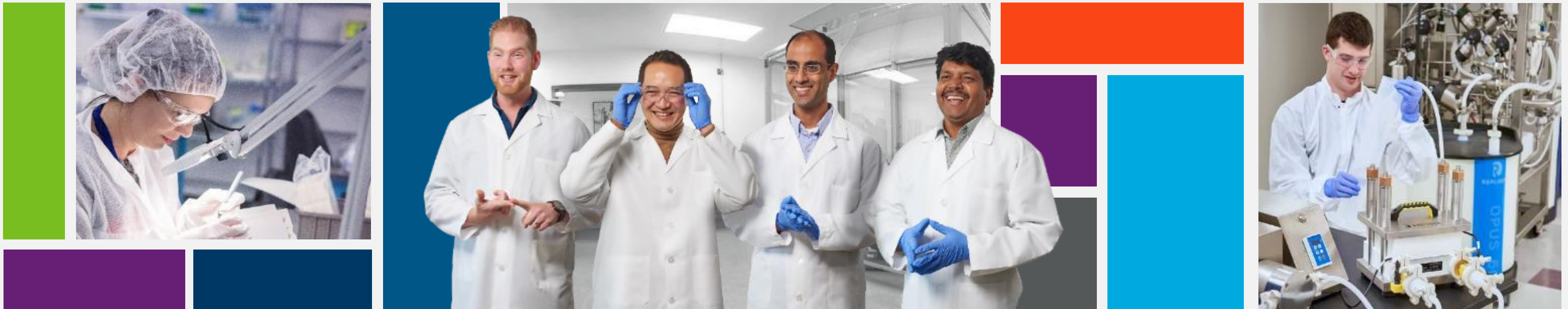


600+ LMH Single-Use Clarification for CHO and Viral Vector Applications by TFDF



October 8th, 2019



Repligen

Transforming bioprocessing

First to market with responsive solutions enabling high productivity and efficiency

OPUS® R
Pre-packed Columns



XCell™ ATF
Single-use Systems



KrosFlo® Integrated
Single-use TFF Skids



TangenX™ Single-use
Flat Sheet TFF Cassette



C Technologies
Spectroscopy Analytics



High-impact technology innovation

Agenda



TFDF™ Technology

How it Works

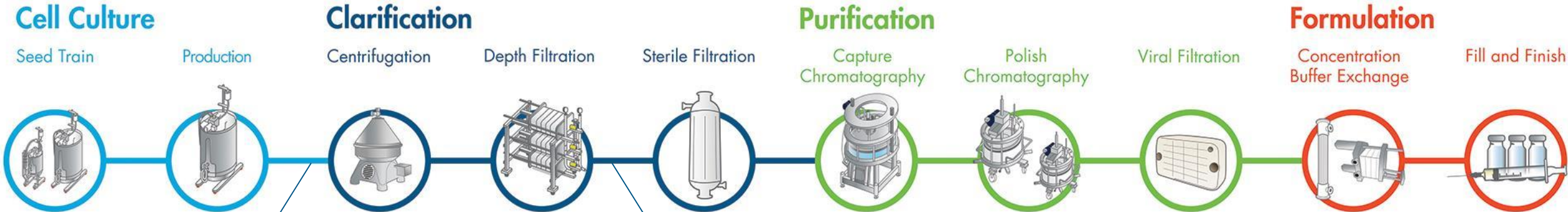
Application Data

Hardware, Software and Flow Paths

Clarification technology for bioprocessing

Technology	Advantages	Disadvantages
Centrifugation	proven technology at low volumes can handle high solids economical at low scales closed system	high maintenance expense sensitive to capacity sensitive to particle size/ density incomplete separation limited suitable machines-high capital cost at scale up
Dead-end Filtration	proven technology at low volumes low capital cost wide range of pore sizes wide range of filter products suited for dilute solutions closed system	sensitive to capacity sensitive to solids concentration expensive to scale up difficulty to collect retentate
TFF	proven technology insensitive to capacity insensitive to solids concentration wide range of cut-offs wide range of filter products suited for dilute solutions closed system	Limited to solids concentration (~50vol%) high yield may require dilution of feed requires periodic cleaning

Clarification technology for bioprocessing



Eliminate centrifugation

OR



Reduce depth filter requirements

- **Smaller footprint**
- **Simpler components**
- **Increased capacity**
- **Faster flux**

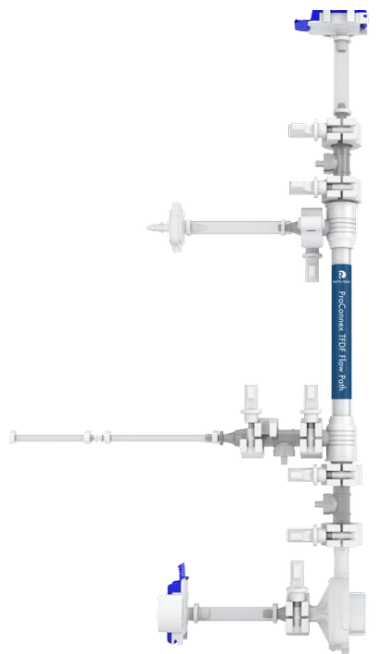
TFDF™
Technology is an
integrated
solution

TFDF™ Depth Filter



Tubular depth filter
Scales from 1 to 2000L

ProConnex® TFDF™ Flow Path



**Closed, Gamma-irradiated,
customizable configurations
complete with sensors**

KrosFlo® TFDF™ System

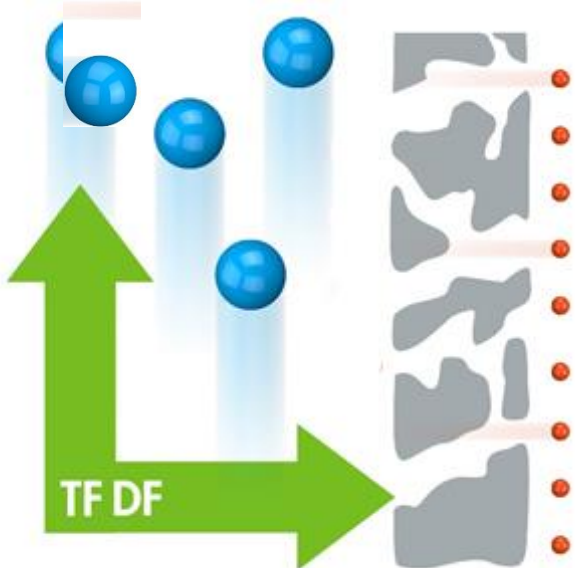
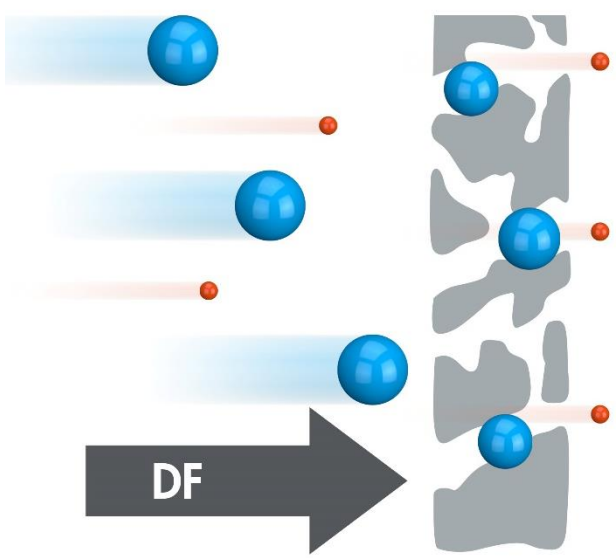
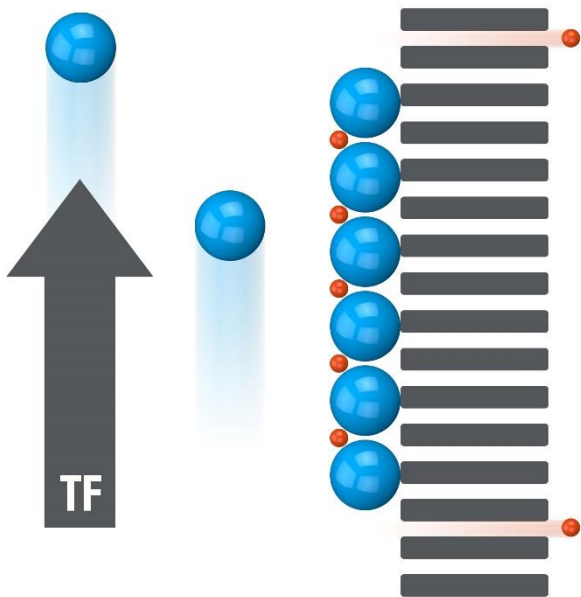


