Improved cattle through genomic selection, assisted reproductive technologies, and gene editing

Alison Van Eenennaam

Professor of Cooperative Extension

Animal Biotechnology and Genomics

Department of Animal Science

University of California, Davis, USA

Email: alvaneenennaam@ucdavis.edu

Twitter: **@BioBeef**

BLOG: https://biobeef.faculty.ucdavis.edu

WEBSITE: https://animalbiotech.ucdavis.edu

Genomic selection | **Assisted Reproductive Technologies** | **Gene editing**

Mueller, M.L., and Van Eenennaam, A.L. 2022. **Synergistic power of genomic selection, assisted reproductive technologies, and gene editing to drive genetic improvement of cattle.** CABI Agriculture and Bioscience. *3:13.*

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

The rate of genetic gain depends upon the four components of the breeders' equation

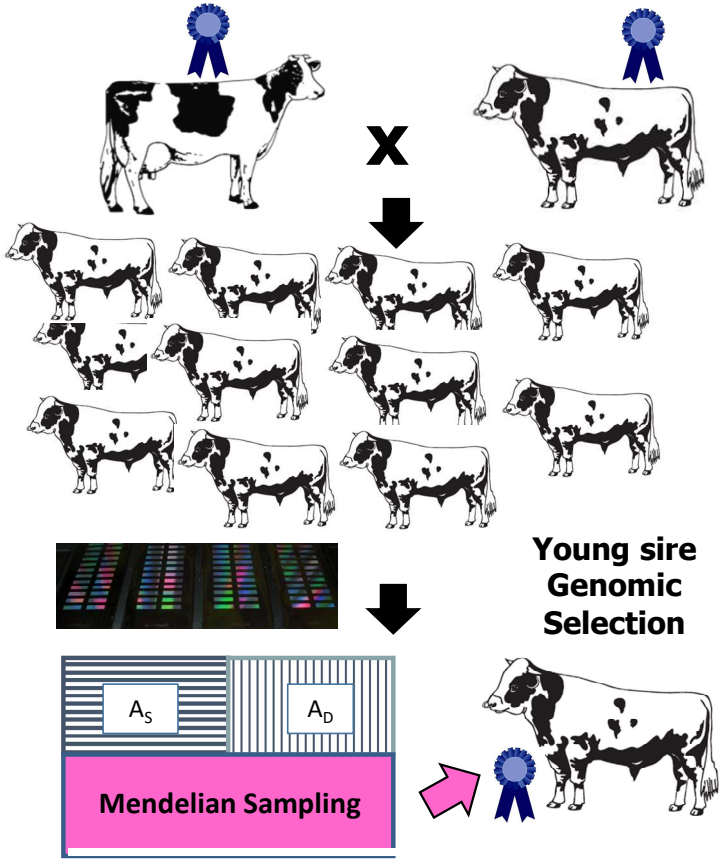
Genetic change per year =

$$\frac{\text{Genomics} \quad \text{Reproduction} \quad \text{Biotechnology}}{\text{(Accuracy x Intensity x Genetic Variation)}}{\text{Generation Interval}}$$

Genomics, Reproduction, Biotechnology

Accuracy = how certain we are about an animal's true genetic merit
Intensity of selection = fraction of animals selected as parents
Genetic variation = variation available in the population
Generation interval = time between generations

Genomic selection enables an early look at who got lucky in the roulette of Mendelian sampling



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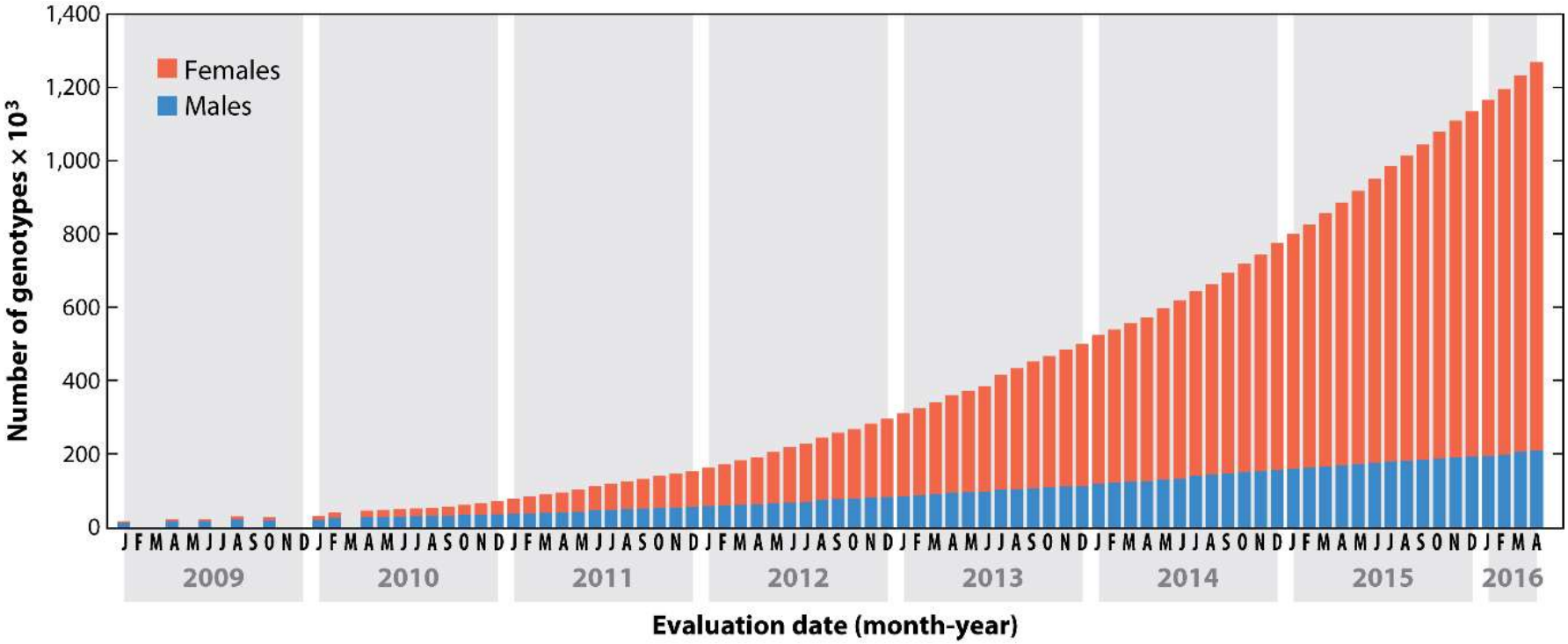
Over one million genomic tests were performed on female dairy cows in the U.S. last year.



