Global Status of Gene Edited Food Animals and their Products

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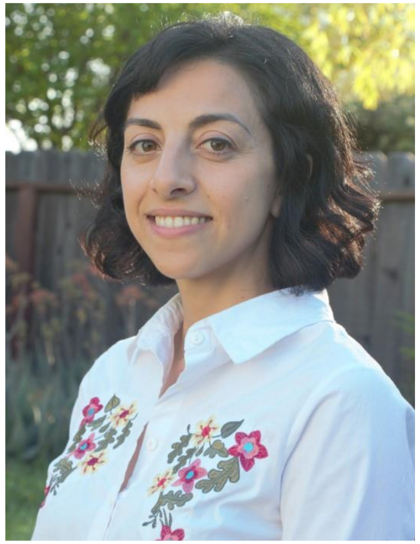
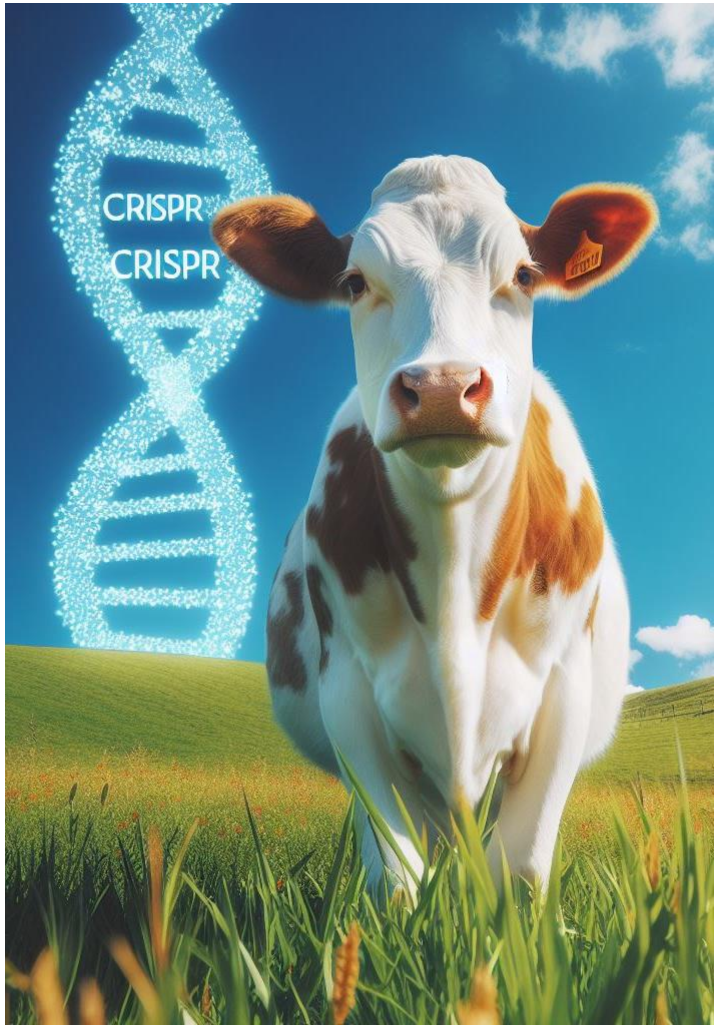
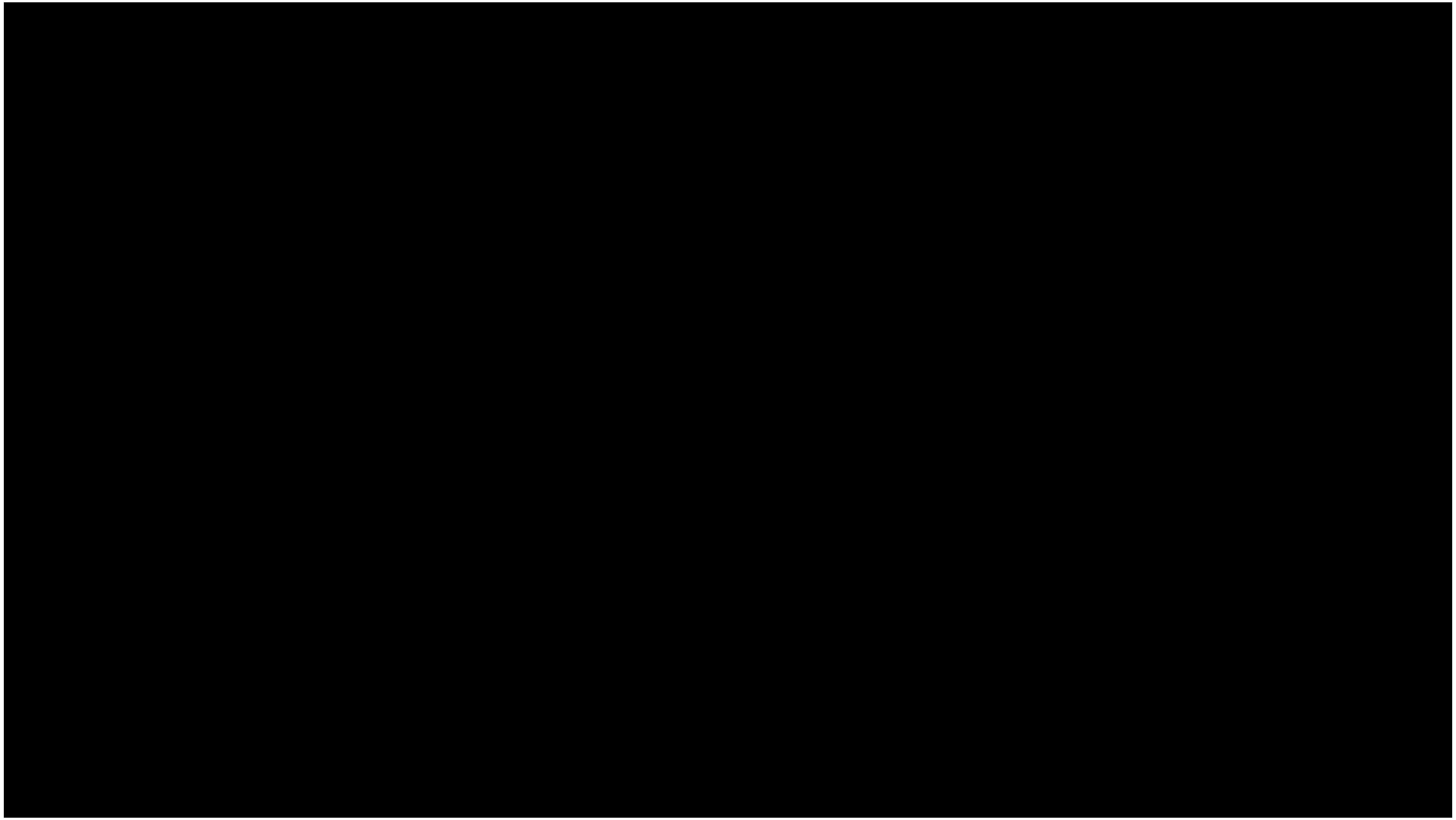




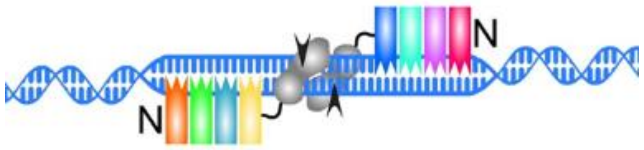
Breeders have selected for desired changes to companion animal populations based on naturally-occurring variation

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

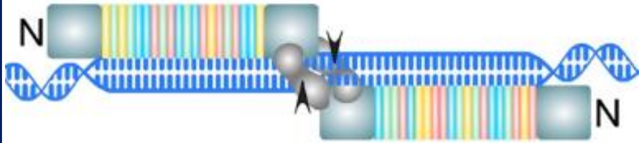
https://youtu.be/bM31E_LRszc



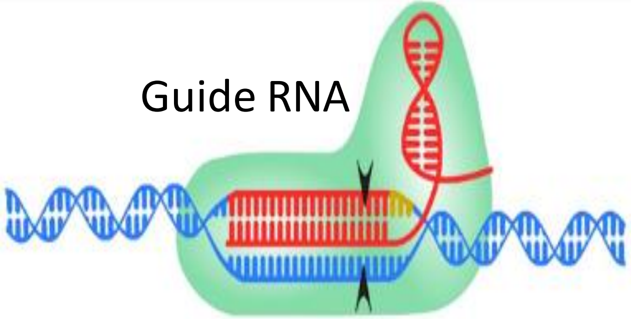
Gene editing allows the introduction of targeted double-stranded breaks in the genome



