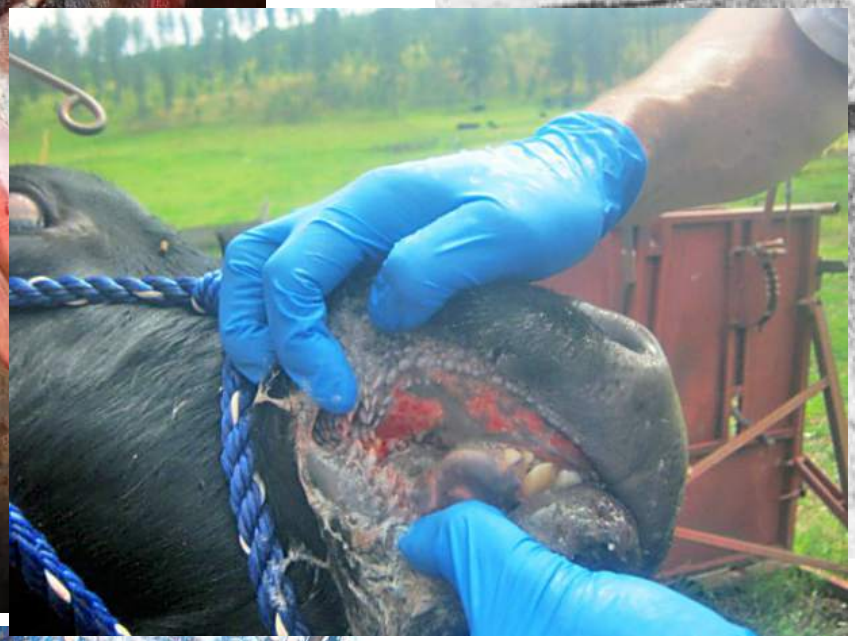
VIROLOGY

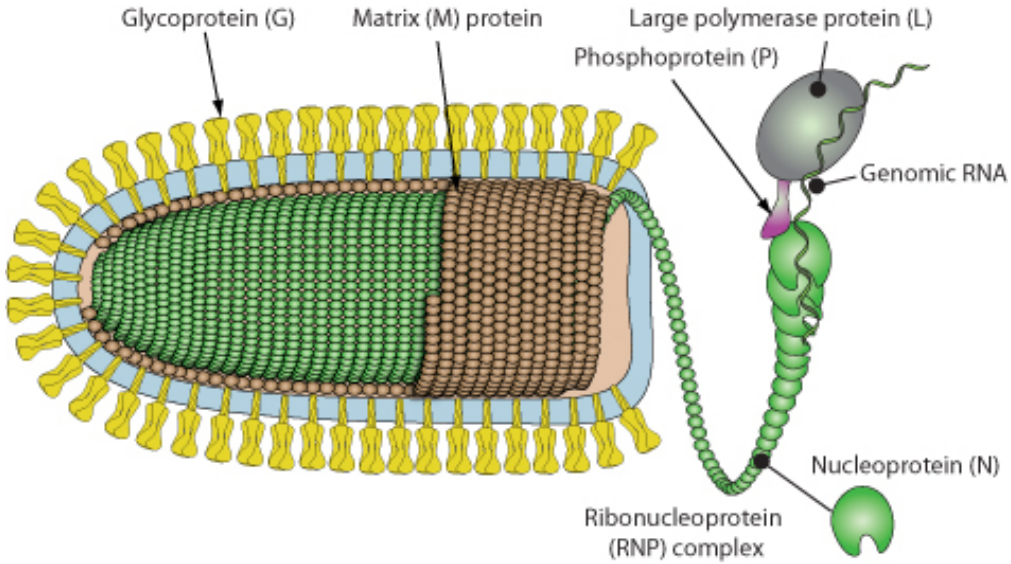
Engineering Viral Genomes: VSV Vectors

Viral vectors

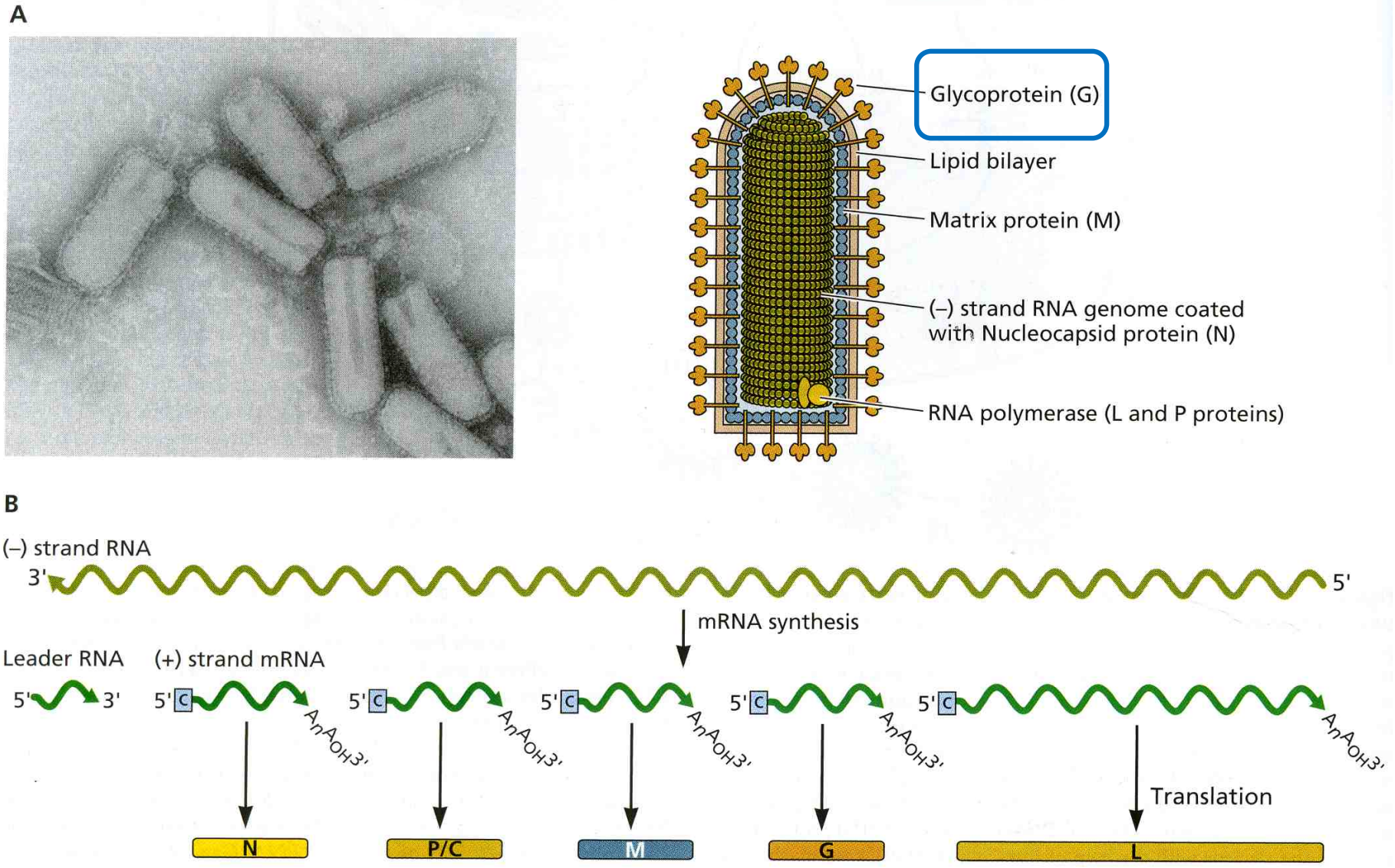
Virus	Insert size	Integration	Duration of expression	Advantages	Potential disadvantages
Adeno-associated virus	~4.5–9 (?) kb	Low efficiency	Long	Nonpathogenic, episomal, infects nondividing cells	Immunogenic, toxicity, small packaging limit
Adenovirus	2–38 kb	No	Short	Efficient gene delivery, infects nondividing cells	Transient, immunogenic
Alphavirus	~5 kb	No	Short	Broad host range, high level expression	Virulence
Epstein-Barr virus	~120 kb	No; episomal	Long	High capacity, episomal, long-term expression	
Gammaretrovirus	1–7.5 kb	Yes	Shorter than formerly	Stable integration	May rearrange genome, insertional mutagenesis require cell division
Herpes simplex virus	~30 kb	No	Long in central nervous system, short elsewhere	Infects nondividing cells; neurotropic, large capacity	Virulence, persistence in neurons, immunogenic
Lentivirus	7–18 kb	Yes	Long	Stable integration; infects nondividing and terminally differentiated mammalian cells	Insertional mutagenesis
Poliovirus	~300 bp for helper-free virus; ~3 kb for defective virus	No	Short	Excellent mucosal immunity	Limited capacity; reversio to neurovirulence
Rhabdovirus	Unknown	No	Short	High-level expression, rapid cell killing	Virulence, highly cytopathic
Vaccinia virus	At least ~25 kb, probably ~75–100 kb	No	Short	Wide host range, ease of isolation, large capacity, high-level expression	Transient, immunogenic

Vesicular Stomatitis Virus (VSV)





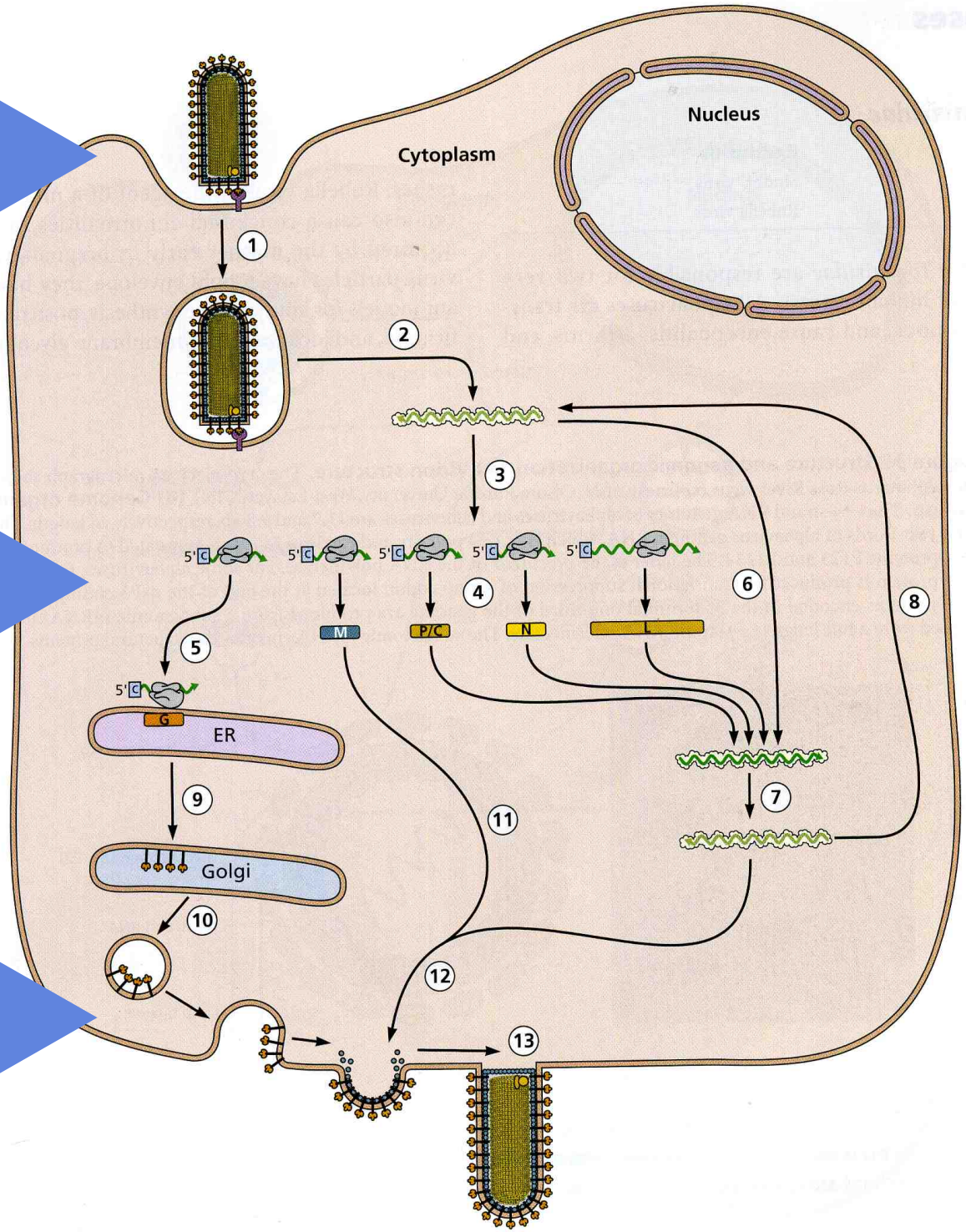
Vesicular Stomatitis Virus (VSV): structure and genomic organization



