

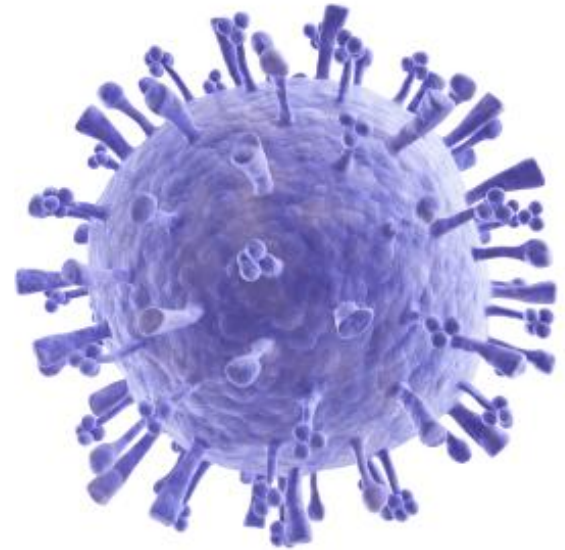
Biosafety Concerns of Viral Vectors—Focus Lentivirus

Debra Brickey PhD CBSP

May 5, 2011

Biosafety Risks of Lentivirus and Other Viral Vectors

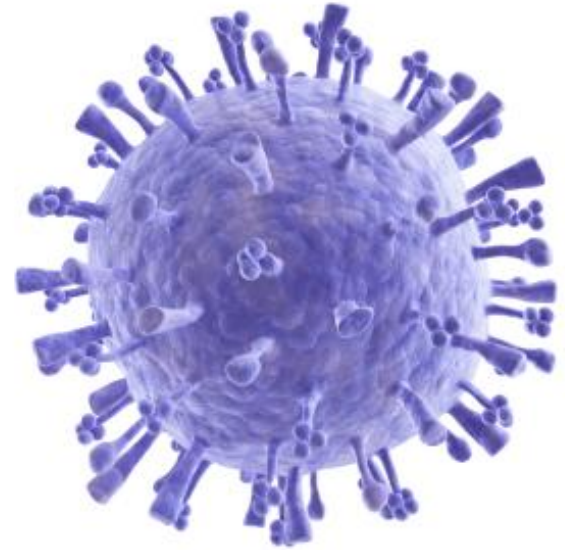
- Recombinant DNA Advisory Committee (RAC)
- 2006 reviewed biosafety of Lentivirus System



Biosafety Risks of Lentivirus and Other Viral Vectors

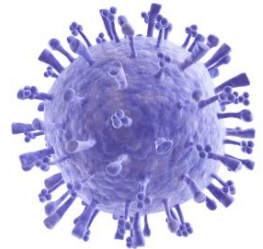
- ▣ Potential for generation of replication competent virus
- ▣ Potential for oncogenesis

Risks are mitigated by the nature of the vector system and its safety features or exacerbated by the nature of the transgene insert encoded by the vector.



Criteria For Risk Assessment of Lentivirus Vectors

- ❑ The nature of the vector system and the potential for regeneration of replication competent virus from vector components.
- ❑ The nature of the transgene insert (e.g. known oncogenes or genes with high oncogenic potential merit special care)
- ❑ The vector titer and the total amount of vector.
- ❑ The inherent biological containment of the animal host, if relevant
- ❑ Negative RCL testing



Nature of the Vector System

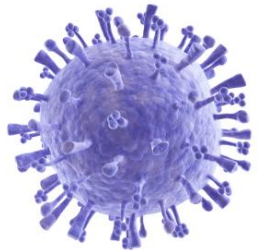
▣ General containment considerations

■ HIV-1, HIV-2, and SIV

- Wild-type: BSL2+ to BSL3
- Vector Systems: BSL2 (rarely BSL2+)

