

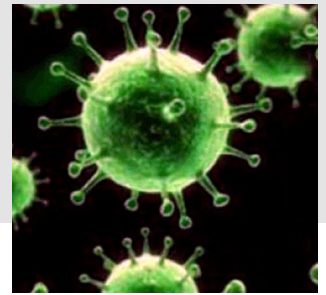
Large scale production of a lentiviral vector reference material – Upstream process

Anja Rodenbrock & Aziza Manceur

October 30, 2023



Goal of the project and background



1. Goal:

- Make lentiviral vector (LV) reference material that could be used by different members of the LV community to benchmark their own standards (in order to harmonize quantification methods across different laboratories)

2. Background:

- The project was initiated by a working group that met at the ISBioTech Conference
- The NRC volunteered to produce the LV material at no charge, but all the consumables were obtained through donations
- Similar initiatives have been undertaken for other viral vectors (Adenoviruses, AAV, etc.)

Viral Reference Materials

VIRAL REFERENCE MATERIALS



[Home Page](#) » Status: Completed and Available

Adenoviridae Image: Q-One BioTech / now part of [BioReliance](#)



[Home Page](#) » Status: List of Participants

HIV Lentivirus Image: Q-One BioTech / now part of [BioReliance](#)



3000 vials
0.5E8 - 1.0E8 TU/mL
0.5 mL/vial



[Home Page](#) » Status: Project In Progress

rAAV2 Image: [Dr. Richard O. Snyder et. al., University of Florida,](#)
[article](#) published in [BioProcessing Journal](#) Vol. 7/No 2.



[Home Page](#) » Status: List of Participants

Baculovirus Image: The Baculovirus Facility,
Department of Biochemistry, [University of Cambridge](#)



Status: [List of Participants](#)

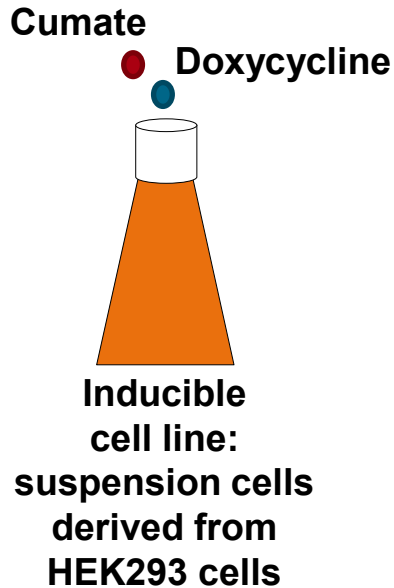
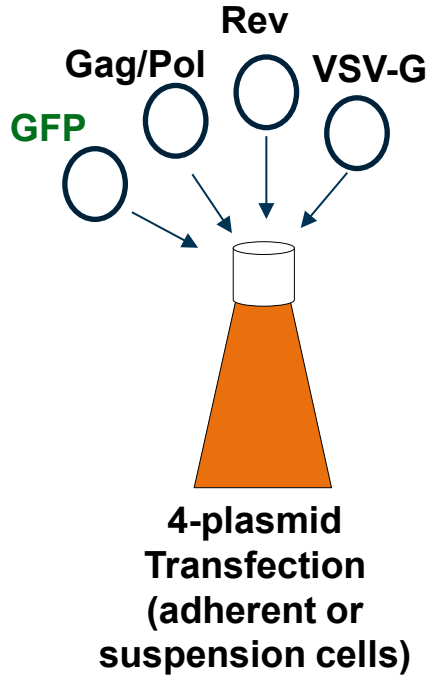
PERV Image: Dr. Klaus Boller / [Science Photo Library](#)