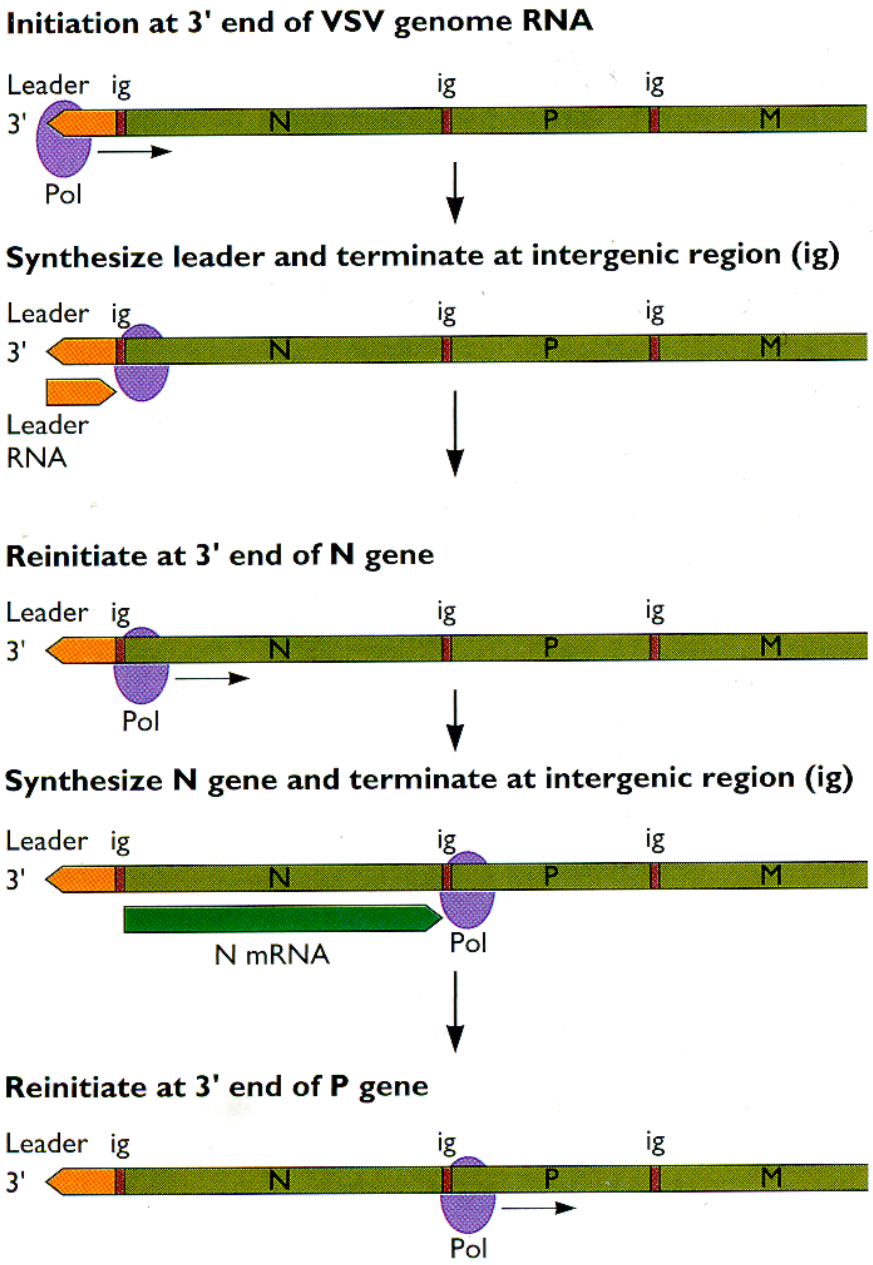
Attachment
Entry
Uncoating

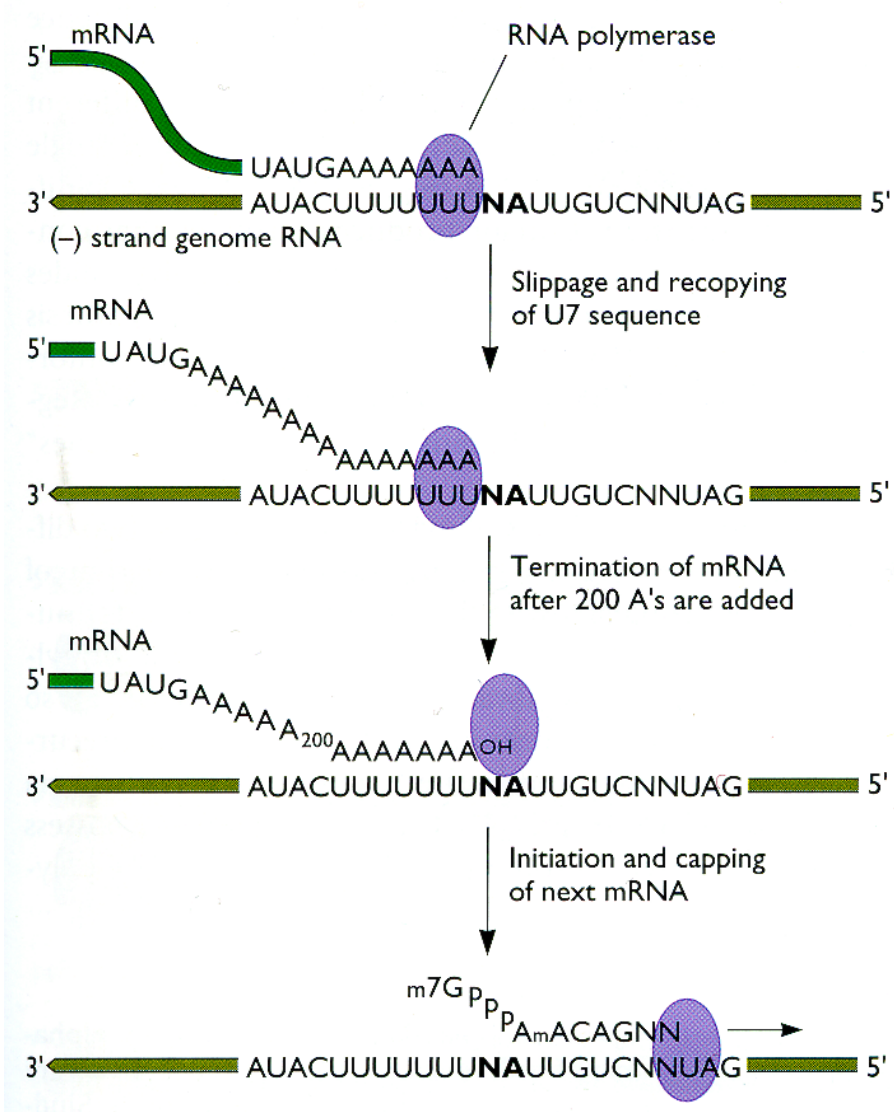
Viral
Synthesis

Assembly
Maturation
Exit

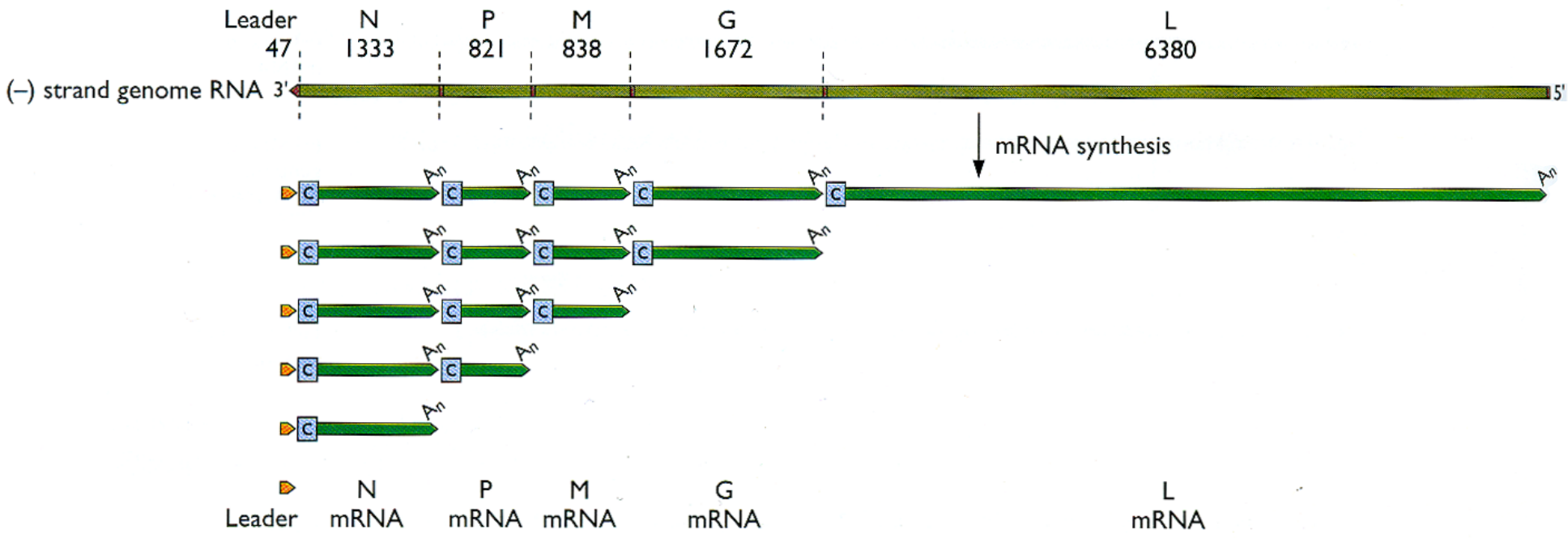


VSV mRNA synthesis and function of RNA pol at an intergenic region

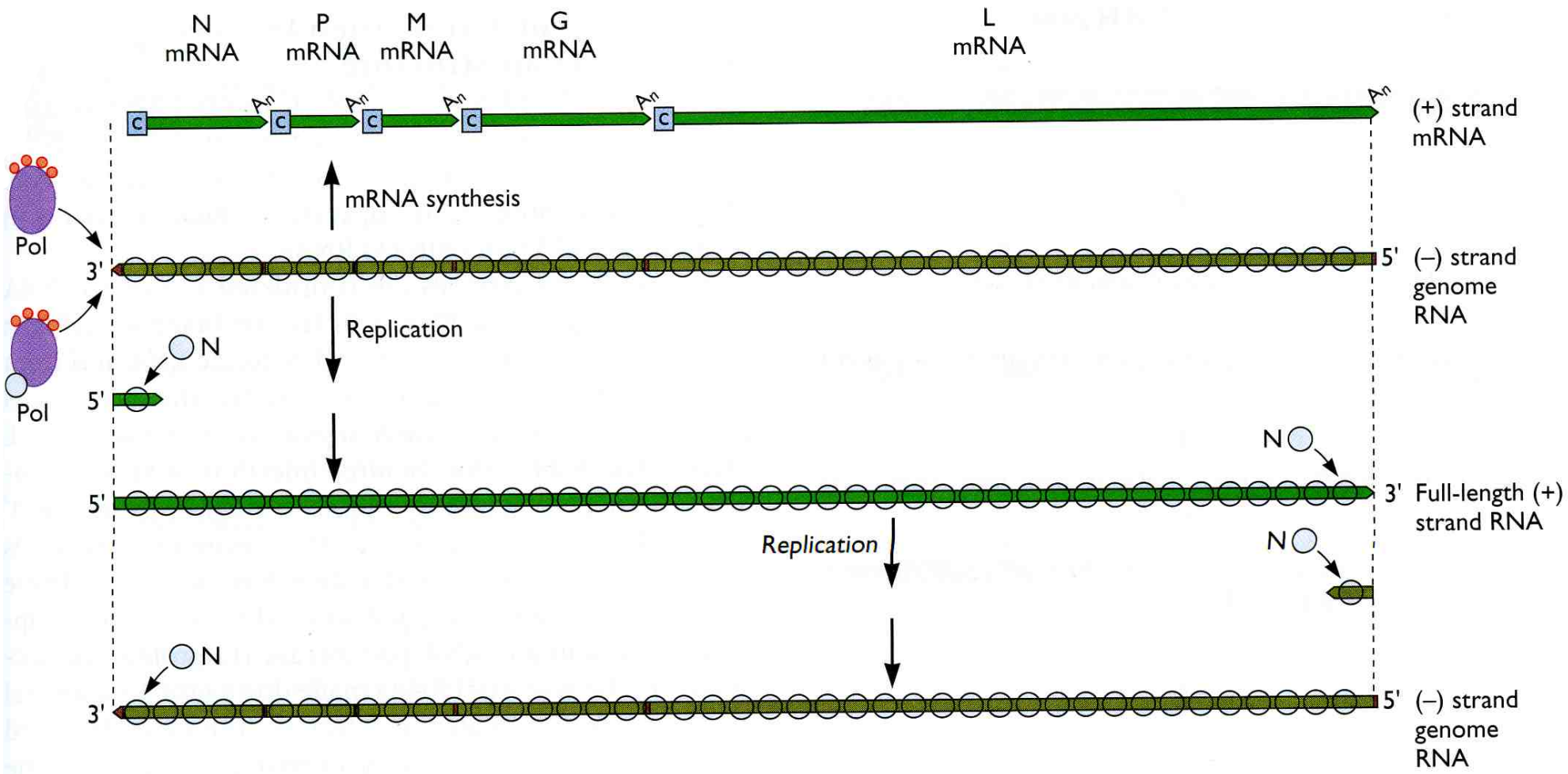




Vesicular stomatitis virus mRNA map



Transcription and replication of the VSV genome



Infectious VSV can be produced by reverse genetics

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 8388–8392, August 1995
Biochemistry

Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones

SEÁN P. J. WHELAN, L. ANDREW BALL, JOHN N. BARR, AND GAIL T. W. WERTZ*

Department of Microbiology, The Medical School, University of Alabama at Birmingham, BBRB 17 Room 366, 845 19th Street South, Birmingham, AL 35294

ABSTRACT **Infectious vesicular stomatitis virus (VSV), the prototypic nonsegmented negative-strand RNA virus, was recovered from a full-length cDNA clone of the viral genome. Bacteriophage T7 RNA polymerase expressed from a recombinant vaccinia virus was used to drive the synthesis of a genome-length positive-sense transcript of VSV from a cDNA clone in baby hamster kidney cells that were simultaneously expressing the VSV nucleocapsid protein, phosphoprotein, and polymerase from separate plasmids. Up to 10^5 infectious virus particles were obtained from transfection of 10^6 cells, as determined by plaque assays. This virus was amplified on passage, neutralized by VSV-specific antiserum, and shown to possess specific nucleotide sequence markers characteristic of the cDNA. This achievement renders the biology of VSV fully accessible to genetic manipulation of the viral genome. In contrast to the success with positive-sense RNA, attempts to recover infectious virus from negative-sense T7 transcripts were uniformly unsuccessful, because T7 RNA polymerase terminated transcription at or near the VSV intergenic junctions.**

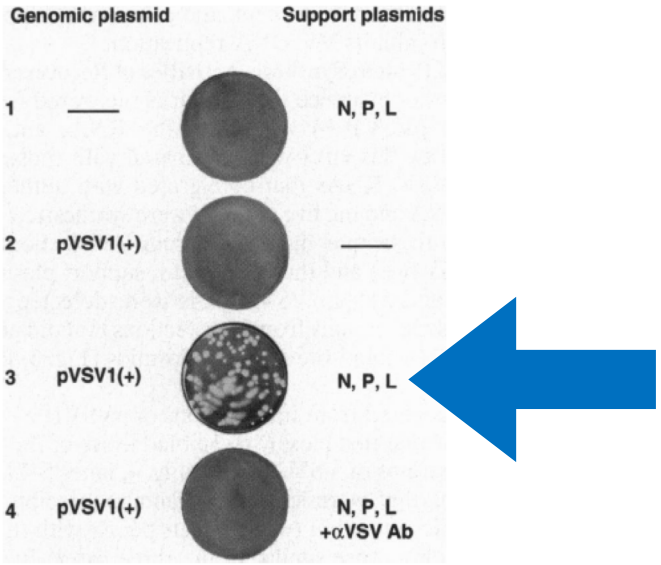


FIG. 2. Plaque assays of recovered virus. Monolayers of BHK21 cells were infected with vTF7-3 and transfected with pVSV1(+) and the N, P, and L support plasmids as indicated. After 45 hr of incubation at 37°C, the culture media were harvested and diluted 100-fold, and the infectious virus in 0.1-ml aliquots was determined by plaque assay using fresh monolayers of BSC40 cells. araC (25 μg/ml) was included in the agarose overlay to suppress the replication of VV. After 30 hr of incubation to allow VSV plaque formation, the monolayers were fixed, stained with crystal violet, and photographed. Shown are plaque assays of medium from cells that received N, P, and L support plasmids only (plate 1); pVSV1(+) only (plate 2); pVSV1(+) and N, P, and L support plasmids without (plate 3) or with (plate 4) subsequent incubation of the medium with anti-VSV antiserum (αVSV Ab).

Infectious VSV can be produced by reverse genetics

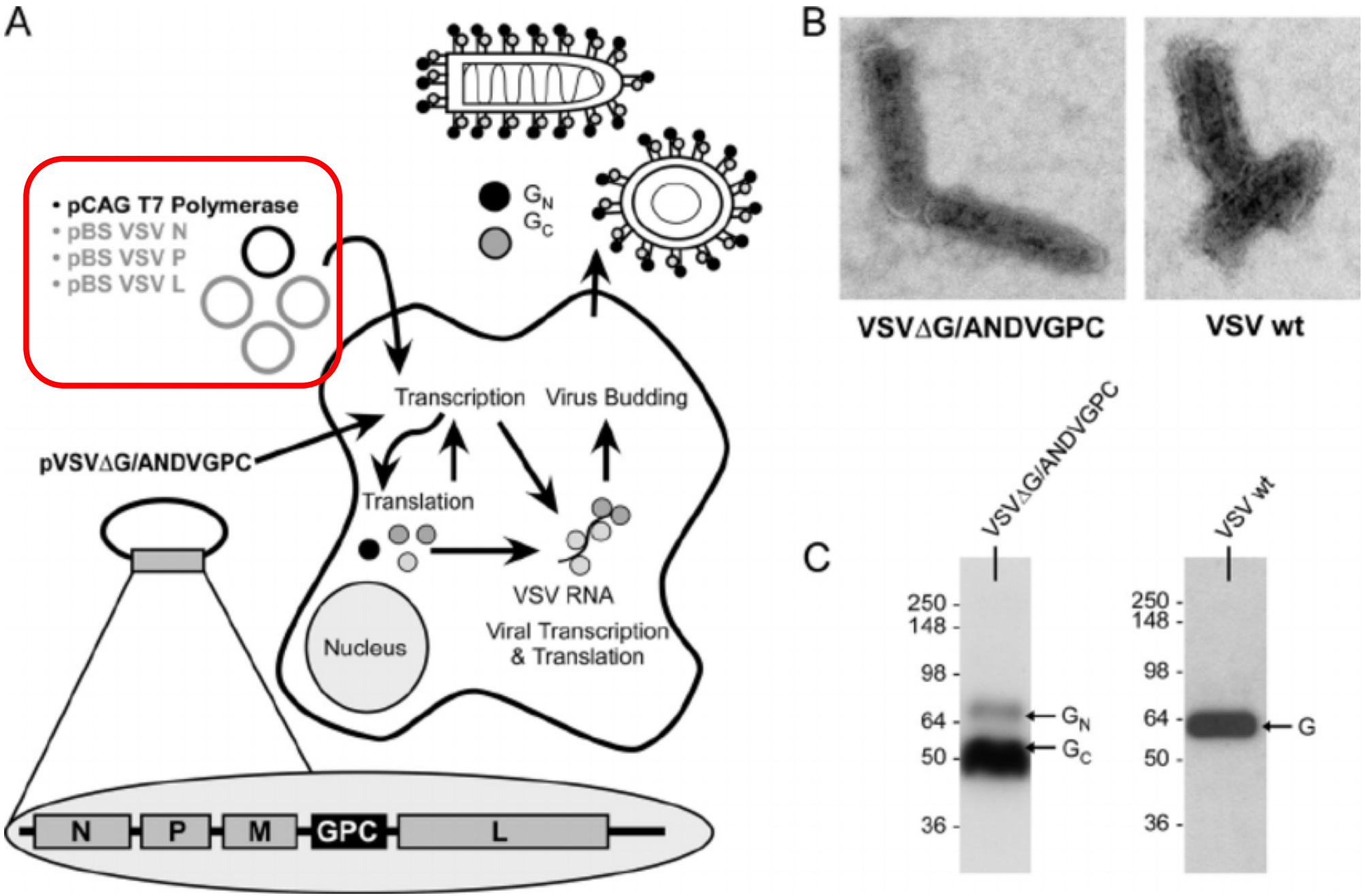


