

LV Reference Material Project

Project Update

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February 2019



What?

- Produce reference material for LV
- Industry working group led by Keith Carson (IS Biotech conference series)
- Info on: isbiotech.org
- Similar work was done for AdV, AAV in the past
- Working group published several requests for proposal (RFP) for the different process steps (production, sterile filtration and vialing/repository, characterization/testing)
- NRC-HHT was mandated to produce LV reference material batch

Can update based on WG comments for clearer context



NRC mandate

- **NRC (A.Manceur/S.Ansorge) replied to proposal in May 2018**
- Stable producer cell line, batch mode, basic purification process was proposed
- NRC to produce, purify, sample, freeze and ship to repository site (now identified as ATCC)
- All information will be made publicly available + publication of production is goal

How much?

According to RFP:

Approximately 3000 vials will be needed, and each vial should contain 0.5 mL with a LVV RM concentration between $0.5E8$ and $1.0E8$ infectious genomes per mL (ig/mL).

→ Total yield needed: $7.5E10$ - $1.5E11$ tu

→ **At 10-20% DSP recovery, we need approx. 70-200 L batch size (see next slide)**

LV yield estimation

Batch Mode	LV upstream yield (tu/mL)	1.00E+06	5.00E+06	1.00E+07	5.00E+07	1.00E+08
LV DSP recovery (%)	Manufacturing Scale (L, bioreactor)					
10	5		5.00E+09	1.00E+10	5.00E+10	
20	10		1.00E+10	2.00E+10	1.00E+11	
30	20		2.00E+10	4.00E+10	2.00E+11	
40	50		5.00E+10	1.00E+11	5.00E+11	
50	100		1.00E+11	2.00E+11	1.00E+12	
60	150		1.50E+11	3.00E+11	1.50E+12	
70	200		2.00E+11	4.00E+11	2.00E+12	
80	500		5.00E+11	1.00E+12	5.00E+12	
Batch = single harvest						
	LV reference project					
volume	titer min	titer max	vials	LV purified	LV purified max	
	0.5	5.00E+07	1.00E+08	3000	7.50E+10	1.50E+11

- Upstream titers are 1E7 on average* in batch mode at NRC
- NRC prepares production scale of 100-200L for LV RM

* Manceur et al, 2017

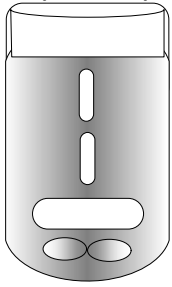
Note: Cell line history incorrectly described in paper 2



How to?

- **See next 2 slides for process flow**
- **Use single-use STR workflow at NRC, batch mode, serum-free, suspension, stable producer cell line**
- **Scale-up strategy:**
 - perform at least 1 ≥ 3 L pilot production including purification prior to LV RM production at scale

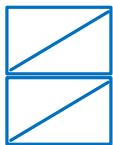
Test run: 10-200 L Batch production
(Clone 18)



10U/ml Denarase or Benzonase addition
(60min before harvest)

6% Sucrose addition (15min before harvest)

