Global status of gene edited animals for agricultural applications

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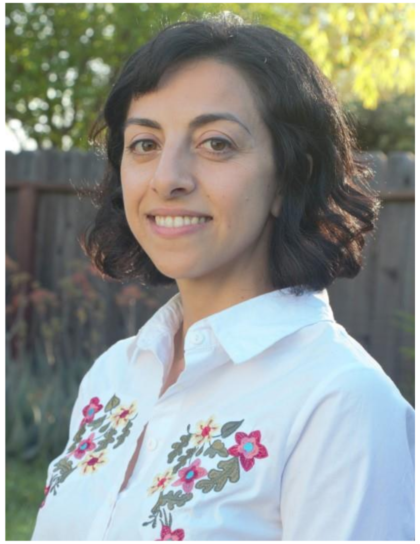
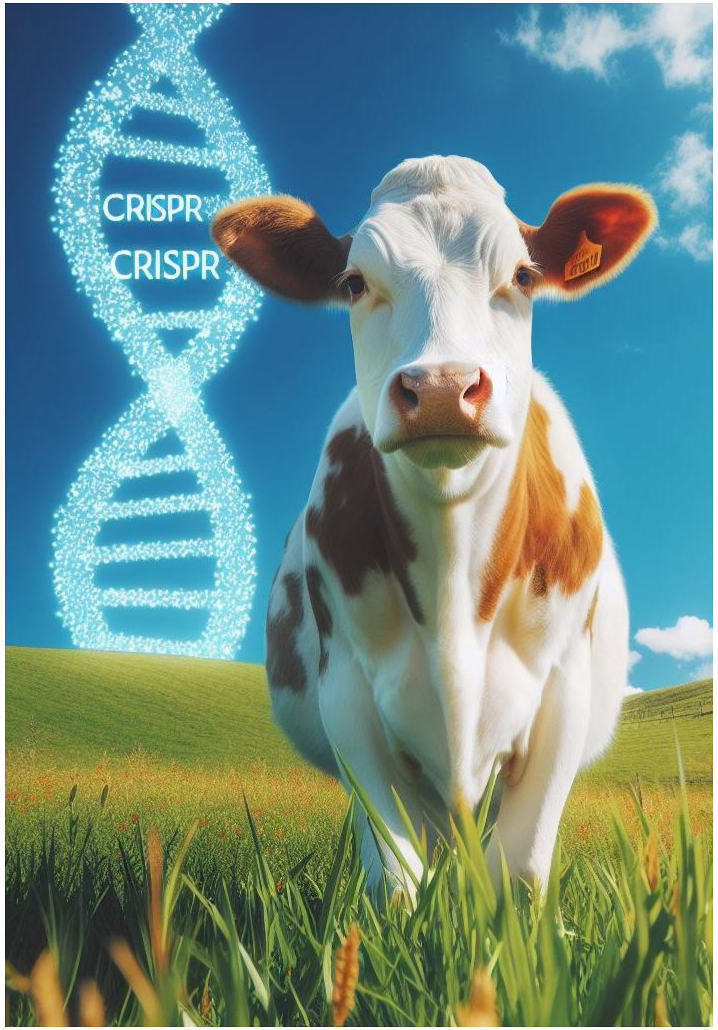
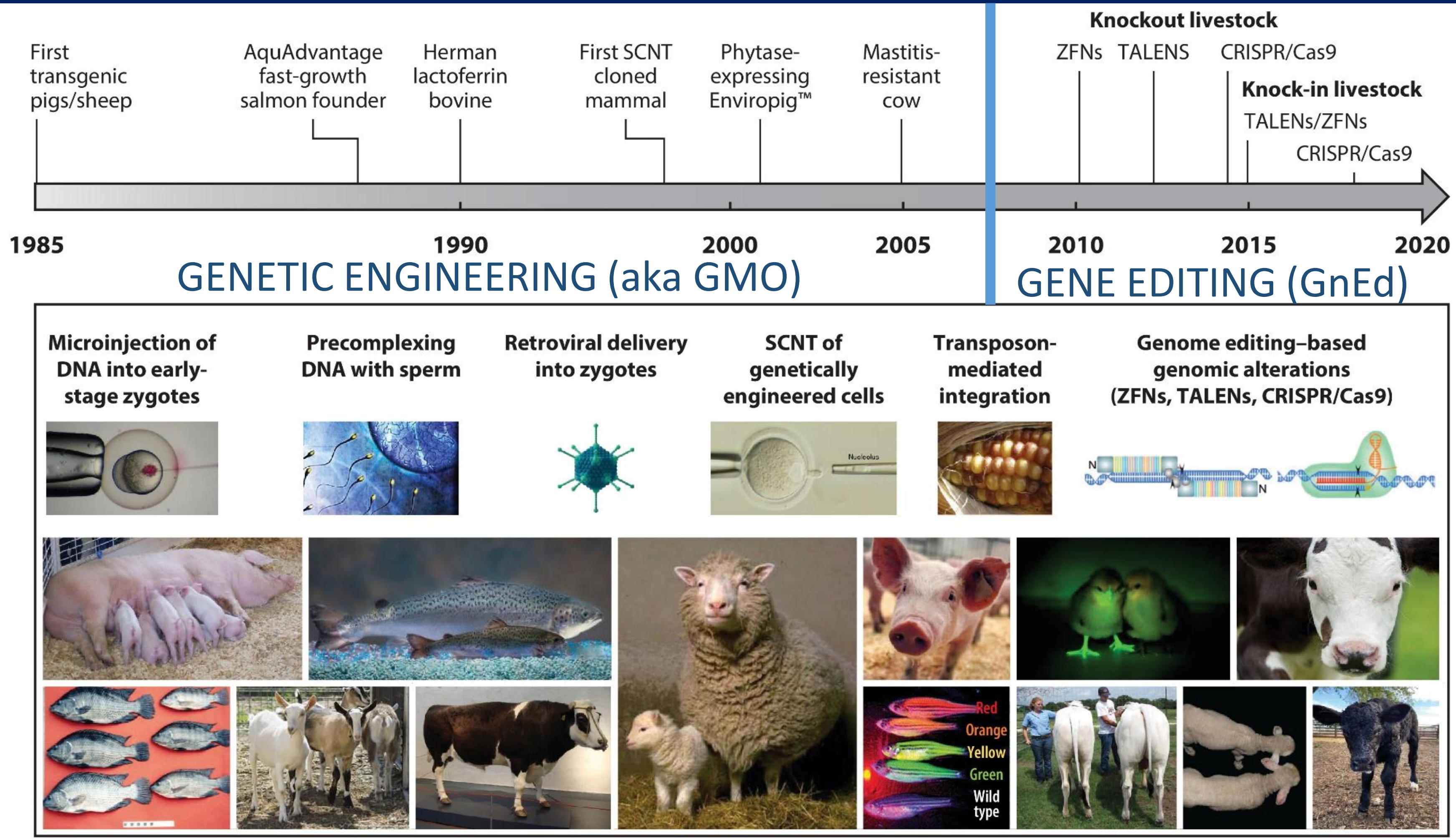




Powered by DALL·E 3 Van Eenennaam 8/19/2024

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

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

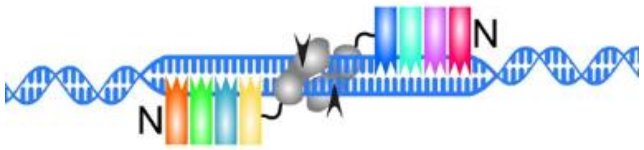


Van Eenennaam, A.L. *et al.* **2021. Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay**. Ann Review of Animal Biosciences.

