# Lentivirus Vector Reference Material Initiative

ISBioTech, Washington DC, March 7th 2017

# Background/History

- 2014 - Industrial partner bluebird bio seeking to form an LVV Working Group contact Keith Carson at ISBioTech
- 2015 - ISBioTech Spring meeting – 1st LVV RSM meeting to gauge general interest in public discussions
- 2016 - ISBioTech Spring meeting – Driving parties bluebird bio, Généthon and Lentigen held 2nd LVV RSM meeting to gauge general interest in public discussions
  - Among next steps it was decided that a survey would be conducted

# Goals

- The global availability of reference standard materials (RSMs) permits the **standardization of quantification techniques** across research and manufacturing organizations.
- The LVV reference material is intended for **use in calibrating internal (product-specific) reference materials** used for quantitation assays, plus the qualification of assays.
  - Well-characterized reference material preparations are important for the establishment of appropriate pre-clinical and clinical dosing, making data from different pre-clinical and clinical studies easier to compare.

# Approach

- Follow existing examples for the generation of other Viral Vector Reference Materials available today through ATCC (Ad5, AAV2, AAV8, etc.)

# Selected references for existing RM

## › Ad5 ATCC VR1516


1. Hutchins B Development of a reference material for characterizing adenovirus vectors Bioprocess. J. 125-28, 2002 Available from: BioProcessing Journal
2. Hutchins B, et al. Working toward an adenoviral vector testing standard. Mol. Ther. 2: 532-534, 2000. PubMed: 11124052
3. Summary of presentations can be found at http://www.isbiotech.org/ReferenceMaterials/Adenovirus.html


## › AAV2 ATCC VR1616


1. Lock M, et al. Characterization of a recombinant adeno-associated virus type 2 Reference Standard Material. Hum. Gene Ther. 21(10): 1273-1285, 2010.


## › AAV8 ATCC VR1816


1. Ayuso E., et al., 2014 Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material. Hum Gene Ther. 2014 Nov;25(11):977-87

# Expected LVV RM Workflow

A

- Establishment of a Reference Material Working Group (RM WG)

B

- Manufacturing of the Reference Material
- Generating the Reference Standard Material aliquots

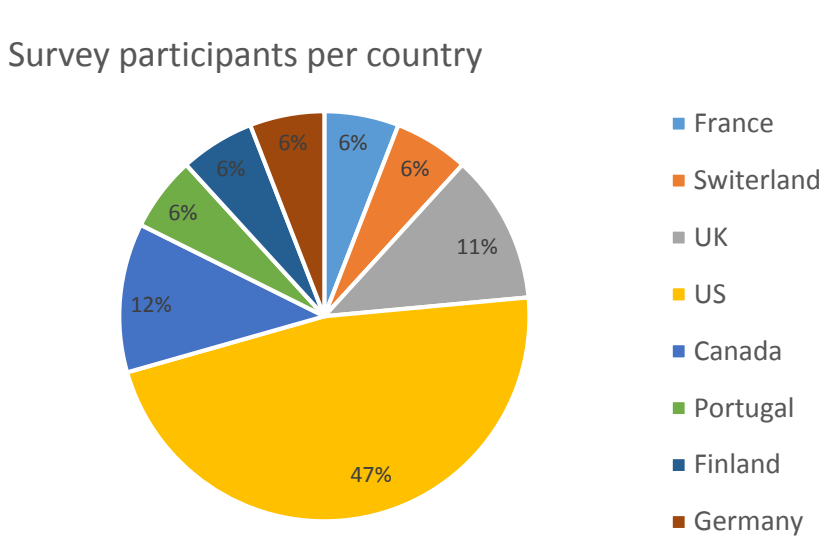
C

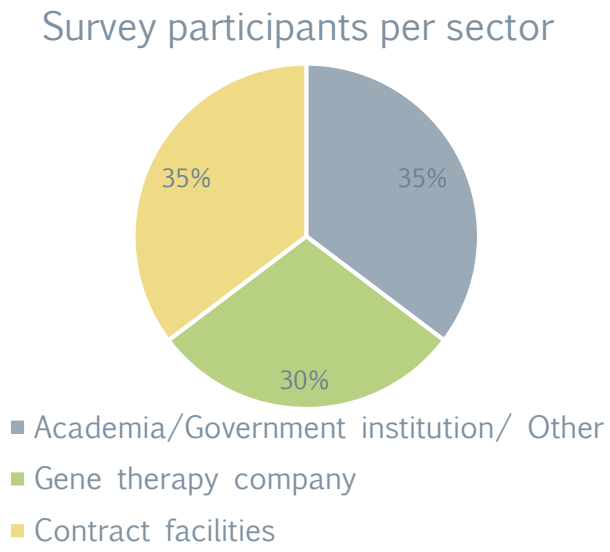
- Testing the Reference Material aliquots (worldwide)
- Report results in peer reviewed journal in the field