**Automated process control logic
from lab to process**

Filter, flow path and system work together as one solution

TFDF™
Technology
combines
benefits of
tangential flow
and depth
filtration

● cells and cell debris ● protein product



Tangential Flow

+

Depth Filtration

=

**Tangential Flow
Depth Filtration**

+++ Cell Density
 + Product Transmission

+ Cell Density
 +++ Product Transmission

+++ Cell Density
 +++ Product Transmission

TFDF™ Technology processes high cell densities with high product transmission

Synergy brings unprecedented benefits

Tangential flow through the lumen

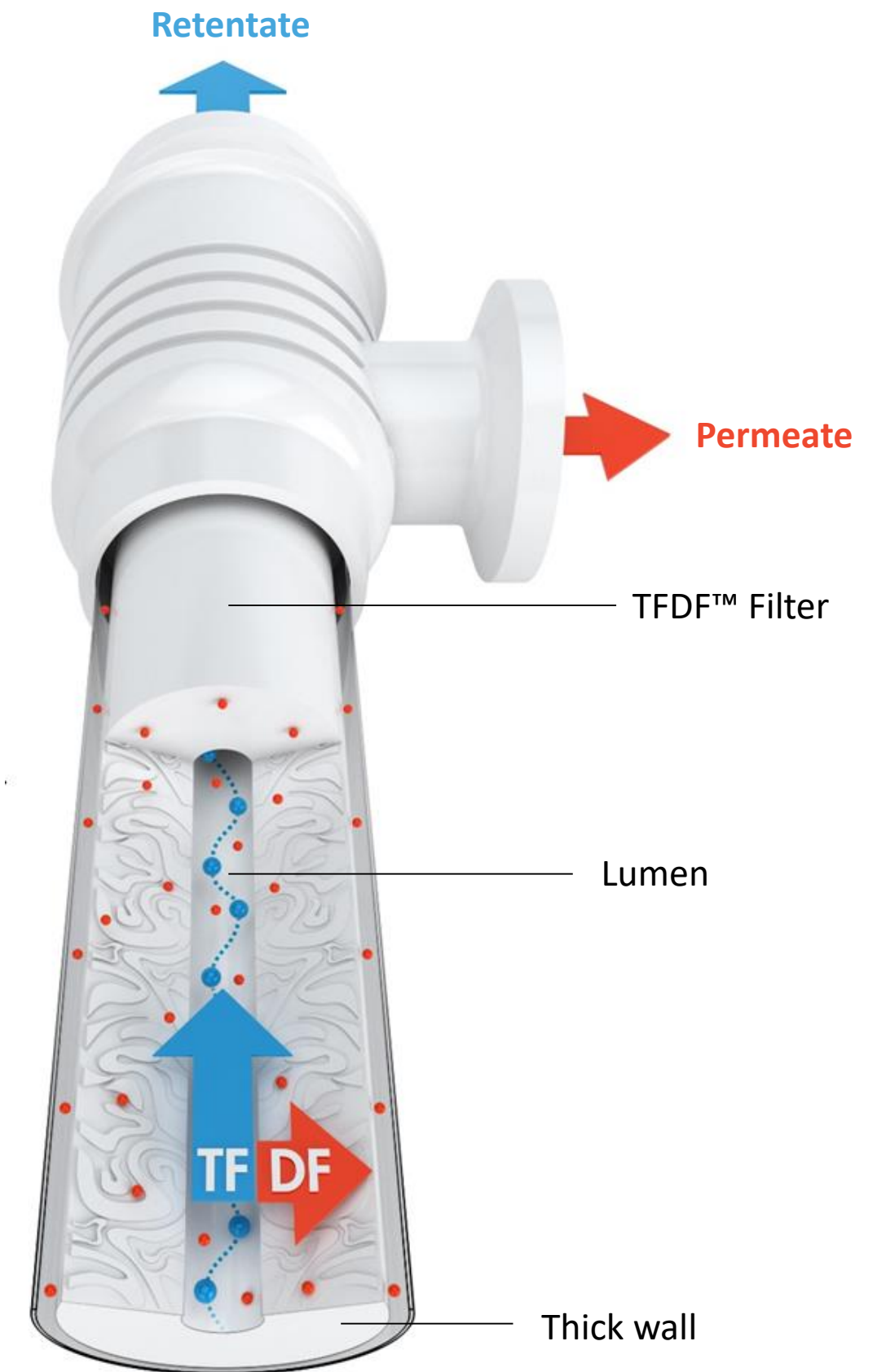
- Low concentration polarization minimizes fouling and turbidity breakthrough
- Robustness for challenging feed stocks

Thick wall depth filter

- High filtration capacity
- Short, highly interconnected paths do not retain product

Synergistic Benefits

- High flux > 1000 LMH
- Low hold-up volume
- Small footprint
- Simple setup and disposal
- Scalable from 1-2000 L
- Process in 3-6 hours





