

## Risk Assessment Summary – V-006378 PRLR-SLICK cattle

### I. Executive Summary

#### 1. Product Definition

The intentional genomic alteration (IGA) in the prolactin receptor gene (called the *PRLR* gene) truncates (or shortens) the prolactin receptor protein (called the PRLR protein) in *Bos taurus* cattle and results in a short, slick haircoat. The cattle with the IGA are referred to as PRLR-SLICK cattle. The IGA is a heritable alteration that was introduced using a genome editing technique known as CRISPR in two “founder” beef calves. Because the IGA conferring the slick haircoat trait is heritable, it can be passed on to offspring, allowing the trait to be propagated through breeding into a line of PRLR-SLICK cattle.

The IGA is the equivalent to the naturally occurring slick mutations that occur in several breeds of conventionally raised cattle where they likely developed as an adaptation to being raised in tropical or subtropical environments. The slick mutations confer a short, “slick” haircoat, and cattle with the slick phenotype have been reported to be better at withstanding hot weather (Dikmen et al., 2008, 2014; Hammond et al., 1996, 1998; Littlejohn et al., 2014; Olsen et al., 2003).

IGA Established Name	Firm
SLICK alteration disrupting <i>Bos taurus</i> g.(NC_037347.1) fs(39099129-39099368) in exon 9 of the <i>PRLR</i> gene in <i>Bos taurus</i>	Acceligen, Inc. a wholly owned subsidiary of Recombinetics, Inc.

#### 2. Summary

Acceligen submitted genomic data and other information to FDA to demonstrate that the IGA contained in PRLR-SLICK cattle is the equivalent to naturally occurring mutations that occur in conventionally raised cattle with a history of safe use as a source of human food. These mutations result in the same short, slick haircoat seen in cattle with the IGA, and people have safely eaten food products derived from cattle with the slick haircoat for years.

##### *Molecular characterization*

Several different slick mutations developed via selective breeding over the years in multiple cattle breeds as they naturally adapted to sub-tropical and tropical environments. Even though the slick mutations identified to date vary in their specific genomic sequences, current literature suggests these mutations all result in functionally equivalent truncated PRLR proteins (within a defined range of amino acid residues) and confer the same slick phenotype.

PRLR-SLICK cattle have an IGA that mimics genomic sequences found in conventionally raised cattle with a history of safe use in animal agriculture food production.

Both Acceligen’s and FDA’s analyses of the genomic data found evidence of unintended mutations in the genomic sequences of the founder calves. However, based on the types of unintended alterations identified and available information about their genomic locations, they are not expected to result in changes to protein expression. Based on the molecular characterization and animal health data, FDA concluded that they do not pose any safety

concerns for the PRLR-SLICK cattle or for people who consume food products derived from these cattle.

*Phenotypic data and animal safety*

PRLR-SLICK cattle have the same phenotype (i.e., short, slick haircoat) as conventionally raised cattle with naturally occurring slick mutations. In addition, the PRLR-SLICK cattle appear healthy based on visual observations and animal health records. The IGA causes no changes to the usual characteristics of the cattle.

Based on FDA's review of the molecular characterization of the IGA, the phenotypic data, and animal health records, as well as a history of no known safety risks to conventionally raised cattle with the slick phenotype, FDA determined that the IGA contained in PRLR-SLICK cattle poses no animal safety concerns.

*Human food safety*

FDA did not identify any human food safety concerns associated with the IGA contained in PRLR-SLICK cattle. In addition, there is a history of safe human consumption of food products derived from conventionally raised cattle with slick phenotypes. FDA determined that food products derived from PRLR-SLICK cattle are as safe as food products derived from conventionally raised cattle with the slick haircoat that are commonly consumed by the public.

*Environmental risk*

FDA did not identify any environmental hazards with regard to the IGA and concluded that the phenotype produced by the IGA contained in the PRLR-SLICK cattle is a well-established trait in several breeds of conventionally raised domestic cattle in the United States (US). In addition, the likelihood of escape and establishment of the PRLR-SLICK cattle in the US environment is low due to the ability to rapidly recover escaped animals and the lack of feral cattle populations in the US. Therefore, the risk to the environment resulting from the IGA contained in the PRLR-SLICK cattle is low.

