**Optimized Genetic Testing for Polledness in Multiple Breeds of Cattle**

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**Table S1** Comparison of SNP-based current poll testing (CPT) and the optimized poll testing (OPT) assays on 20,636 samples for the frequency of “No results” in various cattle breeds.

|  |  |  |  |
| --- | --- | --- | --- |
| **Breed** | **Number tested** | **No results with CPT** | **No results with OPT** |
| Brahman | 2,819 | 492 | 0 |
| Wagyu *a* | 9,050 | 60 | 0 |
| Droughtmaster | 558 | 9 | 0 |
| Shorthorn | 67 | 5 | 1 |
| Santa Gertrudis | 136 | 5 | 0 |
| Brangus | 72 | 6 | 0 |
| Hereford | 3341 | 1 | 0 |
| Angus | 1,602 | 0 | 0 |
| Charolais | 900 | 0 | 0 |
| Limousin | 207 | 0 | 0 |
| *Holstein-Friesian* | *31* | *0* | *0* |
| *Braford* | *14* | *0* | *0* |
| *Murray Grey* | *8* | *0* | *0* |
| *Boran* | *7* | *0* | *0* |
| *Poll Hereford* | *7* | *0* | *0* |
| *Red Angus* | *4* | *0* | *0* |
| *Red Wagyu* | *2* | *0* | *0* |
| *Simmental* | *2* | *0* | *0* |
| *Gelbvieh* | *1* | *0* | *0* |
| *Composite* | *35* | *1* | *0* |
| *Angus x Brahman* | *33* | *7* | *0* |
| *Angus x Simmental*  | *2* | *0* | *0* |
| *Wagyu Cross* | *2* | *0* | *0* |
| *Ultrablack x WAGX* | *2* | *0* | *0* |
| *Cross Breed* | *2* | *0* | *0* |
| *Brahman Cross* | *1* | *0* | *0* |
| *Unknown breeds b* | *1,731* | *1,413* | *8* |
| **Total** | **20,636** | **1,999** |  **9** *c* |

*a* Wagyu includes F1-F4 classes as well as Fullblood population in Australia.

*b* The data includes several breeds of European, Zebu and crossbreds. However, data provided to the testing laboratory does not identify the breed of the sample. These samples are primarily acquired for the “No Results” output of CPT and suspected to be of Brahman and Brahman-infused cross-bred cattle.

*c* Out of 9 samples, which remain as “No results” with OPT, the Shorthorn sample failed at **rs797088784** and the remaining 8 samples of unknown breeds failed at **rs383143898** (the only predictor of Celtic allele), meaning that these samples required a re-run in the laboratory and the “No results” were not because of the OPT predictions.

**Table S2** List of 14 microsatellites mapped within the Poll locus on chromosome 1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Microsatellites** | **Haplotype assay** | **ARS-UCD2.1** *a* | **UMD3.1** *a* | **Btau4.0** |
| CSAFG26 | 1 | 2341080 | 1617119 | 1441734 |
| CSAFG27 | 1 | 2368949 | 1646009 | 1470624 |
| CSAFG28 | 1 & 2 | 2394182 | 1670889 | 1495504 |
| CSAFG29 | 1 & 2 | 2406659 | 1683376 | 1507991 |
| RP42-351B8\_MS1 | 1 | 2410219 | 1686934 | 1511549 |
| RP42-351B8\_MS2 | 1 | 2432556 | 1709169 | 1533784 |
| CSAFG30 | 1 & 2 | 2435911 | 1712696 | 1537311 |
| CSAFG31 | 1 & 2 | 2457207 | 1733999 | 1558614 |
| CSAFG33 | 2 | 2512705 | 1790169 | 1614784 |
| CSAFG34 | 2 | 2575232 | 1852525 | 1677140 |
| CSAFG35 | 2 | 2588208 | 1865512 | 1690127 |
| CSAFG37 | 2 | 2704710 | 1984932 | 1809547 |
| CSAFG38 | 2 | 2735847 | 2016159 | 1840774 |
| CSAFG22 | 2 | 3014463 | 2294700 | 2119315 |

*a* Positions on ARS-UCD1.2 and UMD3.1 are approximated based on the nearest rs-SNP to each MSAT.

