




# final report

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# Evaluation of gene editing technologies for the red meat industry

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# Executive Summary

The demand for animal protein is increasing globally. This is a result of several factors including, but not limited to, population growth, shifting of socio-economic positioning of a large population, and limited possibility for expansion of agricultural area. It is postulated that the broad adoption of reproductive and genetic biotechnologies could assist productivity improvements from on-farm operations to meat processing plants, with potential for reducing animal welfare concerns. This review focuses mainly on gene editing biotechnologies, including cloning, the current regulatory framework, potential benefit and risks to the red meat industry.

Gene editing refers to biotechnologies that allows precise changes to the genome (DNA) of an organism (e.g. animal, plant, and microbe). As the name suggest, these methods allow researchers to delete, add or replace letters (nucleotides) in the genetic code, similarly to the spell check of a writing software.

Gene editing is different from traditional genetic engineering. During traditional genetic engineering a “foreign DNA”, or a gene from a second species, is added to the genome of the target species, defining a transgenic animal since it carries the gene(s) of more than one species. On the other hand, gene editing normally only adds, delete or replace some nucleotides, and does so at very precise location of the genome.

Australian research or commercial production that involves gene technology is primarily regulated by the Office of the Gene Technology Regulator (OGTR) and Food Standards Australia and New Zealand (FSANZ). Recently, the regulation of gene editing has received considerable attention, with regulatory agencies considering if and/or how editing technologies align with existing legislation. The current regulatory system in Australia remains unchallenged as no commercially relevant products have been put to the authorities for consideration. Similarly, key trading partners are considering the regulatory status of editing technologies. Further, genetically modified and cloned animals may also be subject to state and territory government animal welfare legislation applicable to animals used for scientific purposes, as well as the Australian code for the care and use of animals for scientific purposes.

There are opportunities for improvement across the whole food chain via applications of gene editing technologies. Areas of recognised opportunities include, but are not limited to, improvement of productivity (on- and off-farm), animal welfare, disease prevention and for the use of gene editing during the development of other livestock products (e.g. livestock feed and supplements). This highlights some “short-term”/realistic applications relevant to industry, in the sense that these are currently subject of active research. It is worth noting that international genomics projects based on next-generation sequencing of whole-genome are delivering many causative targets that could be further explored using gene editing for livestock improvement.

There are a vast number of possibilities offered by novel technologies such as gene editing to the livestock industry. However, industry leaders, such as the Meat and Livestock Australia (MLA), who want their stakeholders to have access to the best available technologies that increase productivity, competitiveness and maximise profitability have a

difficult task. Formulating appropriate strategies that encourage new opportunities with limited financial resources and few mechanisms geared to understand exactly what is required along the value chain is a challenge. In particular, biotechnologies have raised significant concerns amongst both producers, key markets and domestic consumers. As such, it is important that industry leaders consider the value chain dynamics and develop strategies that articulate the benefits along the value chain, provide transparency in decision making, address stakeholder concern and build trust and trustworthiness of the industry as whole.

MLA have an opportunity to focus on industry-orientated priorities that have a greater likelihood of adoption and acceptance as well as providing leadership in providing the framework for value chain and market acceptance.

The most relevant risk for adoption of gene editing technologies by the red meat industry relates to potential negative public perception, and its local and international activism against the use of the technology. As it stands in April 2017, due to an unclear regulatory environment, its broad application across the industry could lead to commercial/trade barriers that will certainly impact negatively the industry. Nevertheless, regulatory bodies around the world are working to stablish the framework for dealing with these technology. There is a great opportunity to learn from the experience learned from the release of transgenic organisms and move forward with a unified message. There are potential benefits to the industry, and some risks and weaknesses associated with them.

Gene editing is a potential revolution in biotechnology. It gained this position becasue it influences virtually all fields of life sciences from “basic” sciences to commercial applications. Commercial use depends on several factors including resolution of the intellectual property ownership and definition of a country’s regulatory process. As the biotechnology is applied broadly, it is expected that it will be used during the development of improved pastures e.g. higher nutritional value or higher digestibility, and also on the development of feed supplements. It is appears inevitable that cattle will be fed with gene edited feedstock in the near future.

The use of the term “disruptions” to define some of the potential impacts of gene editing within the industry might be a too rash a term. Assuming that the technology is broadly adopted, several industry practices will need to adapt to the new reality. However, these should not be seen as negative or disruptions. A parallel could be drawn with the industry adoption of cattle cloning, where breed societies nowadays already register cloned cattle developed locally and internationally. The optimistic view is that gene editing could invigorate the industry and yield welfare benefits.

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# 1 Glossary and abbreviations

| | |
|---|---|
| Allele | A genetic variant |
| APVMA | Australian Pesticides Veterinary Medicines Authority |
| Cloning | Method where by a mature somatic cell, such as a skin cell, is removed from an elite animal to be copied. They then transfer the DNA of the donor animal's somatic cell into an egg cell that has had its own DNA-containing nucleus removed. The egg is then implanted into a surrogate mother who gives birth to an animal that is a clone of the donor animal. The cloned animal is then bred with other animals to pass on its desirable characteristics. Cloning is different to genetic modification |
| CRISPR | Clustered regularly interspaced short palindromic repeats. Type of SDN to facilitate double-stranded DNA breaks at a precise and predetermined location of the genome |
| Epigenetics | Gene modulation without alteration of the genetic code itself |
| FDA / US FDA | Food and Drug Administration from the USA |
| FSANZ | Food Safety Australian New Zealand |
| Genome | The whole DNA sequence of an organism |
| GMO/GM/GE | Genetically Modified Organism, Genetically Modified/Genetically Engineered. An organism that has had its DNA altered or modified in some way through gene technology. GMOs may have been altered with DNA the same species or from another organism. GMOs are sometimes referred to as "transgenic" organisms |
| HR | Homologous recombination uses a template for repair during the process to re-join the ends of the double-stranded DNA breaks |
| Intracytoplasmic | Refer to the inner cell environment, but outside the cell nucleus |
| Introgression | Repeatedly backcross |
| NBT | New Breeding Technologies, normally refers to gene editing approaches, but can also include cloning |
| NHEJ | Non-Homologous End-Joining uses an error prone process to re-join the ends of the double-stranded DNA breaks |
| OGTR | Office of the Gene Technology Regulator |
| RNAi | RNA interference. A method that can be used to knock-down gene function |

| | |
|---|---|
| RSPCA | Royal Society for the Prevention of Cruelty to Animals |
| SDN | Site directed nuclease. Enzymes that creates double-stranded DNA break at precise and predetermined location of the genome |
| SNP | Single Nucleotide Polymorphism - a genetic variant |
| TALEN | Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. Type of SDN to facilitate double-stranded DNA breaks at a precise and predetermined location of the genome |
| TGA | Therapeutic Goods Administration |
| Transcription | Gene expression |
| Transgenesis | Relates to genetic manipulation which DNA (or genes) from more than one species is combined in a single organism |
| ZNF | Zinc Finger. Type of SDN to facilitate double-stranded DNA breaks at a precise and predetermined location of the genome |
| Zygote | One-cell embryo |

# 2 Background and objectives

Biotechnology has been applied to livestock production for more than 50 years, particularly through the development of reproductive biotechnologies. One of the first biotechnologies that revolutionized animal breeding was the development of artificial insemination in the early 1950's. These were followed by embryo technologies such as multiple ovulation embryo transfer, ovum pickup followed by *in vitro* fertilization and embryo transfer, and later in mid-1990's, animal cloning via somatic cell nuclear transfer. Importantly, all of these biotechnologies breed livestock regarded as safe for consumption.

The generation of elite cattle for advancement in Australian beef and dairy productivity has been done via animal cloning. The cloning of elite cattle has been undertaken in Australia since the early 2000's; it is estimated that around 8-10 dairy and 20-25 beef cattle clones were born in Australia in the last five years. In the dairy industry, animals that were cloned have been selected mainly on estimated breed values, while for beef production they were generally selected to extend the life of a valuable dam or sire.

In parallel, the application of gene technologies that create a genetically modified organism (GMO) have been developed for both animal and plant species (Garas *et al.* 2015; Jez *et al.* 2016). These technologies involve the introduction or modification of novel traits within the DNA of the target animal or plant. Typically, the genetic material introduced has been identified and characterised, sometimes from a different species, using an engineered 'gene construct' and genetic modification process.

Unlike earlier biotechnology approaches, gene technology is one of the most controversial applications of biotechnology. The application of genetic modification (GM) has raised significant concern amongst various sectors of the community and, as such, many governments have developed and introduced biosafety policies and procedures around the safe use of gene technology, particularly with respect to food safety and environmental safety. Products developed though the process of gene technology are regulated in Australia and cannot be introduced in to the market unless they have been rigorously assessed.

GM animals have been produced for multiple agricultural purposes (Van Eenennaam 2017), but to date, only one GM animal, AquAdvantage® Atlantic salmon[1], has been approved for food consumption by the United States Food and Drug Administration (FDA). Approval has not been sought or granted in any other jurisdiction. On the contrary, on the plant side, a number GM plants have been approved for cultivation, food and feed consumption. These include, for example, varieties of maize, soybean, rice, canola, and cotton[2]. In general, GM plants have been developed to improve productivity, increase nutritional components of their products, or to make them more resistant to production challenges such as biotic and abiotic stresses.

More recently, there have been significant advancements in animal and plant biotechnology through the application of *Gene editing* technologies. Application of this technology has wider applications than GM as the technique allows the highly specific direct interrogation and genetic manipulation of targeted DNA sequences within the genome of an organism.

[1] https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/ucm473238.htm
[2] http://www.isaaa.org/

The process of gene editing refers to the use of a set of reagents to precisely find and alter a specific part of the genome, analogous to the cut and paste function of a word processing computer program. Much of the intended applications in animal and plant genetics uses this approach to turn off (knock-down), turn on (knock in), or provide a different form (alternate combination “haplotype”) to the function of a gene.

Importantly, gene editing differs from the traditional GM approaches as it does not necessarily integrate a novel DNA sequence or engineered gene construct into the genome of the targeted plant or animal. Rather, there are multiple approaches to effect an alteration to the function of an existing gene or add a new function/gene to the target (see Section 3).

This is a rapidly evolving scientific field that has the potential to step change agricultural and food production and as such, governments around the world are struggling to determine if and how such technologies could or should be regulated. This is especially relevant to animal production that has not benefited from the commercial application of earlier gene technologies.

The demand for animal protein is increasing globally. The combined factors of population growth, shifting of socio-economic positioning of a large population, more frequent climatic disturbances, limits to expansion of agricultural area, and product competition within the agricultural area are already putting pressure on livestock production. Additionally, there is a growing interest from the general population to where their food comes from and how crops and livestock are bred, which draws focus on how industries address issues such as sustainability, quality and animal welfare. The social licence (“community approval”) in food production is expected to increase and demand that the values of agricultural producers are in sync with those of the community.

The broad adoption of reproductive and genetic biotechnologies offer a great opportunity for the livestock industry, assisting in productivity improvements from on-farm operations to meat processing plants as well as addressing animal welfare and biosecurity concerns.

This review focuses on gene editing technologies, its potential benefit and risks to the red meat industry. The benefits relate to its adoption and application, while the perceived risks are mainly related to the use of technology *per* se and to public perception (nationally and internationally) on the safety of potential products and potential impacts on international trade.

# 3 Review the current and possible future technologies for gene editing in cattle, sheep and goats

## 3.1 Overview

Gene editing refers to biotechnologies that allows precise changes to the genome (DNA) of an organism (e.g. animal, plant, and microbe). As the name suggest, these methods allow researchers to delete, add or replace letters (nucleotides) in the genetic code, similarly to the spell check of a writing software. The biotechnology is comprised of two main components, an enzyme that cuts the DNA and a guide that brings the enzyme to a predetermined exact position in the genome. Additionally, some applications also use a repair template in the reaction. If used, the nature of a repair template (small or large) will define the category of gene editing that the procedure aligns to.

Gene editing is different than traditional genetic engineering. During traditional genetic engineering of an animal, DNA (e.g. a gene) from another breed or species, is added to the genome of the target animal, resulting in a transgenic animal since it carries the gene(s) of more than one breed or species. On the other hand, gene editing targets the addition, deletion or replacement of one or a few nucleotides, and does so at very precise location of the genome. These changes may result in an animal with desired characteristics (see Section 3.4).

There are many potential applications for gene editing within animal science. For example, it can be used to correct for known genetic diseases. It could also be used to change a less desirable form of a gene to a form that will positively impact the desirable trait. Moreover, this method allows the introgression of a favourable form of a gene without the need of outcrossing followed by backcrossing, which disrupts long term animal selection.

Gene editing applications for livestock does, however, require integration with advanced reproductive biotechnologies to ensure delivery of the desired changes. The combination of these two biotechnologies has the potential to rapidly advance high genetic merit animals and positively impact the broader red meat industry.

## 3.2 Gene editing: introduction

Gene editing refers to biotechnologies that allow small and precise changes to the genome (DNA) of an organism (e.g. animal, plant, and microbe)[3]. It uses site-directed nucleases (SDN), which are enzymes that creates double-stranded DNA break at precise and predetermined location of the genome. There are at least three generations of site-directed nucleases (SDN) that allows gene editing in mammalian species: zinc-fingers nucleases (ZNF), transcription activator-like effector nucleases (TALENS), and, the latest, clustered regulatory interspersed short palindromic repeats (CRISPR).

