Cattle Molecular Marker and Parentage Testing (CMMPT)

Organised by a Standing Committee: YES

Meeting information

Date: **Monday, July 21**	
Time: **1:30 PM - 5:30 PM**	
Number of participants: 55	

Chair

Name: Jiansheng Qiu	
Affiliation: Neogen Genomics	
Contact email: jqiu@neogen.com	

Co-Chair (optional)

Name: Emiliano Lasagna	
Affiliation: Universitá degli Studi di Perugia, Italy	
Contact email: emiliano.lasagna@unipg.it	

Agenda

- 1:30 pm: Welcoming Remarks
- 1:40 pm: Presentation by Duty Lab
- 2:00 pm: Presentation of STR results
- 2:40 pm: Presentation of SNP results
- 3:00 pm: Presentation of the Next Comparison Test (2025-2026)
- 3:30 pm: Coffee Break
- 4:00 pm: OP116 A Case-Parent Trio WGS Study Reveals Genetic Risk Factors for Abortion in Hanwoo (*Bos taurus* coreanae). **J. Seo** et al., *Seoul National University Seoul, Republic of Korea*
- 4:20 pm: OP117 ISAG Bursary Award: Utilisation of genomic parentage verification and discovery techniques in the South African Beefmaster cattle breed. **J. J. Reding** et al., *University of Pretoria, South Africa*
- 4:40 pm: OP118 ISAG Bursary Award: Application of Variation Graphs for Genotyping Structural Variants in 14 French Cattle Breeds. **M. M. Naji** et al., *Université Paris Saclay, France*
- 5:00 pm: Business Meeting and Closing Remarks

<u>Summary of the meeting</u>

1. Welcoming Remarks by the CMMPT Chair (Jiansheng Qiu)
Introduction of the current CMMPT committee members and outlines of the agendas.

2. Duty Lab Report (Guillermo Giovambattista)
One hundred and eight laboratories from 47 countries participated in the comparison tests (CTs): 87 labs for STR only, 53 labs for SNP only, and 32 labs for both STR and SNP. However, only 82 labs reported STR results (~94%) and 43 labs reported SNP results (~81%). Fifty-six additional batches of DNA samples were sent to participants upon their requests due to different issues such as custom clearance delays (mainly due to expired import permits and/or additional document requirement by a few countries), carrier problems, and sub-optimum DNA quality (degradation and/or low DNA quantity). The CMMPT committee members held a meeting on the tenth of February 2025 following complaints from several labs regarding poor DNA. The committee reviewed preliminary results/emails and decided to exclude two DNA samples (BCT-08 and BCT-11) from ranking calculation by FASS but recommend the participants to submit results of the two DNAs. The decisions were notified to all participants by an email send by Jeremy Holzner at FASS on behalf of the CMMPT committee.

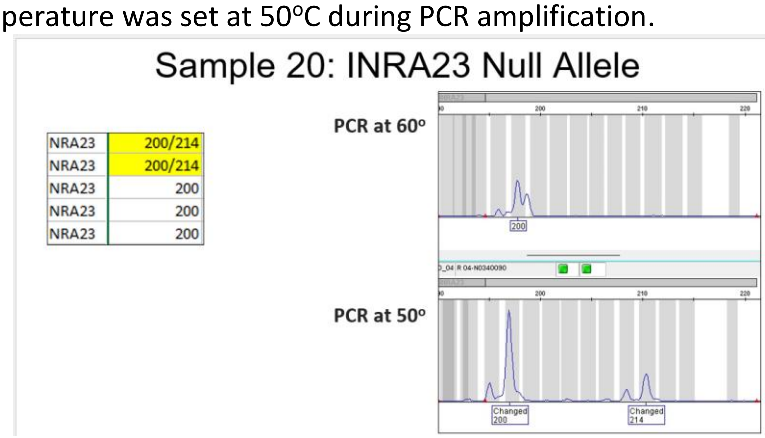
3. STR CT Report (Rosina Fossati)
All 12 required STR markers performed well (see a summary table below).

