
An Introduction to Viral Vectors: Safety Considerations

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Learning Objectives

- Recognize hazards associated with viral vectors in research and animal testing laboratories.
- Interpret viral vector modifications pertinent to risk assessment.
- Understand the difference between gene delivery vectors and viral research vectors.

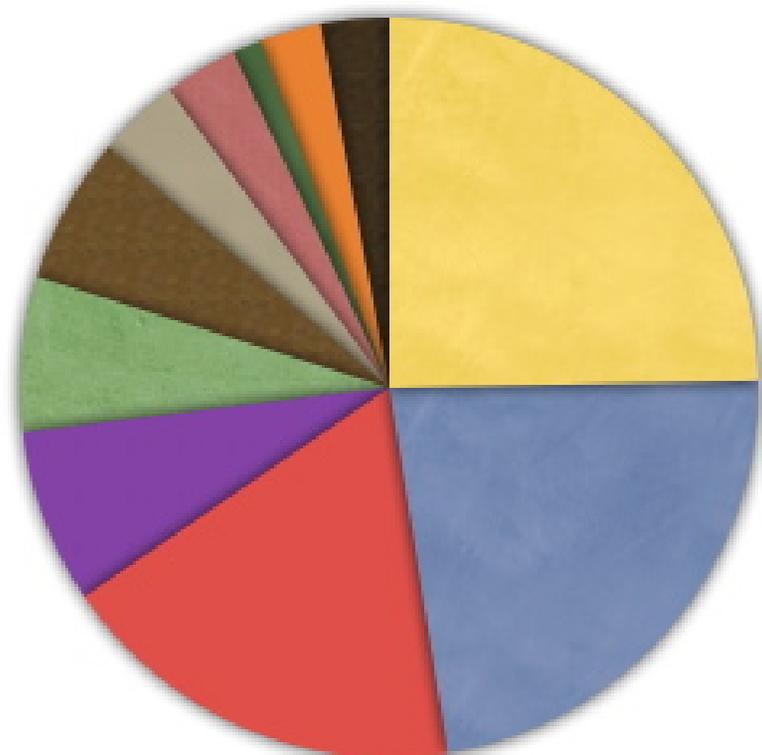
Outline

- Introduction to Viral Vectors
- Retroviral & Lentiviral Vectors (+RNA virus)
- Adeno and Adeno-Assoc. Vectors (DNA virus)
- Novel (-)RNA virus vectors
- NIH Guidelines and Other Resources
- Conclusions

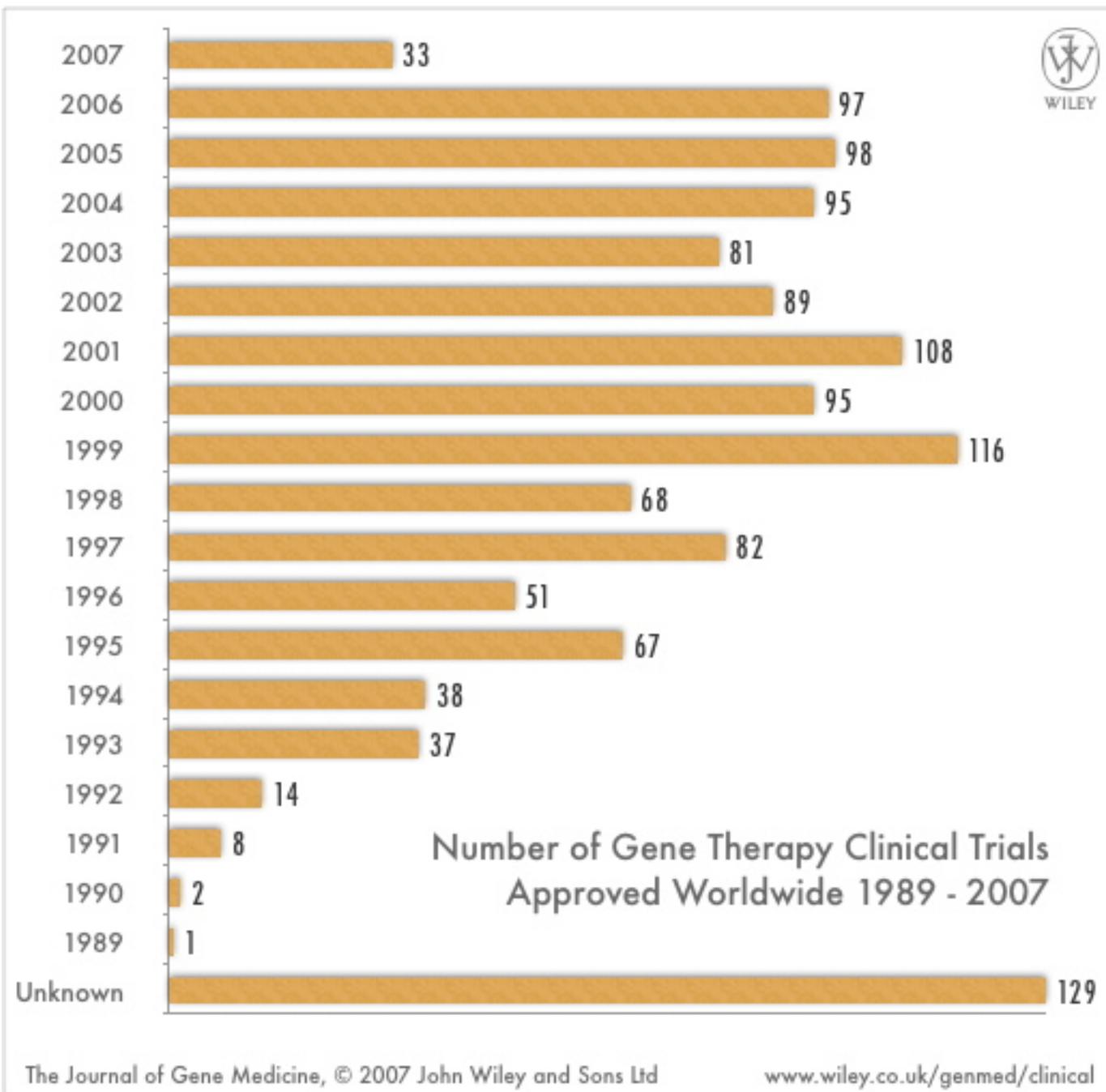
Increased Use of Viral Vectors in Research

