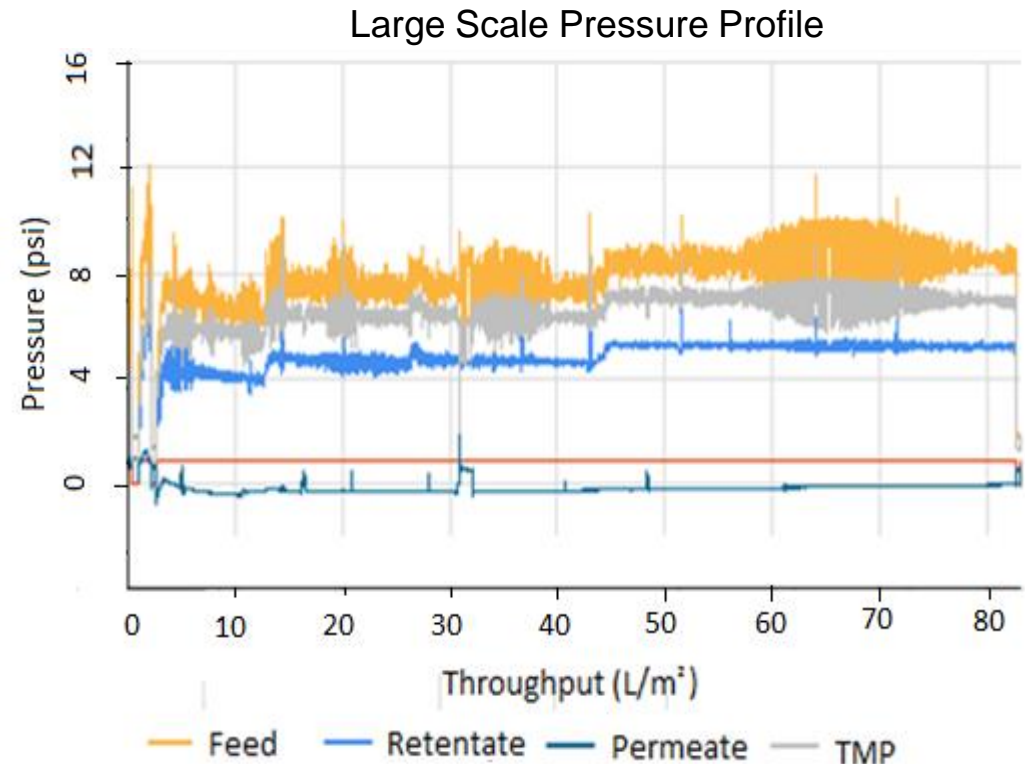
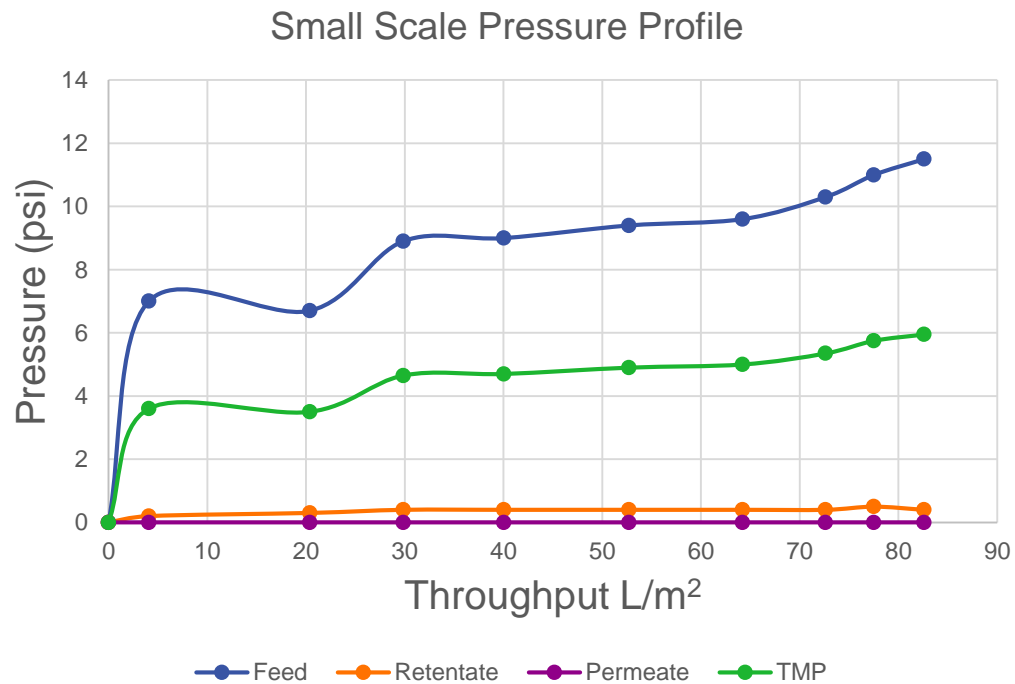
CS1000 System	
Tube ID	½ in.
Cassette area	0.5 – 2.5 m <sup>2</sup>
Recirc. Pump capacity	20 – 1000 L/hr
Typical batch volumes	500 L
Hold-up volume (volume in feed and retentate)	0.6 L
Operation	Automatic



