

# **Future Woollscapes:**

## **The Potential Impact of Biotechnology on the Wool Industry in 2029**

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Executive summary- the table summarizes key animal biotechnologies which are likely to have an impact on the wool industry over short (5-10), medium (10-15), and long term (15-25) time horizon. Prerequisite developments and social hurdles are listed.

Platform technology	Impact on the wool industry	Prerequisites for use (or more efficient use)	Expected societal hurdles	Timeline for introduction to wool industry	Comments
<b>Advanced Reproduction Technologies (ART)</b>					
Artificial insemination ( preservation of sperm cells for wide spread distribution)	increase rate of genetic gain (increased selection intensity)	improve survival rate of frozen semen, non surgical AI method	low	short term	a proven and powerful technology- needs improvement on cost
Sexed semen (separating X and Y chromosome bearing sperm for single sex offspring)	creation of single sex offspring in specific industry applications-first cross ewes, elite top sires, male prime lambs	increase throughput, improve survival rate of sorted semen, improve insemination technique, combine with IVP	low	short term	likely to be most effective if at low cost or in specialist applications
Use of sperm stem-cells	effective if coupled with GM modified stem cells	transfer to sheep, combine with GM	medium	medium-long term	very early technology - no early applications
Multiple ovulation and embryo transfer	increase rate of genetic gain (increased selection intensity)	improvement non-surgical methodology for high volume	low	short term	efficiency generally not cost effective vs gains/benefits
In vitro production of embryos ('test-tube embryos')	increase rate of genetic gain (increased selection intensity and shorten generation interval)	improve efficiency and reduce animal welfare impact	medium	short-medium term	potential for very high number of progeny from female donors. Need to be able to identify elite ewes.
Cloning via somatic-cell nuclear transfer (production of multiple copies of elite sheep)	increase rate of genetic gain (widespread use of elite genetics)	improve efficiency and reduce animal welfare impact	medium	medium term	needs major reduction in cost and improvement in efficiency
<b>Genetic Technologies</b>					
DNA markers and genetic selection (use of DNA tests for production traits in existing breeding programs)	increase of genetic gain-selection options for difficult and costly traits	identification of ovine QTL, improve genetic tools (better understanding of ovine genome)	low	short-medium term	low cost pedigree analysis can have immediate impact, gene based selection will be longer term

DNA diagnostics of pathogens	improved animal health and disease control	develop DNA tests	low	short term	high probability of successful application-linked to QA
Gene-expression profiles (understand interaction of genes and gene products in living animals)	selection based on complex metabolic pathways, research application	develop a comprehensive ovine micro-array	low - medium	medium - long term	can for platform for selection tools based on gene function and potentially greater efficiency if at low cost
<b>GenART- combination of genetic and reproductive technologies</b>	ultra high genetic gain, accelerated dispersal elite genetics	dependant on progress in bort ART and DNA technologies	low	medium	likely to be most effective use of technologies
<b>Genetic Modification (GM)</b>					
Somatic gene transfer (gene therapy, correction of genetic defects, bypass production limits)	depending on chosen application: improved productivity	improve technologies and identify useful application	medium	medium - long term	major spin-off expected from biomedical area in next 10-15years
DNA vaccination (use of DNA plasmid/vector to create an immune response)	improved animal health and disease control, some production applications	adopt and improve existing technology	low - medium	short-medium term	likely to be one of the most effective biotechnology for animal health applications
Gene silencing (method to switch of specific genes)	depending on chosen application: improved productivity, quality assurance	improve technologies and identify useful application	medium	medium - long term	long term technology under development for effective delivery
Germ-line GM (genetic modification that is passed on to offspring)	depending on chosen application: improved productivity, novel traits	improve technologies, identify useful application, reduce animal welfare impact	high	medium - long term	only realistic mechanism to incorporate new traits and pathways in target species
<b>Stem Cells</b>					
Stem Cells (cells with the ability do divide and differentiate into different types of cells)	Tool for many GM applications	identify ovine embryonic or tissue specific stem cells	medium - high	medium - long term	needs to be coupled with GM technologies for specific target applications

## 1 Setting the scene

The first Future Woolsapes meeting defined key factors and constraints that directly influence the future of the wool industry. Biotechnology as a set of platform technologies is potentially well positioned to change the impact of many of these key factors by essentially decreasing cost structures in the wool industry and/or improving income through superior product range and quality. There will be no generic single biotechnology that will achieve a change in all key factors. The approaches required will be highly specific for each of the key factors to be targeted. In addition, the first Future Woolsapes identified the environmental forces that may accelerate some of the primary external drivers for change over which the wool industry will have no direct control. It is highly likely that biotechnology will have an indirect impact on some of these environmental forces by changes in other (competing) livestock industries, environmental land use and global climatic conditions. It is not sensible to view the impact of biotechnology on the wool industry in isolation without its possible impact on other agricultural and environmental systems. Finally the application of biotechnology in the wool industry should be seen in the context of a sheep industry where increasing pressures may shift from a specialist production system (wool and meat) to a multipurpose sheep commodity system (wool, meat, milk), reference is therefore made to wool industry and sheep industry alike where appropriate.

### 1.1 Biotechnology and the link to bioscience

Developments in biotechnology arise from scientific discovery and, in particular, from discoveries in the field of Bioscience. The latter specifically deals with a deep and integrated molecular understanding of the biological processes of all life forms on earth. The enormous investment in human bioscience and specifically biomedical science, fuels the global investment in biotechnology. An investment in sheep-directed biotechnology will automatically obtain substantial leverage from such investments, and should focus on the adaptation of generic biotechnologies for sheep-specific applications. Nevertheless, a deep investment in ovine bioscience is required to ensure that maximum benefit from biotechnology can be obtained to increase the competitiveness of the wool industry.

## 2 Summary of platform of biotechnologies 2004 - 2029

Biotechnologies in the livestock industry are based on the combination of advanced reproduction technologies (ART), advanced selection and diagnostic tools based on genetic technologies, and genetic modification (GM). In order to exploit the sheep genome, we need to develop a deep understanding of the genetic makeup of sheep and need to have cost-efficient tools to disseminate superior and/or GM animals. A simulation study by Smith et al (2000) showed that the lag phase in dissemination of improvement and/or new genetic material through current sheep breeding structures can be drastically reduced using ART (in this study from 26 years to 7 years).