Table 1. The growing arsenal of VSV-based therapeutics for use against infectious and malignant disease[a]

VSV application	Features	Refs
Vaccine vectors		
VSV–HA	Insertion of influenza hemagglutinin gene into VSV genome; hemagglutinin antigen is expressed in VSV-infected cells and on viral surface	[27]
VSV-ΔG–HA	Improved influenza vector; attenuating deletion of VSV glycoprotein increases safety and prevents stimulation of VSV-specific humoral immunity	[4]
VSV–GagEnv	Insertion of HIV *Gag* and *Env* genes into VSV genome; VSV-infected cells express Env and Gag proteins to induce HIV-specific $CD8^+$ CTL and neutralizing antibody responses	[29,32]
VSV–MV-H	Insertion of measles virus hemagglutinin gene into VSV genome; elicits protective MV-specific neutralizing antibody despite the presence of circulating maternal antibody	[28]
VSV-ΔG–RSV-F	Insertion of respiratory syncytial virus fusion gene into VSV genome; RSV-fusion antigen is expressed in VSV-infected cells and on viral surface; attenuated by deletion of VSV-G	[31]
VSV–HCV-C/E1/E2	Insertion of Hep C gene encoding contiguous C/E1/E2 proteins; HepC antigens are expressed in VSV-infected cells	[33]
VSV-rearranged genome	Rearrangement of genes generates a stably attenuated vector	[2]
Natural oncolytic strains		
WtVSV[g]	High sensitivity to anti-viral interferons; selective replication and cytotoxicity in tumor cells exhibiting compromised interferon response	[35,37,40]
VSV-AV1 or VSV-AV2	Highly attenuated replication in normal cells but conserved tumor killing; enhanced therapeutic index	[16]
Recombinant oncolytic strains		
wtVSV–GFP	Expression of green fluorescent protein transgene	[16,39,48]
VSV–Δ51M	Deletion of Met-51 of matrix protein; highly attenuated replication in normal cells but conserved tumor killing; enhanced therapeutic index	[16]
Oncolytic VSVs expressing immunostimulatory cytokines		
VSV–IL-4	Expresses IL-4 gene; enhanced therapeutic index	[36]
VSV–IFN-β	Expresses IFN-β gene; enhanced therapeutic index	[45]
Oncolytic VSVs expressing a suicide gene		
VSV–TK	Expresses thymidine kinase gene; killing of infected and bystander cells with gancyclovir treatment	[36]
VSV–CD/UPRT	Expresses cytosine deaminase (CD)/uracil phosphoribosyltransferase gene; killing of infected and bystander cells with 5-fluorocytosine treatment	[41]
Receptor-targeted VSVs		
VSV–CD4	Expresses CD4 and can infect cells expressing HIV gp120	[43,44]
VSV–Sindbis-ZZ glycoproteins	VSV pseudotype coated with a Sindbis virus glycoprotein/protein A fusion; targeting to tumor-specific antigens when co-administered with a monoclonal antibody	[42]

[a]Abbreviations: AV, attenuated virus; CD/UPRT, cytosine deaminase (CD)/uracil phosphoribosyltransferase; CTL, cytotoxic T lymphocyte; G, glycoprotein; HA, hemagglutinin; HCV-C/E1/E2, hepatitis C virus capsid/envelop 1/envelop 2; IFN, interferon; IL, interleukin; MV-H, measles virus hemagglutinin; RSU-F, respiratory syncytial virus fusion; TK, thymidine kinase; VSV, vesicular stomatitis virus; Wt, wild type.