Number of genotyped animals included in US genomic evaluations for dairy cattle since January 2009

Wiggans, G. R., Cole, J. B., Hubbard, S. M., & Sonstegard, T. S. (2017). **Genomic selection in dairy cattle: the USDA experience.** *Annual review of animal biosciences*, *5*, 309-327.

Over 6 million US dairy animals genotyped

Official US genomic evaluations were first released to the dairy industry in January 2009 for Holsteins and Jerseys, in August 2009 for Brown Swiss, in April 2013 for Ayrshires, and in April 2016 for Guernseys

TOTAL	6,031,684
Ayrshire	16,390
Brown Swiss	66,722
Guernsey	7,902
Holstein	5,214,918
Jersey	724,637

From CDCB (https://queries.uscdcb.com/Genotype/cur_freq.html): Current as of March 21, 2022

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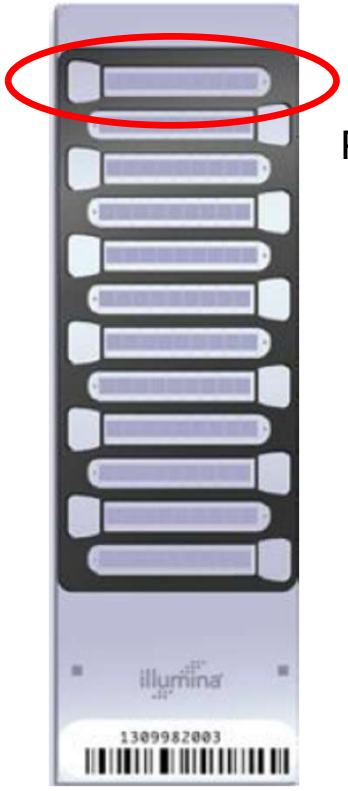
What's a 50K SNP genotype worth?



Pedigree is equivalent to information on ~7 daughters

For protein yield (h^2=0.30), the SNP genotype provides information equivalent to an additional ~32 daughters

