Use of regulatory process for commercialization/determination of GnEd animals (Argentina and Brazil experience)

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Argentinian experience on GnEd animals

GMO definition (Cartagena Protocol)

Genetically Modified Organism:

Organism that has a ***combination of genetic material*** obtained through the application of ***modern biotechnology".***

Modern biotechnology:

- *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles or:

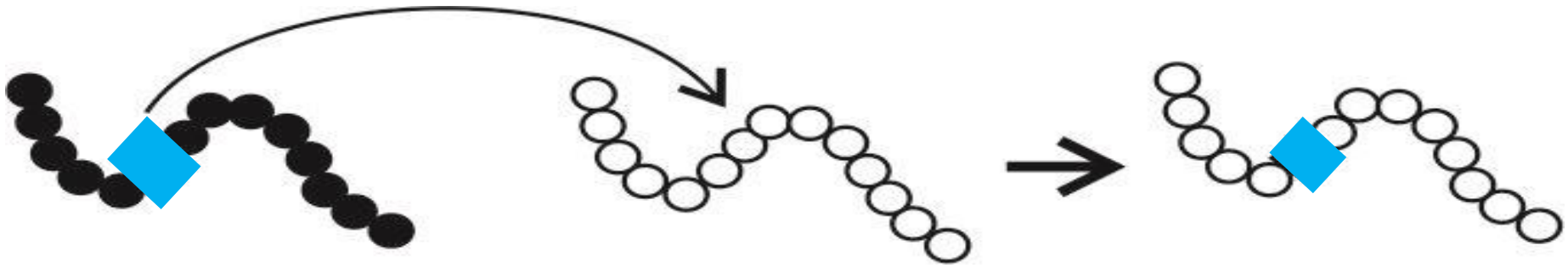
- Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.

“GMO”

Modern biotechnology application

New combination of genetic material

Transgenesis



DNA 1

1 Donor organism **Desired feature**

DNA 2

2 Receiving organism

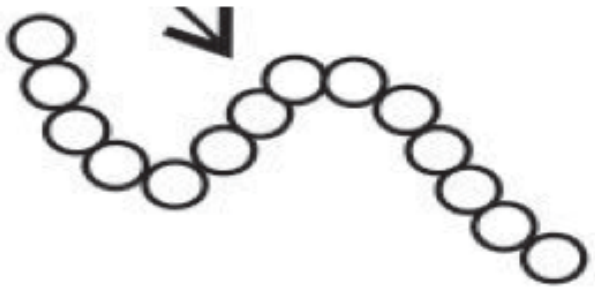
DNA 2 + gene DNA1

3 **GMO product**

“**Genome** editing”

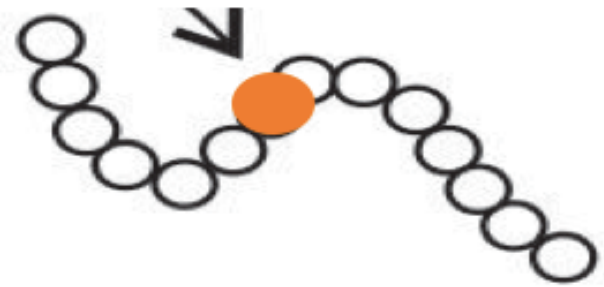
Modern biotechnology application

There is **NO new** combination of genetic material



1	Organism ***receiver***

Gene editing techniques



2	Gene editing ***product***

Cases presented in Argentina

ORGANISM	PHENOTYPE	DEVELOPMENT STAGE	TECHNIQUE
Cow	Hypoallergenic Milk	Design stage*	knockout of an endogenous gene
Tilapia	**Performance increase**	**Real**	**knockout of an endogenous gene**
Cattle	Performance increase	Design stage*	knockout of an endogenous gene
Cattle	Thermal tolerance	Design stage*	knockout of an endogenous gene
Cattle	Thermal tolerance + Hornless	Design stage*	knockout of an endogenous gene