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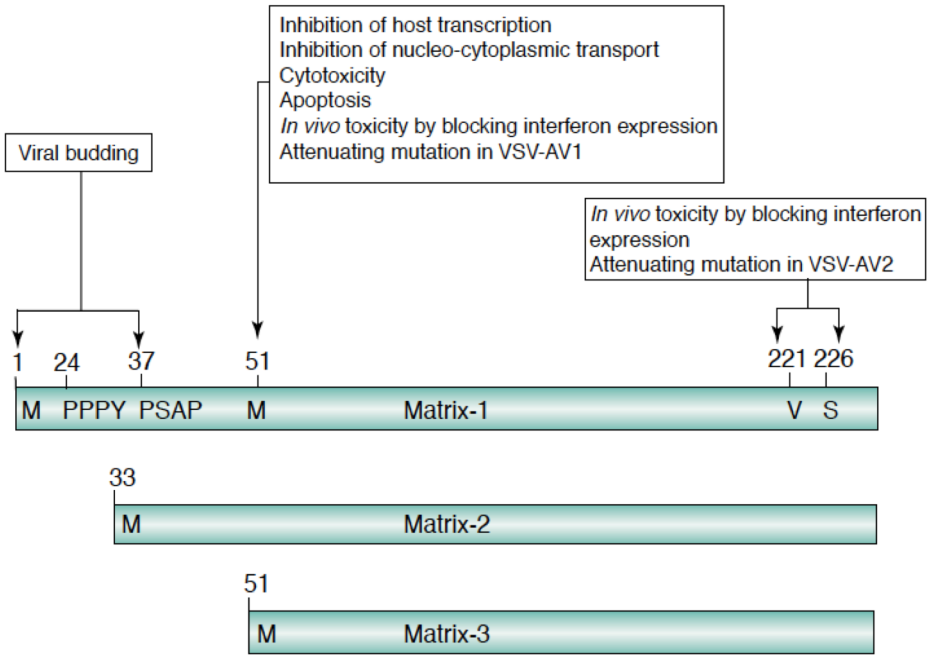
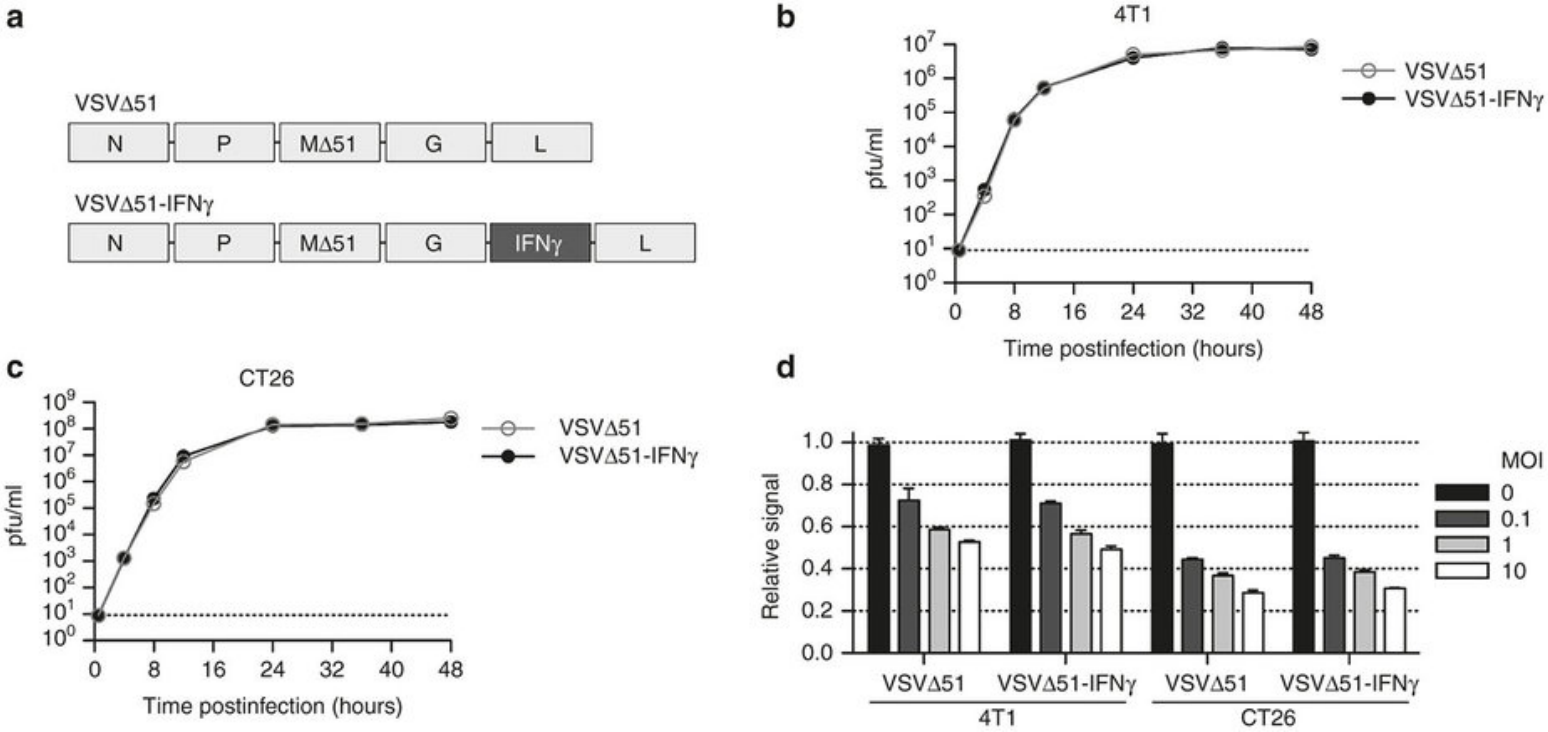
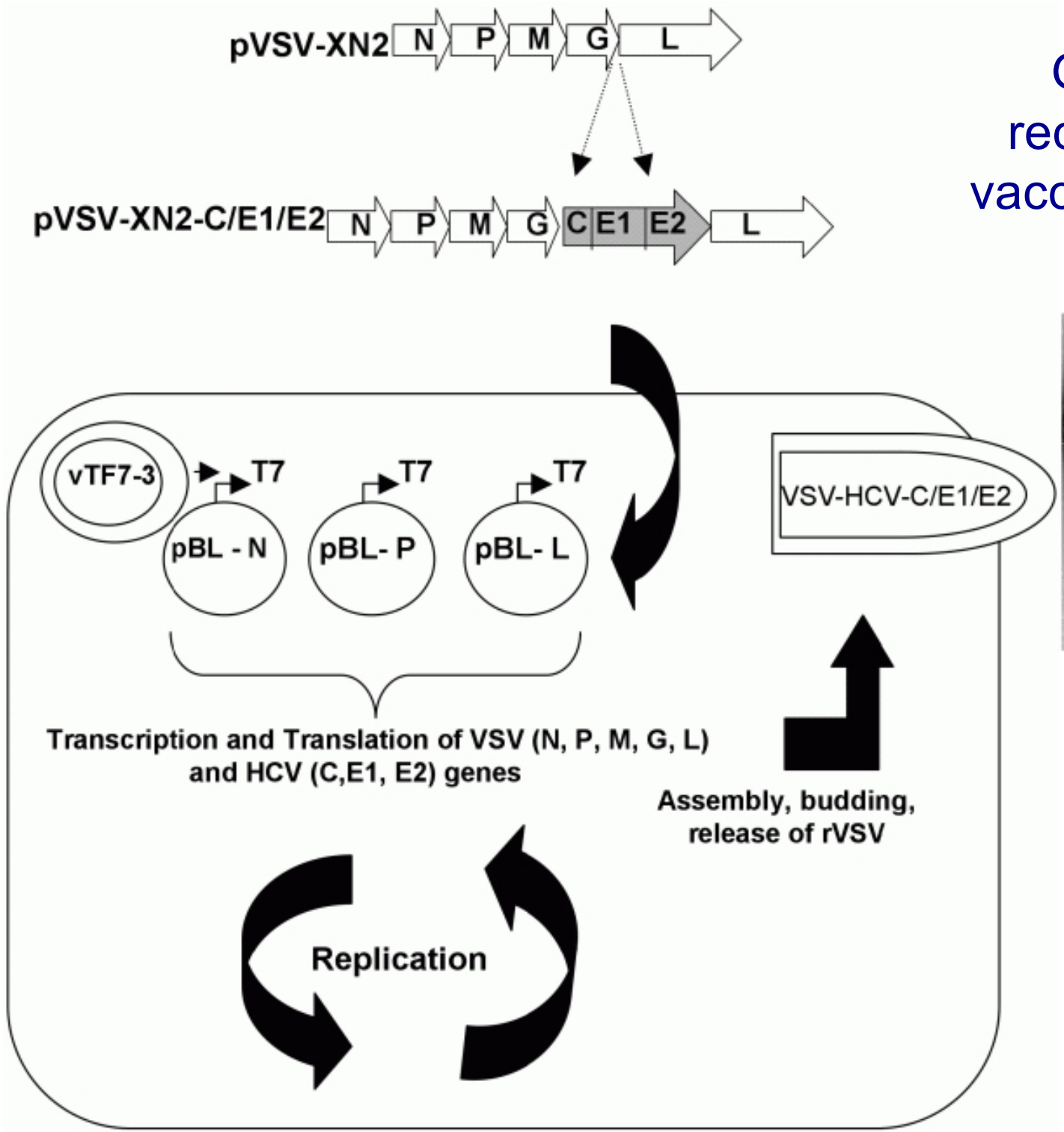
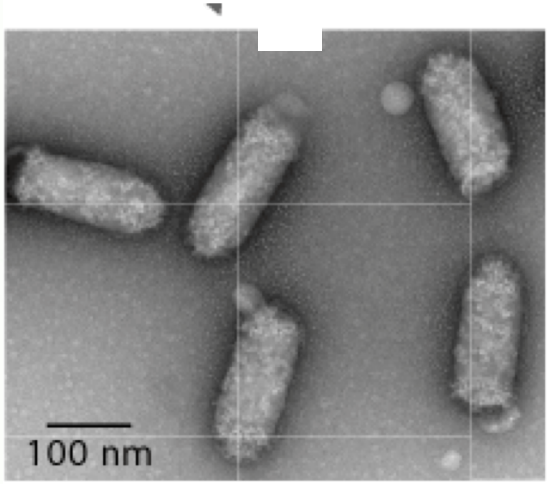
Generation of recombinant oncolytic VSV vector expressing IFN





