From pathogenic viruses to effective vaccines: The power of reverse genetics

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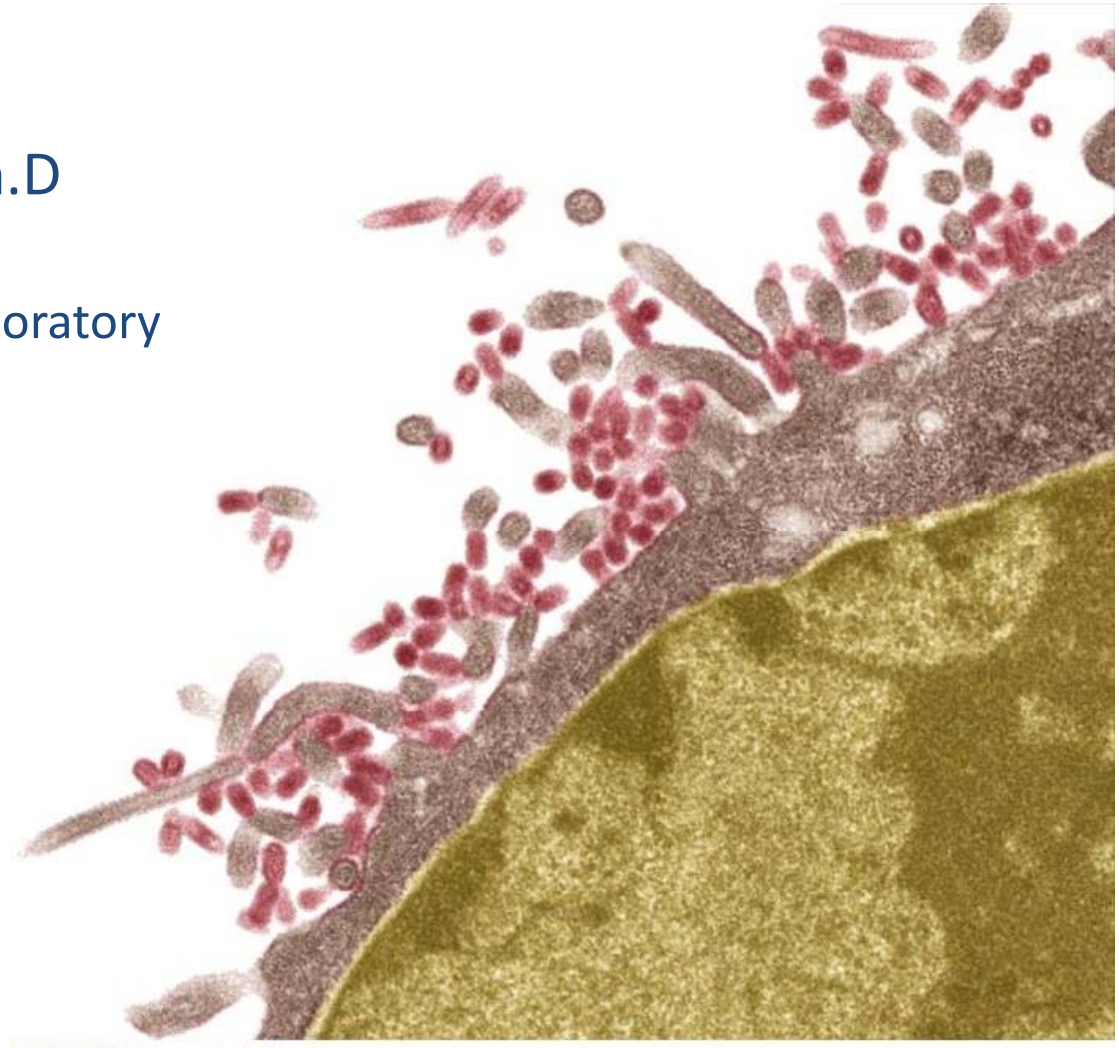
Virology and Cell Technology Laboratory
BIOTEC, NSTDA

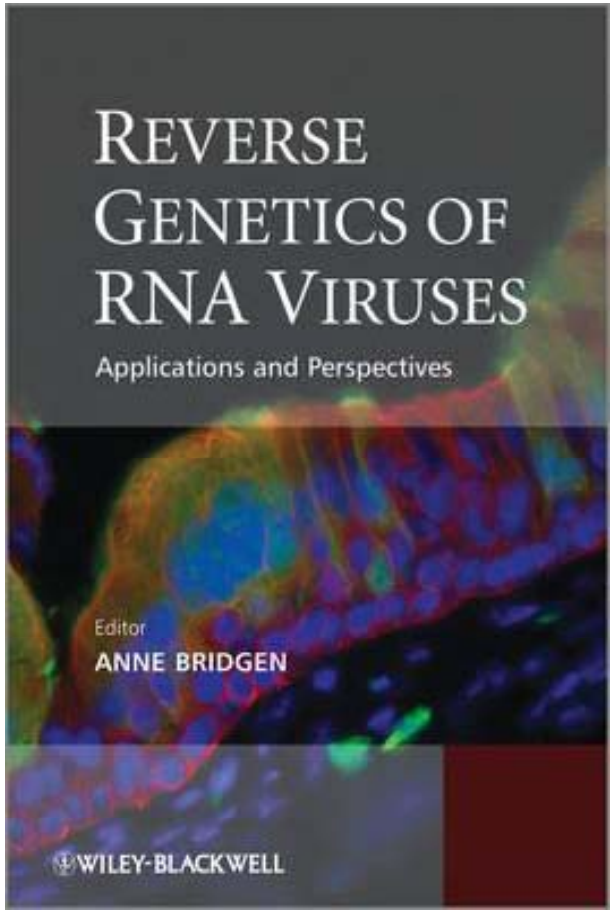


30th Anniversary BIOTEC a member of NSTDA

From Spanish Flu to MERS and H7N9

ZOONOSES

Understanding the Animal Agriculture and Human Health Connection



What is reverse genetics in a virologist's point of view?

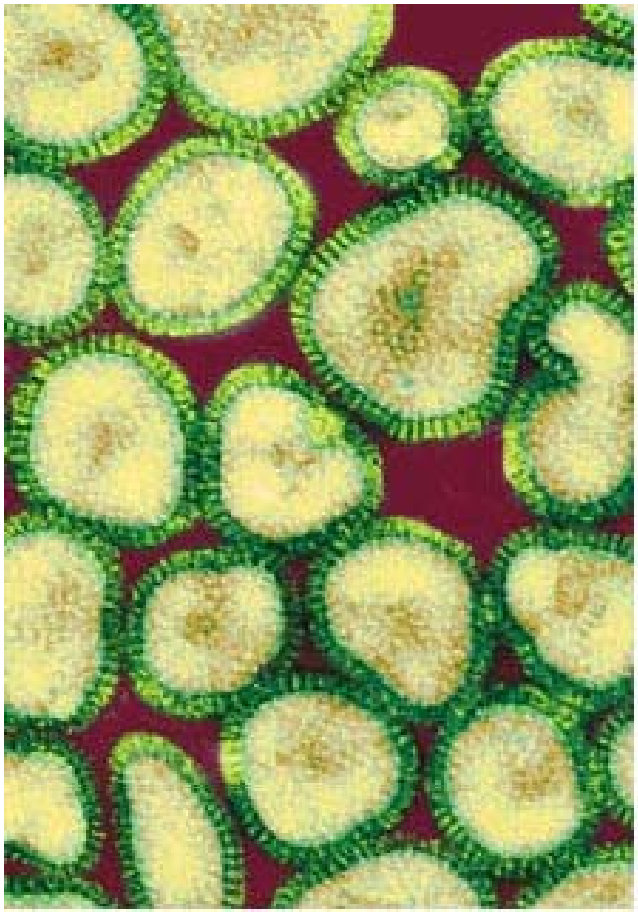
"Reverse genetics is the genetic manipulation of RNA viruses to create a wild-type or modified virus…"

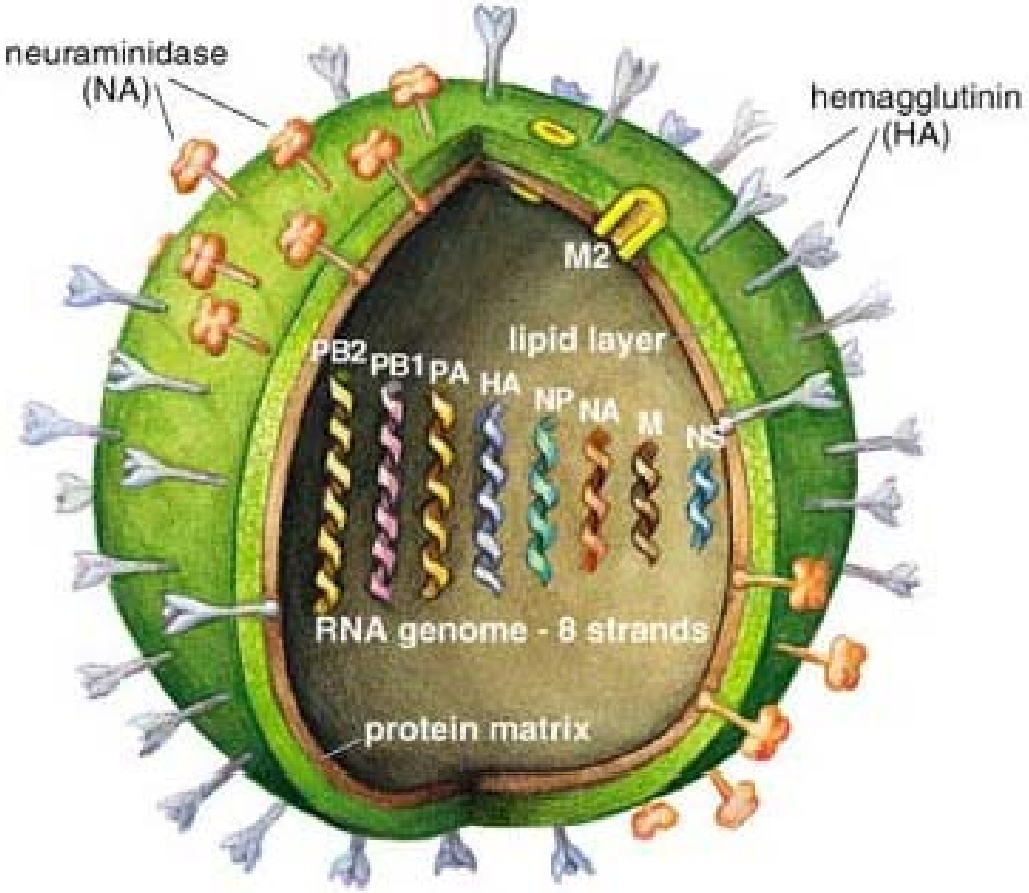
If we are to create viruses by reverse genetics, what information do we need?

