Lentiviral Vectors and Biosafety

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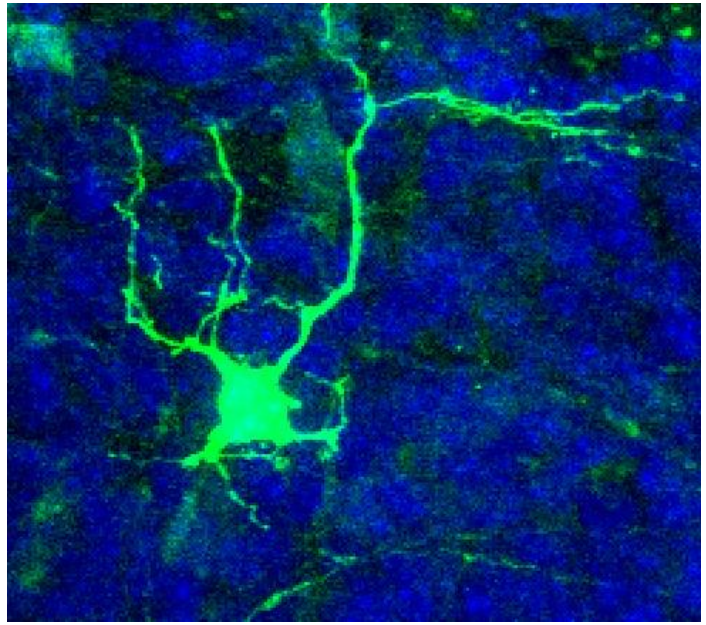
What is a Viral Vector?

- A viral vector is a genetically modified virus that can be used as a research tool.
- Part of the virus's genome is removed and replaced with genes of interest in research.
  - The removed material typically includes the genes responsible for replication (the creation of new virus) and other virulence genes.
  - An "attenuated" virus has been weakened so that it can't cause a disease in an immunocompetent individual.
  - A "replication defective" virus is one that has been modified so that it can't replicate inside a cell, and thus can't cause an infection.
- When the modified virus "infects" a target cell, it inserts the gene of interest into the cell's genome.

Viral Vectors in Research

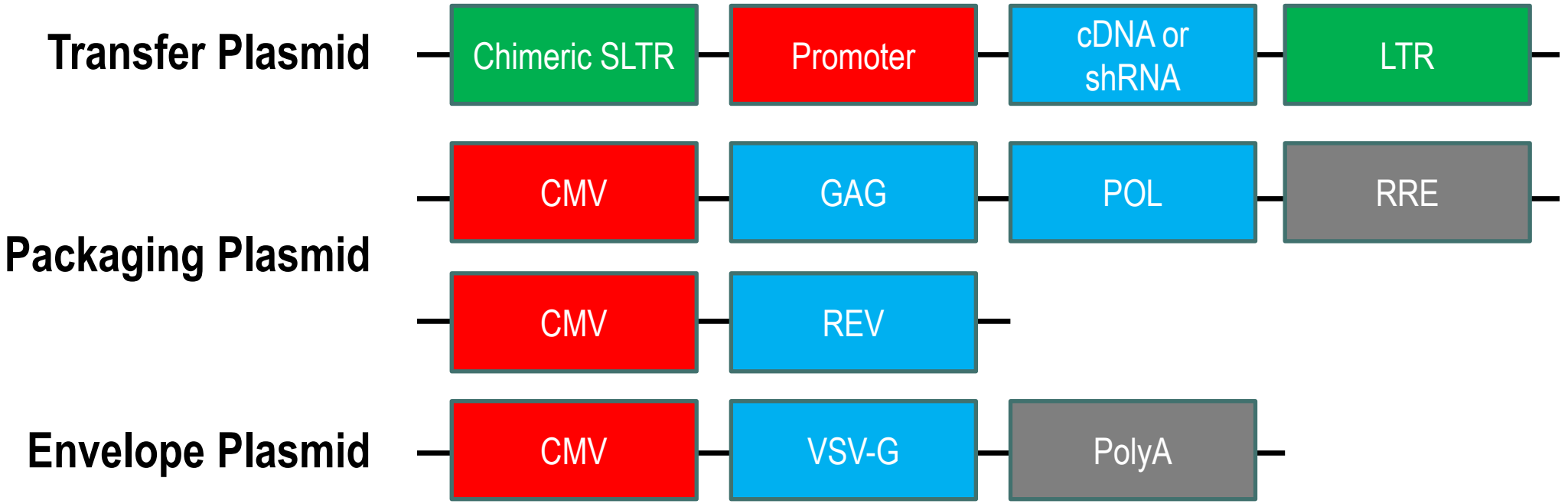
- Gene Expression/Overexpression
  - Transferring a foreign gene into a cell or organism, or inducing overexpression of a gene
  - Can be used to study gene function, identify therapeutic or harmful genes, or create gene products
- Gene Silencing
  - Interruption or suppression of gene expression
  - Can be used to identify genes of interest, study the potential effects of gene suppression and expression
- Reporting/Tracing
  - Insertion of a fluorescent protein
  - Can be used to report gene expression or trace molecular pathways



