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Performance of different SNP panels for parentage testing in two East Asian cattle breeds

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Summary

The International Society for Animal Genetics (ISAG) proposed a panel of single nucleotide polymorphisms (SNPs) for parentage testing in cattle (a core panel of 100 SNPs and an additional list of 100 SNPs). However, markers specific to East Asian taurine cattle breeds were not included, and no information is available as to whether the ISAG panel performs adequately for these breeds. We tested ISAG's core (100 SNP) and full (200 SNP) panels on two East Asian taurine breeds: the Korean Hanwoo and the Japanese Wagyu, the latter from the Australian herd. Even though the power of exclusion was high at 0.99 for both ISAG panels, the core panel performed poorly with 3.01% false-positive assignments in the Hanwoo population and 3.57% in the Wagyu. The full ISAG panel identified all sire–offspring relations correctly in both populations with 0.02% of relations wrongly excluded in the Hanwoo population. Based on these results, we created and tested two population-specific marker panels: one for the Wagyu population, which showed no false-positive assignments with either 100 or 200 SNPs, and a second panel for the Hanwoo, which still had some false-positive assignments with 100 SNPs but no false positives using 200 SNPs. In conclusion, for parentage assignment in East Asian cattle breeds, only the full ISAG panel is adequate for parentage testing. If fewer markers should be used, it is advisable to use population-specific markers rather than the ISAG panel.

Keywords false-positive, Hanwoo, panel, paternity, Wagyu

Correct parentage assignment is a fundamental requirement for a successful breeding program so that production performances can be linked back to the correct families to improve estimates of breeding values. However, in commercial breeding programs, pedigree problems can occur due to missing data, human error or even willful forgery. In any of these cases, a DNA-based parentage test can clarify the ancestry and help improve the breeding program.

The International Society for Animal Genetics (ISAG) established a set of single nucleotide polymorphisms (SNPs) for parentage testing of *Bos taurus* cattle that should be used internationally to make results comparable between laboratories. The panel consists of a core 100 SNPs and an additional set of 100 markers (CMMPT 2012). The core panel was mainly derived from European taurine breeds and thus may not be ideal for parentage assignment in distantly related breeds (Werner *et al.* 2004; Lachance & Tishkoff 2013). To address this, ISAG proposed the additional marker panel to increase the exclusion power in indicine and synthetic breeds and added these breeds to the society's comparison tests (http://www.isag.us/comptest.asp?autotry=true&ULnotkn=true).

We tested the core ISAG panel with 100 markers as well as the full ISAG panel with 200 markers in populations of pure black Australian Wagyu and Korean brown Hanwoo. Not all markers were segregating in these populations (99 and 199 SNPs in the Australian Wagyu; 95 and 195 markers in the Hanwoo; Tables S1 and S2). The Wagyu population was genotyped with the 50K BovineSNP50 v2

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BeadChip (Matukumalli *et al.* 2009; Illumina, Inc.), and genotypes were available for 27 offspring as well as for five sires with offspring ranging between two and 13 per sire. The Hanwoo population was genotyped with the 700K BovineHD BeadChip (Illumina, Inc.) and consisted of 290 offspring from 36 sires with six to nine offspring per sire. Information about the relationship between sires was not available; however, due to the small population size of these breeds it can be expected that sires are related to a certain extent.

As a first evaluation of how well the marker panels can be expected to work, we calculated the exclusion power for the scenario of one known parent according to Jamieson and Taylor (1997). Both the core and the full ISAG panel achieved a total exclusion power of 0.99 in both populations, which suggested that the ISAG panels would work very well to identify wrong sire–offspring assignments.

Further, for each population, we used the marker genotypes to calculate the number of opposing homozygotes of every animal against all others using hsphase in the R package (Ferdosi *et al.* 2014). An opposing homozygote, for any given marker, is defined as one individual being homozygous for an allelic variant and the other individual being homozygous for the alternative allele. This is an easy way to identify Mendelian inconsistencies, which should not occur in true sire–offspring relationships, except for genotyping errors or an unlikely mutation. On the other hand, opposing homozygotes should occur more frequently between unrelated animals and can be used to exclude a parentage relationship (Hayes 2011).

