

**TRACEABILITY
IMPLICATIONS FOR GENETIC IMPROVEMENT AND CONSUMERS
DNA-BASED TRACKING OPPORTUNITIES**

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Introduction

With all the recent publicity for BSE and avian influenza, coupled with the risks of bioterrorism and pandemics, traceability continues to receive increasing attention. For the pig industry, which has survived on its ability to rapidly adopt new technology, traceability represents a golden opportunity to differentiate by directly addressing concerns for food safety assurance. The information coming out of traceability can also open the door to a new phase of improvement in quality of the product itself. This paper reviews the case for traceability, and describes an initiative on DNA traceability now being adopted by Maple Leaf Foods in Canada.

Why the time is right for traceability

To retain public confidence, the responsibility of the pig industry needs to go well beyond the physical form of the product. As well public accountability for food safety, growing medical knowledge will dictate increasing responsibility for human dietary health. As well as an ageing consumer population, the incidences of cardiovascular disease, obesity, diabetes, cancer and neurodegenerative disorders are all on the increase. Claims that add value to the finished product will also need to be defended, for example “high welfare, organic, barley-fed, omega-3, animal protein-free or antibiotic-free”. For Maple Leaf the case for traceability is based on the following.

- Need for absolute food safety assurance.
- Need for zoning in the event of epidemic animal disease (eg foot and mouth).
- Tracking the source of drug residues.
- Recall in the event of contamination (eg pesticides).
- Feedback to allow quality control and improvement.
- Protection against bioterrorism.
- Proof of origin for export markets.

Today the demand for traceability provides a golden opportunity for Canada to *differentiate* its products. The industry is relatively small in terms of key players, communicates well, and already has high standards of production and health. In the wake of BSE and foot and mouth, Canada will also need to demonstrate to its export customers that it could maintain supply of product in the event of a major disease outbreak. Reliable tracking systems would be a key requirement for zoning that would allow unaffected geographical regions to continue exports.

Traceability as a key *point of difference* can therefore be a major competitive advantage for Canada, as its cost advantages are lost to subsidies and gains in efficiency in other countries. At

Maple Leaf traceability forms an integral part of its commitment to food safety, of which the major components are *prevention*, *preparedness*, and *proof* through investment and innovation.

Methods of tracing

Tracing in the live animal is relatively cheap and easy, and a variety of options exist. While ear tags and tattoos are straightforward, RFID tags and transponders are very expensive and can be unreliable. Subcutaneous transponders raise questions of welfare and the risk of entering the food chain. Some elegant ideas such as injecting a unique antigen into the pigs from each farm to give readable antibody in the meat may find even less favour with the consumer.

Tracing inside the slaughter and processing plant is much more expensive. The simpler options include paper bar codes that can be read and reprinted at each point where a cut is divided into smaller portions. Batches can be identified by some form of “marker” or interruption that passes through all lines within the plant. More expensive options include RFID or “smart” credit card type systems. In future it may be possible to create some form of spray-on biochemical bar codes that might identify a particular cutting line or hour of the day. The main requirement is that any code must be readable very quickly. One long-term idea is to read innate DNA sequences using some form of high-energy radiation.

What to trace?

The ultimate objective will be to track every piece of meat back to the farm through each step in the value chain: retail, distribution, processing, slaughter, production, breeding and genetics. The value chain can be divided into three main components: live animal, processing, and distribution and sales. Although it has yet to be implemented in most countries, live animal tracking using tags and tattoos is relatively easy. Many countries are already arranging to track slaughter pigs on a batch basis. Similarly tracking the packed retail product is relatively easy, and in many cases the necessary machine-readable labels already exist.

Tracing through slaughter and processing is an order of magnitude more complex, since each carcass may be broken into hundreds of items through several different production lines. Hams, loins and wieners may each be manufactured at different plants. Both the pathway and the technology are complicated in a high speed and often-dirty environment. For a high-speed slaughter plant, the cost of introducing full traceability could be as high as CA \$15 million dollars with a further \$4 million development costs, resulting in an impossible \$4.50 extra cost per carcass.

Why choose DNA tracing?

The great advantage of DNA tracking is that it can link meat back to the farm of origin, bypassing the expensive step of tracking through the plant. The attraction of DNA is that it requires little capital investment, because it makes use of existing DNA-typing services. DNA typing is very accurate, and relatively free of the human error from hand-labelling systems. It can therefore be used to audit and verify other tracking systems that are vulnerable to human error. DNA can be detected in cooked as well as fresh product, and if necessary in stomach contents.