■ FIV, BIV, and EIAV

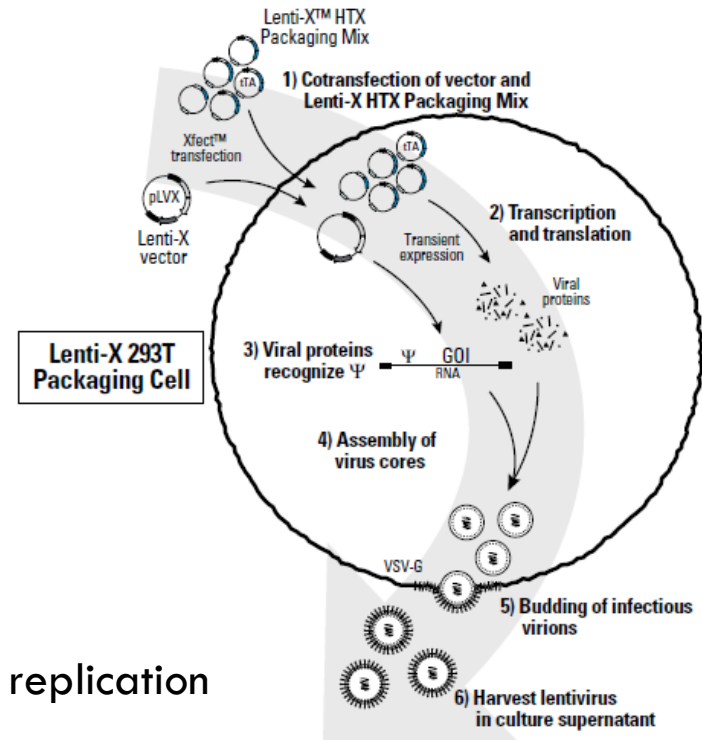
- Wild-type: BSL1
- Vector Systems using VSV-G or other coat protein which broadens tropism to include mammalian: BSL2



Nature of the Vector System

■ 2nd, 3rd, or 4th generation viral systems

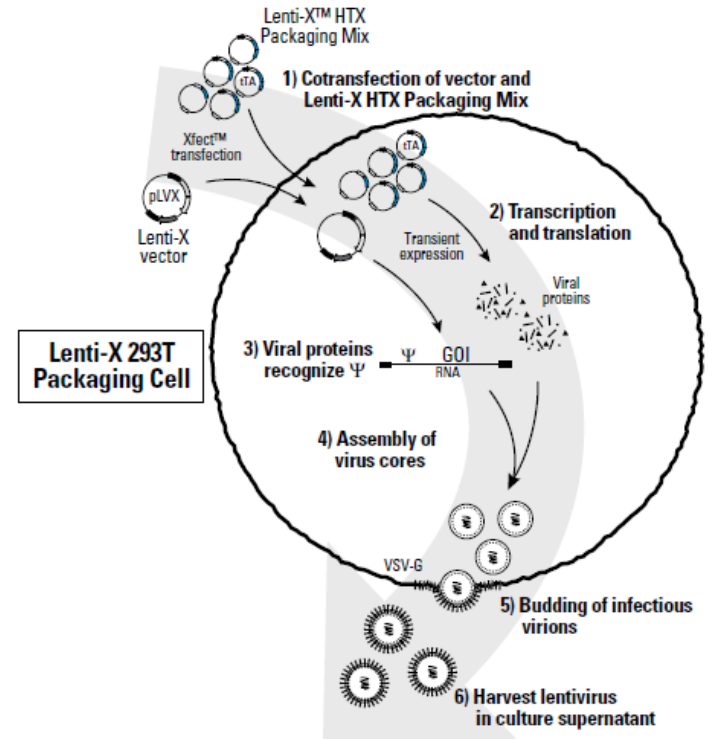
- Self-inactivation (SIN) decreases possibility of RCL and insertional mutagenesis
- Deletion of viral proteins
 - 2nd generation (5 of 9 HIV-1 genes eliminated)
 - 3rd generation (only gag, pol, and rev remain and a chimeric 5'LTR)
 - 4th generation additional changes improving safety and viral production (many proprietary)
- Separate packaging onto multiple plasmids (3 to 5)
- Elimination of TAT expression that is required for viral replication



Nature of the Vector System

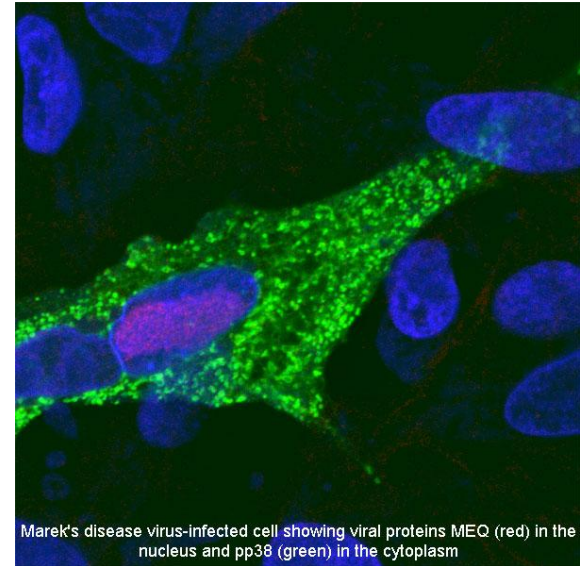
Concerns

- Mixing and matching systems, generations, and packaging cell lines
- Experience of Lab personnel working with virus and at BSL-2
- Special cases using 2nd generation viral system