# Lentivirus Vector Reference Material

## Survey Monkey results

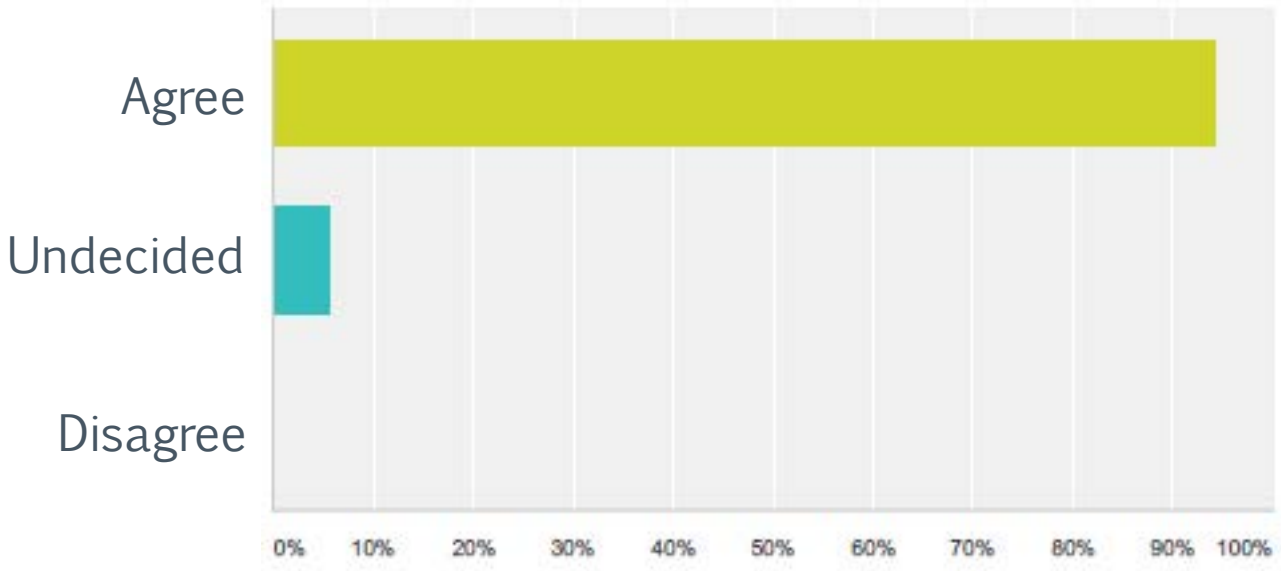
# Response analysis from 18 respondents



# Survey Results

› Question 1: A 3rd generation HIV-1-based VSV-G pseudotyped LVV coding for a gene that is easily detected by FACS (GFP or equivalent) is proposed. Do you agree with this choice or would like to suggest an alternative?




| Answer choices | Responses |
|---|---|
| Agree | 94.44% (17) |
| Undecided | 5.56% (1) |
| Disagree | 0% |
| Total | 18 |

# Survey Results

› Question 2: Please provide your estimate for 1) how many vials should be produced, 2) what the volume per vial should be and 3) what the vector concentration should be in each vial.

| Participant estimates | #vials[b] | Volume (mL)[b] | Titer (TU/mL)[b] |
|---|---|---|---|
| # respondents[a] | 11 | 14 | 8 |
| Min | 500 | 0.05 | 1.0E+06 |
| Max | 10000 | 100 | 1.0E+08 |
| Mode | 1000 | 1 | 5.0E+07 |
| Mean | 3045 | 8 | 4.7E+07 |
| | | | |
| RECOMMENDED | 5000 | 0.5 | 5.0E+7 to 1.0E+8 |

