Introduction to Viral Vectors and the Institutional Biosafety Committee (IBC)

This presentation is intended to serve as an aid for researchers that are new to viral vectors for use with mammalian systems and would like assistance writing IBC applications.

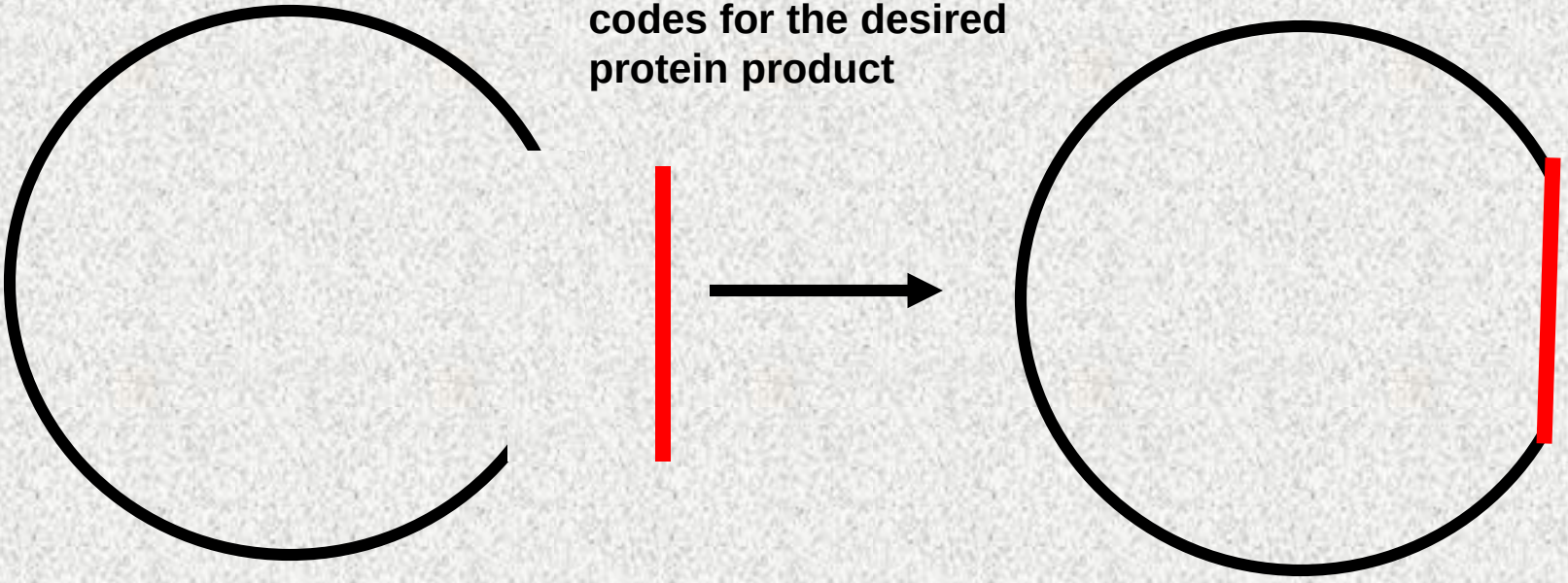
Daniel Eisenman, PhD
Biosafety Officer
Medical University of South Carolina
eisenman@musc.edu
(843) 792-4304

*NIH's D*efinition of Recombinant DNA (rDNA)

- **Molecules that are constructed outside living cells by *joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell*.**

- **Molecules that result from the replication of those described above.**

Insert: Piece of DNA that codes for the desired protein product



Plasmid: Circular DNA Molecule

Recombinant DNA

An institution's IBC is charged with ensuring compliance with NIH guidelines for research involving rDNA.

Any institution receiving funds from NIH to perform research involving recombinant DNA must have an IBC.

The IBC must review the recombinant DNA research conducted at the institution.

NIH guidelines refer to CDC guidelines for Biosafety in Microbiological and Biomedical Laboratories (BMBL)

Failure to comply with these guidelines can result in loss of NIH funding by the institution and additional fines.

What can go wrong? "Seven Experiments of Concern"

Proposed by NIH's National Science Advisory Board for Biosecurity (NSABB)

1. **Demonstrate how to render a vaccine ineffective**
   This would apply to both human and animal vaccines. Creation of a vaccine resistant smallpox virus would fall into this class of experiments.

2. **Confer resistance to therapeutically useful antibiotics or antiviral agents**
   This would apply to therapeutic agents that are used to control disease agents in humans, animals, or crops. Introduction of ciprofloxacin resistance in *Bacillus anthracis* would fall in this class.

3. **Enhance the virulence of a pathogen or render a nonpathogen virulent**
   This would apply to plant, animal, and human pathogens. Introduction of cereolysin toxin gene into *Bacillus anthracis* would fall into this class.

4. **Increase transmissibility of a pathogen**
   This would include enhancing transmission within or between species. Altering vector competence to enhance disease transmission would also fall into this class.

5. **Alter the host range of a pathogen**
   This would include making nonzoonotics into zoonotic agents. Altering the tropism of viruses would fit into this class.

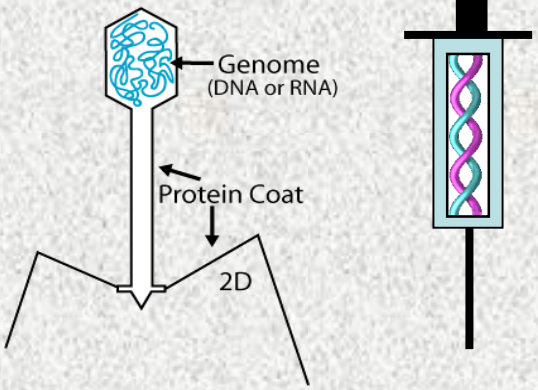
6. **Enable the evasion of diagnostic/detection modalities**
   This could include microencapsulation to avoid antibody-based detection and/or the alteration of gene sequences to avoid detection by established molecular methods.