- Difficulties in DNA delivery to mammalian cells
 - <50% with traditional transfection methods
 - Up to ~90% with viral vectors
- Increased knowledge about viral systems
- Commercialization has made viral vectors more accessible
- Many new genes identified and cloned (transgenes)
- Gene therapy

Vectors Used in Gene Therapy Clinical Trials



- Adenovirus 24.7% (n=331)
- Retrovirus 22.8% (n=305)
- Naked/Plasmid DNA 18% (n=241)
- Lipofection 7.6% (n=102)
- Vaccinia virus 6.8% (n=91)
- Poxvirus 6.4% (n=86)
- Adeno-associated virus 3.5% (n=48)
- Herpes simplex virus 3.2% (n=43)
- RNA transfer 1.3% (n=17)
- Other categories 2.7% (n=36)
- Unknown 3% (n=40)



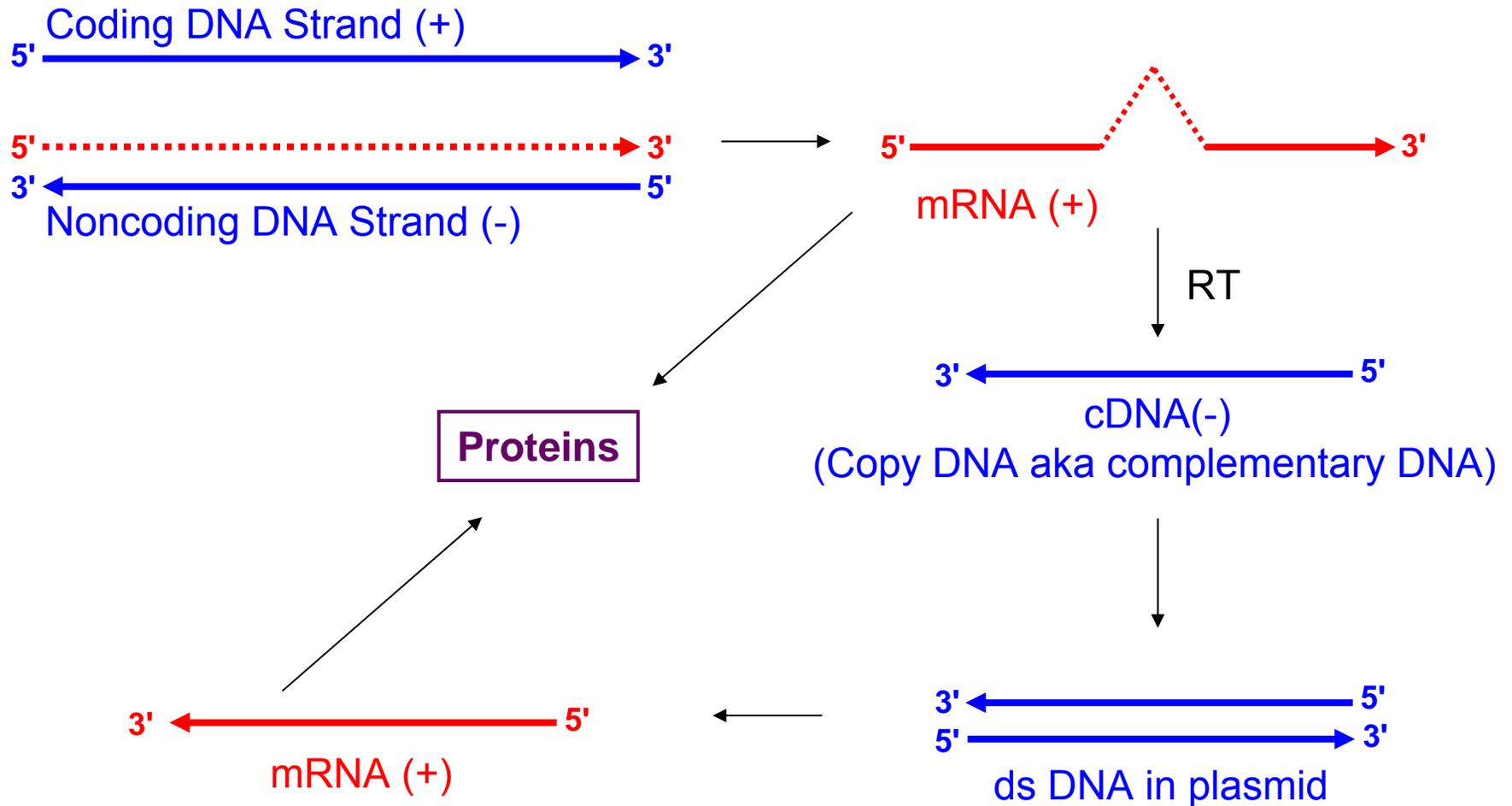
What is a Viral Vector?