I. “Tilapia” FLT -01 – Gene Editing” – Introduction

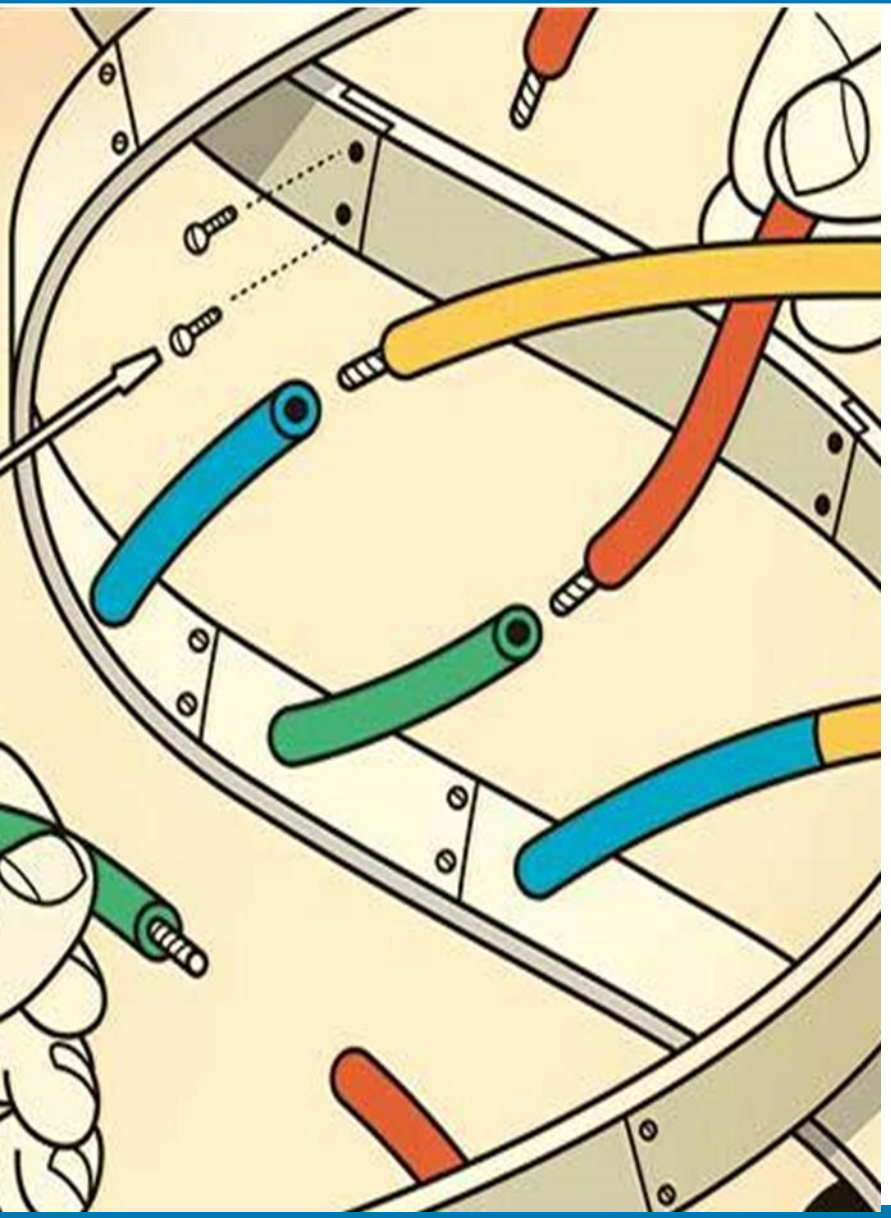
- The tilapia of the Nile, species *Oreochromis níloticus*, is a fish of the Family of the Cíclidos.
- The world production of tilapia approaches 6 million tons per year.
- The production of tílapia is expected to be one of the fastest growing segments of aquaculture production and will be expanded to 8.7 million tons per year by 2025.

