Australian Government

Department of Agriculture, Fisheries and Forestry

The Value of Biotechnology

Applications to Australian Agriculture

A review of non-GM biotechnology—its ability and potential to improve Australian agricultural productivity

2007

DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

Australian Government
Department of Agriculture, Fisheries and Forestry

The Value of Biotechnology

Applications to Australian Agriculture

A review of non-GM biotechnology—its ability and potential to improve Australian agricultural productivity

2007

DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

Prepared for the Department of Agriculture, Fisheries and Forestry
June 2007

ISBN 978-0-9804204-0-1

Acknowledgements
The authors would like to thank the time spent by the case study companies in providing information for use in this report and their contribution to editing of the case studies that were prepared as a result. The authors would also like to thank the industry association representative and researchers who attended the workshops which were an integral part of data gathering for this project.

Authors
The authors of this report are Dr Lyndal Thorburn and Ms Shahana Parbeen of Innovation Dynamics Pty Ltd; and Ms Denise Ironfield of ACIL Tasman; with input from Dr Ian Edwards of Edstar Genetics, and Mr Paul Lloyd and Ms Miranda Bailey of Innovation Dynamics.

Innovation Dynamics Pty Ltd
ABN: 77 109 316 949; ACN 109 316 949
Internet: **www.innovationdynamics.com.au**
Email: contact@innovationdynamics.com.au

Canberra
3/16 Iluka Street
Narrabundah ACT 2604
PO Box 7066
Canberra Business Centre ACT 2610
Telephone: (+61 2) 6239 9500
Facsimile: (+61 2) 6239 9599

Melbourne
4 Laviah Court
Templestowe Vic
Telephone: (+61 3) 9846 1277
Facsimile: (+61 3) 9846 1966

Sydney
Suite G.01
Biomedical Building
1 Central Avenue
Australian Technology Park
Eveleigh NSW 1430
Telephone: (+61 2) 9209 4231
Facsimile: (+61 2) 9209 4242

Perth
145 Stoneham Road
Attadale WA 6156
Telephone: +61 413 322 057
Facsimile: +61 26239 9599

Brisbane
24 Laurette Avenue
Thornlands QLD 4164
Telephone: (+61) 408 155 803
Facsimile: (+61 2) 6239 9599

Contents

List of tables

List of figures

List of boxes

Abbreviations

CRC	Co-operative Research Centre
CSIRO	Commonwealth Scientific and Industrial Organisation
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assays
GM	Genetically modified
GMO	Genetically modified organism
HAL	Horticulture Australia Ltd
R&D	Research and Development
RIRDC	Rural Industries Research and Development Corporation
RNA	Ribonucleic Acid
SARDI	South Australian R&D Institute

Glossary

(for additional terms, see www.biotechterms.org)

Amino acids	The building blocks of proteins.
Antibody	A large defence protein made by the immune system.
Biobleaching	Treatment of paper, pulp or wood fibres with enzymes
Biodesulfurisation	The removal of organic and inorganic sulfur (a pollution source) from coal by bacteria and soil micro-organisms
Biofiltration	The removal and remediation of compounds from air using micro-organisms – usually to control odour and/or toxic emissions
Bioinformatics	The generation/creation, collection, storage in databases, and efficient utilisation of data and information from genomics, chemistry, screening, proteomics, and DNA sequencing
Bioleaching	A process to recovery precious metals from ores using micro-organisms
Bioleaching	The removal of metals from ore using biological processes
Biopharming	The production of pharmaceuticals (or intermediate chemicals utilized to manufacture pharmaceuticals) in crop plants that have been genetically engineered.
Bioprocessing	The use of any living cells (bacteria, plants, animals) for the production of chemicals
Biopulping	Treatment of paper, pulp or wood fibres with enzymes
Bioremediation	The use of plants, bacteria, fungi, etc. to help remove toxic chemical wastes, metals, etc. from a contaminated site.
Biodesulfurisation	removal of sulphur from fuel oil using enzymes
Chromosomes	Discrete units of the genome carrying many genes, consisting DNA wrapped tightly by histone proteins. Found in the nucleus of every plant and animal cell
DNA	Deoxyribonucleic acid – The chemical building blocks of which genes are constructed.
ELISA	Enzyme-linked Immunosorbent Assays
Functional foods	Foods or dietary components that may provide health benefit beyond basic nutrition, usually marketed for these health benefits e.g. omega-3 fortified margarine
Gel Electrophoresis	A methodology to separate the various proteins, DNA or RNA within a given biological sample, prior to their analysis. In 2-D gel electrophoresis the proteins are moved by applying an electrical field in two distinct directions.
Gene Expression	Conversion of the genetic information within a gene, into a protein (or RNAs).
Gene	A natural unit of the hereditary material, consisting chain-like molecules of nucleic acids in a linear arrangement that (in part) constitutes a chromosome.

Gene Sequencing	The process used to obtain the sequential arrangement of genes in the DNA.
Germplasm	The total genetic variability to an organism, represented by the total available pool of germ cells or seed.
Genetic modification (GM)	The selective, deliberate alteration of genes (genetic material) by man.
Marker-assisted selection	The use of DNA sequence "markers" by breeders to select the organisms (e.g., crops) which possess gene(s) for a particular performance trait desired; for subsequent breeding/propagation
Microbial Fermentation	A process in which chemical changes are brought about in an organic substrate through the actions of enzymes elaborated (produced) by micro-organisms
Molecular Markers	A specific sequence of DNA that is associated with a specified trait, because of "linkage" between that DNA sequence (the "marker") and the gene(s) that cause that particular trait.
Nanobiotechnology	Refers to the application of biotechnology within the fields of nanotechnology.
Nanotechnology	Technology in which man manipulates objects whose dimensions are approximately 1 to 100 nanometres.
Nutraceutical	A component of food (e.g., a vitamin, essential amino acid) that possesses medical or health benefits and is delivered in tablet or capsule form.
Peptides	Two or more amino acids joined by peptide bonds.
Phytoremediation	The use of specific plants to remove contaminants or pollutants from either soil (e.g., polluted fields) or water (e.g., polluted lakes).
Polymerase Chain Reaction	A reaction that uses a DNA enzyme to multiply DNA strands in a sample.
Proteins	Compounds composed of a variety of amino acids joined together. Each protein is the ultimate expression product of a gene.
Proteomics	The scientific study of an organism's proteins and their role in its structure, growth, health, disease etc.
Recombinant DNA	DNA formed by the joining of genes into a new combination
RNA	A long-chain nucleic acid whose primary function is to translate the genes into proteins through a series of steps in the cell.

Executive Summary

This study gathered information on the range of non-genetically modified (non-GM) biotechnology tools and techniques (i.e. those excluding genetically modified organisms as a final product) which are applied (or are likely to be applied) in Australian agricultural industries. The report documents, through six case studies and other examples, current successful non-GM biotechnology applications and, where possible, comments on the degree to which biotechnology adds value to these industries. The report also provides general comment on the extent to which Australia currently uses biotechnology tools and techniques, with reference to the situation in Australia's agricultural competitor countries.

The study was based on desk research and a series of workshops held in capital cities during 2006 and attended by representatives of industry associations and scientific researchers. The study also gathered information on projects undertaken in R&D institutions and details of products available commercially in Australia. Case studies were developed through face to face interviews, mainly with companies with products in the market.

The main categories of biotechnology examined were:

- **DNA and RNA technologies**—used to study genes and gene expression;
- **protein technologies**—used to study protein production and function in different species and varieties;
- **cell and tissue culture and engineering**—used to understand and manipulate cellular processes, including immune reactions and embryonic development; and
- **process biotechnology**—using micro-organisms or their chemicals for the purpose of transforming a materials into a product.

Major findings

The degree to which the various categories of non-GM biotechnology are used at different points in the agricultural supply chain is summarised in Table A. Non-GM biotechnology is only currently used extensively in Australia for growing and husbandry, which includes plant and animal breeding and disease management. This is due to the value this technology can add to animal and plant industries; the long-established systems in Australia for developing and commercialising new varieties of plants and improved livestock; and the major role of agricultural research institutions in developing diagnostic tests and strategies for disease management. Non-GM biotechnology was used in a limited way in other supply chain positions; however some valuable and relevant examples were found in most categories in all areas of the supply chain.

Table A: Scale of use of technologies along supply chain

Technology	Supply chain position			
	Growing and husbandry	Logistics and support	Processing	Waste management
DNA and RNA	Extensive	Limited	Limited	Limited
Proteins and other molecules	Intermediate	Limited	Intermediate	Limited
Cell and tissue culture engineering	Intermediate	Not found	Limited	Limited
Process biotechnology	Not applicable	Limited	Limited	Limited
Sub-cellular organisms	Limited	Limited	Not found	Not found

Based on project reviews and comments from workshop participants

Some of the most relevant examples of non-GM applications discussed in the report include:

Growing and Husbandry:

Breeding: Marker-assisted selection (MAS) is widely used, particularly in the grains and cattle industries. MAS helps breeders obtain detailed genetic information about breeding stock and allows new, more productive or higher value varieties of plants and breeding lines of livestock to be developed more quickly than through use of conventional technology. Examples include development of strawberry varieties with superior flavour and flowering characteristics (Case study 1), rapid development of new barley varieties with improved quality and disease resistance, and use of genetic markers to identify cattle with improved meat quality (e.g. marbling and tenderness).

New, more rapid and less expensive, genome screening techniques are being developed (Case Study 2) which may enable a greater proportion on agricultural industries to capture the value of this technology.

Disease diagnosis: The use of biotechnology for disease diagnosis is well established and Australia has the capacity to develop new diagnostics for emerging threats. Examples detailed in this report include use of DNA technologies: to measure the genetic diversity of sugar cane smut and enable production of Australian sugarcane smut-resistant cultivars; to screen soil to identify and semi-quantify the presence of certain fungi and nematodes which allows choice of the most suitable rotation crops and increases returns to farmers (Case Study 3); and to develop a vaccine against bovine herpes virus-1, a cause of sickness and death in Australian feedlot cattle (Case Study 5).

Logistics and support:

Commercial applications of both protein and DNA techniques are being developed to improve product quality and meet increasing domestic and international requirements for verification and traceback. Examples include: the WheatRite® test which allows identification and separation of wheat and barley damaged by preharvest sprouting, the commercial availability of tests to verify grain variety and purity (Case Study 4); the availability of genetic tests that enable cattle feed efficiency to be maximised; and the development of genetic identification systems to prevent product substitution in the fish industry.

Processing:

There is limited use of biotechnology in processing of agricultural products in Australia beyond traditional food manufacture. A few Australian companies are developing functional food or nutraceutical ingredients, mainly those processing dairy or bovine ingredients as this development is an important way of adding value to some agricultural products or waste.

Waste management:

There are only a small number of biotechnology applications in waste remediation in Australia. Examples include the development of Landguard™ OPA to speed remediation of organophosphate chemicals used on crops and in sheep dip (Case Study 6) and research by the Environmental Biotechnology CRC on a demonstration processing plant that uses bacteria to remove excess nitrogen and phosphorus from heavily contaminated abattoir wastewater.

Australia's use of biotechnology in agriculture in relation to competitors

In many cases, Australia's competitors are also placing considerable effort in developing biotechnology applications for agriculture. Australia is considered generally to be ahead of competitors in the use of genetic markers and artificial insemination for breeding, and is on par with competitors in the use of DNA-based and protein-based diagnostic tests and disease treatments. However, Australia's agricultural competitors are well ahead in many other areas, including:

- some genome sequencing projects involving Australian species, e.g. *Eucalyptus spp*;
- proteomics for agriculture (Australia being on par regarding proteomics for human health);
- development of new vaccines (e.g. sub-unit vaccines) for livestock applications;
- biofuels;
- functional foods and nutraceuticals (based on waste products from agriculture); and
- use of biotechnology in the fibre industry.

Further, competitors that have been considered behind Australia in the past, including Brazil and China, are catching up fast. Australia needs to ensure that efforts continue to be applied to developing biotechnology applications where domestic challenges are presented (e.g. high boron soils, salinity, drought, region specific disease strains, and biosecurity). Australia also needs to ensure it captures international developments which are relevant to Australian agriculture (e.g. vaccines and diagnostic tests for diseases which are common elsewhere).

Further applications of biotechnology to Australian agriculture

Many of the successful applications of biotechnology identified in the case studies may be applicable in other Australian agricultural sectors. These include:

- the development of high throughput automated DNA sequencing and low cost genotyping (Case Study 2) could reduce costs, making it economically viable for smaller agricultural industries to capture the benefits of marker assisted selection and identification;

- the expansion of soil diagnostic tests to incorporate other diseases for soil-borne pathogens (Case Study 3) could allow more productive use of land and increase returns to farmers;
- greater utilisation of the technology to verify crop identity and purity (Case Study 4) could improve the accuracy and reliability of agricultural product certification and create increased export market opportunities for Australian agricultural produce, including in speciality high value products;
- extension of the pesticide cleanup technology (Case Study 6) to include other intractable pesticides in conjunction with greater uptake of the technology could provide economic, environmental and health benefits to the farming industry.

There is considerable potential for non-GM biotechnology to be applied more broadly, but there are also a number of limitations. Limitations are due to a mix of technical, regulatory and commercial issues and can be summarised as follows;

- for disease diagnosis, biotechnology techniques are already established .New techniques are often quicker but more expensive. It will take time for slower, but commercially well-established laboratory-based techniques to be replaced by field based methods;
- for disease prevention, new vaccine development techniques (e.g. sub-unit vaccines, peptide vaccines) are just emerging and technical limitations are still apparent. It is likely that these limitations will be overcome in development of new vaccine types for humans (and then be extended to domestic animals);
- in waste management, there have been few domestic regulatory drivers compared to overseas. This may change with increased interest in recycling of water and the potential to value add to waste streams.

Conclusions

The examples presented in this report demonstrate that there have been benefits from use of biotechnology (excluding GMOs as final products) in Australian agriculture. These benefits often accrue to producers through improved speed to market; reduced environmental damage; healthier and more valuable livestock and crops: and maintenance of or improvements in productivity. Where biotechnology contributes further down the supply chain, then the benefits will usually accrue to those later in the chain. Regardless the economy as a whole may benefit.

Australian agricultural industries appear currently to be using commercially available biotechnology-based tools and techniques in a limited manner. Applications may be extended to the benefit of other Australian agricultural industries, the uptake of these technologies as commercial products will depend on cost and efficacy, rather than technical capacity.

Many of Australia's competitors are at a similar stage of development to Australia but seem to be pursuing additional, more valuable, opportunities in plant or animal processing with downstream applications in human medicine. Research and development is very expensive so Australia will need to continue working in major international consortia to remain fully informed and capture information required to develop biotechnology applications specific to Australian conditions.

1 Introduction

Agricultural biotechnology is much more than genetically modified (GM) crops or animals. It covers a whole range of tools and techniques which can be utilised to increase the competitiveness of our primary industries. It is this non-GM biotechnology which is of interest in this report. The purposes of this study were to:

- collate and present information on the full range of biotechnology tools and techniques (excluding genetically modified organisms as final product) which could be applied in Australian primary industries;
- develop short case studies of examples of current successful application of the tools and techniques in Australian primary industries;
- provide general comment on the extent to which Australia currently uses biotechnology tools and techniques, with reference to the situation in competitor primary industries exporting countries;
- identify further opportunities for application of these tools and techniques in Australia; and
- indicate where current and potential non-GM biotechnology add value to Australian primary industries.

1.1 Definitions and conceptual framework

The broad conceptual framework developed for this project was based around three key factors: the type of biotechnology, where in the supply chain biotechnology is used, and the agricultural sub-sector in which it can be applied.

The method used for the study is summarised in Box 1-1.

Box 1-1: Method

The method used for the project was based on a combination of literature reviews, desk research and industry consultation.

The project team reviewed published information and the Australian Agricultural and Natural Resources Online website to compile information on 213 non-GM biotech projects in Australian agricultural industries

The team also gathered information about the types of use of these tools and techniques internationally. This was achieved initially by desk and internet research, consultation with key individuals and research organisations overseas. A series of workshops was also held in major capital cities. At these, representatives of industry associations, State governments and R&D institutions discussed the scope of information gathered in the first phase of the project. They contributed comments and suggestions on Australia's position relative to competitors internationally and discussed barriers to uptake of agricultural biotechnology tools and techniques by Australian primary industries.

The study included a number of case studies on the use of biotechnology and its economic impact on agriculture. By necessity these covered only those technologies which had been used commercially for some time so that impact on agriculture could be addressed. The focus of these case studies was on technologies applied in the cereals and cattle industries, because these are the largest agricultural sub-sectors in Australia and so they offer large markets for companies that develop new technologies for agricultural industries.

1.1.1 Type of biotechnology

The definition of both biotechnology and agriculture can be varied according to the audience and their understanding of the sector and related issues. For this report, we are concentrating on so-called "modern" biotechnology. As a basis, we adopted the basic definition used by the OECD (2005), which defines biotechnology as:

> *The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services.*

There is also a more detailed definition of biotechnology used by the OECD. This definition was used to classify the technologies reviewed during the project and in the chapter by chapter analysis (Box 1-2).

Box 1-2: OECD List-based definitions of biotechnology

DNA/RNA: Genomics, gene probes, genetic modification (engineering), DNA/RNA sequencing (gene sequencing) DNA/RNA /synthesis/amplification, gene expression profiling.

Proteins and other molecules: Sequencing/synthesis/engineering of proteins and peptides (including large molecule hormones); improved delivery methods for large molecule drugs; proteomics, protein isolation and purification, signalling, identification of cell receptors.

Cell and tissue culture and engineering: Cell/tissue culture, tissue engineering (including tissue scaffolds and biomedical engineering), cellular fusion, vaccine/immune stimulants, embryo manipulation.

Process biotechnology techniques (bioprocessing): Fermentation using bacteria, bioprocessing, bioleaching, biopulping, biobleaching, biodesulfurisation, bioremediation, biofiltration and phytoremediation.

Gene and RNA vectors: Gene therapy, viral vectors.

Bioinformatics: Construction of databases on genomes, protein sequences; modelling complex biological processes, including systems biology.

Nanobiotechnology: Applies the tools and processes of nanotechnology and micro-fabrication to build devices for studying biosystems and applications in drug delivery, diagnostics etc.

1.1.2 Where in the supply chain is biotechnology used?

Biotechnology has potential to be used at all points along the agricultural supply chain. As detailed in Table 1-1, activities have been grouped along the supply chain into four main positions (growing/husbandry; processing; logistics and support; and waste management). Each of these is divided into several more detailed sub-positions.

Table 1-1: Supply chain positions and sub-positions

Supply chain position	Supply chain sub-position	
Growing/Husbandry		
	Breeding/selection	Pre-harvest testing
	Agronomy	Pre-slaughter testing
	Husbandry	Harvest
	Soils/feeds etc	Slaughter
	Diagnosis of disease	Other growing/husbandry
	Treatment of disease	
Processing		
	Processing/milling	Processing for therapeutics
	Post harvest treatments/extraction	Fibre processing
	Traditional food processing	Biofuels
	Nutraceutical/functional food processing	Industrial chemicals
	Processing for cosmetics	Other processing
Logistics and support		
	Extension	Marketing
	Grain accumulation	Export
	Feedlotting	Border control
	Transport	Other logistics (incl. tracking)
Waste management		
	Waste reduction	Waste treatments
	Energy reduction	Other waste management

1.1.3 Agricultural sectors to which biotechnology can be applied

The agricultural sub-sectors where biotechnology may be applied are shown in Table 1-2. Technologies can be applied to the main sectors of plants; animals; either plants or animals; or to micro-organisms. Traditional agricultural sub-sectors are grouped into these main sectors.

Table 1-2: Agricultural sector classes and sub-classes

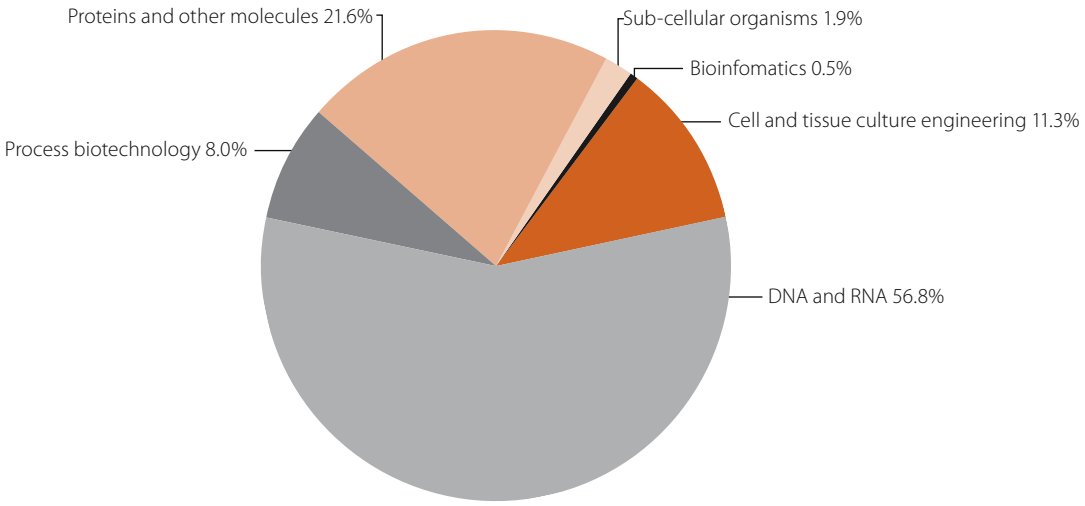
Agriculture sector	Agricultural sector sub-class	
Plants		
	Grain crops	Forestry
	Cotton or other fibre crops	Marine plants
	Sugar	Plant biosecurity
	Horticulture	Other plant crops
	Viticulture	All Plants
Animals		
	Beef	Sheep
	Wool	Fisheries/aquaculture
	Dairy	Other animals
	Pork	Animal biosecurity
	Poultry	All animals
Either plants or animals		
	Either Plants or Animals	
Micro-organisms/fungi		
	Fungi/mushrooms	Other micro-organisms
Other		
	Other	

1.2 Overview of the technologies identified

A major part of this project was the collection of information from publicly available sources on projects currently underway in both research institutions and companies which use biotechnology, as defined in Box 1-1. The project reviewed the current activities of all universities, all R&D Corporations, all State government agriculture departments, the CSIRO and approximately 30 Co-operative Research Centres; together with approximately 80 agricultural biotechnology companies in Australia. The authors believe the findings are indicative of the scope of current agricultural biotechnology activity as well as how frequently the technologies are being used. Nevertheless, the information collected cannot be used to provide a definitive indication on absolute levels of effort.

The technological focuses of projects identified for the study are shown in Figure 1-1.

Figure 1-1: Projects identified by technology type



Source: public information on current projects; See box 1-2 for definitions of each technology type

Projects using "DNA/RNA technologies" made up approximately 57 per cent of the identified projects and products. These projects apply mainly to plant and animal breeding (discussed in Chapter 2).

Projects using "proteins and other molecules" are the second most common project type representing 21.6 per cent of projects. Almost all of the research and development in this area is being undertaken in publicly funded R&D centres, and relatively little has been fully commercialised through private companies. In many instances, publicly funded research institutions or State-funded laboratories undertake the projects through extension services or contract research. These companies and centres offer services to growers and breeders.

Eleven per cent of all projects involved plant or animal "cell and tissue culture and engineering". The most common of these were in-vitro fertilisation services and vaccines, both of which are well established in agricultural industries and are in wide commercial use.

Eight per cent of projects reviewed were "process biotechnology" projects. These largely related to enzyme use in food processing.

