Agricultural applications in China to enhance sustainability

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Gene editing helps sustainable development of animal husbandry

China’s agriculture has made great progress in recent years，however, the sustainable development of agriculture, especially animal husbandry, still faces many challenges. Among them, the development and utilization of rich indigenous livestock breed resources, the prevention and control of epidemic diseases are the two major ones.

The gene editing technology provides us with more solutions for the efficient use of local germplasm resources and the prevention and control of major diseases.

Contents

PART 1

MSTN gene edited for improving the meat production of Chinese local livestock breeds

MSTN gene edited for improving the meat production of Chinese local livestock breeds

Jiaxing Black sow
(Records of livestock and poultry genetic resources in China. Records of pigs, 2011)

Jiaxian Red cattle
(https://kepu.gmw.cn/agri/2021/04/27/content_34802673.htm)

Sunit sheep
(https://cn.bing.com/images/search?view)

There are 83 local pig breeds, 55 local cattle breeds, and 104 local sheep and goat breeds in China, accounting for about one third of the world. Chinese local varieties have many advantages, such as excellent meat quality and flavor, and resistance to rough feeding. However, mainly due to the low meat production, most of the local breeds of domestic animals have become extinct or are on the verge of extinction.

***MSTN* gene editing provides a simple and rapid method to improve the meat production performance of Chinese local varieties and preserve the excellent meat quality. Here I briefly introduce the research work on *MSTN* gene deletion to improve meat production of pigs and cattle in China.**

Discovery of *Myostatin* (*MSTN*)

MSTN **was first identified in mice by McPherron *et al*. in 1997.**
It plays a key role in regeneration and development of skeletal muscle.

Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member

Alexandra C. McPherron*, Ann M. Lawler† & Se-Jin Lee*

* *Department of Molecular Biology and Genetics, and † Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205, USA*

McPherron *et al*. Nature. 1997 May 1;387(6628):83-90.

Conservation of *MSTN*

Percent Identity

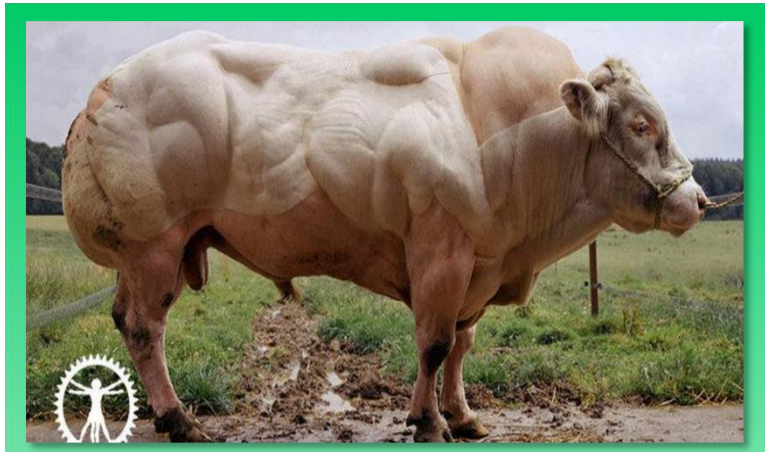
Divergence

	1	2	3	4	5	6	7	8	9	10	
1		99.1	97.2	96.3	96.3	99.1	99.1	99.1	99.1	99.1	1 pig
2	0.9		98.2	97.2	97.2	100.0	100.0	100.0	100.0	100.0	2 human
3	2.8	1.9		95.4	95.4	98.2	98.2	98.2	98.2	98.2	3 cattle
4	3.8	2.8	4.7		100.0	97.2	97.2	97.2	97.2	97.2	4 sheep
5	3.8	2.8	4.7	0.0		97.2	97.2	97.2	97.2	97.2	5 goat
6	0.9	0.0	1.9	2.8	2.8		100.0	100.0	100.0	100.0	6 rabbit
7	0.9	0.0	1.9	2.8	2.8	0.0		100.0	100.0	100.0	7 rat
8	0.9	0.0	1.9	2.8	2.8	0.0	0.0		100.0	100.0	8 mouse
9	0.9	0.0	1.9	2.8	2.8	0.0	0.0	0.0		100.0	9 bat
10	0.9	0.0	1.9	2.8	2.8	0.0	0.0	0.0	0.0		10 chicken
	1	2	3	4	5	6	7	8	9	10	

The MSTN is highly homologous among species at the nucleic acid and protein levels.

Evolutionary conservation of MSTN mature peptide

Natural Mutations of *MSTN* Gene in Animals



Belgian blue:

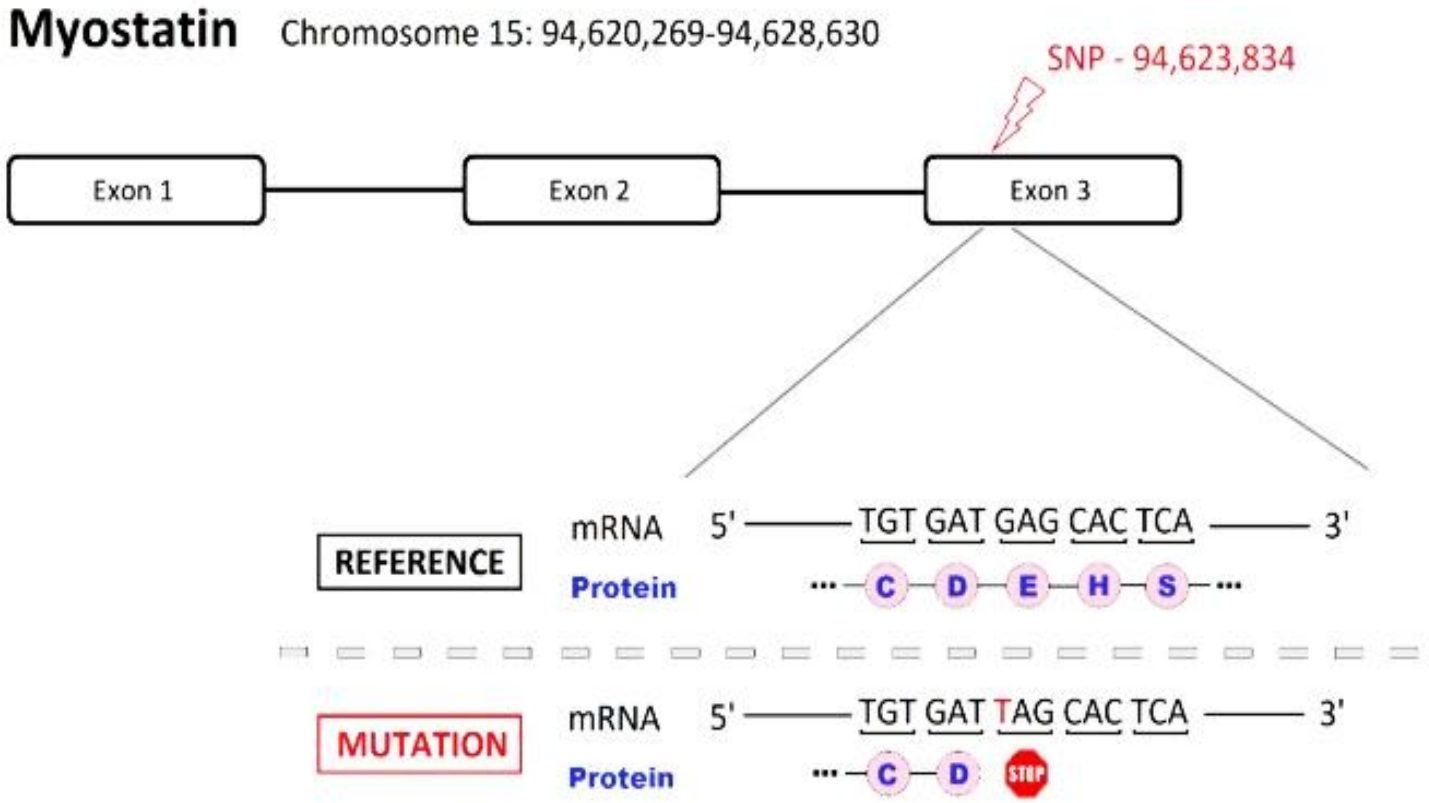
A frame-shift mutation caused by an 11-nucleotides deletion in the 3rd exon, resulting in early termination of translation.