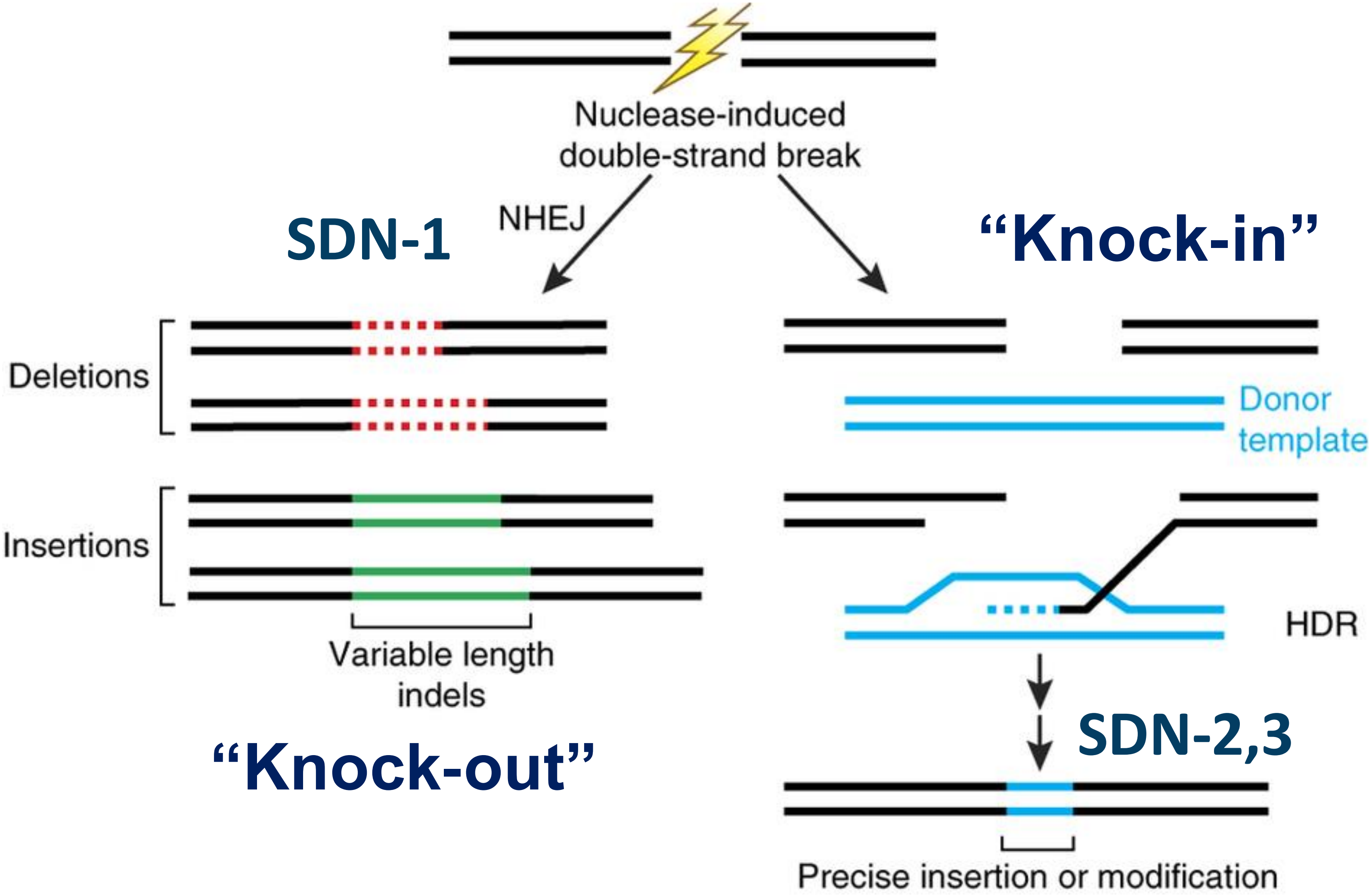
Zinc Finger Nucleases



TALENS

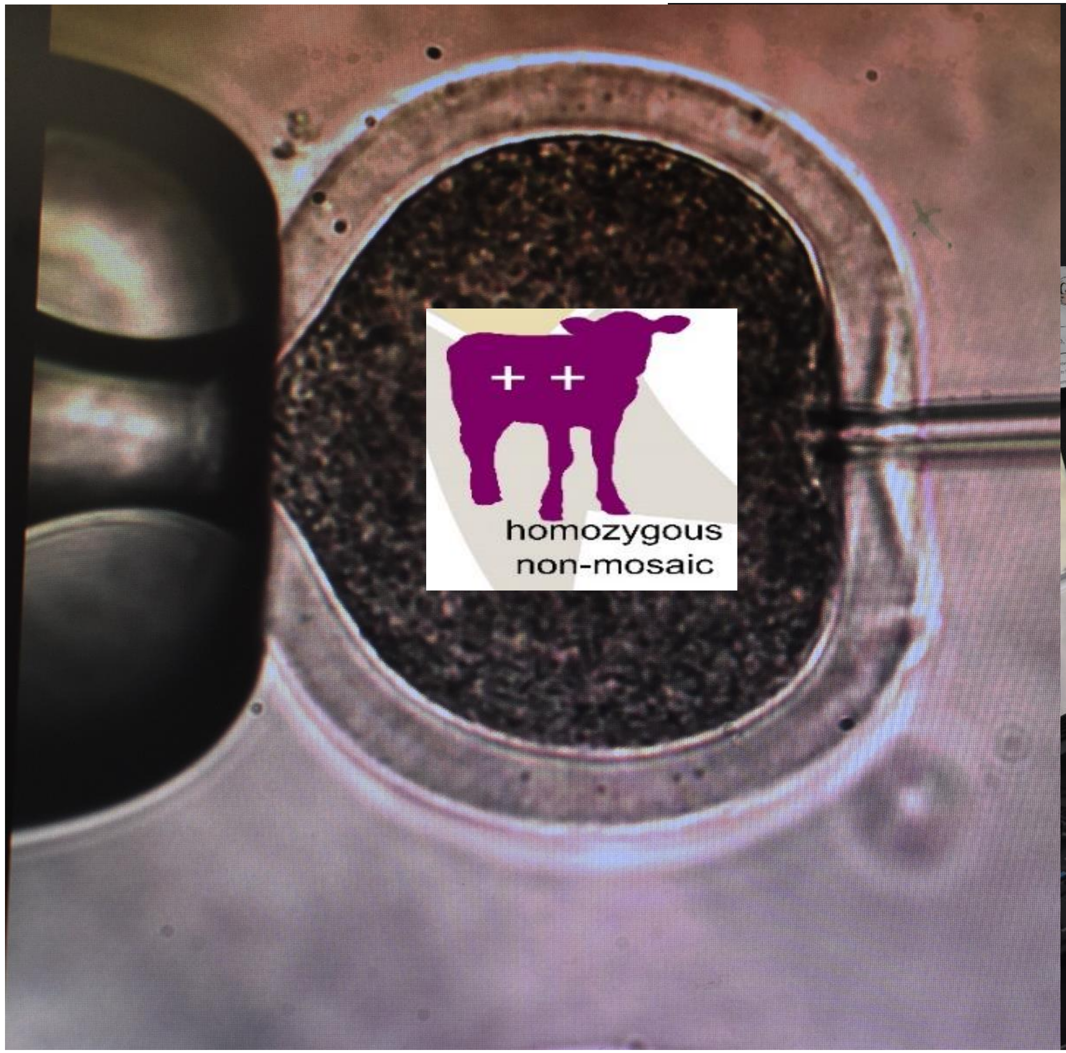


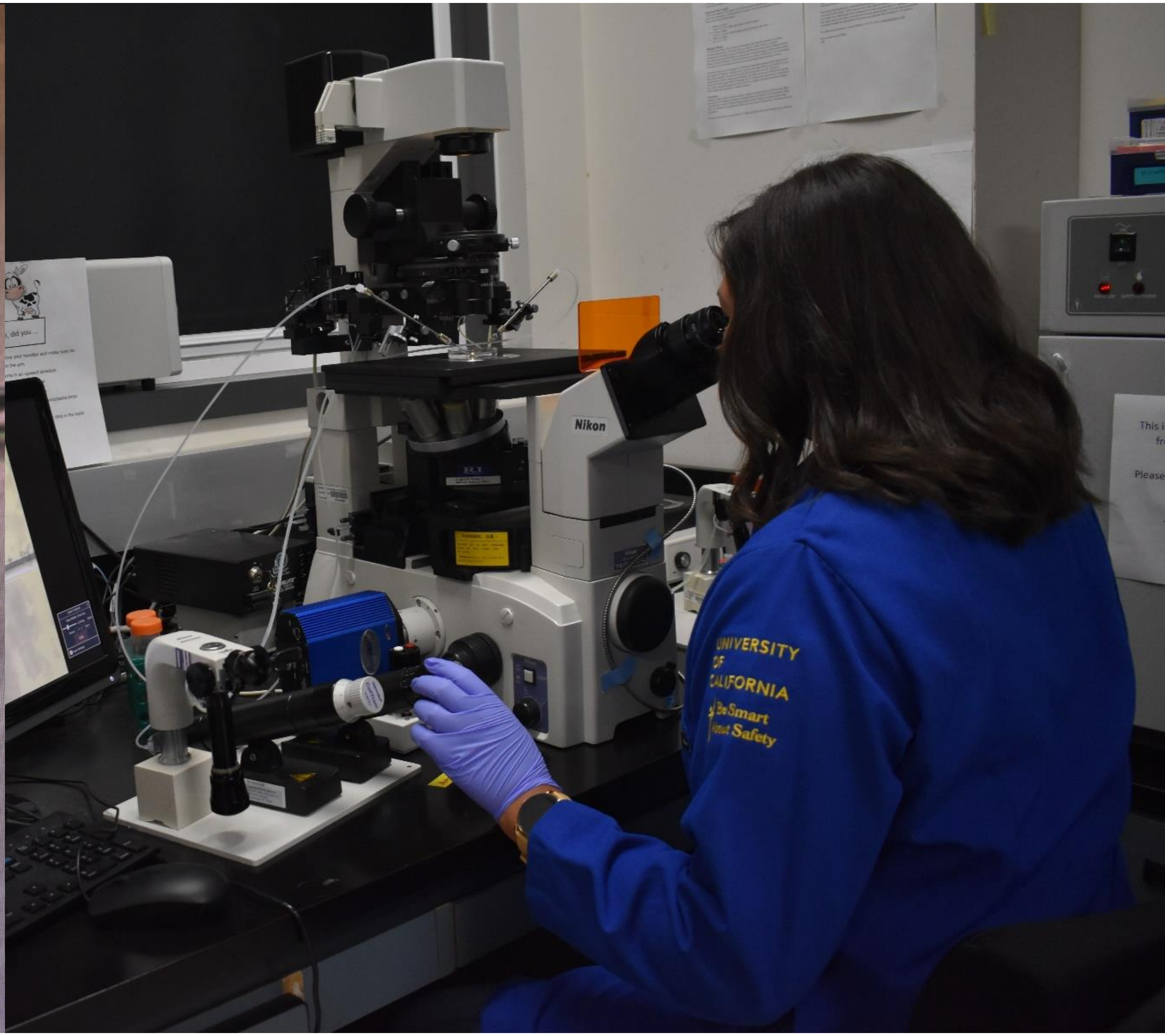
CRISPR/Cas9



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

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.





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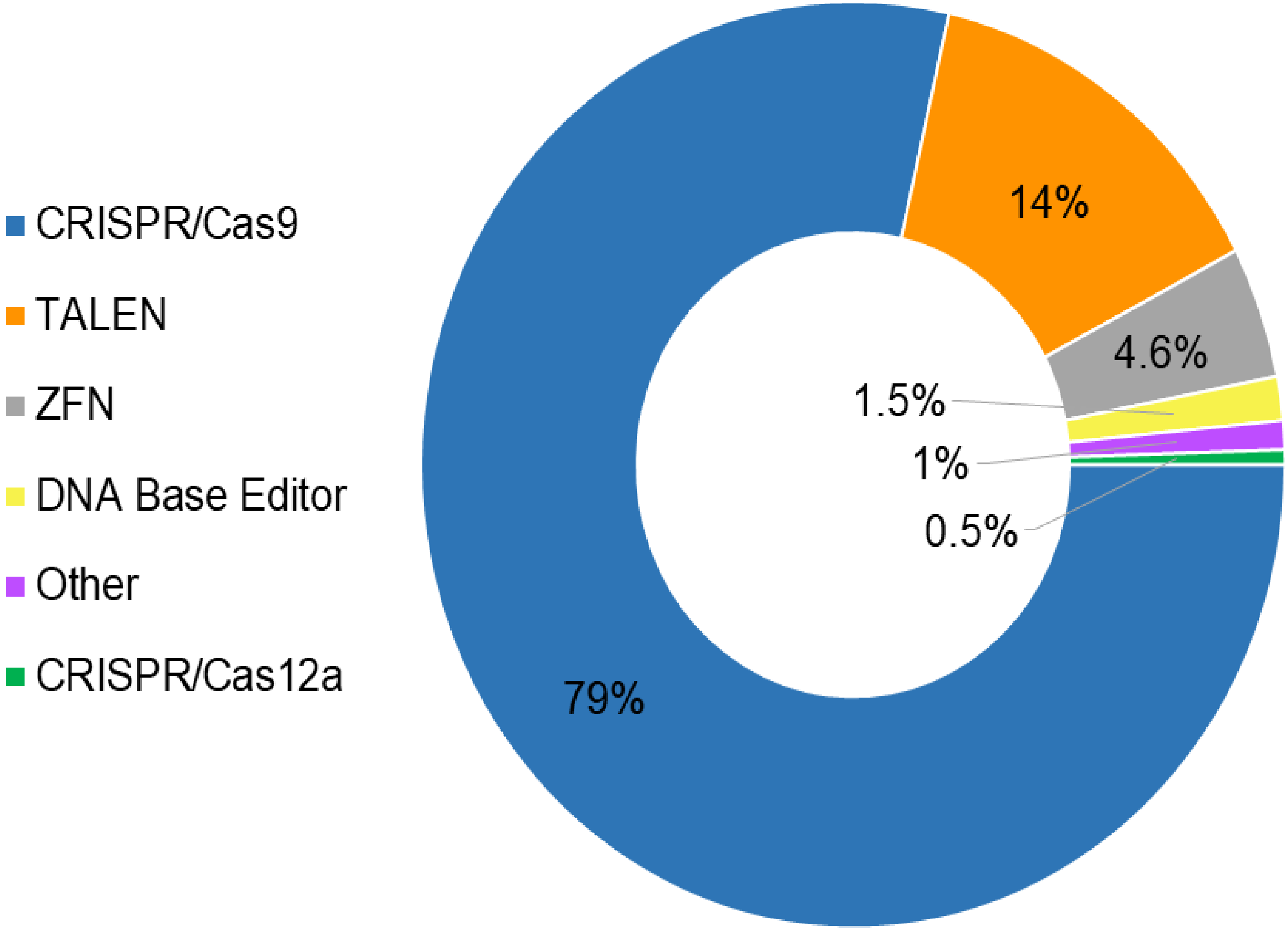
Overview of literature review

- A search using Gene Editing in the Medical Subject Heading (Mesh), or gene edit*, or genome edit* or base edit* in the title or abstract and targeted to agricultural animals was performed in PubMed on July 21, 2023; and resulted in over **1,200 publications**.
- After reviewing each publication, those that were exclusively for biomedical purposes, or where the edits were performed only in cells, or embryos that did not result in a live animals, or where edited animals did not survive beyond birth were excluded.
- The remaining 195 publications were categorized by editing system, species, purpose, type of edit (SDN-1,2,3), & country of first author.

NGTs used in the animal applications identified in the database (n=195)



- 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.

Trait purpose categories used for NGT animal products in the database

Trait category	Description
Abiotic stress tolerance	Resistance to abiotic stressors such as high or low temperature
Biotic stress tolerance	Resistance to biotic stressors such as bacteria, viruses and other pathogens
Color	Altered fur, hair, or skin color
Hypoallergenic	Reduced production or elimination of allergens in food products
Multiple	Applications that target more than a single trait category due to multiple target genes, or target genes with pleiotropic effects
Reproductive characteristics	Including changes in sexual characteristics such as sterility or the ratio of male to female offspring
Quality	Altered meat quality
Yield	Improved meat and fiber yield
Other traits	Traits not classified in the above categories, including welfare traits such as hornlessness and hypogonadotropic hypogonadism as a pig castration free trait.

