GENOME EDITING REGULATION AND DECISIONS IN BRAZIL

comissão técnica nacional

CTNBio

de biossegurança

Luiz Sergio A Camargo

- Researcher at Brazilian Agricultural Research Corporation – Embrapa

- CTNBio's member

E-mail: luiz.camargo@embrapa.br

National Biosafety Technical Commission

CTNBio

CTNBio

- A multidisciplinary collegiate body, created by the Law No. 11.105, of March 24th, 2005, whose purposes are:

  - Provides **technical advisory support** and advice to the Federal Government in the formulation, updating and implementation of the National Biosafety Policy on GMO.

  - Establishes technical safety standards and technical reports **relating to the protection of human health, living organisms and the environment,** for activities involving the construction, experimentation, cultivation, handling, transporting, marketing, consumption, storage, release and disposal GMOs and derivatives.

http://ctnbio.mctic.gov.br/en/inicio

Biosafety's organogram (Law No. 11.105, of March 24th, 2005)

Embrapa

REGISTRATION AND INSPECTION

National interest and socioeconomic factors (not an obligation)

CNBS

CTNBio

CNBS = National Biosafety Council
CTNBIO = National Biosafety Technical Commission
CIBIO = Internal (local) Biosafety Committees
Registration and Inspection Bodies

MAPA	Agriculture/Livestock
ANVISA	Human Health
IBAMA	Environment

CIBio (within institutions and companies):
- First level projects approval;
- Monitoring of activities and accidents;
- Biosafety training and inspection
- Annual report to CTNBio

CIBIO CIBIO CIBIO CIBIO
CIBIO CIBIO CIBIO CIBIO CIBIO
CIBIO CIBIO CIBIO CIBIO CIBIO CIBIO

Adapted from Maria Lucia Dagli

CTNBio's composition

27 Professionals with Ph.D. Degrees
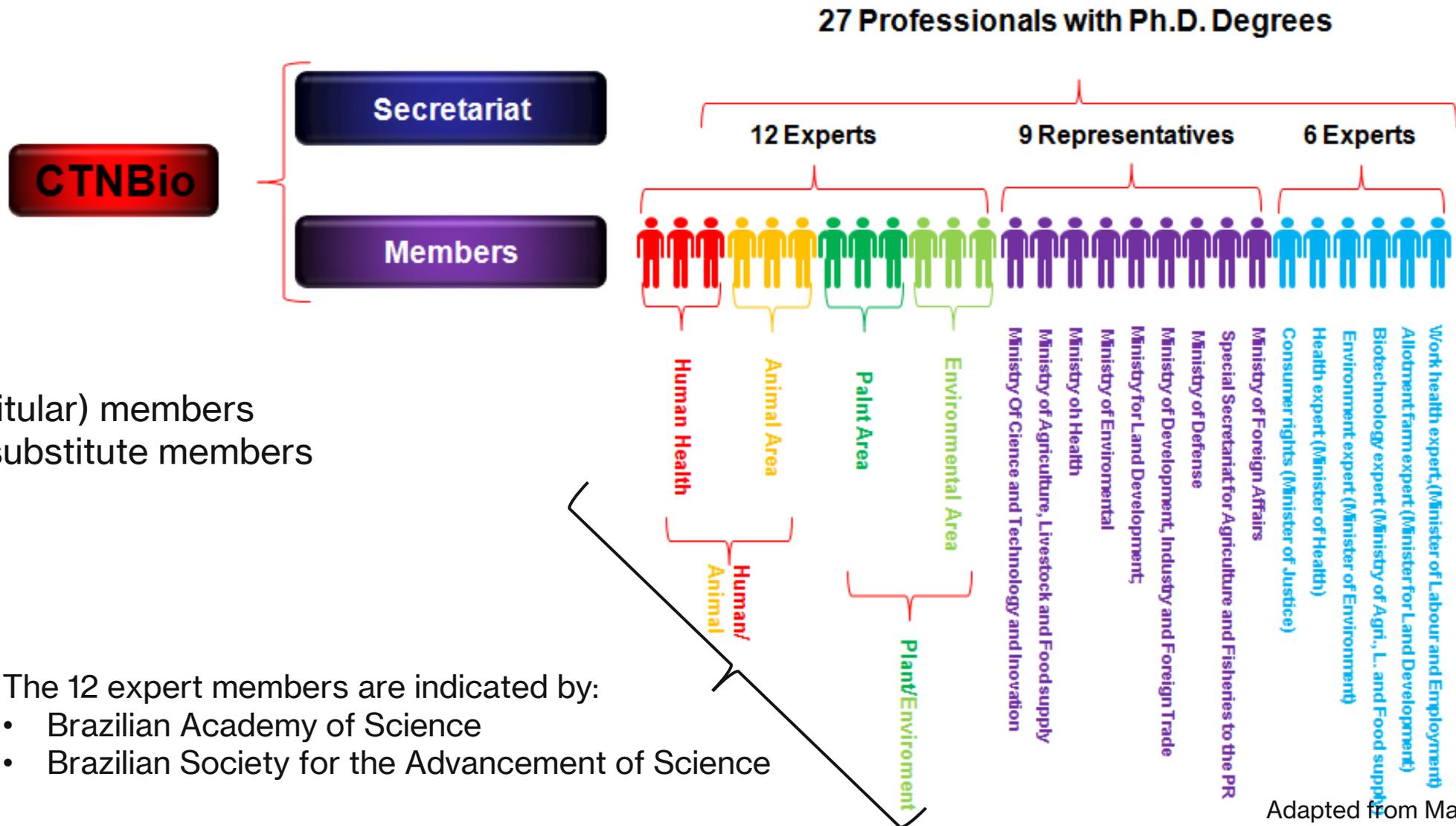
- 27 full (titular) members
  - 27 substitute members

