



Final report

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A comprehensive report on the viability and projected R&D Costs for development of a rigorous tracking and verification system to protect the meat and livestock industry

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Abstract

Meat and Livestock Australia (MLA) has identified the requirement for a comprehensive tracking and verification system (including Feed Forward tracking) to protect the meat and livestock industry in Australia. The requirement is driven by the need to protect the industry from loss of trade through disease or pathogen outbreaks and competitive threats.

ID-DNA has previously identified that the implementation of a transparent and auditable tracking with secure identification requires the use of a verification system comprising of both DNA technology as the ultimate identification system and RFID as the gold-standard method-of-choice for cost-effective tracking. Such a system has been patented by id-DNA.

However although this id-DNA system is currently available, there are two main limitations. Firstly the technology for DNA analysis and verification is both too slow and expensive to allow widespread and cost-effective implementation, secondly the development of an integrated RFID/LIMS software/hardware solution specifically for the livestock industry.

The creation of a near real-time DNA verification device coupled with an integrated data capture and management system will create a comprehensive and foolproof Australian meat tracking and verification system. The inclusion of a built-in comprehensive screening system for livestock pathogens and genetic disease will provide the Australian industry with:

1. Comprehensive, foolproof Australian meat tracking and verification system
2. Quality product certified free from specific genetic disease and pathogens
3. an exceptionally strong platform for World-Leading industry protection
4. a unique opportunity not only to exceed international standards but to become the World's gold-standard for quality product.

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1 Current DNA Analytical Processes

1.1 Genetic identification (DNA fingerprinting)

A "**genetic marker**" is meant any locus or region of a genome. The genetic marker may be a coding or non-coding region of a genome. For example, genetic markers may be coding regions of genes, non-coding regions of genes such as introns or promoters, or intervening sequences between genes such as those that include tandem repeat sequences, for example satellites, microsatellites and minisatellites, although without limitation thereto.

Preferred genetic markers are highly polymorphic and display allelic variation between individuals and populations of individuals.

In particular embodiments, preferred genetic markers are short tandem repeat sequences (STRs), such as are used in a variety of genotyping applications such as DNA fingerprinting, forensic DNA analysis, pre-implantation genetic analysis and foetal genotyping.

The term "**nucleic acid**" as used herein designates single-or double-stranded mRNA, RNA, cRNA and DNA, said DNA inclusive of cDNA and genomic DNA.

Preferably, genetic marker information is produced, at least initially, by amplification of the genetic markers present in a nucleic acid sample obtained from one or more individuals.

Nucleic acid amplification techniques are well known to the skilled addressee, and include polymerase chain reaction (PCR) and ligase chain reaction (LCR) as for example described in Chapter 15 of Ausubel *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons NY, 1995-1999), which is incorporated herein by reference; strand displacement amplification (SDA) as for example described in U.S. Patent No 5,422,252 which is incorporated herein by reference; rolling circle replication (RCR) as for example described in Liu *et al.*, 1996, J. Am. Chem. Soc. 118 1587 and International application WO 92/01813 and by Lizardi *et al.*, in International Application WO 97/19193, which are incorporated herein by reference; nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan *et al.*, 1994, Biotechniques 17 1077, which is incorporated herein by reference; and Q-R replicase amplification as for example described by Tyagi *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93 5395 which is incorporated herein by reference.

A preferred nucleic acid sequence amplification technique is PCR.

The skilled person will also be aware of still further variations of nucleic acid sequence amplification technology that may be useful in amplifying genetic markers for the purposes of genotyping.

As used herein, an "**amplification product**" refers to a nucleic acid product generated by a nucleic acid amplification technique.

A "**primer**" is usually a single-stranded oligonucleotide, preferably having 12-50 contiguous nucleotides, which is capable of annealing to a complementary nucleic acid "template" and being extended in a template-dependent fashion by the action of a DNA polymerase such as *Taq* polymerase, RNA-dependent DNA polymerase or Sequenase™.

In preferred embodiments, the genetic markers are amplified by "**multiplex PCR**", which involves a reaction utilizing a plurality of different primer sets (for example, primers for CF and sex) to amplify a plurality of genetic markers so that simultaneous diagnoses can be performed. Preferably, multiplex PCR produces a plurality of different sized products thereby facilitating discrimination between genetic markers and allelic forms thereof.

PCR reactions utilizing a single set of primers amplifying one specific fragment are referred to herein as a "**singleplex PCR**".

A preferred PCR system is "**fluorescent PCR**". This system uses fluorescent primers and an automated DNA sequencer to detect PCR product (Tracy & Mulcahy, 1991, *Biotechniques* 11 68-75).

Fluorescent PCR has improved both the accuracy and sensitivity of PCR for genotyping (Ziegler *et al.*, 1992, *Genomics*, 14 1026-1031; Kimpton *et al.*, 1993, *PCR Methods and Applications* 3 13-22).

Fluorescent amplification products are electrophoresed using gel or capillary systems and pass a scanning laser beam, which induces the tagged amplification product to fluoresce. The DNA sequencer combined with appropriate software is generally known as a "**Genescanner**". Stored data can then be analysed to provide product sizes and the relative amount of amplification product in each sample.

Fluorescent PCR is highly sensitive, approximately 1000 times more sensitive than conventional gel analysis, (Hattori *et al.*, 1992, *Electrophoresis* 13 560-565.).

This allows the detection of a signal far below the threshold that can be obtained from conventional methods, this results in highly accurate and reliable detection even when the signal is very weak or much lower (<1%) than that of the other allele.

An advantage of fluorescent PCR is that several primers can be multiplexed together since different fluorescent dyes can be simultaneously identified even if the amplification size product ranges overlap each other (Kimpton *et al.*, 1993, *supra*). These different dyes allow identification of one amplification product from the others even if the product sizes are within 1-2 bp of each other. This method has been applied to multiplexing as many as fifteen sets of primers although relatively high amounts of DNA are required.

Fluorescent PCR has already been successfully applied to genetic screening for cystic fibrosis (Cuckle *et al.*, 1996 *British Journal of Obstetrics and Gynaecology* 103 795-799), Down syndrome (Pertl *et al.*, 1994), muscular dystrophies (Schwartz *et al.*, 1992, *American Journal of*

Human Genetics 51 721-729; Mansfield *et al.*, 1993a. Human Molecular Genetics 2 43-50) and Lesch-Nyhan disease (Mansfield *et al.*, 1993b. Molecular and Cellular Probes 7 311-324).

As fluorescent PCR provides accurate quantitative measurements, it is therefore possible to determine the product ratio of one allele relative to the other. These quantitative measurements allow difficulties of single cell PCR such as allelic dropout and preferential amplification to be investigated. These quantitative measurements from each allele can also be compared with each other, which may give an indication of relative numbers of chromosomes

"Quantitative PCR" is where the amount of PCR product from each allele is compared, allowing a calculation of the relative number of chromosomes. This method has been applied to the detection of trisomies by utilising fluorescent PCR with polymorphic small tandem repeats (STRs; Adinolfi *et al.*, 1995, Bioessays 17 661-664). These DNA markers have unclear exact genomic function, are found throughout the genome. STRs can also be used to determine the origin of the extra chromosome and, if maternally derived, whether the extra chromosome is derived from meiosis I or meiosis II (Kotzot *et al.*, 1996, European Journal of Human Genetics 4 168-174).

The method of the invention may be particularly useful for the purposes of **"DNA fingerprinting"**, otherwise referred to as STR profiling. Preferably, STR amplification products are produced by fluorescent multiplex PCR as hereinbefore described.

DNA fingerprinting has been used by forensic science utilizing DNA markers. These STRs are similar to those used for trisomy detection. Their wide variation in length and their distribution between individuals makes STRs preferred genetic markers. In addition, their small size makes them more likely to survive degradation and allow PCR amplification. These STRs are used to build up a series of identifying markers which are then combined to determine the DNA 'fingerprint' (Zeigle *et al.*, 1992, Genomics 14 1026-1031).

