Equine Genetics and Thoroughbred Parentage Testing Workshop

Organised by a Standing Committee: YES

Meeting information

Date: July 6th
Time: 2:00 p.m. to 5:30 p.m. CDT
Number of participants: ~ 90

Chair

Name: Marcela Martinez
Affiliation: Laboratorio de Genética Aplicada. Sociedad Rural Argentina. Argentina
Contact email: mmartinez@sra.org.ar

ISAG INTERNATIONAL SOCIETY FOR ANIMAL GENETICS

Agenda

2:00 PM		**Welcoming Remarks.**
2:10 PM		**Horse Comparison Test.**
2:30 PM		**Donkey Comparison Test.**
2:50 PM	89726	**Contribution of STR genotyping to animal clinical cytogenetics.** Terje Raudsepp*, Josefina Kjöllerström, and Rytis Juras, *School of Veterinary Medicine, Texas A&M University, College Station, Texas, USA.*
3:10 PM		**Election of CT Duty Labs, Election of Committee and Any Other Business.**
3:30 PM		**Lunch Break, Exhibition and Poster Viewing.**
4:00 PM	89947	**Invited Workshop Presentation: Improving parentage verification, transiting from STR to SNP and beyond from a bovine perspective.** Matthew McClure*, *ABS-Global*, *Deforest, Wisconsin, USA.*
4:20 PM	89455	**Development of a Robust Across Breed Equine Parentage SNP Panel for ISAG Approval.** R.R. Bellone*[1,2], T.A. Mansour[2,3], E. Esdaile[1], B. Wallner[4], T. Raudsepp[5], B. Till[1], A. Kallenberg[1], S. Hughes[1], S. Chadaram[6], S. Shrestha[6], R.A. Grahn[1], Equine ISAG SNP Panel Consortium[9], F. Avila[1], M. McCue[7], P. Flynn[8], [1]*Veterinary Genetics Laboratory, School of Veterinary Medicine, UC Davis*, *Davis, CA, USA*, [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, UC Davis*, *Davis, CA, USA*, [3]*Department of Clinical Pathology, School of Medicine, Mansoura University*, *Mansoura, Egypt*, [4]*Institute of Animal Breeding and Genetics, Veterinary University of Vienna*, *Wien, Austria*, [5]*Veterinary Integrative Biosciences, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University*, *College Station, TX, USA*, [6]*Thermo Fisher Scientific*, *Austin, TX, USA*, [7]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota*, *St. Paul, MN, USA*, [8]*Weatherbys Scientific*, *Kildare, Ireland*, [9]*Various Affiliations*.
4:40 PM		**Open Panel to discuss several aspects of SNPs in horses (Panel and next CT, transition between techniques, others).**

Summary of the meeting

Including votes, decisions taken and plans for future conferences

1. Welcoming Remarks

The agenda of the workshop included the discussion of Comparison tests (CTs) results for Horse STR and Donkey STR CTs carried out during the period 2022-23. In this occasion, the Horse SNP CT was deferred until to define the best Panel to be officially adopted by the community.

2. Horse STR CT Discussion

Duty Laboratory: Dr. Rebecca Bellone, UC Davis, Veterinary Genetics Laboratory (USA).

Samples: 23 DNA samples (3 references) representing 9 breeds – Thoroughbred (11), Quarter Horse (4), Appaloosa (1), Arabian (1), Friesian (1), Paint Horse (1), Percheron (1), Trakehner (2) and Warmblood (1). Extractions were done with Gentra® Puregene®-Qiagen procedure. Two labs reported issue with sample ECT14 to complete testing (replacement sent to one of them).
All reference samples were used in the previous CT (2021). They were selected due to some marker issues found in each one (ASB2 alleles B and C, HMS3 allele M for M/N genotype and TKY337 _Back up Panel_ allele P).

Participants: One-hundred and seven labs requested samples and 100 labs reported results. Five labs did not receive the samples and required a second or third (1 case) shipment. There was also delay with 3 of 4 South Africa's labs due to importation documents required.

