Contributed paper

EFFICIENCY OF OPTIMIZED POLL TESTING ASSAY IN AUSTRALIAN BEEF CATTLE

I.A.S. Randhawa[1], K.M. Schutt[2], M.R. McGowan[1] and R.E. Lyons[3]

[1] The University of Queensland, School of Veterinary Science, Gatton, QLD 4343, Australia
[2] Neogen Australasia, University of Queensland, Gatton, QLD 4343, Australia
[3] Agri-Genetics Consulting, QLD 4074, Australia

SUMMARY

Genetic testing for the presence of POLL gene in cattle has been proposed in Australia because it helps avoid dehorning and disbudding in young calves. Animals can be true polled if they carry two copies of either Celtic (PcPc) or Friesian (PfPf) mutations, or one of each (PcPf). Optimized poll testing (OPT) – a 5 SNPs based assay to detect both type of mutations – was developed to improve efficiency of commercial tests, which are used in selective breeding for rapidly increasing the poll gene frequency in herds. This study evaluates the efficiency of OPT assay across various breeds by using a high number of commercial test results (n=98,744). Overall, OPT consistently showed high success rate of 99.56% in commercial tests, which is consistent with previous results (99.60%) based on experimental data. The OPT has been rapidly adopted across the industry leading to greater accuracy and more confidence. OPT has been equally efficient for the taurine (99.50%) and indicine (99.63%), Zebu and other indicus-influenced composite breeds.

INTRODUCTION

The genetics of horns and polledness (absence of horns) is complex in the bovidae family. In cattle, inheritance of polledness (P) is dominant to horns (H); however, the underlying genes and causal mutations display an array of genetic heterogeneity and phenotypic diversity (Medugorac *et al.* 2012; Wiedemar *et al.* 2014). Genetic control of the polledness – the so called "POLL gene" – has been mapped on the starting end of bovine chromosome 1 (BTA1) (Long and Gregory 1978). To date, four different genetic mutations that can cause polledness have been identified in cattle worldwide, all of which are physically located in a narrow neighbourhood on BTA1 (Figure 1, (Aldersey *et al.* 2020)). The known mutations are named according to their geographic origin in cattle (Capitan *et al.* 2011; Tetens *et al.* 2015; Medugorac *et al.* 2017; Utsunomiya *et al.* 2019) e.g., Celtic (Pc), Friesian (Pf), Mongolian (Pm) and Guarani (Pg). Of those, only Pc and Pf have been found prevalent in Australian cattle herds. Animals can be true polled if they carry two copies of either Celtic (PcPc) or Friesian (PfPf) mutations, or one copy of each (PcPf). Due to the genetic complexity the heterozygous animals (HP: HPc or HPf) which carry one copy of horn (H) and one copy of a poll (Pc or Pf) can be polled or may develop small size and unattached horn-like-structures called scurs (Aldersey *et al.* 2020; Gehrke *et al.* 2020).

As a consequence of rising concerns about animal welfare and the costs of bruising and dehorning (Huertas *et al.* 2015), increasing the polled cattle population is a way forward for a sustainable beef industry. Identification of true polled cattle has been a challenge (Connors *et al.* 2018), given that some HP animals can be polled but can potentially pass on an H (horn) allele to its offspring. Therefore, two phenotypically polled animals can produce a horned offspring. Genetic testing for the presence of POLL gene in cattle has been proposed in Australia because it helps avoid dehorning and disbudding in young calves (Prayaga 2007). Poll gene testing has been in practice since 2012 and has evolved through the use of different types of genetic markers, initially based on microsatellites and more recently based on single nucleotide polymorphisms (SNPs). Optimized poll testing (OPT) – a 5 SNPs based assay to detect both type of mutations – was developed to improve efficiency of commercial tests (Randhawa *et al.* 2020), which are used

in selective breeding for rapidly increasing the poll gene frequency in Australian herds. This study evaluates the efficiency of OPT assay across various breeds by using a high number of test results.