"DNA fingerprinting", otherwise referred to as STR profiling. Preferably, STR amplification products are produced by fluorescent multiplex PCR as hereinbefore described.

The STR profiling system has several advantages over alternative earlier methods (Jeffreys *et al.*, 1985, Nature 316 76-79) using single locus probes (SLPs). It is more sensitive and requires only ~1ng of DNA compared to upwards of 50ng for SLPs. It can also be used for highly degraded DNA as it amplifies 100-400 bp compared to the 1,000-20,000bp lengths produced by SLPs. It can be performed in a single tube; hybridisation such as in Southern blotting or Northern blotting is not required; and since alleles are discrete and can be sized precisely, the binding of alleles, a necessity in SLP analysis, is not required.

As used herein **"amplification failure"** is where a genetic marker fails to be amplified.

The reasons for amplification failure of genetic markers obtained from single cells are unclear but are likely to be numerous. They may include problems with sample preparation; e.g. failure to transfer the cell, degradation or loss of the target sequence and/or problems associated with the

PCR. The major cause of PCR failure however is probably due to inefficient cell lysis. This is reflected by the fact that failure varies with cell type used (Li *et al.*, 1988), probably because different cell types, with their different structure and nature, require different lysis procedures.

In the majority of unique sequences examined, PCR amplification failure occurs in the region of 15-30% of single cells (Li *et al.*, 1988. *Nature* 335 414-419; Holding & Monk, 1989, *Lancet* Sep 2 532-5; Boehnke *et al.*, 1989, *American Journal of Human Genetics* 45 21-32; Monk *et al.*, 1993, *Prenatal Diagnosis* 13 45-53). Amplification failure from blastomeres from preimplantation embryos can be even higher. Pickering *et al.*, 1992, *Human Reproduction* 7 1-7, for example, reported very low rates (45%) of R-globin gene amplification using single blastomeres in comparison with single cumulus cells and oocytes (83%). Lesko *et al.*, 1991, *American Journal of Human Genetics* 49 223, also reported high efficiency of amplification of the AF508 locus for cystic fibrosis using nested primers in lymphocytes, but lower efficiency when single blastomeres were used.

As used herein, "allelic dropout" (also known as allele dropout) is failure to amplify one of two heterozygous alleles or the failure of one allele to reach the threshold of detection (preferential amplification).

Potential problems with the diagnosis of heterozygous individuals using PCR include the possibility of total amplification failure of one of the two heterozygous alleles whilst the other allele successfully amplifies (allelic dropout), or the failure of one allele to reach the threshold of detection (preferential amplification). The concept of allelic dropout has been considered, for example, in microsatellite-based detection of cancers (reviewed by Cawkwell *et al.*, 1995, *Gastroenterology* 109 465-471).

The rate of allelic dropout increase appears to be inversely proportional to the amount of template in the sample and directly proportional to the number of primers contained in the PCR.

At the single cell level previous work showed an allelic dropout rate of 25%-33% in cells from heterozygote human embryos (Ray & Handyside, 1994 *Miami Bio/Technology Short Reports: proceedings of the 1994 Miami Bio/Technology European symposium Advances in Gene Technology: Molecular Biology and Human Genetic Disease* 5 46.).

This suggests that some of the inaccuracy of CF diagnosis in single cells may have been due, at least in part, to the allelic dropout of either the affected AF508 or the unaffected wild-type CFTR allele.

The question of allelic dropout remains controversial as although most groups describe allele dropout, since some groups have reported no allelic dropout even in large numbers of single cell analyses (Verlinsky & Kuliev, 1992 *Preimplantation diagnosis of genetic disease: A new technique in assisted reproduction*. Wiley-Liss, New York.; Strom *et al.*, 1994, *Journal of Assisted Reproduction and Genetics* 11 55-62.). In general though, the concept of allele specific PCR failure in single cells is relevant.

In light of the foregoing, it will be appreciated that "**locus dropout**" is where neither allele is amplified to a detectable level.

As used herein "**preferential amplification**" is the failure to amplify one allele of a heterozygous pair of alleles to reach a threshold of detection. In other words, one allele is amplified preferentially over another.

The issue of preferential amplification has not been widely addressed in the literature, since conventional detection systems are generally unable to quantify the amount of PCR product from each allele. However, fluorescent PCR is an ideal system to identify preferential amplification for two reasons. Firstly, it provides highly accurate and reliable detection of signals even when signal strength is very weak or many times lower (to <1%) than the other allele. Secondly, it is quantitative. It is possible to use these quantitative measurements to accurately determine the ratio of signal intensity between the two alleles and thus determine the degree of preferential amplification.

Differences in signal intensity in sister alleles can be either preferential amplification or allelic dropout. If the PCR produced allelic dropout rather than preferential amplification, no signal would be obtained with either technique and misdiagnosis of a carrier cell would occur.

Amelogenin is a sex marker and a highly conserved gene (for tooth protein) found on both the X and Y chromosome, but is 6 base pairs longer on the Y chromosome (step 5). If the sample is male (with both X and Y) there will be a result of two peaks of 106bp (for gene on X chromosome) and 112bp (gene on Y chromosome); a female (2 copies of X) results in a single peak at 106bp.

As used herein, "**multiplex amplification**" or "**multiplex PCR**" refers to amplification of a plurality of genetic markers in a single amplification reaction.

Multiplex Fluorescent PCR (MFPCR) has been shown to be a reliable and accurate method for determining sex (Salido *et al.*, 1992, *Am. J Human genetics* 50 303; Findlay *et al.*, 1994a, *Human Reproduction*, 9 23; Findlay *et al.*, 1994b, *Advances in Gene Technology: Molecular Biology and Human Genetic Disease*. Vol 5, page 62. Findlay *et al.*, 1995, *Human Reproduction* 10 1005-1013; Findlay *et al.*, 1998c, *supra*) diagnosing genetic diseases such as cystic fibrosis (Findlay *et al.*, 1995, *supra*), detecting chromosomal aneuploidies and in genetic analyses for genetic identification, such as typically referred to as DNA fingerprinting (Findlay *et al.*, 1997, *Nature* 389 355-356).

With regard to genetic markers for genetic analysis, preferred genetic markers are STR and/or single nucleotide polymorphisms (SNP) markers. There is an extensive array of STR markers and primers together with MFPCR methodology (e.g. International Application PCT/AU02/01388) to successfully amplify multiple STR markers from limiting amounts of nucleic acid template.

Although from the foregoing a preferred method of genetic analysis is PCR, nucleic acid sequence amplification is not limited to PCR.

Nucleic acid amplification techniques are well known to the skilled addressee, and also include ligase chain reaction (LCR) as for example described in Chapter 15 of Ausubel *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons NY, 1995-1999); strand displacement amplification (SDA) as for example described in U.S. Patent No 5,422,252; rolling circle replication (RCR) as for example described in Liu *et al.*, 1996, J. Am. Chem. Soc. 118 1587 and International application WO 92/01813 and by Lizardi *et al.*, in International Application WO 97/19193; nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan *et al.*, 1994, Biotechniques 17 1077; and Q-R replicase amplification as for example described by Tyagi *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93 5395.

The abovementioned are examples of nucleic acid sequence amplification techniques but are not presented as an exhaustive list of techniques. Persons skilled in the art will be well aware of a variety of other applicable techniques as well as variations and modifications to the techniques described herein.

As used herein, an **“amplification product”** refers to a nucleic acid product generated by a nucleic acid amplification technique.

Although the invention also contemplates use of nucleic acid other than DNA, preferably the nucleic acid is DNA.

More preferably, the nucleic acid is genomic DNA.

Isolation of cellular nucleic acids is well known in the art, although the skilled person is referred to Chapters 2, 3 and 4 of Ausubel *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons NY, 1995-1999), for examples of nucleic acid isolation.