The sub-group termed "gene and RNA vectors" is relatively limited as it excludes the use of whole organisms for applications such as biological control of pests. The main application in agriculture is the use of viruses to carry vaccines or immune stimulants to livestock. The majority of applications in this area involve production of genetically modified organisms, which are outside the scope of this report.

Only 0.5 per cent of projects were classified directly as "bioinformatics". These techniques are used quite often, but usually underpin other technologies described above. Projects using bioinformatics have not been recorded separately, which explains the small number of projects in this category.

1.3 Structure of this report

The next four chapters detail the types of non-GM technologies which have the greatest application along the agricultural supply chain – DNA and RNA (Chapter 2), proteins and other molecules (Chapter 3), cell and tissue culture and engineering (Chapter 4) and process biotechnologies (Chapter 5). Each chapter commences with an introduction to the technology and its broad applications and then discusses applications along the supply chain, highlighting applications in the agricultural sub-sector (plants and animals) as appropriate. Where relevant, examples are provided comparing traditional methods with those used in biotechnology-based applications. Each chapter then comments on the use of these technologies in Australia compared to overseas countries and concludes with a discussion of emerging uses in Australia.

It is important to recognise that many of these technologies do not stand alone. Thus, the chapters cross-reference areas where the technologies are used together, or where they rely on each other for particular applications. Chapter 6 discusses overall findings and conclusions.

2 DNA/RNA technologies

2.1 What are DNA/RNA technologies?

DNA and RNA technologies are used, in broad terms, to identify the location, purpose and activity of particular genes in a plant or animal species. DNA/RNA technologies enable genes to be *sequenced*, *synthesised* and manipulated for specific purposes. *Genomics* is the study of all the genes in a particular organism. *Gene expression profiling* enables researchers to understand when, where and at what level genes are expressed.

Once a particular gene has been identified in one species, similar genes in other species are likely to have comparable functions – the level of similarity depends in part on the evolutionary relationship between the species being studied. The analysis of genes, rather than the physical characteristics which result from expression of a gene/s, enables scientists to identify genes which give a plant or animal particular economic value – for example disease resistance, drought tolerance or desirable production characteristics.

In Australia, DNA technologies play a significant role in plant growing and breeding and animal husbandry (the first supply chain position) including diagnosis and management of disease. There are emerging applications in logistics, such as those associated with border control, and limited applications in the processing and waste management positions in the supply chain.

RNA technologies appear to be limited to research into RNA interference, which is not yet commercially available (but is discussed at the end of the chapter). For this reason, most of the discussion in this chapter focuses on DNA technologies and is referred to as such.

2.2 Where are DNA and RNA technologies used in the supply chain?

2.2.1 Growing and husbandry

Traditional plant and livestock breeding usually requires a considerable amount of time and resources to produce a new breeding line of animal or variety of plant, and is largely dependant on the organism's reproductive cycle. Using traditional approaches it typically takes many years to produce a new variety – for example, breeding a new malting quality barley typically takes 14 years from the first cross-breeding to when the cultivar is released – and success rates are low, from 1 per cent to 2 per cent of plants originally cross-bred (Langridge and Barr 2003).

In traditional livestock breeding, producers select breeding animals based on their appearance or other observable characteristics such as the amount of fleece or milk produced. Animals with valuable traits are mated and the breeding offspring selected on the basis of observable and desirable characteristics.

Tracking the lineage of key performers in any animal species, be it sheep, cattle, or other livestock, can be of high value to the animals' owners, who can charge higher stud fees for animals which have proved to have valuable characteristics. However, even with an understanding of basic genetics, it can take a long time for producers to breed a flock or herd with particular strengths – for example, the dairy industry estimates it takes six years to prove, using traditional methods, an elite sire bull. Further, as highlighted in other studies (Cregan and Noble, 2000), it can be difficult to separate the impact of changes in genetics and breeding outcomes from other influences such as other technologies (e.g. pasture improvement) and agronomy.

DNA technologies allow genes and/or organisms to be identified quickly and desirable characteristics to be analysed more accurately at the genetic level. This speeds up breeding or disease diagnosis and may be less expensive than traditional approaches. Often, DNA or "molecular markers" are used as signposts to

analyse for characteristics of interest; hence the use of DNA technologies in breeding is termed "marker-assisted selection" (MAS).

The value of MAS to plant and livestock breeders is that it allows animal and plant species to be investigated at the level of their DNA. The knowledge generated in this way can be used to manage genetic variation and diversity in livestock and plants, and makes it possible to speed up the selection process. For example, a desired trait may only be observable in the mature animal or a plant, but MAS allows scientists to screen for the trait at a much earlier stage of development. Markers also make it possible to select simultaneously for more than one characteristic in an animal or plant. MAS can also improve animal welfare and increase safety, as it can be used to identify individual animals or plants that are resistant to particular diseases, without exposing the host to the pest or pathogen in question (adapted from Seedquest, 2006).

Today, MAS is one of the standard components used in estimating the breeding value of plants or livestock, the others being appearance (phenotype) and pedigree (Van der Werd, 2000). One particular beneficial use of MAS in breeding is to find the genetic location of characteristics which are difficult to identify from the appearance of the animal or plant (Holland, 2004). Examples of this application include:

- characteristics which "skip" one generation before showing up again in later generations;
- genes that confer resistance to rare diseases; and
- when one characteristic is controlled by many genes (e.g. drought tolerance in plants).

The disadvantage of MAS is that it may be more expensive than traditional approaches. The cost effectiveness of MAS depends on the:

- direct cost of screening using appearances compared to use of genetic testing;
- time saving achieved through genetic testing; and
- economic benefits of bringing new, higher value varieties to market more quickly.

Generally MAS has required sequencing of the organism's DNA to identify the gene or organism of interest. High throughput sequencing methods have been developed to decrease the time required for this step.

Increasingly, screening technologies are being developed which reduce the reliance on DNA sequencing. This technique can be used to identify individual genes or complete organisms. To identify a sample, its DNA is tested against known gene sequences using microarray technology. Protein-based tests developed for a similar purpose are discussed in Chapter 3.

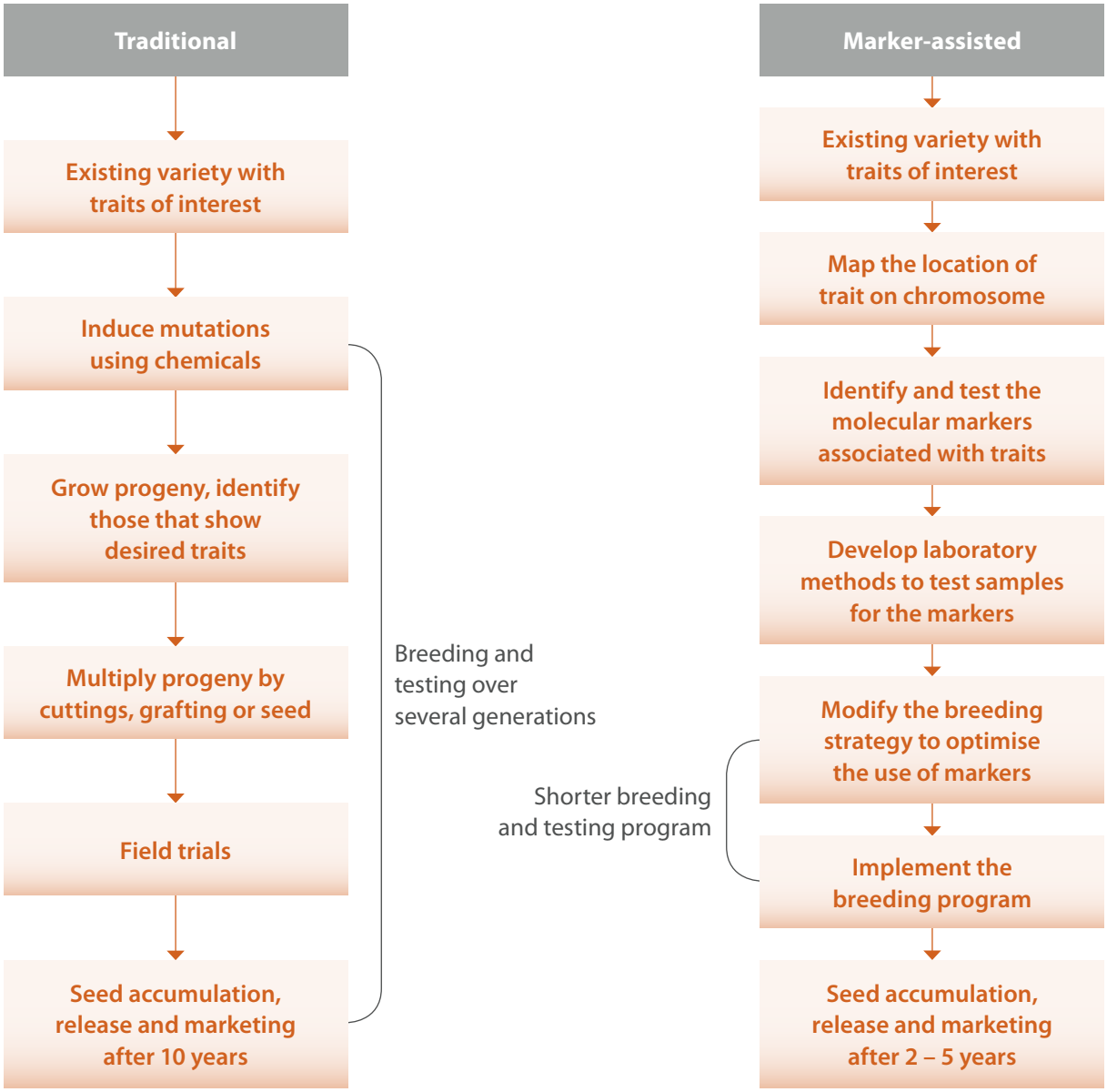
Plant Breeding

DNA markers increase the speed and improve the accuracy and focus of plant breeding programs. The advent of these technologies is said to have halved the time for development of new plant varieties in Australia. For example, in rice it has been claimed that marker-assisted selection saved 2 – 5 years time over conventional approaches to developing a new variety, which would have taken 10 years (Harden, 2000).

The differences between traditional plant breeding and MAS are shown in Figure 2-1.

In plants, MAS has been in use commercially since 1991 (Barr and Langridge, 2001). Based on the projects reviewed, coupled with comments from participants at the workshops, the main use of these technologies in plants in Australia is in the cereals industry and Australia is now recognised as a world leader in the application of molecular markers to cereal breeding. At present, over 2000 markers derived from published sequences are available to cereal breeders, along with a marker database, bioinformatics tools and streamlined procedures that support the technology (Bancroft, 2002).

Figure 2-1: Traditional and DNA-based approaches to plant breeding



Source: Derived from Barr and Langridge, (2001)

The National Barley Molecular Marker Program, a research program operating since the 1980s, is credited with providing the industry with the means to overcome serious yield limitations due to disease (Langridge and Barr, 2003). While these advances may have been possible with traditional approaches, progress would have been much slower. It would normally take up to 15 years to release a new variety of malting barley (Langridge, 2002). In that time, a disease could seriously damage the industry. However, between 1994 and 1999, use of markers enabled the fast track development of the "Sloop" barley variety resistant to cereal cyst nematode (Langridge and Barr, 2003).

Of particular benefit is the ability of DNA markers to allow breeders to select for many desirable traits at one time, and these traits may be suitable for specific markets. The barley cultivar 'Flagship' is a successful example of this with the variety particularly suited for the malting barley market in South East Asia. The variety has outstanding malting quality as well as excellent early vigour and weed competitiveness, in addition to a broad spectrum of disease resistance which gives it significant agronomic advantage (GRDC 2005a). Use of markers has enabled the development to be very focused, rather than relying on these characteristics emerging by chance in random crosses.

Many R&D Corporations (including those in grains, sugar and rice) and Co-operative Research Centres have also funded MAS to address similar issues (disease, environmental stress and quality) in their crops (Table 2-1).

Table 2-1: Examples of current marker-assisted plant breeding programs

Species	Program	Funding agency
Cereal crops	Australian Winter Wheat Molecular Marker Program National Barley Molecular Marker Program	Grains R&D Corporation
Rice	Marker-assisted selection in rice improvement	Rural Industries R&D Corporation
Sugar	Application of molecular markers to sugar cane breeding	Sugar R&D Corporation
Cotton	DNA marker projects – fungal resistance	Cotton Catchment Communities CRC

Source: public sources

The Rural Industries R&D Corporation's rice marker program, for example, has announced development of markers for:

- fragrant and non-fragrant rice (important because consumers will pay more for fragrant rice);
- rice starch gelatinisation temperature (an important component of rice eating quality); and
- resistance to blast disease, which is not present in Australia but has caused crop losses of 60 per cent overseas (Henry and Waters, 2006). The latter is important because of the potential impact on the rice crop of blast disease entering Australia.

Box 2-1 provides a case study of the use of DNA technologies in strawberry breeding in an R&D Corporation funded program.

Box 2-1: Case Study 1 – High throughput sequencing of strawberry plant genomes for variety improvement

R & D Corporations have funded use of high throughput DNA sequencing technologies to develop markers for emerging crops. Strawberries are one such crop, and about 40,000 tonnes are grown annually in Australia. The majority of the crop is sold on the domestic market, returning some $200 million p.a. at the farm gate. Until recently, all varieties grown in the temperate areas of Australia initiated their flower buds during the short days of late autumn and early winter ('short day' varieties). This led to a short flowering period and associated production peak during late spring to early summer with no fruit after January. A small autumn crop was occasionally produced during April from new plants established during the heat of summer.

During 1988 'day neutral' varieties first became available in Australia. This new type of strawberry initiated flower buds throughout the year, so fruit can be marketed continuously for nine months. Unfortunately these new day neutral strawberries lack the flavour of the old short day varieties and are susceptible to many pest and diseases.

In response, the Department of Primary Industries in Victoria, together with Horticulture Australia Ltd and Strawberries Australia Inc developed a program to develop high-flavoured, day neutral varieties adapted to a range of environments in southern Australia. They solicited the assistance of AgGenomics Pty Ltd, a joint venture between Genetic Technologies Ltd and Department of Primary Industries in Victoria. This project commenced in 2003 and was funded by Horticulture Australia Ltd and aimed to develop markers for Australian strawberry breeders (Genetic Technologies Corporation, 2003). At the time, this was the largest private sector genetic marker discovery program in Australia, and was worth $2.1 million. AgGenomics retains the exclusive right to commercialise Intellectual Property generated by this project (AgGenomics, 2004). The research also involved the Victorian Bioinformatics Consortium and has resulted in 14 primary markers that can be associated with functional genes (Keniry, et al., ,2006).

As part of the same project, researchers at Latrobe University have also developed a library of 23,600 expressed sequence tags (short DNA strands) from a range of strawberry DNA samples to help identify genes associated with fruit characteristics (e.g. firmness, flavour, taste, aroma and colour) as well as flowering times and sensitivity to day length (www.hornbill.cssp.latrobe.edu.au/strawberry.html).

The Victorian Department of Primary Industries, in conjunction with Strawberries Australia Inc, is now trialling a range of new day neutral varieties which produce heavy crops of large, firm, flavoursome strawberries which were developed using conventional phenotyping. Varieties recently released include the short day varieties Kiewa, Millewa and Bunyarra, and the day neutral variety Kalinda. The department believes the new varieties will "set a new benchmark for strawberry quality" (Morrison, 2006). It is expected that the day neutral markers, when combined with markers currently being developed for flavour, will constitute a large step forward in technology allowing the advantages of day neutrality to be fully realised throughout the temperate areas of Australia.

The Queensland government is also using genetic markers in strawberries to distinguish between closely related strawberry cultivars in a quality control process for runner production. Correct identification of varieties is critical to the industry as any mistakes will not be discovered until the field is planted and the strawberries begin fruiting, which can be very costly to the producer.

As mentioned previously, the benefits and costs of developing markers for breeding and disease management need to be carefully weighed up. For example, although Horticulture Australia Ltd (HAL) co-funded MAS programs with the strawberry industry, the stone fruit growers (also part of the HAL portfolio) recently decided against funding a project to identify molecular markers suitable for peach, apricot, cherry and almond breeding. This is due to insufficient investment capacity in the industry as well as lower priority on breeding technologies in this industry (Horticulture Australia, 2005).

A case study of an Australian company providing molecular marker services to support Australian crop breeding programs is detailed in Box 2-2.

Box 2-2: Case Study 2 - Using whole genome profiling to support plant breeding programs

Triticarte provides a genome profiling service to wheat and barley breeders in Australia and world-wide. Triticarte Pty Ltd is a joint venture formed by Diversity Arrays Pty Ltd (DArT®) and the Value Added Wheat CRC Ltd. In 2004/2005 DArT® supplied cereal breeders with information on one million molecular markers. Although Triticarte concentrates on cereal crops, the parent company DArT® offers technology development and genotyping services in other areas including crops like rice, sorghum, chickpea, sugarcane and apples.

Before the DArT® technology was developed, it was difficult to profile whole-genomes in a cost- and time-efficient manner and tests were very expensive. Production of whole genome maps took many months or years and cost hundreds of thousands of dollars. It was usually not possible to produce genome profiles on short notice. DArT® can now produce a genome profile in two days and three genome maps per week, which is ten times faster than standard approaches. This provides an enormous cost and time saving while providing high quality data which enables rapid identification by breeders of the genetic makeup of particular varieties.

The DArT® technology is a novel genotyping method that provides low cost, high throughput genotyping that does not rely on breeders having any pre-existing knowledge of the genome sequence. Each DArT® marker is hybridised on a genotyping array and high throughput screening then gives a result showing the presence or absence of each marker (Triticarte, 2004). Any standard genetic analysis software can be used to interpret the data. Clients are sent either the raw data or a report which provides interpretation and analysis of the raw results. Clients are located in Australia and overseas.

An important benefit from the use of DArT® technology is that it can simultaneously select for more than one characteristic and can also speed up the breeding selection process. The maps produced by DArT® are "equivalent to, if not superior to" genetic maps produced by restriction fragment length polymorphism (Wenzl, 2004).

When DArT® was launched, most clients sought DNA profiles in order to characterise breeding parents, clean up seed stocks and create genetic maps. For example, DArT® assisted a rice supplier to identify rice cultivars that had been mixed up. This ensured the correct variety was grown and optimum returns gained. However, clients now also request more information on marker sequences and the locations of specific genes which may enable them to development superior varieties. The technology can give breeders guidance on how to develop better varieties more quickly as DArT® methodology can provide analytical results more quickly than previously possible.

DArT® technology has also been used to assist with biosecurity issues. Sugarcane Smut was first identified in northern Australia in 1998, with the expected source being Indonesia. This smut spread through susceptible varieties grown in the region. DArT® technology was used to measure genetic diversity in 95 smut samples, with further statistical analysis showing that only six isolates were genetically different. The Bureau of Sugar Experiment Stations then identified smut-resistant cultivars and these have been imported for cross-breeding with Australian varieties to impart smut-resistance into the Australian cultivars (Braithwaite, 2005).

Disease diagnosis in plants

DNA technologies are used to identify plant pathogens and to determine the effectiveness of treatments for disease. For example, the Queensland Department of Primary Industries is using molecular diagnostic techniques to identify different strains of sunflower rust and to map variability in rust strains – this enables the Department to identify when new strains of rust overcome the natural resistance of different varieties of sunflowers. This also allows the Department to identify rust infestation in seeds or seedlings in the absence of symptoms, as the disease is normally difficult to identify until the infestation is well established (Qld Dept of Primary Industries, 2006). The Department is also using genetic markers to identify which strains of the pathogen are resistant to certain treatments. In the longer term, this information can also contribute to breeding programs for development of varieties with resistance to these particular strains.

Box 2-3 provides a case study on the use of DNA testing technologies to identify plant pathogens. This technology has been developed by two R&D organisations and successfully commercialised to the benefit of cereal growers.

Box 2-3: Case Study 3 - Identifying soil-borne pathogens

A range of soil-borne pathogens and nematodes live in the soil and infect plant crops reducing their vigour and yield. Cereal crops in Australia are affected by a number of fungal diseases including Takeall, Rhizoctonia and Crown Rot and are also affected by pathogenic nematodes. These infestations can decrease yield by 5 per cent to 60 per cent depending on the level of crop infection.

Crown Rot is a major disease of wheat in Australia and North America. Up until 1999, the most serious crop losses in Australia usually occurred in northern NSW and Queensland, where Crown Rot disease in wheat and barley was caused by *Fusarium pseudograminearum*. However, the distribution and incidence of Crown Rot changed with the introduction of new wheat and barley varieties – the disease has now become prevalent in southern and Western Australia in association with the rapid expansion into these regions of susceptible durum wheat varieties (Backhouse, 2004). While heavy fungal infestations cause visible damage to the crop above the ground, light infestations may have little visible impact above ground, but yield can still be affected.

Cereal cyst nematodes, also called eel worms, are parasitic nematodes that attack and multiply on the roots of susceptible cereals such as wheat, barley and oats. They cause major crop losses - in Western Australia, root lesion nematodes (RLN) cause yield reductions of at least 5 per cent per annum with peak losses of up to 20 per cent in some areas (Vanstone, 2007). Two species of RLN are commonly found in southern Australian soils and these have increased in recent years due to changes in crop rotations that favour them. It is not so much a question of whether RLN is present, but how many nematodes are present in the soil.

Traditionally, farmers try to manage fungal and nematode infestations in crops through crop rotation. As most commercially damaging pathogens are species-specific, crop rotation removes the host plant, resulting in the pathogen population in the soil decreasing significantly. The host species can be reintroduced some time later, (usually one or more years later) and as the pathogen takes time to build up to its former numbers in the soil, the crop can be grown successfully in this soil until this occurs.

While cereal crop rotation does help to reduce the impact of cereal diseases, different diseases may also afflict the rotation crops themselves. A common rotation crop for cereals in South Australia is peas, a legume which returns nitrogen to the soil. However, peas are susceptible to another fungal disease, Blackspot. Blackspot is caused by a group of closely related fungal pathogens. The fungus survives in soil, infecting crop residues and seed for several years. Spores are spread within the soil, by rain splash and by wind from adjacent paddocks (GRDC, 2005b).

Box 2-3: Case Study 3 - Identifying soil-borne pathogens (continued)

Bayer CropScience has licensed from the CSIRO and the South Australian R&D Institute (SARDI) a diagnostic product (Predicta B) which tests soil samples for DNA specific to each of the main cereal pathogens as well as pea Blackspot. The test uses a standard method to amplify small amounts of DNA until there is enough of the DNA to be tested for the species to which is belongs. Predicta B is offered to farmers by local agronomists, who have been trained to interpret the results of testing. Soils samples are mailed to SARDI's laboratory in Adelaide, where each pathogen is identified and its prevalence in the sample is measured – fungal DNA is measured in picograms per gram of soil, and nematodes are measured as whole organisms per gram of soil. Different amounts of DNA represent low, medium or high levels of infection and can be correlated with likely impact on yield. Thresholds have been set, below which the organism is undetectable and the soil sample can be declared relatively free of that particular disease.

The use of Predicta B means that farmers and their advisers can sample a crop grown across a wide area, send in samples, and receive information on the potential impact (low, medium or high risk) of a particular pathogen on crop yield. To provide more extensive coverage, Bayer CropScience is now developing a test for *Bipolaris spp.* another cereal fungal pathogen.