In this Chapter, we describe the key technologies in the areas of ART, genetic technologies and GM, and the potential impact of these techniques on biotechnology in general. We highlight the areas that require further improvement in the next 25 years before commercial exploitation. The following Chapter will identify specific opportunities for the wool industry.

## **2.1 Advanced reproduction technologies (ART)**

The status of ART in ruminants, its problems and limitations, as well as the potential impact on future animal biotechnology, are summarized by Kane (2003), Galli et al (2003) and Evans and Maxwell (2000). Most of these methods are established for use in research and stud breeding, and further commercialisation depends on economics, societal values (animal welfare, environmental concerns, consumer acceptance) and regulatory agencies (animal health and food safety) (Faber et al 2003).

### **2.1.1 Semen technologies**

#### Artificial insemination (AI)

In the sheep industry, fresh semen is used in combination with cervical/vagina insemination, whereas frozen or chilled semen needs to be deposited directly into the uterus (involving laparoscopic AI or transcervical techniques). The use of frozen or chilled semen is less favourable in terms of cost, time, animal welfare and efficiency, but it allows transport and/or storage of semen. Improvement of semen processing to improve survival rate of frozen or chilled sperm, and development of a more efficient non-surgical AI method would lessen the impact of these problems.

#### Sexed sorted semen

Fluorescence activated cell sorters (FACS) are currently used to sort sperm depending on their amount of DNA – Y-chromosome-bearing sperm (which will cause the resulting offspring to be male) carry less DNA than X-chromosome-bearing sperm (which will cause the resulting offspring to be female). The method works reliably in sheep, but only low numbers of sperm cells can be sorted and thus special insemination techniques (involving oviductal insemination) are required. Improvement of sperm-survival and increase of the number of sperm sorted, and combining this technology with other reproductive techniques (see below) will make this technology more useful for the industry.

#### In vivo and in vitro culture of spermatogonial stem cells

This is a rather new technology which has been developed in mice, but preliminary results indicate that it can be used in livestock. The technique involves collection and *in vitro* culture of spermatogonial stem cells (cells that give rise to sperm cells) and their subsequent transplantation into the testes of other males that have previously been denuded of their own spermatogonial stem cells. Prolonged storage of spermatogonial stem cells is possible. During cell culture, the spermatogonial stem cells can be genetically modified, and the recipient male would then produce GM sperm, which could be collected and used in an AI program. However, rearrangement of DNA occurs during meiosis, and sperm cells will not all be identical.

This procedure is still in early stages of its development, and further research will concentrate on methods to achieve pure preparations of stem cells and increase understanding of *in vitro* spermatogonial stem-cell proliferation. The technology has enormous implications for genetic manipulation of sperm. Some of the side effects that are currently seen in transgenic animals (see below) are likely to be overcome

with this procedure, as it enables normal epigenetic development of the embryo to occur.

### **2.1.2 Female reproductive technologies**

#### **Multiple ovulation and embryo transfer (MOET)**

MOET has been well established in the ruminant industry for 20 years. Adult sheep are superovulated by hormonal treatment, followed by insemination (laparoscopic AI or natural) and the resulting embryos are recovered surgically. Embryos can be transferred directly to hormonally-synchronized recipients using a method similar to laparoscopic AI or can be cryopreserved for use at a later date. In cattle, MOET can be performed as a non-surgical procedure and is therefore commonly used by the industry (lower cost and animal welfare impact). The surgical procedures involved in sheep allow fewer repeat cycles of MOET, and therefore only a limited number of offspring can be produced per ewe. Despite many attempts, it has not been possible to adapt the bovine non-surgical technique to sheep.

#### **In vitro oocyte maturation (IVM), fertilization (IVF) and embryo production (IVP)**

IVP is a newer and more flexible procedure, but is more demanding technically and more expensive. It is based on the collection of immature oocytes from abattoir ovaries or living animals via ovum pick up (OPU). The oocytes then undergo *in vitro* maturation (IVM) and *in vitro* fertilization (IVF). The resultant embryos are cultured (*in vitro* culture or IVC) before being transferred to recipients. The combination of IVM and IVF is known as *in vitro* embryo production (IVP). If OPU is performed in lambs, the process is called juvenile *in vitro* embryo transfer (JIVET).

The basic technologies exist, but research on fine-tuning is required to improve the processes (improve efficiency and decrease cost). To improve the viability of embryos, a better understanding of apoptosis, signal transduction and cell-cycle regulation is required, as well as a further optimisation of conditions for maturation and culture. A main concern in IVP is the occurrence of abnormal fetal and neonatal development and growth (large-offspring syndrome). This syndrome is characterized by dystocia, increased perinatal death and disturbed growth and development of bogy organs, and is considered as an animal-welfare issue. It is believed to be caused by exposure of pre-elongation embryos to unusual environmental conditions. Better optimisation of media conditions might overcome this problem in the future. The main impact of improved IVP lies in the increased supply of embryos either from high genetic-merit females for direct transfer or for embryo cloning and/or transgenesis. IVP can also assist in progeny testing of females, which is so far limited by small number of offspring.

### **2.1.3 Cloning**

Cloning is the production of genetically identical animals. This can occur naturally in identical twins, during embryo splitting in combination with MOET or IVP, or by using more advanced technologies such as nuclear transfer. Cloning by nuclear transfer from undifferentiated embryonic cells has been possible for many years, but this approach generates only a limited number of clones. In 1986, a breakthrough was achieved when Campbell et al successfully applied the nuclear-transfer technique to cultured undifferentiated embryonic cells, thereby opening up the prospect of creating an unlimited number of clones from an individual embryo. A second breakthrough

was achieved the next year with the birth of Dolly (Wilmut et al 1997) , who was created by so-called somatic-cell nuclear transfer involving the transfer of a nucleus from a cell from a culture of differentiated adult cells. This approach allows the creation of multiple clones from animals with known genotype and phenotype.

### Somatic-cell nuclear transfer

In somatic-cell nuclear transfer differentiated cells of an animal of interest are collected and cultured. The diploid nucleus of a cultured cell is transferred to an enucleated oocyte (IVM oocytes from abattoir animals, from which the nucleus has been taken). An electronic pulse fuses the nucleus with the oocyte and activates the resultant “zygote”, and the resulting embryo is then transferred to a recipient.

