Genome editing in ruminants

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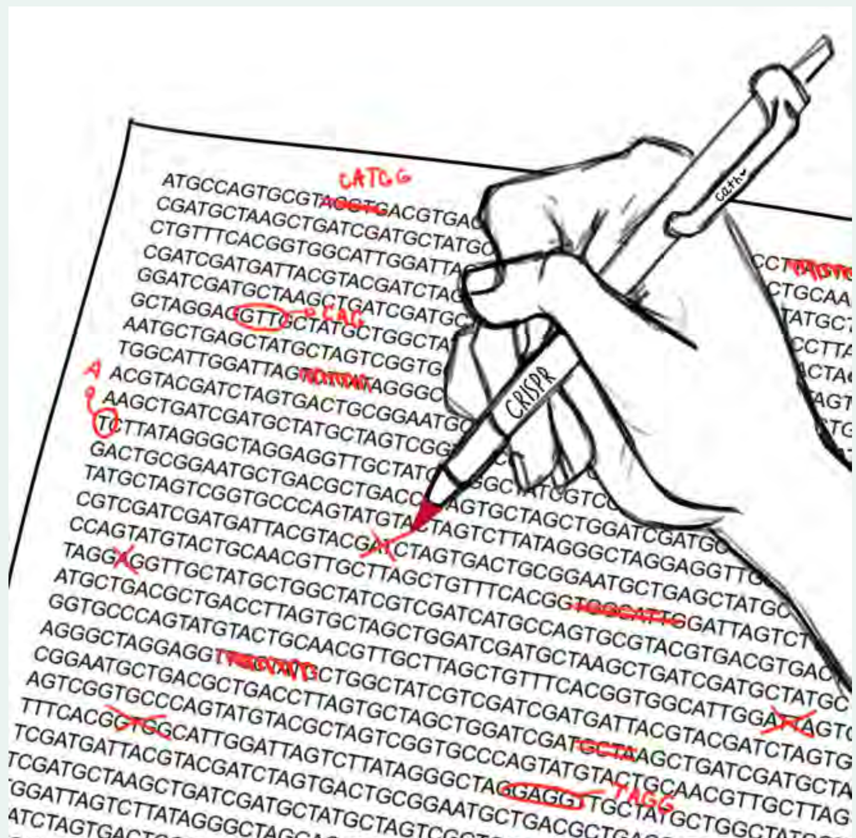
BRAZILIAN GOVERNMENT

Genome editors

- Techniques that allow precise manipulation of the gene
  - Based on site-directed nucleases (SDN) to break the DNA
  - DNA sequence can be deleted, inserted or modified.
  - Similar a text editor



Genome editors

- Like text editors, there are different genome editors
  - With different features and levels of complexity