It takes only 8 – 10 days from taking the soil sample to receiving the report back by mail, so farmers can use the test to make decisions on what crops to plant in what paddocks year by year. By way of example, agronomists in South Australia have used Predicta B on land that had been planted with durum wheat, bread wheat and malting barley in a three year cycle and had suffered unexplained yield declines of 30 per cent to 40 per cent. The testing found heavy infestations of cereal cyst nematode and as a result crop rotations were changed. These farms now grow a nematode-resistant variety of barley and, as a result of the information provided by the biotechnology tests, may be able to plant higher value crops in more rotations and plant lower-valued pulse crops less frequently between cereal crop rotations.

The cost of the test to the farmer is about $250 per sample submitted, with the resulting reports covering the following nine major pathogens: cereal cyst nematode, Take-all, *Rhizoctonia*, *Pratylenchus neglectus*, *Pratylenchus thornei*, Blackspot, Take-all Oat Race, Crown Rot *Fusarium pseudograminearum* and Crown Rot *Fusarium culmorum*). The reports identify the pathogens and also provide a risk analyses which differentiates between high priority issues (for example potential yield loss in per cent from different pathogens if a cereal crop is planted in the paddock from which the sample was taken); and low priority issues (for example, where risk of yield loss in cereals is minimal).

It is expected that, over time, farmers will use the test to establish a benchmark and to identify those paddocks that have higher risks from season to season. This may eventually lead to changes in cropping on a farm basis and more profitable returns per hectare as the farmer could choose to plant something else in the paddocks classed as high risk in any one year.

Bayer CropScience is now conducting further research with SARDI on the use of Geographic Information Systems and satellite data to provide more objective measures of crop damage by soil-borne pathogens. This will provide a farm-wide measure of visible damage caused by soil-borne fungal and nematode attack. Bayer CropScience can use the data being gathered on incidence and prevalence from its existing customers to help develop a deeper understanding of longer term trends within regions and within crops as part of this product development.

Prior to development of similar tests for other crops, R&D needs to be completed to develop appropriate genome markers for each pathogen, and to validate density of infection in the field against laboratory measures. Recently Meat and Livestock Australia funded further development for pasture applications. Research into the detection of soil-borne pathogens such as Black Dot and Later Blight in potatoes is also underway (co-funded by Bayer CropScience, HAL and SARDI).

Biotechnology-based diagnosis can provide a measure of insurance for agriculture and can supplement quarantine procedures. One example of this is the biotechnology disease work undertaken for the Australian banana industry. Bananas are susceptible to a number of diseases including *Fusarium* wilt, Black Sigatoka and Banana Bunchy Top Virus. Since the 1980s, the Australian banana industry has benefited from a range of biotechnology tools including the use of plant tissue culture and a wide range of viral diagnostic tools that allow the industry to maintain virus-free planting material. Through the use of new resistant varieties (developed with the aid of DNA and RNA technologies) and excellent diagnostic tests (Hamill, 2005), Australia has been able to eradicate Black Sigatoka disease from the industry. Therefore there is now no need to apply a weekly fungicide application to control this pathogen, an additional cost faced by banana industries in other countries.

Livestock Breeding

Major industry research groups including Meat and Livestock Australia (MLA) and Australian Wool Innovations Ltd (AWI) are currently utilising and developing DNA markers. For example research is being conducted to identify markers that will enable wool growers to breed sheep with better growth and carcass traits, and is also being used in dairy cattle breeding and aquaculture programs (Australian Wool Innovations Ltd, 2007). In general, work in animal breeding is concentrating on traits that:

- are difficult to measure in other ways (e.g. parasite resistance);
- are expensive to measure (e.g. staple strength);
- are usually measured post-slaughter (e.g. meat quality); and
- may be usually measured later in life (e.g. sire quality or dam fertility).

One of the issues still to be addressed is how to deliver this new information in a cost-effective way to growers. For example, AWI is developing a set of standards that must be achieved before pedigree information can be included in the Sheep Genetics Australia database. Other markers need to be validated in commercial flocks before they can be released commercially.

Aquaculture, like the livestock sector, is also benefiting from biotechnology. The application of biotechnology techniques to determine distinct genetic populations and monitor growth studies have facilitated the development of a selective breeding program for improved growth which has assisted in the development of yabby aquaculture in Australia. The program is based on knowledge of the yabby gained through DNA sequencing of selected genetic regions (de Nys, 2005).

The Innovative Dairy CRC fused genetics and breeding with information, communications (through bioinformatics) and assisted reproductive technologies to develop new generation breeding techniques. The CRC used bovine marker-assisted selection to improve production efficiency by; increasing milk protein levels, increasing milk output, enhancing fertility, maintaining udder health and lengthening the time of lactation (Donnelly, 2006).

The work in lactation, for example, compares bovine biology with a range of species that have extreme lactation characteristics (e.g. goats, mice, seals and wallabies). The CRC used gene expression arrays, gene sequencing, gene function tests, bioinformatics databases and other techniques to develop a combined quality trait genetic map for dairy cattle which is available through the University of Sydney. The CRC has also worked to develop markers for a range of inherited characteristics including resistance to mastitis and it has created a database of 24 million gene markers based on 1500 bulls and 15000 markers of small genetic changes (some provisional patents relating to this have been filed). Markers for milk protein levels are correlated with higher average breeding values, meaning that dairy producers can use these to select higher value animals.

Disease management is another issue being targeted through the use of DNA technologies. There are several hundred disorders in cattle and the genetic causes of around 10 per cent of these have now been identified (Tammen, 2006). DNA-based markers are being developed to test for and identify individual animals which

have inherited particular cattle diseases, or a genetic tendency towards diseases (e.g. mastitis and milk fever in dairy cows).

Overall, DNA testing is being used to identify desirable traits in cattle, including quality traits such as high volume milk production, high fat milk production, high fertility and disease resistance (CRC for Innovative Dairy Products, 2006). This testing is completed using hair samples and the improved productivity gains are expected to add around $20 million in value to the industry every year. It has been predicted that these technologies may be available to farmers as early as 2008.

2.2.2 Processing

There are currently few applications of DNA technologies in other supply chain positions. In plants, the limited current applications identified in processing relate to the use of MAS to identify the different genes involved in control of amylose content of grains. The focus on amylose is related not only to processing characteristics but to health applications of the grain, as high amylose varieties resist digestion and are therefore suitable for low glycaemic index foods (CSIRO, 2006). Research projects have been conducted in this area by the CSIRO, the CRC for Sustainable Rice Production and the Rural Industries Research and Development Corporation (RIRDC). Although the CRC for Rice ceased operations in 2006, RIRDC continues to support projects to identify markers for determining variations in genes for fragrance, gelatinisation temperature and disease resistance in rice (Henry and Walters, 2006).

With regard to the potential applications of high amylose wheat, including nutraceuticals, the GRDC is now a partner in a high amylose wheat joint venture with the French company Groupe Limagrain and the CSIRO. The current project on high amylose wheat, funded by the GRDC, is being co-ordinated through the Value Added Wheat CRC and uses the Triticarte technology (see Box 2-2) for DNA profiling (Value added Wheat CRC Ltd, 2005).

In animals, Genetic Solutions Pty Ltd sells MAS tests for meat marbling (distribution of fat through the carcass) and tenderness (GeneSTAR Marbling4 and GeneSTAR Tenderness4). As with the other GeneSTAR test, these are a valuable genetic selection tool for cattle breeders. However, they also provide the ability to draft or sort cattle at the feedlot or abattoir on marbling and tenderness potential. Cattle selected for particular properties could be managed differently, for example, cattle with greater marbling potential would be suited to a longer feeding program for export sale than those with less potential (**www.geneticsolutions.com.au**).

2.2.3 Logistics

There are three main applications of DNA technologies in the logistics supply chain position.

The first is in identity tracking ("paddock to plate") of animals which is becoming increasingly important worldwide due to food safety considerations, environmental and animal welfare issues. The SureTRAK® identity system developed by Genetic Solutions Pty Ltd is a DNA verification traceability system which links the end product to the carcass, the supplier and sometimes the live animal. It is used to prevent product substitution, to protect branded products and to track food sources for safety reasons. The SureTRAK test uses a paper-thin, fingernail-sized carcass sample taken at slaughter or grading which is stored for future use if required.

A variation on the system applies to live animals, where hair samples can be taken on-farm and stored to enable livestock to be traced back to their herd of origin. The same company, working with Allflex, has also created an animal-based DNA tag which can be combined with the electronic National Livestock Identification System (NLIS) tag, and a visual management tag.

Identity tracking in plants uses protein technologies rather than DNA technologies and is covered in Chapter 3.

The second application of DNA technologies in logistics is in biosecurity. DNA tests are widely used to identify plant and animal varieties and can be used to track movement of pathogens within and between countries. These tests also serve the purpose of identifying diseases and were covered in more detail in Section 2.2.1 and Box 2-2.

The third application is in feed efficiency. The Australian Company Genetic Solutions Pty Ltd supplies a 'feed efficiency test' (GeneSTAR Feed Efficiency4). The test screens livestock for four separate DNA markers that affect feed efficiency. The four DNA markers are independent and additive, meaning that the result for each marker has an individual value and the total potential effect of the markers is gained by adding the four individual results. The greater the score, the less an animal will eat to gain the same amount of weight as its peers. Test results can be utilised at the feedlot level to sort cattle into groups for different feeding programs.

2.2.4 Waste management

There were few applications found of DNA techniques in agricultural waste management and none in commercial use. It is possible to create DNA profiles and libraries for a range of organisms including those that are active in waste decomposition. This is the focus of research being undertaken at both the Environmental Biotechnology Co-operative Research Centre (EBCRC) and Flinders University. Flinders University created a spin-off company, Flinders Bioremediation Pty Ltd, which offers commercial bioremediation services. The company and the CRC use DNA technologies to identify microbes in the environment, with the aim of understanding their growth and interaction during remediation.

2.3 Australia's use of DNA technologies compared to use in overseas countries

Australia's focus to date in DNA and RNA technologies appears to have been on breeding and disease management in major plant crops (cereals) and major livestock (cattle). This section discusses the use of DNA technologies by competitor countries focussing first on plants, then animals.

At the sectoral level, the other countries examined also focus on crops that are important to their economy. For example, Brazil has invested heavily in sugar genomics. The Brazilian government's project is called SUCEST – the Sugar Cane Expressed Sequence Tag Genome Project (Vettore, da Silva, Kemper and Arruda, 2001). The project commenced in 1998 and involved more than 200 scientists from 22 R&D organisations. The project also involved development of state-of-the-art data-mining tools and resulted in the full sequencing of the sugar cane genome. This was then used as the basis for an alliance with Belgian company Crop Design NV in 2001. The knowledge gained from the project has helped to develop the country's bio-ethanol industry which, as of 2005, drew raw materials from 330 sugar mills. Growers of sugar cane are now paid on an end-use formula according to whether their sugar cane is to be used as ethanol feedstock or for food (Martines-Filho, Burnquist and Via, 2006).

China is also investing large amounts of money and resources in particular sectors. According to workshop participants, it is heavily involved in the International Cotton Genome Initiative, whose members also include Brazil, India and the United States and has over 400 active members across more than 30 countries on four continents (but not Australia). Workshop participants also reported that China has also been developing techniques to use microsatellite loci from DNA to identify the impact of sea aquaculture cages on the local environment. Waste from fish held in aquaculture cages concentrates on the sea floor immediately under the cage and induces significant changes in fauna and flora naturally found on the sea floor.

The International Eucalyptus Genome Consortium was an Australian initiative founded in 2004 to obtain funding to sequence the *Eucalyptus globulus* genome. Although the funding bid was unsuccessful, the Oji Paper and the Kazusa DNA Research Institute of Japan commenced a project to sequence *E. camaldulensis*.

Funding was subsequently obtained from the US Department of Energy for sequencing *E. grandis*, with the aim of identifying markers that relate to pulp yield. The project is supported by 15 countries including Australia (Ingram, 2004).

In relation to livestock, other countries appear, in the main, to have a similar focus on gains through breeding programs. Table 2-2 presents examples of the use of these technologies in the United States. The table shows that tests to support breeding and growing predominate, with a more limited range of tests available for processing and a few tests available in logistics. The situation is similar in Australia.

Table 2-2: DNA-based tests for cattle in the United States

Company	Breeding and growing	Processing	Logistics
Biogenetic Services	Parentage, coat colour, leptin hormone levels*, freemartin**	Meat quality	
Bovigen Solutions	Parentage	GeneSTAR marbling***, GeneSTAR tenderness***	
Genaissance Pharmaceuticals	Parentage, coat colour	Tenderness	Identity tracking
Genmark	Parentage, coat colour, BLAD, citrullinemia, MSUD	Kappa-Casein, beta lactoglobulin, alphaS1 casein	
GeneSeek	Parentage, coat colour		
Genetic Visions	Coat colour, BLAD,> citrullinemia, DUMPS,> CVM>	Kappa casein, beta lactoglobulin	
Igenity	Parentage, double black coat colour	Leptin, TenderGENE	
Immgen	Parentage, CVM,> BLAD,> DUMPS,> Pompe's disease	Kappa casein, beta lactoglobulin	
ReproTec	Cattle fertility associated antigen		
Maxxam	Parentage		
MMI Genomics (MMIG)	Parentage, MMIG double black coat colour, polled/horned		
Veterinary Genetics Laboratory	Parentage, freemartin		
Viagen	Breed identification		Animal identification (tracking)

Source: http://animalscience.ucdavis.edu/animalbiotech/Biotechnology/MAS/index.htm
* leptin is a hormone that regulates feeding;
** a freemartin is a female cow that behaves like a male;
*** these are produced by Genetic Solutions Pty Ltd in Australia
> DUMPS = Deficiency of Uridine Monophosphate Synthase;
BLAD = Bovine Leukocyte Adhesion Deficiency;
CVM = Complex Vertebral Malformation

Despite these sectoral focuses there was general agreement amongst workshop participants that all Australia's major competitors (the United States, European Union and Canada) were ahead of Australia in DNA and RNA technologies. In addition, participants considered that countries which have been considered behind us in the past are catching up fast - Brazil was thought to be at about the same level as Australia and China and Argentina were thought to be slightly behind Australia overall. The increased adoption of these technologies in developing countries has been supported by a number of aid programs and is now extending to Africa (Reifschneider, 2006).

Participants at the workshops also discussed emerging competition from China and India, indicating that in the past these countries have been hampered by poor quality cold chain management and high levels of pesticide residues so their products have not been competitive with those from Australia. China and India are now both investing in cold chain infrastructure and have resolved many spoilage issues. As a result, they are now able to compete with Australia on quality. Some workshop participants suggested that the only way that Australia will be able to compete effectively with these countries will be through widespread use of biotechnology and marker-assisted selection to develop improved varieties for which consumers are willing to pay a premium, or to develop products to which we can value-add (such as functional foods) or which can be utilised in other markets (such as biofuels).

2.4 Further potential applications of DNA technologies in the Australian supply chain

The use of DNA technologies is currently focussed on breeding as well as identification and management of disease. Few applications were recorded in processing, logistics and waste management. Further applications in each of these areas are discussed in turn.

2.4.1 Growing and husbandry

Development of markers necessary for crop improvement is expensive, because usually the whole genome of a species needs to be mapped before markers can be identified. In Australia, the cost of genome mapping has meant that these techniques were first used in the more significant agricultural sectors such as grains crops and dairy and beef breed cattle. Crops that are less significant in agricultural terms have had less research effort spent on such developments. In some cases, including cotton, it appears that markers are being imported from overseas rather than developed in Australia. This is likely to have a positive economic impact for those markers which relate to characteristics important in different countries and common in different varieties. However, reliance on these common characteristics may not meet Australia's needs for characteristics unique to Australia such as high levels of soil boron, drought and salt tolerance.

DArT Pty Ltd (see Box 2-2) has developed a novel genotyping method that provides low cost, high throughput genotyping. This technology is already being used for the genome analysis of approximately 20 crops including lupins (in conjunction with the Department of Agriculture and Food, Western Australia); oats (with an international consortium from North America and Europe); sugarcane (supported by local industry and the International Consortium for Sugarcane Biotechnology); sorghum (for private Australian clients) and hops (with an international consortium and HAL). This technology could also be used to identify cultivars of other internationally important crops which may be suited to Australia's climate, for example peanuts, pigeon peas or other legumes.

Diagnosis and Management of Disease

Research is being conducted into how RNA may be used to induce disease resistance in plants, and how to use RNA technologies to develop methods of inducing resistance of plants to diseases. CSIRO has developed a method of using specialised RNA to "vaccinate" plants against viruses before they attack (CSIRO, 2001). RNA which matches that produced by a virus is introduced to the plant and is destroyed by the plant's defence mechanisms. This primes the plant to attack the RNA produced by the virus when it is infected at a later date (discussed further in Chapter 4) as it involves cell culture.

2.4.2 Processing

It is likely that there will be other applications of DNA technologies in breeding programs associated with the development of the fibre and oil processing industries. In particular there are potential applications in the identification of genes in fibre crops (such as cotton) that influence fibre traits such as strength (Australian Cotton Shippers Association, 2003). Also, RIRDC has funded development of DNA libraries for tea tree, with the intention of using them to identify oil processing characteristics (RIRDC, 2001).

2.4.3 Logistics

The applications of DNA technologies in logistics are just emerging and are based around tracking of individual animals and crop varieties along the supply chain. Traceability is important for a number of reasons, including:

- international customers (e.g. the European Union) want to be able to ensure that agricultural products have been raised or produced in accordance with their requirements and regulations (e.g. avoiding antibiotics in livestock production, livestock welfare issues); and
- the capacity to trace and recall batches is required if the food supply chain is compromised.

The red meat industry has implemented a National Livestock Identification System (NLIS) for the identification and tracking of livestock. NLIS is a permanent, lifetime system that enables individual animals to be tracked. NLIS uses either radio frequency tags or DNA technologies to track individuals. There are likely to be applications for these technologies in other domestic animal species beyond cattle. The Pig Traceability Project is currently auditing the efficacy of traceability in the pork industry (Australian Pork, 2006).

The main focus of plant traceability systems is on identification of plant varieties for the purpose of paying end point royalties – these mainly use protein technologies and are discussed in Chapter 3. In the United States the Homeland Securities Presidential Directive 9 of 30 January 2004 highlights the need to develop "systems that, as appropriate, track specific animals and plants, as well as specific commodities and food" in order to protect agricultural and food systems against terrorist attacks, major disasters and other emergencies (Bush, 2004). Traceability systems for agricultural and food products may be required in the future and biotechnology may have a role in this.

2.4.4 Waste management

In the longer term these technologies will have application in waste management, particularly in intensive agriculture, where waste products are highly concentrated and can have significant environmental effects. For example, EBCRC and the SARDI are working together on a $1.2 million project researching the use of integrated biosystems for organic waste and waste water treatment in livestock and food processing (SARDI, 2006).

SARDI is also developing novel methods for cost-effective assessment of the environmental impact of cage aquaculture, under a research project funded by the Aquafin CRC and the Fisheries R&D Corporation

(**www.sardi.sa.gov.au**). The project is developing a system based on DNA techniques to rapidly identify the organisms in sediment samples under sea cages. The aim is to be able to characterise the impact of waste on sea floor flora and fauna as part of environmental management strategies. The technique, when developed, will replace current laborious laboratory identification methods.

2.5 Conclusions

In Australia, DNA technologies are mainly being used in the development of new plant varieties (particularly the grains industry) and breeding of livestock (particularly cattle). As these are Australia's largest agricultural sectors greater funding opportunities are available for research and development of the genetic markers which until recently have been required before these technologies can be transferred to commercial use. As more genomes of important agricultural species are sequenced it will be possible to build DNA marker libraries that can be used in other species. Other technologies are also emerging that will not require prior development of a genome sequence. Such technologies will reduce costs and may be viable tools in smaller sectors.

The benefits and costs of developing markers for breeding and disease management need to be carefully evaluated. Some R&D corporations have considered and rejected molecular marker programs when these initiatives are competing with other promising projects for scarce funding. However, other R&D Corporations and Co-operative Research Centres have strongly supported marker-assisted selection and have used this technology in plant and animal varieties to identify:

- genetic traits for disease resistance;
- genetic markers associated with drought tolerance; and
- genes which confer resistance to pests and diseases.

Cost and the need for highly skilled technical expertise has been a major barrier to the use of this technology. However, recent innovations have seen the cost fall dramatically and there have been significant commercial outcomes in major agricultural sectors.

There are also benefits apparent in terms of quality and returns to growers:

> *We are starting to see DNA-based diagnostics for productivity and product quality traits. In cattle, as an example, the two major price determinants for a piece of meat are tenderness and intramuscular fat distribution (marbling). Increasingly we have genetic tests for those in beef cattle, and they have been commercialised successfully* (Willadsen, 2005).

At present, the use of DNA markers for breeding is limited to traits that have a large effect and which are inherited in a simple way (Holland, 2004). Many inherited traits are controlled by a number of genes and use of DNA markers in this instance is complex and is unlikely to influence productivity or yield. Development of markers may not be technically viable for these characteristics, or may be too expensive for the benefit obtained. Further research is needed to develop techniques to extend DNA and RNA technologies to more complex situations in genetic analysis (Willadsen, 2005).

While Australia is working strongly in this area, and has been a leader in development of DNA technologies in some agricultural sectors, many of Australia's competitors are catching up. This may affect Australia's ability to maintain the productivity gains that have benefited its producers in the past. Many commentators believe Australia has already gained all we can in yield improvements using current methods (Higgins, 2007). Competitors are also sequencing genomes of species of interest to Australia and have a similar capacity to use these technologies to improve productivity through selective breeding. Hence, Australia cannot afford to stop

work in these areas. According to workshop participants, the emphasis should be on the development or use of technologies that remove the need to undertake a full genome analysis first. The use of such technology would provide productivity gains at the research stage.

There is clearly the potential for applying DNA and RNA technologies in processing, logistics and waste management. It is expected that demand will come first in the logistics of animal tracking and identification, because of the introduction of the National Livestock Identification System. However, demand is also growing for reliable trace-back systems, and with emerging emphasis on resource use there will also be growing demand for these technologies in environmental management.

3 Proteins and other molecules

3.1 What are Proteins and other molecules?

The genes in an organism provide instructions to the cell for the manufacture of proteins. Proteins are used by cells in a number of ways: they may influence the rates of chemical reactions (enzymes), be used for structural purposes or be used for defence (immune system). Proteins can be used as drugs, but may be susceptible to digestion if taken by mouth. Research into proteins therefore often includes new methods of drug delivery across the skin or mucous membranes, as well as ways to isolate and purify proteins or part of proteins (peptides) from complex mixes.

The identification and measurement of proteins in an organism is called *proteomics*. An important aspect of proteomics is; understanding how proteins interact and their function in gene regulation. There are many more proteins than there are genes as the product of a gene is a long chain of peptides that may be cut at different points to make different proteins. Hence, the 30,000 genes in humans can code for more than 100,000 proteins (Australian Academy of Science, 2007).

A better understanding of the interactions between proteins and the genes that produce them can help researchers to understand the link between genes and metabolism, feed conversion and an organism's response to disease and infection. Much of the early work in proteomics has been conducted on 'model' organisms such as *Arabidopsis spp* (plants), rice (cereals) and mice (animals). The genomes of these model organisms are well-characterised and serve as reference species for most plants and animals (APAF, 2006).

Proteomics is complementary to DNA and RNA technologies and both technologies may be used for similar purposes. Thus, there is overlap in the application of proteomics and DNA technologies in areas such as breeding, and disease, variety and breed identification (see section 3.2).

3.2 Where are protein technologies used

In Australia, protein profiling is used in several areas in the agriculture supply chain. The main applications are in growing and husbandry (pathogen identification) and in logistics (purity or variety testing). As noted in the previous chapter, DNA and RNA technologies can also be used for these applications. Currently there is limited use of protein technologies in processing or in waste management.

