Emerging Biotech Solutions for Livestock Production

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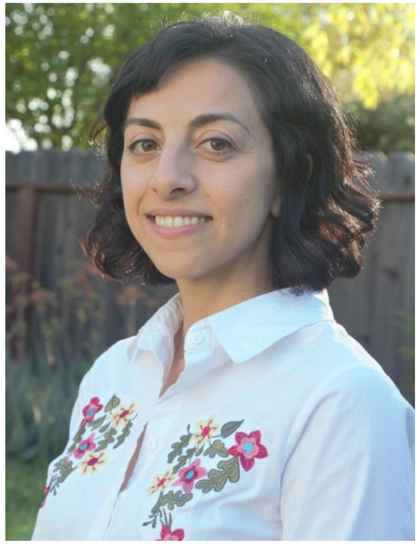

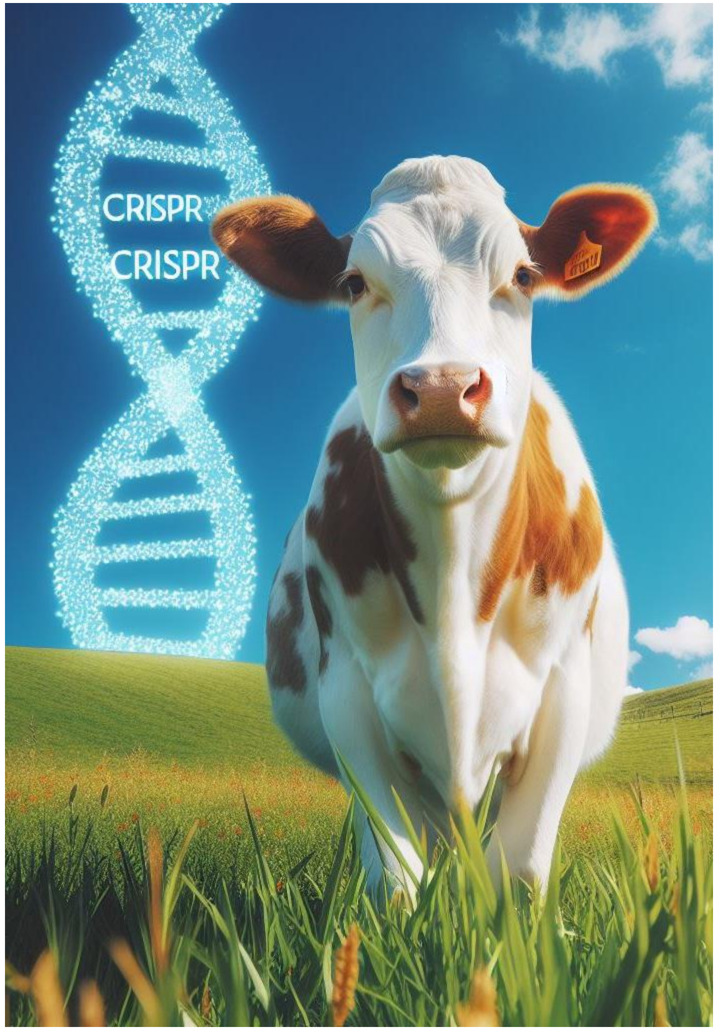

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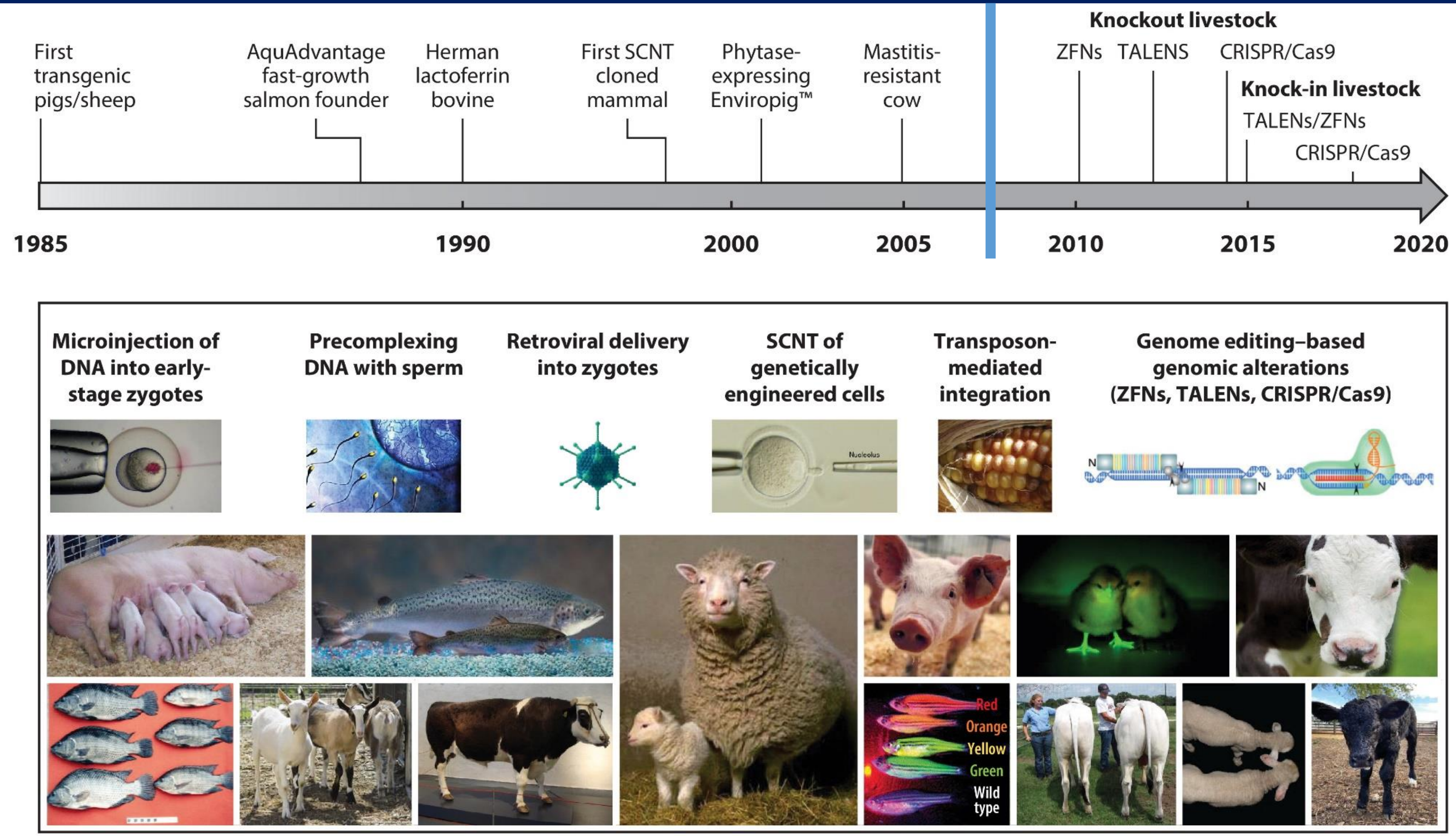
Breeders have selected for desired changes to companion animal populations based on naturally-occurring DNA variation

An abbreviated schematic history of 35+ years of biotechnology food animals for agriculture

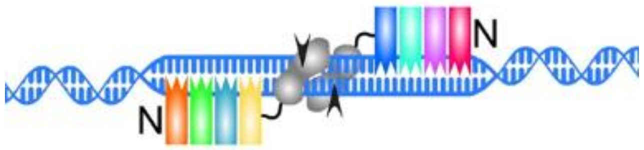


Van Eenennaam, A.L. *et al.* **2021. Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay**. Ann Review of Animal Biosciences.