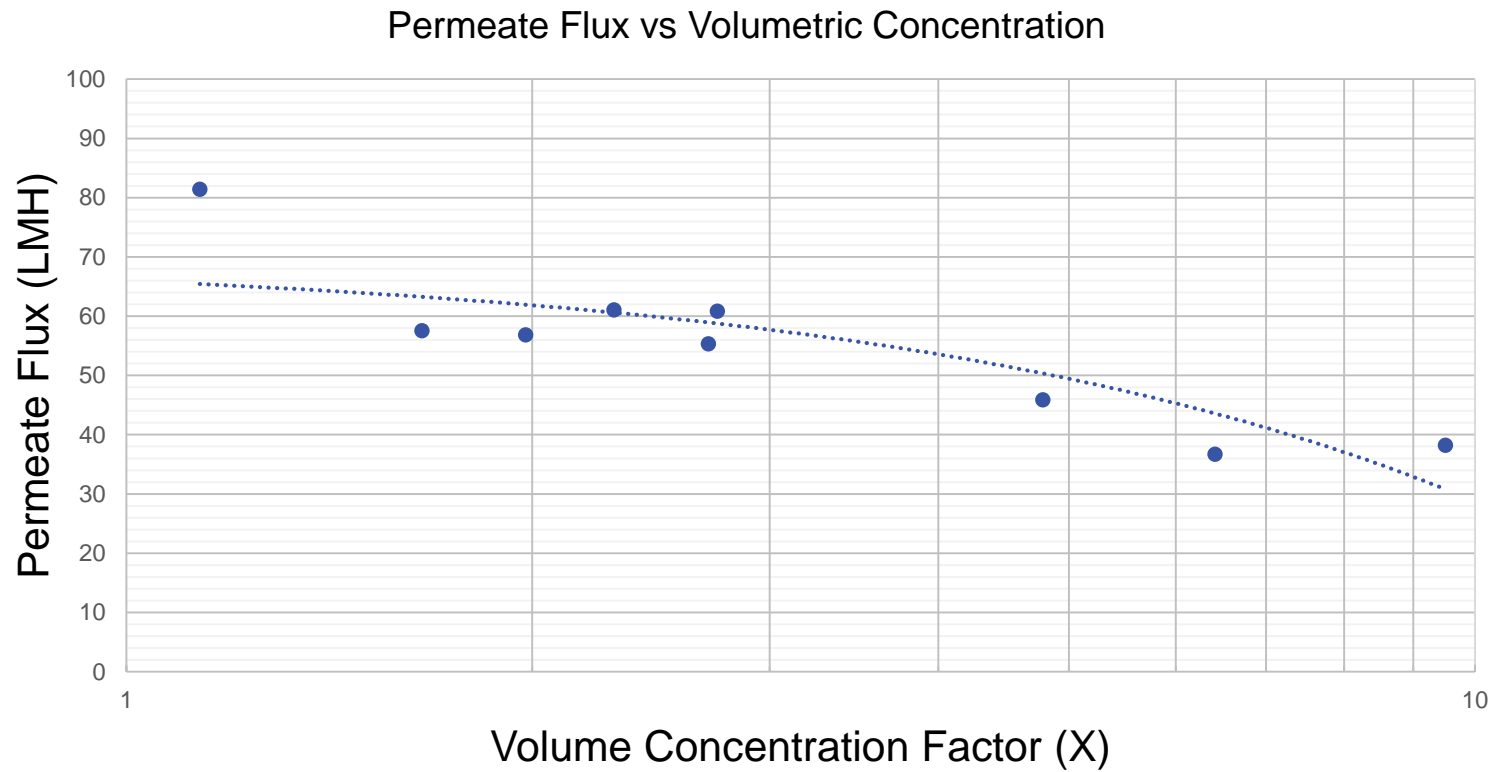
- Pall scale-up TFF systems processing range: 20 L to 800 L
- Manual assemblies are usually used to accommodate volumes below 20 L
- The CS1000 system used here
- Intuitive and easy to use user interface
- Methods are easily configurable and automated – alarms in place
- Pressure monitors, flow monitors, and conductivity monitors available to characterize process performance