Piedmontese:

A missense mutation in the 3rd exon, resulting in replacement of a conserved cysteine by tyrosine in the mature peptide.

Kambadur *et al.* Genome Res. 1997 Sep;7(9):910-6.

Natural Mutations of *MSTN* Gene in pigs



In 2019, a research group from the Roslin Institute scanned a Large White high-lean population by genome resequencing, which identified a natural mutation located in the 3rd exon （G820T）, leading to a stop codon.（The 274th AA）

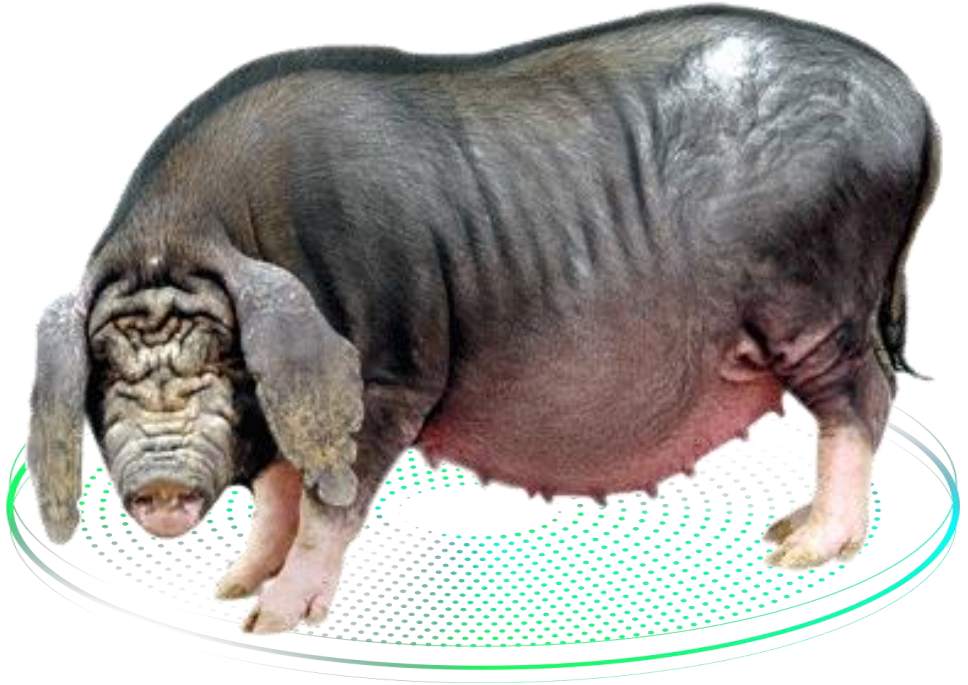
Matika et al. PLoS Genet. 2019 Jan 30;15(1):e1007759.

Improvement of Meishan pig by MSTN gene editing

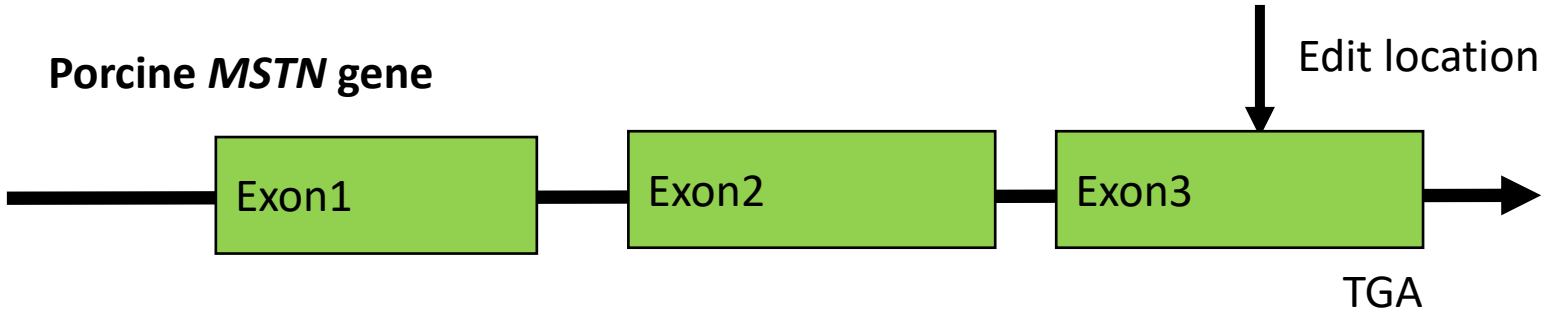
Meishan pig is a famous local pig breed with excellent reproductive ability, good meat quality and flavor, but its lean meat rate is less than 40%.

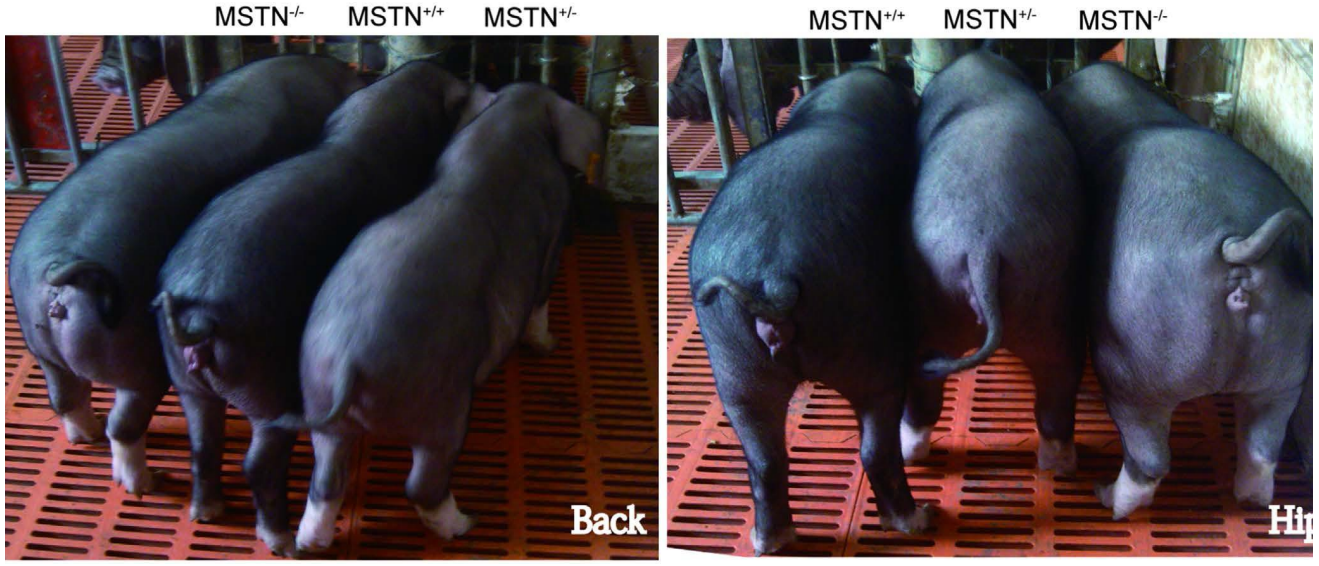
The *MSTN* gene editing was used for the genetic improvement of Meishan pig.

- Preparation of *MSTN* gene editing Meishan pig
- *MSTN* gene edited Meishan pigs were obtained by TALEN technique.