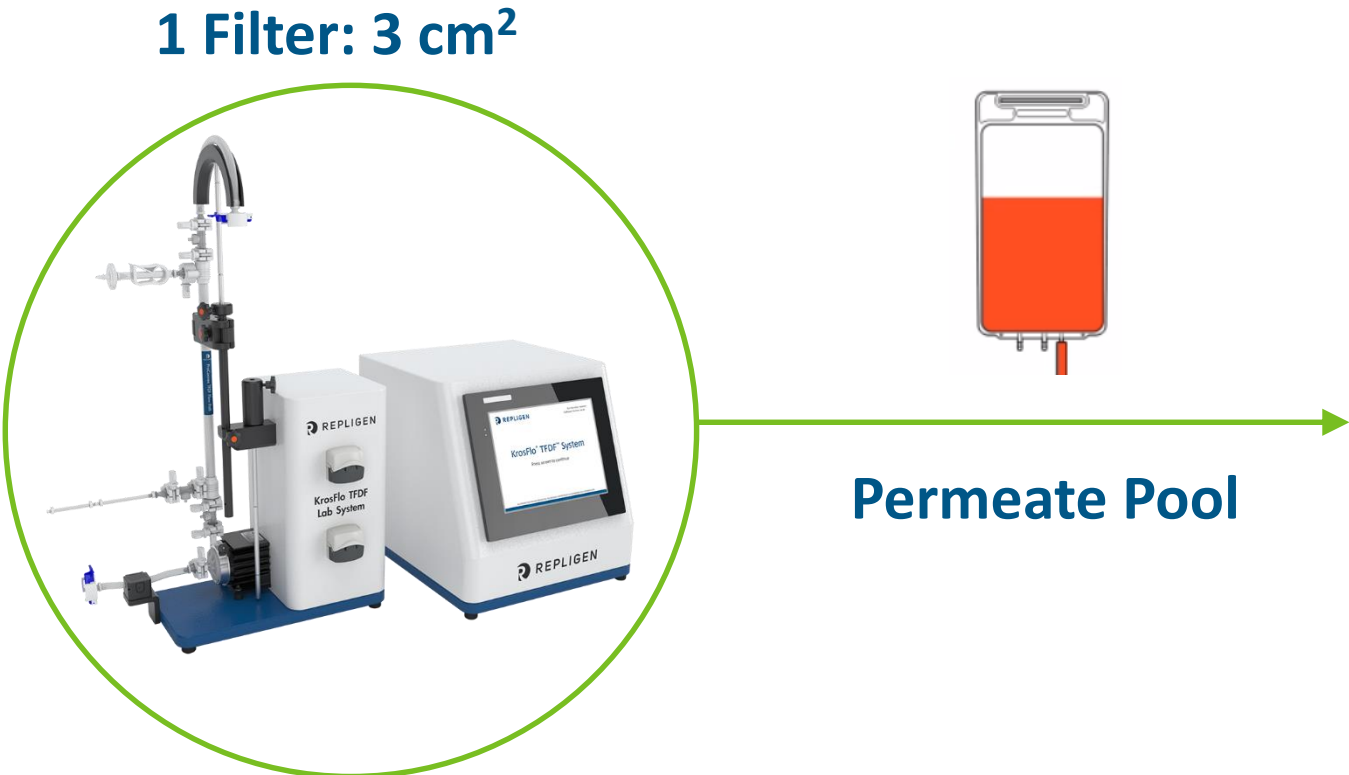
REPLIGEN

INSPIRING ADVANCES IN BIOPROCESSING

Application Data



Rapid clarification at 650 LMH with high yield



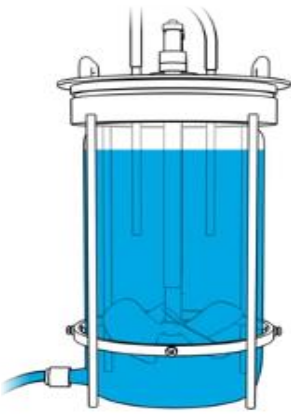
1 Filter: 3 cm²

Cell Culture Feed

Pool Volume	1.2 L
Process Time	6 hours
Yield	> 90%
Turbidity	88 NTU

**Flux 650 LMH
92% NTU reduction**

Volume	1.0 L
%PCV	6.6%
VCC	2.5 x 10 ⁷
Viability	80%
Titer	3.0 g/L
Turbidity	1196 NTU



OR



Increase from 650 to 1300 LMH maintains low turbidity

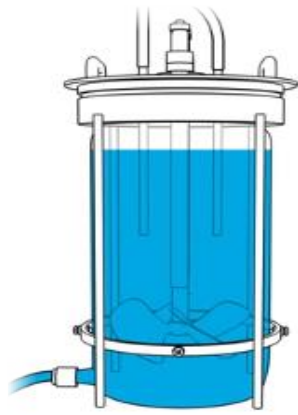
1 Filter: 3 cm²



Pool Volume	1.2 L
Process Time	3 hours
Yield	> 90%
Turbidity	94 NTU

Cell Culture Feed

Volume	1.0 L
%PCV	6.6%
VCC	2.5 x 10 ⁷
Viability	80%
Titer	3.0 g/L
Turbidity	1182 NTU



