基因重組材料生物安全之認知

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103/10/22

Two types of containment

- **Biological Containment**
  (Host-Vector system; B1/EK1 and B2/EK2 level)

  To avoid the following types of escape:
  - **survival of the vector in its host outside the lab is minimal;**
  - **transmission of the vector from the propagation host to other non-lab hosts is minimal.**
- **Physical Containment (BSL1-BSL4/P1-P4)**

  To avoid the following types of escape:
  - **to confine organisms containing rDNA molecules;**
  - **to reduce the potential for exposure of the lab worker, people outside the lab , and the environment to organisms containing rDNA molecules.**

Personnel and Environment are equally important!!

您知道答案嗎？

一、 小王以基因合成方式合成了HIV-1 infectious全基因體雙股DNA，並克隆到 pcDNA3-based vector作為研究材料。

Q: 請問以DH5α細菌操作此合成 DNA 並在 P1 實驗室進行實驗，實驗安全嗎？為什麼？

二、 小王將此含HIV-1的載體在Laminar Flow/P2房轉染到human T4 cells中研究其基因表達的profile。

Q: 請問此操作條件符合生物安全規範嗎？為什麼？

三、 小王將此表達載體之HIV-1 所有構造蛋白及 polymerase ORF完全disabled，以發展成 gene transfer 載體用來表達 ras oncogene，並在P2房轉染到 293T細胞測試表達能力。

Q: 請問此實驗條件有生物安全疑慮嗎？如果有，是什麼？

Biohazard

The three regulatory sets of biohazards

Definition of biohazard:

An agent of biological origin that has the capacity to produce deleterious effects on human; i.e. microorganisms.

1. **Potentially infectious materials**
2. **Infectious agents**
3. **Recombinant DNA**
   - **biohazards created *de novo either* by constructing or synthesizing**

Scope of laboratory biosafety program

- ✓ Biohazard recognition
- ✓ Risk assessment
- ✓ Hazard mitigation

Outline

- ✓ Definition of rDNA
- ✓ An overview of molecular cloning and host-vector system in rDNA technology
- ✓ An overview of viral vectors used in the lab
- ✓ Replication competent genome/ virus
- ✓ Main way and type of biosafety incident
- ✓ Risk assessment
- ✓ Case study

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Definition of Recombinant DNA Molecules

(in the context of the NIH guidelines)

- Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cells;
- Synthetic nucleic acids;
- Or, molecules that result from the replication of those described above.

- **Viral Vector (usually in the form of shuttle vector):**
  Viral vectors are genetically modified viral genome that function as a transfer vector to deliver genetic material into cells. This process can be performed inside a living organism or in cell culture.

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What Does It Mean: “To Clone”?

Clone: a collection of molecules or cells, all identical to an original molecule or cell

- **To "clone a gene" is to make many copies of it, for example, by replicating it in a culture of bacteria.**
- **rDNA technology makes manipulating genes possible.**

Recombinant DNA Technology

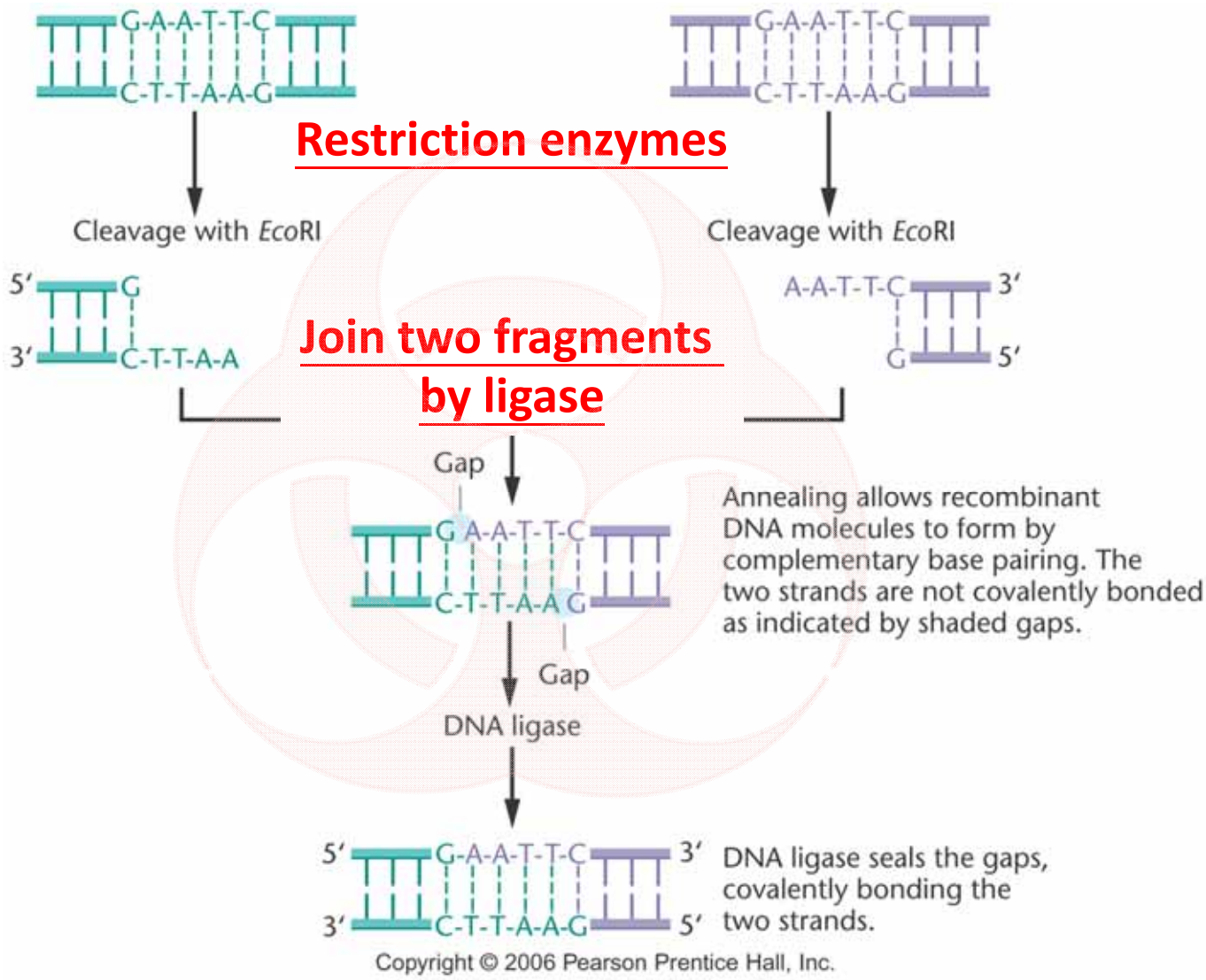
- 1971 paper by Kathleen Dana and Daniel Nathans described isolation of enzyme that cleaved DNA at specific sequences

  : restriction endonuclease

- 1978 Nobel Prize to Nathans, Smith and Arber for restriction endonuclease discovery

Recombinant DNA Molecules

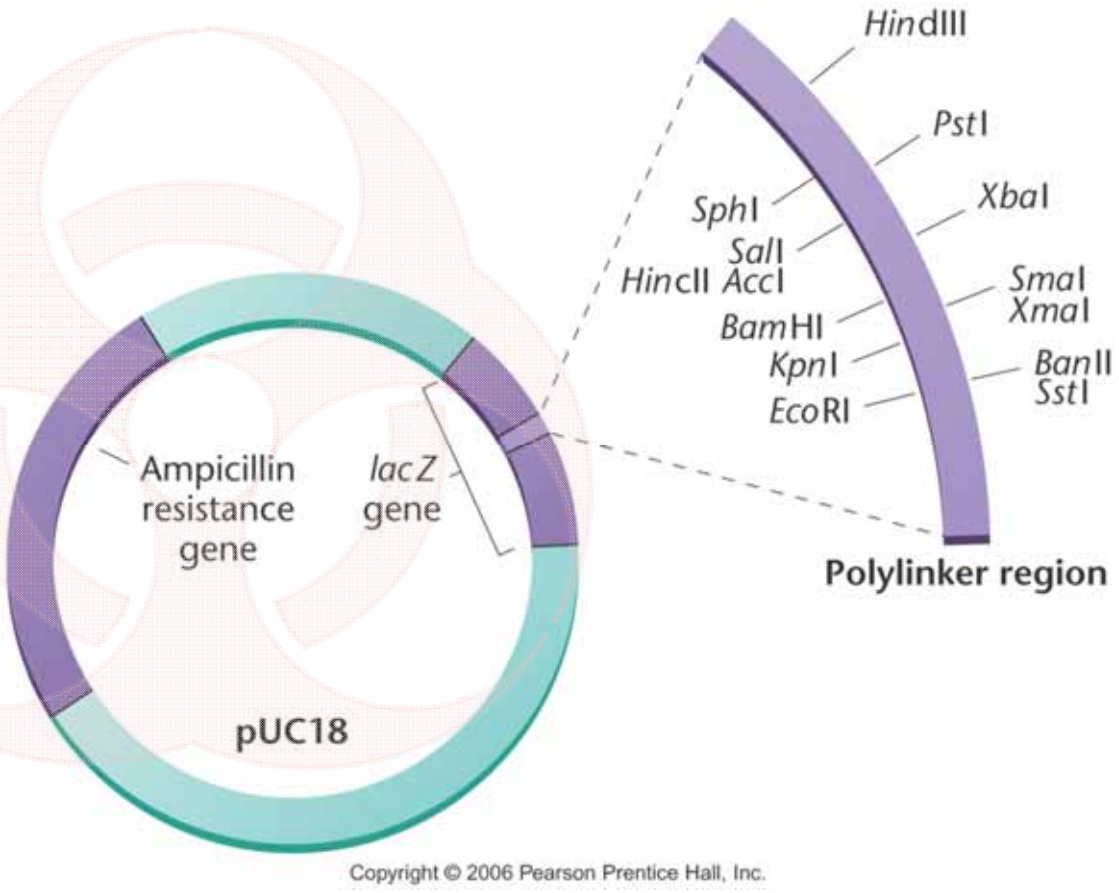
Enzymes



Cloning Vectors

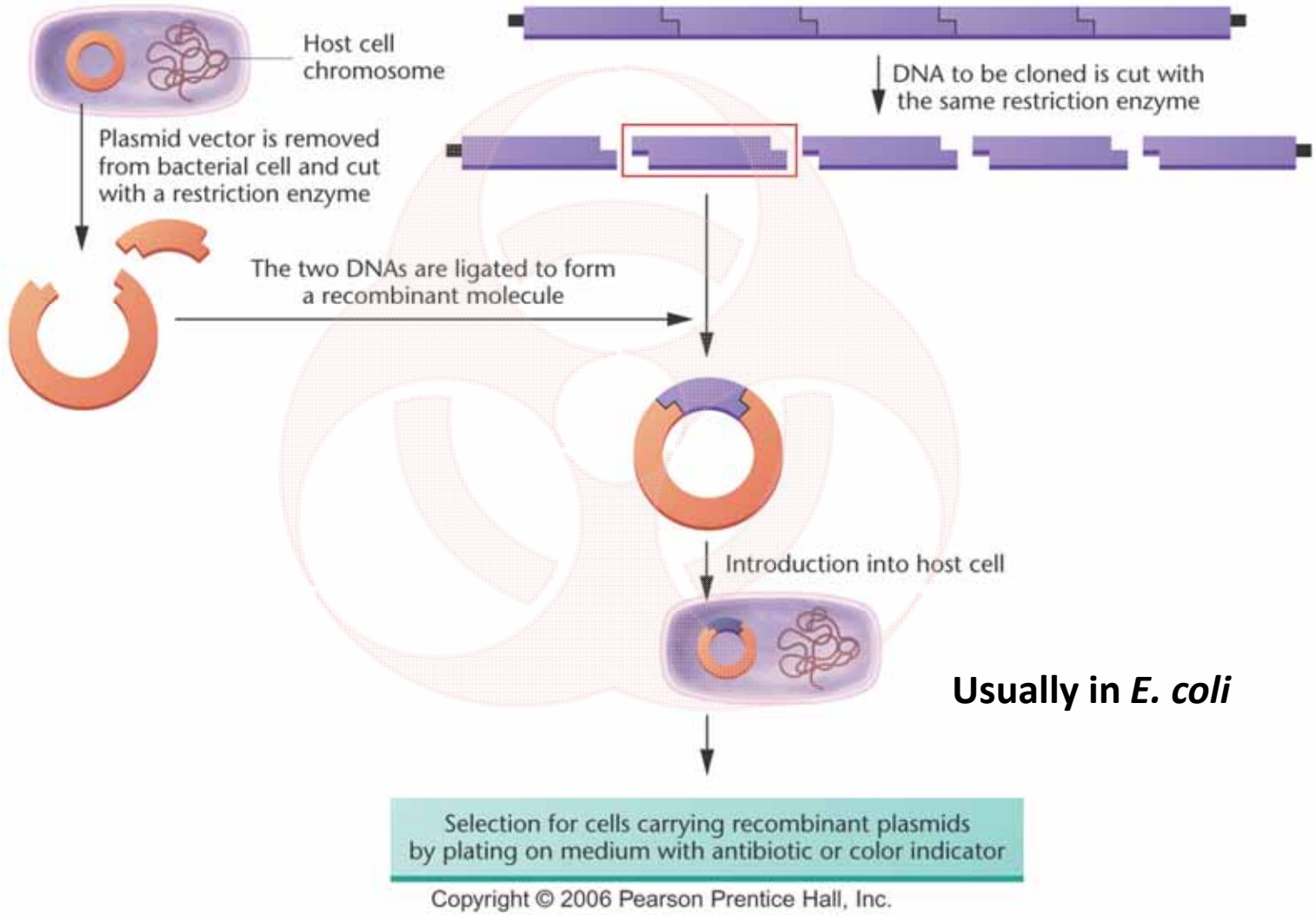
Vector

- *ori*
- **Selectable marker(s)**
- **Multiple cloning site (MCS)**
- **Reporter function useful**