Slide from John Cole, USDA ARS

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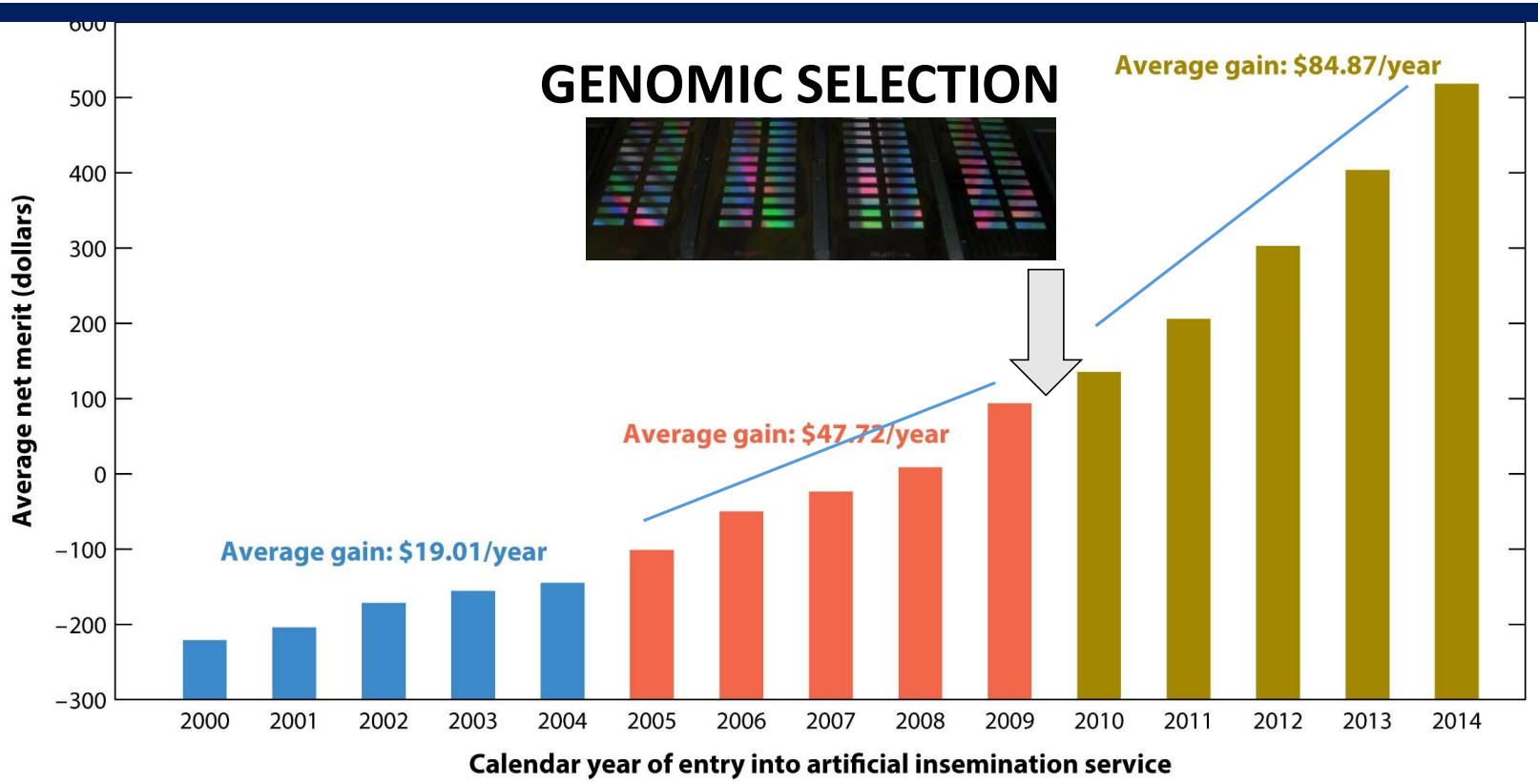
What's a 50K SNP genotype worth?

And for daughter pregnancy rate (h^2=0.04), SNP = ~181 daughters

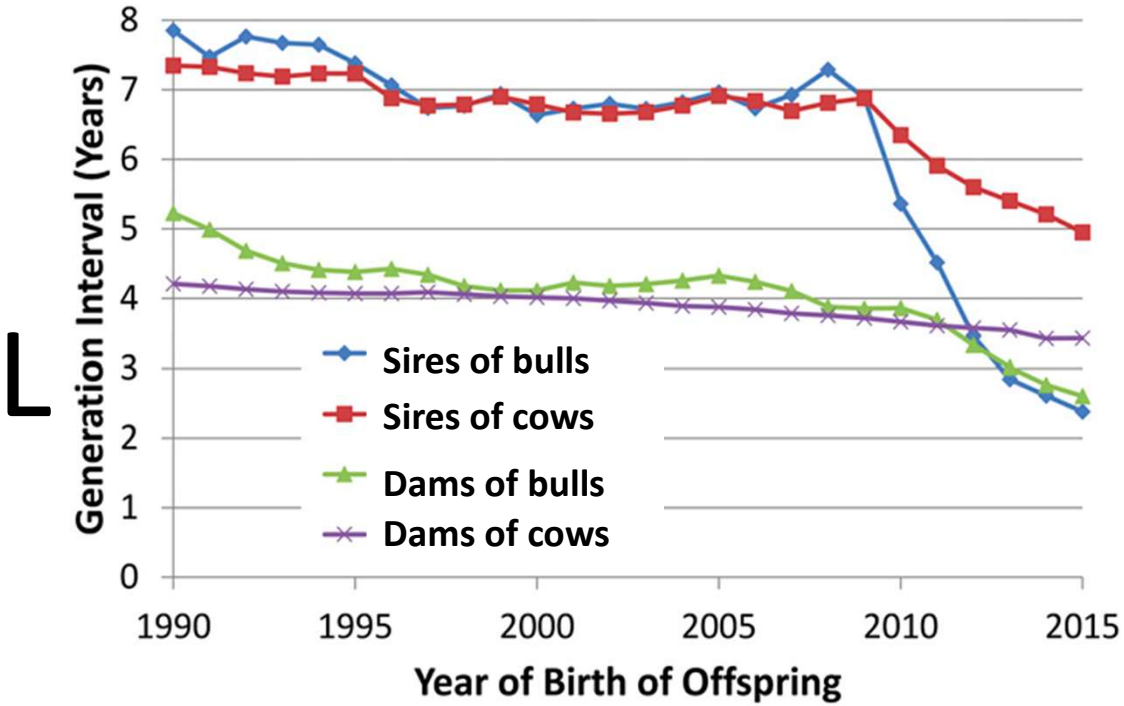
Slide from John Cole, USDA ARS

The rate of improvement in average net merit has nearly doubled for Holstein bulls since the implementation of genomic evaluation in 2010.



