recombinetics

15 December 2016

Dr. Raj Bhula
Gene Technology Regulator
Regulations Review
Office of the Gene Technology Regulator
GPO Box 9848
Canberra ACT 2601

Re: Discussion Paper - Options for Regulating New Technologies

Dear Dr. Bhula,

Recombinetics, Inc. (RCI), St. Paul, Minnesota, appreciates the opportunity provided by the Office of the Gene Technology Regulator from the Australian Government's Department of Health to submit comment and information on the "*Discussion paper: Options for regulating new technologies*". As your department moves forward in this effort to clarify current roles and responsibilities described in the discussion paper, RCI strongly recommends the OGTR's long-term strategy is that gene editing is logically outside the coverage of the Gene Technology Regulations of 2001, because we believe gene editing and precision breeding based on site-specific nucleases (SDN) are excellent examples of creating allelic variations that do not comport with the definitions of the original need framed in the Gene Tech Regulations of 2001. Hopefully, the route to regulatory clarity can be reached swiftly, because the current situation from our perspective is impeding opportunities for innovation and investment from abroad to support Australian agriculture.

Framing Our Response

Founded in 2008, RCI is the premier gene-editing company in livestock, with applications in food animal breeding and care, and therapeutic development and testing of biomedical models. For food animals, RCI focuses on adding value to animals and the protein production chain, by accelerating genetic improvement for animal health and welfare while maintaining sustainability and productivity. Relative to biomedical models, RCI develops precise swine models of patient congenital and progressive diseases, including neurodegenerative diseases, heart disease, diabetes, and cancer. The use of RCI's proprietary pig models is facilitating more rapid commercialization of safe and effective drugs and medical devices, with lower costs.

It is our belief that gene editing and precision breeding are the first biotechnology methods that truly provide economically feasible opportunities to revolutionize genetic improvement of food animals. In the past, livestock breeders could not practicably deploy advanced breeding tools used by crop breeders to affect genetic improvement in a single generation. This is supported by the fact that none of the varieties or breeds of food animals currently on the commercial market

for human food consumption were developed using human-deployed mutagenic techniques. Comparatively, more than 3200 accessions have been developed by crop breeders for improving grain, vegetable and fruit varieties. In contrast, the most productive animal seedstocks were developed by artificial selection, where animal breeders and producers employed modern animal husbandry and statistics to develop phenotype recording systems, which could be evaluated for selection of both type and performance. Ultimately, all of the mutations or causal sequence variants enriched by these selective processes were derived from naturally induced processes.

Artificial selection under the supervision of professional animal breeders and breed association guidelines has led to tremendous genetic progress for production across the spectrum of food animals, and was enhanced by advanced reproductive technologies (i.e. artificial insemination and in vitro fertilization), quantitative genetics in the 20th century, and more recently by DNA marker-based genomic selection. Through genomic breeding, scientists have now identified many of the wrong moves made by conventional selection, which could be corrected by application of genome editing (http://aipl.arsusda.gov/reference/recessive_haplotypes_ARR-G3.html).

Up until now, producers have also attempted to introduce the production alleles from elite animals into more challenging and extensive production environments either directly or by crossing native, adapted populations with breeds from non-tropical, developed countries to make composite or hybrid animals. Direct importation has mostly failed due to heat and disease stress. The conventional cross-breeding strategies are costly in resources and generation interval time; and require large base populations to avoid eventual bottlenecks in diversity caused by selection for a single trait or traits with additive gene action.

Gene editing has the potential as an alternative breeding method to overcome all these challenges, which limit animal protein production in the developing world. These methods can be applied without sacrificing local diversity or the incremental gains accumulated through centuries of artificial selection. Furthermore, like crops, selection of deployable animal traits by gene editing will mostly be derived from genome-based discovery of important, naturally occurring sequence variants (i.e. Genome wide associations studies, next generation resequencing, etc.) that reveal underlying causal variants for important qualitative and quantitative traits (1).

Based on these tenets, the Technical Review of the Gene Technology Regulations 2001 is an opportunity for reinvention of the current regulatory framework, and the OGTR is to be commended for recognizing this need. We understand that this modernization must be approached in a careful and pragmatic manner so as to avoid any unnecessary impediments to technology development or application, while still being transparent to consumers and producers in the marketplace. Many of our viewpoints laid out in the subsequent pages are shared by our scientific colleagues in Australia and New Zealand as published in recent review

(2). Our response is intended to fill the gaps between this review and the OGTR's request for specific comments.