The applications of protein technologies in each of the main areas along the supply chain are discussed in turn in the following sections. Comparisons between traditional approaches to applications are provided where relevant.

3.2.1 Plant growing and animal husbandry

Protein analysis can help researchers identify and quantify disease organisms and to understand the responses of different livestock species to bacteria and viral infections. This has implications for livestock and crop management as well as quarantine and biosecurity.

The most common approach is the use of Enzyme-linked Immunosorbent Assays (ELISA) tests. ELISA tests determine the presence of a protein in a sample through use of a specific antibody linked to an enzyme. A colour change (or fluorescence) is induced when the antibody binds to the antigen. ELISA tests may be very sensitive, can be quantified and may measure the presence of as little as a nanogram of a protein.

ELISA tests were used to confirm the incidence in Australia of the wheat streak mosaic virus (Ellis et al., 2003). In 2002, CSIRO's Division of Plant Industry observed streaking on plants growing in their glass houses in Canberra. CSIRO used DNA technologies to identify the virus and then confirmed this with an ELISA specific for the virus.

ELISA tests can be conducted much faster than traditional methods such as faecal culture. For example, ELISA tests for Bovine Johne's disease (Table 3-1) take 1 – 2 days. In contrast, it can take 21 weeks to confirm a negative result from a faecal culture test in solid medium and 9 weeks from a bacterial culture test in liquid medium. Further, any species testing positive from bacterial liquid culture must be identified using a polymerase chain reaction (Eamens, 2007). Table 3-1 shows that ELISA tests are less sensitive than the culture tests, however the real value of the ELISA test is the ability to test large numbers of animals inexpensively and quickly, so diseased animals can be removed from the herd, thus limiting spread of disease. Calculations by the NSW Department of Primary Industries have shown that the traditional approach costs up to $21 per sample, whereas the ELISA test costs approximately $8.

Table 3-1: Summary of approaches to test for Bovine Johne's Disease

Test	Lab cost	Time	False positives	Clinical sensitivity	Subclinical sensitivity
Faecal culture solid medium	$19-$22	21 weeks	0%	90%-100%	30%-50%
Bacterial culture liquid medium	$39 (+$82 for PCR if required)	9 weeks	0%	90%-100%	30%-50%
ELISA blood test	$6-$9	1-2 days	1%	90%	25%-30%

Source: Eamens, 2007. "Subclinical" refers to animals that are infected but show no outward signs of illness. Animals with clinical signs of BJD may shed 100 million bacteria per gram of dung.

A similar example is the testing of livestock for internal parasites such as liver fluke. Faecal tests, based on a sample of between five and 10 animals, cost from $30 - $70 per sample, and can show the level of infestation as well as presence or absence of infection (Love, 2006). As the samples are pooled, the results apply to the herd as a whole and does not identify infected individuals. An ELISA test specific for liver fluke is available at a cost of $19 per animal. The test uses an individual blood sample and can detect infected animals before they shed eggs in their faeces – this means that infected individuals can be identified much more quickly and separated from others in the herd.

There is also substantial research into proteins being undertaken to understand host-pathogen interactions in both plants and livestock. For example, a research group led by the University of Melbourne and CSIRO Livestock Industries is using proteomics to detect Johne's Disease (JD), an intestinal disease affecting cattle, sheep and other ruminants, and to understand how bacterial strains cause disease. The project aims to better understand virulent strains of bacteria and to identify useful protein markers able to indicate the presence or absence of JD infection. JD infection is difficult to detect and the research is expected to lead to new methods of JD detection in infected animals (Dairy Food Safety Victoria, 2006).

CSIRO by building a database of proteins in blood and tissue from healthy and poisoned animals is also using proteomics to examine plant toxins that cause staggers and annual ryegrass toxicity in grazing animals (Davidson, 2003). The Dairy CRC also commenced projects on proteomics in dairy cattle as part of a larger program to understand the genetics and control of lactation (CRC for Innovative Dairy Products, 2006b).

3.2.2 Processing

Protein technologies are also used in processing where enzymes are often used to control or influence chemical reactions.

In wine making, a range of enzymes is used to improve processing, to promote clarity, to enhance extraction of colour from grapes, to assist filtration and to reduce the activity of bacteria. The Australian Wine Research Institute, for example, has examined the addition of enzymes in conjunction with varying levels of the preservative sulphur dioxide, in order to reduce hazing of the finished wine (Pocock , et al 2003).

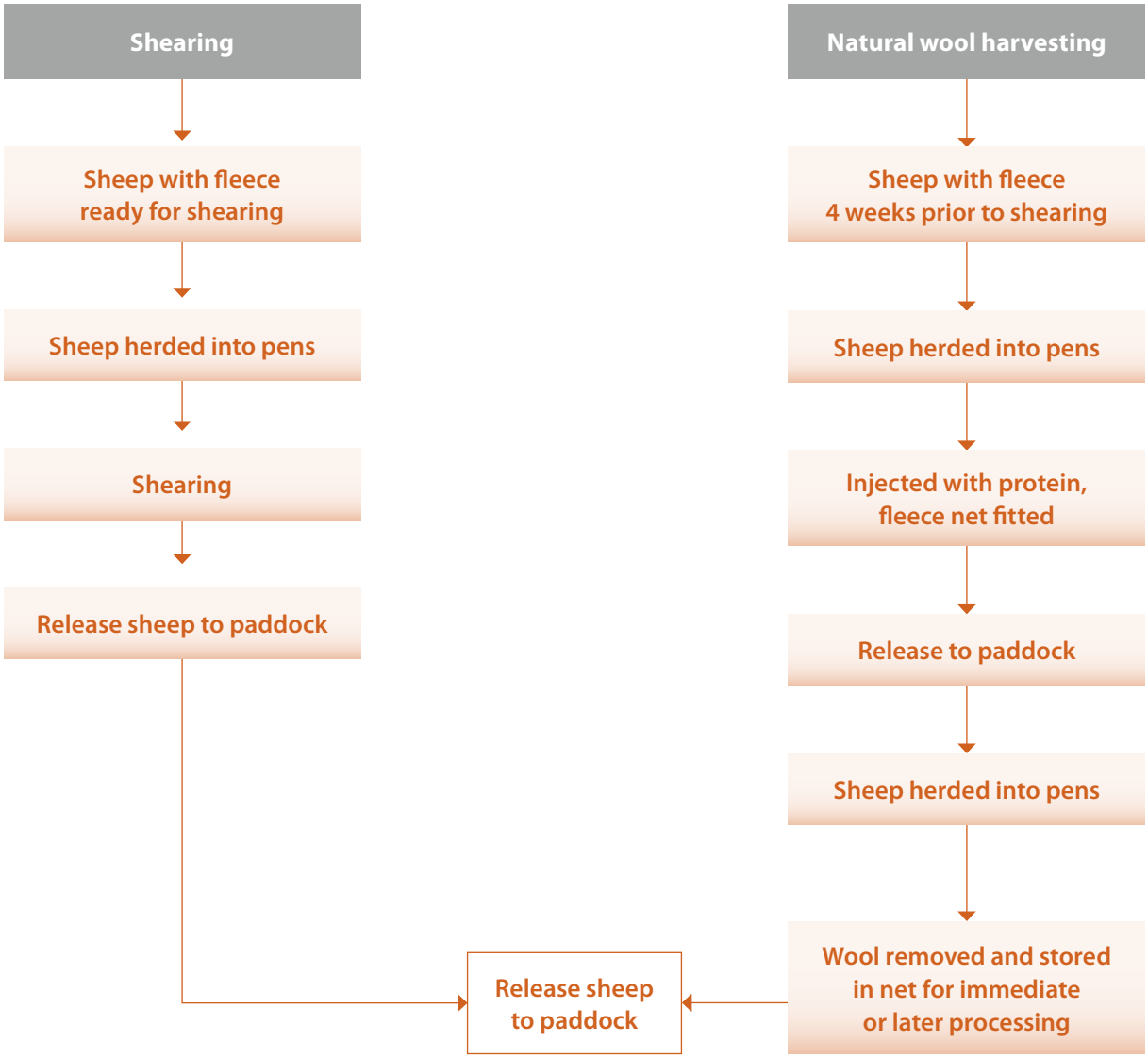
Proteomics can also be used to identify the particular proteins which contribute to beneficial processing characteristics of grains and other crops. Research led by the Australian Proteomics Analysis Facility and published in 2005 compared the proteins in two biscuit-making wheats with different processing qualities (Skylas, et al., 2005). The analysis measured total wholemeal proteins, extracts of starch granule proteins and extracts of other storage proteins. The aim was to characterise, identify and catalogue cultivar-specific proteins that could be used for segregation. The research identified 23 proteins that could be used to distinguish between closely related cultivars and could explain the differences in their processing characteristics.

One commercial use of protein technologies in Australian processing is BioClip®, a natural wool harvesting system. Developed by CSIRO in research that spanned from the 1950s to the late 1990s, BioClip® was launched nationally in 2005 after several years of trials. A specific protein enzyme is injected into sheep when their fleece has grown. The protein disrupts the structure in the wool so that it breaks and can be peeled off without needing shearing (see Figure 3-1). A specially-designed net is placed over the sheep after the injection of the enzyme. Several days later the wool is removed by simply pulling it off, leaving the sheep with a short, even coat. Wool harvesting using the system (Shepherd, 2003):

- provides a longer and more consistent staple length, up to 10-15 mm extra;
- is easier and safer on operators;
- avoids skin damage to the sheep – hence no skin pieces in the wool tops;
- reduces stress on the sheep; and
- enables producers to harvest the wool 5-7 weeks earlier in spring than usual, and hence offers better control of grass seed and fly strike. Grass seeds can contaminate the clip and reduce its value and fly strike can be a serious threat to animal welfare.

Uptake of the technology has been slow due to its relative expense (an extra $2 to $3 per head). In 2006 BioClip® was used to harvest wool from about half a million sheep (FarmOnLine, 2007). The first designated BioClip® wool sale was held in Melbourne on 8 March 2007 (Ballan News, 2007). FarmOnLine quoted potential Taiwanese buyers as saying that BioClip® produced high yields and fewer short or defective wool fibres, meaning more profit for the manufacturers.

Figure 3-1: Protein-based technology and traditional approaches to sheep shearing



3.2.3 Logistics

Protein technologies applications in plants

Approximately 50 countries (including Australia) protect plant breeders' rights, which are an exclusive commercial right to a registered variety. In Australia, owners of plant varieties have the right to produce or reproduce the material; condition the material for the purpose of propagation (conditioning includes cleaning, coating, sorting, packaging and grading); offer the material for sale; sell the material; import or export the material; and stock the material for any of these purposes (IP Australia, 2006).

In order to be protected, a variety must be new, measurably distinct from other varieties and must breed stably from generation to generation. The basis of distinctness is "an objective comparison of the variety with the most similar variety (ies) of common knowledge" (IP Australia, 2006). Quantitative and qualitative differences between the new and existing varieties must be established and recorded.

The limitations of this approach are that it is based on visible and/or measurable physical characteristics. As some valuable varieties may be based on their processing characteristics there are limitations in the registration system. The advent of protein profiling and proteomics has enabled some of these limitations to be overcome and plant breeders' rights systems now allow information on comparative DNA or protein profiles to be submitted as evidence of varietal characteristics (IP Australia, 2006).

The ability to distinguish one variety from another is important for reasons other than being able to claim ownership. Different plant varieties or their crops have different commercial value and the ability to identify one variety from another means that a grower who produces a higher value seed crop can obtain higher payments for that seed. As many seeds look the same, it is easy to confuse the seeds of one variety over another and verification testing is required.

Purity testing using protein analysis has been available for some years, using electrophoresis. An electric current is passed through a medium containing the sample, and proteins travel through the medium at a different rate, depending on its electrical charge and size. Separation is based on these differences. Electrophoresis is used to identify the presence or absence of particular proteins and to identify when different groups of proteins have increased or decreased in a sample. However electrophoresis can only be used to certify samples to a 20 per cent impurity level and is insufficient to meet the demands of overseas customers.

More recent protein-based technologies are used in assays to determine if protein profiles are characteristic of particular varieties and as the following case study highlights how protein technologies can be used to verify the quality or purity of a crop. Purity can be crucial to meet importing country requirements (see Box 3-1).

Box 3-1: Case study 4 - Protein profiling of grains for purity testing

There are thousands of different wheat varieties in the world and each has subtle genetic differences. Growers choose the specific varieties that best suit their farm conditions and market requirements. Market forces are increasingly demanding pure (or single) varieties of grains for specific purposes. Thus varietal testing has become standard practice in order to guarantee sale of grain.

Western Australia grows over 40 per cent of Australia's wheat (www.cbh.com.au). Much of the crop is sold through Grain Pool Pty Ltd, a wholly-owned subsidiary of Co-operative Bulk Handling (CBH), Western Australia's grain storage, handling and marketing organisation. Grain Pool sells Western Australian grain to a range of overseas markets, including Japanese Shochu markets (Shochu is a distilled spirit most often produced from barley) (Long and Washington, 2004). Varietal purity is a crucial requirement for barley exported to Japan for the manufacture of Shochu.

Grain Pool operates a Quality Assurance program for barley in order to ensure grain meets the standards required by customers. Moisture, protein or oil content, weight, colour, adventitious presence of seed or foreign material, insect presence and levels of chemical residues may be measured. The requirements depend on the grain, the grade involved and market access conditions.

Grain Pool uses a crop variety testing service marketed by Saturn Biotech Ltd. The crop variety and purity testing is based on protein profiling using mass spectrometry. Grain samples are analysed and the resulting data compared with the database to identify the grain variety and its purity. The company has protein profiles for all the main varieties of barley, wheat and oats grown in Australia and many varieties of potato, canola and olives. Saturn continually updates its database with introduced varieties of agricultural and horticultural crops because, to remain competitive, new varieties are constantly being introduced into commercial cultivation. Most varieties currently being grown in Australia were introduced in the last two years.

The advantage of protein profiling for variety identification is its speed (results available in 24 hours), low cost when compared to electrophoresis (about 25 per cent of the cost), and high accuracy. Protein profiling is very specific and the Saturn Biotech variety test is now the standard test country-wide. One of the significant advantages of this test is that export claims of purity can now be substantiated at the 98 per cent purity level (as required by the Shochu market) using a sample of only 150 grains. Protein profiling testing allows for routine high throughput testing to the 0.1 per cent impurity level (requires 300 grains for a 95 per cent confidence level). The availability of this testing has enabled Australia to increase its position in global barley markets.

One of the long term users of protein profiling has been the Department of Agriculture and Food, Western Australia (DAFWA). DAFWA uses the technology to check the purity of seed stock for its seed certification scheme. When DAFWA releases a new seed variety it requires purity certification to the 10-5 level, meaning that no more than one in 10,000 seeds can be a different variety. DAFWA also uses protein analysis to help farmers who may have confused their seeds in storage and therefore need varietal and purity tests to identify their stocks.

DAFWA reported that this profiling technology has the advantage of high accuracy. Protein profiling has increased the accuracy and reliability of protein analysis considerably, compared to previous electrophoresis methodologies.

A further application of protein technologies in plants is the use of antibody-based tests to identify pre-harvest sprouting in wheat and barley. Pre-harvest sprouting occurs when there is significant rain on ripe grain prior to harvest. If grain has sprouted, the price paid to the grower is usually significantly reduced because sprouting lowers the market value of the grain. Grain with even mild sprouting cannot be used for bread or noodle manufacture. Hence, being able to identify what parts of a crop have sprouted prior to harvest means that this grain can be separately stored and handled, so the unaffected portion of the crop maintains its value.

The CSIRO and the Value Added Wheat CRC developed the test to detect high levels of alpha amylase enzymes which are produced when wheat starts to sprout before harvest, because of late seasonal rain (Skerritt and Heywood, 2000). The test is reliable on a sample of 20 spikes from a field of wheat and provides a colour indicator that is correlated with the level of alpha amylase present. The test is suited for rapid screening on-farm prior to harvest and for use at silos and elevators. Results are obtained in less than five minutes and the precision of the test is as good as, or better than, the traditional Falling Number test. The Falling Number test measures the time taken for a plunger to fall to the bottom of a glass tube filled with a standardised mixture of wheatmeal and water – the quicker the plunger falls, the more the wheat has sprouted. It must be conducted in a laboratory and hence is not as convenient or quick as the alpha amylase test. The new enzyme test has been commercialised through C-Qentec Pty Ltd and is sold as WheatRite®.

Protein technologies applications in animals

Commercial variety testing using protein technology in Australia currently appears to be concentrated on major commercial crops such as grains. However, applications relating to species or variety identification are emerging in the fish and meat industries. For example, a survey by Food Standards Australia and New Zealand (FSANZ), found that approximately 14 per cent of fish sold as barramundi was actually other species, and over 40 per cent of fish sold as Red Emperor was another species (FSANZ, 2003). Researchers at Murdoch University are working with WA company, Proteomics International Pty Ltd, to combine proteomics with bioinformatics to provide a method for on-the-spot testing of up to 450 fish species using a biometric card (Amalfi, 2005). This will help consumers ensure that when they pay a premium for particular varieties of fish that they are not receiving cheaper substitutes. The new system will replace current DNA-based laboratory-based techniques which take several days to provide a result and are more limited in their scope. The work is expected to save the industry tens of millions of dollars in mislabelled and substitute fish products.

3.2.4 Waste management

In waste management only one application of protein technology was identified in Australia. The research is investigating the understanding of the protein mix in biofilms (these are films such as those bacterial films that line water pipes) and bacterial mixes. Studies of biofilms, such as those underway at the University of NSW (www.babs.unsw.edu.au) can provide insights into signalling between bacteria and are thought to be important to their effectiveness in bioremediation of waste including waste from agricultural operations.

3.3 Australia's use of protein technologies compared to overseas countries

Workshop participants indicated Australia has had significant involvement in proteomics. The government funded the Australian Proteomics Analysis Facility (APAF) in 1995 (O'Neill, 2004). Most of the applications of proteomics have been in human health e.g. the government of Canada has a major program funding proteomics in human health, investing CA$114 million in 14 projects (**www.genome.canada.ca**). On the other

hand, the use of proteomics in agriculture is in its infancy. This is because proteomics tests work best when the full genome sequence in known, however this information is not available for many agricultural species. There can also be technical difficulties such as the preparation of suitable protein from many plant species can be problematic (APAF, 2006).

Other governments are aggressively pursuing proteomics research and its resulting commercial applications. The increasing complexity of proteomics analysis has led to increasing automation of various analytical processes and development of information technologies including biochips (Siitar and Koivistoinen, 2004).

The United States and Japan dominate this area in terms of equipment, with major corporations producing specialised laboratory machines for protein separation, protein identification and high throughput screening. Such equipment is also needed for measuring the levels of proteins in particular cell or organ types (in animals).

Japan's National Institute for Agricultural Science has a major program on proteomics in rice. The program involves a number of other universities and research groups in Japan including the universities of Tsukuba, Tohoku and Niigita. CSIRO scientists have expressed concern that Japan will move ahead of Australia because Japan's five-year US$88 million proteomics initiative (Davidson, 2003).

Canada established the Alberta Network for Proteomics Innovation in 2002, which includes the universities of Alberta, Calgary and Lethbridge. Funding was provided through a three year, CA$6.21 million grant by Western Economic Diversification Canada and CA$10 million in funding from the Alberta Science and Research Authority. The Network provides funding and access arrangements for advanced proteomics research infrastructure in Canada. This initiative has enabled advances in a number of crop research programs which includes crops grown commercially in Australia. For example, Canadian work to diversify its pulse industry through adaptation of European varieties to produce food grade lupins may compete with Australia's export success in food grade lupins (Blade, 2004).

In terms of work on specific species, the US Department of Agriculture is funding a bovine protein sequencing project which aims to, in part, enhance understanding of fertility, disease and metabolism in these species for agricultural applications (Coussens, 2005). While Australia is part of the International Bovine Genome Initiative, workshop participants reported that Australia was limiting its interest to improving the output of our beef industries and was not using the work to diversify into other applications.

New Zealand is applying proteomics in the processing of agricultural products. Researchers there, for example, have compared raw and treated hides to identify the proteins present in both and their impact on leather quality (Choudhury, 2006). Applications of proteomics in deer husbandry and the growth of deer antler (the latter for processing applications) are also a focus of proteomics research in New Zealand (Barling, 2004).

Although workshop participants indicated China is likely to be behind Australia in its proteomics research, proteomics has been part of the country's National High Technology Research and Development Program since at least 1991 and has included the development of an on-line proteomics database (High Technology Research and Development Program of China, 2001). Chinese scientists have announced the results of a number of major research programs in proteomics recently, including:

- genomics and proteomics for silk worms, as part of the Silkworm Genome Project at the Beijing Proteomics Institute (Xia, 2004);
- genomic and proteomic analysis of chickens by the Beijing Proteomics Institute (Wong, 2004); and
- animal nutrition, by the National Key Laboratory of Animal Nutrition at the China Agricultural University in Beijing (Wang, 2006).

3.4 Further potential applications in Australia

The development of new applications of proteomics in Australia will rely heavily not only on proteomics itself but on bioinformatics, which provides a mechanism for interpretation of the vast amounts of data that are generated in genetic analysis. Early proteomics research concentrated on understanding changes in protein expression at different stages of the life cycle or between healthy and diseased organisms. Hence the focus was on separation and characterisation of proteins. While this is still important, new trends in plant and animal proteomics include (Rampitsch and Srinivasan, 2006):

- the analysis of proteins in parts of the cell outside the nucleus (e.g. chloroplasts of plants, which have their own genome);
- analysis of protein changes in response to stress; and
- symbiotic plant-microbe and animal-microbe interactions.

3.4.1 Growing and husbandry

In growing and husbandry the main applications emerging are in understanding responses to environmental stresses, which is of increasing importance due to climate change. The University of Melbourne has research programs on drought tolerance in wheat using proteomics, as well as projects understanding the effects of environmental factors (such as heat, cold and wind) in other cereal crops.

There are also use of protein technologies to improve the understanding of plant-microbe interactions, particularly the role of symbionts, and the interactions between bacteria and the species that they infect. The end point of this work is likely to be development of mechanisms for plants to resist pathogen attack, although commercial application is probably some years away. As an example, the Australian Centre for Necrotrophic Fungal Pathogens at Murdoch University in Perth is using a mix of genomics and proteomics to investigate an important pathogen of wheat. However, the genome map for this pathogen was only released in July 2006 so the work is at an early stage.

3.4.2 Processing

There may be a range of applications of proteomics in fibre processing. This is of particular importance to the wool industry because of existing competition from synthetic fibres. The application of proteomics in the analysis of wool has been hampered until relatively recently by difficulties in staining and electrophoresis (Plowman, Bryson and Jordan, 1999). The Wool Research Organisation of New Zealand (WRONZ), which has sponsored extensive research into this issue, has since used proteomic analysis of high sulfur proteins in wool fibres to understand variations between sheep breeds and the impact of these proteins on fibre crimp (Flanagan, Plowman and Bryson, 2002). WRONZ is currently funding research into the variability in protein composition in relation to other wool traits.

Emerging applications include the use of protease enzymes on wool to improve its washability and reducing its tendency to felt (developed in Finland and sold under the brand of Washwool®); and mould-derived keratin-degrading enzymes which selectively break down wool fibre cuticles and help to reduce felting (developed in Japan) (Ferguson, 2004). Researchers internationally are also developing methods to understand the changes in collagen that occur during the processing of skins with the aim of improving skin quality (Choudhury et al., 2006).