Wiggans, G. R., Cole, J. B., Hubbard, S. M., & Sonstegard, T. S. (2017). **Genomic selection in dairy cattle: the USDA experience.** *Annual review of animal biosciences*, *5*, 309-327.

Genomic selection decreased generation interval which increased the rate of genetic gain



L

Genetic change per year =

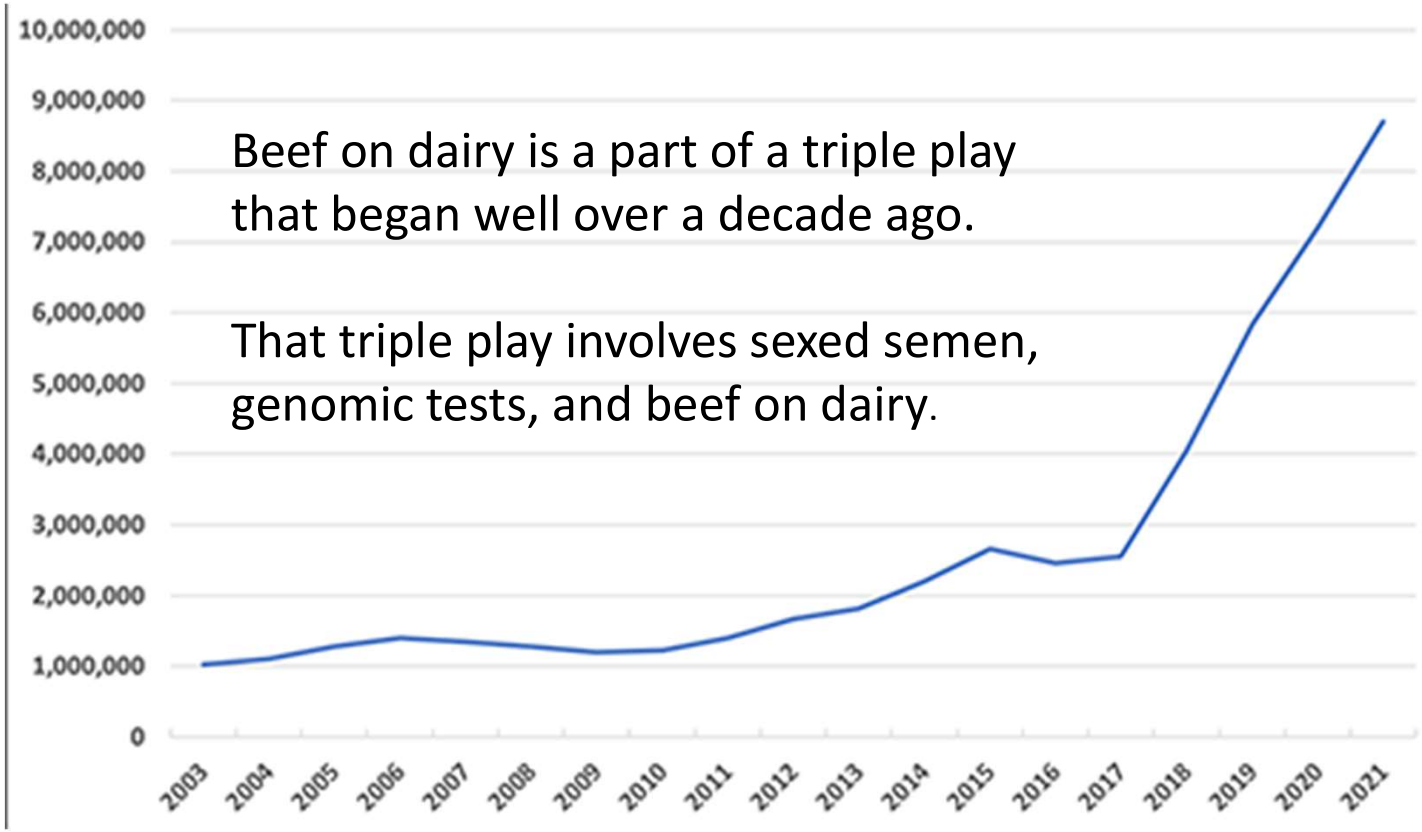
Accuracy (**r**) x
Intensity (**i**) x
Genetic Variation (σ_A)

Generation Interval (L)

García-Ruiz et al. (2016) **Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection**. Proc. Natl. Acad. Sci. U.S.A. 113: E3995-4004.

