Novel FMD vaccine research in China

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Dr. Zhang Qiang

Lanzhou Veterinary Research Institute of CAAS
China National Foot and Mouth Disease Reference Laboratory
86-931-8343725,qzhang1616@sohu.com
Xujiaping1#,Lanzhou,Gansu,P.R.China（730046）

Abstract

- Development of FMD Vaccines
- 1. Mya-98 lineage selected
- 2. Synthetic Peptide Vaccine
- 3. Empty Capsid Vaccine
- 4. Attenuated goatpox virus Live carrier vaccine
- 5. transgenic plant vaccine
- 6. Virus-like Particles based vaccine (Eco.li system)
- 7. Gene modify vaccine
- 8. Others

Development of FMD Vaccines

- **1949: found P. R. China**
- **1957 found Lanzhou Veterinary research Institute**
- **1958: Soviet Union help China to research FMD**
- **1960s-:Research on inactivated and activated vaccines**
- **1970s-:exploratory research on gene engineering vaccine**
- **1990s-:New type vaccine**
  - **synthetic peptide vaccine (23years)**
    - FMDV VP1 (links expression 141-160, 200-213) 14aa-20aa-14aa, have been use)
  - **live carrier vaccine**
    - Pseudorabies(P12A3C)
    - Adenovirus (P12A3C)
    - Capripox virus (Dr. ZHANGQIANG)
  - **DNA vaccine (Dr. Guo hui chen, Dr.Lu Zengjun)**
  - **transgenic plant**
    - Tobacco, tomato, Arabidopsis thaliana

1. Mya-98 lineage selected

– For serotype O, a monovalent vaccine be used on pigs before . After the outbreak affected with O/Mya-98 strain, a new vaccine strain from Mya-98 lineage had been selected successfully by LVRI in 2010.

Field isolates of Mya-98 matching with Inactive vaccine

Inactive vaccines	Protected rate
vaccine 1	87.5％ (14/16)
vaccine 2	81.3％ (13/16)
vaccine 3	81.3％ (13/16)

PD_{50} value per dose of new vaccine strain O/Mya-98/2010 on pigs and cattle

Vaccine Batch No.	PD_{50} value for pigs		PD_{50} value for cattle	
	O/Mya98	O/China99	O/Mya98	O/China99
2010-1	10.81	7.49	7.08	11.84
2010-2	13.59	10.81	10.81	9.00
2010-3	10.81	11.49	/	/

2. Synthetic Peptide Vaccine

1990s-：New type vaccine (gene engineering vaccine)

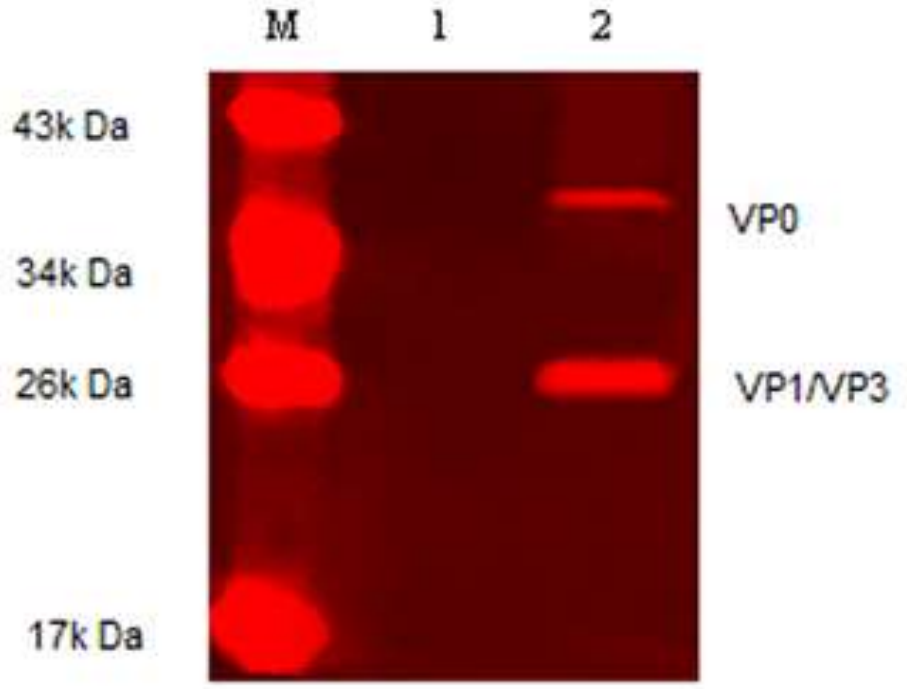
-FMDV VP1 (**links expression 141-160, 200-213**) 14aa-20aa-14aa

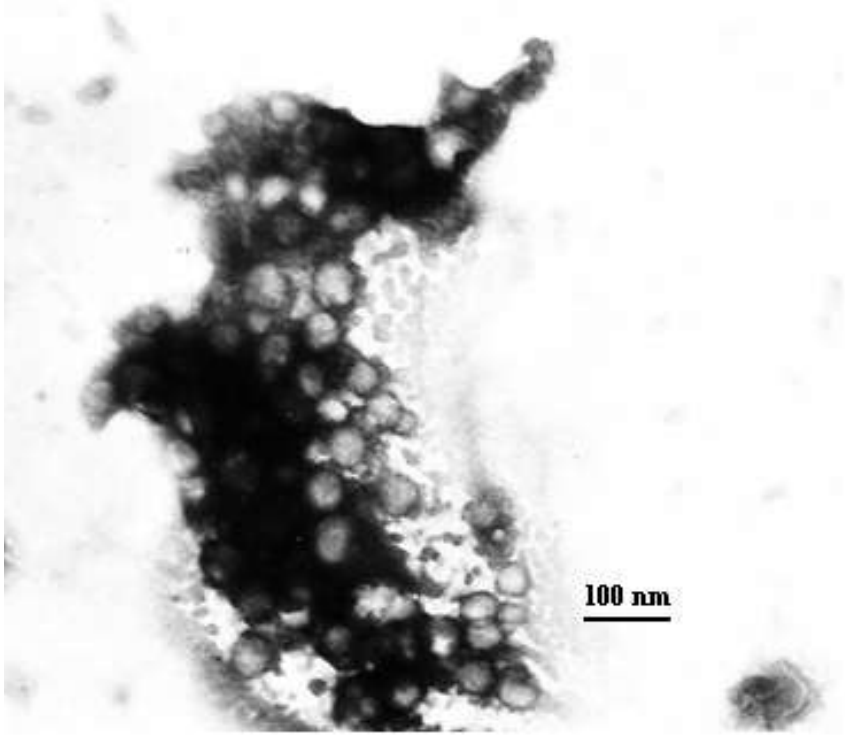
-A new synthetic peptide vaccine was developed in 2006. The vaccine researched by Fudan University , Shanghai Academy of Agricultural Sciences, Zhejiang Academy of Agricultural Sciences and Lanzhou Veterinary Institute of the Chinese Academy of Agricultural Sciences. A "New Veterinary Medicine Registration Certificate" of China has been issued to this vaccine. This means that the vaccine will hopefully be the first FMD gene project vaccine to be launched into manufacture and market.