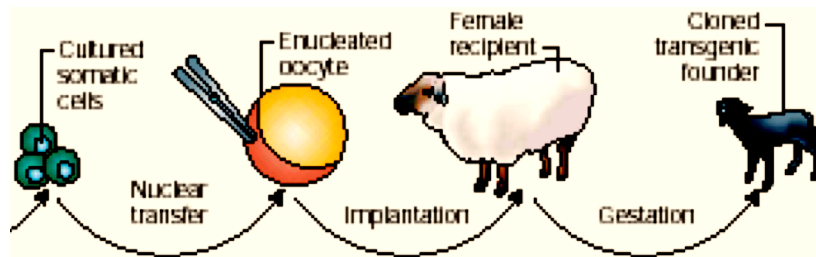


Fig. 1: Somatic-cell nuclear transfer (modified from Clark and Whitelaw, 2003)

Cloning is now feasible in many livestock species, but as yet it has not been conducted at commercial scales. Current methods are relatively inefficient (0.5-4% viable offspring) and are linked to animal-welfare concerns: a high rate of pregnancy losses, problems with offspring survival of newborns and increased incidence of abnormal development due to incorrect reprogramming of the nuclear DNA (epigenetics) and the inevitable un-natural conditions during *in vitro* culturing (see IVP) make this still a highly experimental technology. There are clear indications that the epigenetic abnormalities are not passed on to offspring of cloned animals, and significant progress is being made in improving efficiency. In combination with genetic modification, this approach has great potential for the livestock industry by producing genetically identical copies of superior genotypes or by producing and clonal propagation of transgenic animals.

## 2.2 Genetic technologies

### 2.2.1 DNA markers and genetic selection

Rohrer (2004) provides a detailed overview of the potential impact of genetic technologies on livestock production. For a detailed description of tools used in genome mapping see <http://www.ncbi.nlm.nih.gov/About/primer/mapping.html>. During the past decades we have developed quantitative and molecular genetic tools (e.g. genome maps and DNA libraries) that allow us to identify single genes which cause inherited disease (in sheep, e.g.: spider lamb syndrome: Cockett et al 1999; Neuronal Ceroid Lipofuscinosis: Tyynela et al 2000; glycogen storage disease V: Tan et al 1997) and genes which have a major impact on production traits (in sheep fecundity genes: Inverdale: Galloway et al 2000 and Booroola: Wilson et al 2001; and scrapie resistance: O'Rourke et al 1997). Several ovine studies have aimed to find quantitative trait loci (QTL: genome regions containing genes with a major impact on quantitative traits) for production traits to do with growth, meat, wool, milk; and parasite resistance (e.g. Diez-Tascon et al 2001, Beh et al 2002, Cavanagh et al

2003, Raadsma et al 2004). Once a QTL has been identified, specific DNA markers located in the area of the QTL can be used for selection in an indirect DNA test; referred to as marker-assisted selection (MAS). Once the gene that causes the variability for the trait has been identified within the QTL, a more accurate DNA test can be developed - a so-called direct DNA test, which is used in gene-assisted selection (GAS). So far, progress from experimental QTL results to the utilization of QTL in MAS-supported breeding programs has been slow. However, it is expected that the whole sheep genome will be sequenced in the next 10 years - the National Institute of Health is now aiming to reduce the cost of sequencing by at least four orders of magnitude in that time period. In combination with comparative information about the function of genes, we will see a dramatic increase in the identification of the important genes which are the source of important variation in all production traits. In parallel, we will see a rapid development of tools that allow low-cost, high-throughput genotyping. Thus, the simultaneous analysis of hundreds of genetic loci with known impact on production traits will become feasible.

### **2.2.2 DNA diagnostics of pathogens**

In addition to their potential for improving the effectiveness of animal breeding, DNA technologies are currently being used in the diagnosis of several pathogens – e.g. Q fever and Johne's disease. Similar tests will be available in the future for more pathogens and will become easier and cheaper to perform.

### **2.2.3 Gene-expression profiles**

The rapid development in genomics and proteomics allows us to move from a simplistic approach of looking at single genes and proteins to a more holistic approach in which we are able to evaluate large numbers of genes and proteins, their degree of activity and their interaction. The tools and technologies for these methods are rapidly evolving, with microarrays being the most commonly used tool at the moment (see <http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html> for more detail). Microarrays are small membranes or glass slides that contain samples of many thousands of genes arranged in a regular pattern. They are useful to identify the extent to which a single gene is active in an organism at a certain time point in a specific tissue, i.e. if genes are switched on/off or are highly activated (gene expression). They represent an important tool to increase our understanding of genomes - we will be able to ask increasingly complex questions and develop a deep understanding of how genes function and interact. This understanding of metabolic pathways will enable us to manipulate/control these pathways using genetic (genetic modification or selection) or pharmacological approaches.

## **2.3 Genetic modification (GM)**

Genetic modification or genetic engineering refers to the manipulation of the original DNA content of an organism. This includes insertion of unrelated or foreign DNA and duplication or elimination of existing genes. There are different technologies available to perform these changes; most of them are based on the use of a vector (e.g. a specially-modified virus) that can carry a DNA construct (the gene of interest, a promoter (on/off switch), an enhancer, and a selectable marker gene) into cells. Some methods are used to modify reproductive tissue (germ-line cells), in which case the changes are passed on to the offspring of the GM animal. Other GM technologies change somatic cells, and thus are unlikely to affect all cells of the

individual and are not heritable. The first GM livestock was reported in 1985 (Hammer et al 1985). Future developments, particularly those made in the biomedical fields, e.g. in human gene therapy, will provide us with safe and efficient delivery systems for the insertion of new genes or the manipulation of existing genes (allele switching, knock in or knock out, silencing). In addition, with ART in livestock, we will be likely to efficiently and safely produce transgenic animals for a range of different reasons.

### **2.3.1 Non-germ line GM**

#### Somatic gene transfer (gene therapy)

Somatic gene transfer (introduction of an additional gene) has had some limited successes in treating human inherited diseases (Hacein-Bey-Abina et al 2002, Aiuti et al 2002). The gene transfer can be *ex vivo* (cells are treated in a lab and then injected into the body) or *in vivo* (gene vectors are given directly to the patient). A range of physical (e.g. electroporation, microinjection, particle bombardment, lipofection) as well as viral (e.g. retrovirus, adenovirus, adeno-associated virus, lentivirus) delivery systems are under investigation. Each technique has a range of advantages and disadvantages (e.g. insert capacity, integration, site of integration, duration and level of transgene expression, ease of administration, tissue specificity, efficiency of transfection) and each disease might require a different approach due to these differences. Although the techniques have primarily been developed to correct genetic disorders in humans, it is highly likely that such techniques can be used to increase performance in other traits, including production traits in livestock.