Clarification



Depth filter 1

Depth filter 2

Concentration



UF/DF (20x)
100 or 750 KDa

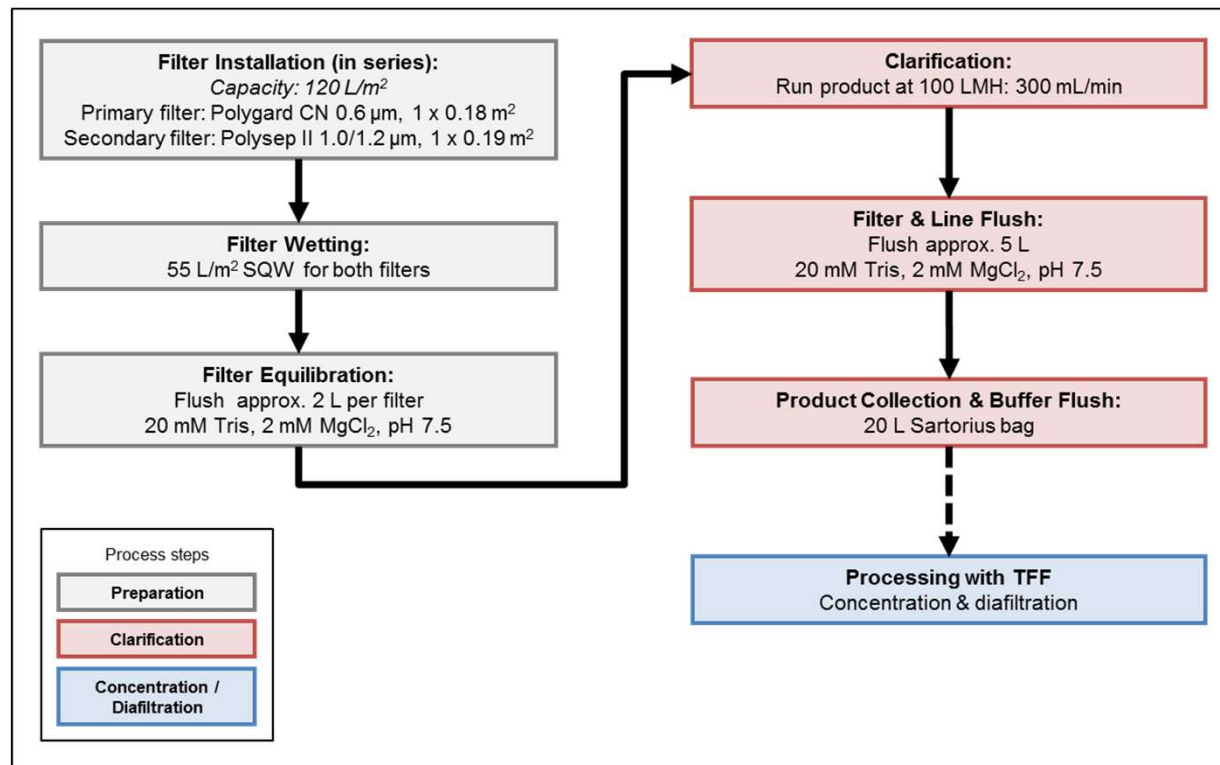
Titer, freeze and ship

Current Proposal

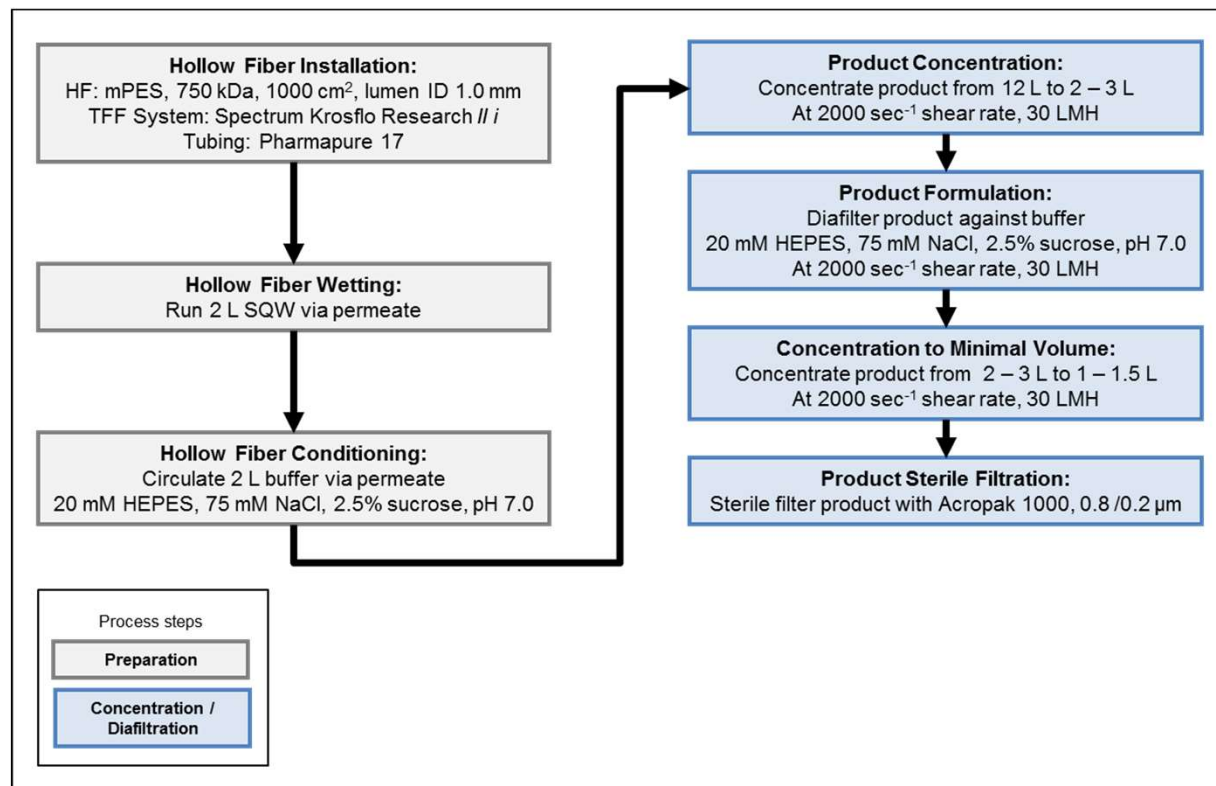
Updated
needed =
study plan
(by Anja)

