SYNTHGENE

Animal mRNA Vaccine Platform

mRNA Technology for Veterinary Vaccines

www.synthgene-bio.com

Synthgene's Five mRNA Technology Platforms

mRNA Sequence Design & Optimization Platform

- Antigen design, mRNA sequence design and optimization, including cap, 5' UTR, CDS, 3' UTR, Poly A, and base modification

Nucleic Acid Chemistry Platform to Support Low-cost mRNA Raw Materials

- Royalty-free cap analogs with seven patents covering novel cap analogs and formulations
- Animal-origin-free NTPs, modified NTPs and cap analogs
- Drug Master File (DMF) on selected cap analogs, NTPs and modified NTPs
- 150+ modified NTPs to meet the needs of different projects

mRNA In-Vitro Transcription (IVT) and LNP Encapsulation Platform

- IVT synthesis up to 1L
- LNP encapsulation up to 1L

Analytical Testing Platform

- In-house mRNA analytical testing, including LC-MS based capping and tailing assays
- In-house LNP analytical testing, including HPLC-based lipid identity and concentration, particle size, PDI and zeta potential

Animal Testing Platform for mRNA Vaccines

- Cell based protein expression analysis
- Evaluation of humoral and cellular immune response in mice
- Animal model evaluation

CONTENT

Pain Points in Animal Disease Prevention and Control

Diversity of pathogens and issue with constant mutation → **Difficulty in isolating and culturing certain pathogens** → **Poor vaccine effectiveness** → **Poor vaccine safety** → **Urgent need to control cost**

- **Foot-and-mouth disease vaccine (FMDV):** There are many strains of the virus, and current vaccines offer insufficient cross-protection. Additionally, synthetic peptides and inactivated vaccines cannot fully activate the immune response.
- **Rabies vaccine (RV):** Inactivated vaccines require multiple doses to be effective, and existing commercial rabies vaccines are expensive.
- **Porcine reproductive and respiratory syndrome vaccine (PRRSV):** The PRRS virus exhibits high mutation rate, and existing vaccines offer poor cross-protection. Inactivated vaccines show poor protection, and live attenuated vaccines carry the risk of ADE effects and recombination with field strains.
- **African swine fever vaccine (ASFV):** The virus is highly pathogenic and possesses a complex genome with intricate invasion, pathogenicity, and immune escape mechanisms. Inactivated vaccines offer poor protection, and the development of safe live attenuated vaccines remains challenging.

Animal mRNA Vaccines

New Approach for Animal Disease Prevention and Control

	Inactivated Vaccine	Attenuated Live Vaccine	Recombinant Subunit Vaccine or Synthetic Peptide Vaccine	Recombinant Live Vector Vaccine	DNA Vaccine	RNA Vaccine
Strong humoral immune response	◉◉	◉◉◉	◉◉	◉◉◉	◉◉	◉◉◉
Strong cellular immune response	◉	◉◉	◉	◉◉◉	◉◉	◉◉◉
Rapid development and production	◉◉	◉	◉◉	◉◉	◉◉◉	◉◉◉
Discrimination between infected and vaccinated animals	◉	◉	◉◉◉	◉◉◉	◉◉◉	◉◉◉
Ease of synthesizing multivalent vaccines	◉	◉	◉◉	◉◉	◉◉◉	◉◉◉
Infectivity	✕	◉◉	✕	◉◉	✕	✕
Risk of genetic integration or mutation	✕	◉	✕	◉	◉◉	✕
Is adjuvant needed?	◉	✕	◉	✕/◉	✕/◉	✕

- Short R&D Cycle
- Safety
- Low Manufacturing Cost
- Easy to Manufacture (No cells involved)
- High Potency
- Elicit both Cellular Immunity and Humoral Immunity

Synthgene Provides Comprehensive Solution for Animal mRNA Vaccines

Synthgene has years of experience developing veterinary mRNA vaccines with vaccine candidates, such as RV, IBRV, BVDV, PRRSV, PEDV, FMDV, PRV, FIPV, FCV, FPV, and FHV. Our solutions include vaccine candidate development, raw materials for mRNA synthesis, vaccine manufacturing process development, analytical development and pre-clinical testing and development.