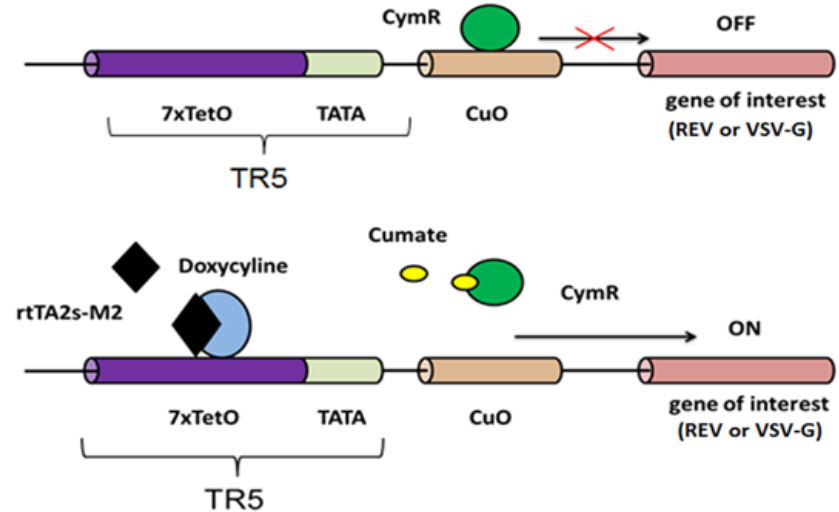
[Home Page](#) » Status: Project Ended

HERV Image: Russell Kightley / [Science Photo Library,](#)
as published in [NatureNews](#), 31 October 2006

Choice of production platform: Transfection vs. inducible system



Molecular switches: LV production is triggered by the addition of inducers



NRC's RFP: LV-production & purification

USP:

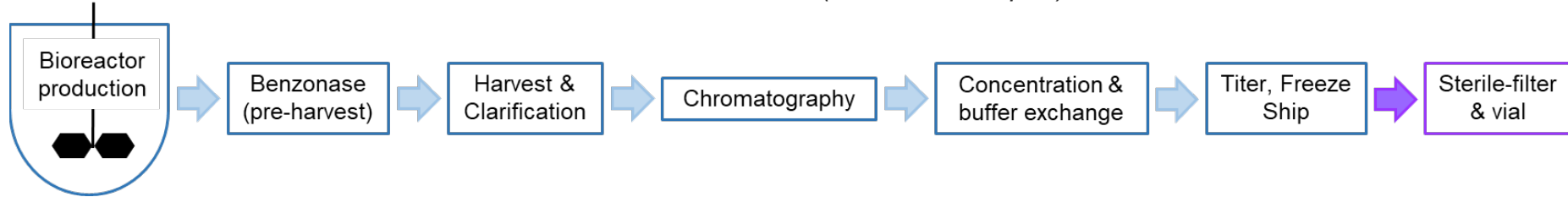
- Stable producer cell-line
- Expression of LV-GFP
- Serum-free / suspension culture

(summarized in this report)

DSP:

- Clarification
- Chromatography
- Concentration / buffer exchange
- Determine titer
- Freeze and ship for final filtration, vialing

(refer to DSP report)



Process optimization and scale-up strategy

Process selection & optimization at different scales:



Shake flask

- ✓ Testing of feeding schedules (VVP*)
- ✓ Confirmation & selection of final process (CCSU**)



BioBLU 3c

- ✓ Confirm process conditions and titer



BioBLU 10c

- ✓ Lock-in process conditions for XDR-200 and DSP
- ✓ Up to 4 runs

Production run:



Wave bioreactor

- ✓ Grow 25 L of inoculum for XDR-200



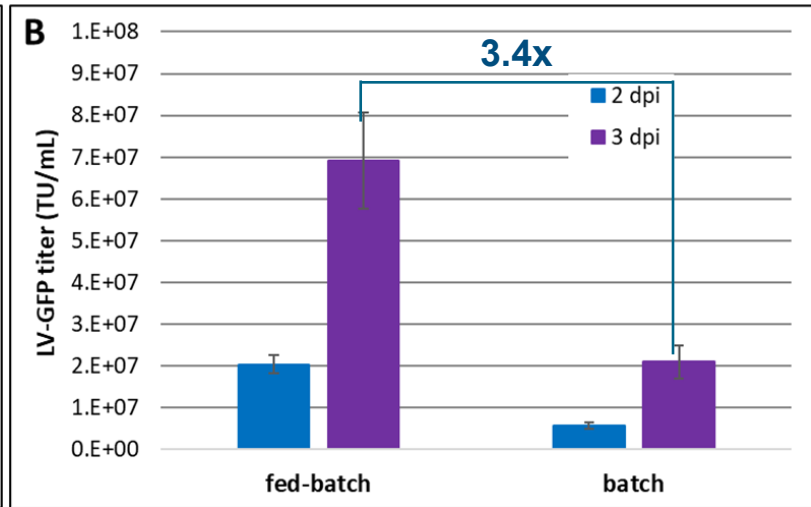
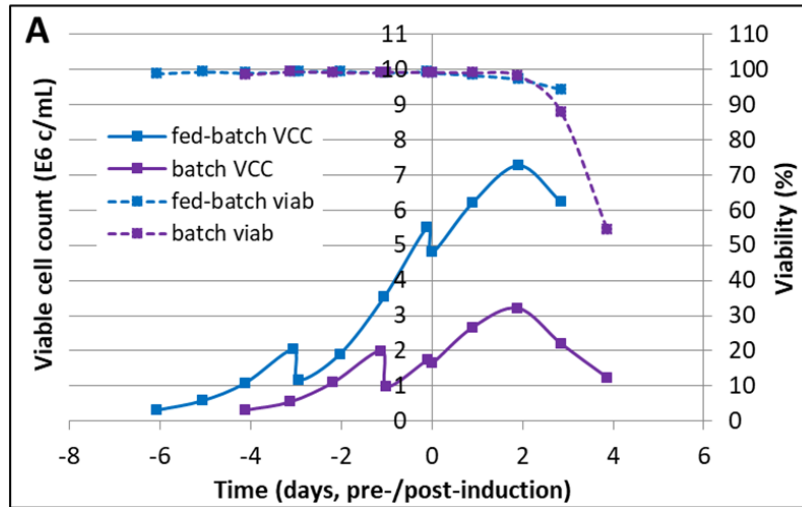
XDR-200

- ✓ Production of LVV-RM

* VVP: Viral Vector Production team at the NRC
** CCSU: Cell Culture Scale Up Team at the NRC

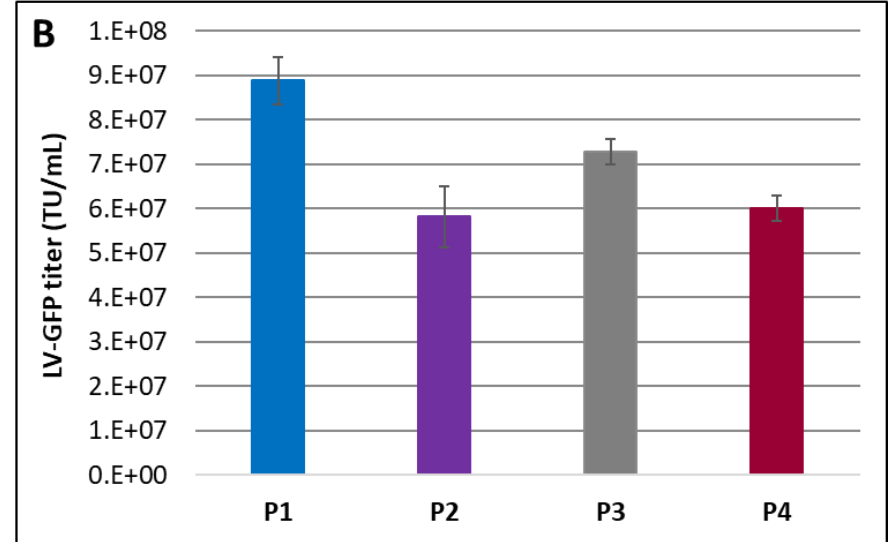
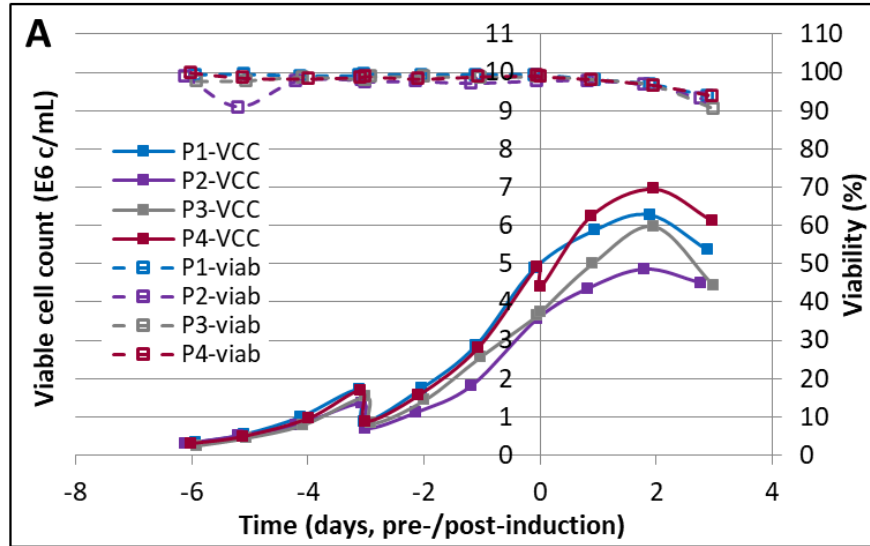
Fed-batch process validation in 3 L bioreactor