3.4.3 Logistics

The main logistical commercial application of protein technology is currently related to variety and/or purity testing in cereal crops, a process which is being driven by the introduction of end-point royalties in the cereals industry (GRDC, 2006) and other sectors such as the stone fruit industry(Low Chill Australia Inc., 2005). Variety testing is particularly important where the variety of fruit or grain cannot easily be distinguished by visual inspection and a premium is being paid for a particular variety.

In animals, the introduction of the National Livestock Identification System for tracking "paddock to plate" may generate demand for new techniques. While there are currently commercial applications being offered based on DNA technologies (see previous chapter), proteomics-based applications are being developed, for example, to identify whether a fish sample is what it is claimed to be (see Section 3.2.3). The new methods provide a much more detailed analysis than do the existing DNA techniques, because there are many more proteins in an organism than there are genes and gene products can be changed after they have been transcribed from the DNA by the action of other molecules in the cell, including RNA (Proteomics International, nd). These applications are an important way of tracking produce as it moves down the supply chain and reaches the consumer.

3.4.4 Waste management

Proteomics is being developed as an analytical tool in waste management. The main applications are in understanding the ways in which microbial populations change as they break down pollutants (Singh, 2006). However this research area is in its infancy and bioinformatics will play a major role in interpretation of the data so that an organism's metabolism in contaminated environments can be predicted (Singh and Nagaraj, 2006).

3.5 Conclusions

Protein biotechnology such as proteomics is a younger science than DNA technologies and as such there are fewer commercially available applications. The two technologies complement each other and are often used together to enhance and deepen the understanding of genetic changes in an organism. The main commercial applications in variety testing and purity testing are likely to become very important in Australian agriculture and need to be extended to crops other than grains. Further, with increased market competition and more stringent market access conditions, Australia's ability to deliver the right product will depend heavily on enhanced use of proteomics tools in the future.

Australia's competitors are at a similar stage of development but seem likely to be pursuing additional, more highly value added, opportunities in processing and medical applications of agriculture. As was noted in the previous chapter, commercial applications to date have relied on development of genome maps, and the development of these has not been cost effective in some of Australia's smaller crops (especially if Australian researchers work in isolation). It is important for Australia to continue to work in major international consortia to gain access to the information needed to develop agricultural applications specific to Australian conditions.

4 Cell and tissue culture and engineering

Cell and tissue culture and engineering include techniques for growing living cells in laboratory conditions as well as related techniques for combining cell culture with engineering to build organs (tissue engineering). Cell and tissue culture also includes embryo manipulation including in-vitro fertilisation and the fusion of different types of cells (cell fusion) to form new cell types including cells which are hybrids of two species, and development of vaccines and immune stimulants for prevention of disease.

4.1 Where are cell and tissue culture used?

In Australia, cell and tissue culture are used mainly in breeding (including artificial insemination) and in vaccine development. Both of these applications are classed under growing and husbandry.

There are substantial complementary uses of DNA technologies in these applications and the general traditional approaches to plant breeding which are reviewed in Chapter 2 are relevant here.

4.1.1 Plant Growing and animal husbandry

Plant breeding

Plant breeding through cloning (the taking of cuttings from parent plants and growing them by inducing the formation of roots) is a very traditional and long-established method of breeding plants. Many of our important horticultural crops, for example Cavendish bananas, have been grown by cloning for many years. This method has the advantage of maintaining the desirable characteristics of a particular plant line. However, as has happened with bananas, it also means that the entire domestic population could be of the same genetic background, and the industry is susceptible to devastation if a new disease emerges.

In the last 50 years, techniques developed for tissue culture of plant cells have enabled the grower to take only a few cells from plants rather than a whole stem or part of stem. The cells are grown in sterile conditions on agar-based cell culture media. Using various plant hormones, the cells can be stimulated to regenerate into plantlets, which can be grown-on in a glasshouse once they reach a certain stage of maturity. Tissue culture is very common in the production of ornamental species of plants because it is faster than growing by seed and it is possible to ensure that unusual colour or leaf forms are maintained (plants grown by seed are more variable).

Animal breeding

Unlike plant breeding, animal breeding by cloning has been a relatively recent development in livestock industries, with the first mammal cloned in 1996 (Dolly the sheep). Animal cloning is still rare and, in Australia, only occurs in a research environment. On the other hand, livestock breeding using artificial insemination technology is common. Animals are bred by using stored, frozen semen to impregnate a female of the same species. Artificial insemination enables breeders to easily bring together sires and dams with good pedigrees, even if the animals are physically distant.

Traditional artificial insemination enables farmers to use a wider range of sires than would normally be available from natural mating and can help to increase conception rates because insemination occurs under controlled conditions at the most beneficial time in the oestrous cycle. Artificial insemination can also help farmers to

selectively produce male or female offspring and/or to meet market specifications for particular stock. However, reliance on traditional (non-DNA) pedigree information alone to select parents is often unreliable and does not take account of genetic variations including traits that may skip generations.

As discussed in Chapter 2 the use of genetic markers adds further value to artificial insemination, because it provides the opportunity to select the preferred characteristics in the parents based on their individual genes, rather than appearance and bloodlines (Lucy, 2005). Marker-assisted selection has been coupled with further developments in the management of artificial insemination and embryo handling and storage, such as:

- The development of hormonal treatments which can be applied to the mother to manage the process of egg maturation. This decreases the amount of handling required per animal and increases the frequency of fertilisation. Egg maturation and hence breeding of a whole herd of animals can be synchronised, thus reducing breeding costs further (see below);
- The ability to hold embryos in suspension after fertilisation. These embryos can then be brought to term in unrelated surrogates, which enables high value donor mother animals to produce many more offspring than they would normally be able to do in their lifetime;
- Ultrasound and/or blood tests for pregnancy-associated proteins, thus allowing pregnancy to be detected with greater certainty.

Figure 4-1 illustrates the traditional and modern biotechnology approaches to artificial insemination. While the costs of synchronised artificial insemination are higher, the benefits to producers are considerable and include (Johnson, 2005):

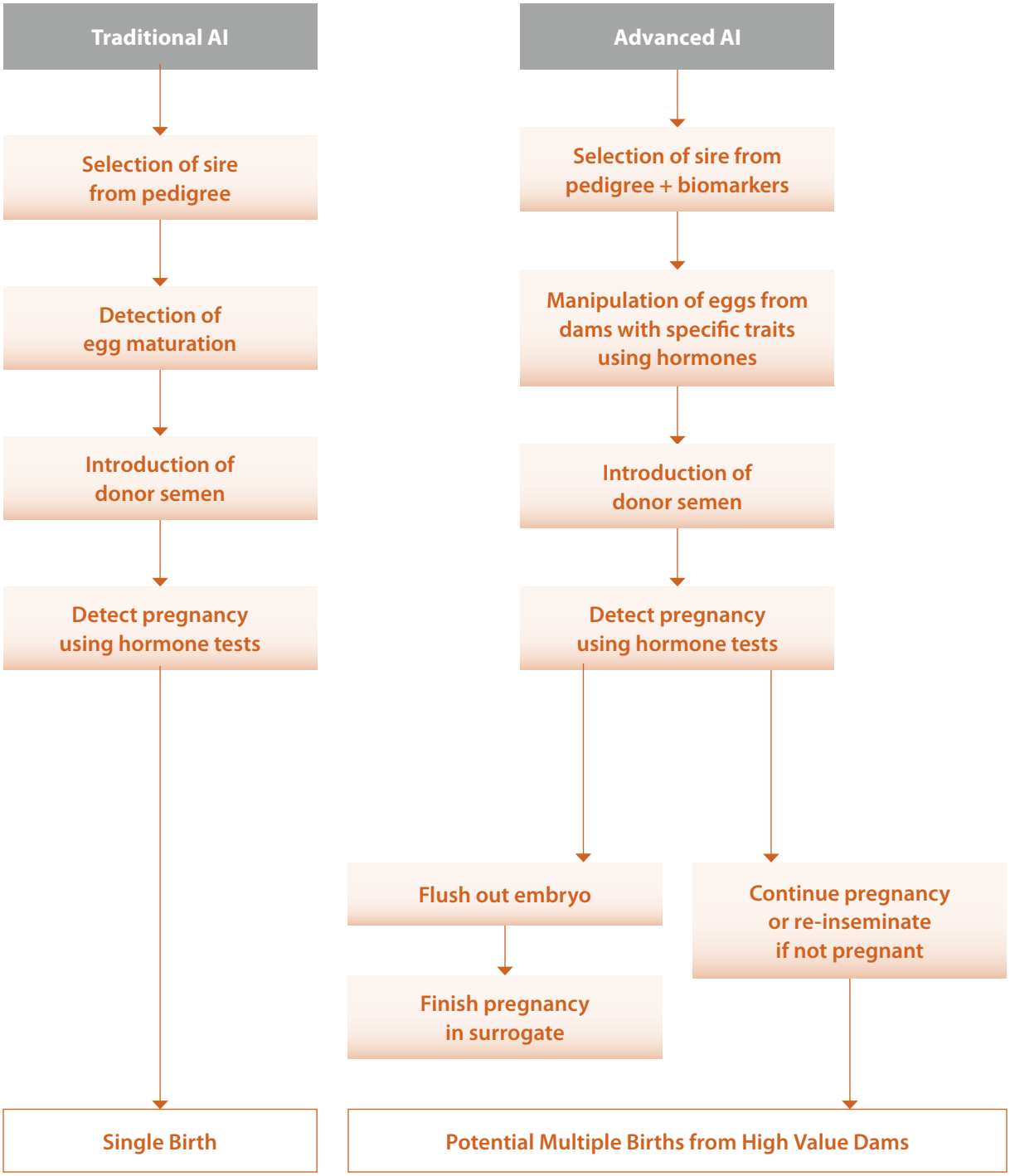
- synchronisation of births – animals' oestrous cycles can be coordinated so that all animals are ready for insemination at about the same time, all fall pregnant within a short time of each other and all give birth at about the same time. This approach reduces late-season offspring which may be more likely to die and helps farmers to manage their breeding herds more efficiently;
- improved management of maternal nutrition;
- improvements in pregnancy rates;
- quicker development of specialised herds; and
- the ability to breed out undesirable traits across the herd more quickly

Diagnosis and management of disease

Vaccines have long been used to provide protection against infectious disease for humans and animals. Biotechnology is being used to develop and deliver new vaccines which could be of value to Australian agriculture.

Traditionally, vaccines have been developed using attenuated (weakened) bacteria or viruses which takes a long time to develop. To produce the attenuated vaccine, the disease organism is bred over successive generations in the laboratory and the weaker strains are selected for further breeding, eventually producing one which cannot cause sickness because it is too weak; however it can still induce natural immunity when used to infect a susceptible animal. Alternatively, the toxins produced by the disease organism may be used to form the vaccine. The value of vaccines to the poultry industry is detailed in Box 4-1.

Figure 4-1: Traditional and modern biotechnology approaches to AI



Box 4-1: Vaccines for the poultry industry

Vaccines are an important mechanism for limiting the spread of disease in intensive livestock operations such as the poultry industry where the animals are in close proximity all the time and diseases can spread through the group more easily than if they were spread out over a larger area. A range of vaccines has been available to the poultry industry for many decades with Australian vaccine companies researching, developing and supplying live vaccines to the livestock industry over that time. An attenuated vaccine is available for chronic bacterial (*Mycoplasma*) respiratory diseases in poultry. Live viral vaccines are available for Marek's Disease (which causes US$1 - $2 billion economic loss globally p.a. – Morrow and Fehler, 2004), infectious bronchitis, infectious bursal disease and Newcastle disease, all of which can cause severe production loss and, in some cases, high levels of mortality.

Vaccine manufacturers must comply with good manufacturing practice and be licensed by the government. The Australian company Bioproperties Pty Ltd has been manufacturing domestic animal vaccines since 1989. The company conducts extensive research & development in Australian institutions and has in-house laboratories for further product development of live vaccines for poultry, swine, cattle and sheep. The company is a core member of the Australian Poultry CRC, together with the Rural Industries R&D Corporation, the University of Melbourne, the University of New England and the Australian Egg Corporation. This collaboration provides an avenue for commercialisation of CRC research so that it reaches and benefits producers. Bioproperties Pty Ltd has seven further vaccines in late stage development and nine in the early R&D stage. The company expects to launch a live vaccine against salmonella in poultry and an additional Marek's Disease vaccine in the next 12 months.

Source: www.bioproperties.com.au and www.poultrycrc.com.au

Many vaccines have been developed for diseases specific to Australia or for diseases which have major economic effects in Australian agriculture (see examples in Table 4-1).

Table 4-1: Selected vaccines for sheep and cattle industry in Australia

Disease		Common vaccine approach
Tick fever	Cattle	Live vaccine active against Anaplasmosis virus, carried by ticks
Ovine Johne's disease	Sheep	Killed whole organisms vaccine active against Mycobacterium paratuberculosis
Calf scours	Cattle	Killed whole organism vaccine active against E. coli
Footrot	Sheep	Antigens (proteins) of *Dichelobacter nodosus*

Source: published sources

Ovine Johne's Disease (OJD), for example, is a serious wasting disease of sheep and has a range of impacts including:

- impediments to the trade of live sheep;
- mortalities of up to 15 per cent p.a. in flocks of adult sheep;
- decreased wool production;
- decreased viability of sheep producers; and
- decreased demand for sheep meat products due to consumer concern.

It has been estimated that OJD infection costs about $17,000 per year for a typical flock of 2,000 ewes and results in a reduction in gross margins of 6.4 per cent per farm (Meat and Livestock Australia, 2005b). It is caused by the bacterium *Micobacterium paratuberculosis* and vaccines against it have been shown to reduce deaths by 90 per cent and delay the onset of shedding of OJD bacteria in the dung by about 12 months (Meat and Livestock Australia 2005a). It

4.1.2 Logistics

The transport and holding of stock provides opportunities for infection that may have a major impact on the ability of growers/handlers, for example, feedlotters, to obtain an economic return. As a result applications of tissue culture and engineering are an important adjunct to stock management and logistics.

Bovine Herpes Virus-1 (BHV-1) is an example of a disease which is a significant source of sickness and death in feedlot cattle. Development of a vaccine to protect against BHV-1 has contributed to reductions in incidence of this disease in cattle feedlots. A brief case study of this vaccine and benefits it provides is discussed in Box 4-2.

Box 4-2: Case study 5 - Benefits of vaccinating cattle against Bovine Herpes Virus-1

Beef is one of Australia's major industries. Beef cattle are sold by farmers to feedlotters, who feed them to maximize weight gain prior to sale. Feedlotters then sell to processors in Australia and overseas. The top 20 feedlotters control about 50 per cent of the Australian market. Large feedlots may hold up to 80,000 head of cattle.

The animals come into these feedlots from many places and may carry a range of infections in with them. One of the most common diseases is bovine respiratory disease (BRD), a syndrome associated with infection from a range of different viruses and bacteria. Bovine herpes virus-1 (BHV-1) is one of the viruses associated with BRD. While cattle can be infected with BRD on the farm, such infections usually result in only mild disease however, when cattle are brought into feedlots they experience a variety of stresses (from transport, mixing with unfamiliar animals and finding feed and water in unfamiliar places) which contributes to an increased susceptibility to disease.

BRD is believed to be the cause of 50 to 90 per cent of sickness and death in Australian feedlot cattle, with a 2001 survey of 72 feedlots reporting that 64 per cent of all illness and deaths were due to the disease (Meat and Livestock Australia, 2001). Annual cost to the industry due to deaths and slow weight gain is estimated at $60m a year (Pettiford and Gaden, 2005).

There are two major sub-types of BHV-1 which contribute to BRD. These are designated Sub-type 1.1 and Sub-type 1.2. In most other parts of the world both sub-types exist, however in Australia only sub-type 1.2 has been isolated. Overseas BHV live vaccines are all based on sub-type 1.1 and hence are not effective against the BHV sub-type 1.2, which is prevalent in Australian cattle.

In 2001, Q-Vax Pty Ltd, a private company situated in Queensland, launched Rhinogard, a new vaccine to protect cattle against BHV-1. Q-VAX was the first company to sell a vaccine against BHV-1, sub-type 1.2, in Australia.

Rhinogard is a one-dose live attenuated intra-nasal vaccine. The decision to develop an intranasal vaccine was based on the need to develop immunity quickly. North American research previously showed that an intranasal vaccine provides some level of protection within 48 hours of administration. An intra-nasal vaccine is more difficult (and therefore more expensive) to administer than an injection. However, it is effective more quickly and is cheaper in the long run, because the alternative is a two-dose dead vaccine. The first dose of this dead vaccine must first be administered on-farm, and hence is an added cost to producers; the second dose is administered at the feedlot.

Box 4-2: Case study 5 - Benefits of vaccinating cattle against Bovine Herpes Virus-1 (continued)

As it produces nasal mucosal immunity very quickly, Rhinogard can be used on feedlot entry, or prior to feedlot entry, if the operators are practised and suitable yards and handling facilities are available (Pettiford and Gaden, 2005).

The benefits to feedlotters of using Rhinogard are reduced losses from death of cattle due to BRD; reduced administration of antibiotics; reduced losses from delays in moving cattle through the feedlot due to weight loss or failure to thrive as a result of BRD; increased export prospects due to compliance with customer demands on weight profile of animals and improved animal welfare through reduction of incidence of BHV-1.

There was no treatment for BHV-1 in Australia prior to the introduction of Rhinogard into the Australian market. The comparison is thus between unvaccinated and vaccinated cattle. Q-VAX's pen and field trial data reveal that the average daily gain in vaccinated cattle is 1.81kg compared to 1.52kg in cattle given a placebo (i.e. unvaccinated); and that feed conversion is 8.3kg of feed per kg liveweight gain in vaccinated cattle, compared to 9.9kg of feed per kg liveweight gain in cattle given a placebo (Q-VAX product brochure).

The company has calculated that these benefits provide an additional income of $20 per head, as against a cost of under $3 per head for the vaccine plus staff time in managing vaccinations. However, cattle are routinely treated for a number of diseases on arrival at the feedlot hence the additional staff time for Rhinogard vaccination is minimal. QVAX estimates a feedlot of 80,000 animals would incur costs of approximately $240,000 for vaccination and benefits of $1.6 million.

Q-VAX is also exploring potential for an injectable form of the vaccine (only one dose required because it is still a live vaccine). This is being developed in response to moves by the industry to vaccinate cattle on-farm, prior to arrival at the feedlot, and would overcome the difficulties of administering an intranasal vaccine.

Two other companies (Intervet and Pfizer) have subsequently offered vaccines against other organisms which cause BRD to the Australian market: Intervet has an injectable two-dose dead vaccine (Bovillis MH) against *Mannheimia haemolytica* (one bacterial disease complication of BRD) and Pfizer sells Pestigard, a dead two-dose vaccine against bovine viral diarrhoea virus (Pettiford and Gaden, 2005)

4.2 Australia's use of cell and tissue culture compared to use in overseas countries

Artificial insemination is well established in Australia and there are many commercial service providers who travel from farm to farm to provide insemination services. Use of artificial insemination has become the normal breeding method in some livestock sectors, particularly horse breeding and dairy cattle. Artificial insemination is supplemented by MAS as markers for particular traits become available. For example, in the Australian dairy industry the Australian Dairy Herd Improvement Scheme (ADHIS) maintains a national database of performance and pedigrees. This database is used by artificial breeding companies to help assist genetic improvements in the industry (Australian Dairy Farmers, 2006).

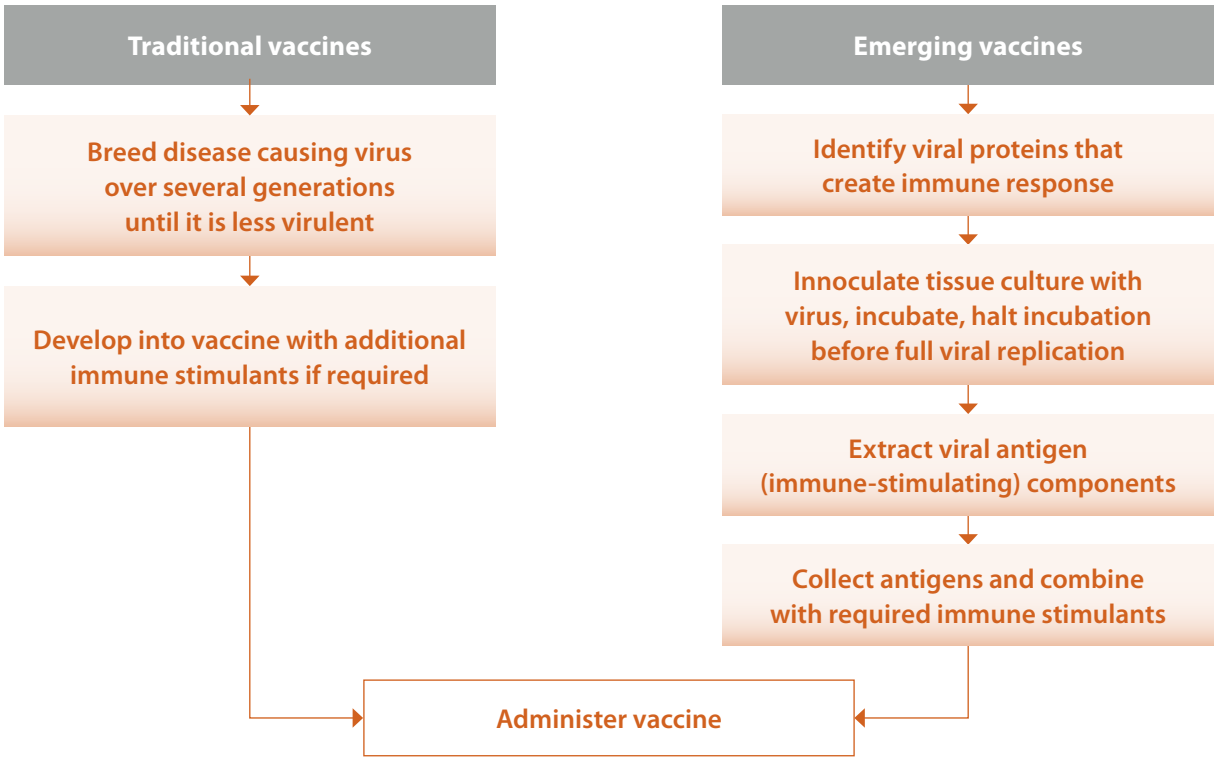
Most of Australia's competitors are investing heavily in improvements to artificial insemination techniques. It is clear that it is necessary to marry these closely to livestock management and record keeping in order to maximise producer benefits. Reflecting the importance of this information, in 2002 the United Kingdom Government awarded its largest ever agricultural development grant to a consortium comprising the BASCO joint venture (owned by the Limousin Cattle and Suffolk and Texel Sheep Societies) and MLC Signet. The BASCO online herd-book database will provide a single source of pedigree and performance information and will provide a means of recording additional traits, including genotypes, to support breeding decisions (British Texel Sheep Society, 2005).

Similarly in Japan, beef cattle breeding trends have shifted in the last 50 years from visual inspection in the 1960s to genetic evaluation of progeny in test stations using carcass field records in the 1980s (Sasaki, 2006). The latter has significantly enhanced the speed of development of advantageous carcass traits such as marbling (the spread of fat in thin "veins" throughout the meat cut, a trait of high value in Japan). Finally, studies on the use of a combination of breeding values and animal management in South Africa have shown that the culling of inferior milk-producing cows and using bulls with high breeding values has produced an improvement of 1200kg of milk per cow over 13 years from the mid-1980s (Muller, 2003).

