Global overview of animal biotechnology applications in the livestock industry

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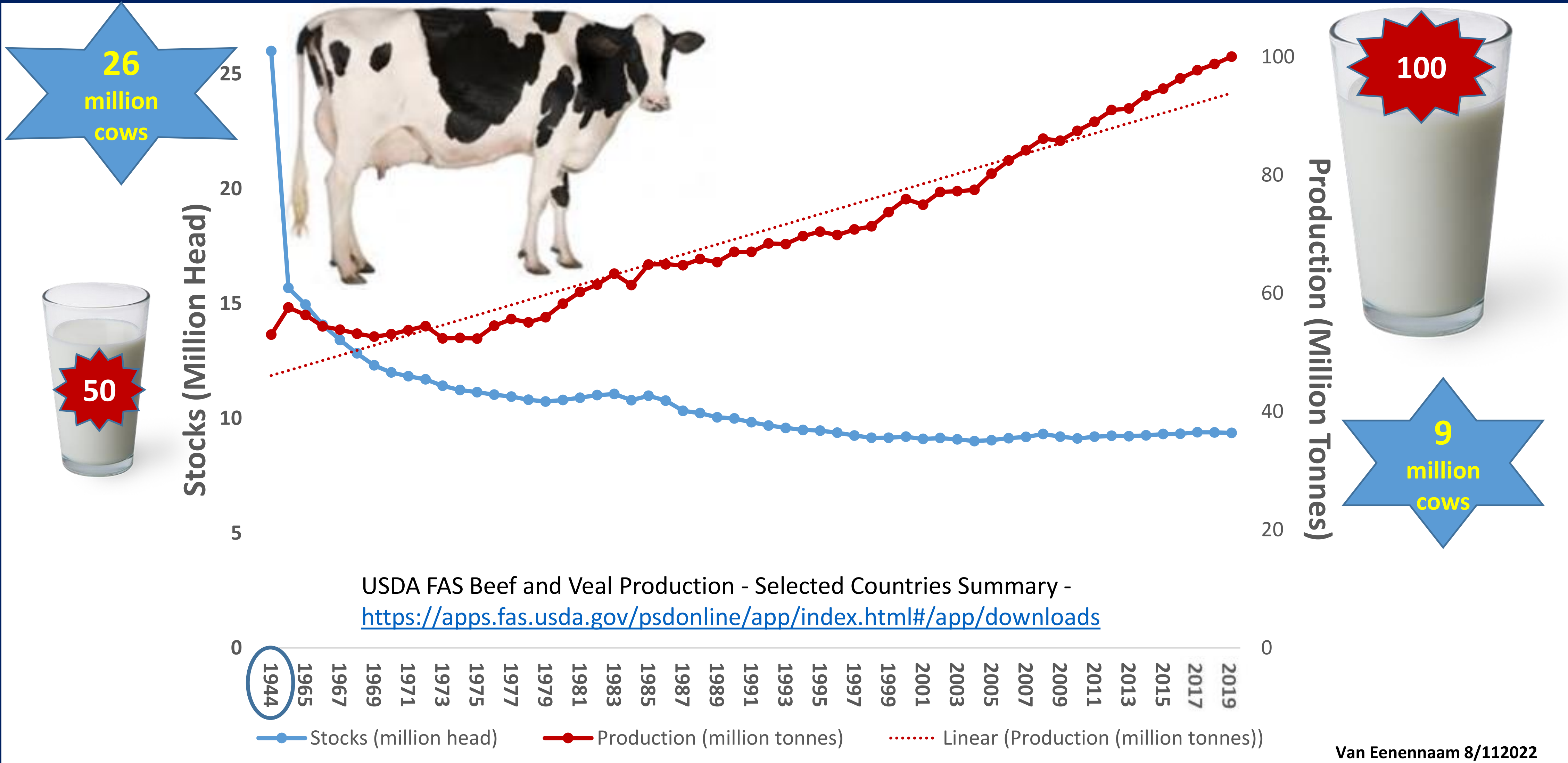
Breeders have selected for desired changes to our food and companion animal populations