#### DNA vaccination

DNA vaccines consist of a foreign gene of interest and a promoter, which are cloned into a bacterial plasmid. After administration of the 'plasmid or naked' DNA vaccine into mammalian cells, the gene is expressed and produces a protein which induces an immune response. DNA vaccines are promising alternatives to conventional vaccines (live attenuated, killed or protein subunits) due to their advantages in terms of type of immune response, antigen presentation and immune memory (Gurunathan et al 2000). DNA vaccines are more cost-effective, easier to produce, easier to store (no need for a 'cold chain') and are believed to be safer than conventional vaccines. Current research is focusing on improving immunogenicity (Sasaki et al 2003). To achieve stronger, longer-lasting and optimised immune responses, different routes of inoculations (intramuscular, intradermal, intranasal, particle bombardment by gene gun or powder jet administration) and different adjuvants (conventional and genetic: expression vectors of cytokines and immunomodulatory molecules) are being investigated. Although DNA vaccines have primarily focussed on health applications, it is likely that such vaccines can also be targeted to enhance production traits in livestock, including reproductive cycles.

#### Gene silencing

McManus and Sharp (2002) and Sioud (2004) review the use of RNA interference (RNAi) as a technique to specifically silence or knock-down gene expression in mammals. RNAi is a mechanism of gene-specific post-transcriptional gene silencing where small RNA molecules bind to a targeted messenger RNA (mRNA), which initiates the degradation of that mRNA. The production of the protein encoded by the mRNA can be reduced by up to 90%. This technique is extremely useful in the

analysis of mammalian gene function, as a tool to block the expression of disease-causing genes, and could develop into a tool that allows external down-regulation of specific gene products in livestock.

### **2.3.2 Germ-line GM**

#### Pro-nuclear injection

Pro-nuclear injection includes the direct injection of a DNA construct into either the male pronucleus or female pronucleus of the fertilized egg. Due to the low efficiency (only 3-5% of animals born carry the transgene, and some animals are mosaic), the lack of control of expression (depending on how many copies of the transgene insert in the genome, the expression of the gene differs) and the random insertion (construct can interrupt important other genes), this approach has not been very successful. Furthermore, researchers often tried to modify rather complex biological traits without having a full understanding of all biological impacts. Not surprisingly, the full potential of this technique has yet to be realised.

#### Embryonic stem cells

In mice, germ-line-competent embryonic stem cells can be cultured and then modified, e.g. by using viral vectors that randomly integrate in the genome or via homologous recombination, which allows site-specific or targeted integration. The nuclei of modified cells are transferred to enucleated blastocysts, which are transferred to recipients. This approach is used routinely to create knock-in (introduce a new gene and its gene product or alter quantity or activity of a protein) or knock-out (eliminate a gene and its product) mice for gene-function studies. Germ line-competent ES cells have not yet been identified in livestock.

#### In vitro transformation & nuclear transfer

The development of somatic-cell nuclear transfer provides an alternative to the pro-nuclear injection method of GM. Somatic cells can be cultured, modified (random or targeted integration) and only those cells that have been successfully transformed are then used for nuclear transfer. This approach overcomes the problems with pro-nuclear injection, and therefore has great potential. However, the undesirable side effects of nuclear transfer (described above) and the limited control of amount and tissue specificity of expression still raise animal-welfare issues. However, offspring of the transgenic animal should inherit the genetic modification without suffering from the side effects of nuclear transfer, homologous recombination allows site-specific integration and increased understanding of functional genomics will lead to the development of a broader range of tissue-specific promoters, which can be activated or regulated via external stimuli.

### **2.3.3 GM plants and microbes**

Similar approaches of gene modification can be used to manipulate plants or microbes but these will not be discussed in this report.

## **2.4 Stem cells**

An interesting introduction to stem cells by Alison et al (2002) attempts to describe different types of stem cells, their potential to differentiate and highlight some of the issues related to current stem cell research (e.g. stem cell markers, stem cell disease and molecular control of stem cell behaviour). Different types of stem cells exist and

are subdivided by their ability to differentiate into different cell types: zygotes are totipotent, i.e. able to differentiate into any other cell type, embryonic stem cells are pluripotent, i.e. form most cell types but can not form trophoblast cells, tissue specific stem cells are multipotent, (i.e. differentiate to cells appropriate for the specific tissue), and committed progenitors are unipotent (i.e. they are capable of generating a specific cell type. Recent studies indicate that it might be possible to manipulate tissue specific stem cells to differentiate into a cell of another tissue. This is called transdifferentiation or adult stem cell plasticity and has great potential to overcome the lack of embryonic stem cells in livestock.

Applications of stem cell research focus mainly on human therapeutic approaches (e.g. regenerative medicine) and use in ART and GM (see above). The sheep industry might benefit from the intensive research into human hair follicle proliferation and adult hair stem-cells related to the cosmetic application of control of hair growth human population (Morris et al 2004, Tumber et al 2004)

### 3 Underpinning technologies

In order for the sheep industry to capitalize on the global pool of biotechnology capability and the under-pinning bioscience, sheep-specific information is required for adaptation of generic technologies. In many cases, this information is scant and unlikely to develop without stimulus from the wool industry, since sheep are seldom targets for mainstream scientific discovery. Some of the specific prerequisites and investment targets for generic tool development over next 25 years are listed below.

**genome information- sheep specific.** In the first instance, the human and bovine genomes will be a suitable reference for the sheep genome. Over time, as DNA-sequencing costs decrease and with significant spare global capacity in sequencing, a multi-coverage DNA sequence of the sheep genome is critical. When it does become available, the genome-sequence information will be appropriately annotated to cross-reference sheep genes and their putative function by alignment with human, mouse and other genomes. Multi-sequence coverage is critical to understand and mine the sequence diversity in the genome for functional polymorphisms and high-density genetic markers.

**genome screening at industry level.** Although generic platforms exist for ultra-high capacity screening of DNA and RNA sequences, the specific targets (polymorphisms/markers, and RNA probes) will need to be sheep-specific. The availability of a genome-wide DNA screening tool and a transcriptome tool for gene expression will enhance further exploration of the sheep genome. The additional requirement to have such platforms operate at ultra-low cost would allow genetic diversity in the commercial sheep sectors to be characterised and exploited. If cost was no limit, this would ideally happen for each animal from which commercial product was obtained, and this information could be integrated into high-level management, marketing and genetic improvement systems.