Flux 1300 LMH
91% NTU reduction



OR



Scalable performance to 1950 LMH

1 Filter: 3 cm²

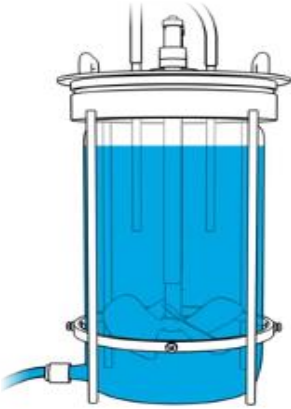


Permeate Pool

Pool Volume	1.2 L
Process Time	1.5 hours
Yield	> 90%
Turbidity	101 NTU

Cell Culture Feed

Volume	1.0 L
%PCV	6.6%
VCC	2.5 x 10 ⁷
Viability	80%
Titer	3.0 g/L
Turbidity	1182



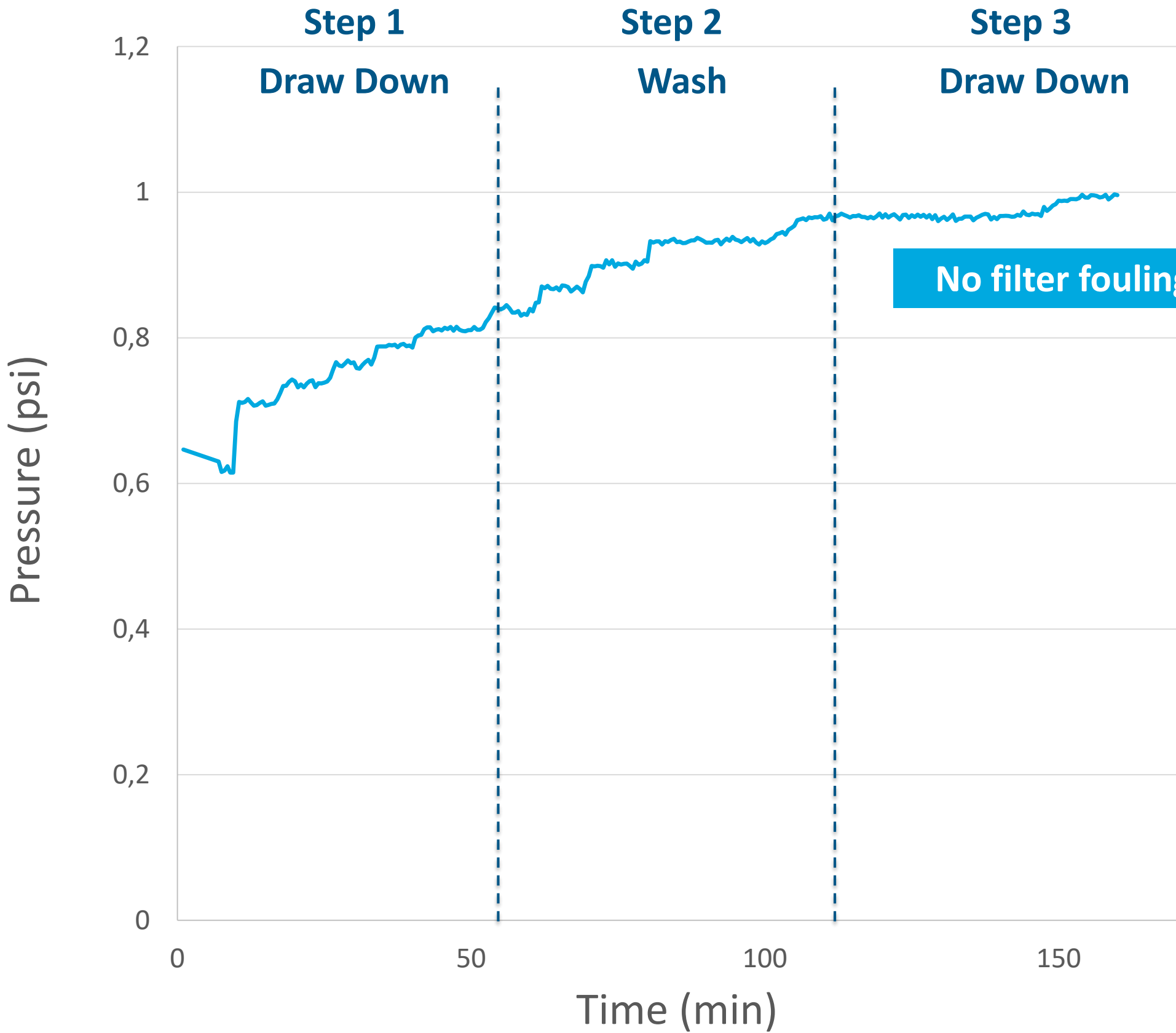
Flux **1950** LMH
91% NTU reduction



OR



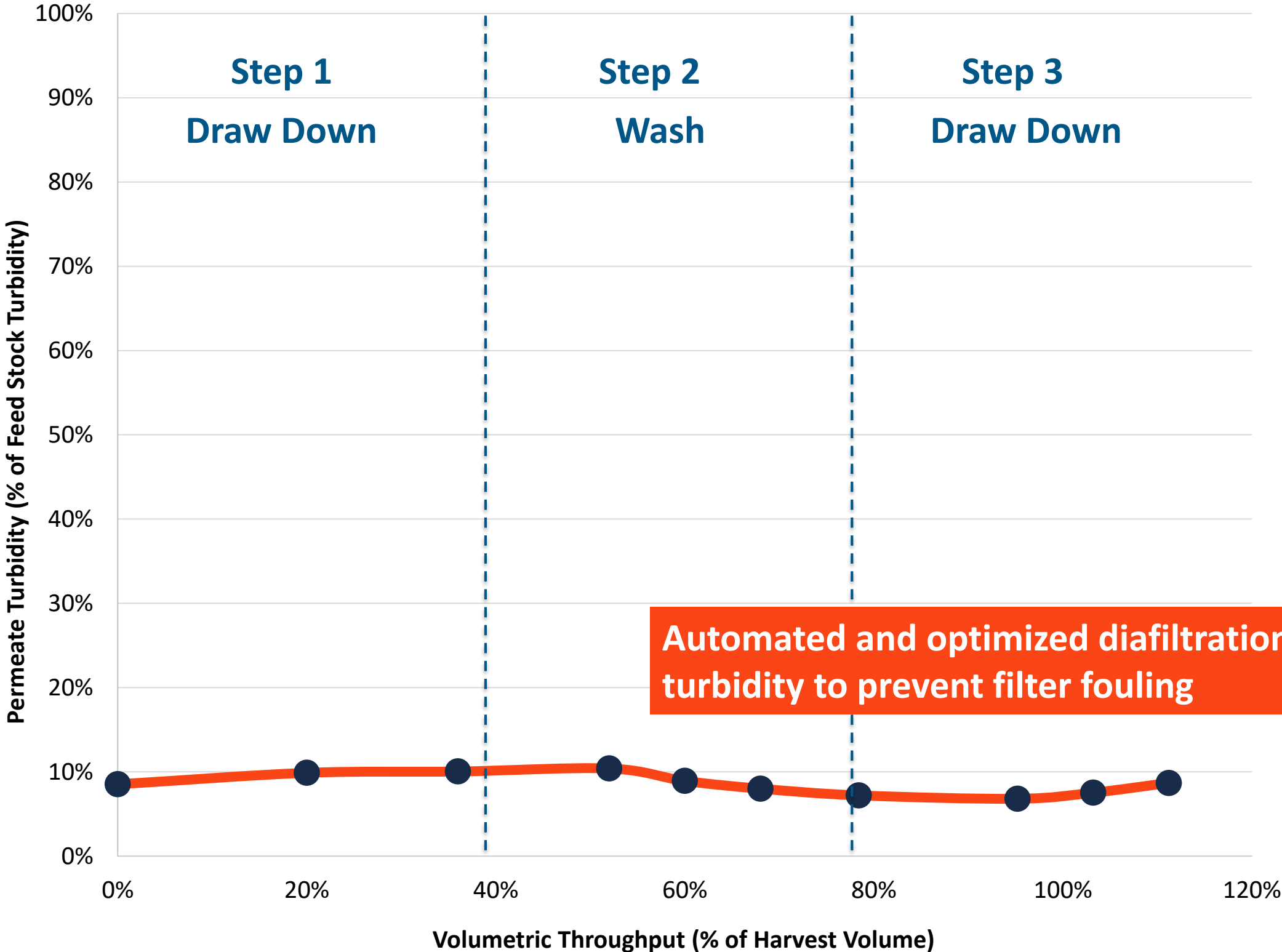
Low TMP with high throughput



No filter fouling with high LMH

Volume	1 L
%PCV	6.8%
VCC	2.3×10^7
Viability	92%
Titer	5.9 g/L
CC Feed Turbidity	954 NTU
Permeate Pool Turbidity	92 NTU
Flux	1300 LMH

Diafiltration optimization prevents turbidity breakthrough



Automated and optimized diafiltration maintains low turbidity to prevent filter fouling