After the DNA break, the cell uses its repair system in one of two ways, non-homologous end joining (NHEJ) or homologous recombination (HR), which uses a nucleic acid template that is homologous at both sides of the DNA break. The outcome of these processes result in random mutations or precision gene edit, respectively (Figure 1). The cell's repair system

[3] A good definition within the animal science application can be found at: http://articles.extension.org/pages/73389/what-is-gene editing

is highly efficient, but it is error prone. Although the DNA is cut at a precise location, the cell will repair it at random using non-homologous end-joining often generating different minor outcomes in the form of minor insertions and deletions. However, if a designed template for repair is provided (homologous recombination) a precise “edition” can be introduced as small deletions, insertions, or a DNA base substitution. Using these versatile gene editing tools, scientists can find a specific gene in a genome, precisely cut the DNA within that gene and “edit” it, making the desired change in the DNA without leaving any foreign material (Barrangou & Doudna 2016).

As described above, transgenic approaches differ from gene editing methods. By definition, transgenic biotechnologies involves the incorporation of DNA (or gene) from one organism into the genome of another species. There are reports in the literature of transgenic cattle, goat, sheep, fish species and chicken, all aimed at improving production-related traits (Laible *et al.* 2015; Lievens *et al.* 2015). The scientific transgenic community is also making use of these modern biotechnologies to add a gene or a gene construct to a precise genomic location of an organism. Thereafter, it is important to differentiate the applications leading to transgenic organisms to those that do not incorporate foreign genetic material, not leading to transgenic organisms.

## 3.3 Gene editing: types of genetic manipulation currently under consideration for application in animal breeding

Independently of the editing method of choice (e.g. ZNF, TALENS or CRISPR), outcomes can be grouped into three broad categories that are named: Site-Directed Nuclease type 1 mutations (SDN-1), Site-Directed Nuclease type 2 mutations (SDN-2) and Site-Directed Nuclease type 3 mutations (SDN-3) (Figure 1).

**SDN-1**

**Description:** Usually derived by the internal repair system of a cell through non-homologous end-joining. This relates to the random repair of a double-stranded DNA break without a template. This method often creates deletions or insertions of some DNA bases at the break/repair site.

**Outcome:** The repair system in this case can be error prone, often leading to small deletions or insertions.

**Application:** If the break/repair site is at a gene, this method could lead to a not-functional gene, in another words, this method can be used to inactivate or “turn off” a target gene.

**SDN-2**

**Description:** Usually derived by homologous recombination, which uses a precise template during the repair of a double-stranded DNA break. Since this template is designed by the user, it can be made in such way that it leads to small precise insertions such as single or a small number of DNA base substitutions.

**Outcome:** Precise allele substitution, small known insertions or small known deletions.

**Application:** This method can be used to introduce a known favourable sequence change or new section into a gene or to replace/fix a known detrimental allele (e.g. disease-related allele) with an allele associated with a healthy status.

**SDN-3**

**Description:** Similar to SND-2, this process utilises homologous recombination, and uses a template during the DNA repair. However, the difference lies on the size of the genetic template. In some instances, the template may be an entire gene (or an active DNA sequence).

**Outcome:** Precise insertion of a relative long DNA fragment, which could incorporate or replace an entire gene.

**Application:** This method can be used to introduce or replace a whole gene into the genome of a target. It is important to note that most of the scientific community consider a product from SDN-3 as genetically modified or transgenic and not a gene edited product.

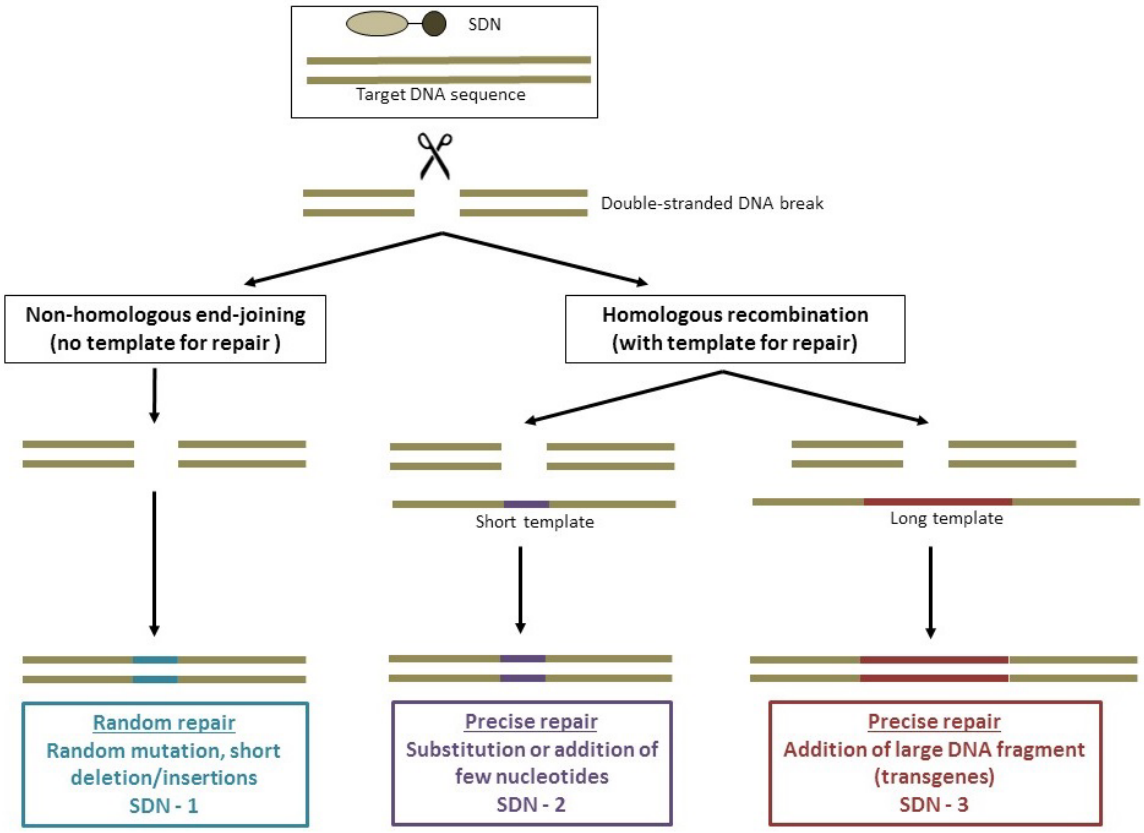



**Figure 1. Schematic representation of three systems used by cells to repair the double stranded DNA break made by site-directed nucleases (SDN).** Non-homologous end-joining uses an error prone process to re-join the ends of the double-stranded DNA breaks, leading to a called SDN-1 mutation, while homologous recombination uses a template for repair that leads to SDN-2 or SDN-3 depending on the type (length and content) or the template used (adapted from Tan *et al.* (2016); Van Eenennaam (2017)).

## 3.4 Gene editing: current and future applications

### *3.4.1 Current applications in livestock*

There are many potential applications for gene editing within the red meat industry. Most of them relate to improving animal/carcase productivity, disease resistance, animal welfare, and meat composition. For example, it be used to “turn off” a gene known to impact productivity, or to correct alleles causing a specific disease or genetic disorder, or to introduce specific alleles into the population. It is worth noting that most of the applications intend to introgress known genetic variations that have been identified and characterised from a population. Further, the outcome may also be obtained by mating or chemical/radiation mutagenesis, however, these methods also have genome wide non target changes that may lead to detrimental effects.

A key feature is that the use of gene editing allows the introgression of favourable alleles without disrupting long-term, long-standing animal selection. An often cited example is for the presence or absence of horns, horned – polled in dairy cattle. For many generations dairy cattle have been selected to improve their dairy attributes from the milk composition to their health-related traits, but little attention was given to the presence of horns, resulting in a majority of cattle being horned. Using classical animal breeding it is possible to introgress the *POLLED* allele into a dairy population; first dairy animals would have to be crossed to polled cattle to bring the allele causing polled, then a series of backcrosses would have to be performed to recover the “loss” in dairy attributes that the first cross had caused. Even though this process is possible, it is impracticable due to the impact in dairy attributes, and the time in generations to its recovery, likely decades. Using gene editing, on the other hand, it is possible in one generation to introgress the polled allele without disrupting the dairy attributes. Here a dairy example was given, but wherever there is long-term selection, and backcrossing is not the best option for the introgression of a known allele, gene editing can contribute.

More than 300 gene-edited individual livestock have been generated in the last few years (Proudfoot *et al.* 2015; Tan *et al.* 2016). The successful introgression of the *POLLED* allele might be the most publicised one (Tan *et al.* 2013; Carlson *et al.* 2016). However, there are other success examples, aiming at increased carcase yield, not surprising, the master regulator of muscle development – *Myostatin* gene, was targeted in cattle, goats, sheep and pigs. The edited animals were reported as being normal with visually increased muscularity (Ni *et al.* 2014; Proudfoot *et al.* 2015; Wang *et al.* 2015). Increased disease resistance is another desired trait that has been targeted in livestock, in a recent proof-of-principle work it has been demonstrated the possibility for the development of lineage of cattle genetically resistant to pneumonia caused by *Mannheimia haemolytica*, a causative agent of respiratory disease and calves (Shanthalingam *et al.* 2016). More examples are listed in Section 4, where other applications are presented and discussed together with the evaluation of their potential benefits to the industry.

The areas to which gene editing have been considered to assist improvements are not new. Breeders have been aiming in improving livestock productivity, disease resistance, animal welfare, and meat composition for many generations, with variable success in one or the other area. Classical animal breeding has assisted and guided this process toward breeding

objectives. It is likely that gene editing will complement those traditional animal breeding programs, and not replace or disrupt them (Van Eenennaam 2017).

### *3.4.2 The benefit of combining gene editing with reproductive biotechnologies*

Gene editing will only create an impact in the red meat industry if it is integrated with animal breeding programs and applied together with advanced reproductive biotechnologies. The efficiency of producing an edited animal is still low, so only a small number of animals will be generated. These edited sires and dams will have to be well managed so that their genescann be rapidly disseminated through use of an efficient reproductive program. Examples of these technologies are semen collection for artificial insemination, multiple ovulation embryo transfer, oocyte pick-up, *in vitro* fertilization or embryo transfer. Reproductive biotechnologies are paramount for gene editing success, from the generation of edited animals to the dissemination of their genetic potential into populations

Currently, two methods have been used in the gene editing process to generate l livestock; cloning by somatic cell nuclear transfer and zygote direct editing. Each approache has positive and negative points. During the cloning process, the edited cell line that will be the nucleus donor, can be genotyped (and/or sequenced) to confirm the editing efficiency before its use to generate an embryo, and pregnancy, which is a great advantage. However, the cloning process is known to have low efficiency, high early embryonic loss, postnatal deaths and birth defects. Zygote editing is normally done by intracytoplasmic injection of gene editing reagents, it has higher efficiency than cloning, and generates fewer reproductive problems. The main drawback is the inability to genotype or screen the embryo before implantation. More embryos, and offspring are produced, but not all of them will be correctly edited. Additionally, a proportion of them will be mosaics, which mean that some but not all cells of the animal will be edited. A mosaic bull, for instance, will potentially generate both edited and non-edited sperm cells (Proudfoot *et al.* 2015). The improvement of protocols for more efficiently generating edited livestock, either via cloning using edited cell lines, or direct editing of zygotes is a highly active field of research.

### *3.4.3 Future applications*

Gene editing is a fast evolving scientific field with direct implications on human health, animal and plant breeding, pharmaceutical industry, etc… These interests combined are pushing the field forward rapidly. Cutting edge experiments using human cells and laboratory animals, will positively impact livestock breeding resulting in benefits to food production. A list of some key future applications is presented below.

**Different types of genetic manipulation.** Future advances in gene editing will make the process more robust, efficient, user-friendly, and with reduced error rate. These improvements will come mainly as better laboratory protocols are developed, and new enzymes (site-directed nucleases) are discovered and fine-tuned. Nowadays gene editing contemplates the following genetic manipulations: deletion, insertion, allele substitution, and gene knockout. These manipulations will certainly be improved with more precise and efficient protocols. Additionally, there are already preliminary protocols for transcriptional activation and repression (increase or decrease the expression of a gene), the fusion of fluorophores on the enzymes allowing real time visualization of the genome, and the possibility to engineer epigenetic changes in the genome (Barrangou & Doudna 2016).

Beyond site-specific genomic control, these technologies extend to both transcriptional and epigenetic changes, giving great control of the functional genome.

**Gene editing of multiple targets in a single procedure.** In dealing with polygenic traits or traits influenced by many genes, a system allowing gene editing of multiple targets at once needs to be developed. Working towards this objective, an orthogonal approach using a guide RNA - Cas9 system was developed, it potentially allows users exploit different CRISPR tools for distinct applications in the same cell (Briner *et al.* 2014). It is expected that many research groups are working towards these goals.

Gene editing offers the possibility to better utilize scientific resources that are already available, and to fill gaps of knowledge on gene function. There is large amount of genomic information that should be better exploited, including results from multiple genome-wide association studies, discovery of causative mutations, and the detection of millions of genetic variants derived from whole-genome sequencing projects, in theory all of those variants that impact production traits could be interrogated using gene editing tools. However, it is known that most production traits are influenced by multiple gene variants. Because not all of them can be targeted at once or even in a few generations, so prioritisation would is required so that genetic variants with larger effects would be targeted first (Jenko *et al.* 2015). In recent study it has been shown that combining gene editing with traditional genomic selection could improve response to selection four-fold after 20 generations (Hickey *et al.* 2016). Traditional animal breeding, genomic selection based programs and gene editing are all complementary.