The 12 expert members are indicated by:
- Brazilian Academy of Science
- Brazilian Society for the Advancement of Science

GMO approvals

GMOs approved from 1998 to 2025

Four GMO animals approved: three insects and one fish
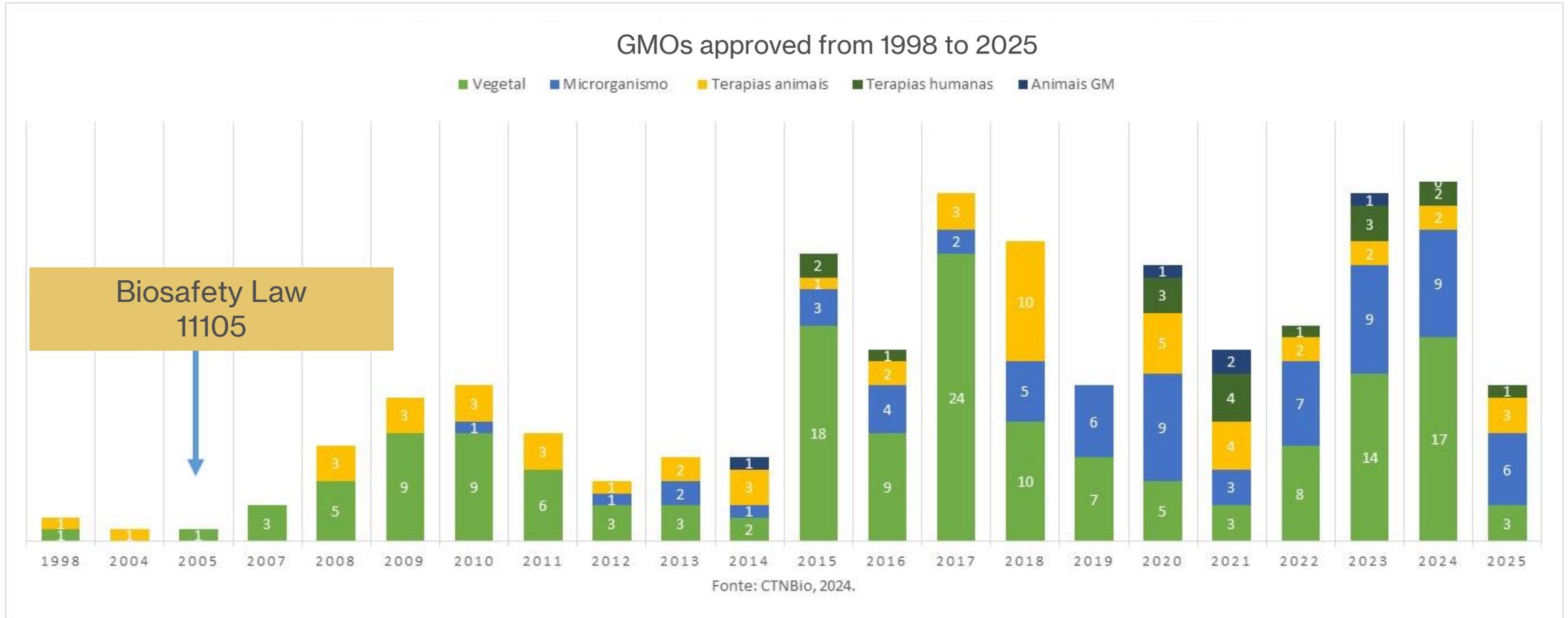
Adapted from Rubens Jose do Nascimento

Gene Editing - normative resolution (RN) #16

Establishes the technical requirements for submitting a consultation to CTNBio on New Breeding Technologies (NBT)

- the principle is to determine, through a case-by-case consultation system, whether a product generated by NBTs should or not be classified as GMO by CTNBio

Table 2 Criteria for classifying biotechnological products as GMO and non-GMO by CTNBio, according to RN16.

