2017 UST Global Research Internship

The Study of the Host Factors Required for Influenza Virus Replication & Sequence Analysis

[redacted]

Respiratory Viruses Research Laboratory

2017.08.22

Institut Pasteur Korea

We are Pasteurians fighting disease in Korea for all mankind

Overview

I. Introduction

- Influenza Virus
- Flu Prevention & Treatments
- Host Factors

II. Identification of Host Factors via siRNA Screening

III. Sequence Analysis

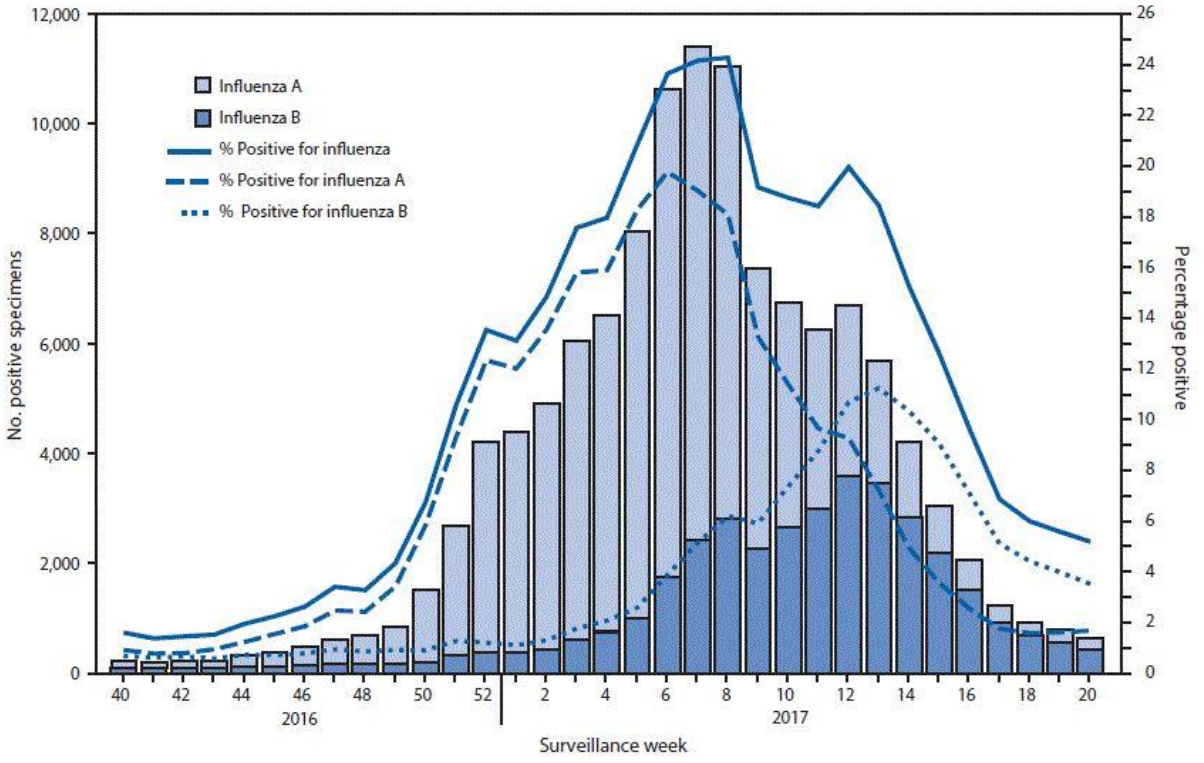
IV. Conclusion

Influenza Viruses

Influenza viruses:

- Can cause infections in the upper and lower respiratory tract, which can lead to an illness – influenza (or flu)
- Belong to the family *Orthomyxoviridae*
- Are negative-sense, single-stranded, segmented RNA genome
- There are 3 types (A, B & C)
- 10-20% of the worldwide populations are affected each year[1]

FIGURE 1. Number* and percentage of respiratory specimens testing positive for influenza reported by clinical laboratories, by influenza virus type and surveillance week — United States, October 2, 2016–May 20, 2017†



* Specimens from 121,223 (14.0%) of 865,168 persons tested positive during October 2, 2016–May 20, 2017.
† As of June 9, 2017.

Source: CDC. 2017. Morbidity and Mortality Weekly Report.

[1] World Health Organization. Influenza (seasonal) fact sheet N°211. 2014.

Flu Vaccine

1) Seasonal flu vaccine:

- The vaccine works by creating antibodies after about two weeks of vaccination against infection
- World Health Organization (WHO) predicts the most common influenza for the upcoming season
- Trivalent or quadrivalent vaccines are available; 2 influenza A viruses (H1N1 and H3N2) and 1 or 2 influenza B virus(es)
- Used to prevent diseases

Drawbacks:

- It is difficult to guess what will be circulating the next season
- Frequent antigenic drifts of the viruses
- “Predictions” may not be correct → Low vaccine effectiveness
- Possible side effects like fever, soreness, aches, and etc.

Anti-influenza Drugs

2) Antiviral drugs:

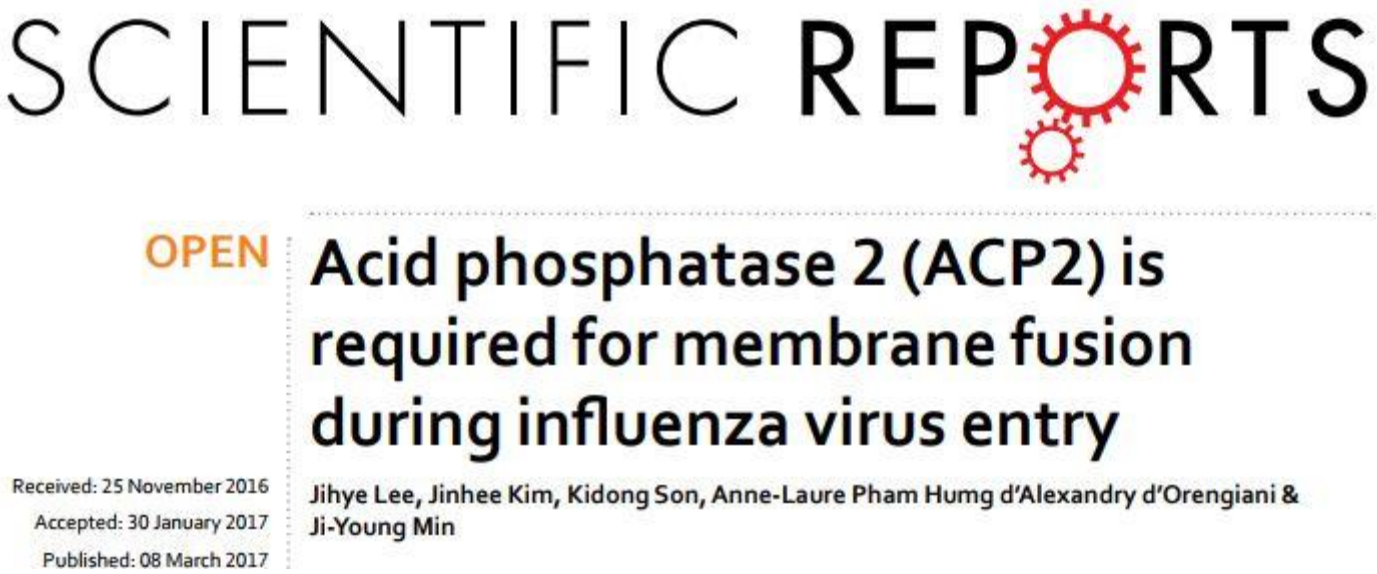
- Generally, only those who are severely sick or in a high risk group are treated with antiviral drugs
- They can be used to treat flu complications (e.g. pneumonia)
- The currently available FDA approved antivirals are NA inhibitors: Oseltamivir, Zanamivir & Peramivir
- Another class of antivirals that blocks M2 ion channels, Adamantanes, are no longer recommended due to high resistance and severe side effects, like neuronal diseases

Drawbacks:

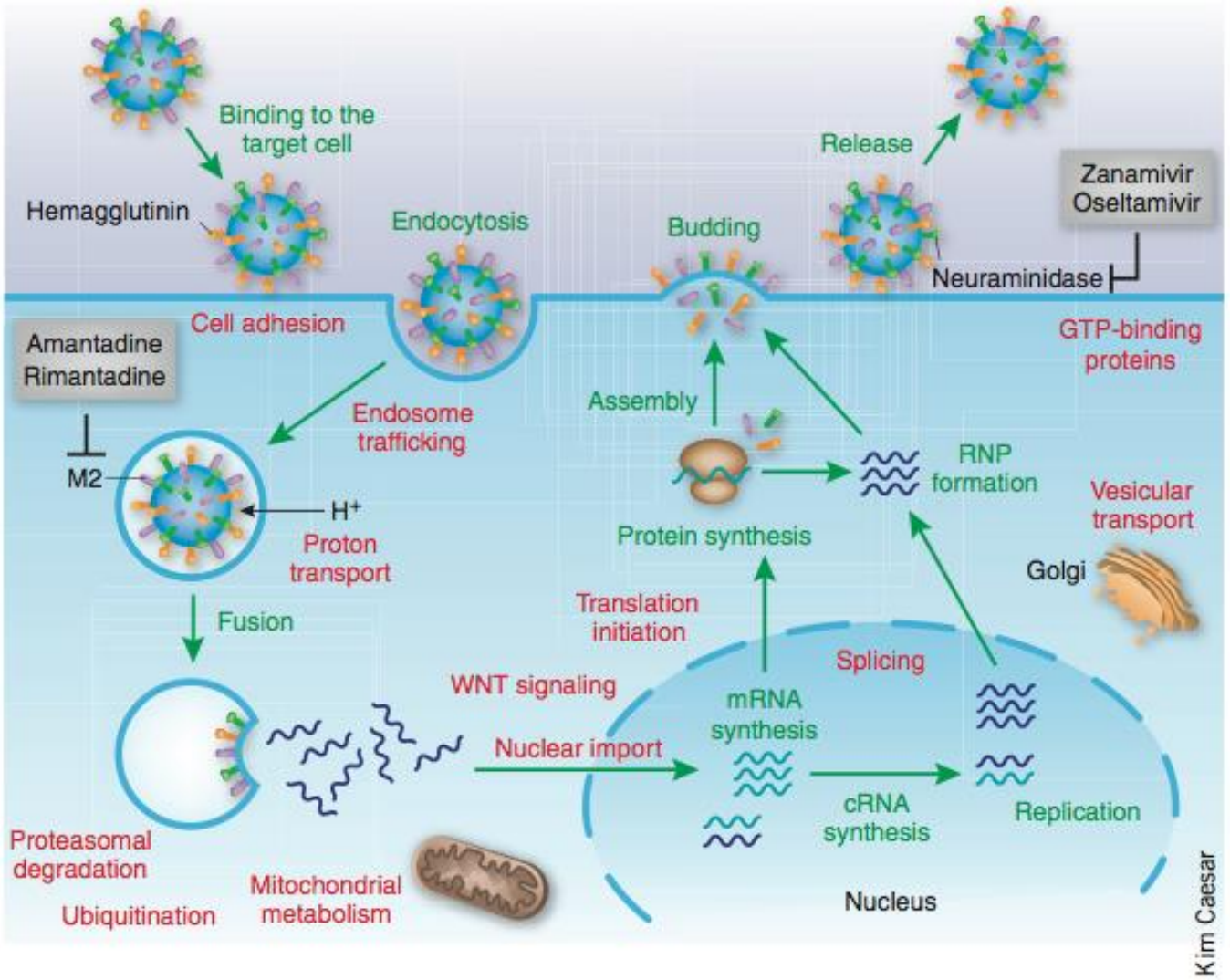
- Possible side effects like nausea, vomiting, diarrhea, and etc. (although these are uncommon)
- Resistance

