Lentiviral Vector Reference Material (LVV RM)
Request for Proposal – RFP 1.0
Vector Production and Purification

1.0 Introduction

This document is a Request for Proposal (RFP 1.0) for any organization to submit a detailed plan to produce and purify a Lentiviral Vector that will be used as a Reference Material (LVV RM). Other aspects of the production and characterization work will be addressed in subsequent RFPs. This RFP is being distributed to individuals who have chosen to serve on the Working Group (WG) for this project, and whose names and affiliations can be found on the International Society for BioProcess Technology (ISBioTech) website at: http://www.isbiotech.org/ReferenceMaterials/lentivirus-home.html.
However, RFPs may be sent to a wider distribution of individuals and organizations known to be invested in the production and use of Lentiviral Vectors. ISBioTech has been selected to coordinate / facilitate this project due to its long-term commitment to viral vectors, plus its involvement in the Ad5 reference material project in 2001. Questions and comments should be directed to lvwg@isbiotech.org.

2.0 Purpose / Use of the Lentiviral Vector Reference Material

The FDA Center for Biologics Evaluation and Research (CBER) has encouraged product sponsors to use a viral reference material to which the infectious titer and particle concentration of their products can be compared. These values are particularly dependent on the variance of the assays being used and will determine the number of replicates required to obtain an accurate measurement. Therefore, the reference material could be used to validate the methods a laboratory uses to determine particle concentration and infectious titer. It could also be used to validate the infectious titer of the positive control virus used in replication-competent lentiviral assays. Please note that ongoing validation work should be performed with the laboratory's internal reference material, as the availability of the reference material will be limited. Sponsors of lentivirus-related INDs should consult with FDA CBER for further guidance, but it is not the intent of the FDA 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 in the U.S. to follow LVV RM procedures when assaying the particle concentration or infectious titer.

3.0 Project Description

3.1 Material Requirements

- 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).
- The LVV RM must be a third generation HIV-1-based VSV-G pseudotyped LVV that will encode a transgene easily detected by FACS (GFP or equivalent).
- The LVV RM must be suitable for use in a cGMP laboratory for QA/QC that supports the production of lentivirus used in clinical trials and commercial manufacturing.
- Additional testing will be performed by several industry and academic partners under detailed SOPs that will be provided to them. Data will be compiled and analyzed for the purposes of publishing papers and preparing oral presentations.

- The cell line must be well-characterized, as well as sterile and free of mycoplasma.
- If used, plasmids must be well-characterized.
- All animal-derived materials used in the process (such as serum and trypsin) must be certified as to their country of origin, as well as documented to be free of adventitious agents including bovine spongiform encephalitis (BSE).

3.2 Table 1: Release Testing for Each Lot (One Lot Preferred): To be conducted by the selected manufacturer to release the LVV RM lot(s)[1]

Attribute	**Recommended Methods**	**Specification**
Infectious titer	Reported in infectious genomes per mL by either ddPCR or qPCR via classical TaqMan of transduced target cells such as HCT116 or HEK293.	0.5E8 – 1.0E8 ig/mL
Identity	Commercial p24 ELISA (expressed in ng p24 per mL) and Flow-Based Titer by GFP expression.	Confirm identity
Purity [2]	Residual plasmid DNA by qPCR or ddPCR.	Report results

[1] Tests must be conducted by the manufacturer prior to shipping: 1) the bulk material for filling elsewhere; or 2) the final LVV RM vials for storage. If the bulk is being shipped for filling elsewhere, these assays would need to be repeated by the manufacturer post-vialing. The fill and finish process will be guided by a separate RFP.

[2] If a transient transfection-based process is used, plasmid DNA residual concentration must be reported and should be at a level such that it does not interfere with the infectious titer assay.

3.3 Table 2: Release Testing by Others: The following tests are to be conducted by selected contract testing facilities, or other collaborators, to further define the LVV RM lot(s)[3] Safety testing, as well as testing for replication competent lentivirus (RCL) must comply with current regulatory guidance for cell and gene therapies. Assay methods other than those listed may be accepted to generate orthogonal data.

Attribute	**Recommended Methods**	**Specification**
Identity	Western Blot for viral gag proteins and confirmation of vector sequence	Confirm identity
Safety testing3	Endotoxin	Less than 0.15 EU/mL
	Sterility	Pass
	Mycoplasma	Negative
	Adventitious agents (*in vitro* methods)	Negative
Replication-Competent Lentivirus (RCL)	qPCR Assay for VSV-G[4]	Negative
Purity	Residual plasmid DNA, host cell protein, host cell DNA, total protein, residual BSA and/or residual Benzonase	Report result

[3] The testing will be governed by separate RFPs

[4] Escarpe P et al. (2003) Mol. Ther. 8: 332-341. http://dx.doi.org/10.1016/S1525-0016(03)00167-9

3.4 Table 3: Additional Characterization: These tests are to be conducted by several academic and industry contributors to characterize the LVV RM lot(s). Tests will be conducted with SOPs supplied by the Working Group in separate RFPs. Data will be compiled and analyzed for publication purposes.