- Viral Vector: A viral genome with deletions in some or all essential genes and possibly insertion of a transgene
- Plasmid: Small (~2-20 kbp) circular DNA molecules that replicates in bacterial cells independently of the host cell chromosome

Molecular Biology Essentials

- Flow of genetic information
- Nucleic acid polarity
 - Infectivity of viral genomes
- Understanding cDNA
- *cis-* vs. *trans*-acting sequences
 - *cis* (Latin) – on the same side
 - *trans* (Latin) – across, over, through

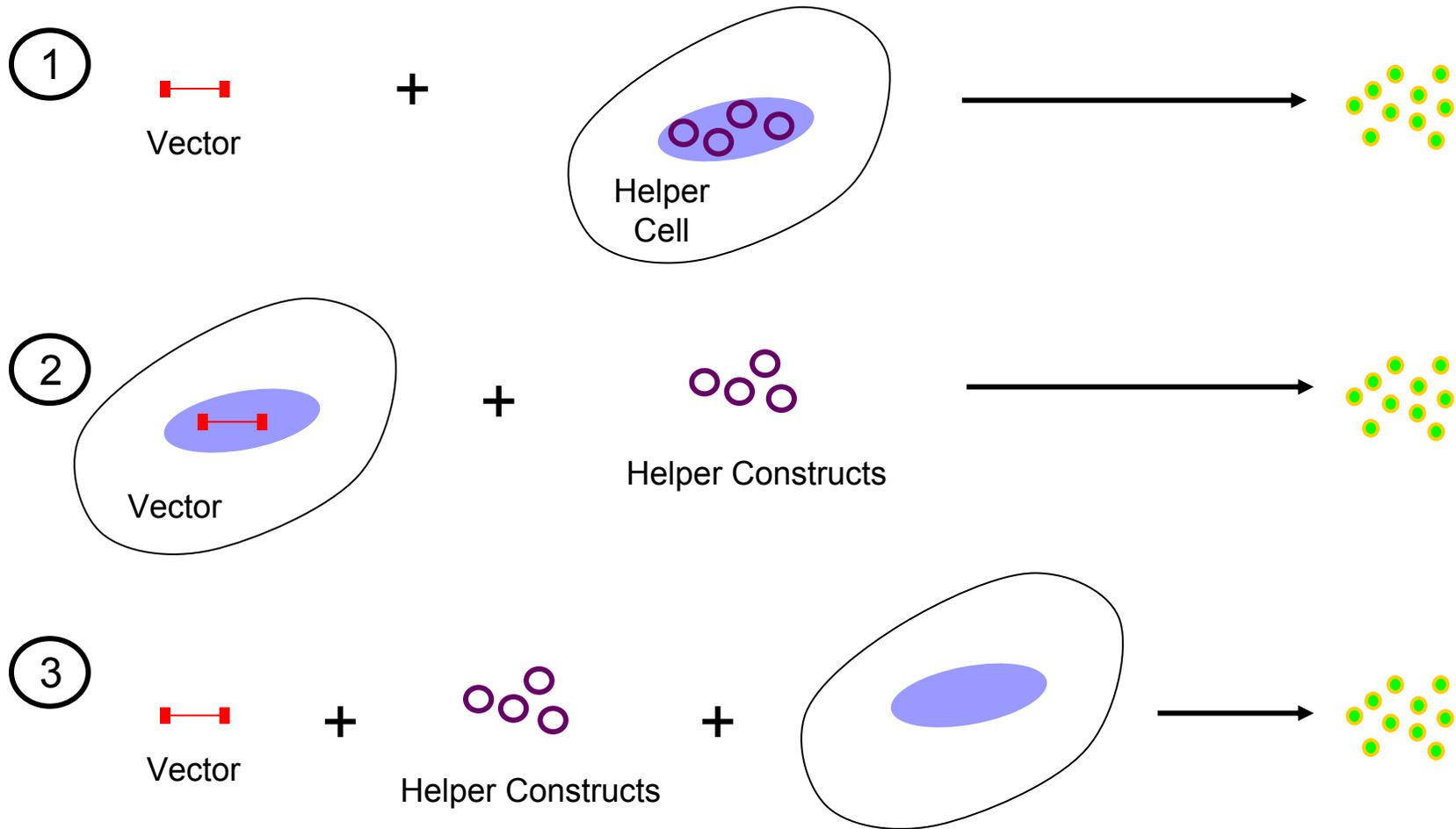
Genetic flow & nucleic acid polarity



Virology Essentials

- Replication-defective vs. infectious virus
- Helper virus vs. helper plasmids
- Pathogenesis
 - Original disease
 - Disease caused by transgene
- Mechanisms of cancer
 - Insertional mutagenesis
 - Transduction

Viral Vector Design and Production



Note: These viruses are replication-defective but still *infectious*.

Features of Retroviral Vectors

- (+) RNA virus with dsDNA intermediate, enveloped
- Naturally integrate into chromosome
 - Long term persistence
- Do not harm target cells as they enter
- Up to ~8 kb of foreign gene sequences can be packaged
- Relatively convenient packaging systems
- Can be manufactured in large quantities

Retroviridae Family (7 genera)

- *Alpharetrovirus*
 - ALV, RSV
- *Betaretrovirus*
 - MMTV
- *Gammaretrovirus*
 - MLV, FeLV, SNV
- *Deltaretrovirus*
 - HTLV I and II, BLV
- *Epsilonretrovirus*
 - WDSV
- *Lentivirus*
 - HIV
- *Spumavirus*
 - HFV, SFV