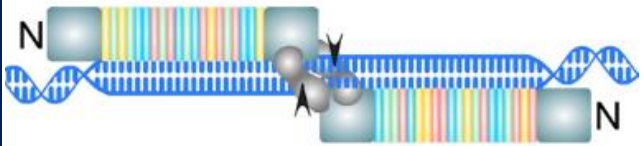
Van Eenennaam AL, et al. 2021
Annu. Rev. Anim. Biosci 9:453–78

WHAT

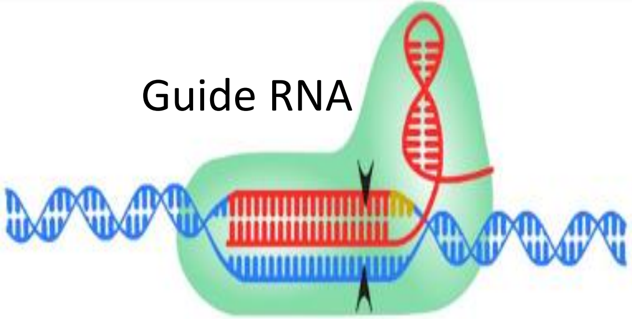
Gene editing (GnEd) allows the introduction of targeted double-stranded breaks in the genome



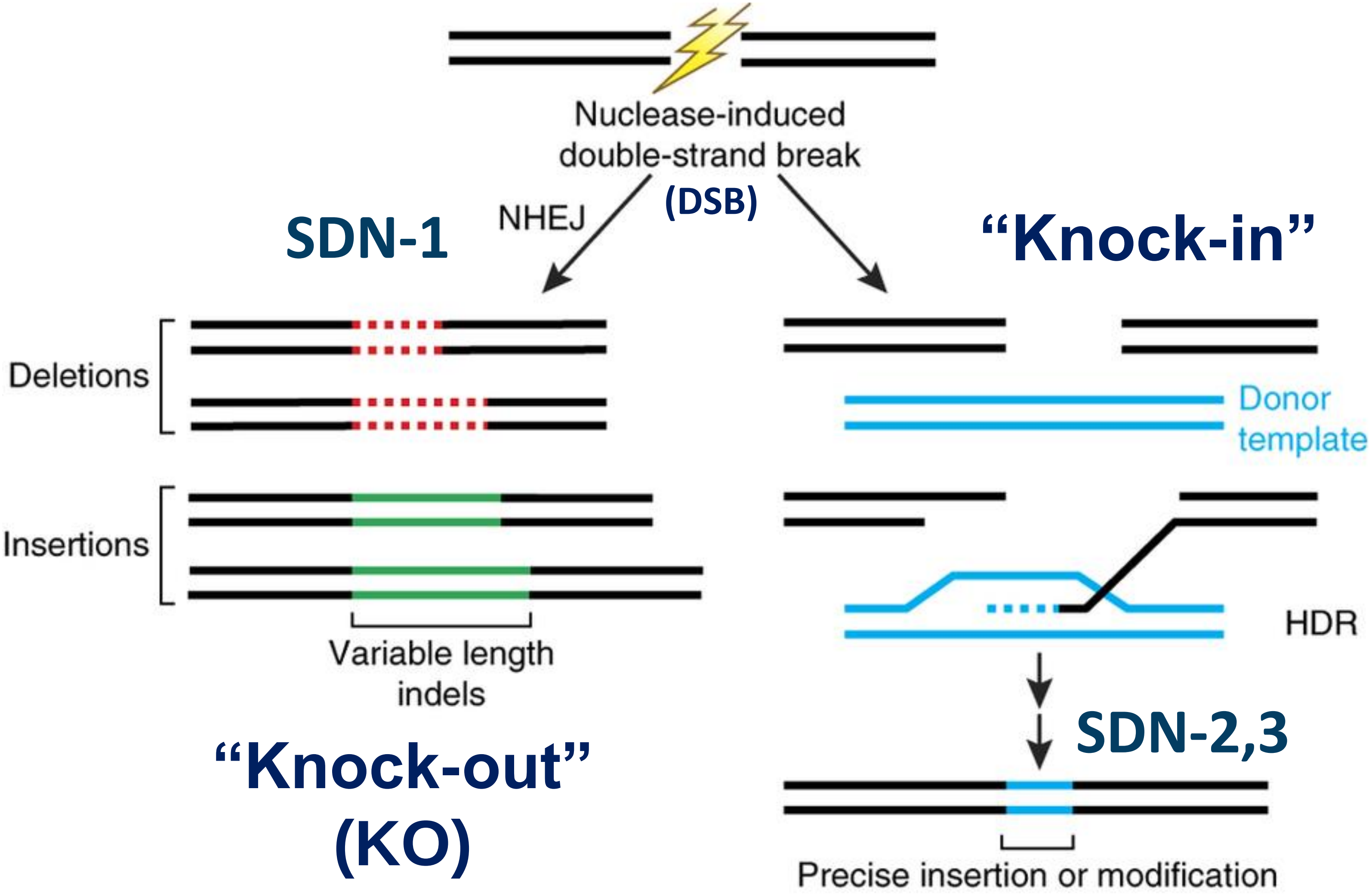
Zinc Finger Nucleases



TALENS



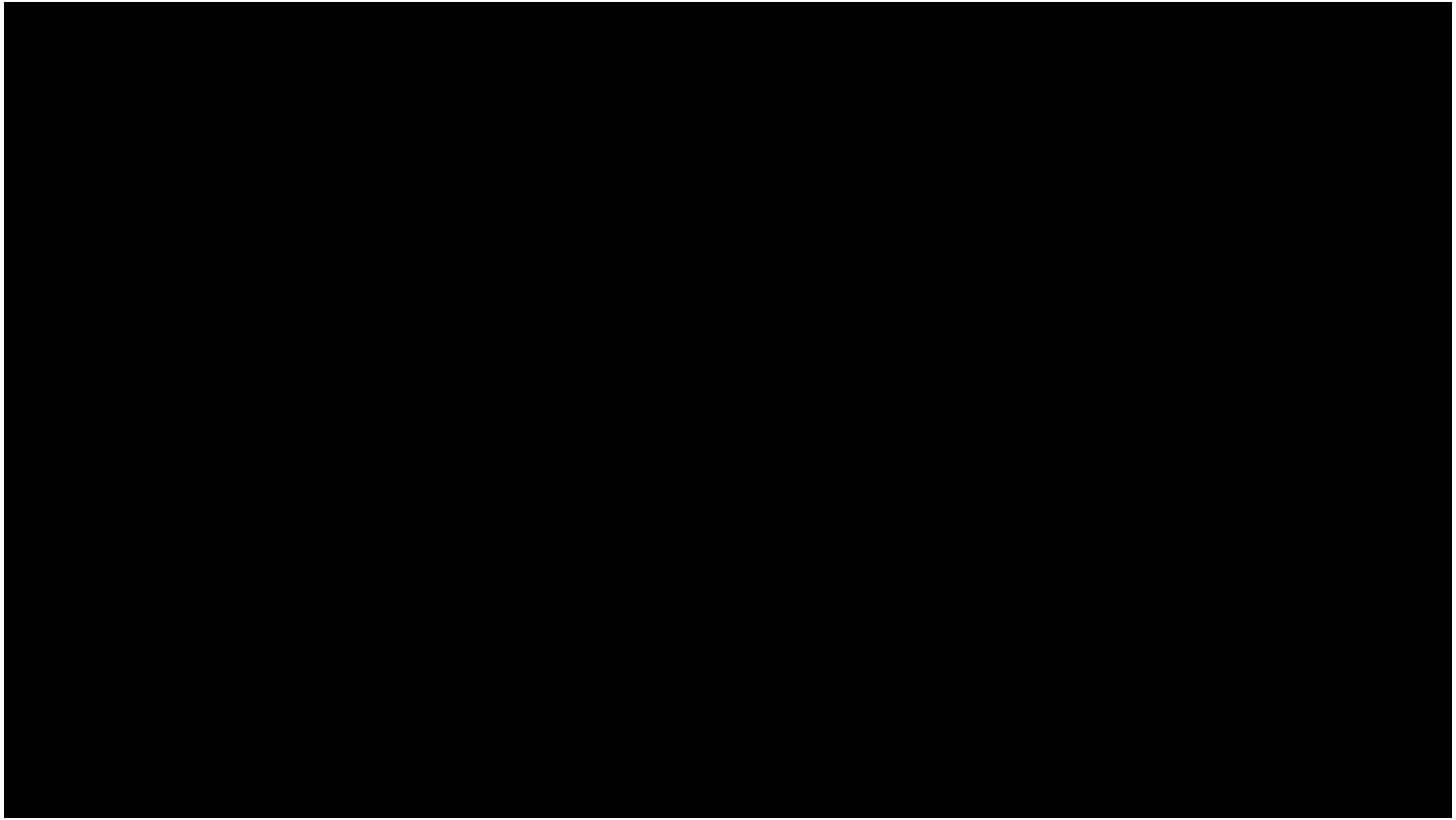
CRISPR/Cas9



