

# Tide Motion® : An Adherent and Scalable Platform for the Production of Viral Vectors



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## Introduction

Viral vectors such as adeno-associated virus (AAV), lentivirus (LV) and retrovirus (RV) are the most commonly used delivery vehicles for gene therapy applications. Current methods of viral vector production have low yields and are limited in terms of their scalability. Protocols to produce viral vectors are largely empirical and the processes generally not well-characterized. These process vulnerabilities translate into high costs for commercially marketed gene therapies.

Esco Aster has developed the TideMotion® Bioreactors for scalable culture of adherent cells. Esco Aster has also established a robust and scalable platform with the flexibility to produce any viral vector-of-choice. For illustration, we present in this poster the upstream portion of production of 3rd generation lentivirus on the TideMotion® Platform.

Esco Aster provides upstream and downstream process development services for viral vectors production using the TideMotion® Platform.

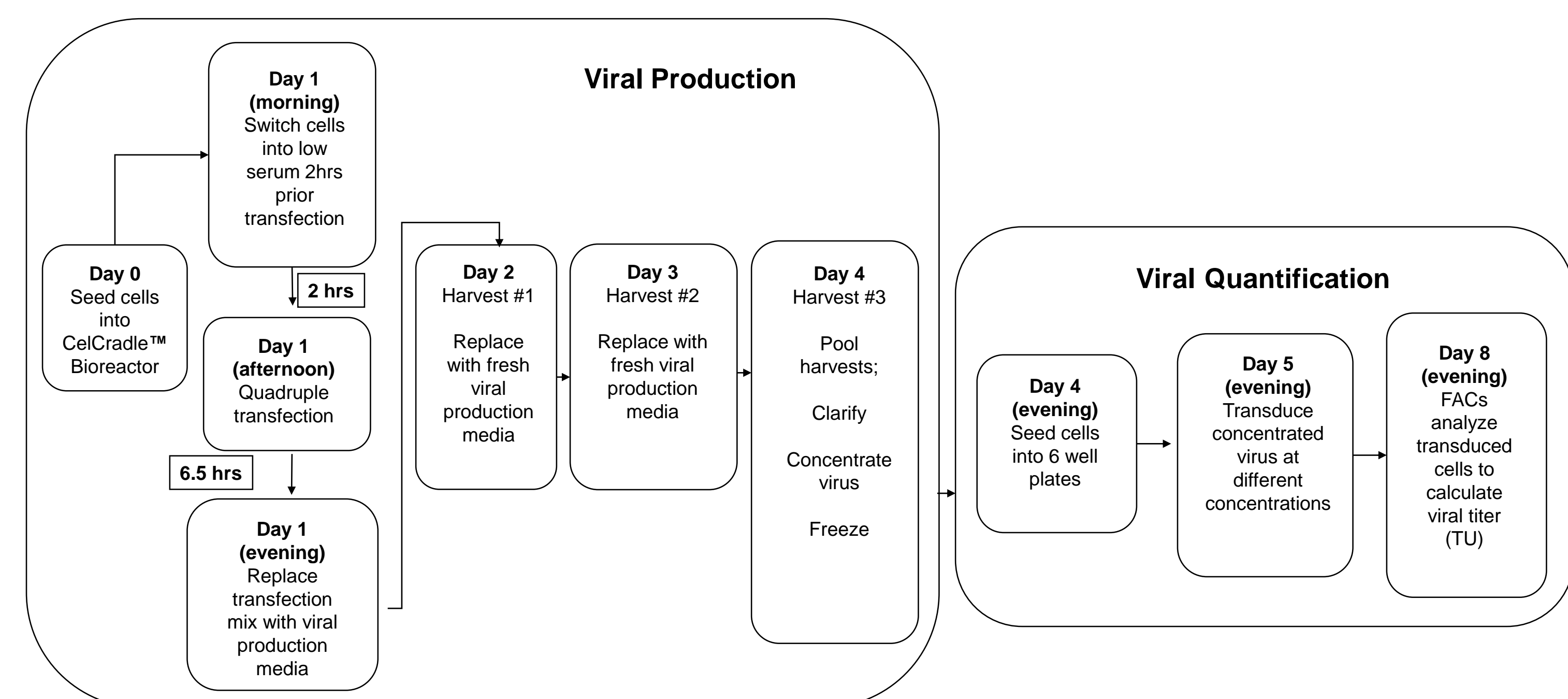
## Challenges Faced During Viral Production When Using Adherent Bioreactors