Single Nucleotide Polymorphisms (SNP) are the most frequent form of variation found in the genome, estimated to occur every 1000 bases. SNP genotyping has multiple applications such as predictive medicine, personal medicine, forensic identification and pharmacogenomics. SNP genotyping has already been used to investigate a number of disorders such as cystic fibrosis, Factor V Leiden mutation, and factors such as A, B, O and Rh blood grouping. However conventional SNP analysis is limited by the relatively high amount of extracted DNA usually required (up to 100ng) for analysis. However in genomic analysis, there is increasing demand to both maximize data by performing multiple analyses and secondly to analyze minimum amounts of sample, even to the single cell level.

Preferred sources of nucleic acids are mammals.

The invention particularly contemplates genetic analysis of human and non-human samples such as from cows, sheep, horses, pigs and any other mammal including companion animals, sporting animals and livestock, although without limitation thereto.

Genetic identification is usually undertaken on samples which contain plentiful amounts of robust DNA such as blood, tissue and bacterial samples etc. Such samples are relatively easy to amplify as they usually contain many thousands of cells and often only involve a single test.

Although the immense potential for an enormously wide variety of scientific disciplines is obvious, studies involving genetic analysis or identification on small or difficult samples have been severely limited as sample analysis and interpretation becomes increasingly problematic as sample size decreases or substrates vary. This is due to three main reasons. Firstly, the extremes of sensitivity required at low copy or single cell level amplification; secondly the difficulty in maintaining high levels of reliability and accuracy and thirdly the difficulties inherent in multiplexing multiple primers to obtain maximum information. These difficulties include characteristic phenomena specific to low copy PCR such as allele dropout (ADO), preferential amplification (PA) and whole locus dropout (WLD), which severely limit diagnostic value and applications.

Most studies on low copy DNA amplification have been undertaken in the human forensic arena where exclusion specificity is usually several billion to 1 using large samples. Although attempts have been made to obtain profiles from low cell samples such as cigarette butts and car keys etc, reliable and accurate results either still depend upon similarly large (>500) numbers of cells and/or markedly decreased discriminating power and reliability. Even at ~100 cells, ADO occurs in more than 20% of samples. At levels below 500pg, reliability is reduced to less than 50% due to frequent ADO and the intensity of the signals being too low to interpret correctly.

DNA fingerprinting at the single cell level has also been attempted using other methods, however these techniques are again severely limited as either

1. PEP (Primer extension pre-amplification) must be used which can cause massive PA which results in misidentification,
2. several days are required,
3. Relatively uninformative markers are used significantly limiting value, and/or
4. Forensic validation is not possible.

However a breakthrough in 1994 demonstrated the first DNA fingerprinting of single cells (Findlay I. et al, 1994. Human Reproduction, 9 (3) 23; Findlay I., et al. 1994b Advances in Gene Technology: Molec. Biol. and Human Genetic Disease. Vol 5, Published by IRL at Oxford University Press. Findlay I., et al, (1995) Human Reproduction 10 (4) 1005-1013).

In 1997 the first ever forensic identification of single cells was published (Findlay I., et al., (1997). Nature 389, 355-356). The use of such single cell DNA fingerprinting systems to definitively identify cells of interest has the following advantages:

- Unlike conventional fingerprints or signatures, DNA fingerprints cannot be rubbed off, smudged, interfered with, or obscured.
- A person cannot erase or alter their DNA fingerprint unlike physical fingerprints or signatures. Their DNA fingerprint will remain with them throughout life and potentially forever even after death.
- Unlike conventional fingerprints, DNA fingerprints cannot be duplicated, manufactured or modified.
- Every single cell from a person contains their unique DNA fingerprint. Person cannot hide.
- DNA fingerprints can be obtained even after death unlike signatures or physical fingerprints. In fact, the inventors have demonstrated that DNA fingerprints can be obtained from samples many thousands of years old.

As used herein, a "**genetic marker**" is meant as any locus or region of a genome. The genetic marker may be a coding or non-coding region of a genome. For example, genetic markers may be coding regions of genes, non-coding regions of genes such as introns or promoters, or intervening sequences between genes such as those that include tandem repeat sequences, for example satellites, microsatellites and minisatellites, although without limitation thereto.

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In particular embodiments, preferred genetic markers are short tandem repeat sequences (STRs), such as are used in a variety of genotyping applications such as DNA fingerprinting, forensic DNA analysis, pre-implantation genetic analysis and genotyping.

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2 The Laboratory Process:

The following steps represent the in laboratory stages of the DNA analysis process from any supplied sample.

Process:

Sample Receipt

Every sample that enters the lab will require the following

- Individual barcode generated
- Chain of custody form & evidence receipt documented by manual entry
- QLD Health formal paperwork scanned

(All information needs to be linked to individual sample tags)

Sample Storage/Evidence Description

Every sample held in storage will require the following

- Formal description manually entered
- Photographic documentation
- Storage conditions and location

(All information needs to be linked to individual sample tags)

Sample Storage/Evidence Description

Every sample held in storage will require the following

- Formal description manually entered
- Photographic documentation
- Storage conditions and location

(All information needs to be linked to individual sample tags)

DNA Extraction

Every sample to be processed for DNA analysis will require the following

- Extraction batch logged (i.e. what samples are extracted with each other for contamination control)-normal batch size is 24 samples
- Extraction kit linkage to individual samples
- Extraction protocol used, person, date, time
- Amount of original sample used
- Amount of sample returned to storage

(All information needs to be linked to individual sample tags)

DNA Quantification

Every sample to be processed for DNA analysis will require the following

- DNA quantified individually
- Amount of extracted sample used in quantification
- Sample concentration manually entered and dilution factor calculated
- DNA working solution created
- Storage and location of original extract and working solution

(All information needs to be linked to individual sample tags)

Plate Format / Run Setup

Completed (working) extraction batches require the following for run setup

- Sample sheet generated for direct import to 3100 (sample information plus run parameters)
- Individual samples / batches transferred into 96 well plate format-according to sample sheet
- Barcode / Run name generated for each 96 well plate to be PCR'd

(All information needs to be linked to individual sample tags)

PCR

The following is required for each run, which also links back to all individual samples within a particular run

- Method used-full volume, half volume
- Chemistry used-profiler plus, identifier
- PCR block, person, date, time
- PCR reagent linkage to plate
- Storage and location of amplified PCR products

(All information needs to be linked to individual sample tags)

3100 Run

The following is required for each plate to be run on the 3100

- Barcode / run name generated for each 96 well plate to be run
- Run reagent linkage to plate (POP4, 1xbuffer, size standard, capillaries)
- Sample sheet selection
- Person, date, time
- Storage and location of run plate

(All information needs to be linked to individual sample tags)

Analysis

The following is required for each sample that has been processed for DNA analysis

- Primary analysis-plate report generated. Person, date, time
- Secondary analysis-plate report generated. Person, date, time
- Above 2 reports compared for discrepancies
- Screen all results for staff contamination
- Raw data linked to individual sample
- Analysed data to final report which contains all information previously linked to GMS sample barcode and customer barcode identifier

(All information needs to be linked to individual sample tags)

2.1 Critical DNA Factors in the Process

Sampling Techniques:

Collection of biological samples from livestock has always been a time consuming and costly process for producers and researchers. Due to this cost, many producers have simply elected not to carry out genetic analysis and diagnostics, resulting in poor production traits and genetic abnormalities remaining within their herds over successive generations.

The tissues that have been used conventionally for DNA analysis are blood, hair and semen samples.

2.1.1 Using Blood Sampling

Blood has been the traditional method of sample collection for decades. This collection method was very time consuming and costly, primarily due to the fact that a veterinarian must be onsite during the whole bleed process. When herd sizes exceed several hundred animals, this collection process can take over several days.

The cost of hiring a veterinarian can easily range between \$150 - \$300 for a standard working day. As a vet can normally process only approximately 100 – 200 animals per day, this means that the average sized herd of 1000 animals may take up to five days to bleed, costing a significant amount of money and time. For example 5 days @ \$300 per day is \$1500 for 1000 animals is \$1.50 per animal solely for vet fees.

Blood must be placed directly into blood tubes containing EDTA buffer to inhibit coagulation during the bleed and transportation to the laboratory. The blood tubes must also be stored on ice before, during and after the bleeding of each animal, and should be maintained at 4°C until processing. These stringent transport requirements means require an additional fee of approximately \$50 - \$200 per batch of samples.