7. **Enable the weaponization of a biological agent or toxin**
   This would include the environmental stabilization of pathogens. Synthesis of smallpox virus would fall into this class of experiments.

Infectious Viruses: A Genetic "Syringe"

Viruses are composed of genetic material encapsulated in a protein coat.

Viruses inject their genetic material into target cells.

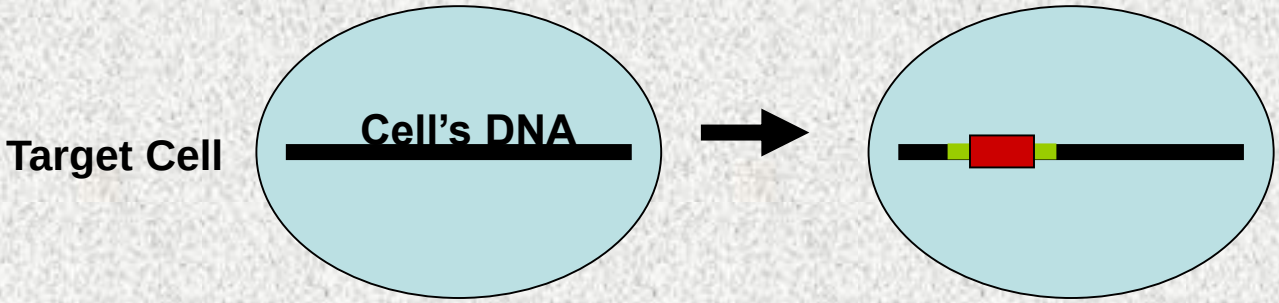


DNA Loaded Syringe

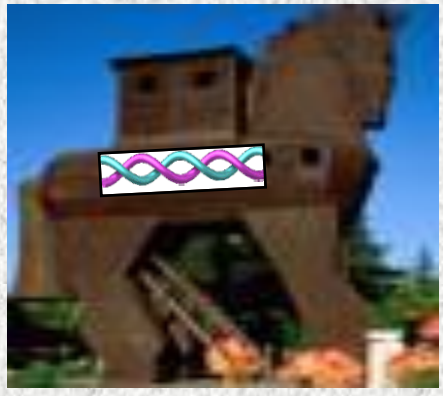
Viruses infect target cells with their genetic material.

The viral DNA can be altered to contain a gene of interest (rDNA) to infect that gene into the target cell.

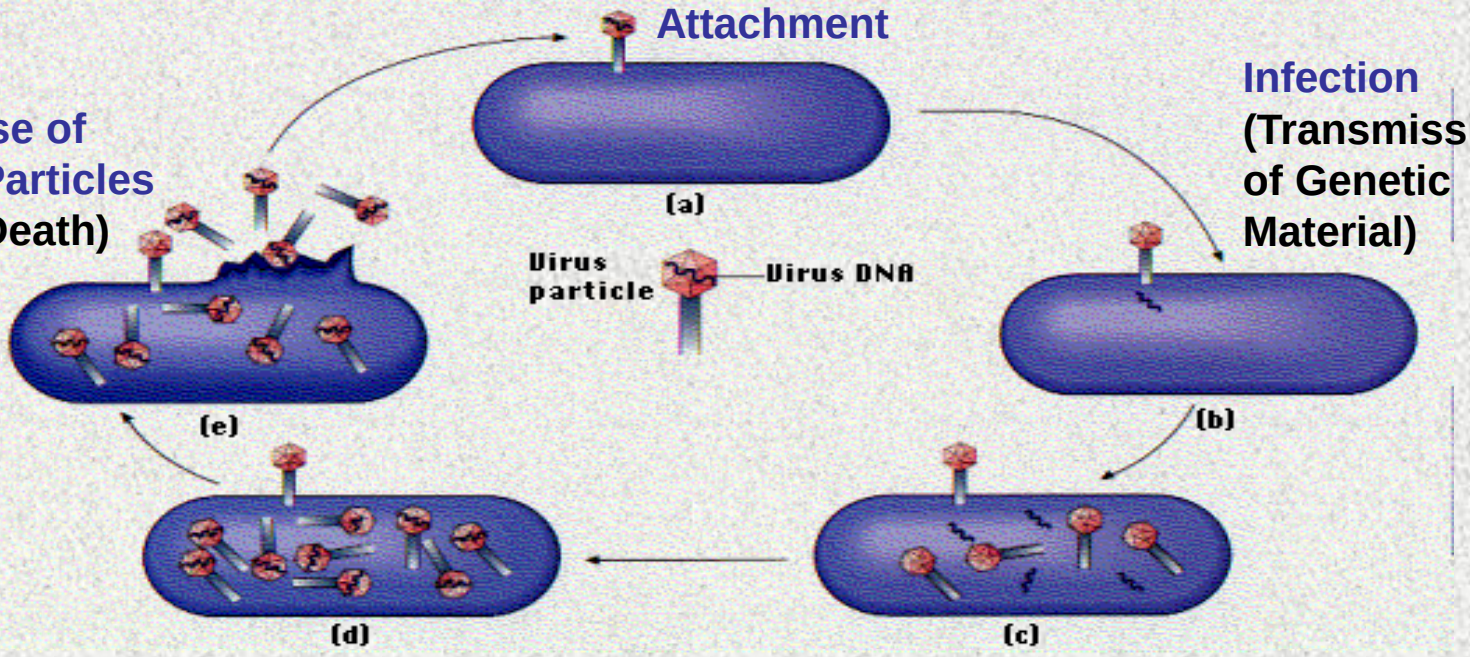
Target Cell Infected With Viral DNA Containing The Gene of Interest



Safety Concerns Related to Infectious Viruses: A Genetic "Trojan Horse"