A straightforward approach to evaluate the effectiveness of a parentage marker panel is to calculate the difference between the smallest number of opposing homozygotes found across all false sire–offspring relations (i.e. all pairwise combinations except the real sire–offspring pairs) and the maximum number of opposing homozygotes in the correct sire–offspring pairs (Hayes 2011). This difference can be divided by the total number of SNPs in the panel to allow comparisons between different panel sizes. Herein, we refer to this difference as the 'separation value'. Intuitively, the larger the value, the better the panel is at resolving parentage assignments, and if the value becomes zero or negative, a perfect separation between true and false sire–offspring relations is impossible. It should be noted that the actual separation values are only meaningful within a data set, as the numbers of opposing homozygotes are panel, population and animal specific.

For the 100 SNPs from the ISAG core panel, the separation value was zero in both populations (Fig. 1), indicating difficulties in separating true from wrong sire–offspring relations, which is in contrast to the high power of exclusion. False-positive rates were then calculated as the proportion of wrongly assigned sire–offspring relations from the total number of identified relations from all pairwise sire–offspring combinations. False-negative rates were calculated as the proportion of wrongly excluded sire-offspring relations from the total number of possible true relations. Following ISAG guidelines and to accommodate genotyping errors, we allowed for up to one opposing homozygote in accepted parentages. As expected from the separation values, the false-positive rates in the core panel were 3.57% for the Wagyu and 3.01% for the Hanwoo. False-negative rates were all zero (Table 1). The contradicting expectations between the power of exclusion and the separation value might be due to the fact that exclusion power (1) does not accommodate for potential genotyping errors or new mutations and (2) only considers the power to exclude a sire–offspring relation and cannot detect a falsely accepted sire–offspring assignment, which was the limitation of the ISAG panel. Thus, power of exclusion is not very well suited for practical parentage testing.

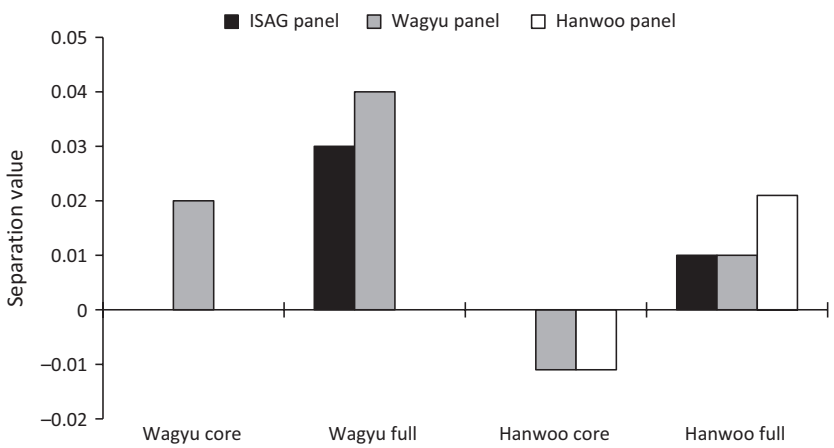

Figure 1 Separation values between true and false sire–offspring relations in the ISAG core and full panels. Separation value is defined as the difference between the maximum number of opposing homozygotes in true relations and the minimum number of opposing homozygotes in false relations, divided by the total number of markers in the panel. Core: 99 markers in the Wagyu population and 95 markers in the Hanwoo population; full: 199 markers in the Wagyu population and 195 markers in the Hanwoo population.

Table 1 Description of panels for parentage test in two different populations with results for a maximum of one or two opposing homozygote mismatches allowed in true sire–offspring relations

				Core panel[2]		Full panel[2]	
Population (#sires/offspring)	Panel	MAF[1]	He[1]	fp% 1M/2M	fn% 1M/2M	fp% 1M/2M	fn% 1M/2M
Wagyu (5/27)	ISAG	0.29	0.39	3.57/3.57	0/0	0/0	0/0
	Wagyu	0.39	0.48	0/3.57	0/0	0/0	0/0
Hanwoo (36/290)	ISAG	0.34	0.43	3.01/11.04	0/0	0/0	2.00/0
	Wagyu	0.35	0.44	7.64/22.46	0/0	0/0	2.00/0
	Hanwoo	0.45	0.49	2.03/7.35	0/0	0/0	0/0

MAF, average minor allele frequency of all markers; He, average heterozygosity of all markers; fp, false-positive rate; fn, false-negative rate; 1M/2M, one or two opposing homozygote mismatches allowed