Amplifying Plasmid in Bacteria

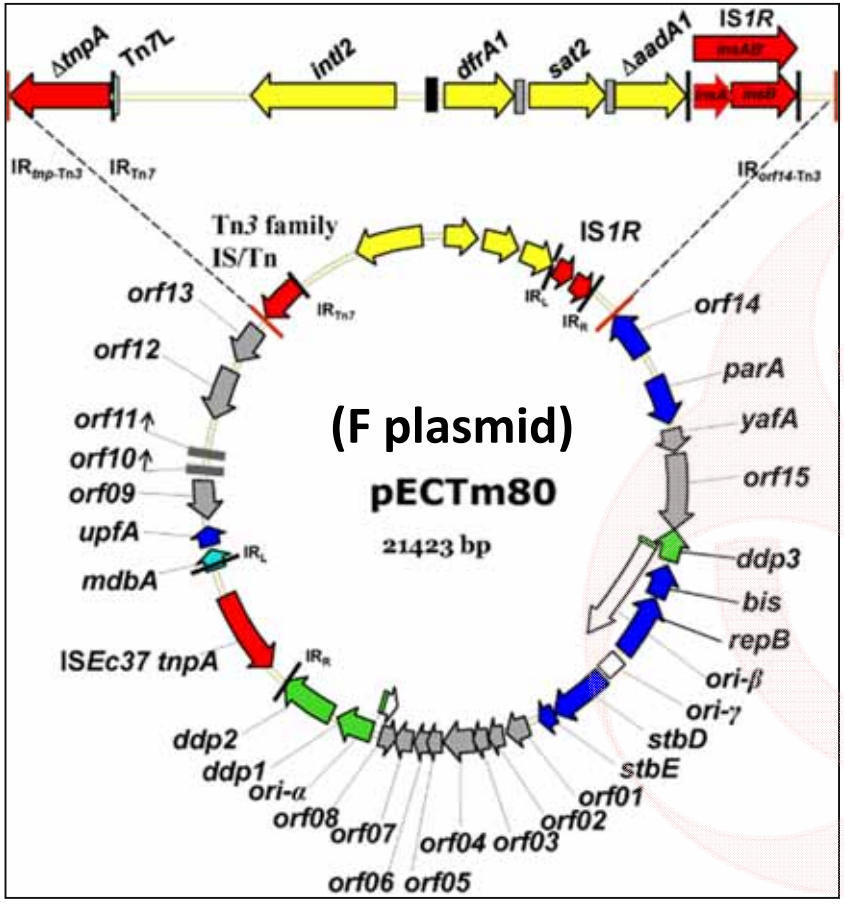
Host

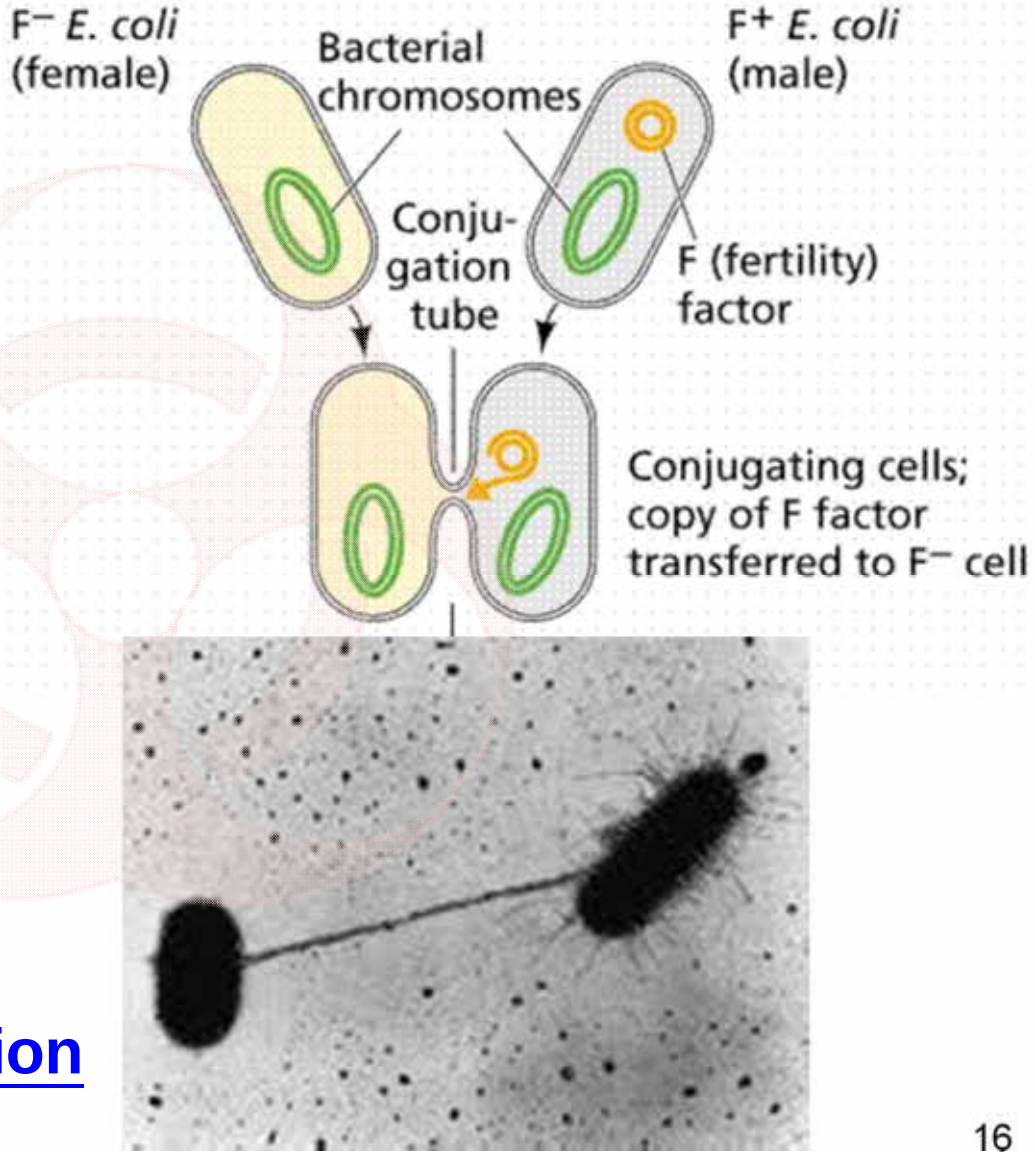


Usually in *E. coli*

Host-Vector System

Plasmid conjugation system





PLoS One. 2012;7(4):e34718. doi: 10.1371 (*E. coli*)

Carry elements for:
transposition and conjugation

Host-Vector System

Host:

1. **B1 system**
   - EK1: *E. coli* K12
   - SC1: *S. cerevesiae*
   - BS1: *Bacillus subtilis*
   - 以動物及植物之培養細胞為宿主之宿主-載體系統（但不會分化至成體）
   - Baculovirus為載體所構成之宿主-載體系統

DH5α:
F´ gyrA96 recA1 endA1 thi-1 hsdR17 glnV44 deoR D(lacZYA-argF) U169 [f80d D(lacZ)M15]

2. **B2/EK2 system**

   For E. coli K12 host-vector system in which the vector is a plasmid/phage, no more than **$1/10^8$ host cells/ phage particles** shall perpetuate (永存) a cloned DNA fragment under the specified non-permissive conditions that similar to natural environment.

Vector: Vectors that sexually move to “unsafe” bacteria was prohibited **(no conjugation system)**.

The First “Safe” Bacterium

- Released in 1976 by Roy Curtiss III at the University of Alabama
- *E. coli* χ1776
  - Required diaminopimelic acid (DAP)
  - Fragile cell walls (low salt, detergent sensitive)
  - Difficult to work with
  - Slow grower
  - Poor receptor for transformation

Examples of genome-type of EK2 host

Plasmid host: **c1776**

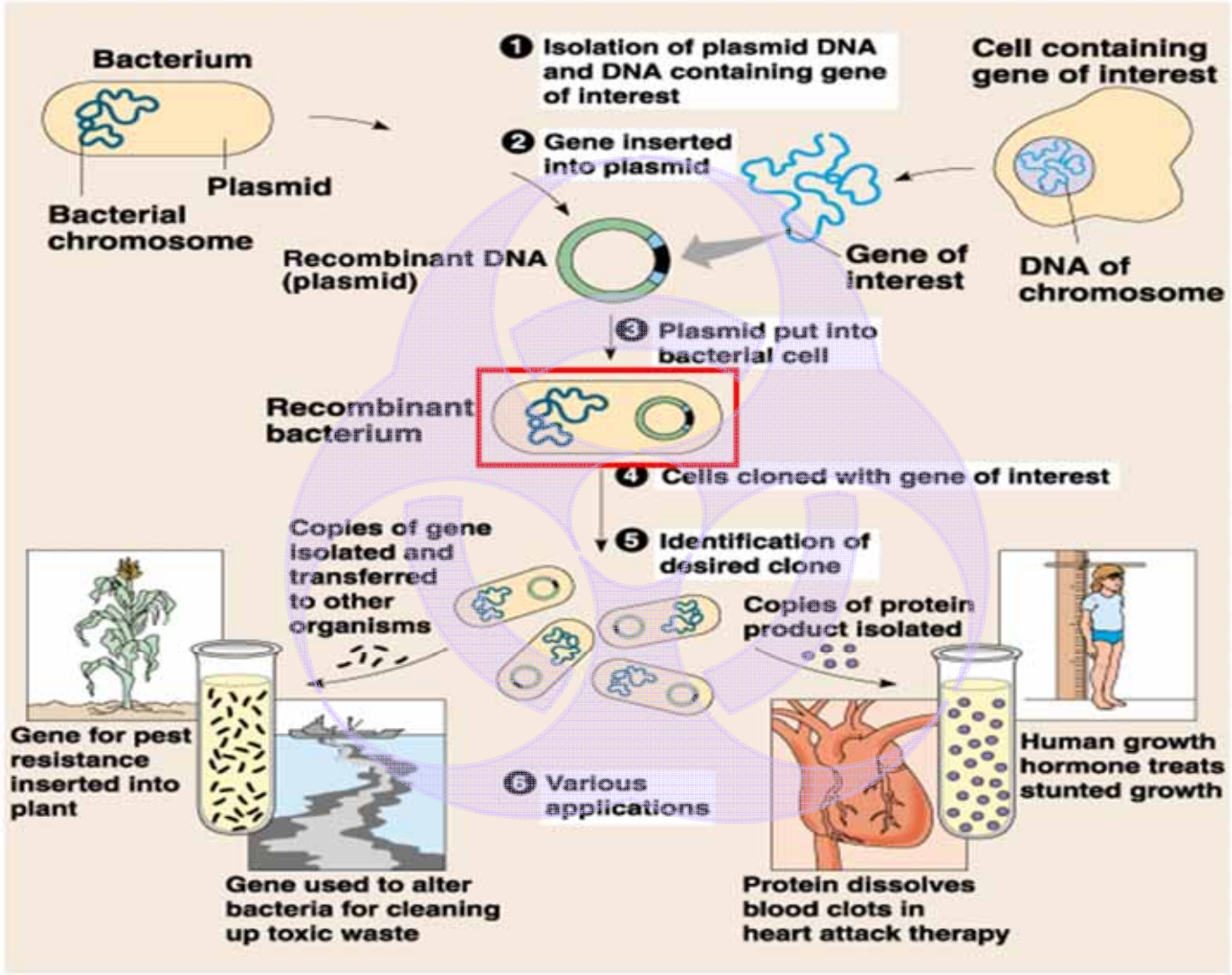
F⁻ fhuA53 dapD8 minA1 glnV42(supE42) D(gal-uvrB)40 minB2 rfb-2 gyrA25 thyA142 oms-2 metC65 oms-1 D(bioH-asd)29 cycB2 cycA1 hsdR2

Phage host: **DP50 *supF***

F⁻ tanA58 dapD8 lacY1 glnV44(supE44) D(gal-uvrB)47 ltuT58(supF58) gyrA29 D(thyA57) hsdS3

Suppressor of amber(UAG) (*supX*)

Summary of cloning rDNA and its applications



Outline

- ✓ Definition of rDNA
- ✓ An overview of molecular cloning and host-vector system in rDNA technology
- ✓ **An overview of viral vectors used in the lab (manipulate virus genome into safer transfer vector)**
- ✓ Replication competent genome/ virus
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- ✓ Risk assessment
- ✓ Case study

Gene Delivery Technologies for Eukaryotic Cells

- **Chemical transfection**
  - **Calcium phosphate**
  - **Liposomes**
- **Electroporation**
- **Micro-injection**
- **Virus-like particles (VLP)**
- **Viral-mediated**

Chemical methods

Physical methods

Biological Methods (rVirus)

Concerns About rViruses

- Insert characteristics, i.e. toxin gene, antibiotics resistant gene (using stringent containment)
- Pathogenicity of parent virus
  - Can engineer to be replication incompetent
- Cytopathogenicity of vector
  - eg. Spike proteins on Adenovirus
- RG level of recombinant virus may be raised or lowered dependent on the characteristics of the insert, etc….. (inserts, scale-up consideration)
- Production of replication competent genome or virus
- Requirements for specialized facilities
- Training requirements

Commonly Used Viral Vectors

BSL 1	BSL 2	BSL 2/3
Baculovirus (insect cells)	**Adenovirus**	**Retrovirus/ Lentivirus** (HIV, SIV, HTLV)
Adeno-Associated Virus	**Poxvirus** (vaccinia, fowlpox)	**Alphavirus** (semliki forest, sindbis, VEE)
	Herpesvirus (Epstein-Barr, Herpes viruses)	**Influenza Virus (rescue plasmids)**