New biotechnological approaches to vaccine development centre mainly on the development of sub-unit vaccines, peptide vaccines and DNA vaccines. These types of vaccines are being developed to elicit immune responses without the risk of using a whole virus. This technology can increase the range of diseases that can be targeted and can reduce unwanted side effects. The vaccines have greater stability and hence a longer shelf life. Sub-unit vaccines and peptide vaccines are discussed further here. DNA vaccines are genetically modified and are not within the scope of this report as they are defined as a GMO.

Sub-unit vaccines contain purified proteins that cause an immune response. They are not infectious and cause fewer side effects than live attenuated vaccines in the vaccinated animal. However, the immune response generated by a sub-unit vaccine may not be as effective as that produced using a vaccine based on whole organisms, and the sub-unit vaccines are more expensive to manufacture because of the levels of purity of the active protein required. To increase effectiveness, sub-unit vaccines are often administered with other molecules that are known to enhance the immune response, termed "adjuvants". In vaccines for human use these molecules are likely to be common chemicals such as alum. In livestock vaccines, components of bacteria such as cell walls are sometimes used for this purpose. Sub-unit vaccines are also being developed for treating all types of diseases, not only infectious diseases. Figure 4-2 illustrates the paths used in the development of traditional and sub-unit vaccines.

Figure 4-2: Development of traditional vaccines and Sub-unit vaccines



Source: MJ Biologics, 2006, www.mjbio.com

Peptide vaccines create immunity by exposing the organisms to parts of proteins of the disease-causing organism.

Many major drug and animal health companies are involved in the development of vaccines for animal applications. International vaccine manufacturers are trialling a range of new-style vaccines for animal use (Table 4-3). This research may be developing improved vaccines for diseases that are currently treated by traditional vaccines; or new vaccines for diseases that are currently untreated.

Table 4-3: Selected emerging vaccines for sheep and cattle internationally

Disease		Emerging vaccine approach
Bovine diarrhoea	Cattle	Sub-unit vaccine to prevent transmission of bovine diarrhoea virus across the placenta from pregnant cows to their unborn calves (Bruschke, 1999)
Bovine herpes virus	Cattle	Sub-unit vaccine for bovine herpes virus (Wisconsin Alumni, Research Fdn, 1995)
Foot and mouth disease	Cattle	Peptide vaccine boosted with cholera toxin (Beignon, 2005)
Scrapie	Sheep	Peptide vaccine (Harmeyer, 1998)

Source: published sources – see references for details.

Improvements to existing vaccines may be targeted at a range of benefits, including (Beard, 1998):

- **longer shelf-life**—vaccine vials can be held for longer and there is less wastage of out-of-date vials;
- **ability to vaccinate against more than one disease**—animals need to be handled less often, thus reducing stress and improving animal welfare, and the cost of vaccination "per disease" is reduced;
- **greater efficacy**—vaccines are effective against their target disease more quickly;
- **reduced risk of conversion of attenuated virus to a virulent form**—there is a lower chance of unwanted resurgence of a disease in a population; and
- **reduced viral shedding in animal droppings**—there is less chance of animal-to-animal transmission.

It is likely that Australia will continue to rely on vaccines developed overseas for global diseases but will need to develop vaccines for those diseases (or disease strains) which are endemic.

Australian research groups and private companies are improving vaccines against diseases which currently affect Australian agricultural productivity, whether they be cosmopolitan or endemic. For example, the Australian Poultry CRC's vaccine development programs draw on a range of biotechnologies to improve vaccines against a number of poultry diseased including *Pasteurella* (gene sequencing), chicken anaemia virus (RNA, molecular markers), Eimeria, (subunit vaccines) and Marek's disease (gene sequencing) (www.poultrycrc.com.au). The commercial application and success of these vaccines will depend not only on their efficacy but on whether they are to be used in livestock for meat production (immediate immune protection required for a short time) or in breeding stock (long term immunity required).

4.3 Further potential use of cell and tissue culture in Australia

4.3.1 Growing and husbandry

Breeding

The ability of livestock producers to benefit from developments in cell and tissue culture probably depends more on the associated use of these technologies with farm management practices and database development than biotechnical developments alone. R&D corporations are aware of the need for integration of a range of biotechnology techniques with management practices and are funding several initiatives. For example:

- Rural Industries R&D Corporation (RIRDC) – development of the Crusader® program for farmed rabbits, which aims:
  - to develop a disease identification and control package for farmed rabbits, including identification of genetic merit for resistance to bacterial infection;
  - to investigate environmental and genetic means of reducing pre-weaning mortality, including development of superior breeding stock with increased litter size;
  - to improve management practices to reduce disease and improve profitability; and
  - to make the information available to rabbit farmers through a central website (Eady, 2005).
- RIRDC – MOPLAN genetic improvement system for angora goats (Mohair Australia Ltd, 2000). The system records breeding performance and links performance of close relatives in assessing breed value for each individual – the systems enables high performance animals within flocks to be identified and recorded.
- Southern Tree Breeding Association and the Animal Genetics and Breeding Unit – TREEPLAN® for *Pinus radiata* and *Eucalyptus spp.* Treeplan® estimates breeding and genetic values for tree species using pedigree data from close and distant relatives, different sites, multiple genetic groups, and multiple traits. The system is web-based and can produce single lists of genetic values for each trait given different production environments. The system has produced cost and time savings while enhancing genetic gains (McRae, et al., 2004).

There has been considerable interest in the development of immuno-castration methods to reduce the loss of carcass condition in males of many livestock species; however the early commercial attempts were unsuccessful due mainly to high costs of production and lack of efficacy. For example, in the mid 1990s, CSIRO successfully developed a vaccine (Vaxstrate®) for vaccinating cattle against luteinising hormone. However, an analysis of the benefits of immunological spaying compared to surgical spaying indicated that there was no net benefit in either growth rates or the price received for the carcass, making the vaccine unsuccessful for economic reasons (Jeffery et al., 1997).

More recently, another vaccine, Improvac®, has been successfully launched for treating boar taint in male pigs. The vaccine is active against Gonadotrophin-Releasing Factor (GnRF), a breeding hormone, and replaces castration as a treatment for maintaining weight gain and improving boar flesh taste. The GnRF vaccination increases weight gain and fat deposition when compared to unvaccinated animals (McCauley, 2003). Launched by CSL Animal Health after funding from the Pig Industry Program, Improvac was found to provide a benefit to cost ratio of 5.4 to one with a net present value (as at 2004-05) of $1.91 million against project outlay of $350,000 (Gaffy, 2005). Improvac® is now distributed world-wide by Pfizer Animal Health.

Disease management and prevention

There are a number of research programs underway to explore further development of vaccines against livestock diseases, for example, farmers in South Australia are trialling vaccines against specific strains of Ovine Johne's Disease, which as discussed previously, is a serious disease of sheep which causes major mortality and flock losses (also see Section 4.2.10). MLA is sponsoring research to monitor the spread of the disease in vaccinated sheep flocks (Meat and Livestock Australia, 2005b).

Many research groups internationally have been attempting to develop effective vaccines against foot and mouth disease. Recently, researchers have demonstrated effective vaccination against foot and mouth disease using a peptide vaccine approach (Wang, 2002). However, it may yet be several years before an effective vaccine reaches the market, due to the need to carry out large scale trials and to develop cost effective manufacturing and vaccine delivery methods.

4.3.2 Processing

While applications of cell and tissue culture in processing in Australia were not identified, there are some applications being developed overseas which may be relevant in Australia. The applications relate to the safety of processed meat for human consumption, and in particular to the threat posed to humans by particular strains of bacteria such as *Escherichia coli*. Research in Scotland found that *E.coli* colonises the lower portion of the gut in cattle and can be transferred to the meat during slaughter and processing (University of Edinburgh, 2004). The research suggested that development of vaccines to prevent colonisation of cattle by particular strains of *E. coli* may provide a method for reducing the potential food poisoning hazards of these bacteria to humans.

4.3.3 Logistics

Further applications of cell and tissue culture and engineering may be desirable in livestock industries where there are diseases which cannot yet be treated by vaccines and where the process of livestock collection and transport exposes animals to increased likelihood of disease. The application of new vaccine development methods will depend in part on the cost and the efficacy of new products under development.

4.4 Conclusions

Cell and tissue culture have been used in agriculture for many years and the general approach for development of new varieties of plants (by tissue culture) and vaccines (by use of whole cells or parts of cells) is well established commercially. In terms of breeding, the main benefits of adoption of newer technologies accrue in the data collection and management and the interplay with agronomy or husbandry. Australian producers and their overseas competitors all recognise that several technologies and practices are complementary and can be brought together to improve productivity in the short and longer term.

The main application in vaccines is also well established commercially. Australia has the skills to develop new sub-unit and peptide vaccines. However these technologies are not yet well established and there are few commercial products available. Success of these vaccines in Australia, for both animal husbandry and in logistics management, will depend more on efficacy and cost than technical capacity.

5 Process biotechnology (Bioprocessing)

5.1 What is process biotechnology?

Process biotechnology (bioprocessing) is any large-scale operation involving the use of whole micro-organisms; animal or plant cells; or chemicals (such as enzymes) produced by animal or plant cells to transform a raw material (biological or non-biological) into a product. The use of whole micro-organisms to process chemicals is usually termed *fermentation*.

Bioprocessing using micro-organisms and enzymes is applied in a number of industries. For example, bioprocessing processes include:

- **bioleaching**—the removal of metals from ore using biological processes;
- **biobleaching or biopulping**—treatment of paper, pulp or wood fibres with enzymes;
- **biodesulferisation**—removal of sulfur from fuel oil;
- **bioremediation and phytoremediation**—the removal or break down of pollutants using micro-organisms, or plants respectively; and
- **biofiltration**—purification of fluids using micro-organisms.

The main applications of process biotechnology are in the manufacture of food, fibres, certain chemicals (including drugs), the extraction of minerals from ore bodies and the conversion of wastes to useful products or to harmless chemicals.

Modern process biotechnology (often termed bioprocessing) often involves use of genetically modified organisms. These GM applications are not within the scope of the report.

5.2 Where is process biotechnology used?

In researching Australian and overseas activity in this area of biotechnology, we found many process biotechnology applications in use overseas. As yet, applications in Australia are limited but include:

- functional food development;
- biofuels; and
- biotechnology-based fibre processing.

In Australian agriculture, process biotechnology is used mainly in agricultural processing and waste management. Twelve projects under process biotechnology were identified, of which seven were examples of process biotechnology in food and fibre manufacture and five were applications in bioremediation.

5.2.1 Growing and husbandry

Process biotechnology is not used in growing and husbandry, except perhaps in the production of animal feeds. However, no examples of such applications were identified in Australia.

5.2.2 Processing

The main applications of process biotechnology in Australian agriculture were in processing of food for humans. The applications discussed here are processing aids and functional foods.

Enzymes and manufacturing aids

Food manufacturing involves a range of technologies including methods for fragmenting, processing, filtering, mixing, storing and maintaining food products so that they are safe, taste good and are attractive to consumers. Traditional food manufacture has relied on enzymes, around 60 of which are in common use. Traditionally, these enzymes are extracted from plants or animals. For example rennet, which is used in cheese making, was traditionally extracted from the stomachs of calves, lambs and goat kids.

The main modern applications in agriculture of process biotechnology appear to be in food manufacture. This includes the development of specialised yeasts for wine processing, starter cultures for foods which require microbial fermentation such as bread, yoghurts, cheese and beer, and use of specialty enzymes to improve food processing. Food Standards Australia New Zealand (FSANZ) approves individual enzymes for particular applications and has to approve their inclusion in the Australia New Zealand Food Standards Code before they can be used in food for human consumption (Table 5-1). Although used in Australia, many of these enzymes are imported.

Table 5-1: Recent selected enzymes considered for approval by Food Standards Australia and New Zealand

Enzyme	Source	Use
Triacylglycerol lipase	Yeast *Hansenula polymorpha*	In bread making to improve dough stability, dough handling and crumb homogeneity
Phosholipase A1	Fungus *Aspergillus orizae*	Cheese manufacture – improves efficiency and yields
Triacylglycerol lipase	Fungus *Mucor javanicus*	In cheese - breaks down fats to improve flavours in cheese
Hexose oxidase	Yeast *Hansecula polymorpha*	In bread making - increases dough strength and bread volume; and in cheese and tofu aids curd formation
Alpha amylase	Bacterium *Bacillus stearothermophilis*	Greater thermal stability of the enzyme, and produces a different chemical profile when breaking down sugar

Source: draft assessment reports issued by FSANZ, 2005 and 2006

Functional foods and Nutraceuticals

In addition to enzymes and food additives there is growing interest in Australia in functional foods (foods which have additional health benefits beyond the nutritional value of the food itself) and nutraceuticals. The main benefits of functional foods to agriculture and food processing arise from capture of higher prices for raw materials as part of a higher value-added supply chain. However, these compounds need to be extracted, purified, formulated and included in final products and value is added at each of these points. In many cases, the return to the original grower is the same as that grower would receive for the product for its use as "normal" food. The main benefits accrue to others in the supply chain, who receive higher prices for the final product, and consumers, who can enjoy health benefits.

By way of example, cartilage (e.g. trachea) is normally a waste product of meat processing. Chondroitin sulphate is derived from the trachea of cattle or pork, deer antlers and shark cartilage and is used as dietary supplement.

Chondroitin sulphate has been shown to influence central nervous system development, wound repair, infection, cell growth and cell division (Trowbridge, 2002). When combined with glucosamine, (also a waste product from meat processing) and administered orally, chondroitin sulphate is claimed to play a role in the protection of joints, by inhibiting enzymes which degrade cartilage, while glucosamine is claimed to speed up collagen synthesis (Michel, et al 2005). Chondroitin sulphate is a component of many products aimed at easing joint and ligament injuries, arthritis and joint inflammation. It is estimated that up to 70 million people in the United States alone have some form of arthritis or joint inflammation (CDC, 2007) and the current US market for products containing chondroitin sulphate is up to $1 billion per annum. Chondroitin sulphate is also an active ingredient in veterinary products, and has a role in moisture retention in some cosmetics. Development of products containing chondroitin sulphate and glucosamine has required the use of biotechnology in extraction of the base molecule, formulation and testing of effects in trials.

The amount of active ingredient in preparations containing chondroitin sulphate ranges from 250mg to 1,200 mg and the final retail prices range from hundreds to thousands of dollars per kg chondroitin sulphate in the final product. The price to farmers for the raw ingredient is about $4 per kg. Ingredients suppliers sell the product for $65 to $150 per kg and the rest of the mark up is at the manufacture and distribution phases. Hence, while economic benefits along the chain are high, many of the benefits accrue to industry players beyond the farm gate. However, in the longer term an important benefit to the economy and society of functional foods may be lower health costs and a healthier Australian community.

Functional foods containing a range of ingredients are marketed for their added value health benefits. For example, the marketing of margarine with omega-3 fatty acids added to reduce absorption of cholesterol. Australia and many other countries have regulations which limit the range of depth of health claims that can be made in food products.

Only a few Australian companies manufacture functional ingredients or finished functional food products. Most of these companies manufacture or sell products which contain dairy or bovine ingredients as development of functional food or nutraceutical ingredients is an important way of adding value to particular agricultural product fractions (in the case of milk) or to waste streams (in the case of red meat and grains). Table 5-2 provides examples of ingredients from several sources.

Table 5-2: Sample applications of functional food ingredients in Australia

Source	Molecule	Application
Milk	Growth factors	Wound repair and sports health
Milk	Lactoferrin, lactoperoxidase	Immune stimulation and enhancement
Milk	Peptides	Anti-hypertensives (lowering of blood pressure)
Bovine blood, soybeans	Ferritin	Bioavailable iron
Wheat bran	Phytate	Muscle growth supplement

Sources: Playne et al., , (2003)

8.2.3 Waste management

Waste has traditionally been either burned or chemically treated to reduce it to less harmful substances which can then be buried in landfill. However, many products of modern living cannot be treated this way because they are very toxic, last a long time in the environment or are not amenable to chemical treatment. Examples of such products are:

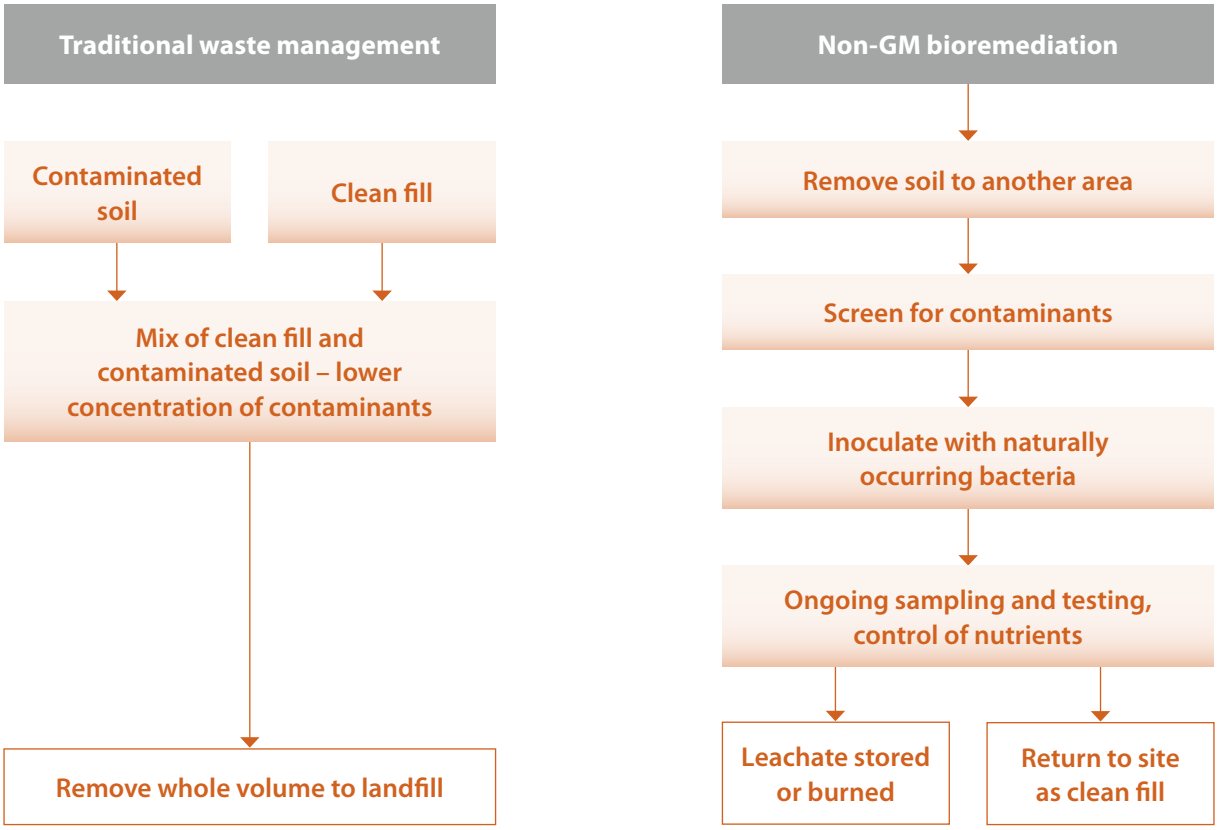
- pesticide residues including DDT (Dichloro-Diphenyl-Trichloroethane - soil half-life 150 years), organochlorines (several months up to 2 years), organophosphates (days to months); and
- petroleum based products.

While these products may eventually break down in the environment, they may be toxic to both humans and animals during the time that they remain active.

Many of the more toxic or long-term residues are treated by burial. Burial sites have to be selected very carefully to ensure groundwater does not percolate through them, resulting in leaching of toxic residues. While burial removes toxic waste from the immediate local area, it may make the burial site unusable for some time.

The main application of biotechnology in waste management is the use of enzymes (either naturally occurring or genetically modified) to break down intractable chemical wastes. Figure 5-1 summaries the general approach to traditional waste management and non-GM bioremediation.

Figure 5-1: Bioprocessing approaches to toxic waste remediation



Source: Diagram derived from text in Dayet al., 1997.

Waste remediation is a well-established application of biotechnology in Australia. It has been applied in decontamination of industrial sites, where it helps to remove intractable waste and to return sites to community use. It has also been used in water recycling, which is particularly important in farm applications given the regular occurrence of drought in Australia's rural areas. The main research group active in the area is the Environmental Biotechnology CRC, which is currently developing remediation applications specifically for the meat processing industry (Box 5-1).

Box 5-1: Using biotechnology to remediate and add value to waste from feedlots

The Environmental Biotechnology CRC is working on recovery and re-use of resources and has a program dedicated to bioprocess engineering, including development of specialised biomass for processing of industrial waste (Environmental Biotechnology CRC, 2002).

Under a collaborative research agreement with MLA, the CRC is developing a demonstration processing plant to deal with excess nitrogen and phosphorus in heavily contaminated wastewater from abattoirs. The technique involves treating waste in batches and will replace existing chemical methods (McGlashan, 2005). Comparative costs of the two approaches are not yet known, however there are environmental benefits from the biotechnology-based approach.

The new treatment method will help to remove 99 per cent of organic carbon, 98 per cent of nitrogen and 99 per cent of phosphorus, all of which can cause toxic algal blooms if they enter waterways. It is expected that the nutrients removed will be developed into other products including fish food. Development of fish food will provide a value-added use for the waste and should also provide a valuable input to Australia's growing aquaculture industry.

Other applications of bioremediation to agricultural waste management are being introduced to help remediate persistent chemical residues (Box 5-2). These applications also have environmental and occupational health and safety benefits.

Box 5-2: Case study 6 - Bioremediation of pesticide residues using biotechnology

To remain competitive and increase yield, Australian farmers use a range of pesticides, fungicides and herbicides to control insect pests, diseases and weeds. However, some of these chemicals break down slowly and some can have undesirable effects on the environment. One such group of chemicals is organophosphates, which are broad spectrum, water soluble insecticides. Organophosphates are used in sheep dips as well as in many plant and crop insecticides.

There are catchment management issues associated with the use of organophosphates and local councils test for residues in their waterways because of the potential impact on both fish and people. Organophosphate residues are also a public health issue in some residential areas – subdivisions built on land previously used for agriculture and contaminated by organophosphates can have impacts on residents' health (Bowes, 2003).

In the 1990s CSIRO scientists identified an enzyme in fruit flies which could break down organophosphates. Supported by Orica Watercare and Horticulture Australia Ltd this research was developed into a number of products which break down organophosphate insecticides, which would otherwise persist for a long time in the environment.

Box 5-2: Case study 6 - Bioremediation of pesticide residues using biotechnology (continued)

The first commercial product from this research is active against organophosphates and was launched in September 2004 as Landguard™ OPA. The enzymes in Landguard™ OPA are able to reduce pesticide concentration to very low levels in minutes unlike natural degradation where organophosphate breakdown takes up to six months. The Landguard™ enzymes split the organic part of the molecule from the phosphate and the breakdown products are two orders of magnitude less toxic than the original pesticide. The product is currently offered as a powder that can be easily added to water to decontaminate waterways and water reservoirs.

Prior to the launch of Landguard™ OPA no other product was available to address the organophosphate residue. Farmers waited several months for the organophosphates to break down naturally. These chemicals could therefore potentially end up in waterways and cause problems outside the farm boundary. Access to this new technology has potential to improve environmental outcomes and improve farm productivity.