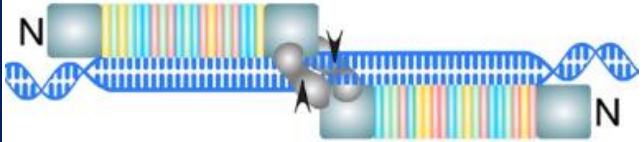
Van Eenennaam AL, et al. 2021
Annu. Rev. Anim. Biosci 9:453–78

WHAT

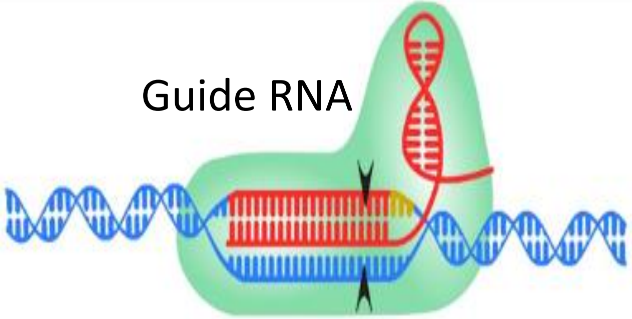
Gene editing allows the introduction of targeted double-stranded breaks in the genome



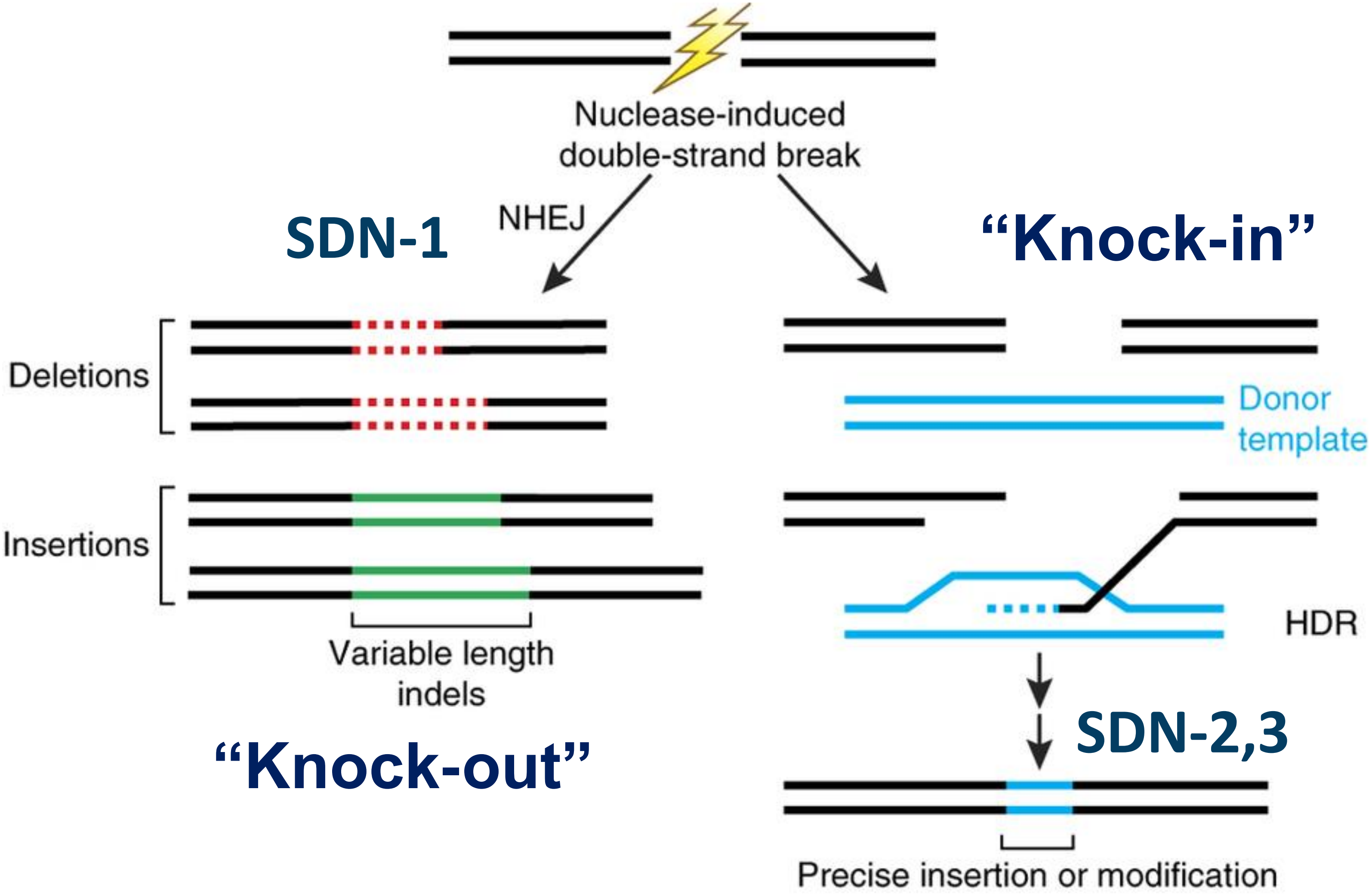
Zinc Finger Nucleases



TALENS



CRISPR/Cas9

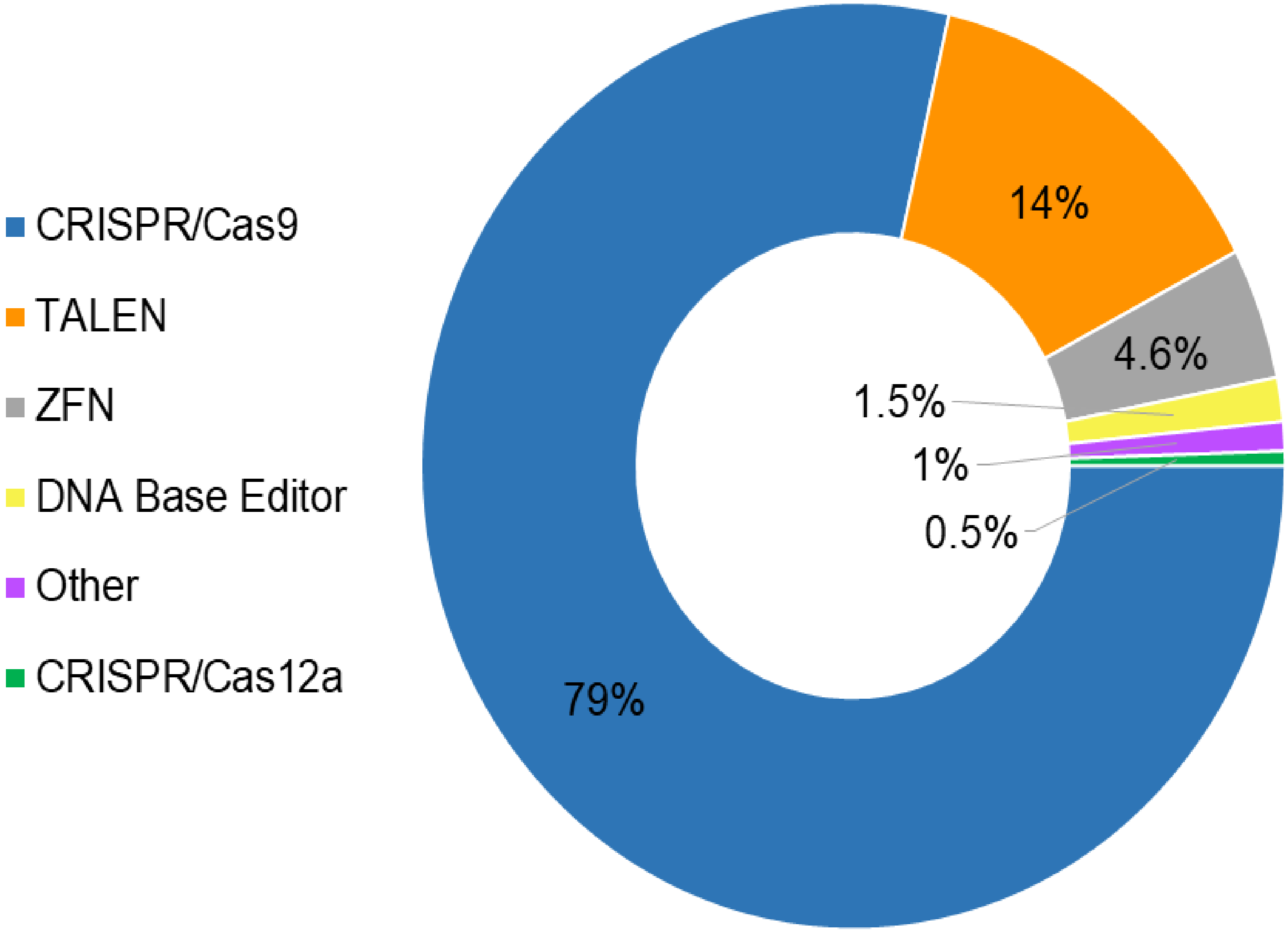


Sander JD, Joung JK. **CRISPR-Cas systems for editing, regulating and targeting genomes**. *Nat Biotech* 2014;32:347-355.

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

https://youtu.be/bM31E_LRszc

Most peer-reviewed gene edited animal research papers (n=195) used CRISPR/Cas9, and most edited embryos followed by cloning of somatic cells. Avian examples use a different route.



