

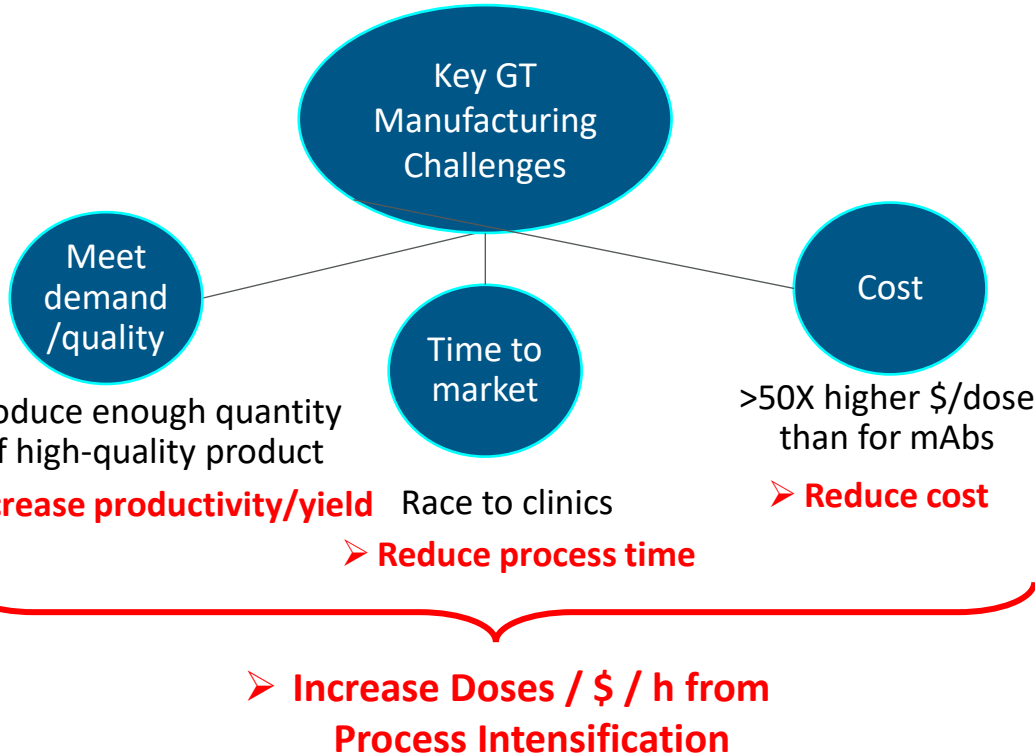
# Better, faster, cheaper: Technology innovation to overcome Gene Therapy manufacturing challenges

Keen Chung Ph.D.  
Associate Director,  
Advanced Bioprocess Applications



# What problem are we solving?

Process improvements needed in Gene Therapy manufacturing

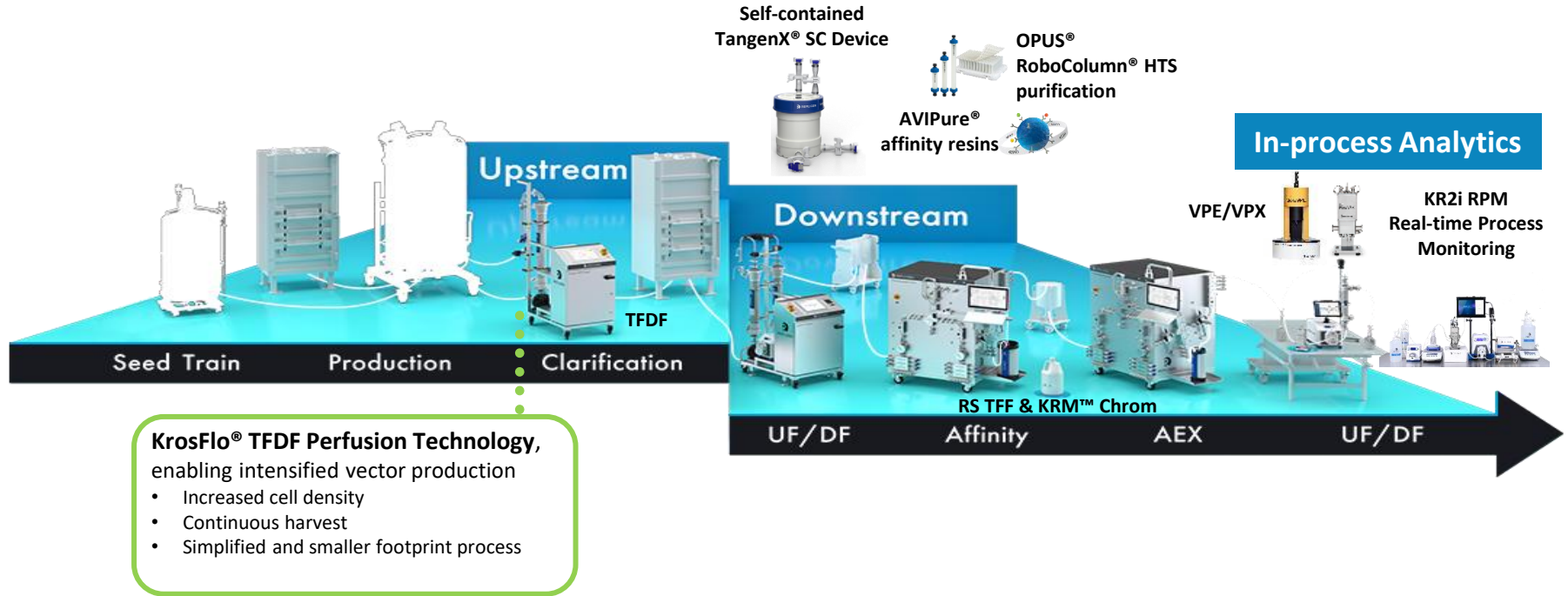


Therapy	Dosage/patient	Doses/L bioreactor	Mfg cost (\$/dose)
mAb	1 g	3-5	<\$100
AAV	1E15 VG	~0.1	>\$10,000
Lentivirus	1E10 TU	~0.5	>\$5,000

All data shown as examples only

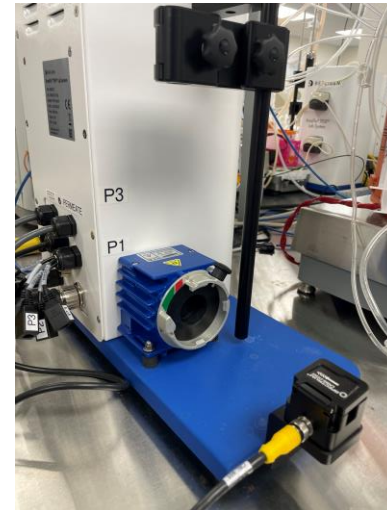
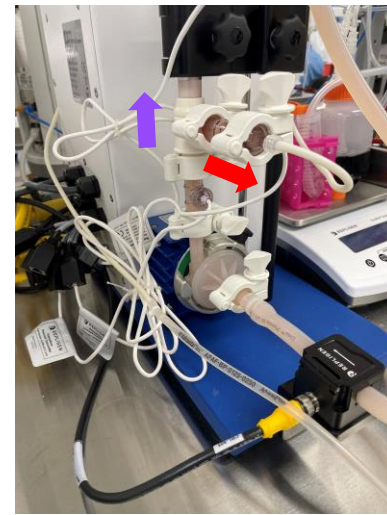
# Process intensification strategies to meet demand for viral vectors

Do MORE with LESS MONEY, TIME, SPACE, EQUIPMENT, CONSUMABLES...





