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/lentivirus-home.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 $1x10^8$ infectious genomes per mL (ig/mL)

1.4 Vector Characterization

The recommended tests are specified in **Table 1.0**, which we intend to be conducted by several academic and industry investigators to characterize each lot of the LVV RM. Where noted, tests will be conducted with SOPs that are supplied by the Working Group. The data will be analyzed by a professional statistician, posted to the ISBioTech website, and used for publication.

Table 1.0

Attribute	Recommended Methods	Report
Identity	- Western Blot for viral gag proteins - Vector sequence - Restriction enzyme mapping - RP-HPLC - SDS-PAGE	Confirm identity
Purity	Residual plasmid DNA, host cell protein, host cell DNA, total protein, residual Benzonase	Results
Safety testing[1]	Endotoxin	EU/mL
	Sterility	Pass
	Mycoplasma	Negative
	Adventitious agents (*in vitro* methods)	Negative
Infectious titer	Either ddPCR or qPCR via classical TaqMan of genomic DNA from transduced target cells such as HCT116 or HEK293	ig/mL
Particle concentration	Commercial p24 ELISA	Nanograms p24 per LVV RM
RNA genome copies	RT qPCR	Number of copies
Replication-competent lentivirus (RCL)[1]	qPCR Assay for VSV-G[2]	Negative

NOTES:

[1] Safety testing methods and RCL need to comply with current regulatory guidance for cell and gene therapies. However, other assays may be approved, or used to generate orthogonal data.

[2] Escarpe *et al*. (2003) *Mol Ther,* 8: 332-341; or Skrdlant *et al*. (2017) *Mol Ther Methods Clin Dev,* 8, 1-7.

1.5 Your Proposal

Please specify the attributes (i.e., purity, particle count) and test methods you would like to contribute. Although we would prefer that you agree to perform all of the tests listed, your proposal can specify a limited number.

- List the tests / methods you plan to use, preferably in the form of a Standard Operating Procedure (SOP), or published references.
- This viral vector must be characterized under GLP conditions, and both methods and materials must be disclosed. **Please note that you may not designate methods or materials as proprietary,** since the Working Group may need to produce more of this material in the future, and it is intended that the same methods and materials will be used.

- If you propose testing methods other than those listed in **Table 1.0**, you must provide qualification data and published references regarding their suitability for the stated purposes.
- Provide a statement describing your experience and capacity to perform the proposed characterization methods, along with the qualifications of the personnel involved in running the assays and reviewing the data.
- Specify the amount of LVV RM you will need per lot (number of vials) to perform the analyses you propose.
- Provide a list of the equipment that will be used, and the calibration status of each.
- Once you receive the vials of LVV RM you request, specify how long it will take for you to run the assays and report the results.
- While not covered in this RFP, we may ask you to help us determine short-term and long-term stability of the LVV RM

1.6 Project Management

The LVV RM project is being managed by the WG 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 concerning the LVV RM. In addition, a nine-member Executive Committee will draft and manage documents, call meetings, and issue reports.

Executive Committee Members:

- Mercedes Segura Gally – bluebirdbio
- Otto-Wilhelm Merten – Miltenyi Biotec
- Vladimir Slepushkin – Kite Pharma
- Kate Bergmann – Eurofins Lancaster Labs
- Xiaobin "Victor" Lu – ICT Bio
- Jakob Reiser – FDA/CBER
- Sven Ansorge – NRC Canada
- Jenny Gronemus – ATCC
- Keith L. Carson – BioProcessing Journal / ISBioTech

1.7 Proposal Submission

Electronic submissions are encouraged, and acceptance of your proposal will be communicated on, or near, March 31, 2019. Please provide complete contact information for the corresponding individual in your organization, attach the documentation specified above, and submit your proposal for receipt at:

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