ZFN

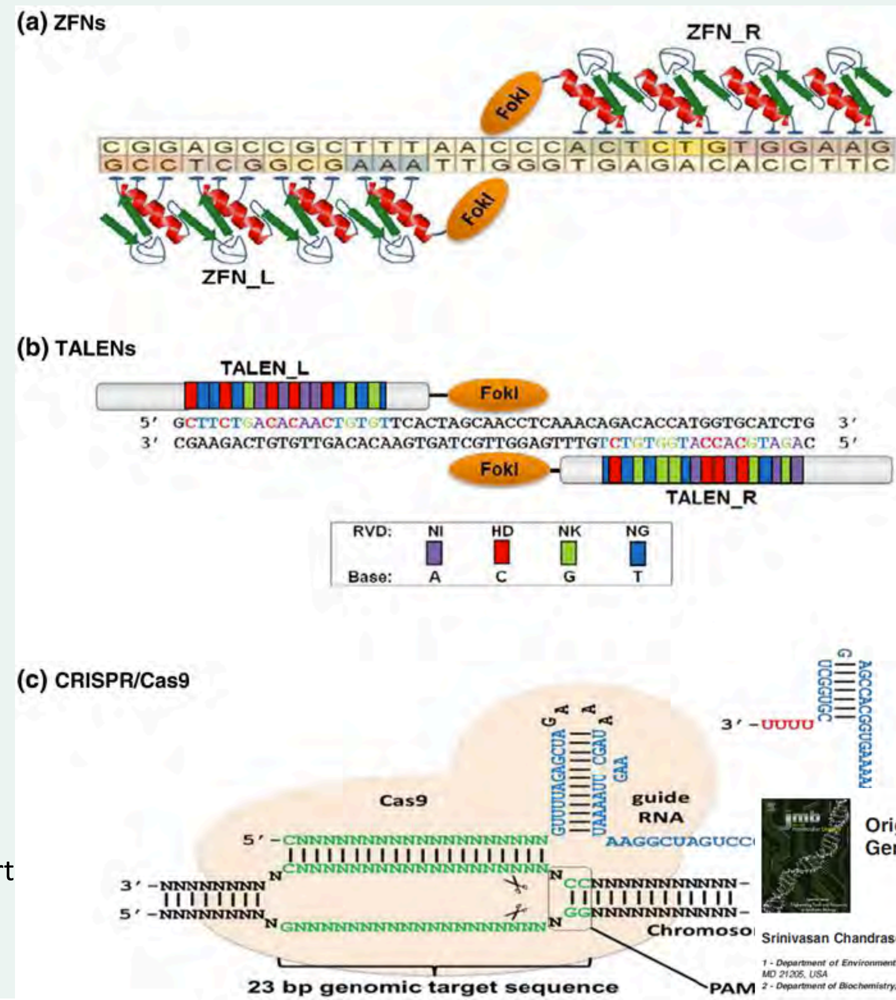
Zinc finger nucleases

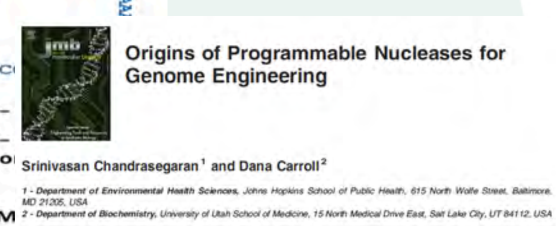
TALENs

Transcription activator-like effector nucleases

CRISPR/Cas9

Clustered regularly interspaced short palindromic repeats




Origins of Programmable Nucleases for Genome Engineering

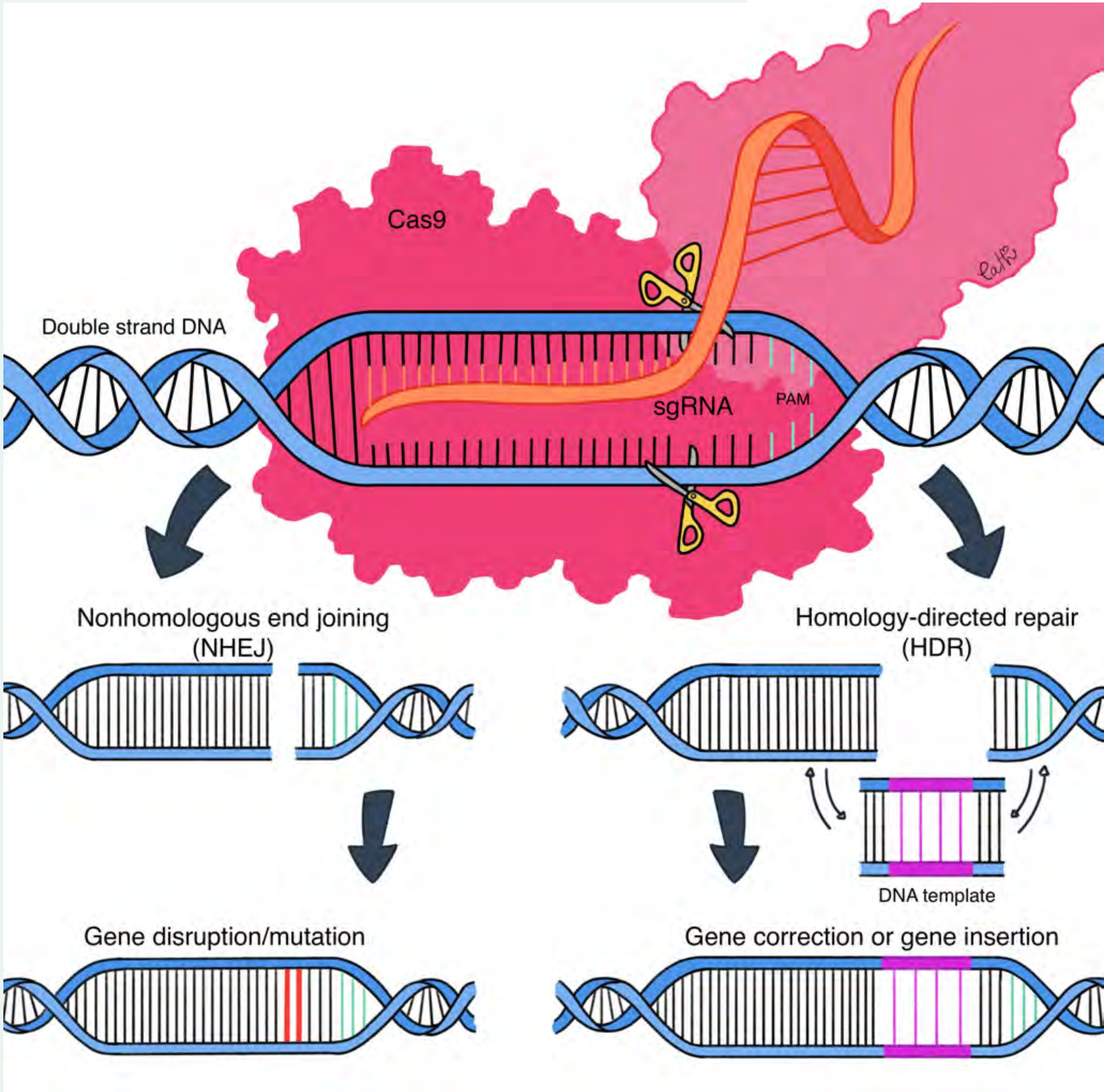
Srinivasan Chandrasegaran[1] and Dana Carroll[2]

