



# Development of next-generation vaccines through mRNA and gut microbe

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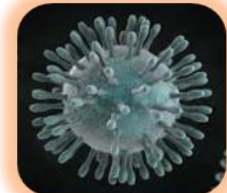
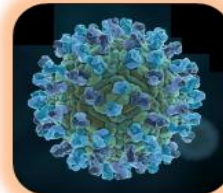
Jeonbuk National University Medical School

Republic of Korea

# Emerging infectious diseases: Constant threats



The significance of  
vaccine development  
cannot be overstated



# Our vaccine development approach



1. **Development of a prophylactic mRNA vaccine for SFTS**
2. **Development of a prophylactic gut microbe vaccine for COVID-19**

# Project 1

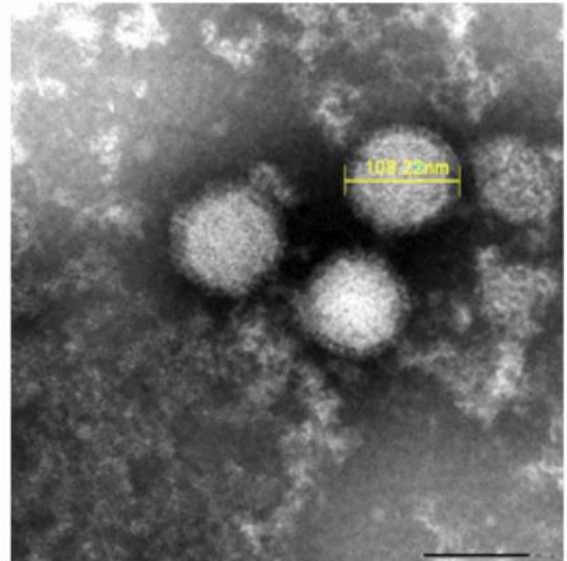
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## Development of a prophylactic mRNA vaccine for SFTS

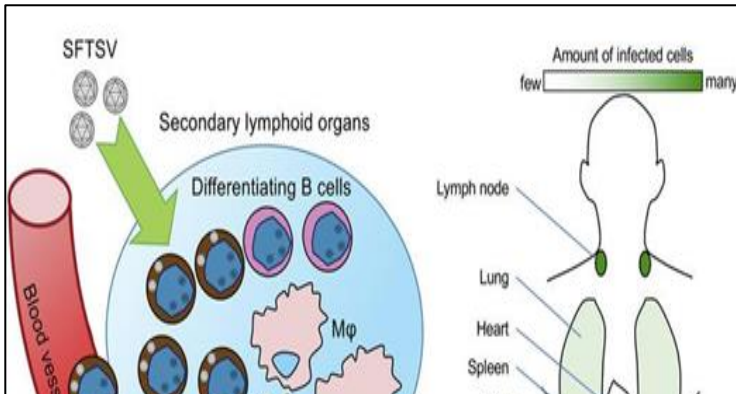
# Severe fever with thrombocytopenia syndrome (SFTS)

## Severe fever with thrombocytopenia syndrome (SFTS)

- ✓ Caused by SFTS virus
- ✓ A tick-borne viral disease



# SFTS : Serious infectious disease



- ✓ High fever
- ✓ Vomiting
- ✓ Diarrhea
- ✓ Multiple organ failure

To overcome the current SFTS threat,  
development of a prophylactic vaccine  
for SFTS is urgently needed

thrombocytopenia

leucopenia

elevated liver enzyme

levels

endemic in Far East

# The Current Trend of Vaccine Development: mRNA

## THE COVID-19 VACCINE RACE

PRE-CLINICAL

**184**

vaccines

are being explored in lab experiments and animals

PHASE 1

**30**

vaccines

are undergoing safety tests in healthy young individuals

PHASE 2

**28**

vaccines

are being tested in broader groups of people

PHASE 3

**21**

vaccines

are in large international trials to test their impact on COVID-19

APPROVED

**13**

vaccines

are approved and licensed for general use

PHASE 4

**5**

vaccines

are being monitored in the wider population after being approved

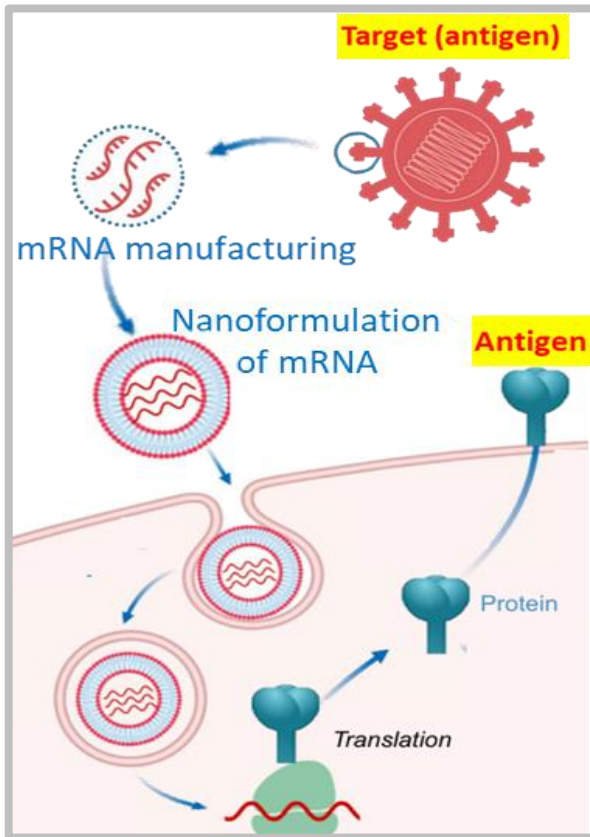
mRNA 백신

Source: COVID-19 Vaccine Tracker

Inactivated virus vaccines  
weakened virus vaccines  
Protein-based vaccines  
Viral vector vaccines  
DNA vaccines

**mRNA vaccines**

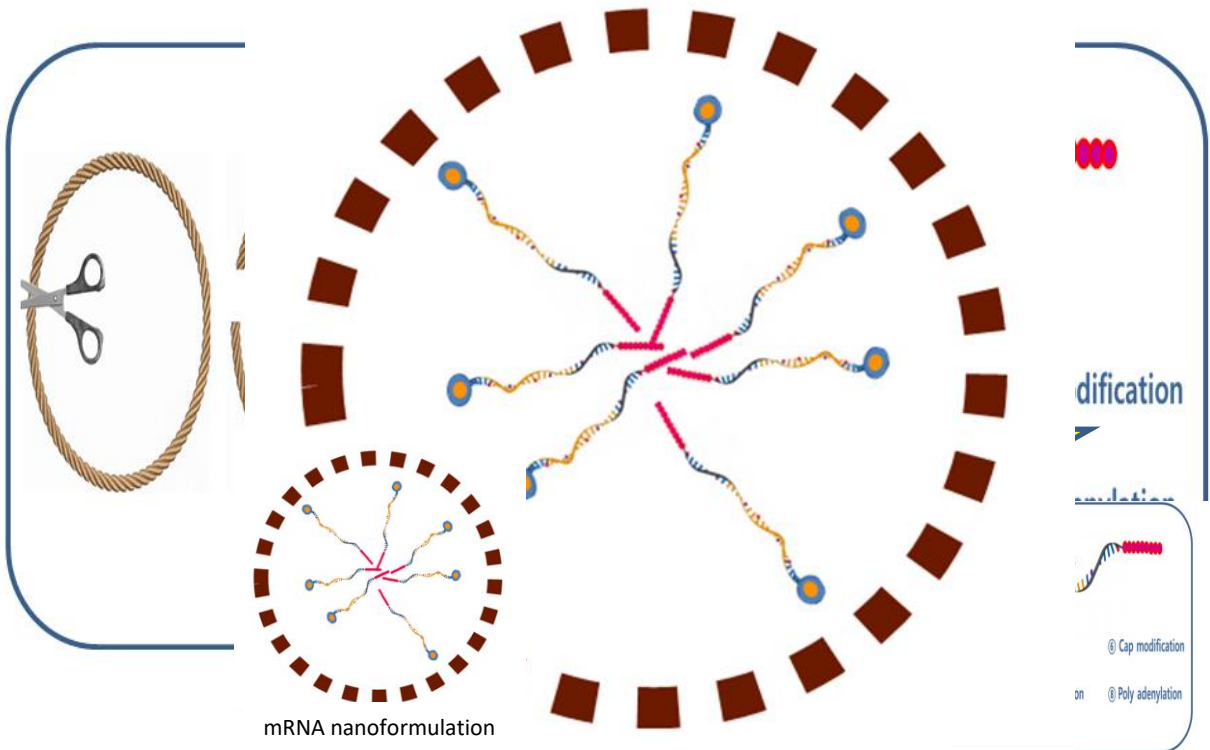
# The Principle and Advantage of mRNA Vaccine



- ❑ Development period: **Much faster** than those of traditional vaccines
- ❑ Prophylactic efficacy: **Much better** than those of traditional vaccines