MATERIALS AND METHODS

Genetic markers for the prediction of Celtic (Pc) and Friesian (Pf) types of poll associated SNP alleles (Table 1) are available on commercial bovine BeadChip assays (Illumina) including Neogen's proprietary GGP Bovine 100Kand GGP Indicus 50K assays (Neogen Corporation, Lincoln, NE). The Pc genotype is predicted by translating a single SNP marker rs383143898 (ARS-UCD1.2 position on BTA1: 2,429,319) based on its horn or poll allele (Table 1). The Pf genotype is predicted based upon four markers associated with Pf (Table 1, Figure 1). Pf associated markers include: rs801127025 (BTA1: 2,372,456), rs799403053 (BTA1: 2,486,811), rs210350155 (BTA1: 2,491,161) and rs797088784 (BTA1: 2,578,598). Results of OPT represent reconciled outcomes from both Pc and Pf predictions to generate genotypes such as HH, HPc, HPf PcPc, PcPf or PfPf. However, if the Pc-associated SNP or more than one Pf-associated SNPs fail during genotyping, or one or more SNPs differ in predicted genotype (H versus Pf) then the result is considered ambiguous and termed as a "No Result". For this study, OPT results on commercial samples (n=98,744) were obtained to check the efficiency of mutation predictions. In addition, call rate, genotyping error and prediction efficiency of the OPT and an additional SNP: rs799920960 (BTA1: 2,748,715), which is also available on the above mentioned commercial genotyping assays, were investigated by using a subset of the commercial tests and previous data (Randhawa *et al*. 2020).

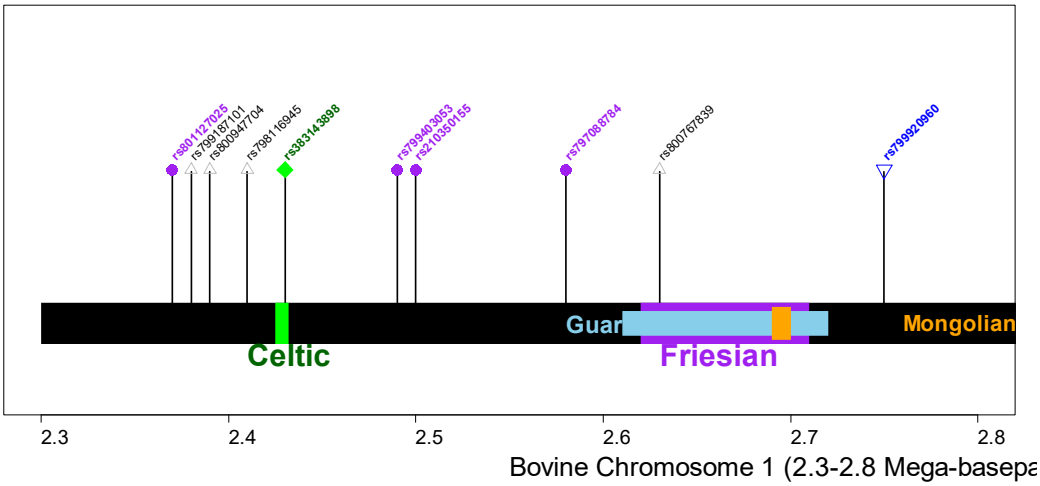

Figure 1. POLL region on chromosome 1 (Bovine assembly: ARS-UCD1.2) showing locations of four known insertion-deletions (Celtic, Friesian, Mongolian and Guarani) associated with polledness across various worldwide breeds of cattle. The Optimized Poll Test (OPT) is based on the 5 coloured SNPs (1-green to predict Celtic and 4-purple to predict Friesian mutations). The blue SNP is localized close to Friesian and have shown strong linkage with Pf. The remaining SNPs have been used in poll testing assays previously and are available on most SNP chip assays

Table 1. Single nucleotide polymorphisms (SNP) on BTA1 for predicting the Celtic (Pc) and Friesian (Pf) mutations

SNPs	Positions*	Mutations	Poll alleles	Predicting mutation
rs801127025	2,372,456	P_{5ID}	T	Friesian (Pf)
rs383143898	2,429,319	P_{202ID}	T	Celtic (Pc)
rs799403053	2,486,811	T>C	C	Friesian (Pf)
rs210350155	2,491,161	C>A	A	Friesian (Pf)
rs797088784	2,578,598	G>A	A	Friesian (Pf)

* Genomic positions based on bovine genome assembly ARS-UCD1.2 (GCA_002263795.2).