Rationale of the Study of Host Factors

- These drawbacks can be enhanced by identifying host cell factors, which are used by influenza viruses to replicate, and lead to development of potential drug targets without resistance
- Therefore, further study has been done to identify the host factors that are involved during the replication cycle of influenza viruses

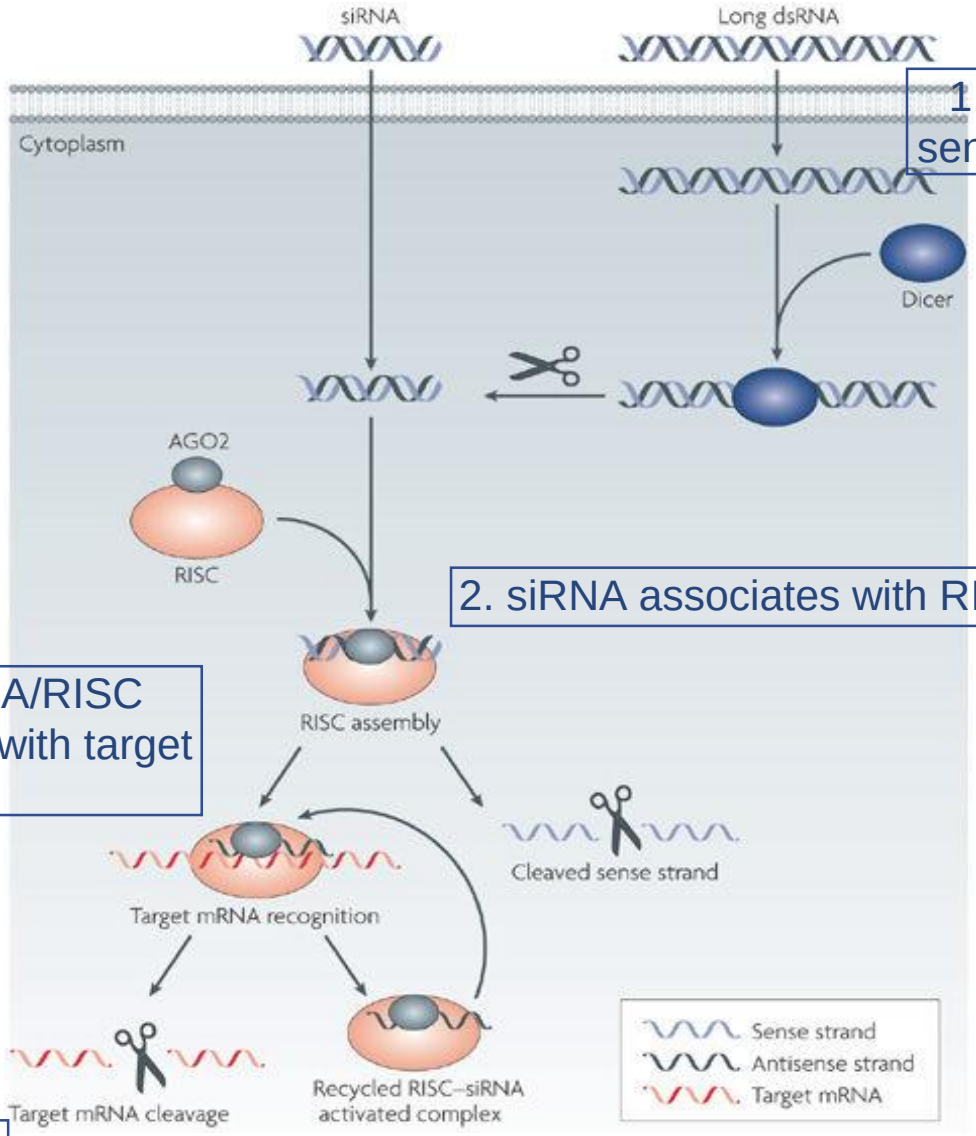


Cellular Targets for Influenza Drugs & Replication Cycle of Influenza Virus



Min JY and Subbarao K. 2010. Nat Biotechnol

The Mechanism of RNA Interference



1. dsRNA is processed into sense/antisense RNA by Dicer

2. siRNA associates with RISC

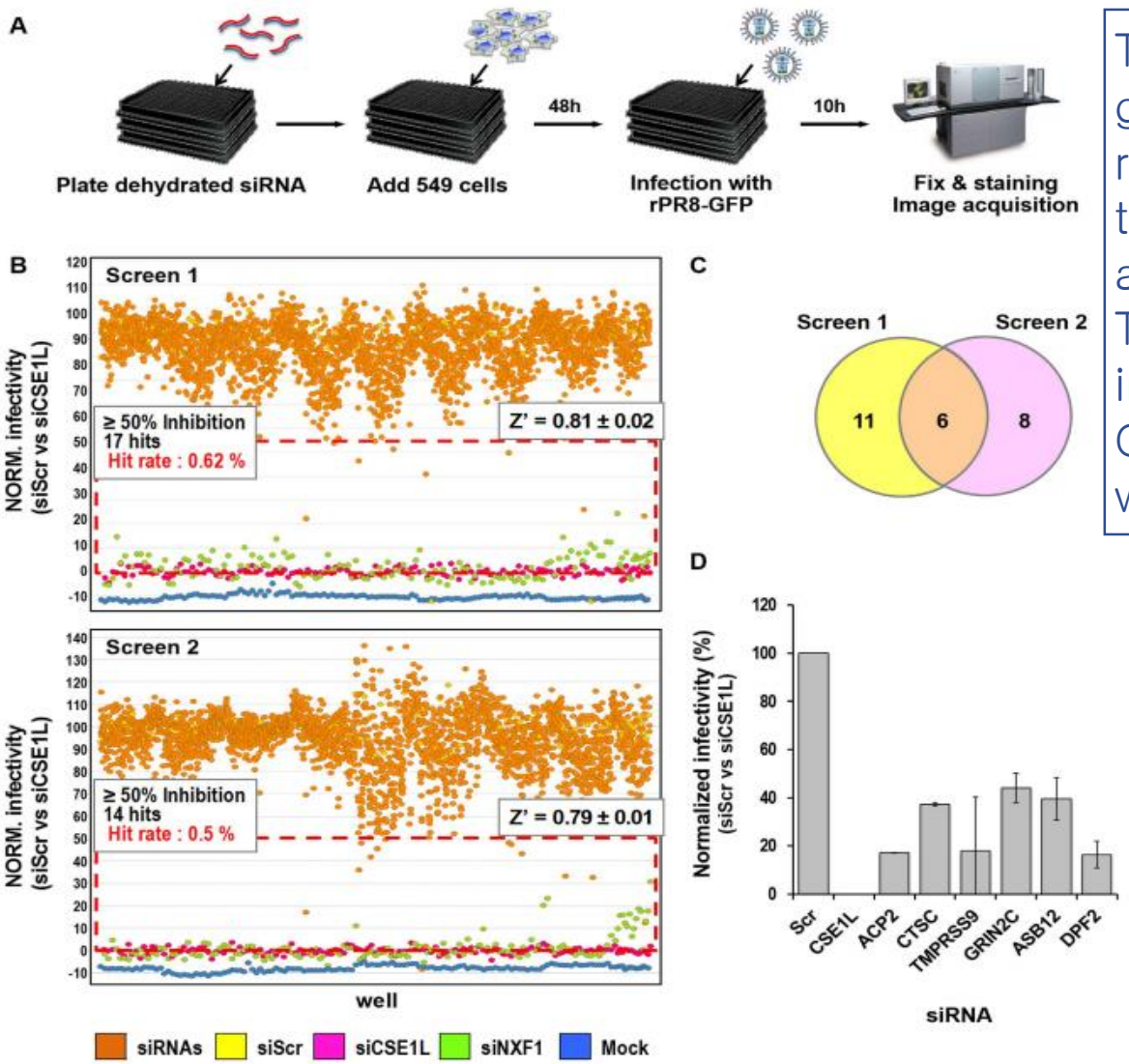
3. Antisense siRNA/RISC complex associates with target mRNA