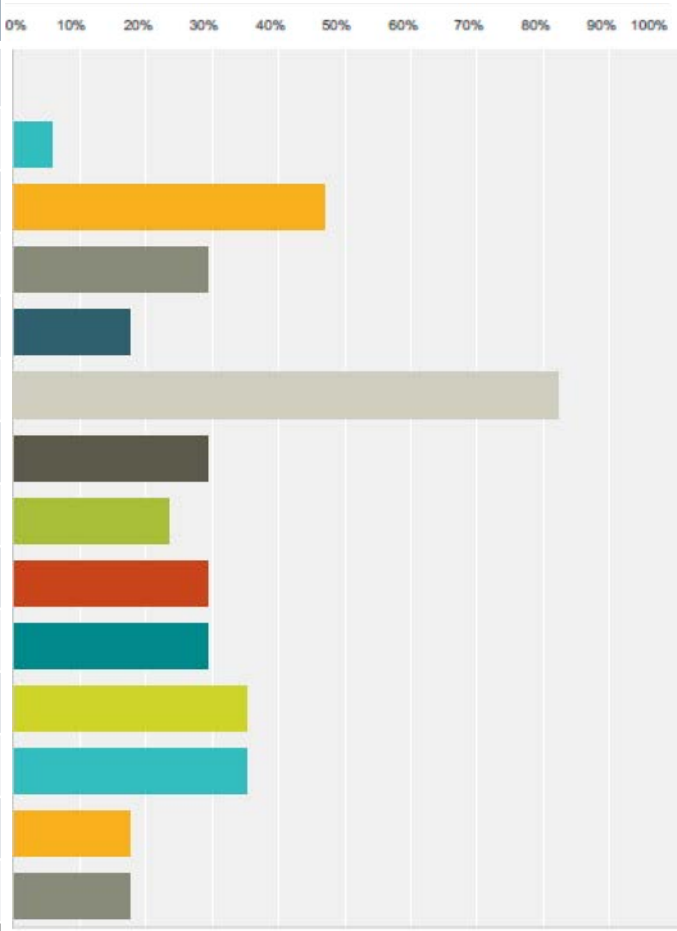
[a] Only responses providing numerical data included

[b] Used average for ranges provided

# Survey Results

› Question 3: Please indicate the type of support your institution could potentially provide to support the LVV RSM initiative

| Answer choices | Responses |
|---|---|
| Cell line donation (well characterized and not proprietary) | 0% (0) |
| Vector construction and plasmid donation (well characterized and not proprietary) | 5.88% (1) |
| LVV manufacturing | 47.06% (8) |
| LVV purification | 29.41% (5) |
| LVV Formulation Development and Short-Term Vector Stability Testing | 17.65% (3) |
| LVV characterization (p24 ELISA, flow, cell-based) | 82.35% (14) |
| Safety testing (myco, sterility, bioburden, RCL) | 29.41%(5) |
| Fill and Finish (Vialing) | 23.53(4) |
| Stability testing | 29.41%(5) |
| Data analysis with statistics | 29.41%(5) |
| Drafting of testing assay SOPs | 35.29% (6) |
| Drafting of publications to compile results | 35.29% (6) |
| Raw Materials supply | 17.65% (3) |
| Repository/Repository management | 17.65% (3) |
| Total respondents | 17 |



# Survey Results

| Answer choices | Responses | Institute |
|---|---|---|
| Cell line donation (well characterized and not proprietary) | 0% (0) | |
| Vector construction and plasmid donation (well characterized and not proprietary) | 5.88% (1) | LentiGen (BD) |
| LVV manufacturing | 47.06% (8) | Catapult (ES); LentiGen (BD); iBET (PA); Lonza H (RJ); bbb (MS); Finvector (HL); Wuxi App Tec (AM); NRC (SA) |
| LVV purification | 29.41% (5) | LentiGen (BD); iBET (PA); bbb (MS); Finvector (HL); Wuxi App Tec (AM) |
| LVV Formulation Development and Short-Term Vector Stability Testing | 17.65% (3) | LentiGen (BD), Bbb (GV); Finvector (HL) |
| LVV characterization (p24 ELISA, flow, cell-based) | 82.35% (14) | Novartis (MJ); Catapult (ES); LentiGen (BD); McGill (AK); Mass Biologics (GS); iBET (PA); Lonza H (RJ); bbb (MS); bbb (GV); Finvector (HL); CEVEC (NF); Wuxi App Tec (AM); NRC (SA); NIBSC (YZ) |
| Safety testing (myco, sterility, bioburden, RCL) | 29.41%(5) | LentiGen (BD), Bbb (GV); Lancaster (KB); Wuxi App Tec (AM); NIBSC (YZ) |
| Fill and Finish (Vialing) | 23.53(4) | LentiGen (BD); Lonza H (RJ); Finvector (HL); NIBSC (YZ) |
| Stability testing | 29.41%(5) | Mass Biologics (GS); NRC (SA); LentiGen (BD); Lonza H (RJ); Finvector (HL); |
| Data analysis with statistics | 29.41%(5) | INSERM (NC); LentiGen (BD); bbb (MS); bbb (GV); NIBSC (YZ) |
| Drafting of testing assay SOPs | 35.29% (6) | Novartis (MJ); Catapult (ES), INSERM (NC); LentiGen (BD); Mass Biologics (GS); bbb (MS); |
| Drafting of publications to compile results | 35.29% (6) | Novartis (MJ); INSERM (NC), LentiGen (BD), McGill (AK); bbb (MS); Wuxi App Tec (AM) |
| Raw Materials supply | 17.65% (3) | LentiGen (BD); bbb (GV); ISBioTech (KC); |
| Repository/Repository management | 17.65% (3) | Catapult (ES); LentiGen (BD); NIBSC (YZ) |

