

4th International Workshop on Regulatory Approaches for Agricultural Applications of Animal Biotechnologies

Regulatory experiences with genome edited animals

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CTNBio – GnEd Cattle



Three cases presented to CTNBio

- 2018 – Polled bull - semen from a bull (Holstein crossbred) generated by TALENs technology
 - Trait: no horns
 - Strategy: repair by homologous recombination
 - Template: plasmid with Celtic allele derived from Red Angus
 - Somatic cell from Holstein crossbred male – edited cells used as nuclei donors for somatic cell nuclear transfer;
 - Off-target analysis: whole genome sequencing – parents and reference genome;
 - Consultation was retracted – detected the presence of plasmid backbone in the bull's genome



Production of hornless dairy cattle from genome-edited cell lines

nature
biotechnology

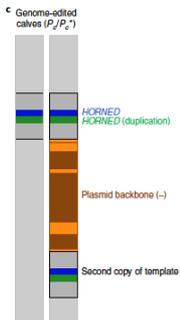
MATTERS ARISING

<https://doi.org/10.1038/nbt.41587-019-0394-6>

There are amendments to this paper

Template plasmid integration in germline genome-edited cattle

Alexis L. Norris^{1,2}, Stella S. Lee^{1,2}, Kevin J. Greenlees², Daniel A. Tadesse², Mayumi F. Miller² and Heather A. Lombardi^{1,2}



Myostatin knockout

- 2021 – Semen from a bull (Nelore) generated by TALENs technology
 - Trait: double muscle (muscle hypertrophy)
 - Strategy: indels in the myostatin gene (growth and differentiation factor 8)
 - Template: no template (TALENS mRNA)
 - TALENs injection into the cytoplasm of IVF zygotes;
 - Off-target analysis: whole genome sequencing – parents and reference genome;
 - Presence of DNA sequence from the plasmid used to synthesize the mRNA was also checked
 - Mosaicism
 - Product considered non-GMO

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



Slick allele

- 2021 – Male (Slick04) and female (Slick03) Angus generated by CRISPR/Cas9 technology
 - Trait: slick hair (thermotolerance)
 - Strategy: indels (Slick04) and HDR (Slick03) in the prolactin receptor (PRLR) gene
 - Stop codon (Slick03)- truncated protein
 - Template: no template - ribonucleoproteins
 - CRISPR/Cas9 injection into the cytoplasm of IVF zygotes;
 - Off-target analysis: two in intron or intergenic regions (Slick03) – no effect on protein expression
 - Mosaicism
 - Product considered non-GMO



Thank you

4th International Workshop on Regulatory Approaches for Agricultural Applications of Animal Biotechnologies

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The logo for Embrapa, consisting of the word "Embrapa" in a bold, blue, sans-serif font, with a large green leaf-like shape behind the letters "r" and "a".