Advantages:

- A large volume of sample can be collected for immediate use and blood storage.
- The blood can be used for other diagnostic applications.

Disadvantages:

- Blood must be kept at 4 °C during the entire process
- Blood tubes must be handled with extreme care in both the bleed (on farm) and transporting to laboratory.
- A registered veterinarian must be present to do the bleeding of animals. This may be inconvenient to the farmer.
- Very messy process, generally with a high risk of contamination both during the bleed process and transportation.
- Significant levels of stress in animals.
- Time consuming and costly.

Transportation

Bloods must be sent by courier in a chilled condition i.e. 4°C. The tubes must be placed in foam racks, surrounded by plenty of packing material to reduce the risk of breakage. Due to the temperature requirements most samples will be sent via air courier.

Advantages:

- Receive very fresh sample stocks

Disadvantages:

- Need for expensive chilled environment.
- Use of air courier service is expensive.
- Large containers for transport to protect samples.
- Sample vials susceptible to breakage.
- Need special transportation requirements.

DNA extraction

There are a number of methods that can be used to extract DNA from blood samples. Preferentially, commercially available kits are used in modern laboratories because of their reliability and consistency in DNA yield and quality. Examples of commercial kits available are Qiagen's QIAamp DNA extraction kit, Amersham's GFX genomic extraction kit and Roche's Hi-Pure extraction kit. The cost of these kits is normally around \$2.50 per sample. However, there are also several well established methods that can be used by laboratories to extract DNA using in-house prepared reagents. Below is an example of such a method.

DNA Extraction Protocol:

- (a) Add 10 –15ml of Blood to 50ml tube and add 2.5x volume of cold lysis buffer
- (b) Mix by hand and spin at 4000rpm for 20mins at 4 degrees
- (c) Pour off supernatant and wash pellet with 20ml of PBS
- (d) Spin at 2000rpm for 5mins at 4 degrees centigrade
- (e) Add 9ml TE at pH8 and vortex gently to dissolve pellet
- (f) Add 500ul (0.01M?) EDTA pH8, 50µl Prot K (20mg/ml), 500µl 10% SDS
- (g) Incubate for several hours at 37 °C centigrade on a slow rotating platform
- (h) The following morning the sample can be spiked with 10µl of Proteinase K and incubated for a further 2 hours to obtain maximum yield.
- (i) Add 2.5ml of phenol and 2.5ml of chloroform and mix on Coulter mixer for 30 – 60mins at room temperature.
- (j) Carefully collect aqueous phase with wide bore plastic pipette being sure not to disturb the protein interface
- (k) Add 2.5x cold absolute ethanol (ETOH) or 1x propanol, mix on the Coulter mixer for 15min
- (l) Collect the DNA with a wide bore plastic pipette and transfer to an Eppendorf tube, remove as much ETOH as possible with a pipette
- (m) Add 70% ETOH mix and spin on a bench top centrifuge
- (n) Carefully pour off ETOH and air dry
- (o) Add 1 – 2mL of 0.1x TE to each tube to dissolve over night.

Advantages:

- DNA extraction process allows high yields of DNA for analysis.
- Very clean DNA product is produced.

Disadvantages:

- Very time consuming. Approx 2 – 4 hours.
- Use of expensive and dangerous reagents for non-commercial method.
- Cost of around \$2 per extraction.
- Need for specialist equipment and training.
- Difficult to automate for high throughput processing.

Storage

Blood – The collected blood can be frozen at –20 degrees centigrade in either the tube it was delivered in or in a specialized freezer bag. Blood can be stored this way for a considerable period with minimal degradation.

The extracted DNA can be stored indefinitely in 0.1X TE solution.

Advantages:

- Long term storage is a viable prospect at –20 °C.
- Large quantities of blood can be collected and stored for each animal.
- Extracted DNA is very robust for future use.

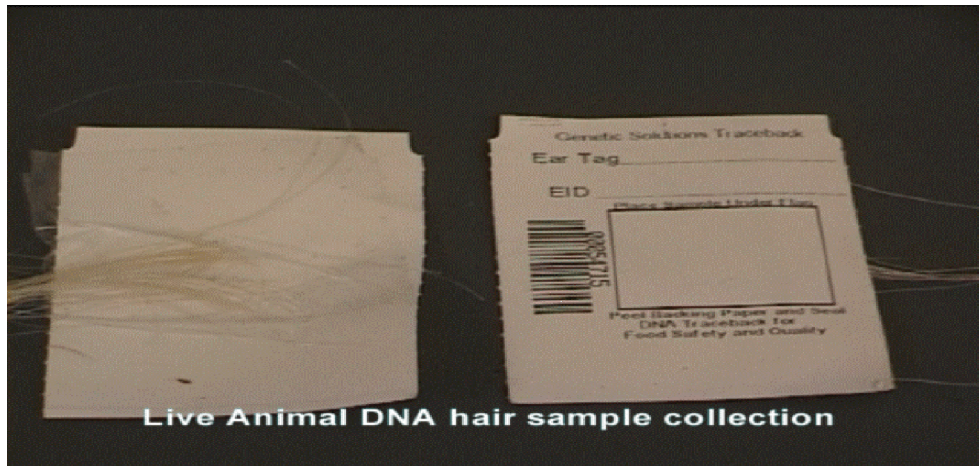
Disadvantage:

- Need a specialised cold room to store blood stocks.
- Need a barcode or filing system for storage of both blood and DNA stocks.
- Freeze/thaw of samples degrades the blood for further extraction processes.

2.1.2 Using Hair Sampling

Hair sampling is now increasingly being performed as a collection method for DNA samples from livestock. Whilst hair samples can often be taken by producers themselves, eliminating the need for a veterinarian, the collection process is usually very messy, with significant risk of cross contamination. This contamination comes from the multiple usage of the same tools for the collection from different animals. Another problem with collecting hair for DNA extraction is that if it not performed properly, resultant DNA extraction can be difficult and time consuming with poor reliability.

Normally the hair sample is placed loose in a bag or is fixed onto a sample card which is posted to the laboratory (see figure). This random placement of hair in the bag or card makes it difficult to acquire the correct amount of hair follicles without handling most of the sample, and therefore introducing outside contaminants.



Advantages:

- Simple cost effective sampling method. Approx \$1 per sample.
- Large quantities of hair can be collected.
- Little training required.
- No special temperature requirements.

Disadvantages:

- Contamination problems, both on the farm during collection and in the laboratory during processing.
- Time consuming. Approx 2 – 4 hours.
- Mildly stressful to the animals

Transport

Hair samples can be collected and placed in an envelope for transport to the laboratory. However significant extra care must be taken to individually wrap each sample to eliminate the chance of hairs becoming free and contaminating other samples. These samples can all be transported at room temperature.

Advantages:

- Samples can be sent via domestic mail, cutting transportation costs for the producer.
- No need to keep samples cold during transport process.
- Multiple samples can be sent at the same time.

Disadvantages:

- Samples can become easily contaminated if not sealed individually.

Extraction

DNA extraction from hair follicles is relatively easy again using either commercial or conventional methods.

DNA Extraction Protocol:

1. Cut or punch follicle rich sample from DNA sample collection or 6 – 10 hair follicles, place in 0.2µl tube or well of 96 well plate
2. Centrifuge tube/plate briefly to collect sub samples into the bottom of the tube.
3. Add 50µl of solution A (200mM NaOH)
4. Incubate at 95°C for 15mins. Mix the contents of the tube 2-3 times during incubation by quickly removing the sample from the heat block
5. Briefly centrifuge to remove condensation
6. Add 50µl of solution B (200mM HCL, 100mM TrisHCL pH8.5)
7. Mix briefly and centrifuge for 2mins at 3000rpm
8. Transfer 50µl to a fresh tube or plate, avoiding the pellet debris. Dilute with 250µl of MilliQ water
9. Store at –20 °C, use 4µl per PCR

Advantages:

- Average yields of DNA for PCR processes.
- Minimal cost and use of reagents needed. Approx \$2 per sample.