|  |   |   |
|--|---|---|
| <b>Challenge #1</b><br>Low Plasmid Transfection Efficiency | <b>Challenge #3</b><br>Difficulty Translating 2D Protocols to protocols used for Stirred Tank Bioreactors | <b>Challenge #4</b><br>Inefficient Harvesting from Cells Lysed In-situ on Macro/microcarriers |
| <b>Challenge #2</b><br>Low Viral Titer                     |   |   |

## Cells, Media and Materials

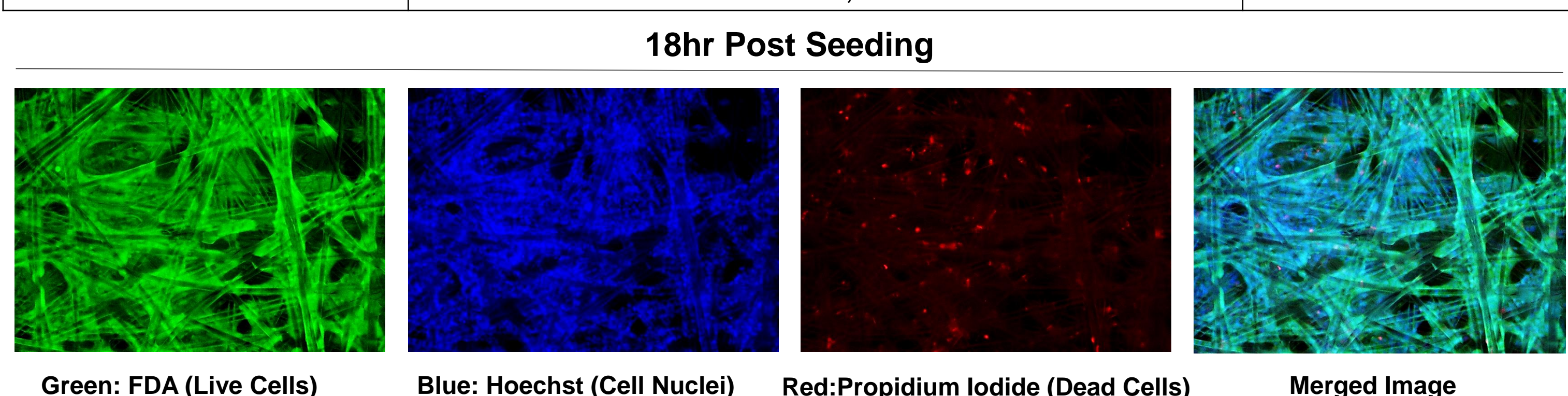
|  |                                       |
|--|---------------------------------------|
| <b>Hardware</b>  | CelCradle™ Bioreactor                 |
| <b>Cell Line</b>                                       | Adherent HEK-293T (ATCC: CRL-3216)    |
| <b>Choice-of-Viral Vector</b>                          | 3 <sup>rd</sup> generation lentivirus |
| <b>Packaging Plasmid(s)</b><br>(obtained from AddGene) | pCMV-VSV-g                            |
|  | pMDL-pRRE                             |
|  | pRSV-Rev                              |
| <b>Expression Plasmid</b>                              |                                       |
| <b>Growth Media</b>                                    | Commercial Complete Media             |
| <b>Transfection Reagent</b>                            | PEI 'MAX'                             |