**Improvement on gene editing scalability.** If gene editing is to have impact on animal production systems, the capacity in producing edited animals needs to be improved. Cloning is currently the method of choice, but, as mentioned before, its efficiency is low, the alternative method of zygote injection while better on scalability, is still labour intensive and has the potential for mosaicism. Considering that cloning has a little more than 20 years of history, including the development of alternative approaches (e.g. Verma *et al.* 2015) to the original method (Wilmut *et al.* 1997), its efficiency has not greatly improved over many years. Therefore , one could tentatively expect that big improvements will occur with zygote manipulation. An alternative method for zygote injection for delivering gene editing reagents is the use of electroporation. There are already some proof-of-principle article published using mice (Qin *et al.* 2015; Hashimoto *et al.* 2016), this method is appealing because it allows the treatment of hundreds of zygotes simultaneously. At the moment its efficiency remains low, but improvements in reagents and laboratory procedures might refine this application. The big driver for improvement in enzymes and protocols is led by the human health and human genetics interests. However if animal scientists are likely to lead the field in scalability which is required for production animals.

**Integration of advanced animal breeding and gene editing to shorten generation interval and speed up dissemination of high merit genes.**.

A potential approach for such combination could be as follows (Figure 2) (Adapted from Kasinathan *et al.* 2015; Van Eenennaam 2017):

1) A cow and sire with high genetic merit are selected and several embryos are produced using *in vitro* fertilization;

2) All of those embryos are transferred to one surrogate mother, and collected by flushing two-weeks from implantation;
3) Recovered embryos are then transferred to the laboratory, where cell lines are developed for each of them;
4) Each cell line is genotyped, and genomic selection is applied to estimate the genetic merit of each line;
5) The best cell line, goes to a gene editing step to further improve its genetic merit. After the procedure, the cell line would be screened for evaluation of gene editing efficiency and quality control;
6) An edited cell is used for cloning via somatic cell nuclear transfer, and transferred to a surrogate mother. Nine months later, calves with high genetic merit are born.

**Figure 2. Gantt chart of a possible combination of advanced reproductive biotechnology, genomic selection and gene editing to breed a high genetic merit animal in less than 1.5 year.**

| | | Months | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Field** | **Activity** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| Reproductive biotechnology | Elite sire and cow selected | | | | | | | | | | | | | | |
| | *In vitro* fertilization | | | | | | | | | | | | | | |
| | Embryo transfer | | | | | | | | | | | | | | |
| | Collect foetuses | | | | | | | | | | | | | | |
| Genomic selection | Establish fibroblast cell lines | | | | | | | | | | | | | | |
| | Genotype each line | | | | | | | | | | | | | | |
| | Genetic merit evaluation - select cell line with best genetic merit | | | | | | | | | | | | | | |
| Genome editing | Genome editing of selected cell line | | | | | | | | | | | | | | |
| | DNA analyses to confirm gene editing and no undesirable effects | | | | | | | | | | | | | | |
| Reproductive biotechnology | Genetically enhanced fibroblast used as nuclear donor | | | | | | | | | | | | | | |
| | Embryos transfer | | | | | | | | | | | | | | |
| Final Product | High genetic merit genome-edited calves | | | | | | | | | | | | | | |

Alternatively, to avoid the cloning step, the gene editing procedure could be applied to *in vitro* produced embryos from a cow and sire with high genetic merit using electroporation or microinjection. Then all embryos could be transferred to surrogate mothers and test editing in the calves born. The main drawback is that not all resulting calves will carry the edited genome. However, if the original parents are of high genetic merit, the offspring will also be of high merit which may compensate for lower than 100% efficiency.

# 4 Evaluate how gene editing and cloning sits within the regulatory framework in Australia and relevant trading partners

## 4.1 Overview[4]

Australian research or commercial production that involves gene technology is primarily regulated by the Office of the Gene Technology Regulator (OGTR) and Food Standards Australia New Zealand (FSANZ).

Recently, the regulation of gene editing has received considerable attention, with regulatory agencies considering if and/or how editing technologies align with existing legislation. The current regulatory system in Australia remains unchallenged with no commercially relevant products put to the competent authorities for consideration. Similarly, Australia's key trading partners are considering the regulatory status of editing technologies and their applications to agriculture and biomedicine.

Further, genetically modified and cloned animals may also be subject to state and territory government animal welfare legislation applicable to animals used for scientific purposes, as well as the Australian code for the care and use of animals for scientific purposes. As such, regulatory implications of potential gene edited animal products should be considered on a case-by-case basis.

## 4.2 Livestock cloning

"Cloning" is a term traditionally used by scientists to describe different processes for the duplication of biological material. In simple terms, livestock cloning refers to somatic cell nuclear transfer where a nucleus from a cell isolated from body tissue is placed into an enucleated egg cell. The resulting cell is implanted into the uterus of a surrogate mother where pregnancy may continue to term and a near copy of the donor animal is produced.

Cloning animals offers breeding programs a reliable way of reproducing superior livestock genetics and ensuring herds are maintained at the highest quality possible. However, there are a relatively small number of cloned animals (all cattle) in Australia, primarily being used for breeding purposes only. No cloned animal products are directly introduced into the food supply chain.

In the preparation of this report, the consultants understand that since 2012 approximately 25 beef cattle (mainly Brahman) and 10 dairy animals (mainly Holsteins) have been cloned. Some of the animals have been registered in accordance with strict industry regulations and guidelines. The rationale for cloning appears to be focused on maximizing and extending superior breeding stock.

Animal cloning is not subject to specific animal cloning legislation. FSANZ have stated that food from cloned animals and their progeny does not require pre-market approval in Australia and New Zealand before entering the food supply and no special labelling

[4] The Productivity Commission from the Australian Government has recently published a comprehensive review on Regulation of Australian Agriculture, which has some references to the use of genetic technologies. The full report can be found at: http://www.pc.gov.au/inquiries/completed/agriculture/report

requirements apply. However, like all food products, foods derived from cloned animals must comply with existing food laws, including relevant standards in the Food Standards Code. Under this Code, State and Territory food laws require that all food sold is safe and suitable. Further, cloned animals used for scientific purposes are also subject to State and Territory government animal welfare legislation including the *Australian code of practice for the care and use of animals for scientific purposes*[5]. RSPCA (Royal Society for the Prevention of Cruelty to Animals) Australia also advocates that all cloning and genetic manipulation of animals should be conducted in accordance with this code (RSPCA Policy D2 Genetic Manipulation).

Internationally, many countries have conducted research on animal cloning including Argentina, Australia, Brazil, Canada, Chile, China, France, Germany, Japan, New Zealand, South Korea, United Kingdom and the United States. However, animal cloning mostly occurs in Argentina, Brazil and the USA in the agricultural sector. More recently, China has indicated plans to clone significant numbers of cattle to meet the growing demand for beef.

The US Food and Drug Administration[6], European Food Safety Authority[7] and Japan Food Safety Commission[8] have all undertaken risk assessments on livestock cloning and have concluded that food products from cloned animals and their offspring are as safe as food products from conventionally bred animals. FSANZ has reviewed these assessments and agrees with the findings.

## 4.3 Regulation of gene editing in Australia

Compared with traditional breeding methods, new technologies such as gene editing provide obvious advantages. However, many governments are struggling to establish a clear distinction between these new approaches and GM technologies and whether they require regulation or not. Many GM regulatory systems, such as the Australian system, are process focused and not product based. This presents a major challenge to many technology developers looking to produce and improve products through new approaches.

There are four key regulatory bodies in Australia that consider both the process of product development and the final product itself:

1. Office of the Gene Technology Regulator (OGTR).
2. Food Standards Australian and New Zealand (FSANZ).
3. Australian Pesticides Veterinary Medicines Authority (APVMA).
4. Therapeutic Goods Administration (TGA).

### *4.3.1 Office of Gene Technology Regulator*

The Office of the Gene Technology Regulator (OGTR) administers the federal *Gene Technology Act 2000*. The object of the Act is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and

[5] Australian code of practice for the care and use of animals for scientific purposes
[6] US FDA (2008) - Animal cloning: a risk assessment
[7] European Food Safety Authority (2012) - Update on the state of play of Animal Health and Welfare and Environmental Impact of Animals derived from SCNT Cloning and their Offspring, and Food Safety of Products Obtained from those Animals
[8] Japan Food Safety Commission (2009) - Risk assessment report on foods derived from cloned cattle and pigs produced by somatic cell nuclear transfer (SCNT) and their offspring (Novel foods).

managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The legislation regulates the process of creating products with 'gene technology' rather than the products themselves (*cf.* Canadian novel foods regulations[9]). As such, the breadth of products that may be covered by the Act is vast and requires case by case assessment.

Within the associated Gene Technology Regulations, Schedule 1 and Schedule 1A provide the current list of organisms that are not considered to be genetically modified (Table 1) and techniques that are not considered to be gene technology (Table 2). Note, Item 1 in Table 2 refers to Somatic cell nuclear transfer (cloning) as a technique that is not considered gene technology and therefore is not regulated under the Gene Technology Act 2000.

**Table 1. Schedule 1: Organisms that are not GM organisms (Regulation 5)**

| Item[1] | Description of organism |
|---|---|
| 1 | A mutant organism in which the mutational event did not involve the introduction of any foreign nucleic acid (that is, non-homologous DNA, usually from another species). |
| 2 | A whole animal, or a human being, modified by the introduction of naked recombinant nucleic acid (such as a DNA vaccine) into its somatic cells, if the introduced nucleic acid is incapable of giving rise to infectious agents. |
| 3 | Naked plasmid DNA that is incapable of giving rise to infectious agents when introduced into a host cell. |
| 6 | An organism that results from an exchange of DNA if:<br>(a) the donor species is also the host species; and<br>(b) the vector DNA does not contain any heterologous DNA. |
| 7 | An organism that results from an exchange of DNA between the donor species and the host species if:<br>(a) such exchange can occur by naturally occurring processes; and<br>(b) the donor species and the host species are micro-organisms that:<br>(i) satisfy the criteria in AS/NZS 2243.3:2010 for classification as Risk Group 1; and<br>(ii) are known to exchange nucleic acid by a natural physiological process; and<br>(c) the vector used in the exchange does not contain heterologous DNA from any organism other than an organism that is involved in the exchange. |

[1] Note: Item numbering is as per the Regulations.

[9] Under the Food and Drugs Act and Regulations, all novel foods (including novel GM foods) must be assessed by Health Canada before they can be sold in Canada.

**Table 2. Schedule 1A: Techniques that are not gene technology (Regulation 4)**

| Item | Description of technique |
|---|---|
| 1 | Somatic cell nuclear transfer, if the transfer does not involve genetically modified material. |
| 2 | Electromagnetic radiation-induced mutagenesis. |
| 3 | Particle radiation-induced mutagenesis. |
| 4 | Chemical-induced mutagenesis. |
| 5 | Fusion of animal cells, or human cells, if the fused cells are unable to form a viable whole animal or human. |
| 6 | Protoplast fusion, including fusion of plant protoplasts. |
| 7 | Embryo rescue. |
| 8 | *In vitro* fertilisation. |
| 9 | Zygote implantation. |
| 10 | A natural process, if the process does not involve genetically modified material. Examples of natural processes include conjugation, transduction, transformation and transposon mutagenesis. |

Schedule 1 and Schedule 1A (Table 1 and Table 2) are clearly out of date and scientifically you could argue that products developed with gene editing technologies are not genetically modified (in a scientific sense). However, consultation with the OGTR indicates that embedded within Australian legislation is the requirement to regulate if there is any level of uncertainty. That is, where there is no clear and obvious alignment with the Schedules, the Regulator is left with no choice but to regulate. As such, the OGTR would currently require a producer of an edited animal to apply for a licenced dealing for any intentional release to the environment of a product.

**So where do gene edited products fit within the current Australian Regulations?**

To determine if products of certain "processes" require regulation, the Regulator must consider each product on a case by case basis. The OGTR is currently reviewing Schedule 1 and Schedule 1A to see if there are opportunities to clarify what is gene technology and what is a Genetically Modified Organism.

In October 2016, the OGTR released a discussion paper "Options for regulating new technologies"[10] under a Technical Review of the Gene Technology Regulations 2001. The primary aim of the review was to provide clarity about whether organisms developed using new technologies are subject to regulation as genetically modified organisms and ensure that new technologies are regulated in a manner commensurate with the risks they pose.

The technical review focused on new technologies and examined:

- cases where the capture or exclusion of these techniques is not clear, and whether those new technologies should be regulated, and
- scientific evidence relating to risks posed as a result of using new technologies.

[10] OGTR (2016) Options for regulating new technologies

The discussion paper examined 4 options for how the Regulator might consider new technologies:

**Option 1.** No amendments to the regulations.

**Option 2.** All oligo-directed mutagenesis and all SDN technologies are regulated.

**Option 3.** Some technologies could be excluded based on whether a template was used or not. As such, technologies that employed an SND-1 approach would not be regulated. Oligo-directed mutagenesis and SDN-2 and SDN-3 would, however, be regulated.

**Option 4.** The use of technologies would be excluded if genetic changes are similar or indistinguishable from products of conventional breeding. This would exclude oligo-directed mutagenesis, SDN-1 and SDN-2 from regulation, but the process of SDN-3 would be regulated.