1 - Department of Environmental Health Sciences, Johns Hopkins School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

2 - Department of Biochemistry, University of Utah School of Medicine, 15 North Medical Drive East, Salt Lake City, UT 84112, USA

How this work

- A RNA guide (sgRNA) identifies the target sequence and the nuclease (Cas9) breaks the DNA double strand in a specific site of the genome (or single-strand break with Cas9 nickases).
- Broken strands can be repaired by nonhomologous end joining (NHEJ) or by homologous recombination (homology-directed repair – HDR)
  - Gene disruption
  - Gene correction (single or several bases) or gene addition



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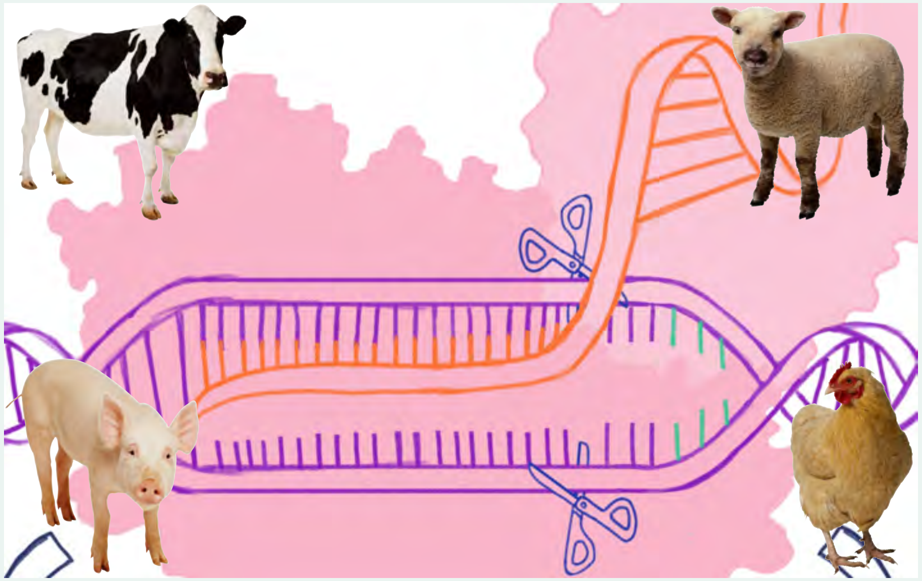
Genome editing in farm animals



Increase resistence to disease or parasites

Improve farm animal welfare

Improve livestock production

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Genome editing in farm animals

- Increase the frequency of favorable trait-associated alleles;
- Promote the introgression of favorable alleles from other breeds (or species);
- Generate new favorable alleles

Tait-Burkard *et al. Genome Biology* (2018) 19:204
https://doi.org/10.1186/s13059-018-1583-1