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

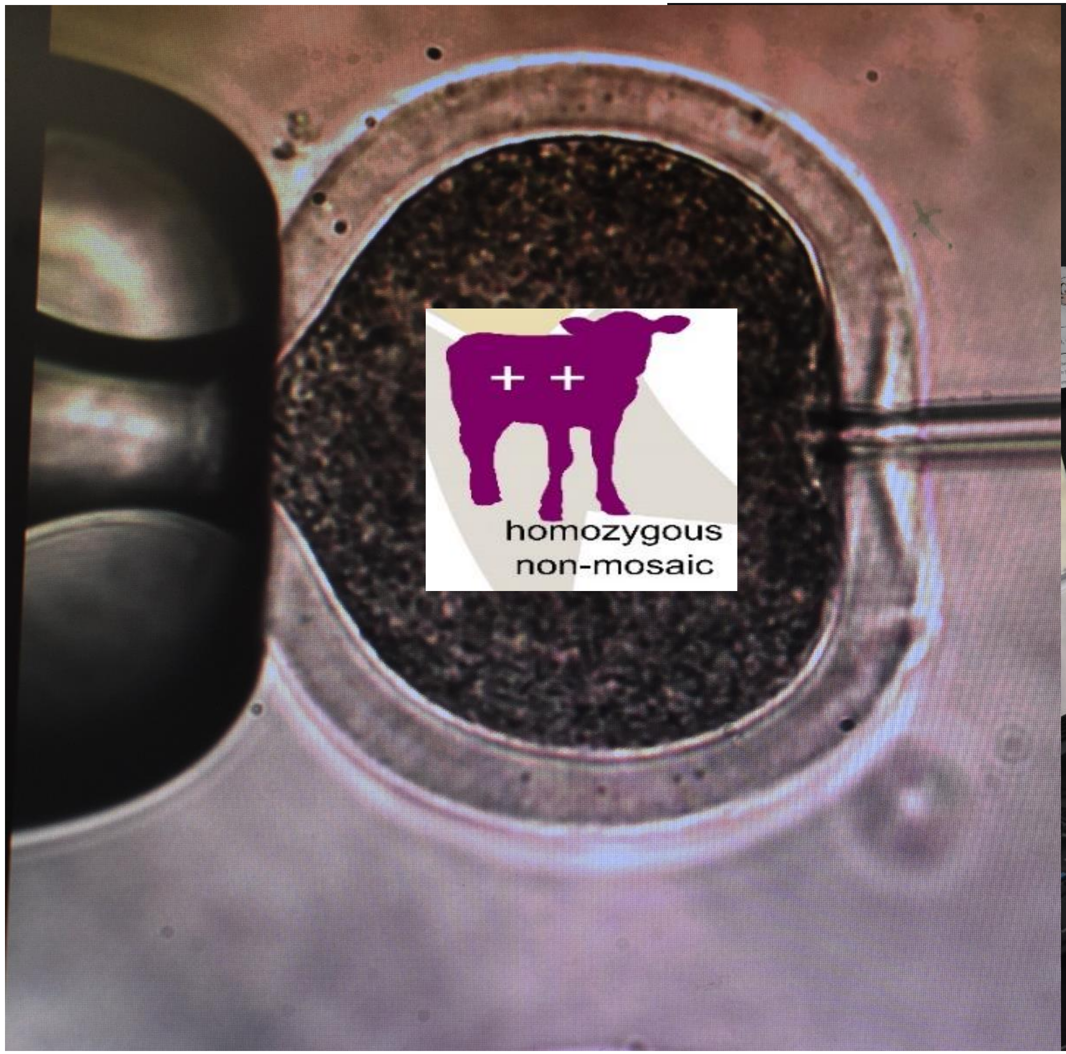
Gene editing (GnEd) involves introducing a double-strand break in the DNA at a targeted location in the genome

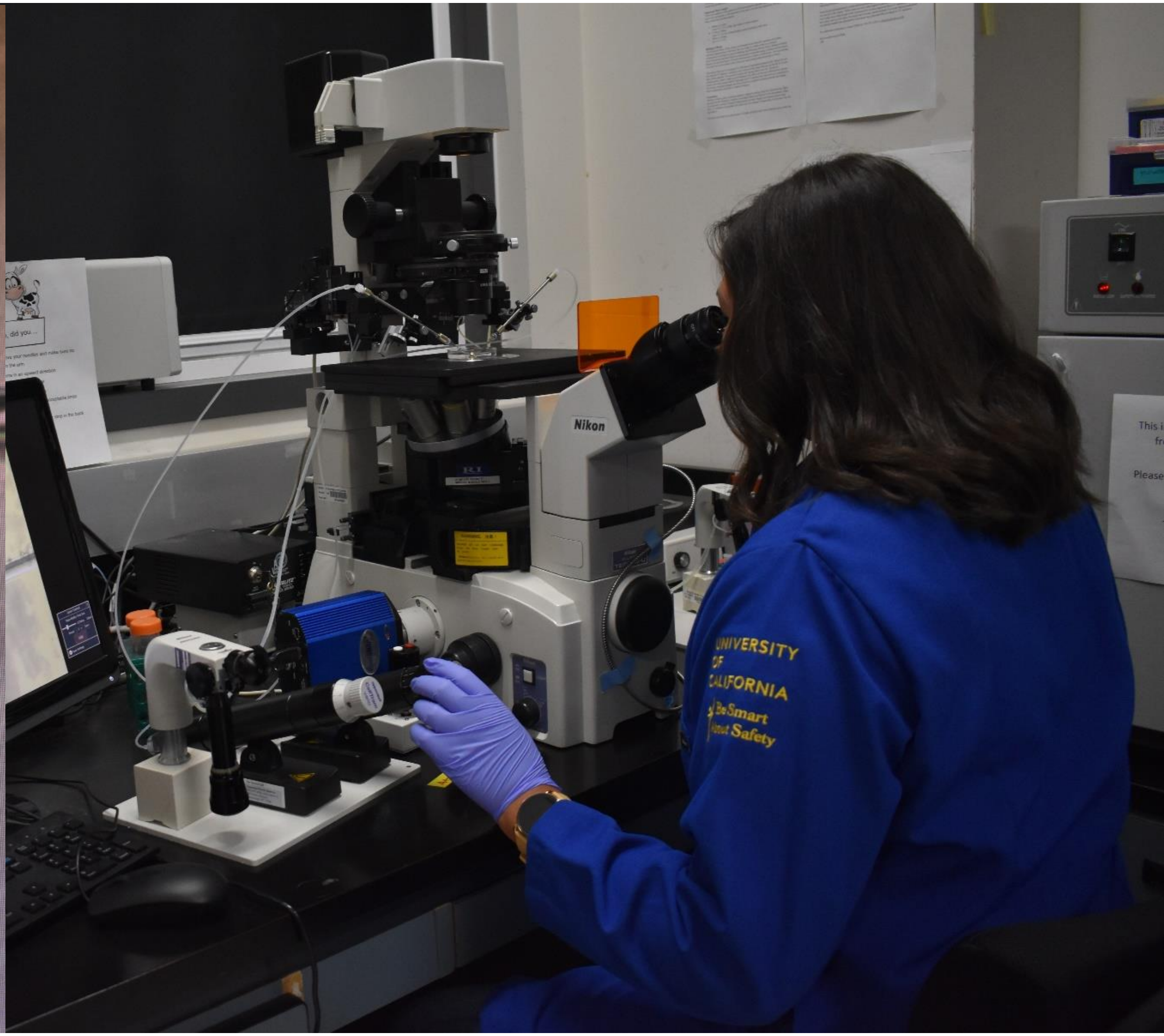
https://youtu.be/bM31E_LRszc

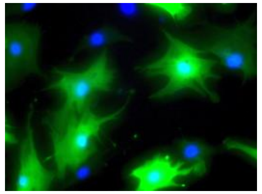


HOW

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.