**animal cell culture and manipulation.** Once again, generic platforms for animal cell culture and manipulation will develop rapidly over next 25 years.

However, sheep-specific cell lines will need to be developed, in particular immortal stem-cell lines for specific tissue targets will require a sheep-specific investment.

**gene control/regulation- RNAi, and inducible promoters.** Many gene regulatory systems are likely to be sheep-specific. To exploit biotechnologies that depend on control of gene function, sheep-specific information on the key regulatory elements will be required (such as promotor sequences, and RNA targets). Most of this will come from comparative information (in the first instance) and later from the sheep genome sequence.

## 4 Impact of biotechnology in the sheep industry in 2029

In the near term, the major impact of biotechnology will be on animal health and specifically vaccine and diagnostic technologies, and animal breeding (genetic and reproductive) technologies. These technologies will, by and large, require no genetic modification (non-GM). The longer-term impact of biotechnology will be in development of products with novel properties, novel products, and more efficient resource use. In this case, biotechnology will take sheep/wool production outside the normal biological boundaries of the sheep genome and require some degree of genetic modification (GM).

### 4.1 Animal breeding, genetic improvement and reproduction

#### 4.1.1 DNA-based diagnostic technologies

Significant global activity has been mounted in both human and livestock to provide tools for diagnostic screening at the DNA level. Such tools are normally based on identifying genetic variants (favourable and unfavourable) of important genes, which affect phenotypic traits of interest. In livestock, the application of such technologies offers enormous scope to predict genetic merit with high level of accuracy, and at a very early age (potentially at the embryo stage). The current state of technology in sheep and most mammalian livestock species is greatly limited by insufficient information of the genome, i.e. of the identity and function of genes that influence the important production traits. Once again, the human genome and now some animal genomes provide excellent reference points for the sheep genome. In particular, the bovine genome sequence will of enormous value. The key aim for the sheep industry is to identify those DNA sequences that specifically impact on sheep traits. It is anticipated that by 2029, the sheep genome will have been sequenced, and that all important production genes and their genetic variants known. In addition it is also expected that the collection of DNA-based information will come at prices amenable for ultra high-throughput analysis in disposable format at point of collection (i.e. on farm, saleyard or abattoir). Some of the applications arising from DNA-based diagnostics include:

#### DNA-based identity testing

This is technically the least demanding application, and is already feasible with current knowledge of the sheep genome. The major advantage will be the capacity to link animal identity to pedigree information and in turn to link this with current systems for prediction of genetic merit, thereby providing a means to accelerate genetic progress. It will be particularly powerful when it is combined with identity

tracking at times when important production information is collected in the commercial sector – e.g. wool production/wool quality assessment of individual fleeces, carcass quality at time of processing. It has the potential to track genetic merit from the seed-stock sector right through to the commercial base, with an integrated information system. This will require parallel technology developments in phenotype assessment of commercially important traits, preferably in real time (i.e. wool processing performance, carcass quality). The potential for value adding is high. The major limiting factor is the present cost of high-volume DNA analysis for a relatively low-value animal. This is changing rapidly, and routine DNA-based identity and parentage tools are likely to be cost-effective within 10 years.

#### DNA-based selection (genetic markers)

This technology has provided much of the impetus to gene discovery for livestock (and human medical applications for that matter). With the development of genome information to DNA-sequence resolution; it is feasible to predict allelic (genetic) variants which have a favourable or unfavourable effect on phenotype (production). The technology to date has been most successful for prediction of inherited disorders determined by a single gene. In these cases, genetic prediction can unambiguously determine if a potential breeding candidate carries none, one or two copies of a specific mutation. For most production traits, however, such diagnostic tests have been disappointing. This is largely due to the fact that most traits are polygenic in nature (i.e. multiple genes of moderate to small effect contribute to the phenotype), and insufficient trait-specific markers are known to make a cost-effective prediction of genetic merit. The interim research position where regions in the genome are known to carry genes of detectable effect – QTL - are imprecise and unlikely to be of practical value in the industry at large until a large panel of such QTL have been resolved to closely-linked flanking markers and, better still, to the specific mutations which are responsible for the production differences. Even in such a scenario, sufficient direct gene markers need to have been identified to make MAS and GAS commercially attractive additions to conventional systems for prediction of genetic merit. DNA-marker-based selection will have greatest commercial utility when it is applied to traits that are expensive and difficult to measure (e.g. isolated pigmented fibres, carcass quality, processing quality, disease resistance), are expressed late in life (e.g. reproductive performance, lifetime productivity), or are expressed in one sex only (e.g. reproductive/maternal performance). The technology could not only allow selection of inferior/superior individuals at a very early age, it could also allow specific introgression (introduction) of new allelic variants (genes) with large effect into target populations (e.g. in relation to ovulation rate, disease resistance). The technology has no real technical barriers and it is only a matter of time and effort to identify most, if not all, of the genetic variants that contribute to important production traits. The main limitation is sufficiently ultra-high capacity genome analysis at sufficiently low cost to link genome to phenotype in either a routine commercial setting (preferred) or research capacity.

#### **4.1.2 Advanced reproductive technologies (ART)**

The importance of the distribution of elite germ plasm is well recognized in all livestock industries. With the advent of AI as one of the earliest and most successful animal biotechnologies to date, there has almost been no limit on the number of progeny a sire can generate. The rate-limiting step to the distribution of elite germ

plasm has largely been with the female, where traditionally a relatively small number of progeny per lifetime are realized. The rapid development and use of IVF and IVM will result in significantly greater capacity for elite females to contribute to the distribution of elite germ plasm. The use of nuclear transfer (cloning) is another avenue where elite animals could potentially be clonally propagated in very large numbers. The use of clonal propagation of elite commercial lines has seen significant changes in the plant industries. The rate-limiting steps and potential niche applications of cloning are discussed below.

#### *Semen preservation and AI technology*

The distribution of elite genetics through AI remains one of the most powerful biotechnologies for livestock industries. The widespread use of AI in sheep is limited by cost exceeding benefits. Lower costs for semen processing and insemination, combined with long-life semen post insemination, would make use of AI more attractive. The proviso for AI to have significant industry impact also rests with the capacity to successfully identify economically superior germ plasm for widespread distribution. The use of advanced sperm-cell technologies such as transgenic manipulation of spermatogonial stem cells is still in its infancy, but scope to distribute GM germ plasm throughout a broad commercial tier remains attractive for traits that bring a quantum leap in profitability (see transgenic impacts below).