Summary of Results:

ISAG Panel:
The relative overall marker concordance among labs was good, ranging from a minimum of 96.30% (ASB23) to a maximum of 99.70% (HTG4). See the table below. For ASB23 there was an error in draft compilation for relative accuracy for ASB23 as several labs did not report this marker in all samples and this was originally counted as wrong instead of missing, when corrected relative accuracy for this marker is 98.2%
Some labs missed the allele "M" of marker HMS3 while six labs had discordant types for ECT7.
Eighty-six percent of the labs ranked 1 (100 – 98% absolute concordance among labs), slightly higher than in the previous CT (83%). Like in the previous CT, only 2% of the labs ranked below 80% of concordance.

Locus	Relative Locus Accuracy
AHT4	98,94%
AHT5	98,3%
ASB17	98,99%
ASB2	97,93%
ASB23	98,33%
HMS2	97,88%
HMS3	97,3%
HMS6	98,03%
HMS7	98,73%
HTG10	97,02%
HTG4	99,70%
VHL20	98,94%

Parentage questions:

Parentage questions concordance was very good. The parentage question one asked if sample #16 qualifies as the offspring of sample #15. Ninety-four percent of the labs answered correctly (Yes), there were 3 wrong answers due to a confusion with the first set of Instructions sent to the labs. Same happens with the second question that asked if sample #10 qualifies as the dam of sample#19. The correct answer is No (97%) with 3 wrong answers.

Back Up Panel:

For the Back Up Panel, the highest discrepancy was shown at marker TKY344 (sample #10) due mainly to one lab that have several miscalls in this marker, while 5 different labs did not identify "K" allele in ECT20 "K/O" sample, but successfully identified it in 2 other K/O" samples. 2 labs called "J/K as "K", and 1 lab called same horse as "J".

TKY337 score improves respect previous CTs probably due to the notes documented in previous CT reports and inclusion of a samples carrying the “problematic” allele “P”, as one of the reference samples.

Discussion Points:

The ST Committee advised the participants to check previous workshop reports to look for frequent causes of marker discrepancies in the CTs. Thus, there was a proposal to send the last workshop report to the participants of the next CT. In addition, it was also advised to be careful with data transcription to the excel report as several labs requested changes in the final score due to this kind of errors. Finally, it was suggested to include as reference for the next CT, a sample with the “M” allele in HMS3.

Dr. Rebecca Bellone raised a motion to carry out an unofficial CT of diagnostic markers, starting with Agouti (Indel type), MC1R (SNP) and Grey (SV) tests, including control samples for them. It

was advised to develop a consistent nomenclature for reporting and to collect the methodology of testing. An extra option in the parentage application form will be include for the new CT.

3. Donkey STR CT Discussion

Duty Laboratory: Katie Martin, Etalon Diagnostics.

Samples: DNA from 22 DNA samples (2 references) were included. Samples 1-15 were received as genomic DNA provided by Cornell University, at concentrations varying from 10-60 ng/uL, while samples 16-23, were extracted at Etalon from blood using the Qiagen Tissue kit and provided to laboratories at a concentration >12 ng/uL.

Participants: Twenty-seven labs requested samples and 23 labs reported results. Seven labs requested one or more samples (mainly samples 17, 18 and 19) or the full set of samples (1).

Summary of Results:

ISAG Panel:
The relative overall marker concordance among labs was good, ranging from a minimum of 94.33% (TKY343) to a maximum of 100% (ASB23, HMS2). The lower accuracy in marker TKY343 reflects a transcription error from one of the participant labs, which also affected marker TKY337, the second lowest marker score.
Seventy-eight percent of the labs ranked 1 (100 – 98% concordance among labs) and two labs ranked below the 80% of concordance.

Locus	Relative Accuracy
AHT4	99.17
ASB23	100.00
HMS18	99.77
HMS2	100.00
HMS3	96.45
HMS6	99.79
HMS7	98.34
HTG10	98.96
HTG7	95.84
TKY297	97.05
TKY312	99.55
TKY337	95.01
TKY343	94.33

Parentage questions:

Parentage questions concordance was reasonably good. The parentage question one asked if sample #7 qualifies as the parent of sample #12. Ninety-one percent of the labs answered correctly (No). The second question asked if sample #17 qualifies as the parent of sample #22. Ninety-one percent of the labs answered correctly (Yes).

