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

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Introduction

Viral Production Day 1: Quadruple Transfection

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Viral vectors such as adeno-associated virus (AAV), lentivirus (LV) and retrovirus (RV) are $\left[\left[\left$			Conditions	Importance	
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.			Growth Media	Standard HEK293T media	NA
			Transfection Media	Standard HEK293T media with low serum	NA
•	Protocols to produce viral vectors are largely empirical and the processes generally not well-characterized. These process vulnerabilities translate into high costs for commercially		Viral Producing Media	Transfection media with additive(s)	Critical
marketed gene therapies.		Pre-Transfection Preparation	Exchange cells into 450 ml of transfection media	Critical	
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.			Cell # per CelCradle™ on Day of Transfection	confidential	Critical
			Plasmid Ratio	confidential	Critical
			PEI 'MAX' : DNA Ratio	confidential	Critical
			DNA per Cell	confidential	Critical
			TideMotion® Parameters (6.5 hrs Transfection)	Uprate: 0.25 mm/sec; Uphold: 30 min; Downrate: 0.25 mm/sec; Downhold: 1 min	Critical
Challenges Faced Du	ring Viral Production V	Vhen Using Adherent	Transfection Period	6.5 hrs	NA
Bioreactors			Post-Transfection Treatment	Replace transfection mix with fresh Virus Producing Media	Optional
Challenge #1	Challenge #3	Challenge #4	S 18hr Post Transfection	48hr Post Transfection 72hr	Post Transfection
Low Plasmid Transfection Efficiency			The Expression		
Challenge #2	Bioreactors		Insger		
Low Viral Titer					

Cells, Media and Materials

CelCradle™ Bioreactor Hardware Cell Line Adherent HEK-293T (ATCC: CRL-3216) **Choice-of-Viral Vector** 3rd generation lentivirus pCMV-VSV-g Packaging Plasmid(s) pMDL-pRRE (obtained from AddGene) pRSV-Rev Transgene ~ 1.5 kb **Expression Plasmid** pExpression - mCherry - 3' LTR -----— 5' LTR promoter GFP

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

Monitoring of pH and Cell Count*

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Cell Metabolic*

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

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conc. (mg/l)

5000

Expression Plasmid Growth Media	Transgene ~ 1.5 kb pExpression -5' LTR - promoter GFP - mCherry - 3' LTR - Commercial Complete Media	$\begin{array}{c} 7.7 \\ 7.5 \\ 7.3 \\ 7.1 \\ 6.9 \\ 6.7 \\ 6.5 \end{array}$	Sound 4000 3000 2000 1000 Min. glucose conc threshold 0	0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0 0.0
Transfection Reagent	PEI 'MAX'	-24 0 24 48 72 Hours Post Transfection	-24 0 24 48 72 Hours Post Transfection * Live cell count was performed using trypsin harvest	-24 0 24 48 72 Hours Post Transfection * PrestoBlue Assay
Overall Pipeline fo	r Upstream Lentivirus Production	Viral Quantification Day	4 to Day 7: Viral Titer via	FACS
Day 1 (morning) Switch cells	Viral Production	A. Transgene B. Expression	C. % TU per Harvest	Summary
into low serum 2hrs prior transfection Day 0 Seed cells into CelCradle™ Bioreactor Day 1 (afternoon) Quadruple transfection 6.5 hrs Day 1 (evening) Replace transfection	Day 2 Harvest #1 Replace with fresh viral production media Day 3 Harvest #2 Replace with fresh viral production media Day 4 Harvest #3 Pool harvests; Clarify Concentrate virus Day 4 Harvest #3 Pool harvests; Clarify FACs Day 5 (evening) Transduce concentrated virus at clarifor Day 5 (evening) Transduce concentrated virus at clarifor Day 5 (evening) Transduce concentrated virus at clarifor Day 5 (evening) Transduce concentrated virus at clarifor Day 6 (evening) Transduce concentrated virus at clarifor	Expression 100% 90% 90% Virus 100x dil 80% 70% 60% 50% 50% 40% 30% 20% 20%	Harvest 1: Harvest 1: Harvest 1: Harvest 1: Harvest 1: Vessel N Total Ha TU per 0 (CelCrac Equiv. T	e^{TM} $\sim 2 - 4e9^{m}$ carrier Volume100 mlAedia Volume500 mlrvested Volume1,500 mlCell dle TM)5.6 - 9.1Cell (2D) ~ 10
production media		Yeirus 100x dil 10%	T175)	otained from at least 3 independent runs