CLONING

Somatic Cell Nuclear Transfer (SCNT) cloning

Advantages

Germline transmission

Confirmed genotype

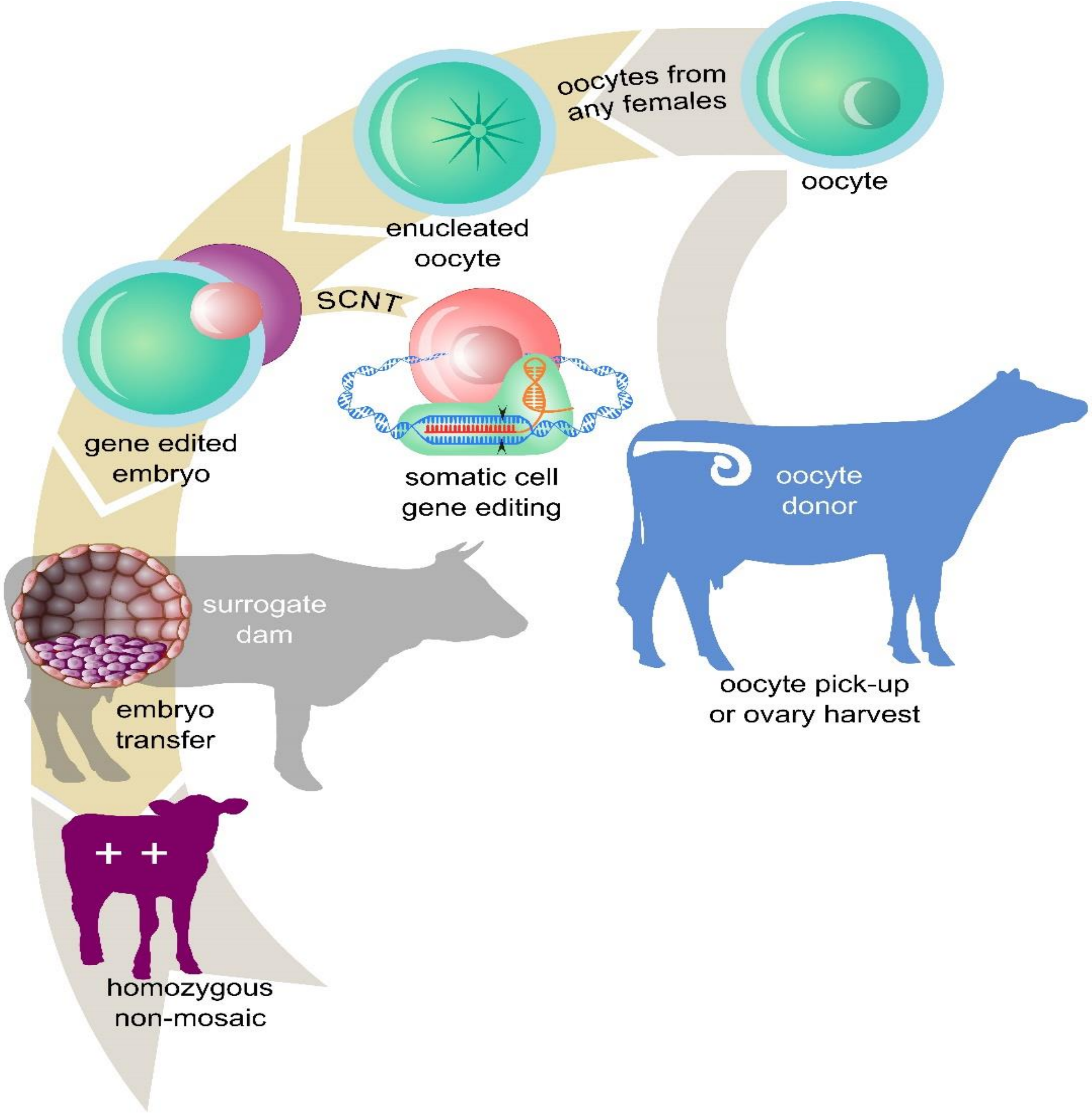
Higher knock-in efficiency in somatic cells

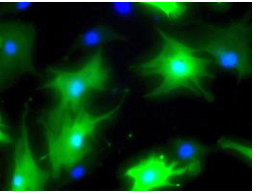
Disadvantages

Cloning efficiency low

Use of a single cell line

Not all cell lines clone well





CLONING

MICRO-INJECTION (CPI)

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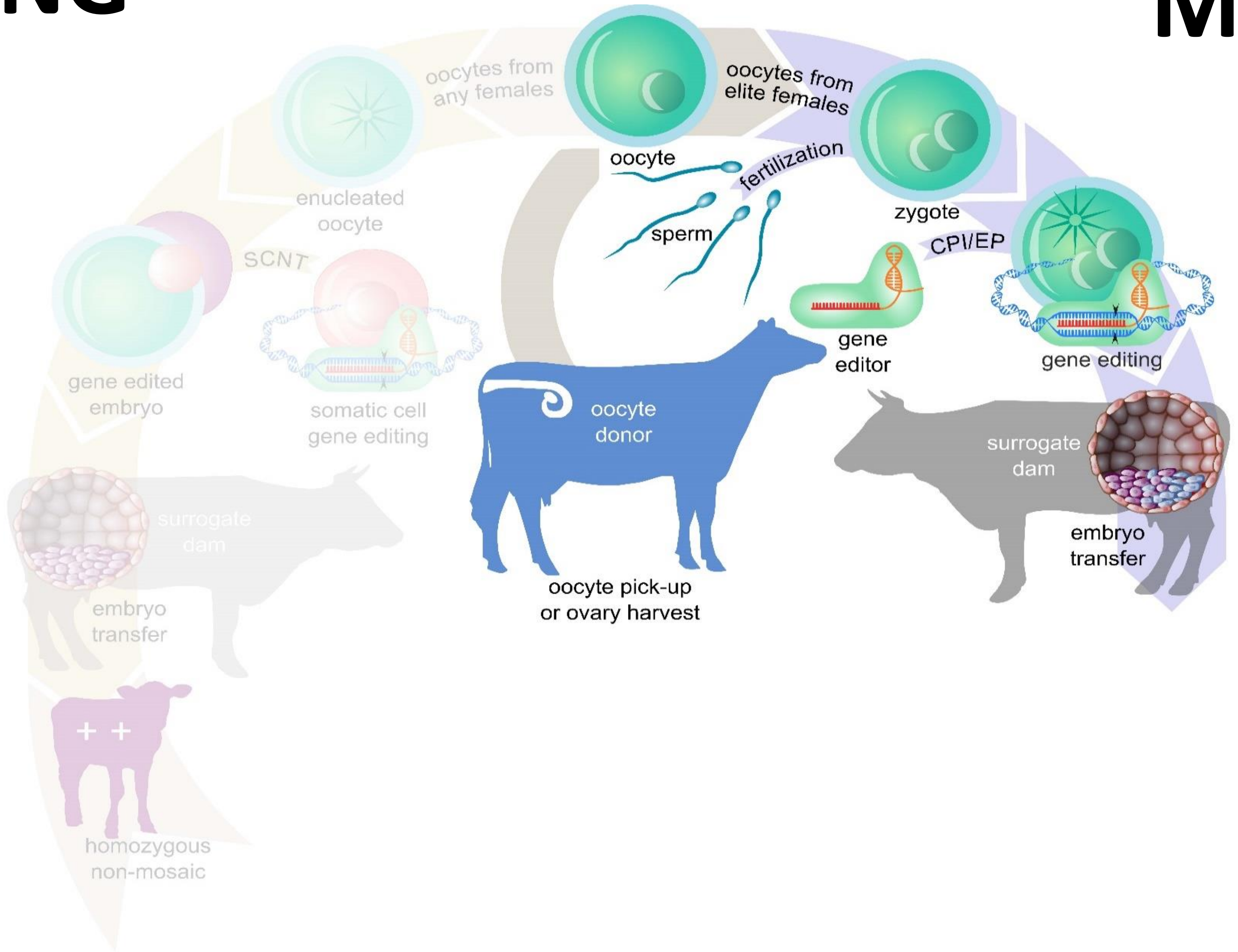