This discussion paper canvases four broad options for how clarity about regulation of specific new technologies could be achieved. The Regulator sought submissions from interested parties on the merits of these options, in particular in response to a set of consultation questions. The submissions[11] have been made available by the Regulator. Outcomes from this review are expected in May 2017.

**Current Regulatory Assessment:** Research and commercial products developed using gene editing technologies would require a licence from the OGTR. The use of gene technology to develop the products means they are regulated items under the current *Gene Technology Act 2000*.

### *4.3.2 Food Standards Australia and New Zealand*

All genetically modified foods intended for sale in Australia and New Zealand must undergo a safety evaluation by Food Standards Australia New Zealand (FSANZ). FSANZ will not approve a GM food unless it is safe to eat. Therefore, if the OGTR determine a product has been developed using gene technology and requires regulation, then FSANZ will also need to consider whether a change to the Australia New Zealand Food Standards Code (the Code) is required. In many cases the data requirements provided to the competent authorities are similar.

In Australia, GM foods are regulated under Standard 1.5.2 – Food produced using Gene Technology, contained in the Australia New Zealand Food Standards Code. The standard (an enforceable regulation) has two provisions – mandatory pre-market approval (including a food safety assessment) and mandatory labelling requirements. This Standard ensures that only assessed and approved GM foods enter the food supply. The safety assessment process used by FSANZ is described in detail in the Guidance Document[12]. Under some circumstances, proponents may also require an application to change Standard 1.5.1 Novel Foods.

[11] Submissions to the OGTR Technical Review "Options for regulating new technologies
[12] Safety Assessment of Genetically Modified Foods

In contrast to OGTR, FSANZ assesses the final product for safety rather than the process itself, all be it the assessment looks at the process of product development. Further, the definitions that FSANZ are guided by differ from those of the OGTR. FSANZ definitions are:

- ***food produced using gene technology*** means a food which has been derived or developed from an organism which has been modified by gene technology
- ***gene technology*** means recombinant DNA techniques that alter the heritable genetic material of living cells or organisms.

At the time of preparing this report, FSANZ have assessed and approved 71 products under Standard 1.5.1. Most companies exporting biotech based products seek approval from FSANZ and other jurisdictions prior to going to market. The FSANZ approval is seen as an import approval and an insurance against adventitious presence with respect to trade. The desirable feature of the FSANZ system is that the assessment process is time bound, therefore for a commercial company there is an element of certainty around regulatory approval. This contrasts with many other systems around the world (e.g. Canada and China).

In March 2016, FSANZ released a new Handbook[13] for applicants that details the requirements and data that must be provided to FSANZ for assessment. Under the requirements for Standard 1.5.1, new sections require applicants to provide safety data around RNAi and siRNA. Previously, the focus of assessment has been on new or novel proteins. This change has been geared to address public concern over the use of RNAi technology.

FSANZ are well respected globally and Australia's key export countries, particularly in Asia, look to FSANZ for leadership in the assessment of biotechnology based products. FSANZ, and OGTR, are well engaged globally through OECD and ASEAN working groups that provide governments with policy advice on the regulation of biotechnology based products.

In 2012 and 2013, FSANZ convened an expert scientific panel to provide advice on how to regulate different plant breeding techniques (New plant breeding techniques workshops[14]).

Participants from the 2013 workshop concluded:

- food produced from plants developed using accelerated breeding following induction of early flowering would be similar to food produced using a conventional plant breeding approach and should not be regarded as GM food
- where targeted mutagenic techniques are used to introduce small, site-specific mutations involving only one or a few nucleotides, and any transgenes have been segregated away from the final food producing lines, derived food products would be similar to food produced using traditional mutagenic techniques and should not be regarded as GM food

[13] FSANZ Application Handbook
http://www.foodstandards.gov.au/code/changes/pages/applicationshandbook.aspx
[14] New plant breeding techniques workshops. Kingston, Australia: Food Standards Australia New Zealand; 2014: Available from: http://www.foodstandards.gov.au/consumer/gmfood/Pages/New-plant-breeding-techniques-in-the-spotlight.aspx

- when targeted mutagenic techniques are used to insert new genes, they are equivalent to transgenesis and, as such, any food products should be regarded as GM
- food products derived from plants using the technique of Agro-infiltration will be purified proteins, and the plants in which they are produced will likely not themselves be used as food. Whether the purified protein products are regarded as GM foods would depend on their use and whether the plants from which they are derived are themselves GM.

Therefore, there are basically 3 categories that could determine regulation of products:

**Category 1:** Comprises cisgenesis, intragenesis, some uses of Site Directed Nucleases and GM rootstock grafting. Products derived from these techniques would be regarded as GM, although a simplified form of safety assessment may be warranted.

**Category 2:** Includes Oligo Directed Mutagenesis and some uses of Site Directed Nucleases, where products derived from them would not be regarded as GM.

**Category 3:** Comprises gene technologies at an early stage that are separated from the final product during the breeding process, such as reverse breeding. For products in this category, the panel concluded that they are not GM, but there is a need to confirm the reliability of the breed out process.

In addition to the expert consultations by FSANZ, the Environmental Protection Agency of New Zealand determined that certain products derived from new technologies were not considered GMO under New Zealand regulatory definitions. This was not a product by product determination, but rather applicable on the technologies in general. However, this administrative decision was defied in the High Court, which ruled that the EPA did not have authority to decide this since it is a legislative matter in New Zealand[15].

The above provide some clues as to how FSANZ may consider certain products with respect to changes to the Food Standards Code.

**Current Regulatory Assessment:** Some products produced using new technologies may require a change to the Food Standards Code. If products are deemed Category 1, as described above, then FSANZ approval would be required. The system is, however, untested and requires a case-by-case assessment.

### *4.3.3 Australian Pesticides and Veterinary Medicines Authority*

The National Registration Scheme for Agricultural and Veterinary Chemicals (National Registration Scheme) was established under Commonwealth and state and territory legislation and ensures that such products are:

- effective on target species
- applied in accordance with relevant and endorsed resistance management strategies

[15] Mokena-Lodge R. Sustainability Council case provides clarity to NZ GM regulations [Internet]. [Wellington(NZ)]: Available from: http://mcguinnessinstituteblog.org/2014/05/22/sustainability-council-case-provides-clarity-to-nz-gm-regulations/

- safe when exposed to humans and non-target species either through direct exposure or residues in treated food stuffs
- not a risk to the environmental
- labelled and packaged correctly.

The Department of Agriculture manages the legislation under which the National Registration Scheme operates. The APVMA, formerly known as the National Registration Authority for Agricultural and Veterinary Chemicals (NRA), sits within the Agriculture portfolio and is an independent statutory authority that administers the National Registration Scheme. The APVMA is responsible for the registration, quality assurance and compliance of pesticides and veterinary medicines up to the point of sale. This includes regulation of agricultural chemicals or medicines produced in, or used on, GM crops. The states and territories are responsible for control of use of pesticides and veterinary medicines.

Companies that seek to register a product for commercial use are required to provide the APVMA extensive data supporting product efficacy as well as the safe and environmentally friendly status of the product. As part of the assessment process, the APVMA receives input from other Commonwealth agencies, including:

- Australian Government Department of Health
- Australian Government Department of the Environment
- Australian Government Department of Agriculture
- Food Standards Australia New Zealand
- Office of the Gene Technology Regulator

Section 14 of the *Agricultural and Veterinary Chemicals Code Act 1994* establishes that the APVMA is required to register an agricultural chemical product when it is "**satisfied**" that a range of issues have been addressed. Prior to granting registration of an agricultural product, the APVMA must be satisfied that a product will:

- be effective for all the uses claimed
- be safe to humans, target and non-target species
- not pose unacceptable risks to the environment or trade with other nations.

New technologies used in crop plants or microbial systems to create bio-active fed to animals may require APVMA approval. Similarly, novel products and some stock feeds may also require APVMA consideration. Direct fed microbial products, enzyme products, pre and pro biotics, nutritional supplements and therapeutic pet foods may also require consideration if they do not meet the End Products[16] test.

**Current Regulatory Assessment:** Some products produced using new technologies may require registration with the APVMA. The APVMA has not made any statements regarding miRNA or gene editing.

[16] APVMA End Products test

### *4.3.4 Therapeutic Goods Administration*

Products developed with new technologies for the livestock industry would generally not require TGA assessment or approval.

### *4.3.5 Regulation of gene editing in key markets*

The novel nature of gene editing has led to discussions on whether the use of such techniques generate products that are, or should, be subject to legislation. As discussed above, there remains uncertainty in Australia surrounding this topic. Similarly, other countries are considering their positions on the regulatory status of gene editing. The resulting regulatory uncertainty creates a barrier into the adoption of these novel techniques.

Many of Australia's trading partners are reviewing and considering the science and regulatory status of gene editing techniques. At this stage, most countries appear to be waiting for other countries or international organisations to take a lead position. This issue is complicated by a lack of harmonisation of existing biosafety legislation, whereby some jurisdictions are process focused (e.g. the EU and Australia etc.) whilst others are product orientated (e.g. US and Canada). Further, the reluctance of countries to openly discuss the topics of gene editing or take a definitive position is likely related to the political and economic effects of an approval in an environment of uncertainty.

Importantly, for the Australian livestock sector, it is a sensitive issue in many trading countries with international trade relations influenced by the position of the government. This is mostly due to the expected difficulty in detection or identification of products derived from gene editing when compared to products obtained through "conventional/traditional breeding" approaches. As such, it is important for the livestock sector to determine if the proposed benefits outweigh any uncertainty and risk to import and export markets and actively contribute to the discussion, and support government at a global level.

Currently, it is not possible to articulate the final positions of key markets, since decisions are not dependent on scientific rationale alone, but also heavily influenced by international relations, and political and public debate. However, what is currently understood is presented in **Section 8, Table 3**.

# 5 List and evaluate the potential benefits to the red meat industry

## 5.1 Overview[17]

There are opportunities for improvement across the whole food chain via applications of gene editing technologies. Areas of recognised opportunities include, but are not limited to, improvement of productivity (on- and off-farm), animal welfare, disease prevention and for the use of gene editing during the development of other livestock products (e.g. livestock feed and supplements). It is highlighted some "short-term"/realistic applications relevant to industry, in the sense that these are currently subject of active research. It is worth noting that world-wide genomics projects based on next-generation sequencing of whole-genome are delivering many causative targets that could be further explored using gene editing for livestock improvement.

## 5.2 Productivity

There are opportunities for improvement across the red meat food chain. In general terms, if costs are controlled, improvement in productivity can be achieved by increasing on-farm production, increasing off-farm production, and increasing quality of the product generating better return.

### *5.2.1 Increasing on-farm productivity*

**Improving female and male reproduction.** There are some candidate SNP with solid association to reproductive traits that could be tested. The gene *BMPR-IB* has been associated to variation in litter size in sheep. In a recent study (Zhang *et al.* 2017), this gene was targeted producing knockout embryos that, if born, would be expected to have higher reproductive rate than their counterparts. This is the first report of gene editing targeting reproductive improvement. More work on this line can be expected in the near future, including the description of these edited sheep.

**Improving feed conversion efficiency.** There are some candidate SNP associated to the traits, but they require further validation. There are research programs in this space, at least, in Australia, USA and Brazil.

**Improving dairy attributes of beef cows.** There are known causative SNP affecting milk composition that could be explored using gene editing. So far no report of their use has been published.

### *5.2.2 Increasing off-farm productivity*

**Improving carcase yield.** Improvement in muscularity increases carcase yield, and a key regulator of muscle growth among several animal species in the *Myostatin* gene. There are several known variation that are known to affect the function of these gene, and consequently the muscularity of the animal. There are already reports of gene edited

[17] A comprehensive list of all livestock ever generated using gene editing, as of beginning of 2016, can be found at Tan *et al.* (2016).

animals targeting this gene in cattle, sheep, and pig (Proudfoot *et al.* 2015; Wang *et al.* 2015; Li *et al.* 2016). These reports are mainly from USA, and China.

**Improving meat quality.** There are some known SNP affecting meat attributes e.g. meat tenderness, and fat colour. There is a report on a gene edited sheep demonstrating the effect of a genetic variation on fat colour (Niu *et al.* 2017).

## 5.3 Animal welfare

**Targeting the *POLLED* locus.** Reducing the requirement of re-horning by fast tracking the generation of high genetic merit polled sires and cows. There are at least two genetic haplotypes that leads to pollness in cattle. One of them was targeted and proven effective in generating polled calves via gene editing (Tan *et al.* 2013; Carlson *et al.* 2016). The generation of these calves was a key proof-of-concept in applications gene edit in cattle, as it is technically more challenging than the *Myostatin* applications for instance. The method is well described and possibly other groups are making use of it, however, so far, only one group has announced their success.

**Targeting adaptation traits.** There is a strong candidate gene affecting thermotolerance (heat stress), the *SLICK* gene, which also affects the hair type in cattle. There is no report on gene edited animal targeting this gene, but considering that it is technically not different than targeting *Myostatin*, it edited animals could be expected anytime now.

The identification of genetic variations affecting traits related to tropical adaptation is the current subject of several research groups around the world (e.g. parasite resistance – ticks and worms). Some candidate genes, and or markers can be expected to be identified in the next few years. Then, once they are identified, these markers could be incorporated into a gene editing project.

