Extending ICAR parentage verification certification to whole-genome SNP comparisons

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Current ICAR/ISAG PV SET approach

- **ISAG PV SET composition**
  - Standard panel of 195 SNPS (recently revised) used internationally for parentage verification.
- **Quality requirements**
  - Requires ≥ 95% call rate on the PV SET (at least 185 successfully called SNPs)
  - Minimum of 175 homozygous common SNP comparisons per test required
- **Classification thresholds**
  - Fixed thresholds specifically tuned to the 195 SNP set (200 original)
  - Duo and trio comparisons

Why whole-genome SNP comparisons?

- **Exclusions due to fixed-panel approach**
  - Many platforms do not guarantee coverage of all 195 ISAG PV SNPs, resulting in “LOWCALL” failures
  - Re-genotyping is often impossible due to unavailability of biological samples
- **Heterogenous genotyping ecosystem**
  - Landscape includes 60+ arrays ranging from 3K to 777K SNPs
  - Sequencing-based technologies growing
- **Need for platform-agnostic standards**
  - An alternative (or complementary) method leveraging all available high-quality SNPs is required

Objectives

- **Assess the situation**
- **Establish empirical thresholds on a whole-genome PV situation**
  - Empirically derived
  - Easy-to-apply thresholds for duo-based, whole-genome SNP comparisons
  - Robust across genotyping platforms
- [desired] **Maintain ICAR classification logic**
  - Mirror the existing ACCEPTED / DOUBTFUL / EXCLUDED logic
- **Validate performance**
  - Quantify performance across breeds and densities
  - Assess the value of trio-based verification in high-density contexts

Materials and methods

Data Source and Quality Control

- U.S. National Cooperator Database
- Autosomal markers
- Pre-exclusion of genotypes with < 90% call rate
- Removed clones, split embryos, and identical twins

Genotyping Platforms

- 56 certified SNP arrays ranging from ~3K to 777K SNPs (currently 60+, including WGS-based systems)
- Diverse technologies representing different densities and manufacturers

Testing Populations

- Balanced representation across 5 breeds: Holstein, Jersey, Brown Swiss, Guernsey, Ayrshire
- ~100,000 DUO pairs: ***Known*** true parent-offspring relationships (max 5 per animal)
- ~100,000 NOT DUO pairs: Unrelated pairs + controlled set of full/half sibs (~10% each)

Analysis Pipeline

- 195 vs. WG performance
- Mendelian conflicts per pair
- Metric: % conflicts over total informative homozygous comparisons

Performance
ISAG PV SET

METRIC	ISAG PV SET (195 SNPS)
Exclusions (LOWCALL)	~25% Excluded 42,758 comparisons failed call rate
DUO Ambiguity	754 Doubtful 1,367 Rejected
NOT DUO False Matches	1,865 Wrongly Accepted Large false positive risk
NOT DUO Ambiguity	5,765 Doubtful

Confirmed: LOWCALL is a concern in the existing commercial landscape

Performance: While the 195 performs relatively well, there are still relatively high wrong calls and doubtful results

Performance and thresholds of WG SNPs

SENSITIVITY (TPR)

99.83%

On declared DUO pairs

SPECIFICITY (TNR)

99.97%

On declared NOT DUO pairs

Key Empirical Findings

- **0.6% Acceptance Threshold:** Selected because virtually no unrelated animals fell below this value (except 2 likely pedigree errors).
- **1.0% Rejection Threshold:** Provides clear separation; only a small number of full-sibs were misclassified as "Doubtful" rather than Rejected.
- **The "Doubtful" Zone (0.6% – 1.0%):** Effectively captures edge cases, often linked to low-density chips (e.g., 3K) or specific array quality issues.

Performance Comparison
ISAG PV SET vs. Whole-genome

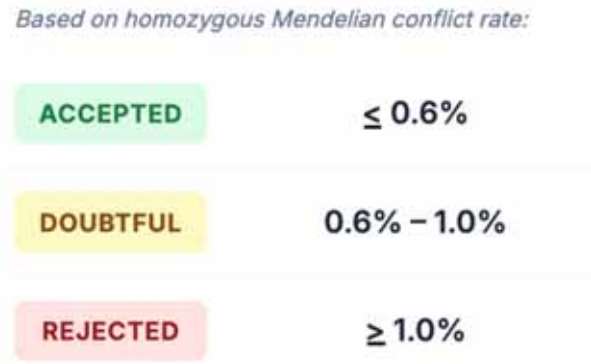
METRIC	ISAG PV SET (195 SNPS)	WHOLE-GENOME (PROPOSED)
Exclusions (LOWCALL)	~25% Excluded 42,758 comparisons failed call rate	Minimal Pre-selected for 90% call rate
DUO Ambiguity	754 Doubtful 1,367 Rejected	161 Doubtful Reduced to 19 with QC SNPs
NOT DUO False Matches	1,865 Wrongly Accepted Large false positive risk	2 Wrongly Accepted High specificity
NOT DUO Ambiguity	5,765 Doubtful	26 Doubtful Reduced to 12 with QC SNPs

Duo comparisons:
Highly discriminative on their own

Trio comparisons:
No benefit

Conclusions and recommendations

- When available, whole-genome comparisons preferred over ISAG PV SET in dairy cattle
  - *Virtually* eliminates "LOWCALL" loss
  - Only use on animals with +7K SNP density or SNP arrays without known QC issues
    - Watch for chromosomal abnormalities!
  - When possible, QC SNPs within chips for better performance
- Included in ICAR guidelines on May 20th, 2026
  - *Section 04 – DNA Technology*,
    - *Chapter 6 – "Parentage Verification Using Full SNP Comparisons"*



Complementary approach to ISAG PV, to be preferred when available.

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- **Purebred breed associations** for providing pedigree data

Thank you for your attention!

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Validation

- Independent(*) population HO/JE
- ~60k comparisons each
- 99.75% sensitivity, 99.95% specificity

DUO comparisons				NON DUO comparisons		
Threshold	Total	Parentage		Threshold	Total	Parentage
0.0%	12745	ACCEPTED		< 0.6%	0	ACCEPTED
0.01% ≥ 0.1%	47454	ACCEPTED		0.61% ≥ 0.7%	0	DOUBTFUL
0.11% ≥ 0.2%	1988	ACCEPTED		0.71% ≥ 0.8%	4	DOUBTFUL
0.21% ≥ 0.3%	280	ACCEPTED		0.81% ≥ 0.9%	8	DOUBTFUL
0.31% ≥ 0.4%	88	ACCEPTED		0.91% ≥ 1%	12	DOUBTFUL
0.41% ≥ 0.5%	39	ACCEPTED		1.01% > 2%	1373	EXCLUDED
0.51% ≥ 0.6%	25	ACCEPTED		2.01% > 3%	3324	EXCLUDED
0.61% > 1%	103	DOUBTFUL		> 3%	53582	EXCLUDED
> 1%	62	EXCLUDED				

- Most of >1% results likely linked to DNA quality or density
  - 40's/50's/60's bulls
  - embryo genotypes (method)
  - Few animals with low density chips (<10k SNPs)

When using QC SNPs **on not desired results**:
not accepted in DUO:
14 DOUBTFUL,
4 EXCLUDED (3 LLD chip, 1 embryo)
not excluded in NON DUO: 2 DOUBTFUL