Introduction to Retro/Lentiviral 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.

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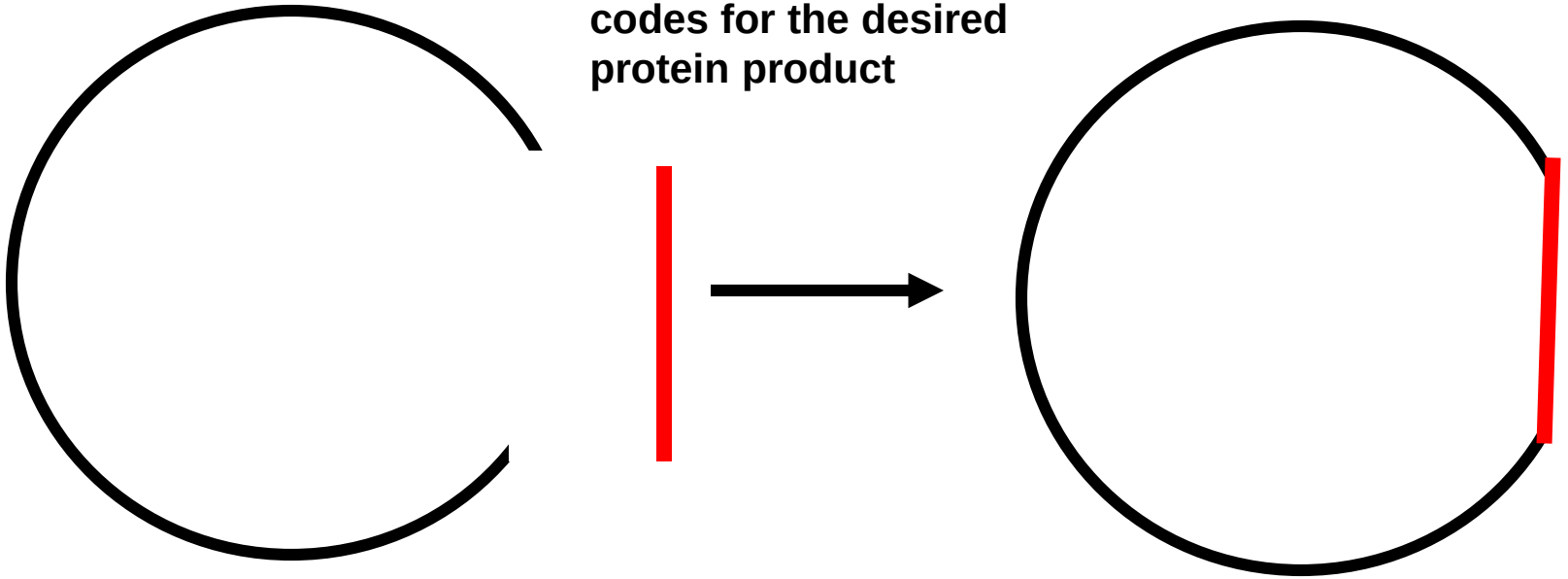
*NIH's D*efinition of Recombinant DNA (rDNA)

- **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.

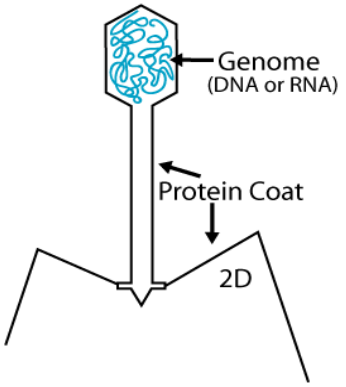
NIH guidelines refer to CDC guidelines for Biosafety in Microbiological and Biomedical Laboratories (BMBL)

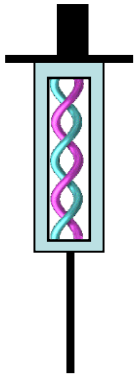
Failure to comply with these guidelines can result in loss of NIH funding by the institution and additional fines.

Infectious Viruses: A Genetic "Syringe"

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

Viruses inject their genetic material into target cells.

