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THERE IS NOTHING ROUTINE ABOUT ROUTINE TESTING. A PERSPECTIVE FROM THE UNIVERSITY OF QUEENSLAND'S ANIMAL GENETICS LABORATORY

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SUMMARY

The following article is a reflection on current trends and challenges in genetic testing across the livestock sector, particularly the cattle industry, from the perspective of a significant genetic testing laboratory based at The University of Queensland.

INTRODUCTION

Much has changed in genotyping technologies since The University of Queensland's Animal Genetics Laboratory (AGL) was first established in 1985. While cattle makes up the single largest species tested at AGL, we also cater for sheep, alpaca, goat and pigs, as well as services and research for the aquaculture industry, fisheries and wildlife ecology research groups. Below are insights into the operations of a successful genetics laboratory.

AGL DOES MUCH MORE THAN SIMPLY GENOTYPE CATTLE.

AGL serves a very wide client base, ranging from research organisations to breed societies, pastoral companies and small to medium-sized livestock producers. Additionally we provide support to the Gatton-based research communities, state police services and others. Hence, it is a requirement for AGL to be both nimble and adaptable. Australian farmers are a unique clientele operating a range of diverse production systems in different terrains and producing cattle for various markets, all whom have specialised requirements and expectations.

Therefore the range of services provided needs to be multi-faceted. While for some clients the experience may be purely transactional (samples in, results reported), many others are looking for a more personalised & ongoing service. AGL's clients are country people that appreciate the ability to discuss testing options and interpretations. In many cases AGL staff have built both rapport and understanding of the herds of many clients, Genotyping results are often merely the beginning, or continuation of, a long and prosperous relationship. In many cases, AGL retains critical herd-specific knowledge that spans many years, and many property managers' tenures.

GROWTH/MARKET TRENDS

The number of samples AGL receives has grown considerably (Figure 1). Looking at the last 5 years (2011-2016) alone, the growth in cattle samples, as measured by case numbers assigned per annum, has averaged 13.4% per annum. This is actually an underestimate of testing volumes given that in the last year or 2 there has been significant client-driven demand for retesting of animals already in the system, and these are not captured in Figure 1.

It is also instructive to look at testing trends over this period. From 2012 - 2016 the number of samples processed on microsatellites (MiP) has remained relatively stable at AGL, excluding a larger than normal demand in 2012 (Figure 2). During this time there has been a rapid increase in the use of genomics and SNP-base parentage (SEQ) requests. In the case of the GeneSeek Genomic Profiler low-density BeadChip (GGPLD), usage was initially for research projects, but the steadily increasing demand for the assay in 2015 and 2016 is primarily due to increased demand from livestock producers.