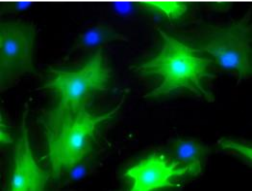

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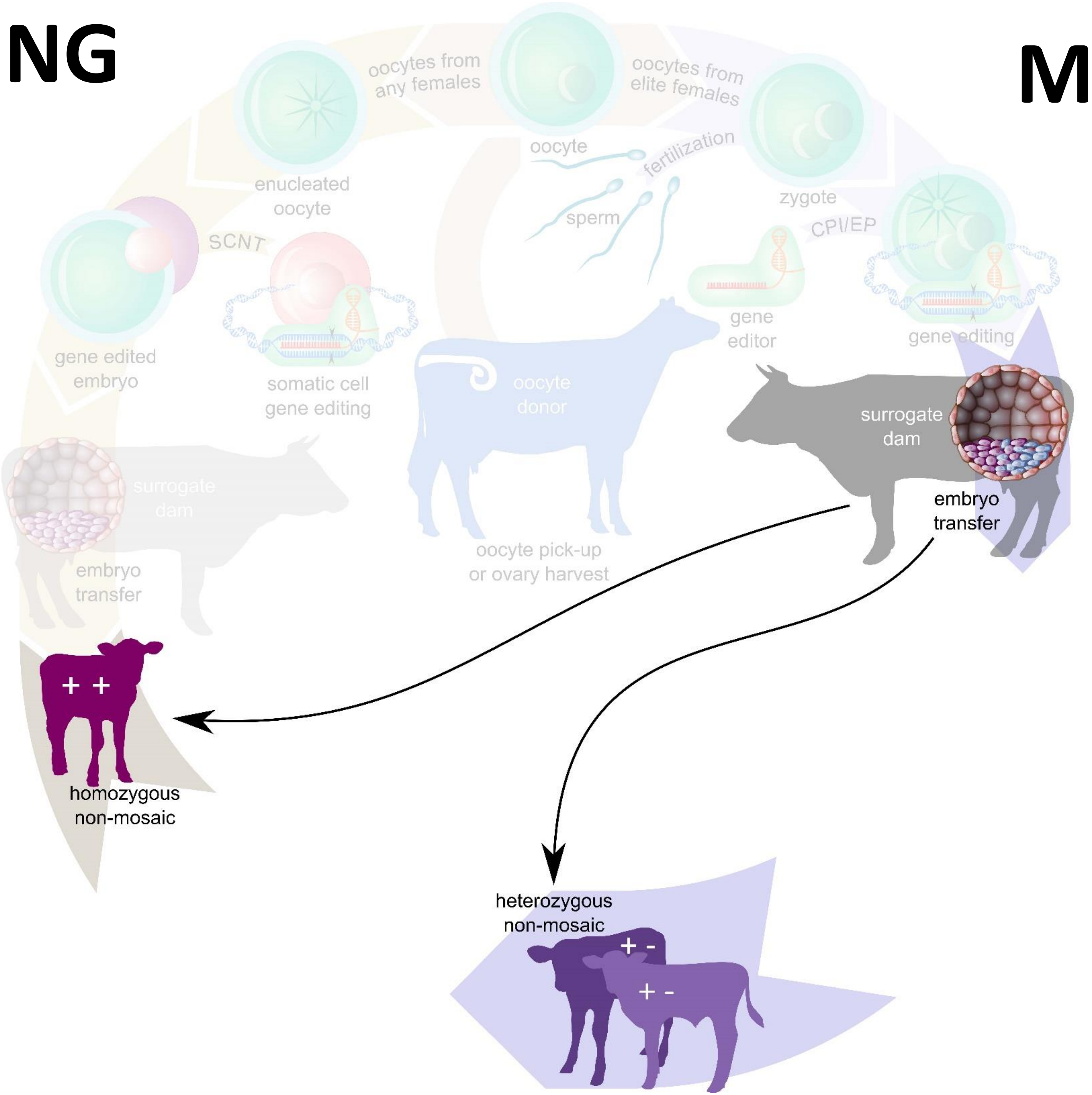

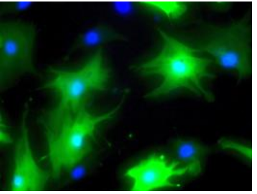

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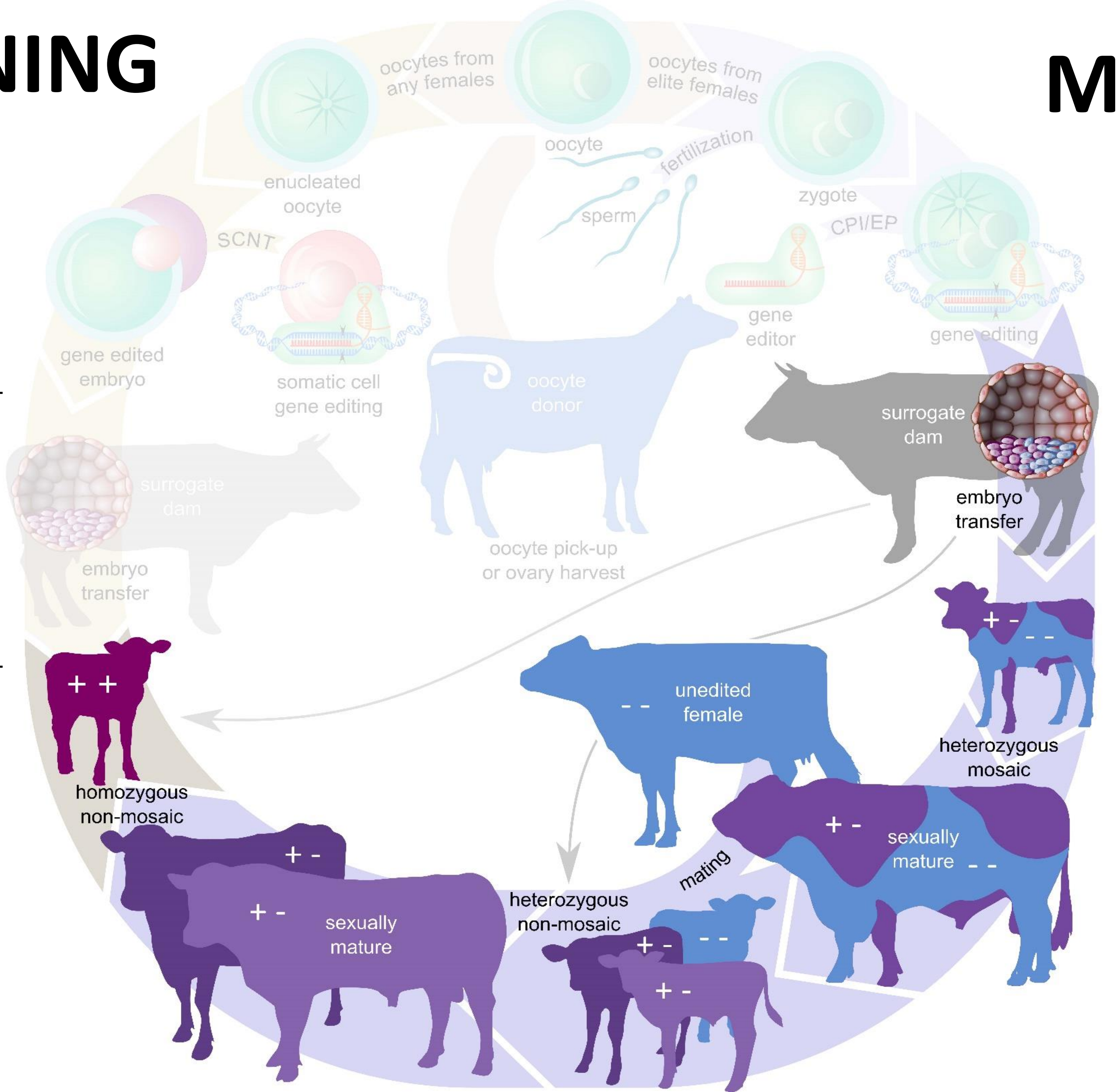

Cytoplasmic Injection (CPI) into embryos

Advantages

No cloning artifacts

Diversity of germplasm

High efficiency for gene knock-outs

Disadvantages

Mosaicism

Low edited/live born

Knock-in is less efficient in early embryos

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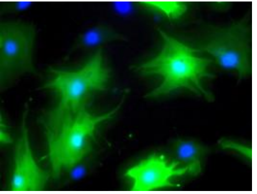