Preparation of gene editing Meishan pig (TALEN) imitating natural mutation of *MSTN* gene of Belgian Blue cattle



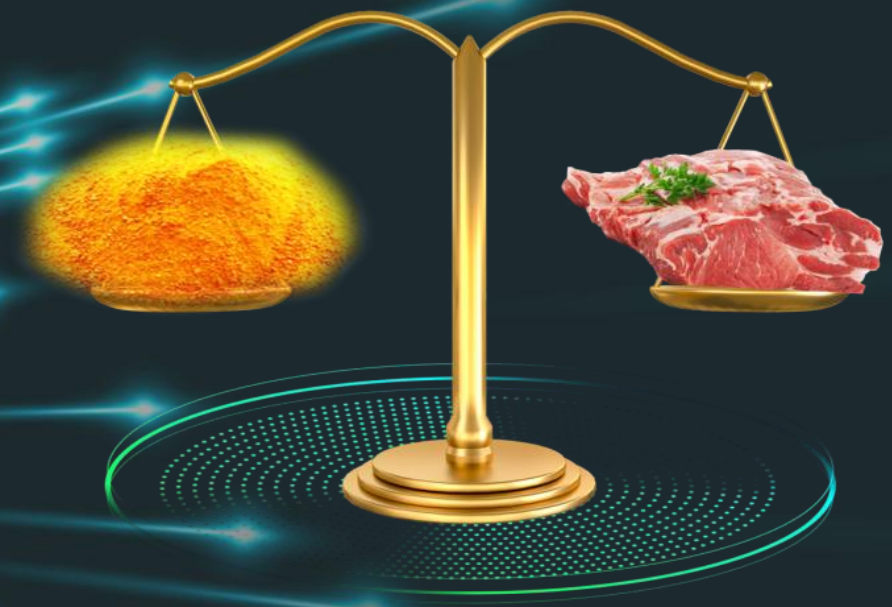


Qian et al, 2015

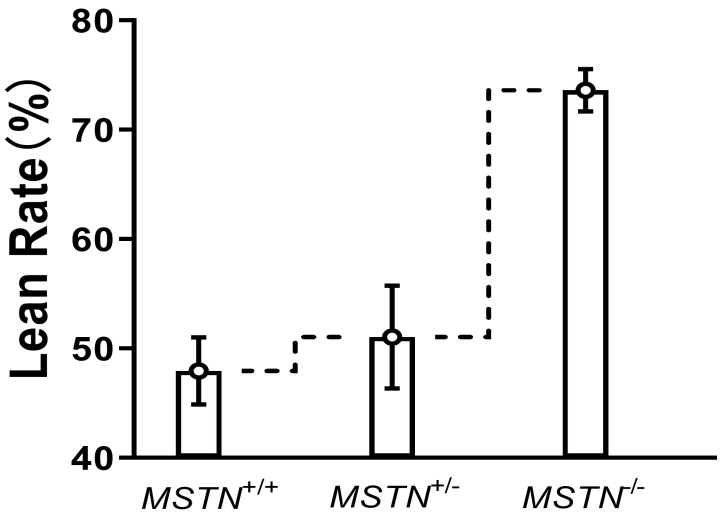
TALEN technology mimics the natural mutation of *MSTN* gene in Belgian Blue cattle in exon 3 of *MSTN* gene, resulting in premature termination of translation.

Production performance of *MSTN* gene editing pigs

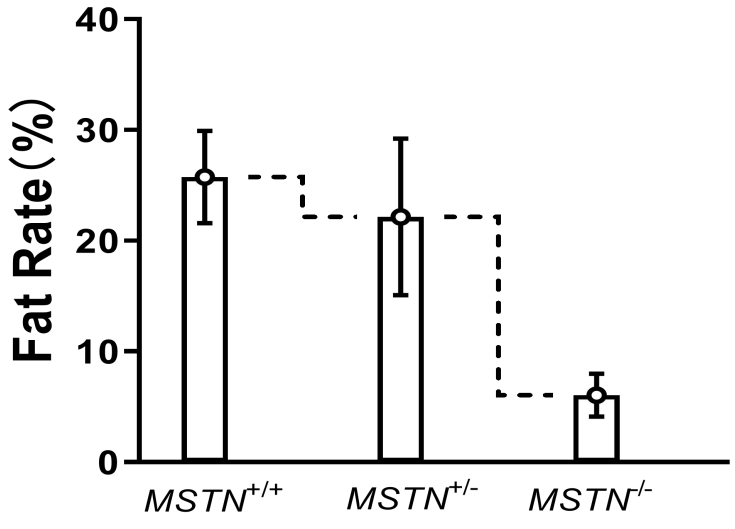
- Slaughter performance
- Reproductive performance
- Feed efficiency
- Meat quality



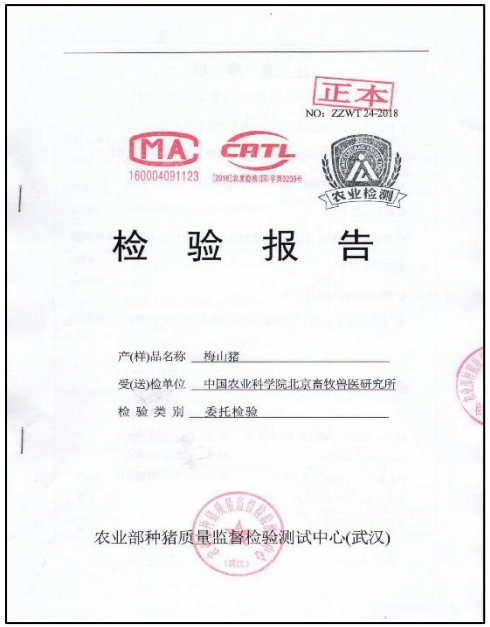
MSTN gene editing can greatly improve meat production performance of Meishan pigs

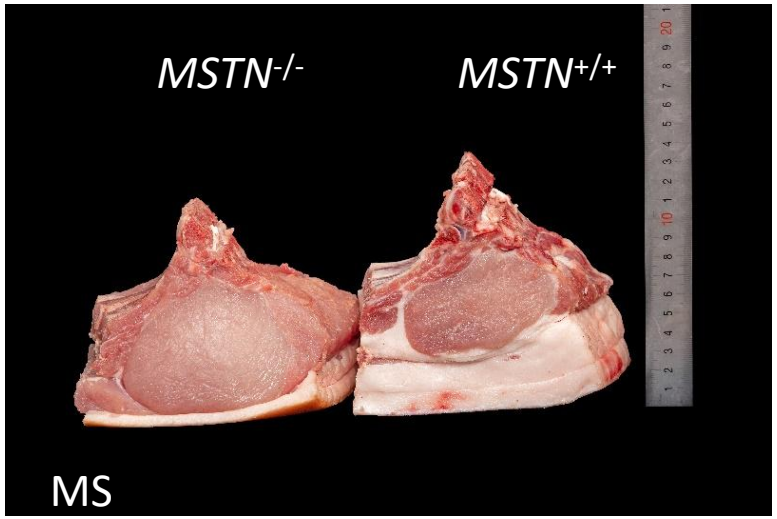


Lean meat percentage



Fat percentage

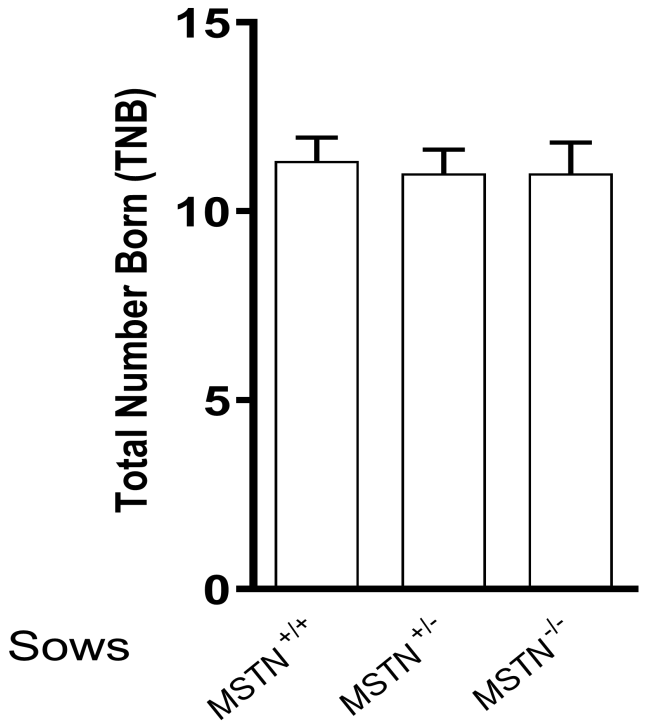

正本
NO：ZZWT 24-2018
160004091123
农业检测
检 验 报 告
产(样)品名称 梅山猪
受(送)检单位 中国农业科学院北京畜牧兽医研究所
检 验 类 别 委托检验
农业部种猪质量监督检验测试中心(武汉)



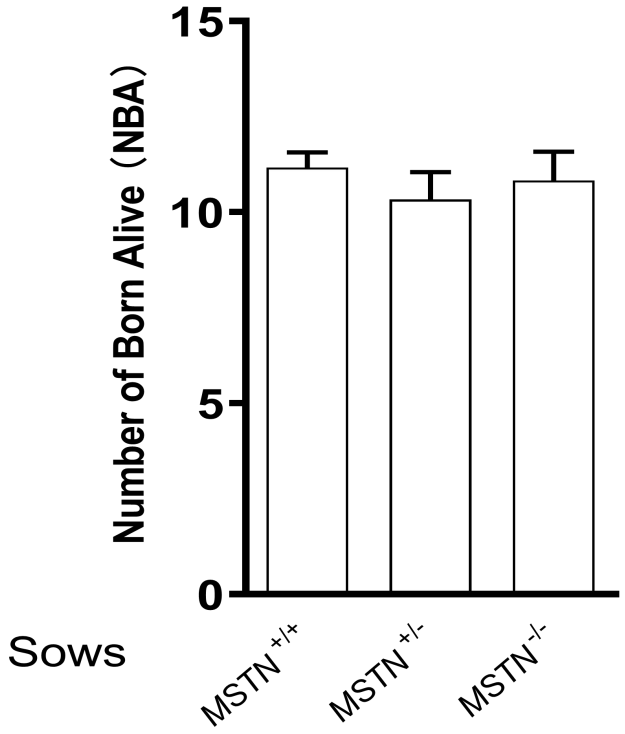
The lean meat percentage： The relative increase of homozygotes was 53%.