#### *Single-sex progeny*

In complex industry production systems such as prime-lamb production, the capacity to control sex of progeny can have significant impact, e.g. generation of first-cross females as prime-lamb dams, or male lambs from terminal sires. Control of sex in complex multi-tier breeding programmes (i.e. rams to breed elite rams, and specialist dam lines) provides for potential greater managerial control and efficiencies of such programmes. Sex preselection at present is only feasible at time of embryo screening, or via use of sex-sorted sperm. Use of sexed semen, in particular with successful freezing and low-dose insemination, is likely to be a near-term technology for specialist applications. The use of sires that produce viable semen only of a single sex is technologically still a holy grail, but would lead to significant practical use of single-sex progeny systems.

#### *In vitro production of embryos (IVP)*

As noted previously, this technology comprises IVM and IVF. Current sources for large number of immature oocytes are derived from mature (MIVET) or juvenile females (JIVET) as early as 3 weeks of age. The capacity to harvest 20-80 oocytes per cycle and combine with IVF, including the use of sexed semen, can generate 5-50 times greater number of progeny per female lifetime. The current cost structure and compounding inefficiencies in the technology pipeline suggest that the major impact for the sheep industry in the short term will be in generation of large numbers of potential elite seedstock. A quantum leap in oocyte culture enabling culture of primordial germ cells would enable many 1000s of oocytes to be harvested from a single donor, making females closer to the male equivalent of AI. Similar constraints on technology and cost structures apply as for MIVET and JIVET.

### Cloning

The simplest forms of clonal propagation through embryo splitting, and use of embryo cells as donor cell lines for nuclear transfer, have had limited commercial success. The primary reason is that limited number of progeny can be generated under this technology. Nuclear transfer from cultured cells of adult proven animals may have enormous impact on the industry if entry cost (suggested price of \$800/viable clone) and efficiency were sufficiently high enough. The primary avenue would be through use of extreme elite sire clones in the commercial sector in field matings (Such sires would effectively become field AI stations of the elite donor sire). If cloning was truly a low cost means for clonal propagation of elite germplasm, ultra-high genetic-merit clonal ewe flocks could be used for low cost amplification of superior male germ plasm in the commercial sector by mating to clonal lines of ultra superior rams. Robust identification of the animals with the greatest genetic merit remains an essential prerequisite for clonal propagation of high impact germ plasm. It could significantly decrease the genetic lag currently seen in the multi-tier industry sector.

#### **4.1.3 Combining genetic and advanced reproductive technologies (GenART)**

Although the two technology platforms of genetic and reproductive technologies each can make a significant contribution to improved sheep productivity, the true benefits are captured when they are used in combination. The ability to make genetic predictions at a very early stage of an animal's lifetime – e.g. at the 32-64-cell embryo stage 4 days after fertilization, means that animals can be genetically evaluated well before their own performance records become available. When genetic screening is combined with juvenile accelerated reproductive technologies, both selection intensity and generation interval are favourably affected to give accelerated genetic gain not possible through conventional selection. The prerequisite requirements are that sufficient genetic markers are available to accurately predict genetic merit for all traits in the breeding objective, and that the procedures are sufficiently robust and cost effective.

## **4.2 Production traits (wool/meat/milk)**

### **4.2.1 Accelerated conventional genetic improvement**

Traits of economic significance that are currently incorporated in breeding objectives can potentially benefit from GenART technology platforms discussed above. It would merely accelerate the rate of genetic gain in the direction already set for conventional selection. The technology could be suitably mature for commercial application within 10 years. A significant investment in identification of sufficient genetic markers for all traits that determine profitability and development of robust reproductive platforms remains the primary prerequisite.

### **4.2.2 Improvement through non-genetic means**

Some of the most eagerly awaited outcomes from the genomics era in human and livestock applications are the discovery of novel drug and vaccine targets. Targets of potential interest in sheep production include control of female reproductive rate (ovulation rate and subsequent lambing rate) and increased muscle mass without loss of wool production. A prerequisite is a detailed understanding of the biological

pathways to be targeted via functional genomics and proteomics research investments, including comparative genomics with other species. The advantage is that application is not dependant on genetic selection or genetic merit of the flock and can be applied to match environmental circumstances. Disadvantages are that results are not permanent and may have to be re-applied through a lifetime, and in each generation of progeny. Furthermore, some of the drug targets for increasing follicle density and adult wool-production characteristics may have to be applied *in utero* via application to the ewe, and may therefore not be amenable to simple delivery routes. Uptake of such applications may be limited, given that prototype drug and vaccine targets are already available for a number of commercial targets (e.g. testosterone precursors for increased muscle mass and fleece weight, Fecundin for ovulation control).

#### **4.2.3 Improvement through genetic modification**

Application of GM technologies at either germline (permanent and passed on to progeny) or non-germline (restricted to each individual only) offer the greatest scope to take production traits beyond their normal biological boundaries. The options are to include control of gene expression/regulation (via knock-out or antisense and interference RNA technologies) to remove genetic control of pathways which have a negative impact on production traits. Examples could be removal of gene action which is responsible for pigmentation, and for limitation of ovulation rate, wool production, chemical composition, muscle development and appetite. Alternatively, transgenic technologies ranging from gene enrichment (to add additional copies of functional sheep genes) to cross-species transgenic routes, may lead to significant changes in fleece chemical composition, processing performance via limiting scale development, shrink proofing, fibre diameter/length strength ratios, increasing resistance to UV damage and weathering, and increasing uniformity of dye uptake. The use of transgenic technologies for sheep to acquire new biochemical pathways to produce their own cystine and glyoxylate cycle for energy supply to the wool follicle, were well ahead of their time and should not be forgotten as transgenic platforms improve over the next 25 years. The major limitations are technological (it has been remarkably difficult to control gene function in animal target tissues with pro-nuclear transgenesis), and lack of identification of suitable gene pathways, which could be added or deleted from the sheep genome. It is likely that once GM consumer products are widely accepted through the food-production system, and technological hurdles have been overcome, GM sheep will be the norm rather than the exception in 2029. The lure to make quantum leaps in breaking biological boundaries will prove irresistible.

### **4.3 Health and management**

The same subheadings for potential biotechnologies applied to animal health apply as for production traits above, with the exception that the balance in the final mix of biotechnologies will be different. In particular, the use of genomics to identify new drug and vaccine targets will be important for future animal health programmes.