Criterion	Classification
(I) Absence of recombinant DNA/RNA	non-GMO
(II) Presence of genetic elements that could be obtained by crossing	non-GMO
(III) Presence of induced mutations that could also be obtained by standard techniques	non-GMO
(IV) Presence of mutations that could occur naturally	non-GMO
(V) Presence of mutations by SDN-1 *	non-GMO
(VI) Presence of mutations by SDN-2 *	non-GMO
(VII) Presence of mutations by SDN-3 *	GMO

* According to case-by-case assessments.

APTER 5
egulatory fram
f genome editi
razil and worldwide

Hugo Bruno Correa Molinari
Edivaldo Domingues Velini
Eduardo Romano de Campos Pinto
Maria Lucia Zaidan Dagli
Galdino Andrade
Patricia Machado Bueno Fernandes
Liliana Mercia Mertz-Henning

genes

MDPI

Review

Updates on the Regulatory Framework of Edited Organisms in Brazil: A Molecular Revolution in Brazilian Agribusiness

Nicolau B. da Cunha [1,2,3], Jaim L. da Silva Junior [14], Amanda M. M. Araújo [2], Ludmila R. de Souza [2], Michel L. Leite [3,5], Gabriel da S. Medina [1,4], Gustavo R. Rodriguez [6,7], Renan M. dos Anjos [3,8], Júlio C. M. Rodrigues [8], Fabrício F. Costa [9,10], Simoni C. Dias [3], Elíbio L. Rech [8] and Giovanni R. Vianna [8]

https://www.alice.cnptia.embrapa.br/bitstream/doc/1132164/1/Regulatory-framework-of-genome-CAP-5.pdf

SDN-1 or SDN-2

Products obtained by site-directed nuclease (SDN) 1 or 2 could be designated as non-GMO, in a case-by-case analysis

- SDN-1 = random mutation directed to the site that involves the junction of non-homologous ends