# Pressure Profile for Small (0.1m<sup>2</sup>) and Large Scale (2.5m<sup>2</sup>) TFF Runs

- Transmembrane Pressure (TMP): 4-8 psi common
- Large and small scale TFF had similar pressure profiles
- Pressure limit for safe operation – 4 barg for reinforced manifold sections

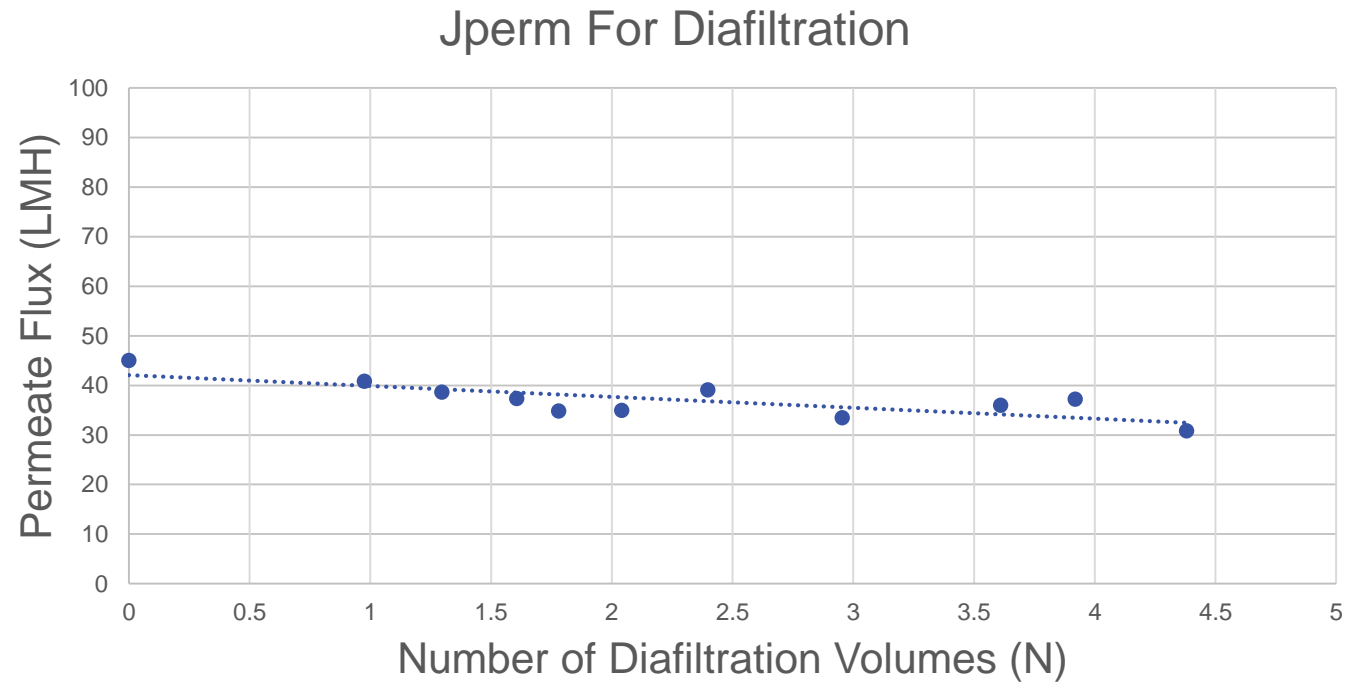


# Obtaining average permeate flux for small scale concentration



**Average permeate flux = 54.8 LMH**

# Obtaining average permeate flux for small scale diafiltration



**Average permeate flux = 40.7 LMH**

# Filter Sizing

- Permeate flux from small scale concentration and diafiltration used to predict filter area for large scale
- Processing time was kept similar for both scales
- Predicted area for large scale = 2.1 m<sup>2</sup>

$$A = \frac{1}{t_T} \left[ \frac{V_c}{J_c} + \frac{V_d}{J_d} \right]$$

$$A = \frac{1}{2.7} \left[ \frac{180}{54.8} + \frac{100}{40.7} \right]$$

A = 2.1 m<sup>2</sup> → 2.5 m<sup>2</sup> with ~20% safety factor

A = area

t<sub>T</sub> = total time

V<sub>c</sub> = volume processed during concentration

V<sub>d</sub> = volume processed during diafiltration

J<sub>c</sub> = avg. permeate flux during concentration

J<sub>d</sub> = avg. permeate flux during diafiltration

**Linear scale-up possible by extrapolating graph and deriving appropriate filter area**

# Small and Large Scale Process Variables Comparison

Process Parameters Table

Parameter	Small Scale	Large Scale
	(~9 L Clarified bulk)	(~200 L)
Processing time (concentration)	~1 h 30 m	~1 h 15 m
Processing time (buffer exchange)	~1 h 10 m	~1 h 5 m
Feed flux (concentration)	500 mL/min (300 LMH)	12.5 L/min (300 LMH)
Feed flux (buffer exchange)	500 mL/min (300 LMH)	12.5 L/min (300 LMH)
AVG Permeate flux (concentration)	54 LMH	60 LMH
AVG Permeate flow rate (buffer exchange)	40 LMH	38 LMH
Membrane surface area	0.1 m <sup>2</sup>	2.5 m <sup>2</sup>
Volume processed	9 L	~200 L
Titer (yield by qPCR)	99%	91%
Flow decay	~ 50%	NA

- Comparable processing times at both scales
- Similar yield at both scales (within assay variability range)