The fat percentage： The relative decrease of homozygotes was 76%.

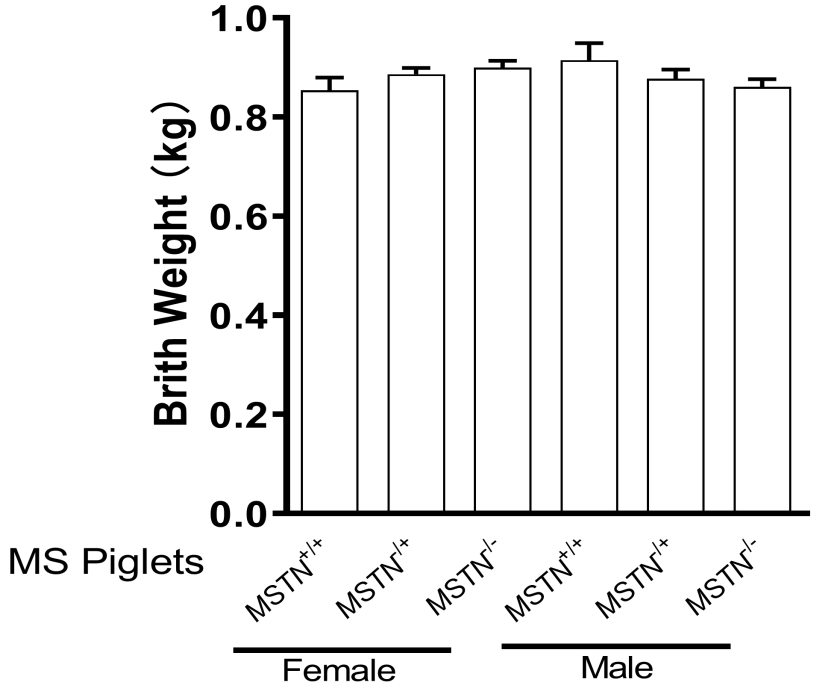
MSTN gene editing had no significant effect on reproductive performance of Meishan sows



Number of litters



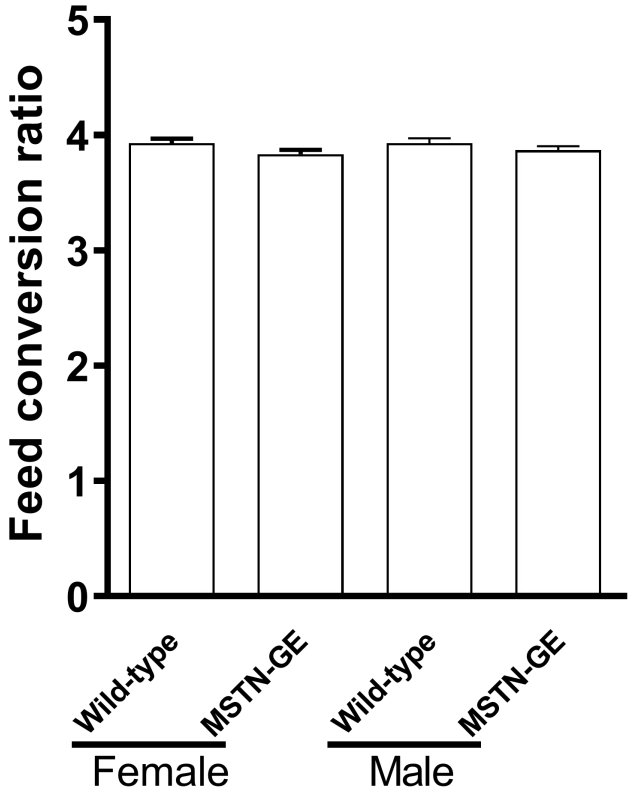
Number of live litters

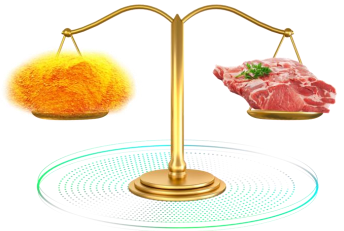


birth weight

The statistics involved sows including *MSTN* + / + (n = 6), *MSTN* + / - (n = 6), *MSTN* - / - (n = 6), and all piglets including *MSTN* + / + Female (n = 31), *MSTN* + / - female (n = 56), *MSTN* - / - female (n = 51), *MSTN* + / + male (n = 28), *MSTN* + / - male (n = 50), and *MSTN* - / - male (n = 24). There was no significant difference in the statistical results.

Feed utilization of Meishan pigs with *MSTN* gene editing

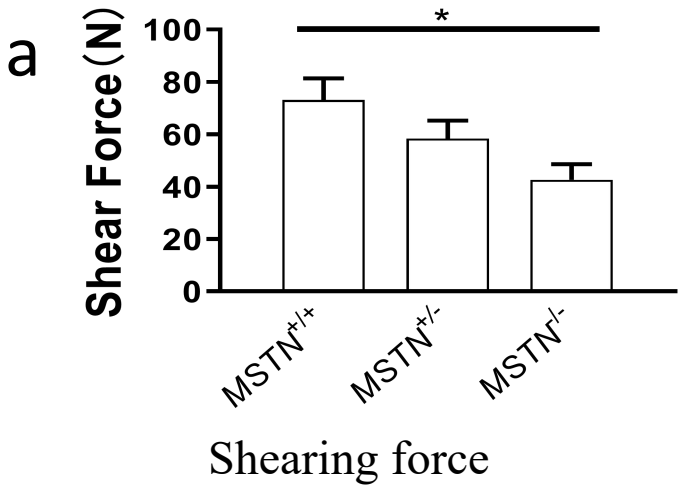




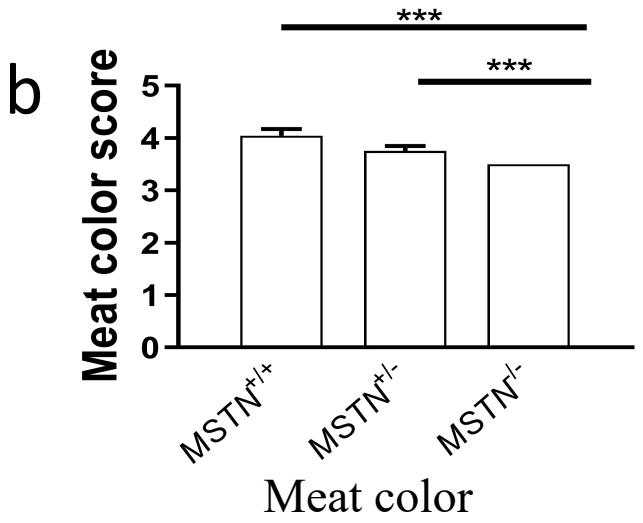
The feed utilization rate of *MSTN* gene edited pigs and wild-type pigs was similar.