-synthetic peptide vaccine were used in China

3. Empty Capsid Vaccine

- A empty capsid vaccine was developed in recent year. The vaccine has a satisfactory immune responses and protected from challenge.
- Contains all protective epitopes present on inactivated vaccine but lacks infectious viral nucleic acid and NSP.
- Allows to distinguish vaccinated animal from infected animals using NSP diagnostic tests.
- Can be safely produced.





Identification of empty capsid-like particles by western blot and electron microscopic examination

Animal #	Vaccine	LPBE-antibody (dpv)				
		-7	7	14	21	28
33	Bm-P12A3C	<8	32	180	360	360
124	Bm-P12A3C	<8	20	90	90	90
122	Bm-P12A3C	<8	20	45	45	45
45	Bm-P12A3C	<8	20	90	90	90
50	Bm-P12A3C	<8	45	360	360	360
2	BmBacPAK-6	<8	<8	<8	<8	<8
11	BmBacPAK-6	<8	<8	<8	<8	<8

FMDV-specific antibody response after vaccination with Bm-P12A3C's

Animal No.	vaccine	Days of onset of pyrexia[a]	Duration of Pyrexia (day)	Lesion scores[b]	Protection [c]
33	Bm-P12A3C	-	-	-	+
124	Bm-P12A3C	-	-	-	+
122	Bm-P12A3C	-	-	-	+
45	Bm-P12A3C	Day 6	2	2	-
50	Bm-P12A3C	-	-	-	+
2	BmBacPAK-6	Day 2	3	4+mouth	-
11	BmBacPAK-6	Day 2	3	4+mouth	-

Protection and clinical signs in cattle after challenge with FMDV Asia1/CHA/05 strain

The result of PD_{50}

Immunization dose of vaccine	Rate of protection	PD_{50}
1	5/5	6.43
1/3	3/5	
1/9	2/5	

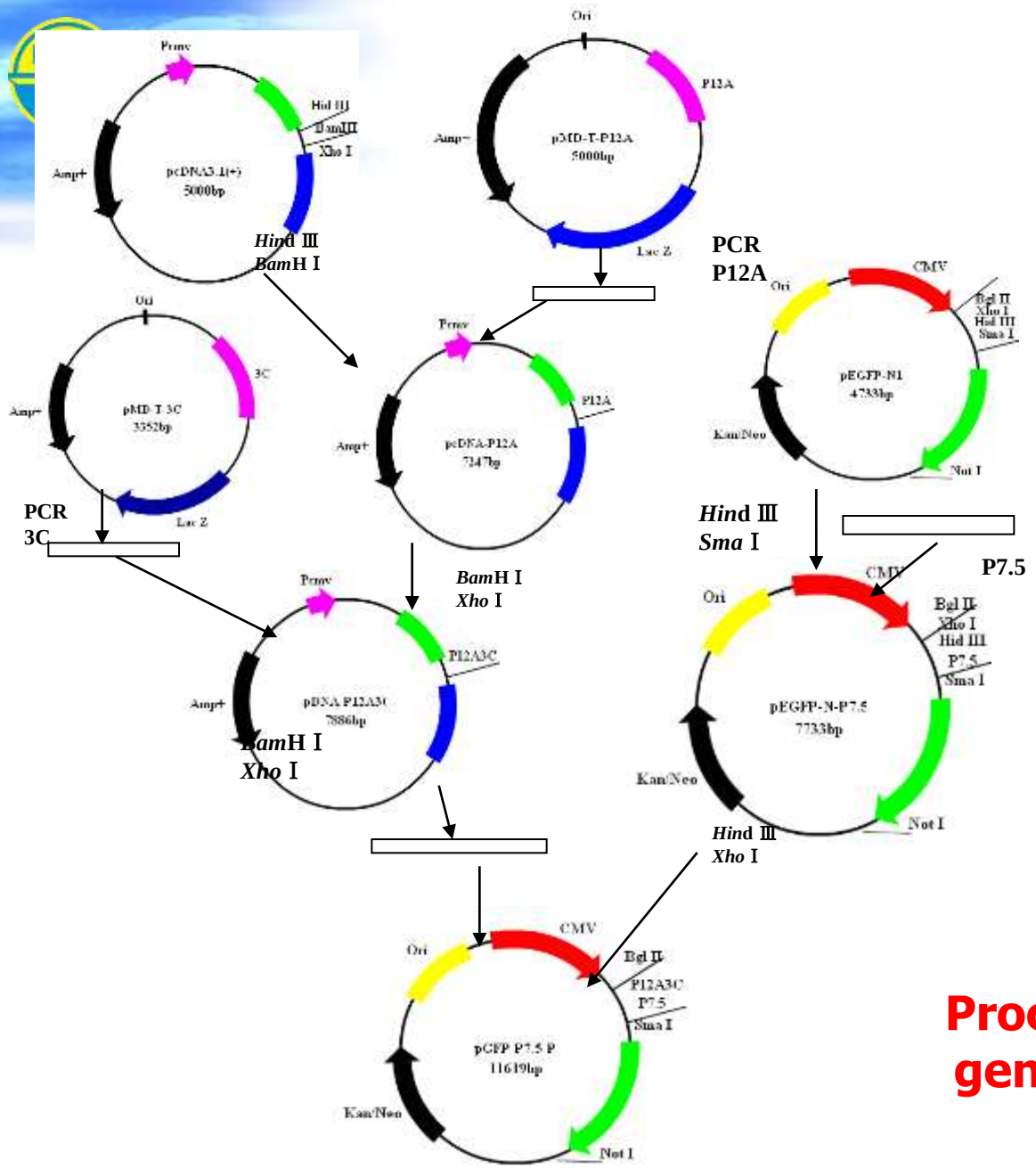
In 2010 used 2.0 million Milliliters

4. Attenuated goatpox virus Live carrier vaccine

Carpripox is a kind of acute fever and contacting infectious disease caused by carpripox virus(CPV)， it is the most heavy pox disease of livestock. CPV can infect all ages of goat and sheep, of which the infection race and death race of lamb reached 100%， causing huge economic loss.