Performance scales to 500 L with comparable LMH and NTU



Permeate Pool

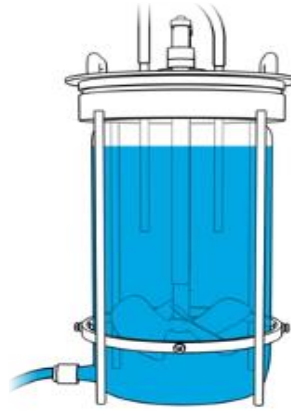


Pool Volume	600 L
Process Time	6 hours
Yield	> 95%

Flux **650** LMH
30 NTU

Cell Culture

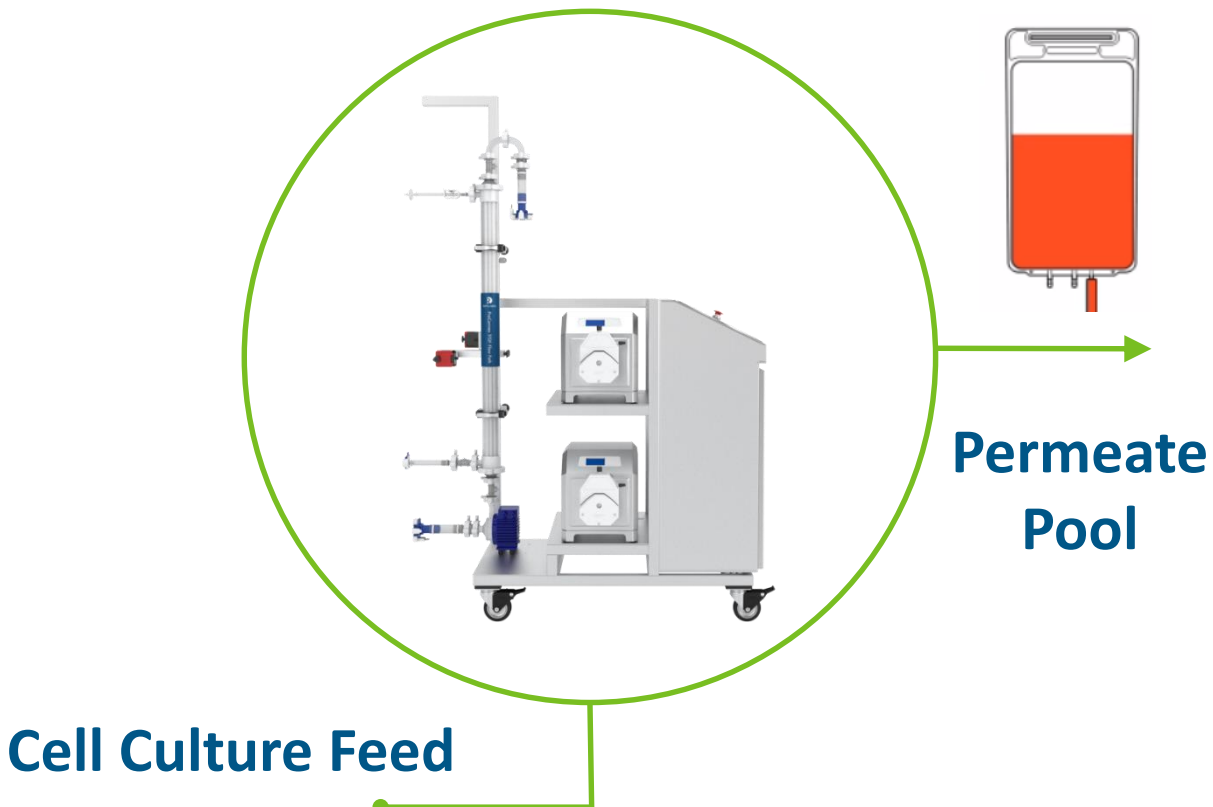
Volume	500 L
%PCV	7 %
VCC	1.5×10^7
Viability	80%



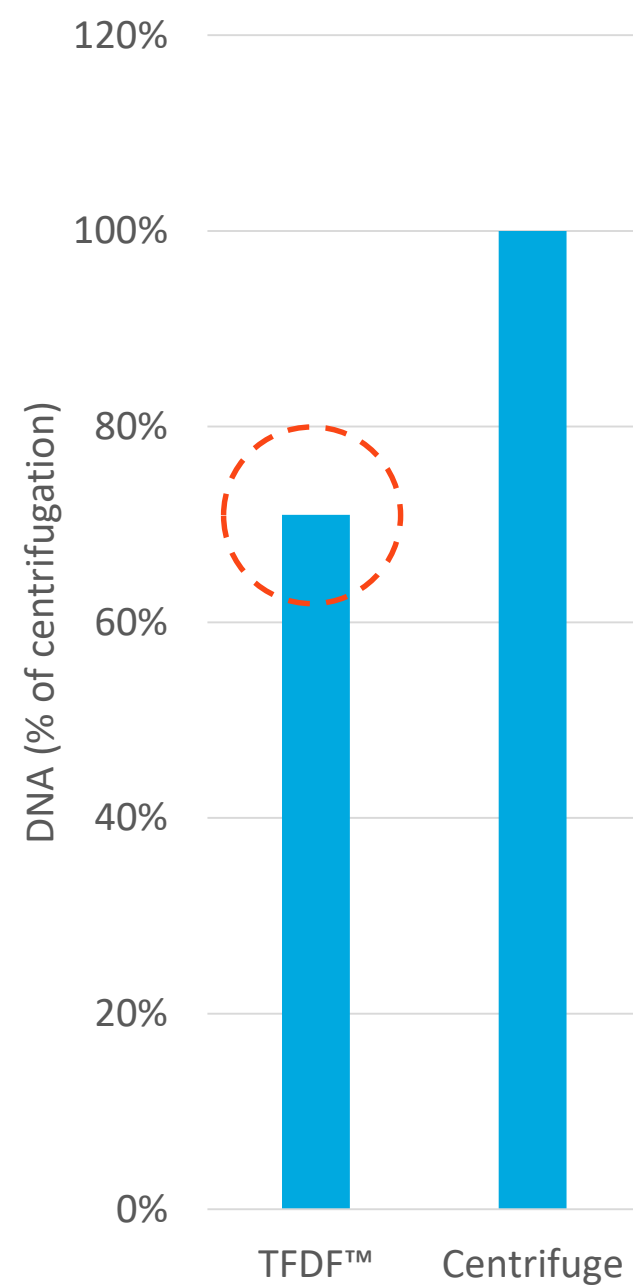
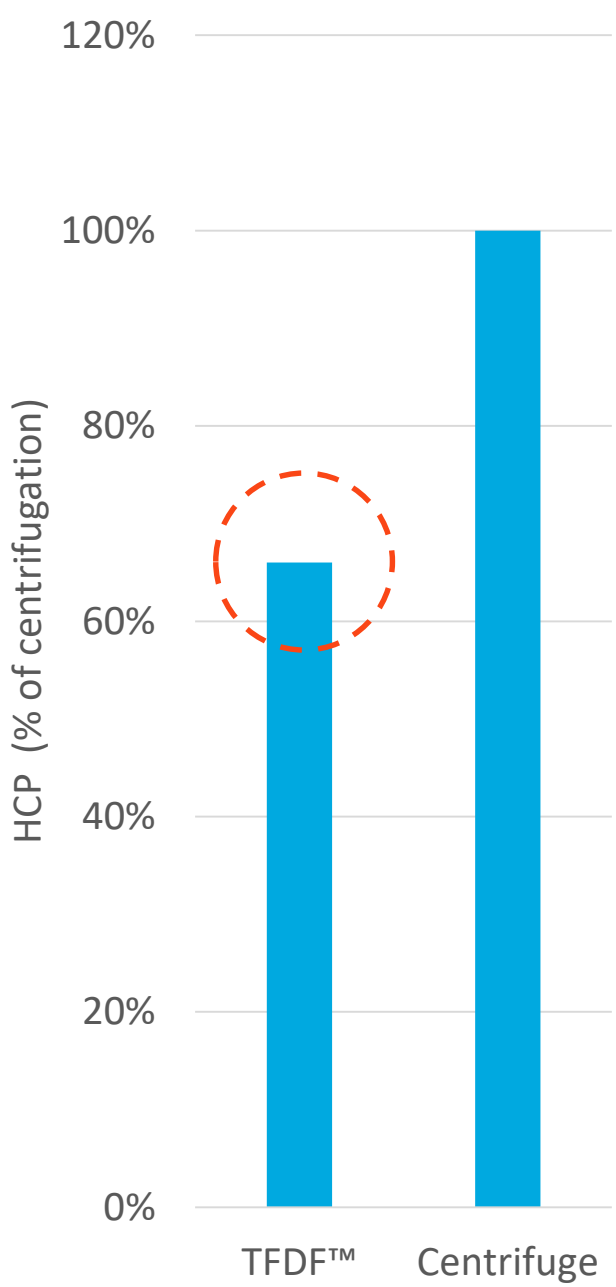
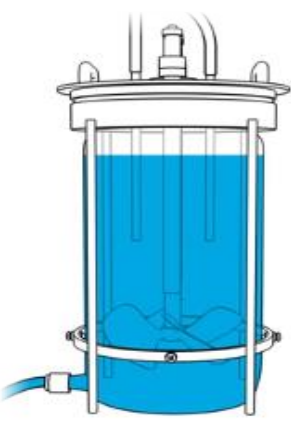
OR