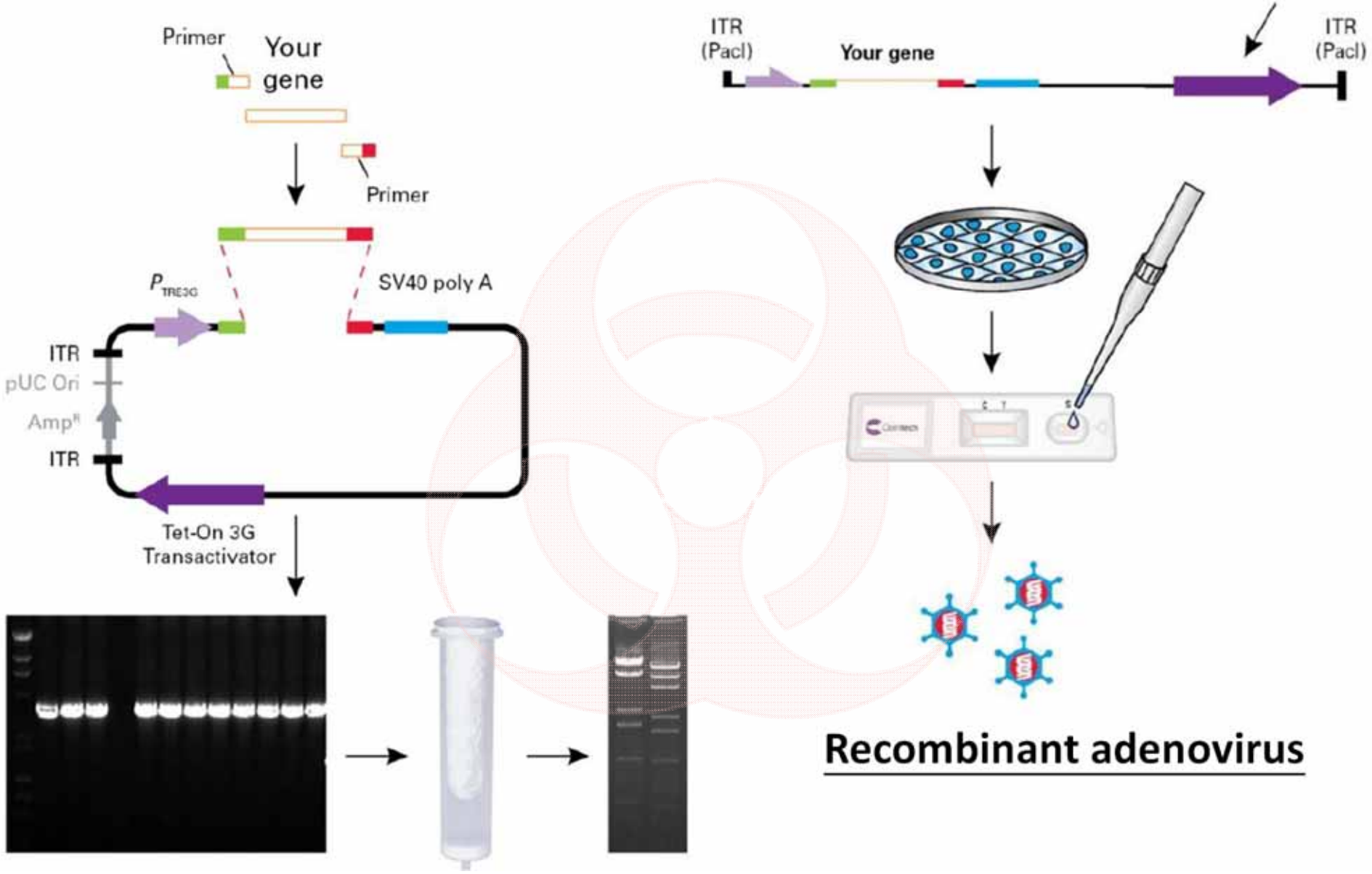
nebiosafety.org/Web%20Data/Flesher.ppt

Adenovirus

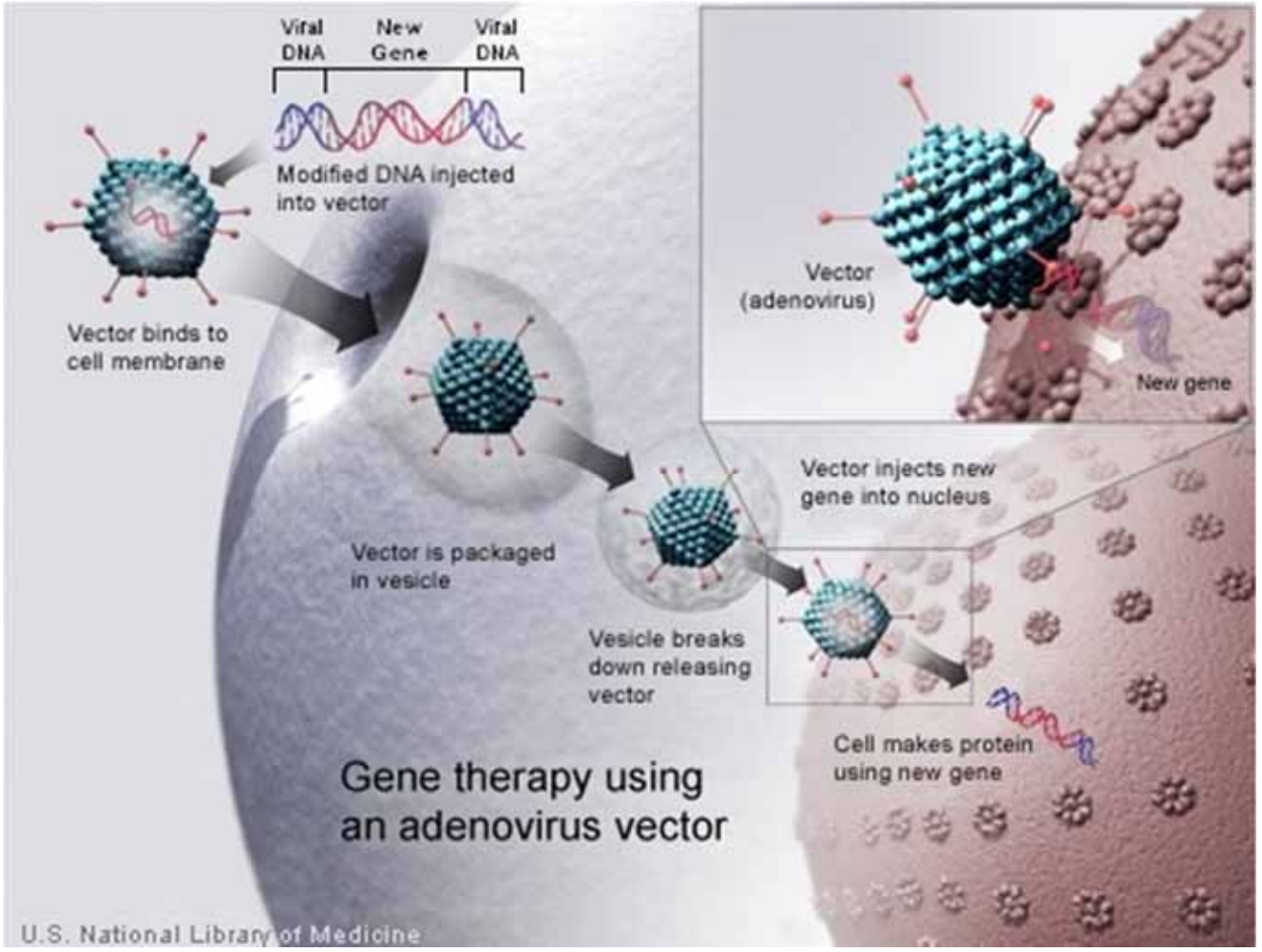
Genome: Linear, non-segmented, d/s DNA, 30-38kbp which has the theoretical capacity to encode 30-40 genes.

Phase:	Gene transcribed:
Immediate early	E1A
Early	E1B, E2A, E2B, E3, E4, some virion proteins
Late	Late genes, mostly virion proteins

Adenovirus System



Gene delivery into cells/animals/human



http://ghr.nlm.nih.gov/handbook/illustrations/therapyvector

Clinical syndromes

Disease:	At Risk:
Acute Respiratory Illness	Military recruits, boarding schools, etc.
Pharyngitis	Infants
Gastroenteritis	Infants
Conjunctivitis	All
Pneumonia	Infants, military recruits
Keratoconjunctivitis	All
Acute Haemorrhagic Cystitis	Infants
Hepatitis	Infants, liver transplant patients

http://www.microbiologybytes.com/virology/Adenoviruses.html

Summary of Ad Vectors

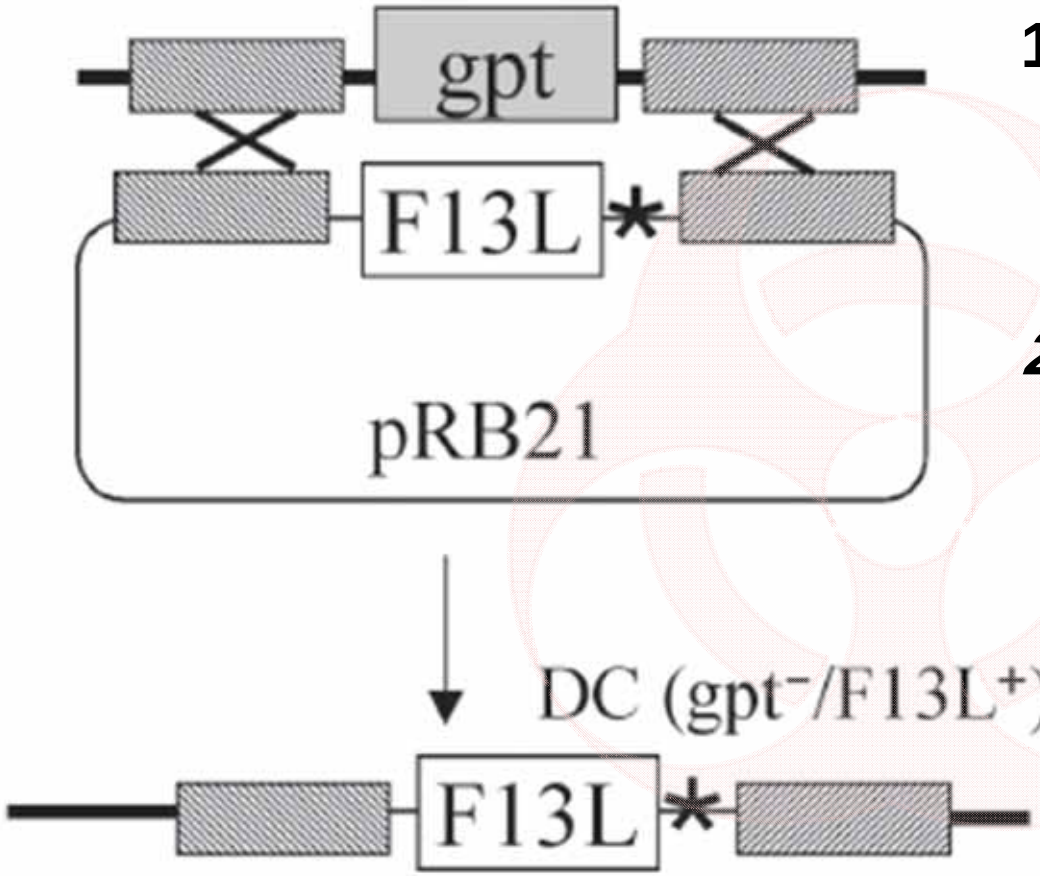
Advantages:

- Higher titer
- Efficient transduction of non-dividing cells in vitro and in vivo

Disadvantages:

- Toxicity
- Immunological response
- Prior exposure

Recombinant vaccina virus selected by gpt method

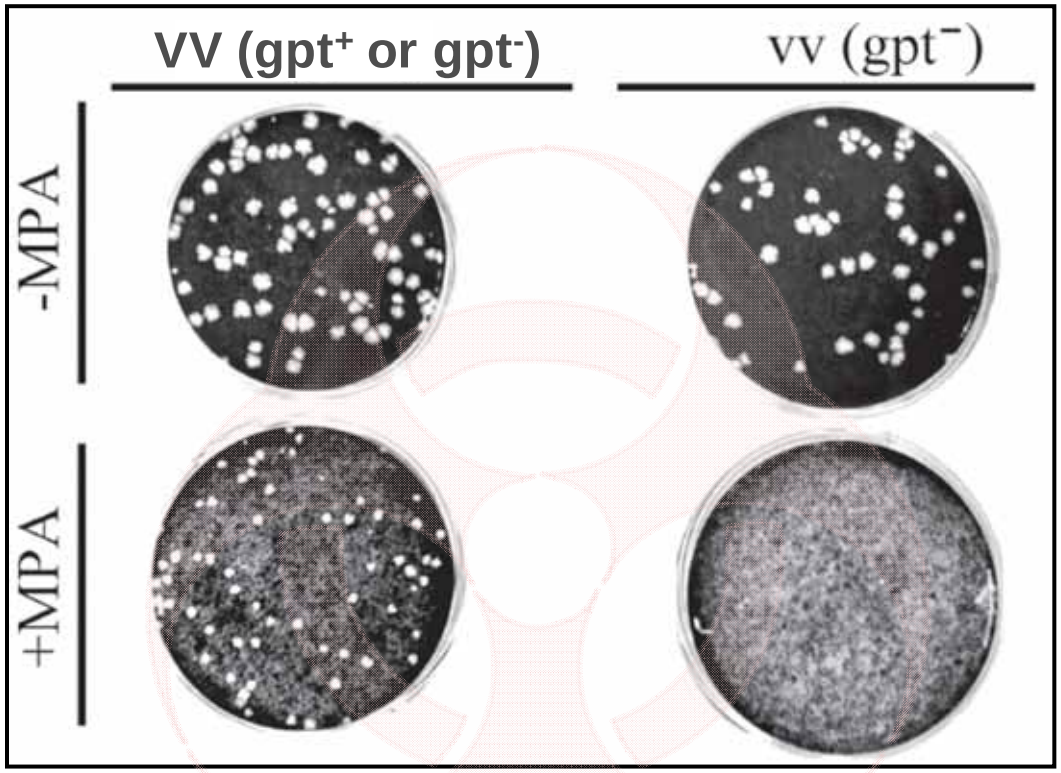


1. **F13L-deleted vaccinia produces tiny plaques (**envelope protein p37**).**

2. ***gpt*** **expression confers resistance to MPA.**

Methods in Molecular Biology, Volume 269, chapter 2

Recombinant vaccina virus selected by gpt method



Mycophenolic Acid (MPA) is an antibiotic useful in research for the selection of animal cells that express the *E. coli* gene coding for GPT (xanthine guanine phosphoribosyltransferase).