In China,the attenuated goatpox vaccine was used to prevent goat and sheep pox disease. The attentuated virus vaccine strain(AV41) which was widely used in China since 1984.

In this study, we used the attenuated vaccine strain(AV41) as live vector to express FMDV and PPR gene. The results showed that P12A and 3C genes of FMDV and H and F genes of PPRV were expressed successfully.



.Using PCR amplification technique, FMDV P12A and 3C gene was obtained.

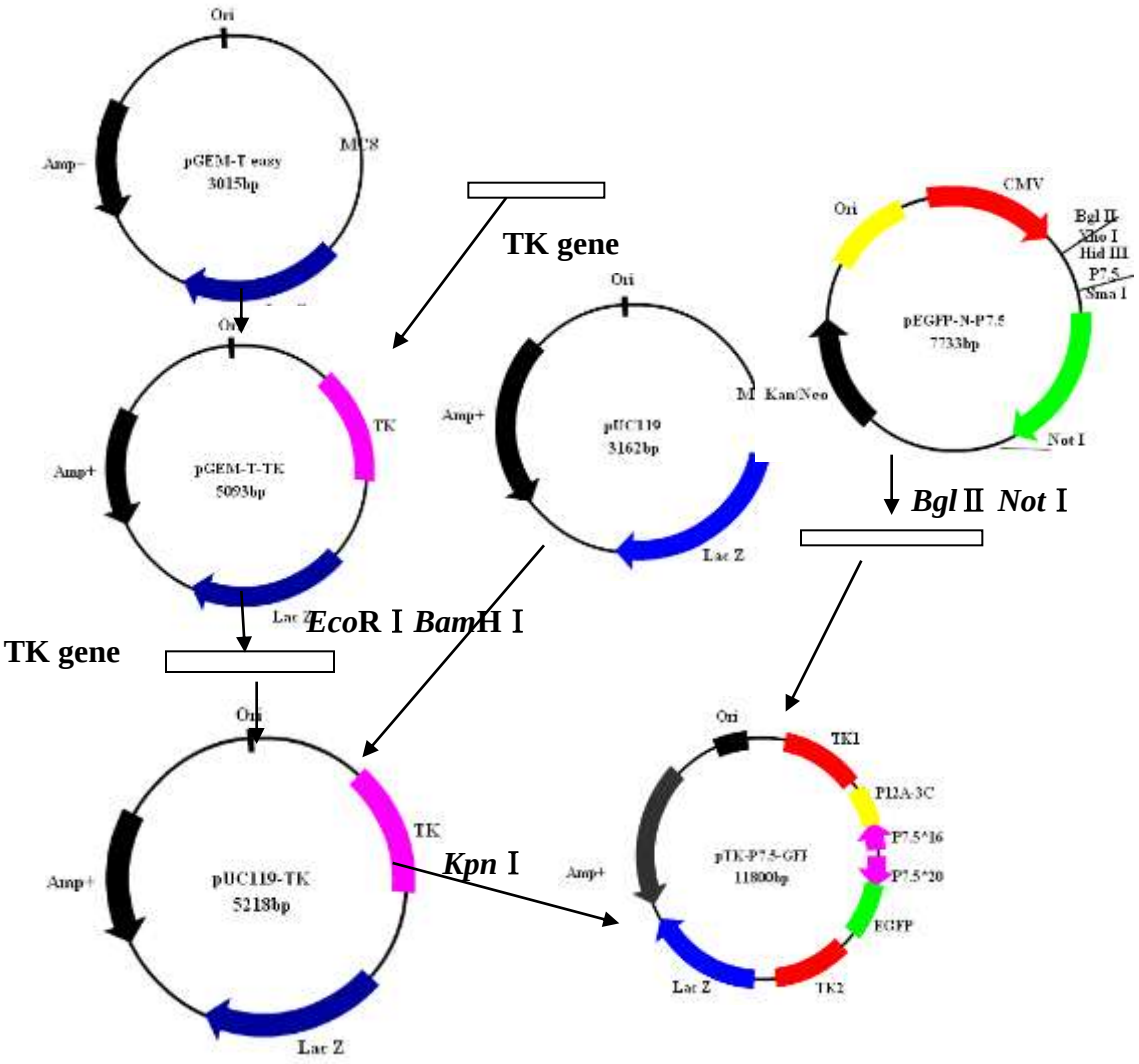
.Then ligated into pMD18-T vector

.The recombinants were di by NheI and Hind III and ligated into pEGFP-N1-P7.5, yielding the recombinant pEGFP-N1-P7.5-P12A3C.

Procedure of constructing gene expression cassette

.CPV TK gene ligated into pUC119 vetor, and obtained the vetor pUC119-TK

. Next the expression cassette EGFP-N1-P7.5-P12A3C was first released from recombinant vector by double digestion of Hind III and Nhe I and ligated into pUC119-TK that was digested by Kpn I, yielding the transfer vector pUC119-TK-EGFP-P7.5-P12A3C