# TFDF run in action





## TFDF-intensified Lentiviral vector production

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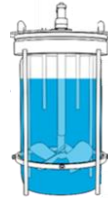
# Transient LV production at 2L scale from batch and TFDF-intensified cell cultures

## Materials and methods

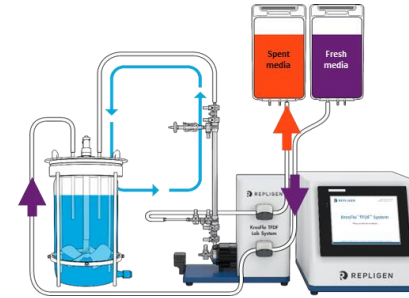
- BioBLU® 3c single-use vessels with macrosparger and two pitched blade impellers (Eppendorf)
- Culture conditions: 200 rpm agitation, 37° C, pH at  $7.2 \pm 0.2$  controlled with CO<sub>2</sub>
- Dissolved oxygen (DO) strategy: 50% air saturation, supplemented using 3-gas auto mixture of air, O<sub>2</sub>, and CO<sub>2</sub>.
- Total constant gas flow rate: 0.1-0.3 L/min
- Intensification starts  $\sim 3 \times 10^6$  cells/mL viable cell density (VCD)
  - KrosFlo® TFDF® Lab System (Repligen) equipped with a TFDF® -30 ProConnex® TFDF® Flow Path (30 cm<sup>2</sup>)
  - 0.5 L/min cross flow
  - Perfusion rate: 1 vessel volume per day (vvd)

## Cell growth phase

### Batch cell culture

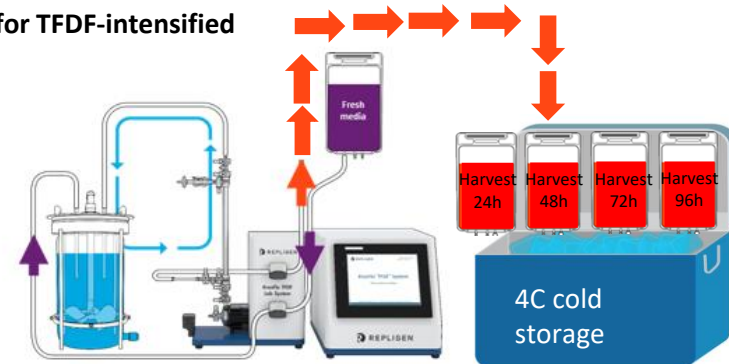


### Perfusion cell culture

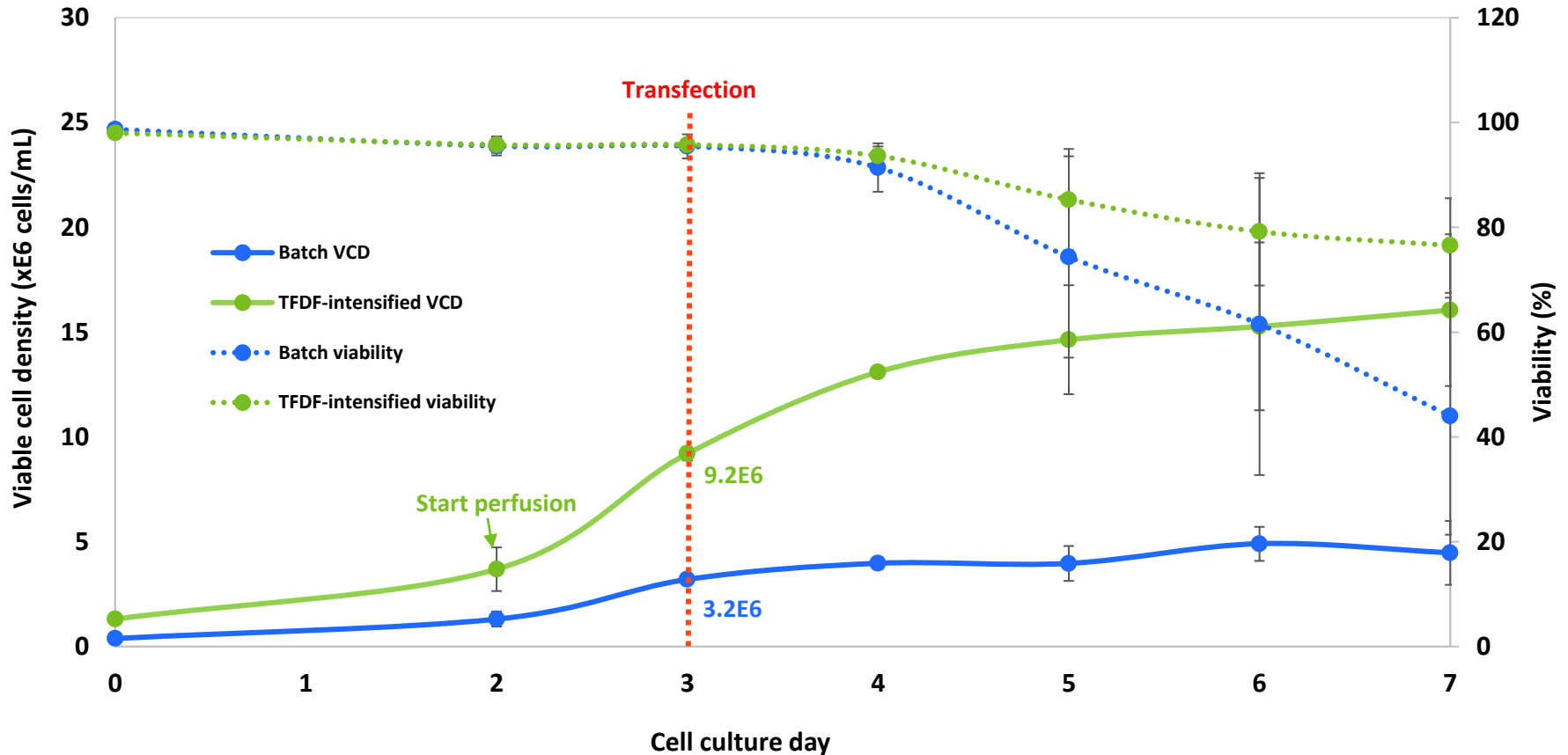


## LV production phase

- VCD at transfection:
  - $3 \times 10^6$  cells/mL for batch
  - $9 \times 10^6$  cells/mL for TFDF-intensified



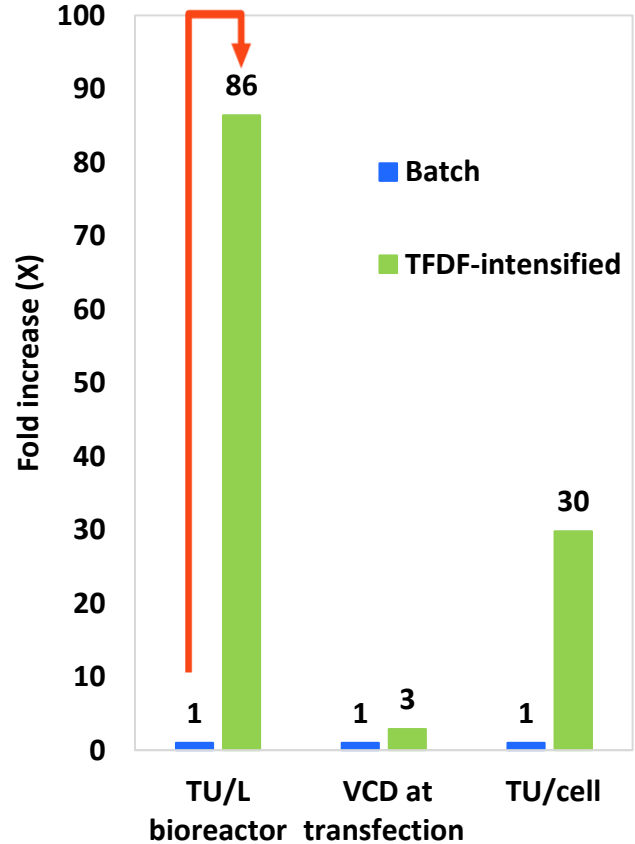
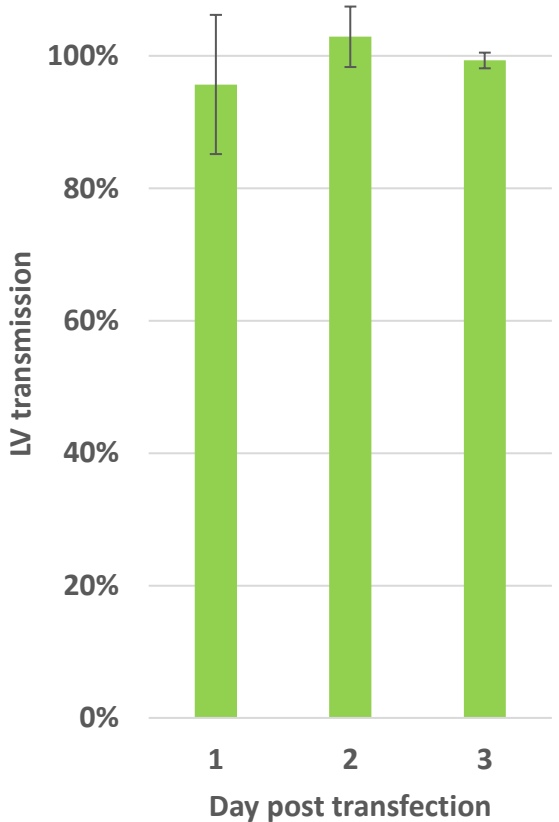
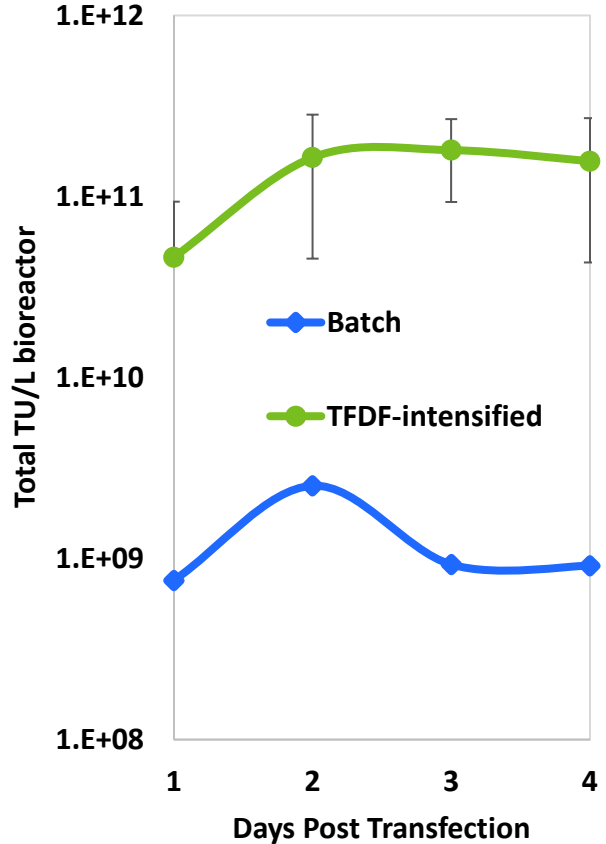
# TFDF intensifies VCD at transfection with identical viability to batch