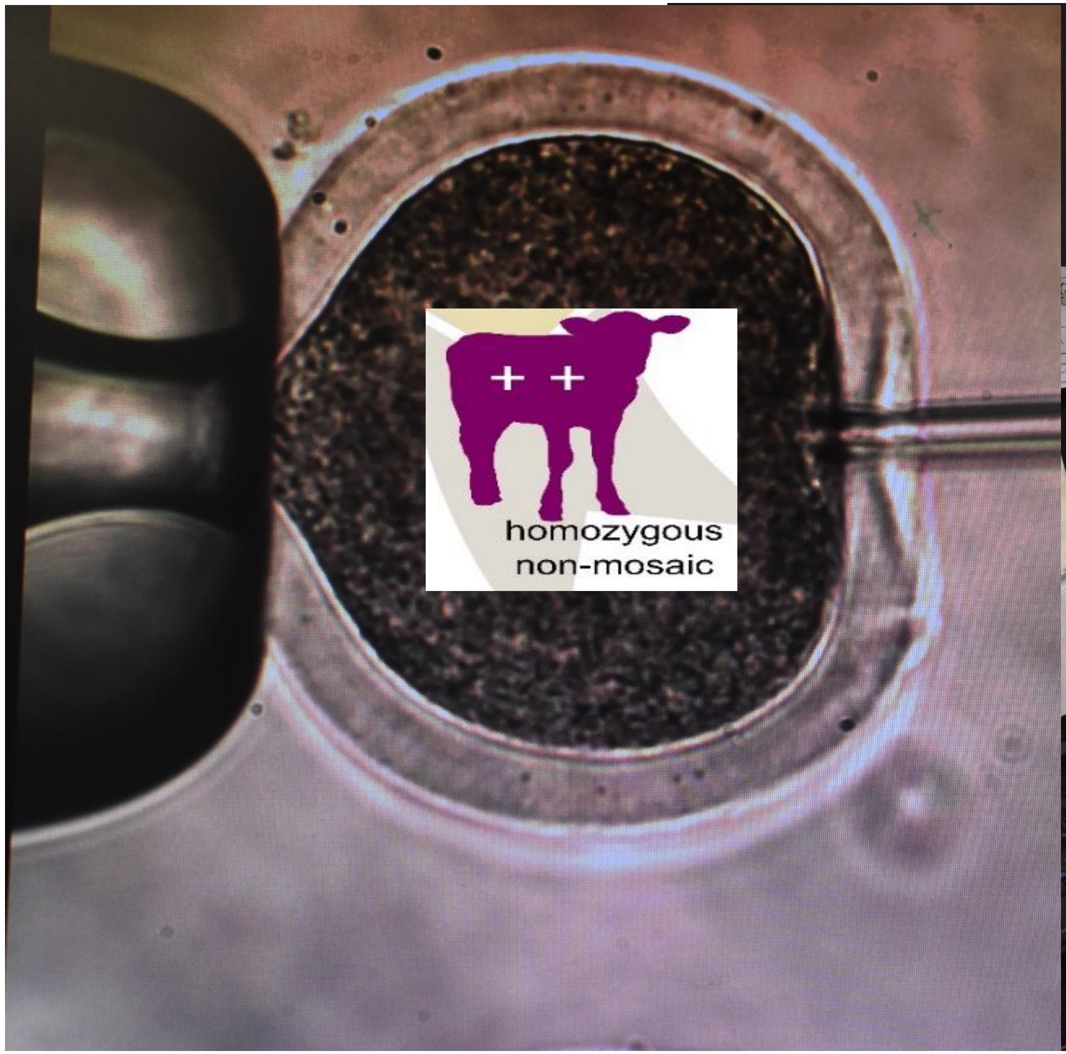
- There were 59 applications (30%) where the editing was done in cell lines followed by cloning to produce an animal, all in mammals;
- 118 publications (61%) that edited developing embryos,
- 18 “other” approaches (9%) to editing, the majority of which were publications with avian species where editing was done in primordial germ cells

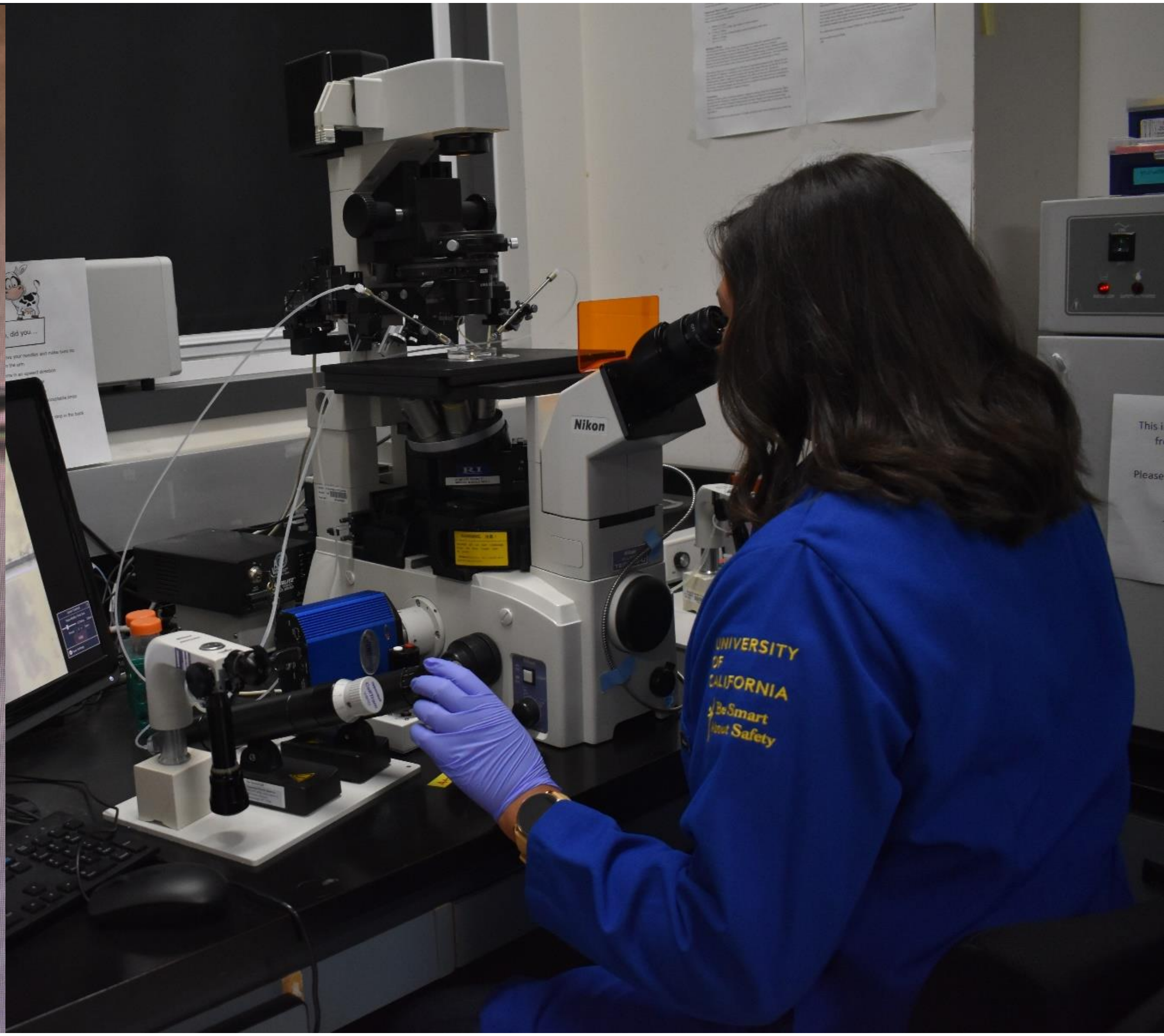
- The majority ~ 75% of these applications were SDN-1 (147) aka knockouts; with 18 SDN-2, and 30 SDN-3 applications.

Van Eenennaam. 2023. *EFSA supporting publication* 2023: 20(9):EN-8311. 82 pp.

HOW

Introducing useful genetic variation into the germline of selected parents such that genetic improvement is inherited by the next generation is the ultimate goal of animal breeding.





WHY

What might we inactivate? (SDN-1)

Genes associated with:

- Allergens (e.g. galactose-alpha-1,3-galactose)
- Thermo tolerance (e.g. SLICK Prolactin receptor)
- Sex ratio skew (e.g. all-female pigs SRY KO)
- Unwanted development (e.g. boar taint)
- Increased yield (e.g. Myostatin KO)
- Disease susceptibility (e.g. PRRS virus CD163)

What traits might we introduce?

DNA variants associated with

- Disease susceptibility (e.g. Tuberculosis (TB))
- Unwanted development (e.g. horns)
- Thermo tolerance (e.g. lighter coat color)
- Improved food quality/nutrition (e.g. high omega-3 pigs)
- Sex ratio skew (e.g. all-female layer chicken)

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.