Nature of the Insert

- Concerns
 - Accidental exposure—Infectious and Integratable
 - Accidental Injection
 - Splash to mucous membranes, cuts
- Non-Oncogene, non-viral inserts
 - Insertional Mutagenesis
- Oncogene or Potential Oncogene and viral inserts
 - Insertional Mutagenesis
 - Integration and Expression of Oncogene



Titer, Amount, and Animal Use

Virus Titer and Total Amount of Virus

- Concerns
 - High titer
 - exposure more likely to result in infection and expression of insert
 - Large volume (greater than 10L)
 - exposure due to handling of large volumes during production
 - RCL event

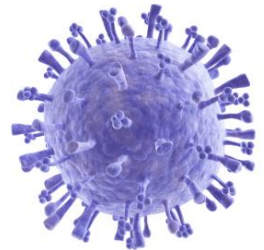
Biological Containment of Animal Host

- Concerns (post-injection, injections at BSL2)
 - Non-permissive host—ABSL1
 - Special cases will increase standard ABSL containment—ABSL2
 - Host permissive for lentiviral replication
 - Host engrafted with human cells

Replication Competent Lentivirus Testing

□ RCL testing

- Required by FDA for use in human clinical trials
- Not required for non-clinical, small volume, and low-risk genes
- Based on risk assessment, the IBC may require RCL testing if larger volumes, high-risk genes, or vector system increase risk of RCL
 - 2006—Had to consider laboratory expertise for working with infectious lentivirus as positive control may increase risk to the investigator compared to the test material
 - P24 Elisa



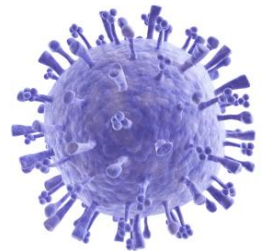
Spills

▣ Disinfectants—

- Choose disinfectant based on agent sensitivity and contact time
- 10% Bleach or phenolic disinfectants for a VSV-G pseudotype

▣ Inside Biosafety Cabinet (BSC)

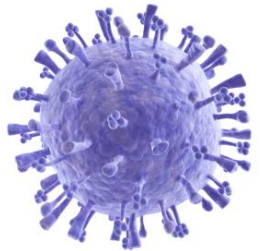
- Spray with disinfectant and wipe up spill with paper towels
- Dispose of paper towels to biohazard container (inside BSC)
- Change to new gloves before continuing work
- Disinfect thoroughly upon completion of work



Spills

▣ Outside BSC

- Cover with paper towels, pour disinfectant over spill, allow a 10' contact time, clean, and repeat (Dispose of materials in Biohazardous Waste Container)
- Inform others in vicinity that a spill has occurred ASAP, do not leave spill unattended
- Wash hands and replace any PPE that has been exposed
- Report to Supervisor, consult BSO if exposure possible
- Evaluate exposure risk



Exposures

❑ IBC Research Accidents and Spills

policy <http://www.ohsu.edu/xd/about/services/integrity/policies/policy-detail.cfm?policyid=1511935>

❑ Splash to mucous membranes

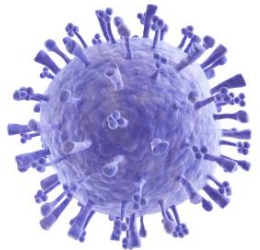
- Rinse eyes, mouth and nose depending on exposure site with water for 10 minutes

❑ Accidental Injection

- Encourage bleeding and wash site with soap and water

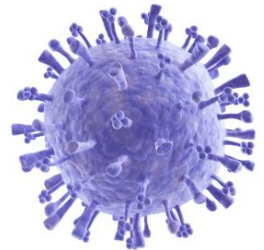
❑ After Initial Response

- Report to Supervisor and BSO
- Go to Employee Health or ED for further evaluation
- Complete accident report: <https://ozone.ohsu.edu/wsirs/>



NIH rDNA Guidelines, BMBL, and Your Lab

- Guidelines used by IBC and the BSO to evaluate protocols and lab practices
 - NIH guidelines
 - http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf
 - BMBL 5th edition
 - <http://www.cdc.gov/biosafety/publications/bmb15/>
- Template for Laboratory Specific Lentiviral Manual
 - <http://www.ohsu.edu/xd/about/services/integrity/policies/policy-detail.cfm?policyid=1534831>