NC Nathalie Cartier, MJ Margit Jeschke; ES; Elena Sokolskaja; BD Boro Dropulic; AK Amine Kamen; GS, Greg Saia, PA, Paula Alves; RJ, Ricardo Jimenez, MS, Mercedes Segura; GV, Gabor Veres; KC, Keith Carson, KB; Katherine Bergmann; HL, Hanna Lesch; NF, Nicole Faust; AM, Andrea Moore; SA, Sven Ansorge, YZ, Yuan Zhao

# Points agreed and points for discussion

# Summary of points agreed so far…

- Vector type: A 3rd generation HIV-1-based VSV-G pseudotyped LVV coding for a gene that is easily detected by FACS (GFP or equivalent)
- Manufacturing of 5000 vials containing 0.5mL/vial of LVV at a concentration between 0.5E8 to 1E8 TU/mL
- Process steps, raw materials and other manufacturing details required to understand the LVV RM characteristics **need to be fully disclosed**

# Summary of points agreed so far…

- The testing would be performed by a number of industrial and academic partners in the Working Group under detailed SOPs that would be distributed along with LVV RM samples. Data will be compiled and analyzed for publication purposes.
- A well characterized cell bank and plasmids (or transformed bacteria) stocks should be kept at ATCC for reproducing another RSM
- The LVV RM will be made available from an unbiased repository like ATCC at minimal cost

# Points for discussion

- Lentiviral Vector Manufacturing
- Lentiviral Vector Purification, Formulation and Fill Finish
- Lentiviral Vector Characterization and Safety Testing
- Drafting Requests for Donation
- Stability studies: short and long-term
- Distribution
- Cost
- Other points of discussion