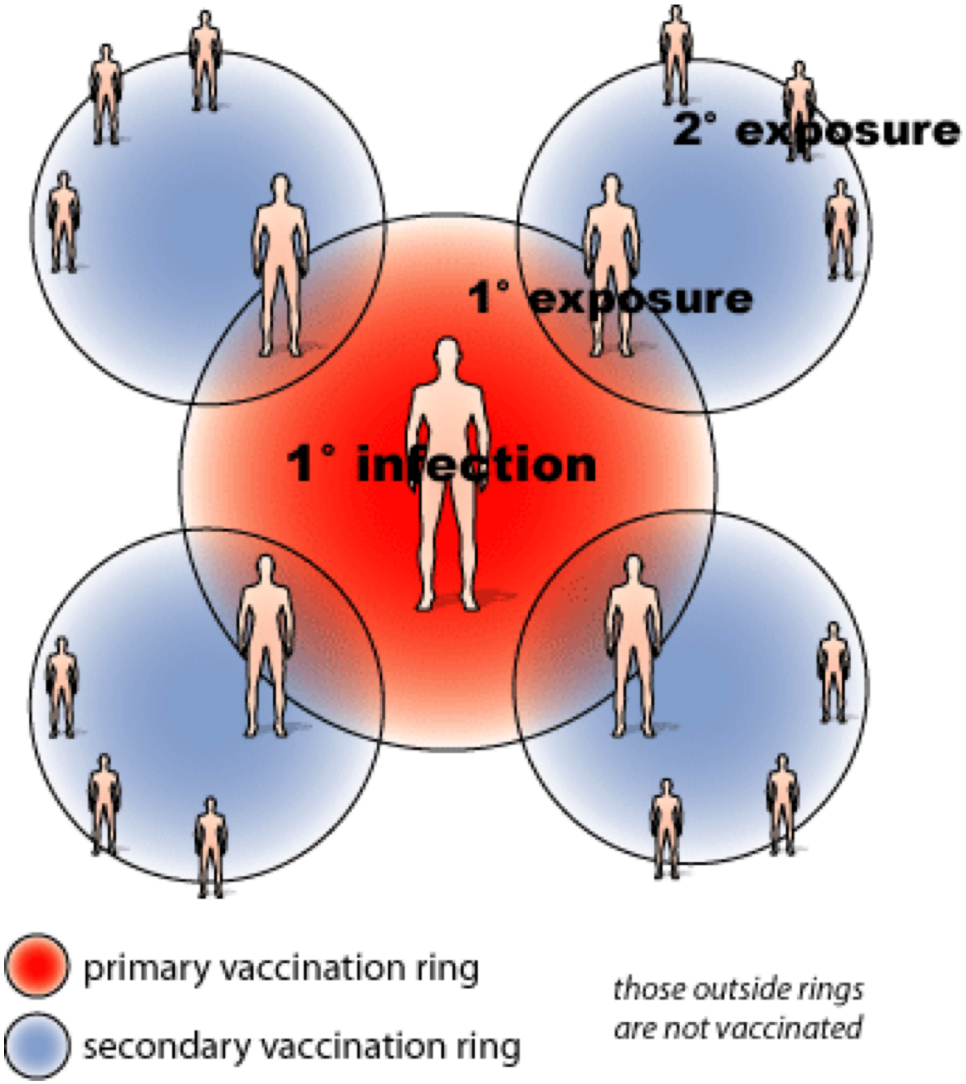
Generation of recombinant VSV vaccine against HCV



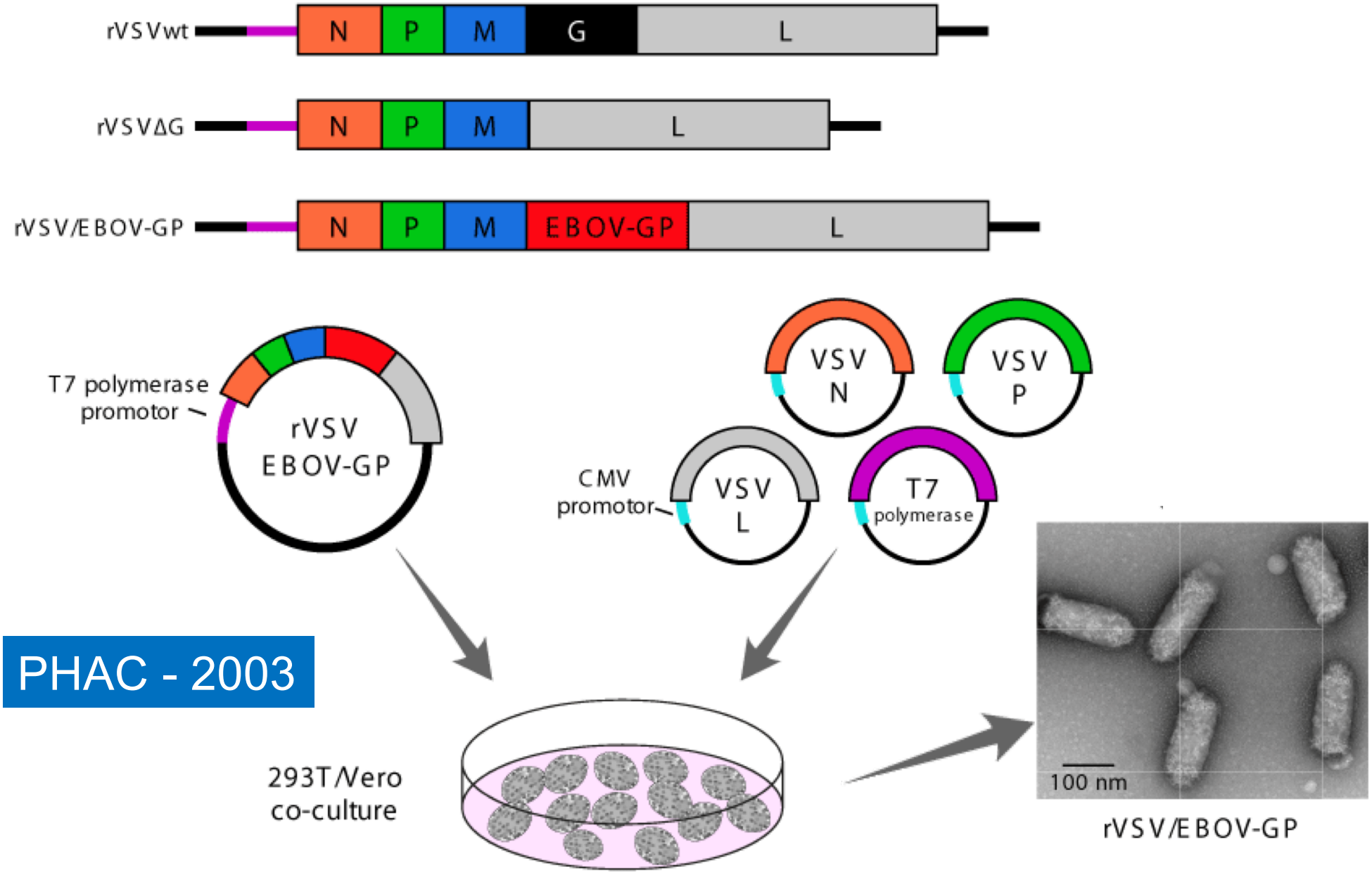


VSV-HCV-C/E1/E2


scientific
hazard
genetic
congo
microbiology
factors
outbreak
protection
toxin
harmful
science
contamination
progressing
caution
respiratory
prevention
biological
patients
deadly
terror
viral
bacteria
germs
traffic
vomiting
symptoms
hemorrhaging
warning
infected
people
health
ebola
virus
epidemic
disease
infections
medical
fever
death
africa
medicine
precaution
virulence
outbreaks
effects
pathogen
microbe
technology
illness
risk
mask
infectious
sickness
morphology
healthcare
hemorrhagic
fatal
biology
serum
headache
cells
danger
migraine
problem
alert
cure
threat
safety
mortal
human

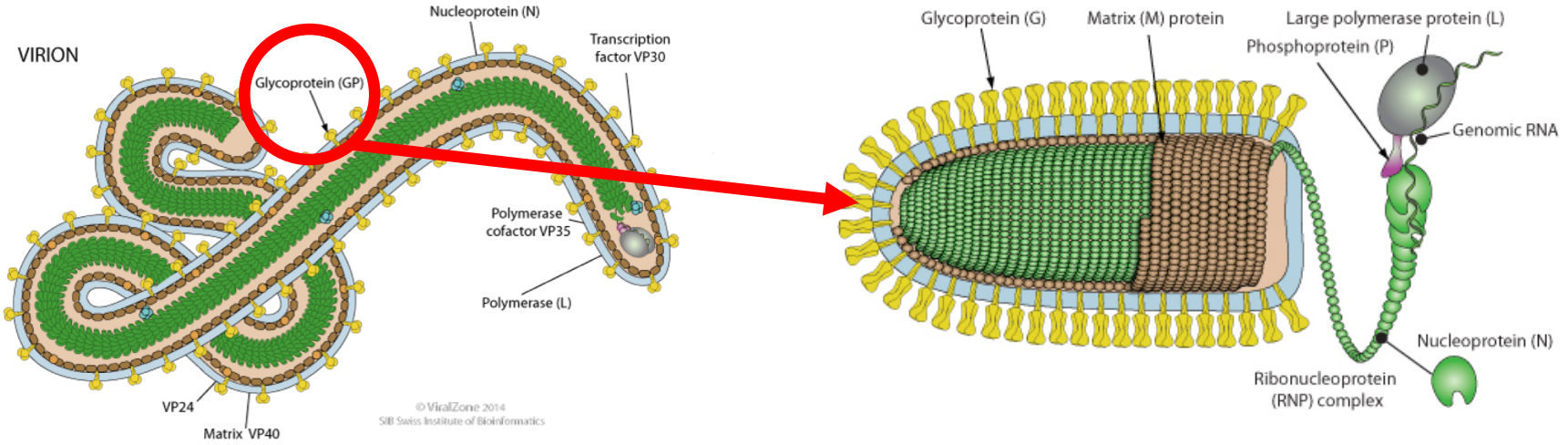

2° exposure
1° exposure
1° infection
primary vaccination ring
secondary vaccination ring
those outside rings
are not vaccinated

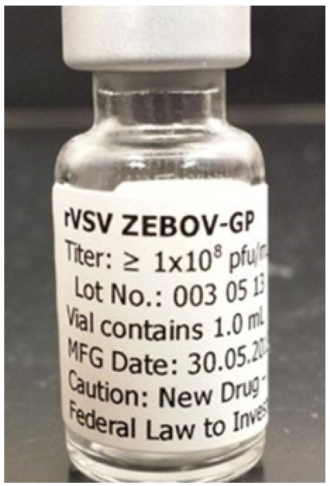
VSV as a vector: generation of rVSV/EBOV

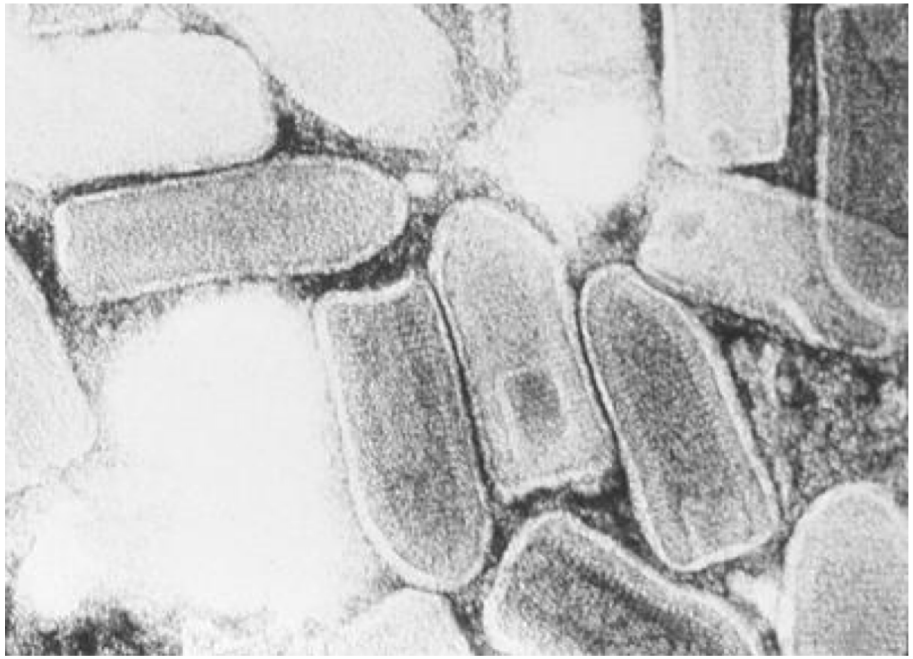


PHAC - 2003

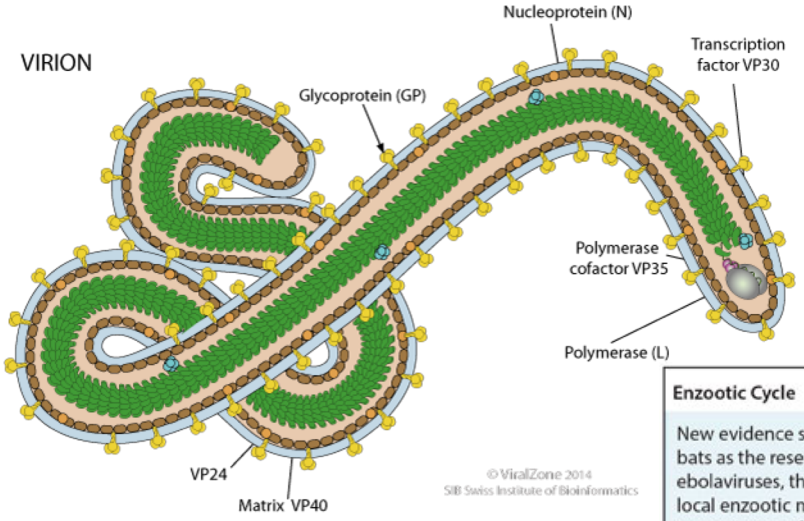
rVSV/EBOV: a virus that can be used to fight other virus infections

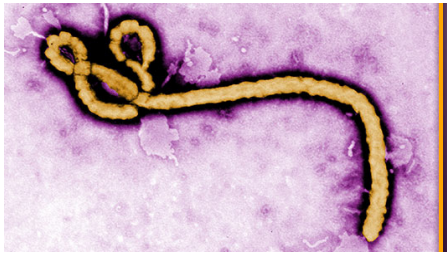






Ebola Outbreak in West Africa 2014





Ebola Update

CDC and Texas Health Department Confirm First Ebola Case Diagnosed in the U.S.