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Batch	Seeding	-	-	Dilution (1/2)	Induction	Butyrate	-	Harvest		
Fed-batch	Seeding	-	-	Dilution (1/2)	Feeding	-	Feeding + induction	Butyrate	-	Harvest



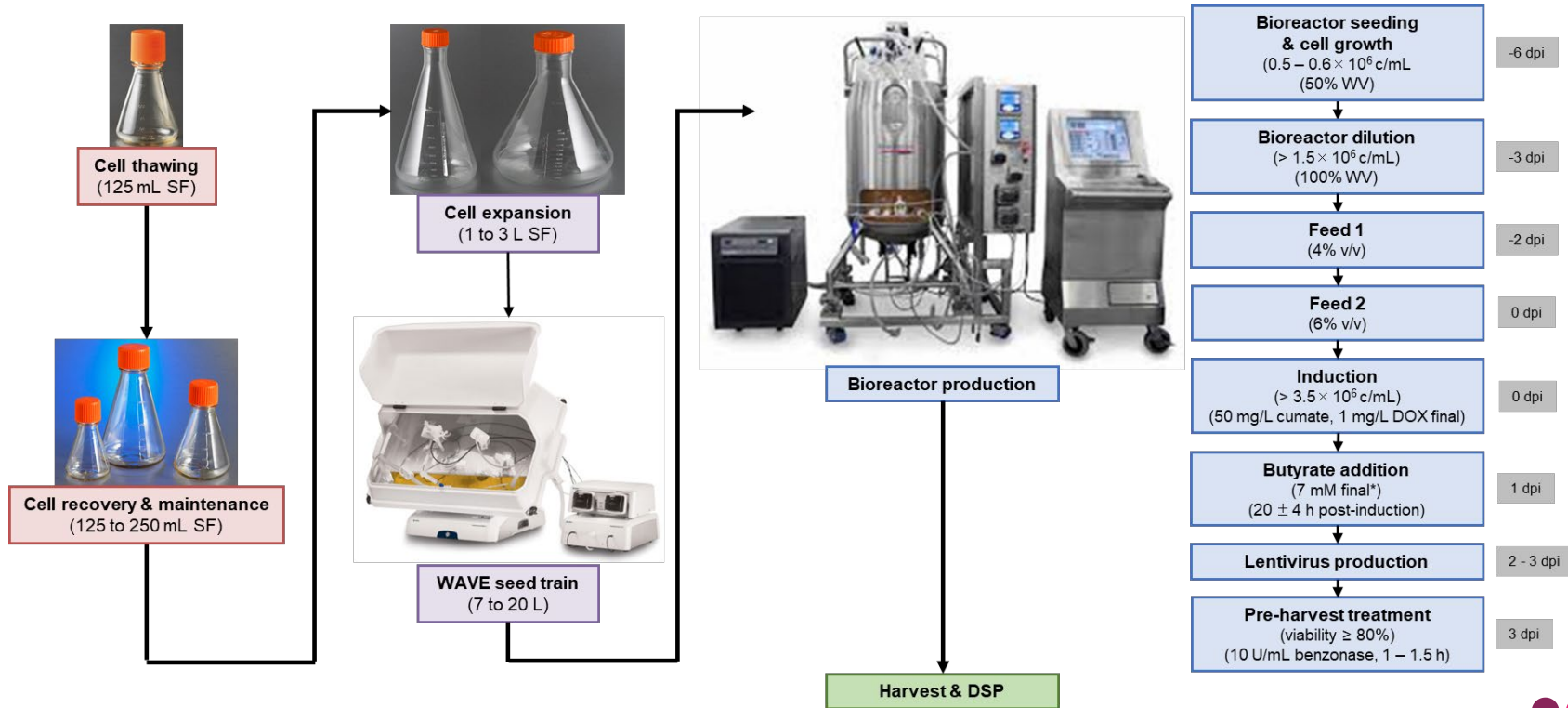
Viable Cell Count (VCC) increased by 2.3-fold (Fig. A) and infectious titer increased by 3.3-fold at 3pi (Fig. B)
 Note: Bioreactors executed in parallel using the same inoculum