Genome Biology

REVIEW Open Access

Livestock 2.0 – genome editing for fitter, healthier, and more productive farmed animals

Christine Tait-Burkard, Andrea Doeschl-Wilson, Mike J. McGrew, Alan L. Archibald, Helen M. Sang, Ross D. Houston, C. Bruce Whitelaw and Mick Watson*

Image source: http://www.estadaomg.com.br/2015/12/raca-de-gado-belga-azul-bos-belgian-blue_23.html

Meat quality

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

- Myostatin – restrain muscle growth
  - Some cattle breeds have natural mutations that causes loss-of-function
  - muscle hypertrophy called double-muscle
    - Superior carcasses (less bone and low fat) – leaner and tendered meat
- Knockout of myostatin
  - TALENS
    - Cattle (Nelore zebu) and sheep
  - CRISPR/Cas9
    - Sheep

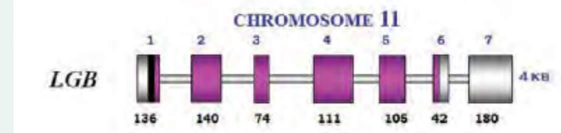
PLOS | ONE

RESEARCH ARTICLE

Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes

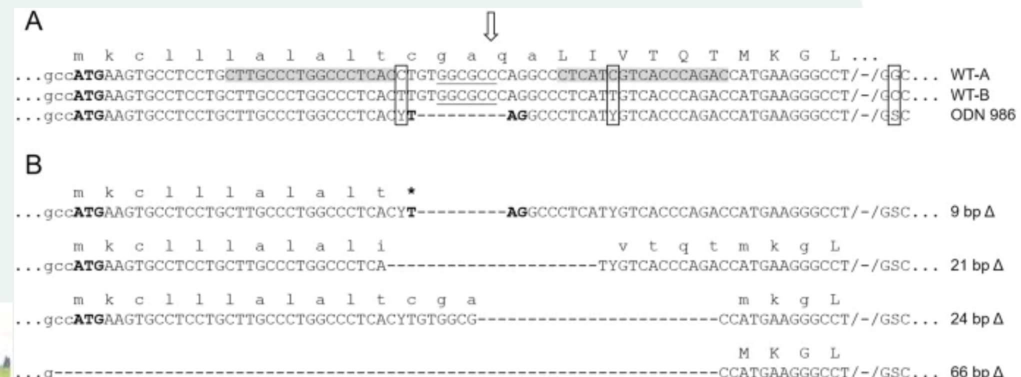
M. Crispo[1]*, A. P. Mulet[1], L. Tesson[3], N. Barrera[2], F. Cuadro[2], P. C. dos Santos-Neto[2], T. H. Nguyen[3], A. Crénéguy[3], L. Brusselle[3], I. Anegón[3]*, A. Menchaca[2]*

1 Unidad de Animales Transgénicos y de Experimentación (UATE), Institut Pasteur de Montevideo, Montevideo, Uruguay, 2 Instituto de Reproducción Animal Uruguay, Fundación IRAUy, Montevideo, Uruguay, 3 INSERM UMR 1064, Center for Research in Transplantation and Immunology-ITUN, Nantes,



Cow milk allergy

- Beta-lactoglobulin (BGL) is the major allergen in the cow milk
- Knock-out the BGL gene - TALENs
  - Achieved by introducing INDELs downstream start codon
  - 9 and 21 pb deletions
  - BGL-free milk



1601 1602


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]

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BGL knockout

- CRISPR to insert indels in BGL gene
  - Target: exon 2
  - Two alleles: Al1 (WT) and Al2 – monoallelic
    - mosaicism

sgRNA

Unpublished data

CRISP-ID

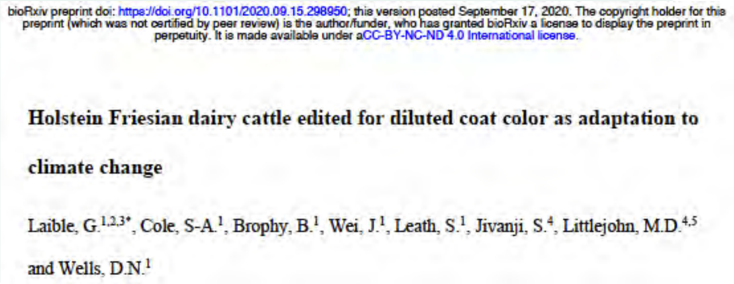
```
Wt-  GTCCAGCAGGGAGATGTCGCTGGCCGCCATGGCCAAGGAGTACCAAGTCCCCGCCACCTGG
Al1- GTCCAGCAGGGAGATGTCGCTGGCCGCCATGGCCAAGGAGTACCAAGTCCCCGCCACCTGG
Al2- GTCCAGCAGGGAGATGTCGCGGGTCTACATGAACAAATAATACCAAGTCCCCTCTTCCTGG
```