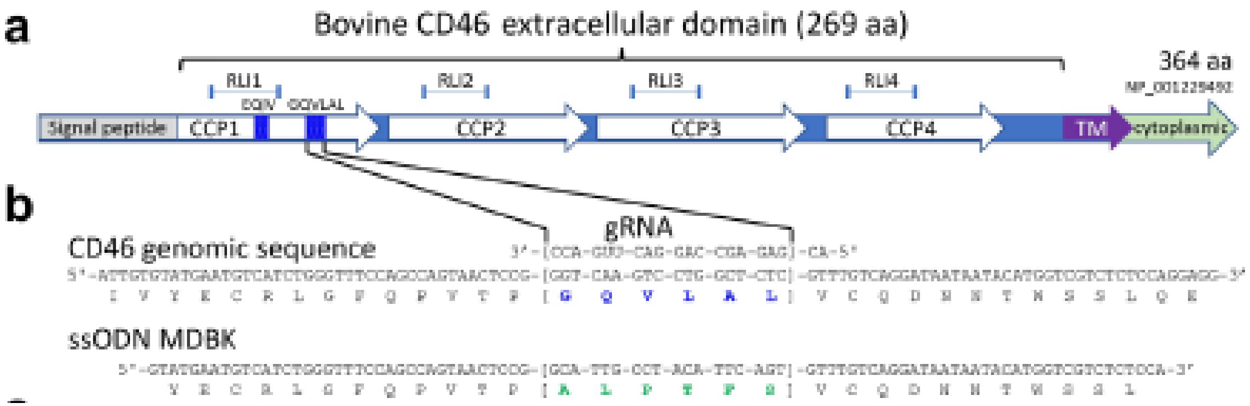
Wu et al. 2015. ***SP110*** **knockin endows cattle with increased resistance to tuberculosis**. Proceedings National Academy of Sciences. 112(13):E1530-E9.

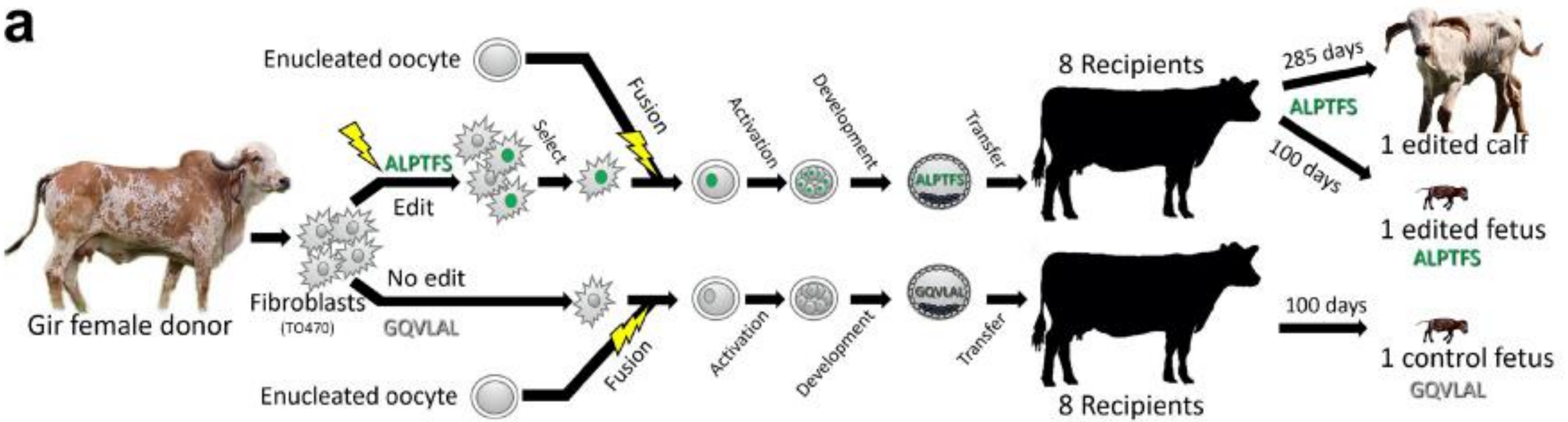
Kindly provided by Prof Yong ZHANG of Northwest A&F University

Northwest A&F University, Yangling, China

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.

First gene-edited calf with reduced susceptibility to a major viral pathogen (BVD)





Aspen M Workman et al. 2023. PNAS Nexus, Volume 2, Issue 5, May 2023, pgad125,
https://doi.org/10.1093/pnasnexus/pgad125

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 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.
Wei, J., et al. 2023. **Production of light-coloured, low heat-absorbing Holstein Friesian cattle by precise embryo-mediated genome editing**. *Reproduction, Fertility and Development*.

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

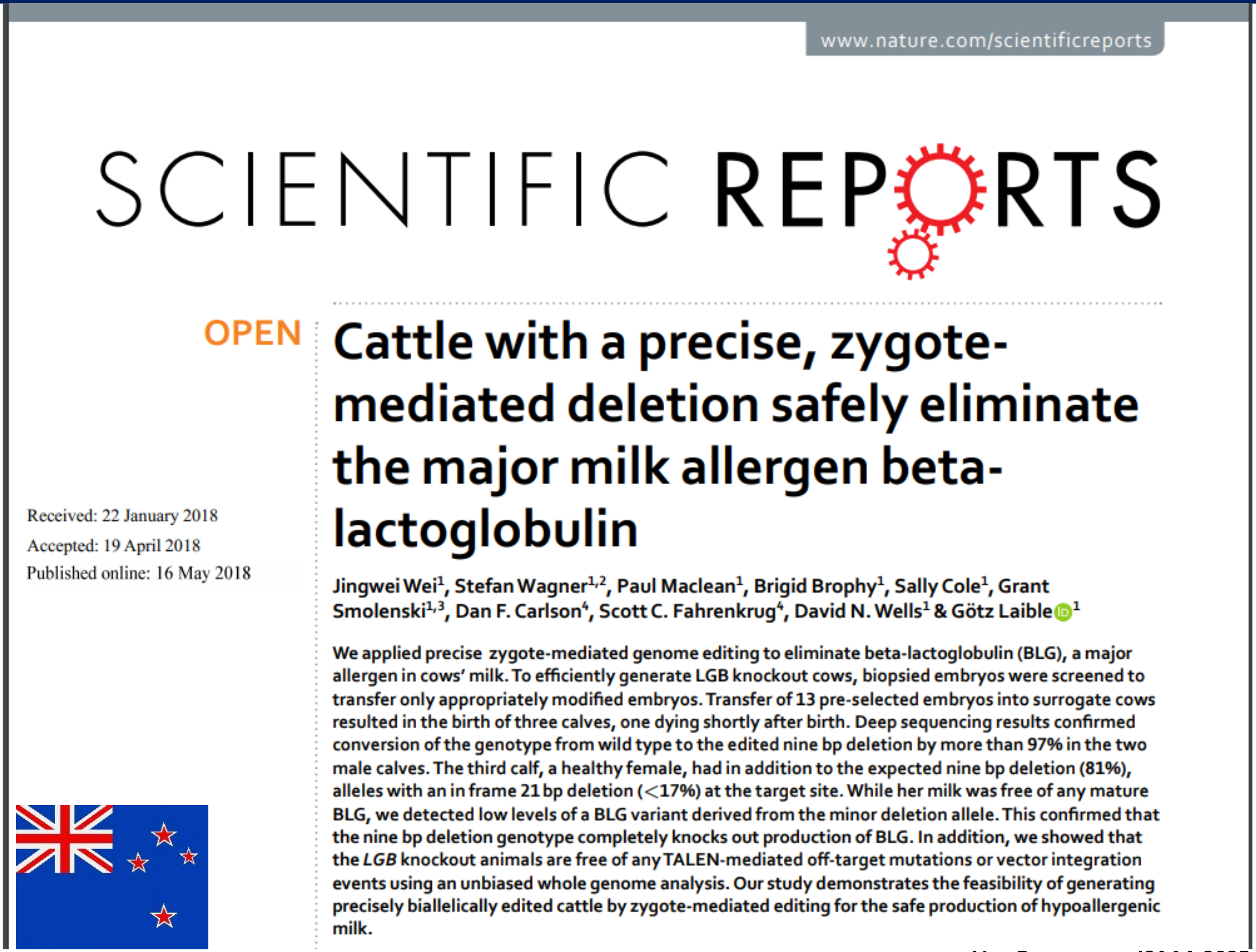
The animal pictured on the left (a) carries the PRLR p.Leu462* mutation; the animal on the right (b) is wild-type

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 to remove the major milk allergen: beta-lactoglobulin protein

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 https://doi.org/10.1038/s41598-018-25654-8



www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

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

Received: 22 January 2018
Accepted: 19 April 2018
Published online: 16 May 2018

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.