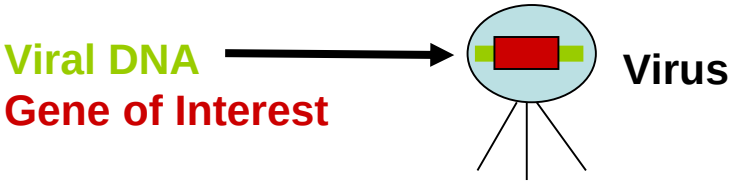


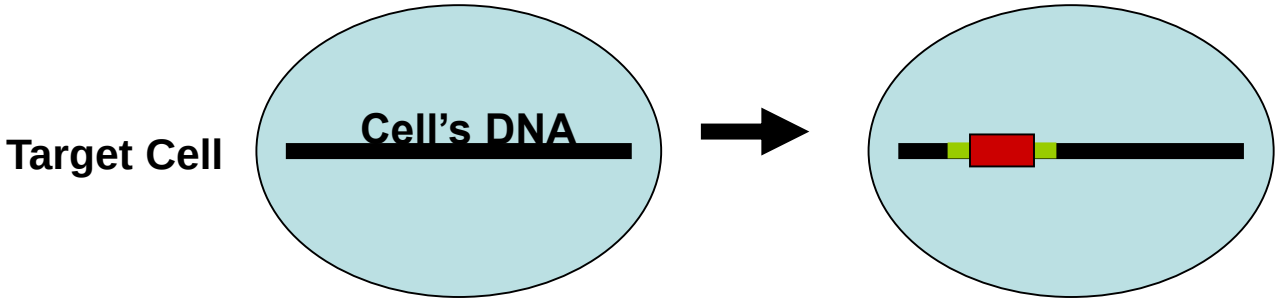


DNA Loaded Syringe

Viruses infect target cells with their genetic material.

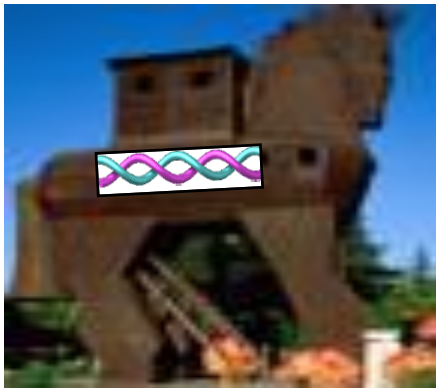
The viral DNA can be altered to contain a gene of interest (rDNA) to infect that gene into the target cell.