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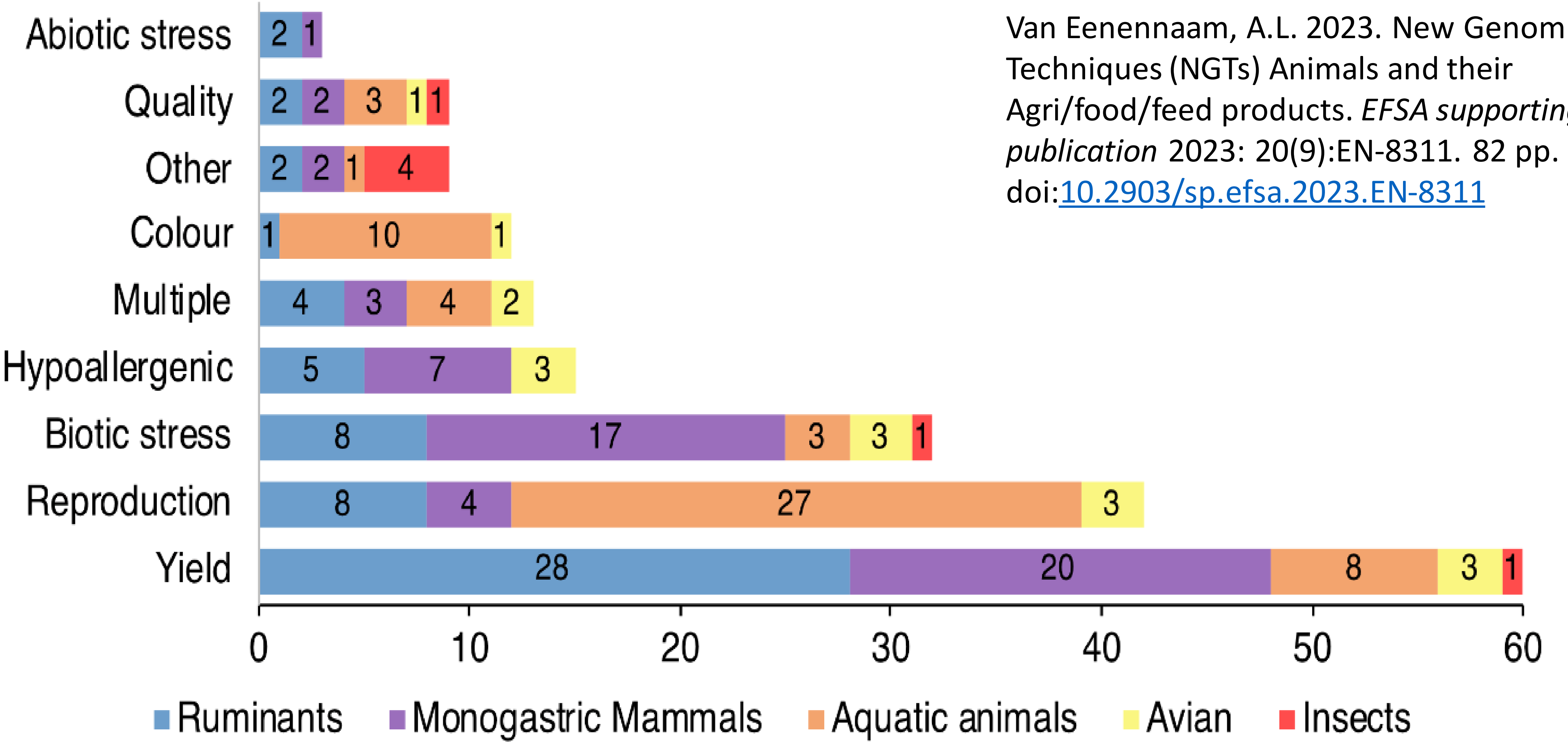
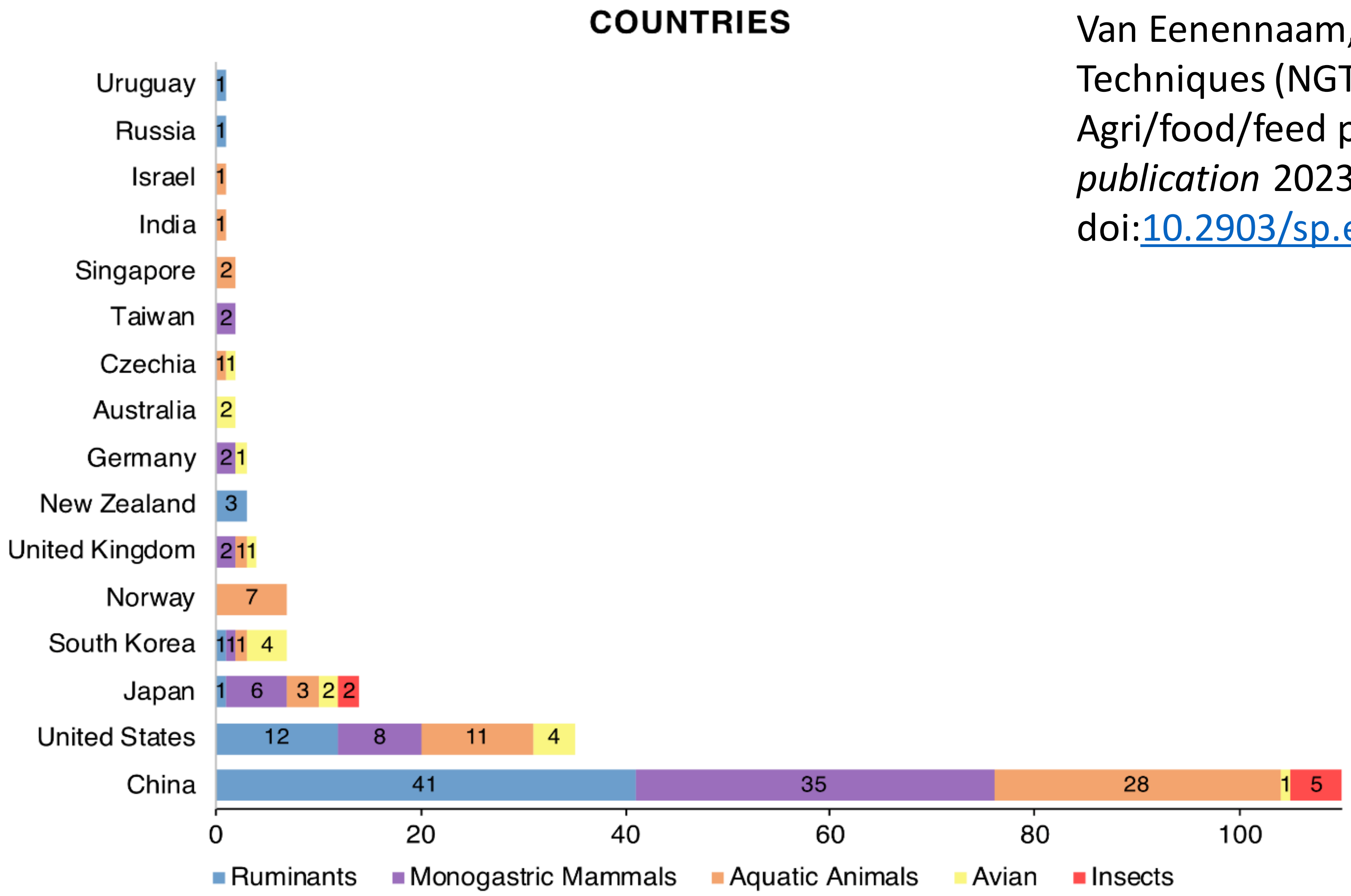
A recent literature review found 195 English-language category peer-reviewed publications producing gene edited food animals for agriculture – the purpose breakdown is below



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

Organism	Common name	Species name	Number (N=195)	Yield	Reproduction	Biotic Stress/ Abiotic Stress	Hypoallergenic/ Quality	Multiple Traits	Other
Mammals (59%)	Pigs	*Sus scrofa*	52	16	4	18	9	3	2
	Cattle	*Bos taurus taurus* *Bos taurus indicus*	23	4	4	10	4		1
	Sheep	*Ovis aries*	20	13	2		2	2	1
	Goats	*Capra hircus*	17	11	2		1	2	1
	Rabbits	*Oryctolagus cuniculus*	4	4					
Avian (8%)	Chickens	*Gallus gallus*	13	2	3	3	4	1	
	Japanese Quail	*Coturnix japonica*	2	1					1
	Duck	*Anas platyrhyncos*	1					1	
Aquatic Animals (29%)	Nile tilapia	*Oreochromis niloticus*	18		16			1	1
	Atlantic salmon	*Salmo salar*	7		3		2		2
	Common carp	*Cyprinus carpio*	4					2	2
	Farmed carp	*Labeo rohita*	1			1			
	White crucian carp	*Carassius auratus*	1						1
	Mozambique Tilapia	*Oreochromis mossambicus*	1						1
	Gibel carp	*Carassius gibelio*	2		2				
	Olive flounder	*Paralichthys olivaceus*	2	2					
	Loach	*Paramisgurnus dabryanus*	1						1
	Channel catfish	*Ictalurus punctatus*	7	2	1	2	1	1	
	Southern catfish	*Silurus meridionali*	1	1					
	Yellow catfish	*Pelteobagrus fulvidraco*	2	1	1				
	Sterlet	*Acipenser ruthenus*	2	1					1
	Tiger pufferfish	*Takifugu rubripes*	1	1					
	Red sea bream	*Pagrus major*	1	1					
	Blunt snout sea bream	*Megalobrama amblycephala*	1	1					
	Rainbow Trout	*Oncorhynchus mykiss*	1		1				
	Redhead cichlid	*Vieja melanura*	1						1
	Royal farlowella	*Sturisoma panamense*	1						1
	Oyster	*Crassostrea gigas*	1	1					
Insects (4%)	Silk worm	*Bombyx mori*	3	1		1	1		
	Honeybee	*Apis mellifera*	4						4
TOTAL			195	32%	20%	18%	12%	7%	11%