# TFDF-Intensified lentivirus transient production

>80x Lentivirus production with KrosFlo® TFDF® Technology



# Stable LV production at 2L scale from batch and TFDF-intensified cell cultures

## Materials and methods

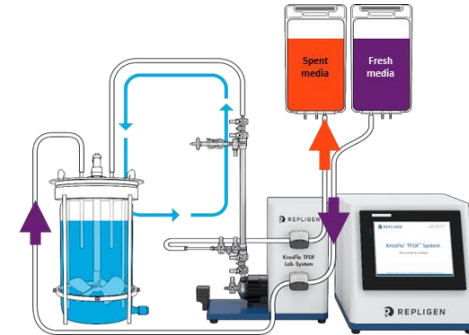
- BioBLU® 3c single-use vessels with macrosparger and two pitched blade impellers (Eppendorf)
- Culture conditions: 200 rpm agitation, 37° C, pH at  $7.2 \pm 0.2$  controlled with CO<sub>2</sub>
- Dissolved oxygen (DO) strategy: 50% air saturation, supplemented using 3-gas auto mixture of air, O<sub>2</sub>, and CO<sub>2</sub>.
- Total constant gas flow rate: 0.1-0.3 L/min
- Intensification starts ~3 X 10<sup>6</sup> cells/mL viable cell density (VCD)
  - KrosFlo® TFDF® Lab System (Repligen) equipped with a **TFDF® -30** ProConnex® TFDF® Flow Path (30 cm<sup>2</sup>)
  - Cross flow 0.5 L/min
  - Perfusion rate: 1 vessel volume per day (vvd)

## Cell growth phase

### Batch cell culture

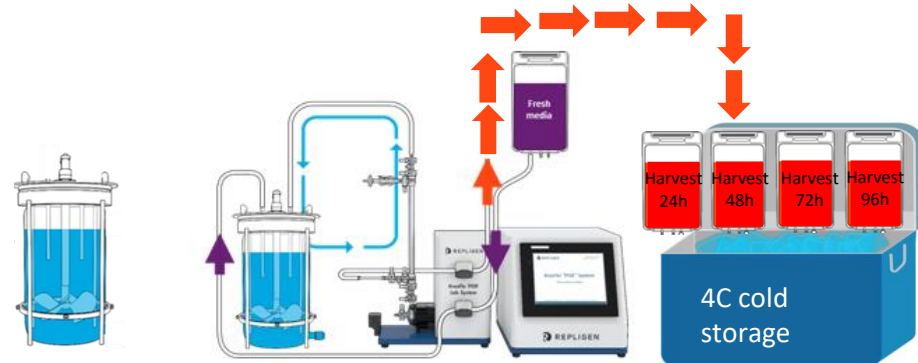


### Perfusion cell culture

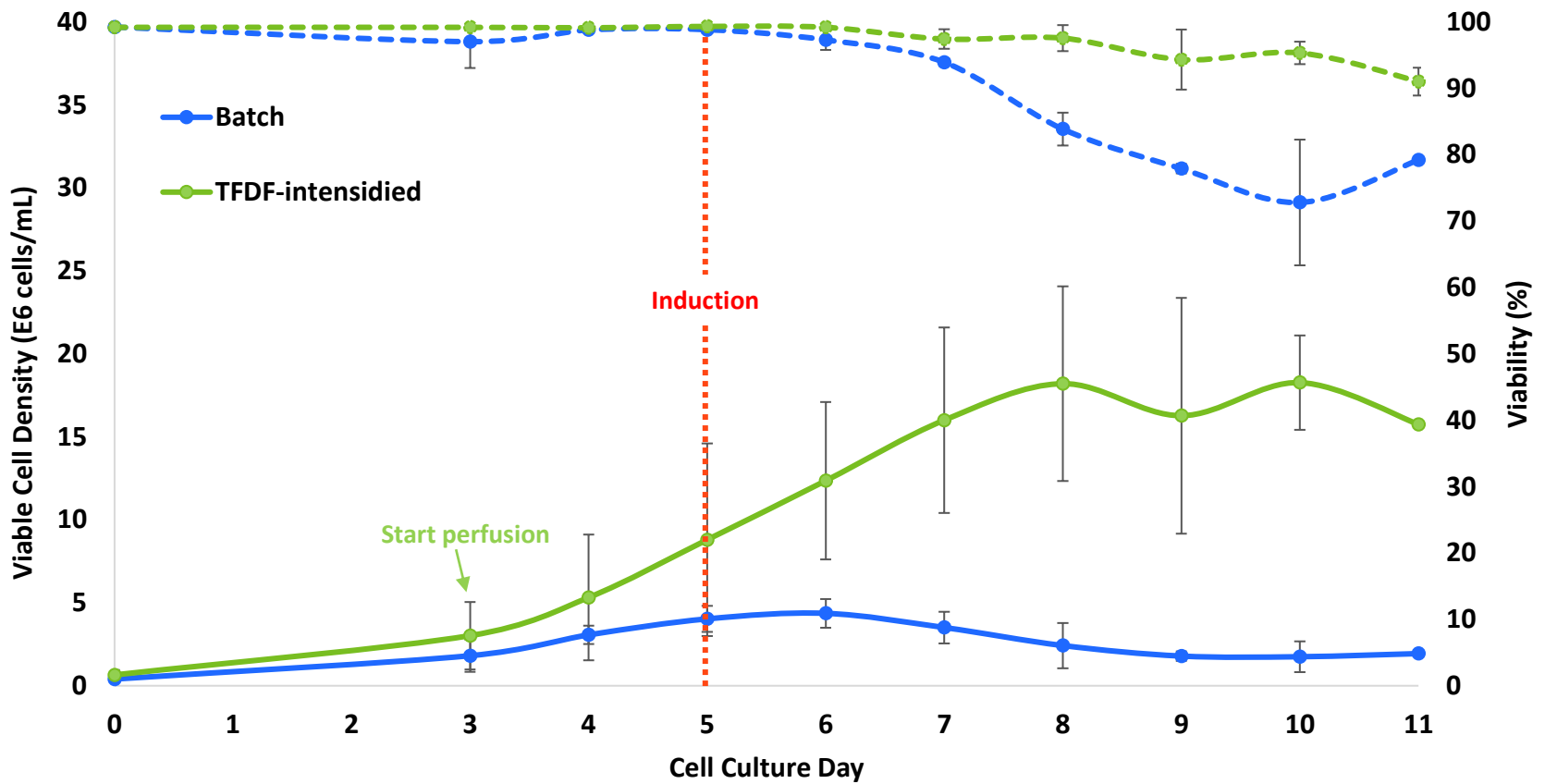


## LV production phase

- VCD at induction:
  - 3 X 10<sup>6</sup> cells/mL for batch
  - 9 X 10<sup>6</sup> cells/mL for TFDF-intensified