#### **4.3.1 Accelerated conventional genetic improvement**

Use of genetic markers to increase natural resistance of sheep for the major diseases, which impact on profitability, is inherently very attractive. Alternatively, the use of genetic markers to eliminate animals/alleles leading to extreme susceptibility

to disease is equally valid for most production diseases, since prevalence is rarely so high that the entire population is affected. It may, therefore, be more sensible to remove the most susceptible tail-end from the flock. Application of genetic markers for disease resistance or traditional genetic means to improve resistance to all major diseases (flystrike, internal parasites, footrot, and ovine Johne's) has been limited. To date, no genes of very large effect have been found. The likelihood of improvement of "generalized" resistance to all diseases via a common set of natural resistance genes is low, given the neutral genetic correlations between disease-resistance traits. The use of genetic improvement to make animals genetically more responsive to vaccines, on the other hand, may benefit from a genetic improvement strategy targeting a common set of host-response genes.

#### **4.3.2 Improvement through non-genetic means**

The use of biotechnology to improve animal health via application of novel drug or vaccine targets warrants serious consideration for high-level investments. The application of vaccines to control flock health is a highly cost-effective strategy for disease control. The use of recombinant DNA technology to produce some of the earliest bacterial vaccines (multi-strain footrot vaccine) shows that the technology can be applied successfully. The use of DNA vaccines to deliver genetic constructs for a complex cocktail of key immunogens to which sheep mount a protective immune response, is worthy of consideration for all complex diseases. This area of biotechnology will benefit from the global momentum in human pharmaco-genomics and vaccine technologies. A strategic investment to improve the understanding of host-pathogen relationships is required to more clearly define the genetic targets for drug or vaccine delivery. It is also likely that vaccines and novel drug targets for reducing animal stress during transport, and management procedures may flow on from human medical genomics applications.

#### **4.3.3 Improvement through genetic modification**

Some of the earlier transgenic applications in sheep have attempted to mimic pest control in cotton, by inclusion of the insecticidal toxins of *Bacillus thuringiensis* (BT), or inclusion of plant chitinase proteins for control of blowfly strike. Although conceptually very attractive, the technological challenges were enormous and to date have not resulted in a disease-resistant strain of transgenic sheep. These applications could be extended to cover bacterial diseases with antibacterial factors secreted in the skin for fleece rot and footrot (although generalized resistance by bacteria to these compounds could also be expected). Use of transgenic technologies for disease control will be a strategy with a long timeline for delivery. Potential impact on environmental non-target species, and development of counter resistance by pathogens are serious considerations. Immediate targets for disease control through the transgenic route may be more modest by providing sheep with a greater capacity to respond effectively to vaccines, or to down-regulate immune suppressors activated by parasites/pathogens for immune evasion.

#### **4.3.4 Novel diagnostic tools for disease control**

The application of biotechnology for development of DNA-, antibody- or protein-based tools for improved diagnostics tests is well recognised. It is expected that protein- and RNA-based disease signatures will be identified by DNA-based technologies. Diagnostic technologies will extend to simple robust test kits for on-

farm use or high-capacity screening of animals in real time in abattoirs or saleyards for QA standards. In addition to sheep-pathogen disease-specific diagnostic tests, kits will include predictive virulence assays and indicators of zoonotic agents. This technology will continue to develop, and will extend relatively unhindered into livestock applications to deliver rapid, low-cost, sensitive and highly-specific disease information.

#### **4.4 Nutrition**

Scope to modify the sheep to better utilize foodstuffs is briefly covered above. Additional biotechnologies, which can lead to greater and more efficient utilization of foodstuffs, are through plant biotechnology (covered elsewhere) or manipulation of rumen micro-flora. Sheep are essentially living fermentation vessels and the possibility to modify rumen micro-flora to increase digestibility of low quality forage, or increase nutrient supply have been research targets for well over 20 years. The limitation to maintain stable modified micro-flora populations and the complexity of the rumen microflora ecosystem have prevented commercial application of the technology.

#### **4.5 Parasites-pathogens**

Biotechnology is equally well positioned to modify important pathogens or pests that adversely impact on profitable sheep production. A full review of strategic initiatives, which control important pests such as blowfly, rabbits and foxes, is beyond the scope of this review.

#### **4.6 Novel products**

The development of human pharmaceuticals through transgenic means has been a primary driver to generate novel products in livestock via a process commonly referred to a “biopharming”. This has now been extended to include industrial products such as “biosteel” - a spider-silk protein produced in the milk of transgenic goats. Sheep are attractive for their highly specialized skin structures to potentially produce novel fibres, or wax/skin secreted lipids (which make up 20-30% of a sheep’s greasy fleece) for cosmetic or industrial purposes. Scope to harvest novel enzymes from GM rumen micro-flora seeded prior to slaughter may lead to high - value by-products from the rumen. Initial investments will be high-risk and for highly specialized niche products most likely via the private sector. The development of novel products through biotechnology requires a strong IP position and high probability of clearance for commercial use before investments are secured in these technologies. Apart from the relevant technical hurdles, environmental impacts and compliance with use of GM organisms are potential barriers for widespread adoption in the short-to-medium term.

### **5 Socio economic impacts & hurdles to adoption**

Commercialisation of any new technology, including biotechnologies, depends on economic value, societal values (animal welfare, environmental concerns, consumer acceptance), regulatory agencies (animal health and food safety) and politics (Faber et al 2003). Most of the information on socio-economic impacts and ethics of agricultural biotechnology relates to GM plants (14 crops with 47 phenotypic GM traits have been commercialised in 2002 (Phillips 2002)). GM livestock have not yet been commercialised for food-production applications. Whereas with GM plants

human health, environmental and regulatory concerns are predominate issues, with GM livestock for research and commercialisation, animal welfare is an additional key issues.

### **5.1 Human safety and welfare**

Both plant and animal GM could be perceived as harmful for human welfare and safety. The main health concerns are related to xenotransplantation (in regards of zoonoses or the creation of new viral diseases – not a focus of this report) and complications with allergic reaction to GM food. Metcalfe (2003) discusses the allergenic and toxic potential of GM foods (due to transgenic proteins or up-regulation of endogenous substances), and describes the possibilities to test for allergic potential as well as the importance of the development of strategies to investigate cases.

Allergen avoidance is the only way to avoid negative effects, thus labelling of GM foods is crucial, which becomes problematic with many processed foods and a constant increase of GM foods on the market. To date, there is no scientific proven case of an allergic reaction in humans from use of GM food, and long-term trials of feeding GM food to animals have shown no negative effects.

