

# Lentiviral Vectors: A Biosafety Primer

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# Topics

- lentiviral life cycle
- features of recombinant lentiviral vectors
- assessing biosafety
  - transgene-specific issues
  - vector-specific issues

# Why do we use lentiviruses in the lab?

- infectious
- can carry foreign DNA (i.e., transgene)
- stably transduce target cells

# Lentiviruses

- lentiviruses are a specific type of retrovirus
- the most famous lentivirus is HIV
- most if not all common lentivirus vectors are based on HIV

# Lentivirus life cycle

- attachment and entry into the cell is mediated by the viral Env (for envelope) protein, encoded by the *env* gene
  - pseudotyping refers to replacing the *env* gene with a gene encoding a different glycoprotein, to change the tropism of the vector
    - tropism = which cell type(s) the virus can infect
    - the Vesicular Stomatitis Virus G (VSV-G) protein is often used because it allows the virus to infect many different cell types

# Lentivirus life cycle

- once the viral RNA genome is delivered into the cell, it is reverse transcribed into a double stranded DNA molecule by reverse transcriptase, an enzyme that is carried into the cell along with the genome

# Lentivirus life cycle

- the double stranded DNA is then transported to the nucleus, where it integrates into the host genome. This is mediated by the viral integrase (also carried in with the viral genome)
  - integration is random, although “open” chromosome is preferred
- the genome is now called a provirus, and is a **permanent** part of the cellular genome

# Lentivirus life cycle

- the provirus is transcribed by the cellular RNA polymerase II using promoter and enhancer elements in the viral LTR (Long Terminal Repeat) to make mRNAs and new viral genomes
- mRNAs go to the cytoplasm, where they are translated into viral proteins
- these viral proteins combine with the viral genome to assemble into progeny virions, which are released from the cell

# Lentivirus vectors

- in a recombinant lentivirus vector, most of the viral genes have been replaced with a transgene(s)
- this vector therefore cannot **replicate** on its own

# Lentivirus vectors

- to produce the vector, therefore, one must provide the viral replication factors in trans
- this is usually accomplished by co-transfecting a plasmid containing the vector genome with other plasmids encoding the replication functions
  - use of multiple plasmids significantly reduces the risk of recombination leading to generation of a virus that can now replicate on its own

# Lentivirus vectors

- the viral particles that are produced still contain the reverse transcriptase and integrase, so that when they infect a target cell, the RNA genome can be reverse transcribed and the DNA product can still be integrated into the host chromosome for expression of the transgene(s)
- in other words, the vector is still **infectious**
  - if it weren't, it wouldn't be of any use

# Lentivirus vectors

- when the vector genome is converted into a provirus, expression is governed by either the lentiviral promoter/enhancer (LTR) or whatever promoter has been engineered into the genome with the transgene coding sequence

# Biosafety risks

- transgene
- vector

# Transgenes

- genes that encode mRNAs
- genes that encode shRNAs

# Transgene considerations

- if a lab worker is accidentally exposed to the recombinant virus
  - possible route(s) of exposure
  - which cells might be exposed and infected
    - tropism of the vector
  - what might happen if the transgene is expressed in these cells
    - special considerations: transgenes that affect cell growth, death, or differentiation, or are otherwise toxic

# Vector considerations

- what is the tropism of the vector
- what might happen when the vector integrates into the host genome<sup>1</sup>
  - insertion into a cellular gene
  - insertion next to a cellular gene
    - provirus has promoters and enhancers
- innate immune response against the infecting recombinant viral particles

<sup>1</sup>there is apparently a new vector system that does not carry the integrase protein, but UM does not have experience with it yet.