Reverse strands 5’-3'

INTA and Universidad de San Martin

Heat tolerance:
diluted coat color

- Holstein cattle: sensitive to high temperature - black and white
  - Black coat can absorb more ligth, retaining more heat - heat stress when under high temperature and humidity
  - 3 bp deletion into pre-melassomal protein 17 gene − using CRISPR
    - Dilution of black coat color (pattern of grey and white − no black areas) − may help to reduce heat stress



Holstein Friesian dairy cattle edited for diluted coat color as adaptation to climate change

Laible, G.[1,2,3*], Cole, S-A.[1], Brophy, B.[1], Wei, J.[1], Leath, S.[1], Jivanji, S.[4], Littlejohn, M.D.[4,5] and Wells, D.N.[1]

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Heat tolerance:
slick hair

- Animals with smooth coat and short hair:
  - can have a better management of body temperature
  - Mutation on prolactin receptor (exon 11) in criollo breeds in Central and South America – heat-tolerant cows but low performance
    - Introduction of mutation using CRISPR in Angus cattle (heat- sensitive)

Caracu cows in Brazil

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Calf image:Acceligen at https://www.acceligen.com/pipeline/

Enhancing thermotolerance in B. taurus cattle

- Bos indicus (zebu) cattle - very tolerant to high temperature and humidity
  - Gir: zebu dairy breed
  - Polygenic trait
  - Genes and/or mutations involved in this process are still unknown

Enhancing thermotolerance in B. taurus cattle

- Genome Wide Association studies with Girolando cattle (Gir x Holstein) – potential targets for thermoregulation