- SDN-2 = homologous repair directed to the site that involves few nucleotides and that meet the conditions established in RN16
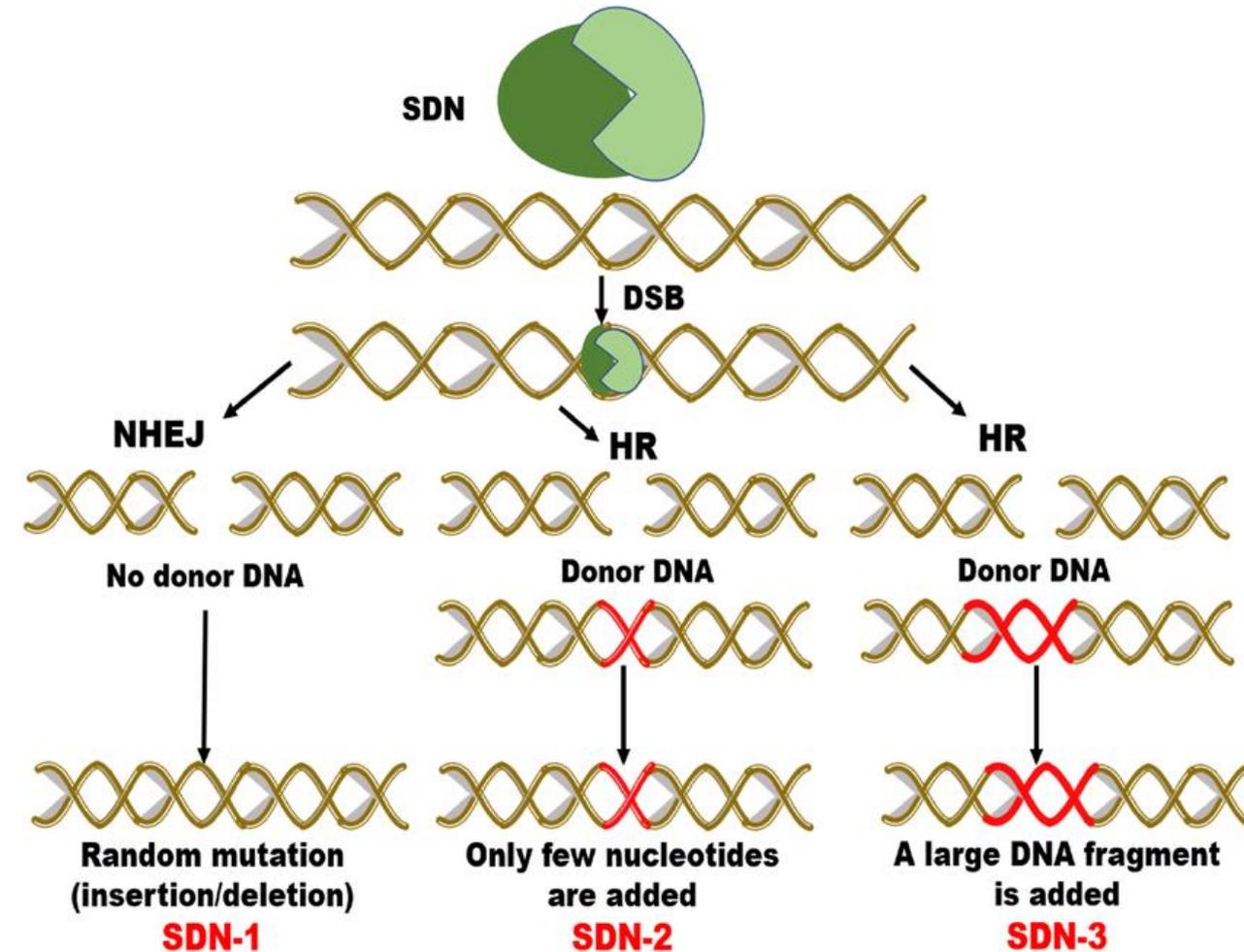
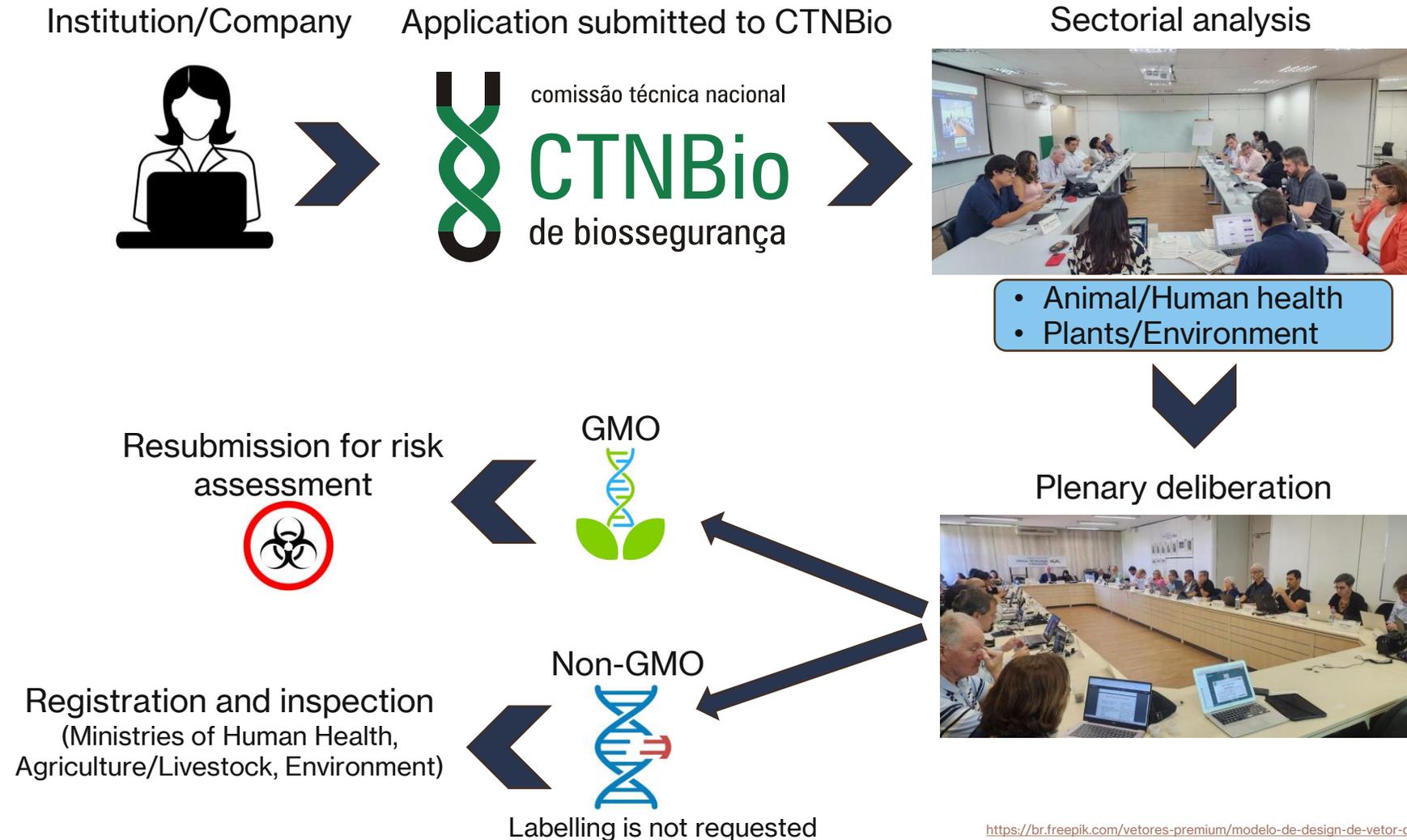# Gene editing - regarding the original organism