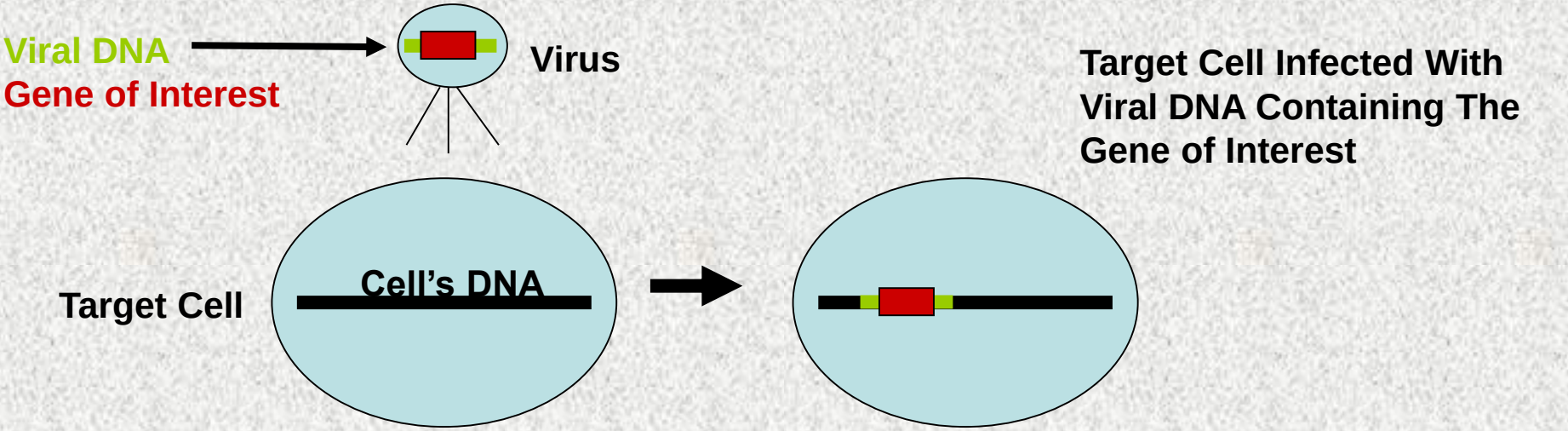


Viruses Cannot reproduce by themselves, so they infect cells with their genetic material to hijack the cellular machinery to produce more viruses. This process can result in cell death, tissue damage or even the death of the infected organism.

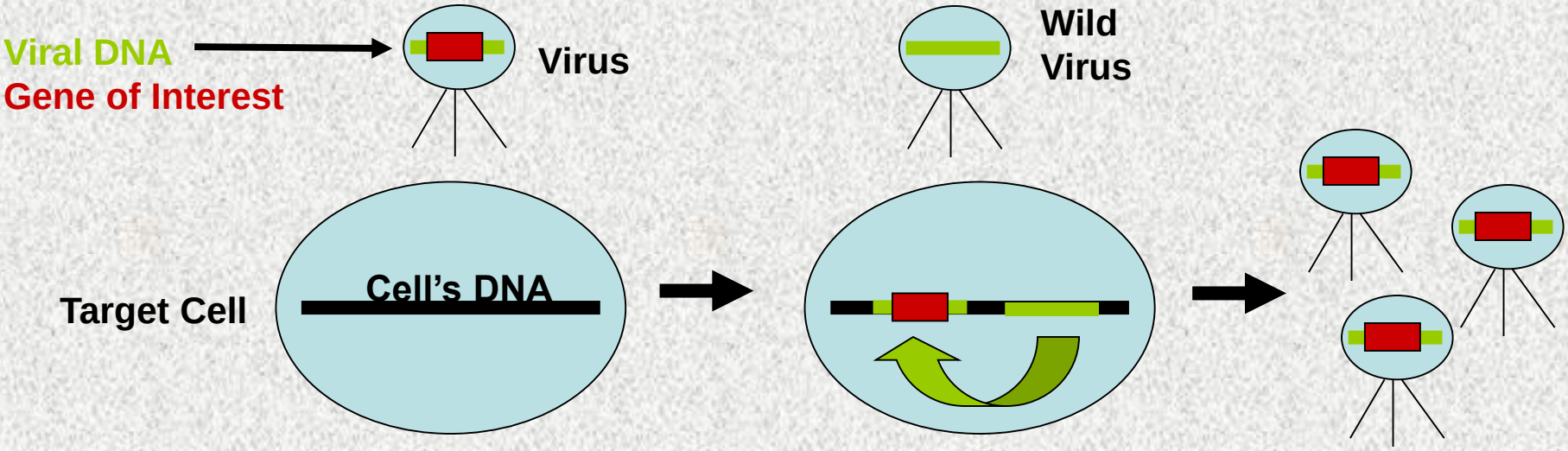


Replication Deficient Viral Vectors: Genetically Engineered So The Viral Infection Cannot Spread

- The viral DNA does not contain the viral genes needed to make more viruses.