Beef Semen Sales Doubles in Three Years

Since 2010, when genomic testing came upon the scene, US beef semen sales climbed from 1.2 million units in 2010 to 8.7 million in 2021



Geiger, C. 2022, Beef on dairy binge continues. https://hoards.com/article-31628-beef-on-dairy-binge-continues.htm

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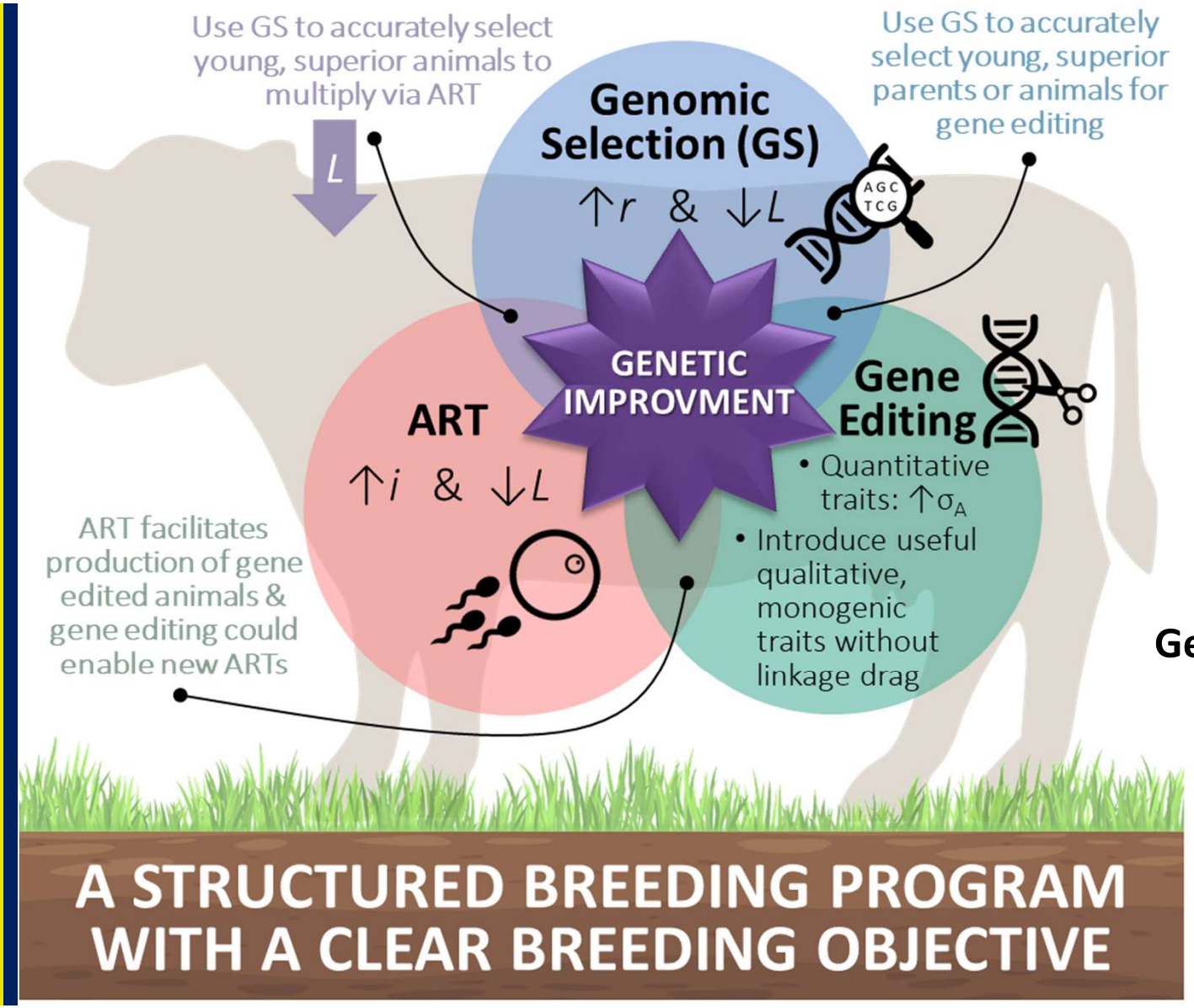

Genetic change/year =

Accuracy (**r**) x
Intensity (**i**) x
Genetic Variation (σ_A)

Generation Interval (L)

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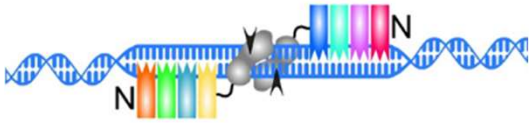
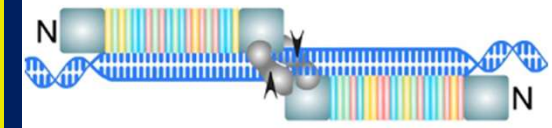
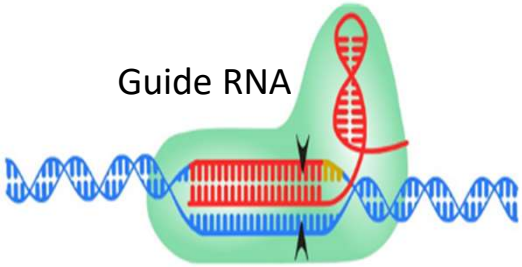
Gene editing allows the introduction of double-stranded breaks at a specific sequence in the genome