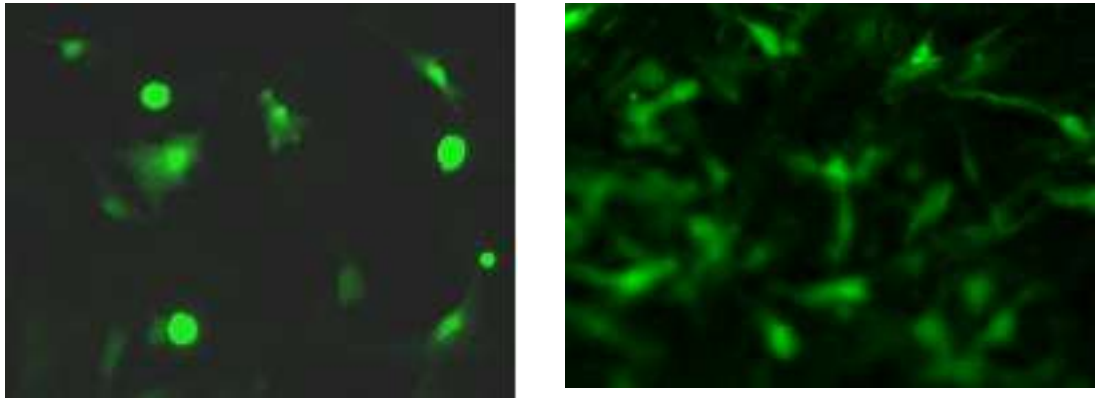


vector

Results and Analysis

Detection of GFP expression

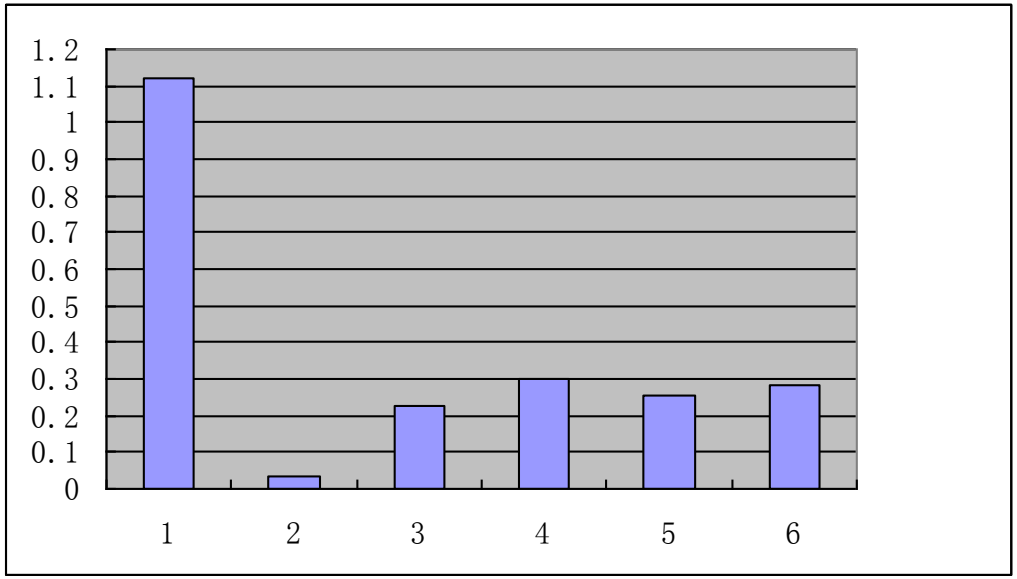
- Then the transfer vector transfecting BHK21 cells which were infected by GTPV AV41 strains. After transfection, the transfected cells were observed under fluo-rescence microscope.
- At the 16-48hs after transfection, plasmid-transfected BHK-21 cells infected with parent virus presented green fluorescence, while the cells without virus infected and those so1ely infected with goat pox virus did not. This result shows that goat pox virus can recognize the promoter and flanking sequences of vaccinia, promoting the expression of EGFP.



The green fluenuence in BHK-21 cells membrane infected(X400)

A.24 h post-infection； B.72 h post-infection

Results and Analysis



Detecting protein by FMDV antigen capture ELISA, the result shows FMDV antigen could be detected in combinant GTPV virus cell culture, show maybe some FMDV virus like particals producing

Analysis of protein by antigen capture ELISA

1. positive control 2. negative control 3-6.the positive sample of RT-PCR

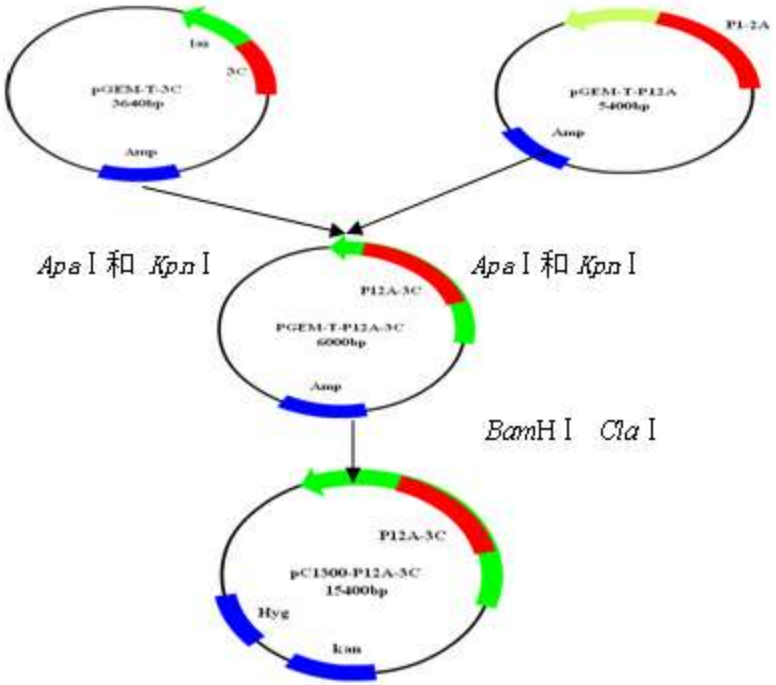
Results and Analysis

	Animal No.	Antibody of FMDV type Asia1 before inculated	Antibody of FMDV type Asia1 after inculated			
			14d	21d	28d	42d
Group1 Inculated 1mL	1	<4	11	11	22	22
	2	<4	<4	<4	6	11
	3	<4	6	8	6	6
	4	<4	6	6	11	8
Group2 Inculated 2mL	1	<4	6	8	22	32
	2	<4	6	6	11	22
	3	<4	<4	<4	<4	8
	4	<4	<4	6	6	6