Disadvantages:

- Need for specialist equipment.
- Need for significant training.
- Time consuming.
- Large risk of contamination.
- Difficult to automate for high throughput processing.

Storage

The hair follicles will remain viable for 1–2 years. However they are difficult to store due to their packaging, often resulting in a filing cabinet system of loose bags and cards which is highly susceptible to future contamination and processing error.

Advantages:

- Large quantities of hair can be collected and stored for each animal.
- Extracted DNA is very robust for future use.

Disadvantages:

- Need a specialised area to store hair samples.
- Hair stocks cannot be stored for an indefinite time.
- Need a barcode or filing system for storage of both hair and DNA stocks to keep track of stocks.
- Stored stocks can become easily contaminated.

2.1.3 Using Semen Sampling

The collection of semen is a labour intensive process that must be carried out by veterinarian. A wide variety of techniques can be used for semen collection varying from animal mounting artificial vaginas to rectal stimulation but all require specialist equipment and take a considerable amount of time. The semen must be stored at 4 °C once the sample has been collected. It must be placed on ice or better still in liquid nitrogen for transportation by a courier to the laboratory. Often semen must also be stored very carefully using slow-cooling to maintain cell viability, particularly if the sample is also to be used for reproductive technologies.

Similar to blood collection, semen collection is a very expensive way of sampling male livestock. Normally costing in excess of \$100 per animal, semen sampling is therefore available to only elite producers and animal studs due to its high cost.

Advantages:

- A single semen sample can be separated into multiple straws.
- The semen can be used in other diagnostic or fertility processes.

Disadvantages:

- Only male livestock can be tested.
- Only a single animal can be treated at a time.
- Need for expensive cold storage facilities.
- Need for liquid nitrogen transportation.
- Need for a specialist courier service.

Transport

Transport of semen samples is normally very difficult due to the requirement of maintaining very low temperatures. Samples are often stored in liquid nitrogen which makes transport of samples not only expensive but hazardous.

Extraction Protocol:

- Centrifuge 100µl of semen for 2 mins
- Add 700µl Semen lysis buffer and vortex to resuspend sperm pellet. Then add 200µl 10% SDS, 100ul 0.39M DTT, 1µl Proteinase K (20mg/ml)
- Incubate at 37°C overnight
- Perform two phenol-chloroform extractions
- Add 2.5X vol absolute ETOH
- Centrifuge 15000g for 5 mins or spool out the DNA
- Wash in 70% ETOH
- Dissolve the DNA from 1 straw in 100 – 200µl MilliQ water or TE buffer.

Advantages:

- Average yield of clean NA.

Disadvantages:

- Labour intensive. 2 – 4 hours processing.
- Time consuming.
- Not susceptible to automation or high throughput techniques.
- Use of dangerous and expensive chemicals. Approximately \$2 – \$5 per sample.
- Need for specialist equipment.

Storage

Semen straws can be stored at –80°C for extended periods of time. They can be stored at lesser temperatures but degrade more quickly. Due to the relatively small size of the straw, many straws can be stored within a small area, but this size does limit the amount of information that can be placed on the storage device. This limited space can make it very difficult to identify the correct straw of interest without a complicated bar-coding system.

Advantages:

- Semen straws can be kept indefinitely at –80°C.
- Many straws can be stored together due to size.

Disadvantages:

- Need for a -80°C freezer or liquid nitrogen facility.
- Need for bar coding system for sample identification.

The inventors have realised that such testing above significantly limits the application of genetic testing due to inconvenience, cost etc. The inventors therefore preferably utilise the methods detailed in provisional patent (Gribbles Molecular Science – 2004904829) to obtain non-invasive samples. Such non-invasive samples provide significant advantages.

2.2 Flesh Collection and Storage for later testing:

This system is currently used by some meat processing companies to ensure a sample for DNA evaluation is held if required to test at a later date. DNA tests are not conducted unless required and then the samples can be referred to.

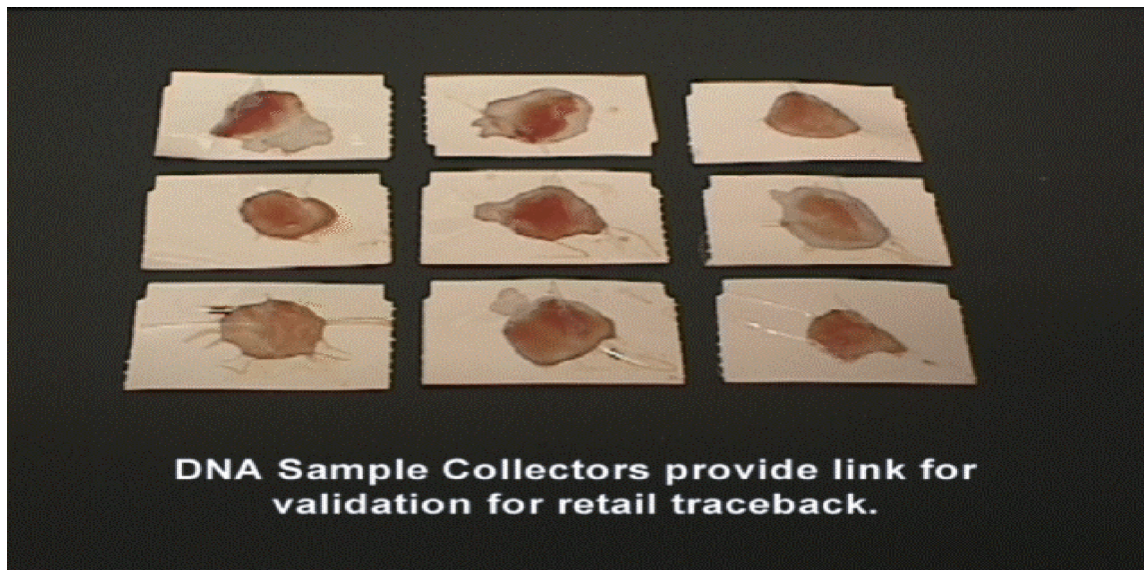
Samples are collected at the abattoir or meat processing plant and can be taken from each animal or from a selection, depending on requirements. The collected issues can be stored in any appropriate receptacle but is best stored in vacuum sealed bags and frozen until required (see figure). Vacuum bags provide the advantage of easy packing for storage and suitable labelling space. Tissue can be kept at 4°C for the short term but should be stored at -20°C for long term storage. Only a small piece of tissue needs to be extracted to obtain adequate DNA for testing.

Advantages:

- Easy to collect
- Collected in a controlled environment in the meat works
- Requires no training

Disadvantages:

- Requires refrigerated or frozen storage
- Risk of contamination during collection
- Need a barcode or filing system for storage.



Transport

Transportation of tissue samples requires either refrigeration or packing on ice. For short distance transportation, tissues can be transported at ambient temperatures in an esky.

Extraction

Tissue extraction can be done following a similar method for the extraction of semen.

- a) Chop about 25mg of tissue sample in small pieces using a scalpel blade and add 500 μ L of saline.
- b) Homogenise using a micro-pestle to obtain an even suspension.
- c) Centrifuge at approximately 10,000g for 5 minutes and remove supernatant.
- d) Resuspend the pellet in 200 μ l 10% SDS, 100 μ l 0.39M DTT, 1 μ l Proteinase K (20mg/ml)
- e) Incubate at 37°C overnight.
- f) Perform two phenol-chloroform extractions
- g) Add 2.5X vol absolute ETOH
- h) Centrifuge 15,000g for 5 mins or spool out the DNA
- i) Wash in 70% ETOH
- j) j. Dissolve the DNA in 100 – 200 μ l MilliQ water or TE buffer.

Advantages:

- Excellent yield of DNA
- Can use the same original tissue sample for multiple extractions

Disadvantages:

- Labour intensive. 2 – 4 hours processing.
- Time consuming.
- Not susceptible to automation or high throughput techniques.
- Use of dangerous and expensive chemicals. Approximately \$2 – \$3 per sample.