Provide:

1. Identification of the genetic technology, purpose, and intended use of the resulting organism and its derivatives;

2. Taxonomic classification, including, where appropriate, subspecies, cultivar, pathovar, strain, and serotype;

3. Risk classification;

4. The gene(s) and/or genetic element(s) manipulated, the source organism(s), and their specific functions;

5. The genetic strategy(ies) used to produce the desired modification(s); the genetic map(s) of the construct(s) used in the process, indicating all genetic elements present;

6. Molecular characterization of the genetic modification in the recipient organism: (1) number of manipulated copies (alleles); (2) location in the genome of the manipulated region; (3) identification of the presence of unintentional genetic modifications (off-target), when applicable.

7. The product resulted from the expression of the manipulated genomic region(s), described in detail, when applicable.

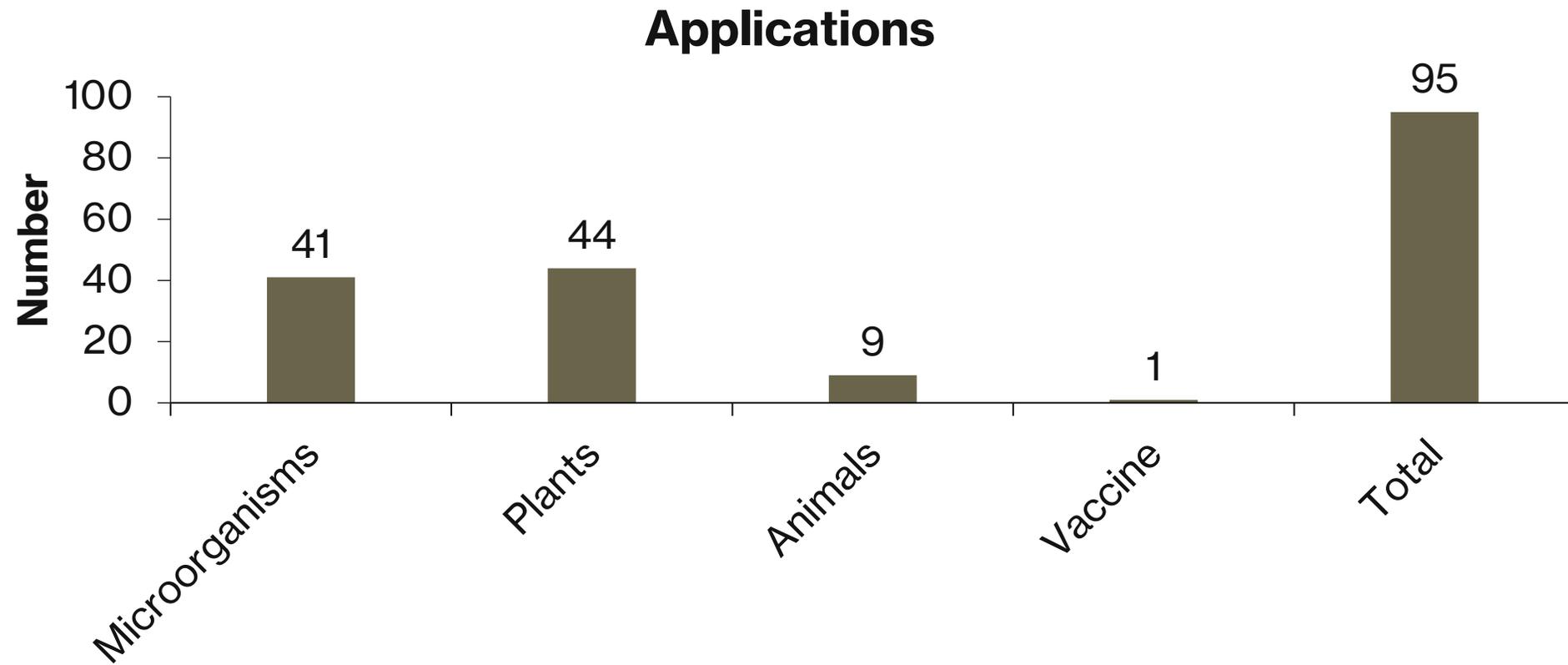# Gene editing - regarding the product (offspring, lineage)

Provide:

1. Proof of the absence of recombinant DNA/RNA molecules, using molecular methods.

2. Whether the product containing DNA/RNA molecules for topical/systemic use has the recombinational capacity to enter the target species and/or non-target species.