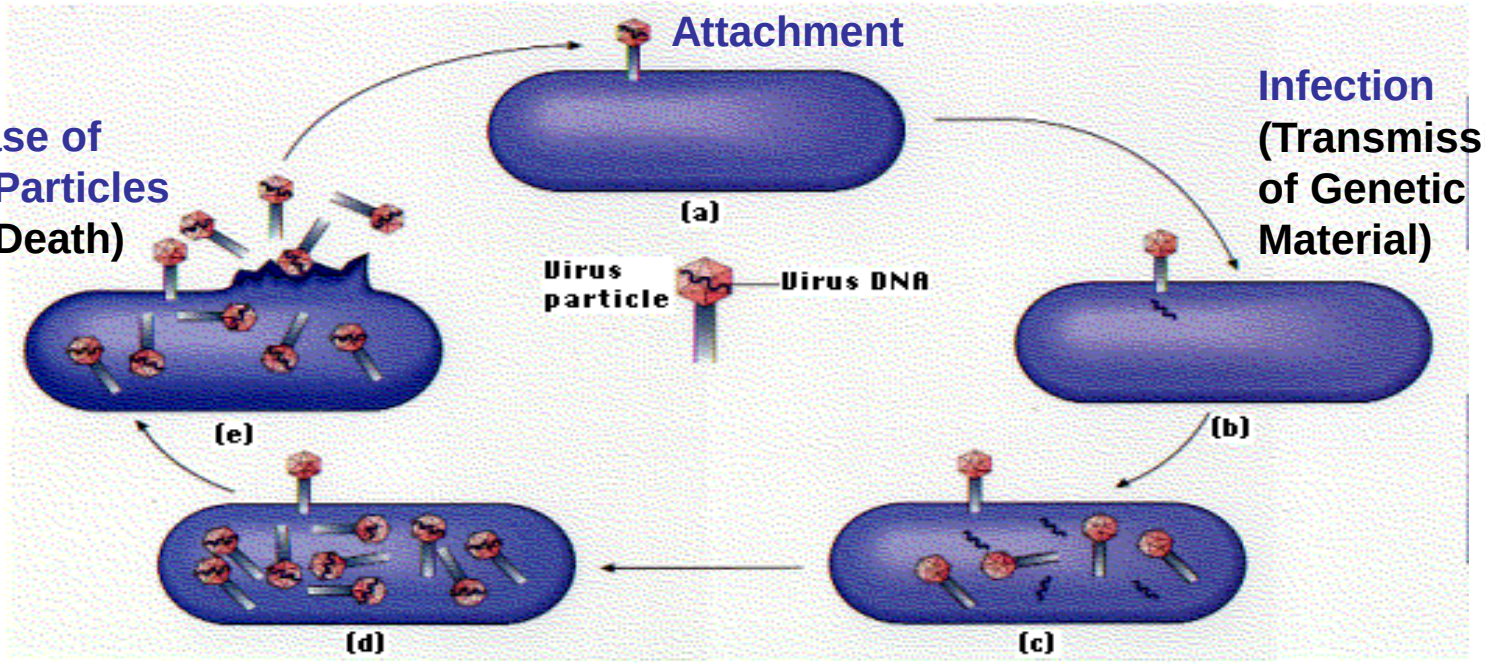


Target Cell Infected With Viral DNA Containing The Gene of Interest

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



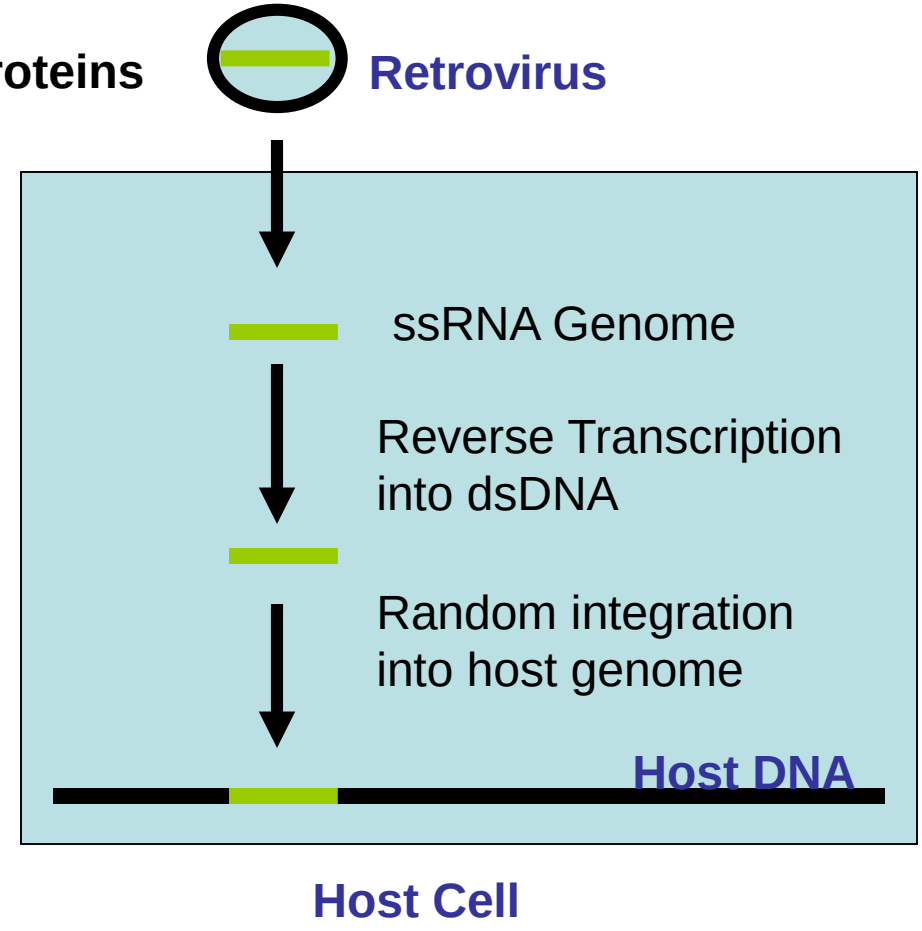
Viruses Cannot reproduce by themselves, so they infect cells with their genetic material to hijack the cellular machinery to produce more viruses. This process can result in cell death, tissue damage or even the death of the infected organism.