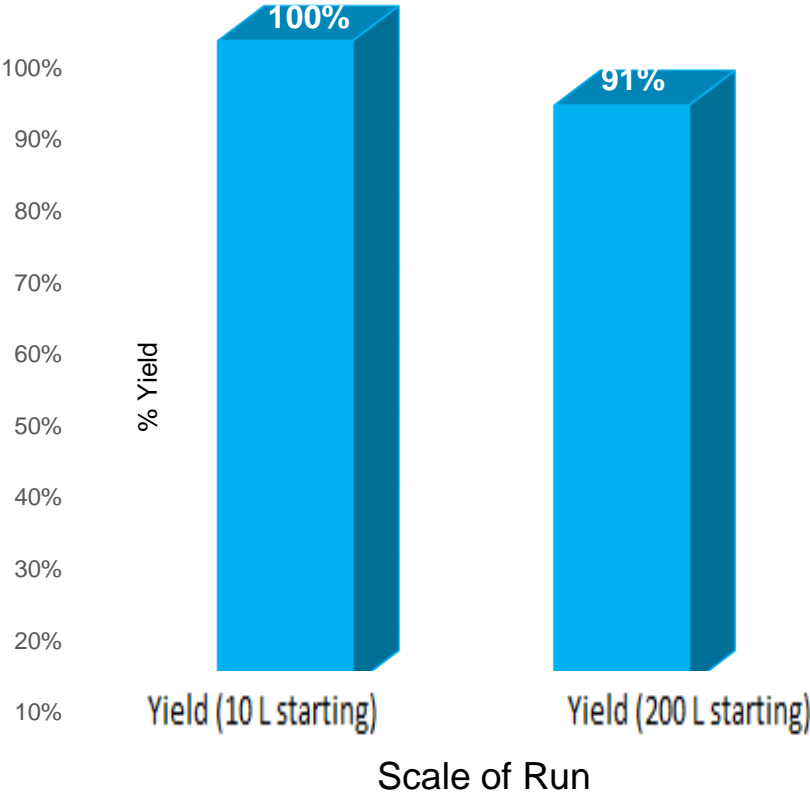
LMH = liters/meter<sup>2</sup>/ hour

LMM = liters/minute/ meter<sup>2</sup>

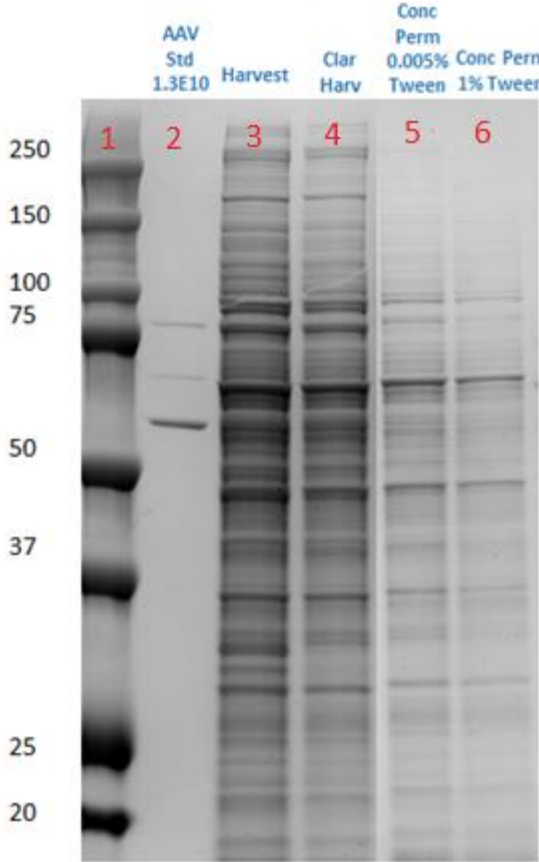
gc/cm<sup>2</sup> = gene copies per centimeter<sup>2</sup>