## 5.4 Disease prevention/resistance

The application of gene editing approaches to develop lineages of livestock resistant to disease is another hot scientific topic in the field. The most commented potential disease targets are Food-and-Mouth disease, prion disease – mad cow, and bovine respiratory disease. The last two have already some published results.

The *PRNP* prion gene, responsible for mad cow disease, was targeted using gene editing in somatic cells and embryos (Bevacqua *et al.* 2016). The authors report the success in knocking out (“turn off”) the targeted gene *in vitro* only as they did not proceed to generate a live animal, but they comment on the possible application of their methodology in generating a herd of “prion gene free” cattle.

Similar approach was applied for the generation of live animals supposedly resistant to the toxin of *Mannheimia (Pasteurella) haemolytica*, which is related to the bovine respiratory disease (Shanthalingam *et al.* 2016). The authors report that leucocytes of edited animals were resistant to *M. haemolytica* leukotoxin-induced cytolysis, giving support to the concept of these animals being more resistant to bovine respiratory disease.

Another promise of gene editing to livestock production refers to the potential reversal of known genetic disorders from offsprings of disease or carrier animals. There are a number of known genetic diseases prevalent in Brahman, Angus, Wagyu, etc… that could be

potentially targeted. However, at this point, to our knowledge, there is no report of gene editing targeting genetic variations that promotes these diseases. Nonetheless, these reports can be expected to the near future.

## 5.5 Use of edited products

The use of gene editing technologies in plant science is more advanced than in animal science. There are scientific activities aiming at improved tolerance to biotic and abiotic stresses and many other traits, of many different crops for oil, food, and fibre. Not surprisingly there are also studding aiming at improving digestibility of livestock fodder. Results of these researches have not been reported yet, but will be in the near future.

Similarly, the use of gene editing technology is much more advance in the microbial research, where the enzyme Cas9 used in the most recent gene editing technology was identified. There are scientific groups trying to develop improved bugs that could be used as probiotic (or supplement) from humans to livestock. In humans, there is growing interest on the bugs we house in our guts as they have been linked to several diseases. In livestock the main interests relate to potential improvement in feed utilization, gas fermentation (potential reduced methane production) and animal health. Both human and livestock applications are ongoing, little has been published in this field, but we should see reports in the near future.

## 5.6 Cattle cloning applications

Cattle cloning seems to be one of the technologies that gain some attraction and stabilised in the sense that never attracted a large number of “end-users”, but always had some. This is the current situation in Australia, but also in more clone-intensive countries like USA and Brazil. One of the key limiting factors for the broader adopting of the biotechnology is its intrinsic low efficiency in generating live animals that did not improve drastically in the last 15 years. Since some of the gene editing approaches requires a cloning step, this reproductive biotechnology might become a bit more used in the near future.

In Australia, as far as we are aware there is only one commercial provider of cattle cloning[18]. It was reported that in the last five years they have cloned between 20-25 beef cattle (mainly Brahman) and 8-10 dairy animals (mainly Holsteins). It seems that in the dairy industry the focus on selection for cloning was based on EBV of young animals, while in the beef industry cloning was used as a way to extend the life of a good cow.

The Australian Brahman Breeders Association (ABBA). Reported that there are seven females registered clones at ABBA, six of them already have registered progeny. It is my understanding that more animals will be registered soon, since it was mentioned that some Brahman were born recently. Angus Australia mentioned that they have capacity and regulation for register clones, but there is none so far in their database. It is their understanding that two bulls from USA that are in their database are clones. He also mentioned that sometimes when semen stocks of “trendy” bull are running low, some more progressive breeders enquire about cloning.

[18] http://www.cloneinternational.com

# 6 Recommend industry research priorities to the red meat industry

## 6.1 Overview

There are vast possibilities offered by novel technologies such as gene editing to the livestock industry (Section 5). However, industry leaders, such as MLA, who want their stakeholders to have access to the best available technologies that increase productivity, competitiveness and maximise profitability have a difficult task. Formulating appropriate strategies that encourage new opportunities with limited financial resources and few mechanisms geared to understand exactly what is required along the value chain is a challenge. In particular, biotechnologies have raised significant concerns amongst both producers, key markets and domestic consumers. As such, it is important that industry leaders consider the value chain dynamics and develop strategies that articulates the benefits along the entire value chain, provides transparency in decision making, addresses stakeholder concern and builds trust and trustworthiness of the industry as whole.

MLA have an opportunity to focus on industry-orientated priorities that have a greater likelihood of adoption and acceptance as well as providing leadership in providing the framework for value chain and market acceptance.

## 6.2 Industry-oriented priorities

There are a few important points to note. The list of priorities below is not intended to be comprehensive nor exclusive. The potential targeted traits were tentatively split into categories following the most perceived benefit, however often a trait would fit more than one category.

**General benefit**

- **Identification of causative targets**
  All gene editing approaches depend on the availability of causative genetic variation to be targeted using the biotechnology. At the moment there are only few causative targets that could be exploited. Thereafter, a key priority should be around the systematic identification of causative targets affecting traits of interest. It is worth noting that the identification of such targets would also benefit approaches other than gene editing, for instance those causative targets could be incorporated into genetic evaluations for the estimation of breeding values.
- **Adjusting methods for generating estimated breed value (EBV)**
  Genetic progress is highly dependent on the estimation of breeding values. If gene editing is to be broadly adopted, projects on the identification of the best approach for dealing with those animals within the genetic evaluation will be necessary. It is still to be defined which quantitative genetic approach will best perform for evaluation of gene edited and non-edited animals at the same evaluation[19].

[19] An example of a research project in these lines was recently presented by Jenko et al. (2015).

**Public and consumer benefit**

- **Animal welfare**
  Considering the general public and meat consumers the traits that should be more appealing relate to animal welfare and meat quality. The flag trait within animal welfare has been suggested to be the *POLLED*.
- **Tropical adaptation traits**
  Adaptation to tropical environment is also related to animal welfare and impact on animal performance. Traits like heat tolerance and parasite resistance could be considered, although causal SNP are not known yet.
- **Meat quality**
  There are some known SNP affecting tenderness and marbling that could be further explored using gene editing approaches.

**Producers/industry benefit**

- **Female and male fertility**
  As mentioned on section 5, there is potential for improvement on- and off-farm productivity. Improvements in cattle fertility can lead to direct positive effects in on-farm activities. There are some known genetic targets affecting male (e.g. scrotal circumference, semen quality) and female fertility (e.g. age at puberty, length of postpartum anoestrus interval).
- **Carcase yield**
  Off-farm productivity could be lifted by improvement in carcase yield, which would benefit producers and processing plants.
- **Feed conversion efficiency**
  Another trait that would impact positively in on-farm productivity is improvement in feed conversion efficiency. There are ongoing studies in Australia and abroad, but causative targets are still to be identified.

**Domestic/International benefit**

- **Disease resistance/ Biosecurity**
  It has been postulated the concept for developing livestock lineages resistant to specific diseases. If developed, these cattle could have positive impact on many commercial aspects nationally and for international trade.
  There are current studies around the world for the generation of edited animals with improved disease resistance, e.g. resistant/tolerant to bovine respiratory disease, prion depleted animal that cannot develop mad cow syndrome. Another likely target is Foot-and-Mouth-Disease. Cattle that are disease resistant/tolerant might also become observed within biosecurity scrutiny.

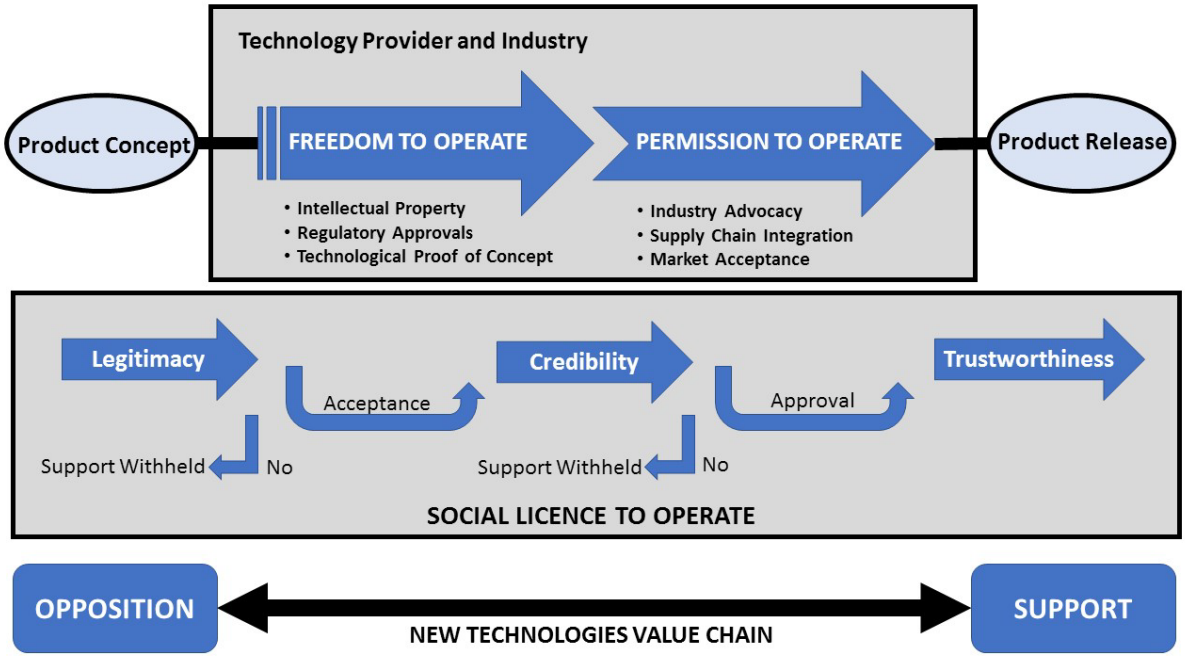
## 6.3 Strategic priorities

Despite the positive attributes of gene editing and significant upside potential for the livestock industry, product development remains inherently risky. In addition to a significant funding requirement and regulatory risk, there is a realisation that the product benefit alone may not be enough to guarantee success. Because of those risk factors, proponents with a desirable trait face significant threats to their profitability and long term viability.

Importantly, industry is often faced with an academic focused science and technology push for beneficial products rather than a value chain desire and pull for a product. Hence the role of industry groups is a vital element in the successful introduction of products developed through new technologies. Further, it is becoming increasingly clear that stakeholders, particularly key markets and consumers, demand that both the producer and industry are legitimate, credible and trustworthy before they will support products derived from new technologies.

The complexity and challenges associated with the value chain for the introduction of new technologies such as gene editing is outlined in Figure 3 and discussed below. There are three strategic priority areas that MLA could focus on to support and exploit new technologies for the livestock industry: Freedom to Operate, Permission to Operate and the Social Licence to Operate.

**Figure 3. New technologies value chain. The complex landscape for introduction of new technologies involves technical elements related to Freedom to Operate and Permission to Operate, and also needs to take into account the public perception, Social Licence to Operate.**



### *6.3.1 Freedom to Operate*

Along the value chain, the freedom to operate of a product addresses the technical elements of the product (i.e. proof of concept in delivering on the proposed benefit) and the intellectual property and regulatory approvals required for market entrance. As discussed in this report, there are currently limited targets identified and validated that have direct benefits to the livestock industry. Further, there is regulatory uncertainty in Australia and in key markets. As such, if MLA is to support the development and introduction of new technologies it must engage with both technology providers and regulators to ensure that the voice of industry is heard. That is, tech providers focus on development and validation of real industry needs and regulators have access to relevant and scientifically validated information to science based risk assessment and decision making.

### *6.3.2 Permission to Operate*

A biotech product cannot get to market without the permission of all industry stakeholders. Fragmentation of support can undermine the legitimacy and credibility of the industry leading to a lack of trust by stakeholders. Experience from the introduction of GM canola into Australia demonstrated the power of a single industry voice calling for choice[20].

Similarly, the introduction of a gene edited product will require industry wide support from a diversity of stakeholders from across the supply chain and key markets. MLA have an opportunity to provide significant leadership in this space through the coordination of industry peak bodies (e.g. Cattle Council Australia, Australian Meat Processors Corporation, Rural Industries Research and Development Corporation, etc.), engagement with state and federal government and the development of policies, best practice and standards that provide confidence in the safety and efficacy of new products through the supply chain.

### *6.3.3 Social licence to operate*

In simple terms, the Social License to Operate (SLO) exists when a product has the ongoing approval of the community and other key stakeholders. It is only granted on the basis of shared values, perceptions and opinions held by the public and other stakeholders with the producer and associated industry.

There are three main components of a SLO that centre around community/stakeholder perceptions of the social legitimacy and credibility of the product, and the presence or absence of trust and trustworthiness of the producer and the industry. These elements are acquired sequentially and are cumulative in building a SLO. That is, a product must be seen to be legitimate before credibility of the benefit and value are accepted and both must be in place before meaningful trust can develop and a product supported.

[20] Delivering market choice with GM canola

The complexities of building a SLO has recently been highlighted[21,22,23,24] particularly in association with disruptive technologies or practices such as mining, agriculture, forestry and renewable energy. Strategies that livestock producers and the industry can adopt are therefore embedded in social science and not necessarily built off rational science based debate or market driven demand. Therefore, it is recommended that MLA engage with the necessary expertise to develop strategies that contribute to enhancing the legitimacy and credibility of desired products derived from new technologies as well as implement those strategies that foster and strengthen the trust and trustworthiness of the livestock industry in general.