# mRNA vaccine synthesis: 4 steps



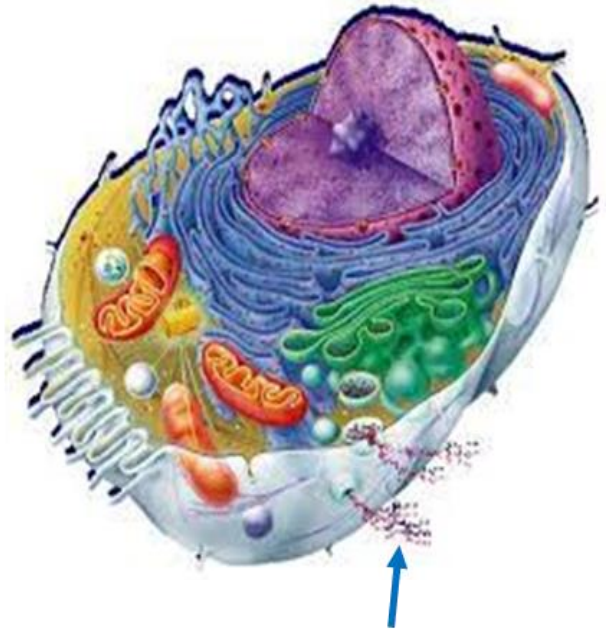
mRNA nanoformulation

mRNA nanoformulation

# The Concept of mRNA vaccine design



Membrane protein



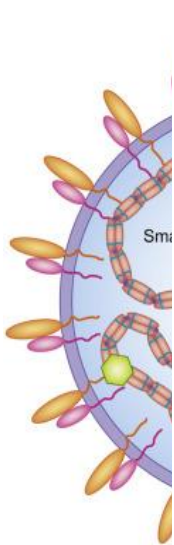
Secretory protein

# The example mRNA Vaccine Design

Moderna Pfizer	ATGTTCTGTTCTCTGGTGTCTGCCCTGGTAGCAGCCAGTGCCTGAACCTGACCACC ATGTTCTGTTCTCTGGTGTCTGCCCTGGTAGCAGCCAGTGCCTGAACCTGACCACC *****	60 60	Moderna Pfizer	CAGCCACCAGGAGCATCTGGTGGTCTCCCAACATCACCAACTGTGCCCTTCGGCGAG CAGCCACCAGTTCATCTGGTGGTCTCCCAATATCACCAACTGTGCCCTTCGGCGAG *****	1020 1020
Moderna Pfizer	CGGACCAGCTGCCACAGCTACACCAACAGCTTACCCTGGGGCGTCTACTACCCCGAC AGAACACAGCTGCCCTCAGCTACACCAACAGCTTACCAGAGGCGGTACTACCCCGAC *****	120 120	Moderna Pfizer	GTGTTCAACGCCACCGGTTGCGCAGGTGTACGCCTGGAACTCGAAGCGGATCAGAAC GTGTTCAATGCCACAGATTGCTCTGTGACGCCTGGAACTCGAAGCGGATCAGCAAT *****	1080 1080
Moderna Pfizer	AAGGTGTTCCGGAGCAGCTCTGCACAGCACCAGGACTGTTCTTCCCTTCTTCCAGC AAGGTGTTCCAGATCCAGCTGCTGCACTCTACCCAGGACTGTTCTTCCCTTCTTCCAGC *****	180 180	Moderna Pfizer	TGGTGGCCGACTACAGCTGCTGTACAAAGCGCCACTTTCAGCATCTCAAGTGTAC TGGTGGCCGACTACTCTGGTGTACAACTCGCCAGCTTACAGCATCTCAAGTGTAC *****	1140 1140
Moderna Pfizer	AACCTGACCTGGTTCACGCCATCACGTGAGCGGCACCAAGCGGCTCAAGCGGTTTCGAC AACCTGACCTGGTTCACGCCATCACGTGTCGGCACAATGGCACAAGAAATTCCGAC *****	240 240	Moderna Pfizer	GGCTGAGCCACCAAGCTGACGACTGTCTTACCAGCTGTACGCCGACAGCTTC GGCTGTCCCTTACCAGCTGACGACTGTCTTACCAGCTGTACGCCGACAGCTTC *****	1200 1200
Moderna Pfizer	AACCCGTGCTGCCCTCAAGCAGCGGTGACTTGCAGCACCAGAAAGCAACATC AACCCGTGCTGCCCTCAAGCAGCGGTGACTTGCAGCACCAGAAAGTCCAACTC *****	300 300	Moderna Pfizer	GTGATCTGGCGACGAGTGGCGAGATCGACCAGCGCAGCAGGCAAGTCGGCGAC GTGATCGGGGATGAGAGTGGCGAGATTGCCCTGGCAGCAGGCAAGTCGGCGAC *****	1260 1260
Moderna Pfizer	ATCCGGGCTGGATCTTCCGACACCCTGGACAGCAAGCCAGAGCTGCTGATCGTG ATCAGAGGCTGGATCTTCCGACACCACCTGGACAGCAAGCCAGAGCTGCTGATCGTG *****	360 360	Moderna Pfizer	TACAACATAAGCTGCCGACACTTACCAGCTGGTGTACGCCTGGAACGCAACAAC TACAACATAAGCTGCCGACACTTACCAGCTGGTGTACGCCTGGAACGCAACAAC *****	1320 1320
Moderna Pfizer	AATAAGCCACCAACCTGGTGTCAAGGTGTGCGAGTTCAGTTCGCAACGACCCCTTC AACAAGCCACCAACCTGGTGTCAAGGTGTGCGAGTTCAGTTCGCAACGACCCCTTC *****	420 420	Moderna Pfizer	CTAAGCCCTTTCAGCGGGACATCAGCAGAGATCTACCAAGCGGCTCCACCCCTTGC CTAAGCCCTTTCAGCGGGACATCTCAGCAGAGATCTACCAAGCGGCTCCACCCCTTGC *****	1440 1440
Moderna Pfizer	CTGGGGGTGTACTACCAAGAACAACAAGAGCTGGATGGAGAGGAGTTCGGGGTAC CTGGGGGTGTACTACCAAGAACAACAAGAGCTGGATGGAAAGGAGTTCGGGGTGTACT *****	480 480	Moderna Pfizer	AACGGGTGGAGGGCTCAACTGCTACTTCCCTTCGACAGCTAGGGCTTCAGCCCAAC AACGGGTGGAGGGCTCAACTGCTACTTCCCTTCGACAGCTAGGGCTTCAGCCCAAC *****	1500 1500
Moderna Pfizer	AGCAGCGCAACAACCTGACCTTGAAGTACGTGAGCAGCCTTCTGATGACCTGGAG AGCAGCGCAACAACCTGACCTTGAAGTACGTGAGCAGCCTTCTGATGACCTGGAA *****	540 540	Moderna Pfizer	AACGGCTGGGTACAGCCCTACCGGCTGGTGGTGTGAGTTCGAGCTGTGCAAGCC AATGGCTGGGATTCAGCCCTACAGAGTGGTGGTGTGAGTTCGAGCTGTGCAAGCC *****	1560 1560
Moderna Pfizer	GGCAAGCAGGGCAACTCAAGAACCTGCGGGAGTCTGTTTCAAGAATCAGCGGCTAC GGCAAGCAGGGCAACTCAAGAACCTGCGGGAGTCTGTTTCAAGAATCAGCGGCTAC *****	600 600	Moderna Pfizer	CCAGC CACCGTGTGTGCCCAAGAAGGACCAACTGGTGGAGCAAGTGGTGAAC CCTGCACAGTGTGCCCTCAGAAAGACCAACTCTGCGAAGCAAAATGGTGAAC *****	1620 1620
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Moderna Pfizer	CTGCTGGCCCTGCAGGAGCTACTGACCCAGGAGCAGCAGCAGCGGGTGGACAGCA CTGCTGGCCCTGCAGGAGCTACTGACCCAGGAGCAGCAGCAGCGGGTGGACAGCA *****	780 780	Moderna Pfizer	ACCTTGGAGATCTGGACATCACCCCTTGCAGCTTGGCGGGGTGAGGCTGATCACCCA ACACTGGAAATCTGGACATCACCCCTTGCAGCTTGGCGGGGTGTGTGATCACCCCT *****	1800 1800

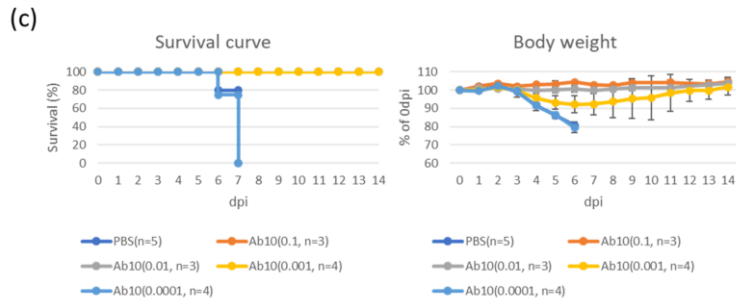
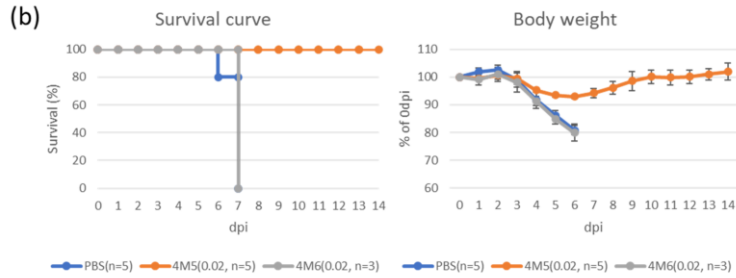
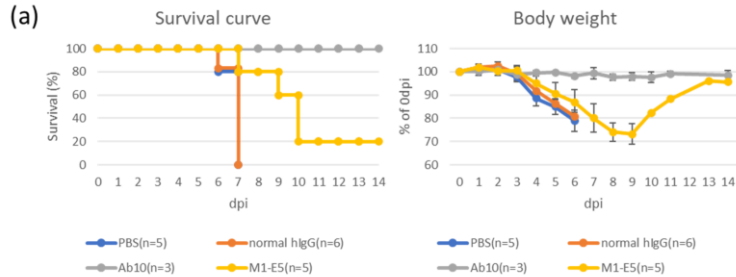
# The Target