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



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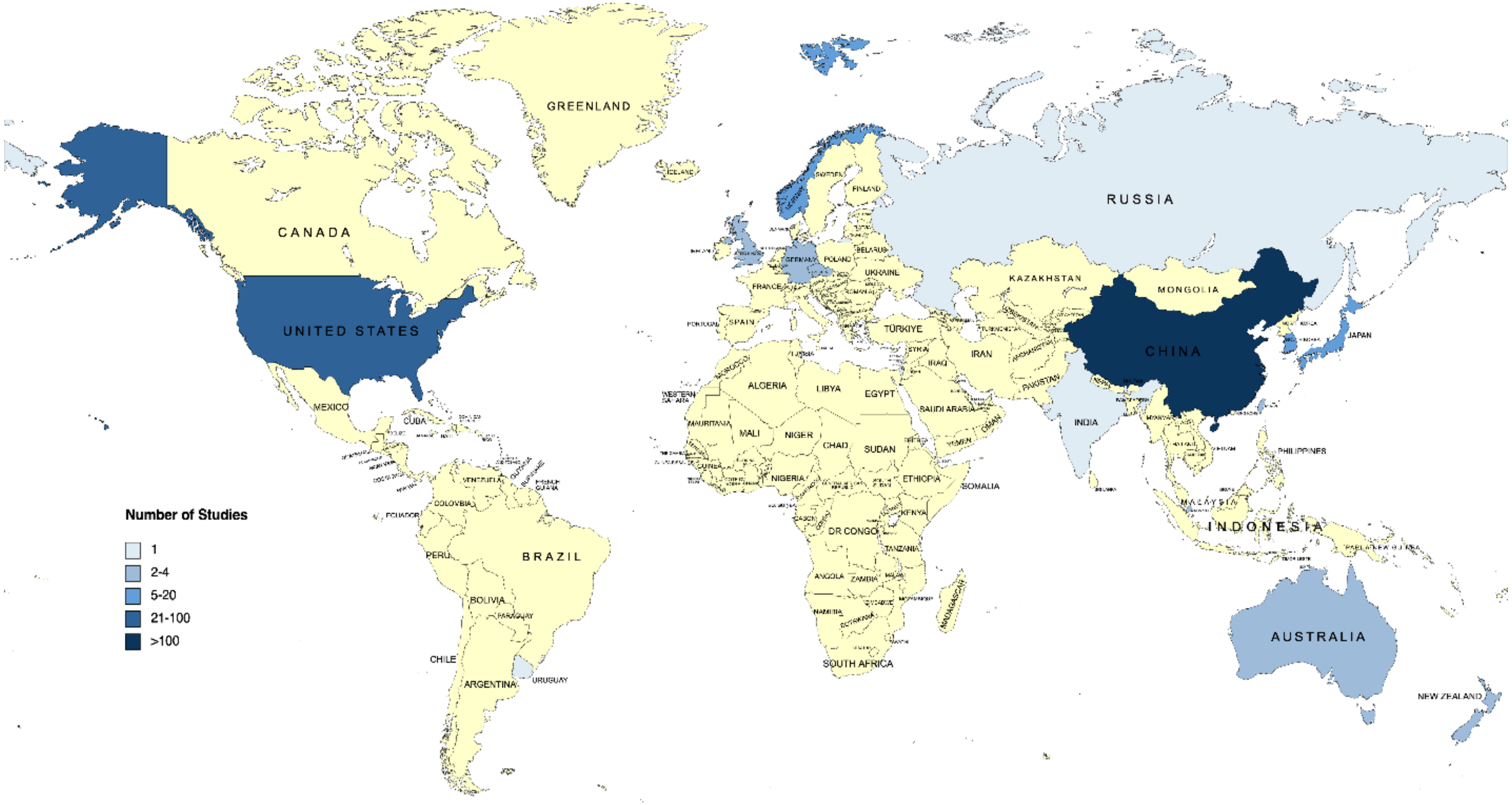
Country of first author on peer-reviewed publications producing Gene Edited Food Animals (and Their Agri/Food/Feed Products)



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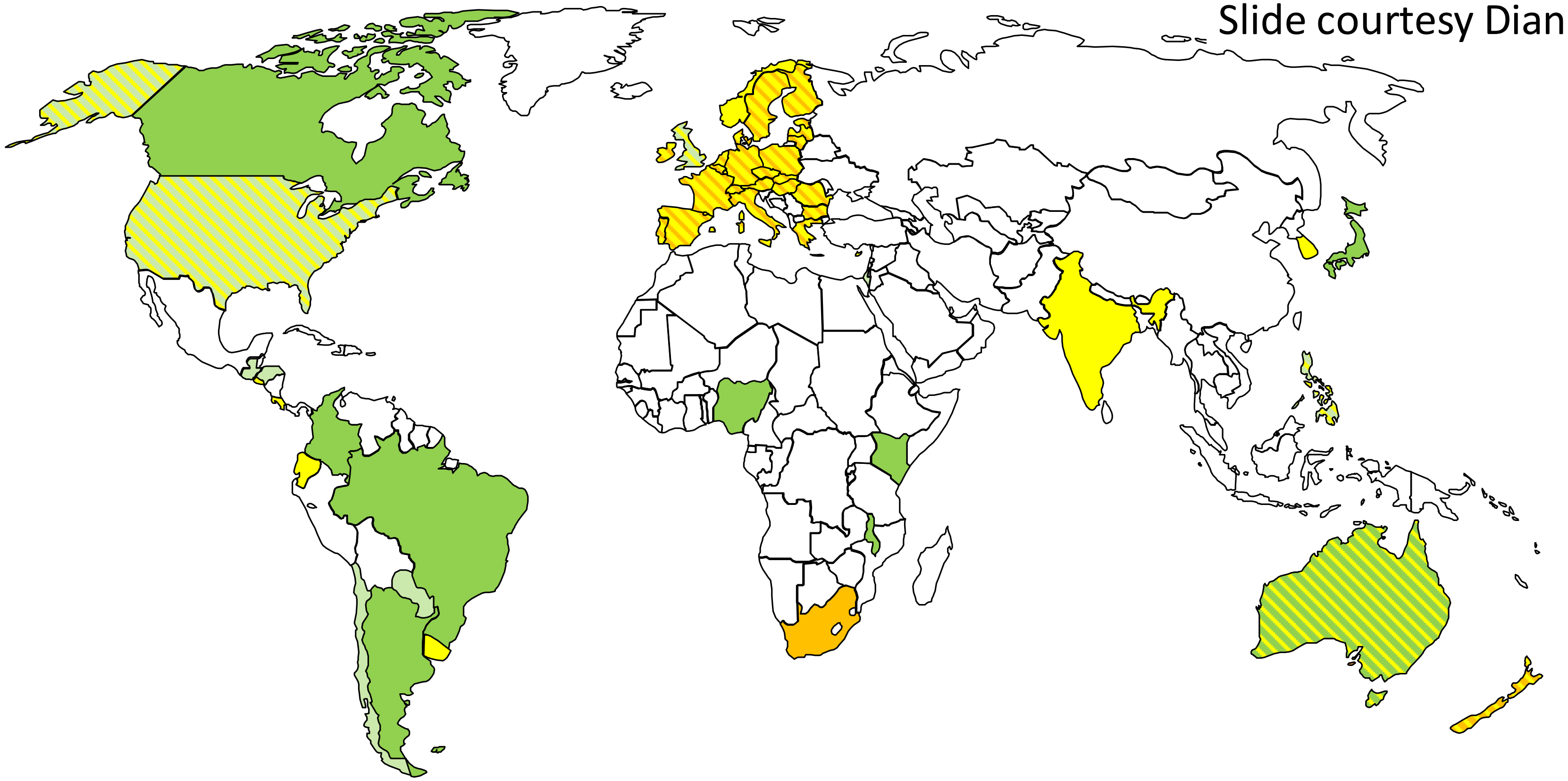
Global Regulatory Landscape for Products of Genome Editing