Generations of Retroviral Vectors

- First Generation
 - Packaging cell lines created with Ψ minus virus
- Second Generation
 - Packaging cell lines use Ψ minus virus with 3' LTR replaced
- Third Generation
 - Three plasmid system (vector and two packaging plasmids with replacement of viral LTRs on helpers)
 - Pseudotyping



Retroviral vs. Lentiviral Vectors

- Non-lentiviruses: Capsids cannot traverse the intact nuclear membrane
 - Cell mitosis required for successful replication
- Lentiviruses: Capsids have a nuclear localization signal on Matrix and Vpr
 - Direct transport through nuclear pore in absence of mitosis

The Marketing of Lentiviral Vectors

ViraPower™ Lentiviral Expression
Systems (Invitrogen)

Lentivirus is a genus, *not* a species

- Family: *Retroviridae*

- Genus: *Lentivirus*

- *Human immunodeficiency virus 1* (human)
- *Human immunodeficiency virus 2* (human)
- *Simian immunodeficiency virus* (monkey)
- *Bovine immunodeficiency virus* (cow)
- *Feline immunodeficiency virus* (cat)
- *Caprine arthritis encephalitis virus* (goat)
- *Visna/maedi virus* (sheep)

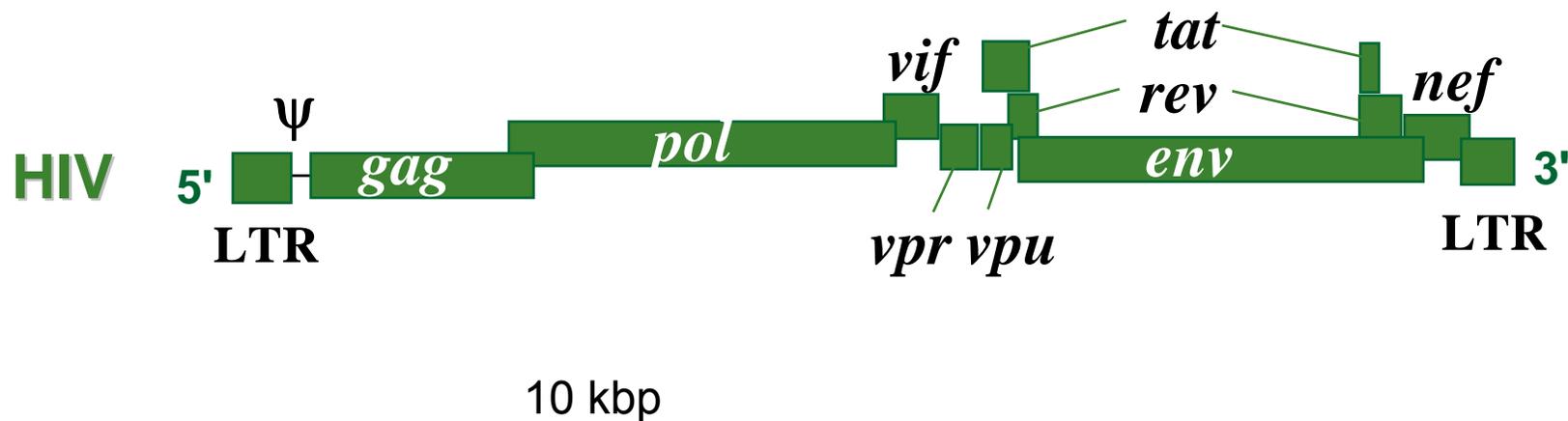
Generations of HIV vectors

■ First Generation Vectors

- Ψ minus helper constructs and split genome (*gag/pol* & *env*); three plasmids with pseudotyping

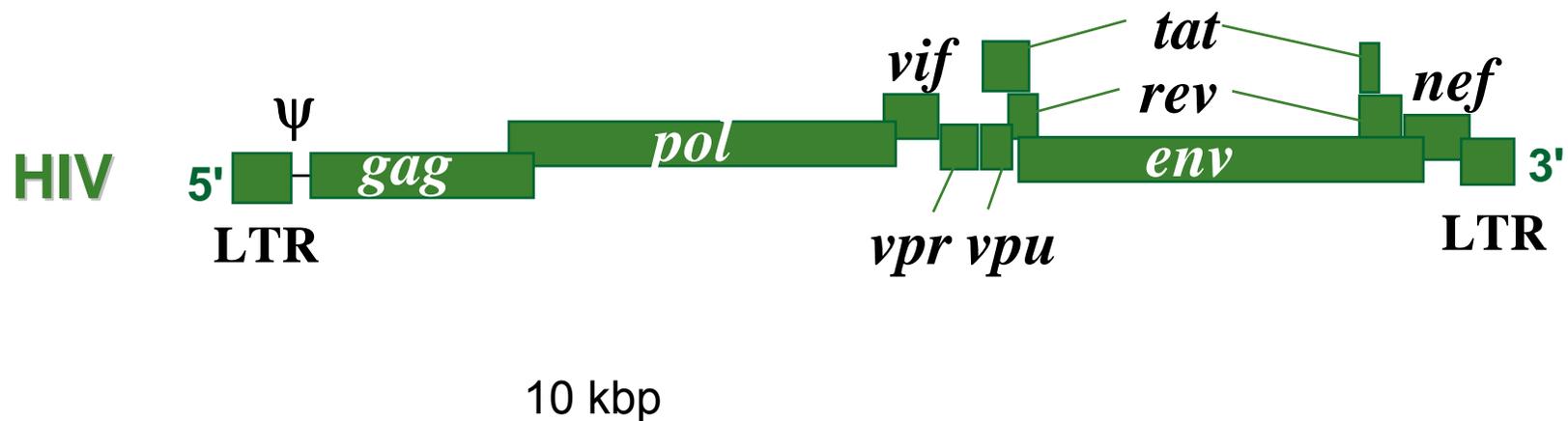
■ Second Generation Vectors

- Deletion of HIV accessory genes *vpr*, *vif*, *vpu* and *nef*



Generations of HIV vectors

- Third Generation Vectors
 - Four plasmids, *tat* eliminated, *rev* supplied *in trans*
- Fourth Generation Vectors
 - Self Inactivating Vectors (SIN); 3 of 9 HIV genes left