GN : the



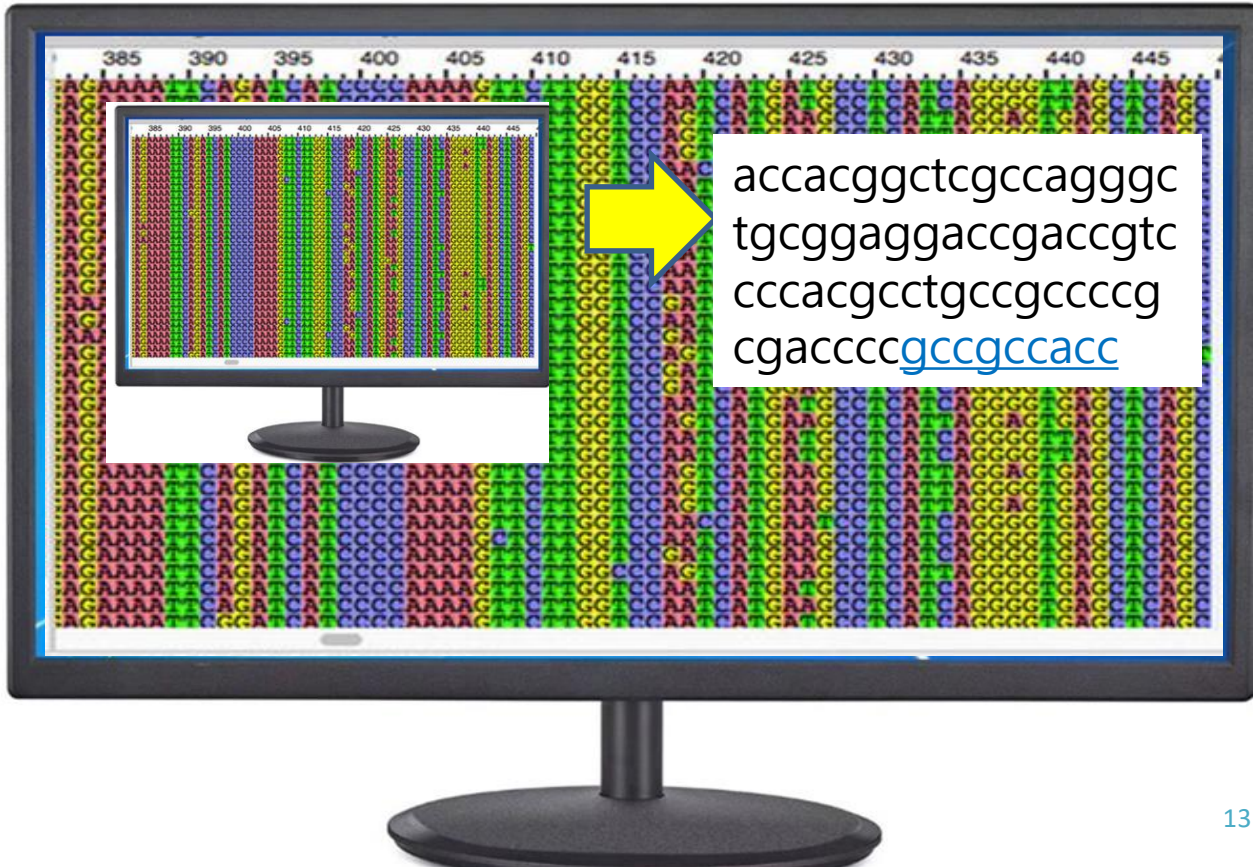
Js: GN

of GN



Shimojima et al., Viruses 2022, 14(8), 1665

# mRNA Vaccine design (1): 5'-UTR design



# mRNA Vaccine design (2): Signal peptide design

**Aliphatic index, positive charge and GRAVY: protParam server**

Aliphatic index    GRAVY

Hydrophobic

Cleavable Site

Positive Charge

n region 3-5 a.a    h region 7-15 a.a    c region 3-5 a.a

Signal Peptide (13-25 a.a)

Nascent Protein

COOH

SignalP 4.1 prediction graph (relative frequency)

SignalP 4.1 prediction graph (probability)

Secretion sorting: PRED-TAT

Sub-cellular localization site: protCompB

n, h and c regions, signal peptide probability and cleavable site: SignalP server version 4.1

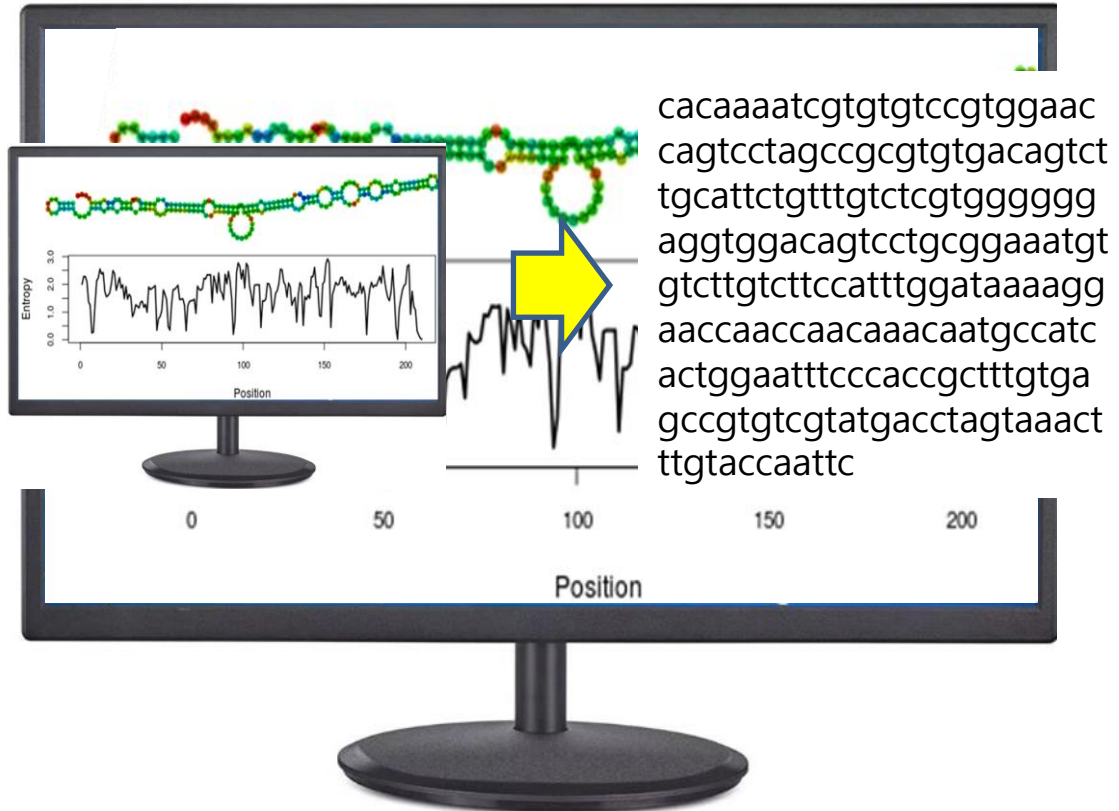
Position

tpB

n, h and c regions, signal peptide probability and cleavable site: SignalP server version 4.1

aagtgggtaacct  
ttattcccttcttt  
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cttattcc

# mRNA Vaccine design (3): 3'-UTR design



# mRNA Vaccine design (4): Codon Optimization

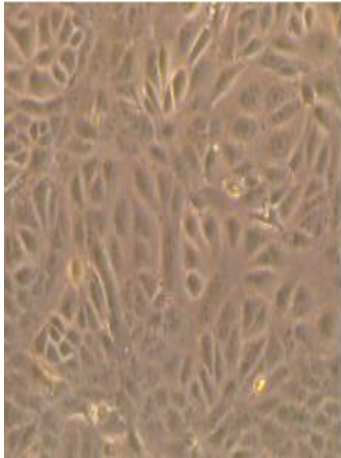
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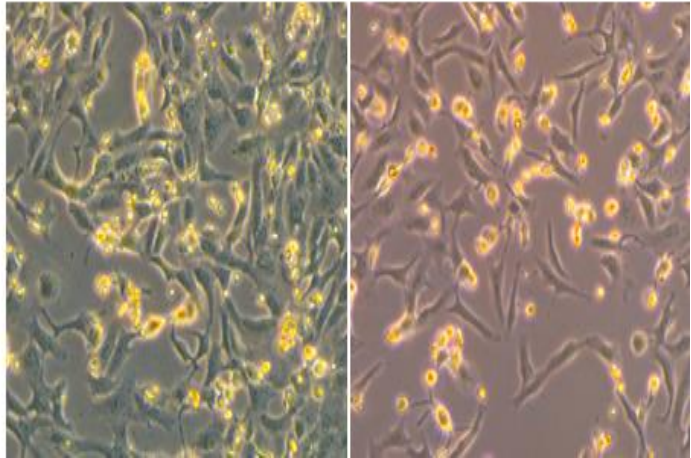