Slide courtesy Diane Wray-Cahen, USDA



Countries with regulatory policy with exclusions

Countries with **pending** policies, regulations, or legal rulings

Countries with GMO only policy with no exclusions

Countries with regulatory policy with exclusions (plants only)

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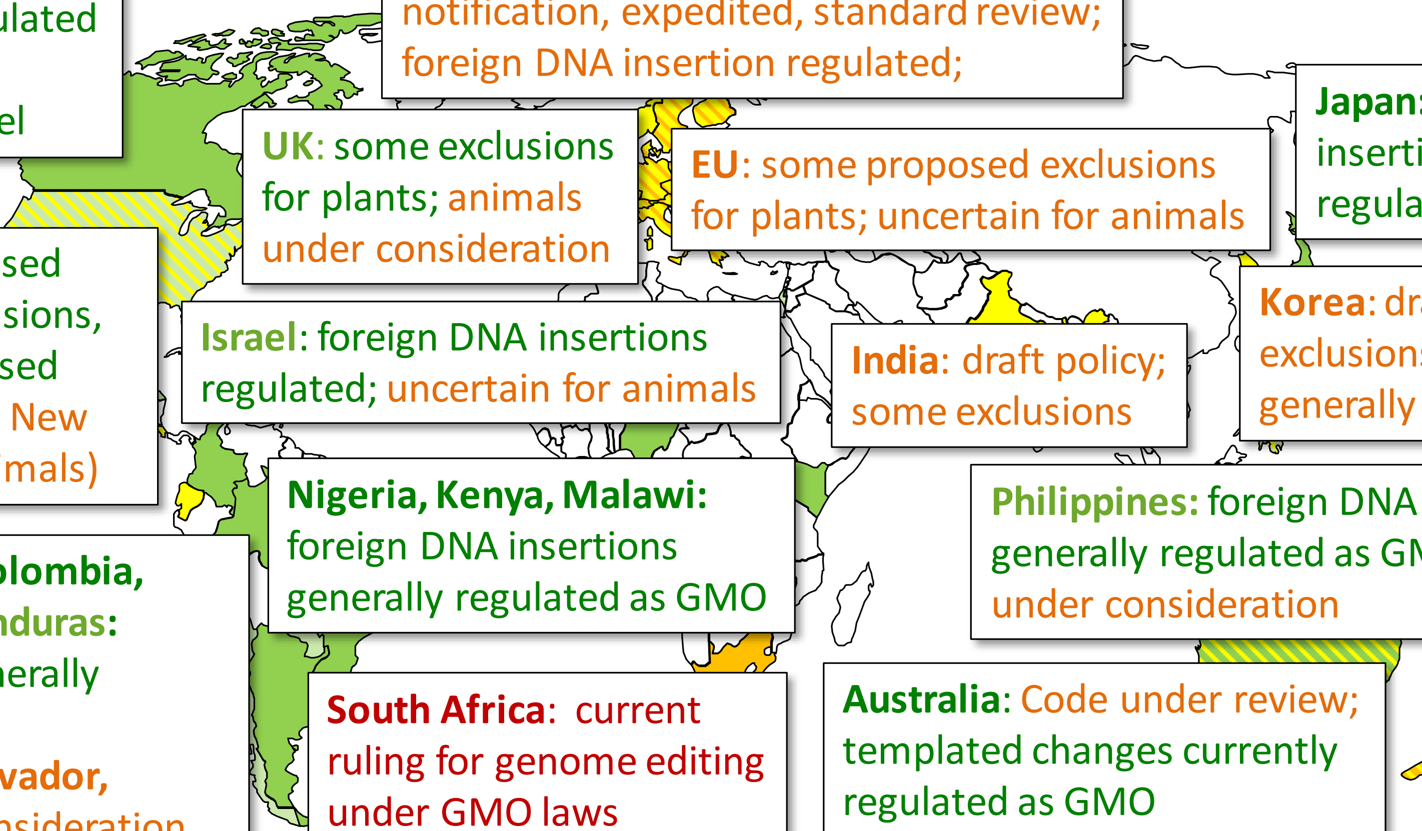
Global Regulatory Landscape for Products of Genome Editing

Modified from Diane Wray-Cahen, USDA



Canada: not regulated unless product identified as novel

Norway: proposed; tiered approach – notification, expedited, standard review; foreign DNA insertion regulated;

UK: some exclusions for plants; animals under consideration

EU: some proposed exclusions for plants; uncertain for animals

Japan: foreign DNA insertions generally regulated as GMO

United States: USDA - revised Rule for plants, some exclusions, ANPR for animals; EPA revised rule, some exclusions; FDA New Animal Drug Approval (animals)

Israel: foreign DNA insertions regulated; uncertain for animals

India: draft policy; some exclusions

Korea: draft policy; all LMO, some exclusions; Foreign DNA insertions generally regulated as GMO

Nigeria, Kenya, Malawi: foreign DNA insertions generally regulated as GMO

Philippines: foreign DNA insertions generally regulated as GMO; animal policies under consideration

Argentina, Brazil, Chile, Colombia, Paraguay, Guatemala, Honduras: foreign DNA insertions generally regulated as GMO

Costa Rica, Ecuador, El Salvador, Uruguay: similar under consideration

South Africa: current ruling for genome editing under GMO laws

Australia: Code under review; templated changes currently regulated as GMO

New Zealand: initial "non-GMO" ruling for some genome editing struck down by courts

- Countries with regulatory policy with exclusions
- Countries with **pending** policies, regulations, or legal rulings
- Countries with GMO only policy with no exclusions
- Countries with regulatory policy with exclusions (plants only)

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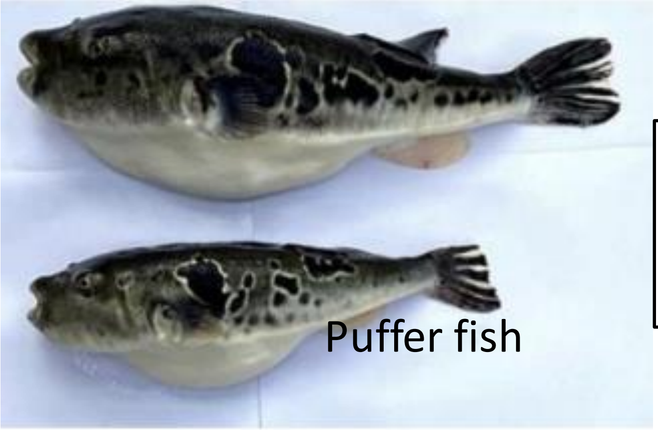
Gene editing myostatin to obtain myostatin (Tilapia, Bream) and leptin receptor (Puffer) KO fish



Puffer fish

Regional Fish

Red Sea Bream

AquaBounty TECHNOLOGIES

Fish (Tilapia)

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

Cattle with simple modifications were determined to be “non-GMO” in Brazil in 2021

Cattle

- Semen from a bull (Nelore) with double muscle
  - TALENs injection into the cytoplasm of IVF zygotes
  - Indels to knockout the myostatin gene
- Male and female with slick hair
  - CRISPR/Cas9 injection into the cytoplasm of IVF zygotes;
  - Mutations inserted in the prolactin receptor
- Both considered non-GMO in 2021

Transgenic Res (2015) 24:147–153
DOI 10.1007/s11248-014-9832-x

ORIGINAL PAPER

Genome edited sheep and cattle

Chris Proudfoot · Daniel F. Carlson · Rachel Huddart · Charles R. Long · Jane H. Pryor · Tim J. King · Simon G. Lillico · Alan J. Mileham · David G. McLaren · C. Bruce A. Whitelaw · Scott C. Fahrenkrug

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

Cattle with simple modifications were determined to be “non-GMO” in Argentina 2020

- *SLICK* edited Red Angus
- Double edited Celtic Pc polled/*SLICK* Holstein

In partnership with Kheiron S.A.

Previous Consultation Instance: product under development

- Produced using TALENs
- 1) Celtic allele: hornless trait. Naturally present in Angus, Simmental, Limousin, Charolais and Galloway
- 2) SLICK allele: improved heat-tolerance trait. Naturally present in Senepol, Carora,Limonero and Romosinuano.