[21] Ankeny,RA, Bray,HJ, 2017, Scourge or Savior? The Complex Relationship between Food and Science, Bloomsbury
[22] Defending the social licence of farming: issues, challenges and new directions for agriculture (2011) edited by Jacqueline Williams and Paul Martin.
[23] The Social Licence to Operate and Coal Seam Gas Development
[24] The social licence to operate: a critical review

# 7 List and evaluate risks to the red meat industry

## 7.1 Overview

The most relevant risk for adoption of gene editing technologies by the red meat industry relates to potential negative public perception, and its local and international activism against the use of the technology. As it stands in April 2017, due to unclear regulatory environment, its broad application across the industry could lead to commercial/trade barriers that will certainly impact negatively the industry. Nevertheless, regulatory bodies around the world are working to stablish the framework for dealing with these technology. There is a great opportunity to learn from the transgenic experience and move forward with a unified message. There are potential benefits to the industry, and some risks and weaknesses associated with them. These are briefly discussed in this section.

## 7.2 SWOT Analysis

The chart below brings a non-comprehensive list of Strengths, Weaknesses, Opportunities and Threats for the application of gene editing and cloning in the red meat industry.

| STRENGTHS | WEAKNESSES |
|---|---|
| Do not disrupt log-term selection;<br>Genetic benefit can be seen in a single generation;<br>Can be integrated with traditional and genomic selection-based animal breeding;<br>Integrated with reproductive biotechnologies – benefit goes beyond the edited animals;<br>Can tackle welfare issues – polled is the classical example;<br>Potentially doesn't change the current supply chain;<br>There are at least three technologies to deliver a solution. | Not many known causative targets;<br>Regulatory environment not clear (nationally and internationally);<br>Low efficiency in producing edited animals; Laboratory protocols are not optimized for livestock applications;<br>Freedom to operate for some technologies is unclear;<br>At the moment only a limited number of "edits" can be made in a single step, limiting its impact for complex traits;<br>"Off target" editing – Is it a real problem? |
| **OPPORTUNITIES** | **THREATS** |
| Systematic identification of functional mutations (adaptation traits, disease resistance);<br>To "correct" for Mendelian genetic disorders;<br>Accumulate favourable alleles in sires and dams;<br>To improve efficiency of protocols for animal editing;<br>Stimulate broader adoption of reproductive biotechnologies;<br>Leverage previous investment in genomic projects;<br>Cloning allows selection of animals based phenotypes obtained after slaughter. | Potential regulatory burden OGTR and FSANZ, State, Territory and Breed Society;<br>"Negative" public perception; including leading to attacks on social media;<br>Potential trade barriers;<br>Inability to disseminate gene-edited genetics – low use of reproductive biotechnology;<br>Lack of transparency or "hidden" adoption;<br>Wrong choice of first traits to market (need to find a "good flag" trait);<br>Low acceptance by industry when competing internationally;<br>Manage "tech push" vs industry need;<br>Imports potentially undetectable. |

**Brief text expanding the points listed at the SWOT analyses chart.**

**Strengths**

1. A favourable allele can be brought to a population without disrupting long-term selection for any other particular trait.
2. If applied in embryos, for example, the calf originated from this procedure will be able to express its “improved potential” (and pass on to other generations).
3. Gene editing should be seen a complementary technology to traditional animal selection. There is no reason for not integrating the different technologies.
4. There is a need for integration with reproductive biotechnologies. Stimulate the adoption of more conventional repro biotechnologies.
5. It is possible to impact on welfare issues – supports “good” public perception.
6. If regulators and food suppliers opt not to differentiate edited beef to conventional beef, supply chain will be unchanged.
7. Gene editing comprises more than one technology (e.g. ZNF, TALENS and CRISPR), sometimes for a specific gene editing one technology will work better than the others. It is good to have more than one approach to deliver a solution.

**Opportunities**

1. Stimulus to identify potential genetic targets.
2. There are highly selected sires/cows that carries alleles for known Mendelian disorders, these “bad” alleles could be potentially fixed for the next generation.
3. Using traditional animal breeding select the best sire and dam, produce IVF embryos and edit them bring additional favourable alleles. Generation after generation accumulate these “good alleles”.
4. Laboratory protocols need to be improved. This is known not only in the livestock research world, also in plant, human and cell biology. On the positive note, there are lots of scientists actively working on this, and we can benefit from the knowledge generated.
5. Since reproductive biotechnologies are essential for the success of gene editing the adoption of one technology implies on the adoption of some others, e.g. IVF or cloning. Moreover, if a great sire is created using gene editing, it is likely that its semen becomes “valuable” and breeders might decide to adopt AI, fixed-time AI, embryo transfer or IVF to make the most of it. Gene editing could stimulate adoption of other technologies.
6. Somatic cell nuclear transfer, or cloning, can be deployed using cells derived from carcases. Thereafter, a selection based on carcase phenotypes is possible to be done, even though the whole efficiency of the method is low (for an example of this application see http://www.texasmonthly.com/food/west-texas-a-m-cloned-cows/).
7. The MLA invest heavily in many research projects involving genetics and genomics. Putting all of these data together, maybe with some strategic investments, this will give an opportunity to support the identification of functional mutations, or in other words, the identification of new targets to be edited.

**Weaknesses**

1. At the moment there is only a limited number of mutations that are known to affect a production trait. If this scenario is not changed, this possibly will limit broader adoption of the technology. It is worth noting, though, that there are several ongoing world-wide genomics projects based on next-generation whole-genome sequencing, these are delivering several causative targets that could be potentially explored using gene editing.
2. Australia (OGTR) reviewing submission to a public consultation, and US (FDA) have just called for a public consultation. Pretty much all other countries are “quiet”.
3. There are some legal disputes on some of the technologies (mainly CRISPR). This should not prevent local development of the technology, but should be considered under commercial applications.
4. I set this also as an “opportunity”. The low efficiency is true not only for livestock; there are lots of researchers working on improvements of efficiency and precision, the livestock community can benefit from these. Nevertheless, protocols optimised to mice and human cells will need to be adapted to livestock – this is not a new approach in livestock science.
5. On top of the low efficiency of the protocols, only few edits can be made in a single procedure. Also similar to “low efficiency”, lots of groups are working on multiplexing gene editing including livestock groups. In the near future we can expect advances in this area.
6. “Off targets effects”. Some SDN applications are not as precise or predictable than others, and might introduce genetic changes others than those first targeted. Such changes can be identified through whole genome sequencing. The potential for “off target” edits needs to be discussed but easily mitigated with quality assurance mechanisms adopted and the evolution of SDN technologies and processes.

**Threats – risks**

1. This relates to the regulatory uncertainties. Depending on the resolution that is adopted, if very strict, the regulatory burden can be prohibitive, empting the scientific community leaving only very large players in the game.
2. How to manage the public perception is one of the main key challenges, which there is no straight answer to which is the best approach.
3. There is potential trade barriers as an issue only for early adopters of the technology, because governments around the world have not decide how to proceed. Taking transgenic plants as an example, after a period of adaptation trade is well stablished, sometimes different crops manage differently, but one the guide-lines are established trade protocols work with them.
4. Due to low efficiency in producing edited improved animals, only a few animals will be produced. If it is intended to impact the broad industry, it will be imperative to drive adoption of reproductive biotechnologies.
5. If there is not a clear regulation, and path for adoption, it is very likely that this technology will be disseminated without transparency (“hidden” adoption), either by the use of imported gene edited semen or developed internally without notification –

this has damaging potential for the industry, and mechanisms should be created to avoid it. At a first glance, most of the genetic changes proposed cannot be detected as those variants could be in nature. Nevertheless, using advanced genetic tools it is possible to scrutinise the potential genetic target to assess its origin.

6. This also relates to the public perception. It will be important to find a trait that is appealing to the public. A welfare-based one would provide wins for the industry and be a credible gain in the eyes of consumers.
7. The industry need should come first, and not what is promptly available by technology companies.

## 7.3 Comments on specific risks

### *7.3.1 Risks related to social perception*

This is by far the most relevant risk for adoption of gene editing within the red meat industry. It relates not only to the local domestic perception, but also should be consider as a key factor under the international trade. This is a relevant topic that requires attention and should be assisted by specialized scientific groups.

Lessons should be learnt from the historical dissociation between traditional genetic engineering creating transgenic animals and the public perception of the biotechnology. In general terms, a proactive approach for engagement with the general public should be taken. Social scientist should get involved. A good “flag trait” should lead the way. Moreover, if scientist, animal breeders, regulators – the industry as a whole supports the use of the technology, the engagement with the public will be easier than if led simply by the industry, especially if led by multi-national corporations.

### 7.3.2 *Intellectual property of the biotechnology and its regulation*

Two out of the three technologies that “gene editing” encompasses (ZNF and TALEN) have their intellectual property more settled, and licenses for commercial applications can be issued. However, the most recent and most appealing technology (CRISPR) still have its IP ownership being dealt at court. The broad commercial application of this technology might be limited until the ownership is settled. Nevertheless, at a scientific level all technologies are available to be used.

The biotechnology evolved faster than the regulation for its application. Now regulatory bodies around the world are trying to update their guidelines to deal with a technology that did not existed at their last update. There are scientific, public and commercial pressures on regulatory bodies, but at the moment it is not clear when a resolution will be announced.

### *7.3.3 Risks related to increasing incidence of genetic disorders*

The risk for increasing incidence of genetic disease by gene editing is not different to the potential risk for increasing incidence of genetic disease associated to use of reproductive biotechnology (e.g. AI). In fact, the risk in case of gene editing applications is only relevant if the genetic material of the sire/cow is spread by reproductive biotechnology, it is not intrinsic of gene editing *per se*, which should have “control steps” to insure the generated animal is suitable for animal production. Due diligence should be in place during all selection of animal to be involved in biotechnologies that have potential to influence multiple herds.

# 8 Evaluate potential local and international perceptions on the use of the technologies and potential market aversion

## 8.1 Overview

The general public perception on the use of gene editing technologies is a key issue that needs to be address proactively. At the time when regulatory framework is under scrutiny for revision, there are questions on the best timing to approach the general public to assess their support of the biotechnology or otherwise – before the regulation is updated, during the discussions or after the regulatory framework is set. All of them have its pros and cons; at this point is not complete clear when the best timing is, but it seems that earlier, and with unified message, the better.

The regulatory framework of many countries are currently under review or have a review planned. Thereafter, a final international perspective can only be expected for the coming years. Here, the current situation of Australia and some key trade partners are presented from interpretations from international reports - Global Agricultural Information Network, USDA.

## 8.2 Local perception on the use of the technology

### *8.2.1 Comments on a survey of community attitudes to gene technology*

So far, no specific survey on the public perception on gene editing technology has been conducted in Australia. It certainly would include surveying the public understanding on the differentiation between traditional gene technologies that creates transgenic organisms and gene editing.

Since there is no reported survey specific on the use of gene editing technologies in agriculture, here are report some key finding on a 2015 survey - “Community attitudes to gene technology” commissioned by the OGTR (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/reports-other).

Although the scientific community clearly differentiate traditional gene technology to the new gene editing technologies, one can expect little differentiation by the general public. Thus, there is an opportunity for educating on this differentiation trying to fill this gap of knowledge.

In general terms, there are more people in support of Genetically Modified Organism (GMO) than were opposed. However, the level of support vary significantly for each application, for instance, medical (e.g. producing insulin) and industrial (e.g. making biofuel) uses have far greater support than the use of the technology in food and crops. Moreover, there were high levels of awareness of GMO (81%) and animal cloning (88%), but these have dropped since 2012, as were the understanding that both would improve our way of life in the future.

The OGTR, other regulators, and possibly other industry representatives (e.g. MLA, ACC, and Breed Societies) can assist addressing public concerns.

“Most support or rejection of GM foods was conditional, and is likely to move based on knowledge of regulation or scientific evidence of safety, indicating that a higher awareness of

the OGTR and other regulators, and their roles, would have some impact on public concerns." Page 4, http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/327437B632158967CA257D70008360B1/$File/Community%20attitudes%20to%20gene%20technology%20Final%20Repor%202015.pdf.

### *8.2.2 Comments on attitudes about science and technology and path form common understanding (interpretations from Cormick and Romanach (2014))*

**A few general considerations**

- We can expect local and international discussions on the use of gene editing technologies in livestock (as edited animal or feed-based).
- Nowhere in the world has a gene edited animal yet been approved for food consumption.
- This is mainly due the fact that Regulatory Institutions around the world need first to "update" their understanding of the new technologies, so that they can Regulate.
- The regulatory framework has to catch up with the scientific advances.
- The submissions to the OGTR Public Consultation gives us some hints on public perception of the technology. As in April 2017, a response from OGTR on the public consultation is expected soon.
    - It should not be a surprise that scientific institutions, in general, recognise gene editing simply as another tool for improvement, and consider it safe to use.
    - On the other hand, there are people and groups opposed to the use of the technology.
- The "social science" around the introduction of the discussion on gene editing in livestock is crucial.

**On the public attitudes towards biotechnology (interpretations from the 2016 symposium "Regulatory Oversight of Products Developed Through New Breeding Technologies (NTBs)" supported by CropLife Australia, The Agriculture Biotechnology Council of Australia, The Australian Seed Federation and AusBiotech)**

- Initial framing of any new biotechnology will largely govern the public debate.
- When information is complex, people make decisions based on their values and beliefs.
- People seek affirmation of their attitudes (or beliefs) – no matter how fringe – and will reject any information or evidence that are counter to their attitudes (or beliefs).
- Attitudes that were not formed by scientific information are not influenced by scientific information.