I. “Tilapia” FLT -01 – Gene Editing” – Introduction

- In the United States a high selling value of fresh tilapia fillet has been established, supplied by producers from Latin America and South America who have been pioneers in the market

- Tilapia is typically harvested for fresh fillets of 1.1 kg in a production cycle of around 13 months.

II. Purpose of the modification



- **Gene Editing**

- The Tilapia line FLT -01 "Extra fillet" has an increase in fillet yield

- Obtained by knockout of an endogenous gene that causes the loss of the function of a negative regulator of muscle growth.

- The edited tilapia exhibits an increase in muscle mass, manifesting a greater weight and yield of the fillet compared to its unedited counterpart.

III. Methodology and technique

- Microinjection w/nuclease mRNA (no DNA involved)
- Small deletion created an early stop codon.
- Homozygous at final product
- Cero off-target sites by design.

“Tilapia” FLT -01 – Final product”

- Tilapia FLT - 01 Phenotype "Extra fillet”
- Deletion of 26 bp in the interest gene

“Tilapia” FLT -01 – Gene Editing” - Conclussions

Resolution 173/15- New breeding techniques (NBTs)

The Product does not contain a **new combination of genetic material** in the genome generated by the application of **modern biotechnology**.

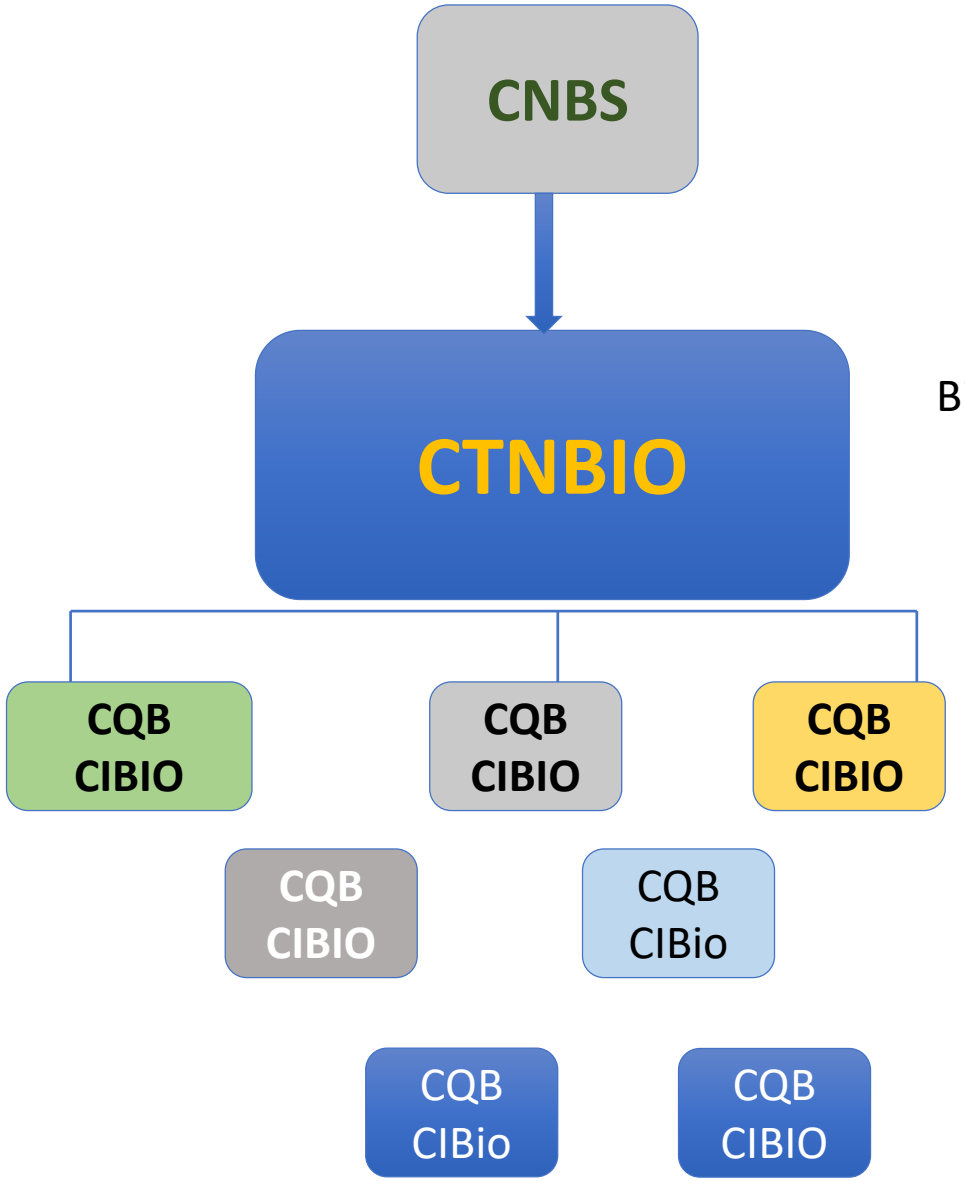
(Res. 763, Cartagena Protocol)