4. Whether the product subject to the request is commercially approved in other countries.

5. If the product utilizes the principle of gene drive, which may allow the phenotypic alteration to potentially spread throughout the population of the recipient organism, explain the precautions for monitoring the organism, using at least two different strategies.

6. How the potential off-targets (that may be present in the product) was assessed.

Workflow – Gene editing (RN #16)

Institution/Company

Application submitted to CTNBio

comissão técnica nacional
CTNBio
de biossegurança

Sectorial analysis

- Animal/Human health
- Plants/Environment

GMO

Resubmission for risk assessment

Plenary deliberation

Non-GMO

Registration and inspection
(Ministries of Human Health, Agriculture/Livestock, Environment)

Labelling is not requested

Photos from author's archives

Applications - normative resolution (RN #16)

Applications

Applications - normative resolution (RN #16)

Application - Animals
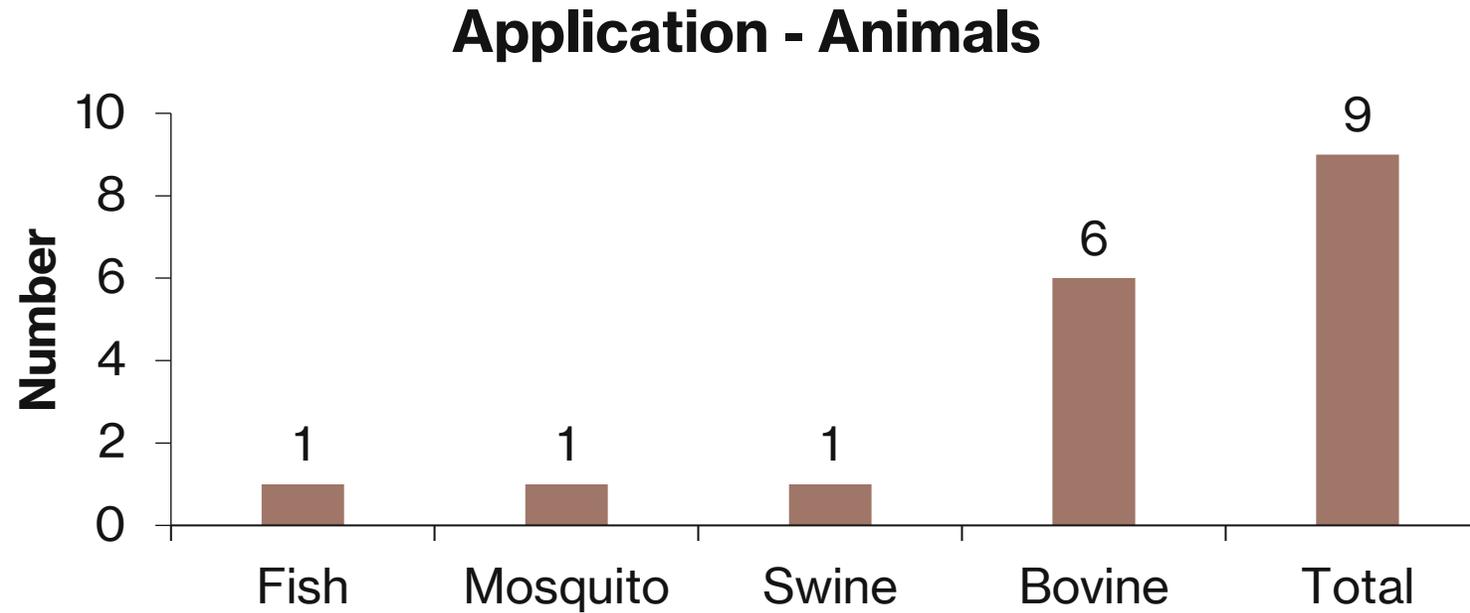
One bovine application was withdrawn

Animals

Bovine:
- Prolactin receptor: mutations to generate a truncated prolactin receptor that results in short and sleek hair (SLICK) - increased resilience to heat stress - (n = 4 applications: 8 Angus and 2 Holstein)
- Myostatin: mutations to knockout the myostatin gene - increased muscle mass (double-muscle) - (n = 1)

Fish:
- Myostatin: mutations to knockout the myostatin gene - increased muscle mass - (n = 1, tilapia)

Swine:
- CD163: deletion of several nucleotides of a target in CD163 receptor gene to prevent virus penetration – increased resistance to Porcine Reproductive and Respiratory Syndrome (PRRS) - (n=1)