MLV and HIV vector hazards

- Integration
- Transgene
- High mutation rate
- High transduction efficiencies
- Amphotropic envelope (broad tropism)
- Recombination
- Endogenous retroviruses
- Seroconversion

Adenoviridae Family

- Genus: *Mastadenovirus*
 - *human adenovirus A-F*
(currently, at least 50 serotypes in humans)

The marketing of Adenovirus vectors

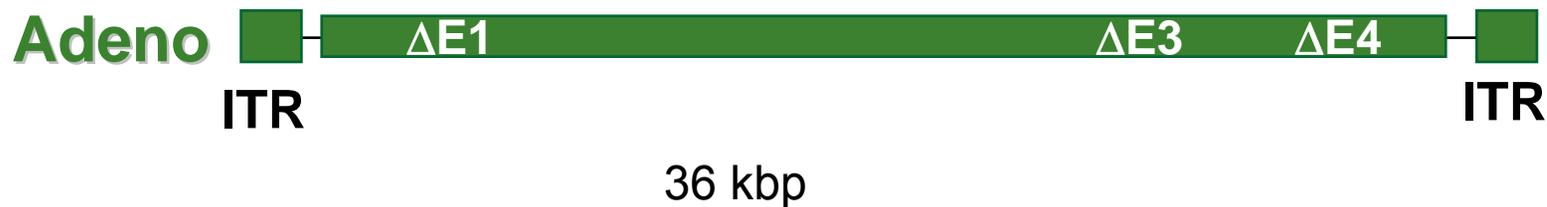
AdEasy™ Adenoviral Vector
System (Stratagene)

Features of Adenovirus Vectors

- dsDNA virus, 35 kb genome, non-enveloped
- Can accept large foreign DNA inserts
- Rarely integrate into the chromosome
- Transient expression only
- Infects many cell types and resting cells
- Aerosol delivery
- Stable and amenable to purification ($\geq 10^{11}$)
- Generate strong immune response
- Relatively Safe (millions of recruits vaccinated)
- Ad2 and Ad5 do not cause cancer

Generations of Adenovirus vectors

- First Generation Vectors
 - Two early genes deleted (E1 or E1/E3)
- Second Generation Vectors
 - Three early genes deleted (E1, E3, E4)
 - Commercialized as AdEasy (He *et al.*, 1998)
- Third Generation Vectors
 - “Gutless vectors”
 - All viral genes deleted; only essential *cis*-acting sequences retained



Adenovirus vector hazards

- Recombination with wild type strains
- Recombination during virus production
- Contamination with helper virus (gutless)
- Transgene
- Immune response
- Difficulties in detecting exposure (prevalence)
- Altered tropism – capsid and fiber proteins

Adeno-associated virus (AAV)

- Example:

Researcher wants to receive mice infected with AAV vector at one week to two months post-infection. He wants to treat these mice as “normal,” uninfected mice because he claims no infectious particles are present. Should he be allowed to do this?

AAV species

Parvoviridae Family

- Genus *Parvovirus* – cats, dogs, mink
- Genus *Erythrovirus* – human; B19
- Genus *Dependovirus* – human; need helper (adeno-associated virus; AAV)

Features of AAV

- ssDNA (+ or -), 5 kb genome, non-enveloped
- Replicate in nucleus of dividing cells (using cellular enzymes and helper virus)
- Relatively stable virion
- Integrates at 19q13.4 and replicates as a provirus
- Not associated with disease

Features of AAV (continued)

- Can infect all cells tested so far
- Can remain latent (in absence of helper)
- High transduction frequency
- Only requires 145 nt ITR for integration
- No superinfection immunity

AAV Vector Hazards

- Specific integration requires Rep protein
- Insertional mutagenesis/cancer
- Deletion/rearrangement during integration
- Multiple copies can integrate
- Reactivation
- Helper viruses: Adeno, herpes, pox

(-) RNA Viruses

■ Nonsegmented

- *Filoviridae* (Ebola)
- *Rhabdoviridae* (Rabies & VSV)
- *Paramyxoviridae* (Hendra & Nipah)

■ Segmented

- *Orthomyxoviridae* (Flu)
- *Bunyaviridae* (Hanta)
- *Arenaviridae* (Lassa)

Minus-Strand RNA Virus Vectors

Generation of biologically contained Ebola viruses

**Peter Halfmann*, Jin Hyun Kim*, Hideki Ebihara†‡, Takeshi Noda†,
Gabriele Neumann*, Heinz Feldmann‡,
and Yoshihiro Kawaoka*†§¶**