Van Eenennaam ISAAA 2025

Gene Edited Polled Calves

Naturally-occurring bovine allele at polled locus

Production of hornless dairy cattle from genome-edited cell lines

To the Editor:
Physical dehorning of dairy cattle is practiced to protect animals and their handlers. Genetic analyses have identified variants that are associated with hornlessness (referred to as 'polled') in cattle, a trait that is common in beef but rare in dairy breeds. We have introgressed a candidate *POLLED* allele into dairy cattle by genome editing and reproductive cloning, providing both evidence for genetic causation and a means to introduce *POLLED* into livestock with the potential to improve the welfare of millions of cattle annually.

In the United States, an estimated 80%[1] of all dairy calves (4.8 million per year) and 25% (8.75 million animals) of beef cattle are dehorned every year. A lower proportion of beef cattle than dairy cattle need to be dehorned because the dominant *POLLED* locus is nearly fixed in beef cattle such as Angus, whereas dairy breeds such as Holstein have a much lower frequency of *POLLED* because of the small number of sires (6%) producing commercially available *POLLED* semen[2]. Physical dehorning of cattle, which is done to protect animals and producers from accidental injury is not only

NATURE BIOTECHNOLOGY VOLUME 34 NUMBER 5 MAY 2016

recombinetics

acceligen

Breeding climate smart cattle for sub-tropical and tropical zones

Tad Sonstegard, Acceligen

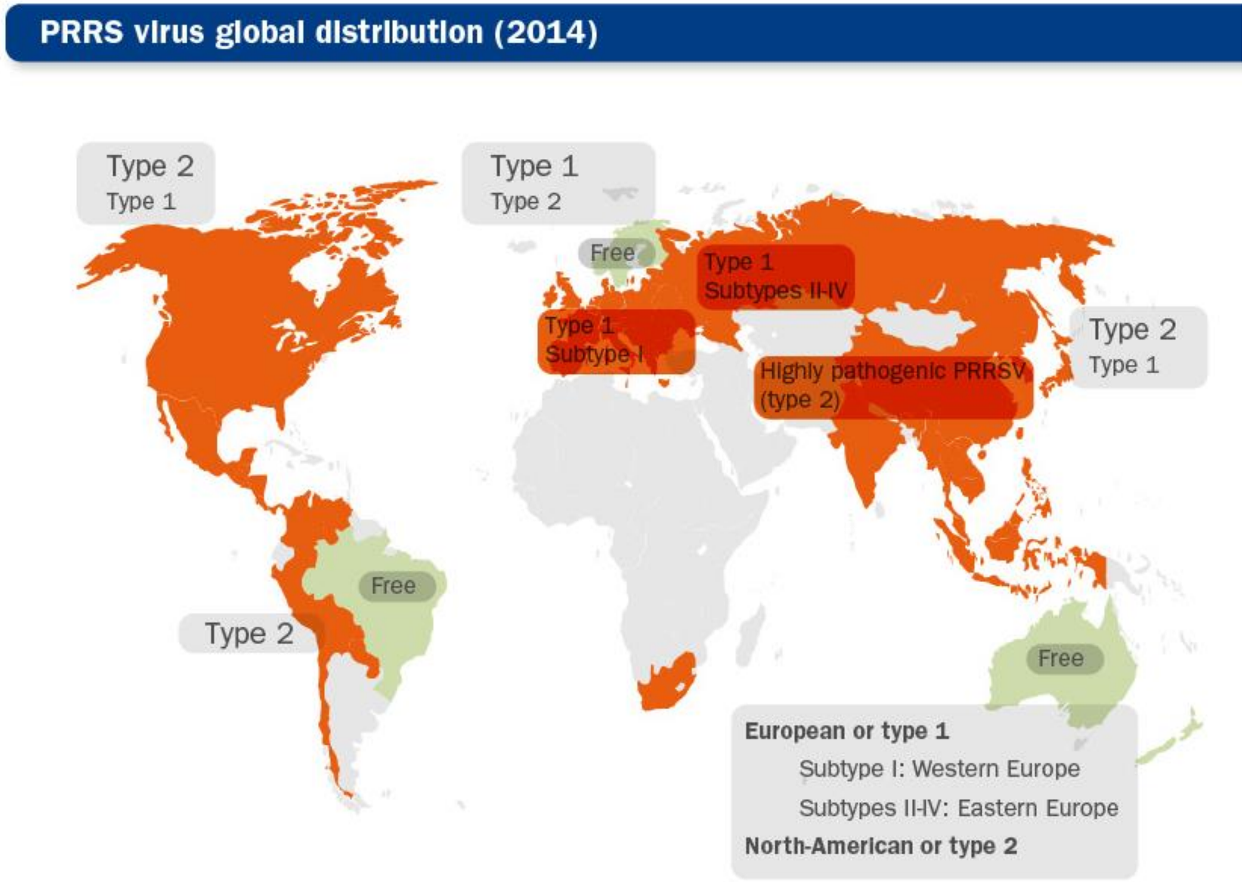
https://www.isaaa.org/kc/proceedings/animalbiotechnology/2022-09-12-4th-intl-workshop/default.asp

Thamani Holstein

- **Thermal tolerant in the Tropics (PRLR)**
- **Trypanosome Resilience (FDX2 & DHRS4)**
- **First Multiplexed – bovine ESC derived clone**
- **Made for Tropical Markets**

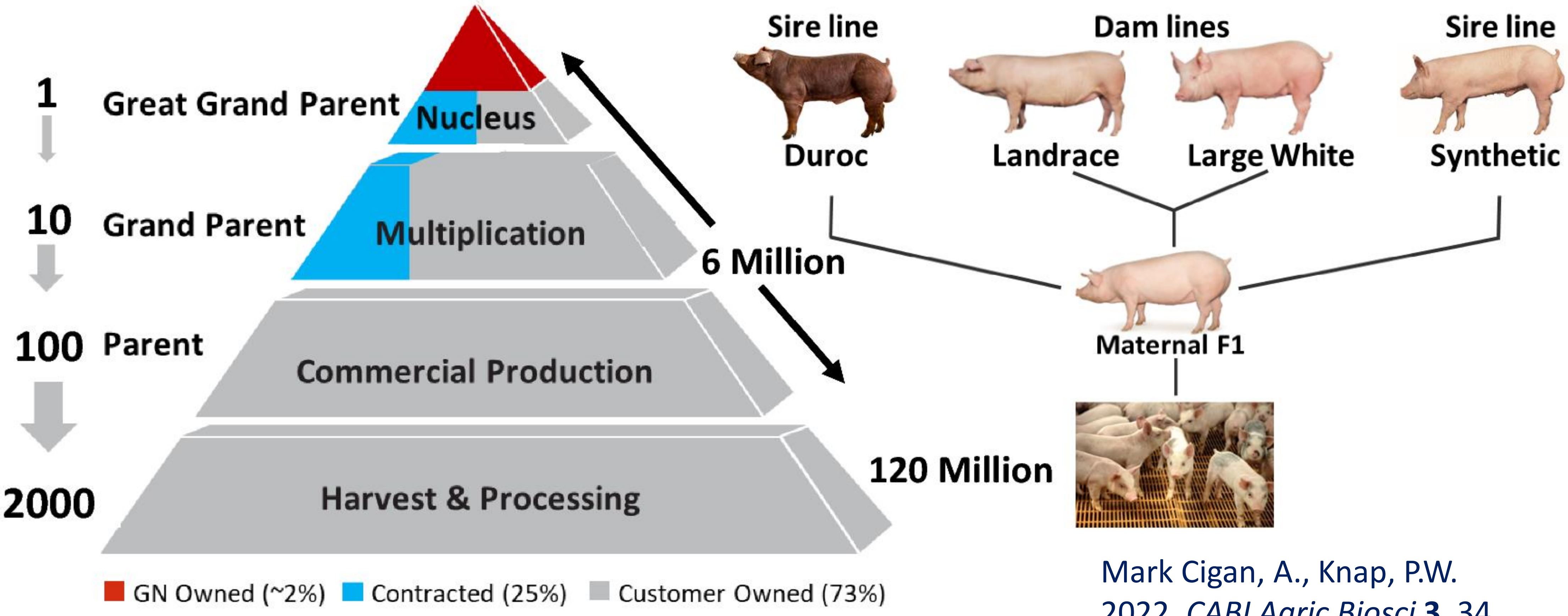
Sonstegard, T., et. al. 2025. Genome-edited livestock to secure sustainability. *Reproduction, Fertility and Development, 37*(1), -. https://doi.org/https://doi.org/10.1071/RD24145

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.