4. RISC cuts the target mRNA

5. mRNA degradation

Kathryn A. Whitehead, Robert Langer & Daniel G. Anderson. 2009. Nat Rev Drug Discov

Identification of Six Host Factors through siRNA Screening



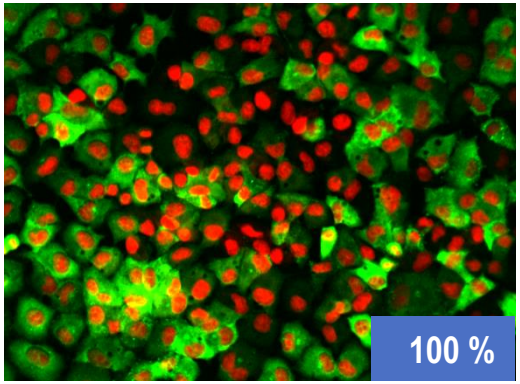
To identify the six human genes required for the replication of influenza virus, the duplicate siRNA screening assays were performed. The overlapping six hits that inhibit the replication of rPR8-GFP virus by more than 50% were identified.

Jihye Lee *et al*. 2017. Scientific Reports.

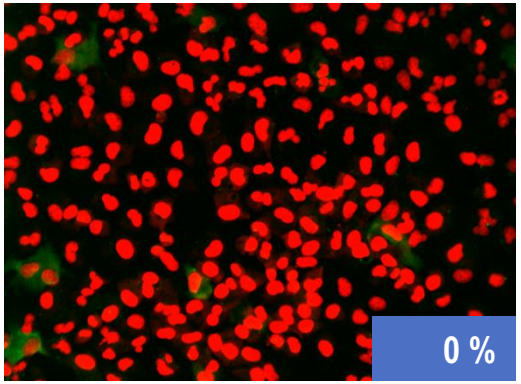
Detection of the Expression Level of NS1-GFP to Confirm Gene X Knockdown Efficiency

(Conducted by Jihye Lee)

Scramble

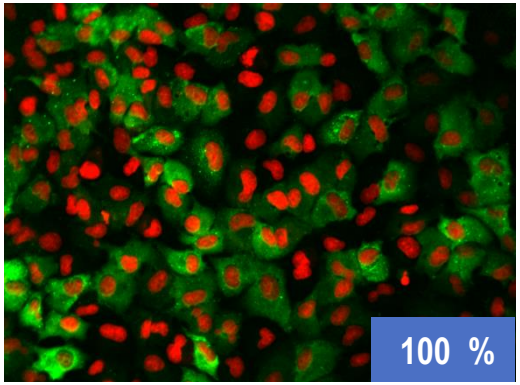


CSE1L

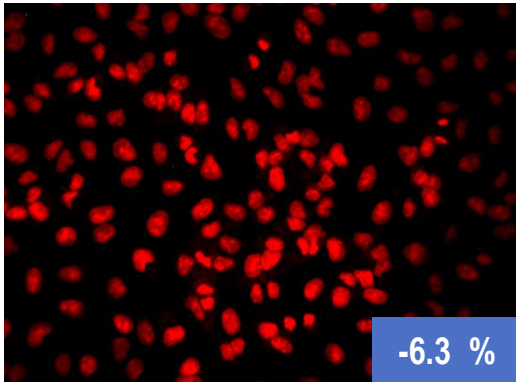


- A549 cells in 384 well plate
- Infection : A/rPR8 NS1-GFP (H1N1) E1, Aug. 29, 2013 at 0.8 MOI (6.1 x 10^6 PFU/ml)
- Positive control siRNAs : 10 nM of CSE1L
- Gene X siRNA concentration : 10 nM
- Determination of inhibitory effect was based on expression level of viral proteins at 10 hpi