For Maple Leaf in the short-term, much of the benefit from traceability is to identify the farm of origin and the slaughter plant. The first step will therefore be to introduce DNA tracking. By connecting with live animal tracking in batches, this will identify the slaughter plant. While this is introduced, the second step will be to comprehensively research systems for tracking through the slaughter and processing plants. The objective will be to introduce tracking through the plant as soon as it is cost-effective.

Single nucleotide polymorphisms (SNPs)

A number of genomics companies are developing DNA tracking systems for meat. The tracking systems exploit natural variation in the DNA code, which is made up of just four units or *nucleotides* (A=adenosine, C=cytosine, G=guanine, T=thymidine). The systems fall into two types: short tandem repeats (STRs), or single nucleotide polymorphisms (SNPs or “snips”). STRs are fragments of DNA of varying length that can be separated by gel electrophoresis. They are made up of repetitive sequences of DNA that are naturally variable, so that for example some individuals may be ‘ACACAC’ while others are ‘ACAC’. Their main advantage is that a single STR may have many variants, so in principle they have a high power of discrimination. The practical disadvantage is that the different variants can be difficult to identify without error, so the system does not lend itself to automated high-throughput genotyping.

SNPs are single units of the code that vary naturally, so that some animals may be A while others are C. The halothane gene (HAL-1843) A to T mutation is actually one such SNP. Unlike STRs there are usually only two variants at a site, and there is no reliance on interpretation. SNPs are therefore like a digital code. The disadvantage is that SNP discovery is expensive, but SNP maps and libraries are being established. With their greater precision and easier high-throughput genotyping, SNPs are therefore the preferred solution for the long term.

Choice of DNA panel

In 2003 Maple Leaf and Pyxis Genomics began to develop a panel of SNPs for traceability. The choices were to trace pork to the individual meat animal, to the sire, or to the dam. Today the ideal of tracing each individual would simply be too expensive. The cheapest option of tracing to the father was ruled out because semen from a single AI sire is used across locations, and therefore gives no information on farm of origin. Therefore the decision was made to trace pork back to the mother of the slaughter pig. SNPs on the maternal chromosomes in meat would be matched back to the mother.

In the Maple Leaf panel the number of SNPs and therefore cost would be minimized in two ways. First, the SNPs would be selected in linked groups of 3-4 that are close together on the same chromosome. This would allow exclusion of non-parents on the basis of haplotypes rather than individual SNPs. Second, maximum use would be made of mitochondrial nuclear DNA that is maternally inherited only in the cytoplasm of the egg. This occurs as a single strand (*haploid* instead of *diploid*) with no recombination and very little mutation.

How DNA tracing works in practice

At farm level DNA tracking works as follows. As each replacement gilt enters commercial production, a blood sample is DNA-typed for the SNP panel and the identity information entered in a database. Producers simply receive bar-coded blood tubes or FTA cards. The breeding female's identity is written on a sheet beside the barcode, and posted with the tubes to the DNA lab. The lab types the sample and enters the dam's DNA genotype and farm into the database. The producer updates the database with farrowing and culling dates.

Meat samples for tracing are sent to the laboratory, and the DNA genotype is entered into the database. Meat is then matched to the mother's identity, which indicates the breeding farm and date of birth of the progeny. The matching is done by a computer search engine, developed for Maple Leaf by IBM. A batch live animal tracking system then links to the nursery, finisher, and from there to transport and the slaughter plant. The live animal tracking system is thus an essential part of full traceability, and is complementary to DNA traceability. The trail stops at delivery to the slaughter plant.

The Lethbridge pilot study

Maple Leaf has established a pilot DNA traceability project at its Lethbridge Alberta plant. This involves progeny from some 25,000 sows from a total of 23 farms. To date some 33,000 sows have been successfully genotyped by Orchid Biosciences for the SNP panel developed by Pyxis. Test runs with the search engine have also been successful, but it has become clear that to achieve the required level of precision in identifying the correct mother on every occasion the number of SNPs on the panel will have to be increased.

There were two main reasons for the shortfall in precision. First the sows of Alberta were more genetically similar than expected. For example 60% of the population shared the same 8-SNP mitochondrial haplotype. Second many of the SNP allele frequencies fell outside the optimum range of 0.4 to 0.5, and differed from the test population frequencies in which the panel was developed. A further practical disadvantage that emerged for the haplotyping approach was that the actual haplotypes existing in a finite population are not known without error. Also, the theory of the optimum size of a SNP panel was not well developed.