### *8.2.3 People most trust those whose values mirror their own. Comments on strategies to enable the adoption of animal biotechnology (based on Tizard et al. (2016)).*

The referred article was based on discussions held during a workshop sponsored by the OECD's Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems.

The final recommendations to enable adoption of animal biotechnologies are listed below. Please refer to the full article for extended comment on each of them.

- The need of a coordinated approach from governments and trade bodies to harmonize the regulatory framework towards the technologies (transgenesis and gene editing).
- The main role that scientist and industry leader have in assisting these new technologies move toward applications in agriculture system.
- Encouraging public acceptance by demonstration of positive benefits of products derived from these technologies.
- The need for clearly differentiating traditional gene technologies, from gene editing and other emerging technologies.

## 8.3 International perception/framework on the use of the technology

At the moment no country in the world has decided how to regulated gene editing in any application from medical to agriculture. In general terms these uncertainty is holding the development or, at least, the announcements of commercial applications of technologies that uses gene editing as part of the protocol. There are many forces pushing for the establishment of regulatory frameworks and the start of trade negotiations, so, being optimistic, it is expected that in one or two years several countries will have some sort of framework to start discussions. Table 3 brings interpretations of the current position of Australia and some selected trade partners for the red meat industry, these are based on reports of the Global Agricultural Information Network, USDA (https://gain.fas.usda.gov/).

**Table 3. Australian and key trade partners perceptions on gene editing technologies (based on Global Agricultural Information Network, GAIN – USDA).**

| Country | Policy Framework | Production and Trade | Comments |
|---|---|---|---|
| Australia | The Gene Technology Act 2000 and the Gene Technology Regulations 2001 form the legislative basis for analysis and regulation of gene editing and genetically modified products. The legislation is process focused rather than product orientated and administered through the Office of the Gene Technology Regulator (OGTR). As such, any process that uses gene technology, (as defined in the Act or listed in the Regulations) are regulated.<br><br>Australia cooperates closely with New Zealand on matters of food safety through Food Standards Australia and New Zealand (FSANZ).<br><br>Other agencies may also play a role in the regulation of products of gene technology, including: the Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA) and Department of Agriculture and Water Resources (DAWR).<br><br>There are no specific regulations around animal cloning, however, both genetically modified and cloned animals are subject to state and territory government animal welfare legislation applicable to animals used for scientific purposes, as well as to the Australian code for the care and use of animals for scientific purposes. | In Australia, cloning of livestock is currently limited to small numbers of breeding cattle, predicted to be less than 100 beef and dairy cattle and a few sheep within a confined research environment. The work is being carried out by public and private research institutions and universities.<br><br>Cloned animals or products from cloned animals are not considered to be an animal health or biosecurity risk and have not been assessed as a hazard in Industry Risk Assessments. There are no additional biosecurity restrictions in relation to the import of embryos derived from cattle, sheep or goats. The same applies for the import of products derived from cloned animals. They are subject to the same quarantine regulations as non-cloned products.<br><br>Food from cloned animals is not regulated in the same way as food from genetically modified organisms. Food Standards Australia and New Zealand consider that food products from cloned animals and their offspring are as safe as food products from conventionally bred animals and do not require any additional regulation.<br><br>Quarantine requirements are the main trade barrier to animal products entering Australia. These requirements would equally apply to any edited animal products. There are no additional biosecurity requirements for cloned animals or animal products.<br><br>This would also apply to the import of semen derived from gene edited animals. | Outcomes from a recent regulatory review undertaken by the Gene Technology Regulator will be made available in May 2017. It is expected an announcement on the review of the Gene Technology Act will also be made around this time.<br><br>No specific market acceptance research has been conducted on the acceptance of food from cloned animals. |
| Canada | The Canadian Regulatory Framework for Biotechnology (1993) and additional acts (e.g. Food and Drugs Act, Seeds Act, Pest Control Products Act) form the legislative basis for analysis and regulation of novel products. Canada's regulatory framework is exclusively product based.<br><br>The Canadian Food Inspection Agency (CFIA), Health Canada (HC) and Environment Canada (EC) are the three agencies responsible for the regulation and approval of products derived from biotechnology. The three agencies work together to monitor development of products with novel traits, novel foods and all products with new characteristics not previously used in agriculture and food production. | Currently, there is no commercial production of a genetically modified animals approved in Canada, and there are no genetically modified animal products approved as feed or as food. Clones, their offspring and the products derived from clones and their offspring would be subject to the same requirements and regulations as those applicable to genetically modified animals and genetically modified animal products. However, there remains the question of whether clones and their offspring and/or the products of clones and their offspring meet the definition of a novel food. The three main governmental bodies with jurisdiction on biotechnology (Health Canada, Environment Canada and the Canadian Food Inspection Agency) have yet to give their opinion on this matter. | Given the legislative framework is product orientated, the current legislation and regulatory framework is considered to adequately cover products derived from gene editing. |

| Country | Policy Framework | Production and Trade | Comments |
|---|---|---|---|
| | The regulatory framework in Canada is product orientated and designed to ensure environmental protection, animal health, plant protection and human health. Provided that these objectives are met, a genetically engineered animal, once approved for environmental release, and a genetically engineered animal product, once approved as food, are treated no differently than the respective conventional animal or animal product. Regardless of the technological process involved in raising, growing, producing or manufacturing, all animals and animal products are subject to the same requirements and regulations when it comes to environmental and plant protection, animal and human health and feed and food safety. | | |
| China | The government of China is in the process of revising laws and regulations governing biotechnology.<br><br>The biotechnology regulatory system for agriculture is outlined in State Council regulations "Food and Agricultural Import Regulations and Standard" and "Agricultural Genetically Modified Organisms Safety Administration Regulations 2001".<br><br>The Ministry of Agriculture (MOA) has primary responsibility for the approval of biotechnology crops for import and domestic production, as well as the creation of agricultural biotechnology policy.<br><br>China has not approved any GE food or feed crops developed by foreign biotechnology firms for domestic commercial production.<br><br>How China will regulate innovative biotechnologies remains uncertain. China has initiated a study to review the regulatory policy for innovative biotechnologies. MOA has established an expert team to analyse whether gene editing is also subject to the existing biotechnology regulatory system. | Biotechnology is designated as a strategic emerging industry in China, and the government invests heavily in biotechnology research.<br><br>Despite years of public research, China has not yet commercialised any genetically modified grains or oilseeds.<br><br>China is a leader in animal biotechnology research. The Key Scientific and Technological Grant of China for Breeding New Biotech Varieties launched in 2008 supports the research of GE animals including swine, cattle, and sheep. Despite the heavy investment and advanced research, China has not yet approved commercialisation of any livestock clones or GE animals or products derived from animal biotechnologies. China does not import GE animals, livestock clones, or products from these animals. | There are no widely accepted studies or surveys available on market acceptance of biotechnology products in China. |
| European Union | At the European Union level, genetically modified products are subject to an authorisation procedure whether for import, distribution, processing, or cultivation for food or feed use. The steps necessary to obtain authorisation for import, distribution, or processing are set out in Regulation (EC) No 1829/2003. Directive 2001/18/EC outlines the procedure that must be followed to obtain authorisation for production/cultivation.<br><br>Three European entities regulate animal biotechnology:<br><br>- The EC's Directorate General for Health and Food Safety (DGSANTE) | Member states that develop GM animals do so predominantly for biomedical purposes<br><br>No GE animal for food use is commercialised in the EU and to date no application has been submitted to EFSA for the release into the environment or placing on the market of GE animals. A French company produces and exports elite cloned horses.<br><br>The Roslin Institute (Edinburgh) have used gene editing to produce piglets designed to be resistant to the African swine fever virus. The Roslin Institute also focuses on using genome editing to enhance resistance to infectious disease in livestock and on producing a chicken that cannot transmit avian flu | There have been no developments or legislative activities on animal produced through innovative biotechnologies<br><br>The main barriers to using animal biotechnology to improve animal breeding are the public and political opposition to it, due to ethical and animal welfare concerns<br><br>The European Commission was expected to publish a legal opinion on whether or not innovative |

| Country | Policy Framework | Production and Trade | Comments |
|---|---|---|---|
| | - The Council of the EU<br><br>- The European Parliament, especially the following committees: Environment, Public Health and Food Safety (ENVI), Agriculture and Rural Development (AGRI), International Trade (INTA) | | biotechnologies fall under the scope of Directive 2001/18/EC. This legal opinion was expected to facilitate the harmonisation of Member States approaches to regulate or not regulate innovative biotechnologies. After long delays, no such opinion has been published. However, it is the sole prerogative of the European Court of Justice to provide a final and binding opinion on the interpretation of EU law. The EC is currently still reflecting on how to proceed with this legal opinion. |
| Indonesia | The Indonesian Government policy on biotechnology is "accept with a precautionary approach" with respect to environmental safety, food safety, and/or feed safety based on scientific approaches as well as taking into considerations of religion, ethical, socio-cultural, and esthetical norms.<br><br>Indonesia's regulatory framework for the evaluation and approval of GM crops was incomplete, however, until August 4, 2016, when the Ministry of Agriculture (MoA) issued regulation 36/2016. Regulation 36/2016 establishes risk assessment guidelines for feed safety, completing the risk assessment framework along with environmental and food safety guidelines. Despite the recent completion of Indonesia's risk assessment framework, approvals for GM products remain on hold due to the MOA's invocation of Government Regulation 21/2005 on the Biosafety of Genetically Engineered Products. This regulation requires that a "monitoring and control" system be implemented in order to regulate approved GM products. The monitoring and control system has yet to be developed, and MOA officials have commented that its creation could take several years.<br><br>Although the Government has several regulations permitting animal biotechnology, there are no clear guidelines for their assessment and approval. | Several GM plants have received food, feed, and/or environmental safety certificates from the Government of Indonesia. However, due to incomplete biosafety assessments, no imported or locally developed GM plants have yet been commercialised.<br><br>There is no commercial production of GM animals in Indonesia. | Indonesia's regulatory framework, and its newly implemented monitoring and control requirements (regulation 21/2005) prevent the commercialisation of GM crops. Indonesia, however, imports GM foods and products.<br><br>The Government of Indonesia has not decided whether the regulations for innovative biotechnologies will follow the regulatory framework of GM products, although Sources report that some government research institutions have conducted research using gene editing and CRISPR technology. |
| Japan | In Japan, the commercialisation of GE products requires food, feed and environmental approvals. Four ministries are involved in the regulatory framework: MAFF, the Ministry of Health, Labour and Welfare (MHLW), the Ministry of Environment (MOE), and the Ministry of Education, Culture, Sports, Science and Technology (MEXT). These ministries are also involved in environmental protection and regulating lab trials. The Food Safety Commission (FSC), an independent risk assessment | As of October 26, 2016, Japan has approved over 309 GM events for food, 150 for feed and 120 for environmental release, including commercial planting for most events.<br><br>Although there is a reluctance to accept GM food and food crops among some consumer groups, Japan remains one of the world's largest per-capita importers of GM crops and has no significant trade barriers. | Most agricultural R&D is operated by the public sector, government research institutes and universities. Recently, however, innovative technologies, such as CRISPR/Cas, has received attention both from public and private sectors, and may influence the future course of |

| Country | Policy Framework | Production and Trade | Comments |
|---|---|---|---|
| | body under the Cabinet Office, performs food and feed safety risk assessment for MHLW and MAFF.<br><br>Although some products and/or approaches of innovative technologies may not fall under the current definition of "genetic engineering", the Tohoku University Gene Research Centre announced that they would manage all "genome-editing technology" in the centre in the same manner as "genetic engineering", and will seek regulatory authorisation for experimental operations (http://www.cgr.tohoku.ac.jp/genome/) | For products from a cloned animal, Japan has a specific labelling requirement that it be labelled as a cloned product. Currently, there is no commercial production of GM animals or cloned animals for the purpose of agricultural production.<br><br>Interest in animal cloning appears to have waned in Japan. As of March 2016, Japan had produced 625 cows by fertilised egg cell cloning, 415 cows by somatic nuclear transfer (SCNT), 638 swine by SCNT, and 5 goats by SCNT. All production has been done in public research institutions. The activity has been steadily decreasing since late 90's when 461 of the 625 cows to date were produced by fertilized egg cell cloning in 1998 alone, and 98 of the 415 cows to date were produced by SCNT in 1999 alone. | biotechnology applications in agriculture in Japan.<br><br>There is no commercial production of GM food crops in Japan. Japan remains a country which receives major benefits from agricultural biotechnology for its food security. Japan relies on imports for almost 100 percent of its corn supply and 95 percent of its soybean supply.<br><br>At this moment, there is no commercial distribution of livestock GM animals in Japan. Moreover, it is not clear how much, if any, public interest there would be in consuming meat from GE or cloned animals.<br><br>There is no significant marketing activity in livestock animal biotechnology |
| Korea | There are numerous government agencies that play a role in administration of the Living Modified Organisms (LMO) Act. However, the Ministry of Food & Drug Safety (MFDS) (under the Prime Minister's Office) is the most relevant. It is the authority for matters related to the import/export of LMOs for food, pharmaceutical, and medical devices; food safety approvals of biotechnology crops; and the enforcement of labelling requirements for non-processed and processed food products containing biotech ingredients.<br><br>As of October 2016, MFDS has granted food safety approval for 164 events including 144 crops, 18 food additives and two microorganisms. RDA has approved 135 events for use in feed out of a total of 158 submissions.<br><br>Korea has not determined the regulatory status of innovative biotechnologies (e.g. genome editing, amongst others). There is growing interest by scientists and regulators in how Korea should approach this issue. Korea is closely watching developments in foreign countries.<br><br>Korea is actively participating in CODEX, IPPC, OIE, APEC and other meetings. Korea tends to loosely follow CODEX regulations in their safety assessment guidelines. | Despite substantial investment, Korea has yet to commercially produce any biotech crops. Korea does not export any biotech crops as Korea does not commercially produce any biotech crops.<br><br>Korea imports biotech crops and products for food, feed and processing, but not for propagation.<br><br>Korea is actively using genetic engineering for the development of animals that produce new biomedicines, bio-organs, etc. Korea is also using cloning technology to expand the number of animals with a high capacity to produce such useful materials and bio-organs. The research is being led by various government agencies and private entities including academia.<br><br>Despite active research by Korean scientists, Korea has yet to commercially produce any genetically-engineered animals. It is too early to estimate how close Korea is to commercial production. As for food use, Korean scientists are relatively unwilling to engage in research as they are concerned about consumer's acceptance of meat from genetically-engineered animals.<br><br>Korea does not export any biotech animal as Korea does not commercially produce any biotech animals | There are contradictory views about biotechnology in the Korean marketplace. The public holds positive views on the use of biotechnology in human and animal research, bio-medicine, and in the treatment of disease while they tend to be negative towards its use in food production.<br><br>Despite the Korean government's support for biotechnology research, the Korean public has a negative perception of crops and foods produced through biotechnology. For meat or food from genetically-engineered animals, it is expected that the public will have even more serious concerns. Consequently, the majority of government funding for biotechnology research is directed toward non-agricultural projects such as biomedicine, stem cell research, cloning, and gene therapy. Koreans in general maintain a positive view towards non-agricultural biotechnology |