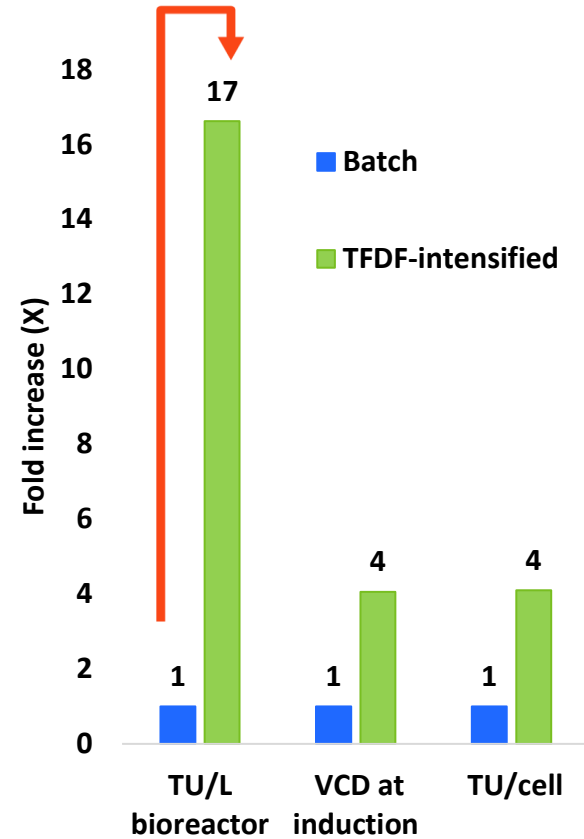
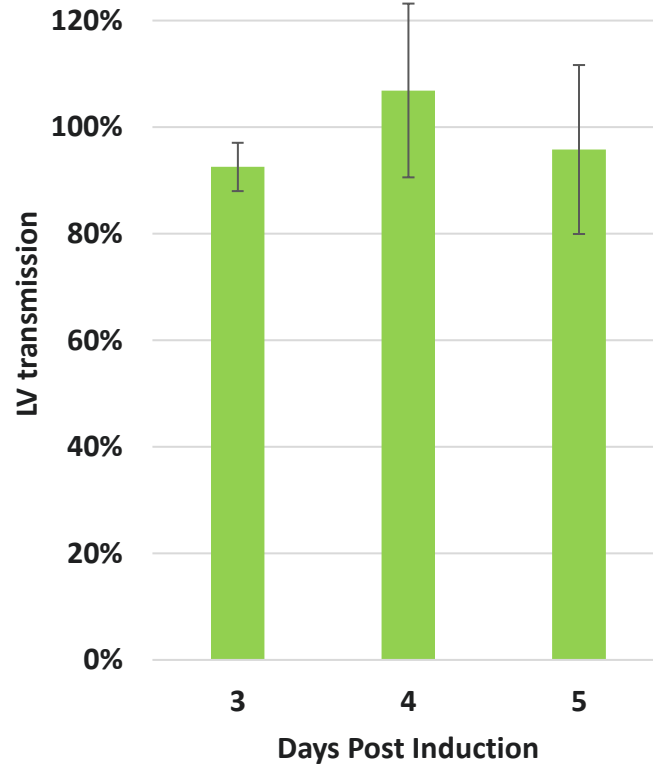
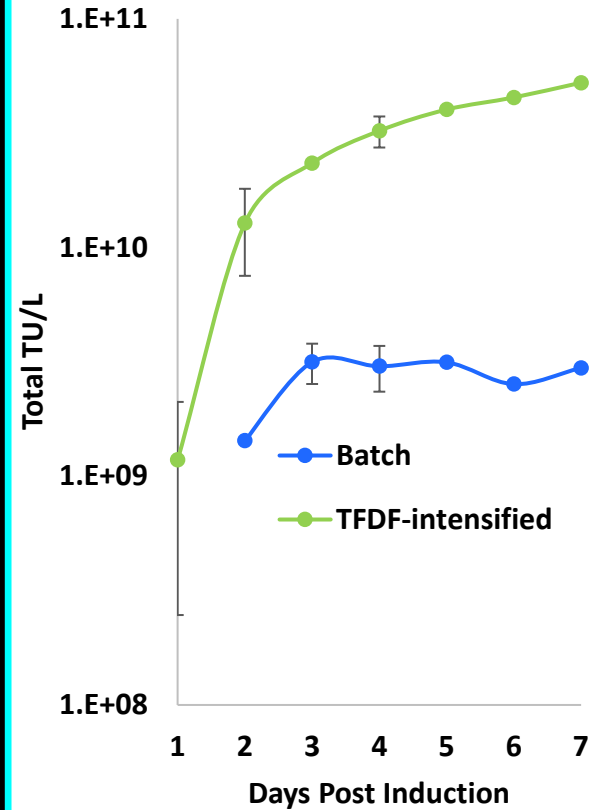


# TFDF based stable cell line culture compared to batch



# TFDF intensified LV stable production

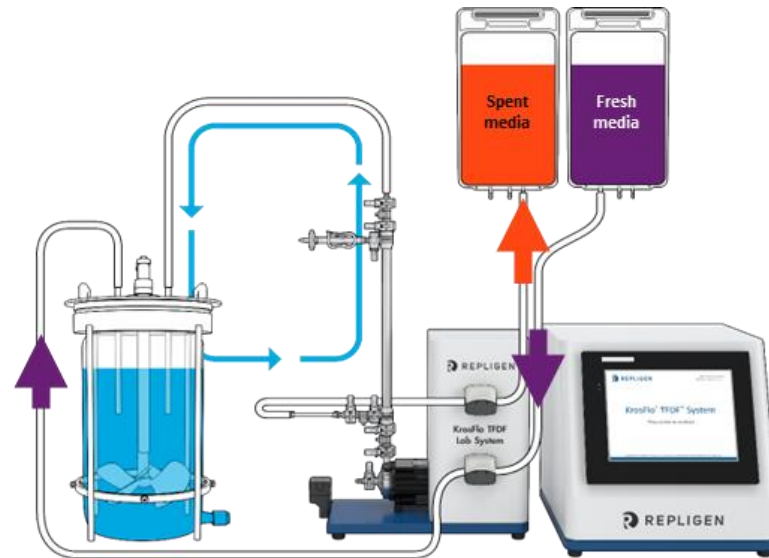
>15x Lentivirus production with KrosFlo® TFDF® Technology