Tilapia” FLT -01

1. Deletion of 26 bp in the targuet gene and no new genetic material combination.

2. No involuntary integration of plasmid DNA in the final product.

Brazilian experience on GnEd animals



Biosafety Law 11105, March 24, 2005

Definition of GMO by the brazilian biosafety law 11.105

- Article 3. Under this Law, it shall be considered:
- I – an organism: each and every biological entity that is capable of reproducing or transferring genetic material, including virus and other classes that may be made known;
- II – deoxyribonucleic acid - DNA, ribonucleic acid - RNA: genetic material which contains determining information about transmissible hereditary characters to progeny;
- **III – recombinant DNA/RNA molecules: molecules manipulated outside live cells through changes made to natural or synthetic DNA/RNA segments that can multiply in a live cell, or yet, DNA/RNA molecules resulting from this multiplication; DNA/RNA synthetic segments equivalent to natural DNA/RNA are also considered;**
- **IV – genetic engineering: the activity of manipulating DNA/RNA recombinant molecules;**
- **V – genetically modified organism - GMOs: an organism the genetic material of which – DNA/RNA has been modified by any genetic engineering technique**;
- VI –GMO by-product: a product obtained from a GMO and that is not capable of autonomously replicating, or that does not contain a feasible GMO form;
- VII – human germinal cell: the mother cell responsible for forming gametes which are found in the female and male sexual glands and their direct progeny in any ploid degree;
- VIII – cloning: an asexual reproduction process, artificially produced, based on a sole genetic patrimony, by using or not genetic engineering techniques;
- IX – cloning for reproductive means: cloning the end purpose of which is to make an individual;
- X – therapeutic cloning: cloning the end purpose of which is to produce embryonic stem cells for therapeutic purposes;
- XI – embryonic stem cells: embryonic cells that are capable of modifying the cells of any organism tissue.

2013
Jeniffer Doudna and Emanuelle Charpentier

The CTNBio and the Precision Breeding Innovation Techniques (“TIMPS”) - the RN16

NATIONAL BIOSAFETY TECHNICAL COMMISSION

NORMATIVE RESOLUTION No. 16, OF JANUARY 15, 2018

Sets forth the technical requirements for submitting an inquiry to the CTNBio concerning Precision Breeding Innovation Techniques (PBI).