Storage

Tissue samples and their respective DNA should be stored at -20°C for medium to long term storage. That said, tissue samples can be quite small allowing for large numbers to be stored in a small space.

Advantages:

- Can be stored for long terms
- Don't require specialised storage facilities

Disadvantages:

- Requires -20°C facility
- Need a filing system to keep track of both tissue and DNA samples.

3 Genetic Tests Relevant to the Meat and Livestock Industry

One of the significant recent developments in DNA technology in respect to the livestock industry is the linkage of SNP (single nucleotide polymorphisms) profiles with various growth and quality attributes. Using micro-array libraries of many thousands of SNPs, it has been possible to demonstrate that the presence of some SNPs in association with others can be linked to beneficial attributes that give an individual an advantage over others.

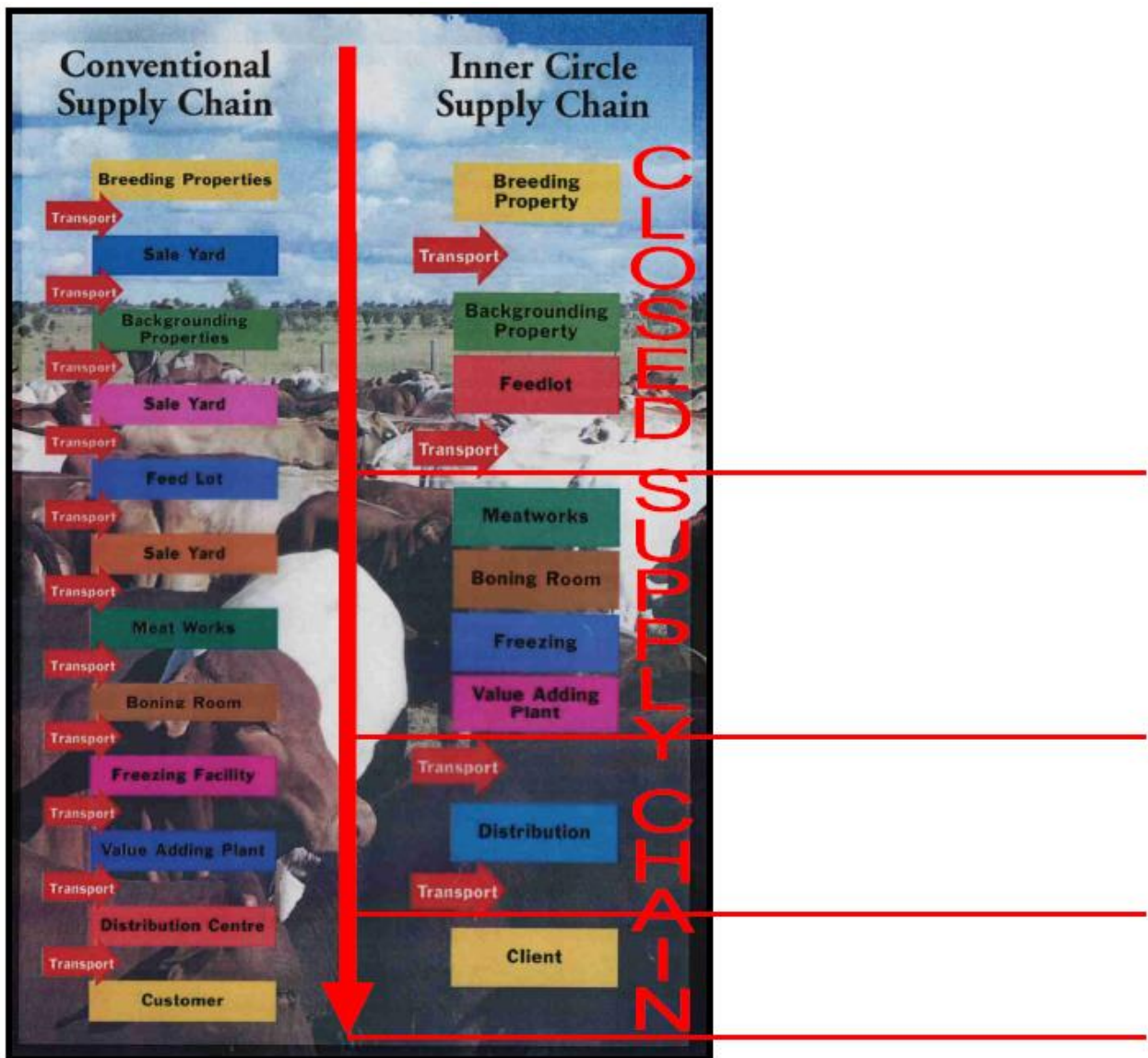
In the livestock industry, these attributes could be high feed conversion ratios, resistance to disease or appropriate marbling of meat. The work of various groups on this field is gathering this information and it is anticipated that within 10 years animal breeding programs will be designed around such SNP analysis of breeding stocks. These SNP profiles will also be able to be used to identify meat products from these herds as they progress through the supply chain. Such analysis will be more reliable than the standard genotyping used for parentage identification as the information will not only link the meat to its origins at conception but could also contain information as to the quality of the product.

Also, like humans, cattle suffer from a number of genetic diseases caused by mutations or SNPs in critical genes in their genomes. For example maple syrup urine disease in Poll Herefords and Shorthorn cattle, congenital myoclonus in Poll Herefords, citrullinaemia and Bovine Leucocyte Adhesion Deficiency (BLAD) in Holsteins and a-mannosidosis in several breeds. Although many of these genetic diseases are now uncommon due to judicious breeding programs to remove the defect, it is still important to maintain the ability to monitor the status of cattle in respect to known mutations, especially in breeding cattle.

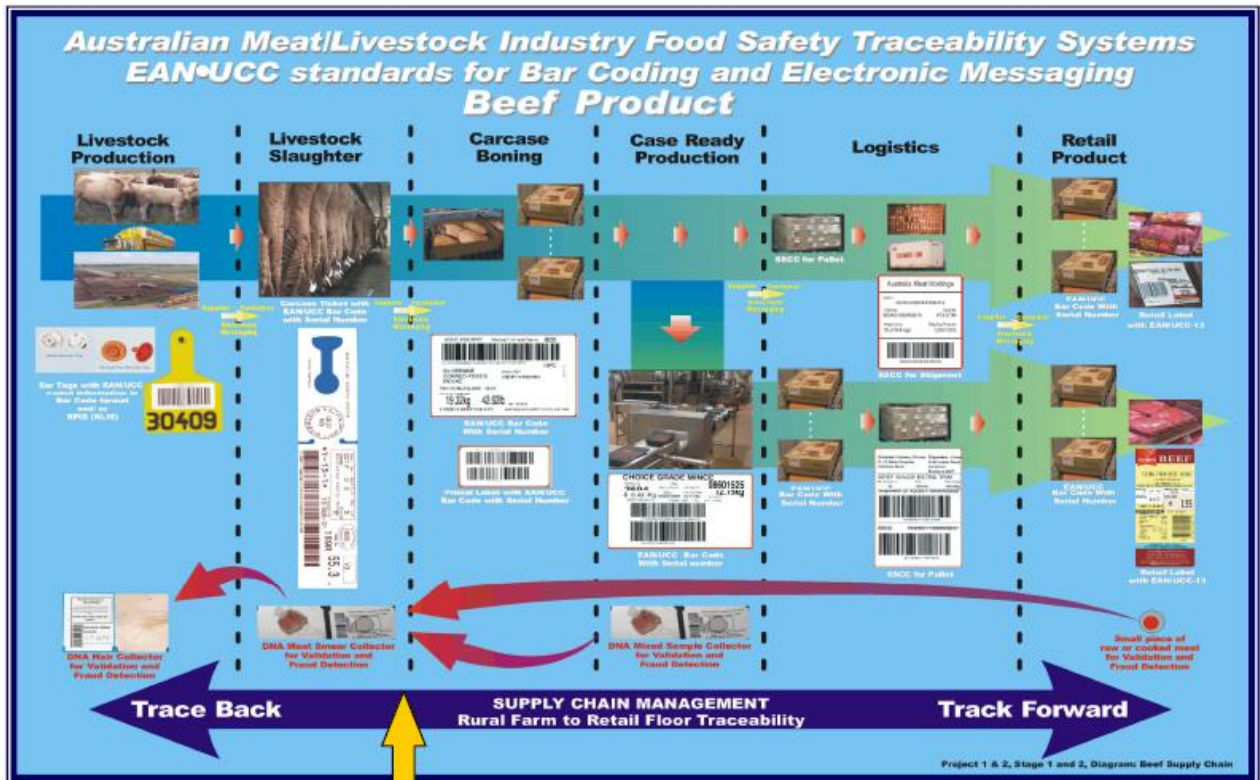
3.1 Current Workflow and Processing Stages from Animal Tagging to Product – Paddock to Plate