1. **Genome of the virus**
2. **Replication cycle**
3. **Permissive cell lines**

Example I

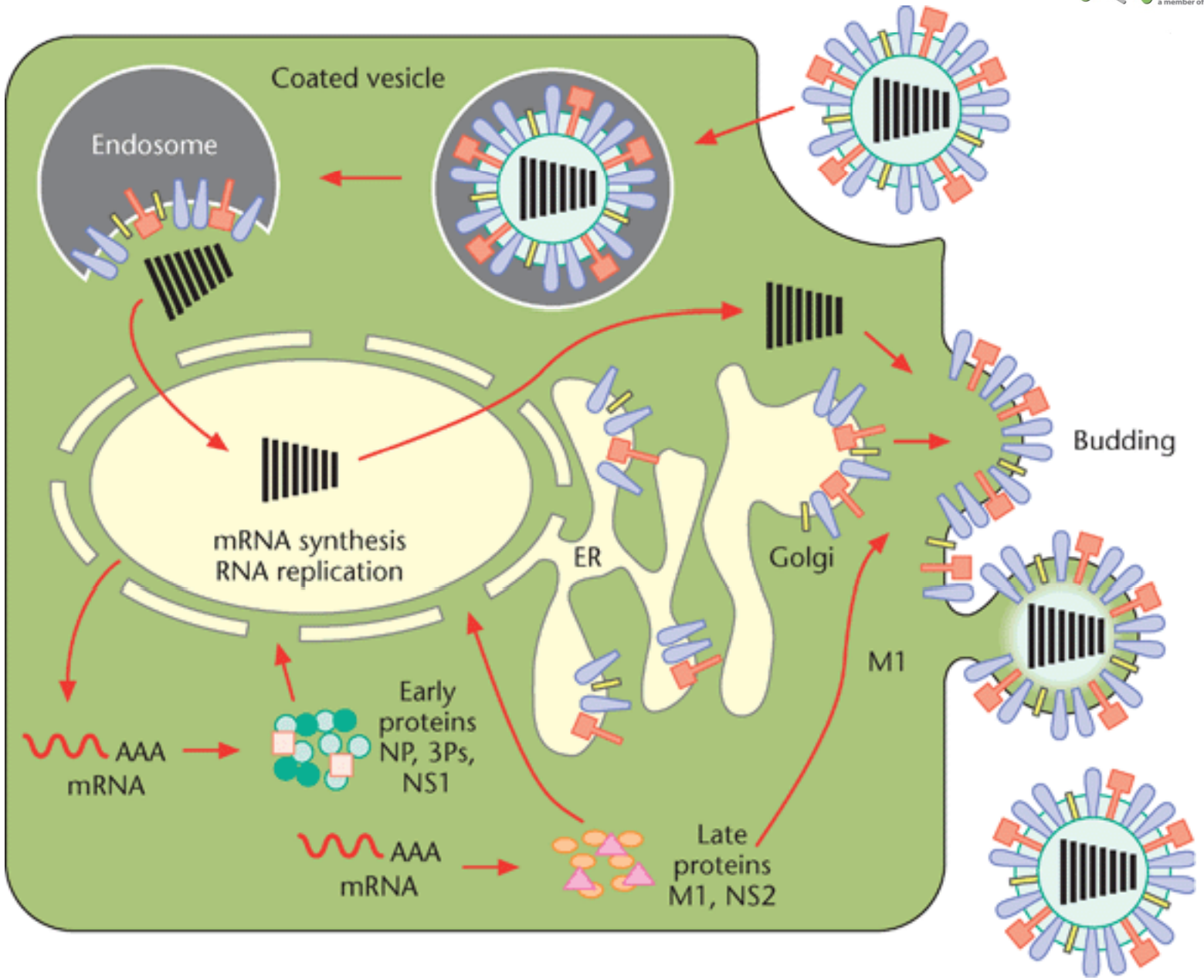
Influenza virus particle

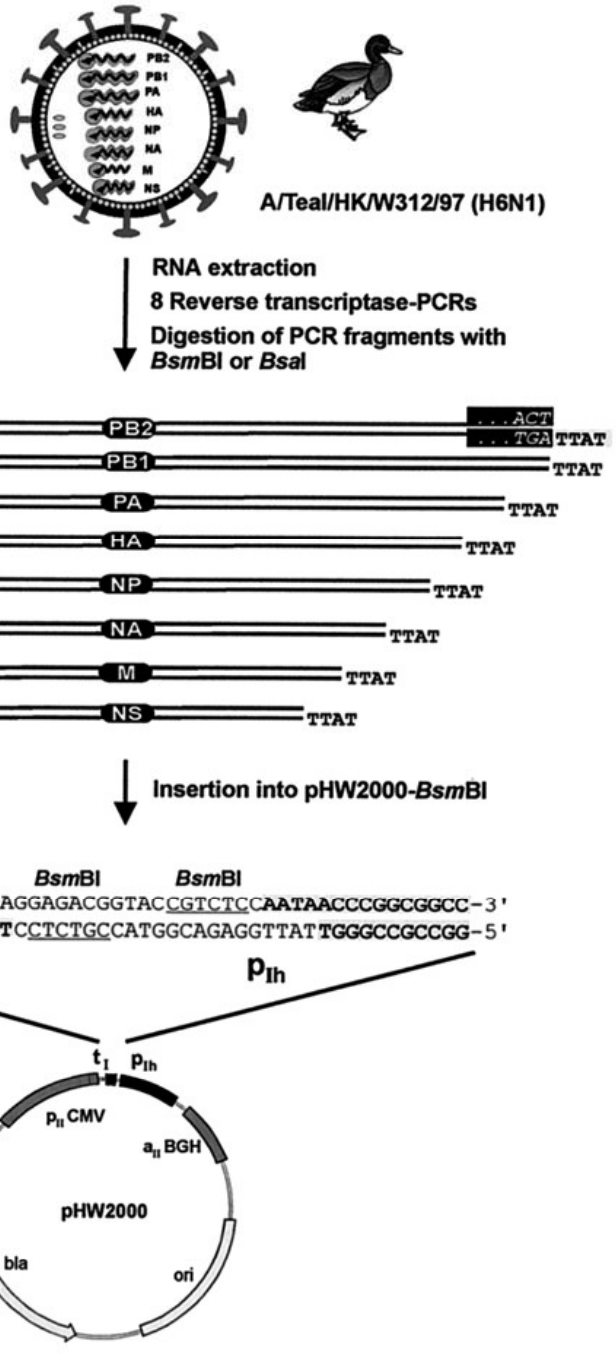


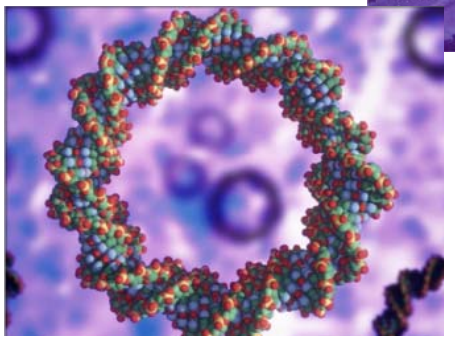


False-color micrograph courtesy of Gopal Murti, St. Jude Children's Research Hospital. Illustration by Emma Skurnick.

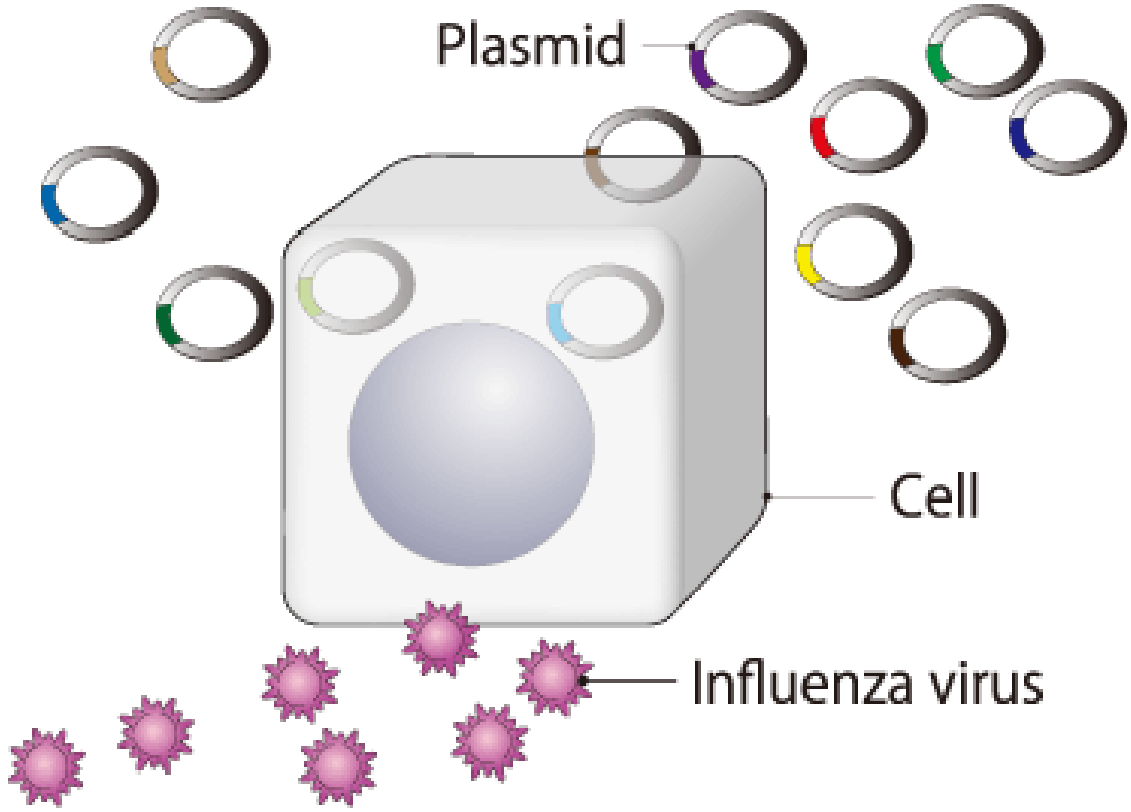
How influenza viruses replicate




PB2
PB1
PA
HA
NP
NA
M
NS
A/Teal/HK/W312/97 (H6N1)
RNA extraction
8 Reverse transcriptase-PCRs
Digestion of PCR fragments with BsmBI or BsaI
dsDNA
GGGAGC...
CG...
...ACT
...TGA
TTAT
GGGA
PB2
PB1
PA
HA
NP
NA
M
NS
Insertion into pHW2000-BsmBI
BsmBI
BsmBI
5'-TCCGAAGTTGGGGGGGAGGAGACGGTACCGTCTCCAATAACCCGGCGGCC-3'
3'-AGGCTTCAACCCCCCCTCCTCTGCCATGGCAGAGGTTATTGGGCCGCCGG-5'
tI
pIh
pII CMV
aII BGH
pHW2000
bla
ori

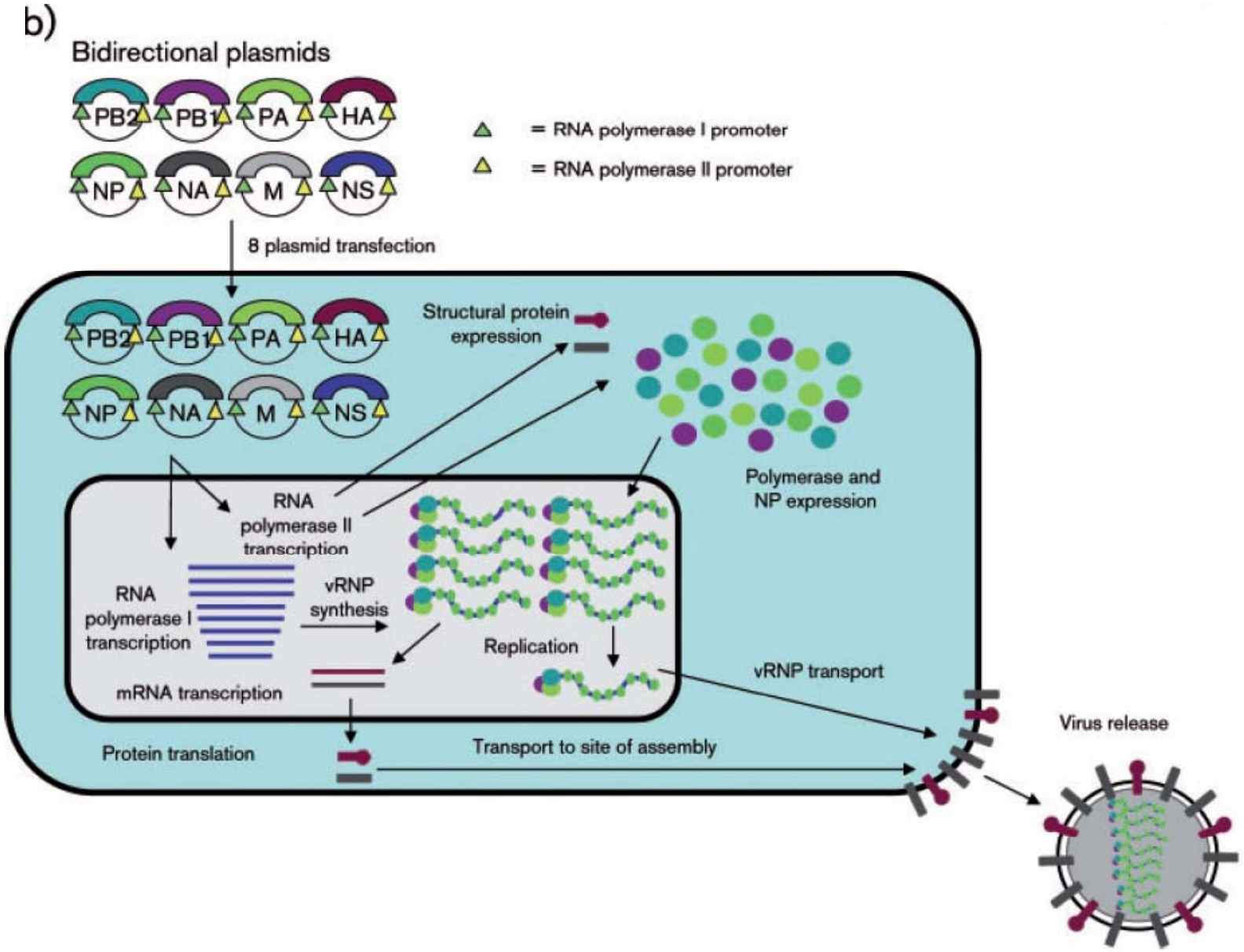


Reverse genetics of influenza virus (simplified)