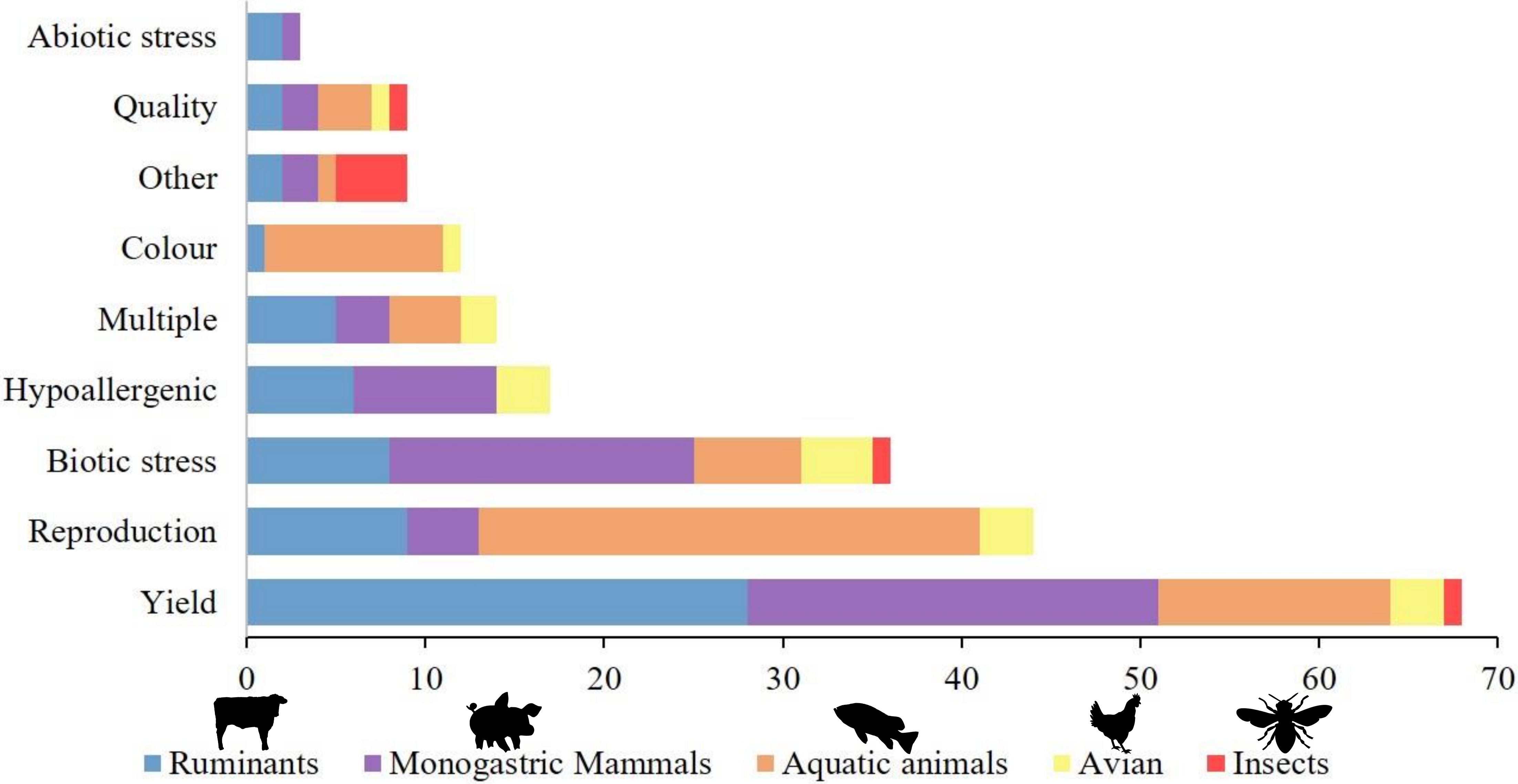
Technical considerations towards commercialization of respiratory and reproductive syndrome (PRRS) virus resistant pigs



Mark Cigan, A., Knap, P.W. 2022. *CABI Agric Biosci* **3**, 34

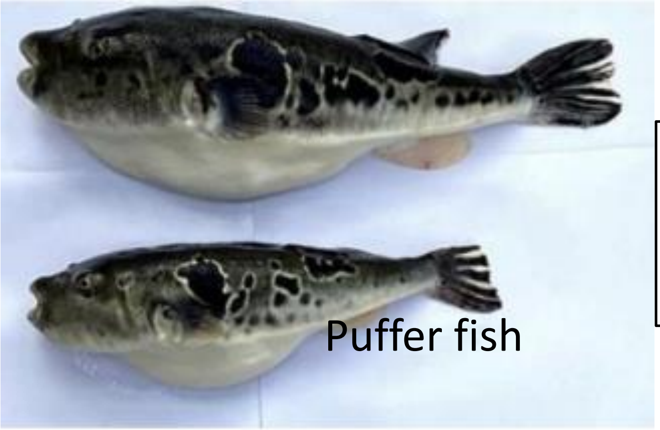
Whitworth et al. 2016. **Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus (PRRSV).** Nature Biotechnology 34:20-22.

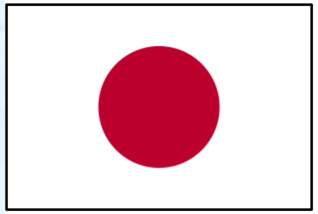
Trait targeted in animal gene editing applications



Ledesma, A.V., and A.L. Van Eenennaam. 2024. Global status of gene edited animals for agricultural applications. The Veterinary Journal. https://doi.org/10.1016/j.tvjl.2024.106142.

Gene editing to obtain myostatin KO (Bream) and leptin receptor KO (Puffer, Flounder) fish





Regional Fish

``22nd Century Olive Flounder" - flounder that might be common on dinner tables in the 22nd century

https://regional.fish/en/

Implications & Lessons From the Introduction of Genome-Edited Food Products in Japan

Matsuo et al. 2022. Front Genome Ed. 4:899154. doi: 10.3389/fgeed.2022.899154.

Gap Between the Anticipated and Actual Public Response

The previous GM food controversies from the late 1990s, which **were driven by a number of major news companies and consumer group coalitions**, questioning their environmental and food safety risk exhorted the Japanese government to implement a mandatory legal authorization scheme for GM products. It was thus anticipated that the introduction of newer technologies would likely meet strong resistance

Given the low public acceptance of GM in Japan, it was anticipated that the societal introduction of genome editing technologies would face a degree of public controversy. A previous consumer perception survey **found more support for tight regulations of genome-editing-derived foods which were designed to reduce the risk to as close to zero as possible rather than scientifically proven regulations and technically reasonable.**

However, even though there were indeed some social actions, for instance, some groups were against the use of genome-editing; petitions were made by some consumer groups; they did not develop into a mass mobilization, and media coverage was mostly positive. After filing the notifications, there were no considerable public reactions, nor did they receive any sustained attention.

WHERE

Gene-edited Animal Database

https://www.isaaa.org/animalbiotechdatabase/default.asp

ISAAA Inc.

Programs | Knowledge Center | Resources | Webinars | GM Approval Database

Species | Countries | Trait | Genes | NGT | SDN Types | Institutes | Editing Methods | Years

Animal Biotech Database

This database was compiled to include research and development of gene-edited (also called New Breeding Techniques) animals for agricultural applications. It was based on a literature review of the peer-reviewed literature that was conducted in 2023 (Van Eenennaam A.L.2023. New Genomic Techniques (NGTs) Animals and their Agri/food/feed products. EFSA supporting publication 2023: 20(9):EN-8311. 82 pp. doi:10.2903/sp.efsa.2023.EN-8311).

Historically genetically engineered organisms have had to receive a "GMO" regulatory approval to be commercialized or sold in any given country. A database of GM crop events approved worldwide is maintained on the ISAAA site (https://www.isaaa.org/gmapprovaldatabase/).

However, gene-edited products are often not considered for regulatory approval, as many countries and jurisdictions are considering edits that could have be achieved using conventional breeding (e.g. knockouts, intraspecies allele substitutions) as conventional breeding. As such these products do not have to go through a "GMO approval", but rather enter into a regulatory process to obtain a determination as to whether they are or are not a "GMO". Depending upon the country, this is sometimes based on the Cartagena protocol definition of an LMO which includes "any living organism that possesses **a novel combination of genetic material** obtained through the use of modern biotechnology". This is often interpreted to mean they are free of any "transgene" or "foreign" DNA.