## Overall Pipeline for Upstream Lentivirus Production



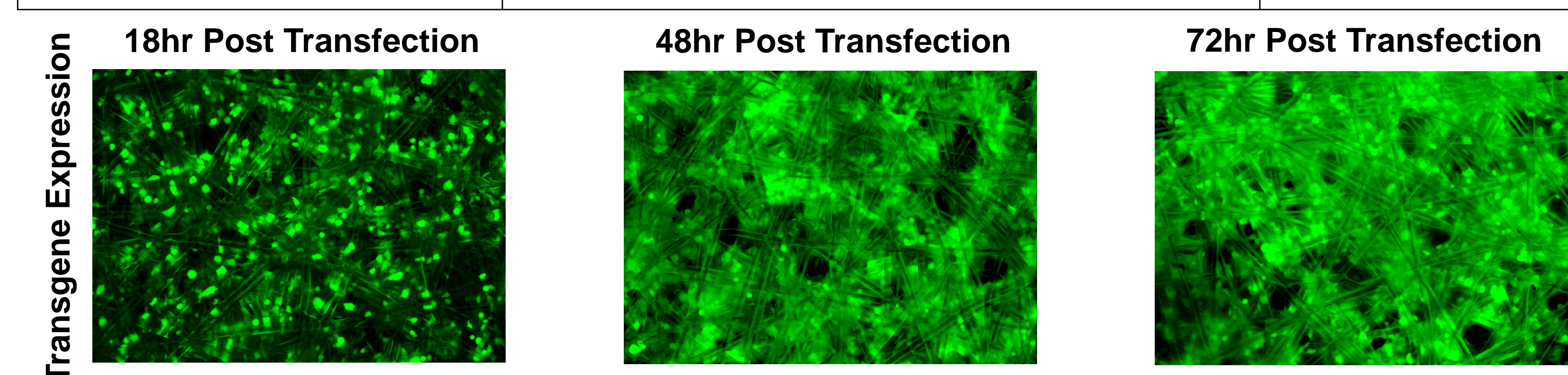
## Viral Production Day 0: Cell Seeding

|  |  |                             |
|--|--|-----------------------------|
| <b>Cell # per CelCradle™</b>               | Conditions: <i>confidential</i>                                      | Importance: <b>Critical</b> |
| <b>Method of Seeding</b>                   | Seeding with Inverted Position of CelCradle™ Bottle                  | <b>Critical</b>             |
| <b>Seeding Volume</b>                      | ~150 ml  | NA                          |
| <b>Seeding Hours</b>                       | ~3-5 hrs   | NA                          |
| <b>Seeding Protocol</b>                    | 30 min interval gentle swirl   | NA                          |
| <b>Seeding Efficiency</b>                  | > 85 %   | NA                          |
| <b>Top up Media</b>                        | To 500 ml with complete growth media                                 | NA                          |
| <b>TideMotion® Parameter (Cell Growth)</b> | Uprate: 1 mm/sec; Uphold: 1 min; Downrate: 1 mm/sec; Downhold: 1 min | NA                          |



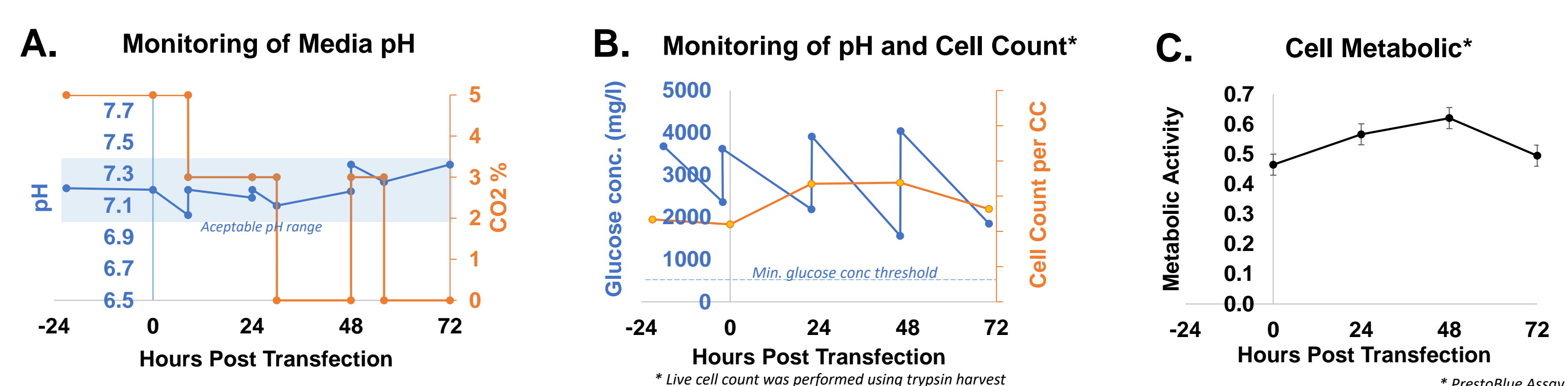
## Viral Production Day 1: Quadruple Transfection