Vaccine Candidate Development

Design mRNA sequences for antigens specific for prevailing viral strains.

To enhance immune response, these mRNA constructs can include multiple antigens, antigen mutations, signaling peptides and co-stimulatory signals.

Our proprietary algorithms then optimize the entire sequence—from UTRs, CDS, to the cap and poly-A tail—ensuring maximum protein expression.

In-house-produced Raw Material
CAP5011, CAP 5 m7G(5')vppp(5')(2'OMeA)pG
CAP1811, CAP m7G(5')vppp(5')(2'-OMeA)pU
CAP2811, CAP m7(3'-OMeG)(5')vppp(5')(2'-OMeA)pG
CAP3411, CAP m7(3'-OMeG)(5')vppp(5')(2'-OMe,m6A)pG
N1-Me-pUTP
5-OMe-UTP
Cy5-UTP
Fluorescein-12-UTP
.....

T7 Co-transcription kit

Biological Validation

Immunofluorescence (IF) assay

Immunoblotting (WB) assay

Flow cytometry (FCM) assay

Tool mRNAs for LNP Formulation Development
eGFP mRNA with N1-Me-pUTP
Fluc mRNA with N1-Me-pUTP
Fluc-eGFP mRNA with N1-Me-pUTP
mCherry mRNA with N1-Me-pUTP
OVA mRNA with N1-Me-pUTP
.....

Tool mRNAs

mRNA Synthesis / LNP Encapsulation / Analytical Development

We have the capability to conduct IVT synthesis in-house, along with comprehensive process development.

We can perform full panel QC testing on plasmid DNA, mRNA and LNP. Our analytical team can develop construct-specific assays such as LC-MS based capping and tailing assay. We have high-resolution Mass Spec on site as well as Zetasizer and HPLC-CAD to support LNP testing.

Pre-clinical Testing and Development

Stage	Test	Measurement	Method
In-vitro Testing	Protein Expression Verification	Protein Expression	Western Blotting, Indirect Immuno-Fluorescence Test
Mouse Testing	Humoral Immunity	Binding Antibody | Detection	ELISA
		Neutralizing Antibody | Detection	Neutralization Test
	Cellular Immunity	IFN-γ and other cytokines | Detection	Elispot / ELISA
	Challenge Protection	Weight, Body Temperature, Survival Rate, Viral Clearance, and Viral Load	Viral Challenge Study
Target Animal Testing	Humoral Immunity	Binding Antibody | Detection	ELISA
		Neutralizing Antibody | Detection	Neutralization Test
	Cellular Immunity	IFN-γ and other cytokines | Detection	Elispot/ ELISA
	Challenge Protection	Weight, Body Temperature, Survival Rate, Viral Clearance, and Viral Load	Neutralization Test

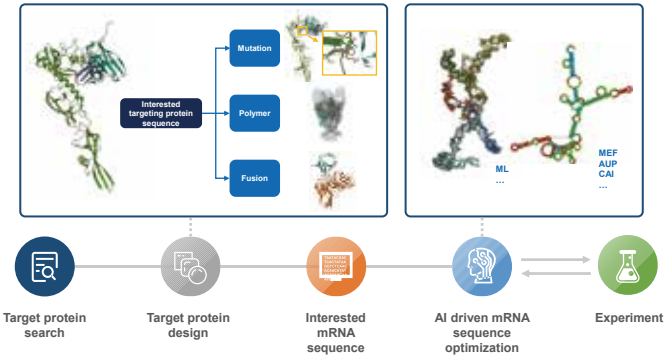
Why Choose Synthgene to Support Your Animal mRNA Vaccine Development?