Automatic feeding system

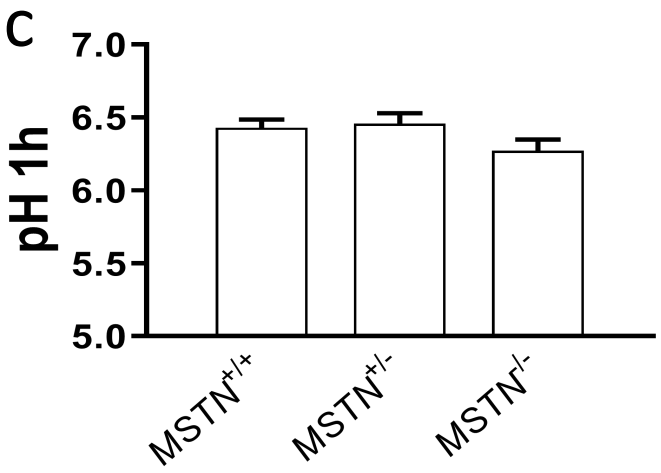
MSTN gene editing makes pork tender and healthier



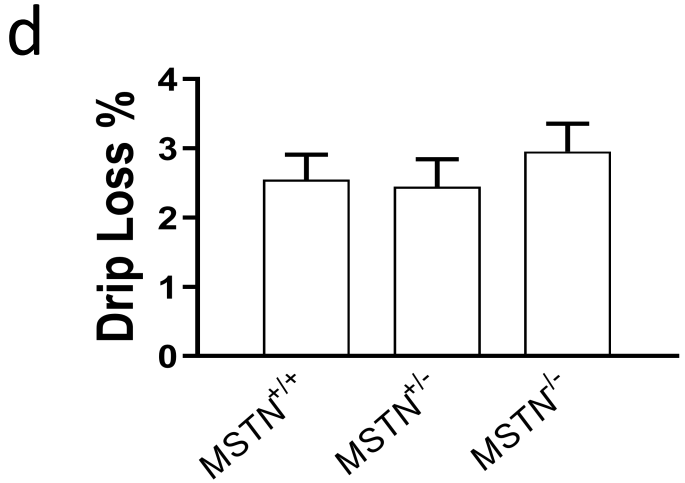
Shearing force



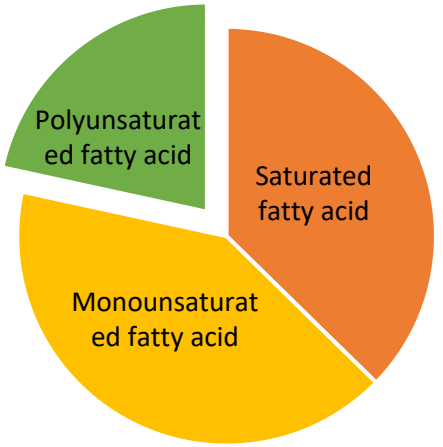
Meat color



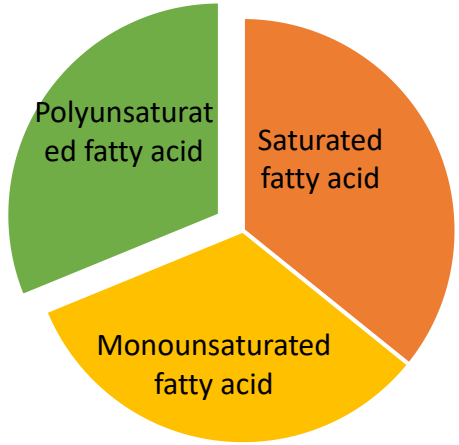
pH 1



Drip loss



Wild type pork



MSTN gene editing pork

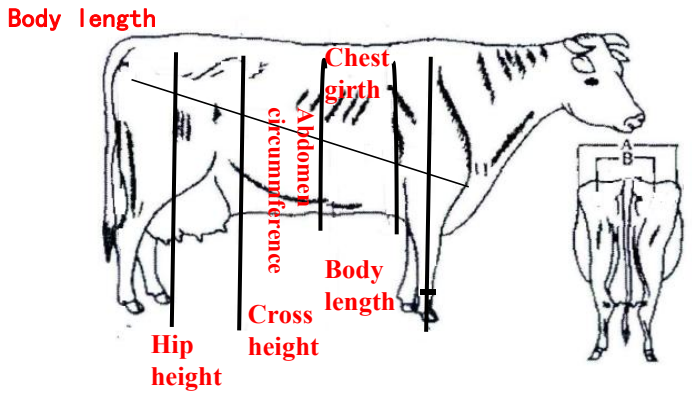
MSTN gene editing of Chinese Local Yellow Cattle

193 Luxi Yellow Cattle and 68 Mongolian Cattle with *MSTN* gene editing have been bred. (Kindly provided by Professor Guangpeng LI of Inner Mongolian University

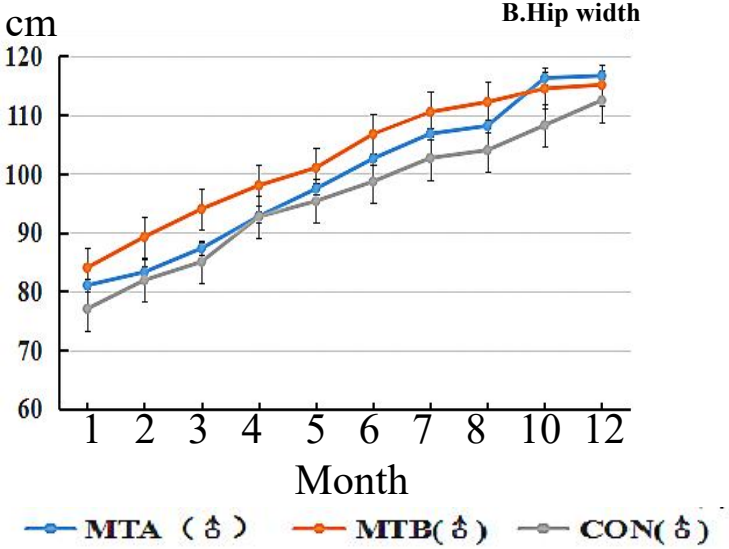
MSTN gene editing F1 Luxi Yellow Cattle

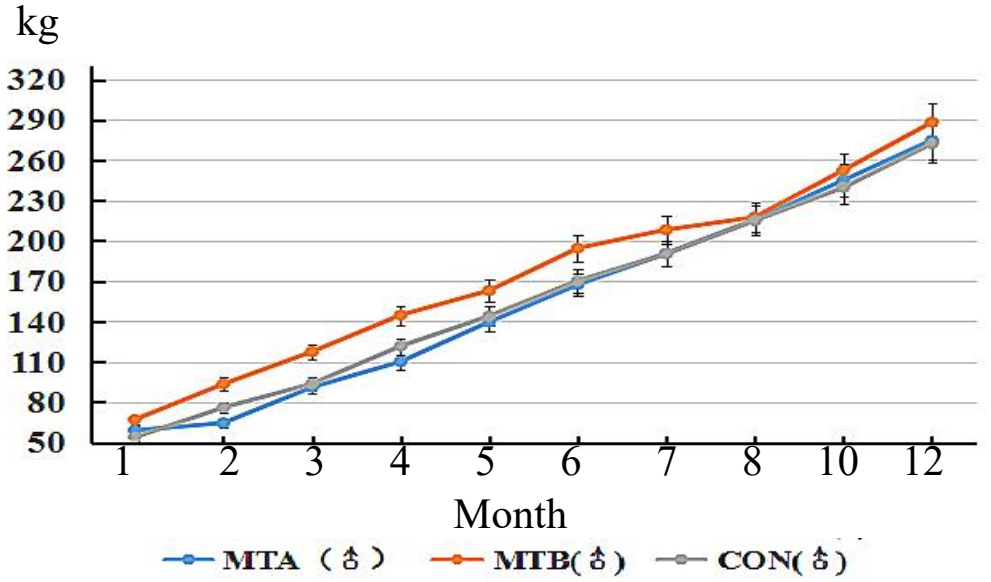
Gene editing F1 Mongolian Cattle

MSTN gene editing of Chinese Local Yellow Cattle



A.Cross width
B.Hip width

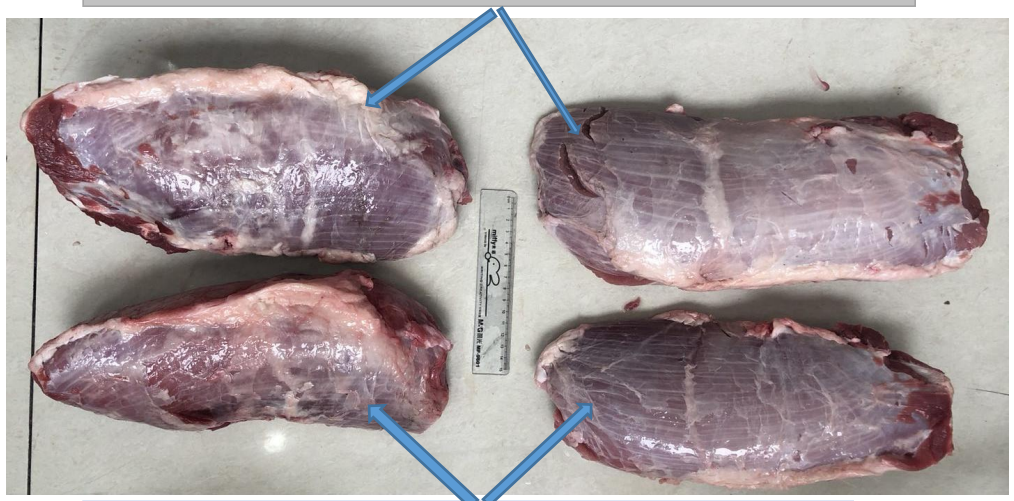




MSTN gene mutation significantly increased the growth and development speed of cattle, and the body weight, body height, chest circumference and body oblique length