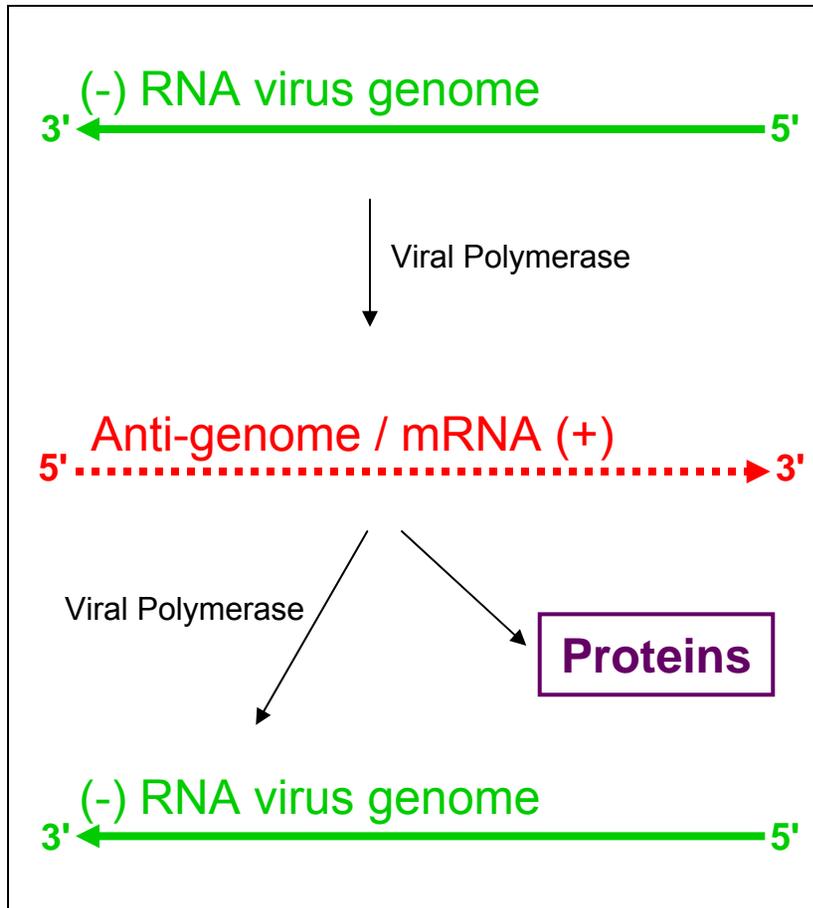
*Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706; §Division of Virology, Department of Microbiology and Immunology, and †International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 113-0033, Japan; and ‡Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada and Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada R3E 3R2

Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved December 12, 2007 (received for review August 25, 2007)

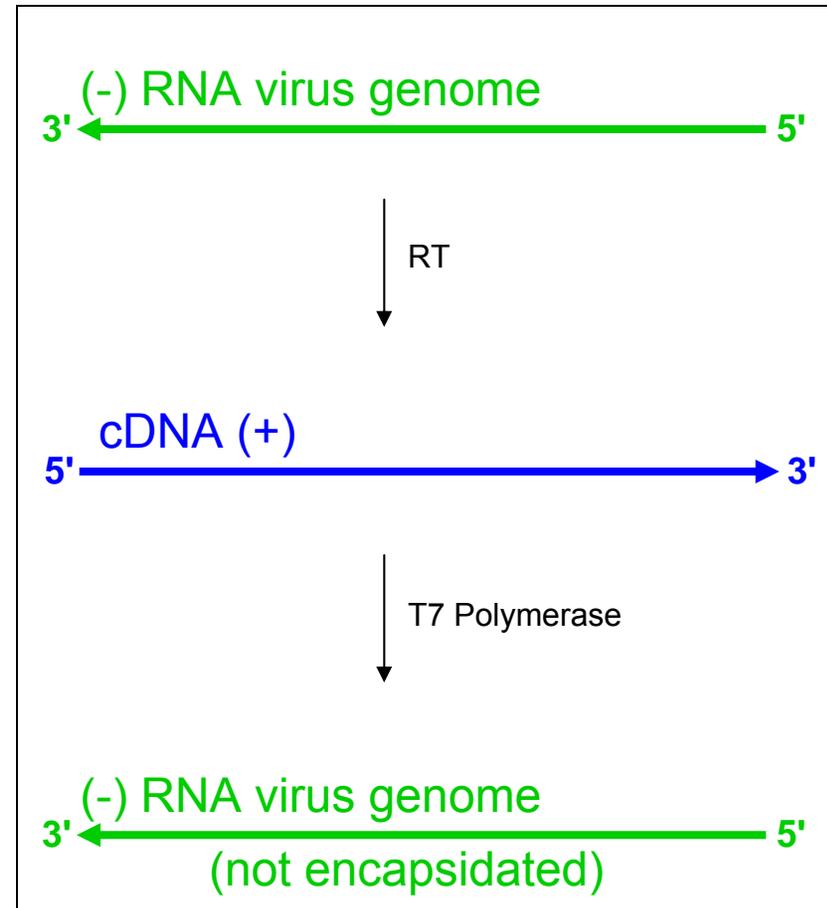
PNAS January 29, 2008 vol. 105 no. 4 1129–1133