RCI Response to OGTR's Consultation questions

1. **Which option/s do you support, and why?**

   Recombinetics supports ***Option 4****: exclude certain new technologies from regulation on the basis of the outcomes they produce.*

   RCI believes the new breeding methods that use SDNs can deliver more predictable outcomes for genetic improvement compared to other conventional and advanced breeding methods, which either rely on selection for naturally occurring variants or synthetically induced mutagenic events (random double strand breaks in DNA – not practiced in food animals), respectively.

   Option 4 is the option most closely aligned to existing best practices for conventional animal breeding. This is because transmission of an allele is first & foremost a genetic concept, not a molecular biology one, alleles must be characterized by phenotypic and genotypic data before deployment by any breeding method. Gene editing or precision crossbreeding are simply breeding techniques used to confer desirable traits by introgression of alleles by gene conversion into a new genetic background (2). From this perspective, we believe oversight of SDN application for genetic improvement is not warranted unless the intended changes could potentially have a negative effect on animal well-being (i.e. deletion of a housekeeping gene needed for any cell's function) or composition of the food products derived from the animal is significantly changed (1, 2).

   For example, an edited or precision bred animal has to clear through several selective processes just like conventionally bred animals must do to get to the commercial marketplace. Today, for conventional breeding, much of this selection is based on information from past performance combined with DNA genotypes or sequence data to better inform decisions. Likewise, edited embryos or cells must still be screened and chosen for proper allele transmission initiated by a double strand break (DSB) event before being brought into production.

   If a company plans to market edited/precision bred genetics, then to remain competitive these animals must also pass through a genome resequencing screen to look for off-target edits and naturally occurring deleterious mutations. Additional phenotype testing could also be warranted to prove economic value to potential buyers. Commercial release of animals with deleterious mutations produced either by natural processes or editing, which could be causative of downstream animal health problems, has been and will continue to be detrimental to the genetics company selling an "improved" animal (see

citation 3 as an example). In other words, the deployment of any breeding method must maintain an animal's genetic integrity, and it is the bottom up pressure from the marketplace that applies the necessary economic and quality assurance pressures for breeders to self-evaluate and regulate to remain competitive.

Finally, RCI recognizes that a potentially important place for regulation of commercial application of gene editing is when the final breeding outcome produces a novel food product. If naturally occurring alleles are precision bred into a new genetic background, then the animal food product still will be "Absolutely Identical" to food components that already exist and have been previously consumed (SDN2 and potentially SDN3). If there is a gene break or knock out (SDN1), then this food product could be missing a protein component, but it would not necessarily be novel to what mutagenic events might have occurred naturally. The rate of natural mutagenesis has been calculated in livestock to be roughly more than 30 novel events in a single generation (2), and clearly this rate of unregulated mutation is much than precision changes made by any category of SDN editing. The 1000 Human Genomes project has also taught us that every living animal is carrying a genetic load of on average more than 10 defects, which is why we avoid inbreeding. For cattle, we also know there are animals carrying mutations within genes involved in recombination that accelerate their rate of natural mutagenesis (4). All combined, although RCI endorses **Option 4**, we truly believe the best option is to ultimately regulate the food product not the biotechnology process (1, 2, 5).

2. **Are there other risks and benefits of each option that are not identified in this document?**

RCI believes there is possible risk in attempting to categorization types of edits as differing processes (Option 2-4). The practicality of edit types can be biased based on species, and already is or will quickly become outdated due to rapid advances in genome editing technology. Below we summarize some cases supporting this supposition.

First, we believe there is already some confusion about the spectrum of different applications for SDNs among scientists and policy makers, and we also will provide some specific examples that might blur distinctions between SDN-2 from SDN-3. Second, there already are methods for editing that do not rely on DSB mechanisms, but rather allow direct base pair editing (6). This next generation of gene editing tools looms on the horizon for production of food animals, yet maybe too new to have been included in this discussion paper. We would argue that based on mechanistic actions (no DSB), such an editing tool does not fit into any SDN category.

Relative to Option 3 – we believe the assumption that a non-homologous end joining (NHEJ) repaired double strand break (DSB) is less risk than a DSB repaired by homology directed repair (HDR) is flawed (i.e. SDN-1 is more like natural mutagenesis than SDN-2;

therefore, SDN-2 should be regulated – option 3). Both SDN-1 and SDN-2 (and potentially SDN-3) have a first step that requires a DSB event. RCI believes that simultaneous presence of a repair template does not constitute genetic modification or a difference in risk, because this template is never introduced or transferred into the target genome. The review by Jasin and Haber (2016) talks about this in the context of genome editing, especially Fig. 2 (7). From this perspective, one could argue that SDN-2 is less risk than SDN-1, because the outcome is a predictable qualitative phenotype from the copied allelic information of an existing animal bred into a different genetic background. In contrast, a gene knockout that has not been found in nature may have a much less predictable phenotype, which means the breeder will need provide its own pre-market characterization to avoid losing market share if the genetic accession fails.