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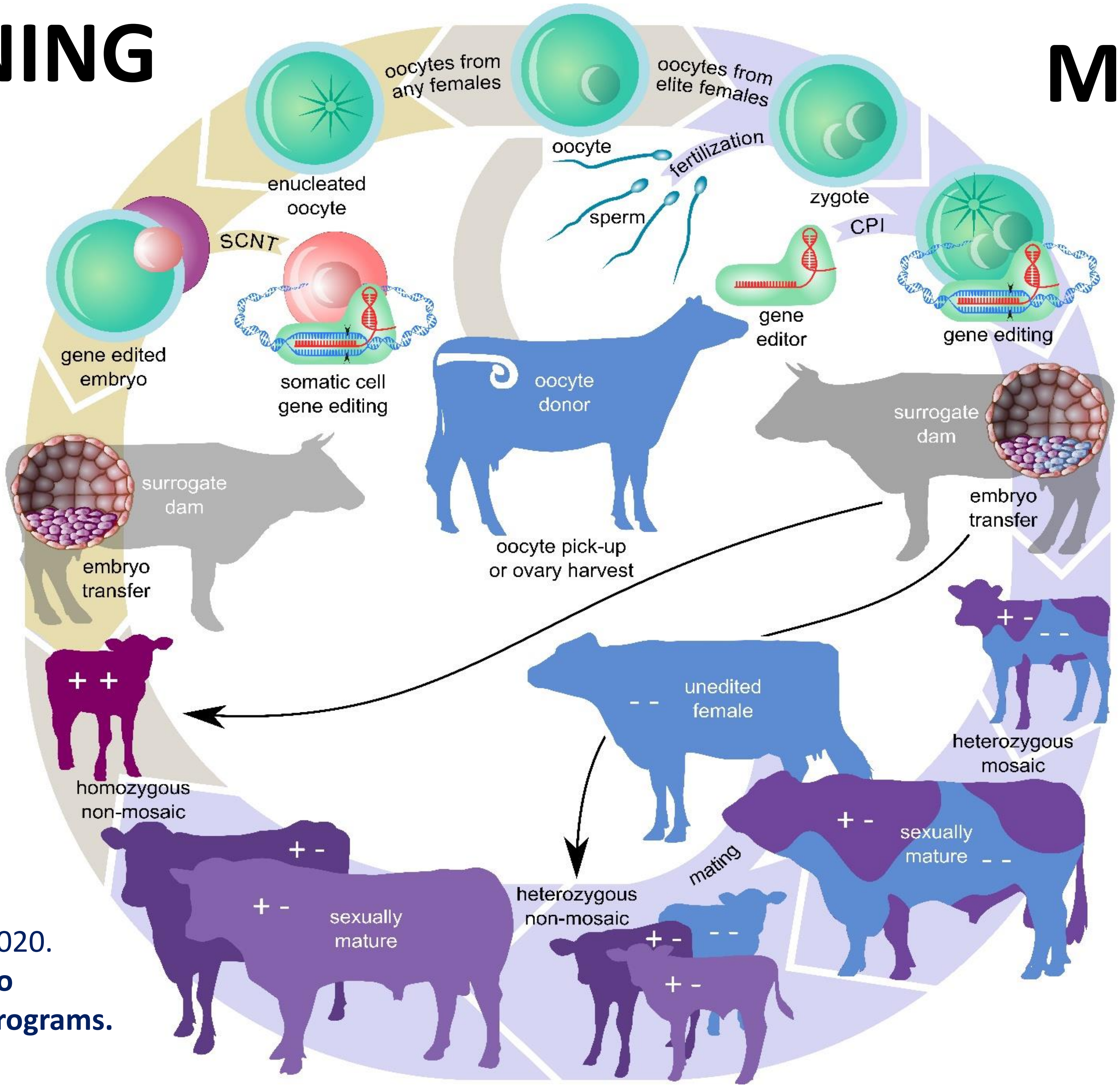

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- Mosaicism
- Low edited/live born
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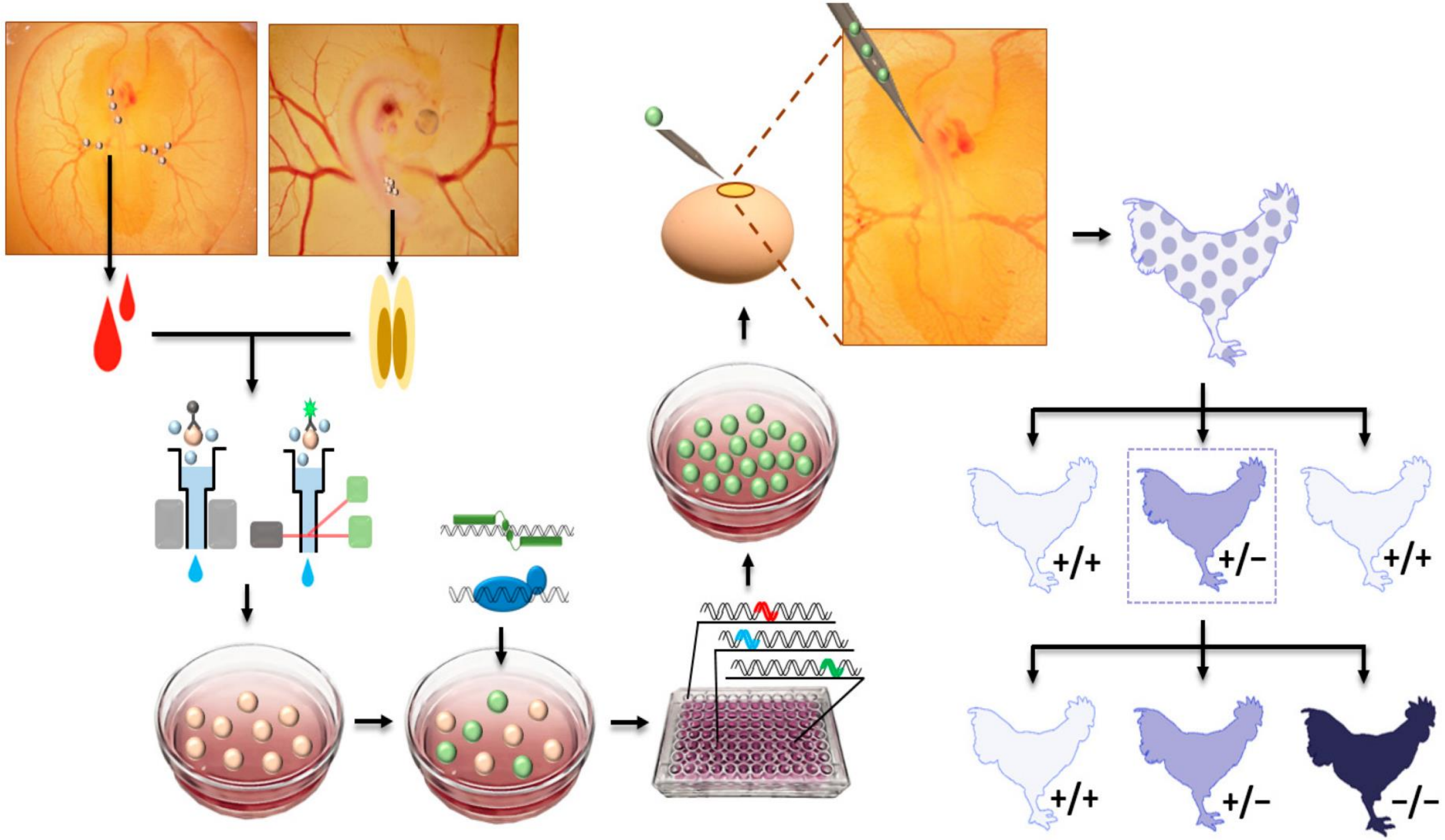
Bishop and Van Eenennaam. 2020.
Genome editing approaches to augment livestock breeding programs.
JEB doi:10.1242/jeb.207159

Graphical summary of primordial germ cell (PGC)-mediated method for genome editing in the chicken



Lee J et al. 2020. Current Approaches and Applications in Avian Genome Editing. Int J Mol Sci. 21:3937. doi: 10.3390/ijms21113937.