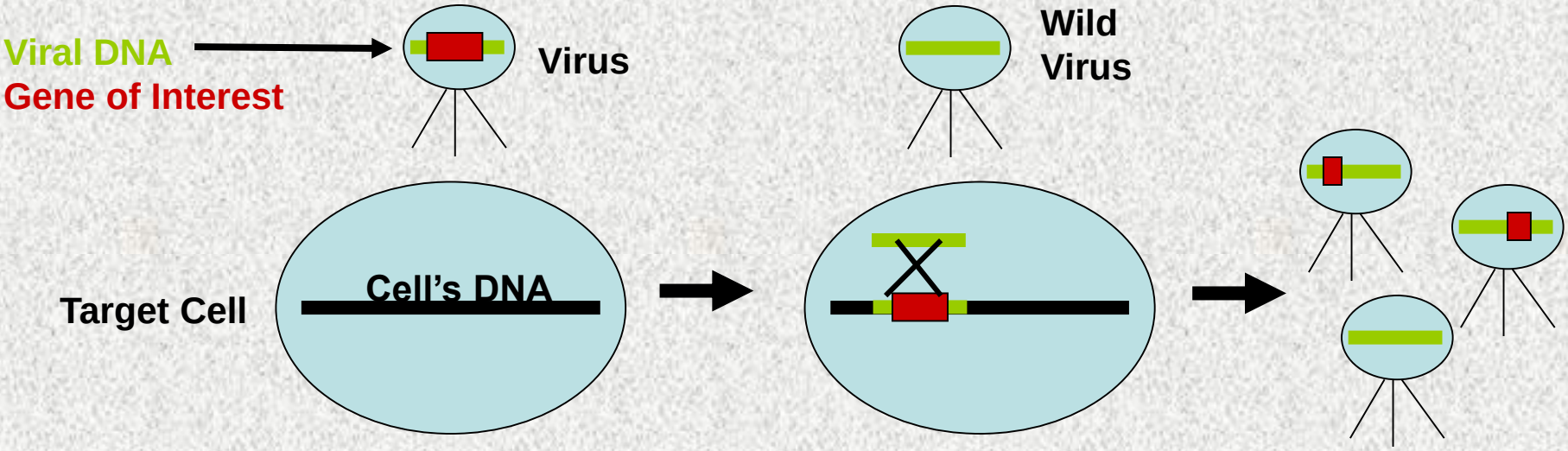
Rescue of Replication Deficient Viruses by superinfection with Wild Viruses



Complementation:

The genome from the wild virus provides the missing proteins needed for the viral vector to replicate. The superinfected cell functions similarly to a packaging line.

Rescue of Replication Deficient Viruses by superinfection with Wild Viruses

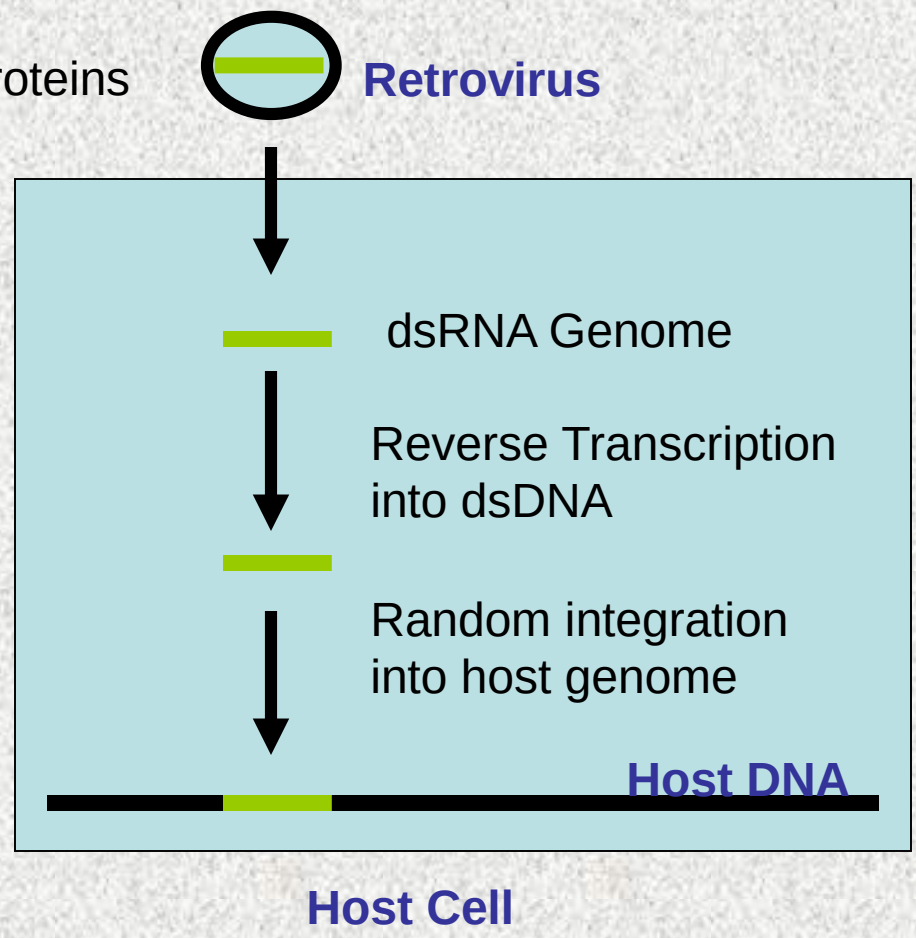


Recombination:

The genome from the wild virus randomly recombines with the viral vector, providing sufficient genetic material for the viral vector to replicate. The resulting rescued virus may possess pieces of the original insert gene. The viral genome is impossible to predict due to random recombination. The virus may exhibit altered virulence.

Retroviruses
(including Lentivirus, HIV and MMLV based vectors)

- Double stranded RNA genome
- Lipid membrane enveloped
- Host range determined by envelope proteins