3. Conclusions

Based on the data and other information submitted by Acceligen, FDA determined that the IGA contained in PRLR-SLICK cattle and the cattle's associated products, including offspring, semen, embryos, and food products derived from them, pose low risk to people, animals, the food supply, and the environment. Therefore, FDA does not intend to object to Acceligen marketing the IGA in PRLR-SLICK cattle or marketing the cattle's associated products. FDA also does not intend to object to Acceligen introducing cattle containing the IGA into the food supply. The agency's decision is limited to the marketed products (for example, live PRLR-SLICK cattle, semen, embryos, and meat) derived from the existing two cattle containing the IGA for which FDA has reviewed data and their progeny. Additionally, FDA intends to treat facilities or farms that are engaged in standard agricultural practices for PRLR-SLICK cattle, including assisted reproduction techniques (such as embryo transfer) or raising them for food production, the same as facilities or farms that are engaged in these practices for cattle without IGAs.

## II. Product Characterization

### 1. Molecular Characterization

FDA evaluated the following molecular data and information to determine if the IGA contains the same sequence found in conventionally raised cattle (for which there is history of safety) and whether there are any safety concerns:

- current literature describing the naturally occurring slick mutations identified in conventionally raised cattle
- experimental design and methodology used to create the IGA in PRLR-SLICK cattle
- whole genome sequencing data to confirm the IGA sequence and characterize any unintended alterations in PRLR-SLICK cattle

Acceligen provided data and information for molecular characterization of the IGA in a total of 4 beef calves. The IGA was introduced into the genome of PRLR-SLICK cattle using CRISPR-Cas9. Acceligen prepared embryos via *in vitro* fertilization and subsequently injected genome editing reagents to create the IGA by disrupting the *PRLR* gene, resulting in truncation of the encoded protein due to introduction of a premature stop codon. These embryos were then transferred into surrogates and calves were born.

Acceligen provided raw whole genome sequencing (WGS) data and their bioinformatics analysis for four edited calves and their un-edited parents. This genotypic data was used to evaluate the actual sequences of the IGA in the animals' genomes and screen for the presence of any unintended alterations that might pose an animal or food safety concern and risk to the environment. FDA independently analyzed the WGS data and confirmed Acceligen's conclusion that of the four calves born, three contained the IGA in exon 9<sup>1</sup> of the *PRLR* gene and one was determined to be unedited ("no-take"). Among the three calves with the IGA, one founder animal died unexpectedly due to a heart defect (attributed to bovine congestive heart failure; BCHF). Acceligen screened for two genetic markers (single nucleotide polymorphisms; SNPs) associated with BCHF and found that the deceased calf was positive for both (discussed more in Section II.2.a). FDA's genomic analysis confirmed that the deceased calf inherited these genetic markers from his unedited parents, suggesting that the SNPs were not newly acquired during the process of introducing the IGA and that the IGA was unrelated to the disease.

Several different naturally occurring slick mutations exist stably in populations of multiple cattle breeds that adapted to tropical environments (Flórez Murillo *et al.*, 2021; Littlejohn *et al.*, 2014; Porto-Neto *et al.*, 2018). While the genomic sequences of these slick mutations vary, they are located within a certain genomic region of the *PRLR* gene and confer the same slick phenotype. They contain premature stop codons resulting in PRLR truncations after certain conserved tyrosine residues and before another highly conserved tyrosine residue. Even though the naturally occurring slick mutations result in truncated PRLR of varying lengths, the current literature suggests that they are functionally equivalent based on their known functionally important domains as the mutations have the same reduced number of encoded tyrosine amino acids and result in the same phenotype. While the molecular mechanism by

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<sup>1</sup> Exon 9 based on the National Center for Biotechnology Information (NCBI) reference sequence NM\_001039726.2.

which slick mutations exert their effects is unknown, tyrosine residues of PRLR are thought to be critical for its function.