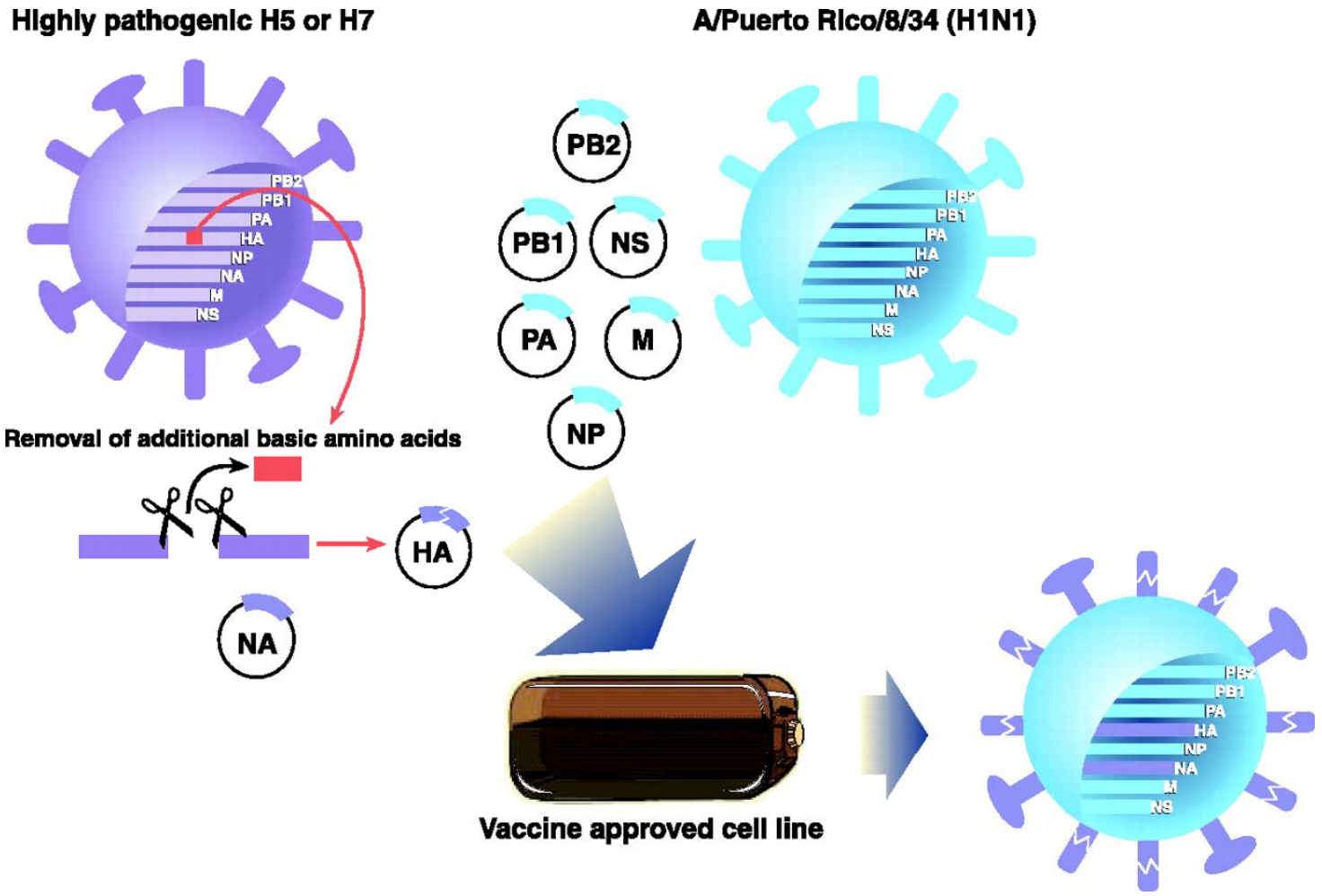


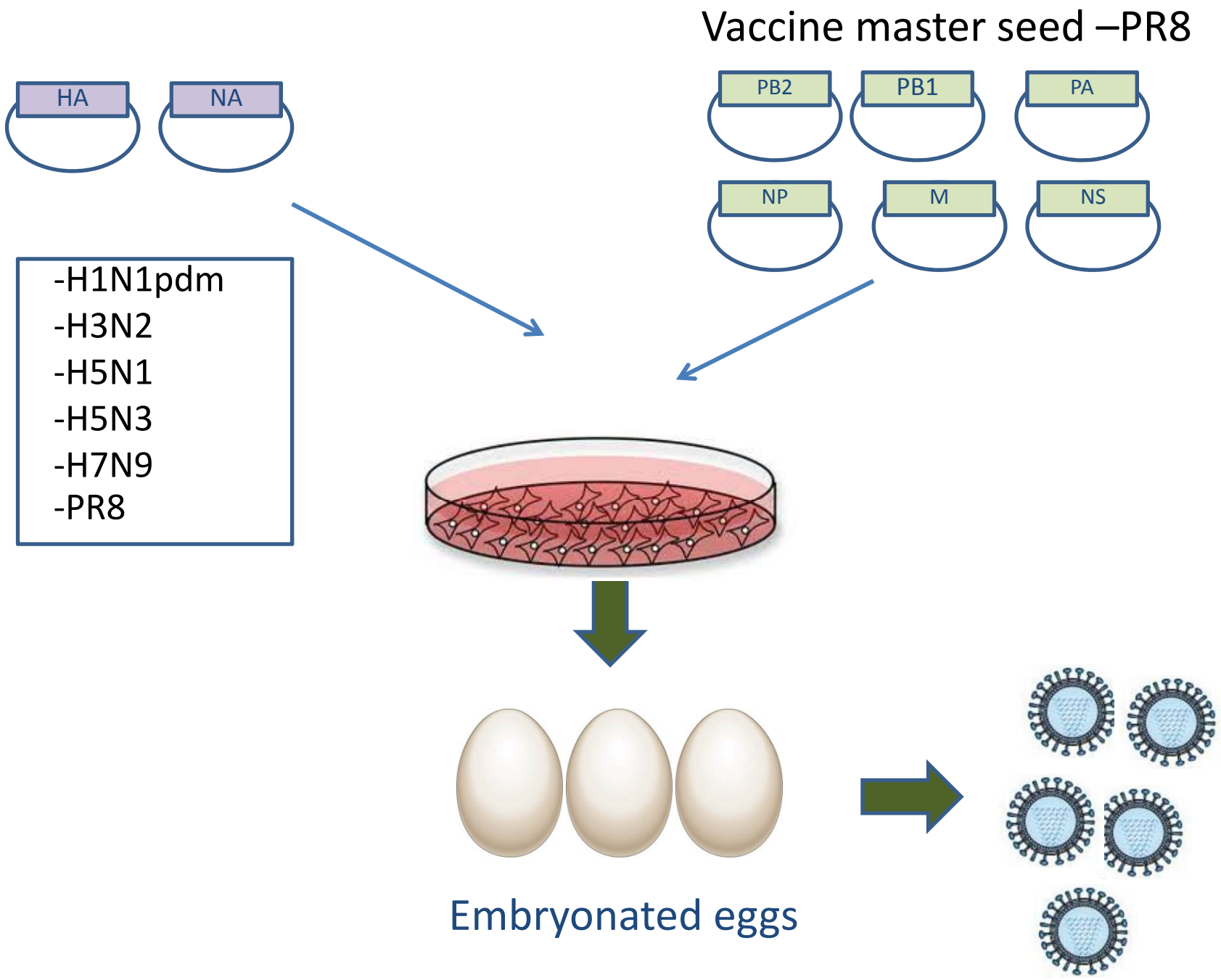
Artificial synthesis of influenza (conceptual diagram)

Reverse genetics of influenza virus (more complicated)

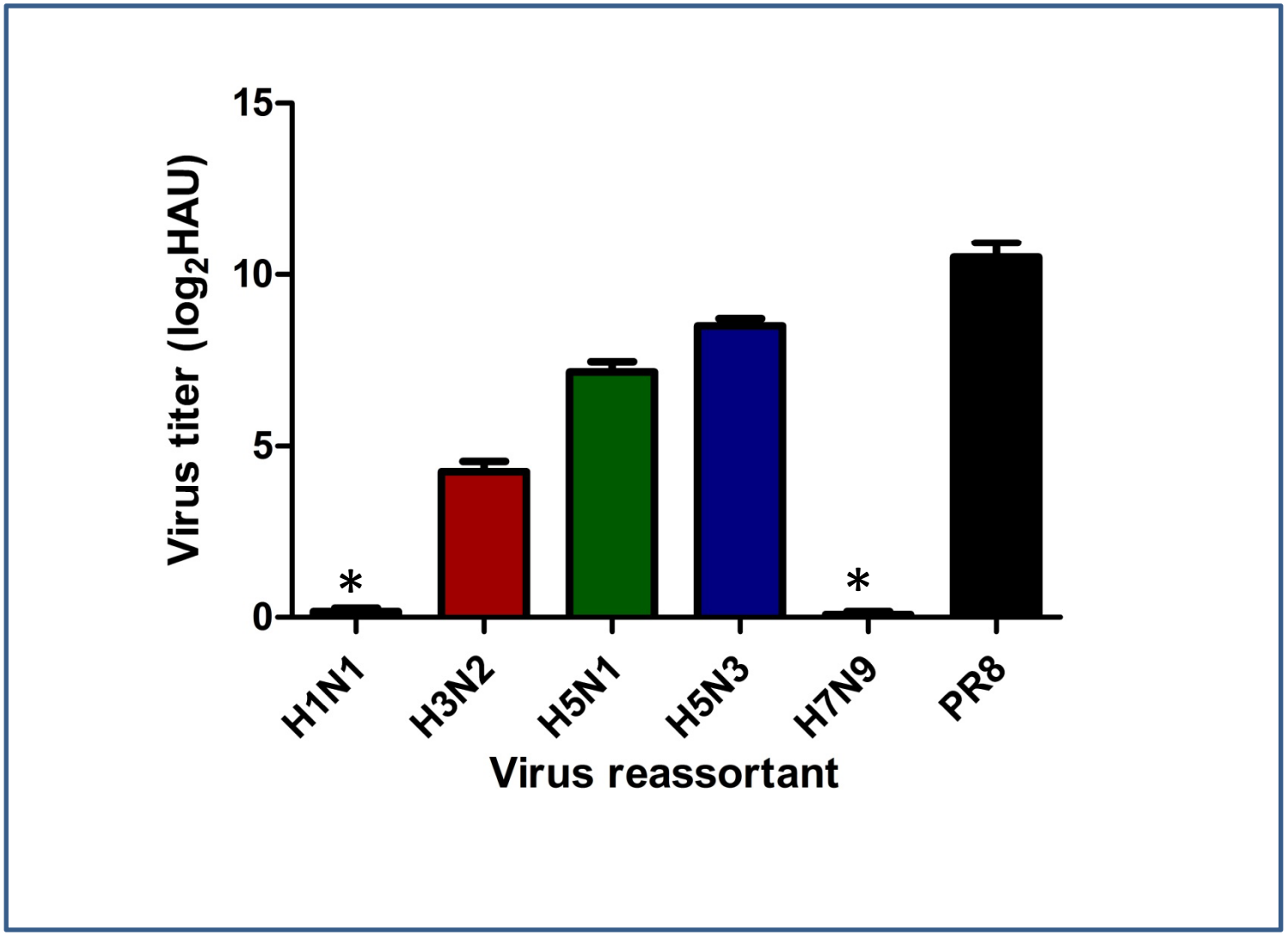


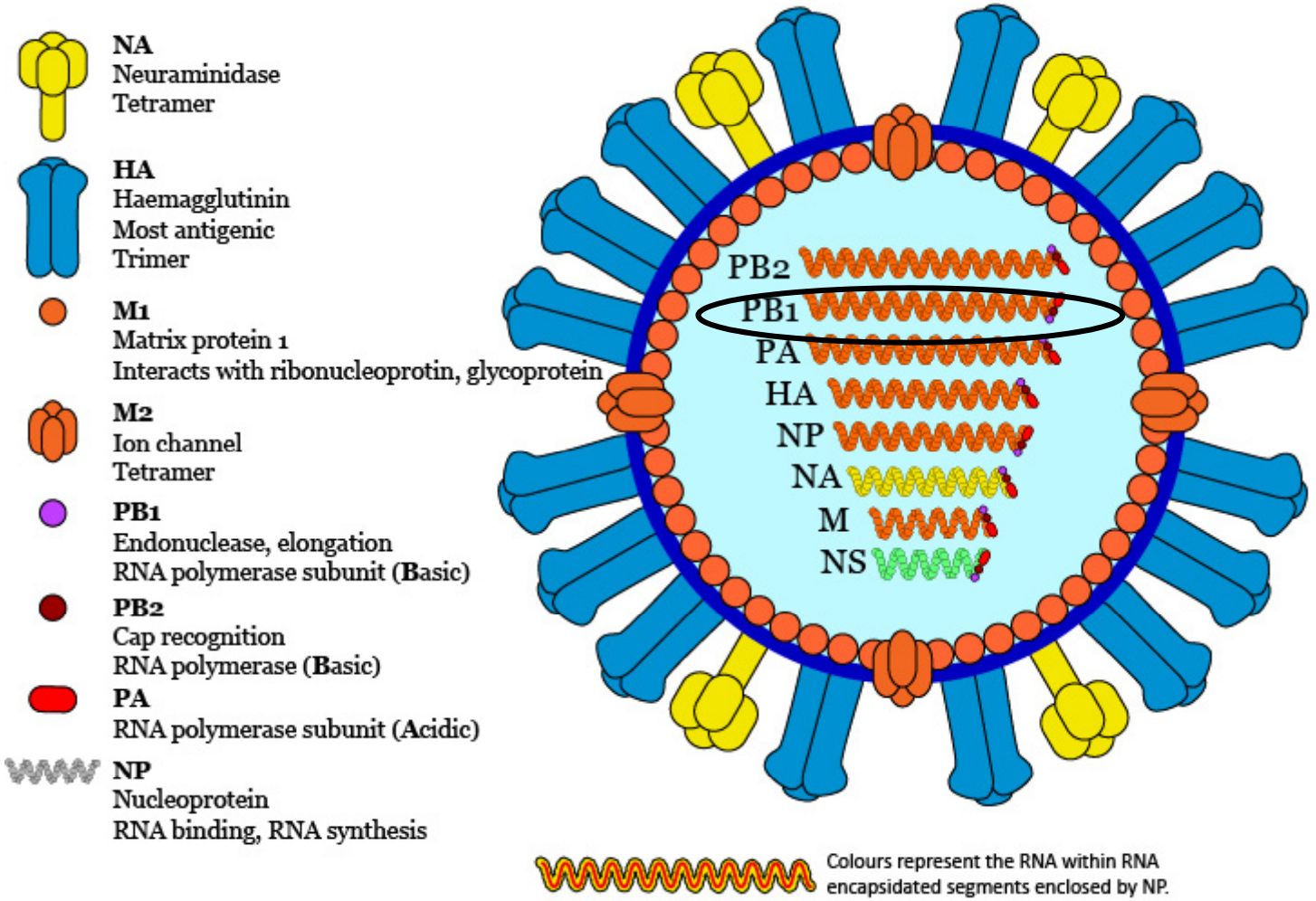
Standard influenza vaccine production by reverse genetics