- **Scale-up strategy: perform at least 1 ~10 L pilot production including purification, in order to:**
 - de-risk yield estimation
 - Generate purity and recovery data
 - Take decision for/against column chromatography step (e.g. Mustang Q)
 - For 10 L runs, material to ship to ATCC to de-risk sterile filtration

Clarification Step



TFF step



NRC Update January 2019

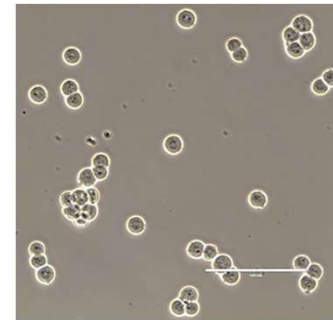
- **We need a minimum production volume of 7-10 L for DSP testing**
→ BioBLU 10 c cultures planned at NRC to de-risk scale-up
- **2x TFF steps, each approx. 10 fold concentration (i.e. from 200 L to 2 L)**
- **Overall recovery and purity: unknown → lab scale needed prior large-scale**
- **Decision point: with 10 L scale results – lock in conditions for scale-up run**

WG Meetings

- **T/C August 31, 2018:**
 - Proposed and decided to perform 3 L pilot runs
- **T/C December 10, 2018:**
 - Cell line history: clone 92 vs clone 18
 - Clone 18 (SIN) was selected
 - Decision on formulation buffer: 20 mM HEPES p H 7.0, 75 mM NaCl, 2.5% Sucrose
 - Based on PIPES patent (provided by V. Slepushkin)

NRC's HEK293 platform

- **Based on suspension-grown basal cell line**
- **Used directly for transient transfection**
- **Starting point for stable cell line generation**
- **Cell line history and cGMP banks available: ATCC (Manassas, VA), Core Cryolab (Toronto)**
 - Available for licensing or for R&D under MTA
- **Clinical and commercial track record**



Questions on Cell Line

- **Performance of clone 18**

- Critical to demonstrate at least equivalent performance in bioreactors for scale estimation
- Ongoing work at NRC
- stability completed: $>1E7$ TU/mL in batch after 7 weeks in culture
- shake flask results confirmed $>1E7$ TU/mL range in batch
- 3 L bioreactor run scheduled by end of April 2019
- Mycoplasma and sterility testing ongoing



NRC-ATCC collaboration for bulk to fill/finish

- Call with ATCC (Reed Shabman and Kurt) on December 11
- Can harvest be split in 2?
- Ship material from TFF#1 and TFF#2 to ATCC?
- ATCC to perform sterile filtration – losses expected, perform test run prior to 200 L production batch
- February 2019: Jenny Gronemus taking over from Reed Shabman at ATCC

NRC-WG-ATCC meeting on Feb 28

1. **Steps and samples of 3L pilot run to determine infectious titer (ig/mL), particle concentration, DNA, and total protein**
 - a. Before addition of Benzonase from bioreactor
 - i. With and without Benzonase?
 - b. Before addition of Trehalose from bioreactor
 - i. With and without Trehalose?
 - c. Just before harvest from bioreactor
 - d. After dead ended filters from dead ended clarification material
 - e. After 1st TFF post-concentration
 - f. After 2nd TFF post-concentration
 - g. After shipment to ATCC
 - h. After sterile filtration
 - i. 0.45 µm or 0.2 µm filter?
2. **Thawing prior to filtration**
 - a. Thaw conditions
3. **Material lists**
4. **Shipping of 3L pilot run material**
 - a. Courier
 - b. Shipping process
 - c. Shipping address
5. **Vialing**
 - a. Label text for vials of product run material

Webinar



Next steps/open questions

- **Review material list – Anja**
- To be finalized in week of February 25
- **Write-up study plan - Anja**
- To be finalized in week of February 25 – draft ready
- **Material/Cell Line Transfer to ATCC**
- For cell line only, we can use standard Salk-NRC MTA approach conditional to deposit at ATCC (not a transfer for distribution purposes)
- No MTA needed for LV product generated using clone 18