# The Culture of SFTS Virus

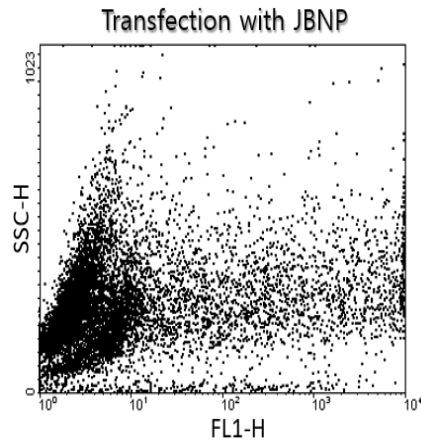
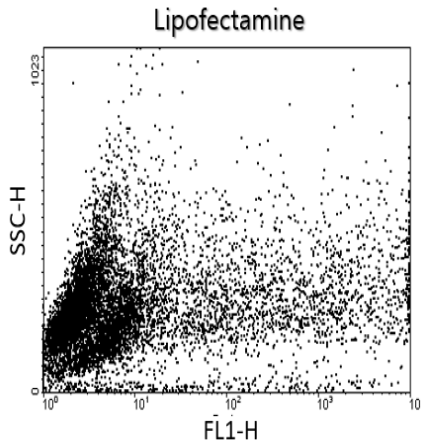
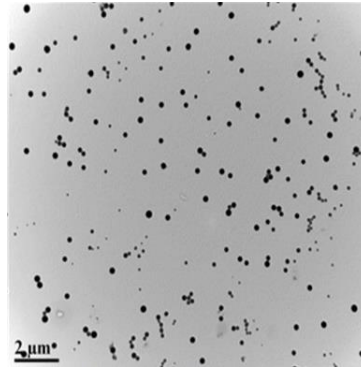
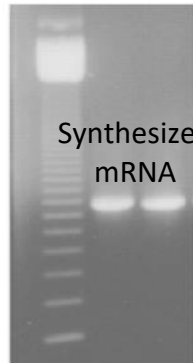
Non-infected  
healthy Vero Cells



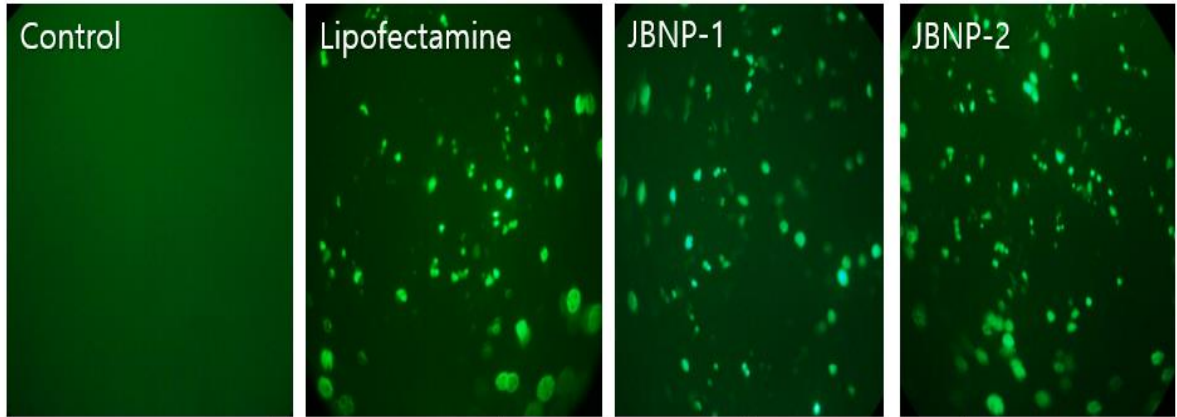
The Vero cells infected  
with SFTS virus



# Nanoparticle for mRNA vaccine

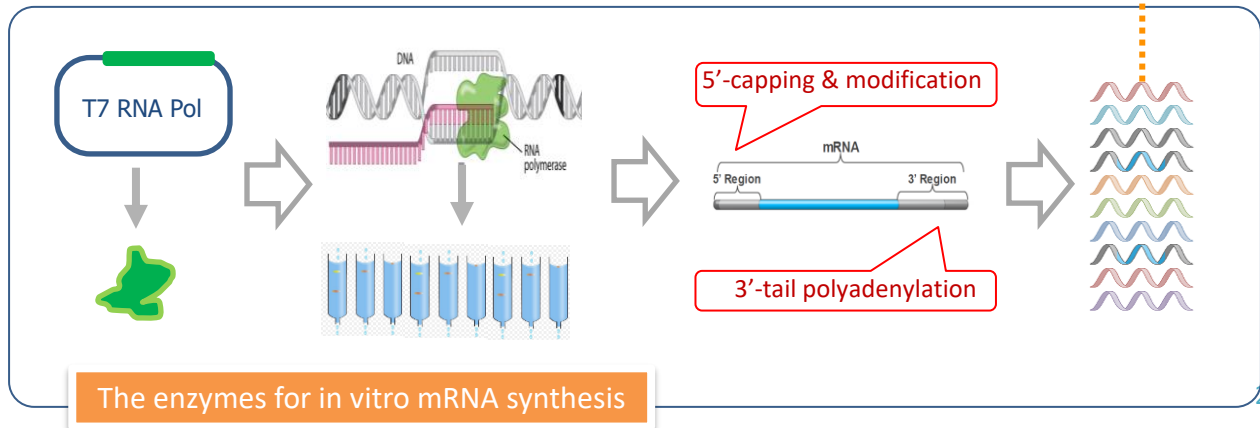
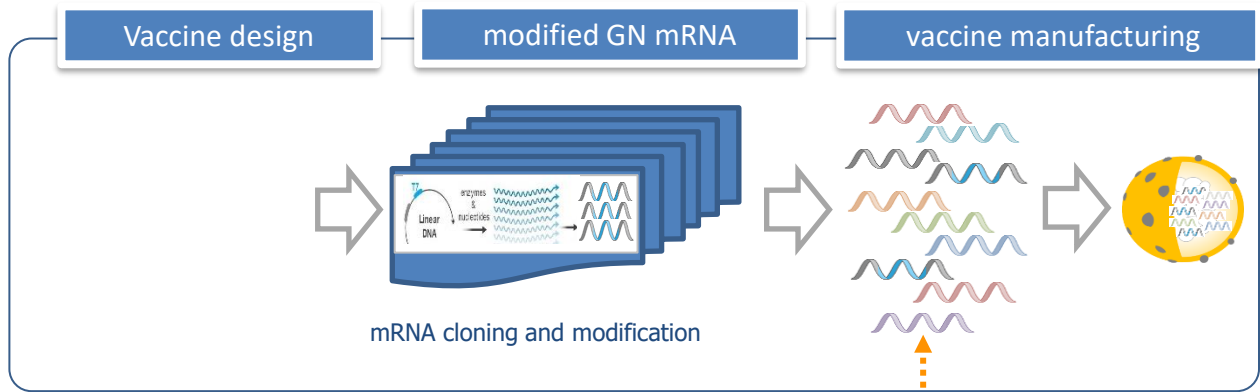


# Transfection Efficiency of Nano-mRNA



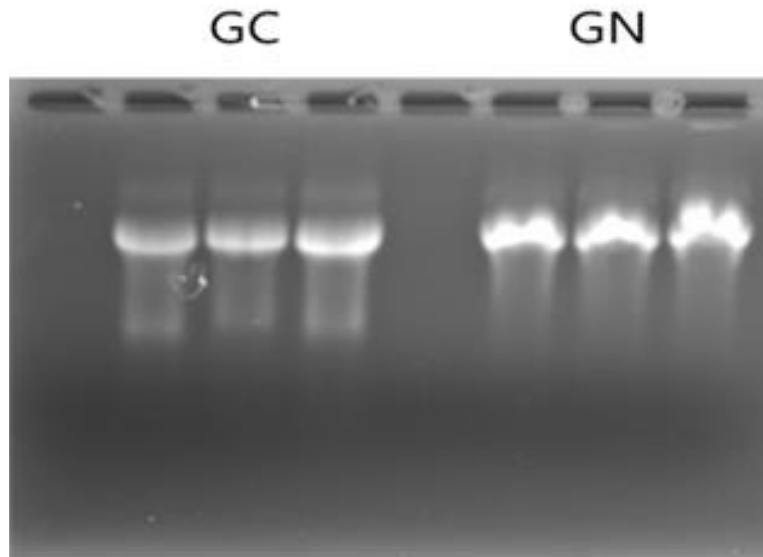
Carrier	Control	Lipofectamine	JBNP-1	JBNP-2
Plating 세포 수	$6 \times 10^4$ /ml	$6 \times 10^4$ /ml	$6 \times 10^4$ /ml	$6 \times 10^4$ /ml
Live cells after 40h	$4.25 \times 10^5$ /ml	$7.5 \times 10^4$	$3.1 \times 10^5$	$2 \times 10^5$
Viability (%)		17%	73%	47%
유전자 전달효율	-	(60~)70%	78%	85%