At Synthgene, we offer end-to-end solutions by manufacturing mRNA vaccines and conducting pre-clinical testing under one roof. This eliminates the need to engage separate companies for manufacturing and testing, streamlining the entire process. With years of experience and hundreds of mRNA vaccine candidates developed, we have the expertise you can rely on. Additionally, since we produce mRNA raw materials in-house, we have greater control over both costs and project timelines.

- **Our novel cap analogs bypass third-party IP, providing a strategic advantage. Additionally, our low-cost GMP-grade cap analogs and NTPs contribute to significant vaccine cost reduction.**

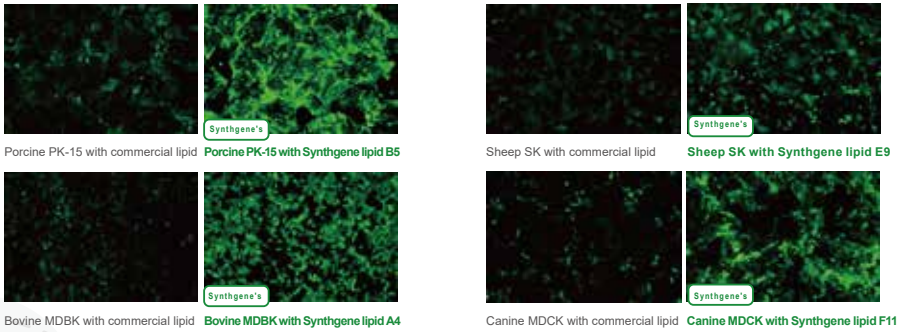
Synthgene's 9 cap analog patents issued in China

- **With years of experience in mRNA sequence design and optimization, we specialize in creating highly effective mRNA constructs tailored to specific therapeutic needs.**



Synthgene can provide mRNA vaccine sequence design and optimization services

- **Extensive capability and experience in LNP formulation development, enabling the creation of efficient and targeted delivery systems for various therapeutic applications.**



Porcine PK-15 with commercial lipid | **Porcine PK-15 with Synthgene lipid B5**

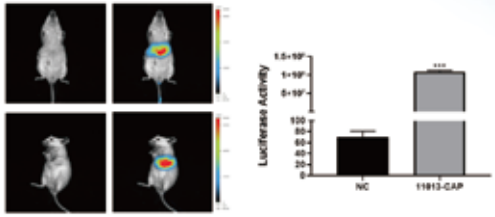
Sheep SK with commercial lipid | **Sheep SK with Synthgene lipid E9**

Bovine MDBK with commercial lipid | **Bovine MDBK with Synthgene lipid A4**

Canine MDCK with commercial lipid | **Canine MDCK with Synthgene lipid F11**

Synthgene's LNP cell transfection results

- **We have the capability to conduct comprehensive pre-clinical testing in both cells and animal models, ensuring thorough evaluation of efficacy and safety before advancing to clinical trials.**



In vivo animal imaging and in vitro results of activity detection in cells

- **Our BSL-3 lab is equipped to safely handle infectious agents, allowing us to test the performance of mRNA vaccines against high-risk pathogens in controlled environments.**

P3 biosafety laboratory helps evaluate the efficacy of animal mRNA vaccines

Case Study

Rabies mRNA Vaccines

Collaborating with an animal health expert in China, Synthgene has designed and manufactured multiple mRNA constructs and tested several LNP formulations. The tables below list the critical quality attributes for five of the mRNA vaccine candidates.