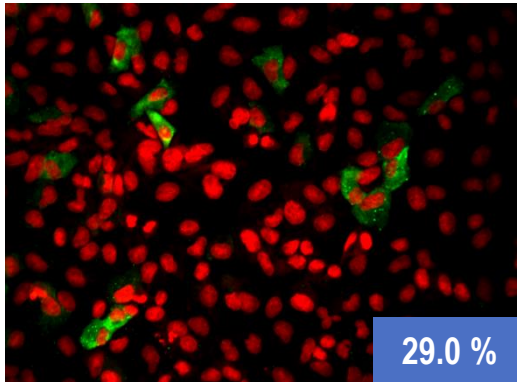
Gene X #1



Gene X #2

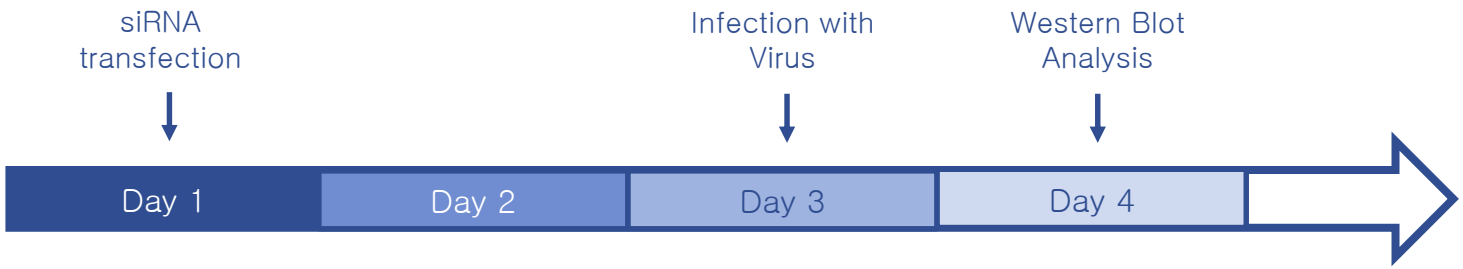


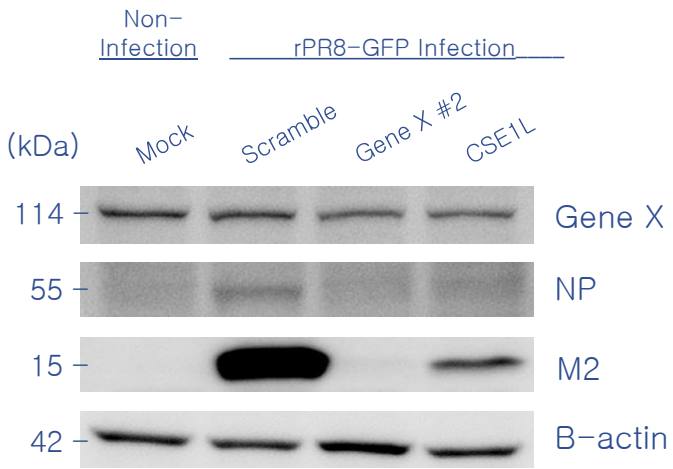
Gene X pool

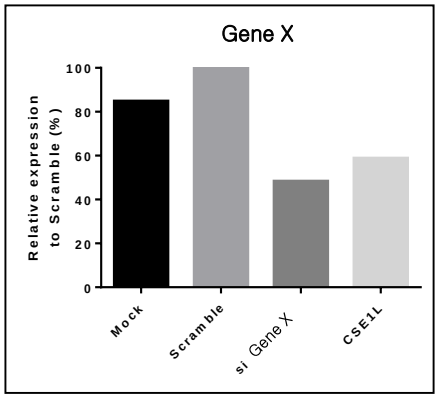


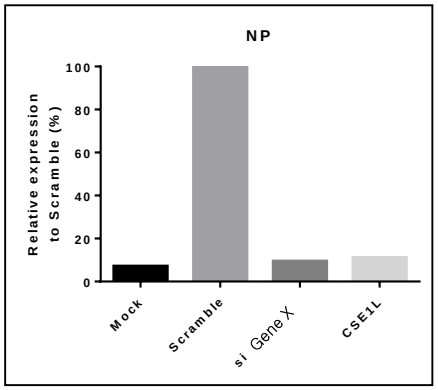
- Low viral infection
- Low cytotoxicity

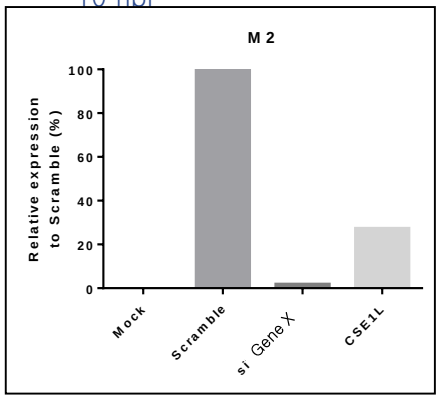
Confirmation of Protein Expression Knockdown by Western Blot Analysis











- A549 cells in 6 well plate
- Infection : A/rPR8 NS1-GFP (H1N1) E1, Nov. 10, 2016 at 2 MOI (1 x 10^6 PFU/ml)
- Positive control siRNAs : 10 nM of CSE1L
- Gene X #2 siRNA concentration : 10 nM
- Determination of inhibitory effect was based on expression level of viral proteins at 10 hpi

Conclusion

- When Gene X was transfected with siRNA, the efficiency of influenza virus infection was reduced
- Further study will have to be done to determine how Gene X affects viral replication
- Identification of the host factors, which are used by influenza viruses to replicate, can lead to the development of new potential drug targets