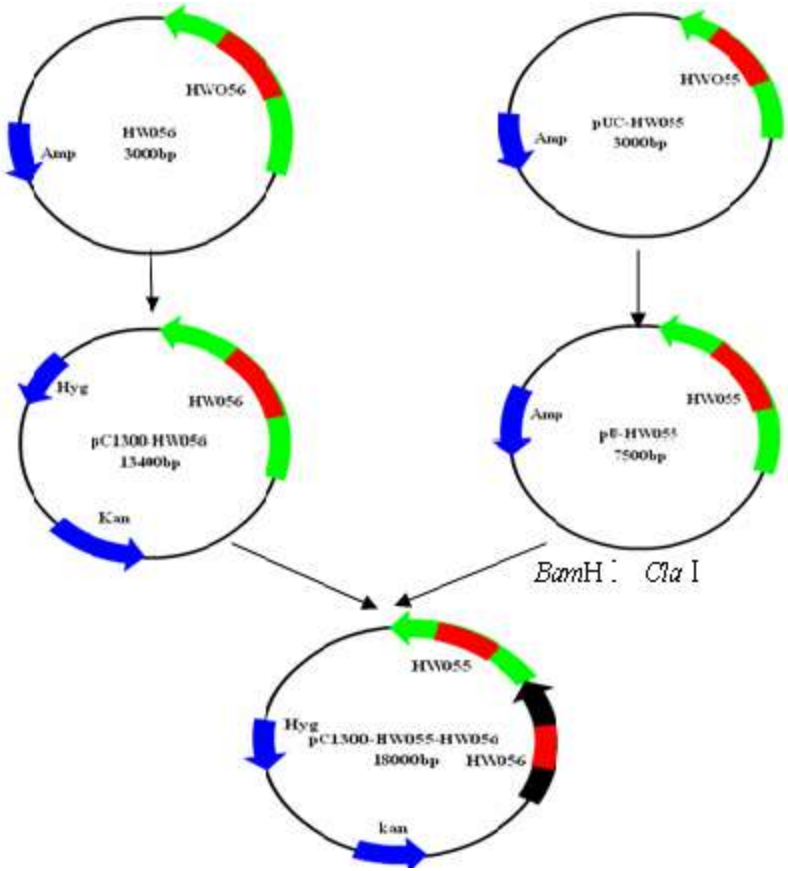
Measurement of antibody against *FMDV*

8 sheep delived into 2 groups. group 1 ,every sheep intradermic inject with recombinant GTPV vaccine 1mL, group2 every sheep intradermic inject with recombinant GTPV 2 mL. After vaccined 14d, some animals' FMDV antibody raised slightly.

5. Research on transgenic plant vaccine



pC1300-P12A-3C recombinant plasmid

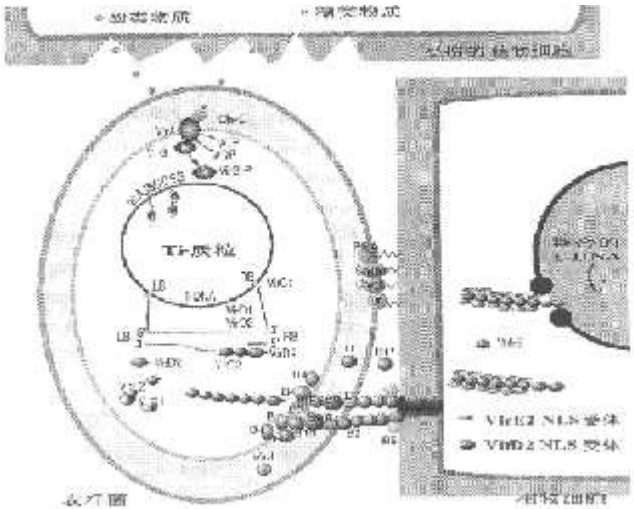


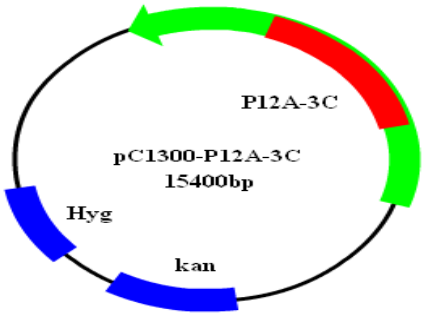
pC1300-HW056-HW055 plant expression vector

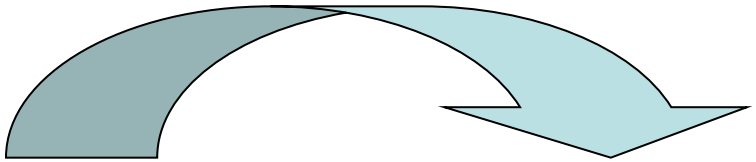
The construction of plant expression vector