### **5.2 Animal ethics and welfare**

Straughan ([http://www.bbsrc.ac.uk/tools/download/ethics\\_animal\\_biotech/Welcome.html](http://www.bbsrc.ac.uk/tools/download/ethics_animal_biotech/Welcome.html)) discusses the complicated issues of ethics, morality and animal biotechnology. He differentiates between the intrinsic and extrinsic concerns about animal biotechnology, and highlights a range of moral and ethical issues (e.g. sentience, speciesism and naturalness). As described above, some ART technologies are currently inefficient and have animal-welfare problems. GM of animals can have unforeseen animal-welfare implications caused by the changing of the normal expression of genes, e.g. pigs with additional growth hormone suffered from a range of welfare problems. Straughan concludes that moral concerns are often complex and controversial but need to be acknowledged. Discussion should not focus on 'What is right and what is wrong', as an agreed moral rule-book does not exist. It is important for scientists and ethicists to work together (as they do in the human genome project) to discuss these issues and maintain a constant review of the relevant issues.

### **5.3 Environmental concerns**

Despite the general public concern of a negative impact of GM plants on the environment (e.g. uncontrolled spread or resistance genes to other species and the use of suicide genes), scientific reviews (e.g. European Commission report) of GM work so far give little cause for concern. On the contrary, they provide mounting evidence of environmental benefits (Kessler and Economidis (2001)). Furthermore, Zechendorf (1999) discusses the positive impact of biotechnology in sustainable development, specifically in the areas of sustainable food production, renewable materials and energies, and could help to counteract climate change. The potential for uncontrolled gene flow in livestock is less of a risk in most intensively managed livestock operations.

#### **5.4 Regulation of biotechnology**

Due to the perceived and real risks, a range of regulatory or policy needs is obvious. Schilter and Constable (2002) describe some of the requirements of regulation of GM in Europe and discuss methods to identify unintended changes, evaluate toxicity and allergenicity, maintain control and post-launch surveillance, and develop traceability and labelling. Biotechnology is regulated on many levels, starting with the careful planning of research projects by highly trained scientists, which are reviewed by peers and funding bodies before any research commences. Research projects can not start without approval of the institutional ethical and risk-management committees. In Australia, regulation at the governmental level exists through a range of agencies such as the Office of the Gene Technology Regulator (OGTR - <http://www.ogtr.gov.au>) for risk assessment and regulation of GM applications, Food Standards Australia New Zealand (FSANZ - <http://www.fsanz.gov.au>) for labelling and food safety, and the Australian Quarantine and Inspection Service (AQIS) for the import and export of GM products.

Considering the lessons learned from other countries (specifically Europe) and the fact that consumer perception is believed to be influenced by regulatory approaches, it appears crucial to align the level of legislation with the level of risk (Cantley 2004). Precautionary regulations should be dynamic and adaptive to scientific evidence to maximise the benefits and minimise the risk of biotechnology. Unfortunately, politics are often driven by emotion and media campaigns – disregarding both scientific opinion and factual benefits and risks associated with new technologies.

#### **5.5 Patents and ownership of biotechnology**

Farnley et al (2004) outline the controversy in patenting of biotechnological inventions, which is mainly based on social and moral concerns (e.g. will developing countries benefit? Can human genes be patented?) and the strain on the traditional criteria for patenting (inventions need to be novel, inventive and show commercial utility). Patents are national rights and often expensive and difficult to enforce if granted for several countries. It appears that the current systems need to adapt further to the rapidly-increasing field of IP in biotechnology. It might be of some concern that the production and IP rights for GM plants are highly concentrated. A single company is estimated to have a market share of 88-91% in this new and potentially powerful business (market value of GM seeds is an estimated US \$3 billion), with only two other companies sharing the remainder (Phillips, 2002).

#### **5.6 Consumer perceptions and acceptance**

Many studies have been performed to analyse consumer perception and to investigate the reasons for different perceptions in different countries (e.g. Gaskell et al 1999 & 2004, Braun 2002, Hails and Kinderlerer 2003, Hoban 2004). Hoban (2004) summarises international public attitudes towards agricultural biotechnology. Consumer perception of biotechnology varies between countries (with America and most of Asia being more positive than Europe, Japan and Australia) and strongly depends on the perceived usefulness of the biotechnological application: medical applications (such as DNA vaccines, pharmaceuticals made by genetic engineering, and DNA diagnostics) are much more acceptable than GM food (Eurobarometer, 2000).

Other aspects that influence consumer perception are believed to relate to the trust in regulatory processes, the source of information, media coverage and the level of education. It appears that issues unrelated to agricultural biotechnology can influence consumer perception: the recent food crises in Europe (e.g. BSE and FMD) have caused consumers in Europe to distrust regulatory authorities and industrial farming processes. Many other socio-economic issues such as globalisation, liberalisation, loss of local tradition, ethical values and US economic dominance are brought up in discussions about biotechnology. However, there is evidence that results of surveys and purchasing behaviour are not always in agreement, i.e. the consumer bought the cheaper GM product in a study in the UK.

Several reports concluded that the future of GM might not depend on future scientific developments, but in consumer acceptance (and its influence on politics). To increase consumer acceptance, different strategies have been discussed. It appears to be essential to have a more open and well-informed dialog between scientists, opinion leaders, educators and the public, to develop an educational program (see EIBE Biotechnology Education <http://www.rdg.ac.uk/EIBE/English/INTRO.HTM>), and to focus on GM products that are commercially and socially beneficial.

## 6 Glossary

The terminology used in biotechnology is extensive. Instead of providing a detailed glossary we have tried to explain techniques in chapter two and refer to two web based glossaries for additional information:

FAO Glossary of Biotechnology for Food and Agriculture:  
[http://www.fao.org/biotech/index\\_glossary.asp](http://www.fao.org/biotech/index_glossary.asp)

Human Genome Project: Genome Glossary:  
[http://www.ornl.gov/sci/techresources/Human\\_Genome/glossary/](http://www.ornl.gov/sci/techresources/Human_Genome/glossary/)

## 7 Related webpages

Animal Biotechnology: <http://www.animalbiotechnology.org/>

Future uses of Agricultural biotechnology: [http://www.animalbiotechnology.org/future\\_uses.pdf](http://www.animalbiotechnology.org/future_uses.pdf)

Livestock to 2020: <http://www.animalbiotechnology.org/livestock%20revolution.pdf>

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