[1]MAF and He were the same in the core and the full panel

[2]Core panel: 99 markers in the Wagyu population and 95 markers in the Hanwoo population; full panel: 199 markers in the Wagyu population and 195 markers in the Hanwoo population

Using the full ISAG panel (200 SNPS) resulted in a better resolution with a positive separation value in both populations (Fig. 1). False-positive and false-negative rates in the Wagyu population were all zero; but 2.00% of true sire–offspring relations were wrongly rejected in the Hanwoo (Table 1). The number of opposing homozygote mismatches allowed to accept a parentage assignment was then increased to two to account for additional genotyping errors due to the increased number of markers used in the full panel. This allowance eliminated the few false-negative assignments in the Hanwoo population (Table 1).

Although the core panel performed poorly for both East Asian populations, the full panel worked sufficiently with only few false-negative results. This improvement reflects the efforts of the ISAG to make their comparison test workable in a wider variety of cattle breeds but also reflects the increased power provided by the larger number of markers (e.g. the false-positive rate for only the additional marker panel is similar to that of the core panel at 2.68% in Hanwoo).

To improve parentage assignment with fewer marker numbers, we selected new population-specific panels (Tables S1 and S2). In total, 245 SNPs were selected for the Wagyu and 257 for the Hanwoo. For comparative purposes with the ISAG panel, these marker sets were subset to match the numbers of (core and full) ISAG markers segregating in each population. SNP markers for the Wagyu were selected as part of a larger study (including 119 Wagyu) to develop a panel suitable for Wagyu and Australian indicine breeds. The Hanwoo SNPs were selected from a population of 265 animals (with some overlap with the animals of this study). Selection was based on high call rates (GC score), SNPs in Hardy–Weinberg equilibrium (to minimize the possibility of marker fixation in the future), and marker diversity as indicated by high minor allele frequency and heterozygosity to achieve a high level of individual differentiation, and were spread across and within chromosomes to reduce linkage between markers.

The power of exclusion for both population-specific core panels (99 SNPs in Wagyu and 95 in Hanwoo) was the same as for the core ISAG panel (0.99); however, the new full panels (199 SNP in Wagyu and 195 in Hanwoo) reached a power of exclusion of 1 in their respective populations and were thus slightly better than the full ISAG panel.

Further, the new Wagyu panels showed a positive separation value and no false-positive or false-negative assignments, making it a better choice than the core ISAG panel (Table 1). The new Hanwoo panel also performed better than did the ISAG panel in regard to the separation value and had lower false-positive rates (2.03%). The still detectable errors were probably due to the small effective population size of Hanwoo, and small panels do not have enough power to exclude potentially closely related candidate sires (Table 1). Additionally, we tested the new Wagyu panel on the Hanwoo population and found that results were worse in the core panel but similar to the full Hanwoo panel (Table 1). The performance of the Hanwoo panel on the Wagyu population was not evaluated, as only 30 markers were also on the 50K chip.

When we used all markers initially selected for the new panels – 245 in the Wagyu and 257 in the Hanwoo – separation values were higher and there were no false-positives or false-negatives in either population for their respective panel when we allowed for a maximum of one or two mismatch. The Wagyu panel used on the Hanwoo population performed similarly to the ISAG panel in regard to false-negative results and showed 2.00% of sires being wrongly excluded as a parent at one allowed mismatch.

We limited the scope of this work to the evaluation of parentage assignment between presumably unrelated sires and their offspring. For this scenario, all full panels with around 200 SNPs performed well. This will not be so clear cut when candidate sires are closely related (e.g. full sibs or half sibs). When the relationships between the half-sib groups (as a proxy for full-sib sires) were included in the

evaluation, false-positive rates in the Hanwoo population were 3.33% (ISAG), 1.36% (Wagyu) and 0.68% (Hanwoo), and only the full panel (257 SNPs) provided perfect separability. On the other extreme, the core ISAG panel had a false-positive rate of 39.96%.

This study demonstrates that parentage tests in breeds that are only distantly related to European taurines should use the full ISAG panel or define population-specific marker panels with a stringent threshold if fewer marker numbers are available to unambiguously assign parent–offspring relations. This is especially advisable for closed breeds, such as the Korean Hanwoo, that do not require an international comparison.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. 50K BovineSNP50 v2 BeadChip (Btau 3.0).

Table S2. 700K BovineHD BeadChip (Btau 3.0).