Mosquito:
- RNA interference to generate sterile mosquitos for population control - (n=1)

Considerations - RN #16

Large number of applications with microorganisms and plants
- Sacharomyces - ethanol production
- Soybean, corn, sugarcane, cotton, sorghum, tomato - disease/parasite control and production

Registration and regular inspection are requested by Ministries before entering in the market (as any conventional product)
- Ministry of Agriculture, Livestock and Food Supply (Secretariat of Animal and Plant Health);
- Ministry of Health (Brazilian Health Regulatory Agency - ANVISA)
- Ministry of Environment (Brazilian Institute for the Environment and Renewable Natural Resources – IBAMA)

Animals
- Just few cases (about 10%)
  - Products still not in the Brazilian market
- International companies – not made in Brazil or Latin America (in contrast to crops and microorganisms)

Gene-edited cattle born in Brazil

SLICK - Prolactin receptors:

- Electroporation of in vitro-fertilized Angus zygotes with RNPs + ssODN harbouring a stop codon

Animal # ID	Editing (%)	HDR (%)*	Frameshift score* (%)	HDR + frameshift score (%)	Phenotype (hair length)
5400	48.3	21.5	25.2	47.1	Wild type
5401	74.8	16.2	48.1	65.3	Slick
5402	81.7	59.8	21.4	81.7	Slick
5403	0	0	0	0	Wild type
5404	0	0	0	0	Wild type