If the presence of a DNA template is not the basis for a pre-market regulatory decision, but rather the length of the template or sequence contained within it is; then the boundary between SDN-2 and SDN-3 must be clearly defined. We realize, some of the intention for SDN3 is to distinguish cis-genesis and trans-genesis events from copying of natural allelic variants into new genetic backgrounds. However, we view all these applied processes of editing as advanced breeding methods.

Again, the selection of **Option 4** as the best practical way forward is also supported mechanistically, because during the editing process (post-DSB) nothing is being introduced or transferred into the target genome (7). Rather, a competition for the selected repair mechanism takes place that is dependent on the availability of cellular factors to initiate non-homologous end joining (NHEJ) or homology directed repair (HDR). HDR is facilitated but still not always favoured when a DNA template is provided to direct allelic information for gene conversion, whereby specified nucleotides can be copied in reverse-complement into the DSB site (7, 8). There is experimental evidence suggesting this template information can be provided as ssDNA or dsDNA with potentially no size limitation just differences in efficiency for the gene conversion (9). One could speculate that RNA templates will be developed for HDR as these DSB repair mechanisms are better defined.

Currently, it is clear that conversion by HDR takes place by one of two components: invasion-mediated synthesis-dependent strand annealing (SDSA) pathway or by single-strand annealing (SSA). This is critical to our view of Option 4, and underscores that editing based on DSB followed by HDR is a completely natural process, and there really is no transferred or introduced recombinant DNA constructs or synthetic DNA placed into the target genome. The transfection/injection of the “molecular scissors” into clone-able fibroblasts/one-cell embryos only increased the frequency of DNA breaks at the target locus billions fold over the natural processes of mutation. The DSBs stimulate gene conversion, which is not something novel or unnatural. In the case of sister chromatid repair, the template is copied, not physically acquired. This is definitely also what’s going

on during gene conversion in meiosis. There has been a lot of evidence in yeast and Drosophila over the years that somatic repair goes by the SDSA mechanism, which involves invasion, copying, then withdrawal of the extended strand and re-pairing with the other end of the break (8). There are also some neat experiments in *C. elegans* that indicate that the whole of a large insert is copied, even in a situation where that need not be true (9). This type of recombineering blurs the distinctions laid out between SDN2 and 3 as something mechanistically different just based on differences in sequence length post gene conversion. The only way to separate it is to say "could not be obtained by conventional breeding". The opportunities now exist to introduce any sequence without the need for a recombinant DNA construct.

As a final example of how SDN-2 and SDN-3 need to be clearly defined, we provide the following for what might be considered borderline, if DNA length changes were set as marks for distinction in regulation. The precision breeding event was to genetically dehorn a cell line derived from horned dairy bulls and then clone these cells into animals (10). The remarks are from Alison Van Eenennaam, Ph.D., Animal Genomics and Biotechnology Cooperative Extension Specialist in the Department of Animal Science at the University of California-Davis. She offers the following regarding US regulations on editing:

"Towards this end, I believe the example of the Polled (hornless) genome-edited cow would be a useful case study to assist in the development of the proposed update to the Coordinated Framework. The Celtic "Polled" allele (PC) is a naturally occurring dominant, allele that occurs with high frequency in some beef breeds, e.g. Angus; however, it is at low frequency in dairy breeds. As a result, horns are manually removed from almost all dairy calves. The polled allele consists of a 202 bp insertion-deletion. Genome editing using TALENs has been used to copy the PC allele into a dairy cell line. The resulting animals carry the same PC DNA sequence that is found in Celtic polled breeds and would be predicted to be phenotypically polled (hornless)."

The outcome of deliberations on examples like these will be critical to the future of biotechnology, as well as on the emerging applications of this science to benefit animal protein production, well-being, and human health.

3. **Is there any scientific evidence that any of options 2-4 would result in a level of regulation not commensurate with risks posed by gene technology?**

Based on our response and previous levels of oversight for conventional animal breeding and advanced crop breeding, options 2 and 3 clearly are forms of regulation that do not match the risk of based on differences in cellular processing of DSB events where there is NHEJ alone or NHEJ and HDR, both of which RCI has argued are natural processes with similar outcomes.