RESULTS AND DISCUSSION

Table 2 shows results from the obtained commercial tests performed using OPT based predictions. The available data were combined into two groups: Taurine (*Bos taurus*) and Zebu (*Bos indicus* and composite), based on the breed information about each sample. A total of 53,310 Taurine and 45,434 Zebu results show that OPT was generally successful with 99.56% efficiency. The remaining 0.44% (438) samples providing "No results (NRs)" are more likely be due to a failure to amplify one or more markers during the genotyping process. Previously, Zebu cattle showed very high number of failure rate with over 10% of NRs by using previously available POLL gene testing assays (Randhawa *et al.* 2020). Hence, we compared the NRs from OPT between the Taurine and Zebu, and respectively found that 0.50% and 0.37% of their samples returned an NR (Table 2). As such, these results are markedly lower than the previous tests and within the expected range of genotyping errors (Wu *et al.* 2019). However, the results suggest that the Zebu (n=170 out of 45,434) had significantly less (Fisher's Exact test, $p<0.001$) NRs than the Taurine (n=268 out of 53,310). This suggests that OPT test has successfully overcome the high rates of NRs in the commercial application, especially for the Zebu and composite breeds. Moreover, results may suggest that the Taurine breeds have an undetectable lack of LD between some of the SNP markers and the POLL mutations or an additional variation within the genotyping probe regions, either of which is causing the decrease in POLL gene prediction. A preliminary investigation of the collected samples and previously available results suggest that one of the SNPs (rs801127025) – to predict Pf – is likely the frequent cause of NRs in several breeds, including genotyping error and mismatch with the rest of Pf predicting markers (Randhawa *et al.* 2020). Note that rs801127025 is located farthest from Pf, rather upstream of the Pc (Figure 1). Given the potential for recombination (~0.2%) between the Pf and Pc, and a slightly higher chance between Pf and rs801127025, there is the possibility that unique haplotypes may exist in some breeds or herds. We emphasise that the rate of NRs (~0.44%) should not be taken as a lack of performance of OPT per se. However, there can be a simple alternative to further reduce the NRs.

We investigated another SNP (rs799920960) in a small dataset, which has not shown genotyping error or discordance with Pf. Hence, rs799920960 can be used either to substitute rs801127025 or as an additional marker for a leverage to accept two mismatches in OPT. The proposed marker is strongly linked to Pf because it is closely localized than any other SNPs being used to predict Pf (Figure 1). However, we suggest that additional research is required to evaluate the utility of SNP marker (rs801127025) of the OPT assay and the proposed increase in the efficiency in some breeds by including the additional marker (rs799920960). Overall, the OPT is performing as expected by providing commercial efficiency (99.56%) concordant to previously reported experimental results (99.6%) used to design the OPT (Randhawa *et al.* 2020). OPT has shown greater accuracy of head phenotype predictions, but phenotyping and sampling errors may deflect. All in all, the OPT has been rapidly adopted – replacing the poll haplotype test (Connors *et al.* 2018) – across the industry for poll breeding to achieve sustainable beef production.

Table 2. Performance efficiency of OPT in the Taurine and Zebu breeds

OPT output	Taurine	Zebu	Total	Percentage
HH	24,011 (45.04%)	19,908 (43.81%)	43,919	44.48%
HPc	14,968 (28.07%)	17,147 (37.74%)	32,115	32.52%
HPf	796 (1.49%)	445 (0.97%)	1241	1.26%
PcPf	1,224 (2.29%)	333 (0.73%)	1557	1.58%
PfPf	185 (0.34%)	12 (0.02%)	197	0.20%
PcPc	11,858 (22.24%)	7,419 (16.32%)	19,277	19.52%
No results (NR)	268 (0.5%)	170 (0.37%)	438	0.44%
Total	**53,310**	**45,434**	**98,744**	**-**

CONCLUSIONS

This study shows that OPT has been very successful (99.56%) for commercial testing of POLL gene in Australian beef cattle, both Taurine (99.50%) and Zebu (99.63%) breeds. Being compatible with genomic products, the test is also available at lower cost than the previous stand-alone tests. The OPT is performing as expected and it has been rapidly adopted across the industry leading to greater accuracy and more confidence to achieve a more sustainable beef industry.

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