Nucleic Acid Polarity for (-)RNA Viruses

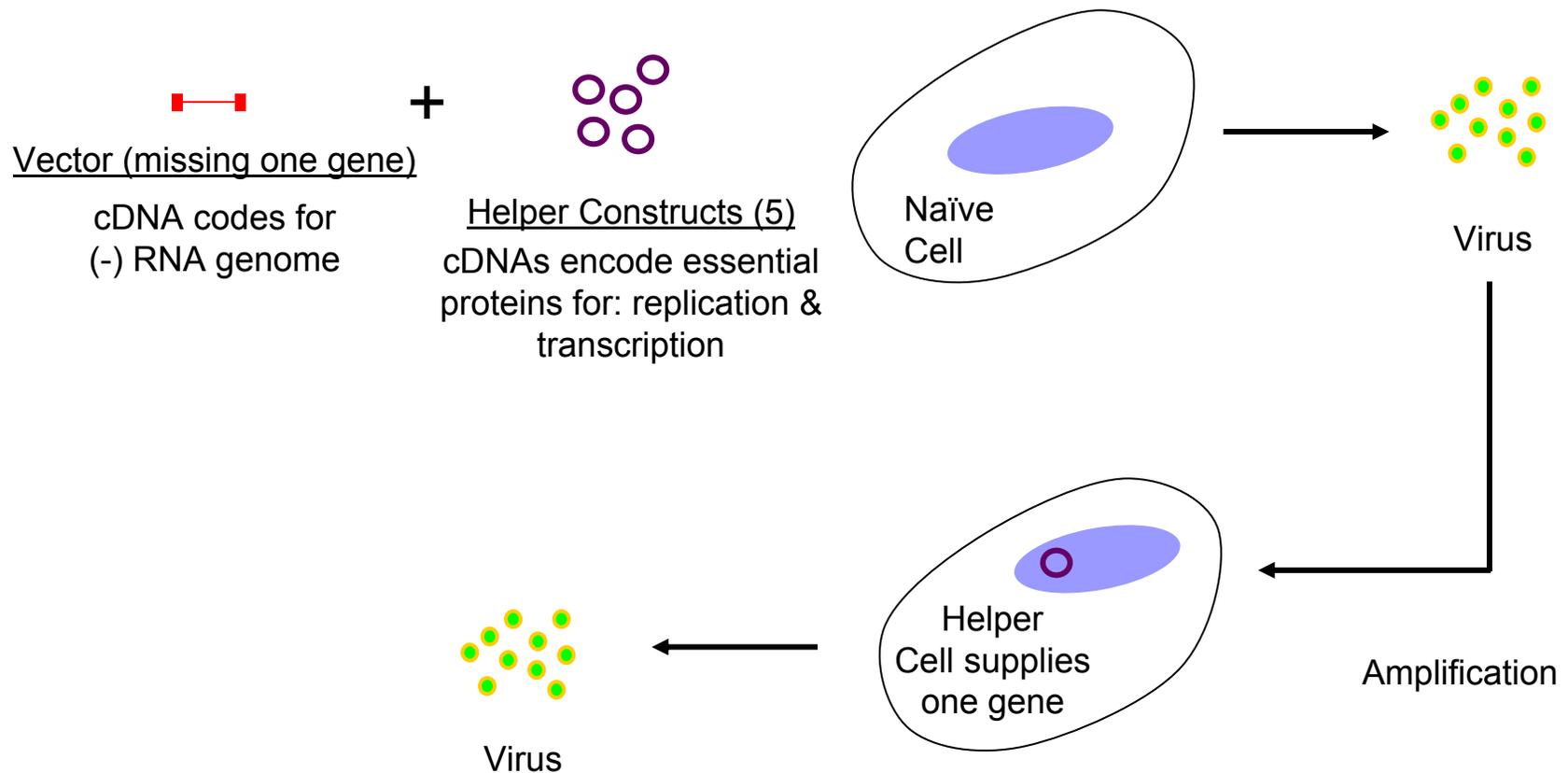
Wild Type Virus



Viral Vector



Viral Vector Design and Production for the Ebola Virus Vector



(-) RNA Vector Hazards

- Includes RG3 and RG4 agents
- Little known about recombination for (-) RNA viruses
- Little known about the replication cycles for these viruses
- Such unknowns create difficulties with regard to accurate risk assessments

Animal Studies

- Push to move animals to lower containment levels
- Excretions and secretions
- Issues of mixed waste (bio and chemical)
- Proper equipment for containment
- Recombination with endogenous sequences or wild type species

Relevant NIH Guidelines

- Section I – Scope
 - Section II – Safety Considerations
 - Section III – Types of Experiments Covered
 - IIIA – IBC Approval, RAC Review, NIH Director Approval Mandatory
 - IIIB – NIH/OBA and IBC Approval Mandatory
 - IIIC – IBC and IRB Approval, RAC Review Mandatory
 - IIID – IBC Approval Before Initiation
 - IIIE – IBC Notification At Initiation
 - IIIF – Exempt Experiments
 - Section IV – Roles and Responsibilities
-

Relevant NIH Guidelines (continued)

- **Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems**
 - **Caution:** Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.
 - **Section III-D-3-a.** Experiments involving the use of infectious or defective Risk Group 2 viruses (see Appendix B-II, *Risk Group 2 Agents*) in the presence of helper virus may be conducted at BL2.
 - **Section III-D-3-b.** Experiments involving the use of infectious or defective Risk Group 3 viruses (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) in the presence of helper virus may be conducted at BL3.
 - **Section III-D-3-c.** Experiments involving the use of infectious or defective Risk Group 4 viruses (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*) in the presence of helper virus may be conducted at BL4.
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Relevant NIH Guidelines (continued)

- **Section III-D-3-d.** Experiments involving the use of infectious or defective restricted poxviruses (see Sections V-A and V-L, *Footnotes and References of Sections I-IV*) in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).
- **Section III-D-3-e.** Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BL1

Relevant NIH Guidelines (continued)

- **Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus**

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus ...may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used....

Relevant NIH Guidelines (continued)

■ Section III – Types of Experiments Covered

- IIIA – IBC Approval, RAC Review, NIH Director Approval Mandatory (Transfer of certain drug resistance genes)
 - IIIB – NIH/OBA and IBC Approval Mandatory (Cloning of toxin molecules)
 - IIIC – IBC and IRB Approval, RAC Review Mandatory (Gene transfer into humans)
 - IIID – IBC Approval Before Initiation
 - IIIE – IBC Notification At Initiation
 - IIIF – Exempt Experiments
-

Relevant NIH Guidelines (continued)

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

- **Appendix B-I. Risk Group 1 (RG1) Agents**

“RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include ...adeno-associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus....”