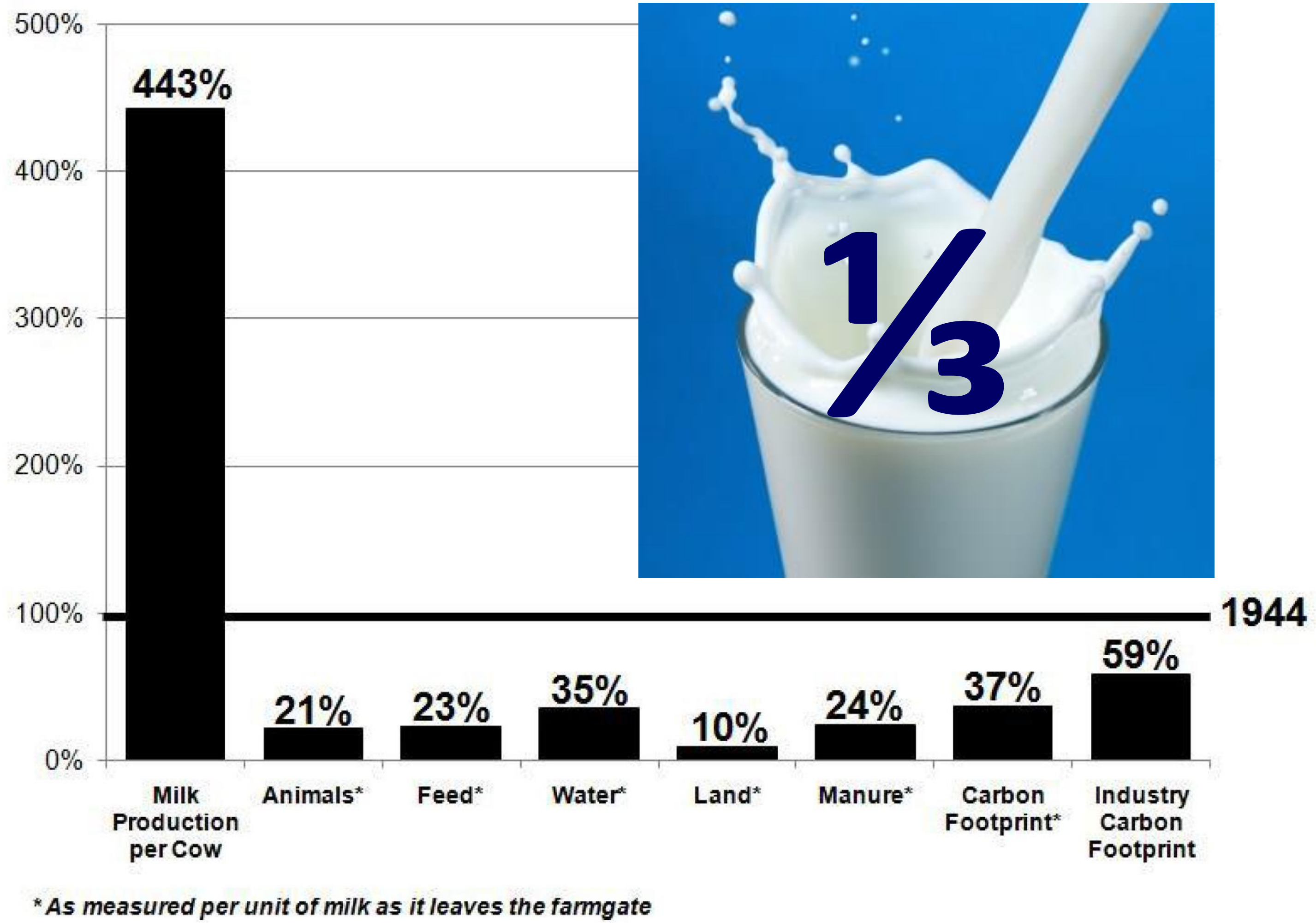
US Dairy Cattle Inventory 1944; 1964 – 2019

Stocks Down (Million head; blue, left)
vs. Milk Production Up (Million Tonnes; red, right)



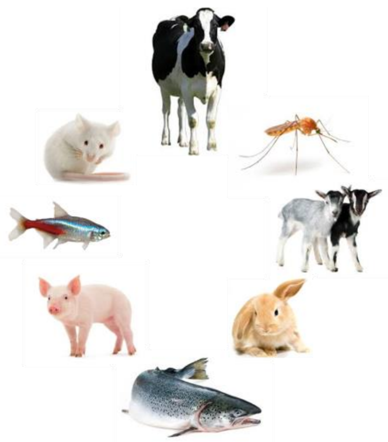
Van Eenennaam 8/112022

The GHG emissions associated with a glass of milk in the US today is ⅓ the 1944 value



Capper, JL and DE Bauman, 2013. Annual Review of Animal Biosciences. 1 pp. 9.1–9.21

Modification of Animal Species



Technologies Used:

- Selective Breeding
- Artificial Insemination
- Embryo Transfer
- Ovum PickUp

} BREEDING TOOLS

- Genomic Selection

} GENOMICS

- Cloning
- Genetic Engineering
- Genome Editing

} MODERN BIOTECHNOLOGY

Research

Billions of genetically engineered Mice/Laboratory Rodents/Zebrafish

Biomedical Products

Pigs – Xenotransplantation; Blastocyst complementation of organs

Pharma products

Rabbit - Ruconest
Goat – ATryn, spider silk;
Chickens – Kanuma
Cows – polyclonal antibodies