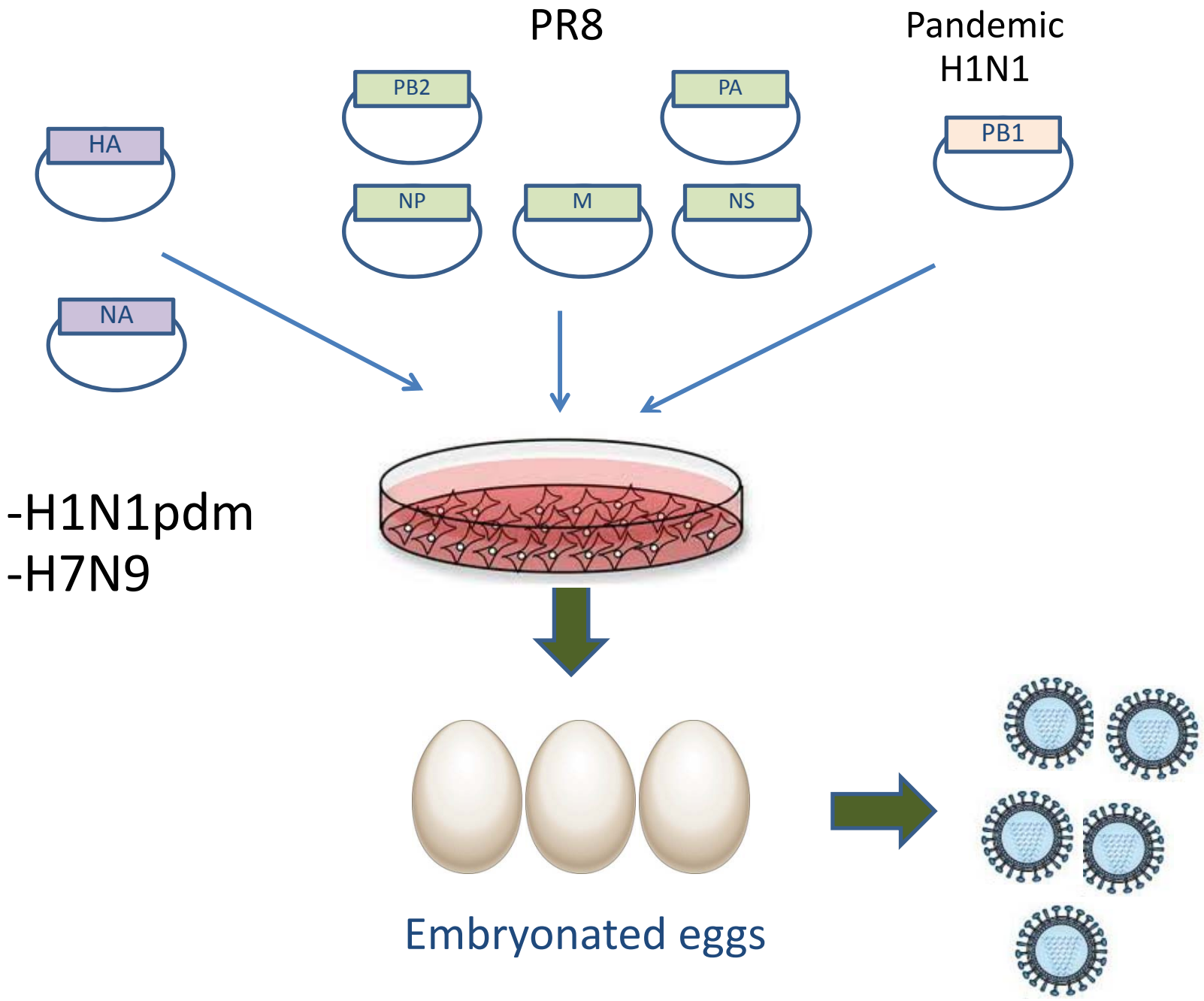



Vaccine master seed –PR8
HA
NA
PB2
PB1
PA
NP
M
NS
-H1N1pdm
-H3N2
-H5N1
-H5N3
-H7N9
-PR8
Embryonated eggs

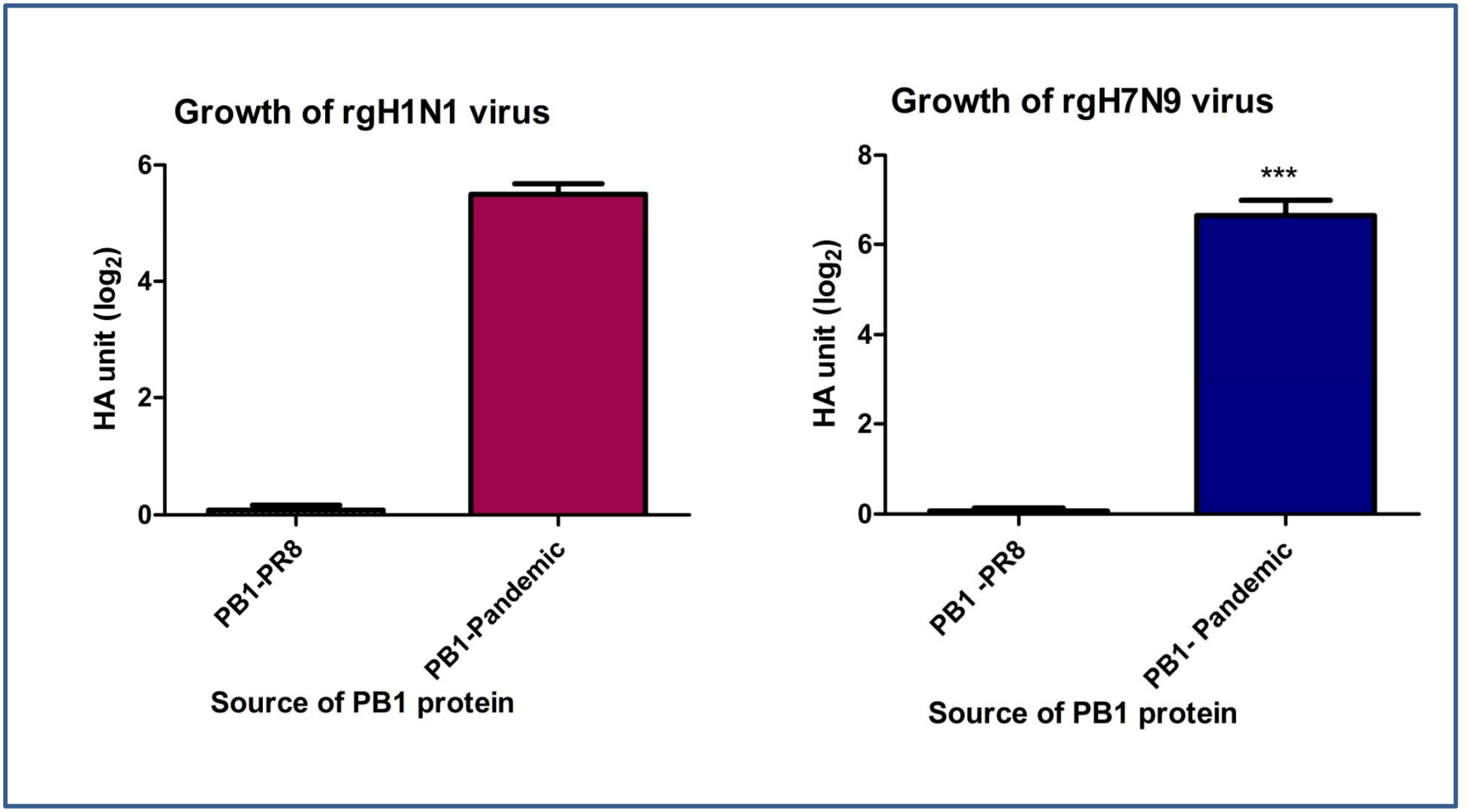
Growth property of various 6PR8+2(HA-NA) viruses