THE NATIONAL BIOSAFETY TECHNICAL COMMISSION (CTNBio), using its legal and regulatory powers and in observance of sections XV and XVI of article 14 of Law No. 11.105 of March 24, 2005;

Whereas there is a need to assess Precision Breeding Innovation (PBI) techniques, which also comprise the so-called New Breeding Technologies (NBTs) in the light of Law No. 11.105 of March 24, 2005;

Plant Breeding Innovation

CTNBio Normative Resolution No 16 of January 15, 2018

1

Petition submission to CTNBio

2

National Gazette Publication

CTNBio's member referendary

90 days, extendable for equal time

3

Referendary final opinion set to CTNBio's subcommittees

4

CTNBio's plenary deliberation

Conventional
Registration as usual

GMO
dossier for GMO risk assessment

ORGANISMS CONSIDERED AS NON-GMOs BY RN 16

- Globalyeast JV CO Brasil S.A.
- **YEASTS FOR ETHANOL**

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- **YEASTS FOR ETHANOL**

- Du Pont do Brasil S.A.
- **WAXY CORN**
- (higher amounts of amylopectin)

- Lallemand Brasil Ltda.
- **YEASTS FOR ETHANOL**

- Ourofino Saúde Animal Ltda.
- **CANINE PARVOVIRUS VACCINE**

- Agro Partners Consulting
- **POLLED CATTLE SEMEN**

- AquaBounty Technologies
- **TILAPIA**

Polled Cattle

- Petition submitted to CTNBio for commercialization of semen of polled bull (Buri) by Recombinetics (USA) and Agropartners (Brazil)
- CTNBio analyzed and approved as non-GMO in October 2018
- In 2019 – FDA sequenced the genome of the bull
- FDA found plasmid sequences and considered as GMO
- Recombinetics and Agropartners **cancelled** the process.

O touro transgênico Buri, em foto de maio de 2018

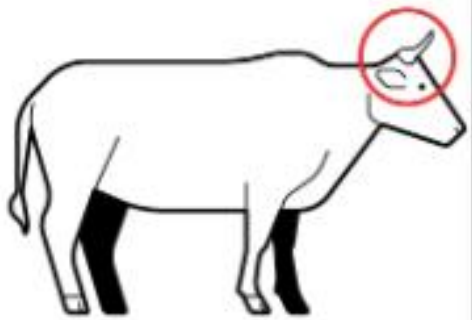
Alison Van Eenennaam/University of California-Davis

Technique by Recombinetics

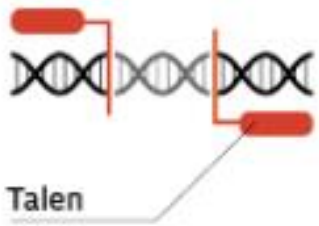
- Fibroblast homozygous for Celtic Allele (polymorphism)
- Deletions of DNA sequences using Talens (transcription activator-like effector nucleases)
- introgression into cells (fibroblasts)
- Insertion of fibroblast nuclei into bovine enucleated oocyte
- Insertion into pseudopregnant female
- POLLED CATTLE

Entenda como o Buri foi feito e por que a edição gênica deu errado

① A Recombinetics retirou células da orelha de um touro mestiço de três raças leiteiras cujos animais normalmente apresentam chifres

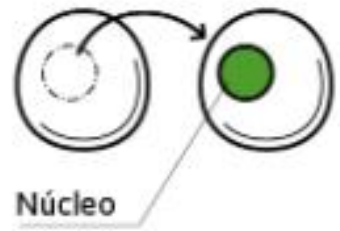


② Em laboratório, essas células sofreram edição gênica por meio da ferramenta Talen. A empresa utilizou enzimas como "tesouras moleculares" para cortar o DNA no local onde ficam os genes responsáveis pelos chifres

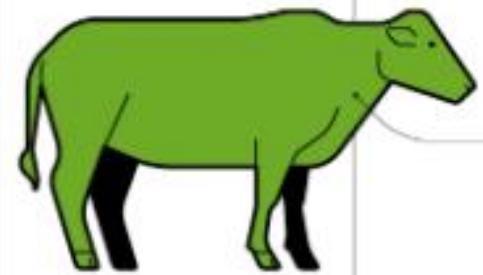


③ No lugar deles, inseriu o alelo céltico, encontrado na raça angus, que confere a ausência de chifre. Um plasmídeo serviu como vetor do material genético