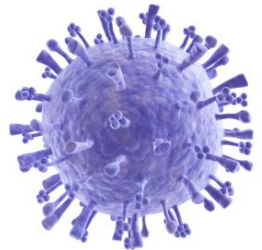
Risk Assessment of Procedures

▣ Work Practices that may require a Risk Assessment (not all inclusive)

- Stereotactic injections

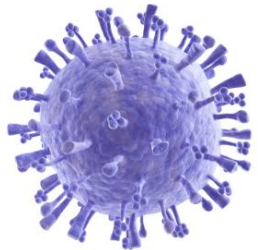
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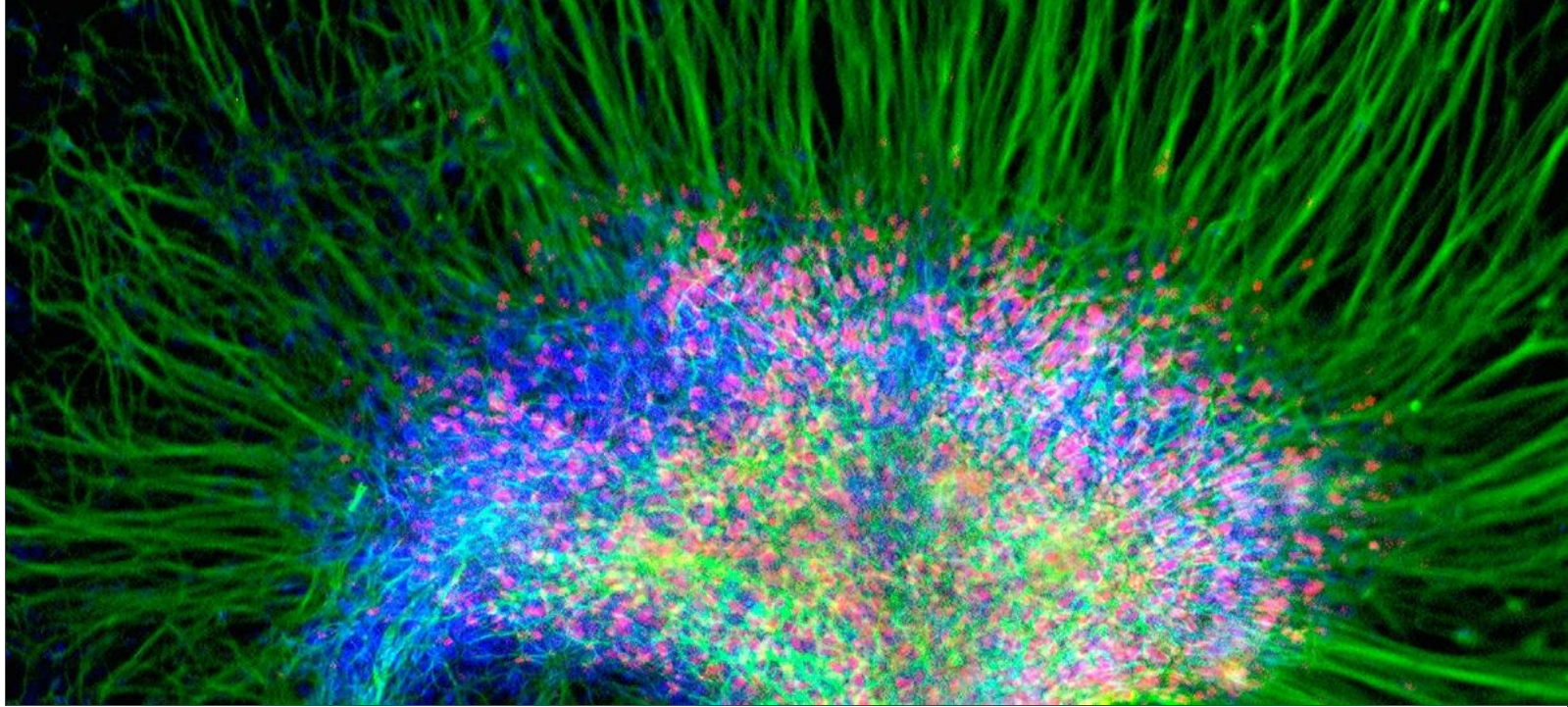
- Animal injections occurring outside BSC
- Work with live virus outside BSC
- Use of sharps



Training

- ▣ BigBrain Courses (<https://bigbrain.ohsu.edu/>)
 - General and Laboratory Safety Course
 - Bloodborne Pathogen Training for OHSU Researchers
- ▣ Group training for BSL2 work practices by request
- ▣ DCM/DAR training for ABSL2 and animal injections





Good Luck and Be Safe With Your Research!