Retroviruses

(including Lentivirus, HIV and MMLV based vectors)

- **Single 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

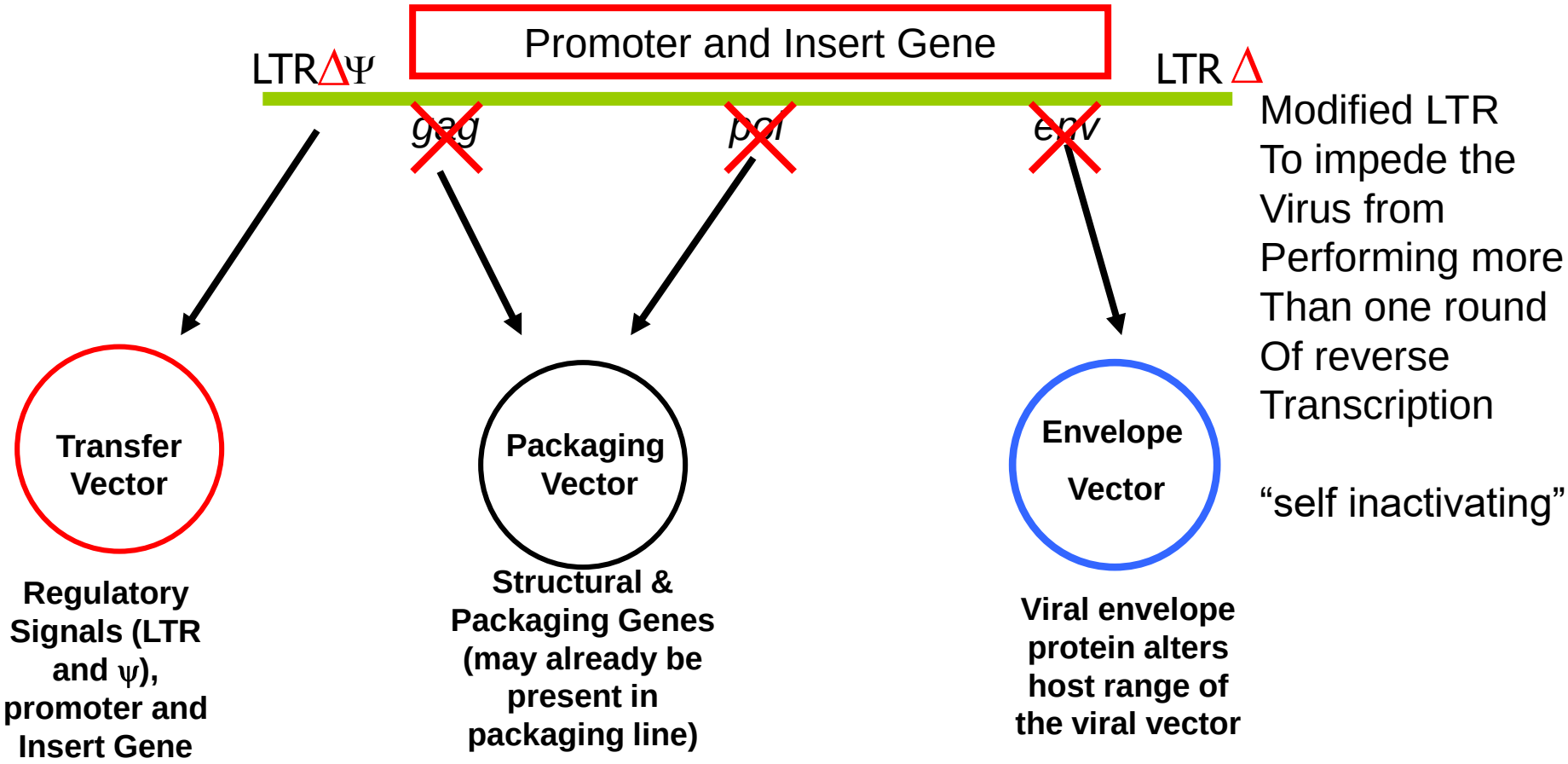
***gag*: Packages viral genome into viral particles**