pC1300-P12A3C transformation mediated by

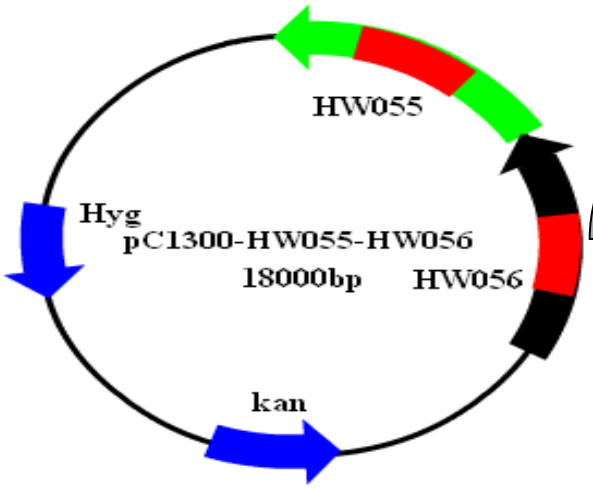
Agrobacterium fumefaciens

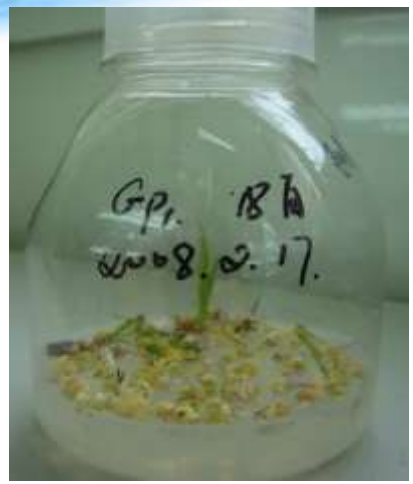






pC1300-HW056 -HW055 transformation mediated by gene gun

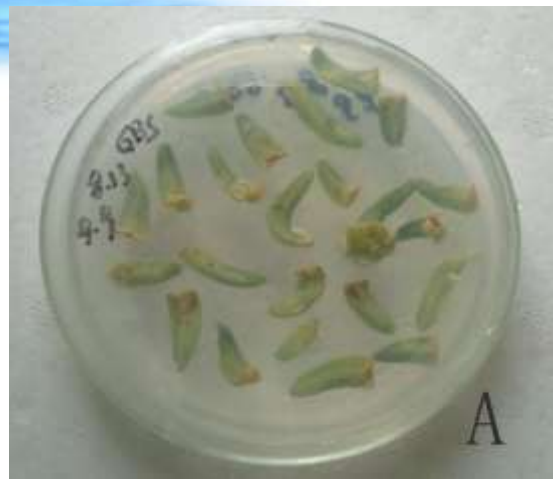




differentiation of maize callus

maize plants transplanted in soil

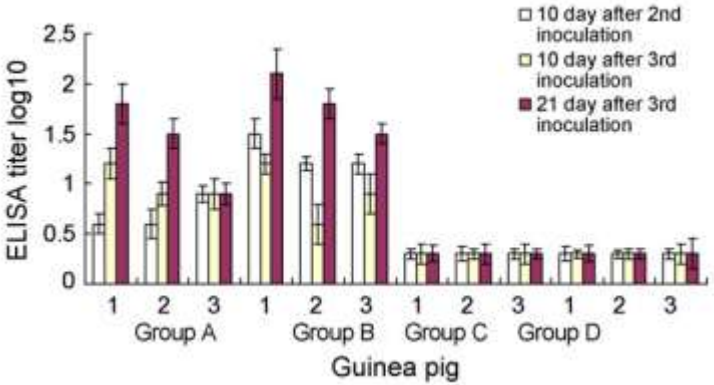
Transgenic tomato plants



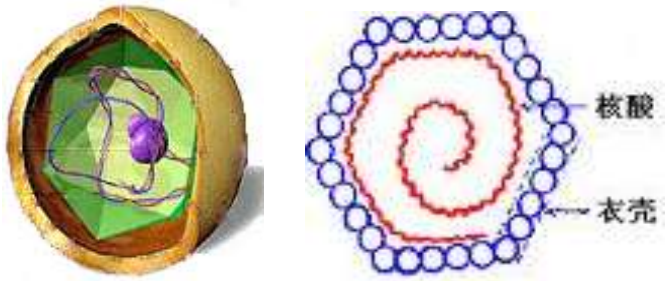
Selection of transgecic tomaco resistant calli and resistant shoots

Transgenic tomato plants transplanted in soil

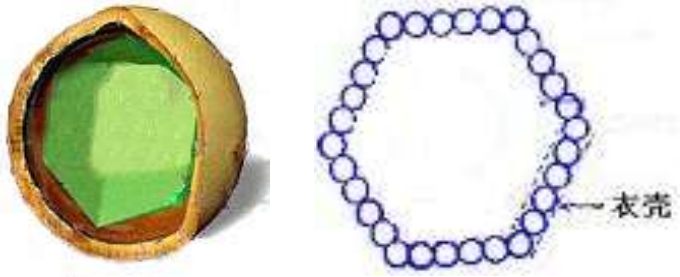
ELISA titer after inoculation in guinea pigs



6. Virus-like Particles based vaccine (Eco.li system)



virus

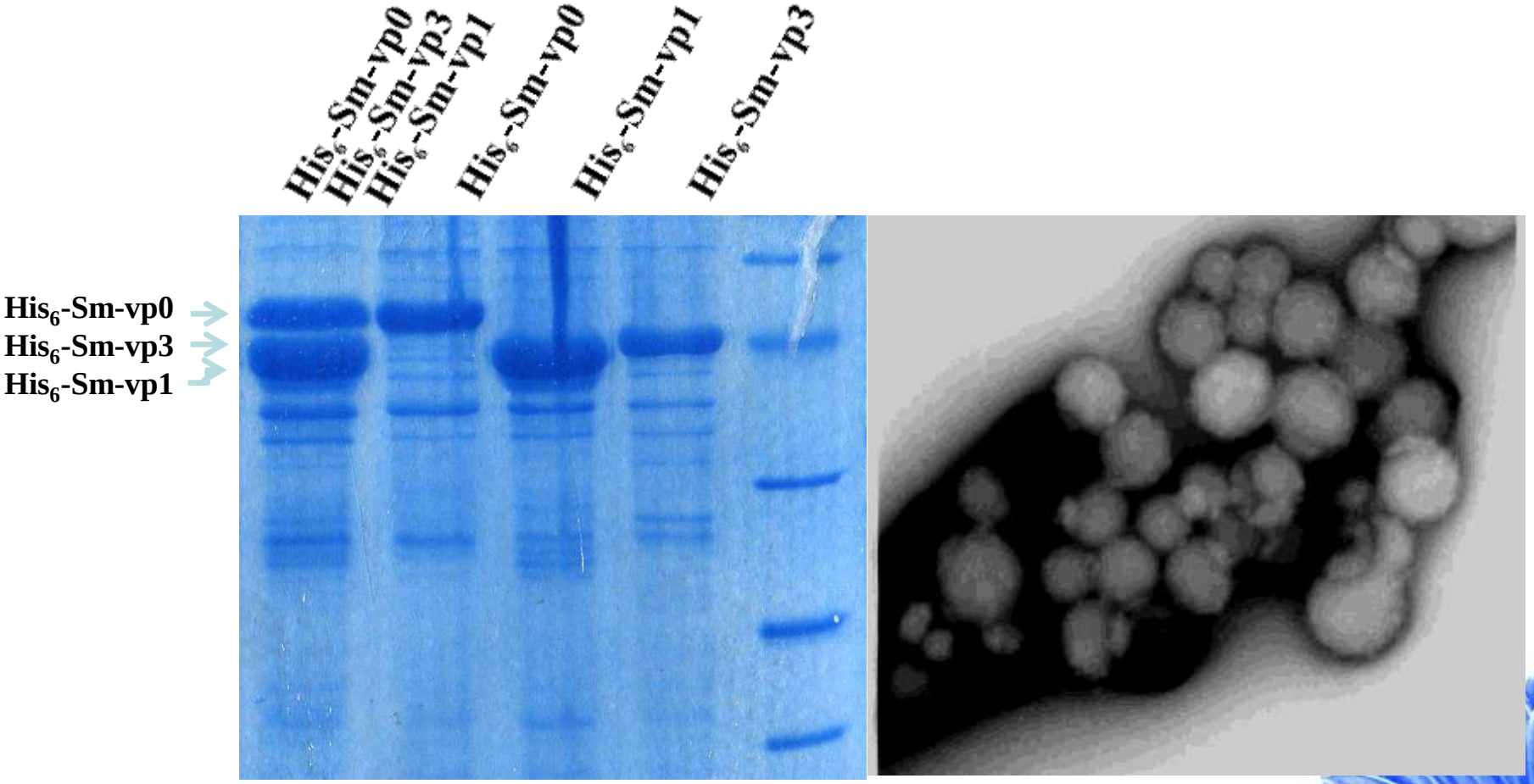


Virus-like Particles

100%(16/16) protection in pig

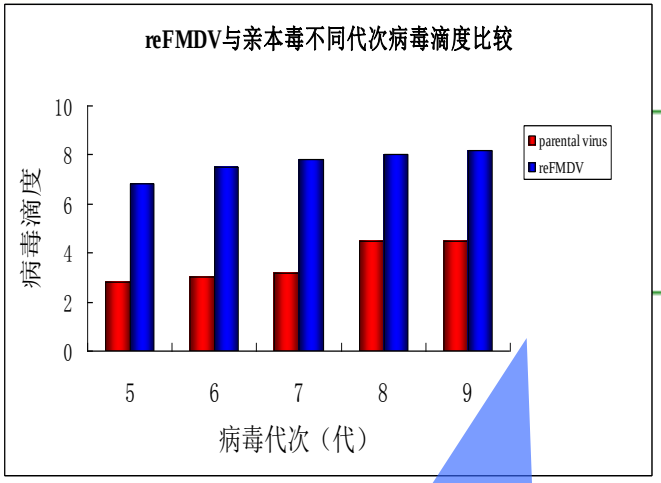
dose	protection rate	PD50 in cattle
1	5/5	6.34
1/3	4/5	
1/9	2/5	
Ctrl	0/3	