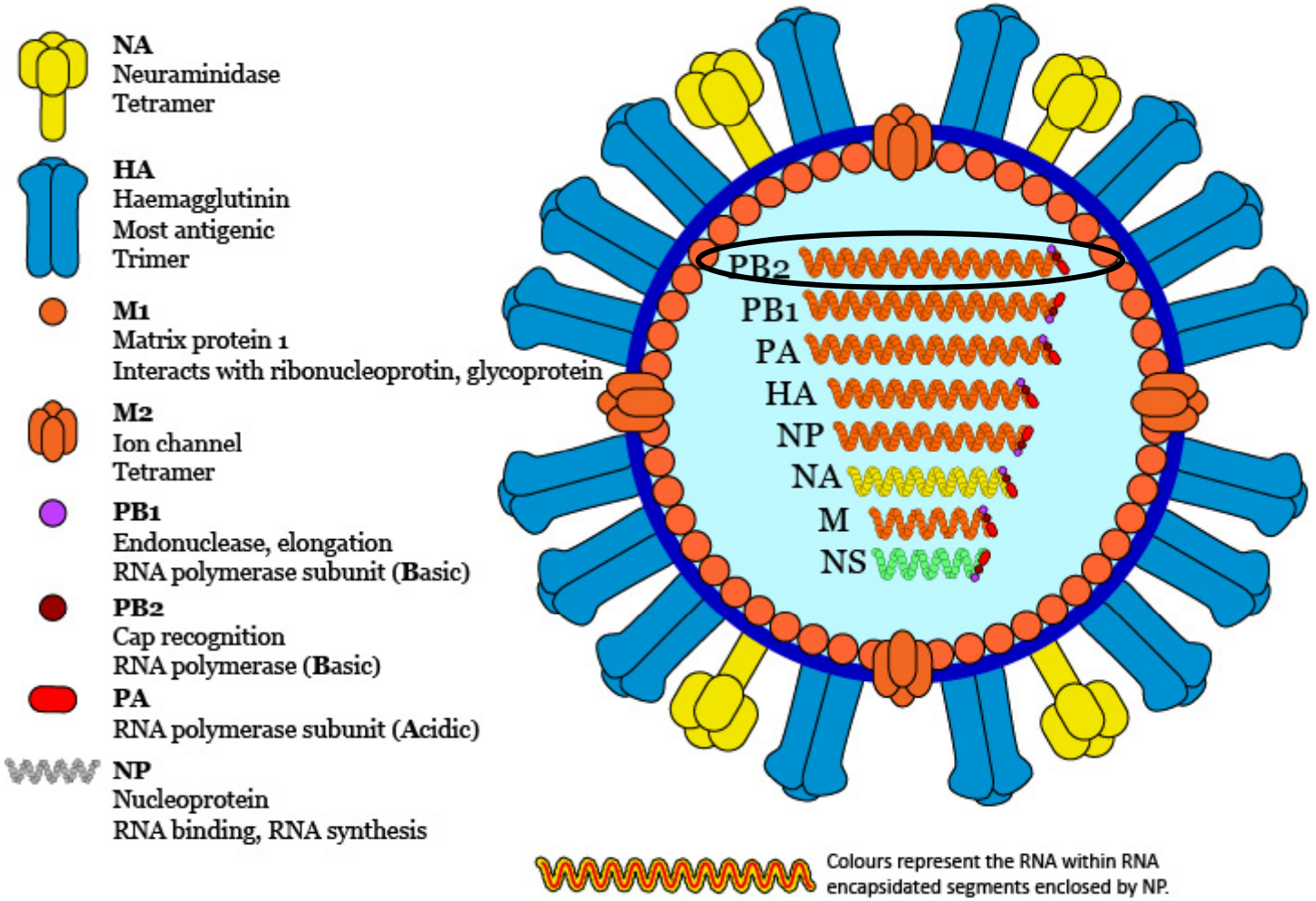


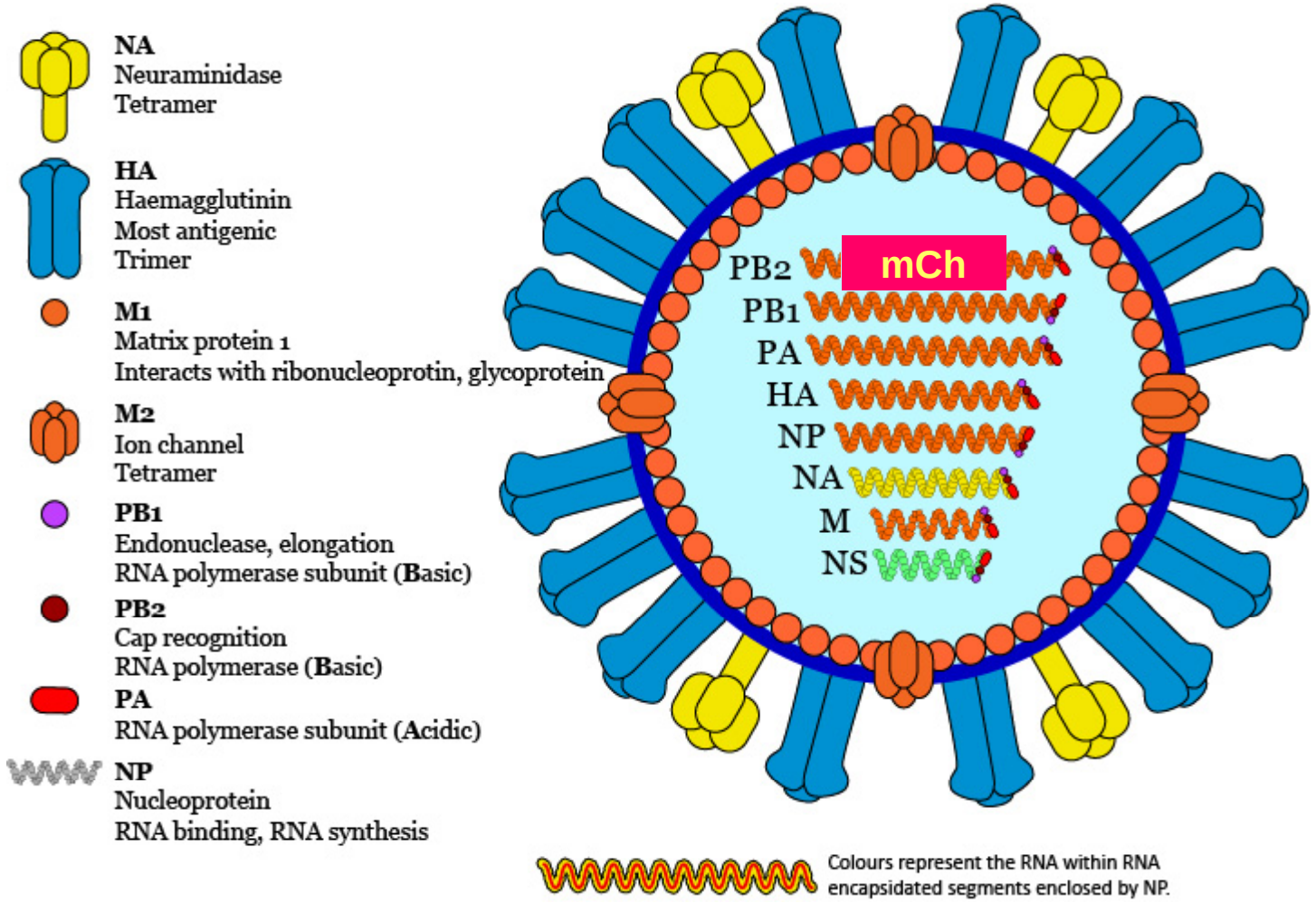

NA
Neuraminidase
Tetramer
HA
Haemagglutinin
Most antigenic
Trimer
M1
Matrix protein 1
Interacts with ribonucleoprotin, glycoprotein
M2
Ion channel
Tetramer
PB1
Endonuclease, elongation
RNA polymerase subunit (Basic)
PB2
Cap recognition
RNA polymerase (Basic)
PA
RNA polymerase subunit (Acidic)
NP
Nucleoprotein
RNA binding, RNA synthesis
PB2
PB1
PA
HA
NP
NA
M
NS
Colours represent the RNA within RNA encapsidated segments enclosed by NP.


PR8
Pandemic
H1N1
PB2
PA
PB1
HA
NP
M
NS
NA
-H1N1pdm
-H7N9
Embryonated eggs

Effect of PB1 source on rgH1N1 and rgH7N9 growth




NA
Neuraminidase
Tetramer
HA
Haemagglutinin
Most antigenic
Trimer
M1
Matrix protein 1
Interacts with ribonucleoprotin, glycoprotein
M2
Ion channel
Tetramer
PB1
Endonuclease, elongation
RNA polymerase subunit (Basic)
PB2
Cap recognition
RNA polymerase (Basic)
PA
RNA polymerase subunit (Acidic)
NP
Nucleoprotein
RNA binding, RNA synthesis
PB2
PB1
PA
HA
NP
NA
M
NS
Colours represent the RNA within RNA encapsidated segments enclosed by NP.


NA
Neuraminidase
Tetramer
HA
Haemagglutinin
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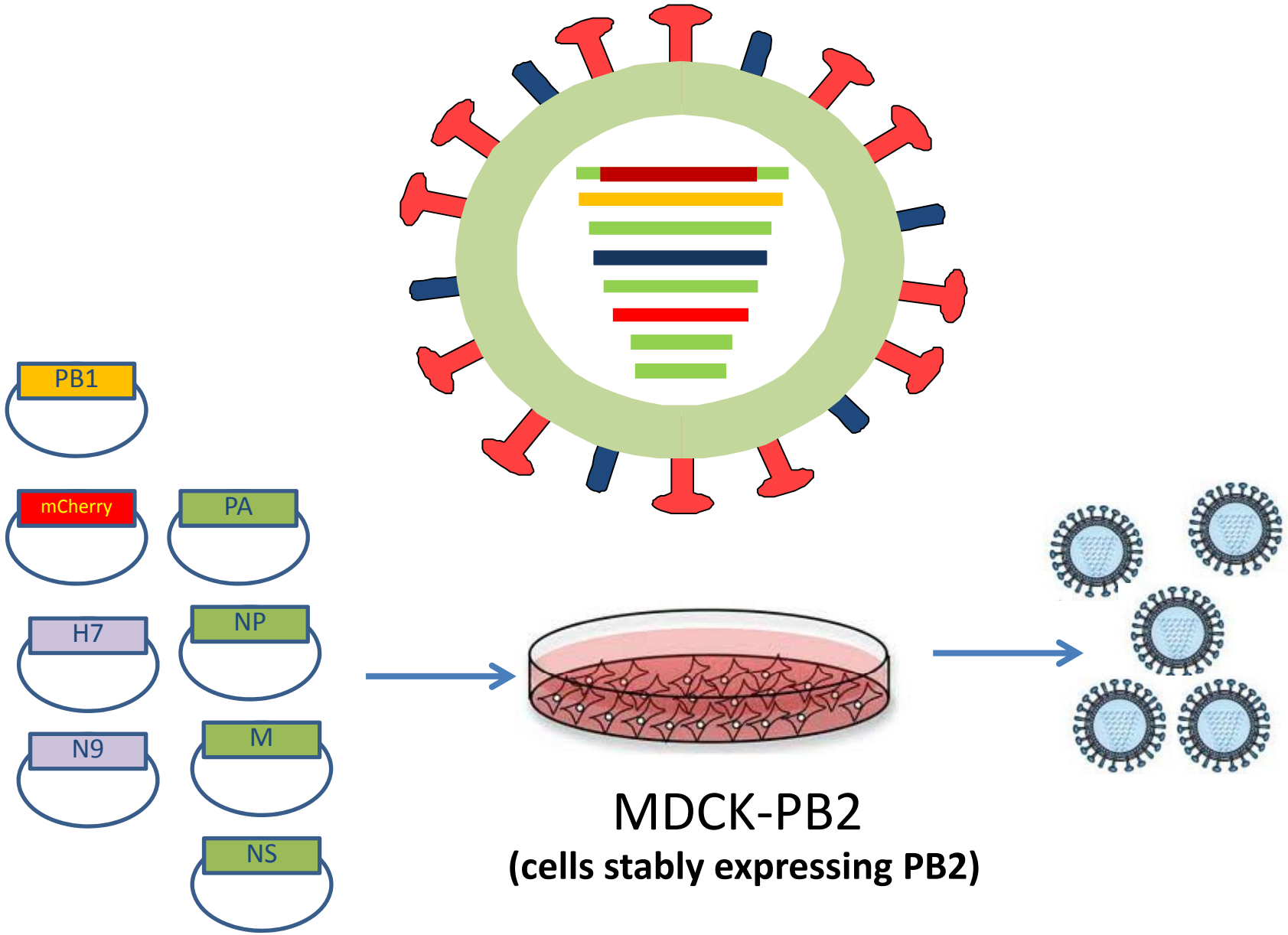
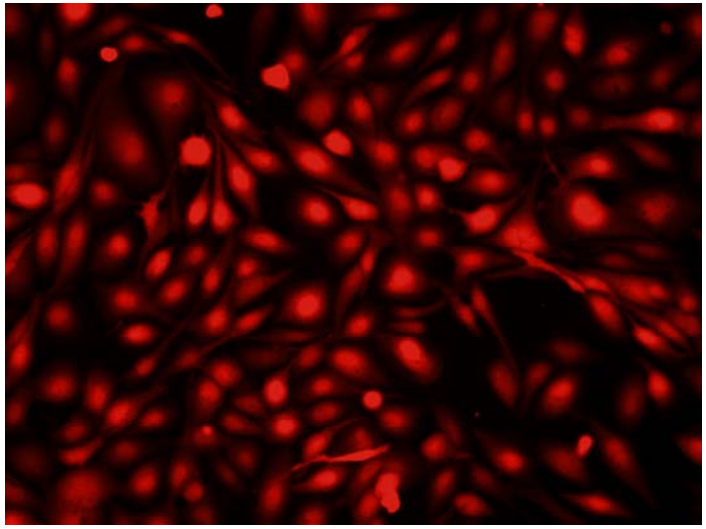
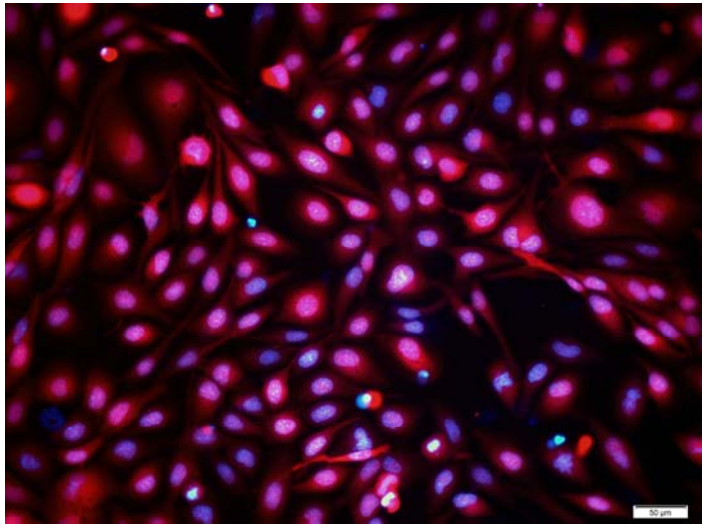
Ion channel
Tetramer
PB1
Endonuclease, elongation
RNA polymerase subunit (Basic)
PB2
Cap recognition
RNA polymerase (Basic)
PA
RNA polymerase subunit (Acidic)
NP
Nucleoprotein
RNA binding, RNA synthesis
PB2
mCh
PB1
PA
HA
NP
NA
M
NS
Colours represent the RNA within RNA encapsidated segments enclosed by NP.

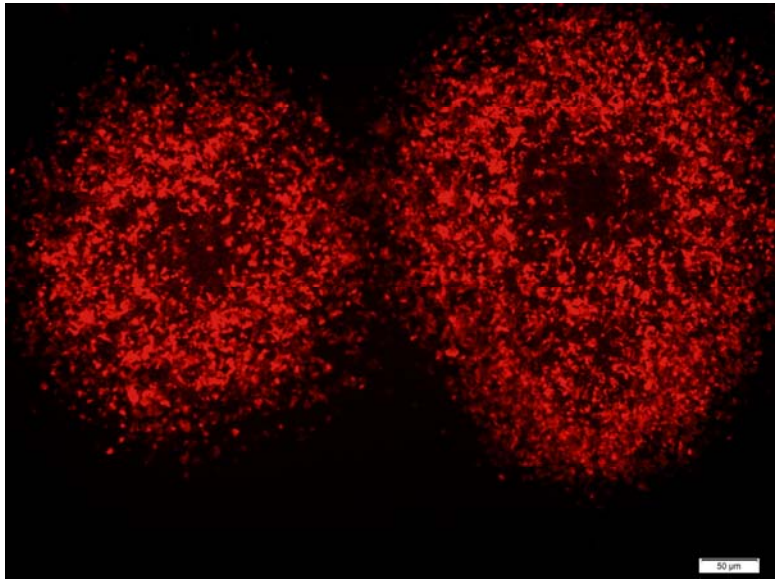
Construction of replication-defective rgH7N9 virus