|  |   |                   |
|--|---|-------------------|
|  | <b>Conditions</b>   | <b>Importance</b> |
| <b>Growth Media</b>                                  | Standard HEK293T media  | NA                |
| <b>Transfection Media</b>                            | Standard HEK293T media with low serum                                       | NA                |
| <b>Viral Producing Media</b>                         | Transfection media with <b>additive(s)</b>                                  | <b>Critical</b>   |
| <b>Pre-Transfection Preparation</b>                  | Exchange cells into 450 ml of transfection media                            | <b>Critical</b>   |
| <b>Cell # per CelCradle™ on Day of Transfection</b>  | <i>confidential</i>   | <b>Critical</b>   |
| <b>Plasmid Ratio</b>                                 | <i>confidential</i>   | <b>Critical</b>   |
| <b>PEI 'MAX' : DNA Ratio</b>                         | <i>confidential</i>   | <b>Critical</b>   |
| <b>DNA per Cell</b>                                  | <i>confidential</i>   | <b>Critical</b>   |
| <b>TideMotion® Parameters (6.5 hrs Transfection)</b> | Uprate: 0.25 mm/sec; Uphold: 30 min; Downrate: 0.25 mm/sec; Downhold: 1 min | <b>Critical</b>   |
| <b>Transfection Period</b>                           | 6.5 hrs   | NA                |
| <b>Post-Transfection Treatment</b>                   | Replace transfection mix with fresh Virus Producing Media                   | Optional          |