# The enzymes required for in vitro mRNA synthesis



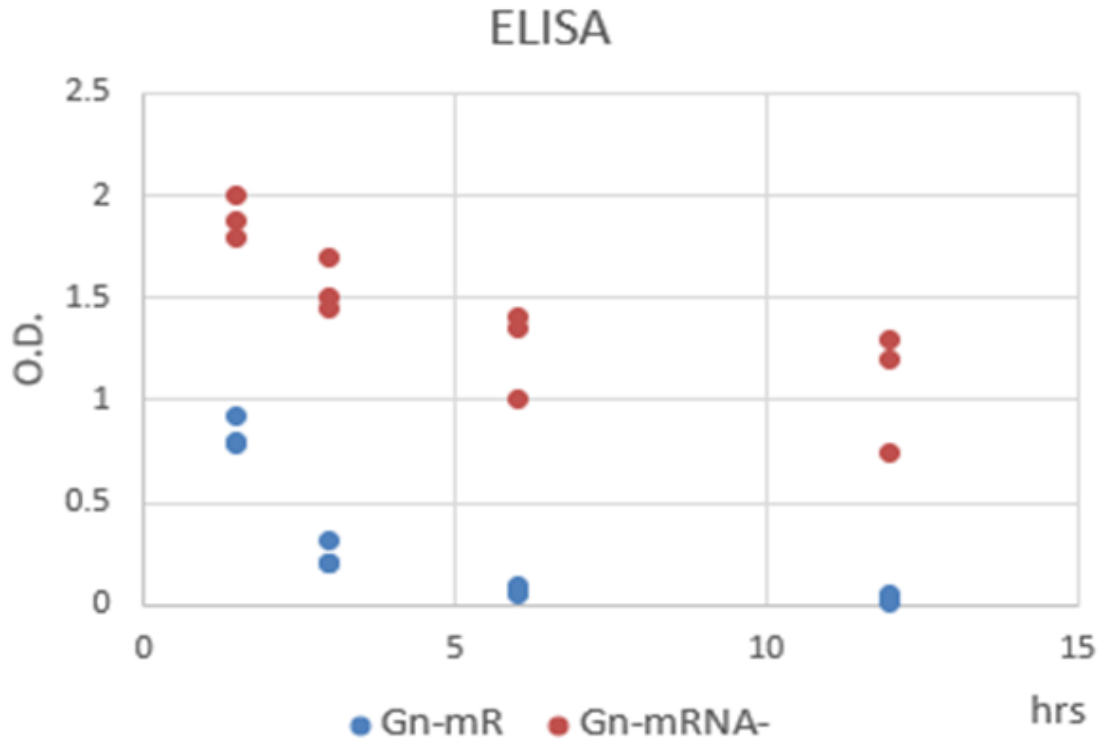
The enzymes for in vitro mRNA synthesis

# The enzymes for in vitro mRNA synthesis

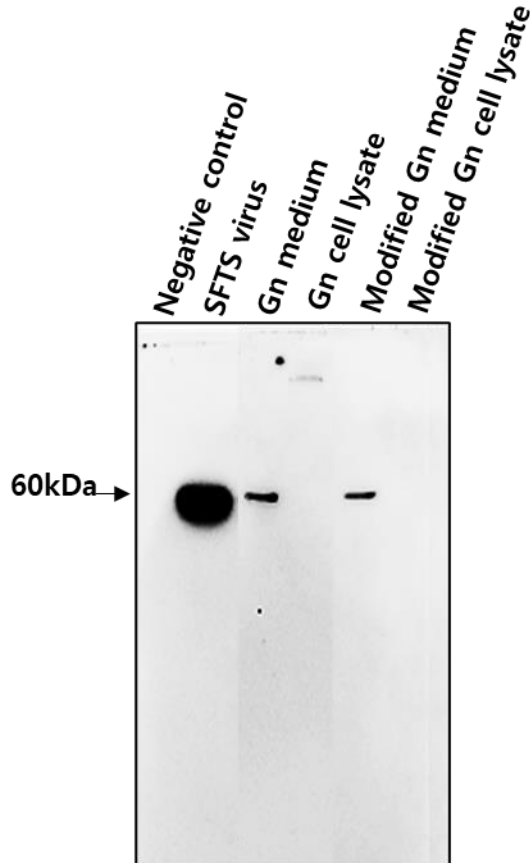


RNA triphosphates  
RNA guanine  
RNA guanine  
methyltransferase

# GN Expression from the SFTS mRNA Vaccine Candidate



# The Expressed GN from the SFTS mRNA Vaccine Candidate



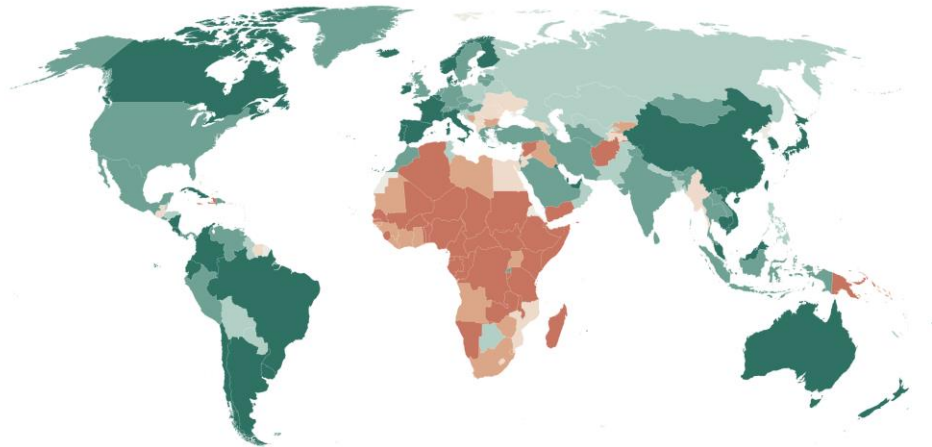
# Project 2

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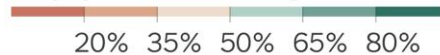
## Development of a prophylactic gut microbe vaccine for COVID-19