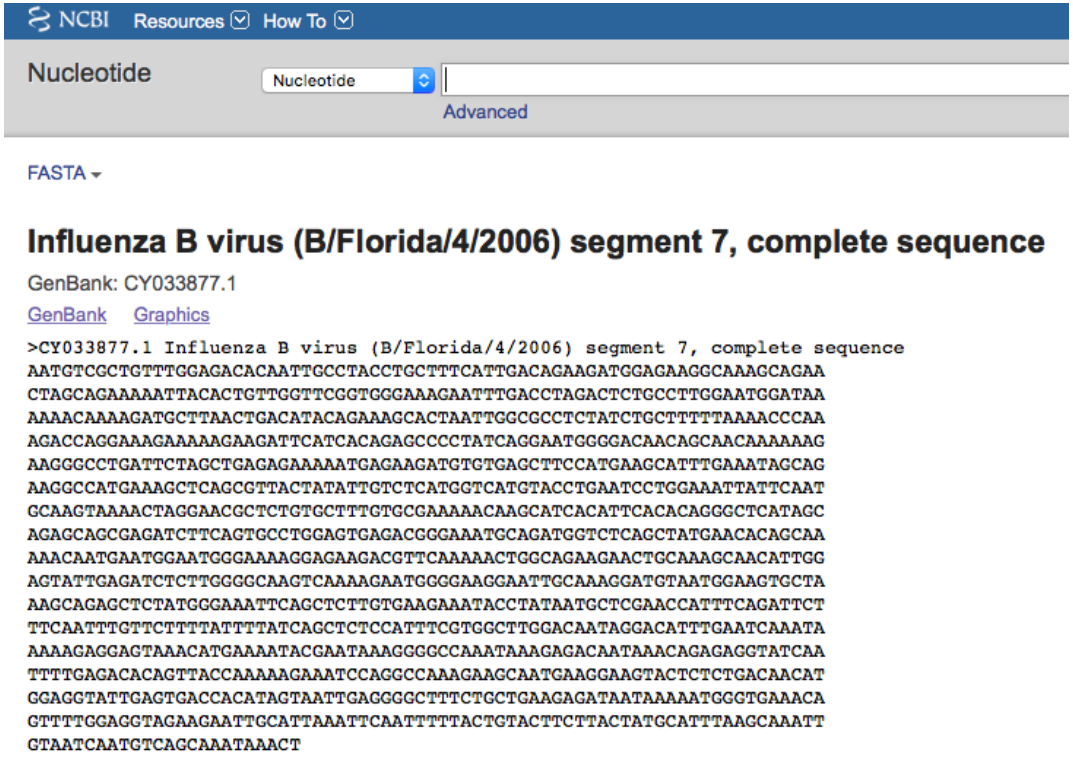
Sequence Analysis

- Influenza virus: B/Florida/04/2006
- Purpose: To check whether the genome of the virus is equivalent to the database (NCBI)
- The sequence of the Influenza B Virus was confirmed using One-step RT-PCR and the software, Sequencher

Sequence Analysis

1) PCR Primer Design

- The segment was divided into 2 or 3 sections of ~700 bp.
- The beginning and the end of each section were selected for primers (Forward & Reverse)
- The criterion like the GC content of 40-60%, primer length of ~18-22 bp, and melting temperature of 52-58 ^{o}C were satisfied.



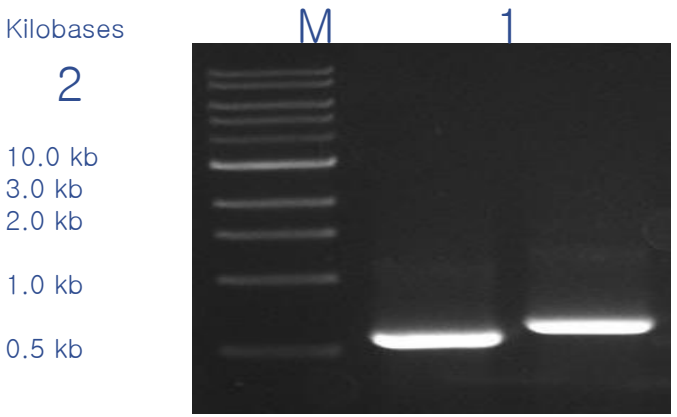
Sequence Analysis

2) One-step RT-PCR

Lanes	1	2
Target genes	M1/BM2	
Primers	2 μl (1 μl F1, 1 μl R1)	2 μl (1 μl F2, 1 μl R2)
Annealing Temp (°C)	54.3	51.1
Virus	Influenza Virus B/Florida	