In addition Landguard™ OPA has also been trialled in a number of other agricultural situations including:

- remediation of sheep dip effluent in a mobile sheep dip operation;
- breakdown of residues from spraying organophosphates on cotton;
- cleaning of equipment used to spray organophosphates on farms. In this case the rinse water contains Landguard™ OPA and because the Landguard™ is only active for 30 minutes after hydration, this same water can be held in the tank overnight and used to mix the insecticide for the next application the following day.
- remediation of organophosphate contaminated water in the horticulture industry. Field trials using LanguardTM OPA to clean a ground spray rig used to spray a pear orchard resulted in the rapid degradation of methyl parathion;
- decontamination of soil to enable turf to be grown on land previously used to grow alfalfa, during which time it was treated with organophosphate insecticides; and
- remediation of tailwaters remaining after cotton irrigation with a 90% reduction in residue levels in 10 minutes in 80,000 litres of tailwater..

Trials of Landguard™ OPA have shown that it succeeds in reducing organophosphate levels to below the NSW statutory minimum in half an hour. In 2005-2006 Orica Watercare and CSIRO won the DuPont Innovation Award in the category of Food, Agricultural Production and Marketing for Landguard™ OPA.

The Landguard™ series of products is expected to be applicable to many agricultural sectors in Australia such as cotton, which uses organophosphate insecticides as sprays or in irrigation waters. There are also potential applications in using the product as a wash on fruit to remove pesticide residues at the packing facility. There are export prospects in the United Kingdom and the United States, both of which have passed laws requiring more complete remediation of many insecticides and industrial chemicals. The strengthening of remediation regulation in Australia could see a substantial increase in demand for Landguard™ in this country.

A range of enzymes against organophosphates, triazines, pyrethroids, carbamates, phenyl urea and neonictinoids are now being developed, based on the same technology platform.

5.3 Australia's use of process biotechnology compared to use in overseas countries

Australia appears to be behind several other countries in its use of process biotechnologies. To some extent Australian food processors' slow uptake in these fields may be related to the more limited role of manufacturing in the Australian economy, compared to many economies overseas. As the use of enzyme technologies in food processing in Australia does not require any specific specialisation (unlike, for example, the need for specialised vaccines to treat unique Australian livestock diseases) it is more economic to import these technologies than to develop them in Australia. However, the Australian Government's Food Innovation Grants Program is supporting value adding to agricultural materials and has funded the development of new, biotechnology-derived, food ingredients.

Internationally, there are several exciting areas where biotechnology is being applied in plant and animal bioprocessing, with downstream applications in human health or energy. As outlined below, there are major investments being made overseas by governments and the private sector. These areas provide opportunities for high levels of value adding to agricultural products, and could be major threats to Australian agricultural industries as overseas growers utilise bioprocessing to build valuable businesses. The major categories discussed here are, functional foods, bioethanol and fibre processing.

5.3.1 Functional Foods and nutraceuticals

Functional foods are major segments of the food market in many developed countries. Global market size has been estimated to be in excess of US$100 billion per year and growth rates are estimated to be at around seven per cent p.a. depending on source and definitions (Datamonitor, 2004). Major product segments include probiotic drinks (in particular milk and sports drinks), cereals and bars, and confectionary (Datamonitor, 2004). Many ingredients, particularly milk bioactives and a number of anti-oxidants, are relatively mature in terms of market acceptance. Other new bioactives are emerging and are being developed (many as nutraceuticals) by Australia's competitors, particularly New Zealand, (Table 5-3).

Table 5-3: Sample bioactives, source and applications

Extract	Bioactive role	Application
Pine bark (various spp)	Anti-oxidants	Extracts from *Pinus pinaster* may relieve high blood pressure, reduce platelet aggregation and low-density lipoprotein (bad)-cholesterol and may enhance circulation. Extracts from *Pinus radiata* are sold as nutraceutical Enzogenol® by a New Zealand company. Extracts from *Pinus sylvestris* are thought to reduce inflammation through their antioxidant activity
Fucoidan (marine algae)	A polysaccharide occurring in algae (*Fucus* spp.)	May play an important role in nerve transmission and long term memory storage. Sold as nutraceutical U-Fn by a US firm and as Modifilan by a company in Russia.
Lycopene (tomatoes)	Carotenoid	Could play an important role in treating cancers, as well as helping to prevent cardiovascular disease. Sold as a nutraceutical Ly-co-mato® by a company in Israel
Lutein (flowers)	Carotenoid	Eye health. Blackmores Australia imports a lutein-based nutraceutical called Lutein-Vision®

Extract	Bioactive role	Application
Cocoa flavenols	Anti-oxidants from cocoa beans.	Have been associated with reducing cholesterol and improving blood vessel function. Mars Corporation (US) is investing heavily in research
Omega-3 (fish)	Essential fatty acid	Added to margarines, cereals and a range of other products and marketed as functional foods

Source: web searching. Company names provided as examples only

Research into functional foods is also being completed in Australia. For example, The National Centre of Excellence in Functional Foods is a consortium of scientists from organisations, including from CSIRO and Food Science Australia, who research, develop, substantiate and assist in commercialising functional foods. One example from this centre is Recaldent which is based on casein polypeptides that have the potential to prevent and repair tooth decay. The development of this product was based on the investigation of the bacteria that are responsible for dental caries and periodontitis, and the identification of the molecular processes that allows the repair of early tooth decay without invasive processes. Recaldent is now included in a range of food products produced by Australian and international companies, including chewing gum and toothpastes.

5.3.2 Bioethanol

There has been substantial interest recently in biofuels because of pressures on existing oil supplies. Biofuels have come to prominence as the cost of traditional oil-based fuels and concerns about global warming have increased.

There are two types of biofuels – bioethanol and biodiesel. Biodiesel is produced by chemical modification of waste vegetable oil or from high density plant oils such palm or soybean. Production of biodiesel does not involve biotechnology and is not within the scope of this report. Bioethanol is a fuel produced by microbial fermentation of starchy plants (e.g. sugar beet, sugar cane, corn, cassava) and can be used in standard internal combustion engines. It can also be used to make ethyl-tertiary-butyl-ether, which is a fuel additive.

Fermentation of biomass to produce ethanol is not a new technology; however recently many countries have developed extensive policies to build their biofuels capacity. In 2001, the European Commission adopted an action plan and two proposals for Directives to foster the use of alternative fuels for transport, starting with biofuels (European Commission, 2001). The action plan outlines a strategy to achieve a 20 per cent substitution of diesel and gasoline fuels by alternative fuels in the road transport sector by 2020.

Brazil has also invested in developing biofuels, concentrating on generating bioethanol from sugar. More than 20 per cent of cars sold in Brazil today are able to run on either petrol or ethanol (Luhnow, 2006). The Brazilian bioethanol program now uses over half of the country's sugar cane crop.

Worldwide, at least 22 countries have biofuel projects (Hamilton, 2004). The American Coalition for Ethanol estimates that in 2006 the US ethanol industry will provide 5 billion gallons of ethanol which equates to about 3 per cent of national sales.

Early bioethanol projects used feedstock such as agricultural residues, livestock waste, energy crops such as sugar and oilseeds such as canola, forestry waste, industrial waste and garden waste which were hydrolysed and fermented to produce ethanol (Nicolau, 2003). Emerging technologies are using biomass crops with high levels of cellulose which is the main energy source for bioethanol production. Hydrogen can also be produced as a result of this process (Biofuels Research Advisory Council, 2006). It has been estimated that between four per cent and 13 per cent of agricultural land in the European Union would be required to produce the amount of biofuel to reach targets set in 2003.

The production of crops for biomass requires higher yields than when the same crops are produced for food. Research has been conducted into improving agronomic practices, breeding high biomass feedstock, improving harvesting methods and processing (including enzyme developments) (US Dept of Energy, 1999). The main focus of breeding studies is increasing cellulose accumulation.

5.3.3 Fibre processing

Internationally, compared to Australia, there appears to be much greater emphasis on the use of biotechnology for fibre processing, particularly the use of enzymes in softwood processing (Nutsubidze and Sarkanen, 1997). Enzymes such as xylanase help to break down the wood pulp more quickly so that it can be processed more efficiently and with less toxic waste than when stronger chemicals are used. The United States is leading in the application of protein technologies to fibre manufacture.

While Australia has well-established wool, cotton and forestry industries and these are our main fibre crops (for clothing and paper respectively), there has been little commercial interest in development of new fibre crops. However, a recent example of new Australian work is Australian Papyrus Ltd, which is building a pilot plant to manufacture paper out of banana fibre (Australian Papyrus Ltd, nd). Also, a workshop on the development of a bagasse-based fibre industry (including consideration of the use of sugar bagasse as a source of paper fibre) was conducted in Queensland in May 2007.

5.3.4 Phytoremediation

While the use of bioremediation is growing in Australia, a further development overseas is the use of whole plants, termed phytoremediation. According to workshop participants, development of both bioremediation and phytoremediation are being driven strongly overseas by the strengthening of regulatory frameworks which limit the amounts of harmful pesticide residues that can be associated with agricultural activities. In addition, land use pressures provide an incentive to quickly restore contaminated lands for agricultural or residential use. An example of the use of phytoremediation overseas is the use of crops such as globe yellowcress (*Rorippa globosa*) to absorb cadmium from contaminated soils (Wei, 2005). The phytoremediation crop is then harvested and removed and the cadmium, which is absorbed into the plant's leaves, is treated off-site in a contained facility.

5.4 Further potential applications of bioprocessing in Australia

The main areas where process biotechnologies have potential application in Australia are in the development of functional foods and bioethanol. These are discussed as types of processing in this section. Emerging applications in logistics and waste management are also reviewed.

5.4.1 Processing

Biofuels, in particular bioethanol, provide potential additional income to farmers for their crops and a number of bioethanol companies have recently been established in Australia. At present, the focus is on development of blends of ethanol and petrol blends, made from agricultural products including wheat and sugar cane biomass by-products. (Australian Farmers Fuel, nd). As with the examples overseas, developments are mainly based on existing technologies.

Despite signs of commercial activity in biofuels, most biofuel projects, both internationally and in Australia, are supported by government because they are not yet economically viable without this support. Biofuels tend to be promoted on their wider economic and environmental impacts, rather than direct commercial arguments. Arguments in favour of biofuels include that they (Hamilton, 2004):

- improve energy efficiency by allowing higher octane ratios, reduce carbon monoxide emissions and support a cleaner combustion system;
- offset demand for imported crude oil and reduce pressure on the balance of trade;
- encourage rural economic development and employment;
- improve the environment; and
- help Australia reduce CO_2 emissions.

5.4.2 Logistics

There are additional applications for biotechnology emerging in logistics, mainly related to food preservation, with applications designed to have an impact on the storage and transport of fresh food. Researchers are developing methods of preserving meat using bacterial films, which prevent spoilage organisms establishing on the surface of food, e.g. cuts of meat (Vermeiren, et al., 2004). Enzymes are also being used to break down spoilage organisms (Galperrin, 2006; Abdou, 2007). Such techniques enable meat processors and retailers to respond to emerging consumer preferences for "natural" and authentic foods while maintaining safety (Altieri, et al., 2005).

5.4.3 Waste management

The main trend in process biotechnology for waste management is the conversion of waste to valuable products rather than simply targeting the breakdown of waste into harmless by-products. Examples of these applications include the use of microbial fuel cells to create energy while at the same time remediating wastes such as sulphur (Angenent, 2006; Rabaey, 2006).

5.5 Conclusions

Process biotechnology is probably one of the areas where Australia is furthest behind other countries in terms of the use of these technologies in agriculture. Australia appears to have relatively limited use of both established and emerging technologies, matters which may be linked to the role of manufacturing in the economy as a whole. The most common applications were found in bioremediation using whole organisms; and food manufacturing using imported enzymes.

Australia has an emerging interest in biofuels. However, compared to many other nations such as Brazil and the United States, biofuel production in Australia is still well behind many other nations. Most biofuel projects, both internationally and in Australia, are supported by government because they are not yet economically viable by themselves. Environmental benefits (reduced greenhouse emissions) and fuel security are cited as major drivers for government decisions to support biofuels.

6 Summary and conclusions

The report presents information on how non-GM biotechnology tools and techniques (excluding genetically modified organisms as final product) are being applied in Australian primary industries and how they benefit users down the supply chain.

6.1 Biotechnology's current application in Australian agriculture

This report is based on a review of 213 agricultural biotechnology projects along the supply chain using biotechnology, including 119 relating to plants, 79 relating to livestock and 11 relating to micro-organisms. Use of biotechnology is more prevalent in the cattle, sheep and grains industries, possibly because there are greater resources to invest in biotechnology research and associated applications in the larger sectors.

Many examples of the utilisation of biotechnology in agricultural research were identified; however, examples of commercial applications were less prevalent. Nevertheless, examples of commercial biotechnology applications along the supply chain were identified. Selected examples that highlight major uses on non-GM biotechnology in the agricultural supply chain are discussed below.

Breeding

The use of marker-assisted selection (MAS) has become widespread and helps to obtain genetic information on breeding stock and to speed up the development of new, more productive or higher value varieties of plants and breeding lines of livestock. MAS can halve the amount of time required to bring a new plant variety to market. MAS can also enable breeders to isolate livestock with unfavourable genetic make-ups so they can be excluded from breeding programs and to identify livestock with favourable characteristics for use in breeding programs.

A limitation of genetic markers is that usually the genome of a species (identification of gene sequences and their function) must be understood before MAS can be applied, This process is expensive and time-consuming, limiting the extension of this technology to less economically significant crops. However, this limitation is being overcome through the development of new genome screening techniques such as that used in DArT technology (Case Study 2) that do not require pre-existing knowledge of a species' genome sequence.

Other detailed examples identified in this report of the use of biotechnology for breeding include:

- use of MAS to develop strawberry varieties with superior flavour and flowering characteristics (Case Study 1);
- use of genetic markers and other biotechnology techniques to improve the management of, and to add value to, artificial insemination and embryo-handling techniques.

Disease diagnosis and treatment

For disease diagnosis, biotechnology techniques are well established and Australia has the capacity to develop new diagnostics for emerging threats. The techniques are used in a range of plant and livestock sectors, and are often incorporated either into or are often based on research in Australia. Examples of the use of biotechnology for disease diagnosis in the report include:

- Use of DNA technologies to measure the genetic diversity of sugar cane smut leading to the identification of six individual strains of smut worldwide. This information was used by the Bureau of Sugar Experiment Stations to identify smut-resistant sugarcane cultivars internationally. These sugarcane varieties were used in cross-breeding with Australian cultivars to produce smut-resistant Australian cultivars.

- Identification of a range of fungi and nematodes in soil using the Predicta-B test, which helps to determine the best crop rotations in the South Australian wheat belt (Case Study 3).
- Development of a vaccine for the Australian strain of bovine herpes virus-1 (BHV-1), which is believed to be a significant cause of sickness and death in Australian feedlot cattle (Case Study 5). Vaccinated feedlot cattle are less likely to lose weight and it is estimated that vaccination of cattle on arrival at a feedlot of 20,000 cattle would result in a net benefit of $1.36 million.

Logistics and support

In logistics, an increase in demand for food security and quality assurance is driving requirements for improved trace back and identification systems for both domestic and export markets. Commercial applications of both protein and DNA techniques are being developed to meet these requirements. Examples of the use of biotechnology in logistics and support in the report include:

- The WheatRite® test used to identify pre-harvest sprouting in wheat and barley. As sprouted grain is of lower value because it cannot be used for bread or noodle manufacture, being able to identify sprouted crops allows the unaffected portion of the crop to be harvested separately and its value maintained.
- The GeneSTAR Feed Efficiency4 test, developed by Genetic Solutions Pty Ltd, screens livestock for four separate genetic markers that affect feed efficiency. Test results can be utilised at the feedlot level to maximise efficiencies by allowing the cattle to be sorted into groups for different feeding programs.
- Prevention of substitution in fish wholesale outlets, using protein technologies to "fingerprint" fish species. Researchers at Murdoch University are working with Proteomics International Pty Ltd to develop a method for on-the-spot testing of up to 450 fish species using a biometric card. This card will help ensure consumers get the species that they pay for and is expected to save the industry tens of millions of dollars in mislabelled and substituted fish products.

Processing

Compared to some other countries there is relatively limited processing of agricultural products in Australia beyond traditional food manufacture. Internationally, use of biotechnology in agricultural and fibre processing is much more advanced. These areas provide opportunities for high levels of value adding to agricultural products, and Australian agricultural industries could be missing out as overseas growers utilise bioprocessing of agricultural products to build valuable businesses.

The main modern applications in agriculture of process biotechnology appear to be in bioremediation and in food manufacture, the latter including development of specialised yeasts and starter cultures. In Australia, most of these enzymes are imported from overseas.

Development of functional food or nutraceutical ingredients is an important way of adding value to particular agricultural product fractions or to waste streams. Only a few Australian companies, mainly those processing dairy or bovine ingredients, manufacture functional ingredients or finished functional food products.

Australia's involvement in biofuels is recent and many other nations such as Brazil and the United States are more advanced in this technology. Environmental benefits (reduced greenhouse emissions) and fuel security are cited as major drivers for government decisions to support biofuels.

Waste management

Compared to some overseas countries, in Australia applications of biotechnology in waste remediation are limited. This may be because, compared to other countries which have stronger legislative requirements,

in Australia there are few regulatory drivers regarding the management of agricultural waste. In the future, Australia may increase its use of biotechnology in this area due to an increased need to recycle water and to add value to waste streams.

Examples of waste management using biotechnology in the report include:

- Development of Landguard™ OPA to speed remediation of organophosphate chemicals used on crops and in sheep dip (Case Study 6). Organophosphates generally break down slowly and some can have undesirable effects on the environment. The enzymes in Landguard™ OPA are able to reduce pesticide concentration to very low levels in less than 30 minutes and have a number of applications, including use by mobile sheep dip operations.
- The Environmental Biotechnology CRC in collaboration with MLA, is developing a demonstration processing plant which uses bacteria to remove excess nitrogen and phosphorus from heavily contaminated abattoir wastewater.

6.2 Biotechnology's impact

While the economic benefits of biotechnology for agriculture and the economy can not always be readily quantified, it seems clear that the existing benefits are significant and future benefits could be large. For example:

- Marker-assisted selection of plants allows breeders to simultaneously select for more than one characteristic and importantly the technology can also speed up the development of new varieties. It has the potential to bring higher yielding and/or more water efficient grains to market relatively cheaply in a much shorter period of time than more traditional breeding methods.
- DNA-based diagnostics by identifying productivity and product quality traits can improve quality and potentially increase returns to growers through price premiums and improving market access.
- Modern biotechnology can speed up the diagnosis and treatment of disease, vaccines can be developed more quickly and with improved specificity reducing disease associated losses.
- A range of biotechnology techniques are being used to develop functional foods, which can produce economic benefits along the chain, although many of the benefits may accrue to industry players beyond the farm gate. However, in the longer term an important benefit to the economy and society of functional foods is likely to be lower health costs and a healthier Australian community.

Many of the technologies being developed are only relatively new, e.g. proteomics and new methods of developing vaccines. As result, their main use is still in public sector research. Over time, more of these will be developed into commercial products and are likely to be taken up in customer sectors including agriculture. Those likely to increase in usage include variety testing and purity testing in crops other than grains and in livestock other than cattle. The uptake of many of these technologies as commercial products will depend on cost and efficacy rather than technical capacity.

6.3 Conclusions

The examples presented in this report demonstrate that there have been benefits from use of biotechnology (excluding GMOs as final products) in Australian agriculture. These benefits often accrue to producers through improved speed to market; reduced environmental damage; healthier and more valuable livestock and crops: and maintenance of or improvements in productivity. Where biotechnology contributes further down the supply chain, then the benefits will usually accrue to those later in the chain. However the economy as a whole may also benefits.

There may be wider social reasons for using biotechnology, including healthier communities, reduced use of scarce resources, regional development and improved environment outcomes, including reduced greenhouse gas impacts.

While there are many examples of biotechnology being developed in the research setting, Australian agricultural industries appear to be using commercially available biotechnology-based tools and techniques in a limited manner (Table 6-1). There are two exceptions to this view. Firstly, the use of biotechnology to develop and release new varieties of plants and breeds of domestic animals is well established. Secondly, research institutions have a major role in using biotechnology to develop new diagnostic tests which if required can be developed relatively rapidly for commercial use. Both of these applications are listed under growing and husbandry in Table 6-1.

Table 6-1: Scale of use of technologies along supply chain

	Supply chain position			
Technology	**Growing & husbandry**	**Logistics & support**	**Processing**	**Waste management**
DNA and RNA	Extensive	Limited	Limited	Limited
Proteins and other molecules	Intermediate	Limited	Intermediate	Limited
Cell and tissue culture engineering	Intermediate	Not found	Limited	Limited
Process biotechnology	Not applicable	Limited	Limited	Limited
Sub-cellular organisms	Limited	Limited	Not found	Not found

Based on project reviews and comments from workshop participants

As noted above, Australian researchers are using biotechnology tools and techniques extensively and are developing new applications which could be applied by industry. However, given the limited number of commercial applications identified in many supply chain positions the uptake of existing biotechnology tools and techniques could be expanded significantly and would have corresponding benefits in those sectors into which it is introduced.

Many of Australia's competitors are at a similar stage of development to Australia but seem to be pursuing additional, more valuable, opportunities in plant or animal processing with downstream applications in human medicine. Research and development is very expensive so Australia will need to continue working in major international consortia to remain fully informed and capture information required to develop biotechnology applications specific to Australian conditions.

7 References

Abdou, A, Higashiguchi, S, Aboueleinin, AM, Kim, M and Ibrahim, HR (2007): *Antimicrobial peptides derived from hen egg lysozyme with inhibitory effect against Bacillus species*, Food Control, 18 (20): 173 – 178

AgGenomics Ltd (2004): *AgGenomics has Achieved a Number of Significant Milestones*, press release, 11 February 2004, **www.aggenomics.com.au**

Altieri, C. Speranza, B, Del Nobile, M and Sinigaglia, M, (2005): *Suitability of bifidobacteria and thymol as preservatives in extending the shelf life of fresh packed plaice fillets*, Jnl Applied Microbiology 99: 1294-1302

Amalfi, C (2005): *Fishy practice stopped by science*, Western Australian Newspaper, 9 August 2006

Angenent, L (2004): *Production of bioenergy and biochemicals from industrial and agricultural wastewater*, Trends in Biotechnology 22 (9): 477 – 485

APAF (2006): *Plant and Agricultural Proteomics*, **www.apaf.org.au**, accessed 24 November 2006.

Australian Academy of Science (2007): *Biology Meets Industry – Genomics, Proteomics, Phenomics*, **www.science.org.au**

Australian Animal Health Laboratory, (2006): *21 years of achievement: Australia's animal health laboratory, Livestock Horizons* 2, pp.6-7.

Australian Cotton Shippers Association (2003): Presentation summaries from their conference on 3 September 2003, **www.austcottonshippers.com.au/news_item.asp?idnews=16**, accessed 30 November 2006

Australian Dairy Farmers (2006), **www.adff.com.au**, accessed December 2006.