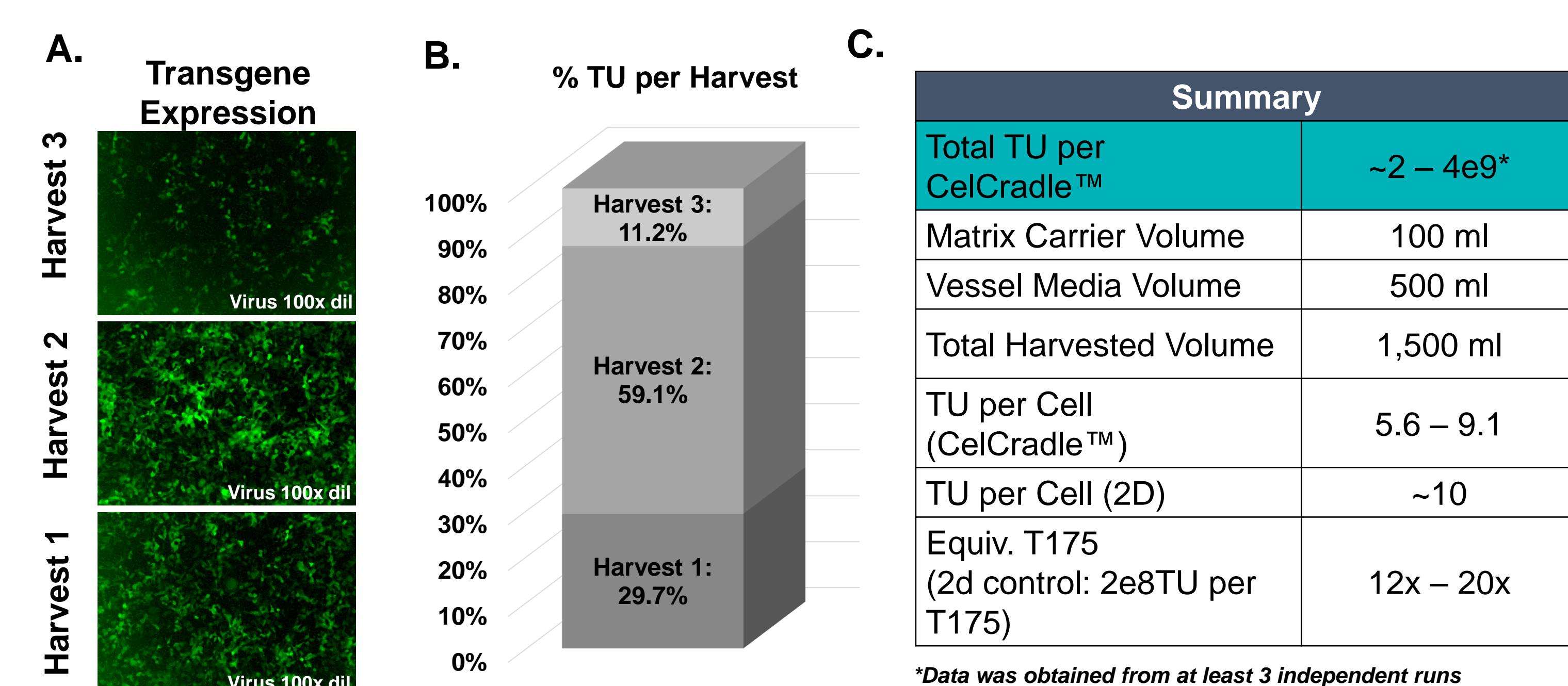


## Viral Production Day 2 to Day 4: Culture Monitoring / Viral Harvest

|                          |                   |                   |
|--------------------------|-------------------|-------------------|
|                          | <b>Conditions</b> | <b>Importance</b> |
| <b>pH</b>                | Maintain pH > 7.0 | NA                |
| <b>Glucose</b>           | > 0.5g/l          | NA                |
| <b>Harvest Timepoint</b> | 24h, 48h, 72h     | <b>Critical</b>   |



## Viral Quantification Day 4 to Day 7: Viral Titer via FACS



## Pipeline Flexibility

| Applicable to Virus Type        | Phase I: Seed | Phase II: Viral Production/Harvest |                         |                         |                         |                     |
|---------------------------------|---------------|------------------------------------|-------------------------|-------------------------|-------------------------|---------------------|
| <b>LV</b> (secreted virus)      | Seed          | Transfect <b>FOUR</b> plasmids     | Harvest 1 (supernatant) | Harvest 2 (supernatant) | Harvest 3 (supernatant) | NA                  |
| <b>RV</b> (secreted virus)      | Seed          | Transfect <b>THREE</b> plasmids    | Harvest 1 (supernatant) | Harvest 2 (supernatant) | Harvest 3 (supernatant) | NA                  |
| <b>AAV</b> (non secreted virus) | Seed          | Transfect <b>THREE</b> plasmids    | Harvest 1 (supernatant) | Harvest 2 (supernatant) | Harvest 3 (supernatant) | <b>Cell Harvest</b> |

## Scale-up Cell and Viral TU Count

|  | CelCradle™ (Lab Scale)    | TideXcell 2L (Pilot Scale) | TideXcell 20L (Production Scale)       | TideXcell 50L (Production Scale)       | TideXcell 100L (Production Scale)      |
|--|---------------------------|----------------------------|--|--|--|
| <b>Carrier Volume</b>                      | 0.1 L                     | 2 L                        | 20 L                                   | 50 L                                   | 100 L                                  |
| <b>Seeding Density</b>                     | 4.7x10 <sup>8</sup> cells | 9.3x10 <sup>9</sup> cells  | 9.3x10 <sup>10</sup> cells (Projected) | 2.3x10 <sup>11</sup> cells (Projected) | 4.7x10 <sup>11</sup> cells (Projected) |
| <b>LV TU per Batch</b>                     | 2-4x10 <sup>9</sup>       | 4-8x10 <sup>10</sup>       | 4-8x10 <sup>11</sup> (Projected)       | 1-2x10 <sup>12</sup> (Projected)       | 2-4x10 <sup>12</sup> (Projected)       |
| <b>Equiv. to T175 (175 cm<sup>2</sup>)</b> | 9.9X – 16.2X              | 198X – 324X                | 1980X – 3240X (Projected)              | 4950X – 8100X (Projected)              | 9900X – 16200X (Projected)             |