**Table S3** Call rate of 10 SNPs in different cattle breeds and cross-bred populations.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Breeds** | **Number tested** | **rs801127025** | **rs799187101** | **rs800947704** | **rs798116945** | **rs383143898** | **rs799403053** | **rs210350155** | **rs797088784** | **rs800767839** | **rs799920960** |
| Angus | 1594 | 99.89 | 100 | 96.96 | 100 | 99.96 | 99.94 | 99.65 | 99.50 | 100 | - |
| Angus X Brahman | 33 | 100 | 100 | 90.91 | - | 100 | 100 | 100 | 100 | - | 100 |
| Boran | 7 | 100 | 100 | 28.57 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Braford | 14 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Brahman | 2604 | 98.27 | 99.92 | 85.71 | 100 | 99.97 | 99.73 | 98.78 | 99.77 | 99.60 | 100 |
| Brangus | 9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Charolais | 370 | 99.19 | 99.73 | 98.92 | 100 | 100 | 99.80 | 99.19 | 98.65 | 100 | - |
| Composite | 35 | 97.14 | 100 | 95.71 | 100 | 99.05 | 100 | 98.57 | 100 | 97.14 | - |
| Droughtmaster | 289 | 96.93 | 99.83 | 89.62 | 100 | 99.88 | 98.70 | 95.85 | 99.65 | 100 | - |
| Hereford | 2820 | 99.88 | 99.98 | 99.66 | 99.96 | 99.81 | 99.95 | 99.59 | 98.09 | 100 | - |
| Holstein-Friesian | 31 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Limousin | 24 | 100 | 100 | 91.67 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Murray Grey | 8 | 100 | 100 | 87.50 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Poll Hereford | 7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Red Angus | 4 | 100 | 100 | 75.00 | 100 | 100 | 100 | 100 | 100 | 100 | - |
| Santa Gertrudis | 81 | 97.88 | 100 | 93.83 | 100 | 100 | 99.07 | 96.91 | 100 | 100 | - |
| Shorthorn | 67 | 100 | 100 | 100 | 100 | 100 | 100 | 93.28 | 64.18 | 100 | - |
| Unknown | 1631 | 100 | 100 | 98.41 | 100 | 99.52 | 100 | 100 | 100 | 100 | 100 |
| Wagyu | 9047 | 99.45 | 100 | 98.03 | 99.99 | 99.99 | 99.79 | 99.27 | 99.93 | 99.99 | - |

**Figure S1** Distribution (%) of MSAT and SNP based poll gene testing results for Horned (HH), Hybrid (HP), Polled (PP) and failing (“Not Determined” or “No Results”) samples across 10 Australian beef breeds (n=16,941).

**Table S4** Results of targeted DNA sequences of 55 Brahman samples showing 60bp genome region around SNP rs800947704[C/T=Y] (marked as 0) and upstream variant SNP rs381418143 [A/G=R] (marked as -3). Two types of errors were observed in the genotyping assays, 1: genotype failed (--) when -3=G (yellow highlights), 2: wrong genotypes when -3=R and 0=Y (purple highlights).

|  |  |  |
| --- | --- | --- |
| **🡻Samples** | **DNA Sequence results around rs381418143 (-3) & rs800947704 (0)** | **Genotypic assay** |
| SNPs 🡺 |  \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\***-3**\*\***0**\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* | rs800947704 |
| 1 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 2 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**C**GGGGTGTCCCATTGA | CC |
| 3 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 4 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 5 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 6 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 7 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**C**GGGGTGTCCCATTGA | CC |
| 8 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 9 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 10 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 11 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 12 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 13 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 14 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 15 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**C**GGGGTGTCCCATTGA | CC |
| 16 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 17 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 18 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**C**GGGGTGTCCCATTGA | CC |
| 19 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 20 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 21 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 22 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 23 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 24 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 25 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 26 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 27 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 28 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 29 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 30 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 31 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**T**GGGGTGTCCCATTGA | TT |
| 32 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 33 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 34 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 35 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 36 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 37 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 38 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 39 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 40 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 41 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 42 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 43 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**C**GGGGTGTCCCATTGA | CC |
| 44 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**C**GGGGTGTCCCATTGA | CC |
| 45 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**Y**GGGGTGTCCCATTGA | TT |
| 46 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 47 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**C**GGGGTGTCCCATTGA | CC |
| 48 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 49 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**C**GGGGTGTCCCATTGA | CC |
| 50 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**Y**GGGGTGTCCCATTGA | CT |
| 51 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 52 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**A**TT**C**GGGGTGTCCCATTGA | CC |
| 53 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 54 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**G**TT**C**GGGGTGTCCCATTGA | -- |
| 55 | GGGGAAAGACTATGCACCAACCCCTTACAGAAGATGTGGCC**R**TT**C**GGGGTGTCCCATTGA | CC |

**Figure S2** Breed-wise distribution (%) of Optimized Poll Testing (OPT) assay results (HH = Horned, HP = Hybrids, PP = Polled, No Results = Failed assay) in first column and Allele Frequencies (AF) of Horn (H), Poll-Celtic (PC) and Poll-Friesian (PF) mutations in column 2 across ten Australian beef breeds (n = 16,903). Note that presence of H alleles in Angus and PC/PF alleles in Wagyu is indicative of cross-bred individuals. Overall, the results show that polled animals of Australian beef breeds predominantly carry PC as compared to PF, except for Shorthorn.