LV Reference Material Project
Project Update
Sven Ansorge
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**

<table>
<thead>
<tr>
<th>Batch Mode</th>
<th>LV upstream yield (tu/mL)</th>
<th>1.00E+06</th>
<th>5.00E+06</th>
<th>1.00E+07</th>
<th>5.00E+07</th>
<th>1.00E+08</th>
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</thead>
<tbody>
<tr>
<td>LV DSP recovery (%)</td>
<td>Manufacturing Scale (L, bioreactor)</td>
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<td></td>
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<tr>
<td>10</td>
<td>5</td>
<td>5.00E+09</td>
<td>1.00E+10</td>
<td>5.00E+10</td>
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<td></td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>1.00E+10</td>
<td>2.00E+10</td>
<td>1.00E+11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>2.00E+10</td>
<td>4.00E+10</td>
<td>2.00E+11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>50</td>
<td>5.00E+10</td>
<td>1.00E+11</td>
<td>5.00E+11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>1.00E+11</td>
<td>2.00E+11</td>
<td>1.00E+12</td>
<td></td>
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<tr>
<td>60</td>
<td>150</td>
<td>1.50E+11</td>
<td>3.00E+11</td>
<td>1.50E+12</td>
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<td></td>
</tr>
<tr>
<td>70</td>
<td>200</td>
<td>2.00E+11</td>
<td>4.00E+11</td>
<td>2.00E+12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>500</td>
<td>5.00E+11</td>
<td>1.00E+12</td>
<td>5.00E+12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Batch = single harvest

**LV reference project**

<table>
<thead>
<tr>
<th>volume</th>
<th>titer min</th>
<th>titer max</th>
<th>vials</th>
<th>LV purified min</th>
<th>LV purified max</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5.00E+07</td>
<td>1.00E+08</td>
<td>3000</td>
<td>7.50E+10</td>
<td>1.50E+11</td>
</tr>
</tbody>
</table>

- 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
Current Proposal

• 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

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

Updated needed = study plan (by Anja)
Clarification Step

Filter Installation (in series):
Capacity: 120 L/m²
Primary filter: Polygard CN 0.6 μm, 1 x 0.18 m²
Secondary filter: Polysep II 1.0/1.2 μm, 1 x 0.19 m²

Filter Wetting:
55 L/m² SQW for both filters

Filter Equilibration:
Flush approx. 2 L per filter
20 mM Tris, 2 mM MgCl₂, pH 7.5

Clarification:
Run product at 100 LMH: 300 mL/min

Filter & Line Flush:
Flush approx. 5 L
20 mM Tris, 2 mM MgCl₂, pH 7.5

Product Collection & Buffer Flush:
20 L Sartorius bag

Processing with TFF
Concentration & diafiltration
TFF step

**Hollow Fiber Installation:**
- HF: mPES, 750 kDa, 1000 cm², lumen ID 1.0 mm
- TFF System: Spectrum Krosflo Research II
- Tubing: Pharmapure 17

**Hollow Fiber Wetting:**
- Run 2 L SQW via permeate

**Hollow Fiber Conditioning:**
- Circulate 2 L buffer via permeate
  - 20 mM HEPES, 75 mM NaCl, 2.5% sucrose, pH 7.0

**Product Concentration:**
- Concentrate product from 12 L to 2 – 3 L
  - At 2000 sec⁻¹ shear rate, 30 LMH

**Product Formulation:**
- Diafiltrate product against buffer
  - 20 mM HEPES, 75 mM NaCl, 2.5% sucrose, pH 7.0
  - At 2000 sec⁻¹ shear rate, 30 LMH

**Concentration to Minimal Volume:**
- Concentrate product from 2 – 3 L to 1 – 1.5 L
  - At 2000 sec⁻¹ shear rate, 30 LMH

**Product Sterile Filtration:**
- Sterile filter product with Acropak 1000, 0.8/0.2 μm
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 pH 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
Cell Line History

HEK293SF-3F6
RCB
Transfection with Gag-pol, rev, VSV-G from Salk Institute

293SF-PacLV#29-6
packaging cell line

Transduction with LV ‘Tet07CSII-CMV-GFP’

Clone 92
Expressing a cSIN-LV

Transfection with CSII-CMV-GFP

Clone 18
Expressing a SIN-LV

Selected by WG for LV reference material

1: Broussau et al, 2008
2: Manceur et al, 2017
Note: Cell line history incorrectly described in paper 2
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
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
THANK YOU
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.

<table>
<thead>
<tr>
<th>Where</th>
<th>When</th>
<th>What</th>
<th>Why</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioreactor</td>
<td>Before addition of</td>
<td>- Infectious titer in g/mL</td>
<td>Determine titer, etc before Benzonase</td>
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<tr>
<td></td>
<td>Benzonase</td>
<td>- Particle Concentration</td>
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<td></td>
<td></td>
<td>- DNA</td>
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<td></td>
<td></td>
<td>- Total protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioreactor</td>
<td>Before addition of</td>
<td>- Infectious titer in g/mL</td>
<td>Determine titer, etc after Benzonase</td>
<td>Is Benzonase really needed?</td>
</tr>
<tr>
<td></td>
<td>Trehalose</td>
<td>- Particle Concentration</td>
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<tr>
<td></td>
<td></td>
<td>- DNA</td>
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<td></td>
<td></td>
<td>- Total protein</td>
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</tr>
<tr>
<td>Bioreactor</td>
<td>Just before harvest</td>
<td>- Infectious titer in g/mL</td>
<td>Determine titer, etc after Trehalose</td>
<td>Is Trehalose really needed?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Particle Concentration</td>
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<td></td>
<td></td>
<td>- DNA</td>
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<td></td>
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<td></td>
<td></td>
<td>- Total protein</td>
<td></td>
<td></td>
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<tr>
<td>Dead Ended</td>
<td>After Dead Ended</td>
<td>- Infectious titer in g/mL</td>
<td>Determine titer, etc after clarification</td>
<td>See how much we’ll lose from this step</td>
</tr>
<tr>
<td>Clarification</td>
<td>Filters</td>
<td>- Particle Concentration</td>
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<td></td>
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<td>- Total protein</td>
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<tr>
<td>Post</td>
<td>After First TFF</td>
<td>- Infectious titer in g/mL</td>
<td>Determine titer, etc after first concentration step</td>
<td>See how much we’ll lose from this step</td>
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<tr>
<td>Concentration</td>
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<td>- Particle Concentration</td>
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<td>- Total protein</td>
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<tr>
<td>Post</td>
<td>After Second TFF</td>
<td>- Infectious titer in g/mL</td>
<td>Determine titer, etc after 2nd concentration step</td>
<td>See how much we’ll lose from this step</td>
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<td>- DNA</td>
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