B/Florida/04/06

Seg7_M1/BM2

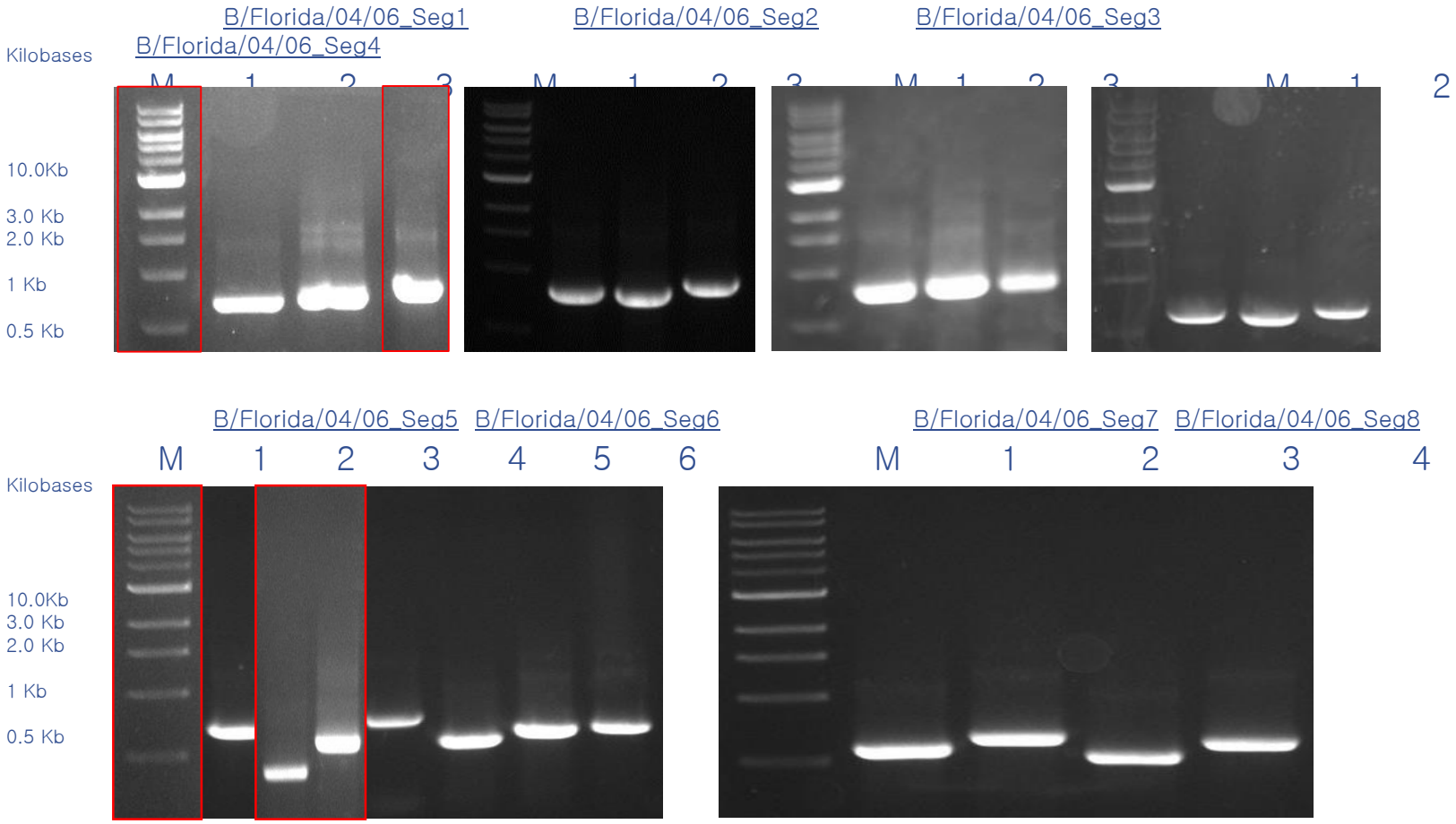


PCR Platform	50°C/1hr	
	95°C/15min	
	94°C/30sec	39 Cycles
	47°C-55°C (gradient) /1min	
	72°C/3min 30sec	
	72°C/10min	
	4°C/infinite	

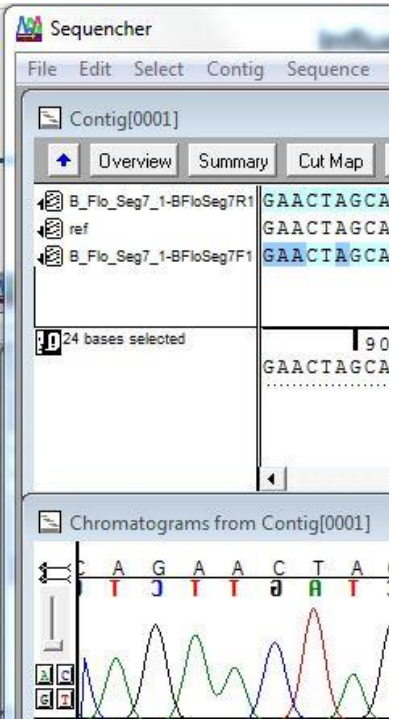
	PCR tubes	
	Seg 7_M1/BM2_1	Seg 7_M1/BM2_2
Primers	1 μl F1 + 1 μl R1 = 2 μl	1 μl F2 + 1 μl R2 = 2 μl
DEPC H2O	17 μl	17 μl
Sample (RNA)	1 μl	1 μl
Annealing temperature (°C)	54.3	51.1