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

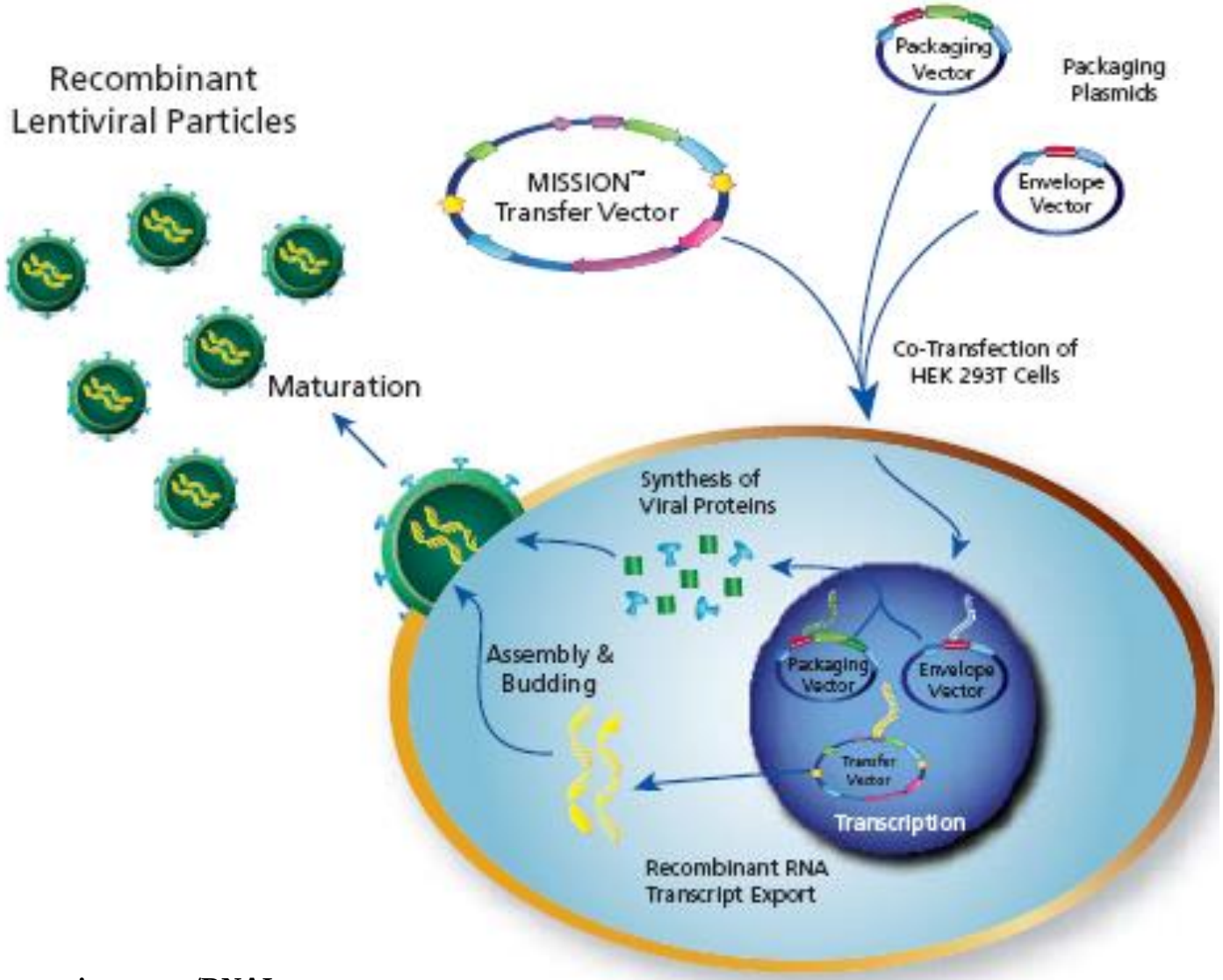
***env*: viral envelope proteins, necessary for entry into host cells, dictate host range**

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



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.