Scale-up to 10 L bioreactor (Pilot runs P1 to P4)

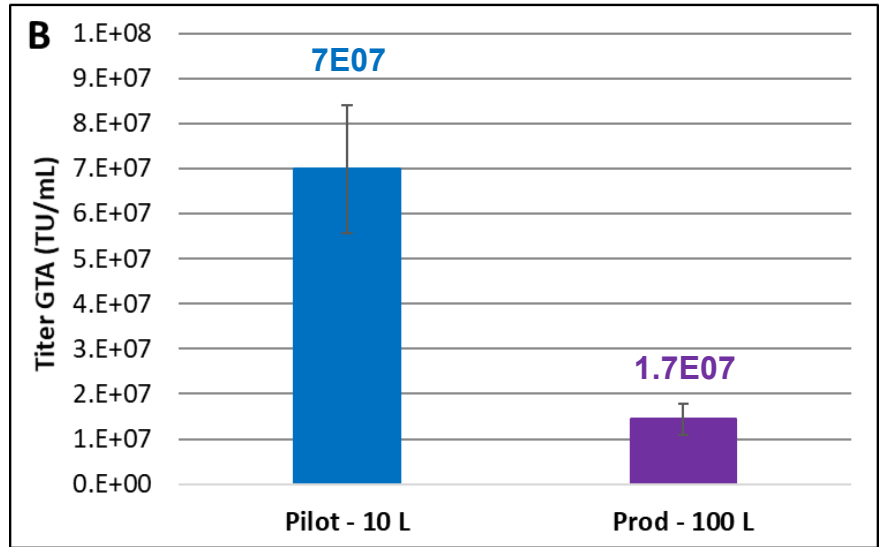
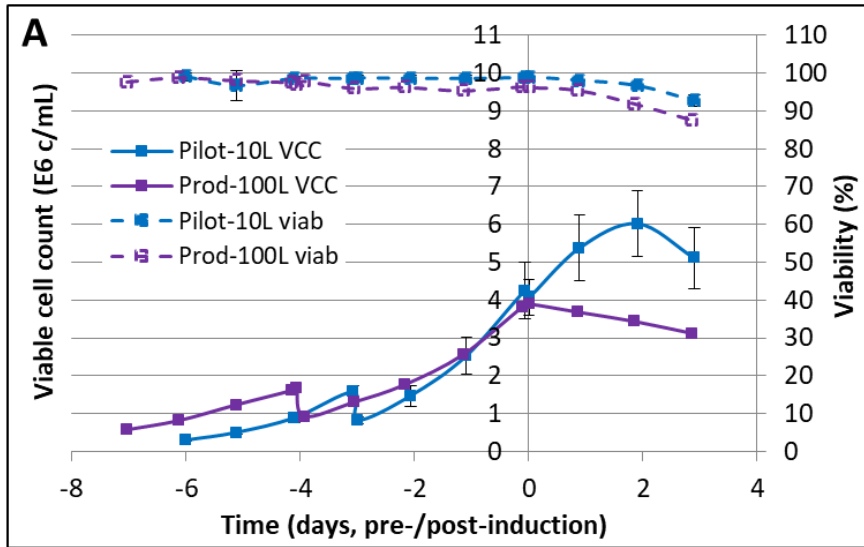


- Slightly different seeding densities: P1 = $3.3E05$ cells/mL P2/P4 = $3.0E05$ cells/mL P3 = $2.51E05$ cells/mL
- Similar profiles for VCC, viability, infectious titer (3 dpi); 91 - 94% viability at harvest
- Lower cell growth for P2 most likely due to slower cell growth of the seed train
- Infectious titers of $\geq 6E07$ TU/mL for all runs; lowest titer (P2 = $5.8E07$ TU/mL) most likely due to lower cell growth