Finally, there are practical reasons why post-market regulation in animals defines the commercial space for editing applications to breeding, as animals are quite different as a risk compared to microbes and plants. RCI believes, traditional food animals with edits by their very nature, pose no arguable difference in risk to consumer food safety, the environment, or other wild animal populations nearby or overlapping with production zones. In the developed world, animal husbandry is a very controlled practice with multiple levels of standards for making food from animals. Some of the essence of this is based on fundamental differences in domestication history and animal breeding practices for selection and sexual reproduction compared to microbes and crops. For the most part, the wild founding populations of most livestock are extinct. Therefore, unlike plants and aquaculture, the threat of breeding with non-domesticated ruminants is limited by accessibility and speciation, which allows no truly viable escape path for novel alleles. One could argue even if new alleles found their way into other sexually compatible species, these alleles like disease resistance or no horns may already exist by natural adaptive processes.

Another limiting factor for genetic distribution is that the animal genetics marketplace for food animals varies across species, but none of the commercial genetics companies monopolizes the marketplace. Cattle, especially for meat production, are in non-vertically integrated systems with many seedstock producers. So there are always many choices. The closest example to seed companies for crops would be poultry, where only a few companies provide most of the world's genetics for broiler and egg laying production in chickens. These genetics are highly proprietary, and one would imagine kept under strict control from distribution.

4. How might options 2-4 change the regulatory burden on you from the gene technology regulatory scheme?

RCI views that any additional regulatory standards should be based on the risks inherent to the animal product, not the process used to develop it as stated in our citations (2, 5). A regulatory change to Option 4 still requires the animal breeder to obtain prior genetic knowledge of naturally occurring alleles within the target species and biological information on their effects. In our business model, this information is a prerequisite for precision breeding/editing. Hence, an exemption from regulatory requirement would still be based on previous research (application of SDN-1 and SDN-2).

The scientific literature demonstrates that editing technologies offer a lower risk to the environment, animal health, and human health compared to mutagenesis techniques with a safe use history. If the OGTR plans to adopt Options 2 or 3, then RCI requests that risk assessment should be proportionate to proven lower level of risk that has already been demonstrated in multiple species. Placing genome editing for known alleles within

the same species under regulatory control, would be a significant burden for the livestock industry, and may create trade and other competitive barriers for the Australian livestock industry. Import of edited germplasm into Australia could occur undetected, if edited individuals carried natural variants that were indistinguishable from conventionally bred animals.

5. **How do you use item 1 of Schedule 1, and would it impact you if this item was changed?**

   RCI recommends that Item 1 should be clarified to provide the framework whereby the regulation of new technologies is commensurate to the risks.

6. **Might contained laboratory research on GM gene drive organisms pose different risks to other contained research with GMOs, and how could these risks be managed? Supporting information and science-based arguments should be provided where possible.**

   No comment for this question as our company has no current intentions to use these methods for genetic improvement of animals intended for food consumption.

7. **What RNA interference techniques are you using, and are there RNA interference techniques that you believe have unclear regulatory status? Please provide details of the techniques and science-based arguments for whether these techniques pose risks to human health or the environment.**

   No comment for this question as our company has not yet used or evaluated these methods for genetic improvement of animals intended for food consumption.

8. **Do you have proposals for amendments to any other technical or scientific aspects of the GT Regulations? All proposals should be supported by a rationale and a science-based argument.**

   There are currently only two proven ways to deploy gene editing technology in food animals. These are through transfection of fibroblasts destined for nuclear transfer cloning (primordial germ cells in poultry) or by microinjection of mammalian one celled embryos (2). This later method has a spectrum of efficiencies depending on the input parameters of the editing tools, such that in most cases, many of the resultant animals from microinjection of IVF embryos have no edits or are mosaic for the edit (11). This means animals produced from IVF embryo injection have to go through a Mendelian transmission test to confirm commercial viability as a germ plasm product. RCI would

strongly suggest that any animals produced by injection treatment of IVF embryos, which are found to carry no edits or cannot transmit the edit by sexual reproduction, should be treated as conventionally bred animals. In essence, the mutagenic treatment failed as if there were no treatment applied. Furthermore, any recipient animals carrying edited clones or IVF embryos should have no restrictions under conventional animal quality measures relative to entering the food chain for human consumption.

In conclusion, RCI agrees that the time is right to revisit, refine and modernize the previous regulatory framework. The technical facts of the processes of SDN clearly demonstrate that Option 4 is the best choice for oversight at this time, because a boundary placed between processes applying NHEJ and NHEJ and HDR is arbitrary in relation to the nature of the processes and the final animal outcomes. RCI shares the OGTR's objective, namely to ensure public confidence in the regulatory system and to prevent unnecessary barriers to innovation and competitiveness through improved transparency, coordination, predictability and efficiency of the system, while protecting health and the environment.

Thank you for consideration of our views. Please feel free to contact me if you there are any questions or information I can provide.

Sincerely,

Tad Sonstegard Ph.D.
Chief Scientific Officer of Acceligen
The Food Animal Subsidiary of Recombinetics

Citations

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