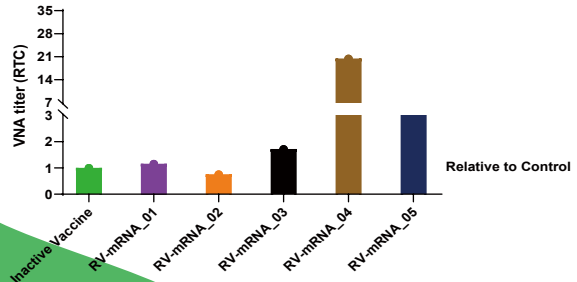
Table 1. RV-mRNA_01~05 mRNA CGE purity ≥ 80%; capping efficiency ≥ 99%

mRNA Name	Con. (ng/μl)	260/280	CGE purity	Capping efficiency	PolyA
RV-mRNA_01	1017.2	1.96	90.6%	99.20%	30A, 69-86A normal distribution
RV-mRNA_02	1118.0	1.99	80.5%	99.70%	30A, 69-83A normal distribution
RV-mRNA_03	984.7	1.95	81.5%	99.70%	30A, 70-83A normal distribution
RV-mRNA_04	994.2	1.95	86.5%	99.60%	30A, 69-83A normal distribution
RV-mRNA_05	967.6	1.95	80.8%	99.10%	29-32A, 70-79A normal distribution

Table 2. RV-mRNA_01~05 mRNA-LNP: particle size 80~120nm; PDI≤0.14, encapsulation efficiency ≥90.0%

Name	Particle size (nm)	PDI	Total mRNA con. (μg/ml)	Free mRNA con. (μg/ml)	Encapsulation efficiency (%)
RV-mRNA_01-LNP	113.5	0.07	99.76	8.14	91.84
RV-mRNA_02-LNP	114.6	0.14	119.25	8.49	92.88
RV-mRNA_03-LNP	94.76	0.09	120.71	3.15	97.39
RV-mRNA_04-LNP	88.16	0.01	128.33	3.22	97.49
RV-mRNA_05-LNP	95.25	0.13	138.50	3.50	97.48

Of the five vaccine candidates, RV-mRNA_04 produced the highest levels of neutralizing antibodies, with the titer being 20.46 times higher than that of the commercial inactivated rabies vaccine.



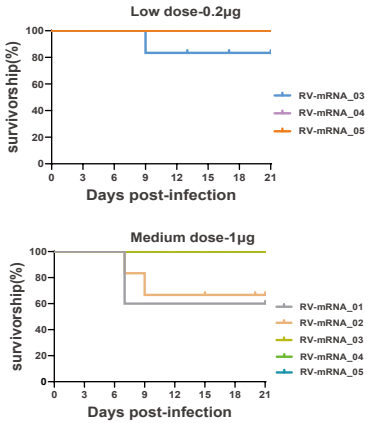
No.	Neutralizing Antibodies VNA (Relative to Control)
Inactivated rabies vaccine	1.0
RV-mRNA_01	1.16
RV-mRNA_02	0.76
RV-mRNA_03	1.72
RV-mRNA_04	20.46
RV-mRNA_05	6.83

Results of neutralizing antibody test of RV-mRNA/inactivated rabies vaccine in mice.

* The neutralizing antibodies (VNA) of the rabies virus are highly correlated with the protective effect of the vaccine, so the antibodies induced by the vaccine can effectively reflect its efficacy.

We then lowered the vaccine dosage from 2ug to 0.2ug and performed viral challenge study with the CVS-24 strain. With RV-mRNA_04 and RV-mRNA_05 vaccines, even the low dosage level showed 100% survival rate.