Viral Psuedotyping: A Double Edged Sword

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

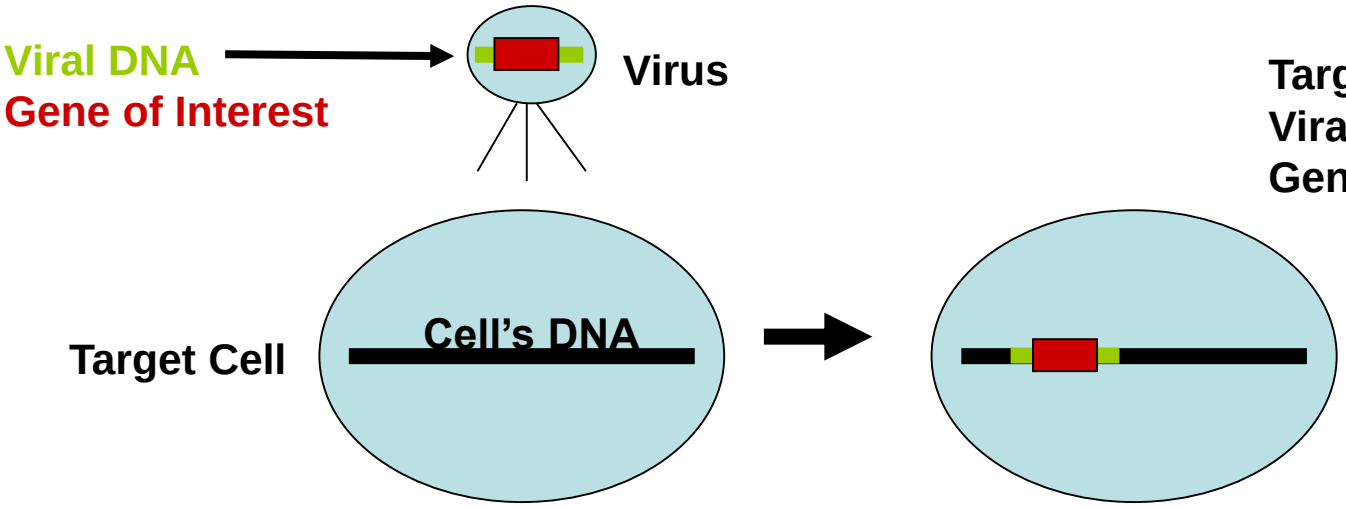
Psuedotyping: Altering the viral envelope protein to alter tropism, thus allowing the virus to infect cells it originally could not

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 which can infect humans!

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

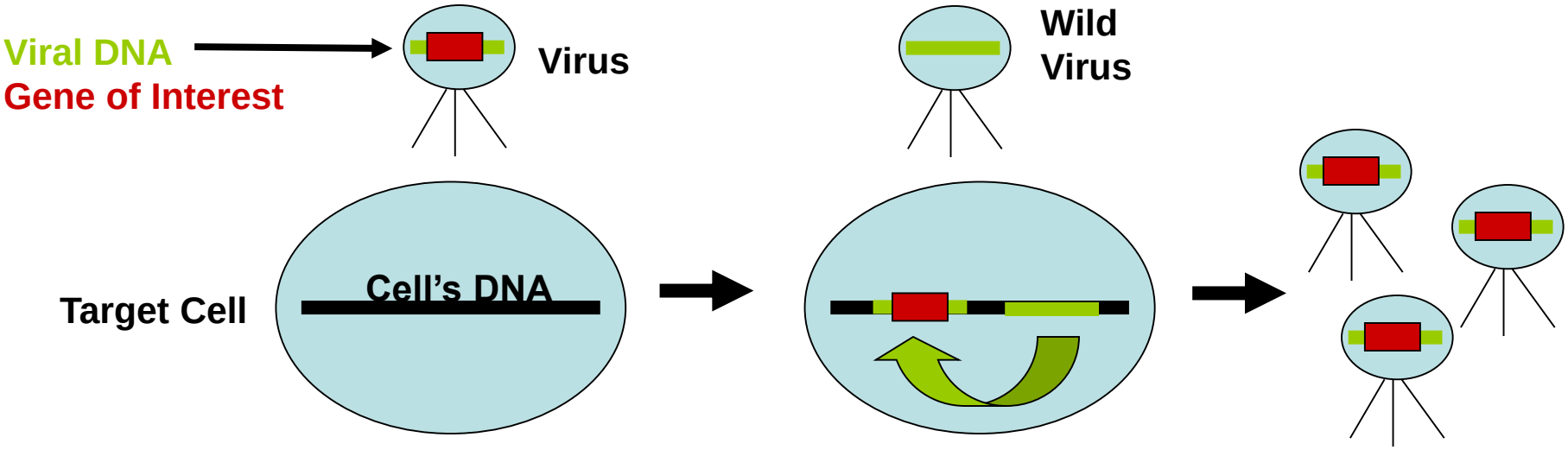
- **The viral DNA does not contain the viral genes needed to make more viruses.**