Growth performance was significantly improved

Gastrocnemius muscle of test cattle



Control calf gastrocnemius muscle

Soleus muscle of test cattle

Soleus muscle of control cattle

Growth performance was significantly improved: body weight（18.6%）， daily gain(19%)， slaughter percentage(11%)， carcass weight(13.6%)， meat production(20%) than the control cattle.

MSTN gene is an ideal gene for improving Chinese local livestock breeds

Higher lean meat percentage, healthier meat

PART 2

Gene editing enhances livestock resistance to infectious diseases

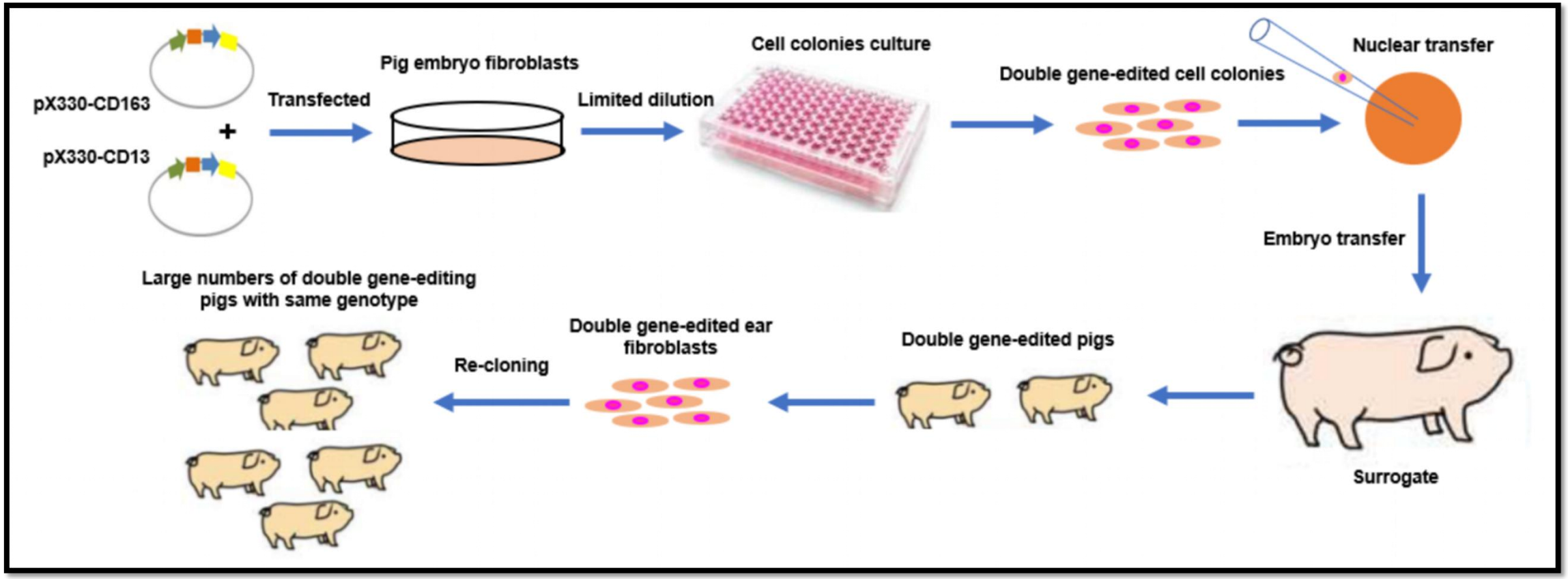
Gene edited for diseases resistance of domestic animals

Epidemics cause hundred billion dollars economic losses to China's animal husbandry every year (personal communications) . Swine epidemics mainly include **African swine fever**, Porcine reproductive and resporatory syndrome**(PRRS)** and **diarrhea.**

There have been many attempts to create new germplasm resistant to African swine fever by gene editing technology in China, but there has been no successful report as yet. But as we all knew, there were several reports about the **gene edited pigs** fully resistance to **PRRS** and Transmissible gastroenteritis virus (**TGEV**) .

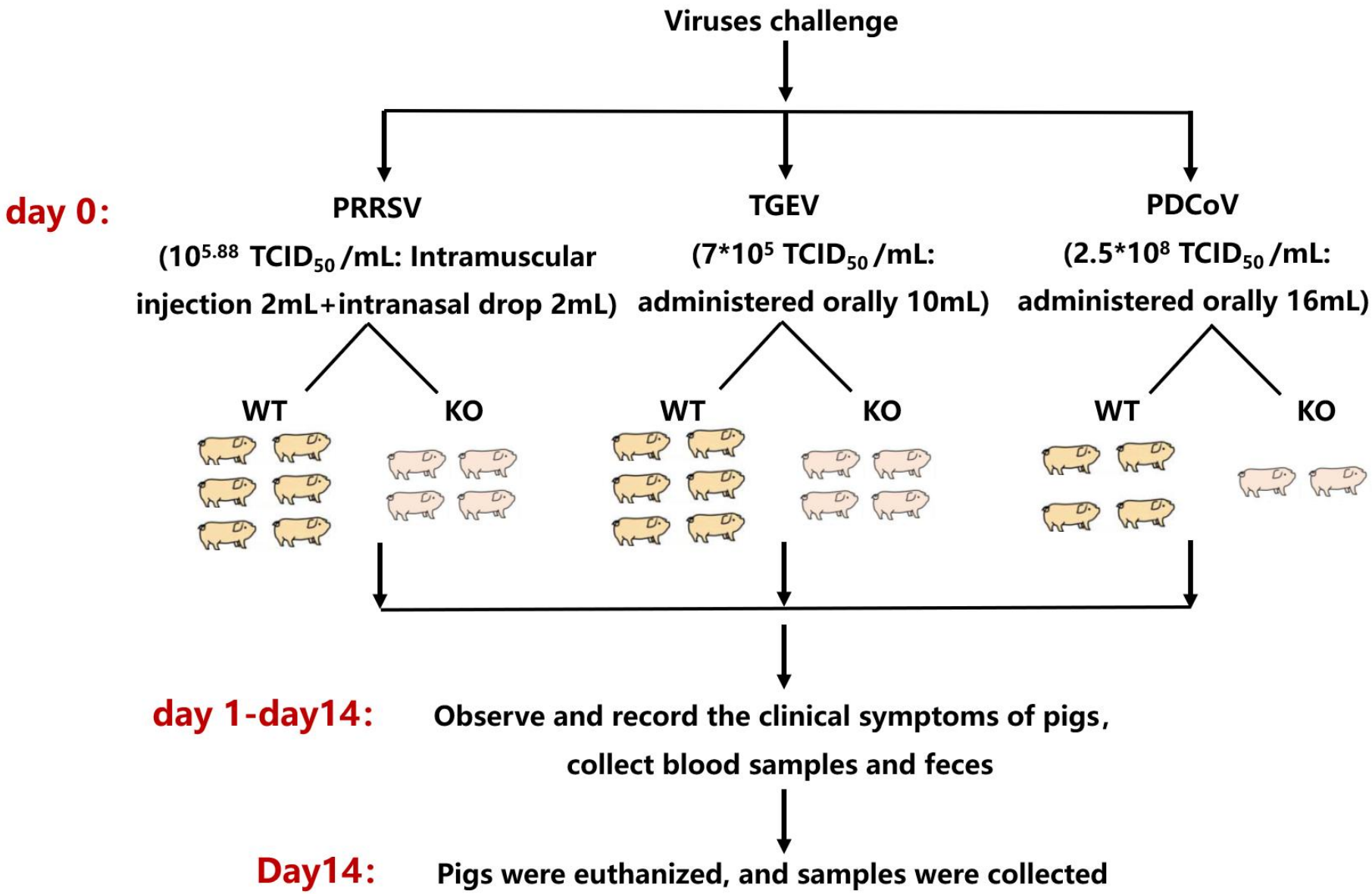
CD163 and *pAPN* double-gene edited pigs