Gene-edited Animal Database

Species –

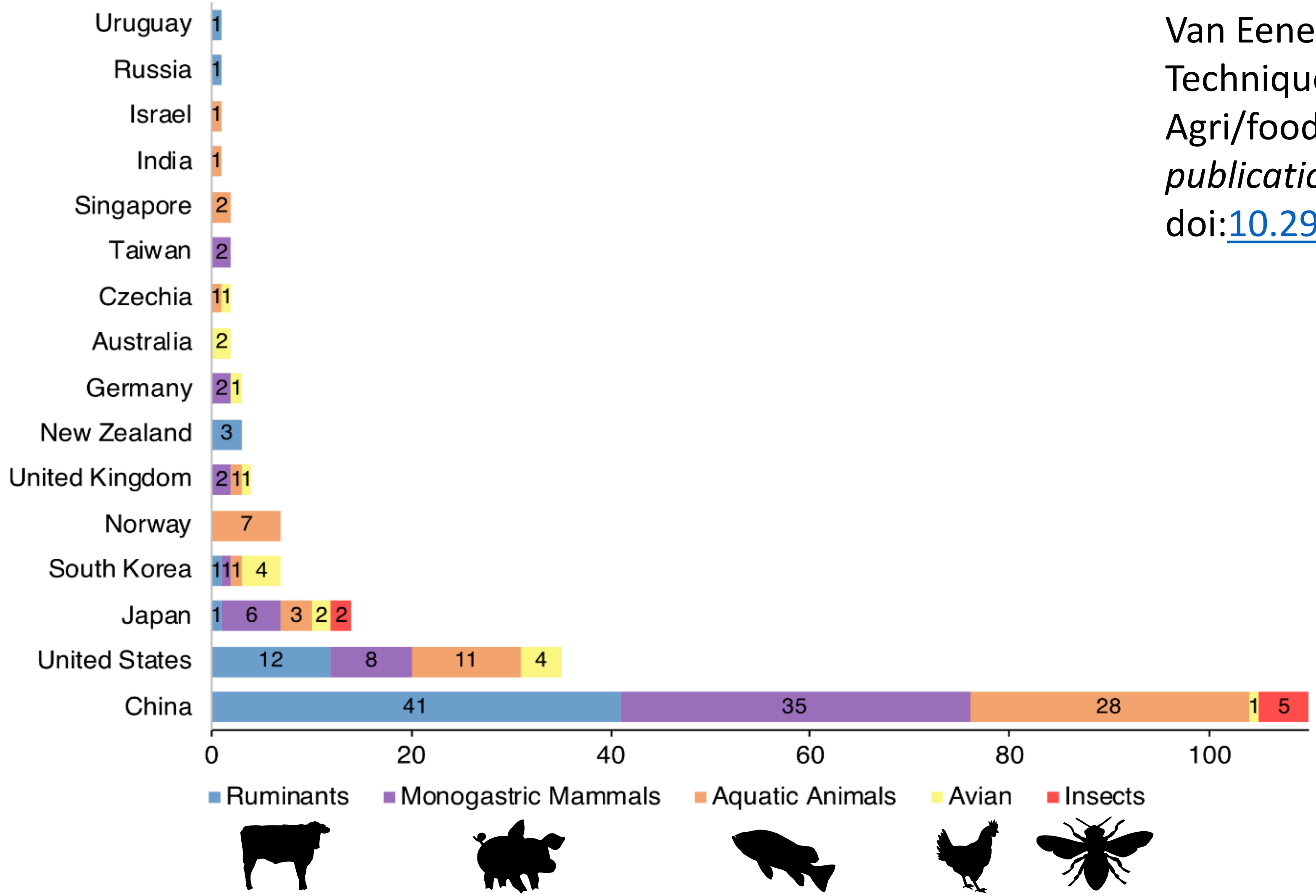
- Atlantic Salmon (*Salmo salar*)
- Blunt snout sea bream (*Megalobrama amblycephala*)
- Cattle (*Bos taurus*)
- Cattle (*Bos taurus x Bos taurus indicus*)
- Channel catfish (*Ictalurus punctatus*)
- Chicken (*Gallus gallus*)
- Common Carp (*Cyprinus carpio*)
- Duck (*Anas platyrhyncos*)
- Farmed Carp (*Labeo rohita*)
- Gibel carp (*Carassius gibelio Bloch*)
- Goat (*Capra hircus*)
- Honeybee (*Apis mellifera*)
- Loach (*Paramisgurnus dabryanus*)
- Nile tilapia (*Oreochromis niloticus*)
- Olive flounder (*Paralichthys olivaceus*)
- Oyster (*Crassostrea gigas*)
- Pig (*Sus scrofa domesticus*)
- Quail (*Coturnix japonica*)
- Rabbit (*Oryctolagus cuniculus*)
- Rainbow trout (*Oncorhynchus mykiss*)
- Red sea bream (*Pagrus major*)
- Redhead cichlid (*Vieja melanura*)
- Royal farlowella (*Sturisoma panamense*)
- Sheep (*Ovis aries*)
- Silkworm (*Bombyx mori*)
- Southern Catfish (*Silurus meridionalis*)
- Sterlet (*Acipenser ruthenus*)
- Tiger pufferfish (*Takifugu rubripes*)
- White crucian carp (*Carassius auratus civieri*)
- Yellow catfish (*Pelteobagrus fulvidraco*)
- Yellow catfish (*Tachysurus fulvidraco*)

Undergone regulatory process: NO

Sheep (*Ovis aries*)

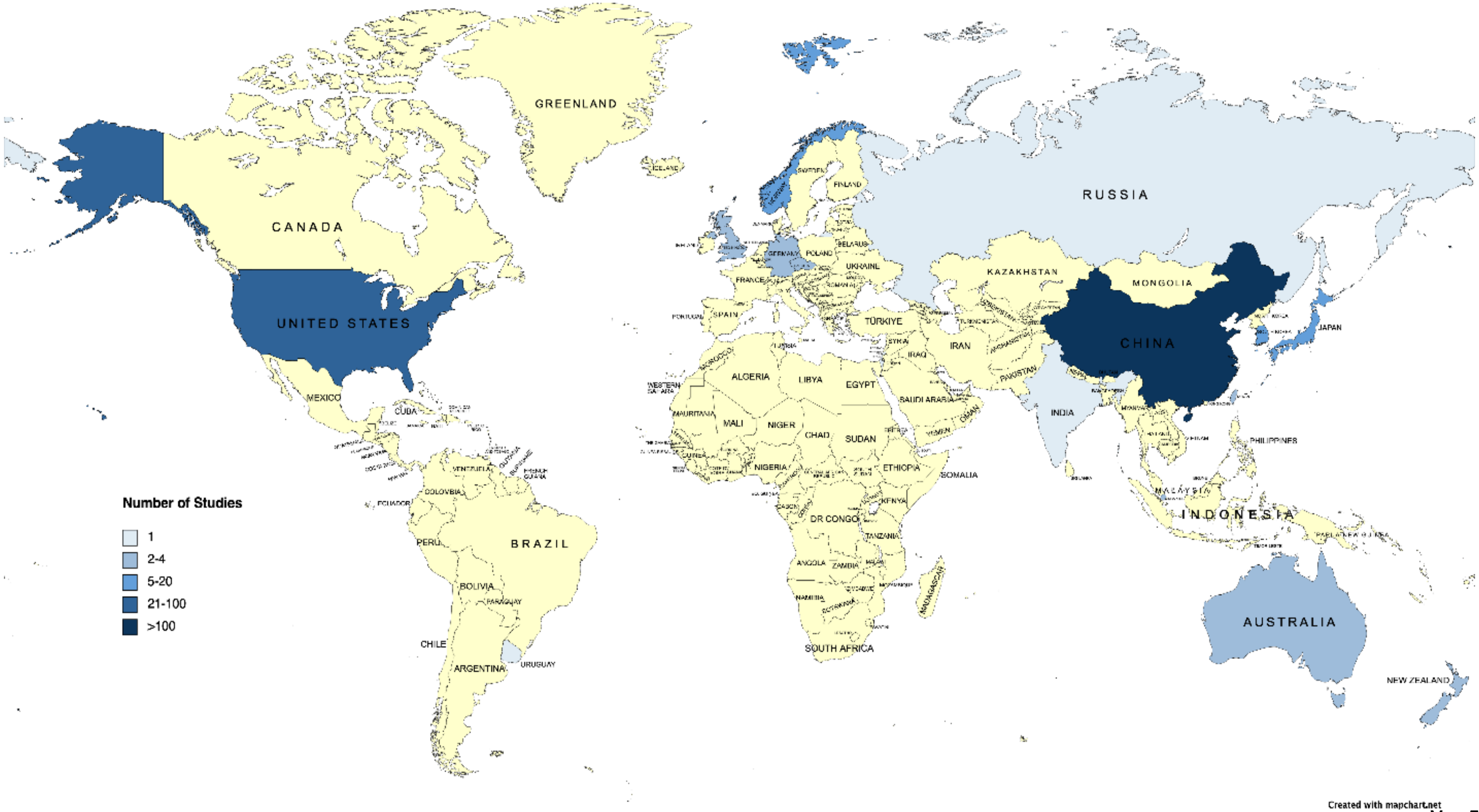
Year	Country of first Author	Species	Genes	
2023	China	Sheep (*Ovis aries*)	MSTN	View Details
	New Zealand	Sheep (*Ovis aries*)	NANOS2, DAZL	View Details
2022	China	Sheep (*Ovis aries*)	MSTN	View Details
	China	Sheep (*Ovis aries*)	MSTN	View Details
	United States	Sheep (*Ovis aries*)	SOCS2, PDX1	View Details
2020	China	Sheep (*Ovis aries*)	MSTN	View Details
	China	Sheep (*Ovis aries*)	BMPR1B	View Details
	China	Sheep (*Ovis aries*)	FGF5	View Details
2019	China	Sheep (*Ovis aries*)	SOCS2	View Details
2018	China	Sheep (*Ovis aries*)	MSTN	View Details
2017	China	Sheep (*Ovis aries*)	AANAT, ASMT	View Details
	China	Sheep (*Ovis aries*)	ASIP	View Details
	China	Sheep (*Ovis aries*)	FGF5	View Details
	China	Sheep (*Ovis aries*)	FGF5	View Details
2016	China	Sheep (*Ovis aries*)	BCO2	View Details
	China	Sheep (*Ovis aries*)	MSTN	View Details
	China	Sheep (*Ovis aries*)	MSTN	View Details
	China	Sheep (*Ovis aries*)	MSTN, ASIP, BCO2	View Details
2015	Uruguay	Sheep (*Ovis aries*)	MSTN	View Details
2014	United States	Sheep (*Ovis aries*)	MSTN	View Details