Expression of mCherry in infected cells

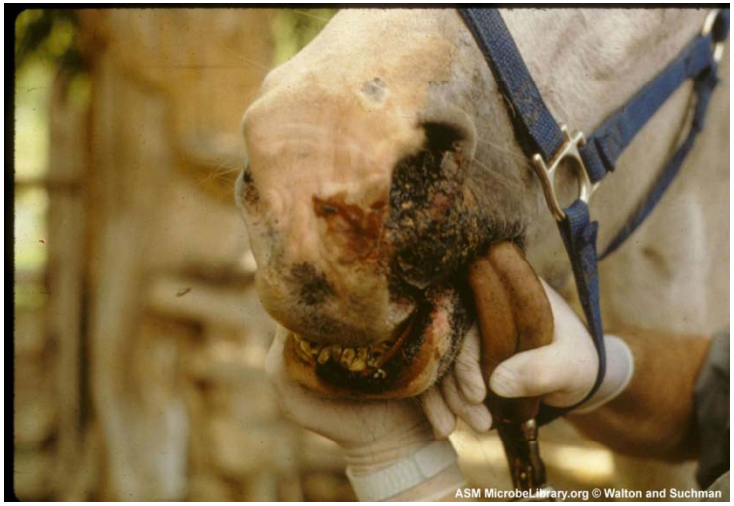


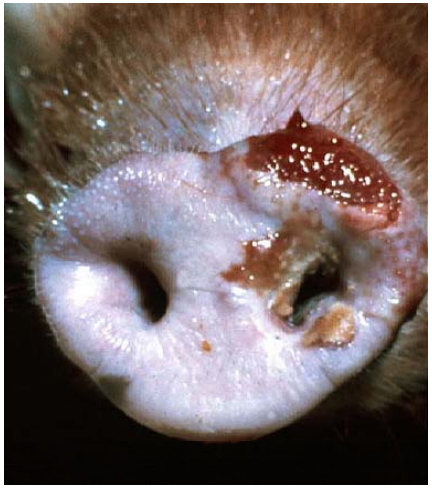


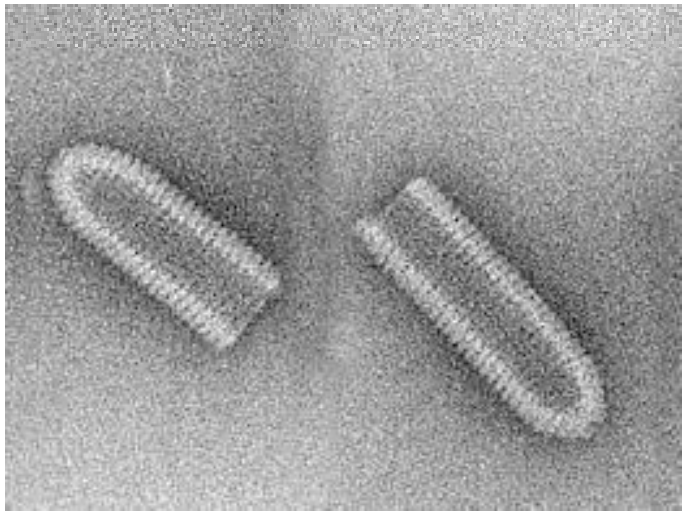


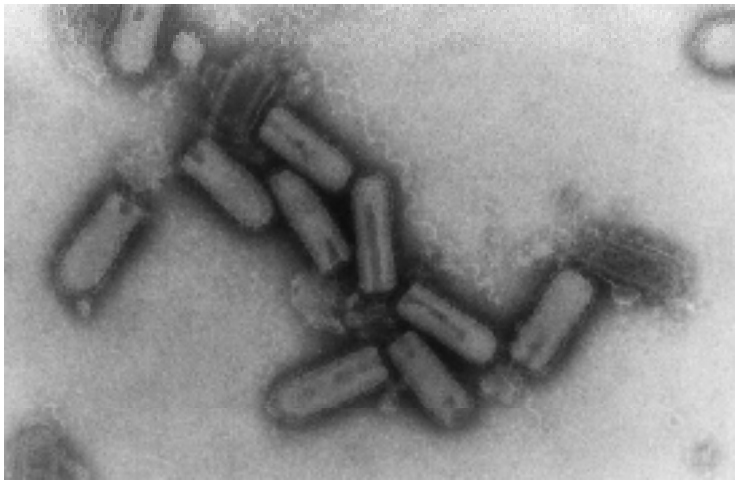
Example II

Vesicular stomatitis virus (VSV)

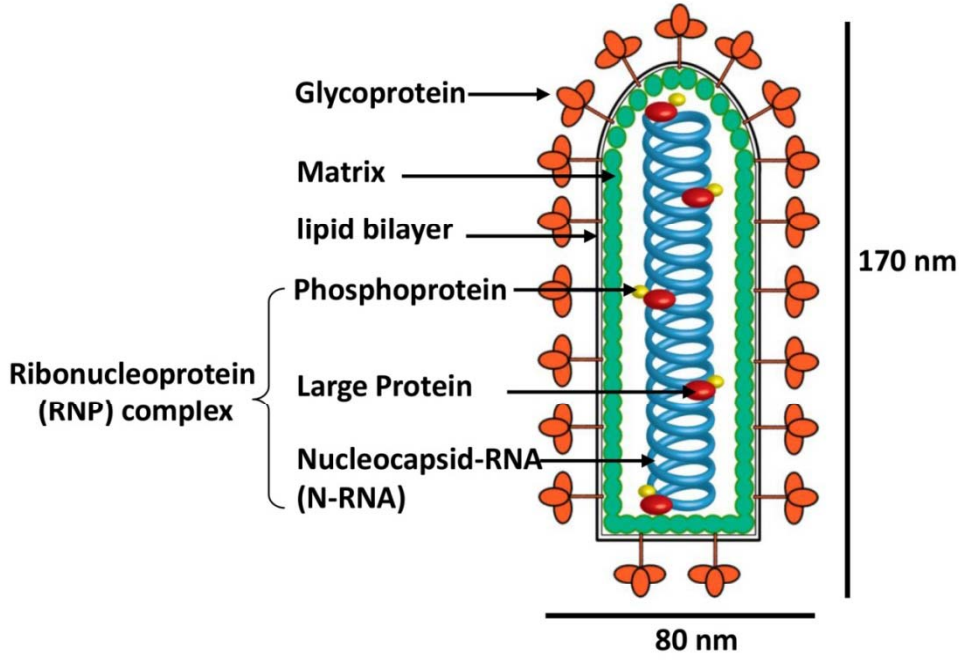








Vesicular stomatitis virus (VSV)