Target Cell Infected With Viral DNA Containing The Gene of Interest

No New Viral Particles are Created
Infection dose not spread

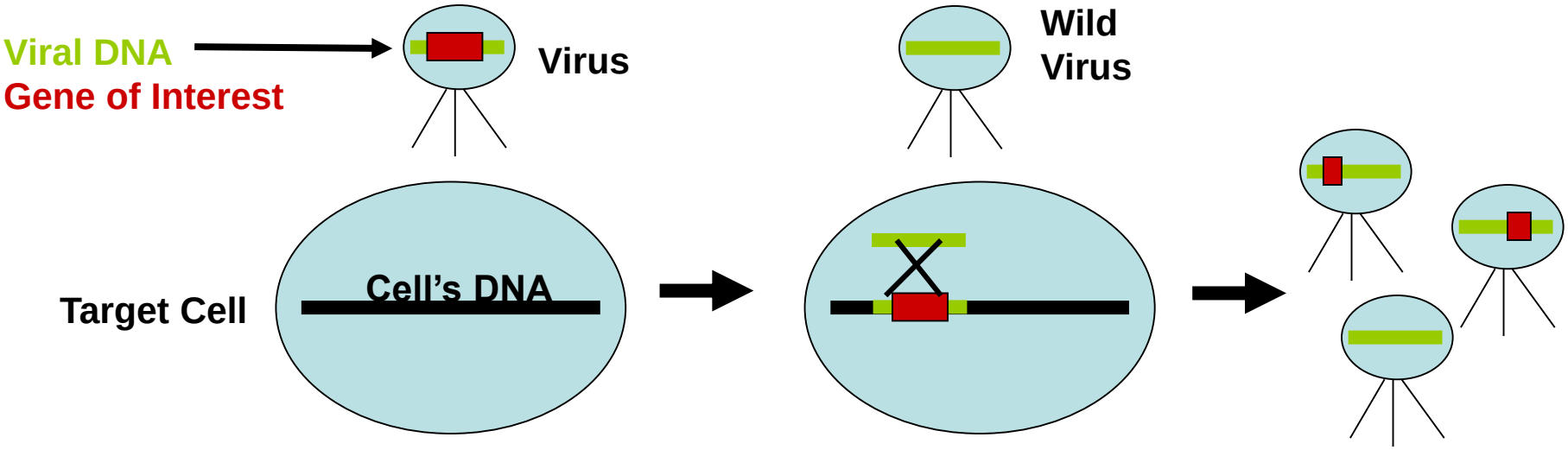
Rescue of Replication Deficient Viruses by superinfection with Wild Viruses



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.

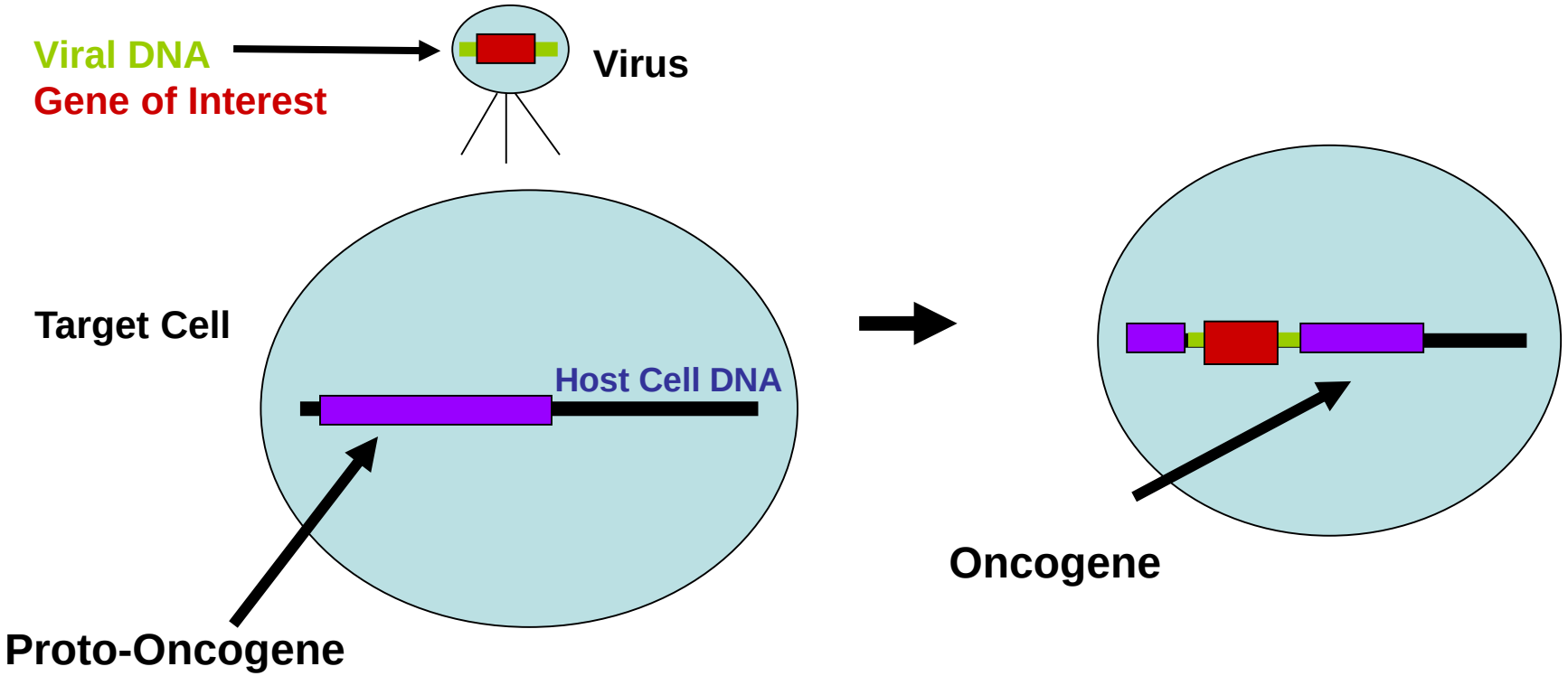
Rescue of Replication Deficient Viruses by superinfection with Wild Viruses