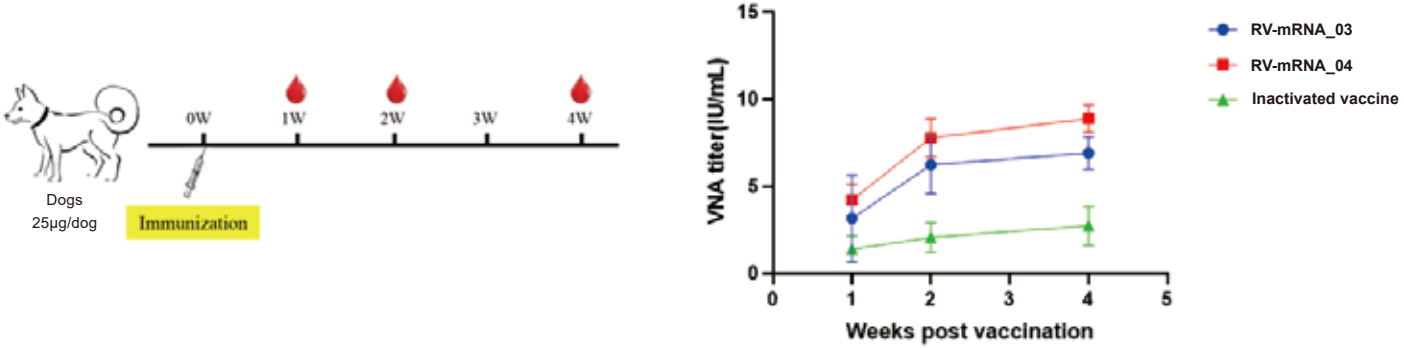
No.	Vaccine protection—Mouse survival rate		
	Low dose immunization (0.2μg)	Medium dose immunization (1μg)	High dose immunization (2μg)
Inactivated rabies vaccines	/	/	100%
RV-mRNA_01	/	66.6%	100%
RV-mRNA_02	/	71.4%	100%
RV-mRNA_03	83.3%	100%	100%
RV-mRNA_04	100%	100%	100%
RV-mRNA_05	100%	100%	100%



RV-mRNA/inactivated rabies vaccine protection rate (mouse survival rate results)

* After animals are vaccinated with the rabies vaccine, the higher the protection rate, the lower the likelihood of reinfection!

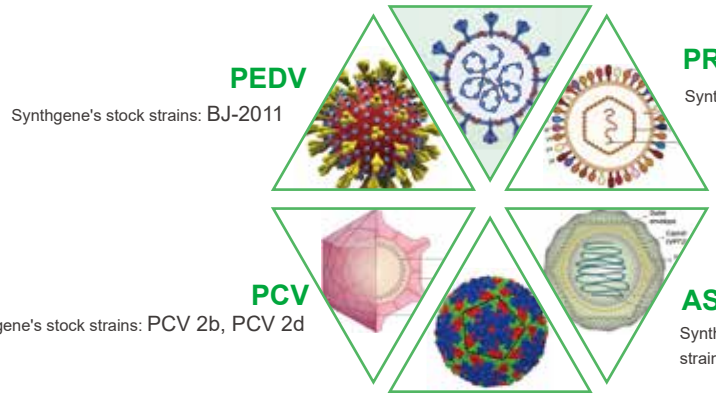
The vaccines were also tested in target animals, where the leading candidates RV-mRNA_03 and RV-mRNA_04 showed 3 times more neutralizing antibody than the commercial inactivated vaccine.



Neutralizing Antibody Detection Results in Animal Studies for RV-mRNA / Inactivated Rabies Vaccine

Animal Infectious Pathogens and Prevalent Strains

Target Animal—Pig



PEDV Porcine epidemic diarrhea (PED) is an acute intestinal infectious disease caused by porcine epidemic diarrhea virus (PEDV), with diarrhea as the main symptom.

PRRSV Porcine reproductive and respiratory syndrome (PRRS), also known as blue ear disease, is a highly contagious disease of pigs caused by porcine reproductive and respiratory syndrome virus (PRRSV).

PRV Porcine pseudorabies (PR), also known as Aujeszky's disease, is a highly contagious disease caused by pseudorabies virus (PRV) that is susceptible to a variety of animals. It has no obvious host tropism and pigs are the only natural host and main source of infection of PRV.

ASFV African swine fever (ASF) is an acute, hemorrhagic, highly contagious disease caused by the African Swine fever virus (ASFV) infecting domestic pigs and various wild boars (such as African wild boars, European wild boars, etc.).

FMDV Foot-and-mouth disease is an acute, febrile, highly contagious disease caused by foot-and-mouth disease virus (FMDV) that occurs in even-toed ungulates such as cattle, sheep, and pigs.