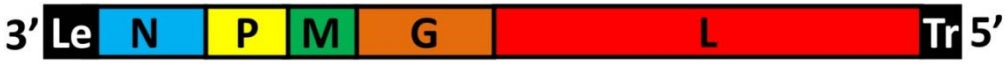
N = Nucleocapsid protein

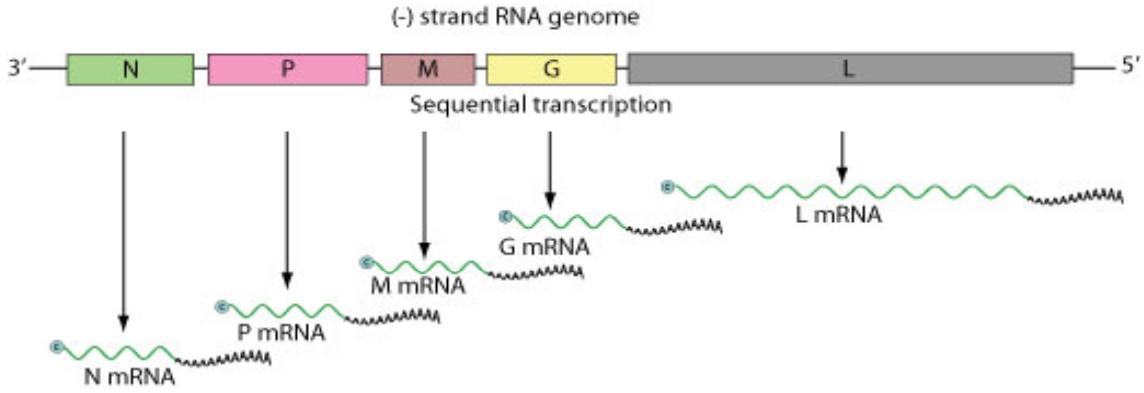
P = Phosphoprotein

M = Matrix protein

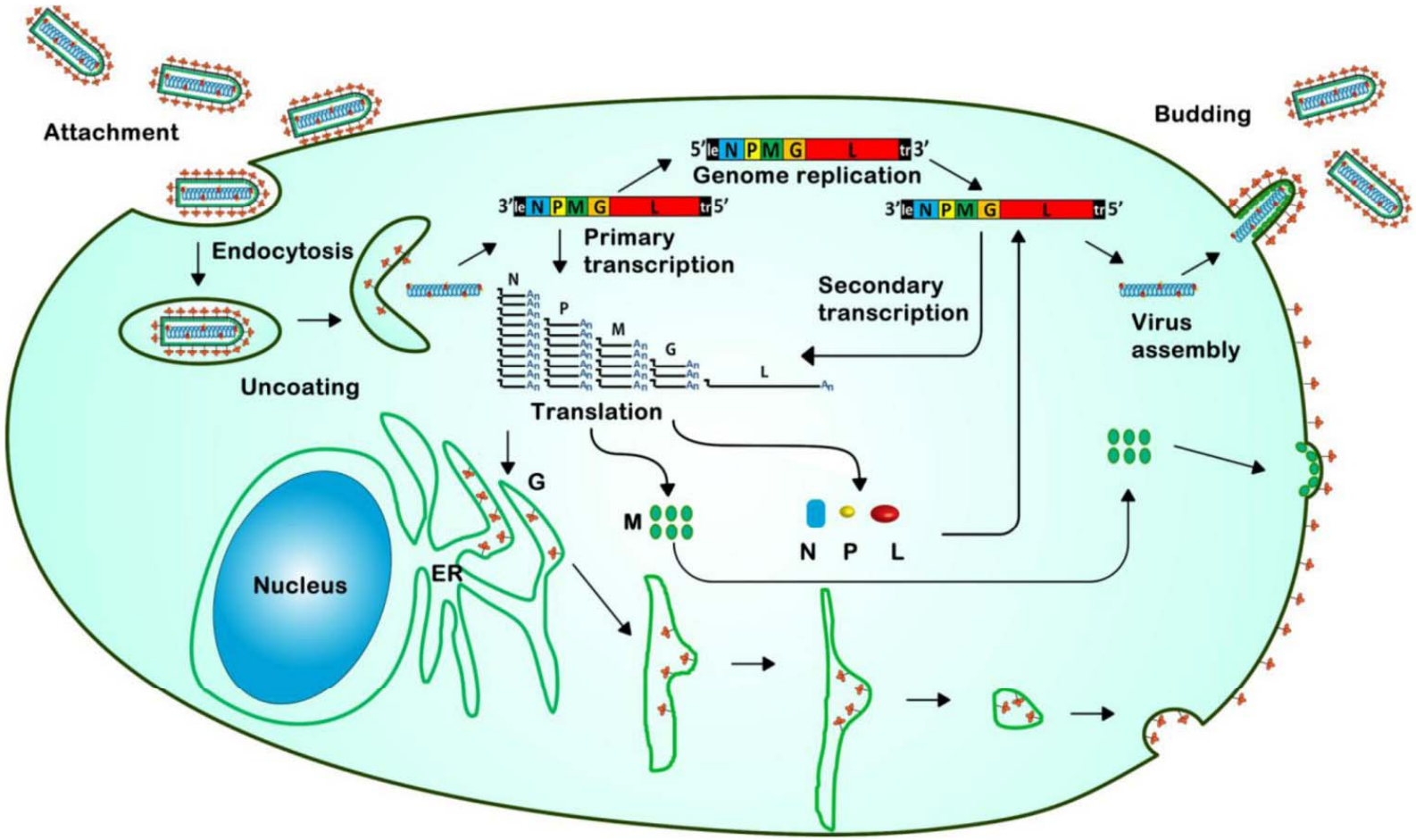
G = Glycoprotein

L = Large polymerase protein

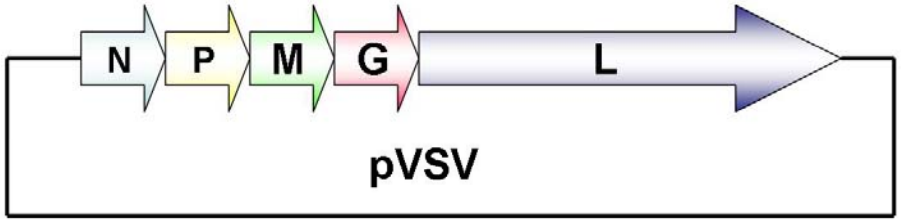


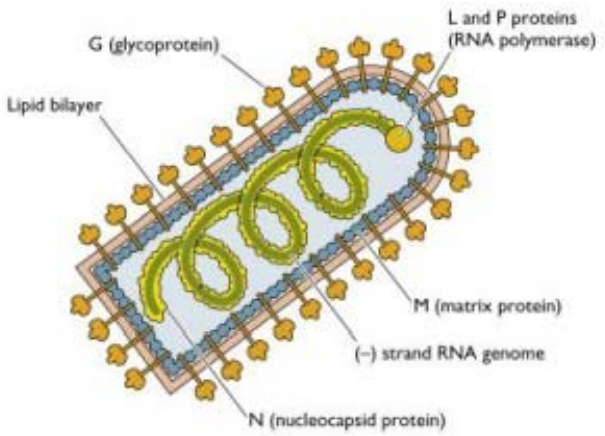


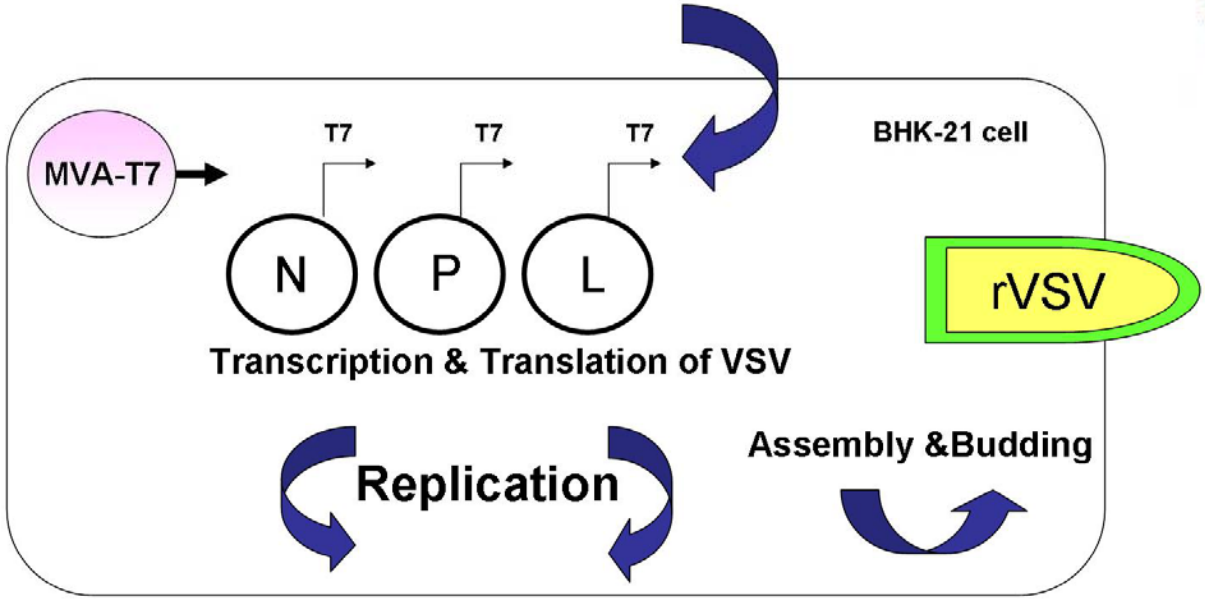
Replication cycle of VSV

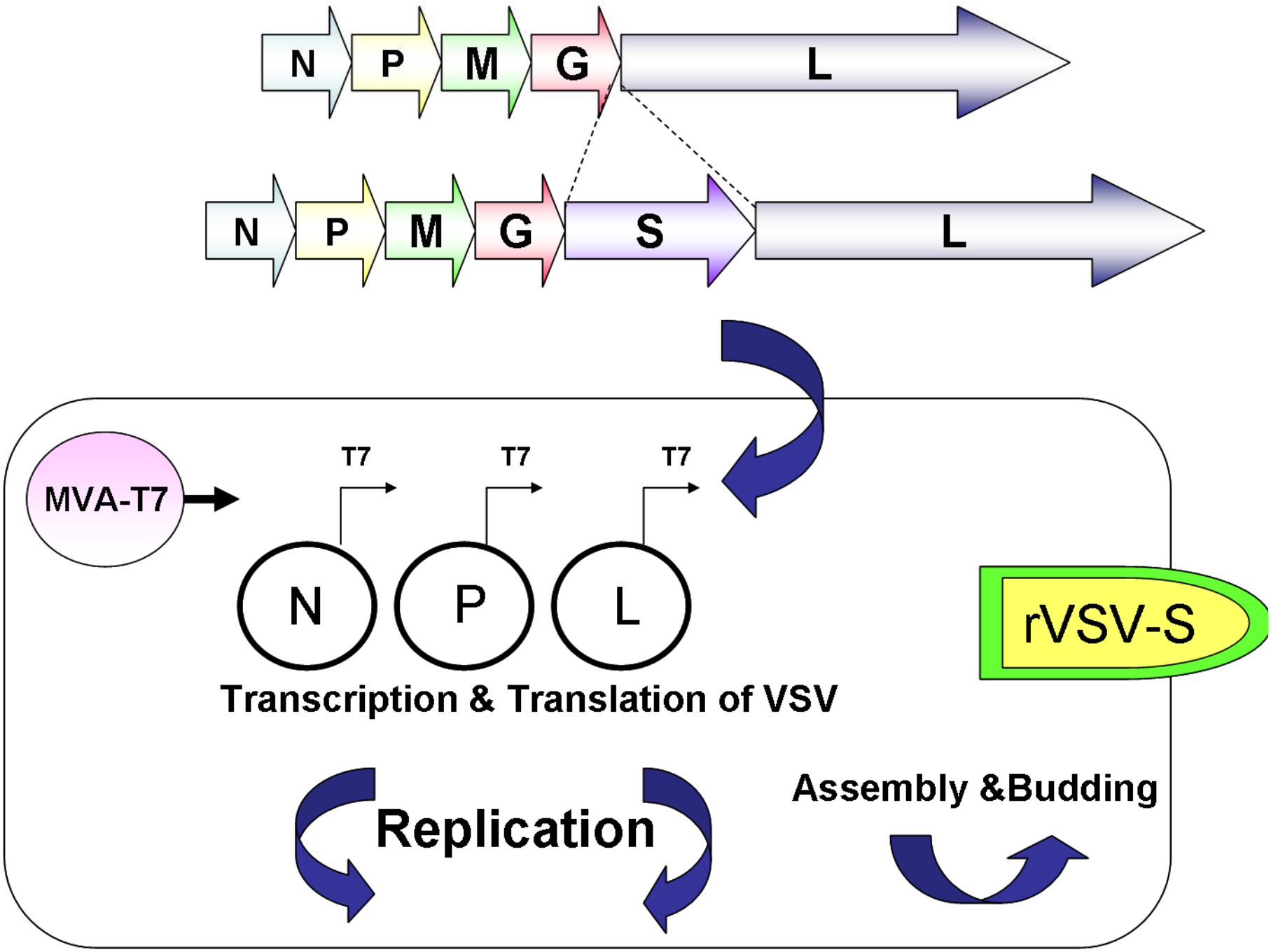


Reverse genetics of VSV-based vaccine

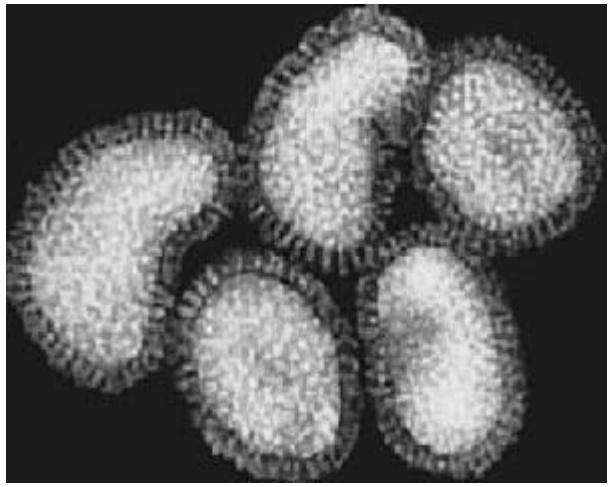


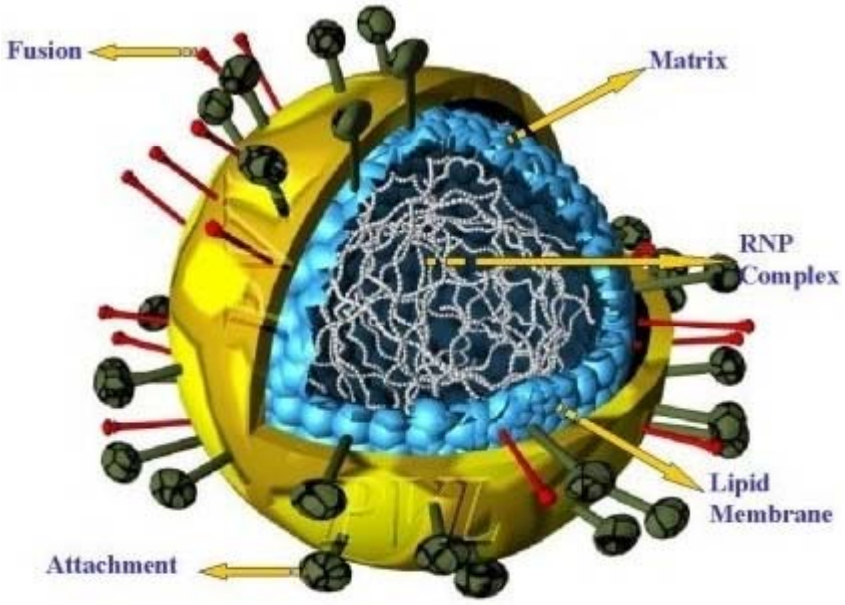


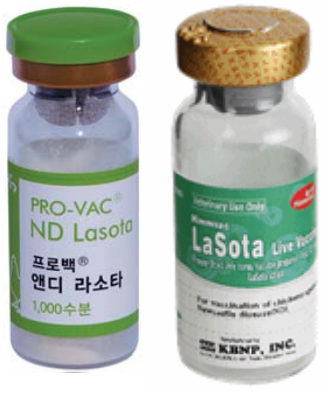


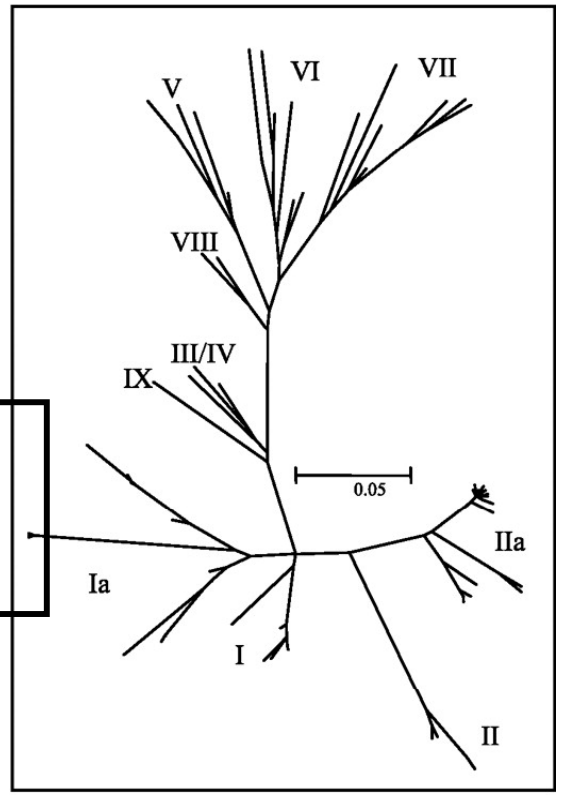

N
P
M
G
L
N
P
M
G
S
L
MVA-T7
T7
T7
T7
N
P
L
Transcription & Translation of VSV
rVSV-S
Replication
Assembly &Budding

Newcastle Disease Virus (NDV)









JVI
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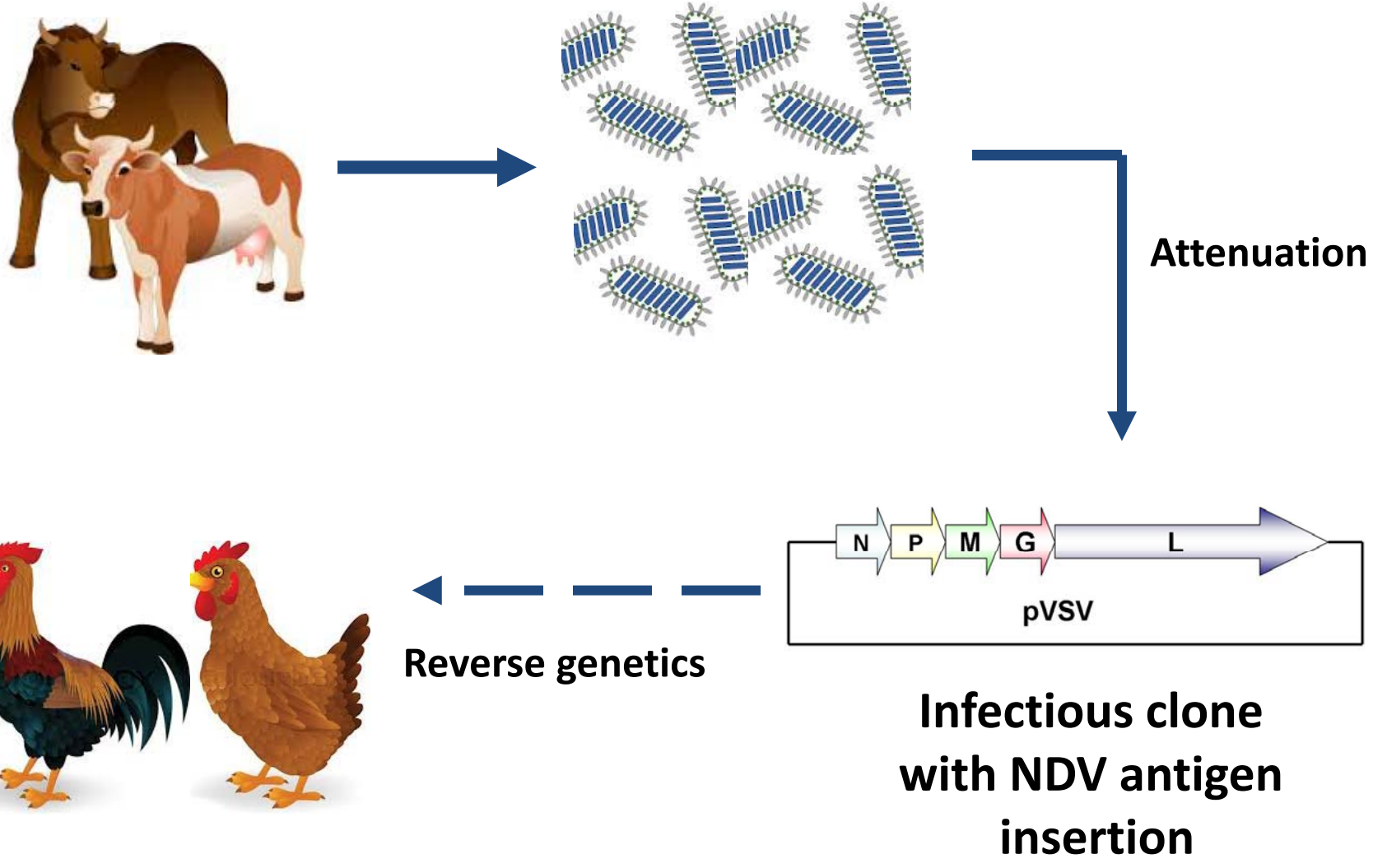
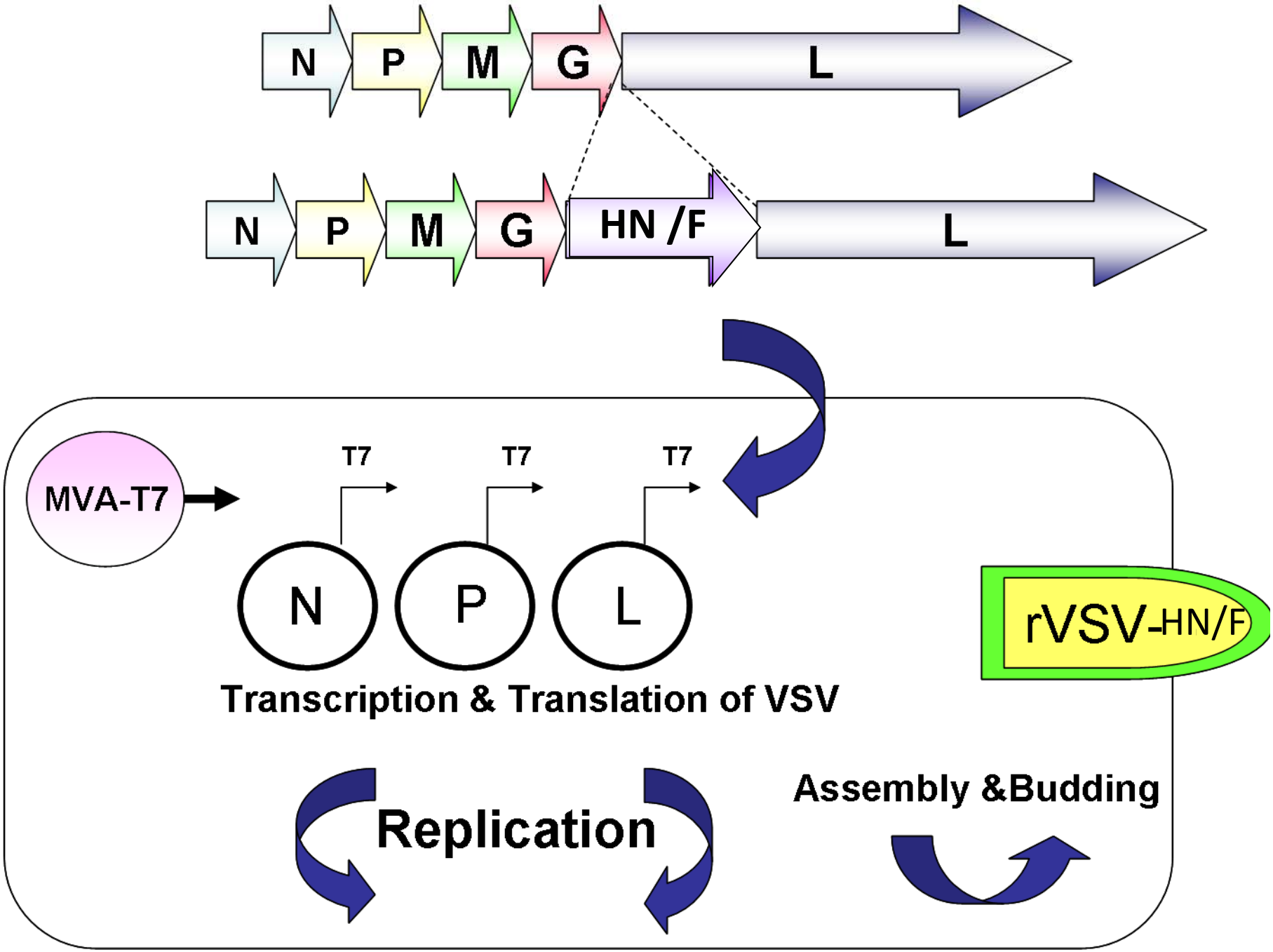
GENOME ANNOUNCEMENT