- **Appendix B-II-D. Risk Group 2 (RG2) - Viruses**

“Adenoviruses, human - all types”

Relevant NIH Guidelines (continued)

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

- **Appendix B-V. Animal Viral Etiologic Agents in Common Use**

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

- **Appendix B-V-1. Murine Retroviral Vectors**

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Resources

- NIH Guidelines for Research Involving Recombinant DNA Molecules:
http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm
- NIH lentivirus guidance document:
http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html
- Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. L. C. Chosewood and D. E. Wilson (ed.). U.S. Government Printing Office, Washington, D.C. Available free at <http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>.
- Centers for Disease Control Fact Sheets: <http://www.cdc.gov/>
- Material Safety Data Sheets (MSDS) for Infectious Substances (Canada):
<http://www.phac-aspc.gc.ca/msds-ftss/>
- American Biological Safety Association Risk Group Classifications:
<http://www.absa.org/riskgroups/index.html>
- Fleming, D. O. and D. L. Hunt (eds.). 2006. *Biological Safety: Principles and Practices*, 4th ed. ASM Press, Washington, D.C.
- Scientific literature
- Microbiology textbooks

Viral Vectors and Transgene Containment

TABLE 4 Viral vectors and transgene containment

Gene transfer vector ^a	Host range ^b	Insert or gene function ^c	Laboratory containment level ^d
MMLV based— <i>gag</i> , <i>pol</i> , and <i>env</i> deleted	Ecotropic	S, E, M, G, CC, T, MP, DR, R, TX, O _v , O _c	BSL-1*
	Amphotropic, VSV-G pseudotyped	S, E, M, MP, DR, T, G, O _v , O _c , R, CC, TX	BSL-2 BSL-2+ BSL-3
Herpesvirus based—nonlytic	Broad host range	S, E, M, MP, DR, T, G, O _v , O _c , R, CC, TX	BSL-2 BSL-2+ BSL-3
		S, E, M, MP, DR, T, G, O _v , O _c , R, CC, TX	BSL-2 BSL-2+ BSL-3
Lentivirus based—HIV, SIV, EIAV, FIV, etc.; <i>gag</i> , <i>pol</i> , <i>env</i> , <i>nef</i> , and <i>vpr</i> deleted	Ecotropic, amphotropic, VSV-G pseudotyped	S, E, M, MP, DR, T, G, O _v , O _c , R, CC, TX	BSL-2 BSL-2+ BSL-3
		S, E, M, MP, DR, T, G, O _v , O _c , R, CC, TX	BSL-2 BSL-2+ BSL-3
Adenovirus based—serotypes 2, 5, and 7; E1 and E3 or E4 deleted	Broad host range, infective for many cell types	S, E, M, T, MP, DR, R, G, CC, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3
		S, E, M, T, MP, DR, R, G, CC, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3
Alphavirus based—SFV, SIN	Broad host range	S, E, M, T, MP, DR, R, G, CC, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3
		S, E, M, T, MP, DR, R, G, CC, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3
Baculovirus based	Broad mammalian host cell range	S, E, M, T, MP, DR, R, G, CC, O _v , O _c , TX	BSL-1* BSL-2 BSL-2+/BSL-3
		S, E, M, T, MP, DR, G, O _v , O _c , R, CC, TX	BSL-1* BSL-2 BSL-2+/BSL-3
AAV based— <i>rep</i> , <i>cap</i> defective	Broad host range; infective for many cell types, including neurons	S, E, M, T, MP, DR, G, O _v , O _c , R, CC, TX	BSL-1* BSL-2 BSL-2+/BSL-3
		S, E, M, T, DR, MP, CC, R, G, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3
Poxvirus based—canarypox, vaccinia ^e	Broad host range	S, E, M, T, DR, MP, CC, R, G, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3
		S, E, M, T, DR, MP, CC, R, G, O _v , O _c , TX	BSL-2 BSL-2+ BSL-3

Fleming D. and D. Hunt (eds.). *Biological Safety: Principles and Practices*, 4th ed. ASM Press, Washington D.C. 2006

Footnotes to Table

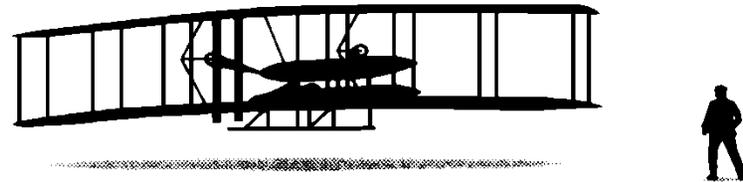
- S = Structural
- E = Enzymatic
- M = Metabolic
- G = Cell growth
- CC = Cell cycle
- DR = DNA replication
- MP = Membrane protein
- T = Tracking protein
- TX = Toxin
- R = Regulatory Protein
- Ov = Viral Oncogene
- Oc = Cell Oncogene

Conclusions

- Viral vectors can be handled safely
- Realize that modern vector systems are becoming increasingly complex
- Understanding all hazards → accurate risk assessments
- Know the purpose and potential uses of the vector with respect to its design
- Pay attention to the transgene, especially when function not fully understood
- Be cautious in prematurely lowering containment levels for novel vector types
- Who's conducting the safety testing?



Questions?



“The difficulty lies, not in the new ideas, but in escaping from the old ones, which ramify, for those brought up as most of us have been, into every corner of our minds.”

John Maynard Keynes (1936)