# Virus Recovery for Small and Large Scale and impurity clearance for small scale

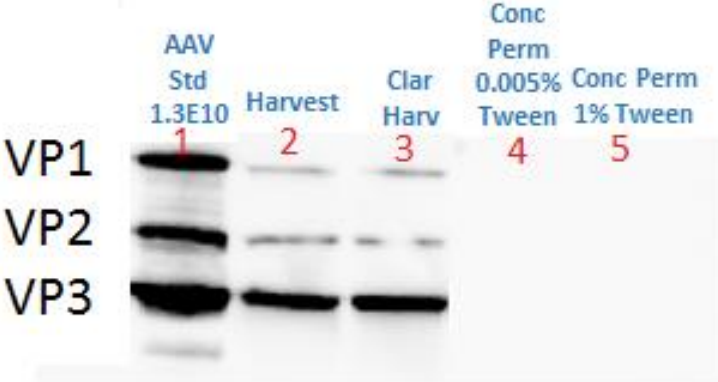
Process Yield for Small and Large Scale



SDS-page for starting load and permeate in small scale



Western for starting load and permeate in small scale



Recovery is in the 90% range for small and large scale TFF as tested by qPCR



# Pall Virus Hardware Platform



**Adherent seed train:**  
Xpansion multiplate bioreactor



**Adherent bioreactor:**  
iCELLis 500 bioreactor



**Clarification:**  
Stax depth filter systems



**Purification:**  
Allegro single-use chromatography system



**Concentration:**  
Allegro single-use tangential  
flow filtration systems



**Suspension seed train and bioreactor:**  
Allegro STR 200, 1000 and  
2000 L bioreactors



**Media/buffer mixing:**  
Allegro 50L mixer, Mag Mixer



**Media/buffer storage/handling:**  
Allegro plastic/stainless steel totes



**Sterile filtration:**  
Allegro MVP single-use system



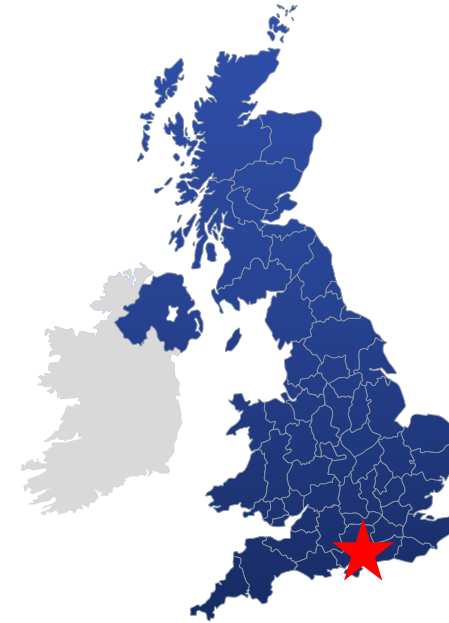
# Conclusion

- TFF linear scalability from 9L to 200 L was successfully demonstrated.
- Pressures, processing times and yields at both scales were kept in the same range.
- Pall has scalable TFF solutions for gene therapy application as well as other biologics.

# Process Development Services (PDS): UK and US Development Laboratories

## Portsmouth, United Kingdom

- 200 m<sup>2</sup> of configurable BL2-grade suites
- Newly designed flexible laboratories
- Equipped with Pall systems for suspension and adherent culture
- Latest innovations in Pall technology for large scale downstream processing (DSP)
- Advanced analytics on site including Q-TOF, H-Class Bio UPLC and Pall's ForteBio Octet<sup>®</sup> systems

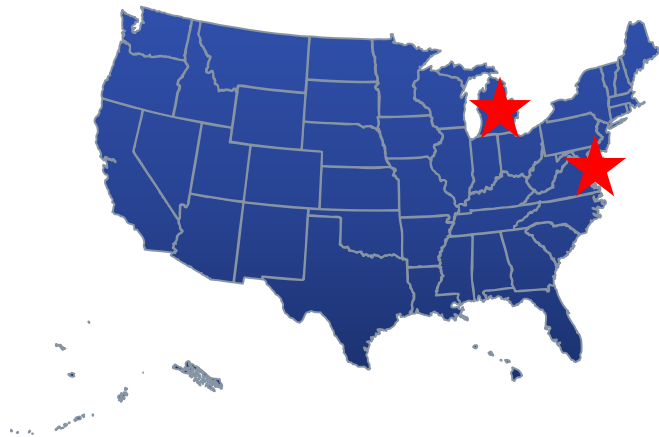


## Westborough, Massachusetts, USA

- 500 m<sup>2</sup> of configurable laboratory space
- Class 10,000 cleanrooms and BL2-grade suites
- Newly designed laboratories with a focus on recombinant protein and cell therapy processes
- Advanced analytics on site including qPCR, Flow Cytometry, HPLC and Pall's ForteBio Octet<sup>®</sup> systems

## Ann Arbor, Michigan, USA

- 200 m<sup>2</sup> of laboratory space
- Three rooms for BL-2 support
- Focus on adherent microcarrier process development and training on techniques



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**Thank You**

**Poster # 62**

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