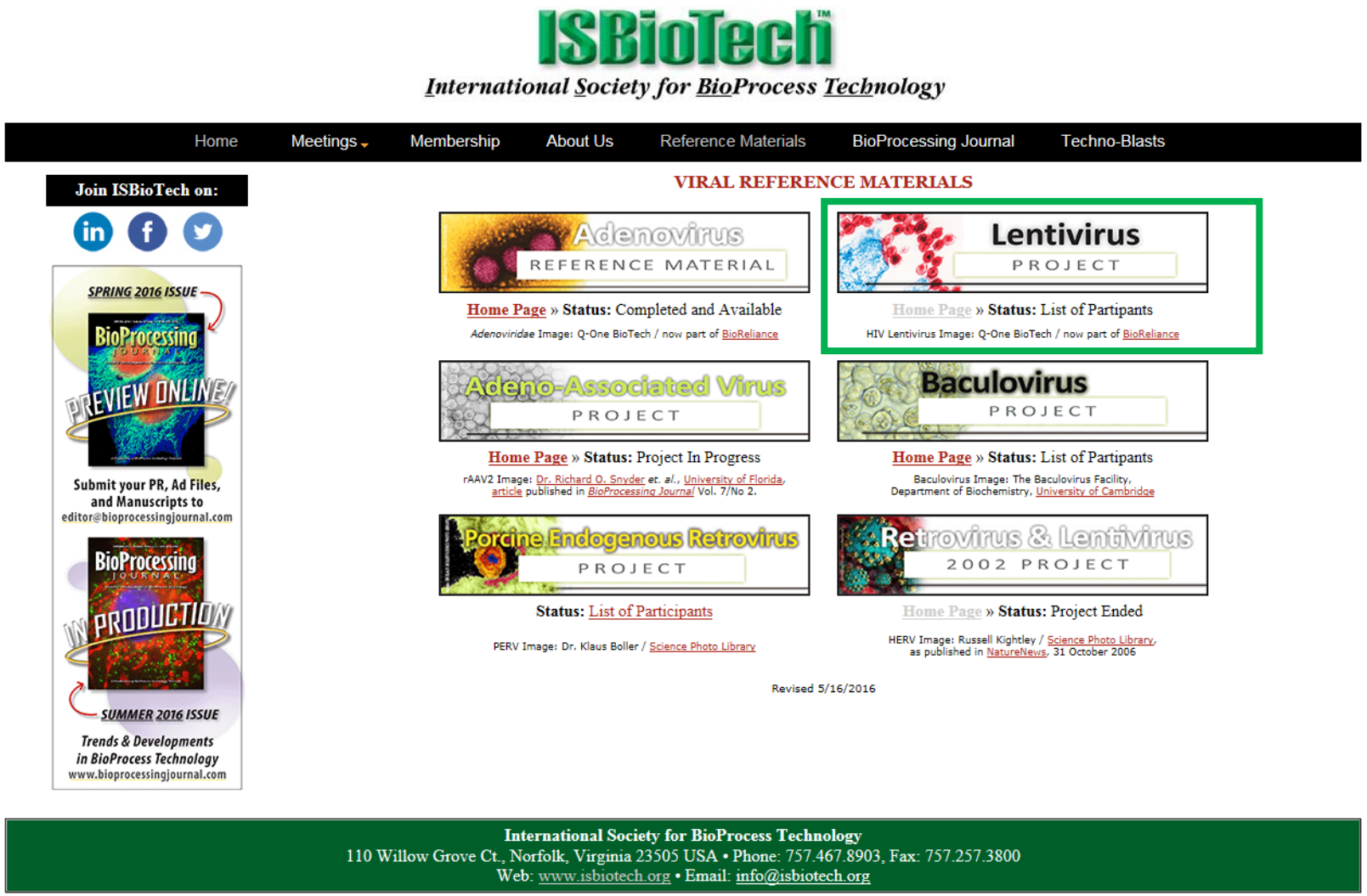
# Next Steps

- Consolidate LVV RM Working Group
  - Please respond to lvwg@isbiotech.org if you'd like to be included as a member of the **Working Group** for this initiative.
  - Please note that there is no limit to the number of individuals who can join from each organization, but there can only be one voting member from each organization.
- Obtain alignment from the Working Group on points of discussion
- We are working to identify a place and time for the next Working Group Meeting
- Comments, please send to lvwg@isbiotech.org

# Working Group so far..(through JoForm)

| # | Name | Institute | Email | Job Title |
|---|---|---|---|---|
| 1 | Hugo Soares | iBET - Instituto De Biologia Experimental e Tecnologica | hsoares@ibet.pt | PhD Student |
| 2 | Amine Kamen | McGill | amine.kamen@mcgill.ca | Professor |
| 3 | Henry George | MilliporeSigma | henry.george@sial.com | Head, Viral Vector Cell Lines |
| 4 | Helen Segal | Stanford University | hsegal@stanford.edu | PD and Manufacturing Scientist |
| 5 | Manjunatha | bub | manjuss168@gmail.com | RS |
| 6 | Martin Wisher | BioReliance | martin.wisher@sial.com | Head of Regulatory Affairs |
| 7 | Andre Sobczyk | Brain & Spine Institute, Paris | andre.sobczyk@icm-institute.org | Head of vectorology core facility |
| 8 | Ales Strancar | BIA Separations | sara.primec@biaseparations.com | CEO |
| 9 | Yuan Zhao | NIBSC/MHRA | yuan.zhao@nibsc.org | Section Head |
| 10 | Rolando DeAngelis | BioMarin | rolando.deangelis@bmrn.com | Scientist 1 |
| 11 | Melina Fan | Addgene | mfan@addgene.org | Chief Scientific Officer |
| 12 | Meghan Rago | Addgene | meghan@addgene.org | Senior Scientist |
| 13 | Janet Chung | Bluebird bio | jchung@bluebirdbio.com | Sr. Scientist |
| 14 | Mercedes Segura | Bluebird bio | msegura@bluebirdbio.com | Associate Director |
| 15 | Nicole Faust | CEVEC Pharmaceuticals GmbH | faust@cevec.com | CSO |
| 16 | Eevi Lipponen | Finvector Vsion Therapies | eevi.lipponen@finvector.com | Head of Process Development |
| 17 | Leyla Diaz | BioReliance | leyla.diaz@sial.com | Principal Sci, Developnt Services |
| 18 | Kerstin Hein | Cevec Pharmaceuticals GmbH | hein@cevec.com | Head Viral Vectors |
| 19 | Andrea Moore | WuXi AppTec | andrea.moore@wuxiapptec.com | Associate Principal Scientist |
| 20 | Xiaobin Lu | FDA | xiaobin.lu@fda.hhs.gov | Biologist |
| 21 | Lexan Lhu | Charter Medical | llhu@chartermedical.com | Sales Engineer |
| 22 | Howard Kim | CCRM | howard.kim@ccrm.ca | Development manager |