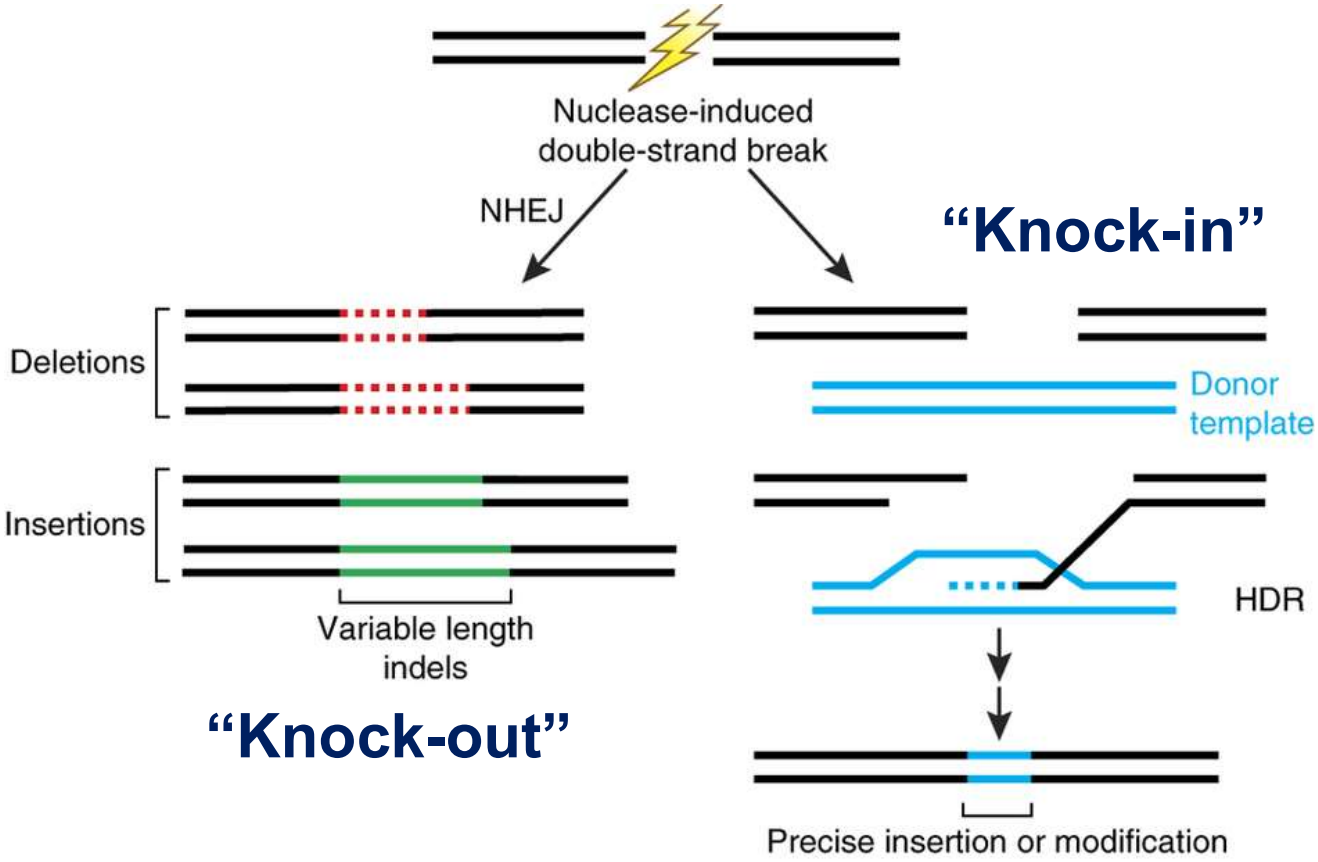
Zinc Finger Nucleases



TALENS



CRISPR/Cas9



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

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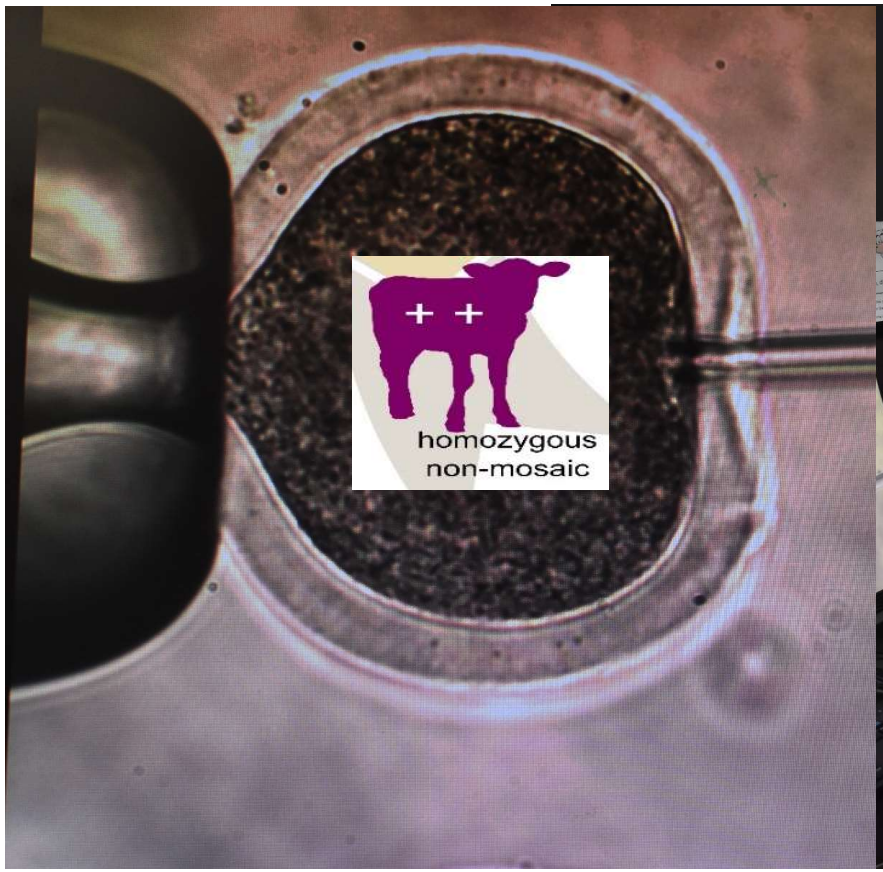
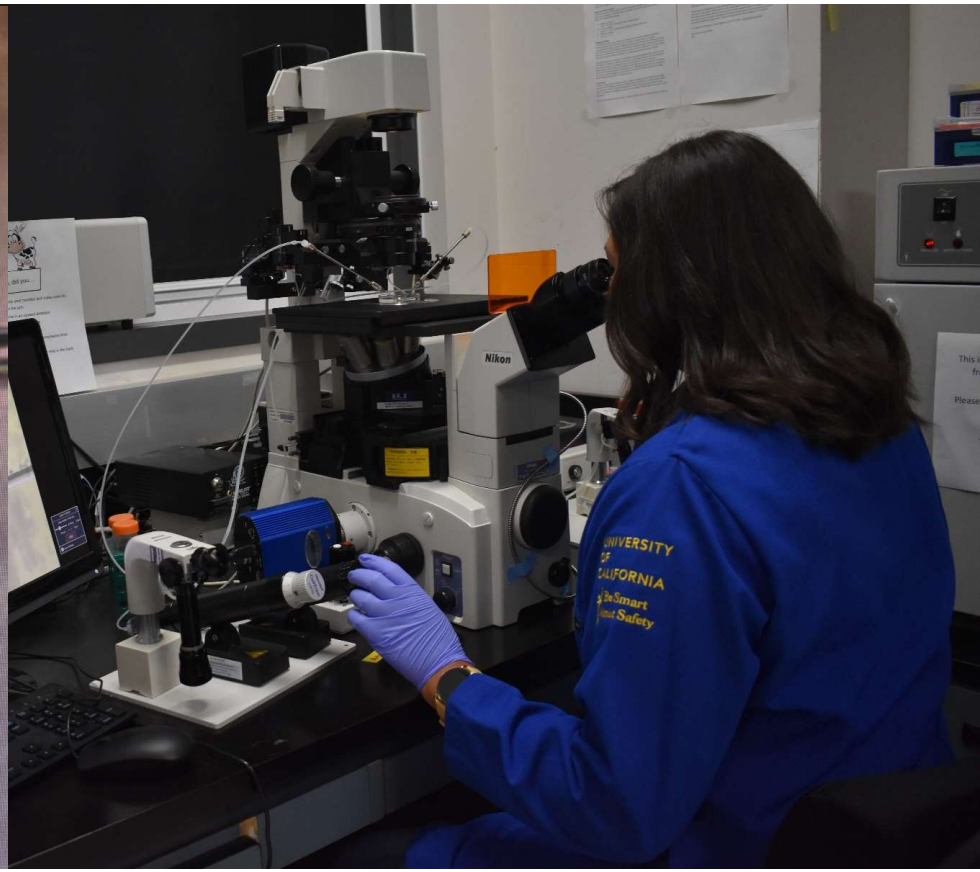
Introducing useful genetic variation into the germline of selected parents such that genetic improvement is inherited by the next generation is the ultimate goal of animal breeding.





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What might we knock-out?

Genes associated with

- allergens
- disease susceptibility
- unwanted development

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

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
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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.

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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 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.

FDA gives enforcement discretion to SLICK cattle submission by Recombinetics

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

March 7, 2022

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

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For Immediate Release: March 07, 2022

Today, the U.S. Food and Drug Administration announced it has made a low-risk determination for the marketing of products, including food, from two genome-edited beef cattle and their offspring after determining that the intentional genomic alteration (IGA) does not raise any safety concerns (low-risk determination). The IGA results in the equivalent genotype (genetic make-up) and short-hair coat trait seen in some conventionally bred cattle, known as a "slick" coat. This is the FDA's first low-risk determination for enforcement discretion for an IGA in an animal for food use.