Pets

GloFish
Micropigs

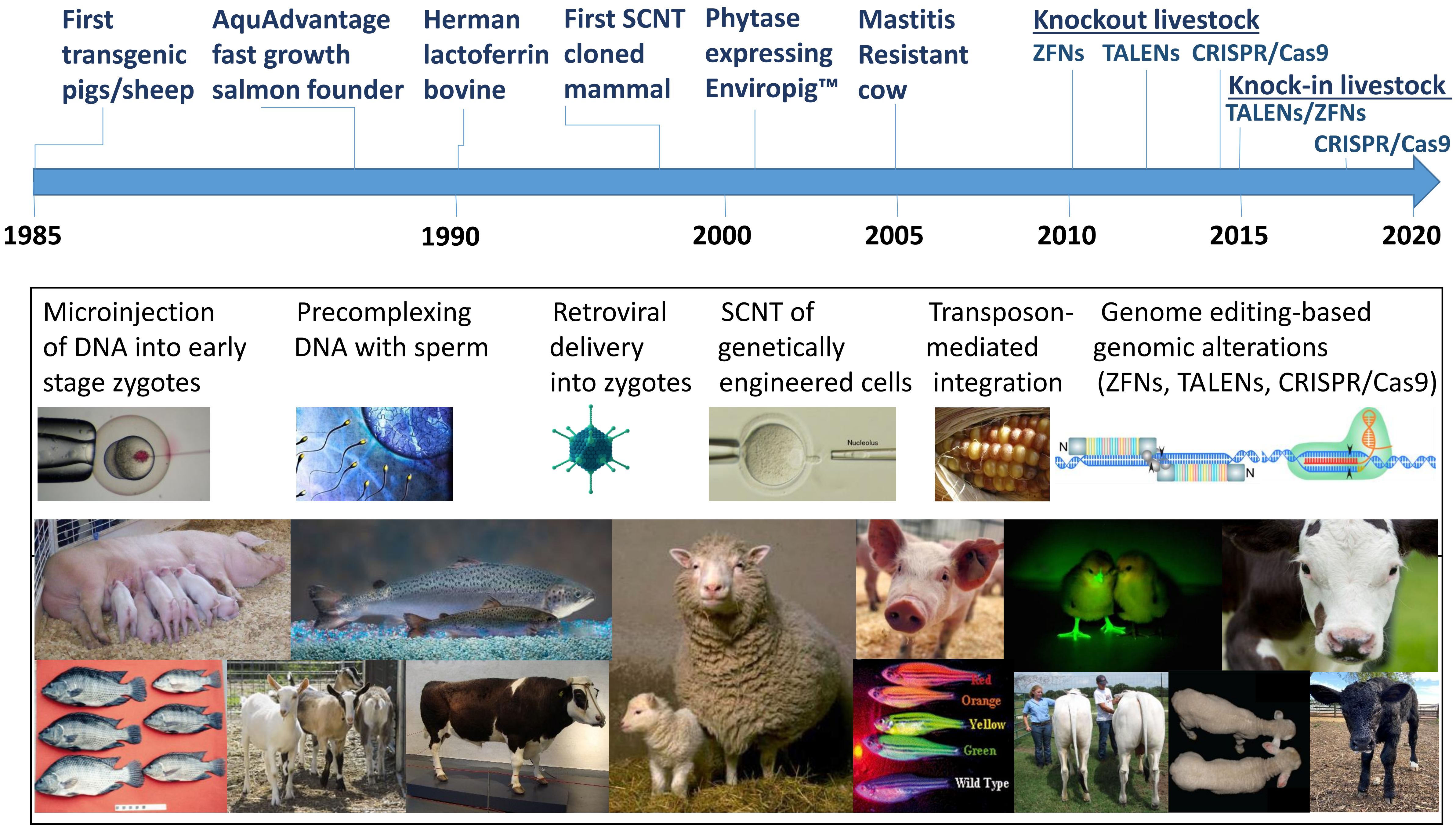
Pest Control

TseTse fly – sleeping sickness
Mosquitoes – zika/malaria resistance
Moths – agricultural pest control

Agriculture/ Food products

AquAdvantage Salmon - fast growth
Disease resistance
Improved product quality
Decrease environmental footprint
Single sex offspring

Van Eenennaam AL. 2018 **The contribution of transgenic and genome-edited animals to agricultural and industrial applications.** Rev Sci Tech 37(1):97-112. doi: 10.20506/rst.37.1.2743. PubMed PMID: 30209426.



Van Eenennaam, A.L. *et al.* **2021. Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay**. Ann Review of Animal Biosciences.

Only 2 approved commercially-available Genetically Engineered (GE) food animals in the US

2

AquAdvantage salmon –
first reported in the
scientific literature in 2002

Galsafe pig –
first reported in the
scientific literature in 2002

Van Eenennaam, A. L., F. De Figueiredo Silva, J. F. Trott, & D. Zilberman. **2021. Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay**. Annual Review of Animal Biosciences. 9:453-478

Gene editing involves introducing a double-strand break in the DNA at a targeted location in the genome

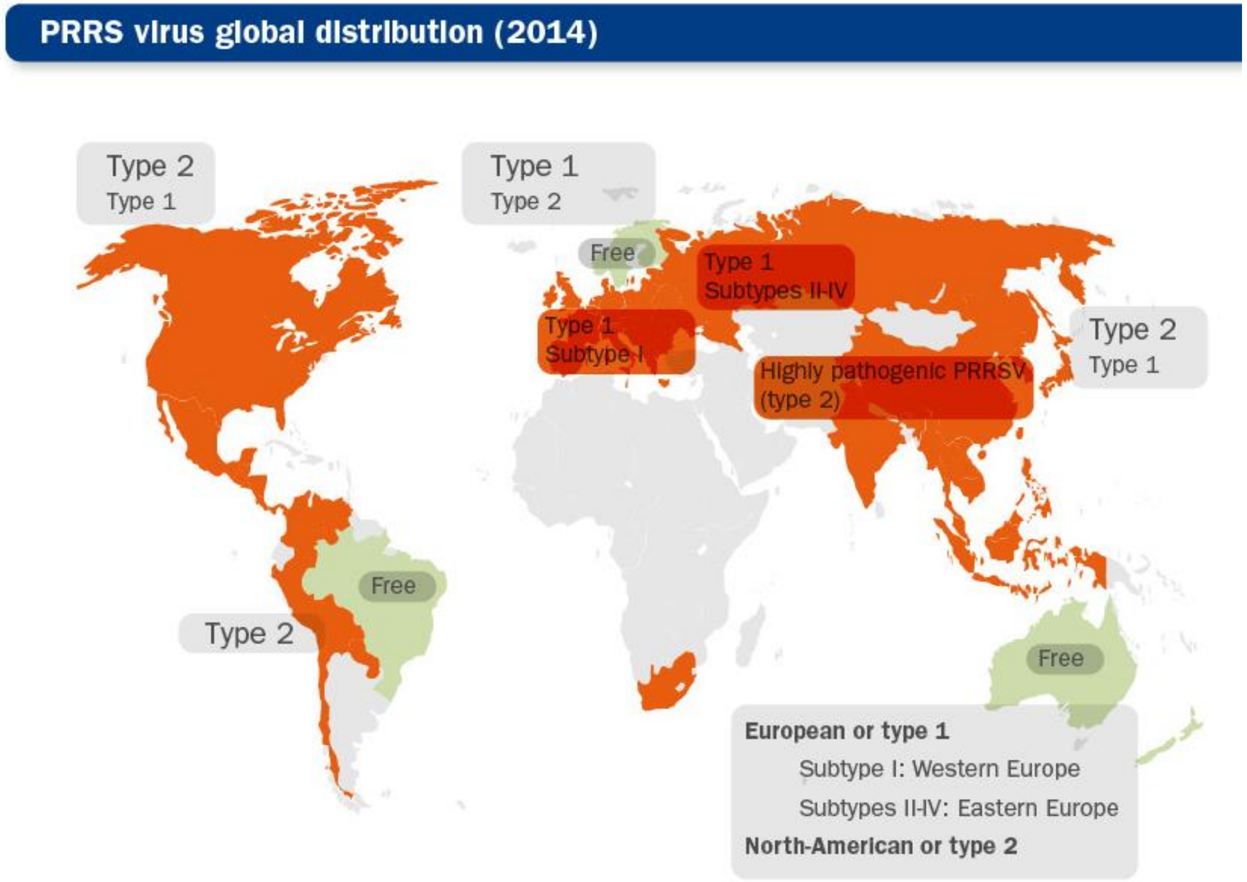
https://youtu.be/bM31E_LRszc

What might we knock-out?