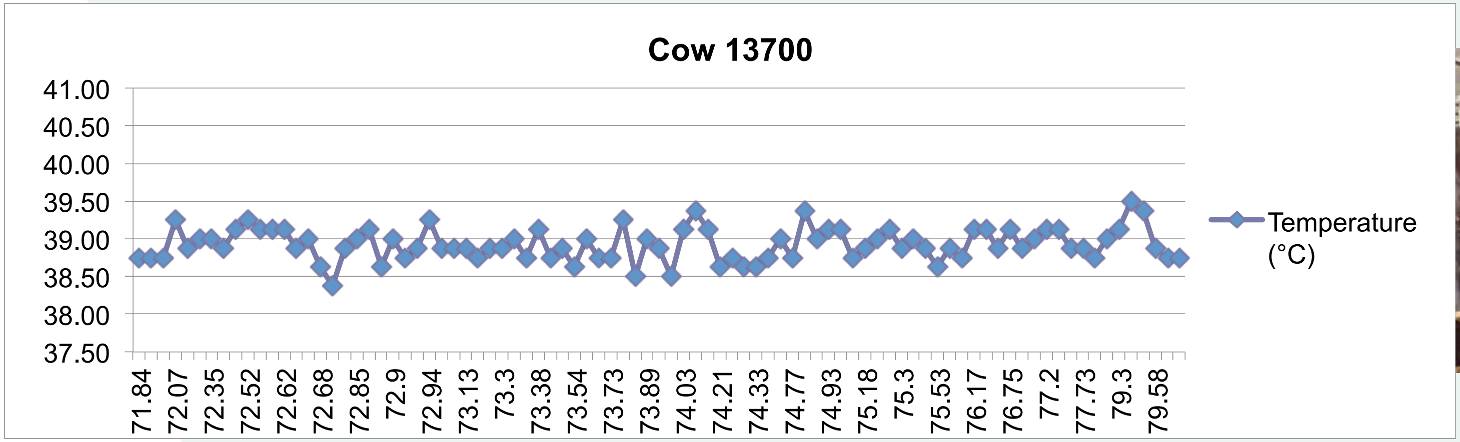
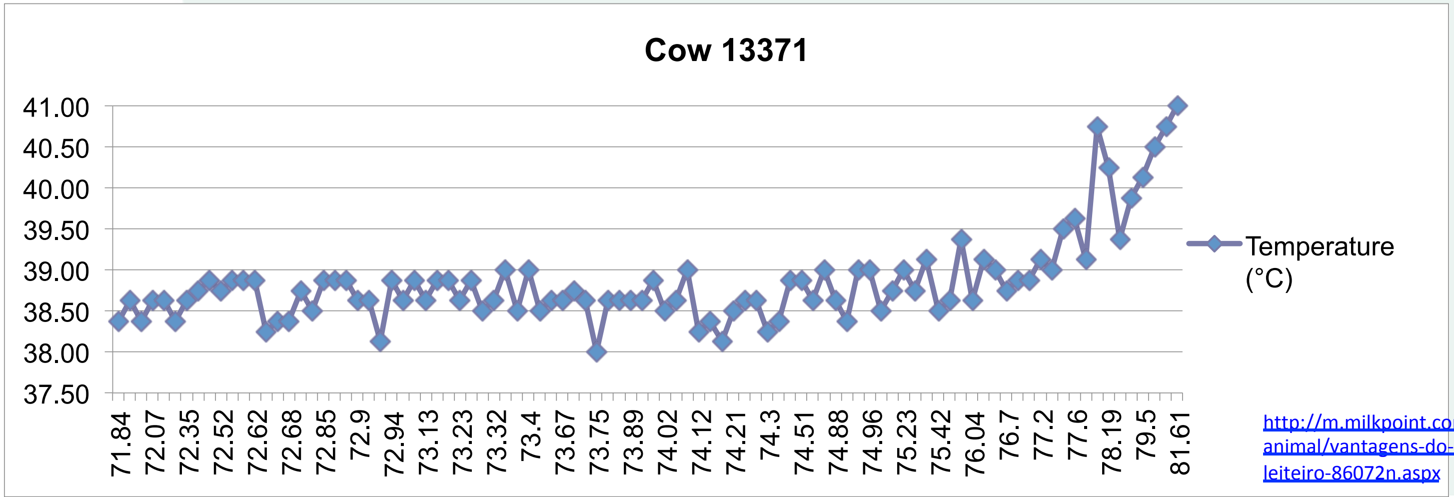