June 2020 – no foreign DNA sequence and as such “no new combination of genetic material” And so considered “non-GMO”

acceligen™

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

FDA gives enforcement discretion to *SLICK* cattle submission by Acceligen (Recombinetics)

FDA Makes Low-Risk Determination for Marketing of Products from Genome-Edited Beef Cattle After Safety Review

Decision Regarding Slick-Haired Cattle is Agency's First Enforcement Discretion Decision for an Intentional Genomic Alteration in an Animal for Food Use

Share

March 7, 2022

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https://www.fda.gov/news-events/press-announcements/fda-makes-low-risk-determination-marketing-products-genome-edited-beef-cattle-after-safety-review

Content current as of:

03/07/2022

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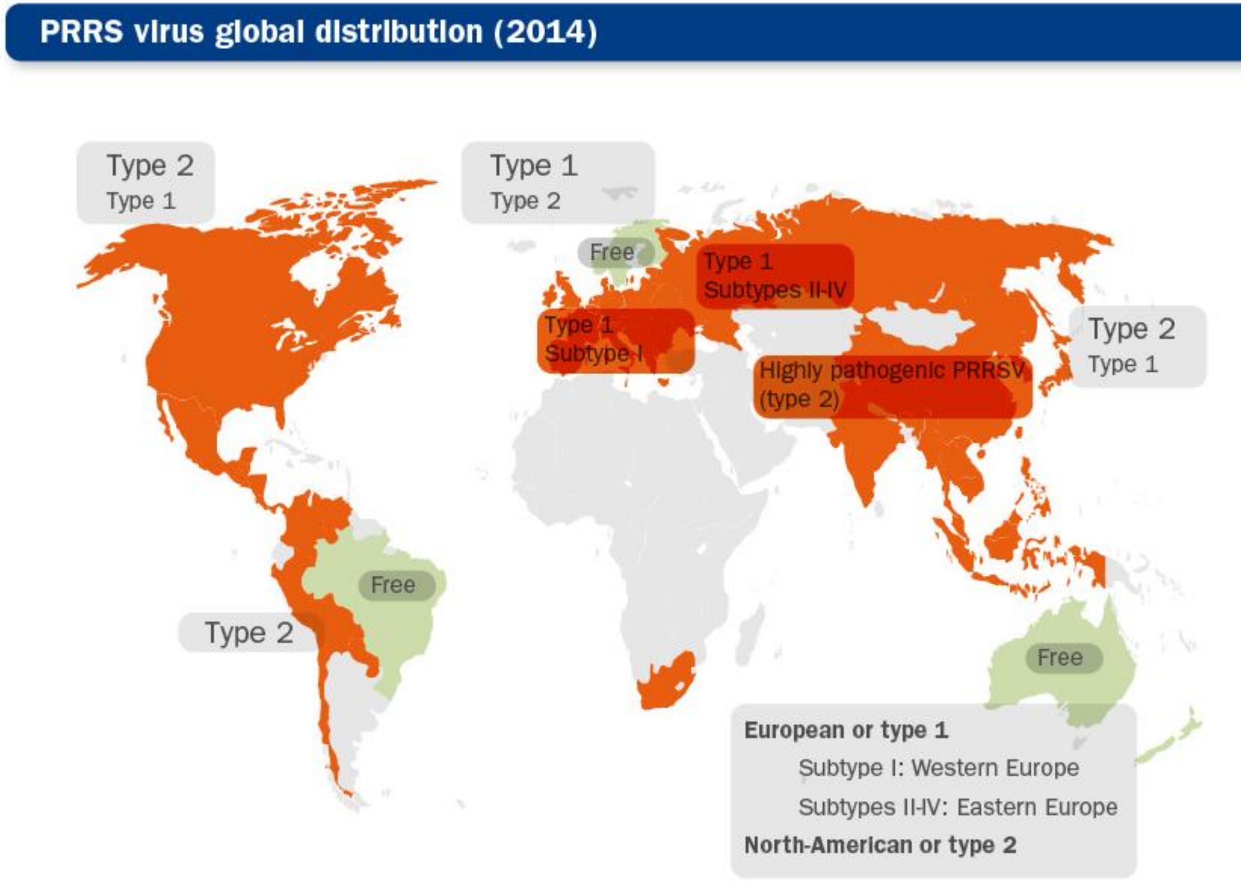
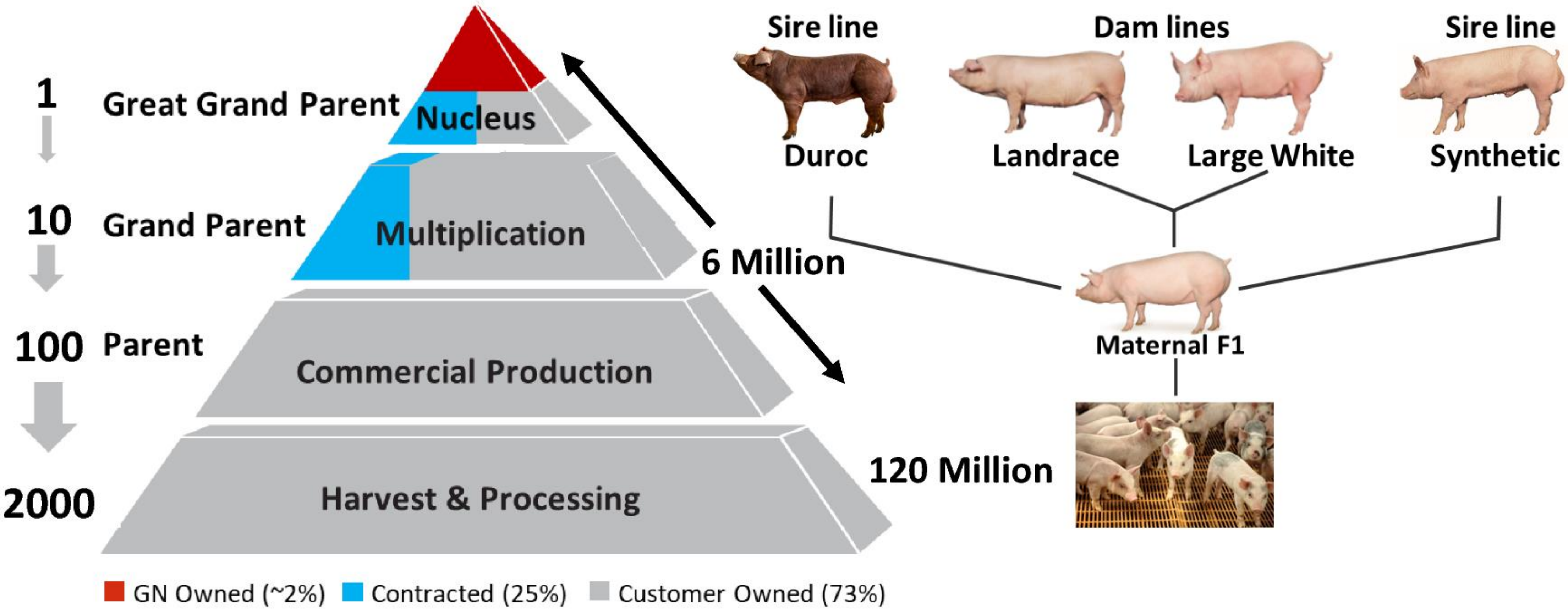
Gene editing to produce Porcine Reproductive & Respiratory Syndrome (PRRS) virus resistant pigs