Based on the current literature and information about the various naturally occurring mutations identified to date and considering normal biological variability (*e.g.*, variability in sequence of the targeted gene resulting in PRLR truncation within a defined amino acid range and yielding the same reduced number of encoded tyrosine amino acids and baseline mutations in the genome), FDA considers the IGA to be equivalent to naturally occurring slick mutations. Based on this criterion, FDA concludes that the genotypic data demonstrate that the cattle with the IGA have a functionally equivalent genotype as conventionally raised cattle with naturally occurring slick mutations, *i.e.*, while the specific sequences of the IGA vary, the IGA contains premature stop codons predicted to result in PRLR protein truncation within the same amino acid range resulting from naturally occurring mutations. Both Acceligen's and FDA's analyses showed that the alleles containing the IGA contain premature stop codons that would result in truncated PRLR protein analogous to the naturally occurring slick mutations. Due to the specific process used to generate the IGA, the cattle containing the IGA were mosaic, meaning that in different cells/tissues of the cattle, different alleles may be present (these cattle could have several, including multiple distinct but equivalent IGA alleles as well as wild-type or otherwise non-IGA alleles). Genetic mosaicism is commonly observed when the CRISPR system is used in embryos due to potential random editing of cells at different stages of embryonic development. Acceligen included a disclaimer in the product label to describe that PRLR-SLICK cattle may have 2 or more genetically different sets of cells and, as a result, first-generation progeny may not all inherit the slick phenotype.

From the WGS data, there is evidence of unintended alterations in the cattle containing the IGA. FDA's analysis confirmed the unintended alterations reported by Acceligen and found evidence for a few additional unintended alterations, including a duplication located in a repetitive intergenic region and indels (short insertions and deletions) in intergenic regions. Except for the duplication, all unintended alterations are indels ranging from 1 to 11 DNA base pairs in length located in intronic or intergenic regions. The discrepancy between FDA's and Acceligen's analysis results are attributable to differences in bioinformatics analysis. None of the identified unintended alterations are expected to result in changes to protein expression based on their locations and available genome annotation. Based on the molecular characterization and animal health data, FDA determined these unintended alterations do not pose any safety concerns for the cattle or for humans consuming food from the cattle.

Based on the review of these data and information, FDA concluded that the IGA in PRLR-SLICK cattle have a genotype equivalent to naturally occurring slick mutations found in conventionally raised cattle used in animal agriculture food production.

Acceligen has used standard genome editing techniques and molecular biology reagents to generate the IGA in cattle. FDA's review of the submitted data indicated that the IGA itself as well as identified unintended alterations do not pose a safety concern and do not contain any sequences that may pose risk to humans, animals, and the environment.

2. Phenotypic Characterization / Animal Health
  - a. Animal Health

The following management considerations were presented with respect to the animals subject to the current evaluation:

- Housing
- Nutrition
- Periodic health observations, veterinary examinations
- Vaccination, anthelmintic treatments, other preventative health measures
- Animal growth
- Confirmation of the slick phenotype in the three animals containing the IGA

Although the cattle subject to the current evaluation were maintained under rigorous physical containment/housing conditions per procedures of the facility where they were reared, management practices such as nutrition, preventative health, health/veterinary observation were representative of typical cattle production practices.

Based on periodic health/veterinary observations, Acceligen indicated that the health and growth of animals with the IGA were similar to conventionally raised cattle (i.e., cattle without the IGA) of the same genetic background. Acceligen confirmed there were no major health abnormalities or defects seen, with animals in excellent health during their upbringing. During the evaluation period, the cattle displayed normal rates of growth of 3 to 3.5 lb. per day.