# Stable LV production at 10L scale from 2L scale TFDF-intensified cell cultures

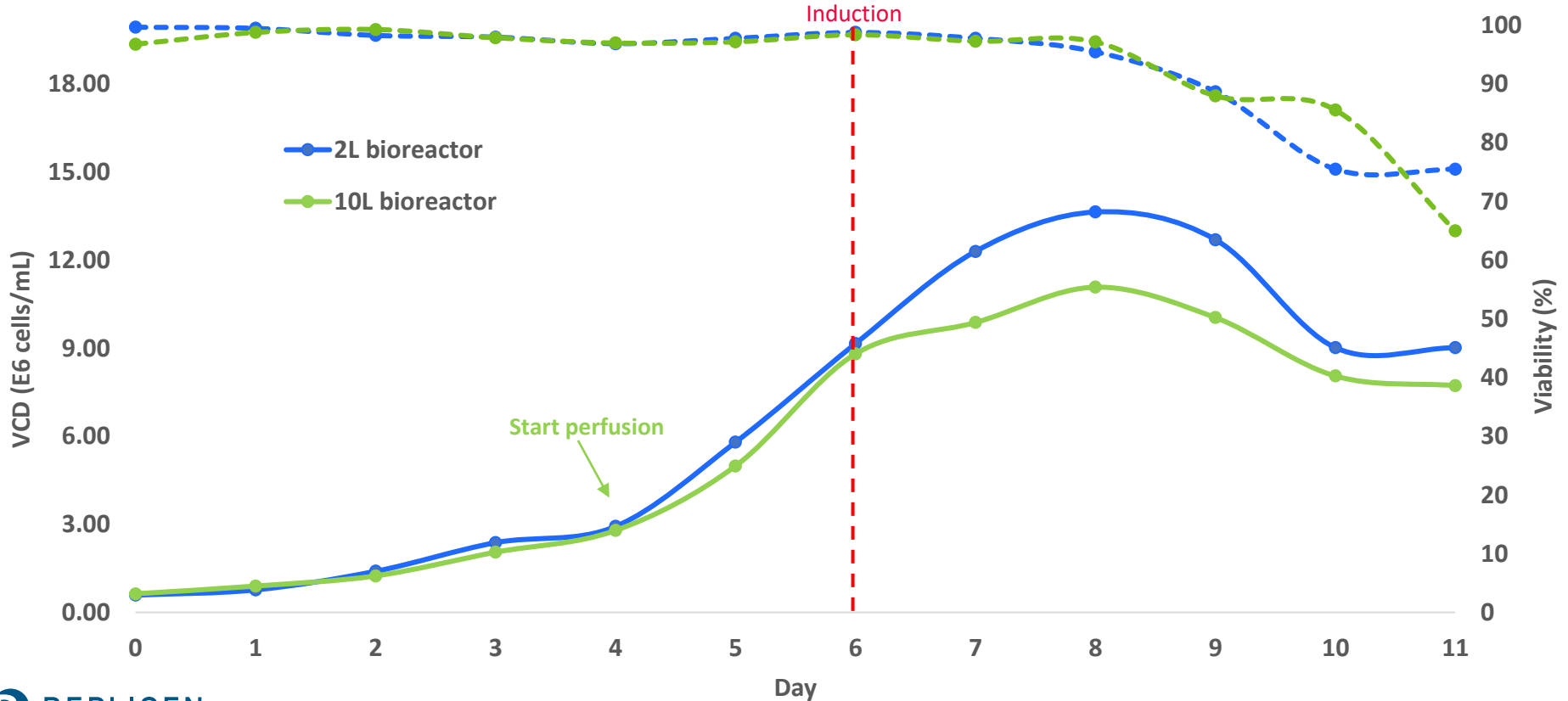
## Materials and methods

- BioBLU® 3c and 10c single-use vessels with macrosparger and two pitched blade impellers (Eppendorf)
- Culture conditions: 200 rpm agitation, 37° C, pH at  $7.2 \pm 0.2$  controlled with CO<sub>2</sub>
- Dissolved oxygen (DO) strategy: 50% air saturation, supplemented using 3-gas auto mixture of air, O<sub>2</sub>, and CO<sub>2</sub>.
- Total constant gas flow rate: 0.1-0.3 L/min
- Intensification starts  $\sim 3 \times 10^6$  cells/mL viable cell density (VCD)
  - KrosFlo® TFDF® Lab System (Repligen) equipped with a **TFDF® -30 (30 cm<sup>2</sup>, 2L bioreactor) or TFDF® -150 (150 cm<sup>2</sup>, 10L bioreactor) ProConnex® TFDF® Flow Path**
  - Cross flow 0.5 L/min
  - Perfusion rate: 1 vessel volume per day (vvd)
- **VCD at induction:**
  - $9 \times 10^6$  cells/mL for TFDF-2L
  - $9 \times 10^6$  cells/mL for TFDF-10L



# TFDF based high VCD induction at 10L large scale

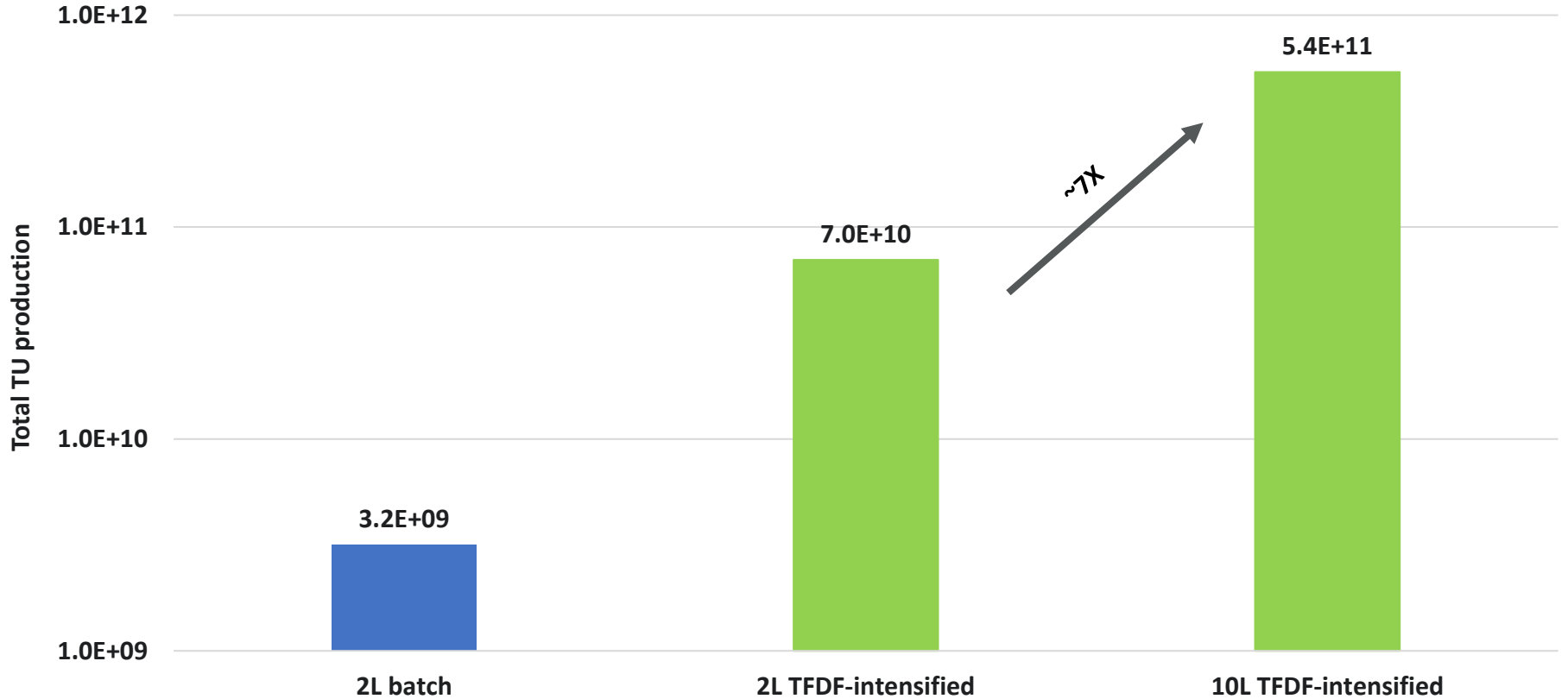
Scale-up from 2 to 10L bioreactor



# TFDF scale up LV production with stable cell line

Scale-up from 2 to 10L bioreactor

~54 doses from 10L bioreactor TFDF-intensified cell culture  
(@1E10 TU/dose, CAR-T application)





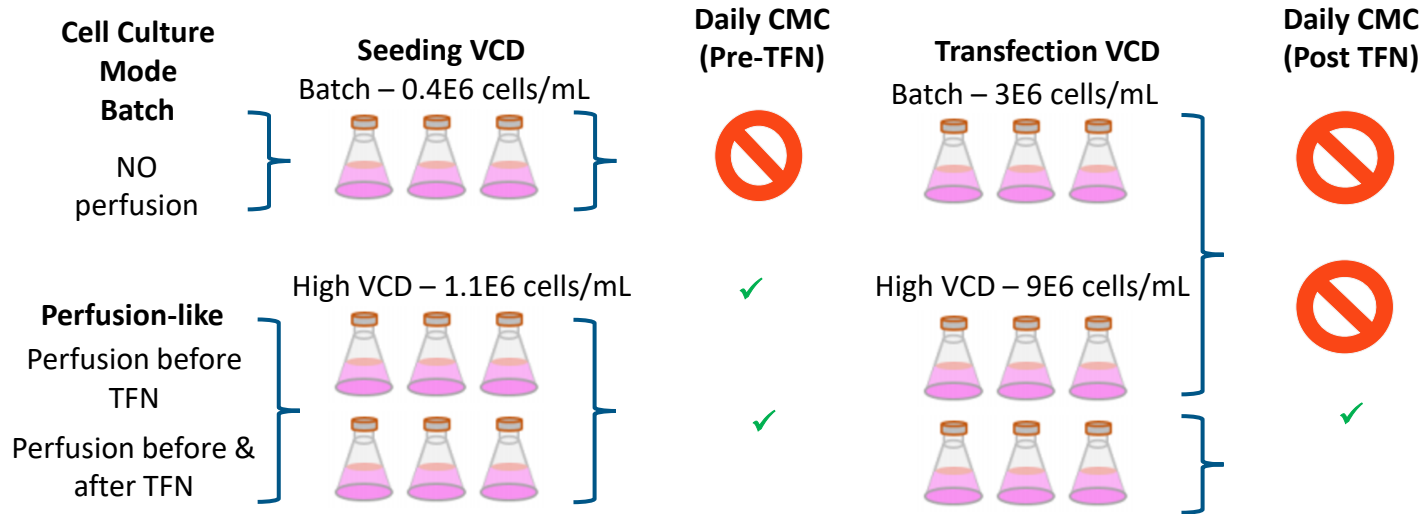
## TFDF-intensified AAV viral vector production