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03/07/2022

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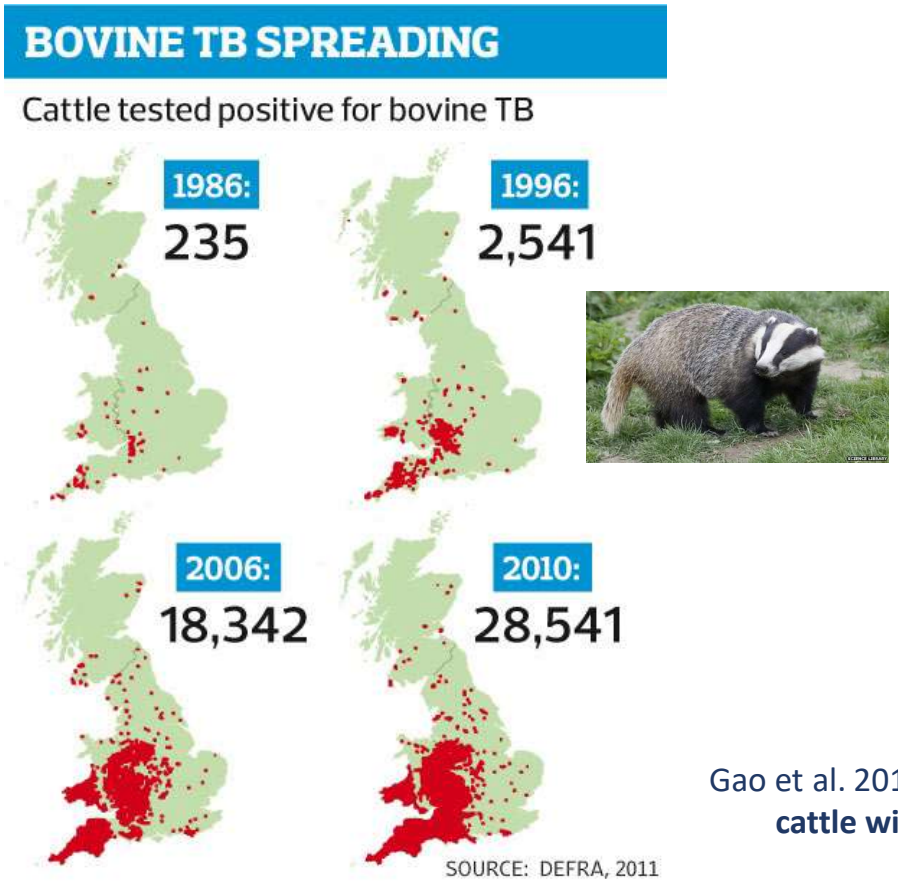
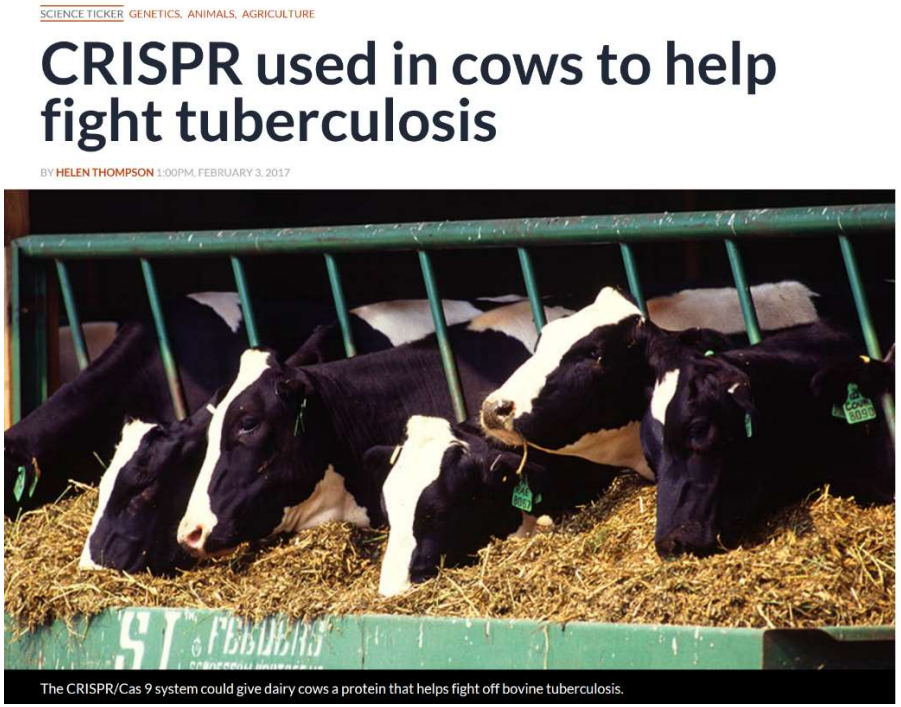
What might we knock-in?

Genes associated with

- Improved food quality/nutrition
- disease resistance
- unwanted development

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.

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.
Northwest A&F University, Yangling, China

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

Gene Edited Polled Calves

Naturally-occurring bovine allele at polled locus

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

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Thanks for inviting me

revive&restore
genetic rescue for endangered and extinct species

FFAR
Foundation for Food and Agriculture Research

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Van Eenennaam Laboratory

My laboratory has received support from the USDA National Institute of Food and Agriculture and the Biotechnology Risk Assessment Grant (BRAG) under award numbers 2015-67015-23316, 2017-33522-27097, 2017-38420-26790, 2018-67030-28360, 2020-67015-31536, 2020-70410-32899