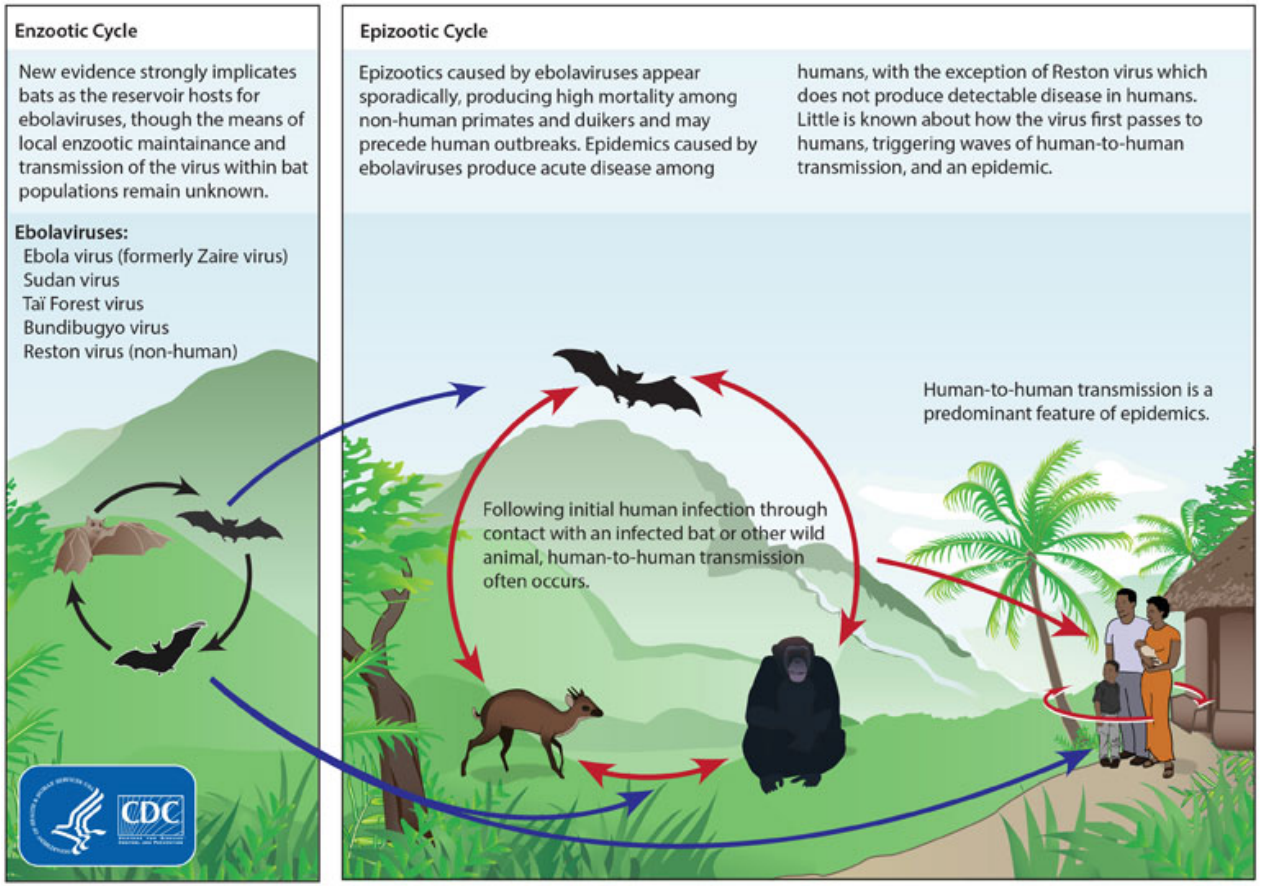
Enzootic Cycle

New evidence strongly implicates bats as the reservoir hosts for ebolaviruses, though the means of local enzootic maintainance and transmission of the virus within bat populations remain unknown.

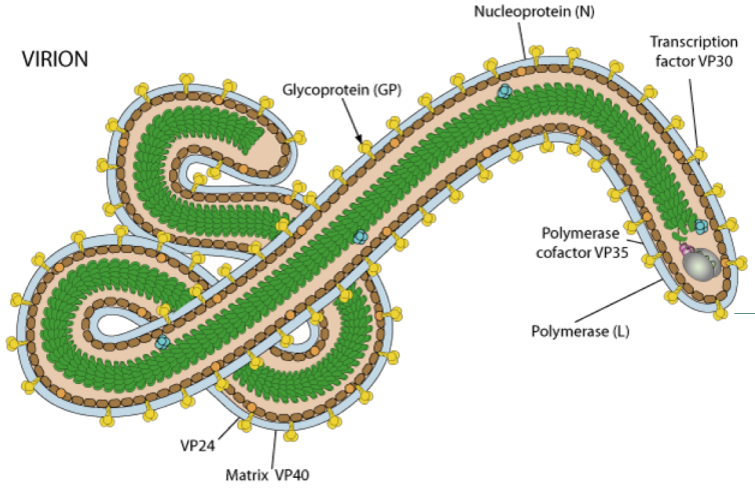
Ebolaviruses:
Ebola virus (formerly Zaire virus)
Sudan virus
Taï Forest virus
Bundibugyo virus
Reston virus (non-human)

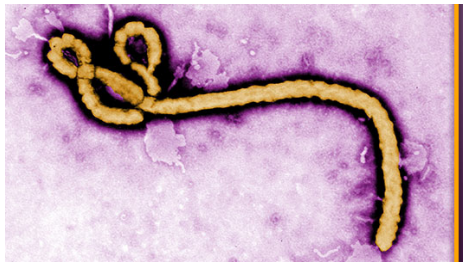
Epizootic Cycle

Epizootics caused by ebolaviruses appear sporadically, producing high mortality among non-human primates and duikers and may precede human outbreaks. Epidemics caused by ebolaviruses produce acute disease among humans, with the exception of Reston virus which does not produce detectable disease in humans. Little is known about how the virus first passes to humans, triggering waves of human-to-human transmission, and an epidemic.



Ebola in West Africa nel 2014-2016





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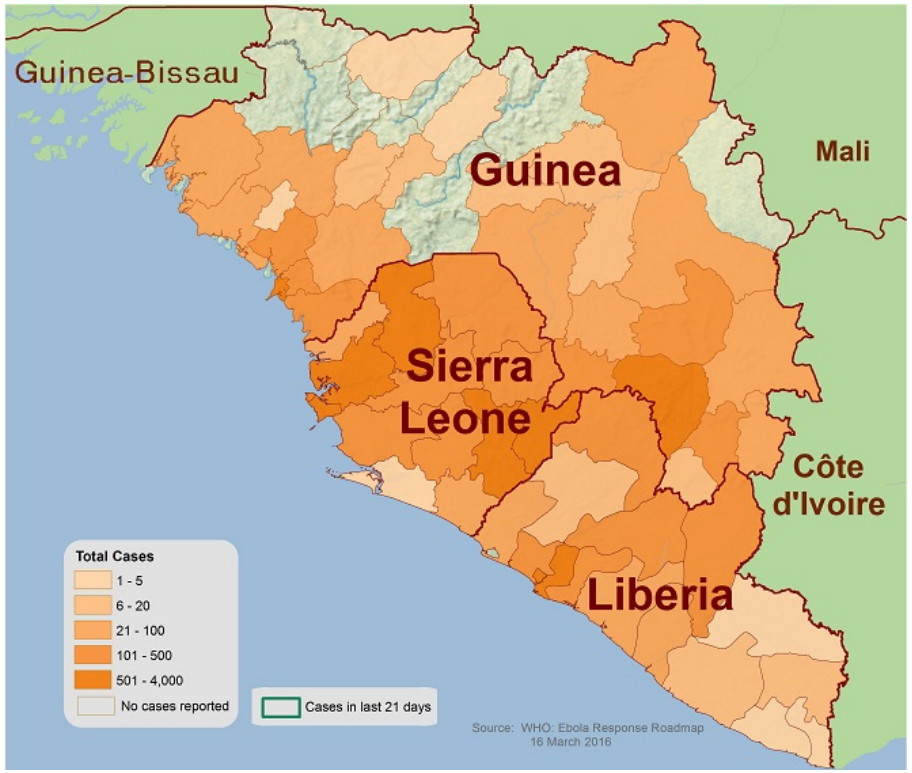


Ebola by the Numbers*

27,000+
Total number of cases

11,000+
Total number of deaths

24,665
Health workers trained by CDC in West Africa

2,471
CDC deployments

4
Patients diagnosed with Ebola in the U.S.

11
Patients with Ebola treated in the U.S.

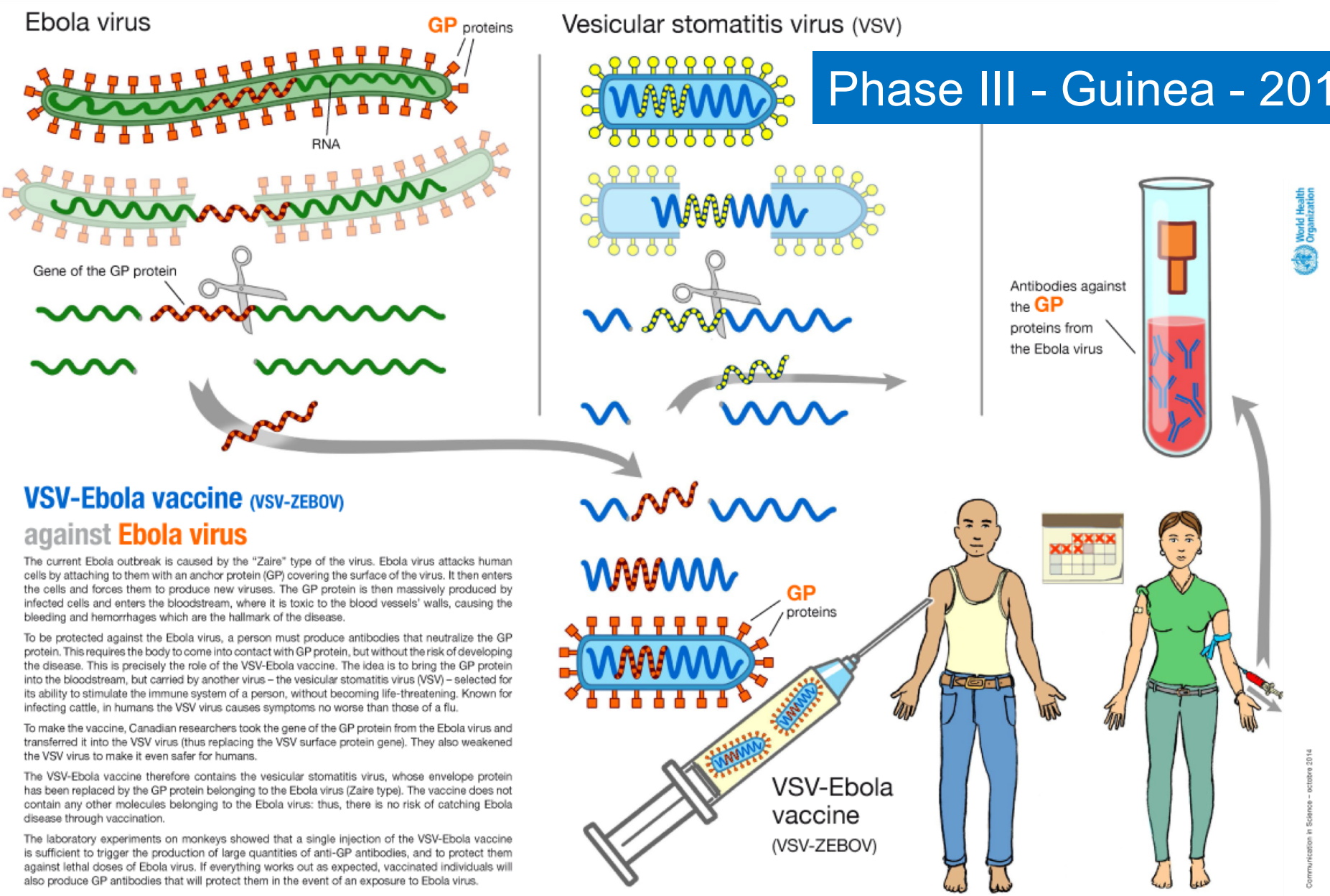
600+
U.S. healthcare workers trained in Anniston