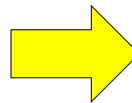
# Vaccination vs COVID-19 Incidence



Share of population receiving at least one dose

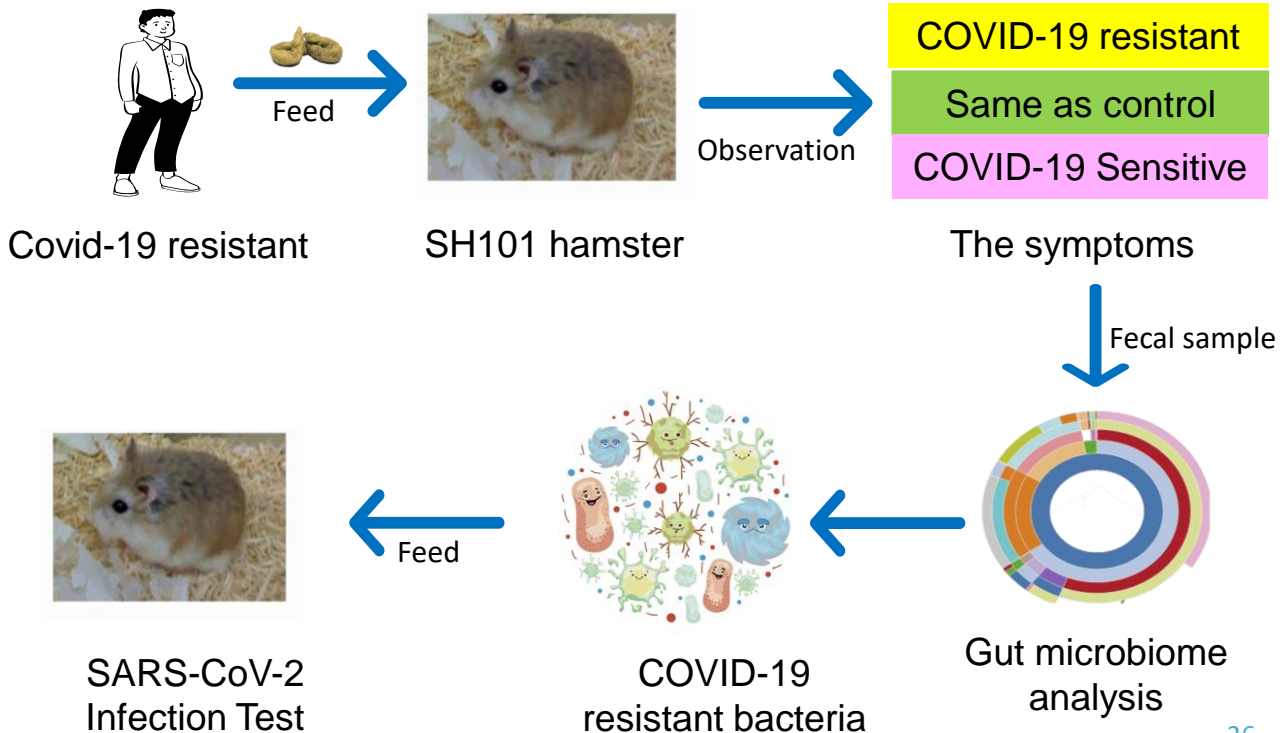


**No correlation  
between vaccination  
& incidence rate of  
COVID-19**

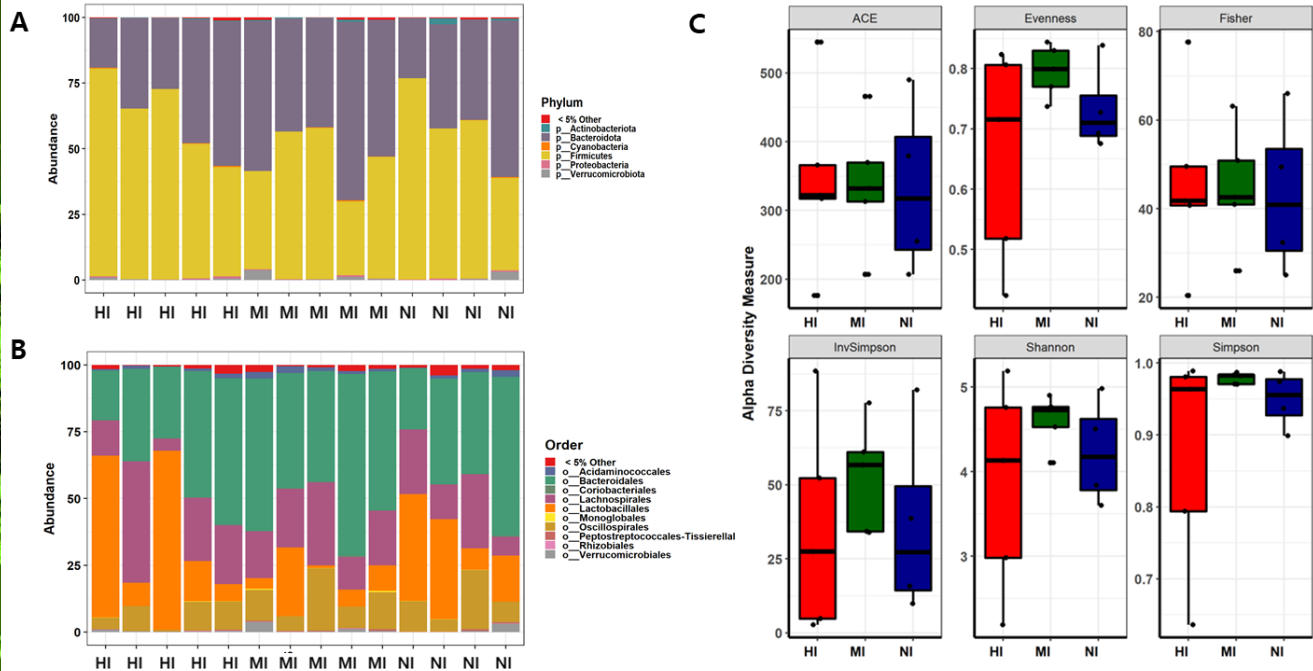


**Gut microbiome  
may play a role in  
protecting COVID-19**

# Development Procedure for The Gut Microbe vaccine

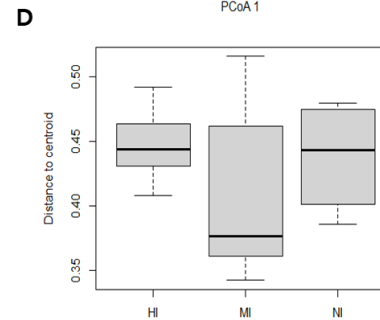
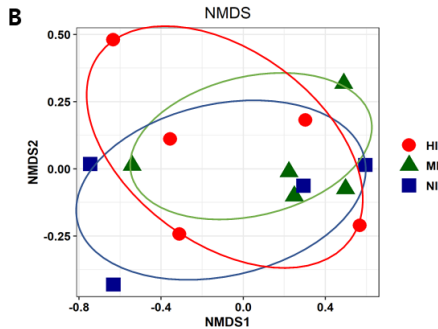
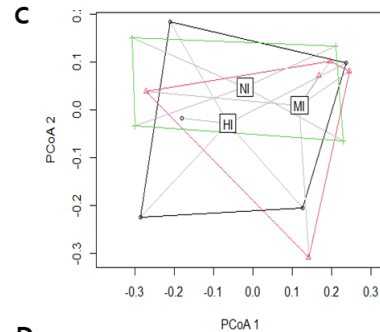
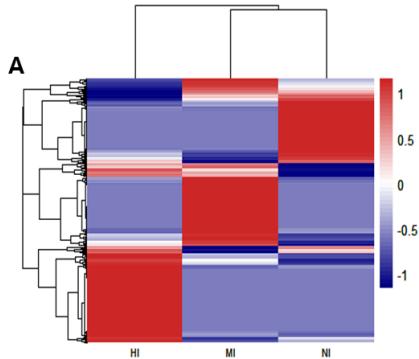


# Abundance and $\alpha$ -diversity



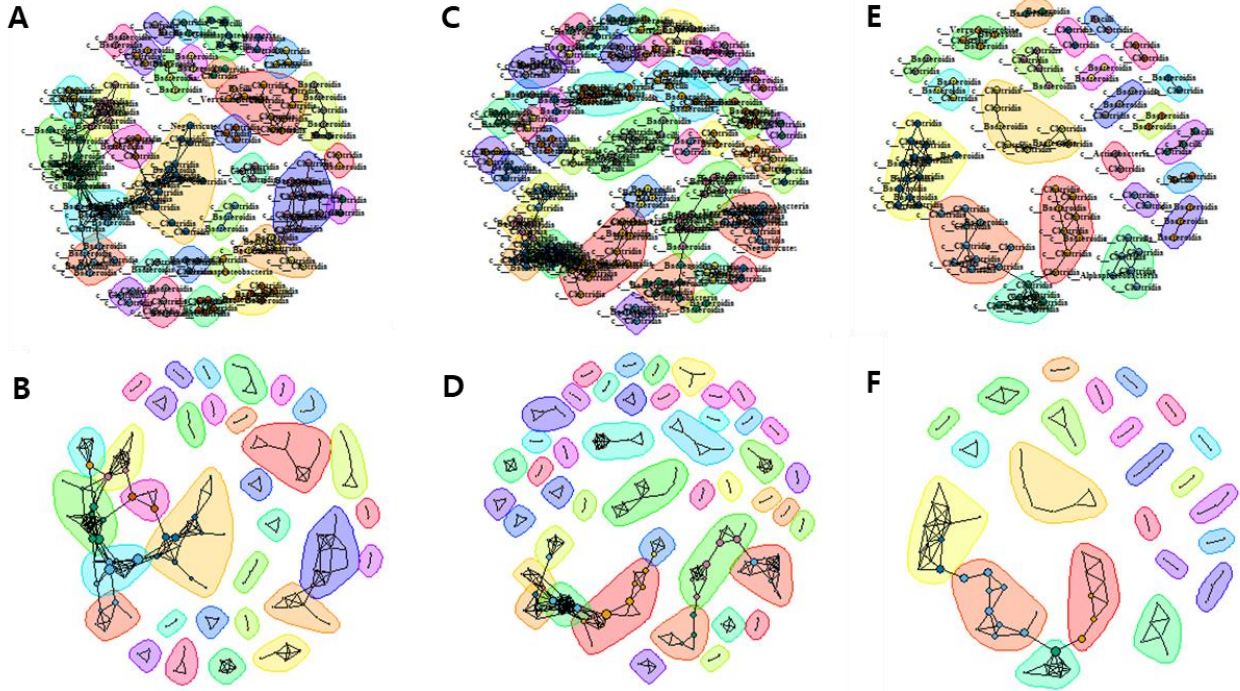
HI, highly infected group; MI, same infectivity with control;  
NI, non infected group

# $\beta$ -diversity



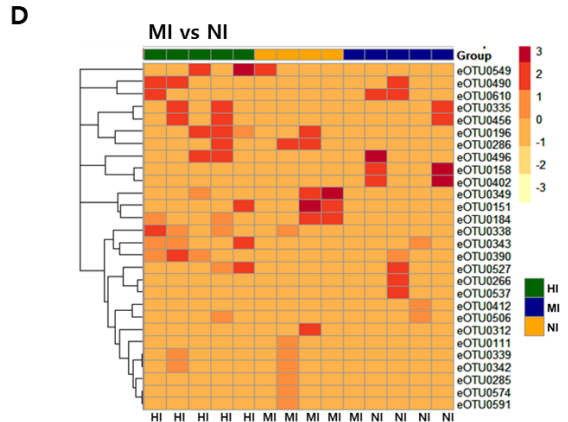
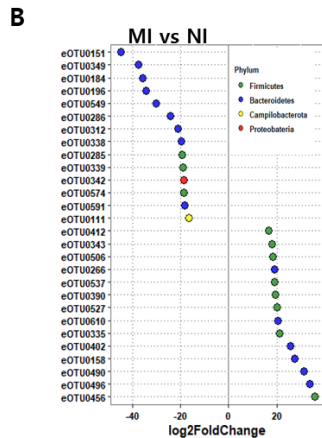
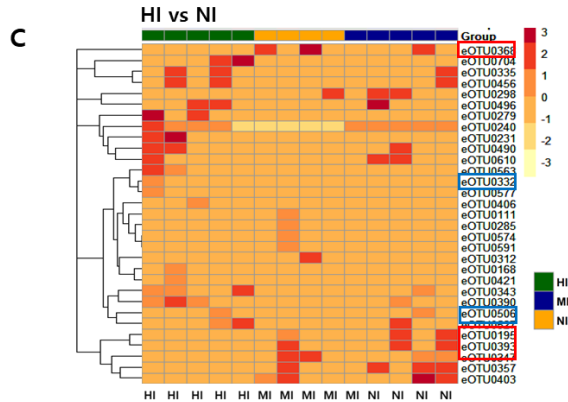
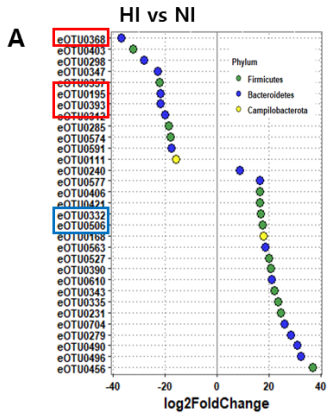
HI, highly infected group; MI, same infectivity with control;  
NI, non infected group