Genes associated with:

- Disease susceptibility (e.g. PRRS virus)
- Allergens (e.g. galactose-alpha-1,3-galactose)
- Unwanted development (e.g. boar taint)
- Thermo tolerance (e.g. SLICK)
- Increased yield (e.g. Myostatin)

Gene editing to produce Porcine Reproductive & Respiratory Syndrome (PRRS) virus resistant pigs



Whitworth et al. 2016. **Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus (PRRSV).** Nature Biotechnology 34:20-22.

Genetic improvement (permanent, cumulative) as a solution to animal disease rather than antibiotics/chemicals

Van Eenennaam, A.L. 2019. **Application of genome editing in farm animals: Cattle.** In *Transgenic Research* 28: 93-100.

Gene editing to remove the major milk allergen: beta-lactoglobulin protein

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Received: 22 January 2018
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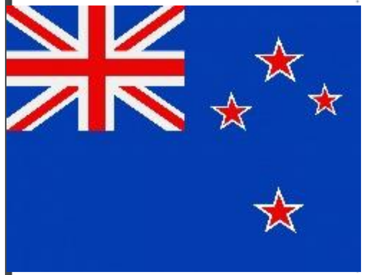
Published online: 16 May 2018



Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin

Jingwei Wei[1], Stefan Wagner[1,2], Paul Maclean[1], Brigid Brophy[1], Sally Cole[1], Grant Smolenski[1,3], Dan F. Carlson[4], Scott C. Fahrenkrug[4], David N. Wells[1] & Götz Laible[1]

We applied precise zygote-mediated genome editing to eliminate beta-lactoglobulin (BLG), a major allergen in cows' milk. To efficiently generate LGB knockout cows, biopsied embryos were screened to transfer only appropriately modified embryos. Transfer of 13 pre-selected embryos into surrogate cows resulted in the birth of three calves, one dying shortly after birth. Deep sequencing results confirmed conversion of the genotype from wild type to the edited nine bp deletion by more than 97% in the two male calves. The third calf, a healthy female, had in addition to the expected nine bp deletion (81%), alleles with an in frame 21 bp deletion (<17%) at the target site. While her milk was free of any mature BLG, we detected low levels of a BLG variant derived from the minor deletion allele. This confirmed that the nine bp deletion genotype completely knocks out production of BLG. In addition, we showed that the *LGB* knockout animals are free of any TALEN-mediated off-target mutations or vector integration events using an unbiased whole genome analysis. Our study demonstrates the feasibility of generating precisely biallelically edited cattle by zygote-mediated editing for the safe production of hypoallergenic milk.

Wei, J., Wagner, S., Maclean, P. *et al.* 2018. Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin. *Sci Rep* **8,** 7661

Gene editing to knock-out a gene called KISSR: which controls sexual development hormones

Castration is done because intact males are aggressive and the smell and the “boar taint” taste of pork caused by excessive testosterone is undesirable.

KISSR is a gene which controls sexual development hormones. Knock-out animals testicles will never enlarge and descend and they will never develop *“that unmistakable ripe smell of an adult boar.”*

Flórez, J.M., et al., 2022. **CRISPR/Cas9-editing of KISS1 to generate pigs with hypogonadotropic hypogonadism as a castration free trait.** Frontiers in Genetics, 13.

Gene editing of prolactin receptor to produce *SLICK* cattle for warmer climates

The animal pictured on the left (a) carries the PRLR p.Leu462* mutation; the animal on the right (b) is wild-type

Image from Littlejohn, M., Henty, K., Tiplady, K. *et al.* 2014. Functionally reciprocal mutations of the prolactin signalling pathway define hairy and SLICK cattle. *Nat Commun* **5,** 5861. https://doi.org/10.1038/ncomms6861

Rodriguez-Villamil P. et al. 2021. **Generation of SLICK beef cattle by embryo microinjection: A case report.** Reprod Fertil Dev. 33(2):114. doi:10.1071/RDv33n2Ab13.

Gene editing myostatin to obtain double muscle cattle/sheep/pigs

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)

Qian et al, 2015

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

From a presentation **"Agricultural applications in China to enhance sustainability"**
September 13, 2022
LI Kui , likui@caas.cn
Agriculture Genomics at Shenzhen, Chinese Academy of Agricultural Sciences

https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshop

Naturally-occurring myostatin knockout in a cow at French cattle show, October 2022

Gene editing myostatin to obtain double muscle fish (Tilapia, Bream, Puffer)

Fish (Tilapia)