The contribution from Australian Country Choice at this point to this report should be noted. They have allowed id-DNA into their process and as such enabled us to obtain a full understanding of the current processes used for identifying meat from DNA. This support has also enabled us to obtain a strong in-sight into how DNA and RFID could be better deployed throughout the chain.

The following ACC diagram depicts the current stages both in standard meat supply chains and also the integrated supply chain model of ACC.



Traceability techniques and data capture methods currently used in the ACC process highlight a number of factors in the Chain of Custody and process of traceability.



3.2 Weaknesses

- Current Hair Collection Techniques are prone to inaccuracy and have inherent flaws in the process and side effects.
- It is critical that DNA traceability occurs at the beginning of the process and doesn't start along the chain
- The system doesn't allow for continuity and a smooth inexpensive flow of data rather than relying upon numerous side processes and is susceptible to loss, mistaken identity and handling contamination.
- The ruggedness of carcass tags is questionable and these are subject to loss
- Further break downs in the systems security and continuity occur along the chain in boning and packaging etc.
- Bar Code labels are inherently prone to damage
- A uniform identification system is not deployed throughout the chain
- DNA availability is not available in real time rather after the event.
- The shear geographical diversification of the supply chain makes data capture challenging and chain of custody

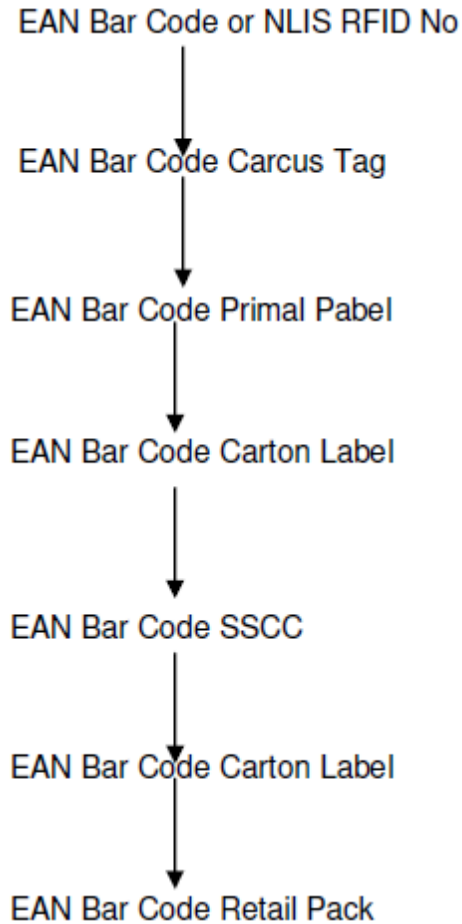
- A centralised database tracking DNA of the beast from paddock to plate does not exist so national introduction of such a solution would be inherently difficult
- There is a lack of verification checks along the way to ensure animal is matched with label etc.
- DNA is not matched along the chain in a formal audited process providing true Quality Assurance
- Further analytical processes and genetic tests are not provided back along the chain to enhance the chain and progress the industry. This is an obvious bi-product of testing.
- Chain of Custody data capture is not standardised along the channel and as such can not be marketed on an industry basis
- Current RFID ear tags do not accommodate read / write technology and do not allow for the storage of DNA information

3.3 Strengths

- The industry has already voluntarily introduced DNA based traceability
- The integrated distribution channel provides for better control and processes that would allow a system like DNA traceability to be readily deployed.
- Labelling facilities and processes are readily deployed throughout each stage in the process supporting data capture and chain of custody
- The value of DNA traceability would not be a hard sell to the industry especially if providing value added benefits across the chain and supporting price maintenance of beef.

3.4 Risks of Data Capture Failure

- In the current process there seems to be a great number of data transfer points. E.g.;



This number of data capture points makes every point a potential risk for break down in the system! What is positive is that entities like ACC have their own QA processes in place and have invested in software solutions to assist their control over the process and quality assurance. Systems like that deployed and invested in by ACC do not seem to assist across the board and smaller less well equipped facilities will not be able to afford such systems.

The crux of this report therefore is to identify how DNA and RFID together can enhance and simplify traceability.

4 Current Technology Limitations on DNA Capture and Tracking

It is surprising that read rates of current RFID Ear Tags are still not 100% - reads can be as low as 90% at the first read position into the Abattoir. Facilities like ACC have back up wand readers to support this process.

Much relies on the success of the back end management solution which in the case of ACC seems to be quite strong. Cross matching is done in the back end data base at ACC where the Carcass ID, Head ID, Hide ID, Offal ID (batch ID).

Offal identification is definitely a tricky one and is currently batched as is Tallow and Meal. Infrastructure costs of the current technology (RFID) are high and competition seems limited.

Carcass labels by their very nature provide the first link to the RFID tag through a back end data base and hook tag systems seem to still be in their infancy so current traceability is provided through software and probability. The data base integrity and accuracy of the facility is an area of reliability where if not in place, traceability will not be successful.

Current DNA processes occur in two areas;

1) Some Breeders are taking advantage of DNA testing of their animals in the field as marketed by Allflex and Genetic Solutions.

DNA Tag

Integration of DNA Technology with Animal ID.

Allflex, in conjunction with Brisbane based biotechnology company, Genetic Solutions, have launched a simple but effective method of collecting animal DNA.

This technology firmly secures the ID wheel, with a mixture of Visual, Electronic and DNA identifiers.



Collection of a DNA sample allows for a DNA profile or "fingerprint", enabling trace forward of the animal from that point. Even after the animal is killed a sample of meat can be taken and traced back to the hair sample collected from the live animal, using Genetic Solutions SureTRAK traceability system

How to Collect Hair Samples

Extract 20-30 strands from the end of the animals tail, ensuring the hair collected has follicles.(hair roots attached)

Ensure the sample is dry and free from dust and dung.

Place the follicles on the sticky portion of the provided sample collector and press the paper backing firmly over the samples.

Cut off the remaining hair shafts and place collector in the reply paid envelope.

This process as outlined in an extract from the Allflex web site seems weak for the reasons outlined in the Hair Collection method of DNA, not too mention the practicalities of collecting the hair from the beast.

Genetic Solutions is also marketing parentage testing through DNA as I'm sure the MLA is aware.

2) The Abattoir is also having DNA tests conducted on animals as they pass the Hot Grading point in the process. Here a DNA sample is taken and stored for evaluation later if required. These samples are associated to the back end database in the case of ACC and cross matched against the other various id verification points.

ACC use RFID in both hook tracking and board tracking in the packaging process. Despite their huge investment in this area – neither are working at this stage.

5 The Future Model

The future model needs to be simpler, available to all producers and fundamentally secure and accurate! This has been one of the driving forces to the id-DNA patent enabling writing of the DNA profile in the field to the cattle ear tag. One could merely recite the patent to cover functionality of the vision for the future but in simple terms we feel that the DNA is best established while the animal is living and tagged with the RFID chip. In this manner, the DNA result travels with the animal, securely embedded within the RFID ear tag, bolus etc. and available at all times to verify and seamlessly transfer to sub batches or to a back end data base for verification of reference.