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Monitoring of Media pH

Pipeline Flexibility

Applicable to Virus Type	Phase I: Seed	Phase II: Viral Production/Harvest
LV (secreted virus)	Seed	Transfect FOUR plasmidsHarvest 1 (supernatant)Harvest 2 (supernatant)Harvest 3 (supernatant)Harvest 3 (supernatant)

Viral Production Day 0: Cell Seeding

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

plasmids Transfect RV Harvest 2 Harvest 1 Harvest 3 NA THREE Seed (supernatant) (supernatant) (supernatant) (secreted virus) plasmids Transfect AAV Harvest 1 Harvest 2 Harvest 3 **Cell Harvest** THREE Seed (supernatant) (supernatant) (supernatant) (non secreted virus) plasmids

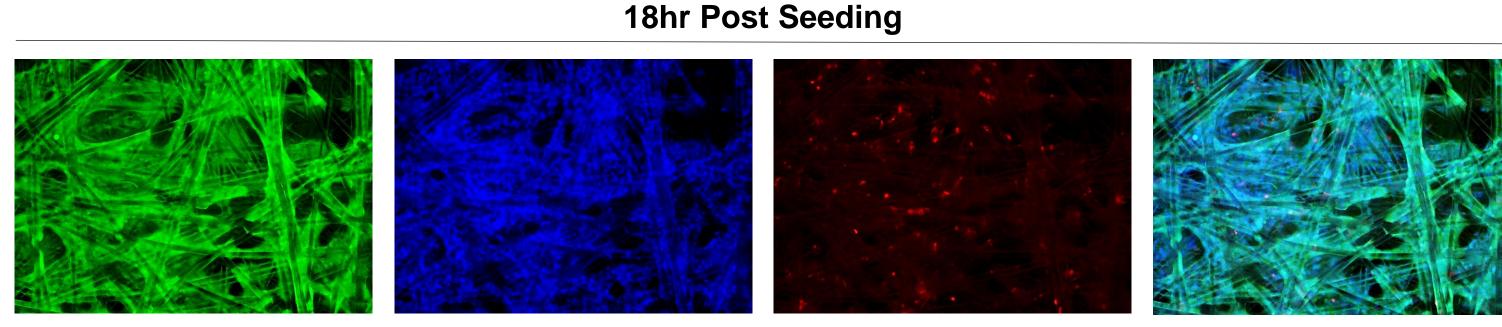
Scale-up Cell and Viral TU Count

	CelCradle™ (Lab Scale)	TideXcell 2L (Pilot Scale)	TideXcell 20L (Production Scale)	TideXcell 50L (Production Scale)	TideXcell 100L (Production Scale)
Carrier Volume	0.1 L	2 L	20 L	50 L	100 L
Seeding Density	4.7x10 ⁸ cells	9.3x10 ⁹ cells	9.3x10 ¹⁰ cells (Projected)	2.3x10 ¹¹ cells (Projected)	4.7x10 ¹¹ cells (Projected)
LV TU per Batch	2-4x10 ⁹	4-8x10 ¹⁰	4-8x10 ¹¹ (Projected)	1-2x10 ¹² (Projected)	2-4x10 ¹² (Projected)
Equiv. to T175 (175 cm2)	9.9X – 16.2X	198X – 324X	1980X – 3240X (Projected)	4950X – 8100X (Projected)	9900X – 16200X (Projected)

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Green: FDA (Live Cells)

Blue: Hoechst (Cell Nuclei)

Red:Propidium lodide (Dead Cells)

Merged Image