The Retroviral Genome

Long Terminal Repeat (LTR): Necessary for integration into host genome

ψ (Psi): packaging signal

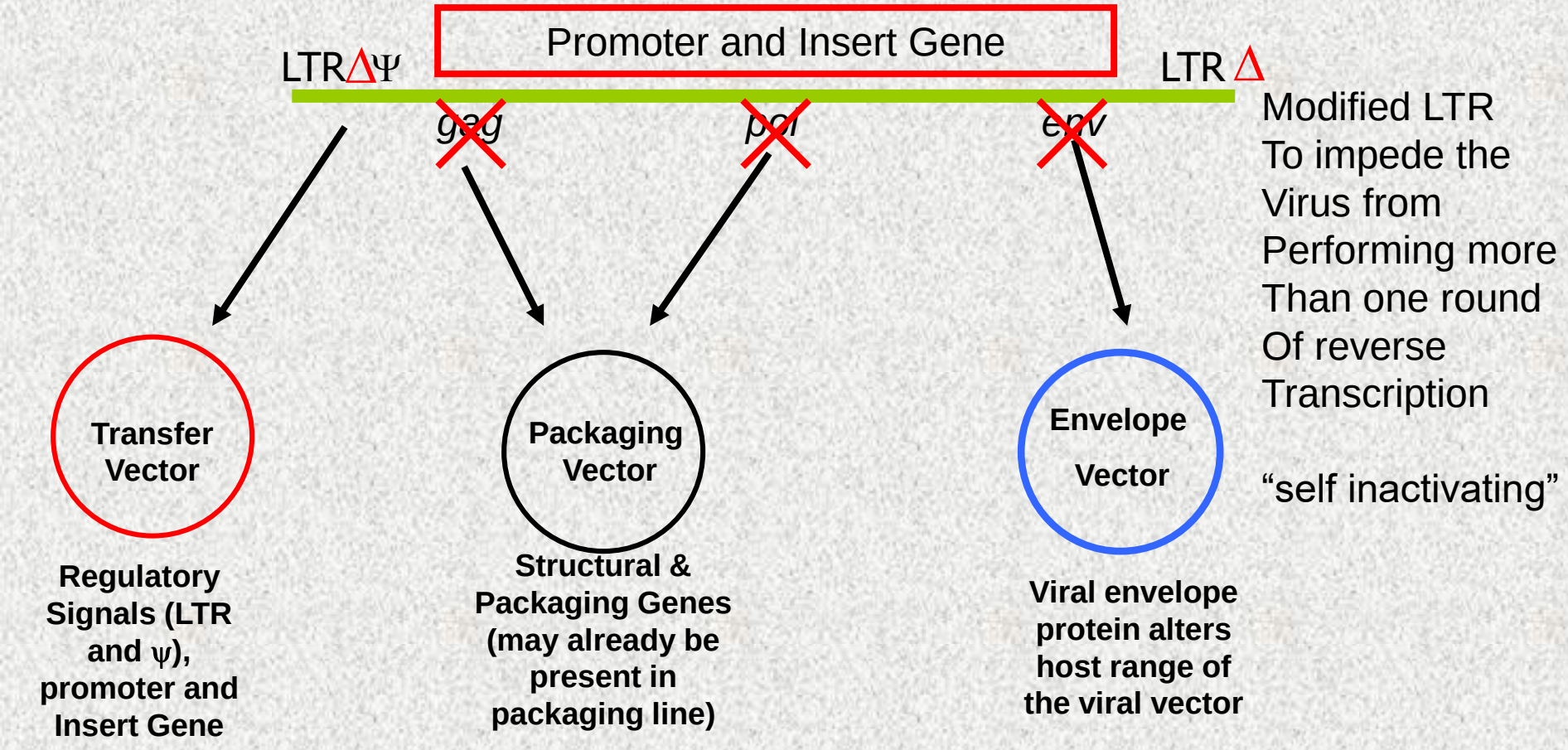
***gag*: Packages viral genome into viral particles**

***pol*: viral polymerase necessary for viral replication**

***env*: viral envelope proteins, necessary for entry into host cells, dictate host range**

Design of Replication Incompetent Lentiviral Vectors (3rd Generation)

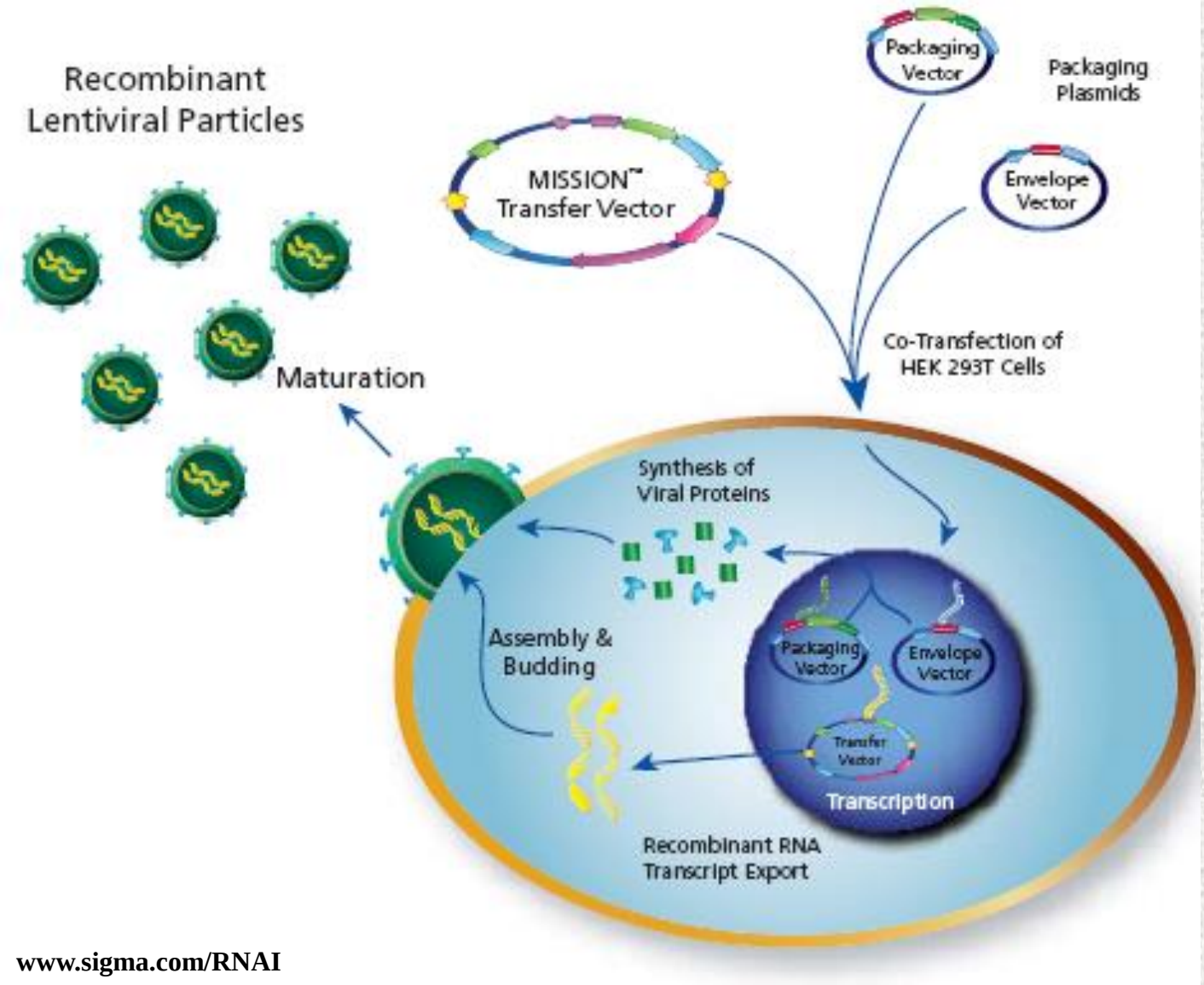
The viral vector is "gutted" as much as possible to create room for the insert gene and to divide the viral genome into cis- and trans- acting regions