Industry 1

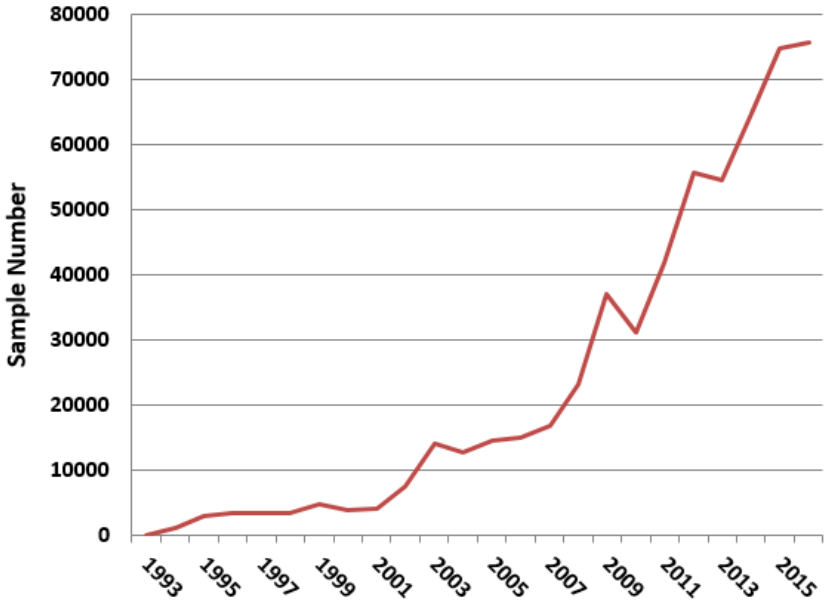

Figure 1. Cattle samples received per full year 1993 – 2016

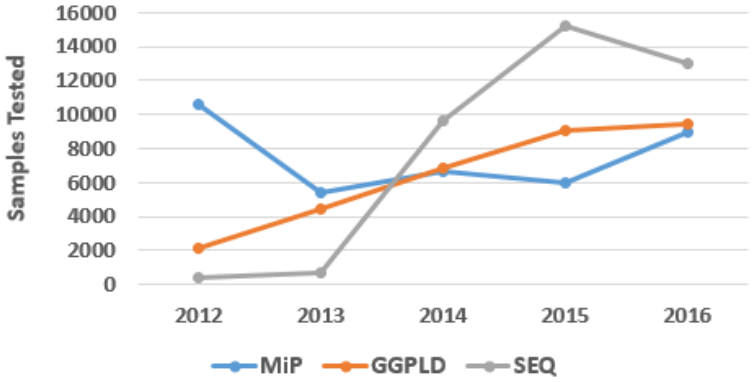

Figure 2. Count of parentage and genomic testing at AGL 2012-2016.

THE CSI EFFECT

The Crime Scene Investigation (CSI) effect is any way in which the exaggerated portrayal of forensic science on crime television shows influences public perception (Cole and Dioso-Villa 2007). It is very relevant to those working in customer-facing roles within the scientific profession.

The CSI effect manifests itself in a multitude of ways at AGL but most commonly in regards to unrealistic expectations of turnaround time or the amount and quality of sample that is required. When parentage does not immediately resolve, it is often assumed that AGL can simply run it against everything in the database to identify the correct parent. This not only assumes that the sire or dam is 'in the system", but also that AGL has the resources to develop the equivalent of a National DNA Data Bank for Australian Cattle and that sufficient markers are available to discriminate every

individual. It is important to get the message out to all users and potential users of genetic and genomic testing services that 'real science' does not happen this way.

PARENTAGE CHALLENGES

From the parentage viewpoint, northern herds tend to be more complex than southern herds. This is due to a number of factors including sire-only parentage, larger overall herd and parent lists, difficulty in providing complete sire lists and a greater chance of uncaptured parents. There are also significant logistical challenges in providing resubmissions for samples that fail genotyping or produce anomalous results.

Success rates of northern parentage verification (PV) analyses can still be maximised, despite these aforementioned constraints, with open and frequent communication between AGL staff and the client. The PV success rate of a large northern herd that used this tactic was considerably improved over a 3 year period (Table 1).

Table 1. Parentage verification success rates for a large Northern herd

	Analysis 1	Analysis 2	Analysis 3
Year 1	46%	71%	89%
Year 2	61%	89%	97%
Year 3	95%	97%	

FROM MICROSATELLITES TO SNP

Much has been written about the promises of SNP-based parentage verification (SNP_PV) in livestock and animal traceability across the supply chain (Heaton et al. 2002, Van Eenennaam et al. 2007, Baruch and Weller 2008). However, costs associated with moving a breed from PV using MiP to SNP_PV are substantial, as are the logistical challenges. Retaining unused samples (with greater than 500,000 hair samples archived) at AGL has helped significantly reduce time spent sourcing new samples for animals, especially when animals are deceased. Once the decision is made to transition across to SNP_PV, experience shows us that very clear communications is essential to avoid issue of incompatible profiles between sires, dams and progeny. For smaller breeds, where there remains a lack of incentive to use genomics, then the change to SNP_PV is uneconomical and PV using MiP will probably remain part of the AGL offerings for many years to come. However as price per SNP test falls, the move to SNP will likely become attractive to even the smaller breeds.