Lentiviral Vector Generations

- Most common backbone is HIV-1
- Often pseudotyped to alter tropism
  - VSV-G is the most common pseudotyping protein
- To increase safety, lentivirus is genetically modified.
  - Split genes required for viral replication into separate plasmids
  - Fully/partially delete some genes involved in replication and pathogenicity
- In 2nd generation vectors genes are split into three plasmids
- In 3rd generation vectors they are split into four plasmids, the LTR is partially deleted, and tat is removed

3rd Generation Lentiviral Vector



Lentiviral Vectors

Advantages

- Can carry moderately sized transgenes (up to 8Kb)
- Can infect both dividing and non-dividing cells
- Efficient gene transfer
- No immunogenic proteins generated
- Stable transgene expression
- Susceptible to most disinfectants
- Not able to survive well outside the laboratory

Disadvantages

- Oncogenic potential
- Accidental infection with harmful gene
- Very small potential for generation of replication competent virus

Working Safely with Viral Vectors

Risk Assessment

Hazard: A material or procedure that has the capability to cause harm to people, animals, or the environment.

- Agent Hazards
  - Pathogens
  - Oncogenes
  - Toxins
- Laboratory Procedure Hazards
  - Sharps use
  - Handling large volumes of infectious materials
  - Equipment that may generate aerosols

Risk: The likelihood that a hazard will cause harm.

- Risks can be mitigated with proper containment.

Risk Assessment

Risk Assessments:

- Identify hazards of a known materials/procedures
- Identify ways that exposures or injuries can occur
- Determine the associated consequences of an exposure or injury.

The Risk Assessment is used to determine appropriate containment levels, microbiological practices, safety equipment use and facility safeguards.

Hazards Associated with Viral Vectors

Research Component	Hazard	Risk Management Strategy
Vector Design	Generation of replication competent virus	Gene deletion, separation of genome to prevent replication, choice of virus
Transgene	Accidental exposure to oncogenes, toxin genes	Restrict sharps use, work in BSC, PPE
Scale	Large scale work has higher risk of accidental release/exposure	Additional containment and spill measures required at large scale
Sharps Use	Self-inoculation	Substitution, safety-engineered sharps, no recapping, immediate disposal into sharps containers
Laboratory Equipment	Aerosolization, spills	Equipment design, work in BSC, use secondary containment
Animal Experiments	Viral shedding, sharps use, bites/scratches	BL2 housing, sedation/restraint, safe sharps use

Risk of Wild-Type Regeneration

Replication-deficient viral vectors can recover the deleted genes required for replication through recombination, enabling them to make wild-type virus, which can cause illness

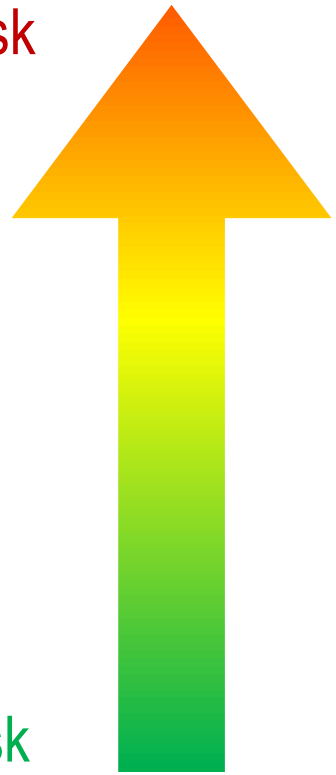
How is this risk minimized?

- The risk of recombination is lower with vector systems that have the vector and packaging functions separated onto three or more plasmids to minimize recombination events.

Nature of Gene of Interest (Transgene)

What is the risk of exposure to the transgene and potential impacts?

Higher Risk

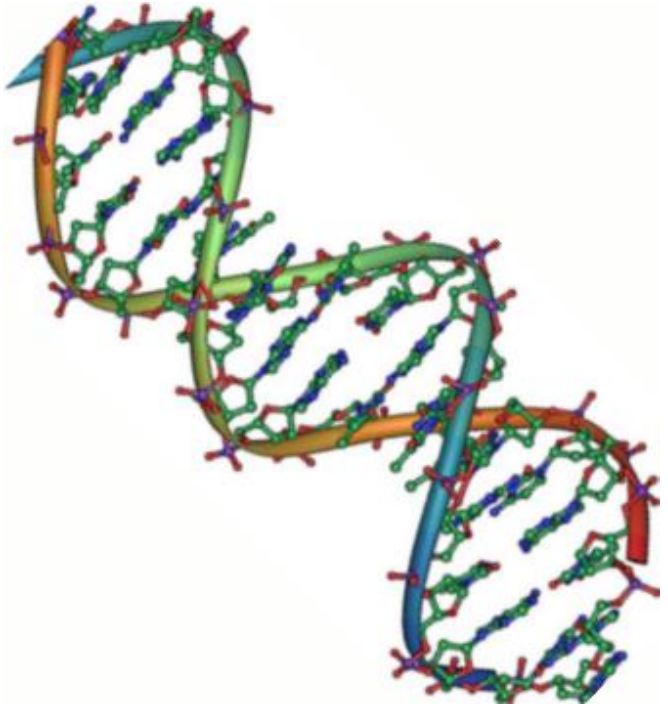


Lower Risk

- Human oncogenes
- Antagonists of tumor suppressors
- Genes coding for toxins
- shRNA, siRNA (potential off-target effects)
- Human genes not associated with disease
- Foreign mammalian genes not associated with disease
- Marker Proteins (e.g. GFP)