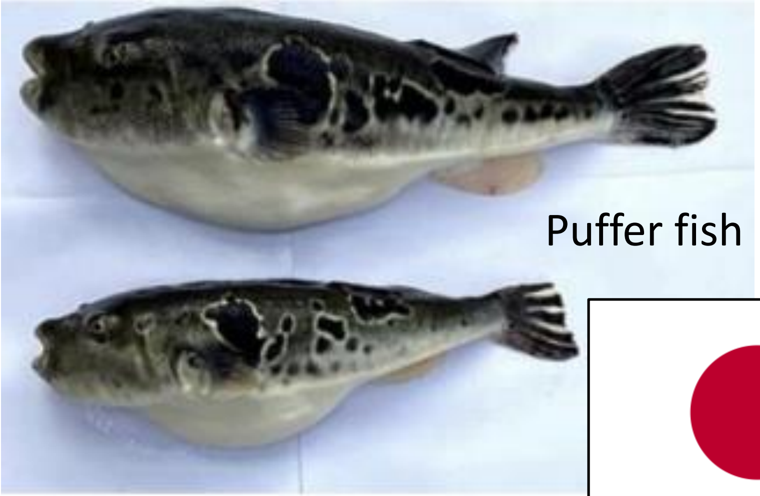
AquaBounty TECHNOLOGIES

Nile tilapia with increased fillet yield

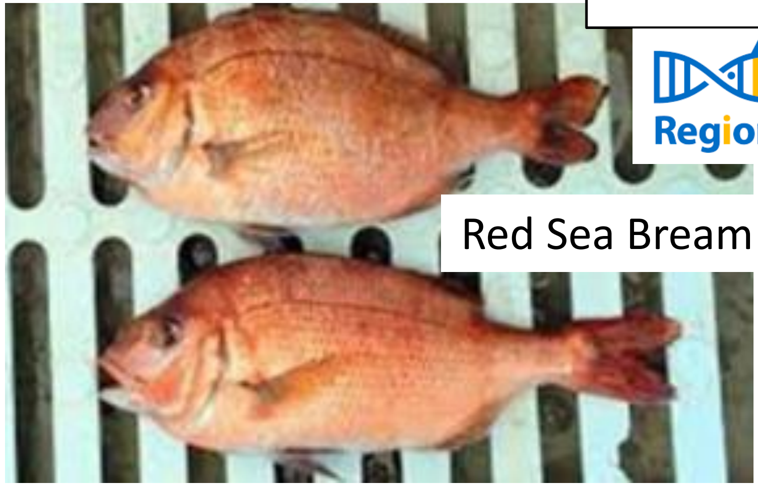
- Fish embryos injected with CRISPR/Cas9 mRNA
- Deletions of nucleotides to knockout the gene
- Increased growth rate and feed conversion
- Product considered non-GMO in 2019

Brazil

Argentina



Puffer fish



Regional Fish

Red Sea Bream

Van Eenennaam 1/31/2023

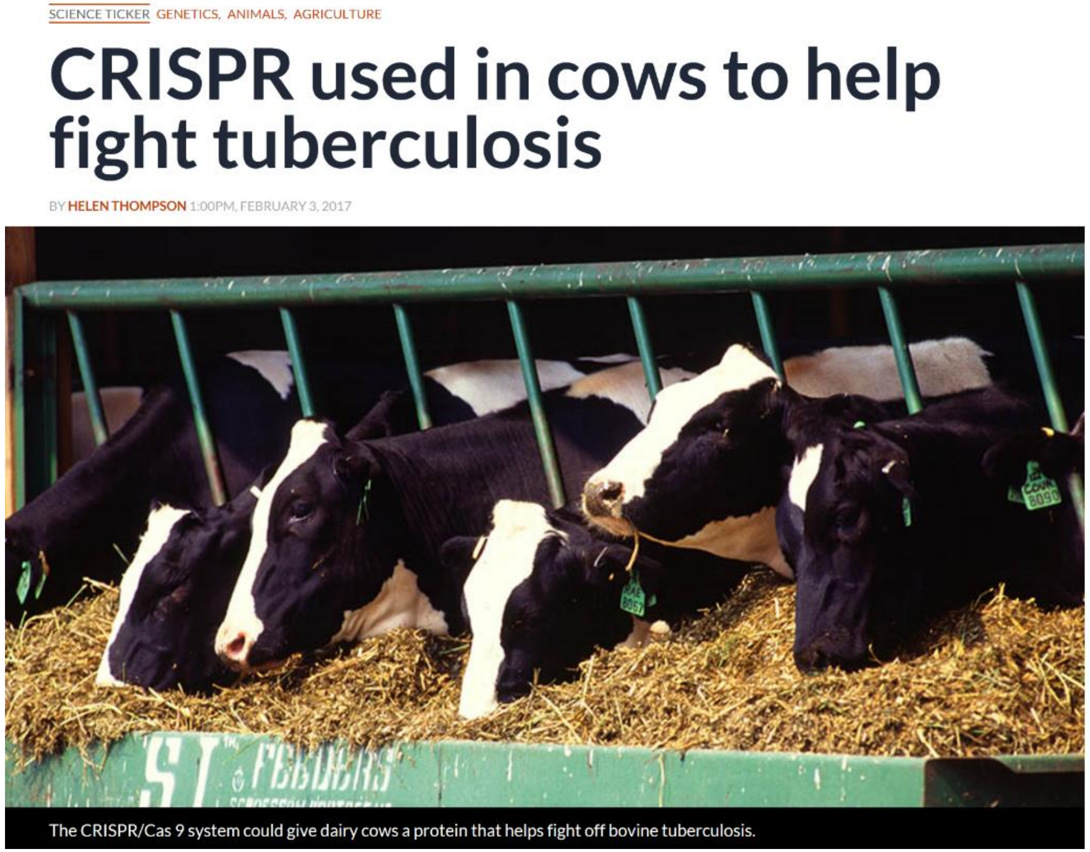
https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshop

What might we knock-in?

Genes associated with

- Disease susceptibility (e.g. tuberculosis)
- Unwanted development (e.g. horns)
- Thermo tolerance (e.g. lighter coat color)
- Improved food quality/nutrition (e.g. high omega-3 pigs)

Gene editing to produce Tuberculosis resistant cattle


SCIENCE TICKER GENETICS, ANIMALS, AGRICULTURE

CRISPR used in cows to help fight tuberculosis

BY HELEN THOMPSON 1:00PM, FEBRUARY 3, 2017

The CRISPR/Cas 9 system could give dairy cows a protein that helps fight off bovine tuberculosis.