CHALLENGES OF SNP REVOLUTION

The challenge in context of the Australian market has been trying to find the sweet spot of sufficient markers for accurate parentage at a price deemed acceptable. In an industry as diverse as the Australian cattle industry this has proven to be no simple task. AGL currently offers 2 SNP-based parentage assays: SEQ1 iPLEX panels contained a total of 138 SNP including 95 ISAG core plus 4 ISAG additional SNP, or SEQ2 consisted of 59 additional SNP for a combined total of 197 markers genotyped and total of 97 ISAG core SNP. These extra markers were developed to be informative in Brahman and Tropical Composite breeds. As reported previously (Lyons et al, 2013), we demonstrated that the ISAG-recommended core bovine SNP parentage panel is not sufficient to provide accurate parentage verification in many common Australia production systems. Further, we acknowledged that these panels were less than ideal. A number of publications over recent years has highlighted the advantage of larger numbers of SNP for parentage (Strucken et al. 2014; McClure et al., 2015), but these rarely take into account the economic reality of the market and current technologies.

PRICING CHALLENGES

Price expectations of the livestock industry do not necessarily align with commercial realities of test prices. Unlike supermarkets or other commodity-based services, and perhaps unlike standard R&D within research organisations, there is much more to be considered than the consumables' cost. Significant challenges and considerations in development and implementation of testing need to be both understood and appropriately costed. For any test performed at AGL, the samples will pass through up to 6 hands from arrival to reporting and beyond. In simple terms, there is reception, cataloguing, sample preparation, DNA extraction and QC, pre-PCR, post-PCR, data analysis and reporting data in a multitude of different formats prone to change regularly. Standardisation of reporting remains a challenge across the industry.. As already discussed, AGL prides itself on doing more than simply churning out data. AGL liaises with clients regularly and has intimate knowledge of herds and breeding regimes based on prior testing. The labour costs at AGL associated with pre- and post-testing consultations and follow-up discussions with are significant.

Other factors often overlooked, but of critical importance to the feasibility of genetic diagnostic labs include: patent and licensing considerations or costs, maintenance and depreciation costs for equipment, newer technology upgrades necessary to remain competitive, the additional costs of validation of novel platforms or assays, data and sample storage, informatics for interpretation of genomic variation, volume discounting options and commercial risk mitigation.

THE FUTURE

Much has been written about the decreasing cost per marker for genotyping and/or sequencing. The large number of high-throughput SNP genotyping technologies available are growing, but this in itself offers many challenges. Capital investments previously made will largely dictate services offered, and at AGL the reliability and reproducibility of the fixed Illumina Infinium platform has been very successful. Minimizing turnaround times and throughput variability remain important factors that have influenced AGL's model of developing in-house facilities rather than outsourcing. Genotype-By-Sequence (GBS) is often suggested as the way of the future, and certainly has a role in R&D or where flexibility is required. However, one major challenge with GBS approaches, especially for high-throughput genotyping facilities, is the considerable investment needed for bioinformatics support to properly analyse, curate and store the massive amounts of sequence data obtained from running GBS.

At the end of the day producer uptake of these technologies is not driven by cost-per-marker statistics. Producers are seeking a reproducible, highly accurate and informative result that can be translated into achieving their breeding objectives and/or a more saleable item. Reduced costs will be welcomed, but only if there is no compromise to results, and to date that has been the challenge. Attaining the 'holy grail' of 1 test per sample for everything you could need including Parentage, Recessives, Trait markers, EBVs, and ultimately the ability to make early selection decisions, is becoming a more realistic goal.

REFERENCES

Baruch, E. and J. I. Weller (2008) *Animal Genetics* **39**: 474.
Cole, S., & A. Dioso-Villa. (2007). *New England Law Review.* **41**: 435.
Heaton, M. P., G. P. Harhay, G. L. Bennett, et al. (2002) Mammalian Genome 13: 272.
Lyons, R.E., Buttsworth S., Waine D. and M. Kelly. (2013) *Proc. Aust. Assoc. Anim. Breed. Genet.*
McClure, M.C., McCarthy, J., Flynn, P., *et al*. (2015) *Proc. Int. Comm. Anim. Rec.* 175.
Strucken, E. M., Gudex, B., Ferdosi, M. H.,. *et al*. (2014) *Animal Genetics* **45**: 572.
Van Eenennaam, A. L., R. L. Weaber, D. J. Drake, et al. (2007) *J. Anim. Sci*. **85**: 3159.