Impurity profile comparable to centrifugation



Volume	500 L
%PCV	7 %
VCC	1.5×10^7
Viability	80%



Comparable HCP and DNA levels after TFDF™ and clarification

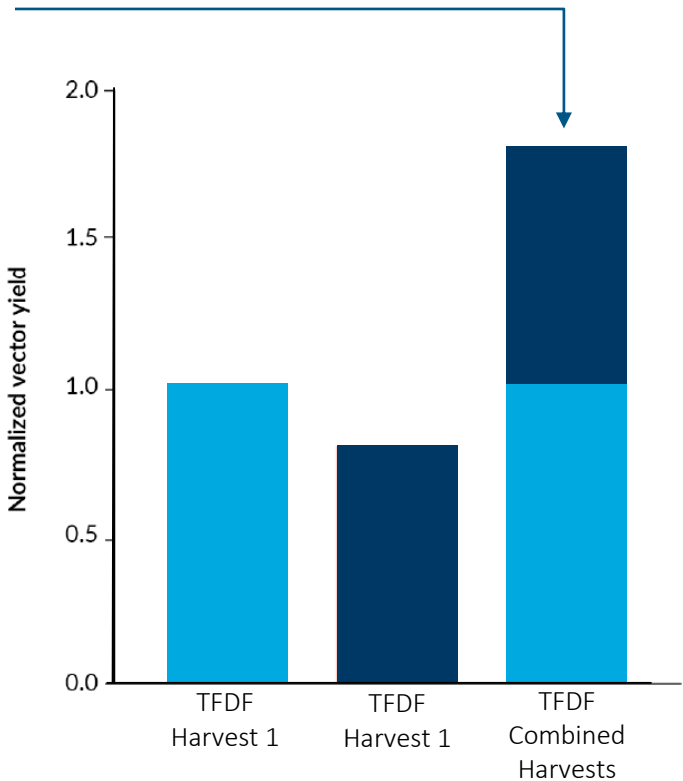
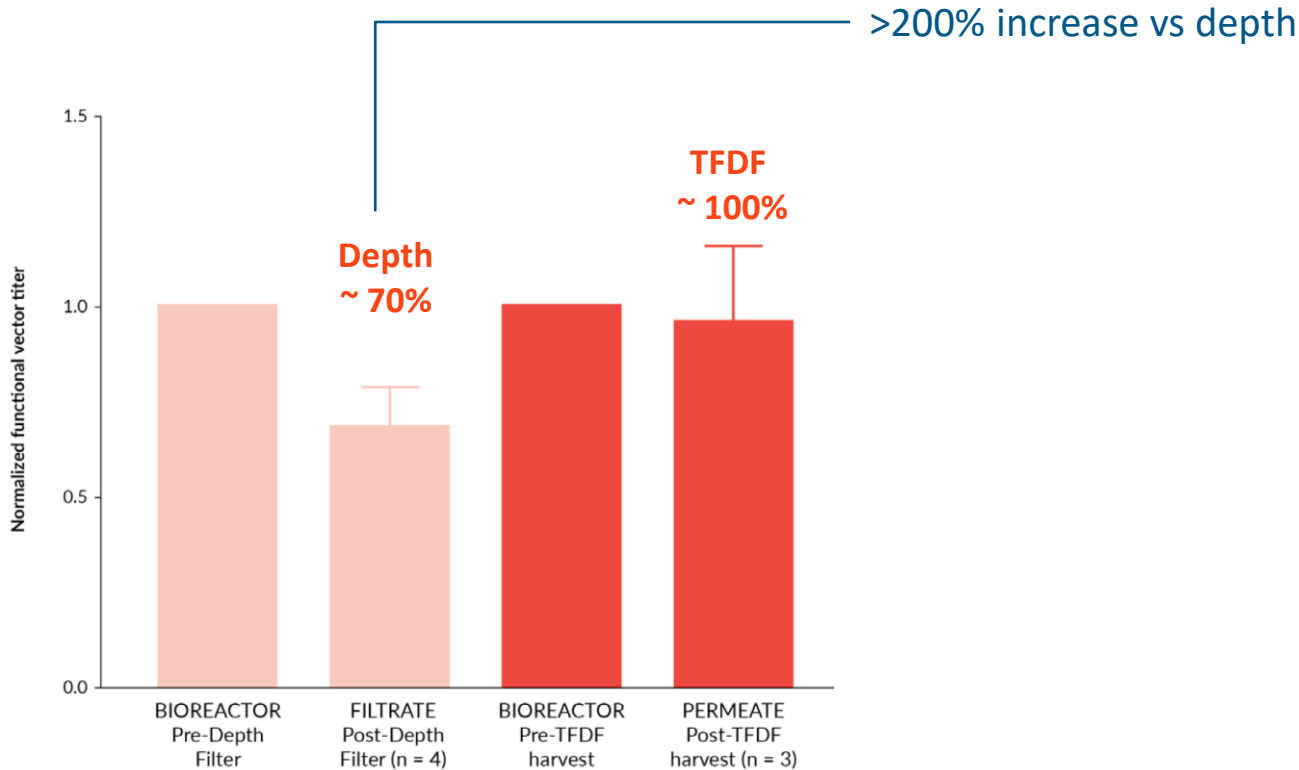
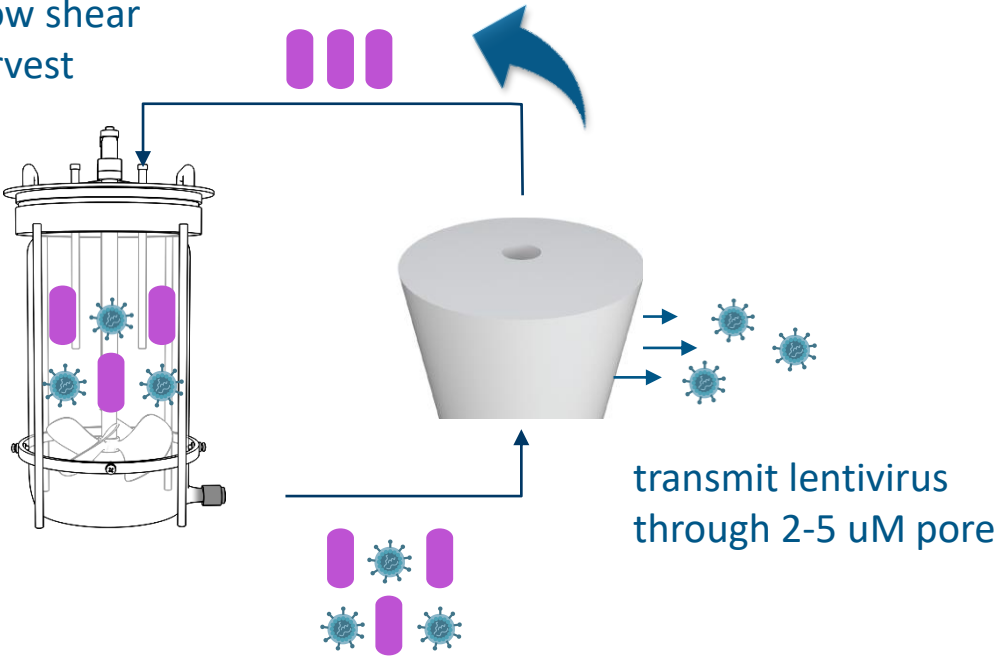
> 200% viral vector yield enhancement with multi-harvest TFDF based method