Generation of *CD163* and *pAPN* double-gene edited pigs

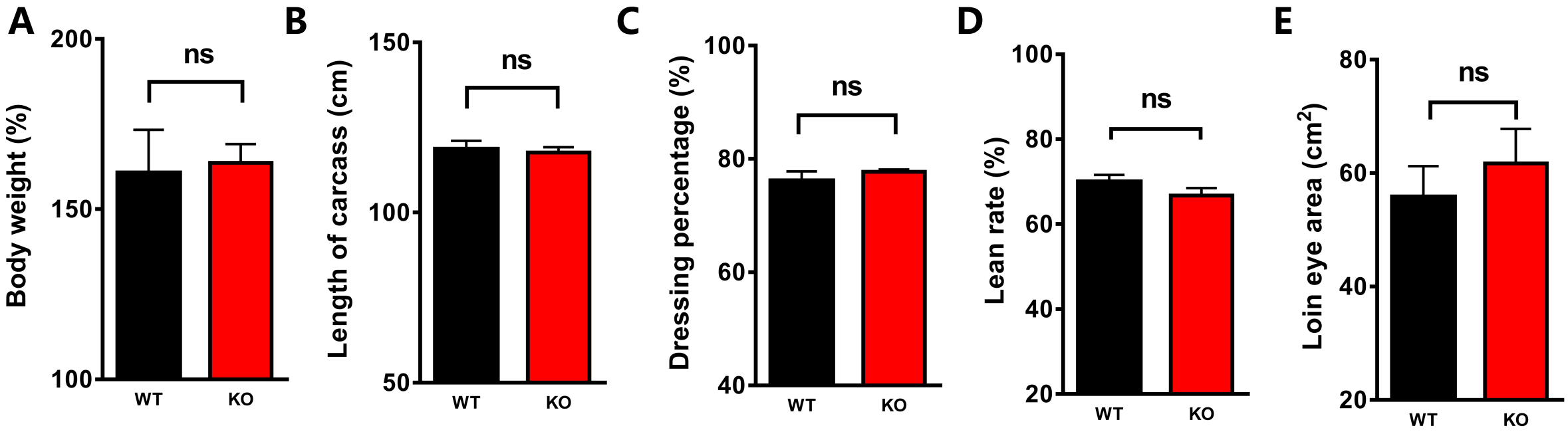


Schematic of the production of double-gene edited pigs

Virus challenge test of disease resistant gene editing pigs



Growth performance and carcass traits are normal in double-gene edited pigs



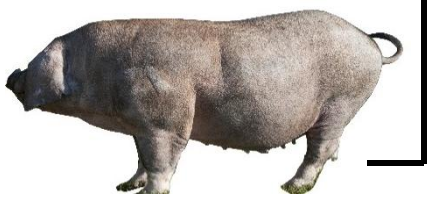
A-E. Growth performance and carcass traits are normal in double-gene edited pigs.

MSTN, *CD163* and *pAPN* three gene deletion pigs were approved for pilot test in China

Ideal breed: 25% Chinese Meishan breed blood, good meat flavor and possibly good reproductive ability. Meat production is similar to world popular commercial breeds, fully anti PRRSV and TGEV.

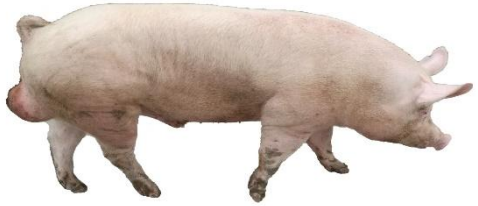
Three gene edited pigs

High lean meat percentage / high polyunsaturated fatty acid *MSTN* edited big white pig



Meishan pig with high lean meat percentage / high polyunsaturated fatty acid (*MSTN*) gene editing

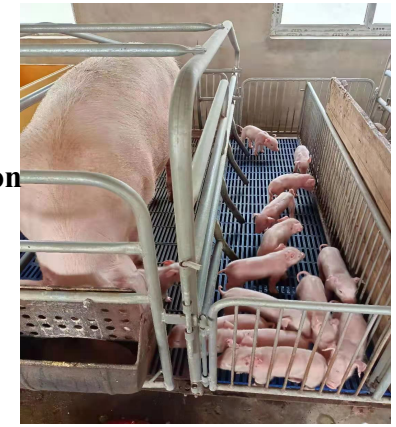
Hybridization



***CD163* / *pAPN* edited pigs**

High lean meat percentage/ high polyunsaturated fatty acid *MSTN* edited Large White - Meishan pig

Hybridization



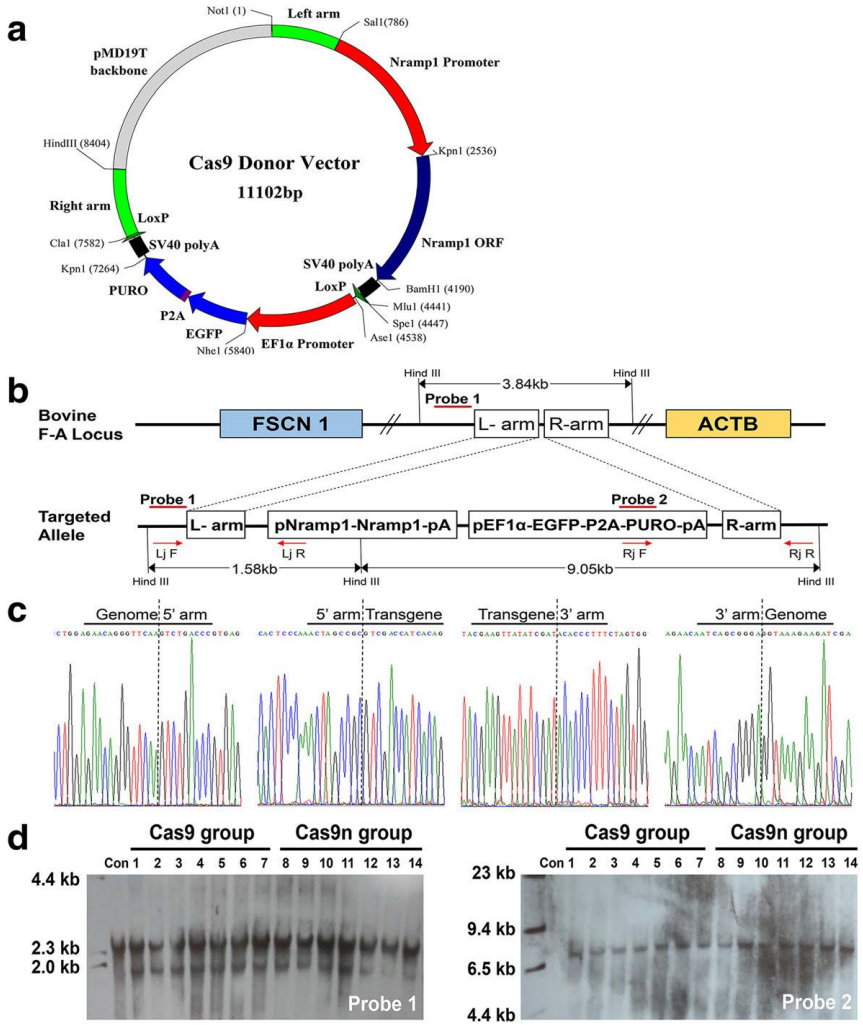
Pigs with high lean meat percentage / high polyunsaturated fatty acid / multiple resistance to major diseases