Complete Genome Sequences of Newcastle Disease Virus Strains Circulating in Chicken Populations of Indonesia

Rationale of new NDV vaccines




N
P
M
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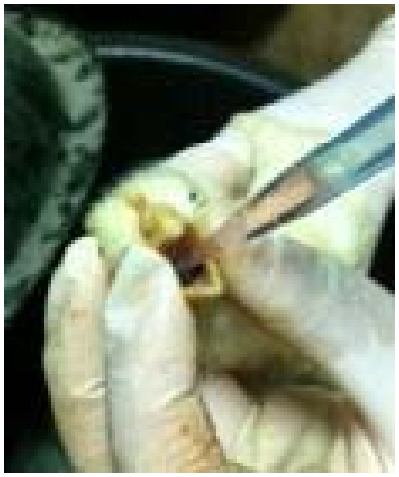
N
P
M
G
HN /F
L
MVA-T7
T7
T7
T7
N
P
L
Transcription & Translation of VSV
rVSV-HN/F
Replication
Assembly &Budding

Animal testing of VSV-based vaccines



Regimen of VSV-based NDV vaccination in chickens

D2 D13 D27 D37

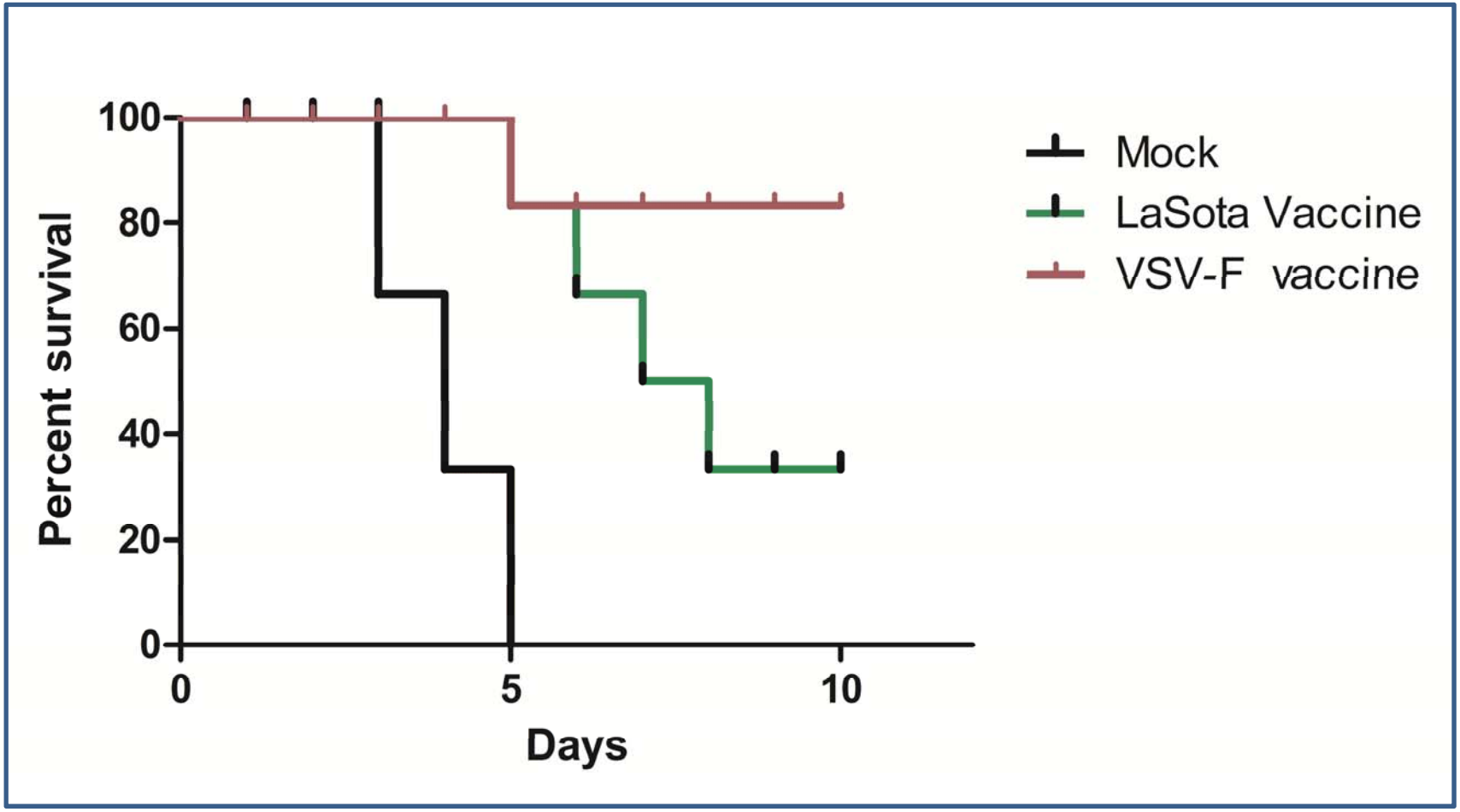
Challenge

Sacrifice

NDV signs : started at 4 d.p.i.

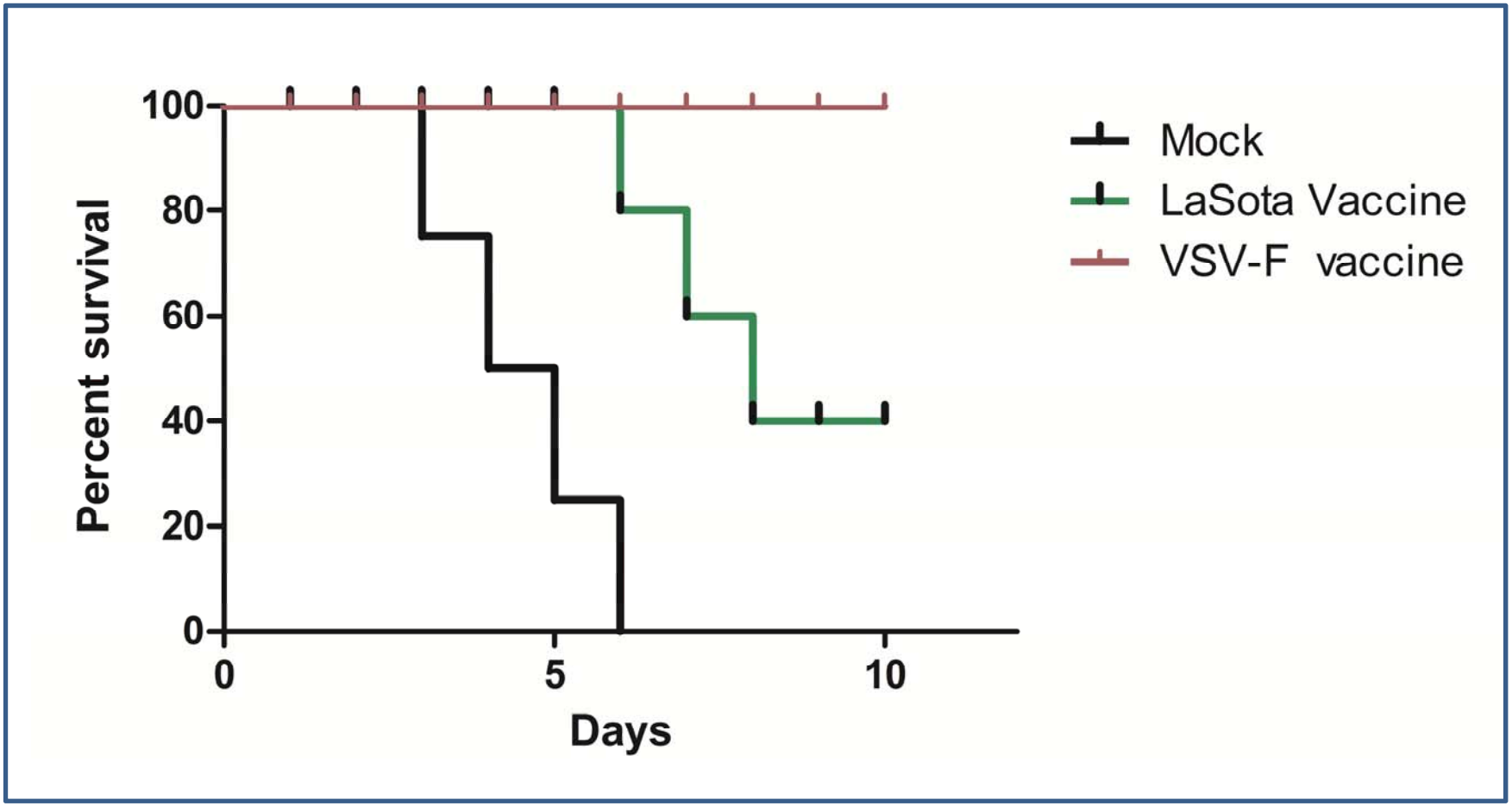
VSV-based vaccine protected chickens from NDV challenge

Efficacy of VSV-based NDV vaccines in challenged chickens



n = 20 / group

Efficacy of VSV-based NDV vaccines in contacted chickens



n = 10 / group

Take-home messages

-Reverse genetics is no doubt a very powerful technique in virology.

-Now we have the potential to conduct reverse genetics on nearly every group of human and animal viruses.

-Reverse genetics are not always easy…Common difficulties are:

1. viral sequence
2. clone generation and stability
3. transfection and rescue
4. complex virus genome
5. inability of the virus to replicate in cell culture

- Ethical issues (Which viruses should be rescued?)

Acknowledgement

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

MAYO CLINIC

Virology and Cell Technology Laboratory

CPF

BETAGRO

MAHIDOL UNIVERSITY
Wisdom of the Land

BIOTEC
a member of NSTDA

สวทช NSTDA

BIOTEC
a member of NSTDA

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NATIONAL CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

Thank you