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# Understanding the importance of continuous perfusion for high VCD transfection

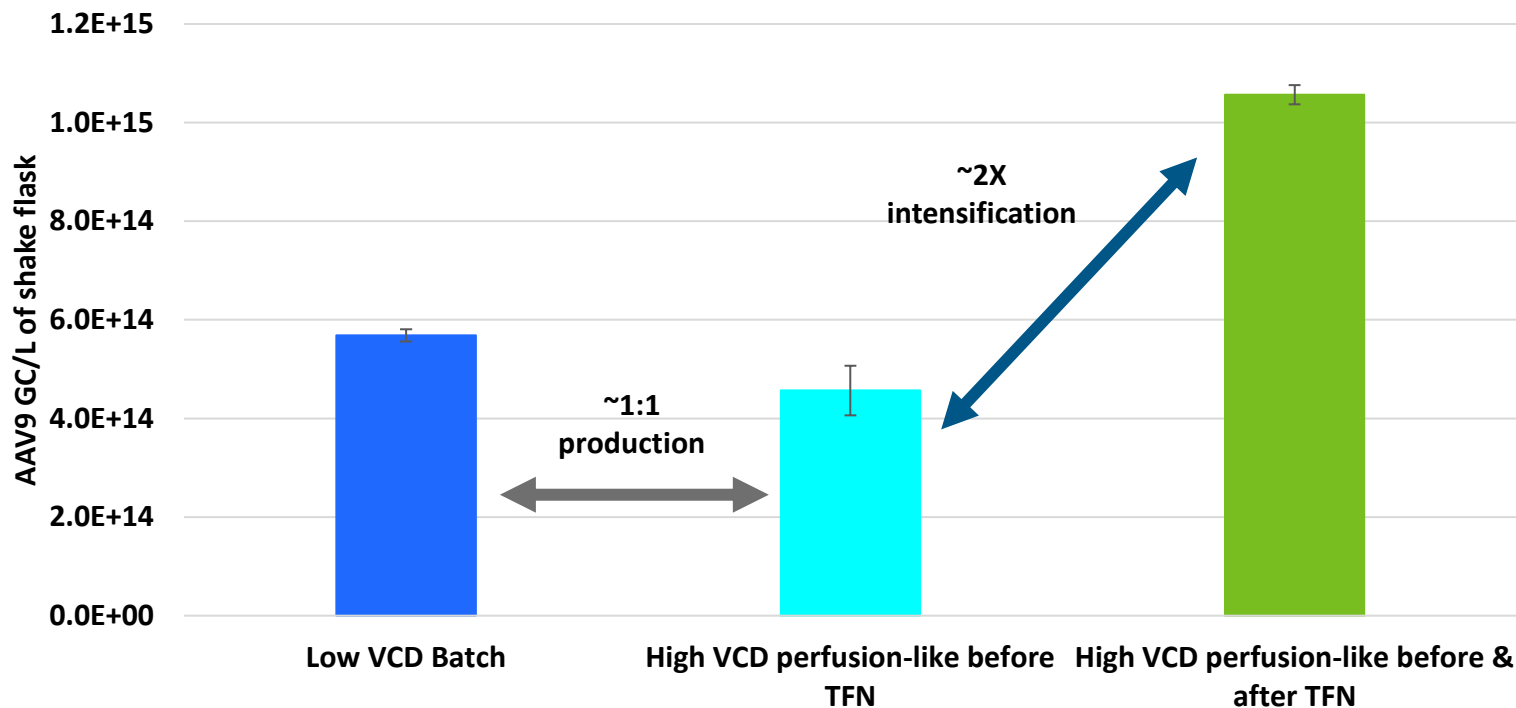
Perfusion-like shake flask experiment



- Batch:
  - Low VCD transfection – 3E6 cells/mL
- Perfusion-like
  - High VCD transfection – 9E6 cells/mL
  - Daily CMC to mimic perfusion

# The importance of continuous perfusion for high VCD transfection

AAV9 production SF data



**Conclusion:** Continuous perfusion post high VCD transfection is necessary for increased viral production yields.

# Transient AAV production at 2L scale from batch and TFDF-intensified cell cultures

## Materials and methods

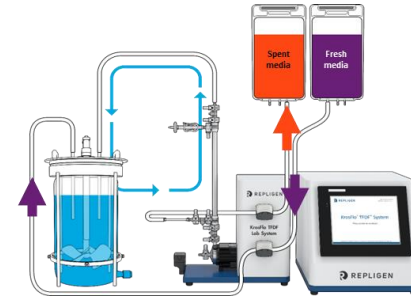
- BioBLU® 3c single-use vessels with macrosparger and two pitched blade impellers (Eppendorf)
- Culture conditions: 200 rpm agitation, 37° C, pH at 7.2 ± 0.2 controlled with CO<sub>2</sub>
- Dissolved oxygen (DO) strategy: 50% air saturation, supplemented using 3-gas auto mixture of air, O<sub>2</sub>, and CO<sub>2</sub>.
- Total constant gas flow rate: 0.1-0.3 L/min
- Intensification starts ~3 X 10<sup>6</sup> cells/mL viable cell density (VCD)
  - KrosFlo® TFDF® Lab System (Repligen) equipped with a TFDF® -30 ProConnex® TFDF® Flow Path (30 cm<sup>2</sup>)
  - 0.5 L/min cross flow
  - Perfusion rate: 1 vessel volume per day (vvd)

### Cell growth phase

#### Batch cell culture

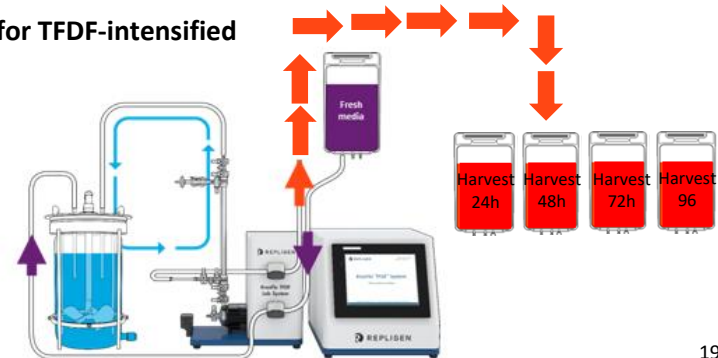
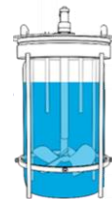