EXPERT INSIGHT

Lentiviral vector manufacturing process enhancement utilizing TFDF™ technology

Thomas Williams, Oliver Goodyear, Lee Davies, Carol Knevelman, Michael Bransby, Kyriacos Mitrophanous & James Miskin

tubular format, low shear enables multi-harvest



- TFDF™ filter successfully transmits lentivirus
- TFDF ~ 100% yield vs 70% Depth

- Low shear TFDF™ preserves host cells
- Enables multi-harvest with > 200% increase vs depth

CELL & GENE THERAPY INSIGHTS
 VIRAL VECTOR BIOPROCESSING & ANALYTICS:
 TODAY'S KEY TOOLS AND INNOVATION
 REQUIREMENTS TO MEET FUTURE DEMAND



TFDF™ Technology



Integrated hardware, software and flow paths

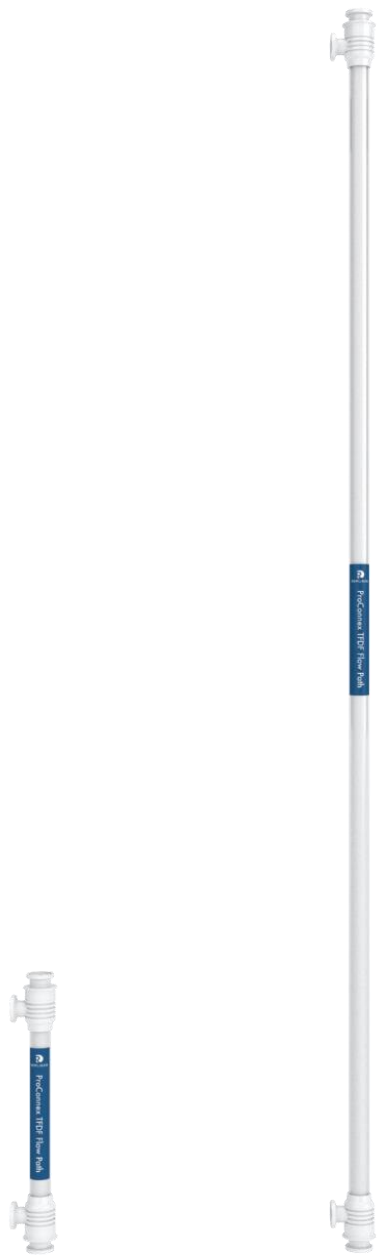
Filter capacity increases with tube count and length

Tube length

Tube count



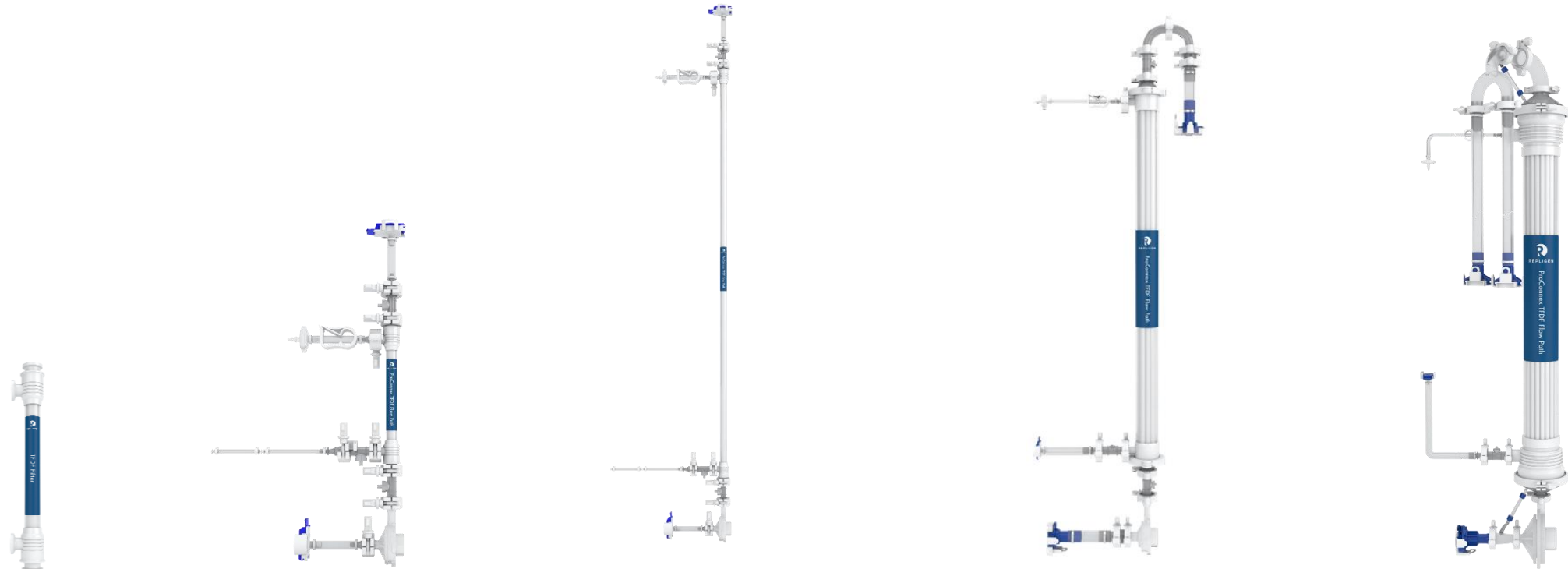
Number of tubes	1	1	10	40
Surface area (cm ²)	3	150	1500	6000
Recommended processing volume (L)	< 1	< 50	< 500	< 2000



Physical length (cm)	20	108
Effective length (cm)	2	108
Surface area (cm ²)	3	150