Classification of GnEd categories relevant to regulatory considerations

SDN-1 involves the unguided repair of a specific double strand break (**DSB**) by non-homologous end joining (**NHEJ**). Spontaneous repair of this break may result in a mutation that modifies a gene's activity, or causes gene silencing or knock-out (KO). Efficient method, with many applications already.

SDN-2 involves a nucleic acid sequence donor, usually short single-stranded DNA, to direct the repair of a specific DSB. The donor is identified as a repair template, enabling the insertion of the mutation(s) at the target site, because it carries one or more minor mutations surrounded by two homology sequences that match sequences either side of the DSB.

SDN-3 involves a sequence donor, usually double-stranded DNA carrying a gene or an even longer genetic element, to direct the repair of a targeted DSB. Given that the donor's two ends are homologous to the DSB ends (often more than 800 bp each), the donor can be introduced at the target site as a repair template since it is recognized by the DSB ends.

Friedrichs, Steffi, et al. **An overview of regulatory approaches to genome editing in agriculture**. *Biotechnology Research and Innovation* 3.2 (2019): 208-220. https://doi.org/10.1016/j.biori.2019.07.001

What might we knock-out? (SDN-1)

Genes associated with:

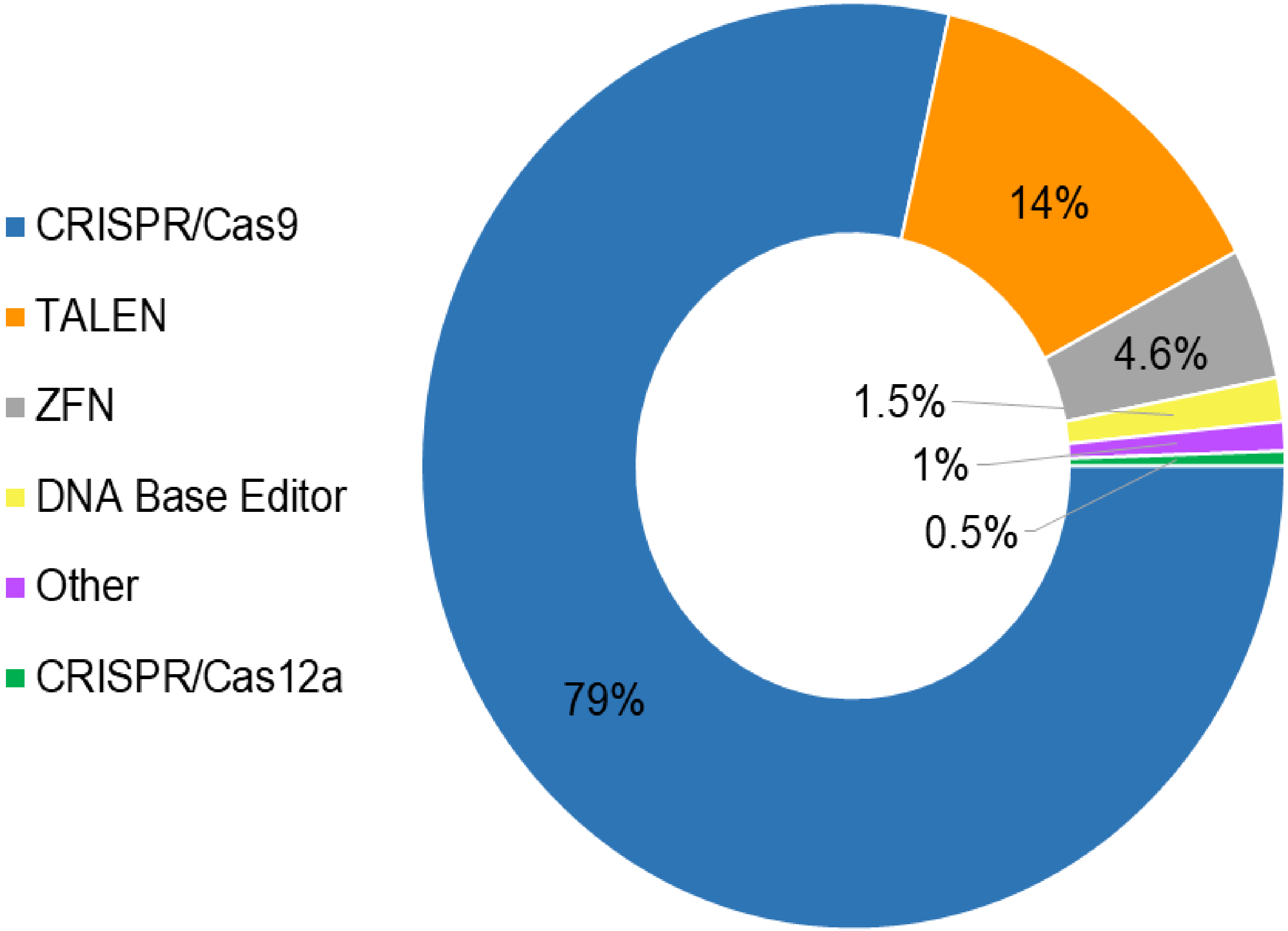
- Allergens (e.g. galactose-alpha-1,3-galactose)
- Thermo tolerance (e.g. SLICK Prolactin receptor)
- Sex ratio skew (e.g. all-female pigs SRY KO)
- Unwanted development (e.g. boar taint)
- Increased yield (e.g. Myostatin KO)
- Disease susceptibility (e.g. PRRS virus CD163)
- Surrogate Germline/Broodstock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

What might we knock-in? (SDN -2/3)

Genes 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)

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.

WHY

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 (e.g. welfare)	Traits not classified in the above categories, including welfare traits such as hornlessness and hypogonadotropic hypogonadism as a castration free trait.

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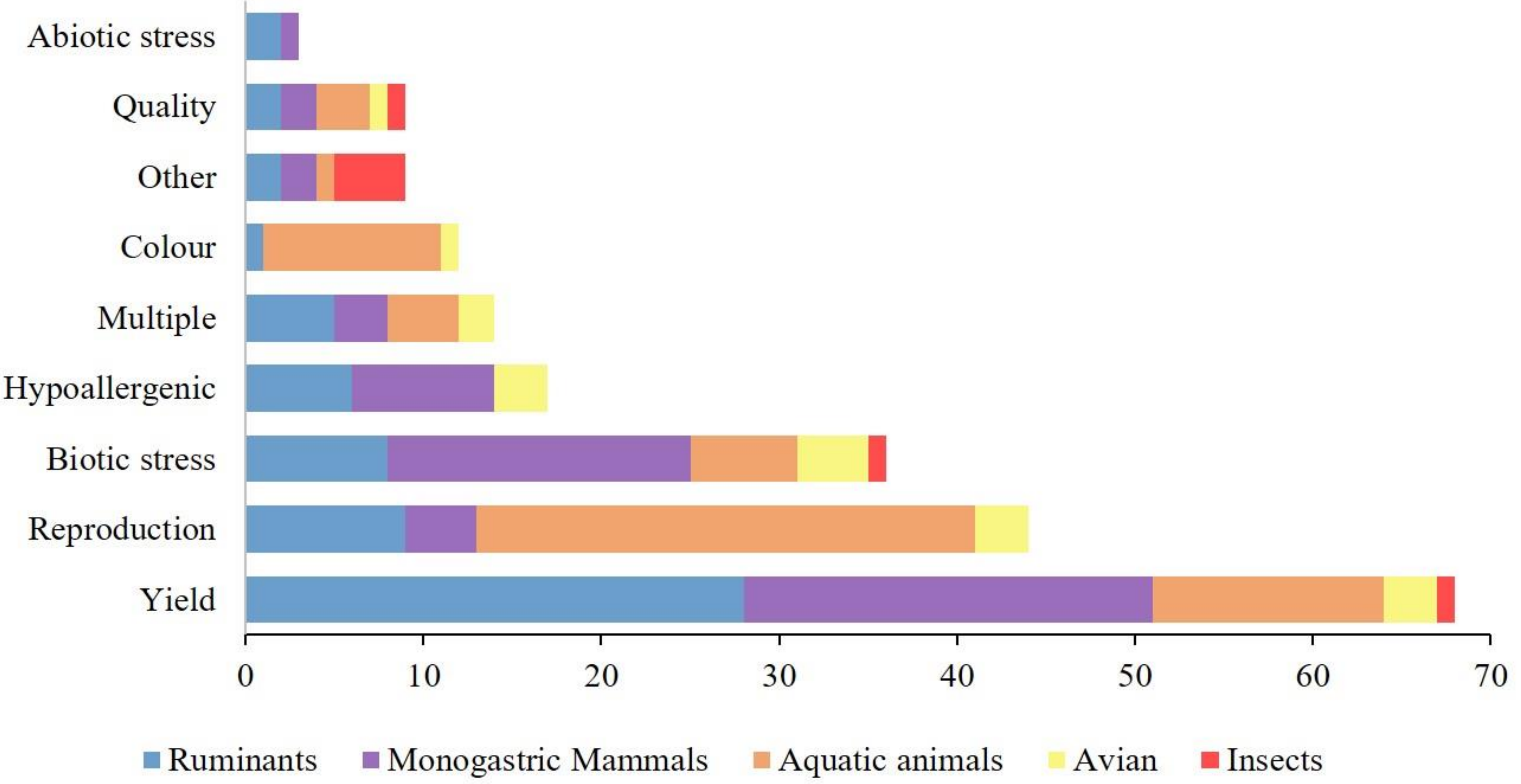
Trait targeted in animal gene editing applications