#### Perfusion cell culture

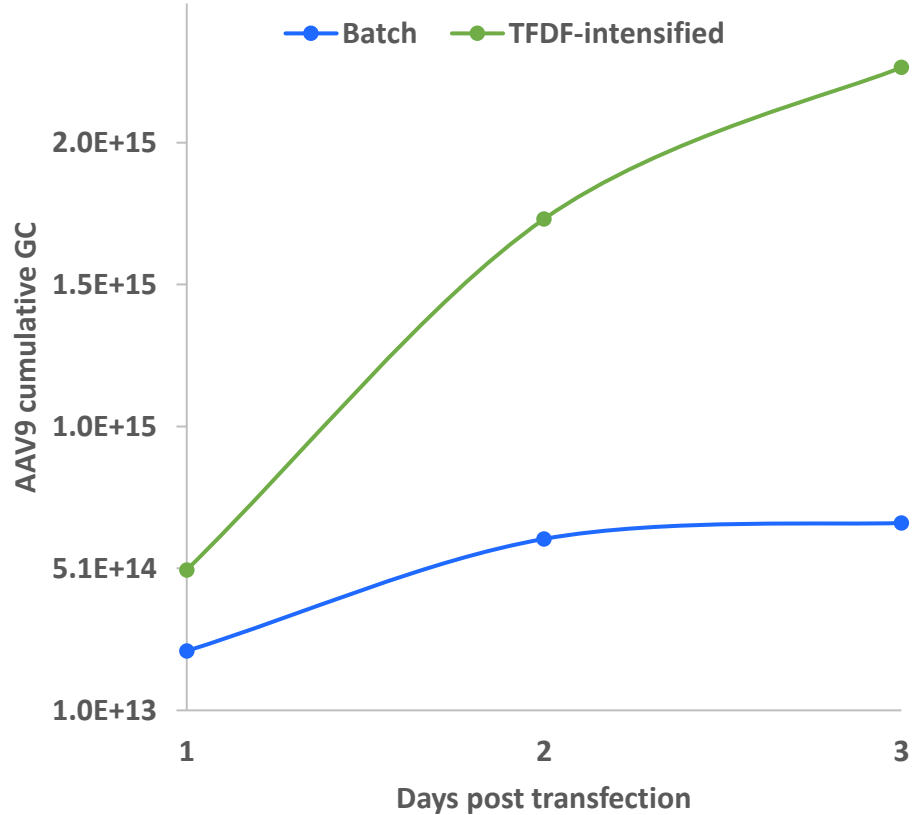
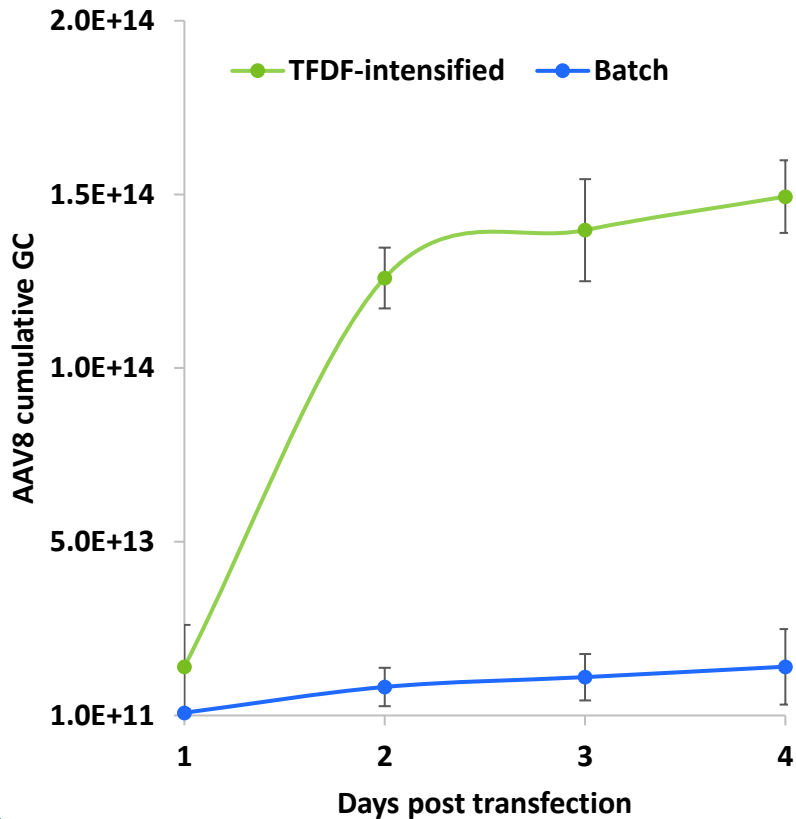


### AAV production phase

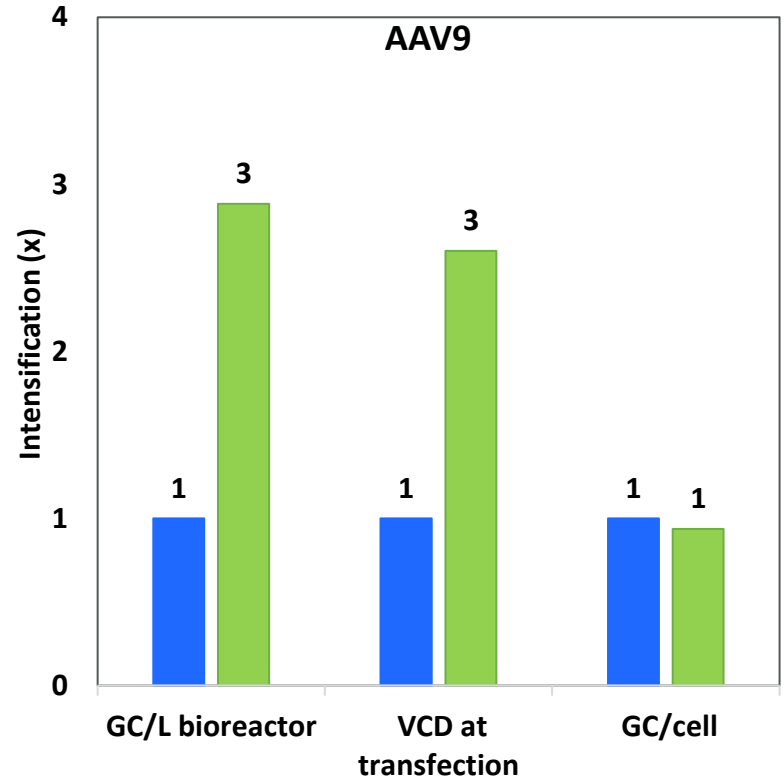
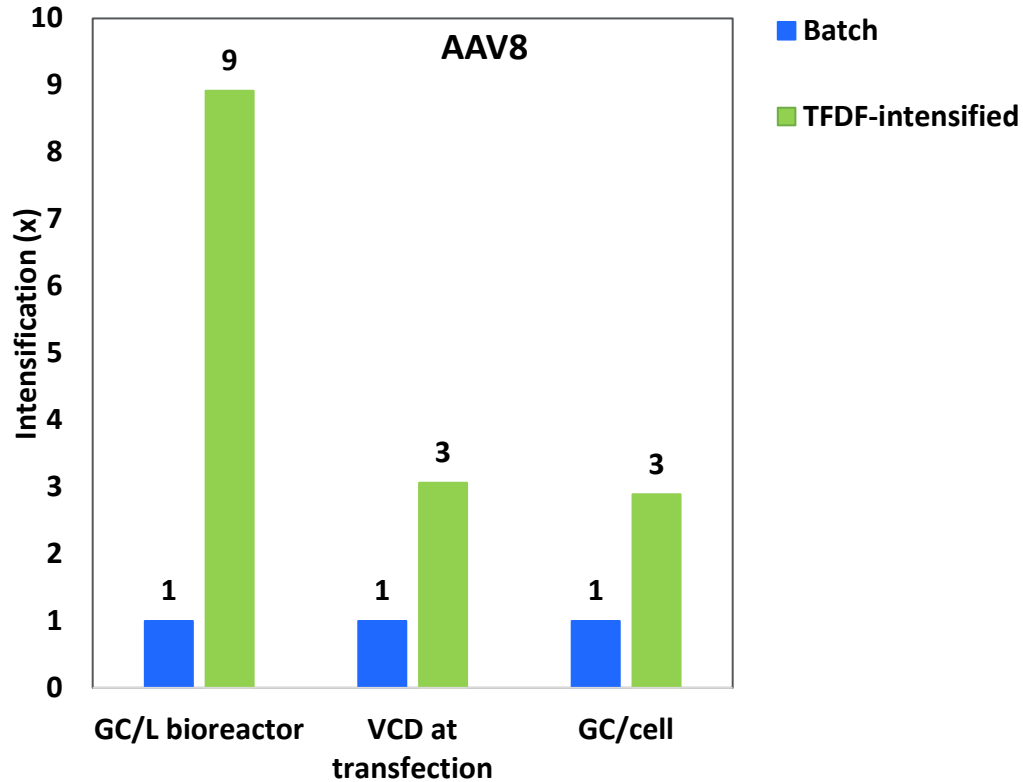
- VCD at transfection:
  - 3 X 10<sup>6</sup> cells/mL for batch
  - 9 X 10<sup>6</sup> cells/mL for TFDF-intensified