Precision for tracing to the dam

A guide to the number of SNPs required can be derived as follows. Assume SNP allele frequencies of q and $1-q$ respectively, with n SNPs in the DNA panel, and m mothers in the population. The probability of excluding a random non-parent in the population P_E based on a single SNP:

$$P_E = 2q^2(1 - q)^2$$

So the probability of non-exclusion P_{NE} on a single SNP is:

$$P_{NE} = 1 - P_E$$

And for a panel of n SNPs the probability of non-exclusion P_{NE_n} is:

$$P_{NE_n} = (1 - P_E)^n$$

The probability of finding a single correct mother P_{CM} in a population of m sows is the probability that all non-mothers are excluded:

$$P_{CM} = (1 - (1 - P_E)^n)^m$$

For tracing to the dam with 25,000 sows at Lethbridge, this would indicate a panel of around 180 SNPs. However, this average number would be the absolute minimum, since it does not account for relatedness within the sow population, and it does not allow for the real non-random distribution of allele frequencies in the population. A stochastic simulation may therefore be needed to arrive at the true panel size. One strategy to reduce genotyping cost is to have a main panel of say 250 SNPs, plus a secondary panel of say 100 SNPs that can be used as a “tie-breaker” only in the event of two or more candidate mothers for the same piece of pork.

Next steps

The next step will be to create a traceability panel with some 500 SNPs with optimal minor allele frequencies of 0.4 to 0.5. For 6-12 months this will be put through a “proof of principle” test by tracing to the *sire* rather than to the dam. During that time AI boars will be segregated by production pyramid so that the sire identity will indicate the pyramid of origin. Over half the farms supplying Lethbridge still use natural mating, while the remainder are served by just two AI studs. The logistics are therefore relatively simple, but the sire tracing system could not easily be transferred to other plants.

Tracing to the sire in this way offers proof of origin for the export customer, but it does not provide the same degree of traceability as the dam. The intention is therefore to switch back to tracing to the dam as soon as the 500 SNP panel has been tested and optimized to a smaller number of SNPs that is cost effective on a higher volume of parents.

Costs of DNA tracing

Today the cost of DNA typing a single animal or meat sample for a 300 SNP panel is in the range CA \$40.00 to \$50.00 including collection. If a sow produces 50 market pigs in her lifetime, the cost is around \$1.00 per carcass. If gilts are typed on entry to the herd, their first progeny will be slaughtered more than eight months later. In view of the long lead-time, large batches of blood samples can be accumulated to take advantage of economies of scale for DNA typing.

The largest cost comes at the start of the scheme when the existing sow population is DNA typed for the first time. After that, only herd replacements are typed, so annual testing costs fall to around 40% of the start-up cost. At Lethbridge for example it would cost around \$1.0 million to type all the 24,000 sows, but the annual cost thereafter would be only \$0.4 million. Within five

years the cost of high speed SNP typing is expected to come down to around \$10.00 per dam, or around 20 cents per carcase. It will make sense therefore to introduce traceability in the very high value markets first, and phase in lower margin markets as the costs descend.

Product quality

Who will pay for traceability? The objective of traceability is to position the pork industry as safe, responsible and proactive. However it is unlikely that consumers will be willing to pay more for traceable pork that protects against bioterrorism or epidemics or sundry accidents. Probably the cost of traceability will never be recovered from increased food safety assurance. Instead it will have to be recovered from improved *product quality*.

The real opportunity for DNA traceability is to improve product quality in two ways. In the short term pork can be tracked to identify the *causes* of poor meat quality. A particular breed type, diet, farm, truck company, cooler or day of the week can then be targeted for improvement. In the long term, the genetic causes of high and low meat quality can be better understood. Samples from high and low quality meat can be accumulated and used in a genome scan to identify possible markers. These markers can then be used at nucleus level in genetic improvement programs.

Summary and conclusions

DNA traceability offers a key point of difference for the pig industry. The ideal goal for the pig industry will be to track every step of production from genetics through to the plate. Tracking every piece of meat through modern high-speed plants represents a major challenge. In the meantime DNA offers a way of tracing from meat back to the farm and circumventing the plant. It is complementary to live animal tracking systems, extending traceability from the point of slaughter to any piece of pork even after cooking.

Maple Leaf is continuing to develop its DNA traceability system at the Lethbridge Alberta plant. The SNP panel is being extended to increase precision, and will be tested in a proof of principle step that will involve tracing to the sire, before reverting to tracing to the dam. In the short term DNA traceability offers proof of origin and food safety assurance. In the longer term the financial gain will come from identifying factors that can be used to improve product quality. Hopefully this will include the genes themselves responsible for better pork and better health of both the animals and the consumer.

Further reading

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