100 L production: Process flow



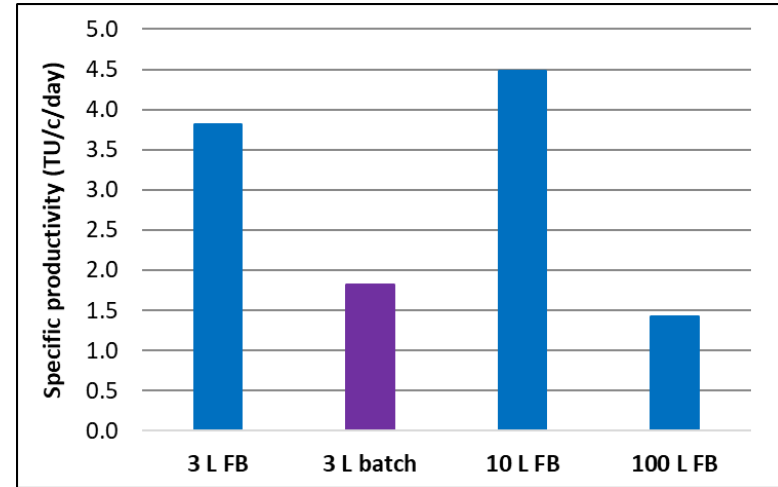
100 L production: Results



- Slower cell growth at production (100 L) vs. pilot (10 L) scale (despite higher seeding VCC)
- Comparable viability during growth phase, but slightly lower viability at harvest (87%)
- Infectious titer at production (100L) is 4 times lower than pilot runs (10L)
- Specific productivity at production (100L) is 3 times lower than pilot runs (10L)

Comparison across scales

Batch	Titer (E7 TU/mL)	Volumetric productivity (E7 TU/mL/d)	Specific productivity (TU/c/d)
Fed-batch, 3 L (n=1)	6.9 (3.3x)	2.3 (3.1x)	3.8 (2.1x)
Batch, 3 L (n=1)	2.1	0.74	1.83
Fed-batch, 10 L (n=4)	7.0	2.4	4.5
Fed-batch, 100 L (n=1)	1.44	0.51	1.4



() = fold increase vs. batch

- 3.3 fold improvement of fed-batch vs. batch in 3 L bioreactor
- Consistent scale-up from 3 L to 10 L bioreactor
- 4-fold lower titer in 100 L bioreactor and 3-fold decrease in specific productivity (vs. 3 – 10 L)
- Lower production yield is linked to lower VCC post-induction and lower specific productivity

General conclusions

- An international LV standard has become crucial for harmonization between institutions and regulatory agencies.
- NRC has developed a fed-batch process using a NRC proprietary stable/ inducible cell line that produces a LVV encoding a GFP-reporter cassette.
- A fed-batch process was developed: The timely addition of feed to our original batch process supported a higher cell density and a titer increase of about 3-fold
- Similar titers were obtained at 10 L pilot scale in four independent runs, demonstrating that the process is reproducible.
- Despite lower titers at 100 L scale, enough material was produced to meet the original goal. This material is expected to be available to the scientific community through ATCC in 2024

Donors & collaborators



Thank you
to NRC
staff!

**Viral Vector
Production team**

Réналd Gilbert
Sophie Broussau
Parminder Chahal

**Cell Culture
Scale-Up team**

Martin Loignon
Anja Rodenbrock
Danielle Jacob
Aziza Manceur
Ricardo Ochoa
Stéphane Lanthier
Elodie Burney
Julien Robitaille
Johnny Montes
Sven Ansorge
Sonia Tremblay

**Quality Attributes
and Characterisation
team**

Mauro Acchione
Julia Transfiguracion
Mimi Simmons
Mathieu Coutu

**Quality Assurance
team**

Nadia Mameri
Karen Roy
Joline Cormier

**Downstream
Purification team**

Krishnaraj Tiwari
Sushma G. Puttaswamaiah
Guillaume Arthus-Cartier
Mehul Patel
Martin Lafrance
Rosalva Ramirez
Smita Upamaka
Brahim Mahfoudi
Allan Matte
July Dorion-Thibaudeau

**Business
Development team**

Eileen Raymond
Alexandre Serrano
Alexandra Sinha

Department head:
Kelley Parato

THANK YOU

Anja Rodenbrock • RCO • anja.rodenbrock@nrc-cnrc.gc.ca
(CCSU Team)

Aziza Manceur • RO • aziza.manceur@cnrc-nrc.gc.ca
(CCSU Team)