Blood sample

F0 population

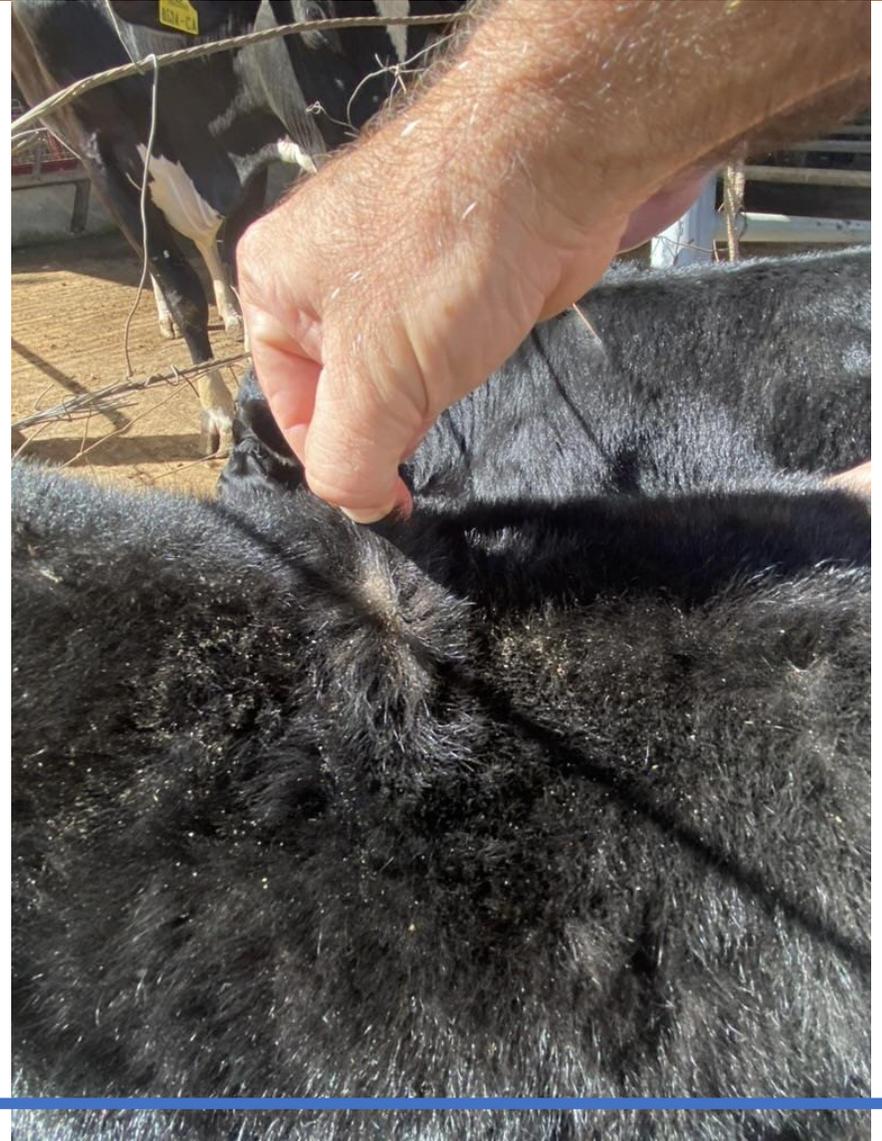In collaboration with

Associação Brasileira de Angus

*Only insertions of the stop codon or indels in a proportion over 5% were considered for calculation of knock-in rate and frameshift score, respectively
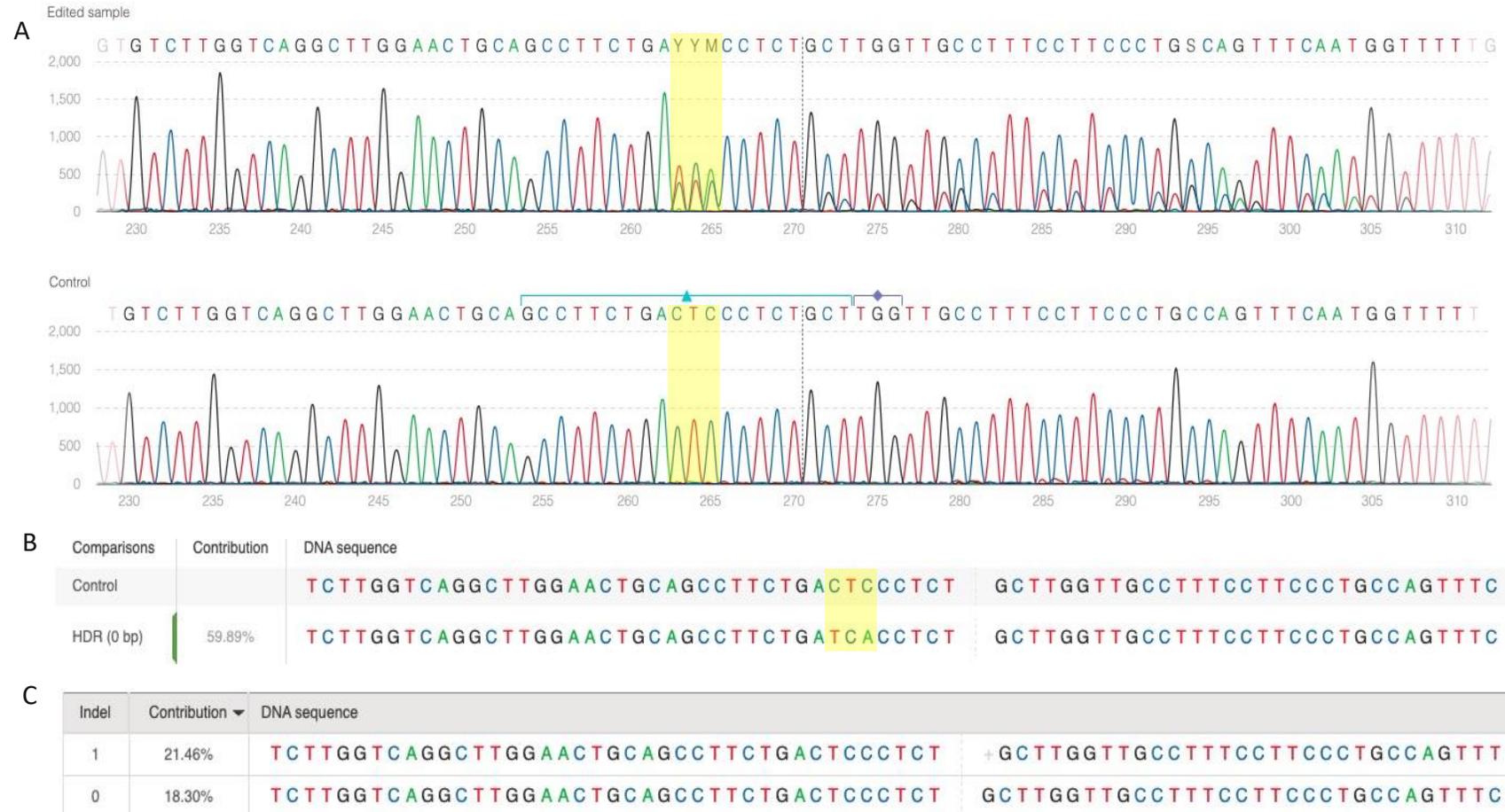
Phenotype

Hair length

Calf #5402

Concluding remarks

Brazilian GMO policy has been considered robust:
- Large number of GMOs approved since 2005 and no critical issues have been reported

Gene editing regulation has worked well so far:
- But are some internal discussion (in animals mainly):
  - Edits that are not found in nature or in a specific or correlate specie
  - Best way to search for off-targets and how to value their impact

- Lack of funds for animal gene-editing research in the country (compete with crops)
  - Despite having facilities and knownledgement
  - Need of collaborations with farmers, breeders and international institutions
    - To put the results on the field and to advance in knowledging

Ackowledments

comissão técnica nacional

CTNBio

de biossegurança