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

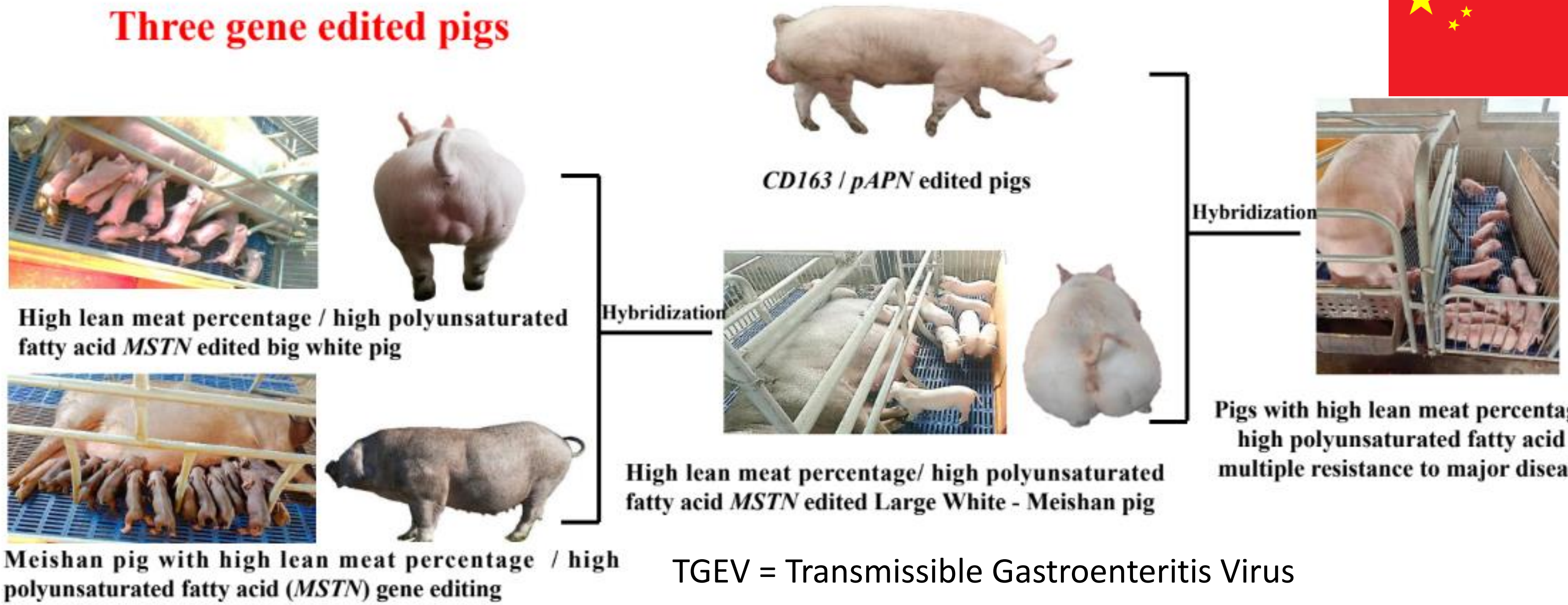
Northwest A&F University, Yangling, China

Wu et al. 2015. ***SP110* knockin endows cattle with increased resistance to tuberculosis**. Proceedings National Academy of Sciences. 112(13):E1530-E9.

Gao et al. 2017. **Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects**. Genome Biol. Feb 1;18(1):13.

Gene editing to produce pigs with three alterations – Myostatin, PRRSV, TGEV

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.



TGEV = Transmissible Gastroenteritis Virus

https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshop

Around ~ 20* gene edited livestock are in the early stage of development and safety evaluation

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

*These numbers are not official numbers they are only estimates.

From a presentation entitled "**Agricultural applications in China to enhance sustainability**"
September 13, 2022
By LI Kui , likui@caas.cn
Agriculture Genomics at Shenzhen, Chinese Academy of Agricultural Sciences.
Available at https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshop

https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshop

Gene editing to produce Classical Swine Fever resistant pigs

PLOS | PATHOGENS

2018

Check for updates

RESEARCH ARTICLE

Genetically modified pigs are protected from classical swine fever virus

Zicong Xie[1☯], Daxin Pang[1☯], Hongming Yuan[1], Huping Jiao[1], Chao Lu[1], Kankan Wang[1], Qiangbing Yang[1], Mengjing Li[1], Xue Chen[1], Tingting Yu[1], Xinrong Chen[1], Zhen Dai[1], Yani Peng[1], Xiaochun Tang[1], Zhanjun Li[1], Tiedong Wang[1], Huancheng Guo[2], Li Li[1], Changchun Tu[2], Liangxue Lai[1], Hongsheng Ouyang[1]*

1 Jilin Provincial Key Laboratory of Animal Embryo Engineering, Institute of Zoonosis, College of Animal Sciences, Jilin University, 130062, Changchun, Jilin Province, People's Republic of China, 2 Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Institute of Military Veterinary, Academy of Military Medical Sciences, Changchun, Jilin Province, People's Republic of China

☯ These authors contributed equally to this work.
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Abstract

Classical swine fever (CSF) caused by classical swine fever virus (CSFV) is one of the most detrimental diseases, and leads to significant economic losses in the swine industry. Despite efforts by many government authorities to stamp out the disease from national pig populations, the disease remains widespread. Here, antiviral small hairpin RNAs (shRNAs) were selected and then inserted at the porcine *Rosa26* (*pRosa26*) locus via a CRISPR/Cas9-mediated knock-in strategy. Finally, anti-CSFV transgenic (TG) pigs were produced by somatic nuclear transfer (SCNT). Notably, in vitro and in vivo viral challenge assays further demonstrated that these TG pigs could effectively limit the replication of CSFV and reduce CSFV-associated clinical signs and mortality, and disease resistance could be stably transmitted to the F1-generation. Altogether, our work demonstrated that RNA interference (RNAi) technology combining CRISPR/Cas9 technology offered the possibility to produce TG animal with improved resistance to viral infection. The use of these TG pigs can reduce CSF-related economic losses and this antiviral strategy may be useful for future antiviral research.