# Co-occurrence Network Analysis

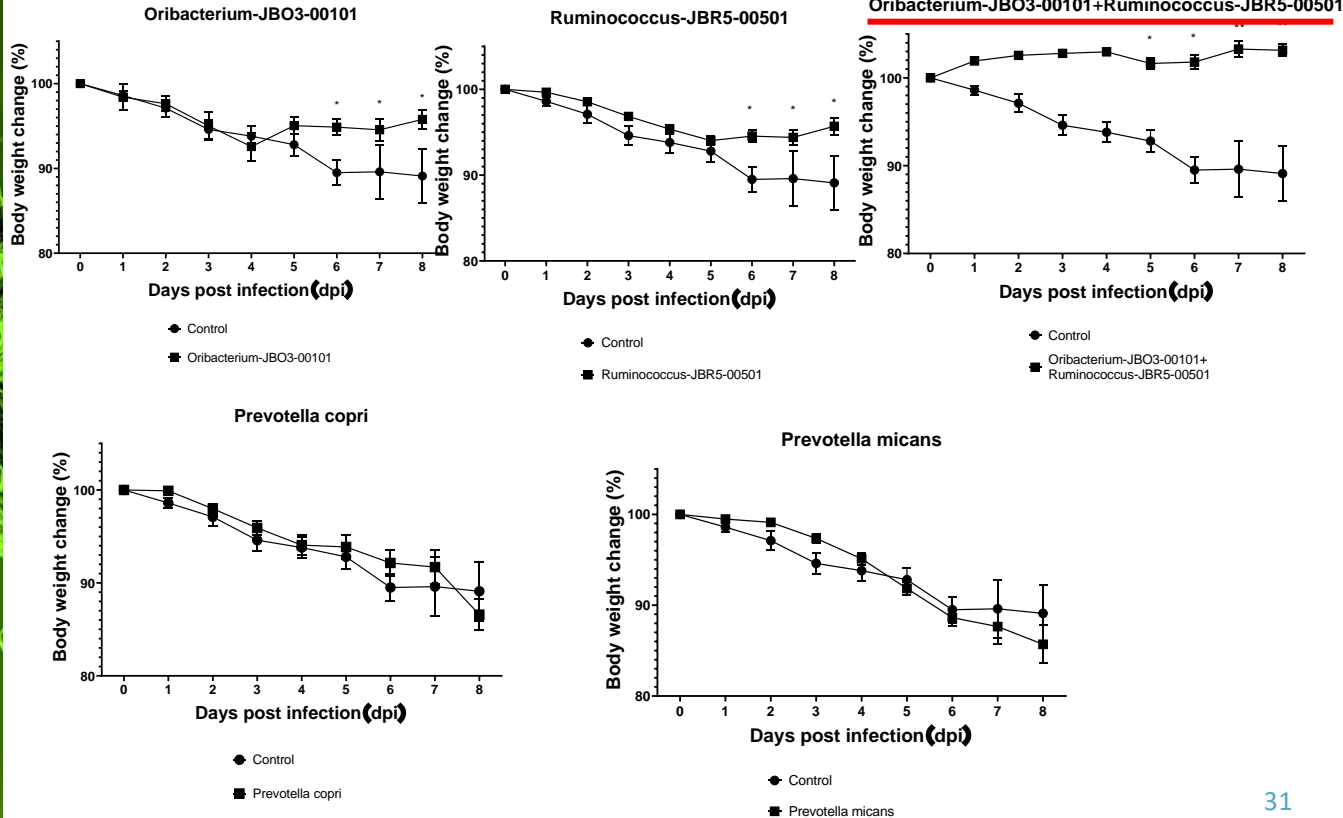


HI (A-B), MI (C-D), and NI (E-F); HI, highly infected group;  
MI, same infectivity with control; NI, non infected group

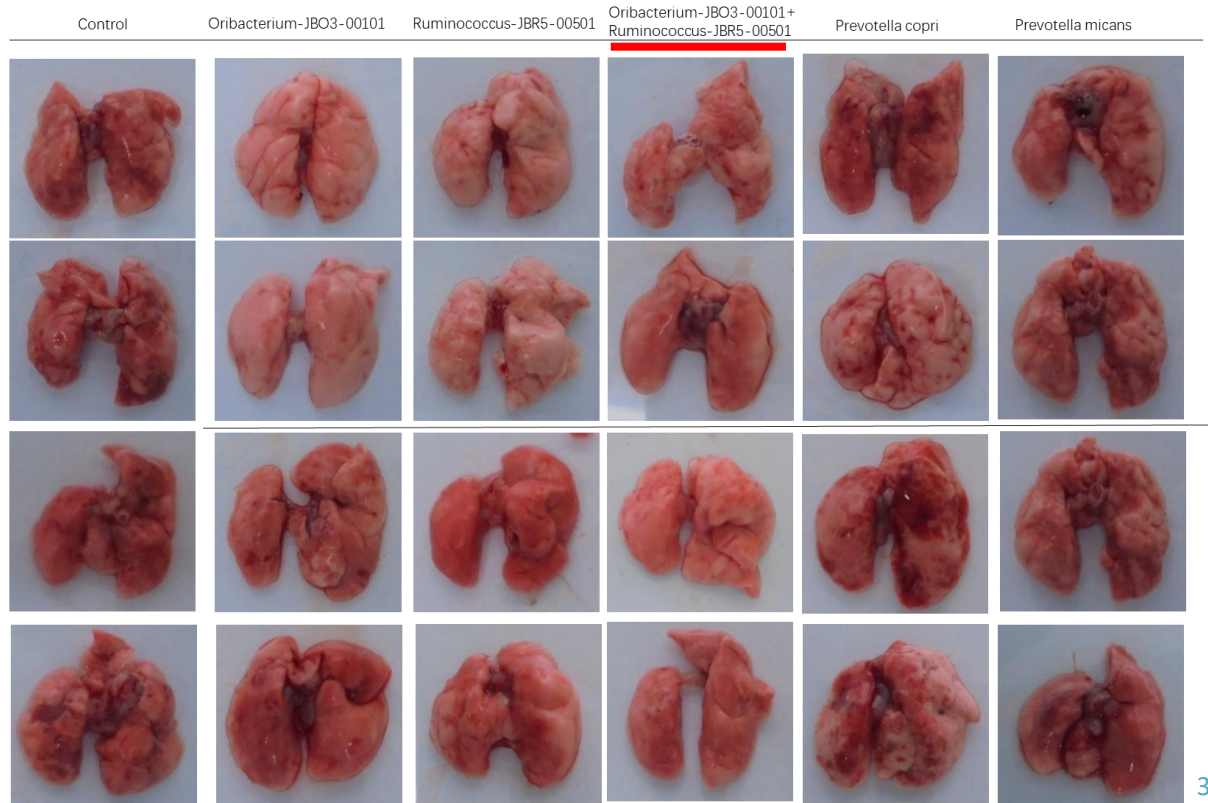
# DESeq2 Differential abundance analysis