Discussion Points:
The ST Committee advised the participants to check previous workshop reports to look for frequent causes of discrepancies in the CTs. Thus, there was a proposal to send the last workshop report to the participants of the next CT. In addition, it was also advised to be careful with data transcription to the excel report as several labs requested changes in the final score due to this kind of errors.

Oral presentations:

1) **Contribution of STR genotyping to animal clinical cytogenetics**. Dr. Terje Raudsepp

2) **Invited Workshop Presentation: Improving parentage verification, transiting from STR to SNP and beyond from a bovine perspective.** Dr. Matthew McClure.

Discussion Points:
After Dr. McClure's talk, the utility of imputation to convert SNP into STR, during the transition between techniques, was discussed. In Dr. McClure's experience and with Weatherbys lab's input, imputation was discouraged for equine migration.

3) **Development of a Robust Across Breed Equine Parentage SNP Panel for ISAG Approval**. Dr. Rebecca Bellone.

The international project lead by Dr. Bellone consisted of a 3-stages plan.

Phase 1: Identify an initial panel of ~ 1000 SNPS for consideration in the parentage panel.

Phase 2: Genotype SNPs from Phase 1 in a reference sample set of 192 horses across laboratories and platforms to select the most concordant for Phase 3.

Phase 3: Evaluate panel of concordant SNPs from Phase 2 for efficacy in parentage testing across breeds and make recommendation on primary and back up panels.

As result of the Phase 1, 1291 autosomal SNPs were selected based on the highest MAF in the largest number of studies/breed. Also, markers in sex chromosomes were selected in order to have a set of polymorphisms able to be used for quality control for sex and for sex chromosome diagnostic anomalies (aneuploidies, sex-reversal). From the non-PAR region, 39 were selected on Y chromosome and 150 in X chromosome, plus 20 X-PAR markers. Both autosomal and sex SNPs showed a good distribution across chromosomes.

In Phase 2, 192 samples from different breeds (QH, TB, Arabian/Barb and their crosses, Warmblood, Argentine Creole, Friesians, Draft Horse, Pony, Standardbred and Icelandic Horse) were tested by 14 laboratories. Three of these labs contributed with data tested on 2 platforms. Eight labs analysed in various SNP arrays (1 GGP Equine V5 (GGP), 6 Equine 80 Select (80K), 1

Axiom Equine 670K array) and 9 labs genotyped with a kit provided by Thermo Fisher Scientific (GBS-1489) in Ion Torrent S5 platform.

From the collected results, the min, max, and average concordance (absolute accuracy) was calculated for each sample and marker across all platforms/replicates.

Results analysed as CT, based on number of markers that could be tested in platform:

Platform	Consensus	Markers in Platform	Number of Possible Results adjusted for Y markers and number if Females	Adjusted Absolute Genotyping Accuracy	Rank
GBS	178806	1489	282250	63.35	5
80K arrray	233641	1456	279552	83.58	4
GBS	253950	1489	282250	89.97	3
75K array	268034	1498	283978	94.39	3
GBS	266656	1489	282250	94.48	3
GBS	268948	1489	282250	95.29	2
GBS	269057	1489	282250	95.33	2
GBS	270742	1489	282250	95.92	2
GBS	271699	1489	282250	96.26	2
GBS	275474	1489	282250	97.60	2
GBS	279030	1489	282250	98.86	1
80K arrray	275980	1456	279552	98.72	1
80K arrray	277019	1456	279552	99.09	1
670K array	276672	1449	278208	99.45	1
80K arrray	278363	1456	279552	99.57	1
80K arrray	278554	1456	279552	99.64	1
80K arrray	279113	1456	279552	99.84	1

The results were adjusted for removing Y markers in females from count.

For autosomes, results analysed based on average concordance:

_ >95% average concordance: 787 SNPs

_ >97% average concordance: 381 SNPs

For Y chromosome: 8 markers were selected that pass 90% concordance threshold in males and have low genotyping rate in female <1%

For X chromosome: non PAR, 73 markers were selected with 95% concordant in females and low heterozygosity in males. PAR: 4 SNPs remained with 95% concordance in males and females combined.