Questions from NRC to WG 1/2

- **WG: cell line transfer needed (or MTA signed) prior to 10 L pilot runs?**
- **ATCC-NRC: Jenny and my colleagues at NRC to come up with an initial proposal to ship material from smaller scale runs to you of course material from your pilot-scale run would go to Jenny at ATCC for sterile filtration, vialing and storage. *Then we can request that ATCC send vials to characterization contributors who can corroborate test results we receive from NRC in order to de-risk sterile filtration? → great idea!***

Questions from NRC to WG 2/2

- Should NRC come forward as characterization site?
- GLP? Vs NRC quality system

Lentiviral Vector Reference Material (LVV RM) Request for Proposal - RFP 3.0

Characterization

1.1 Introduction

This RFP is being distributed to individuals who have chosen to serve on the LVV RM working group (WG), and whose names and affiliations can be found on the International Society for BioProcess Technology (ISBioTech) website at <https://www.isbiotech.org/ReferenceMaterials/working-group.html>. We may also distribute this document to others we know may be interested in participating.

This Request for Proposal (RFP) is intended to recruit labs that will help characterize a Lentiviral Vector Reference Material (LVV RM). Your involvement in this work must be considered a "donation" in which you will not receive monetary compensation. However, we will distribute another RFP in which we will request that your key consumable materials be donated for your use. In addition, we will show your organization as a key contributor on our website, as well as in signs and announcements at ISBioTech meetings.

1.2 Purpose / Use of the LVV RM

The Office of Tissue and Advanced Therapies (OTAT), Center for Biologics Evaluation and Research (CBER), FDA has recommended that sponsors use viral vector reference materials to which the infectious titer and particle concentration of their laboratory's internal reference materials can be compared. Furthermore, the LVV RM can be used to validate internal assays for particle concentration and infectious titer where the results can be compared to those of the reference material. However, ongoing validation work should be performed using your laboratory's internal reference materials, as the availability of the LVV RM will be limited.

Sponsors of lentivirus-related INDs should consult with OTAT/CBER for further guidance. However, it is not the intent of OTAT/CBER to standardize assay methods across the field, or to require that the values assigned to the LVV RM be duplicated during validation studies. There is no requirement that the LVV RM procedures suggested in this RFP be mandatory for particle concentration or infectious titer determinations.

1.3 Vector Specification

- A lentiviral vector containing a GFP transgene, pseudotyped with VSV-G
- Produced by chemical induction of a stable HEK293-based cell line that is grown in suspension with serum-free medium
- Total amount requested is 3,000 vials, each containing 0.5 mL
- LVV RM, with an infectious titer between 0.5 and 1×10^8 infectious genomes per mL (i.g/mL)



NRC-CNRC

NRC.CANADA.CA   

THANK YOU



National Research
Council Canada

Conseil national de
recherches Canada

Canada 

AOB: add sampling plan to study plan

Before the conference call next week, I'd like to establish the steps and samples associated with the 3L pilot run, in which we need to establish where, when, what, and why we'll take each sample.

The following table is a rough, first attempt to identify what we want to do. This really needs simplification, so please start whacking away at it. What else do we want to look for? I suggest that at least two samples be taken for each step, and that we have at least two labs do the analysis.

Then to some degree, we'll be able to evaluate the "cold chain" involved in transporting the material from NRC to ATCC. And we may want to do an accelerated stability test (room temperature, and higher) for X hours.

Where	When	What	Why	Notes
Bioreactor	Before addition of Benzonase	<ul style="list-style-type: none"> Infectious titer in ig/mL Particle Concentration DNA Total protein 	Determine titer, etc before Benzonase	
Bioreactor	Before addition of Trehalose	<ul style="list-style-type: none"> Infectious titer in ig/mL Particle Concentration DNA Total protein 	Determine titer, etc after Benzonase	Is Benzonase really needed?
Bioreactor	Just before harvest	<ul style="list-style-type: none"> Infectious titer in ig/mL Particle Concentration DNA Total protein 	Determine titer, etc after Trehalose	Is Trehalose really needed?
Dead Ended Clarification Material	After Dead Ended Filters	<ul style="list-style-type: none"> Infectious titer in ig/mL Particle Concentration DNA Total protein 	Determine titer, etc after clarification	See how much we'll lose from this step
Post Concentration	After First TFF	<ul style="list-style-type: none"> Infectious titer in ig/mL Particle Concentration DNA Total protein 	Determine titer, etc after first concentration step	See how much we'll lose from this step
Post Concentration	After Second TFF	<ul style="list-style-type: none"> Infectious titer in ig/mL Particle Concentration 	Determine titer, etc after 2 nd concentration step	See how much we'll lose from this step