Sequence Analysis

2) One-step RT-PCR



3) Sequence a



Influenza B virus (B/Florida/4/2006) segment 7, complete sequence

GenBank: CY033877.1

GenBank Graphics

>CY033877.1 Influenza B virus (B/Florida/4/2006) segment 7, complete sequence

AATGTCGCTGTTTGGAGACACAATTGCCTACCTGCTTTCATTGACAGAAGATGGAGAAGGCAAAGCAGAA

GCAGAA

GGAGACACAATTGCCTACCTGCTTTCATTGACAGAAGATGGAGAAGGCAAAGCAGAA

CTAGCAGAAAAATTACACTGTTGGTTCGGTGGGAAAGAATTTGACCTAGACTCTGCCTTGGAATGGATAA

CTAGCAGAAAAATTACACTGTTGGTTCGGTGGGAAAGAATTTGACCTAGACTCTGCCTTGGAATGGATAA

CTAGCAGAAAAATTACACTGTTGGTTCGGTGGGAAAGAATTTGACCTAGACTCTGCCTTGGAATGGATAA

AAJ TGACATACAGAAAGCACTAATTGGCGCCTCTATCTGCTTTTTAAAACCCAA

AAAACA AGCACTAATTGGCGCCTCTATCTGCTTTTTAAAACCCAA

AAAACAAAAGATGCTTA TGGCGCCTCTATCTGCTTTTTAAAACCCAA

AGACCAGGAAAGAAAAAGAAGATTCATCACAGAGCCCCTATCAGGAA CAAAAAAG

AGACCAGGAAAGAAAAAGAAGATTCATCACAGAGCCCCTATCAGGAA

AGACCAGGAAAGAAAAAGAAGATTCATCACAGAGCCCCTATCAGGAATGGGGACAACAC

AAGGGCCTGATTCTAGCTGAGAGAAAAATGAGAAGATGTGTGAGCTTCCATGAAGCATTTGAAATAGCAG

AAGGGCCTGATTCTAGCTGAGAGAAAAATGAGAAGATGTGTGAGCTTCCATGAAGCATTTGAAATAGCAG

AAGGGCCTGATTCTAGCTGAGAGAAAAATGAGAAGATGTGTGAGCTTCCATGAAGCATTTGAAATAGCAG

AAGGCCATGAAAGCTCAGCGTTACTATATTGTCTCATGGTCATGTACCTGAATCCTGGAAATTATTCAAT

AAGGCCATGAAAGCTCAGCGTTACTATATTGTCTCATGGTCATGTACCTGAATCCTGGAAATTATTCAAT

AAGGCCATGAAAGCTCAGCGTTACTATATTGTCTCATGGTCATGTACCTGAATCCTGGAAATTATTCAAT

GCAAGTAAAACTAGGAACGCTCTGTGCTTTGTGCGAAAAACAAGCATCACATTCACACAGGGCTCATAGC

GCAAGTAAAACTAGGAACGCTCTGTGCTTTGTGCGAAAAACAAGCATCACATTCACACAGGGCTCATAGC

GCAAGTAAAACTAGGAACGCTCTGTGCTTTGTGCGAAAAACAAGCATCACATTCACACAGGGCTCATAGC

AGAGCAGCGAGATCTTCAGTGCCTGGAGTGAGACGGGAAATGCAGATGGTCTCAGCTATGAACACAGCAA

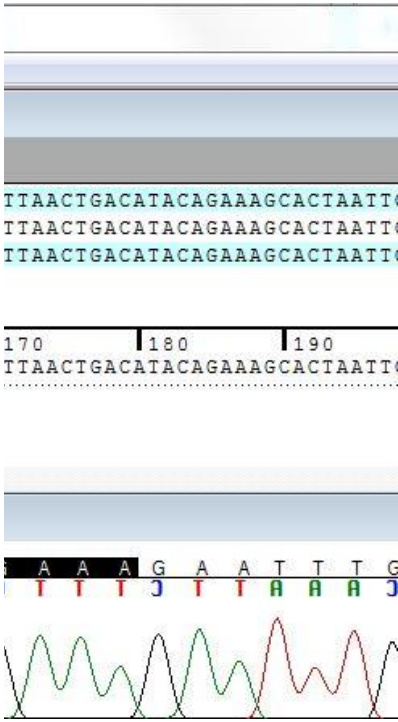
AGAGCAGCGAGATCTTCAGTGCCTGGAGTGAGACGGGAAATGCAGATGGTCTCAGCTATGAACACAGCAA

AGAGCAGCGAGATCTTCAGTGCCTGGAGTGAGACGG

GGTCTCAGCTATGAACACAGCAA

CAGTGCCTGGAGTGAGACGGGAAATGCAGATGGTCTCAGCTATGAACACAGCAA

Sequence identity = 100%



Impressions

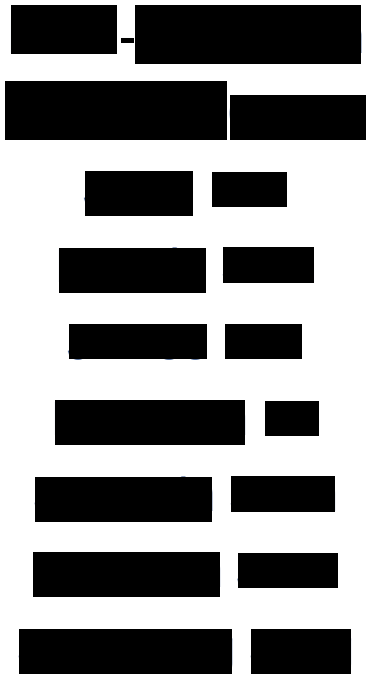
- Great 1:1 mentorship program for scientific discussions, experiments & general guidance and inspiration
- Learned how real research differs from science learned in the classroom
- Various hands-on experiments (e.g. RT-PCR, gel electrophoresis, Western Blot & etc.) helped me improve my laboratory techniques.

Plan After the Internship

I plan to return to my university to complete my last year for a bachelor's degree and participate in research. I expect to utilize my research internship experience at UST-IPK campus in school and research laboratories. Upon the completion of my bachelor's degree, I hope to pursue a career in the field of medicine.

Acknowledgment

Respiratory Viruses Research Laboratory

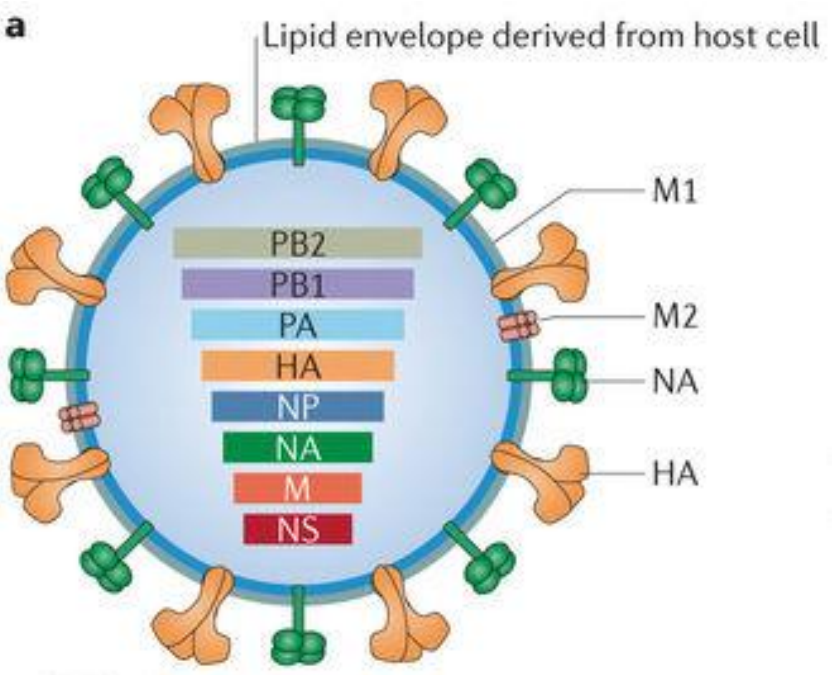


Thank you

Influenza Viruses

Influenza A virus:

- Affects a wide range of mammalian & avian species
- The only type that can cause pandemics – e.g. 1918 Spanish Flu
- Can be divided into subtypes based on their glycoproteins
- Causes severe illnesses
- Highly mutagenic



Yi Shi *et al*. 2014. Nat Rev Microbiol.

Influenza Viruses

Influenza B Virus:

- Has 8 single-stranded RNA segments
- Cannot cause human pandemics but can cause epidemics
- Can only go through antigenic drift
- Mainly affects humans
- Currently circulating influenza B viruses belong to either B/Yamagata or B/Victoria

➢ Influenza A and B viruses circulate and cause epidemics

Influenza C Virus:

- Only has 7 single-stranded RNA segments
- Cannot cause human pandemics/epidemics