Attribute	Recommended Methods
Infectious titer	Reported in infectious genomes per mL by either ddPCR or qPCR via classical TaqMan of transduced target cells such as HCT116 or HEK293.
Particle concentration	Commercial p24 ELISA (expressed in ng p24 per mL)
RNA genome copies	RT qPCR
Reverse Transcriptase activity	RT qPCR, or commercial enzymatic activity PCR-based RT assay (PERT)

4.0 Your Proposal

4.1 Process Description

Please submit a proposal for any process you can demonstrate will produce and purify a LVV that is specified in this document. A cGMP process is preferred, but a well-documented one may be accepted. All methods of LVV production that can meet the yield and purity requirements will be considered, including suspension and adherent-based transient transfection processes or producer cell line-based processes. See acceptance criteria in Tables 1 and 2.

The entire process and testing methods must be provided. **You may not designate methods or materials as proprietary** since the WG may need to produce more of this reference material in the future, and it is intended that the same methods, along with the materials stored by the designated repository, will be used.

If you are selected to produce and purify the LVV RM, you must provide the following materials, along with full documentation, to the designated repository. These materials will be stored, but **they will not be for sale**:

- Minimum of 10 vials of the cell line used to produce the LVV RM. Targeted cell concentration of 10 million cells/vial
- Minimum of 10 vials of each plasmid used to produce the LVV RM. Targeted DNA concentration of 1 mg/mL

4.2 Optional Processing (Formulation and Vialing):

Your proposal may also include a buffer exchange step to adjust the purified bulk material into the final formulation, plus vial filling. But if you are not selected to fill the vials, your proposal must contain provisions for freezing the purified material and shipping it to the organization that is chosen to do the filling.

4.3 Storage

Describe your plan to ensure:

- Purified bulk material, and filled vials, will be maintained at a temperature below minus 60°C in an appropriate freezer, such as a minus 80° freezer.
- Locations in the freezer will be chosen to prevent temperature excursion.
- Redundant storage (backup) will be used in case of catastrophic failure.

4.4 Shipping

The filled vials, or the LVV RM purified bulk material (if a different organization will be doing the vialing), must be shipped for overnight delivery. The material must be frozen at or below minus 80°C and shipped in a manner that will keep the material at no higher than minus 60°C.

4.5 Consumables

After the production and purification processes have been approved, the WG Executive Committee will send out requests for the consumable materials to be donated. In the past, other RM WGs have been successful in acquiring most of the materials that were needed, which helped lower the production costs.

4.6 Documentation

In addition to the documentation requested above, please submit production and purification batch records, or summary information, for similar production lots generated by the proposed methods. You must provide detailed descriptions of the production and purification processes including:

- Methods
- Reagents
- Equipment
- Consumable products
- Cell line
- Plasmids (if used)

In addition, please provide:

- Conditions and procedures for storage and shipment of the purified material
- The QC tests and QA standards for the cell line, plasmids, and crude vector bulk material
- Description of the facility
- Qualifications of the individuals who will do the work
- Information about similar work that has been accomplished in this facility by these personnel.
- Estimated cost and market value for goods and services involved in your proposal

4.7 Proposal Submission

Please submit a brief summary via email and include your full contact information. Then establish a Dropbox™ folder with your documentation and "invite" lvwg@isbiotech.org to "edit" (access) your folder. Proposal decisions will be communicated on or about **May 31, 2018**.

5.0 LVV RM Project Management

5.1 Working Group

The LVV RM project will be managed by the WG that has been established for this purpose. Anyone with a legitimate interest may join this WG, but only one individual from each organization can vote on critical decisions. In addition, an Executive Committee (see below) will draft and manage documents, call meetings, and issue reports.

5.2 Current Executive Committee Members

- Mercedes Segura Gally, PhD – bluebird bio
- Otto-Wilhelm Merten, PhD – Genethon
- Boro Dropulic, PhD – Lentigen
- Yvonne Reid, PhD – Formerly with ATCC
- Katherine Bergmann, PhD – Eurofins Lancaster Labs
- Xiaobin "Victor" Lu, PhD – FDA CBER
- Keith L. Carson, ChE, MBA – ISBioTech

5.3 Contact Information

ISBioTech
Attn: LVV RM WG
7985 Holgate Road
Pensacola, FL 32514 USA
Phone: (617) 686-5426
Email: lvwg@isbiotech.org