Modified LTR
To impede the
Virus from
Performing more
Than one round
Of reverse
Transcription

"self inactivating"

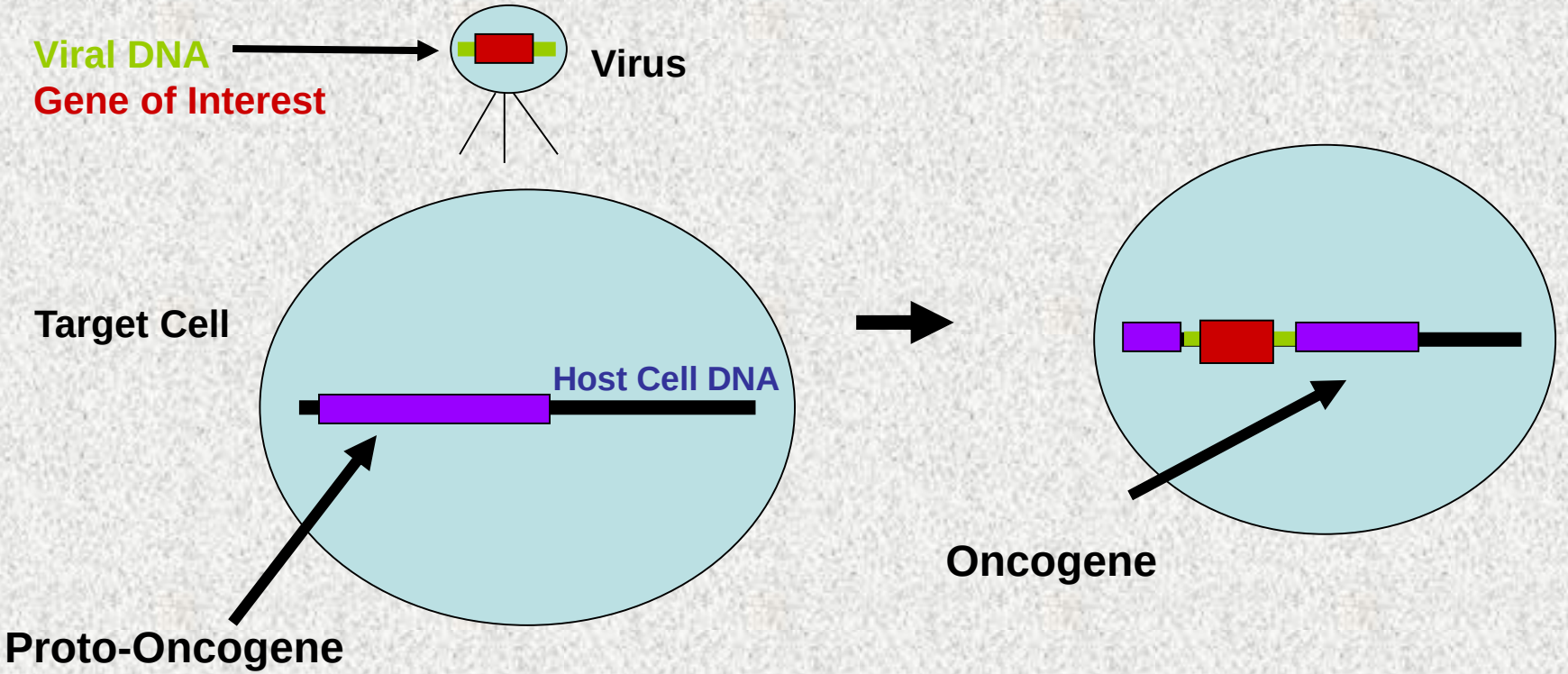
Packaging Recombinant Lentiviral Particles



The three plasmids containing the viral genome components are transfected into the packaging line to create the infectious viral particles.

Multiple plasmids are used so multiple recombination events would be required to reconstitute a replication competent virus.

Risks Associated with Retroviruses: Insertional Mutagenesis



Random integration of viral genome may disrupt endogenous host genes. Of special concern Is disruption of proto-oncogenes, which can lead to increased cancer risk.