One exception included the unexpected death of one male calf with the IGA. At the postmortem examination, the death was attributed to bovine congestive heart failure (BCHF), known to occur in North American cattle operations, affecting Angus-influenced genetics more so than other breeds. United States Department of Agriculture (USDA) researchers have identified two single- nucleotide polymorphisms (SNPs) associated with BCHF that may be used to identify cattle at higher risk for BCHF. Acceligen screened all four calves and found that two of them were positive for both genetic markers, including the deceased calf; one calf was positive for one marker, with the remaining calf negative for both. As described above, FDA's genomic analysis confirmed that the deceased bull inherited the SNPs from his unedited parents. As such, the introduction of the IGA was not an underlying risk factor for BCHF.

Based on the lack of identifiable hazards from genomic/molecular characterization, coupled with the overall health status of the cattle subject to this evaluation, FDA concluded that risks to animal health for animals with the IGA are no greater than for conventionally raised cattle.

b. Intended phenotype

The three animals carrying PRLR truncating alleles were visually observed to have the slick phenotype marked by their short coat, and the one unedited calf without the truncation had normal hair phenotype. Recognizing the limited nature of the dataset upon which this determination is based, published literature documented the association of the slick phenotype with improved thermotolerance during periods of high ambient temperature and humidity (Dikmen et al., 2008, 2014; Hammond et al., 1996, 1998; Littlejohn et al., 2014; Olsen et al., 2003). These reports presented a variety of endpoints (e.g., body temperature, respiration rate, heart rate, sweating rate, hair measurements) in cattle

with and without the slick phenotype, and consistently illustrated improved thermotolerance in slick vs. non-slick cattle. Background genetics included pure Criollo breeds and/or Criollo breeds crossed on a variety of dairy (e.g., Holstein, Brown Swiss) and beef (e.g., Angus, Hereford, Charolais) breed types. Based on this information, FDA determined we can reasonably expect the cattle with the IGA and slick phenotype to be more thermotolerant than their longer-haired counterparts during periods of heat stress.

### 3. Human Food Safety

FDA evaluated human food safety for PRLR-SLICK beef cattle and concluded that there is reasonable certainty of no harm for human consumers of food from the PRLR-SLICK cattle due to the absence of identifiable hazards. The basis for the conclusion is described below.

The primary food safety question is whether there is any difference between the food from the PRLR-SLICK cattle and food from cattle already used in agriculture with a history of safe use. To determine if the food from the PRLR-SLICK cattle is as safe as food from cattle commonly consumed by the public, FDA evaluated the product to identify any potential food safety hazards associated with human consumption of the edible tissues derived from the PRLR-SLICK cattle.

For the hazard identification, the following information was examined:

- the molecular characterization of the IGA
- the phenotypic characterization of animals with the IGA
- the health status of the animals
- any unintended effects, especially those associated with indirect toxicity due to alteration of physiological processes, and
- any potential impact on the composition of the edible tissues due to the IGA

As described above in Section II.1, the IGA was confirmed at the targeted location in the *PRLR* gene. The molecular characterization did not reveal any potential human food safety concerns from a thorough genotypic analysis. FDA concluded that it is unlikely that the truncated protein encoded by the *PRLR* gene is a food safety concern because similar mutations occur in conventionally raised cattle and are safely consumed. DNA sequencing did not identify any changes to the genome that are expected to change protein expression or function other than the intended truncation of PRLR as described above. The phenotypic characterization described in Section II.2, also revealed no indication of direct or indirect toxicity to the animals with the IGA that may cause a potential food safety concern. Additionally, the phenotype is already commonly found in traditional animal agriculture, particularly in tropical regions.

FDA did not identify any animal safety concerns in PRLR-SLICK cattle beyond those that would be expected in comparator cattle without the IGA under conventional cattle management practices (Section II.2). Based on the consistent information from the molecular characterization of the IGA, the phenotypic characterization of the animals, and the animal health records, FDA has not identified any direct or indirect effects on the edible tissues of cattle resulting from the IGA. Conventionally raised cattle with the slick phenotype are routinely consumed as human food and therefore FDA does not expect a change in the compositional or nutritional content of the edible tissues derived from the PRLR-SLICK cattle

because they are similar in genotype, phenotype, and health status of naturally occurring slick cattle. No hazards were identified that required further characterization.