Animal category breakdown X country of peer-reviewed publications producing gene edited food animals for agriculture



Van Eenennaam, A.L. 2023. New Genomic Techniques (NGTs) Animals and their Agri/food/feed products. *EFSA supporting publication* 2023: 20(9):EN-8311. 82 pp. doi:10.2903/sp.efsa.2023.EN-8311

Country of first author on peer-reviewed publications producing Gene Edited Food Animals (and Their Agri/Food/Feed Products)



Van Eenennaam ISAAA 2025

Gene-edited Animal Database

〉 〉 Animal Biotech Database 〉 SDN Types 〉 SDN-1

Undergone regulatory process: YES

SDN Type: SDN-1

Year	Country	Species	Genes	
2024	Brazil	Pig (*Sus scrofa domesticus*)	CD163	View Details
2023	Brazil	Cattle (*Bos taurus*)	PRLR	View Details
	Colombia	Pig (*Sus scrofa domesticus*)	CD163	View Details
	Japan	Olive flounder (*Paralichthys olivaceus*)	LEPR	View Details
2022	Japan	Red sea bream (*Pagrus major*)	MSTN	View Details
	Japan	Tiger pufferfish (*Takifugu rubripes*)	LEPR	View Details
	United States	Cattle (*Bos taurus*)	PRLR	View Details
2021	Argentina	Cattle (*Bos taurus x Bos taurus indicus*)	MSTN	View Details
	Brazil	Cattle (*Bos taurus x Bos taurus indicus*)	MSTN	View Details
	Brazil	Cattle (*Bos taurus*)	PRLR	View Details
	Japan	Red sea bream (*Pagrus major*)	MSTN	View Details
	Japan	Tiger pufferfish (*Takifugu rubripes*)	LEPR	View Details
2020	Argentina	Cattle (*Bos taurus*)	PRLR	View Details
	Argentina	Cattle (*Bos taurus*)	PRLR, Pc POLLED	View Details
2019	Brazil	Nile tilapia (*Oreochromis niloticus*)	MSTN	View Details
2018	Argentina	Nile tilapia (*Oreochromis niloticus*)	MSTN	View Details

GnEd animal products that have undergone a regulatory review/low risk determination

Country	Common name	Trait	Gene Targeted	Year
Argentina	Nile Tilapia	Increased yield	Myostatin	2018
	Beef cattle	Heat tolerance	Prolactin receptor	2020
	Dairy cattle	Heat tolerance/Polled	Prolactin receptor/Pc polled	2020
	Cattle	Increased yield	Myostatin	2021
	Other species (?)	Undisclosed as no notice required for non-GMO products		
Brazil	Nile Tilapia	Increased yield	Myostatin	2019
	Beef cattle	Heat tolerance	Prolactin receptor	2021
	Dairy cattle	Heat tolerance	Prolactin receptor	2023
	Cattle	Increased yield	Myostatin	2021
	Pig	PRRS-resistance	CD-163	2024
Colombia	Pig	PRRS-resistance	CD-163	2023
	Cattle	Heat tolerance	Prolactin receptor	2024
Japan	Red Sea Bream	Increased yield	Myostatin	2021/2022
	Tiger Pufferfish	Faster growth	Leptin receptor	2022
	Olive Flounder	Faster growth	Leptin receptor	2023
USA	Beef Cattle*	Heat tolerance	Prolactin receptor	2022

https://www.isaaa.org/animalbiotechdatabase/default.asp

Editing as a Cherry on Top of the Breeding Sundae

It will be able to introduce useful alleles without linkage drag, and potentially bring in useful novel genetic variation from other breeds

Genome Editing

Somatic cell nuclear transfer cloning

Genomic Selection

Embryo Transfer

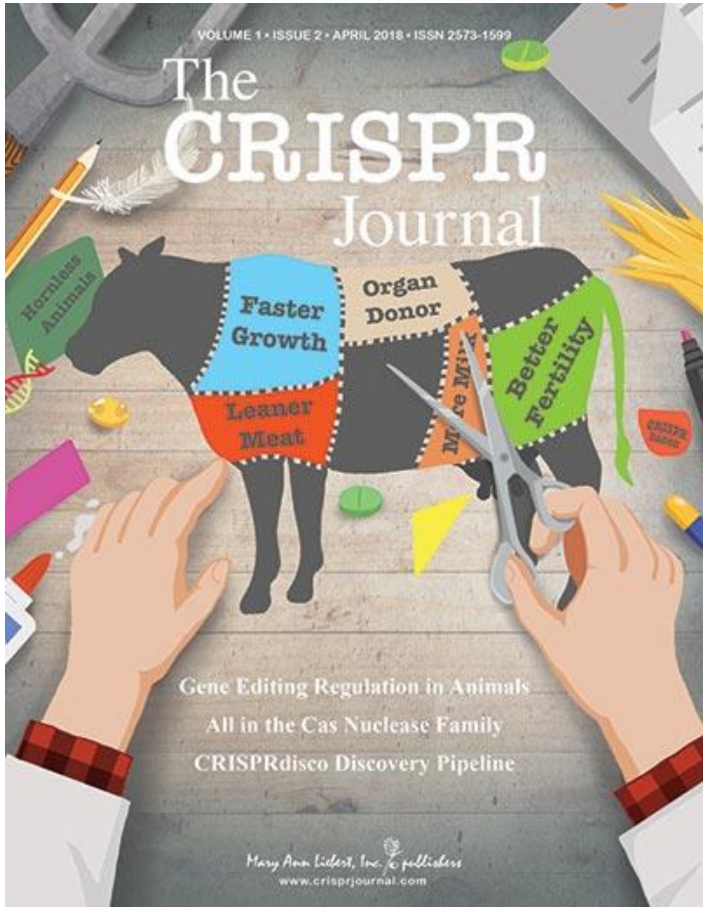
Artificial insemination

Progeny testing

Performance recording

Development of breeding goals

Association of like minded breeders



Van Eenennaam, A. L. 2018. The Importance of a Novel Product Risk-Based Trigger for Gene-Editing Regulation in Food Animal Species. 1 (2): 101-106. https://doi.org/10.1089/crispr.2017.0023

Summary

- Genome editing offers an approach to introduce useful genetic variation and alleles without the linkage drag typically associated with cross-breeding.
- Scaling useful edits to commercial livestock breeding programs will be technically complicated and expensive
- Regulators in many countries consider simple edits (e.g. knockouts, moving allele from one breed to another) with no “foreign DNA” to be “non-GMO”
- The fate of genome editing in livestock will depend upon developing a risk-based regulatory framework

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UC DAVIS ANIMAL SCIENCE

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- Barbara Nitta
- Ross lab members

- Dr. John Cole, URUS Group LP
- Dr. Pablo Ross, ST genetics
- Dr. Tad Sonstegard, Acceligen
- Dr. Bo Harstine, Select Sires Inc.

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

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