Recombination:

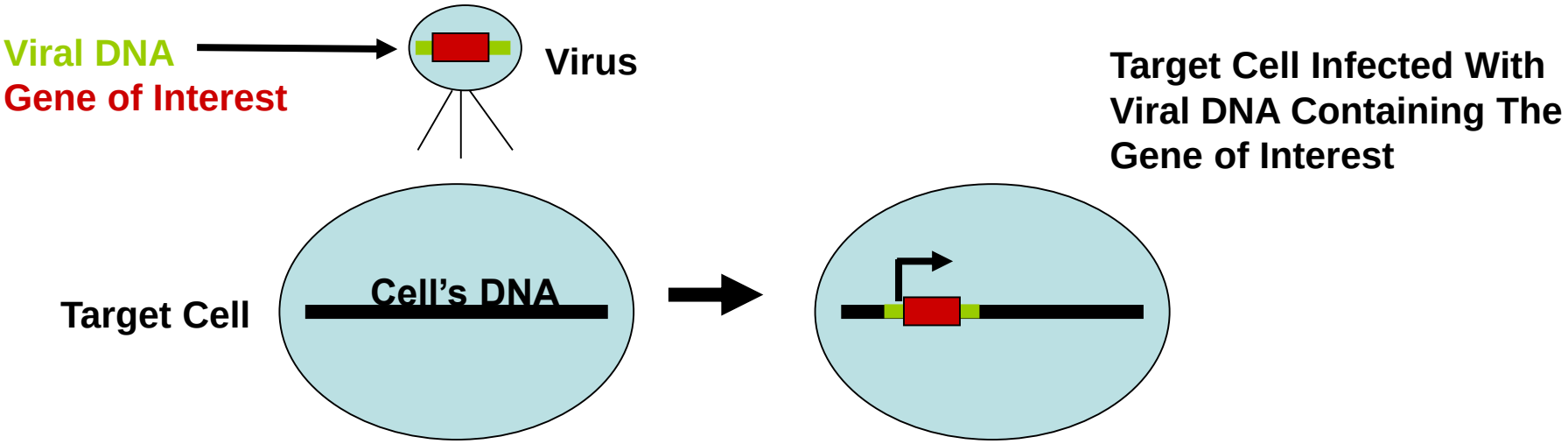
The genome from the wild virus randomly recombines with the viral vector, providing sufficient genetic material for the viral vector to replicate. The resulting rescued virus may possess pieces of the original insert gene. The viral genome is impossible to predict due to random recombination. The virus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

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.

Risks Associated with Retroviral Vectors: Viral Transduction



Individuals infected with the viral vector may express the insert gene at the site of infection.

Likely Symptoms of Lab Acquired Infections with Retro/Lentiviral Vectors

- **Fever / flu-like symptoms**
- **Possible inflammation of infected tissues**
- **Random integration of viral genome into host genome can result in insertional mutagenesis and oncogenesis (see slide 15)**
- **Expression of insert genes in infected tissues (oncogenes, inflammatory mediators and toxins are of special concern) (see slide 16)**

Common Methods of Deactivating Viruses

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

Ethanol ← **Cavicide**
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?

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