Inadvertent Insertional Mutagenesis

- Insertional mutagenesis: Modification of DNA by incorporating extra nucleobases
- Can be an inadvertent side effect of virus-mediated stable transfection
- Has caused leukemia in at least one clinical trial
- Associated primarily with retroviral and lentiviral vectors, although AAV vectors could hold similar risks



Biosafety Level 2 Containment

- Most viral vector work is done at BL2
- Includes all BL1 practices and:
  - Lab specific training & supervision
  - Restricted access to the lab
  - Physical containment (e.g., in a BSC) of
    - all materials or procedures that could generate infectious aerosols or splashes
  - Ensure all work surfaces are decontaminated with an appropriate disinfectant
  - Biological waste disposal procedures

Hierarchy of Controls

First Choice

Last Line of Defense

Elimination
Avoid the hazard

Substitution
Reduce the hazard

Engineering
Move the hazard away

Administrative
SOPs and Training to address the hazard

PPE
Place a barrier between you and the hazard

Viral vector necessary?

Is there a safer vector system?

Physical containment required?

Microbiological technique?

Coat, gloves, safety glasses – other PPE?

Engineering Controls / Physical Containment

Biosafety Cabinet

- Product, personnel and environmental protection
- Proper work area set-up, reduce clutter, don't block grilles
- Disinfect before and after work; after spills
- Wear PPE! This protects you and your research from contaminants.

Centrifuge Safety

- Sealed buckets / rotors

Sharps Safety

- Approved sharps waste containers
- Substitute plastic for glass
- Safe needle systems

Administrative Controls

- Biosafety Committee Approval
- Written Procedures (SOPs)
- Biosafety Training
- Good Microbiological Technique
- Restricted Lab Access
- Effective decontamination
- Spill response plan
- Proper waste treatment / disposal
- Lab hygiene / hand washing
- Sharps minimization
- Aerosol minimization
- Signage and labeling
- Medical surveillance / vaccination

Personal Protective Equipment

- Lab coats
- Disposal vs. non-disposable
- Eye/face protection
- Safety glasses with side-shields
- Face protection (goggles, face shield) for tasks with splash potential
- Gloves
- Disposable nitrile or latex
- Surgical mask (for splashes)
- N95 respirator (for aerosols)

This is the last line of defense from exposure… so wear it!

Viral Vectors and In-Vivo Experiments

Questions to consider:

- Are you generating a stably transduced cell line to inject into animals?
- Will animals be administered viral vector directly?
- How will the animal be safely restrained or sedated?
- What will be excreted/shed and for how long?
- Where should this work be performed?

Viral Vectors and In-Vivo Experiments

Safety Considerations:

- Proper sharps handling
- Appropriate restraint techniques and/or sedation to prevent bites and scratches
- Properly identify hazards associated with cage changing/animal care
- Post the appropriate door and cage signage
- Waste disposal (carcasses and bedding)

Spill Response

Non-Emergency

- **You know** the hazards associated with the spill
- Inhalation is not an exposure concern
- The spill does not generate a hazardous byproduct
- You have the appropriate supplies

Emergency

- **You don't know** what the hazards are
- You don't have supplies/PPE to clean the spill
- There is a personal exposure
- The spill is in a public space (stairwell, elevator)
- **You're not sure what to do**

Biological Spill Cleanup

For Non-Emergencies:

- Wait 15 minutes for aerosols to settle
- Wear personal protective equipment
- Place absorbent material on spill area to prevent spreading
- Slowly add disinfectant and wait 20 minutes
- Place recovered material in biohazardous waste
- Place broken glass in approved sharps container
  - Use forceps/a dustpan and broom to move broken glass!

Exposure Response

If	Then
Chemicals splash in eyes or face	Use eye wash station for **20 minutes**
Splash/spill on skin or clothing	Remove contaminated clothing and flush with water for **20 minutes**
Needle stick or cut with a sharp	Wash wound gently with soap and water
Inhalation of aerosol	Move away from hazard (blow nose)

Report all spills and exposures to EHS, no matter how minor!

In Conclusion

- Remember to register your viral vectors research with the IBC!
- Safe work practices and controls are determined by an effective risk assessment.
- A clear understanding of the hazards and risks associated with specific vectors and tasks is essential!
- Report spills and exposures to EHS.
- Contact the Biosafety Officer with questions or concerns or help with a registration.

Questions?

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