Locus	Results	Consensus	Relative Accuracy
BM1818	1468	1460	99.46
BM1824	1465	1462	99.80
BM2113	1468	1462	99.59
ETH10	1468	1458	99.32
ETH225	1466	1444	98.50
ETH3	1468	1451	98.84
INRA23	1468	1447	98.57
SPS115	1468	1454	99.05
TGLA122	1454	1440	99.04
TGLA126	1468	1464	99.73
TGLA227	1467	1445	98.50
TGLA53	1454	1431	98.42

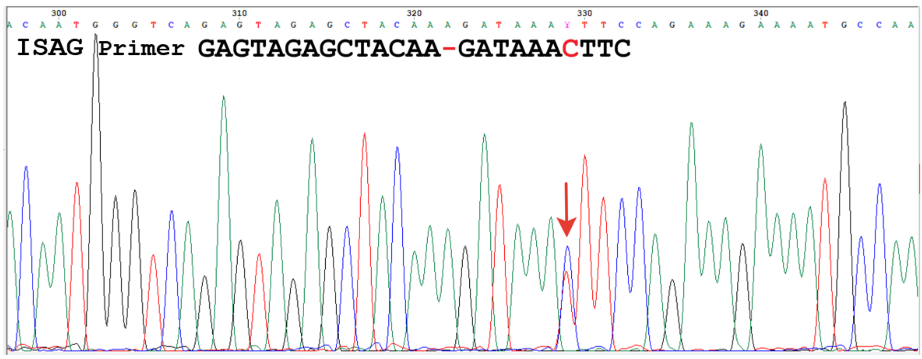
Like the finding from last (2022-2023) STR CT, Tgla53 had the highest error rate among labs.

Laboratory errors for each marker

A null allele (214) of INRA23 was reported on BCT-20, and the allele can only be detected when the annealing temperature was set at 50°C during PCR amplification.



It appears that the current ISAG recommended INRA23 forward primer (GAG TAG AGC TAC AAG ATA AAC TTC) has two mismatched (see a graph below). A new INRA23 forward primer (GAG TAG AGC TAC AAA GAT AAA CTT C) is recommended to enhance mismatch tolerance and prevent the allele dropout. Since this is a new finding, the CMMPT committee approved to accept both 200/200 and 200/214 as correct call for the INRA23 on BCT-20!



Thirty-five labs send comments along with their results, 16 of which indicated DNA quality issues with BCT-08 and BCT-11. A few labs sent emails to FASS indicating they made transfer errors and/or typos and asked for potential corrections.

Parentage questions:
<u>Question 1 - Does sample BCT-11 qualify as the offspring of sample BCT-15?</u>
No effect because sample 11 was eliminated from the CT.
<u>Question 2 - Does sample BCT-17 qualify as the offspring of sample BCT-18?</u>
98.7% answered NO. One lab couldn't respond the question.

4. SNP CT Report (Jiansheng Qiu)
Of 43 labs that submitted results, 31 of them (~72%) used Illumina Infinium Bead-ARRAY, 3 labs used ThermoFisher's Axion arrays, and 9 labs used ThermoFisher's target sequencing platform. As expected, the number of labs utilizing genotype by sequencing was noticeably more than the previous CT (3 labs).

Nineteen labs submitted comments on sample issues (poor DNA quality and/or low total DNA quantity for a few samples). Below is a summary of call rates per each of the 22 samples across all participating labs.