OPEN ACCESS

Citation: Xie Z, Pang D, Yuan H, Jiao H, Lu C, Wang K, et al. (2018) Genetically modified pigs are protected from classical swine fever virus. PLoS Pathog 14(12): e1007193. https://doi.org/10.1371/journal.ppat.1007193

Editor: Shou-Wei Ding, University of California Riverside, UNITED STATES

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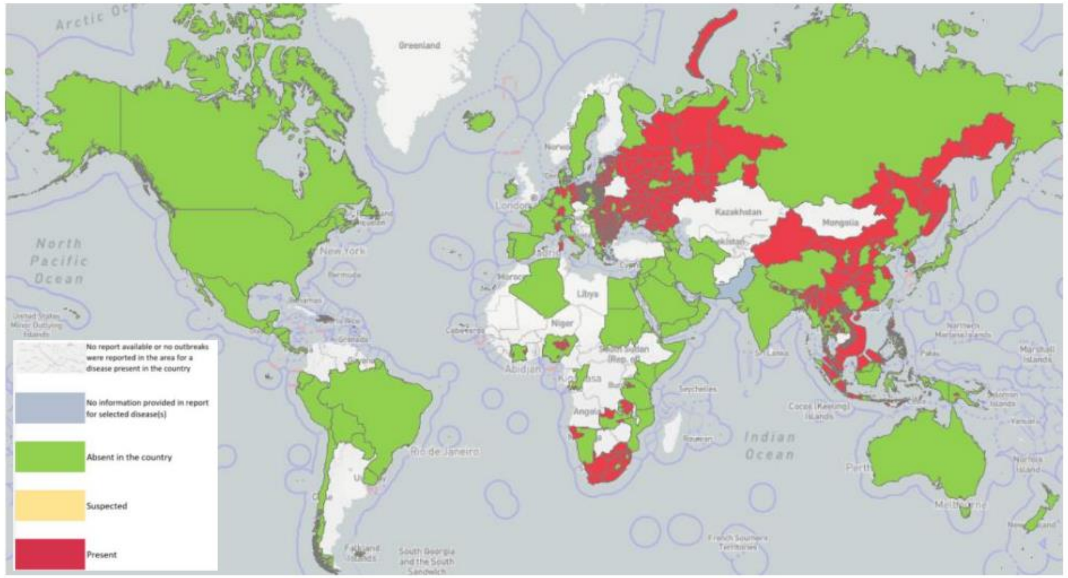
Accepted: October 31, 2018

Published: December 13, 2018

Globally since 2020, and as of 29 September 2022,African Swine Fever (ASF) has been reported in 45 countries including ROK

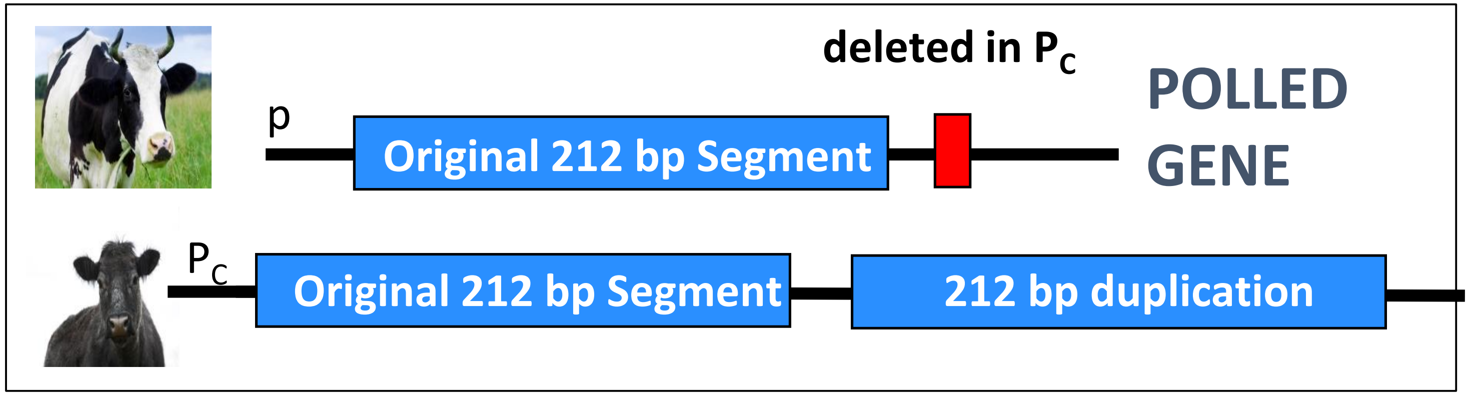


Gene editing to obtain coat color variants better suited to warmer climates

Laible, G., Cole, SA., Brophy, B. et al. 2021. Holstein Friesian dairy cattle edited for diluted coat color as a potential adaptation to climate change. BMC Genomics 22, 856.