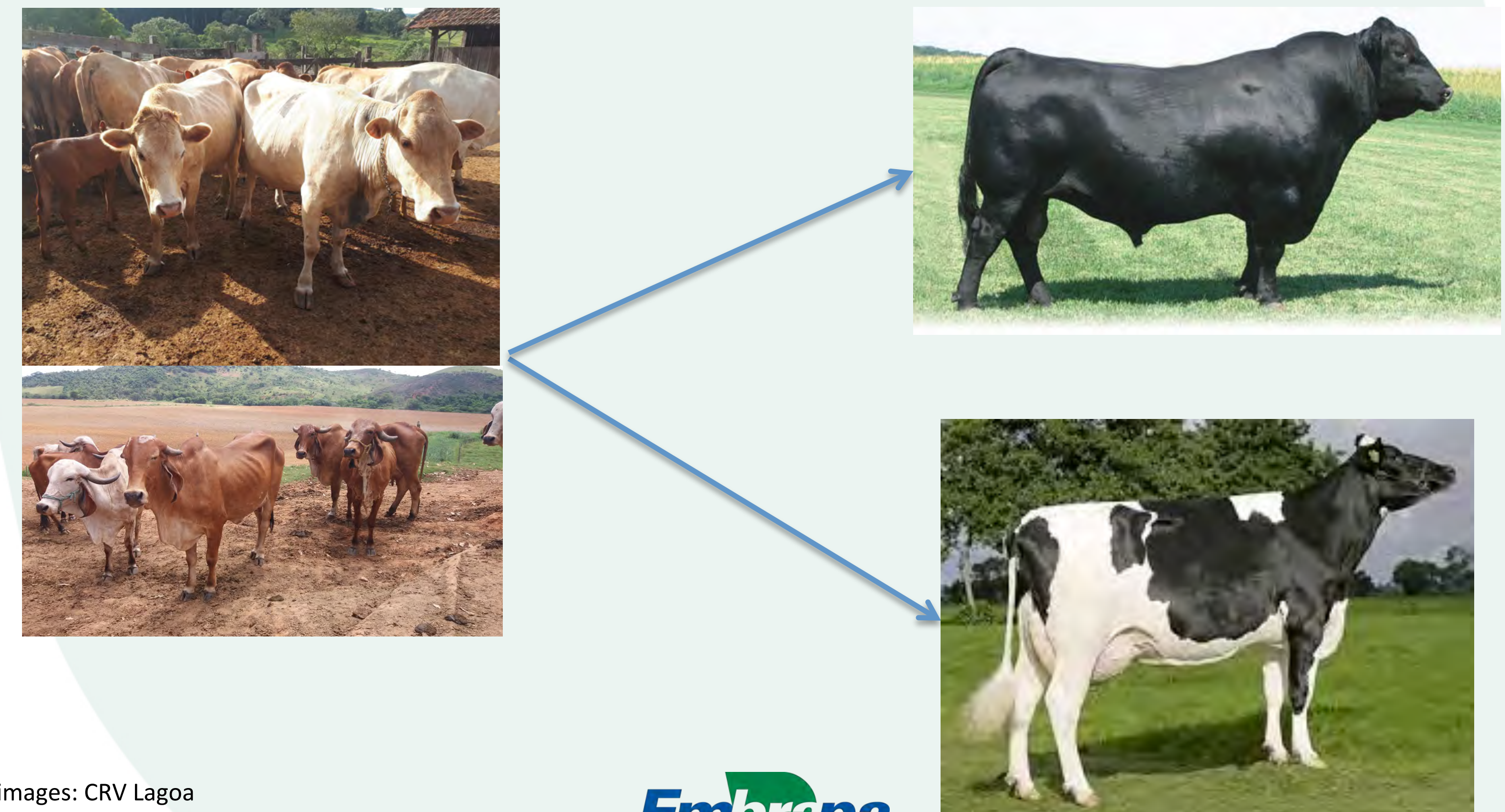
Image: Camargo, LS



http://m.milkpoint.com.br/radar-tecnico/bemestar-e-comportamento-animal/vantagens-do-manejo-do-estresse-calorico-em-gado-leiteiro-86072n.aspx

Enhancing thermotolerance in B taurus cattle

- New targets (from Zebu cows) to edit the genome of European cattle raised in the tropics



Holstein, Angus images: CRV Lagoa
Caracu and Girolando images: Camargo, LS

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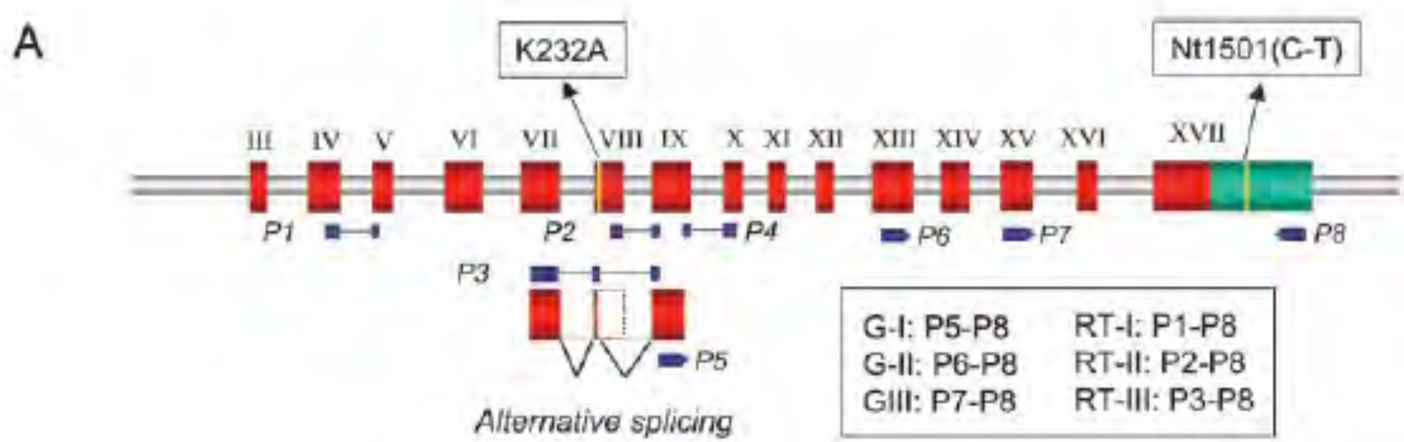
Enhancing milk production in zebu cows