Viral Psuedotyping: A Double Edged Sword

Tropism: The ability of a virus to infect a particular type of host cell

Psuedotyping: Altering the viral envelope protein to alter tropism, thus allowing the virus to infect cells it originally could not

Tropism	Host Range	Viral Envelope Protein	Receptor for Viral Envelope
Ecotropic	Mouse / Rat	Gap70	mCAT-1
Amphotropic / Dualtropic	Mammals	4070A / 10A1	Ram-1 / GALV
Pantropic	All Animals	VSV-G	Phosphotidyl serine Phosphotidyl inositol GM3 ganglioside

Special care should be used when working with pantropic or amphotropic viruses that can infect humans!

Adenovirus

•dsDNA genome

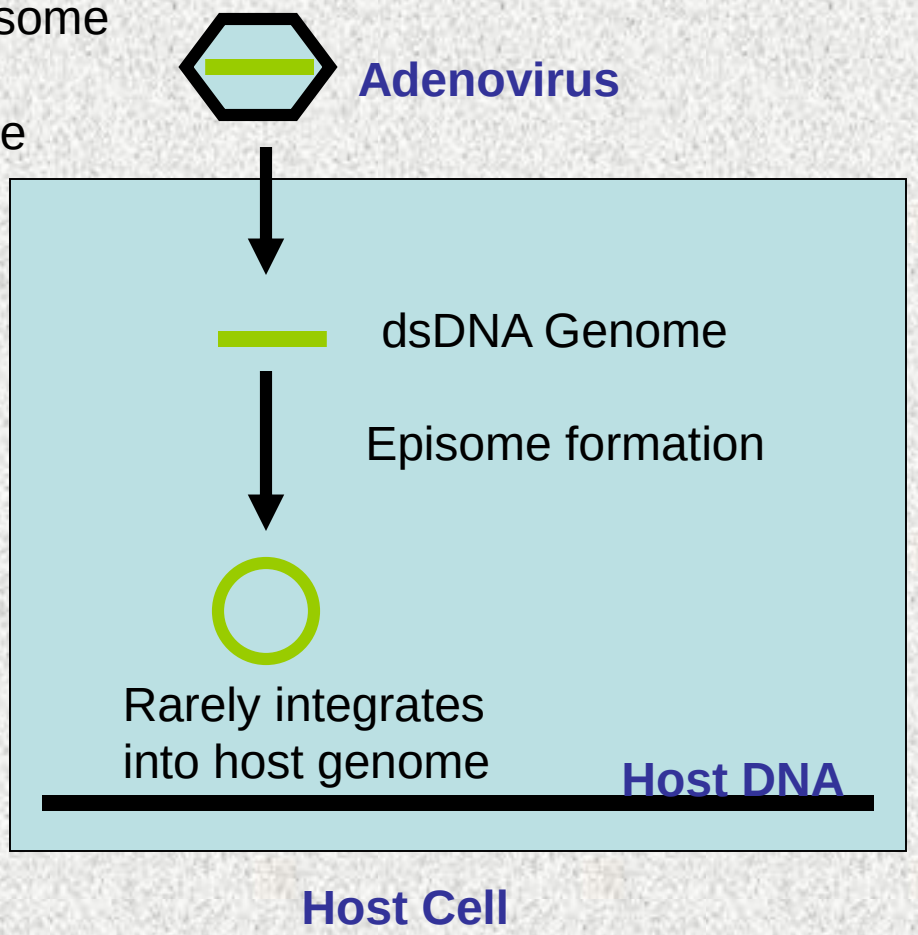
•Non-Lipid Enveloped

•Upon infection, the viral DNA forms an episome

•Episome rarely integrates into host genome

•Fixed host range affecting
Rodents, humans and other animals

•Known receptors:
Cocksacki & Adenovirus Receptor (CAR)
HLA / MHC I



Risks Associated with Adenoviruses

•Adenovirus is transmitted by direct contact with mucus membranes (eyes, nose and mouth), fecal-oral transmission, and occasionally waterborne transmission.

•Adenovirus infections most commonly cause illness of the respiratory system with symptoms ranging from the common cold to pneumonia, croup, and bronchitis. Depending on the infecting serotype, adenovirus infection may also cause other illnesses such as gastroenteritis, conjunctivitis and rash.

•Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection.

•Transplacental infection can occur during pregnancy and can lead to teratogenic effects.

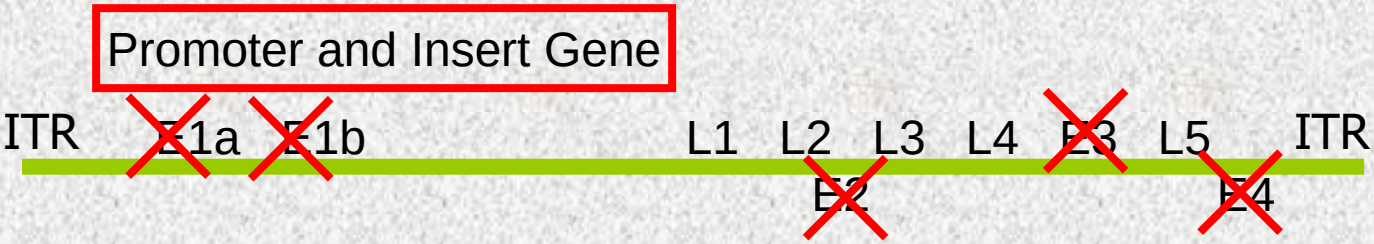
The Adenoviral Genome

ITR – Inverted Terminal Repeat (origin of viral replication)

E – Early Response Genes
- Initiation and activation of viral replication
- Suppression of host cell gene expression and protein synthesis
- Activation of late response genes (L)

L – Late response (viral structural components)

Design of Replication Incompetent Adenoviral Vectors



E1a – Initiates replication, activates adenoviral transcription, stimulates infected host cell to enter S phase