Gene Edited Polled Calves

Naturally-occurring bovine allele at polled locus



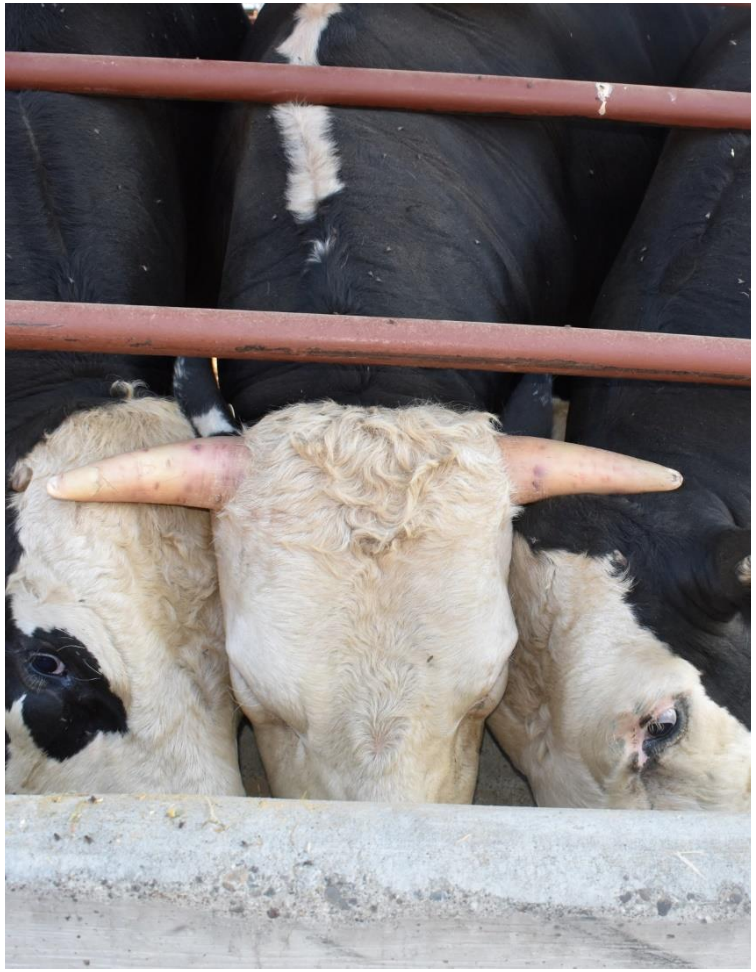
Production of hornless dairy cattle from genome-edited cell lines

Daniel F Carlson[1], Cheryl A Lancto[1], Bin Zang[2], Eui-Soo Kim[1], Mark Walton[1], David Oldeschulte[3], Christopher Seabury[3], Tad S Sonstegard[1] & Scott C Fahrenkrug[1]

USDA United States Department of Agriculture
National Institute of Food and Agriculture
2015-67015-23316

Carlson DF, Lancto CA, Zang B, Kim E-S, Walton M, et al. 2016. **Production of hornless dairy cattle from genome-edited cell lines.** Nature Biotechnology 34: 479-81

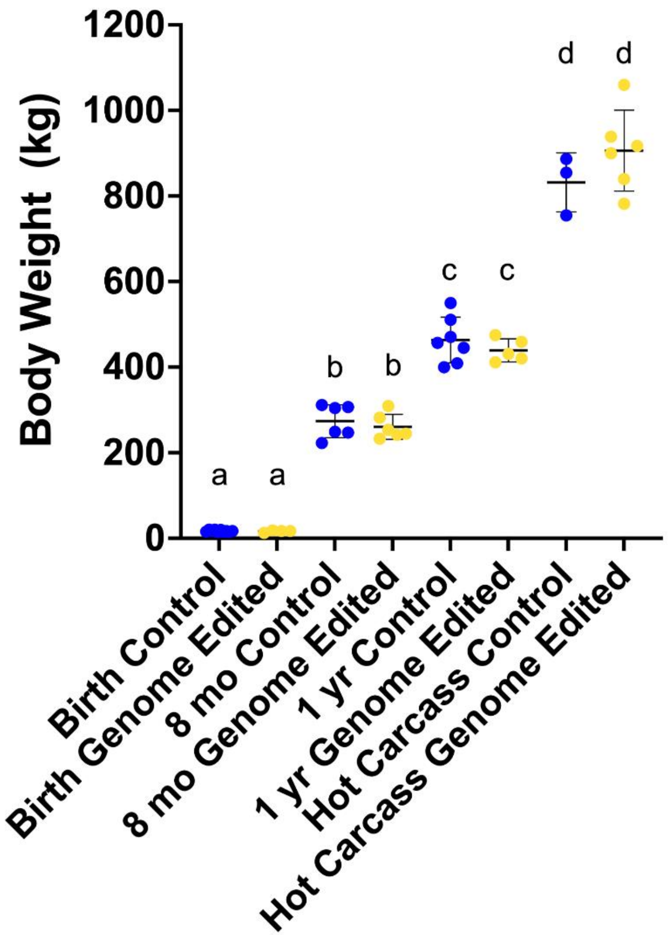
We analyzed the 6 heterozygous hornless offspring of the genome edited bull and controls for several years



Young, A.E. *et al.* 2020. **Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull.** Nature Biotech 38, 225–232

USDA United States Department of Agriculture National Institute of Food and Agriculture
Biotechnology Risk Assessment Grant #2017-33522-27097

The growth & health, and the milk and meat composition of the 6 heterozygous hornless offspring of the genome edited bull were equivalent to contemporary controls



USDA United States Department of Agriculture National Institute of Food and Agriculture
Biotechnology Risk Assessment Grant #2017-33522-27097

Trott, J. et al. 2022. **Animal health and food safety analyses of six offspring of a genome-edited hornless bull**. GEN Biotechnology. *1:2, 192-206*

Summary

- Genetic improvement of both plants and animals has been an important driver of agricultural sustainability.
- Biotechnology offers an approach to introduce useful genetic variation and alleles without the “linkage drag” typically associated with conventional cross-breeding.
- Traits that have been targeted include disease-resistance, heat tolerance, growth, and animal welfare traits that are difficult to address using conventional breeding.
- The fate of animal biotechnology will depend upon developing a harmonized, risk-based regulatory framework that permits international trade of products (milk, meat, eggs), gametes (i.e. sperm & oocytes), and embryos.

Acknowledgements

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- Dr. Tamer A. Mansour
- Dr. Xiang (Crystal) Yang
- Amy Young
- Barbara Nitta
- Ross lab members

revive & restore
genetic rescue for endangered and extinct species

FFAR Foundation for Food and Agriculture Research

SELECT SIRES INC.

USDA United States Department of Agriculture

National Institute of Food and Agriculture

2015-67015-23316 2017-33522-27097, 2017-38420-26790, 2018-67030-28360, 2020-67015-31536, 2020-70410-32899