Vaccina Virus Vector

Advantages

- Broad host range
- Easy to generate viruses
- Accepts large inserts
- High expression level
- Molecular virology well understood

Limitations

- Lytic infections
- Readily transmisible agent
- Vaccination requirement
- Scale-up considerations

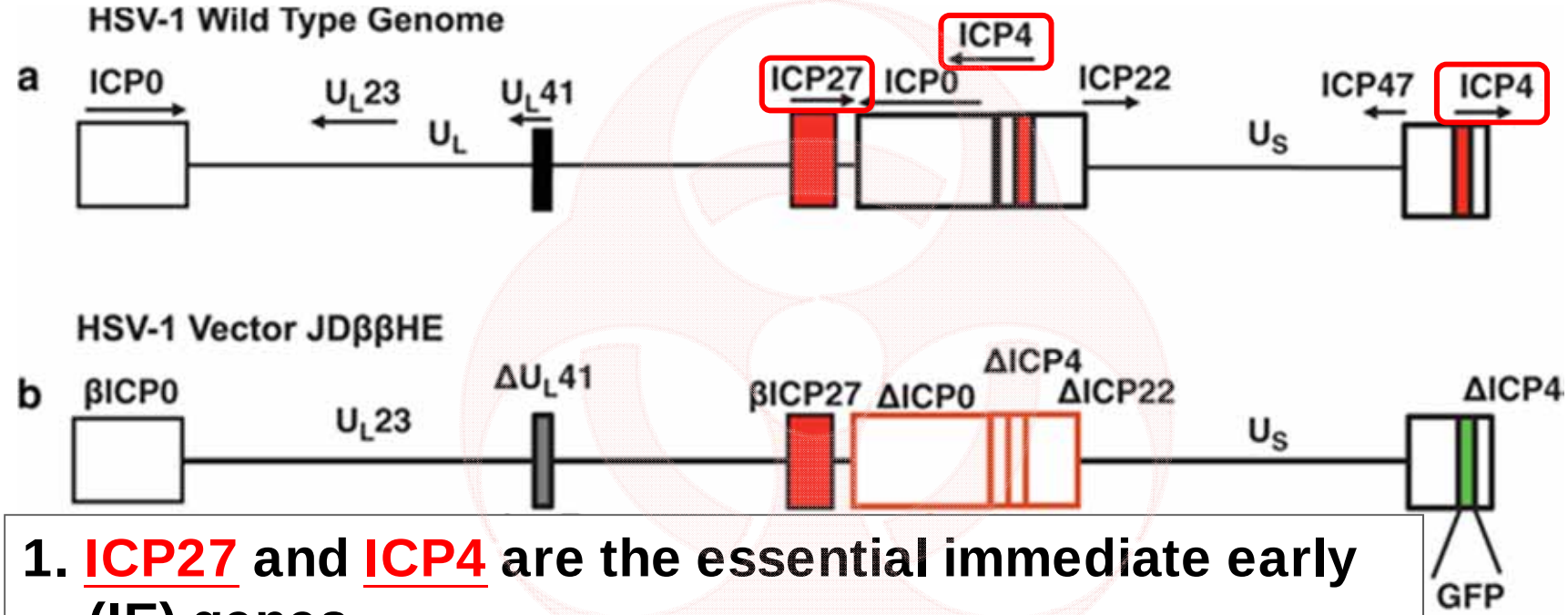
Risks associated with Vaccinia virus

orthopoxvirus exposures reported to CDC, 2005-2008

State	Virus (strain, if known)	Nature of accident	Result in infection?
CA	Vaccinia	Eye splash	No
FL	Vaccinia (rabbitpox)	Eye splash	No
CT	Vaccinia (recombinant WR)	Needlestick	Yes (hospitalization)
PA	Vaccinia (recombinant WR)	Needlestick	Yes
CT	Vaccinia	Eye splash	No
IA	Vaccinia (recombinant WR)	Needlestick	Yes
NM	Vaccinia	Animal care facility	No
MD	Vaccinia (recombinant WR)	Needlestick	No
NH	Vaccinia (recombinant WR)	Needlestick	Yes (hospitalization)
MA	Vaccinia (recombinant NYCBH)	Needlestick	Yes (hospitalization)
MO	Monkeypox	Needlestick	No
GA	Vaccinia	Animal care facility	No
CA	Vaccinia (recombinant WR)	Eye splash	No
NH	Vaccinia (recombinant WR)	Eye splash	No
VA	Vaccinia (recombinant WR)	Unknown	Yes (hospitalization)
FL	Vaccinia	Tube leakage	No

Virology (2009) 385:1-4

HSV1 wild-type genome and configuration of the JDHE vector

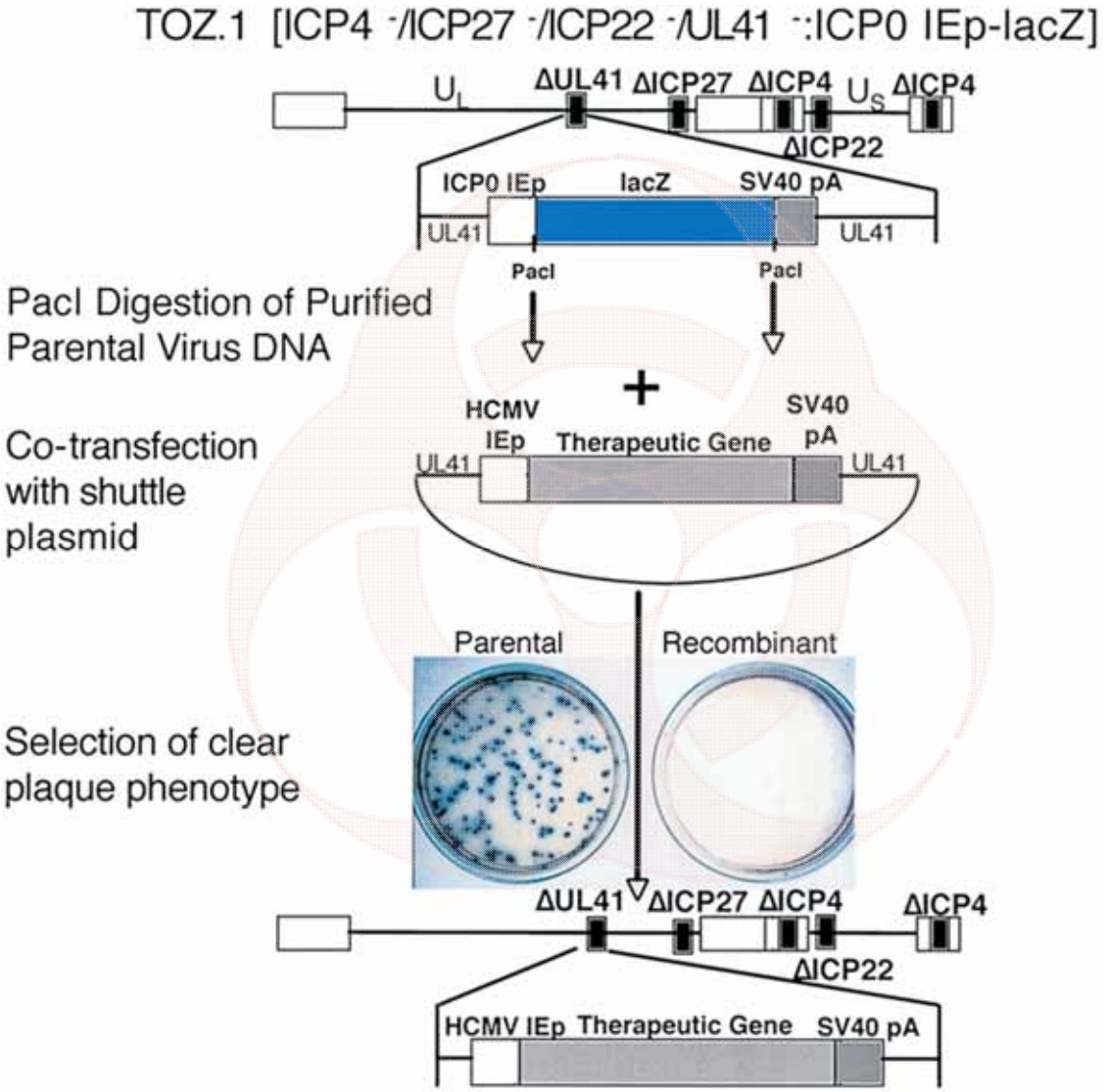


1. ICP27 and ICP4 are the essential immediate early (IE) genes.

2. ICP0, ICP22 and ICP47 are nonessential IE genes.

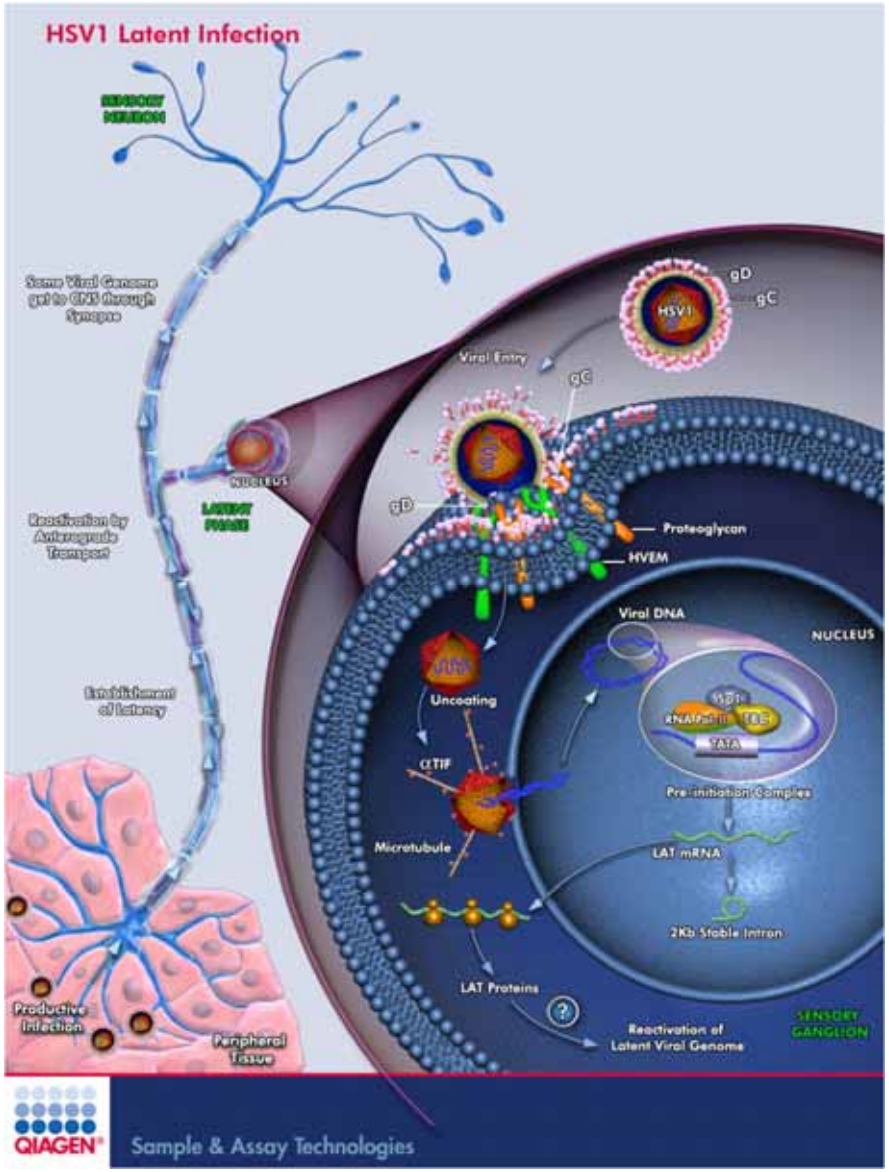
Gene Therapy (2009) 16, 493–501

Construction of replication-defective HSV-1 vector



http://cshprotocols.cshlp.org/content/2011/5/pdb.prot5615/F1.expansion

HSV1 Latent Infection



Summary of HSV1

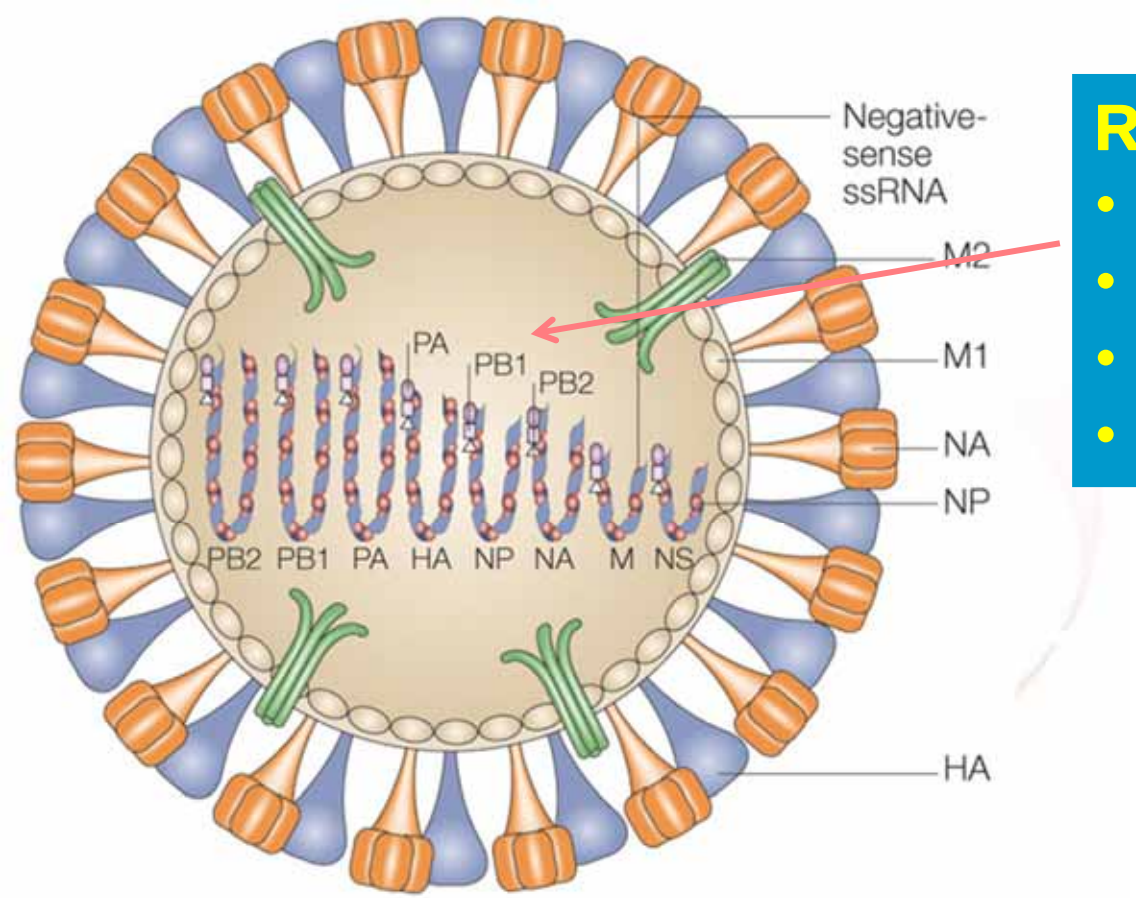
Advantages:

