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Production of Lentiviral Vector Reference Material

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The Production of Lentiviral Vector Reference Material

1. Background and objectives

A request for proposal was distributed by IS BioTech. The goal is to make lentiviral vector (LV) reference material that could be used by different members of the LV community. This material could be used to validate quantification methods across different laboratories.

The material requirements are:

- 3000 vials
- Vials of 0.5 to 1ml
- Titer of 0.5E08 to 1.0E08 ig/mL
- The LV must encode a transgene easily detected by FACS (such as GFP)
- Endotoxin-free and sterile LV material
- A GMP process is preferred, but a well-documented process is acceptable
- The cell line used for production must be well-characterised, sterile and free of mycoplasma

2. Workplan

We propose to prepare the LV material using a producer cell line named clone 92; this clone is derived from HEK293 cells, and grows in suspension in serum-free media [1,2]. The LV produced expresses GFP. The production of LV is induced by the addition of doxycycline and cumate. The proposed production and purification processes are outlined below.

2.1 Description of a 200L production in batch mode

Description of Work

Our current process is performed in a serum-free media from Hyclone (HyClone™ HyCell™ TransFx™-H Media). Cells will be expanded in a 50L wave bag (25L working volume). They will be transferred to a 200L SUB (Xcellerex, GE) along with 75L media. After 48-72hrs, the cells will be further diluted two times, for a final volume of 200L. The next day, they should reach a cell density of about 1E06 cells/ml. We will then induce with cumate and doxycycline, and harvest 72hrs post-induction. The complete production process should take 10 to 11 days. An approximate titer of

1E07 TU/ml is expected at the time of harvest in the supernatant (Figure 1). Please note that the largest LV volume previously produced at NRC in a single batch is 70L in the 200L SUB. However, we routinely produce other viral vectors at the 500L scale.

Budget Estimate

The following items would have to be provided by the LV reference group or through donations:

- One bag for the 200L SUB = 5000$
- One 50L wave bag = 500$
- A minimum of 225L HyClone™ HyCell™ TransFx™-H Media = 22 500$
- 6g Cumate and doxycycline = 500$
- Other reagents (Probe sheaths, tubing, single use sample manifold, etc) = 2500$
- Sub-total production = 31 000$

The NRC will provide the operators and the facility.