Phase 3 consisted of trio testing in diverse breeds, with known results based on previous STR testing. One hundred sixty-seven trios from different breeds (range of 1-23 trios/breed and more than 10 breeds with 7 trios), which were Expected to Qualify, were genotyped using ThermoFisher Ion Torrent Technology. Analysis was done with both markers from 95% concordance Phase 2

and 97% concordance Phase 2. Using 95% concordance markers, 54 exclusions were found in 37 of 167 trios, while using 97% concordance, exclusions were detected in only 8 of 167 trios. In the first case, 3 markers (one on chromosomes 4, 10 and 20) were responsible for 25/54 exclusions.

Based on ICAR parentage rules, autosome markers in 97% concordance panel would qualify all 167 trios analyzed.

https://www.icar.org/Documents/GenoEx/ICAR%20Guidelines%20for%20Parentage%20Verification%20and%20Parentage%20Discovery%20based%20on%20SNP.pdf
Briefly, there 2-steps in the parentage analysis:

Step 1: Investigate offspring/parent combinations- homozygous SNPs with opposing genotype considered a mismatch.

Parentage Accepted: 0 – 4 mismatches 0-1%
Parentage Doubtful: 5 – 10 mismatches 1%+1-2.5%
Parentage Excluded: >10 mismatches >2.5%

Step 2: Trio testing –offspring heterozygous and if parents are same homozygous genotype =mismatch.

Parentage Accepted: 0 – 6 mismatches 0-1.5%
Parentage Doubtful: 7 – 14 mismatches 1.5% +1-3.5%
Parentage Excluded: >14 mismatches >3.5%

On the other hand, the analysis of the trios Expected to be Excluded, using 97% concordance markers (466 autosome and sex chromosomes markers), showed that the exclusion markers range between 10-227 for different breeds.

An additional analysis indicated that the selected X and Y markers should allow for identification of XO and potentially XY sex reversal cases.

Discussion points:

Based on this presentation, an open discussion on ISAG approval was started.

The first point was the motion to use the 97%concordance set of autosome markers as Core Panel (381 SNPs). This motion was approved per unanimity. The set of sex markers (8 markers in Y chromosome and 77 markers in X chromosome), plus the additional 406 markers in the 95% concordance panel, which was proposed as Backup Panel, were commented but not voted.

The next point was the decision to carry out and official vs non-official CT. In favor of the ranked (official) proposal was to avoid a longer period of time without ranking; against the proposal, the lack of experience in this panel that should be tested more extensively by the community. The motion was voted, again by Institutional members, with 11 votes against an official CT and 9 in favor.

Therefore, the next Horse SNP CT will be carried out with a Core Panel of 381 autosome markers and will not be officially ranked. For optimal evaluation, institutional members are encouraged

to participate in this unofficial CT. Participants should inform the platform, methodology (array, GBS kit) used and the threshold values applied for QC filtering in the pipeline.

New Committee chair

Chair: Leanne Van de Goor
Term of service (*add years of first and second term of service*): 2021-2025 (first term)
Affiliation: VHL Genetics
E-mail address: leanne.vandegoor@vhlgenetics.com

New Committee co-chair (optional)

Chair:
Term of service (*add years of first and second term of service*):
Affiliation:
E-mail address:

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

New Committee members

Other committee members	First term of service (from year to year)	Second term of service (from year to year)	Email address
Romy Morrin	ISAG-ISBC Liaison (*ex officio*)		rmorrin@weatherbys.ie
Rebecca Bellone	2019-2023	2023-2027 (confirmed)	rbellone@ucdavis.edu
Guillermo Giovambatista	2019-2023	2023-2027 (confirmed)	guillermogiovambattista@gmail.com

Amparo Martinez	2021-2025		amparomartinezuco@gmail.com
Teruaki Tozaki	2021-2025		ttozaki@lrc.or.jp
Paul Flynn	2021-2025		pflynn@weatherbys.ie
Pedro J. Azor Ortiz	2023-2027		pedroazor@lgancce.com

COMPARISON TEST (2022-2023) YES

Duty laboratory: HORSE STR CT

Contact person: Rebecca Bellone
Affiliation: VGL, UC Davis. USA
E-mail address: rbellone@ucdavis.edu

Comments (issues rising)

See Discussion Points of Horse STR Duty Lab.