Empty capsid subunit vaccine(VLP)

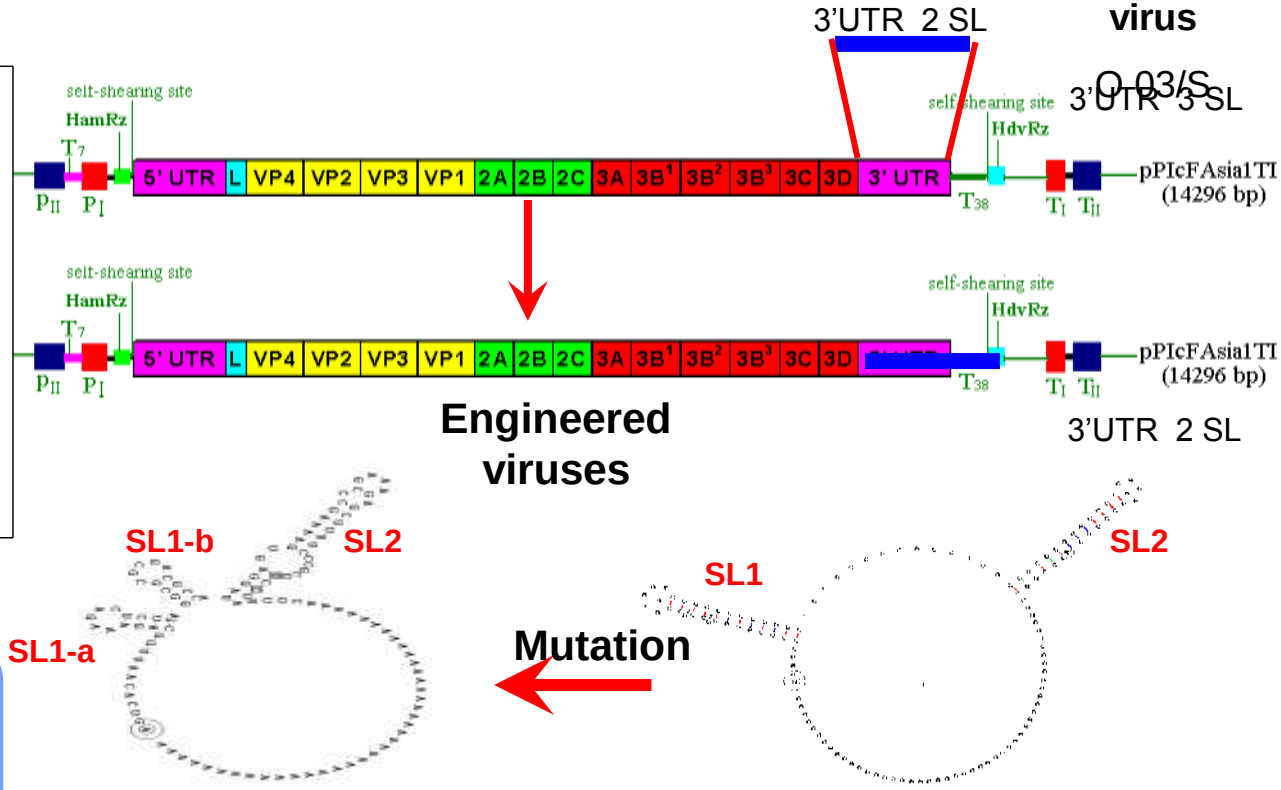


7. Gene modify vaccine based on genetic engineering and FMDV RGS (reverse genetics system)

Engineering FMDV with increased growth properties and antigenic match for the development of improved vaccine



improve the virus reproductive performance in BHK-21 cells by molecule recombination



A Mutation of Stem-loop Structures in the 3‘Noncoding Region Can Attenuate FMDV to Increase Growth Properties

The results of PD_{50} test with engineering FMDV

	Immunize dose	Rate of protection (%)	PD_{50}
Test 1	1	5/5	10.81
	1/3	5/5	
	1/9	3/5	
Test 2	1	5/5	13.59
	1/3	5/5	
	1/9	4/5	