150,000
U.S. healthcare workers trained by webinars and calls

59,665,191
Number of views for CDC's Ebola website

CDC

rVSV/EBOV: a VSV-based vaccine against Ebola

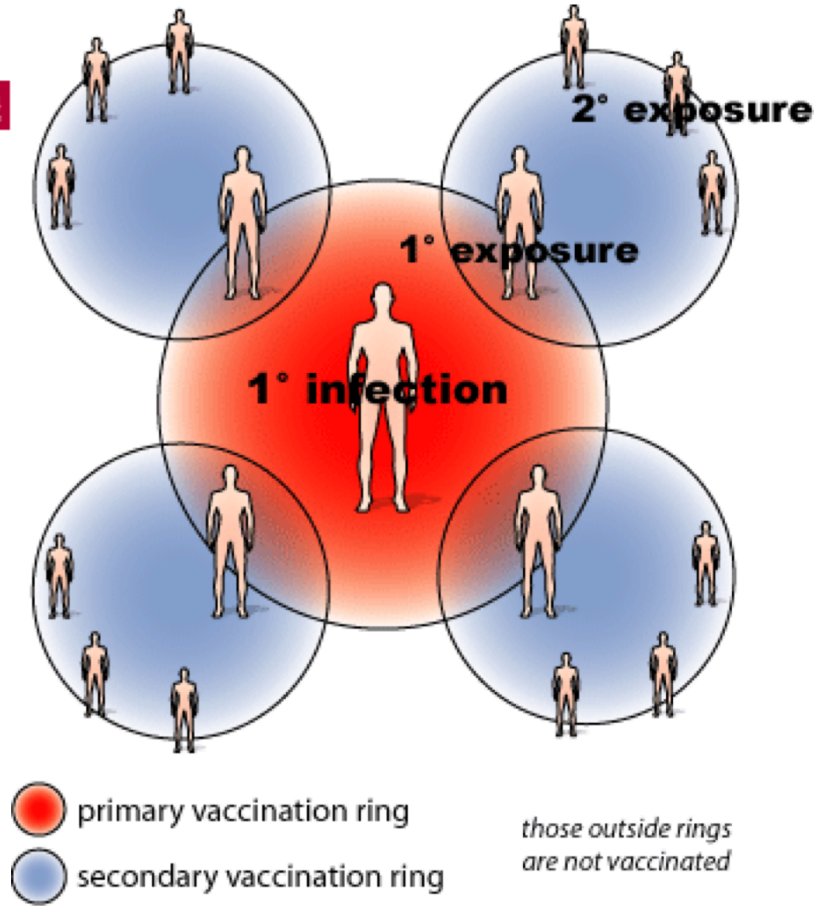


rVSV/EBOV: a VSV-based vaccine against Ebola

Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!)

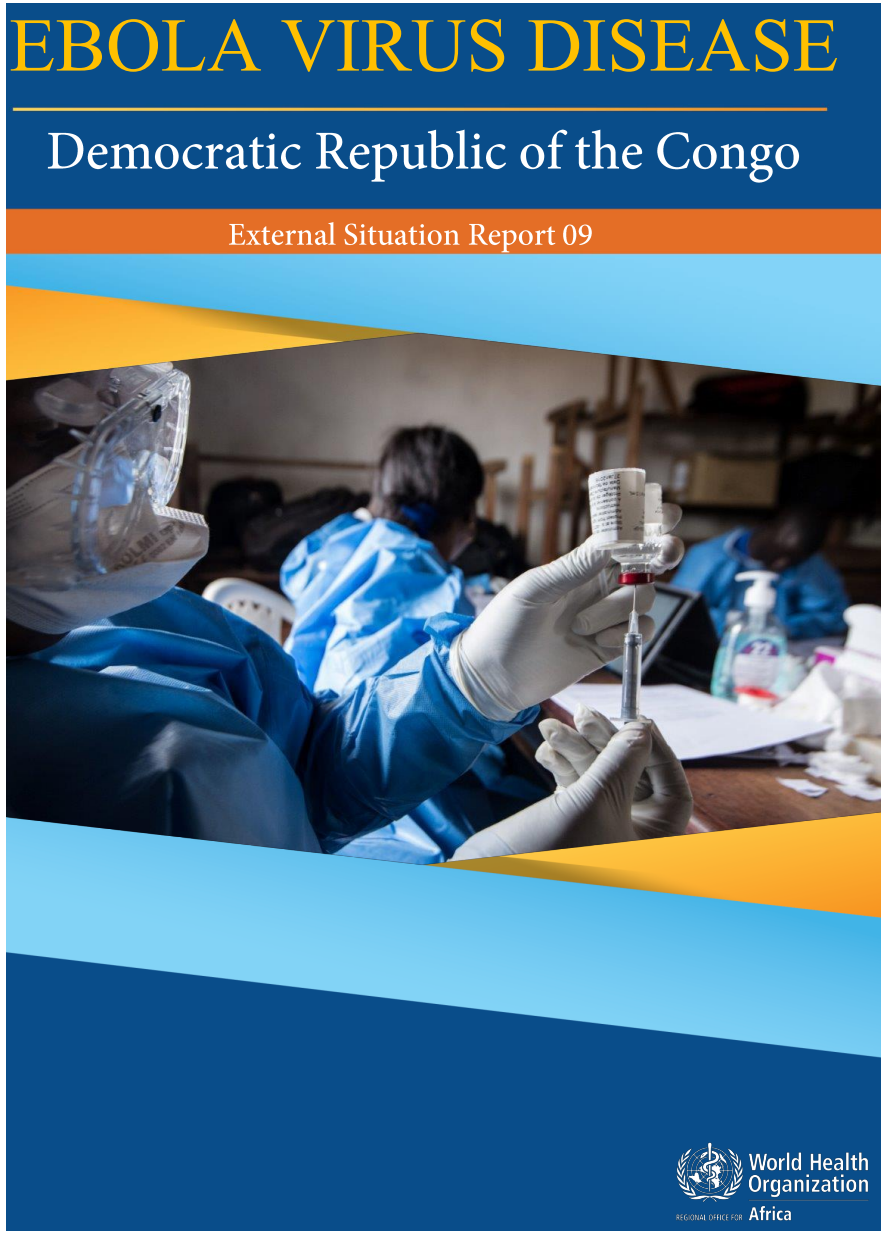
Ana Maria Henao-Restrepo, Anton Camacho, Ira M Longini, Conall H Watson, W John Edmunds, Matthias Egger, Miles W Carroll, Natalie E Dean, Ibrahima Diatta, Moussa Doumbia, Bertrand Draguez, Sophie Duraffour, Godwin Enwere, Rebecca Grais, Stephan Gunther, Pierre-Stéphane Gsell, Stefanie Hossmann, Sara Viksmoen Watle, Mandy Kader Kondé, Sakoba Kéïta, Souleymane Kone, Eewa Kuisma, Myron M Levine, Sema Mandal, Thomas Mauget, Gunnstein Norheim, Ximena Riveros, Aboubacar Soumah, Sven Trelle, Andrea S Vicari, John-Arne Røttingen*, Marie-Paule Kieny*

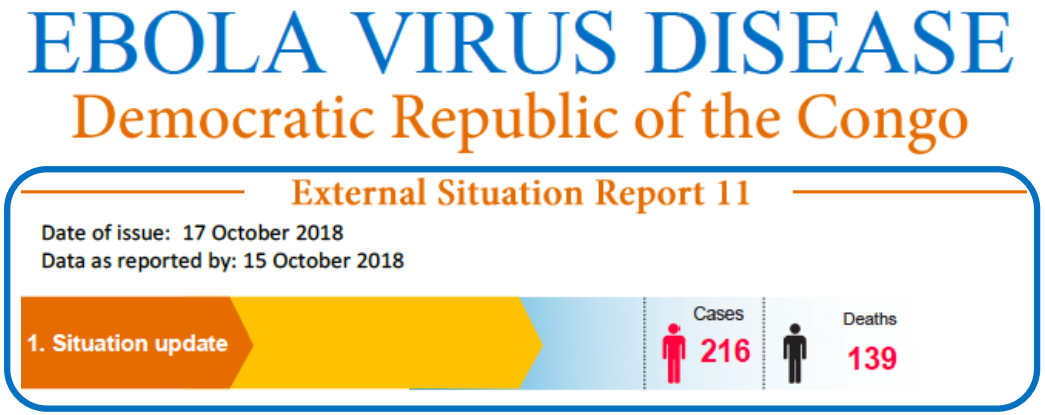
Lancet 2017; 389:505-518



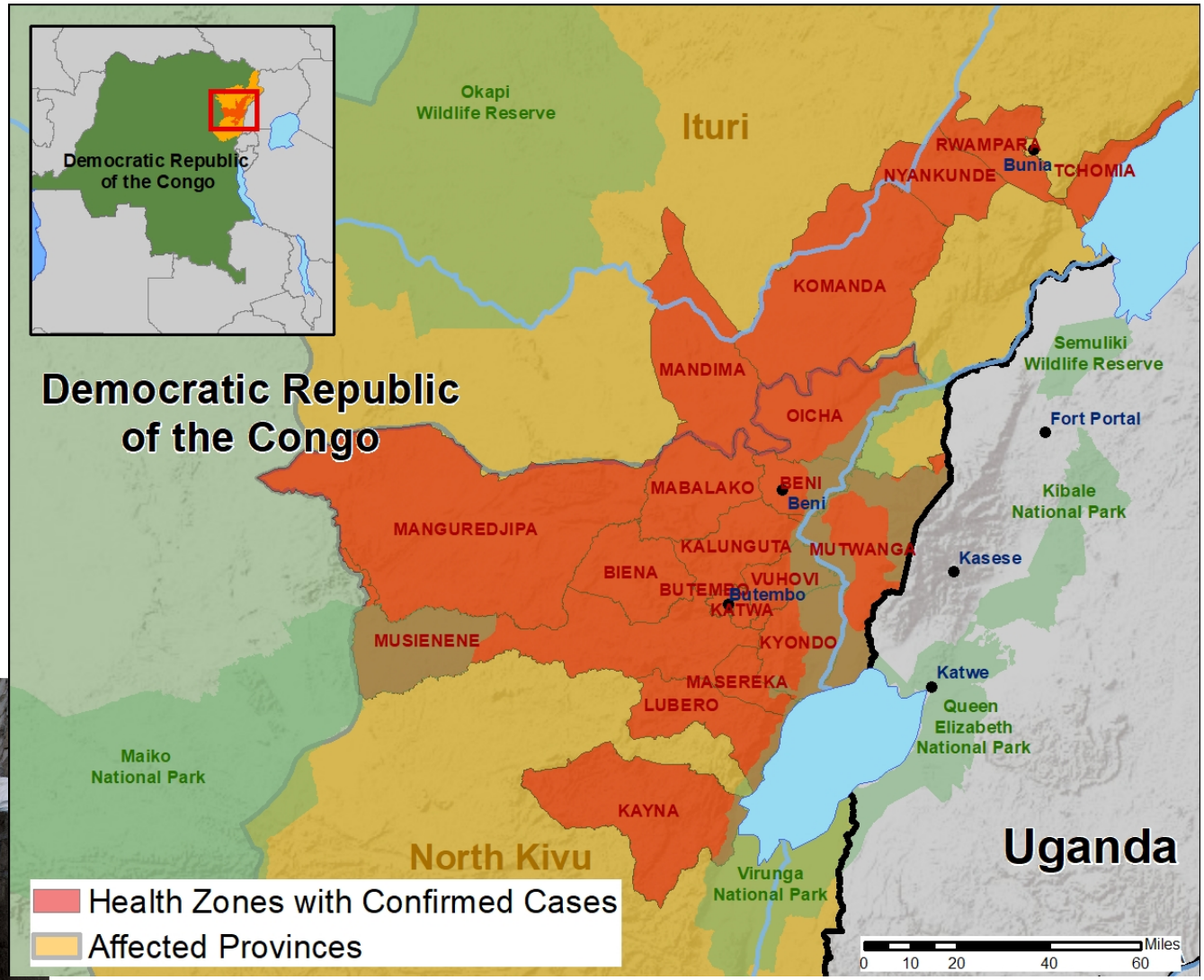
Phase 3 Efficacy Tria l- Guinea – 2015-2016