E1b – Viral RNA transport while blocking host mRNA transport, blocks apoptosis

E2 – Involved in replicating viral genome

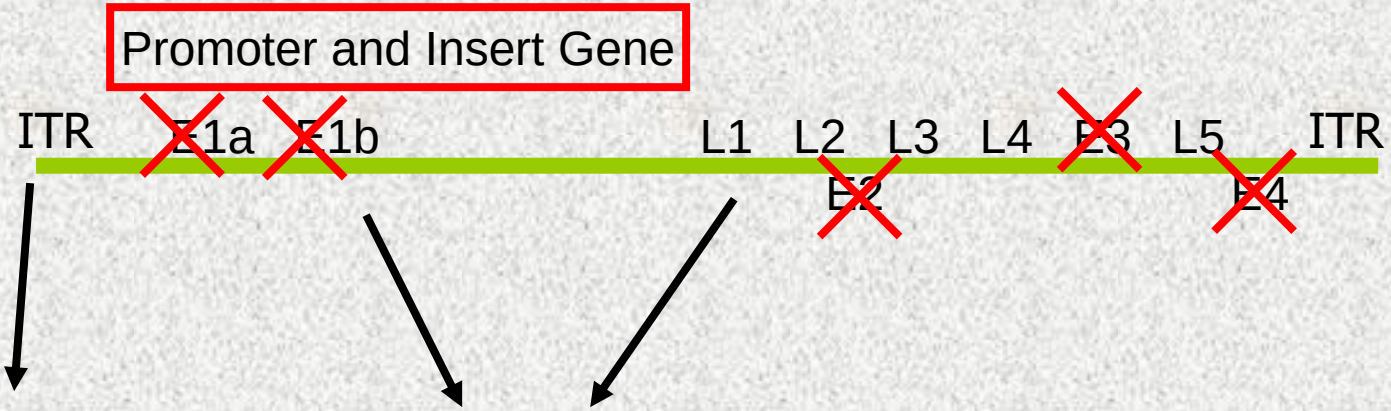
E3 – Limits expression of HLA / MHC I and inflammation caused by TNFα to limit antiviral immunity of the infected host

E4 – Blocks host protein synthesis which can lead to death of host cell

Some adenoviral vectors may have fewer Early Response genes deleted. However, all E1 deficient adenoviral vectors are replication deficient in cells that neither express E1 or E1 like proteins.

Design of Replication Incompetent Adenoviral Vectors

The viral vector is "gutted" as much as possible to create room for the insert gene and to divide the viral genome into cis- and trans- acting regions. The deleted regions are present in packaging cells or are provided by co-transfection completing the viral genome and allowing creation of viral particles.



Adenoviral Plasmid

Viral genome, promoter and Insert Gene

Helper Vector

Deleted Early Response Genes (E1, etc.)

(may already be present in packaging line)

The viral genome is divided to require recombination to reconstitute a replication competent virus. Barring such recombination, the resulting viral particles can infect but cannot replicate.

Common Methods of Deactivating Viruses

Lipid Enveloped Viruses
(Retro, Lenti, MMLV, HIV, Herpes Simplex, Flu, Hepatitis B and C)

Ethanol
Quaternary Ammonium Compounds
Phenol
10% Bleach
Aldehydes (Paraformaldehyde, Gluteraldehyde)
Autoclave

Non-Lipid Enveloped
(Adenovirus, Adeno-Associated Virus)

10% Bleach
Aldehydes (Paraformaldehyde, Gluteraldehyde)
Autoclave

Please note:

Non-lipid Enveloped Viruses are Resistant to weaker disinfectants like ethanol and quaternary ammonium compounds.

10% bleach decomposes over time and has an approximate half life of 2 weeks. Recommend making fresh weekly.

Liquid disinfectants must be allowed the Appropriate contact time to be effective.

Risk Assessment

Risk assessment is a vital part of the IBC review process as required by the NIH.

The purpose of a risk assessment is to determine the risk to researchers, the community and the environment.

Steps to conduct a risk assessment:
Identify hazards
Assess possibility for exposure
Manage the risk

Managing risk involves implementing controls to limit risk.
Example of controls include:

Personal Protective Equipment (PPE): **gloves, lab coat, eye and respiratory protection**

Engineering: **Biosafety Cabinet, centrifuge with sealed rotors or safety caps**

Work Place Practices: **Following the PI's approved biosafety protocol**

Administrative: **Training, supervision, lab inspections, vaccination, medical surveillance**

Containing Risks Associated with Aerosols

Aerosol Producing Procedure	Method of Containment
Splash/Spray	biosafety cabinet, fume hood, splash shield
Vortexing	sealed tubes, biosafety cabinet
Centrifugation	sealed tubes, sealed rotor, safety cups
Homogenization	biosafety cabinet, fume hood, splash shield
Flow cytometry	fixation or BSL2+ containment
Injection/administration Into animals	biosafety cabinet, animal restraint
Cage cleaning (infected animals)	biosafety cabinet, PPE (contact Biosafety Officer to review procedures and PPE)

PPE for BSL2 labs: gloves and lab coats are required,
eye and respiratory protection (as needed)

Factors of a Risk Assessment for Viral Vectors

Vector

Insert Gene

Procedures

Volumes

Examples of Low Risk Work with Viral Vectors

Vector – Replication incompetent and self inactivating vector
Limited tropism (incapable of infecting humans)

Insert Gene – Is Not: toxic, oncogenic, immune modulatory, or increases tropism or pathogenicity

Procedures – limited to cell culture in a biosafety cabinet, centrifugation with sealed tubes and safety caps or sealed rotors

Volumes – 1-10 mL (easy to contain and transport)

Examples of High Risk Work with Viral Vectors

Vector – Replication competent vector, capable of infecting humans

Insert Gene – toxin or toxic at high levels, oncogene, immune modulation, increases viral tropism or pathogenicity

Procedures – aerosol production (homogenization, vortexing in open tubes, centrifugation without sealed tubes, safety caps or sealed rotors) injection/administration into animals

Volumes – Liters (requires bulkier containment and a cart to transport, higher likelihood of a spill)

Additional Questions?

Please contact:

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