ProConnex[®] TFDF[™] Flow Paths

Complete, ready-to-use TFDF[™] flow paths

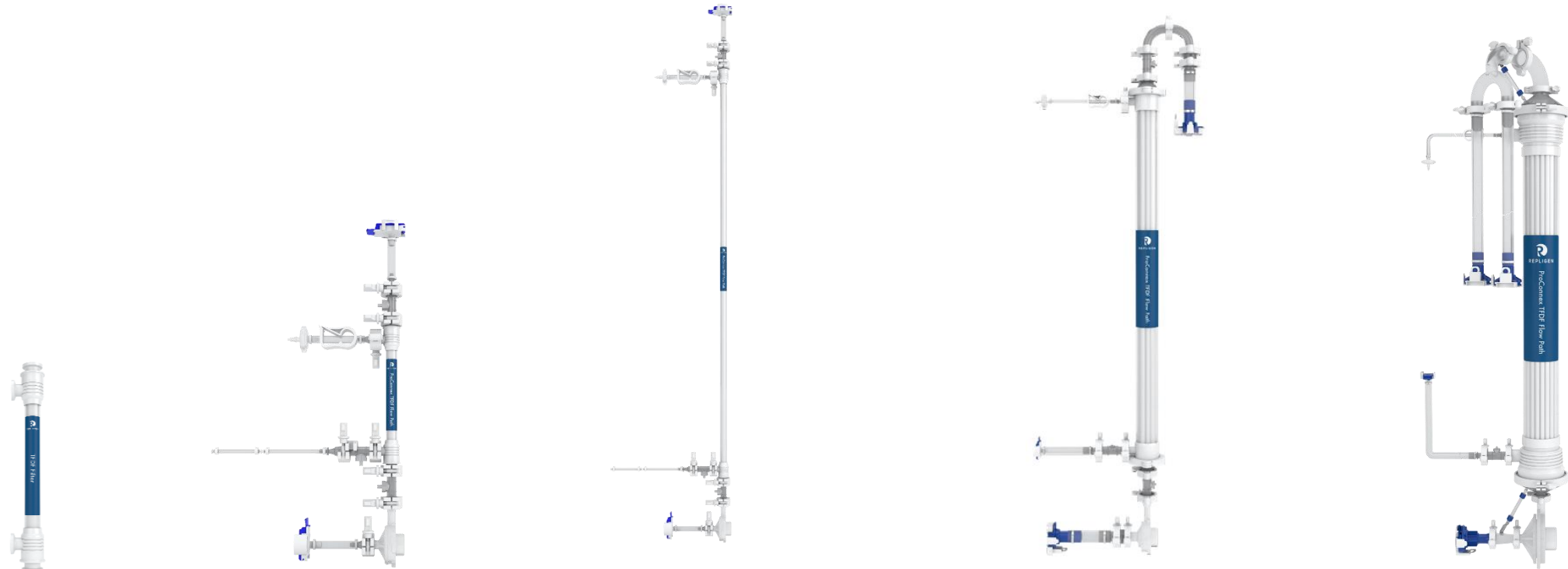


Surface area (cm ²)	3	150	1500	6000
Recommended processing volume(L)	< 1 L	< 50 L	< 500 L	< 2000 L

- Closed
- Single-use
- Gamma-irradiated
- Dry (zero flushing)
- Integrated sensors
- Disposable pump head
- Standard and customizable flow paths

ProConnex[®] TFDF[™] Flow Paths

Complete, ready-to-use TFDF[™] flow paths

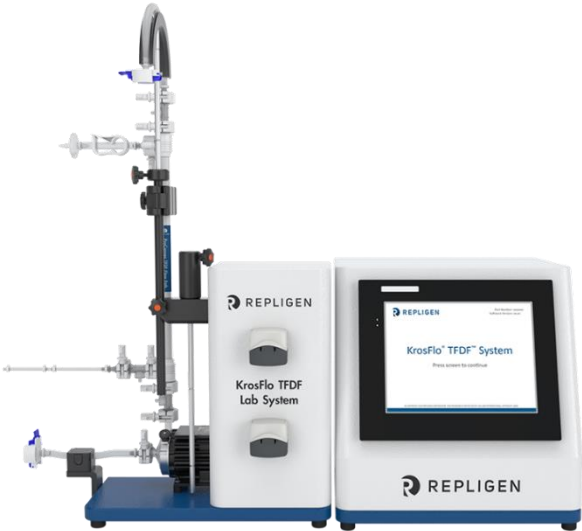


Surface area (cm ²)	3	150	1500	6000
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- Closed
- Single-use
- Gamma-irradiated
- Dry (zero flushing)
- Integrated sensors
- Disposable pump head
- Standard and customizable flow paths

KrosFlo® TFDF™ Systems scale from lab to process

KrosFlo® TFDF™ Lab System



1-50 L volume

KrosFlo® TFDF™ Pilot System



50 L - 500 L volume

KrosFlo® TFDF™ Process System

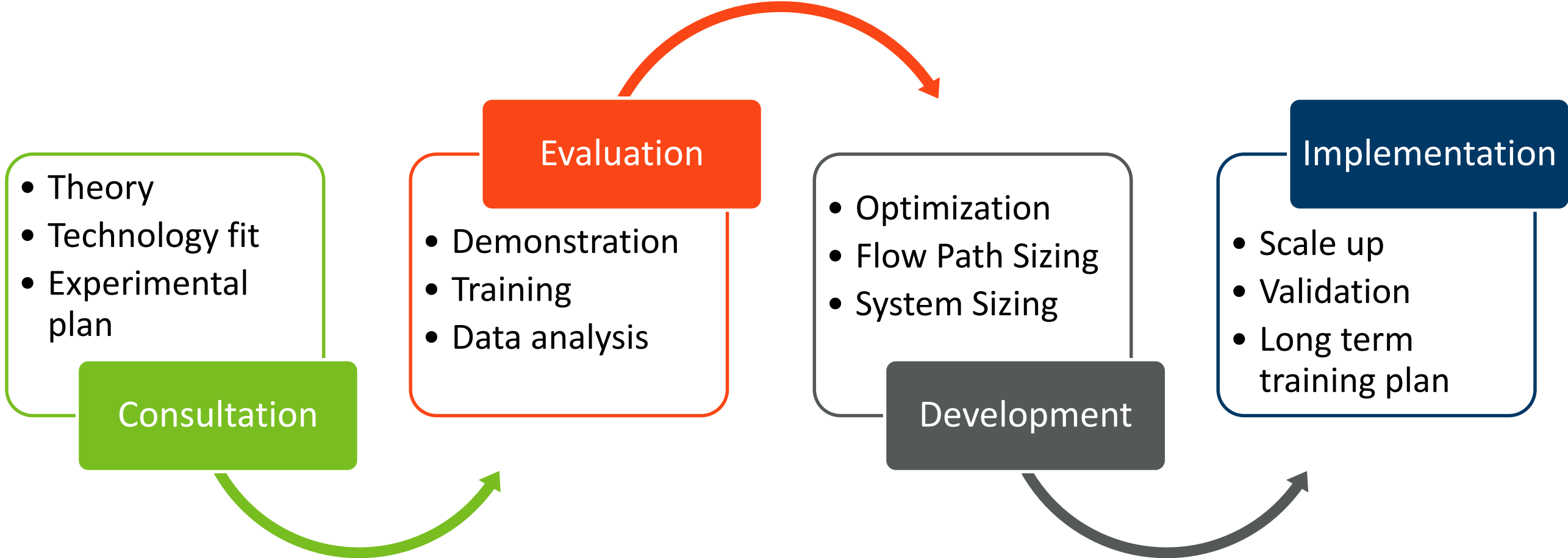


500 L - 2000 L volume

- Walkaway automation of TFDF™ processes
- Small footprint
- ProConnex® TFDF™ Flow Path integration
- Configurable
- Scalable from Lab-scale to Process-scale

Repligen-guided implementation ensures success

Working with Repligen field applications support from consultation to execution



Set new standards in CHO Fed-Batch Clarification

Filter

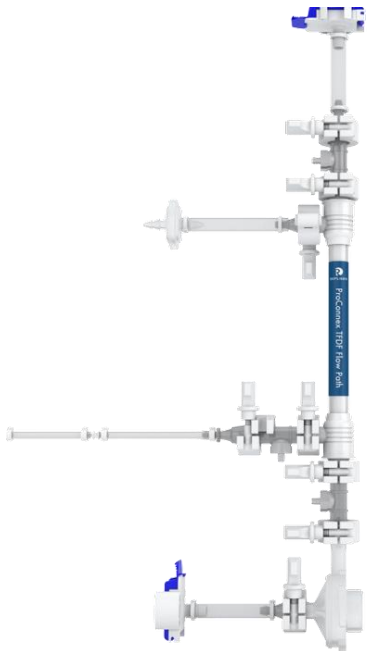
Tubular filter from 1-2000L



Combines the power of tangential flow with depth filtration
 High flux, high capacity, high yield, low turbidity, minimal dilution

Flow Path

Closed, Gamma-irradiated, customizable complete with sensors



Complete consumable with integrated sensors
 Capacity increases with tube number and length

System

Automated process control logic from lab to process



Integrated hardware, software and flow path
 Scalable from lab-scale to process-scale



OR



Thank you!

TFDF™ Technology

repligen.com/TFDF

Visit us at booth 801



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