List of recommended markers with primer information

Horse Core Panel: VHL20, AHT4, AHT5, ASB2, HMS6, ASB23, HTG10, HMS3, HMS2, HTG4, HMS7, HTG10.

AHT4 F: AACCGCCTGAGCAAGGAAGT /**AHT4 R**: CCCAGAGAGTTTACCCT

AHT5 F: ACGGACACATCCCTGCCTGC /**AHT5 R**: GCAGGCTAAGGAGGCTCAGC

ASB2 F: CCACTAAGTGTCGTTTCAGAAGG /**ASB2 R**: CACAACTGAGTTCTCTGATAGG

ASB17 F: ACCATTCAGGATCTCCACCG /**ASB17 R**: GAGGGCGGTACCTTTGTACC

ASB23 F: GAGGGCAGCAGGTTGGGAAGG /**ASB23 R**: ACATCCTGGTCAAATCACAGTCC

HMS2 F: CTTGCAGTCGAATGTGTATTAAATG /**HMS2 R**: ACGGTGGCAACTGCCAAGGAAG

HMS6 F: GAAGCTGCCAGTATTCAACCATTG /**HMS6 R**: CTCCATCTTGTGAAGTGTAACTCA

HTG4 F: CTATCTCAGTCTTGATTGCAGGAC /**HTG4 R**: CTCCCTCCCTCCCTCTGTTCTC

VHL20 F: CAAGTCCTCTTACTTGAAGACTAG /**VHL20 R**: AACTCAGGGAGAATCTTCCTCAG

HTG10 F CCTAATGTCATATGGAAAGCCTTG /**HTG10 R** TGGGCTTTTTATTCTGATCTGTCACATTT

HMS3 F ACATCAGTCAGAAGCTGCGAAC /**HMS3 R** CCCCTCTTGCTCTAAAGCCCCA

HMS7 F: TGTTGTTGAAACATACCTTGACTGT ** /**HMS7 R**: CAGGAAACTCATGTTGATACCATC

** original sequence; can produce null allele. Alternate sequence for consideration: TGTTSTTGAAACATACATTGACTGT.

COMPARISON TEST (2022-2023) YES

Duty laboratory: Donkey STR CT

Contact person: Katie Martins
Affiliation: Etalon Diagnostics, United States.
E-mail address: khoefs@etalondx.com

Comments (issues rising)

See Discussion Points of Donkey STR Duty Lab.

List of recommended markers with primer information

Donkey Core Panel: AHT4, HMS6, ASB23, HTG10, HMS3, HMS2, HTG7, HMS7, HMS18, TKY297, TKY312, TKY337, TKY343.

Primer sequences are the same of those used for Horses (AHT4, HMS6, ASB23, HTG10, HMS3, HMS2, HMS7)

Primers specific for Donkeys:

HTG7-F: CCTGAAGCAGAACATCCCTCCTTG /**HTG7-R**: ATAAAGTGTCTGGGCAGAGCTGCT

HMS18-F: CAACAATGAAAATTTGTCCTGTGC /**HMS18-R**: GTAAATGAGTAGACAATCATGAGG

TKY297-F: GTCTTTTTGTGCCTCTGGTG /**TKY297-R**: TCAGGGGACAGTGGCAGCAG

TKY312-F: AACCTGGGTTTCTGTTGTTG /**TKY312-R**: GATCCTTCTTTTTATGGCTG

TKY337-F: TTTTGAGCAGAGCAGGGTTT /**TKY337-R**: CTTGTGCCCCTCATGTCTTT

TKY343-F: TAGTCCCTATTTCTCCTGAG /**TKY343-R:** AAACCCACAGATACTCTAGA

Duty laboratory for the next comparison test with contact details

Duty laboratory: HORSE STR-SNP CT

Contact person: to be confirmed
Affiliation:
E-mail address:

Duty laboratory: Donkey STR CT

Contact person: to be confirmed
Affiliation:
E-mail address:

SIGNATURES

Chair Marcela Martinez **Horse Duty laboratory**

Robin Everts **Donkey Duty laboratory**