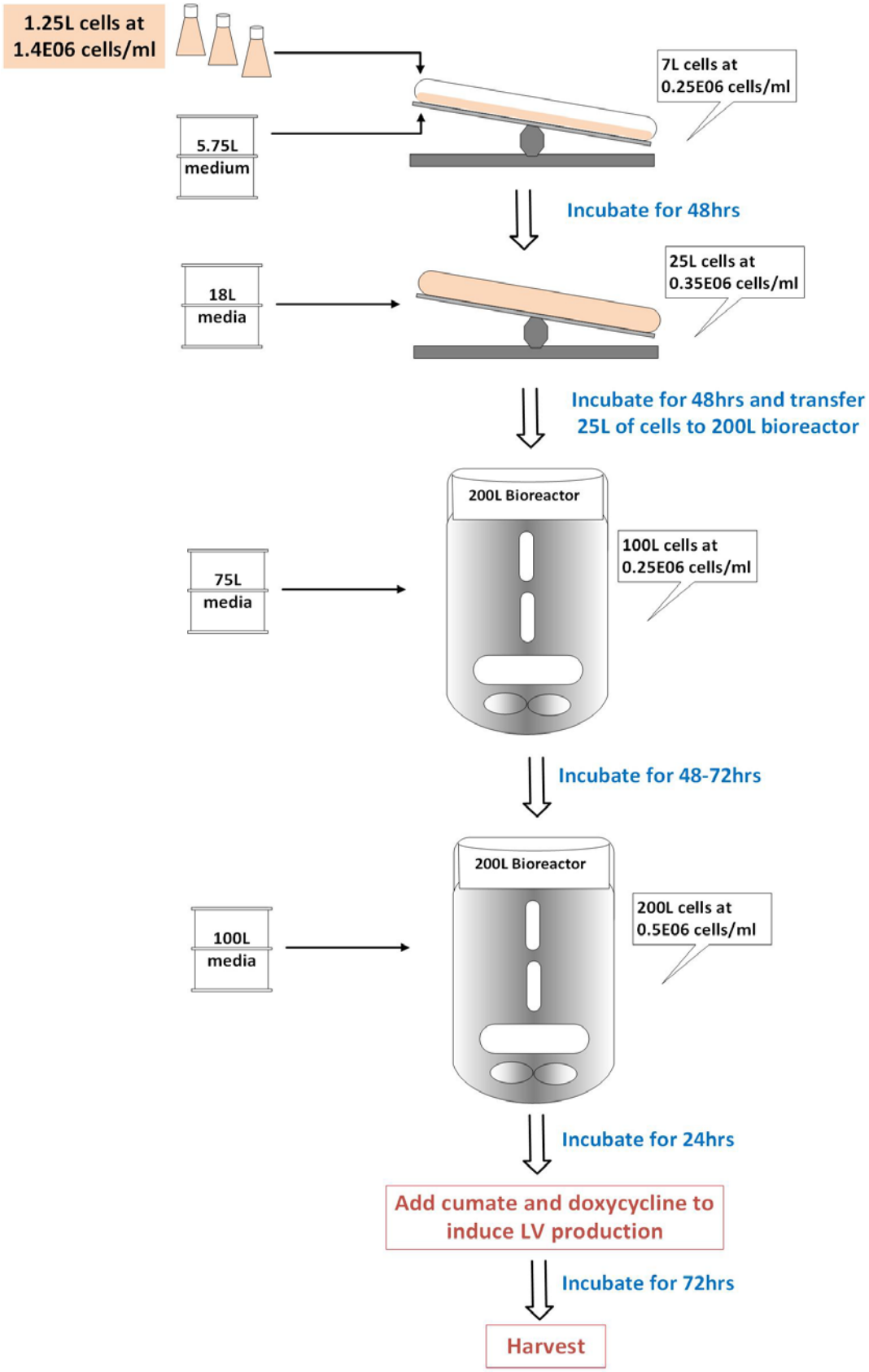

Figure 1: Overview of the production process

2.2 Purification process

Description of work

Our current purification process is composed of the following steps:

1) Nucleic acid digestion will be performed for 1hr at 37°C in the 200L SUB directly.
2) Clarification with filters and supplement: 6% trehalose is added directly into the 200L SUB 15min before harvesting. A train of two depth filters is then used to clarify the supernatant. A recovery of 75% is expected after this step.
3) TFF1: Concentration and diafiltration by ultrafiltration. A 7 to 10x concentration is expected.
4) TFF2: Concentration and diafiltration by ultrafiltration (TFF2). A 7 to 10x concentration is expected.
5) Retain samples to determine the titer (by ddPCR and flow-based titration of GFP expression) and identity by p24 ELISA.
6) Freeze and ship to the next facility, where sterile filtration (0.22µm) and vialing will take place. A 50% loss is expected after sterile filtration.

Before freezing, samples will be retained in order to measure the titer by flow cytometry, ddPCR and p24 ELISA. An overview of the process is shown in figure 2.
Note that the proposed clarification process was tested at a 3L scale only at NRC. The UF/DF was performed at the 70L scale. To de-risk the 200L clarification step, a test run could be performed at the 50L scale.
Alternatively, a different DSP process could be considered. We are currently working on a chromatography-based protocol and we expect to have an optimized process by the fall 2018.

Budget Estimate

The following items would have to be provided by the LV reference group or through donations:

- Enzyme for nucleic acid digestion = 10 000$
- 20L Trehalose at 60% = 12Kg trehalose = 14 000$ (Sucrose could be considered and is a cheaper option)
- Two depth filters for clarification = 6000$
- Two filters for UF/DF = 10 000$
- Tubing and connectors = 3000$
- Bags for buffer preparation and pump head for the concentration scid= 5 000$
- Sub-total DSP = 48 000$

The NRC will provide the operators and the facility.