In summary, FDA concluded there are no identifiable direct or indirect effects of the truncation of the *PRLR* gene or the IGA on the safety of food derived from the PRLR- SLICK cattle. FDA also concluded that the safety of food products made from PRLR- SLICK cattle is no different than the safety of food products made from commercial cattle that do not contain the IGA including those conventionally raised cattle with the naturally occurring slick phenotype.

#### 4. Environmental Risk

FDA evaluated the potential risk to the environment from the marketing of the IGA in PRLR-SLICK cattle or associated products derived from them (i.e., offspring, semen, or embryos). FDA's low risk determination is not limited to animals raised at specific facilities or under certain conditions; therefore, the PRLR-SLICK cattle could be housed on any farm in the US with typical farm containment (e.g., pens and pastures), and carcasses and waste can be disposed of according to typical farm practices (e.g., burial, slaughter, composting, application to agricultural fields, etc.).

As described above, the IGA results in a truncated PRLR protein that produces the slick phenotype. It is a well-established trait that occurs in heritage Criollo cattle breeds present in tropical regions. In addition, Holstein cattle lines with the slick phenotype have been developed, and are present in the US, through conventional crossbreeding of Senepol and other Criollo breeds with Holstein lines (Food and Agriculture Organization of the United Nations' Domestic Animal Diversity Information System). Based on information submitted by Acceligen, no new expression products (e.g., proteins other than the truncated PRLR protein) are expected, and no hazards have been identified with regard to the IGA.

FDA's evaluation assumed that the PRLR-SLICK cattle would be reared under minimal containment practices in the US. If an escape were to occur from a farm, PRLR- SLICK cattle would be expected to be recovered quickly because they are large herd animals that are used to being handled by humans. In addition, establishment of a PRLR-SLICK cattle population in the US environment is unlikely because of a lack of feral cattle populations in the US with which escaped PRLR-SLICK cattle could breed.<sup>2</sup>

In summary, the information provided by Acceligen adequately demonstrated that the introduction of the IGA into the animal results in a slick phenotype that exists in other conventionally raised domesticated cattle in the US and does not pose a hazard to the environment. In addition, the likelihood of escape and establishment of the PRLR-SLICK cattle in the US environment is low. Therefore, the potential risk to the environment is low from the marketing of the IGA in PRLR-SLICK cattle and the cattle's associated products, including offspring, semen, embryos, and food products derived from them.

### III. Conclusions

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<sup>2</sup> Four newspaper articles found during an online search at the time of FDA's review reported that some small herds (<300 head) of feral or wild cattle exist in the western US (e.g., California, Texas, Arizona, New Mexico), and are often removed by land management agencies.

Data and information demonstrate that the IGA in PRLR-SLICK cattle poses low risk to humans, animals, and the environment. The IGA in PRLR-SLICK cattle is equivalent to naturally occurring mutations (with normal biological variability) and results in the same short, slick hair trait as in conventionally raised cattle that have a history of safe use in animal agriculture food production. Although the IGA in PRLR-SLICK cattle is not approved, conditionally approved, or index listed,<sup>3</sup> because FDA has determined the IGA is low risk, at this time the agency does not intend to object to Acceligen marketing the IGA in PRLR-SLICK cattle or associated products derived from them (i.e., offspring, semen, or embryos) or introducing meat derived from the cattle containing this IGA into the food supply. This decision is limited to the marketed products (e.g., live animals, semen, embryos, meat) derived from the existing two cattle containing the IGA for which FDA has reviewed data and their progeny.

#### IV. References

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Porto-Neto LR, et al. (2018) Convergent Evolution of Slick Coat in Cattle through Truncation Mutations in the Prolactin Receptor. *Front Genet.* 9:57.

USDA Agricultural Research Service. Bovine Congestive Heart Failure in Feedlot Cattle. <https://www.ars.usda.gov/plains-area/clay-center-ne/marc/bchf/bchf-main/>

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<sup>3</sup> See sections 512, 571, and 572 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. §§ 360b, 360ccc, and 360ccc-1].