Australian Farmers Fuel (SAFF) (nd), **www.farmersfuel.com.au**, accessed November 2006

Australian Papyrus Ltd (nd), **www.papyrusaustralia.com.au**, accessed November 2006

Australian Pork (2006): *Australian Pork Traceability Audit*, **www.apl.au.com**

Australian Wool Innovations (2007): *Breeding Tools for the Future*, and *DNA Marker Technology*, **www.wool.com.au**, accessed 5 January 2007

Backhouse, D, Abubakar, AA, Burgess, LW, Dennis, JI, Hollaway, GJ, Wildermuth, GB, Wallwork, H and Henry, FJ (2004): *Survey of Fusarium species associated with crown rot of wheat and barley in eastern Australia*, Australasian Plant Pathology 33(2) 255 – 261

Ballan News (2007): *Promising Auction Debut for BioClip Wool*, Ballan News 9 March 2007

Bancroft, I (2002): *Insights into cereal genomes from two draft genome sequences in rice*, Genome Biology, 3(6), online publication

Barling, P, Liu, H, Matich, J, Mount, J, Ka Wai Lai, A, Ma, L and Basford Nicholson, LF (2004): *Expression of PTHrP and the PTH/PRHrP receptor in growing red deer antler,* Cell Biology International, 28 (10): 661 – 673. PTH/PTHrP = parathyroid hormone/parathyroid hormone-related peptide receptor

Barr, A, Eglinton, J, Langridge, P, Warner, P and Chalmers, K (2001): *Marker-assisted Selection – where to now?* Proceedings of the 10th Australian Barley Technical Symposium, Canberra, ACT 16-20 September 2001, ISBN 1 920842 13 6

Barr, A and Langridge, P (2001): *National Barley Molecular Marker Program – Achievements and Future Directions*, 10th Australian Barley Technical Symposium, 16 – 20 September 2001

Beard, C (1998): *Out on the Farm with DNA Vaccines*, Nature Biotechnology, 16: 1325 - 1328

Beignon, A (2005): *A peptide vaccine administered transcutaneously together with cholera toxin elicits potent neutralising anti-FMDV antibody responses*, Veterinary Immunology and Immunopathology, 104 (3-4): 273 – 280

BIO (nd): *Food Biotechnology* **www.bio.org/speeches/pubs/er/food.asp**, accessed 30 November 2006

Biofuels Research Advisory Council (2006): *BioFuels in the European Union – A Vision for 2030 and Beyond*, 14 March 2006

Blade, S, Lopetinsky, K, Olson, M, Laflamme, P and Phillips, C (2004): *High protein lupins: diversifying the pulse industry in western Canada*. New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress, Brisbane, Australia, 26 September – 1 October 2004

Bowes, D (2003): *Body Burden of POPS in Children*, report on organophosphate and organochlorine residues in Coffs Harbour

British Texel Sheep Society (2005): *Beef and Sheep Data – Future Secured in the UK*, press release, 17 October 2005.

Braithwaite, K (2005): *Final Report – SRDC Project BSS258 – Assessing the Impact that Plant Variation has on the Sugarcane Breeding Program*, BSES Ltd, December 2005

Bruschke, C, van Oirschot, JT and van Rijn, PA (1999): *An experimental multivalent bovine virus diarrhea virus E2 subunit vaccine and two experimental conventionally inactivated vaccines induce partial fetal protection in sheep*, Vaccine, 17 (15-16): 1983-1991

Bush, G (2004): *Homeland Security Presidential Directive HSPD-9 - Defense of United States Agriculture and Food*, 30 January y2004

CDC (2007): *Arthritis Surveillance*, **www.cdc.gov/arthritis/state_coordinators/faqs/case_definition.htm**, accessed January 2007, US Centers for Disease Control

Choudhury, S, Allsop, T, Passman, A and Norris, G (2006): *Use of a proteomics approach to identify favourable conditions for production of good quality lambskin leather*, Analytical and BioAnalytical Chemistry 384(3): 723 – 735

Coussens, P (2005): *Determining Gene Function for Agriculture and Using Domestic Species as Model Organisms*. Panel session at the Bovine Genome Project – the Next Phase International Workshop, 29-31 March 2005, Houston, Texas. USDA Co-operative State Research, Education and Extension Service, **www.csrees.usda.gov**

CRC for Innovative Dairy Products (2006a): *Fact Sheet – Using Genetic Markers in Dairy Cattle*, January 2006

CRC for Innovative Dairy Products (2006b): *Fact Sheet - Genomics and Proteomics,* **www.dairycrc.com/www/163/1001127/displayarticle/1001764.html**, accessed January 2007

Cregan, P and Noble, G (1996): *Genetic Improvement – Plants and Livestock.* Summary of proceedings, 25th Riverina Outlook Conference, 1996, **www.regional.org.au/au/roc/1996/roc1996055.htm**

CSIRO (1998): *Bug Puts the bite on Pesticide Problem,* CSIRO Media Release 2 June 1998, 98/131

CSIRO (2001): *Hairpin RNA beats plant viruses*, CSIRO Media Release 2001/150

CSIRO (2006): *New Wheat Has Healthy Potential*, CSIRO Media Release 06/38

Dairy Food Safety Victoria (2006): *Food Biosecurity for Victorian Dairy Products*, MP1/016, **www.dairysafe.vic.gov.au/project016/project016.htm#Project1**

Data Monitor (2004): *Nutraceuticals – Global Industry* Guide

Davidson, S (2003): *Pioneering Proteins,* Ecos, 114: 20 - 24, Jan – Mar 2003

Day, S, Morese, G and Lester, J (1997): *The cost effectiveness of contaminated land remediation strategies*, The Science of the Total Environment, 201: 125-136.

de Nys, Rocky (2005): *The development of yabby aquaculture in Australia*, in Biotechnology and the future of Australian agriculture", Proceedings of the High Flyers Think Tank held at the Shine Dome, Canberra, 26 July

Desai, K and Park, HJ (2005): *Recent Developments in Microencapsulation of Food Ingredients*, Drying Technology 23 (7): 1361 – 1394

Donnelly, P (2006): *Biotech Advances to deliver Next Generation Dairy Breeding*, presentation to Bureau of Rural Science, 2006

Eady, S (2005): *Crusader – a Production system for Farmed Rabbits*, RIRDC Publication 05/038, April 2005. Project number CSA 23-A.

Eamens, G (2007): *Testing for Bovine Johne's Disease*, PrimeFacts 461, February 2007, NSW Department of Primary Industries

Elbehri, A (2005): *Biopharming and the Food System – Examining the potential benefits and risks*, AgBioForum, Jnl of Agrobiotechnology Management Economics, 8(1): article 3, **www.agbioforum.org/v8n1/v8n1a03-elbehri.htm**, accessed 14 December 2006

Ellis, M, Rebetzke, G, Mago, R and Chu, P (2003): *First report of wheat streak mosaic virus in Australia,* Australasian Plant Pathology, 32(4): 551-553

Environmental Biotechnology CRC (2006): *Seven Year Business Plan*, EBCRC Ltd, Sydney

European Commission (2003): *Transport: Commission proposes to encourage alternative fuels, starting with biofuels*, Press release, IP/01/1543, 7 November 2001

European Commission (2007): *Innovation and technical developments in energy*, Directorate General for Energy and Transport, **http://ec.europa.eu/energy/res/sectors/bioenergy_en.htm**, accessed March 2007

FarmOnLine (2007): *Premiums Expected at BioClip Sale*, FarmOnLine 27 February 2007

Ferguson, I (2004): *Project Dolly – Impacts of New Technologies to 2029*, Future Woolscapes Research Brief, Position Paper, June 2004, Ian Ferguson and Associates Pty Ltd

Flanagan, L, Plowman, J and Bryson, W (2002): *The High Sulphur Proteins of Wool – Towards an Understanding of Sheep Breed Diversity*, Proteomics, 2(9): 1240 – 1246

Food Standards Australia (2003): *A Pilot Survey on The Identity of Fish Species as Sold Through Food Outlets in Australia*, Food Standards Australia New Zealand, New South Wales, Northern Territory, South Australia, Queensland and Western Australia. 16pp

Foster, M (2003): *GM Canola: 'What are its economics under Australian conditions?'* Australian Grains Industry 2003, produced for the Grains Research and Development Corporation, **www.grdc.com.au/bookshop/docs/abare_gm_canola_2003.pdf**

Gaffy, J (2005): *Improvac Trials*, in Islam, N (ed.): "Gains from DAWA's R&D Investment - Benefit Cost Analysis 2004-05", Miscellaneous Publication 28/2005, ISBN 0-9758096, August 2005

Galperin, M (2006): *Protecting Sausages with Bacteria Instead of Salt*, Environmental Microbiology 8(2): 185 – 192

Genetic Solutions Website: **www.geneticsolutions.com.au**, Accessed January 2007

Genetic Technologies Corporation (2003): *Annual Report 2003*, **www.gtg.com.au**

GRDC (2004a): *Pre-harvest Sprouting*, GroundCover Special Issue on Weather Damage, Issue

GRDC (2005a): *Ground Cover*, Issue 57, August/September, online **www.grdc.com.au/growers/gc/gc57/barley2.htm**

GRDC (2005b): *Pulse Disease: Environmental influence and management decisions*, Research Update, **www.grdc.com.au**

GRDC (2006): *EPR Debate Defines Crop Breeding Future*, Ground Cover, Issue 63, August 2006

Hamill, S (2005): *Biotechnology benefits banana industry – a successful partnership*

Hamilton, C (2004): *Biofuels Made Easy*, presentation to Australian Institute of Energy, Melbourne Branch, 18 March 2004

Harden, B (2000): *Rice Breeding Gets Marker Assists,* Agricultural Research Magazine, US Department of Agriculture, December 2000

Harmeyer, S, Pfaff, E and Groschup, MH. (1998): *Synthetic peptide vaccines yield monoclonal antibodies to cellular and pathological prion proteins of ruminants*, Jnl of General Virology, 79: 937 – 945

Henry, R and Waters, D (2006): *New Markers for Australian Rice Improvement*, RIRDC report 06/123, November 2006

Higgin, TJ (2007): *Crops of the Future,* BioFutures-BioSolutions Forum, Parliament House, Canberra, 14 June 2007

High Technology Research and Development Program of China, *Annual Report 2001*, **www.863.org.cn/english/annual_report/annual_repor_2001/200210100027.html**

Holland, J (2004): *Implementation of molecular markers for quantitative traits in breeding programs - challenges and opportunities*, Paper presented at New directions for a diverse planet conference: Proceedings of the 4th International Crop Science Congress, Brisbane, 26 September – 1 October 2004, ISBN 1 920842 20 9

Horticulture Australia (2005): *Summerfruit Industry Report 2004-05*.

Ingram. K (2004): *International Eucalyptus Genome Consortium*, ABC Radio, 20 July 2004

IP Australia (2006): *Plant Breeders Rights*, **www.ipaustralia.gov.au/pbr/about.shtml**, accessed January 2007

Jeffery, M, Loxton, I, Van Der Mark, S, James, T, Shorthose, R, Bell, K and D'Occhio, M (1997): *Liveweight gains, and carcass and meat characteristics of entire, surgically spayed or immunologically spayed beef heifers*, Aust. Jnl Experimental Agriculture 37(7) 719 - 726

Johnson, SK (2005): *Possibilities with today's reproductive technologies*, Theriogenology, 64(3): 639-656

Keniry, A, Hopkins, C, Jewell, E, Morrison, B, Spangenberg, G, Edwards, D and Batley, J (2006): *Identification and characterization of simple sequence repeat (SSR) markers from Fragaria× ananassa expressed sequences*, Molecular Ecology Notes, 6(2): 319 – 322, June 2006

Langridge, P (2002): *From Genome Structure to Breeding of Wheat and Barley*, 10th Australian Barley Technical Symposium, 2001

Langridge, P and Barr, A (2003): *Preface* to special issue - *Better Barley Faster: the Role of Marker-assisted Selection,* Australian Journal of Agricultural Research, 54 (12) i – iv

Lawrence, L (2002): *Crop Protection Genes – Building Pest and Disease Defences into plants*, Australian Grain August-September 2002, pp8 – 12

Long, N and Washington, J (2004): *Premium Grains for Sochu*, GRDC Research Update, February 2004

Love, S (2006): *Laboratory Tests for Worms – Prices and Availability*, Primefacts May 2006, NSW Department of Primary Industries.

Low Chill Australia Inc. (2005): *Low Chill Stone,* Fruit Grower Newsletter, No. 3/05, September 2005.

Lucy, M (2005): *New Tools for Managing Reproduction*, Animal Production in Australia 25: 363 – 372

Luhnow, D (2006): *As Brazil Fills Up on Ethanol, It Weans off Energy Imports*, The Wall St Journal, 16 January 2006

Mallik, A, Pinkus, GS and Sheffer, S (2002): McKinsey Quarterly, Number 2

Martines-Filho J, Burnquist, H and Vian, C (2006): *BioEnergy and the Rise of Sugarcane based bioethanol in Brazil*, Choices magazine, 2nd quarter 2006, 21(2), **www.choicesmagazine.org**

McCauley, I, Oliver, WT, Harrell, RJ, Suster, D, Kerton, DJ and Dunshea, FR (2003): *A GnRF vaccine (Improvac®) and porcine somatotropin (Reporcin®) have synergistic effects upon growth performance in both boars and gilts*, Aust. Jnl of Agricultural Research 54(1) 11 - 20

McGlashan, S (2005): *Waste-water cleaning bacteria* – brochure produced by Department of Education Science and Training

McRae, T, Dutkowski, GW, Pilbeam, DJ, Powell, MB and Tier, B (2004): *Genetic Evaluation Using the Treeplan System.* Presentation to IURFO Joint Conference Joint Conference of Division 2 Forest Genetics and Tree Breeding in the Age of Genomics – Progress and Future, Charleston, South Carolina, 1-5 November 2004.

Meat and Livestock Australia (2001): *Controlling Bovine Respiratory Disease in Feedlot Cattle*, On-Farm Tips and Tools Brochure, July 2001, **www.mla.com.au**

Meat and Livestock Australia (2005a): *The role of vaccination in the control of Ovine Johne's Disease*, brochure

Meat and Livestock Australia (2005b): *Ovine Johne's Disease R&D Update*, MLA, Sydney

Michel, B, Stucki, G, Frey, D, De Vathaire, F, Vignon, E, Bruehlmann, P and Uebelhart, D (2005): *Chondroitin 4 and 6 sulphate in osteoarthritis of the knee – a randomized*, controlled trial. Arthritis and Rheumatism, 52(3): 779-786.

MJ Biologics (2006): *Innovation in Animal Biologics*, **www.mjbio.com**, accessed December 2006

Mohair Australia Ltd (2000): *Breeding Angora Goats*, **www.acga.org.au**

Morris, M, Dreher, K, Ribaut, JM and Khairallah, M (2003): *Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection.* Molecular Breeding 11, 235-247

Morrision, B (2006): *National strawberry breeding program developing better tasting strawberries in Victoria.* Information supplied by Horticulture Australia Ltd to the Australian Agricultural and National Resources Online Database, September 2006

Morrow, and Fehler (2004) cited in Walkden-Brown, S, Islam, A, Cheetham, B, Groves, P, Burgess, S and Island, F (2005): *Quantitative Measures of MDV and HVT in Poultry Dust – A new tool for Monitoring Marek's Disease?* **www.jcu.edu.au**, accessed 12 May 2007

Muller, C and Botha, J (2003): *The response to selection during first lactation on the phenotypic and genetic trends in the Elsenburg Holstein-Friesland herd*. South African Jnl of Animal Science, 33(2): 111

Nikolau, A, Remrova, M and Jeliazkov, I (2003): *Biomass availability in Europe*, December 2003

Nutsubidze, N and Sarkanen, S (1997): *Lignin Depolymerase Catalyzed Biobleaching of Softwood Kraft Pulp*. Proceedings of the 9th International Symposium on Wood Pulp Chemistry. G6, 1-5, (University of Minnesota Department of Bio-based Products)

OECD (2005): *A Framework for Biotechnology Statistics*

O'Neill, G (2004): *Paradigm Shift in Proteins*, Australian Biotechnology News, 1 June 2004

O'Neill, G (2005): *GM research heads to warmer climes*, Australian Biotechnology News, 21 April 2005

Pettiford, S and Gaden, R (2005): *New cattle vaccines boost feedlot performance and herd fertility*, **www.beef.crc.org.au**

Playne, M, Bennett, L and Smithers, G (2003): *Functional Dairy Foods and Ingredients*, Aust. Jnl Dairy Technology 58(3): 242-264

Plowman, J, Bryson, W and Jordan, T (1999)\: *Application of proteomics for determining protein markers for wool quality traits*, Electrophoresis 21(9): 1899 – 1906

Pocock, K, Adams, K, Kwiatkowski, M and Waters, E (2003): *Combined heat and proteolytic treatment of white wines reduce haze forming protein content without detrimental effect*, Australian Journal of Grape and Wine Research 9 (1): 56-63

Primary Industries and Resources SA (2007): *Barley variety sowing guide 2007*, Fact Sheet 37/86/07, **www.sardi.sa.gov.au**

Productivity Commission (2002): *Impact of a Foot and Mouth Disease Outbreak on Australia*, Research Report, AusInfo, Canberra

Proteomics International (nd), **www.proteomics.com.au**, accessed November 2006.

Queensland Department of Primary Industries and Fisheries (2006): *Biotechnology and its application to field crops research and development*, **www2.dpi.qld.gov.au/fieldcrops/9020.html**, accessed 1 December 2006.

Rabaey, K, van den Sompel, K, Maignien, L, Boon, N, Aelterman, P, Clauwaert, P, de Schamphelaire, L, Them Pham, H, Vermeulen, J, Verhaege, M, Verstraete, W (2006): *Microbial Fuel Cells for Sulfide Removal*, Environ. Sci. Technol. 40: 5218-5224

Rampitsch, C and Srinivasan, M (2006): *The application of proteomics to plant biology – a Review*, Canadian Jnl of Botany, 84: 883-7892

Reifschneider, F (2006): *Selected to Make Their Mark*, CGIAR News, June 2006

RIRDC (2001): 2001 *Annual Report. Summary of operational plan 2000-02: Essential Oils and Plant Extracts*, **www.rirdc.com.au**

Sasaki, Y, Miyake, T, Gaillard,C, Oguni,T, Matsumoto, M, Ito, M, Kurahara, T, Sasae, Y, Fujinaka, K, Ohtagaki, S and Dougo, T (2006): *Comparison of genetic gains per year for carcass traits among breeding programs in the Japanese Brown and the Japanese Black cattle*. Jnl Animal Science, 84(2): 317-323

Siddique, K (2006): *From the Director*, Beanstalk, Newsletter of the Centre for Legumes in Mediterranean Agriculture, 7(1): 2, September 2006

Siitar, H and Koivistoinen, H (2004): *Proteomics – Challenges and Opportunities in Finland*, TEKES Technology Review 157/2004, April 2004

Singh, OV (2006): *Proteomics and metabolomics: The molecular make-up of toxic aromatic pollutant bioremediation*, Proteomics 6(20), special issue "Proteomics Review", pp5481-5492

Singh, OM and Nagaraj, NS (2006): *Transcriptomics, proteomics and interactomics – unique approaches to track the insights of bioremediation*, Briefings in Functional Genomics and Proteomics, 4(4): 355 – 362.

Skerritt, J and Heywood, R (2000): *A Five-Minute Field Test for On-Farm Detection of Pre-Harvest Sprouting in Wheat*, Crop Science, 40, May-June 2000, pp742-756

Skylas, D, Cordwell, S, Craft, G, McInerney, B, Wu, M, Chin, J, Wrigley C and, Sharp, P (2005): *Proteome Analysis of Two Soft-Grained Wheats of Different Processing Quality: Cultivar-Specific Proteins*. Australian Journal of Agricultural Research, 56(2): 145 - 155

Smith, M (1996): *Antibody Production in Plants*, Biotechnology Advances 14(3): 267-281

Somerville, C (2004): *Genetic Engineering of Cellulose Accumulation*, Stanford University Global Climate and Energy Project, January 2004 – December 2006

South Australian Research & Development Institute (SARDI) (2006): **www.sardi.sa.gov.au**, Accessed November 2006

Taboga, O, Tami, C, Carrillo, E, Nunez, J, Rodriguez, A, Saiz, J, Blanco, E, Valero, M, Roig, X, Camarero, J, Andreu, D, Mateu, M, Giralt, E, Domingo, E, Sobrino, F and Palma, E (1997): *A large scale evaluation of peptide vaccines against foot-and-mouth disease – lack of solid protection in cattle and isolation of escape mutants*, Journal of Virology, 1997, pp2606 - 2614

Tammen, I (2006): *Cattle Diseases – Are they Inherited?* Presentation by University of Sydney Faculty of Veterinary Science at the Provet Partners in Veterinary Education Conference, Sydney, 6-7 July 2006, **www.vetsci.usyd.edu.au/partners/conference06/**, accessed December 2006

Triticarte (2004): *Polymorphism Detection,* **www.triticarte.com.au/content/our-assay.html**, accessed January 2007

University of Edinburgh (2004): *New E. coli research offers safer meat processing*, press release dated 28 July 2004, reported in **www.foodproductiondaily.com**

US Dept of Energy (1999): *Review of the Research Strategy for Biomass Derived Fuels*

Value Added Wheat CRC Ltd (2005): *Annual Report 2004-05*

Van der Werd, J (2000): *An Overview of Animal Breeding Programs in Kinghorn,* B et al., (2000): "Animal Breeding – Use of New Technologies", University of Sydney ISBN: 0 646 38713 8

Vanstone, V (2007): *Root Lesion and Burrowing Nematodes in West Australian Cropping Systems*, Department of Agriculture and Food, Western Australia, Bulletin 4698, February 2007

Vermeiren, L, Devlieghere, F and Debevere, J (2004): *Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products*, Int. Jnl of Food Microbiology, 96(2): 149-164

Vettore, A, da Silva, F, Kemper, E and Arruda, P (2001): *The Libraries that made SUCEST*, Genetics and Molecular Biology, 24 (1-4), December 2001.

Wang, C-Y, Chang, TY, Walfield, AM, Ye, J, Shen, M, Chen, SP, Li, MC, Lin, YL, Jong, MH, Yang, PC, Chyr, N, Kramer, E and Brown, F (2002): *Effective synthetic* peptide vaccine *for foot-and-mouth disease in swine*. Vaccine. 20 (19/20):2603-2611

Wang, J, Li, D, Dangott, LJ and Wu, G (2006): *Proteomics and its Role in Nutrition Research*, Journal of Nutrition, 136: 1759 – 1762

Wei, S and Zhou, Q-X (2005): *Phytoremediation of Cadmium-Contaminated Soils by Rorippa globosa Using Two-Phase Planting*, Environmental Science and Pollution Research International, 13 (3): 151-155

Wenzl, P, Carling, J, Kudrna, D, Jaccoud, D, Huttner, E, Kleinhofs, A and Kilian, A (2004): *Diversity Arrays Technology (DArT) for whole-genome profiling of barley*. Proceedings of the National Academy of Science 101: 9915-9920, 11 June 2004

Willadsen, Dr Peter (2005): *'Biotechnology: Livestock',* in Biotechnology and the future of Australian agriculture, Proceedings of the High Flyers Think Tank held at the Shine Dome, Canberra, 26 July. Page 62

Williamson, S (2005): *Plants, Proteins and Proteomics*, Australian Biotechnology News, 29 November 2005

Wolf, BW, Bauer, L and Fahey, G.C. (1999): *Effects of chemical modification on in vitro rate and extent of food starch digestion: An attempt to discover a slowly digested starch*, Journal of Agricultural and Food Chemistry 47 (10): 4178 – 4183

Wong, G K-S, Hillier, L, Brandstrom, M, Croojmans, R, Ovcharenko, I, Gordon, L, Stubbs, L, Lucas, S, Glavina, T, Kaiser, P, Gunnarsson, U; Webber, C and Overton, I (2004): *A Genetic Variation Map for Chickens with 2.8 million single-nucleotide polymorphisms*, Nature, 12 September 2004, 432 (7018): 717-722

Xia, Q et al. (2004): *A Draft Sequence for the Genome of the Domesticated Silkworm* (*Bombyx mori*), Science, 306 (5703): 1937 - 1940