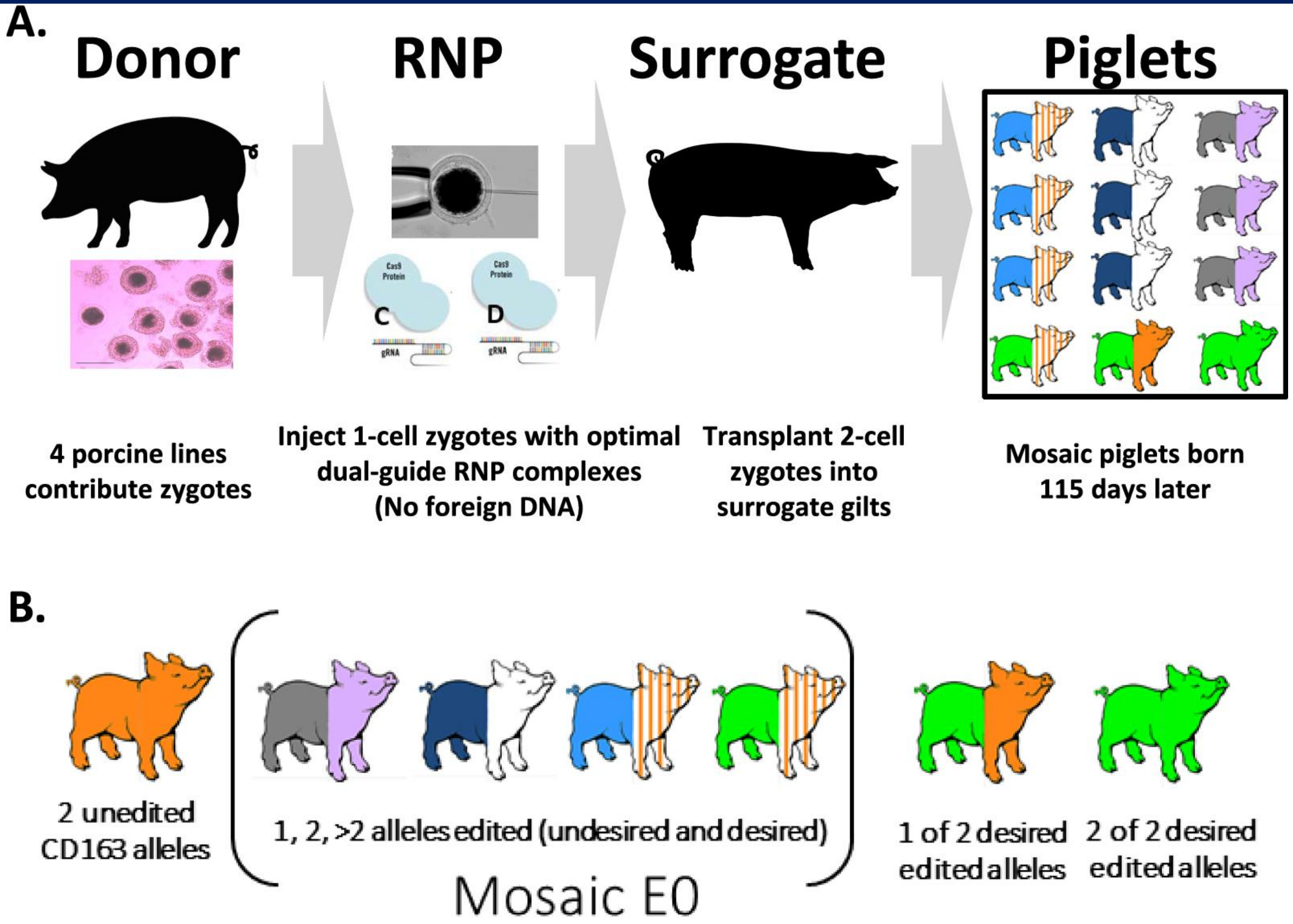
Whitworth et al. 2016. **Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus (PRRSV).** Nature Biotechnology 34:20-22.

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



Mark Cigan, A., Knap, P.W. **Technical considerations towards commercialization of porcine respiratory and reproductive syndrome (PRRS) virus resistant pigs**. CABI Agric Biosci 3, 34 (2022). https://doi.org/10.1186/s43170-022-00107-5

Scaled production of pigs containing modified allele of CD163.



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

A. Advancing PRRS virus resistance allele

1st Generation (E0)

➢ **Mixture of alleles**

- Identify piglets containing desired CD163 using Illumina and Nanopore
- Many pigs contain multiple alleles (mosaic)
- Sequence capture pigs with desired allele
- Pigs with desired allele bred to wild-type line identical mates

2nd Generation (E1)

➢ **Heterozygous alleles**

- Identify piglets with transmitted desired CD163 by Illumina
- Pigs with desired allele screened by sequence capture to sequence CD163 allele and identify transmitted off-target INDELs
- Heterozygous E1 pigs with no off-target INDELs are crossed
- Crossing based on genetic indexes

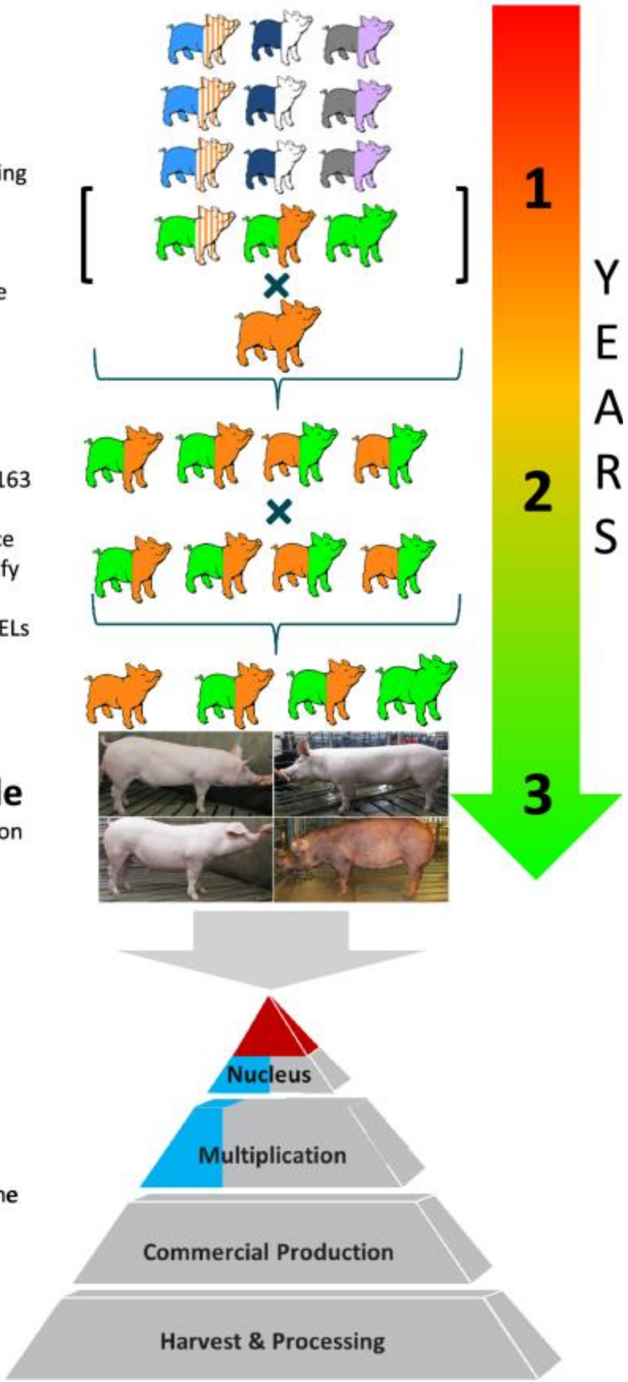
3rd Generation (E2)

➢ **Homozygous CD163 allele**

- CD163 allele segregates 1:2:1 in E2 generation
- Advance homozygous CD163 allele pigs
- No detected off-targets in this population
- Disease, commercial performance testing, regulatory submissions

B. Nucleus and conventional breeding

- 10-20 founder boars for each line used for continued genetic improvement of small gene edited nucleus herd
- Upon regulatory approval distribute PRRSV resistance germplasm though pyramid by breeding



Scaled breeding steps for 1st, 2nd & 3rd generation of pigs to generate gene edited nucleus herd.

*"Approximately 10–20 high genetic merit CD163$^{m/m}$ boars across 2 maternal and 2 paternal lines are used to maintain a small nucleus population for multiplication and genetic improvement. **Upon approval**, these founders would be multiplied and distributed to producers for commercial production and sale using conventional breeding practices."*

Burger et al. 2024. **Generation of a Commercial-Scale Founder Population of Porcine Reproductive and Respiratory Syndrome Virus Resistant Pigs Using CRISPR-Cas**. The CRISPR Journal. Feb 12-28. http://doi.org/10.1089/crispr.2023.0061

Genetically Engineered (rDNA)

Brazil

- 2 Mosquitoes (2014, 2020, population control)
- Fall Armyworm (2021)
- Salmon (2021, somatotropin)

Canada

- Pig (2010, Environment, phytase)
- Salmon (2016, Food, somatotropin; 2013 Environment, somatotropin)

USA

- Salmon (2015, somatotropin)
- Pig (2020, alpha-gal knockout)
- GloFish (2003) Enforcement Discretion

Japan

- 10 Silkworms (various, color, dye retention)

Vietnam

- Silkworms (spider silk)

Argentina

- Various species and traits in Phase 1, but none commercialized/deregulated

Genome Edited *

Brazil

- Tilapia (MSTN KO, 2019)
- Cattle (MSTN KO, 2021)
- SLICK Beef Cattle (2021)
- SLICK Dairy Cattle (2023)

Colombia

- PRRSv-resistant pig (2023)

USA

- SLICK Cattle (2022) Enforcement Discretion

Japan

- Sea Bream (MSTN KO, 2021; 2022 - variants)
- Tiger Pufferfish, Fugu (fast growth, 2021; 2022 - variants)
- Flounder (fast growth, 2023)

Argentina

- Tilapia (MSTN KO, 2018)
- SLICK Beef Cattle (2020)
- SLICK, Polled, Dairy Cattle (2020)
- Cattle (MSTN KO, 2021)
- Undisclosed, various species

Slide courtesy Diane Wray-Cahen, USDA

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 that allows trade of animal products (meat, milk, eggs, and gametes)

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- Dr. Xiang (Crystal) Yang
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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
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SELECT SIRES INC.

USDA
United States Department of Agriculture

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