- Episomal Persistence (dsDNA circular)
- Broad Host Range
- Infects Non-dividing and Dividing Cells
- Large Genome Size (152 Kb)
- High Titers Produced (10^8-10^{10} particles/ml)
- High Transduction Efficiency (1-3 particles/cell)
- Redosing Possible Even in immune Host
- Long-Term Transgene Expression in Neurons

Disadvantages:

- Transient Transgene Expression
- Episomal Persistence
- Residual Cytotoxicity

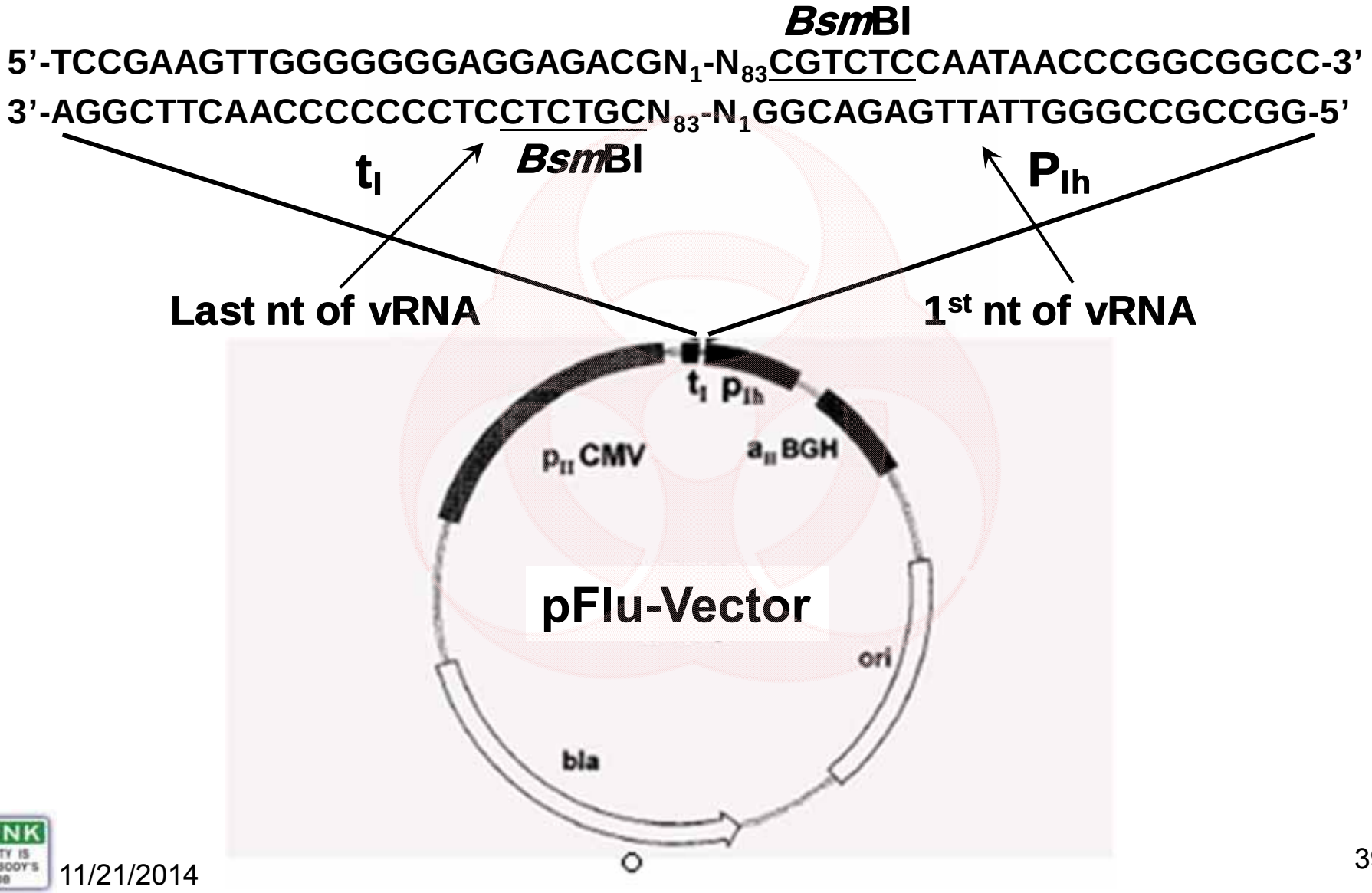
Schematic diagram of an influenza A virus virion



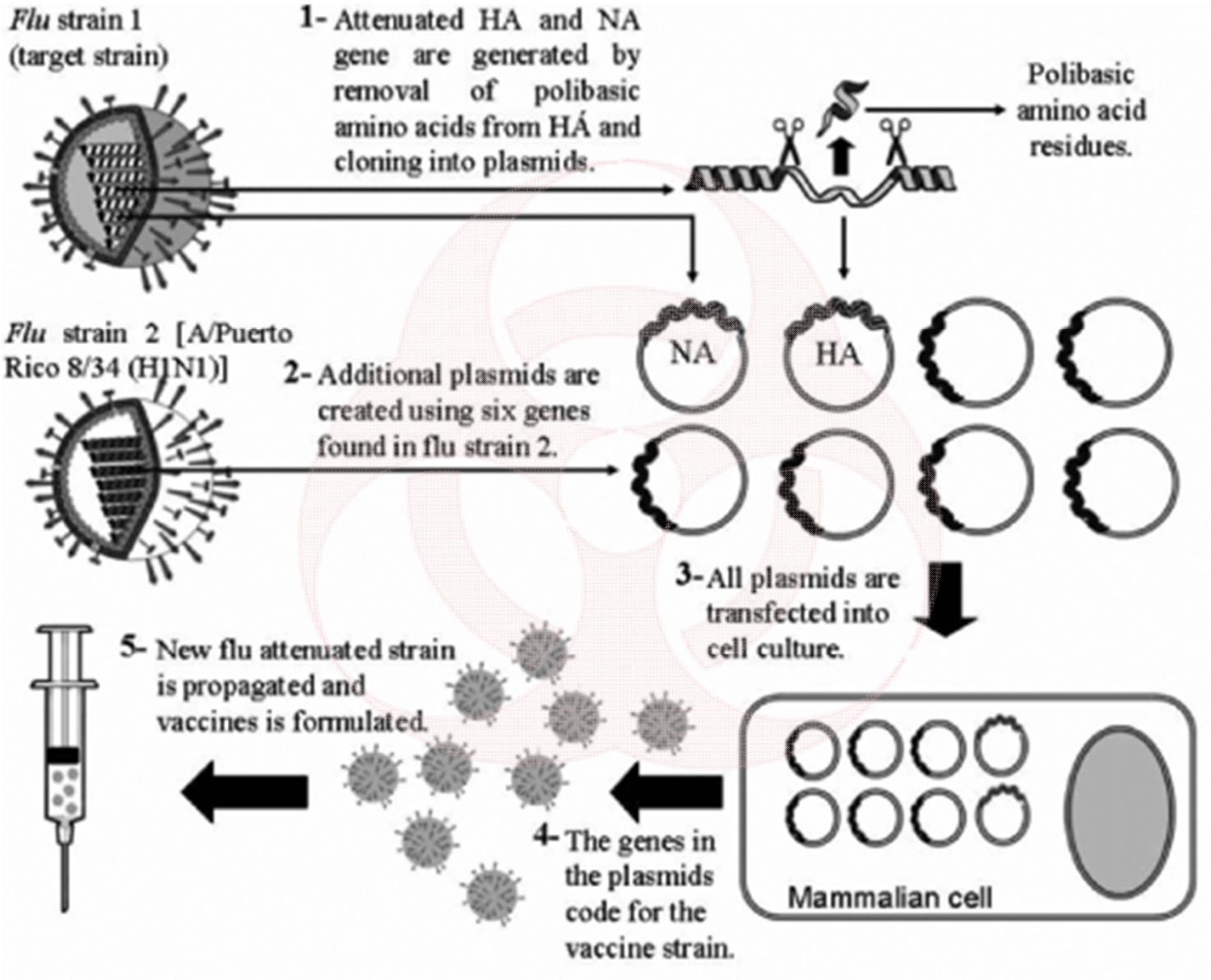
RdRp Complex:

- **PB1**
- **PB2**
- **PA**
- **NP**

Reverse genetic system of influenza virus



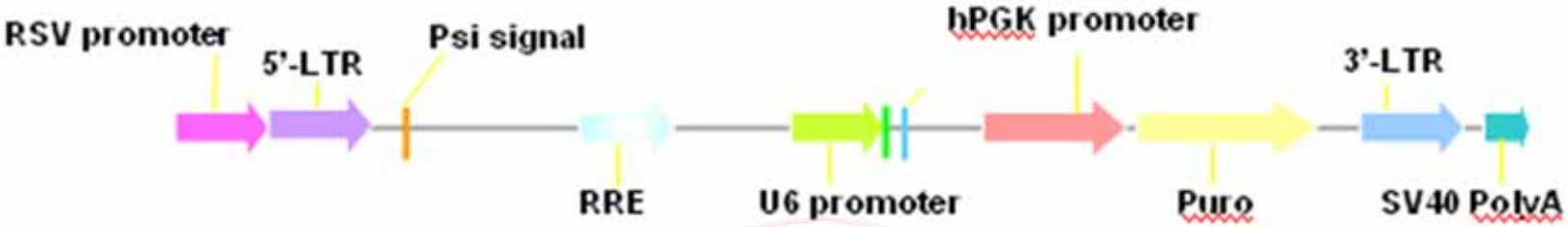
Rescue recombinant influenza virus using reverse genetic system

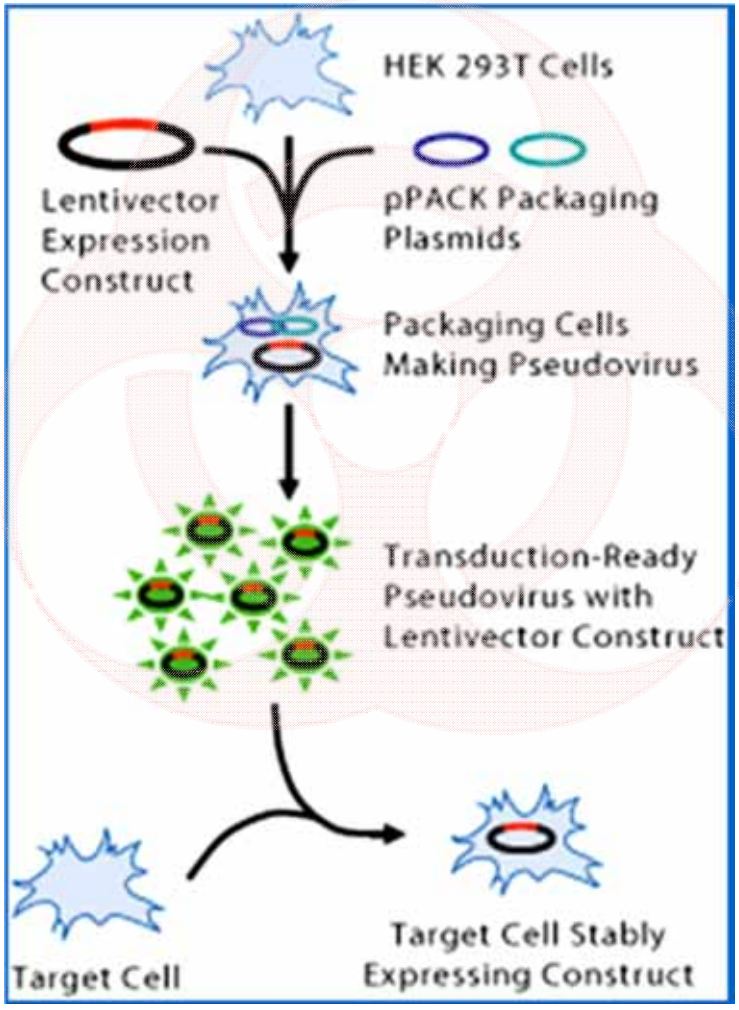


Major concerns about IAV reverse genetic system

- **WSN/33 and PR8 as model systems in the lab**
- **Manipulate virulent strain, such as H5N1**
- **Recombine with strains from different species, such as human, avian, and swine**
- **Contamination of unwanted plasmid(s) during rescue step**

HIV as a Vector for Gene Delivery





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- ✓ **Replication competent genome/ virus (RCV)**
- ✓ Main way and type of biosafety incident
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- ✓ Case study