④ Os cientistas selecionaram a célula que foi transformada e fizeram um clone, a partir de transferência de núcleo. Um embrião foi criado com esse material editado e implantado no útero de uma vaca



⑤ Buri e Spotigy nasceram e cresceram sem chifres. No entanto, o material genético do plasmídeo, que deveria ter desaparecido, permaneceu no genoma dos animais



⑥ A Recombinetics e a UC Davis checaram e viram que não havia alterações fora do sítio da edição no DNA. Mas, no local da edição, houve uma integração com o plasmídeo, o que foi detectado pela FDA em 2019

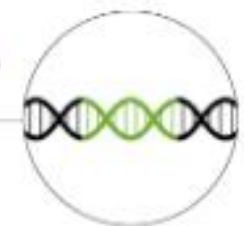


Ilustração Alexandre Affonso

Polled cattle

Use of Gene Editing to Introduce the Polled Trait into Elite Germplasm

by:- Alison Van Eenennaam And Maci Mueller, University Of California-Davis

Genetic Editing Eliminates Dairy Cattle Horns

by Ross Tellam in SPLASH!® milk science update: May 2019 Issue

- Horns on dairy cattle can injure their handlers and other cattle.
- Physical dehorning of cattle is widely practiced, but producers, animal rights activists, and the public want a more acceptable and long-term alternative.
- Genetic editing technology can permanently eliminate horns from dairy cattle while potentially maintaining their hard-won elite dairy production genetics.

Next time you are running with the bulls in Pamplona you may have a moment of vivid, but very brief, clarity and think "*If only the bulls were Polled*." In a significant breakthrough, scientists used genetic editing technology to produce hornless dairy cattle (Polled cattle) thereby potentially eliminating a controversial animal welfare issue, the physical dehorning of dairy cattle, while likely retaining their elite dairy production genetics [1-3].

FDA finds a surprise in gene-edited cattle: antibiotic-resistant, non-bovine DNA

by Sam Bloch
08.15.2019, 4:09pm

Tech

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Genome-Edited Hornless Cattle Found to Have Unintended Antibiotic Resistance Genes

THIRD WORLD NETWORK BIOSAFETY INFORMATION SERVICE

Dear Friends and Colleagues

Genome-Edited Hornless Cattle Found to Have Unintended Antibiotic Resistance Genes

Genome-edited hornless cattle were produced in 2016 by Recombinetics, Inc. of Minnesota. The company reported that "our animals are free of off-target events". But in a paper published online on 28 July 2019, US Food and Drug Administration (FDA) researchers re-examined the DNA of the genetically dehorned calves and found that the two calves' genomes did contain unintended DNA alterations.

One calf was found by FDA to have an unintended duplication of the polled gene locus; while the DNA of both calves contained two antibiotic resistance genes, along with various other gene sequences of bacterial origin. The inadvertently introduced bacterial sequences were found close to the editing site. Of the two antibiotic resistance genes found by FDA, one confers Neomycin/Kanamycin resistance and the other Ampicillin resistance. The unexpected DNA sequences detected by the FDA researchers originate from the plasmid (a DNA carrier) used by Recombinetics to introduce the polled DNA sequence.

The presence of the previously undetected antibiotic resistance genes in genome-edited cattle raises issues of biosafety given that there is a strong global push to limit the spread of genes conferring antibiotic resistance. No research has been carried out on the possible consequences for animal health, or whether these additional genes are biologically active.

The finding that genome-editing techniques can, unbeknownst to the developer, introduce foreign DNA is a significant blow to the no-regulation argument. As the genome-edited cattle *do* contain DNA unnatural to cattle, despite the claims of their developers to the contrary, this makes them subject to FDA regulation. This finding is also a powerful vindication of the EU's stand to regulate genome-edited organisms as GMOs.

https://biosafety-info.net/articles/agriculture-organisms/animalsfish/genome-edited-hornless-cattle-found-to-have-unintended-antibiotic-resistance-genes/

Thank you!
Questions?

O touro transgênico Buri, em foto de maio de 2018

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