Call rates per sample											
	BCT-01	BCT-02	BCT-03	BCT-04	BCT-05	BCT-06	BCT-07	BCT-08	BCT-09	BCT-10	BCT-11
%failed calls	0.027072	0.028026	0.071318	0.025283	0.025164	0.041264	0.02588	0.571974	0.028384	0.116518	0.580561
%sucess calls	0.972928	0.971974	0.928682	0.974717	0.974836	0.958736	0.97412	0.428026	0.971616	0.883482	0.419439
CMMP Decision								excluded		newly excluded	excluded
	BCT-12	BCT-13	BCT-14	BCT-15	BCT-16	BCT-17	BCT-18	BCT-19	BCT-20	BCT-21	BCT-22
%failed calls	0.032439	0.033035	0.044126	0.027072	0.039237	0.027907	0.025403	0.030769	0.027072	0.003816	0.004174
%sucess calls	0.967561	0.966965	0.955874	0.972928	0.960763	0.972093	0.974597	0.969231	0.972928	0.996184	0.995826

Besides two DNA samples (BCT-08 and BCT-11) that showed issues with STR, BCT-10 had ~88% call rate due to low DNA quantity. Based on the preliminary results provided by FASS, the CMMPT held another meeting, and the committee decided to exclude 3 samples (BCT-08, BCT10, and BCT-11) from ranking calculation for the cattle SNP CT.

Since BCT-11 was eliminated from the CT, parentage question #1 was dropped. Forty-two out of 43 labs correctly answered the parentage question #2 whereas 1 lab did not give answer. Note that the answers to the parentage questions are not used for ranking.

5. Next (2025-2026) Comparison Test (Emiliano Lasagna)

Volunteers for duty Lab(s) needed and Why should I work as a duty lab!

- Taking on the duty lab role means actively contributing to ISAG life.
- It's a great opportunity to gain hands-on experience with lab operations and protocols.
- It also allows you to expand your international network and improve your teamwork skills.
- It's an amazing experience!

Practical suggestions for future duty lab(s):

- Collect more blood samples than requested (some problems can occur in the DNA extraction or during the genotyping),
- Prepare extra sets (about 10 more, already officially requested from ISAG due to collaboration with ICAR),
- Use a minimum of 2 samples as reference samples and send them to 2-3 reputable labs for independently generating genotypes (both for STR and SNP CTs),
- Carefully check the quantity and the quality of extracted DNA,
- Carefully read the previous CT reports and contact previous duty labs for advice,
- Generate draft report template(s) and submit to Chair/Co-Chair of the CMMPT for approval.

6. Overall evaluation/comments by the CMMPT committee:

- Great STR and good SNP CT test results!
- DNA quality (mainly low amount of a few DNA) adversely affected the SNP results; email requests were addressed by the Duty lab, CMMPT committee, and FASS promptly.
- No transfer/typo or other clerical errors were allowed to change!
- While fixed arrays appeared to give more consistent SNP results, genotyping by sequencing was adopted by more labs.
- A useful ICAR parentage guideline: https://www.icar.org/index.php/icar-recording-guidelines/
- **Special thanks to**
    - Guillermo from the duty lab for diligently addressing requests from many labs
    - Jeremy Holzner from FASS for generating shipping labels and data reports

7. Business Meeting and Elections

New Committee chair

Chair: Emiliano Lasagna
Term of service: second term of service 2025-2029
Affiliation: Universitá degli Studi di Perugia, Italy
E-mail address: emiliano.lasagna@unipg.it

New Committee co-chair

Co-Chair: Paul Flynn
Term of service: first term of service 2023-2027
Affiliation: Weatherbys Scientific
E-mail address: pflynn@weatherbys.ie

New Committee members

Other committee members	First term of service	Second term of service	Email address
Marcela Martinez	2019-2023	2023-2027	mmartinez@sra.org.ar
Guillermo Giovambattista	2021-2025	2025-2029	ggiovam@fcv.unlp.edu.ar
Rosina Fossati	2021-2025	2025-2029	fossati@genexa.com.uy
Yoshiyuki Miyazaki	2021-2025	2025-2029	y-miyazaki@liaj.or.jp
Kimberley Walsh	2025-2029		kwalsh@neogen.com
Romy Morrin-O'Donnell	Ex Officio		rmorrin@weatherbys.ie

Note: One term runs for two bi-annual conferences (i.e. four years)

Next Comparison test (2026-2027): YES

Next Duty Laboratories for the 2025-2027 CTs: To be determined

SIGNATURES

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Chair **Co-Chair** **Duty laboratory**