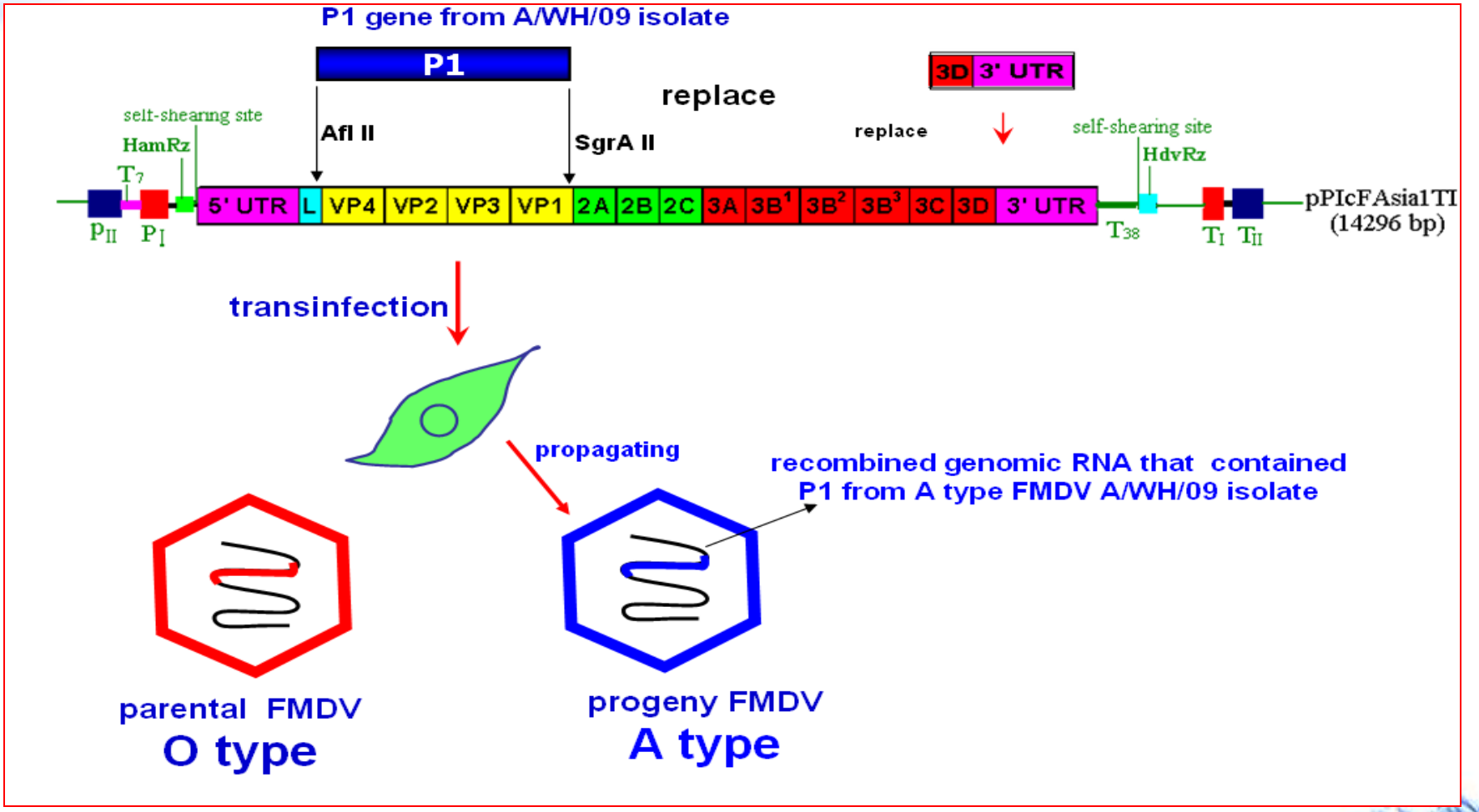
Protective efficacy in cattle

Animals	Dose	Morbidity	Protection	Protection Rate
8	1ml	3	5	5/8
8	2ml	0	8	8/8
2（N.C）	0	2	2	0/2

Engineering Foot-and-Mouth Disease Viruses with Increased Antigenic Match for the Development of Improved Vaccine

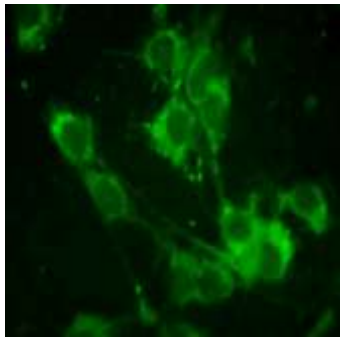
To Increase Antigenic Match

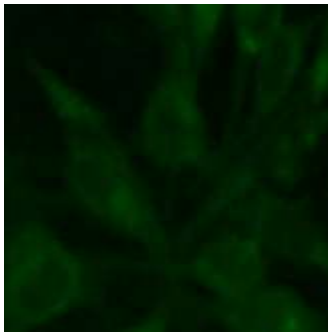
Construction of recombinant genome-length cDNA copy of prA/P1-FMDV

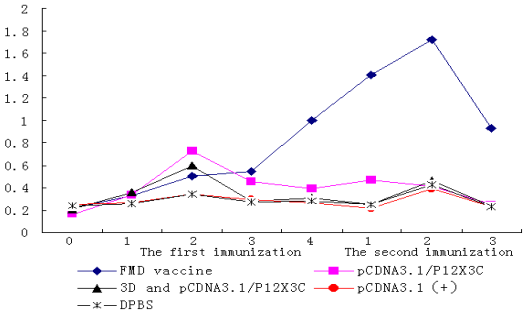


8. Others

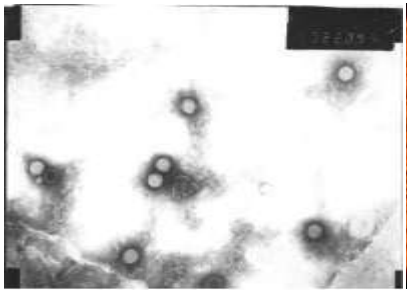
DNA vaccine

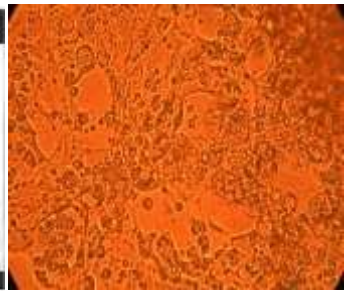


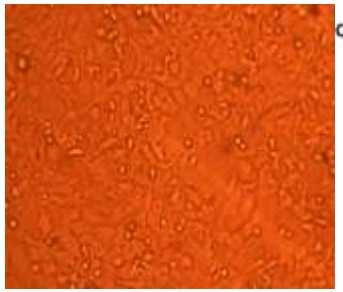


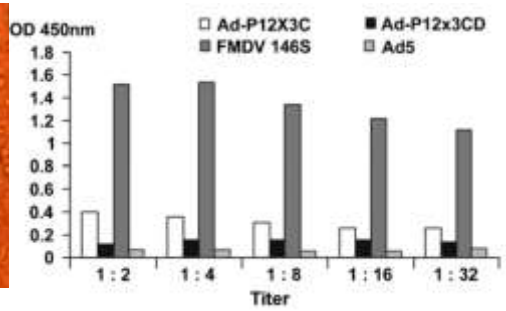


Recombinant live vector(Adenovirus) vaccine





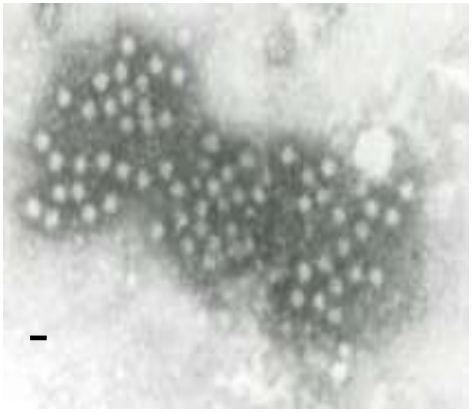




8. Others

- live carrier vaccine
  - Pseudorabies
- Marker vaccine
- Multiple epitopes vaccine

Rescue virus vaccine



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Yin Xiangping
Sun Shiqi


Thanks for your attention
美丽的草原我们的家