# Body Weight Change after Infection



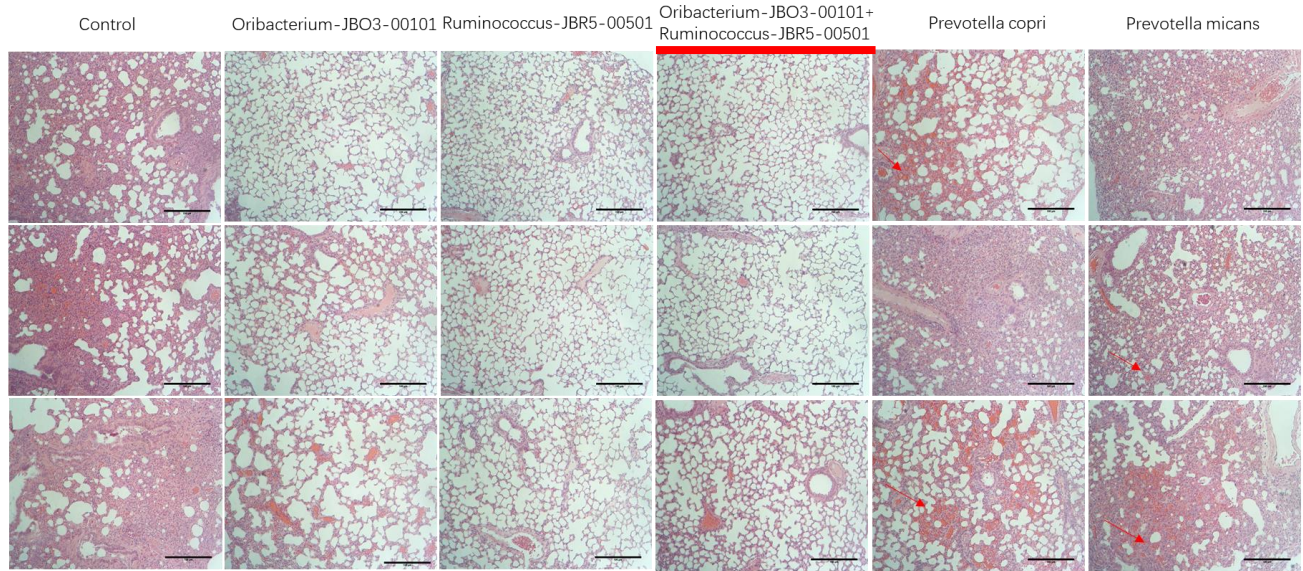
# The lungs of the infected hamsters



8dpi Photographs of the lungs

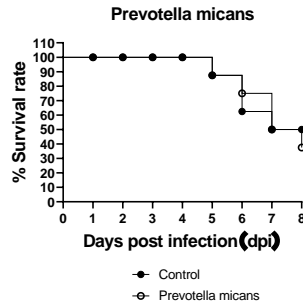
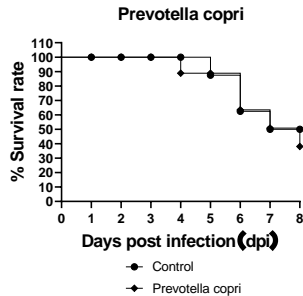
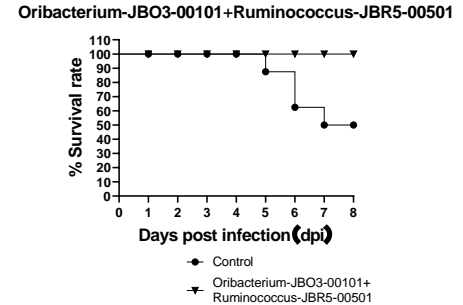
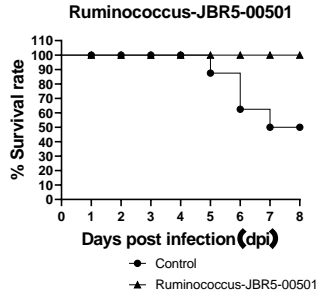
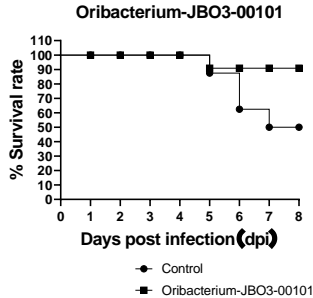


# The histological image of the of the infected hamsters

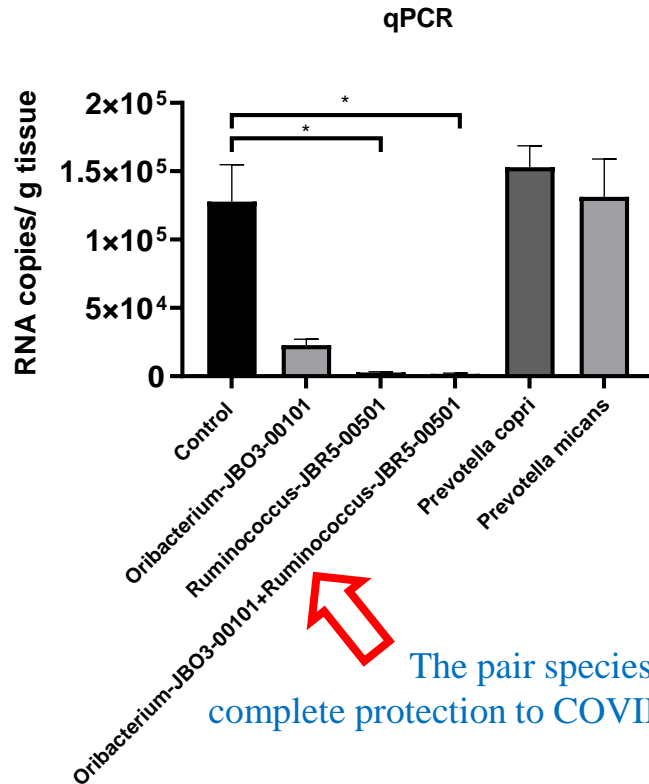


Scale Bar:100um

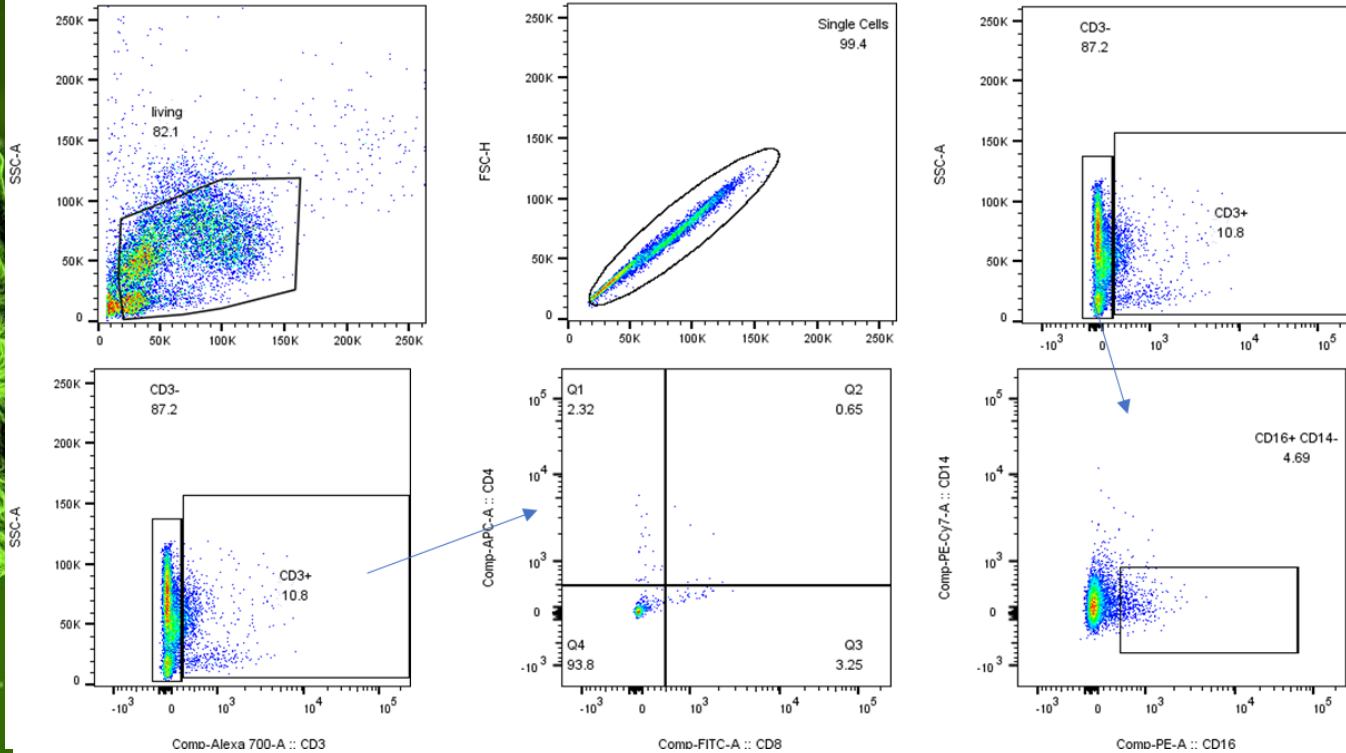
# The survival rate of the of the infected hamsters



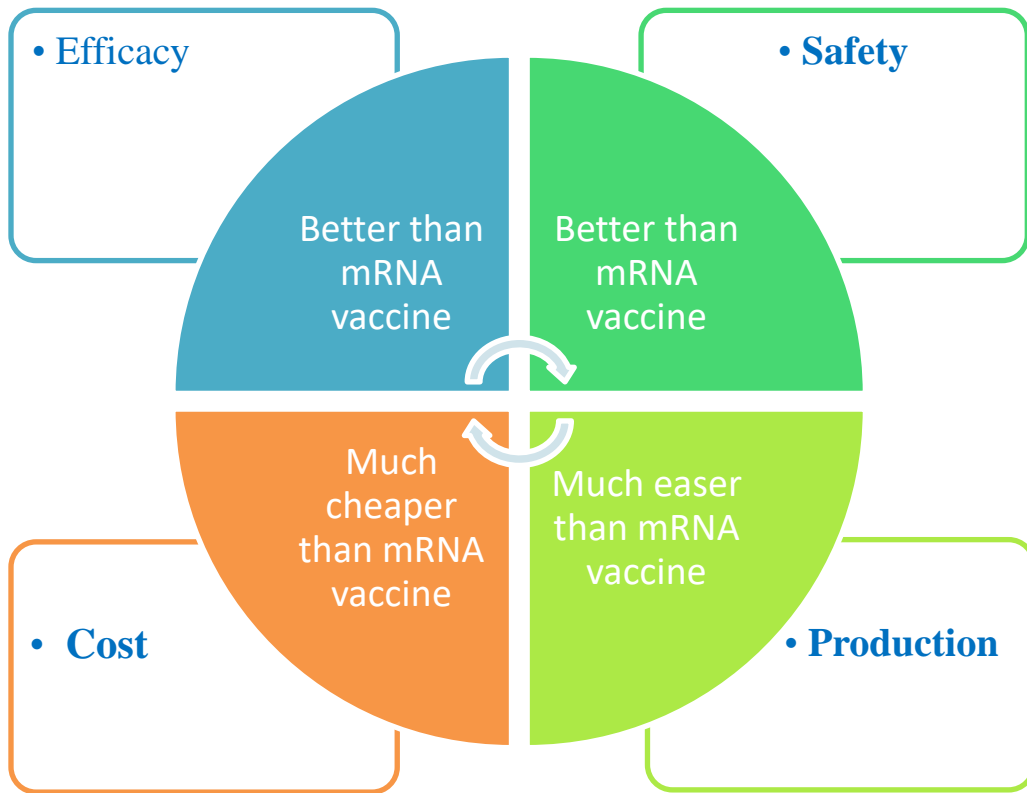
# RT-PCR Result of the infect hamster



# Flow Cytometry Analysis



# Prospect of the COVID-19 gut microbe vaccine



# THANK YOU