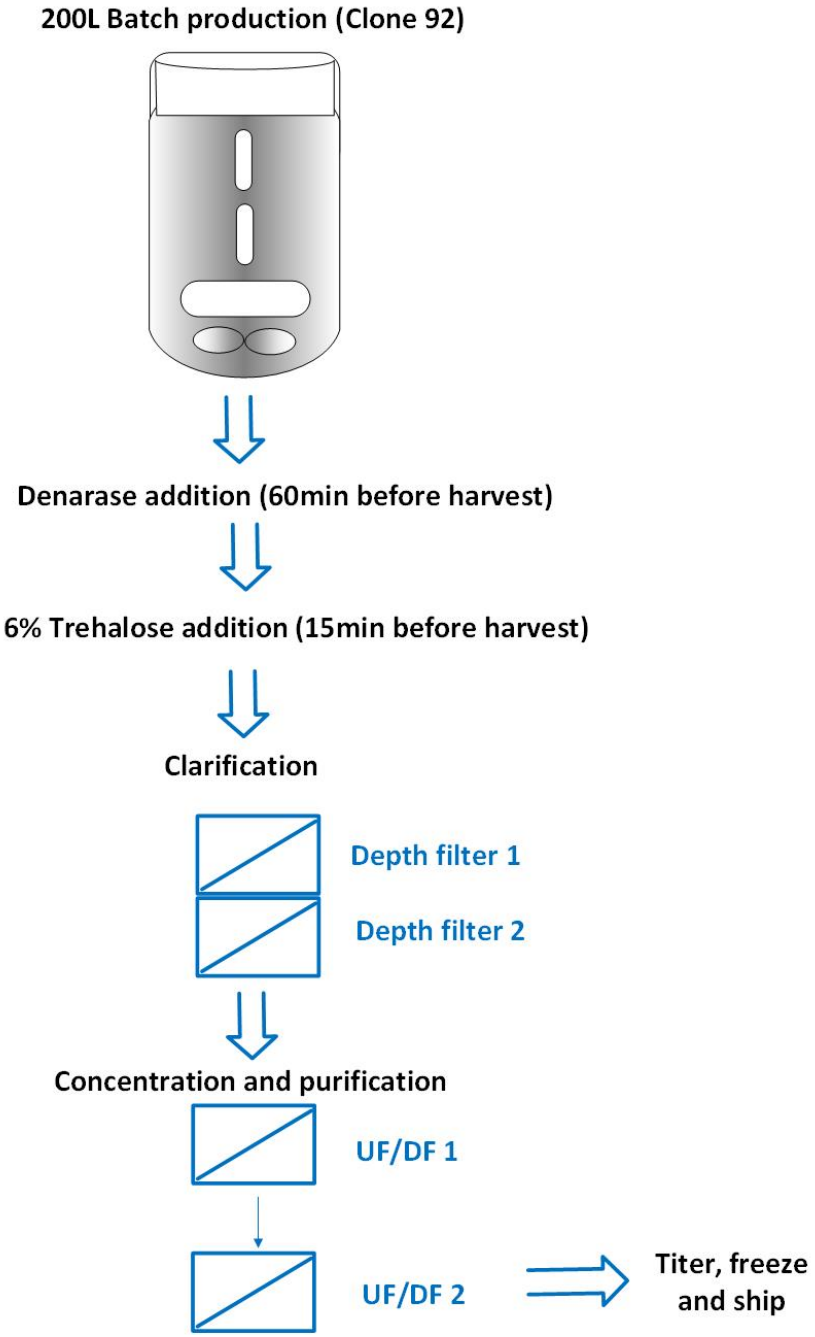

Figure 2: Overview of the concentration and purification process

3. Estimates for volumes and final titer

On average, the titer obtained in the supernatant before clarification is 1E07 TU/ml with clone 92. We expect to lose about 25% in yield after clarification and another 50% after sterile filtration. The final recovery after sterile filtration should be between 7 and 20% based on previous work at 3 to 70L scale. Therefore, the volumes required before clarification to achieve the minimal requirements