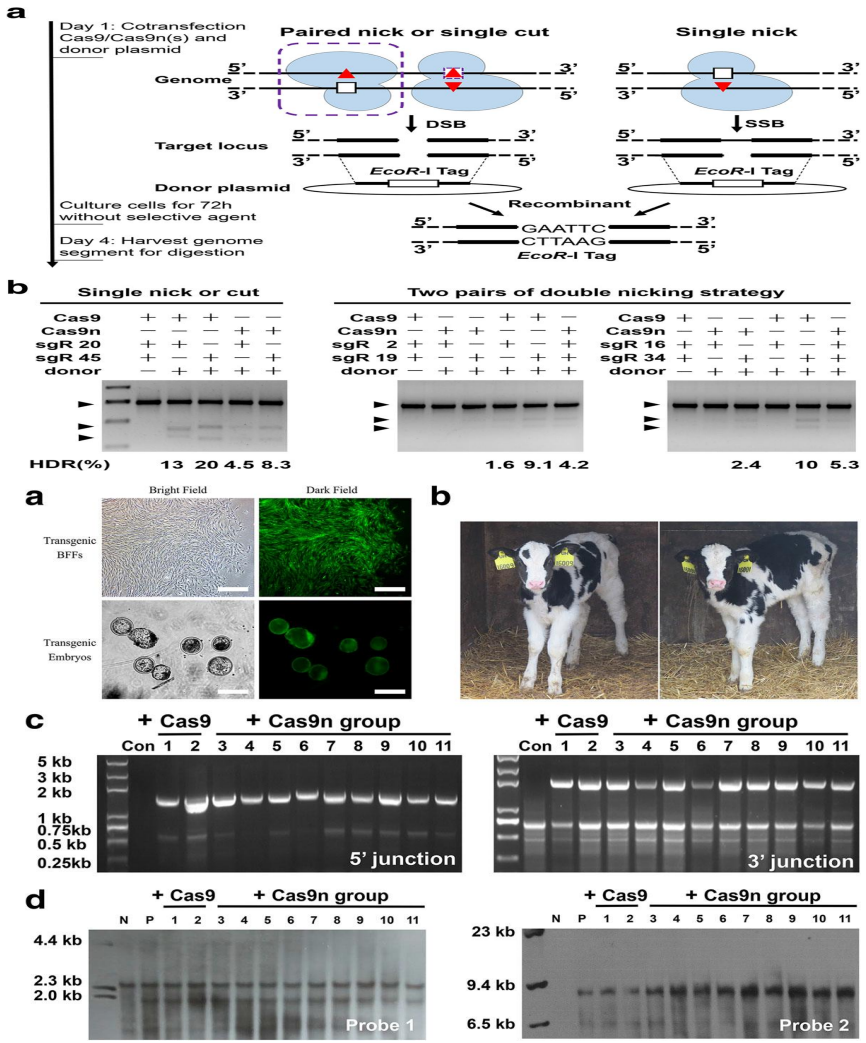
Gene editing technology was used to prepare the anti tuberculosis Dairy cattle

Kindly provided by Prof Yong ZHANG of Northwest A&F University

NRAMP1 gene editing anti tuberculosis Dairy cattle

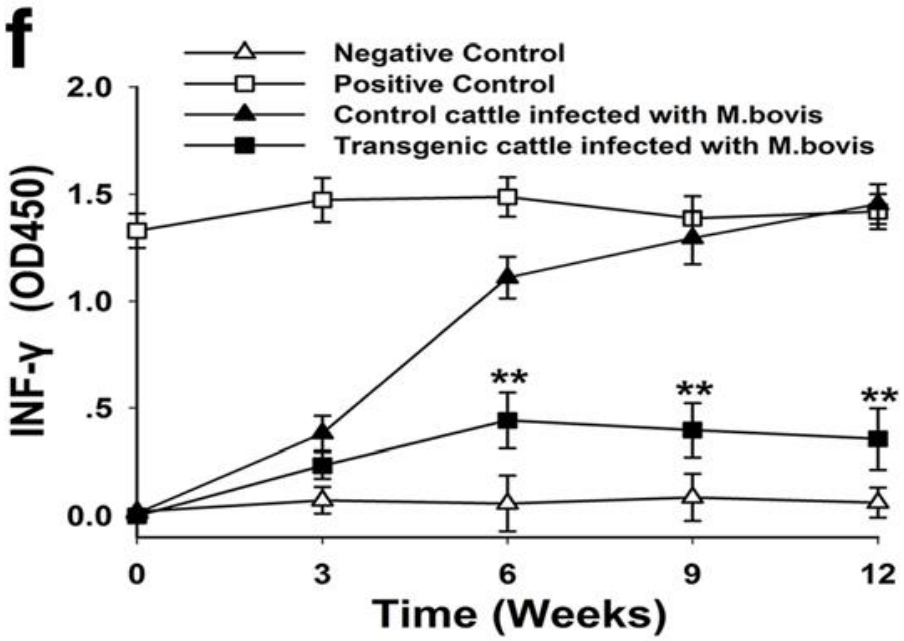
The Cas9 incision enzyme was applied to increase the copy number of bovine *NRAMP1* gene, 11 cloned cows with *NRAMP1* gene were obtained.



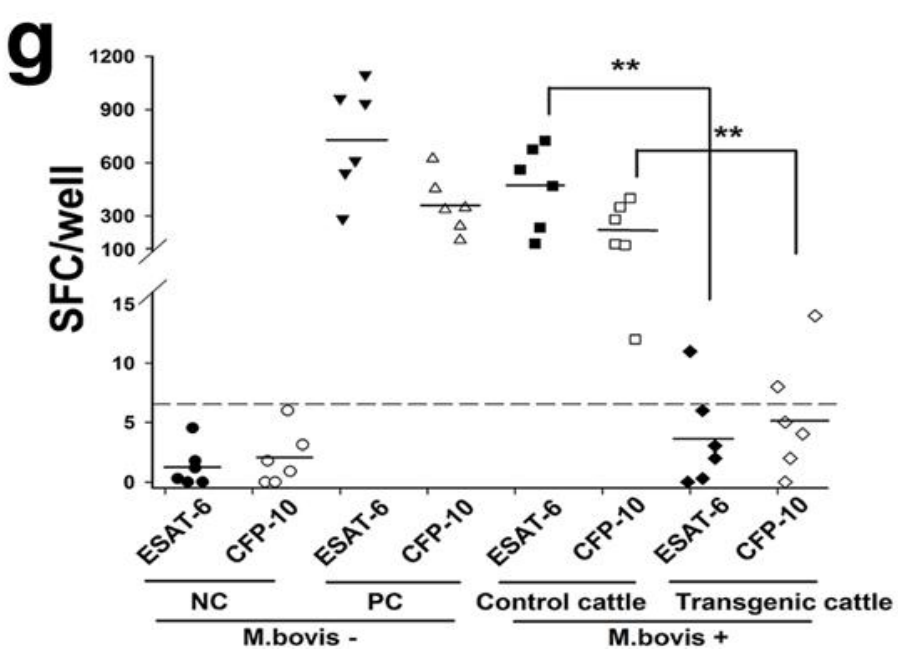


NRAMP1 gene editing enhances tuberculosis resistance in Dairy cows

36 *NRAMP1* gene editing anti tuberculosis cows were bred. The average annual milk output of lactating cows reached 10800 kg. The in vivo challenge test showed that the anti tuberculous bacteria ability of NRAMP1 gene edited cattle was significantly improved， and the anti tuberculosis ability was increased by more than 60% compared with the control group.



M. IFN in transgenic cattle and control cattle within 12 weeks after bovis infection- γ Release level detection



Detection of IFN release from PBMCs stimulated by ESAT-6 and CFP-10 antigen by enzyme-linked immunospot assay- γ Level of

PART 3

Commercialization prospect of gene edited livestock in China

China has issued a simplified review guide for gene edited crops

- China issued a simplified review guide for gene edited crops in Jan, 2022
- The simplified review guide for gene edited animals has been formed in the first draft in China.
- Currently, gene edited animals are still evaluated by five stages, the same as the transgenic animals and crops in China.

GMO safety assessment in China

5 stages :

1. Experimental research
2. Pilot trails
3. Environmental release
4. Production test
5. Safety certificate

About 20 gene edited livestock are in the stage of safety evaluation monitored by MOA (Incomplete personal statistics)

0 at the stage of applied for safety certificate

3 at the stage of the production test

7 at environmental release

10 at pilot trials

Prospect

Gene editing technology will play an important role in the sustainable development of agriculture, but it is essential to simplify the safety assessment procedures and accelerate the commercialization.

Institute of Animal Science,
Chinese Academy of Agricultural Sciences
Northwest A&F University
Inner Mogonian University
Huazhong Agricultural University
University of Guelph
Tianjin Ninghe Pig Breeding Farm
China Agricultural University

Thank you very much!