1° Ebola Outbreak in DRC 2018 (May-June)

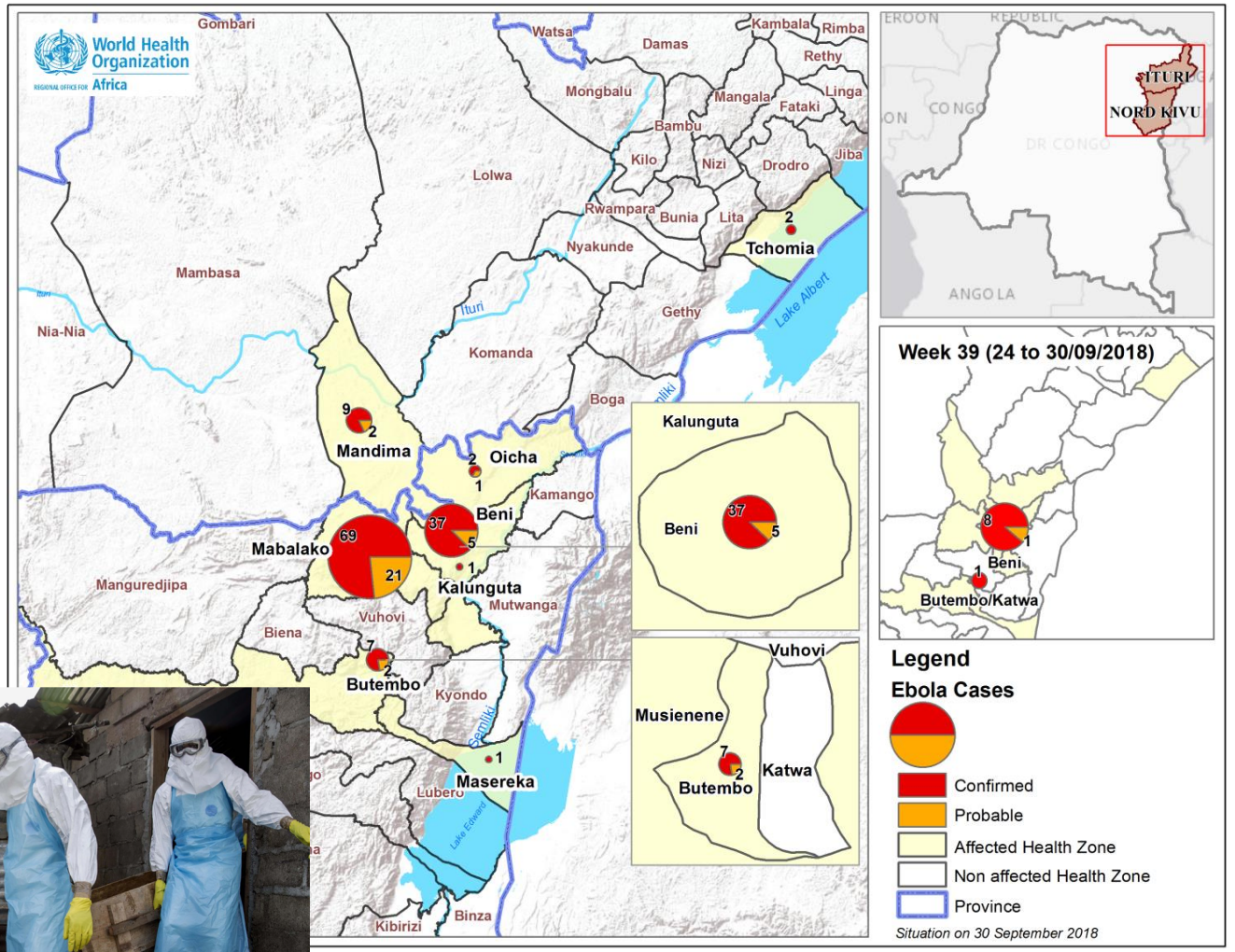




Ebola in DRC (since August 2018)



2° Ebola Outbreak in DRC 2018 (from August)

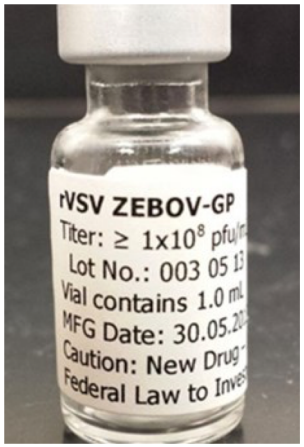


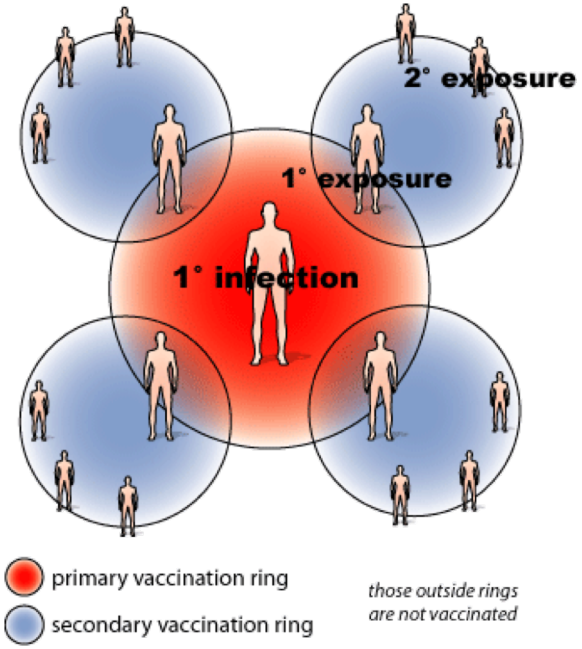
Ebola in DRC April 2019

EBOLA VIRUS DISEASE

Democratic Republic of the Congo

External Situation Report 37





External Situation Report 37

Date of issue: 16 April 2019
Data as reported by: 14 April 2019

1. Situation update

Cases	Deaths
1264	814

World Health Organization

- As of 16 April 2019, 101 195 contacts and contacts of contacts have been vaccinated. Of those 26 613 were contacts and 74 367 contacts of contacts. The vaccinated people at risk included 29 688 HCWs/FLWs, and 26 361 children 1-6 years old. Detailed micro-plans are also in use to monitor the progress and number of cases with and without rings. Table 2 provides an overview of the status as of 15 April 2019.

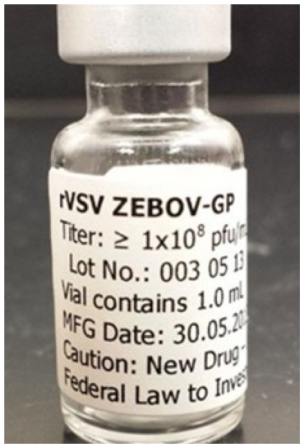
- On 12 April 2019, INRB and WHO published a preliminary analysis of the efficacy of RVSV-ZEBOV-GP emerging from the DRC outbreak data (Please see here for preliminary analysis). The data suggest high efficacy of this candidate vaccine and of the ring vaccination in this outbreak.

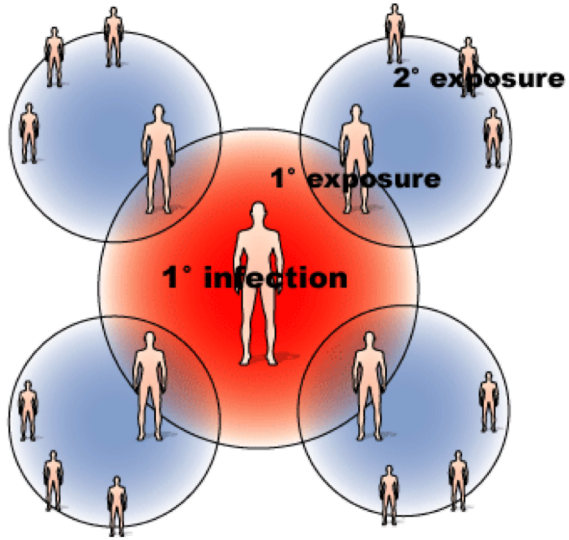
Ebola in DRC July 2019

EBOLA VIRUS DISEASE

Democratic Republic of the Congo

External Situation Report 49





External Situation Report 49

Date of issue: 9 July 2019
Data as reported by: 7 July 2019

1. Situation update

Cases	Deaths
2418	1630

World Health Organization

- As of 6 July 2019, 154 037 people at risk have consented to and received the rVSV-ZEBOV-GP Ebola vaccine. Of those, 37 373 are contacts and 67 756 contacts-of-contacts. The total number of vaccines includes 31 016 HCWs/FLWs and 34 522 children 1-17 years of age.

- On 12 April 2019, INRB and WHO published a preliminary analysis of the efficacy of RVSV-ZEBOV-GP emerging from the DRC outbreak data (Please see here for preliminary analysis). The data suggest high efficacy of this candidate vaccine and of the ring vaccination in this outbreak.

Ebola in DRC November 2019

EBOLA VIRUS DISEASE

Democratic Republic of the Congo

External Situation Report 69

World Health Organization
REGIONAL OFFICE FOR Africa

Date of issue: 26 November 2019
Data as reported by: 24 November 2019

1. Situation update

Cases	Deaths
3303	2199

World Health Organization

Vaccines

- From 8 August 2018 to 23 November 2019, 255 136 persons were vaccinated.
- 3191 persons were vaccinated in the week of 11 to 17 November 2019, compared to 3530 during the week of 4 to 10 November 2019.

Virology AY 2019-20: take-home message…

- Viruses are not solely pathogenic nuisances; they can be beneficial;…
- …they contribute to global ecological homeostasis, keep our immune system active and alerted, and….
- …nevertheless, they can be used to design and develop virus-based therapies….
- …to treat genetic diseases and cancer, and to deliver vaccines to prevent other infectious diseases.
- ***Not all viruses come to harm!!!***

…and for those who do not have enough…

…some interesting websites:

- ***http://viralzone.expasy.org/***
- ***http://www.viprbrc.org/brc/home.do?decorator=vipr***
- ***http://www.virology.net/garryfavwebindex.html***
- ***http://jvi.asm.org/***
- ***https:://asm.org/***

May I remind you that…

Virology A.Y. 19-20 Course Grade Determination

Virology Final Exam

- The final exam will be a Moodle-based test of **11 questions** with different formats (multiple choice, true/false, filling in checklists) and **6 open questions** for a maximum grade of 32/30.

- Grading 31 and 32 will give rise to " 30 cum laude".

- Any additional points obtained by MMT and MRE will be added to the final exam grade of the first exam session (**January 27, 2020 - February 26, 2020; 2.00 pm).**

Virology A.Y. 19-20 Course Grade Determination

To sum up…

- Final exam: **January 27** and **February 26, 2020** (**Morpho Lab. - 2.00 pm**)
- Presentations available on the Moodle platform
- Textbook available at the DBIOS library
- Office hours: **on email appointment**

Dont' forget what you have learned here!!!

VSV as an oncolytic virus

The natural preference of VSV for replication in transformed cells