# TFDF based AAV intensified production



# TFDF intensification improves AAV production and specific productivity (qP)

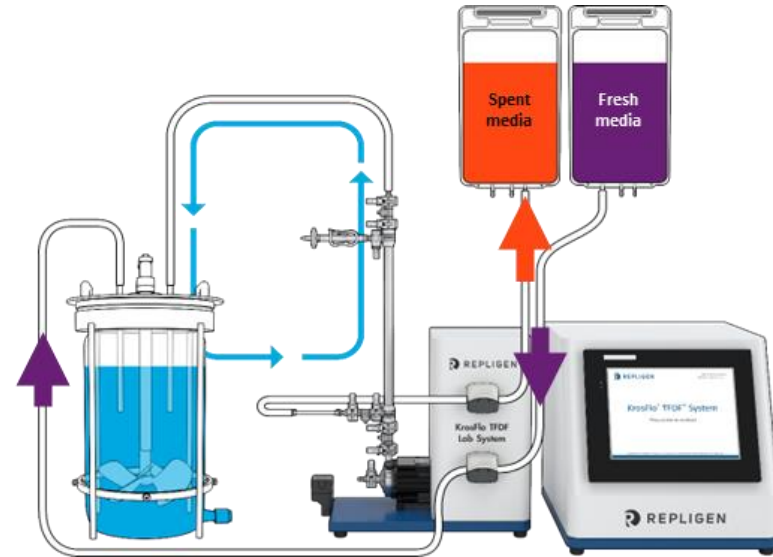


# AAV9 production at 10L scale from 2L scale TFDF-intensified cell cultures

## Materials and methods

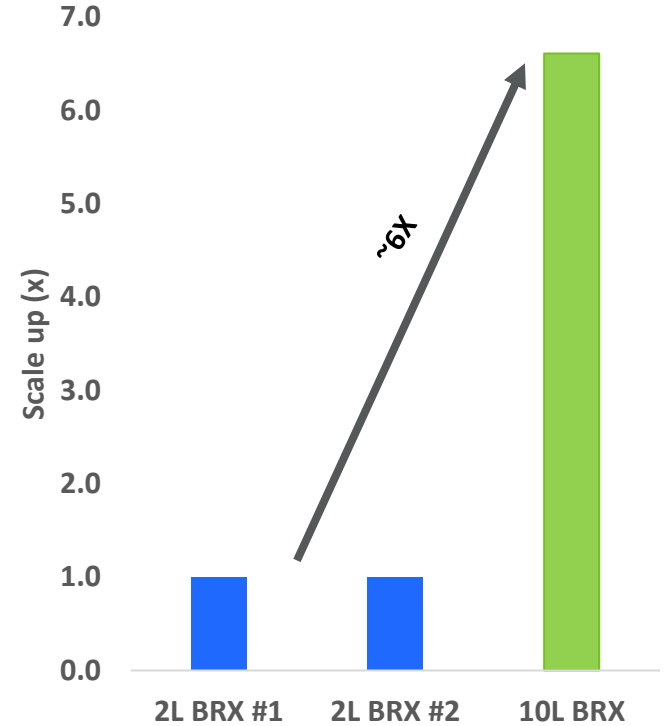
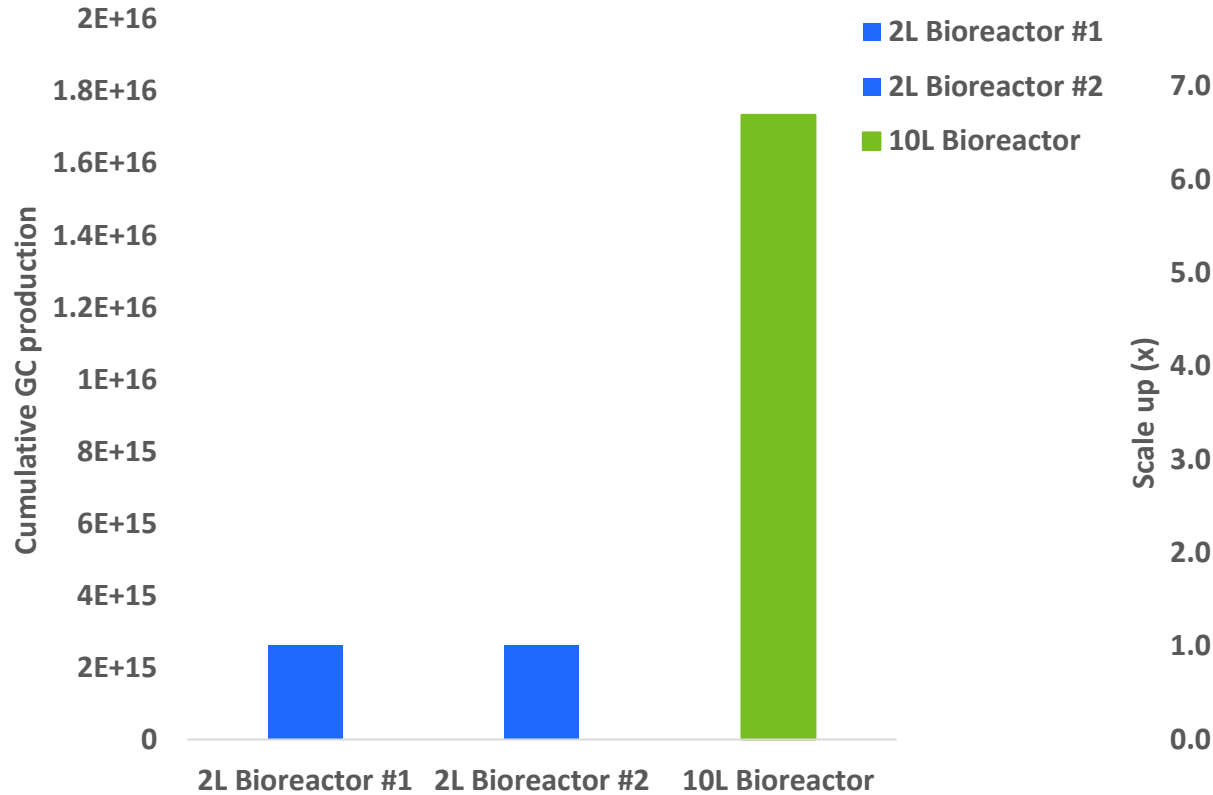
### Bioreactor, culture and production conditions

- BioBLU® 3c and 10c single-use vessels with macrosparger and two pitched blade impellers (Eppendorf)
- Culture conditions: 200 rpm agitation, 37° C, pH at 7.2 ± 0.2 controlled with CO<sub>2</sub>
- Dissolved oxygen (DO) strategy: 50% air saturation, supplemented using 3-gas auto mixture of air, O<sub>2</sub>, and CO<sub>2</sub>.
- Total constant gas flow rate: 0.1-0.3 L/min
- Intensification starts ~3 X 10<sup>6</sup> cells/mL viable cell density (VCD)
  - KrosFlo® TFDF® Lab System (Repligen) equipped with a **TFDF® -30 (30 cm<sup>2</sup>, 2L bioreactor) or TFDF® -150 30 (150 cm<sup>2</sup>, 10L bioreactor)** ProConnex® TFDF® Flow Path
  - Cross flow 0.5 L/min
  - Perfusion rate: 1 vessel volume per day (vvd)
- **VCD at transfection:**
  - 9 X 10<sup>6</sup> cells/mL for TFDF-2L 2X runs
  - 9 X 10<sup>6</sup> cells/mL for TFDF-10L



# TFDF scale up AAV9 transient transfection production

2L to 10 L scale up



# Conclusion

LV:

- Substantial increased LV production from TFDF-intensified process
  - ❑ > 80X production in transient mode, >15 X production from stable cell line
  - ❑ Enabled continuous LV cold harvest to prevent virus inactivation
  - ❑ Demonstrated 10L perfusion run enable ~54 doses production ( equivalent close to a 200L batch production) for CAR-T application

AAV:

- Substantial increased AAV production from TFDF-intensified process
  - ❑ >3-8X AAV transient transfection production
  - ❑ Continuous perfusion is critical to AAV intensification
  - ❑ Demonstrated successful scale up 10L perfusion run

TFDF based intensification as a solution to provide :

- ❑ Better:
  - ❖ high-quality production yield
  - ❖ Simplified and smaller footprint process
- ❑ Faster and cheaper:
  - ❖ Shorten timeline to market
  - ❖ Reduce consumables and scale ups to save time and cost



**Thank you!**

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