of the RFP (3000 vials at 0.5E08 TU/ml) will vary from 215L to 75L depending on the final recovery. We are within the specification of the proposal, but close to the minimal amount required if the recovery is under 10%. To make sure that we achieve the volume of material required, a 300-400L production could be performed in a 500L stainless steel bioreactor. However, the risk associated with using a non-disposable vessel will have to be evaluated.

4. Testing for product release

The infectious titer will be measured using a gene transfer assay (GTA) and flow cytometry. HEK293A cells are used as target cells. Prior to transduction, LV samples are serially diluted in DMEM supplemented with 8 µg/mL polybrene and incubated at 37°C for 30 min. Transduction is performed by removing culture medium, adding 200µl diluted LV to cells and incubating overnight at 37°C. The next day, 800µl culture medium is added to each well and cells are incubated for an additional 48 hours prior to flow cytometry to quantify GFP expressing cells.

If required, we also have the capacity to measure the titer by ddPCR.

Identity will be confirmed using a p24 ELISA kit (Cell Biolabs) according to the manufacturer's instructions. This is a kit we use routinely.

5. Storage and shipping

After concentration and purification (UF/DF 2), we expect a final volume of 3L. The material will be frozen in 1L aliquots at -80oC. The material will then be shipped to the institution that will do the sterile filtration and vialing by World Courier (they offer temperature control solutions such as adding dry ice if the package is blocked at customs for instance). Shipping will cost approximately 1000$.

6. Description of the facility and qualifications of the individuals

NRC does not operate a GMP facility, but we have a quality system in place that supports the writing of detailed documents for IND submissions for instance. Most of our personnel has more than 15 years of experience. With clone 92, we have produced about 20 runs at the 3L scale, 1 run at the 20L scale and 1 run at the 200L scale (70L working volume). The clarification procedure described in section 2.2 was performed with material from shake flasks, and at the 3L bioreactor scale. The concentration and diafiltration steps (UF/DF) were performed at the 200L scale (70L working volume).

7. Other considerations and assumptions

- We assume that the safety testing and vialing (including 0.22 µm sterile filtration) will be performed in other facilities.
- We assume that the consumables, such as culture media, bioreactor bags and filters will be provided to the NRC through donations and coordinated by the working group or IS BioTech.
- Note that NRC cannot be in competition with other Canadian manufacturers/CMOs. We will give priority to other Canadian groups if they express interest in producing the LV reference material, or perform the work in collaboration with them.
- The plasmids used to make clone 92 were obtained from the Salk institute. As a result, we assume that a tri-partite agreement will be put in place before we can provide the cell line to IS BioTech.
- Note that sterility and mycoplasma testing of clone 92 is ongoing.
- This is a preliminary proposal. We assume that we will get approval from NRC higher management to proceed with this project if it is selected.
- The NRC will put all the resources and efforts towards a successful production. However, the NRC cannot be held responsible in case of a failure during the run, due to a mechanical failure or contamination. Contingency material (media, bioreactor bags and filters) would have to be planned and acquired by the Reference Material group if needed.
- The NRC will provide all the relevant laboratory batch records and 20 vials of sterile and mycoplasma-free cell line to IS Biotech.
- <u>The final cost estimate for reagents, consumables and shipping is 80 000$.</u>

References

1. Broussau S, Jabbour N, Lachapelle G, et al. Inducible packaging cells for large-scale production of lentiviral vectors in serum-free suspension culture. *Mol Ther.* 2008;16(3):500-507.
2. Manceur AP, Kim H, Misic V, et al. Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. *Hum Gene Ther Methods.* 2017;28(6):330-339.