# LVV RSM project ISBioTech 2015 and 2016 meeting minutes and list of attendees



# Questions and Information

lvwg@isbiotech.org

**Working Group Executive Committee***

- Keith Carson: kc@isbiotech.org
- Boro Dropulic: boro.dropulic@lentigen.com
- Otto Merten: omerten@genethon.fr
- Mercedes Segura: msegura@bluebirdbio.com

*Placed in alphabetical order

# Points of discussion for this meeting

# Points for discussion this meeting

- Lentiviral Vector Manufacturing
- Lentiviral Vector Purification, Formulation and Fill Finish
- Lentiviral Vector Characterization and Safety Testing
- Drafting Requests for Donation

# Lentiviral Vector Manufacturing

- Vector constructs: Vector construction and plasmid donation (well characterized and not proprietary) was offered by 1 survey participant (Lentigen)
- Cell line: no cell line donation identified

# Lentiviral Vector Manufacturing

- The method of manufacture of such LVV RSM
  - Adherent vs. suspension (FBS or serum free)
  - Transient transfection vs. producer cell line
- Manufacturing scale – Consider a final volume 2500 mL of LVV at 0.5-1E8 TU/mL
- Manufacturing site

Note

- 8 Survey participants offered manufacturing capabilities

# Lentiviral Vector Purification, Formulation and Fill Finish

- Process steps and level of purity needed – Consider Purpose
- Final formulation and short term stability
- Purification and fill finish site

Note:

- 5 Survey participants offered DSP capabilities
- 3 Survey participants offered LVV Formulation Development and Short-Term Vector Stability Testing
- 4 Survey participants offered Fill and Finish (Vialing)

# Lentiviral Vector Characterization Testing

- ☐ p24 ELISA
- ☐ Infectious titer by cell-based assay and PCR
- ☐ Infectious titer by flow cytometry and GFP/equivalent expression
- ☐ Other?

Note:

- 12 Survey participants offered testing capabilities

# Lentiviral Vector Safety Testing

- ☐ Mycoplasma
- ☐ Sterility
- ☐ Endotoxin
- ☐ Bioburden
- ☐ RCL
- ☐ Other?

Note:

– 5 Survey participants offered Safety testing capabilities

# Drafting of Requests for Donation (RFD):

- Need help drafting of RFD for:
  - ❑ Cell Line
  - ❑ Plasmid
  - ❑ Production*
  - ❑ Purification*
  - ❑ Formulation and Short-Term Stability Testing
  - ❑ Safety Testing
  - ❑ Filling
  - ❑ Characterization
  - ❑ Long-Term Stability and Sterility Testing

* Include proposal for how the material, would be processed and why the people involved are qualified to do the work. The Working Group would then vote on the submissions and select one

# Backup slides

# Lentiviral Vector Stability Studies

- Short-term field use & shipping configuration stability
- Long-term stability at -80°C
- Studies to be performed?

Note:

- 5 Survey participants offered Stability testing capabilities

# Distribution

- The LVV RM will be made available from an unbiased repository like ATCC or other at minimal cost
- The repository will restrict the number of vials that can be delivered to users and have all users sign a material transfer agreement (MTA) in which they agree to only use the material for the intended purposes, which are to validate internal reference materials and assays.

# Cost

- Costs to cover repository expenses and manufacturing expenses.

  Note: There are multiple potential mechanisms (sponsors, granting mechanisms, vial cost, etc.) and it should be discussed collectively

# Other points

- Drafting of testing assay SOPs
- Drafting of publications to compile results
- Raw Materials supply
- Repository/Repository management