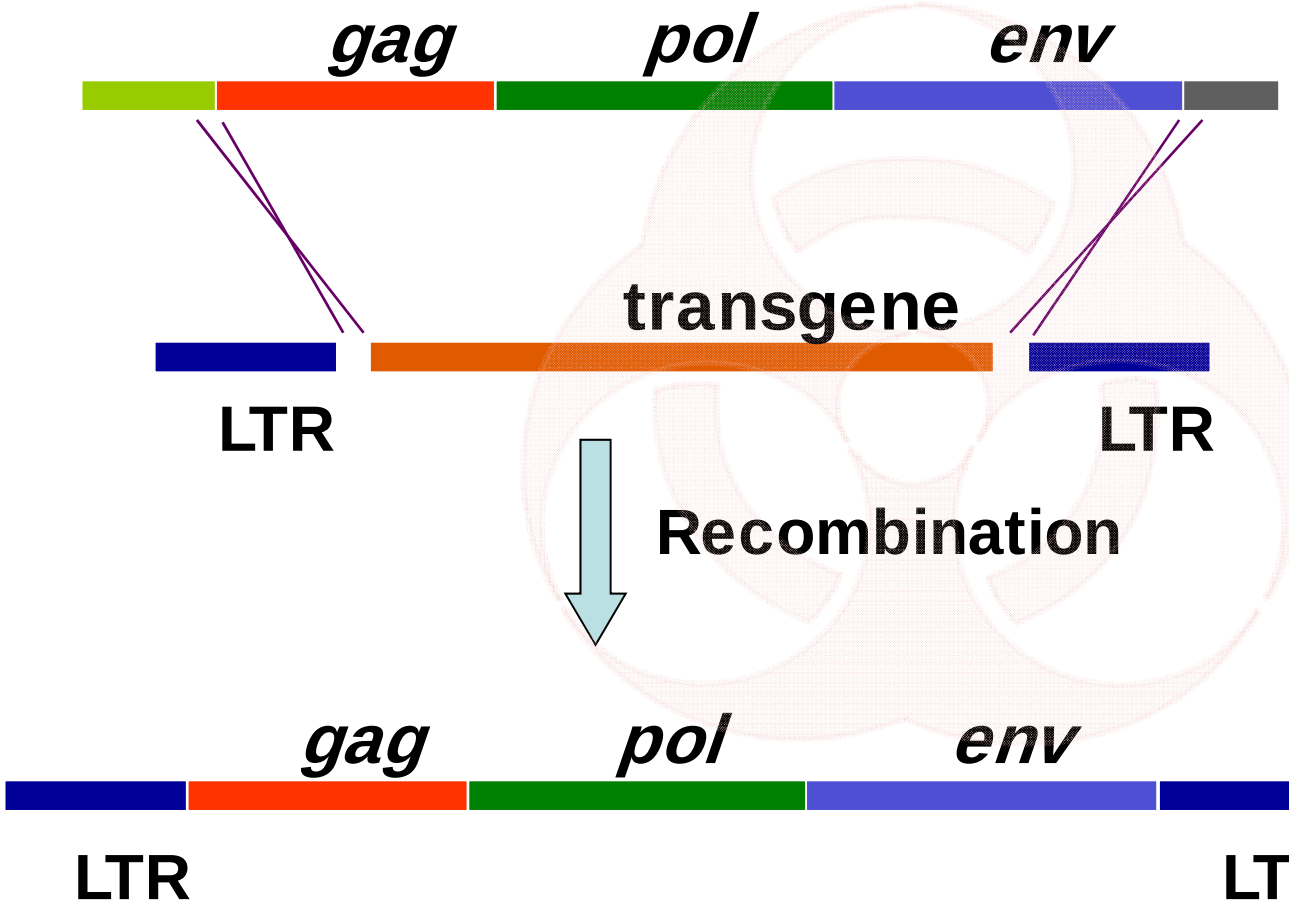
Concerns about Recombinant Virus

- Pathogenicity of parent virus
- Cytopathogenicity of vector
  - viral vector
  - inserted gene/sequence: oncogene, toxin, etc…
- Requirements for specialized facilities
- Scale up considerations (RCV problem)
- Biological safety considerations
- Training requirements

Origin of Replication Competent Virus

- ✓ During amplification and packaging of defective viral vector genomes, Replication Competent Virus (RCV) may generate through a process called recombination.

Reconstitution of RCV
(Retrovirus as an example)



Trans-acting genes

Retroviral vector genome

Replication competent retrovirus

Why Is RCA A Problem?

- Reconstitution of RCV is a rare event
- The contamination is generally at a very low level
- Potential for amplification in culture
- Pathogenicity in vivo
- Replication competent virus can disseminate

Strategies of Avoiding RCV Production

- Split genomes into different DNA constructs (lentiVector)
- Removal of viral regulatory regions (Adeno and HSV1 Vector)
- Production as a transient single batch rather than continuous culture
- Use of human cell lines for virus construction
- Pseudotyping of virus (trans-complementation)

Viral Vector Elements

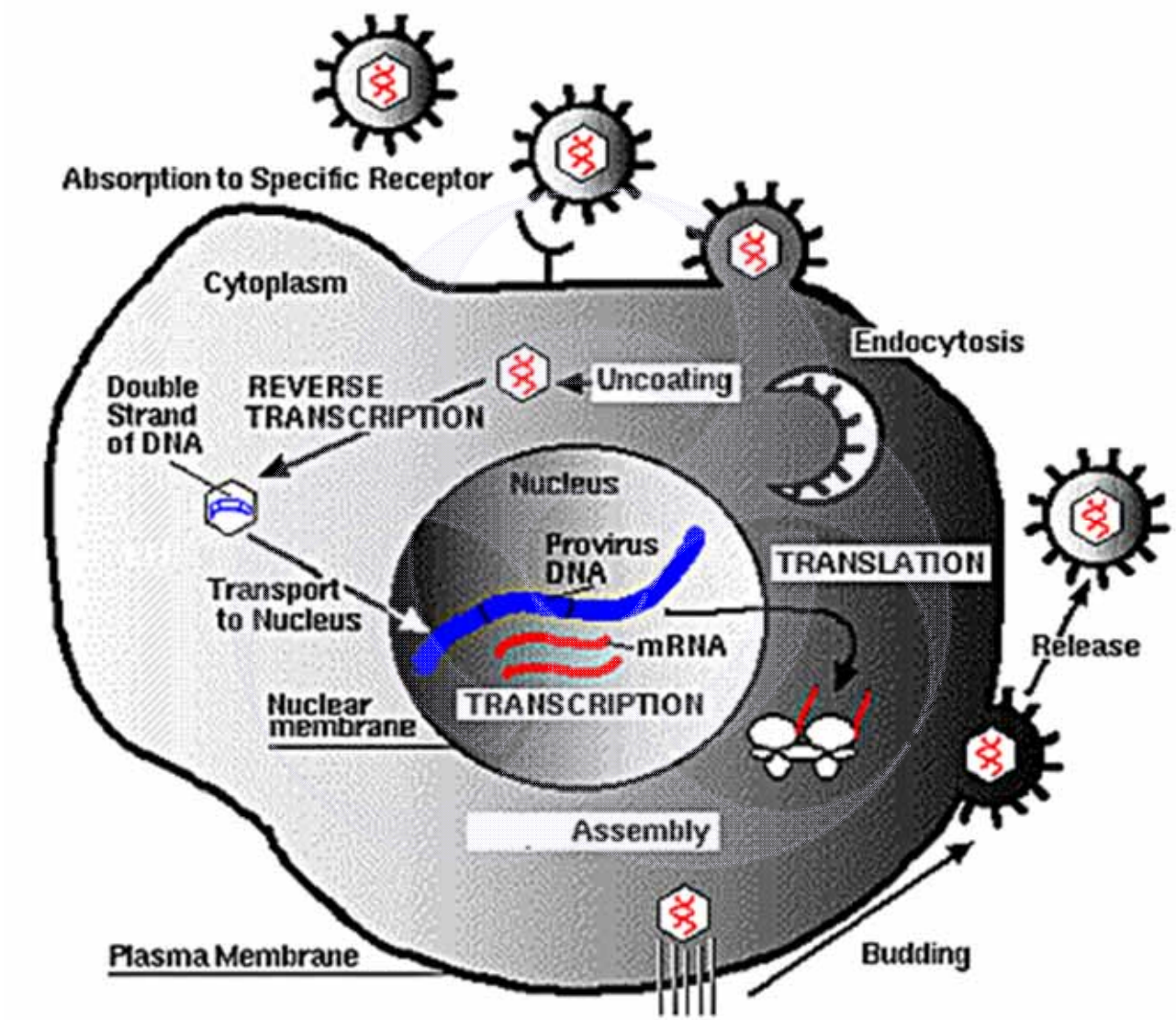
***trans*-acting elements**

- **Replication proteins**
  - **Polymerases**
  - **Proteases**
  - **Replicases**
- **Structural proteins**
  - **Capsid proteins**
  - **Envelope proteins**

***cis*-acting elements**

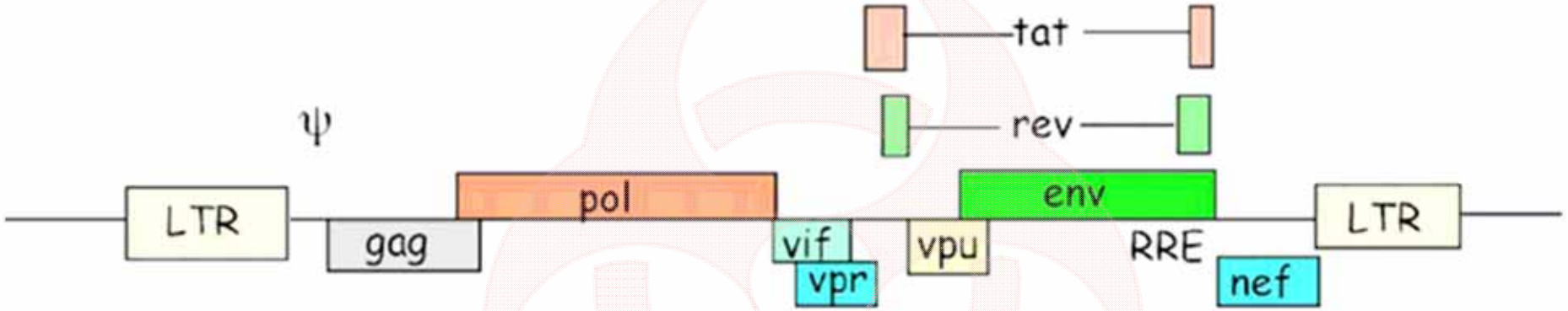
- **End repeats**
  - **ITR**
  - **LTR**
- **Packaging signals**
- **Regulatory sequences**

Replication of Retrovirus (HIV-1)