PCV Porcine circovirus disease (PCVD) is a multi-system dysfunctional disease in pigs caused by porcine circovirus (PCV).

Symptoms in pigs after infection with the epidemic strain

PEDV Strain BJ-2011

- Newborn piglets have watery diarrhea, vomiting, and dehydration within 1 week, and die of dehydration within 2 to 4 days of diarrhea;
- Weaned pigs, fattening pigs, and sows often show clinical symptoms of depression and anorexia;
- Adult pigs show depression, anorexia, and vomiting.

PRRSV Strain NADC30, JXA1

- Infected piglets develop fever, difficulty breathing, obvious abdominal breathing, cyanosis of the extremities, loss of appetite, and depression;
- Infected adult pigs develop fever, mental fatigue, and anorexia.

PRV Strain JX/CH/2016

- Piglets aged 2 to 4 weeks have fever, vomiting, diarrhea, anorexia, and lethargy;
- Pigs aged 2 months and above have fever, cough, constipation, ataxia, and intermittent convulsions.

ASFV Strain G-I

- The pigs have increased body temperature, rapid breathing, loss of appetite, coughing, cyanosis of the body surface and limb extremities, and bleeding spots.

FMDV Strain O/mya, O/Cathy, A/GZCS

- At the beginning of the disease, the body temperature rises to 40–41℃, the patient is listless, and has a reduced or no appetite;
- The main feature is hoof blisters and lameness.

PCV Strain PCV 2b, PCV 2d

- Weaned piglets have elevated body temperature, loss of appetite, muscle weakness, diarrhea, and difficulty breathing;
- Pigs aged 8 to 18 weeks have subcutaneous edema, loss of appetite, depression, and lameness.

After the viral challenge, the infected animals exhibited corresponding clinical symptoms.

To evaluate vaccine efficacy, the vaccinated animals were monitored for changes in weight, body temperature, and survival status. Viral clearance and viral load were measured using qPCR. The humoral immune response was assessed via ELISA, while the cellular immune response was evaluated using ELISpot or ELISA.

Humoral Immunity

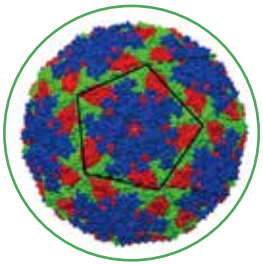
Blood samples were collected to check antigen-specific antibody by ELISA

Cellular Immunity

Blood or spleen samples were collected to check cellular immunity by ELISA or ELISpot

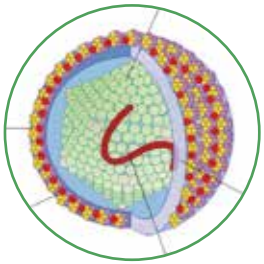
Animal Infectious Pathogens and Prevalent Strains

Animal Model—Cow



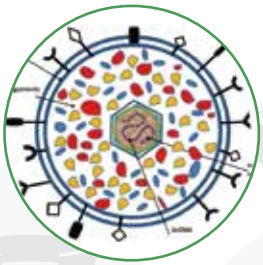
FMDV

Synthgene's stock strains:
O/mya, O/Cathy, A/GZCS



BVDV-1

Synthgene's stock strains:
NM01



IBRV

Synthgene's stock strains:
BHV 1.2

BVDV-1

Bovine viral diarrhea (BVD) is caused by infection with bovine viral diarrhea virus (BVDV). BVDV has three genotypes, BVDV-1, BVDV-2 and BVDV-3.

IBRV

Infectious bovine rhinotracheitis (IBR) is a respiratory infectious disease of cattle caused by bovine infectious rhinotracheitis virus (IBRV), also known as bovine herpesvirus type 1 (BHV-1).