To enable this model to be established there are a few critical elements;

a) Introduction of a read / write RFID ear tag with sufficient memory capability to store genetic data on the tag. From id-DNA's experience we believe a 256bit memory capability would be enough to enable the DNA structure and a property ID etc.

Once the DNA markers have been determined, through either an in lab test or a field test (near real time in the future), a data format for these markers will be stored on the chip.

The DNA markers are always stored within the silicon chip with the level of protection/security and data transfer method the silicon protocol uses.

This means the protection and transfer technique may be different depending on the chip and protocol but regardless of this the DNA markers are stored on the RFID tag and risk of tampering through unauthorised access to the chip data, is minimised.

Chip Data Structure Relevant to DNA:

0 to 512 = 9 bits per marker

16 rows

2 columns

Total no of bits = $9 \times 16 \times 2 = 288$ bits

More accuracy would be achieved using 16 bits per marker

$16 \times 2 \times 16 = 512$ bits (16 pairs of markers)

A choice needs to be made therefore as to what information is stored on the ear tag and what is stored in the back end database. To keep things simple, we see the NLIS number and the DNA markers residing on the Ear Tag Chip.

As a pallet to plate scenario is being sort we would also envisage that the Electronic Product Code (EPC globally managed and marketed through GS1), be catered for in a new chip direction. This would enable the DNA structure and perhaps the NLIS number be incorporated into the packaging label through the global EPC infrastructure – this would then be used as a global code structure for meat. This would be a large exercise to coordinate and standardise upon but a feasible option for the meat industry to enhance traceability and while EPC adoption is in its infancy may be the best time to commence this process.

An explanation of the current EPC structure is attached however the following outlines the current code structure of EPC;

	Header	Filter Value	Partition	Company Prefix	Serial Reference	Unallocated
SSCC-96	8 bits	3 bits	3 bits	20-40 bits	37-17 bits	25
	0011 0001 (actual value)	8 (decimal capacity)	8 (decimal capacity)	999,999 – 999,999,999,999 (decimal capacity)	99,999,999,999 – 99,999 (decimal capacity)	[Not Used]
Eg.	049	3	5	0614141	0000999777	

Which Chip Technology?

We have detected through the limited research conducted in this report a lack of performance in the current chip technology used for RFID ear tags, however, this report is not designed to identify a better technology or critique the existing technologies performance. It is a great achievement that the system is in place!

Id-DNA has had communications with the existing silicon provider Texas Instrument, which indicates a read / write version of the existing protocol is on the drawing board but it doesn't seem to be a priority. It is indeed possible for them to do this as seen in past tag products they produce for other markets with R/W capability and greater memory capability.

We also believe that to create a seamless automated transfer of information throughout the supply chain then a number of different forms of the tag should be available or indeed, a combination of technologies may be used – e.g. a transfer of data at the packaging point may be through a dual reader that reads the hook tag and the carcass tag and marries the 2 to a packaging tag.

b) We see the NLIS Database as key to the success of implementing a strong DNA identification system. We also see the NLIS database as having the capability, in a revamped format, as having the capability to enhance communications from all points in the supply chain in order to accurately simplify and provide inherent value to the producer. Traceability could be dramatically enhanced through a more cross relational database with a strong communications back end enabling field communications with mobile computing devices that can read and transfer data to the ear tag and back to the database either in a batch or real time manner depending upon available communications. This in essence also goes to the basic theme of id-DNA's patent surrounding DNA handling to RFID tags.

The enhancement of the database would also enable a more thorough offering of genetic information to be stored securely and used by the producer and MLA in quality assurance. Following is a list of genetically determined data that could be tested for and stored;

- Coat colour – markers, e.g. black coat.
- Johnes (Cattle, sheep & goats)
- BLAD (Bovine Leukocyte Adhesion Deficiency)
- Bovine Citrullinemia – affected calves can die within 1 week of birth
- BVD (bovine virus diarrhoea)

- Genotyping for milk production (Kappa- Casein, beta-Lactoglobulin, Alpha S1 Casein)
- DNA test for myostatin – improved meat yield
- PSS (Porcine Stress Syndrome) – pigs
- Spider Gene Test - sheep
- Scrapie resistance - sheep
- E. Coli resistance (pigs)

What is interesting to note is that the commercial viability of testing for multiple items would make DNA testing more affordable and justifiable to the producer – they would actually get some real information that they could use to enhance their production and the lab would be able to offer cost reductions for multiple tests. Similar to how a Pathology company now charges for multiple tests done on human blood!

It is likely that a 2 pronged approach to DNA will prove the outcome. The first step being to enhance the back end NLIS data base and quality assurance procedures based around DNA collection, reporting and usage whilst, in parallel, building a platform for enhancement and introduction of RFID chip read / write technology.

We envisage the future model for treatment and the combination of RFID and DNA in the Meat (Resource) Supply Chain will be an automated traceability solution starting, we believe, not in the Paddock but from the breeding! Critical elements to this future “Breed to Mouth” model will be;

- Genetic Management and reporting of Stock
- Database establishment of the National Resource
- Field traceability through mobile computing and communications supported by RFID
- Field reporting and data capture supported by DNA and RFID
- Supply Chain traceability through the development and introduction of a National Standard for Supply Chain Management of the resource
- Smooth RFID enabled data capture at any time in the supply chain through:
 - Effective tag technology and form factor
 - Affordable Reader and Antenna Infrastructure
 - A national hook tag programme that enables automated flow through of Carcus
 - A national packaging standard for meat packaging that incorporates RFID traceability and DNA traceability. This would include an openly available source code programme to support the adoption of a unified back end data base solution. Examples of this type of programme can be provided by Sunshine Technologies Pty Ltd who are currently working with the Federal Government on a programme for Smart Card adoption on a national basis for Access Control Facility management software and tag standards that enabled all elements of the meat to be traced through RFID and verified through DNA
 - Offal, Tallow and Meal identification and verification single cell DNA testing / verification as part of a Quality Assurance programme
 - Tag applications or pre-tagged packaging
- A strong Quality Assurance Programme which would be enhanced through a 3rd party contract with an independent National Testing Authority / Organisation.

6 Near Real Time DNA Authentication

There is a great deal of work being done by companies around the world to achieve the holy grail of near real time authentication. Key to the advancements in the achievement of near real time authentication are 2 things – Nanotechnology / Microfluidic Technology and advancements in processors.

Microfluidics deals with the behaviour precise control and manipulation of microliter and nanoliter volumes of fluids. It is a multidisciplinary field comprising physics, chemistry, engineering and biotechnology, with practical applications to the design of systems in which such small volumes of fluids will be used. Microfluidics has emerged only in the 1990s and is used in the development of DNA chips, micro-propulsion, micro-thermal technologies, and lab-on-a-chip technology. (ref: Wikipedia)

Companies researching this are broken down into groups; Silicon Manufacturers -

Hitachi

Hitachi is developing atomic and molecular devices, single-electron transistors and more.

IBM

They were the first company to write their name with atoms. Numerous departments work on nanotechnology, and one is called The Nanoscale Science Department.

Id-DNA has had initial discussions with IBM regarding our potential application of their technology.

Zyvex

The first molecular nanotechnology development company. "We are creating technology for atomically precise manufacturing."

CALMEC - California Molecular Electronics Corporation

A Silicon Valley company whose business plan is structured to position the Company as a leader in the Molecular Electronics industry.

MITRE Nanosystems Group

A well organized group with big plans for small computers.

Hardware Manufacturers:

There are many hardware manufacturers around the world involved the Life Science areas of Pathology and Genetics including Applied Biosystems and GE Electronics both located in the USA.

Id-DNA are in a Unique position with the combined knowledge of genetics, pathology, micro-electronics and RFID to be able to facilitate the research and development of a near real time DNA Authentication device to be used in the paddock to plate traceability and quality assurance of Australian Meat. It is our opinion formed through this knowledge that the technology is at hand to meet a 3-5 year objective of having a near real time authentication device that can be deployed in the field to secure true authentication through DNA.