http://www.accessexcellence.org/RC/VL/GG/retrovirus.html

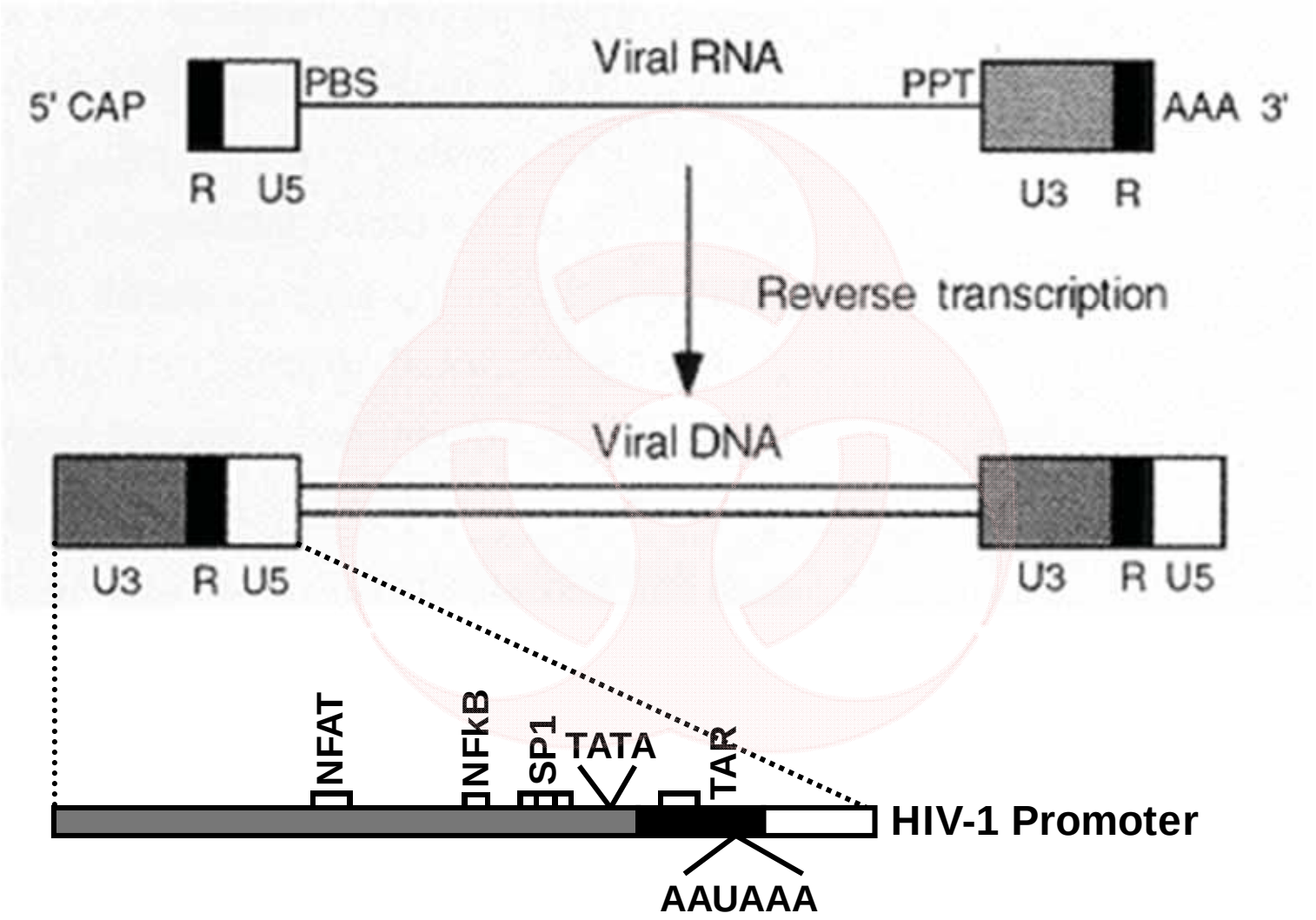
HIV-1 Genome Organization



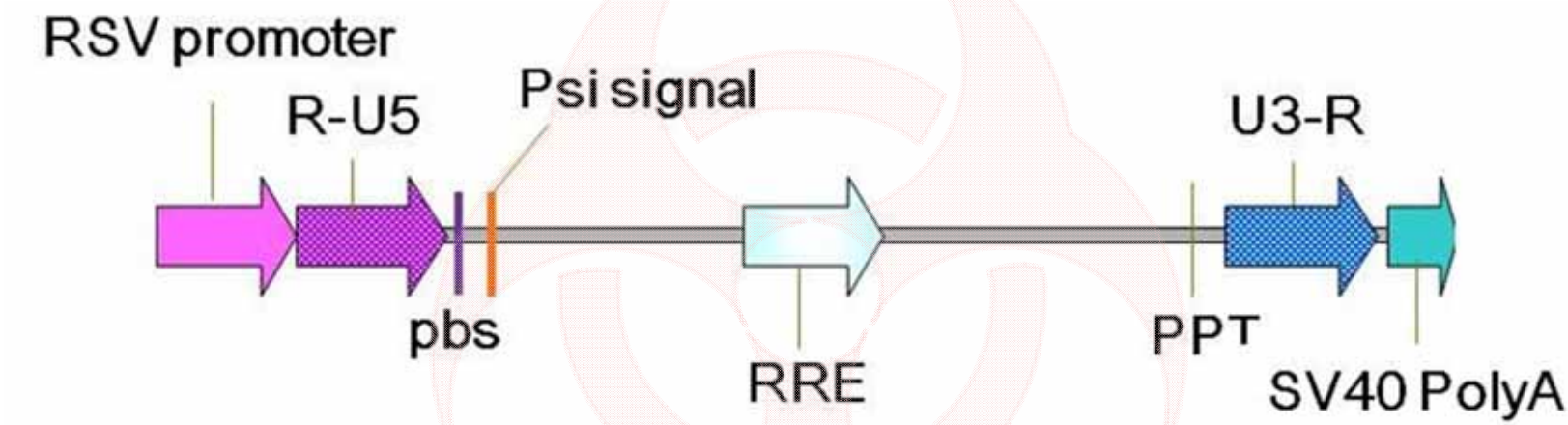
Encode 9 ORFs

http://www.vcu.edu/oehs/chemical/biosafe/lentiviralvectors.pdf

Reverse Transcription and Role of LTR



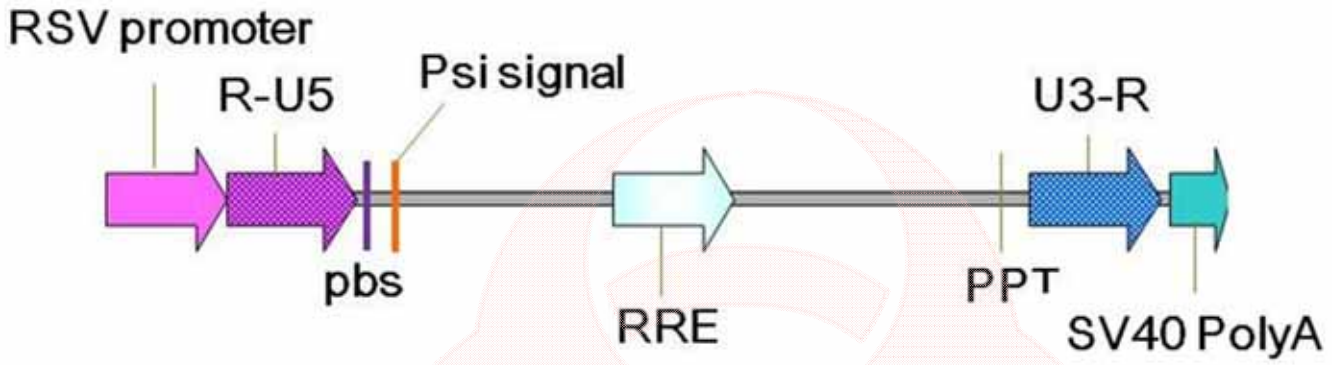
Cis Elements Require for HIV-1 Replication



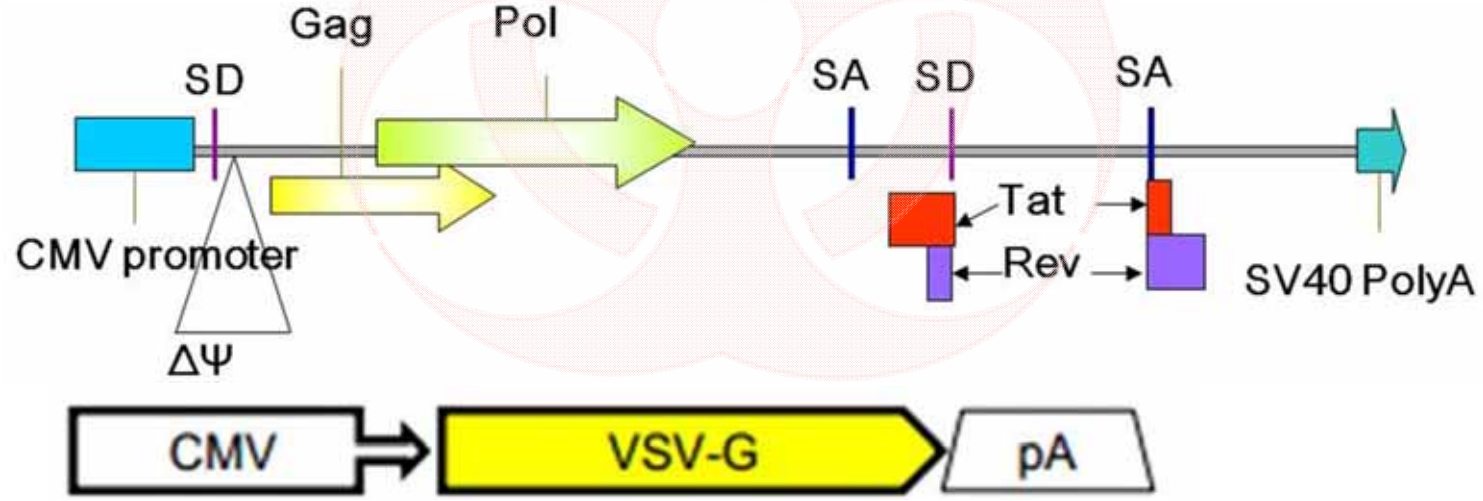
Lentiviral Vector Design

2nd Generation

Transfer Vector



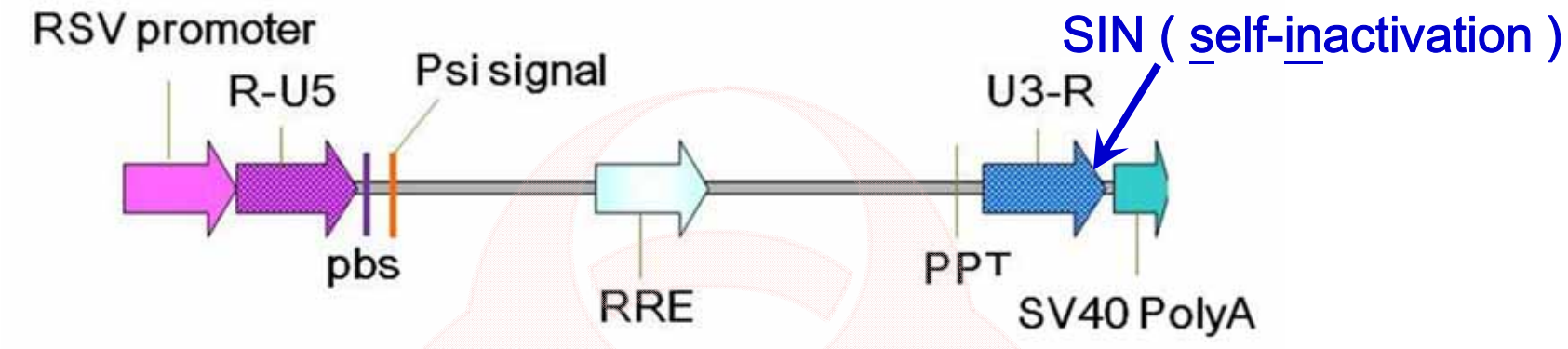
Helper Vector



Lentiviral Vector Design

2.5 Generation

Transfer Vector



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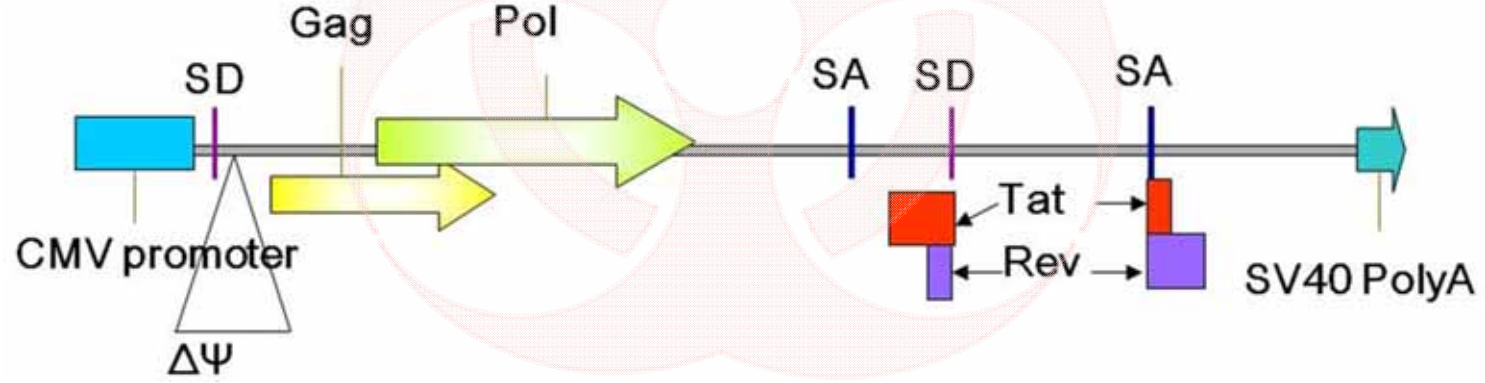
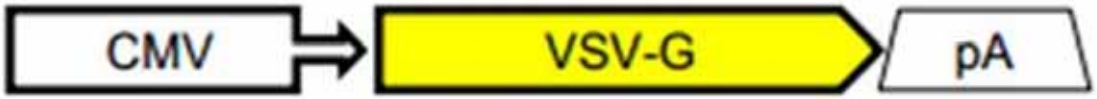
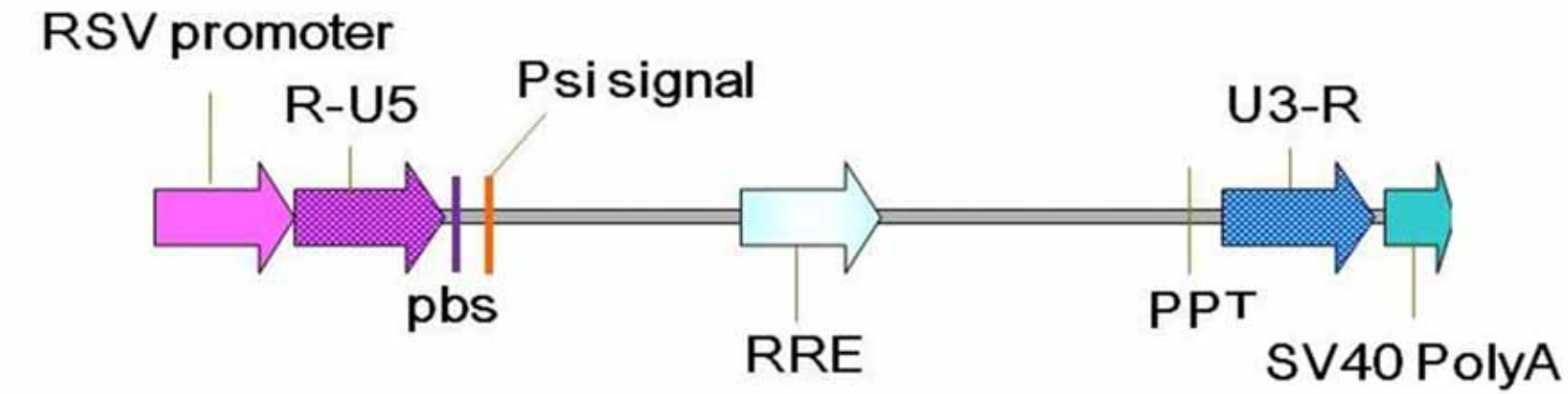




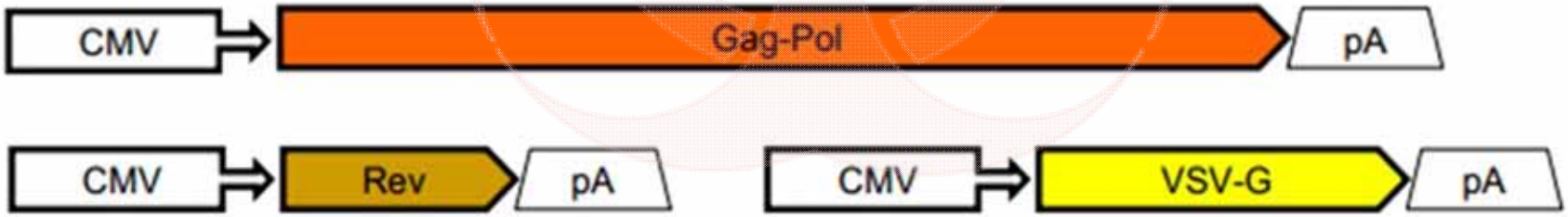
Lentiviral Vector Design

3rd Generation

Transfer Vector



Helper Vectors

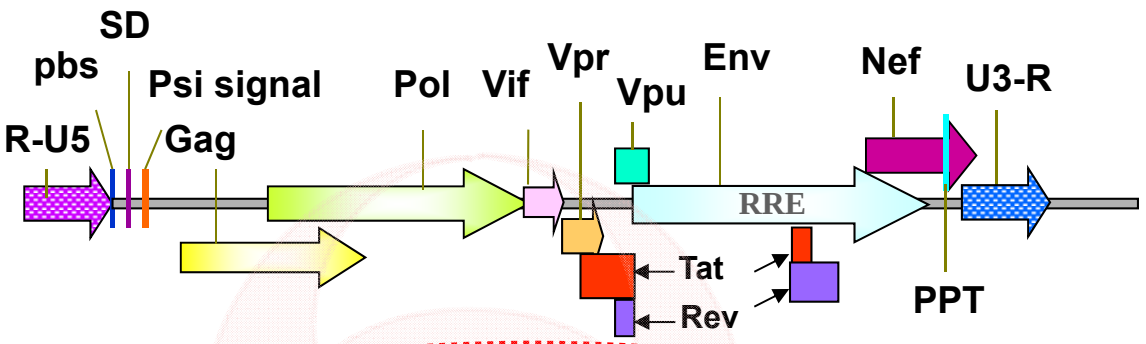


Modified from: http://www.asgct.org/am11/program/presentations/Dropulic.pdf

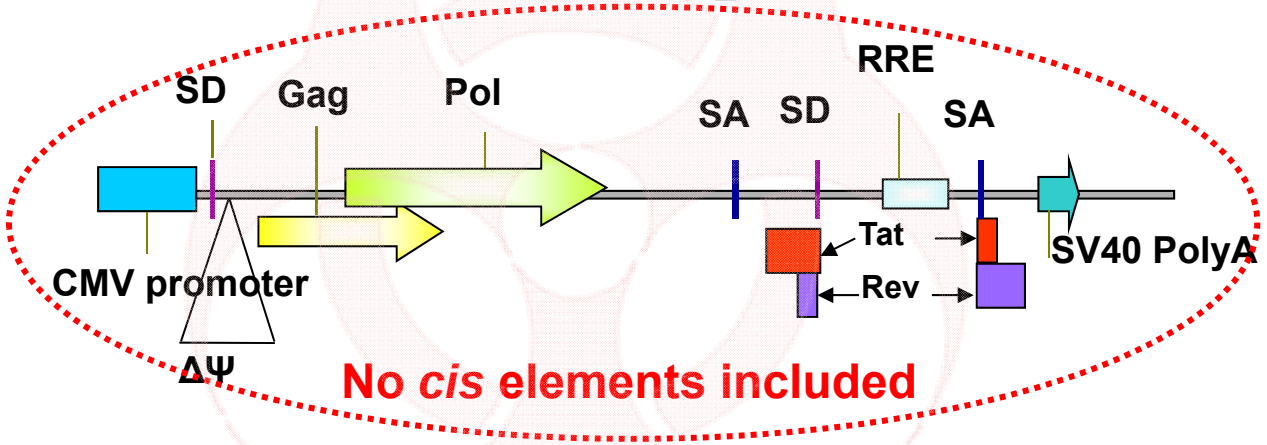
Genome Organization of Lentiviral Vector