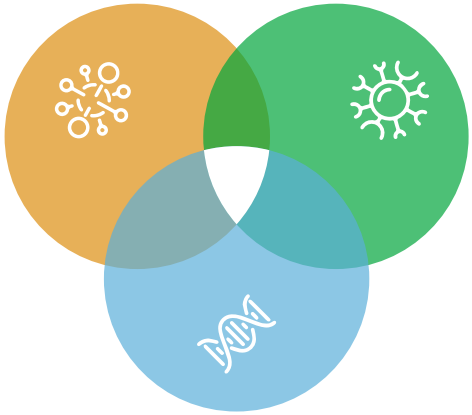
FMDV

Foot-and-mouth disease is an acute, febrile, highly contagious disease caused by foot-and-mouth disease virus (FMDV) that occurs in even-toed ungulates such as cattle, sheep, and pigs.

Symptoms after cattle are infected with the epidemic strain

BVDV-1 Strain NM01

- Fever, reaching 40–42℃, superficial ulcers on the lips, palate, gums, and oral mucosa, profuse drooling, foul-smelling breath, rapid breathing, and persistent diarrhea, which is watery at first and later contains blood and mucus.



IBRV Strain BHV 1.2

- The body temperature rises to as high as 42°C, with a large amount of mucopurulent rhinorrhea, highly congested nasal mucosa, difficulty breathing, ataxia, conjunctival congestion and edema.

FMDV Strain O/mya, O/Cathy, A/GZCS

- The body temperature rises to 40–41℃, the spirit becomes listless, and the dog drools. Blisters appear on the inner surface of the lips, palate, gums, tongue and cheek mucosa. The spaces between the toes and the hoof crown become red, swollen, and painful, and blisters appear rapidly.

After the virus attack, animals can show corresponding typical clinical symptoms.

The weight, body temperature, and survival status of the animals were monitored, and qPCR was used to detect viral clearance and viral load in the target organs.

Humoral Immunity

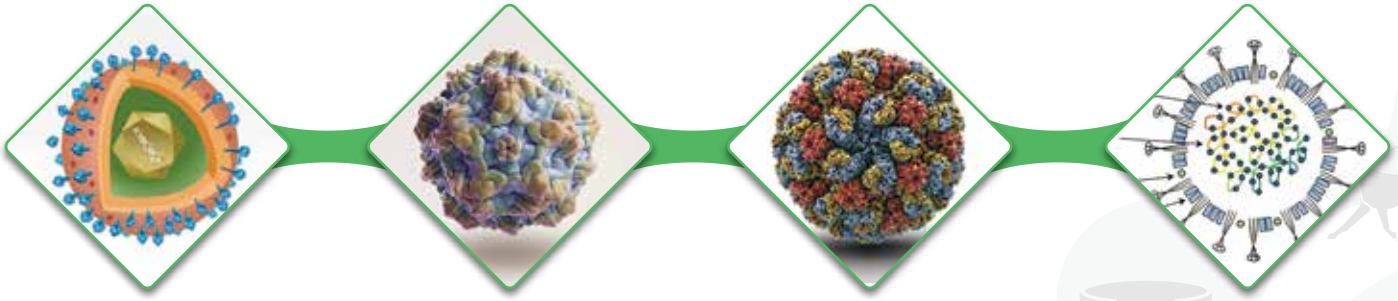
Blood samples were collected to check antigen-specific antibody by ELISA

Cellular Immunity

Blood or spleen samples were collected to check cellular immunity by ELISA or ELISpot

Animal Infectious Pathogens and Prevalent Strains

Animal Model—Cat



FHV

Synthgene's stock strains:
RPVF0304

FPV

Synthgene's stock strains:
RPVF0110

FCV

Synthgene's stock strains:
RPVF0207

FIPV

Synthgene's stock strains:
FIPV-1

FHV Feline viral rhinotracheitis (FVR) is an acute, highly contagious respiratory disease caused by the feline herpesvirus (FHV), characterized by sneezing, tearing, conjunctivitis, and rhinitis.