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| | | | and believe biotechnology will play an important role in the country's economic development.<br><br>There are contradictory views about biotechnology in the Korean marketplace. The public holds positive views about the use of biotechnology in human and animal research, bio-medicine, and in the treatment of disease while they tend to be negative towards the use of the technology to produce food. No market studies are available. |
| Malaysia | The regulatory framework for GE animals is contained in the 2007 Biosafety Act and 2010 Approval Regulations.<br><br>Depending on the particular animal species involved, the Department of Veterinary Services (DVS) and/or Fisheries, as well as NRE would be the key government entities involved with the decision making. | No commercial production of GM or cloned animals.<br><br>No exports of GM or cloned animals<br><br>Malaysia is highly dependent upon imports for genetics in livestock production, particularly for ruminants. It is conceivable that some of these imports may have been derived from clones.<br><br>No trade restrictions related to animal biotechnology issues | Genetic Engineering in animal production has a negative perception among the public and government. Neither government nor private sector conducts research and development using Genetic Engineering in animal production. Although the NBB did approve a controlled field release GM mosquitoes in 2010, opposition to the project at that time has halted further efforts to develop GM mosquitos. The GM mosquitoes were developed to fight dengue by releasing massive numbers of "genetically sterile" male *Aedes aegypti* mosquitoes |
| Philippines | There is currently no legislation or regulations in place covering the development, use, import, or disposal of livestock clones, GM animals, or products derived from these animals or their offspring in the Philippines. | There are no Philippine GM or genome-edited animals or clones under development that are expected to be in the market within the next five years. | Public awareness on GM animals is low. |
| Saudi Arabia | The Kingdom of Saudi Arabia (KSA) follows two GSO issued mandatory agricultural biotechnology regulations - GSO 2141/2011 "General Requirements for Genetically Modified Unprocessed Agricultural Products" and the GSO 2142/2011" General Requirements for Genetically Modified Processed Agricultural Products". The two technical regulations require positive biotech labelling if unprocessed agricultural products, processed food product, feed products or seeds contain more than one percent genetically engineered (GE) plant ingredients. GSO 2141/2011 prohibits the importation of any genetically modified animals, birds, fish and their products. | Saudi Arabia prohibits the importation any genetically modified animals, birds, fish and their products. | Since the establishment of biotech labelling requirements in Saudi Arabia in 2001, no GE retail packed food products have been imported into the country. Major Saudi food importers do not import food products derived in part from genetic engineering and therefore do not put biotech labels on their products. They are concerned that dealing with biotech products could jeopardise their product image and result in losing market. Saudi |

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| | | | consumers have limited knowledge about agricultural biotechnology and, in general, hold negative attitude towards biotech products. On the other hand, some European, Asian and local food producers put the biotech free symbol on their product labels to promote their products. |
| Taiwan | Taiwan has a U.S.-style interagency coordination approach to regulate biotechnology. Taiwan Food and Drug Administration (TFDA) is responsible for food safety assessments, including pre-market approval and GM labelling and traceability. TFDA conducts import inspections and market surveillance inspection on food products, including GE products.<br><br>The Department of Animal Industry, under the Council of Agriculture, is responsible for regulating GM livestock. To date, Taiwan has established only one regulation regarding animal biotechnology, “Regulations for the Field Trial of Transgenic Breeding Livestock (Fowl) and Bio-safety Assessment" in November 2002.<br><br>Taiwan has not issued regulations specific to gene editing in animals | Taiwan does not produce or export any biotech crops.<br><br>Taiwan imported over $3.15 billion dollars of agricultural products from the United States in 2015, roughly one billion of which consisted of GM crops such as corn, soybeans and cotton. Taiwan imports a similar amount of GM crops from Brazil.<br><br>The Council of Agriculture (COA) stopped funding agricultural biotechnology research several years ago and has also stopped accepting applications for field testing. Since then research in all types of agricultural biotechnology has been limited.<br><br>GM livestock for food animals in Taiwan is not foreseen in the near future. Currently, no GM animals are in commercial production. Taiwan does not import or export GM animals. Researchers in Taiwan developed GM ornamental fish, but they are not currently traded due to regulatory challenges. | Taiwan’s domestic policy process, particularly regarding food safety, is highly susceptible to public influence, including from the highly saturated and active media market. Small consumer groups, media outlets, and individual university professors and legislators can have great influence on legislation and regulations. This has resulted in increased restrictions on biotechnology that are not always science based. |
| United States | Regulation of GM crops in the United States is divided among three regulatory agencies: the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA). Each of these agencies regulates GM products from a different perspective.<br><br>The FDA is responsible for regulating the safety of GM products that are eaten by humans or animals. According to a policy established in 1992, FDA considers most GM products as “substantially equivalent” to non-GM products. In such cases, GM products are designated as “Generally Recognised as Safe” under the Federal Food, Drug, and Cosmetic Act (FFDCA) and do not require pre-market approval. If, however, the insertion of a transgene into a food product results in the expression of foreign proteins that differ significantly in structure, function, or quality from natural proteins and are potentially harmful to human health, FDA reserves the authority to apply more stringent provisions of FFDCA requiring the mandatory pre-market approval of food additives, whether or not they are the products of biotechnology. | No GM animals are commercially produced for food consumption in the US.<br><br>After years of detailed study and analysis, the Food and Drug Administration has concluded that meat and milk from clones of cattle, swine (pigs), and goats, and the offspring of clones from any species traditionally consumed as food, are as safe to eat as food from conventionally bred animals. This conclusion stems from an extensive study of animal cloning and related food safety, culminating in the release of three FDA documents in January 2008: a risk assessment, a risk management plan, and guidance for industry. | |

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| | Given that no new or foreign proteins are generally expressed in edited products, the requirement for FDA approval was considered unlikely.<br><br>However, in January 2017, the FDA released a revised draft guidance that expands the scope of existing guidance on genetically engineered animals to include animals intentionally altered through genome editing techniques. It is unclear what impact this will have.<br><br>FDA "Guidance for Industry #187" updates the never finalised 2009 document "Regulation of Genetically Engineered Animals Containing Heritable rDNA Constructs" to the much more expansive "Regulation of Intentionally Altered Genomic DNA in Animals" to expand the scope of the guidance to address animals intentionally altered through use of genome editing techniques. Therefore, the edited DNA would be considered and regulated similar to that of drugs. | | |

# 9 Comment the potential impact of related issues such as consumption of edited plants by livestock, inoculation of edited rumen organisms and use of edited pest species

## 9.1 Overview

Gene editing has been coined as the new revolution in biotechnology. It gained this tittle as it influences pretty much all fields of life sciences from “basic” sciences to commercial applications. The latest depend on several factors including resolution on the intellectual property ownership and definition of country’s regulatory process. As the biotechnology is applied broadly, it is expected that it will be used during the development of improved pastures e.g. higher nutritional value or higher digestibility, and also on the development of feed supplements. It is quite inevitable that cattle will be fed with gene edited feedstock in the near future.

## 9.2 The use of gene editing on animal feed and feed supplements

Gene editing technology will certainly be used in the near future for the development of improved pastures aiming at improved nutritional value, resistance to environmental stressors and low fibre content - improved digestibility. I can also be expected to be used for the development of feed supplements, including microbes, aiming at improved feed utilization for instance.

Under the current regulation, gene edited crop or pasture would be treated as a genetically modified crop, requiring only OGTR approval before feeding livestock, no specific requirement. Similarly, gene edited bugs would require only OGTR approval before feeding livestock, however, this is trickier as it takes into account the “risk level” of the organism. Nevertheless, the regulatory procedures exist, after the regulatory review it might be amended, but the path for utilization and commercialization should still be available, and potentially will be used in the future.

## 9.3 The use of gene editing on pest animals

Together with the control of disease vectors, the control of populations of pest animals using gene editing approaches is a hot scientific topic. There are heated debates on the development of the technology, and mainly under considerations for releasing gene edited animals into nature.

Most of the gene editing applications aiming at controlling populations uses a different approach than what was discussed in this document, they use gene editing approaches to efficiently generate transgenic animals. These transgenic animals can bias genetic inheritance favouring the spread of a gene somehow deleterious to the population e.g. it bias sex ratio, lead to infertility or sub-fertility or to early deaf. Understandably there are lots of points that need to be discussed before the release of such animal, listing just few, the efficiency and sustainability of the method, the potential genetic reversal, the species containment, population monitoring, among many others. However, not to consider this biotechnology as an option, should not be an option.

# 10 List examples of potential disruptions to industry practices

## 10.1 Overview

The use of the term “disruptions” to define some of the potential impacts of gene editing within the industry might be a too rash term. Considering that the biotechnology is broadly adopted, certainly several industry practices will need to be adapted to the new reality. However, these should not be seen as negative or disruptions. A parallel could be drawn with the industry adaptation to the use of cattle cloning, where for instance breed societies nowadays are ready to register cloned cattle originated locally and internationally. With a somehow optimistic view, gene editing could reinvigorate the market and use of reproductive biotechnologies.

## 10.2 Breed societies

Breed societies will need to adapt their registration documents, if gene editing is adopted by the industry. Certainly each breed society will have to deliberate on how to deal with the new biotechnology, and the challenges it imposes on registered cattle, e.g. Mendelian genetic diseases or the presence/absence of horns might not be traceable using pedigree (if gene edited correct the “disease allele” or *POLLED* locus), or how to deal with the fact that there might be full cattle lineages resistant to a specific disease, or how to deal with imported gene edited genetics. These are just few of the points that will need to be discussed.

Nevertheless, considering that the two biggest beef breed societies (Australian Brahman Breeders Association and Angus Australia) have already developed guidelines for dealing with cattle cloning. One could be optimistic that, if needed, the breed societies will deliberate and develop strategies to face the new emerging biotechnology.

## 10.3 Estimating breeding values (EBV)

There are some scientific groups studying the potential impacts of gene editing on the methods for estimation of breeding values and their subsequent use for animal selection. There are several possibilities for dealing with gene edited animals while estimating breeding values, but at this point it is not clear which strategy will work the best. This is an active scientific field that should be further explored.

## 10.4 Import of genetic material

The first step needed before the import of any genetic material of gene edited animals (e.g. semen, embryos) is a clear regulatory framework. As mentioned before in this document, the Australian Government is currently reviewing its regulation of the Gene Act, which includes updating the guidelines considering gene editing. Once this is finalised, consideration on the import of genetic material should be clearer.

If gene editing follows the example of cattle cloning that is not regulated by OGTR and considered safe to eat by FSANZ, the import of genetic material would just have to conform to the current regulation (e.g. semen collected in registered centre, disease free, etc…).

However, depending on the outcomes of the current review run by OGTR, it might be imposed an additional step, which is too uncertain to be discussed at this point.

## 10.5 Gene editing and the adoption of other biotechnologies.

The use of reproductive biotechnologies in general is low in Australia, especially across the commercial beef herd. The use of artificial insemination is growing, but AI herds still represent a very small proportion of the national herd. The use of these biotechnologies, e.g. (fixed-time) artificial insemination, (fixed-time) embryo transfer, *in vitro* fertilization, all of them optimise the use of animals with superior genetic merit, havening the potential to assist the improvement of larger herds. At first instance, gene editing depend on reproductive biotechnologies to allow the breeding of such animals, then to have greater use of this high-tech animals, the combination with other biotechnologies are immediate. Thinking a bit further, this new technology has the potential to reinvigorate the market of reproductive biotechnology, e.g. IA centres, IVF services, FTAI, etc…

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