(Improved biosafety by eliminating non-essential genes or sequences)

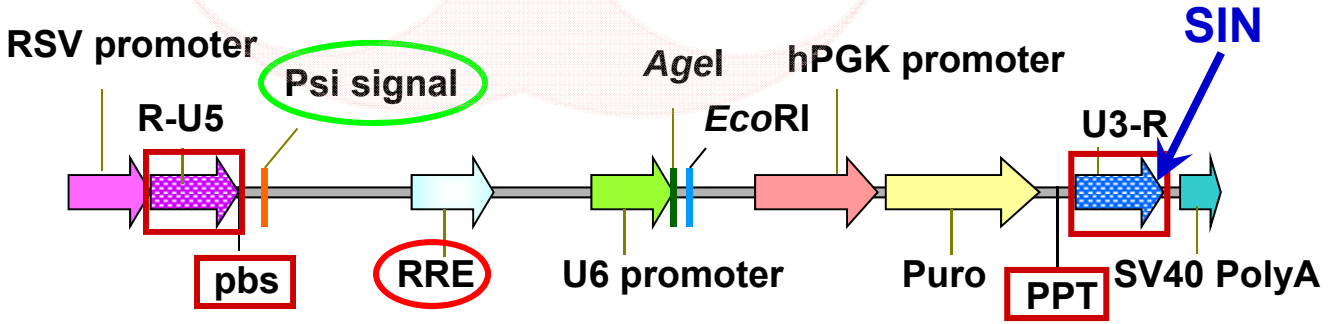
WT HIV-1:



pCMVΔR8.91:



pLKO.1-puro:



Summary of Lentiviral Vectors

- Belong to the retrovirus family but can infect both dividing and non-dividing cells.
- They are more complicated than retroviruses, containing an additional six proteins, tat, rev, vpr, vpu, nef and vif.
- Human immunodeficiency virus (HIV) has been disabled and developed as a vector for in vivo gene delivery; but integration is a safety issue used in human therapy.
- Low cellular immune response, thus good possibility for in vivo gene delivery with sustained expression over six months.
- No potent antibody response.

Outline

- ✓ Definition of rDNA
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- ✓ Replication competent genome/ virus
- ✓ **Main way and type of biosafety incident**
- ✓ Risk assessment
- ✓ Case study

生物實驗意外感染主要途徑及類型

意外感染主要途徑

- 吸入氣溶膠(氣霧; aerosol)
- 通過皮膚接觸
- 黏膜接觸污染物
- 吸收攝入

典型意外類型

- 針頭 /注射器 刺傷
- 感染性物質溢出/噴霧 接觸或吸入
- 碎玻璃/其他銳器 扎傷
- 從移液管吸入
- 動物咬傷/抓傷或外寄生蟲造成人畜共通之疾病感染

氣霧(Aerosol)：產生與特性

會產生氣溶膠(氣霧)的實驗操作步驟

- 攪拌器
- 超音波震盪
- 使用移液管（特別是最後一滴被吹出時）
- 擰開瓶蓋
- 從隔離蓋中抽出針頭
- 組織研磨
- 清潔動物籠舍

氣溶膠(氣霧)需被考量的特性

- 微生物在氣溶膠中的生存力
- 微生物的濃度
- 氣溶膠顆粒的大小（對氣溶膠顆粒進入呼吸道的深度和滯留時間有影響）
- 煙霧的持續性（＜**10μm**的煙霧顆粒持續飄浮在空氣中，**20-30μm**大小的顆粒容易經由通風系統轉移）

氣溶膠[氣霧](Aerosol)的微觀狀況

注射針頭自藥瓶抽出產生

均質機使用產生

使用移液Pipet時擠出
最後殘存的液滴產生

謹慎操作避免氣溶膠[氣霧]的飛濺

最常被報導造成實驗室感染之病原

1930 - 1978	1979 - 2004
Brucella spp.	M. Tuberculosis (肺結核分枝桿菌)
Coxiella burnetii	Arboviruses (蟲媒病毒)
Hepatitis B virus	Coxiella burnetii (Q熱,立克次體(rickettsia))中之伯納特氏柯克斯氏體引起之人畜共通(zoonosis)傳染之急性熱病)
Salmonella typhi	Hantavirus (漢他病毒)
Francisella tularensis	Brucella spp(ex: Brucellosis) (布氏桿菌病)
Mycobacterium tuberculosis	Hepatitis B virus (B型肝炎病毒)
Blastomyces dermatitidis	Shigella spp. (志賀氏桿菌--桿菌性痢疾)
VEE	Salmonella spp. (沙門氏菌)

Harding & Brandt Byers, 2006.

其他常造成實驗室感染之病原

- **Genetically modified infectious agent** 基因改造之感染性病原
- **Vaccinia virus**牛痘病毒
- **Human Immunodeficiency Virus (HIV)** 人類後天免疫不全症病毒
- **Simian Immunodeficiency Virus (SIV)**猿猴免疫缺乏病毒
- **Severe Acute Respiratory Syndrome (SARS) Coronavirus**嚴重急性呼吸道症候群冠狀病毒
- **Rabies virus**狂犬病病毒
- **West Nile Virus** 西尼羅病毒
- **Tick-borne encephalitis virus (TBEV)**蜱媒腦炎病毒
- **Lymphocytic choriomeningitis virus**淋巴球性腦膜脈胳膜炎病毒
- **MRSA Staphylococcus aureus**對甲氧苯青黴素 **(methicillin)**具有抗藥性的金黃色葡萄球菌
- **Escherichia coli O157:H7** 大腸桿菌**O157**
- **Clostridium difficile CD**菌**--**腸道感染
- **Burkholderia mallei**鼻疽伯克霍得菌
- **Parasites**寄生蟲造成之感染症

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分類基準(病原微生物危害性)

等級	說明	例子
第一級危險群 (RG1)	不易引起人類健康成人之疾病	• *Bacillus subtilis* • *Escherichia coli*-K12 • adeno-associated virus Type 1-4
第二級危險群 (RG2)	所引起的疾病很少是嚴重的，通常有預防及治療的方法	• Measles virus • *Salmonellae* • *Toxoplasma spp.* • Hepatitis B virus
第三級危險群 (RG3)	可以引起嚴重或致死的疾病，可能有預防及治療之方法	• *M. tuberculosis* • SARS-associated Coronaviruses • Hantaan virus
第四級危險群 (RG4)	引起嚴重或致死的疾病，通常無預防及治療之方法	• Ebola virus • Marburg virus

Biological Agent: Infectious dose

- The number of microorganisms required to initiate infection

Q fever (a disease caused by infection with *Coxiella burnetii*)	10 organisms by inhalation
E. coli	10^8 organisms by ingestion
Malaria	10 organisms by IV injection
Poliovirus 1	2 pfu by ingestion

Wedum, A.G., Barkley, W.E., and Hellman, A. 1972. *J Am Vet. Med Assoc.* 161:11, 1557-1567.

Biological Agent:
method of transmission

- Direct contact
  - Direct transmission to receptive portal of entry
- Indirect Contact
  - Vehicle-borne such as inanimate materials or objects (fomites)
  - Vector-borne (arthropods)
- Airborne
  - Dissemination of microbial aerosols to a suitable portal of entry

Risk Assessment

Risk assessment is ultimately a subjective process.

The investigator must make an initial risk assessment based on the RG of an agent or recombinant agent.

- Usually, containment level shall be equivalent to RG level of the agent, i.e. RG2 in BSL2
- Parent agent and its recombinant must be considered separately.

RG level may be raised or lowed.

- ✓ **Oncogene ↑**
- ✓ **Originally viral sequences less than 2/3 ↓**
- ✓ **Toxin gene expression (BSL3/EK1 to BSL2/EK2)**
- ✓ **RG2 or RG3 agents are used for animal inoculation or transmission. a higher containment is recommended.**
- ✓ **Large-scale culture (more than 10L) ↑**

Risk Assessment

Factors to be considered:

- Virulence
- Pathogenicity
- Infectious dose
- Environmental stability
- Route of spread
- Communicability (傳染性)
- Availability of vaccine or treatment
- Vectors/ Recombinant properties
- Concentration/volume

實驗操作者如何進行自我風險評估
-四項要領

- 我的實驗操作使用哪些生物材料？
  - 預測潛在之安全考量
  - 明瞭(危害物質)進入身體的途徑
  - 生物醫學研究相關知識
  - 實驗操作者對生物性、化學及物理性危害需具備的知識
- 使用這些材料的潛在危險為何？
  - 危害辨識
  - 風險評估

實驗操作者如何進行自我風險評估
-四項要領

- 我如何避免自己、同事及環境，因實驗操作而暴露於潛在危機中
  - 實驗使用物品的相關參考資料
  - good laboratory practice
  - 行政面的控管
- 我如何維護自身、同事及環境安全
  - 實驗設備的工程控管
  - 個人防護裝備

General guidelines to assess risk : Reviewer point of view

- Biological Agent
  - RG level
  - Insert characteristics
  - Volume
  - Concentration
  - Cytopathogenecity of viral vector
- Proposed practices/procedures
- Proposed location (and environment)
- Training, experience, health status of worker (potential host)

Biosafety Reminders!

- Proper Use of Biosafety Cabinets
- Good laboratory competency reduces biohazards
  - The risk for exposures
  - Laboratory-acquired infections
  - The unintended release of research materials to the environment

Summary

生物安全等級 Biosafety Level

BSL-1/ P1
不會引致健康成人的疾病

BSL-2/ P2
引致疾病但很少會很嚴重,通常能有效預防或處理者

BSL-3/ P3
引致嚴重的疾病,但通常無法有效預防或處理者

BSL-4/ P4
引致嚴重的疾病,並且通常無法有效預防或處理者

風險評估因子

危害性
- 疾病的嚴重性
- 傳染便利性
- 傳染途徑
- 對動植物的影響

特性
- 是否為原生種類
- 數量與分布

反應性
- 是否有藥或疫苗
- 人類的免疫作用

生物風險群組

RG-1
多數細菌、枯草桿菌、酵母菌、大腸桿菌K12型 and derivatives.

RG-2
登革熱、肉毒桿菌、腸病毒、A-E型肝炎病毒.....

RG-3
炭疽桿菌、結核菌、HIV 1、日本腦炎、漢他病毒、 SARS---

RG-4
依波拉病毒、剛果出血熱病、拉沙熱病、Marburg virus

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- ✓ Definition and overview of rDNA
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- ✓ Re-visit

您知道答案嗎？

一、 小王以基因合成方式合成了HIV-1 (RG3病毒)全基因體雙股DNA，並克隆到 pcDNA3-based vector作為研究材料。

Q: 請問以DH5α細菌操作此合成 DNA 並在 P1 實驗室進行實驗，實驗安全嗎？為什麼？

二、 小王將此含HIV-1的載體在Laminar Flow/P1房轉染到 human T4 cells中研究其基因表達的profile。

Q: 請問此操作條件符合生物安全規範嗎？為什麼？

三、 小王將此表達載體之HIV-1 所有構造蛋白及 polymerase ORF完全disabled，以發展成 gene transfer 載體用來表達 ras oncogene，並在P2房轉染到 293T細胞測試表達能力。

Q: 請問此實驗條件有生物安全疑慮嗎？ 如果有，是什麼？

Reference:

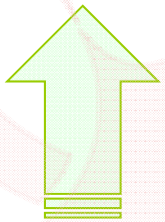
1. http://www.sinica.edu.tw/~biosafe/2-1.html
2. http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm
3. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm
4. 行政院衛生署疾病管制局
   「感染性生物材料管理及傳染病病人檢體採檢辦法」
   94年9月26行政院衛生署署授疾字第0940000614號令訂定發布全文十九條
   95年4月11日行政院衛生署署授疾字第0950000194號令修正發布第二條之一及第十九條條文
6. 行政院環境保護署「有害事業廢棄物認定標準」
   96年7月4日行政院環境保護署環署廢字第0960049171號令修正
7. 國家科學委員會「基因重組實驗守則」
   93年6月 增修版

Thank you for your attention

Questions?

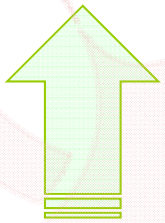
Biosafety issue regarding:

Host-Vector System



Biosafety issue regarding:

Infectious agent's characteristics



Biosafety issue regarding:

1. Vector characteristics;
2. Insert nature,
3. Replication competent genome /virus (RCV) problem;
4. Consideration of experimental environment: contamination issue.