- Zebu cattle − high tolerance to heat stress but low milk yield
- Alleles associated to milk yield in Holstein and Jersey cattle
  - Diacylglycerol o-acyltransferase 1 (DGAT1) gene - triacylglycerol synthesis
  - GC>AA polymorphism (K232A) results in missense mutation
    - Lysine > Alanine − allele A



and functional confirmation of the causality
DGAT1 K232A quantitative trait nucleotide in
g milk yield and composition

, Frédéric Farnir*†, Latifa Karim*, Nadine Cambisano*, Jong-Joo Kim*, Alex Kvasz*, Myriam Mni*,
ean-Marie Frère‡, Wouter Coppieters*, and Michel Georges*§

Genetic and functional confirmation of the causality of the *DGAT1 K232A* quantitative trait nucleotide in affecting milk yield and composition

Bernard Grisart*†, Frédéric Farnir*†, Latifa Karim*, Nadine Cambisano*, Jong-Joo Kim*, Alex Kvasz*, Myriam Mni*, Patricia Simon*, Jean-Marie Frère‡, Wouter Coppieters*, and Michel Georges*§

Enhancing milk production in zebu cows

Online journal ISSN 1676-5680
GMR Genetics and Molecular Research

***DGAT1 K232A* polymorphism in Brazilian cattle breeds**

G.A. Lacorte[1], M.A. Machado[2], M.L. Martinez[2], A.L. Campos[2], R.P. Maciel[2], R.S. Verneque[2], R.L. Teodoro[2], M.G.C.D. Peixoto[2], M.R.S. Carvalho[1] and C.G. Fonseca[1]

- High frequency in Holstein cattle
- Low frequency in zebu cattle

Table 2. *K232A DGAT1* genotypic and allelic frequencies (%) including observed and expected heterozygosity.

Breed	Genotypic frequencies (%)				Allelic frequencies (%)		Heterozygosity (%)	
	KK	AK	AA	EP[1]	K	A	Observed	Expected
Gyr	94.0	4.0	2.0	0.06	96.0	4.0	4.0	7.0
Guzerat	100.0	0.0	0.0	-	100.0	0.0	0.0	0.0
Nellore	100.0	0.0	0.0	-	100.0	0.0	0.0	0.0
Red Sindhi	95.0	5.0	0.0	1.00	97.5	2.5	5.0	5.0
Holstein	14.0	26.0	60.0	0.03*	27.0	73.0	26.0	39.0
Gyr x Holstein F1	30.0	62.0	8.0	0.04*	61.0	39.0	61.0	48.0

[1]Exact probability for Hardy-Weinberg equilibrium testing (Haldane, 1954).
*Significant for EP (<0.05).

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Enhancing milk production in zebu cows

- Introgression of alleles associated to milk production
  - Increase the frequency of allele A in Gir cattle
  - contribute to improve milk yield of Zebu cows (Gir)

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Holstein, Jersey images: CRV Lagoa
Gir image: Camargo, LS

Challenges

- Improve the efficiency of gene editing
  - Increase the efficiency zygote transfection: procedures to replace cytoplasm injection
  - Increase the rate of INDELS and HDR (precise editing) in both alleles;
  - Reduce mosaicism
- Regulation
  - Different views of how to regulate genome editing in farm animals

NATURE METHODS | CORRESPONDENCE

Unexpected mutations after CRISPR–Cas9 editing *in vivo*

Kellie A Schaefer, Wen-Hsuan Wu, Diana F Colgan, Stephen H Tsang, Alexander G Bassuk & Vinit B Mahajan

Nature Methods 14, 547–548 (2017) doi:10.1038/nmeth.4293
Published online 30 May 2017 Updated online 14 June 2017

To the Editor:

REVIEW ARTICLE

WILEY Reproduction in Domestic Animals

Basics of genome editing technology and its application in livestock species

Bjoern Petersen

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Takeaway message

- Genome editing in ruminants
  - Can help to accelerate genetic improvement
    - Benefit farmers and consumers in different parts of the world
  - Animal welfare is a concern
  - Take longer than plants to succeed
  - Partnership

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Acknowledgement

CNPq

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Thank you
Mercy beaucoup
Muito obrigado

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