FPV Feline panleukopenia, also known as feline panleukopenia syndrome (FP) and feline infectious enteritis, is an acute, highly contagious disease caused by feline parvovirus (FPV).

FCV Feline calicivirus (FCV) is a small, membraneless, single-stranded RNA virus that primarily causes acute upper respiratory tract infections (URIs) in cats.

FIPV Feline infectious peritonitis (FIP) is a chronic infectious disease of cats caused by feline infectious peritonitis virus (FIPV) and characterized by the occurrence of peritonitis and ascites

Symptoms of cats infected with the epidemic strain

FPV Strain RPVF0110

Mental fatigue, loss of appetite, body temperature rises to over 40°C, vomiting and diarrhea, watery stools.

FHV Strain RPVF0304

Increased body temperature, coughing, sneezing, foaming at the mouth, increased eye and nasal secretions, depression, and loss of appetite.

FIPV Strain FIPV-1

Loss of appetite, weight loss, elevated body temperature, which can be maintained at 39.7–41°C, distended abdomen, difficulty breathing, corneal edema, and redness of the eye humor.

FCV Strain RPVF0207

Fever (39.5–40.5°C), sneezing, serous or mucous discharge from eyes and nose, oral ulcers. Difficulty breathing, depression, rales in the lungs.

- After the virus attack, animals can show corresponding typical clinical symptoms.
- The weight, body temperature, and survival status of the animals were monitored, and qPCR was used to detect viral clearance and viral load in the target organs.

Humoral Immunity

Blood samples were collected to check antigen-specific antibody by ELISA

Cellular Immunity

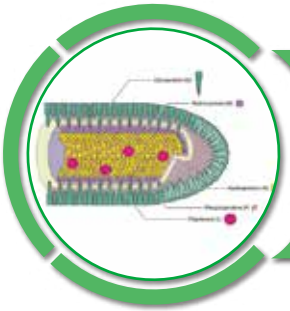
Blood or spleen samples were collected to check cellular immunity by ELISA or ELISpot

Animal Infectious Pathogens and Prevalent Strains

Animal Model—Canine

RV

Synthgene's stock strains:
CVS-11



Rabies is a natural zoonosis caused by infection with the rabies virus (RABV) with severe symptoms and a very high mortality rate, with a mortality rate of almost 100%. Dogs are the main hosts and transmitters of rabies.

Symptoms of dogs infected with the epidemic strain

RV Strain CVS-11

During the depression phase, the dog is mentally depressed, with dilated pupils, hyperreflexia, pica, and gradually increased saliva secretion. During the frenzy phase, the dog is highly excited, behaves violently, and often attacks humans and animals or bites itself. Frenzy attacks often alternate with depression.

- After the virus attack, animals can show corresponding typical clinical symptoms.
- The weight, body temperature, and survival status of the animals are monitored, and qPCR can be used to detect the animal's detoxification and viral load in the target organs.

Humoral Immunity

Blood samples were collected to check antigen-specific antibody by ELISA

Cellular Immunity

Blood or spleen samples were collected to check cellular immunity by ELISA or ELISpot

About Synthgene

Synthgene is a company that provides materials and services to support mRNA and oligonucleotide manufacturing. Founded in 2018 by a group of passionate nucleic acid chemists, the goal of the company is to debottleneck the raw material supply and develop innovative solutions to support customers in the industry. The company has a robust quality system and ISO9001 certification to support cGMP manufacturing of mRNA raw materials that include NTPs, modified NTPs and cap analogs. For the GMP raw materials, Drug Master Files have been filed with FDA. In addition, Synthgene offers analytical development service and mRNA-related impurity standards and detection kits, LNP screening service, IVT process development and IVT kits for mRNA research. For solid-phase DNA and RNA synthesis, Synthgene offers high quality phosphonamidites to support cGMP oligo manufacturing. With over 200 scientists in R&D and AI-assisted discovery platform, Synthgene will continue to bring new products to the market.

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