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-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

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

Filter:

- Species +
- Countries +
- Trait Categories +
- Genes +
- NGT +
- SDN Types +
- Institutes/Developers +
- Editing Methods +
- Year +
- 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*)

〉 🏠 〉 Animal Biotech Database 〉 Species List 〉 Sheep (*Ovis aries*)

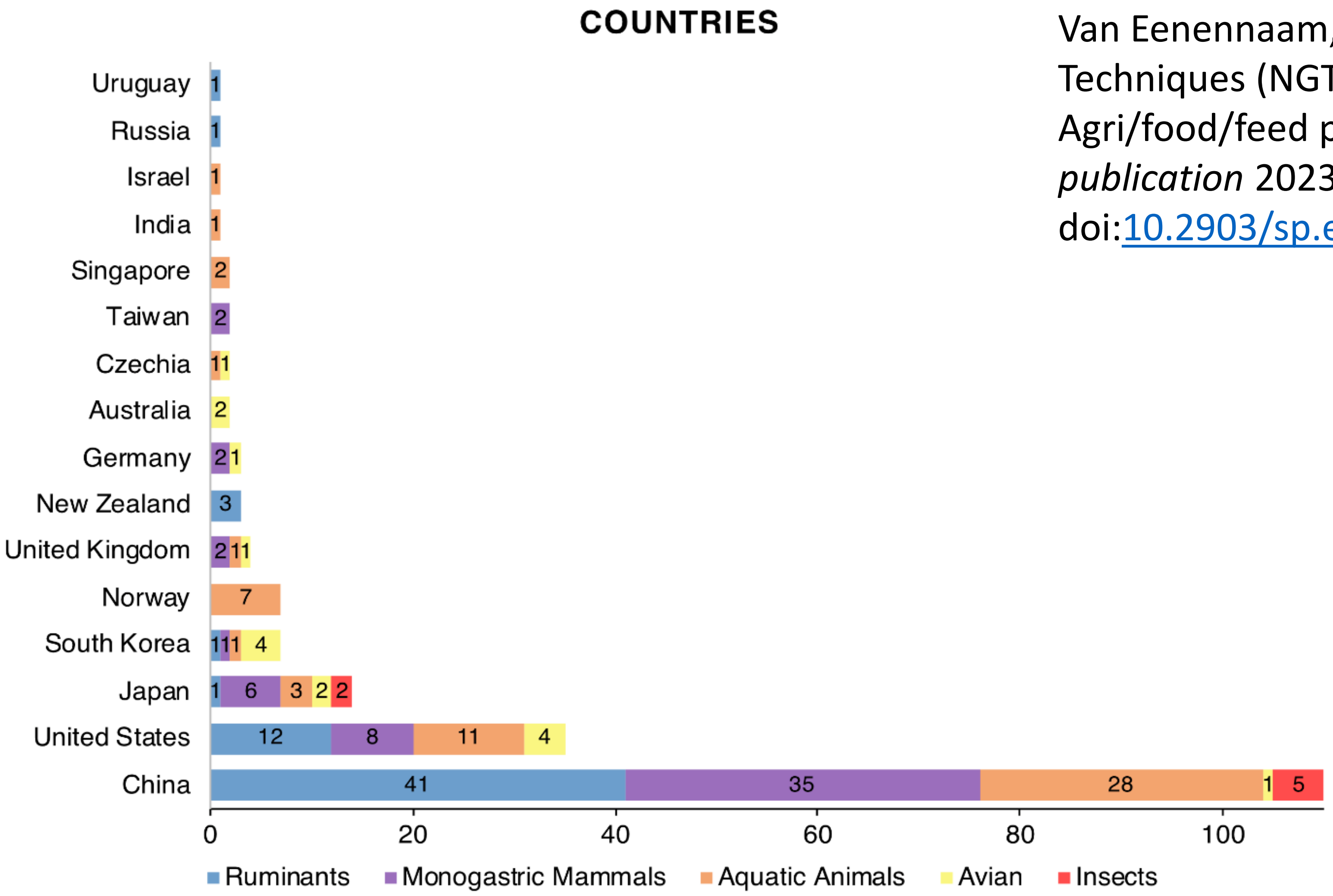
Undergone regulatory process: NO

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

WHERE

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. *USDAsupporting publication* 2023: 20(9):EN-8311. 82 pp. doi:10.2903/sp.efsa.2023.EN-8311

Gene-edited Animal Database

Countries

- Australia
- China
- Czech Republic
- Germany
- United Kingdom
- Israel
- India
- Japan
- South Korea
- Norway
- New Zealand
- Russia
- Singapore
- Taiwan
- United States
- Uruguay

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

Gene-edited Animal Database

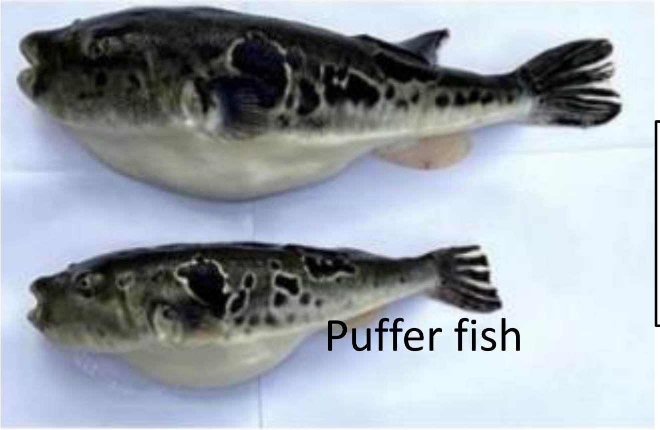
〉🏠〉Animal Biotech Database 〉SDN Types 〉SDN-1

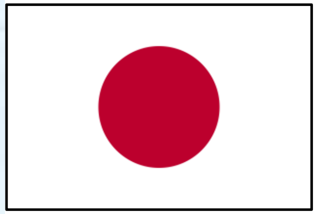
Undergone regulatory process: YES

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

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





Regional Fish

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

https://regional.fish/en/

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Summary

- Genome editing offers an approach to introduce useful genetic variation and alleles for large effect loci such as PRRS virus disease resistance and welfare traits like polled into breeding programs
- The way that NGTs are introduced into different species depends upon the biology of that species, and differs markedly between different classes of animals (i.e. mammals, birds, fish and insects),
- 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, and 18 “other” approaches (9%) to editing, the majority of which were publications with avian species where editing was done in primordial germ cells.
- CRISPR/Cas 9 and SDN-1 edits are the most commonly reported types of edits
- Database **https://www.isaaa.org/animalbiotechdatabase/default.asp**

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

UC DAVIS ANIMAL SCIENCE

- Dr. Josephine Trott
- Dr. Joey Owen
- Dr. James Murray
- Dr. Bret McNabb
- Dr. Elizabeth Maga
- Dr. C. Titus Brown
- 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

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