ISAG
INTERNATIONAL SOCIETY FOR ANIMAL GENETICS

Equine Genetics and Thoroughbred Parentage Testing Standardization Workshop

Organised by a Standing Committee: YES

Meeting information

Date: July 25th, 2025
Time: 8:00 – 11:50 am
Number of participants: 65-70

Chair

Name: Leanne van de Goor
Affiliation: VHLGenetics
Contact email: Leanne.vandegoor@vhlgenetics.com

Agenda

8:00 AM	**Welcoming Remarks.**
8:10 AM	**Horse STR and SNP Comparison Test Report- presented by Dr. Rebecca Bellone**
8:40 AM	**Donkey STR Comparison Test Report- presented by Dr. Rebecca Bellone**
9:00 AM 95375	**Development of a Robust Across Breed Equine Parentage ISAG SNP Panel.** R.R Bellone*[1,2], E. Esdaile[1], F. Avila[1], B.J. Till[1], B. Wallner[3], T Raudsepp[4], S. Hughes[1], J. Hughes[1], Grahn R[1], S Chadaram[5], S. Shrestha[5], A.S. Grulikowski[1], M. McCue[6], P. Flynn[7], T. Mansour[2], [1]*Veterinary Genetics Laboratory, University California Davis, Davis, CA, USA*, [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA*, [3]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, AT*, [4]*Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, School of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA*, [5]*Thermo Fisher Scientific, Waltham, MA USA*, [6]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA*, [7]*Weatherbys, Kildaire, IE.*

9:20 AM	94662	**Evaluation of SNP markers for parentage testing in Taishu horse population.** Taichiro Ishige*[1], Tomoko Yoshihara[2], Koki Kawate[1], Mio Kikuchi[1], Risako Furukawa[1], Teruaki Tozaki[1], and Hironaga Kakoi[1], [1]*Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya-shi, Tochigi, Japan*, [2]*Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima-shi, Kagoshima, Japan.*
9:40 AM		**Election of CT Duty Labs, Election of Committee and Any Other Business.**
10:00 AM		**Coffee Break.**
10:20 AM		**Challenges and practicalities regarding the transition from STRs to SNPs for parentage verification. Romy Morrin, Weatherbys, Kildaire, IE.**
10:40 AM		**Discussion several aspects of SNPs in horses (Nomenclature, Exchange profiles, Next CT, rules parentage verification).**

Summary of the meeting

Horse STR Comparison Test:

Ninety-nine laboratories submitted results for this year’s Horse STR comparison test. There were issues with shipping delays as detailed below. Twenty test samples and three controls were distributed for this CT, with 15 of these being from the Thoroughbred breed. Controls were selected based on genotypes with problematic alleles from the last CT (ASB2 “B” and C”, HMS3 “M”, and TKY337 “F”. Relative accuracy ranged from 97.34 (AHT5) to HMS6 (99.90). Five labs had a discordant type for HCT8 missing the “I” allele. HCT12 had 7 discordant calls and HCT20 had 6 discordant calls for HTG10 where these samples were genotyped as “K/L” instead of “L”. The “B” allele for ASB2 was again missed in HCT3 (3 labs) and HCT7 (2 labs). For HMS3, three out of 12 samples with the “M” allele had discordant calls despite positive control sample with the “M” allele. Eighty six percent of the labs ranked 1. Of the markers tested in the back-up panel ten labs missed “D” allele of TKY394 in HCT8 (relative accuracy- 96.47%).
Three diagnostic markers were selected at the last workshop for inclusion in this CT with specific reporting instructions provided: Agouti (indel), Extension (SNP), and Gray (CNV). Eleven labs participated and the consistent reporting made analysis of these markers possible. Relative concordance was 99.56 for Agouti and 100% for Extension and Gray. The committee will select which diagnostics will be evaluated in the next CT and will work to increase participation as a possible way to enable interlaboratory comparison test for other laboratory accreditations.

Horse SNP Comparison Test

Twenty-seven laboratories participated in the unofficial Equine SNP CT test with markers selected from the Bellone et al. study and approved in the 2023 workshop. Markers tested were posted to the ISAG website with Equcab3 coordinates and alt and ref alleles provided. The same test and control DNA samples were provided for the SNP CT as for the STR CT. The reporting template for the SNP CT was uploaded twice to the ISAG website (once to fix a typo and again to fix the control genotypes for HCT22 and HCT23 that were initially flipped) but these errors did not appear to impact ranking and results. Five different technologies were employed including Aviti Sequencing, Axiom array, Genobaits Sequencing,

Illumina array genotyping (80K Equine select array with at least two different versions used), and Thermos Fisher Ion Torrent Sequencing (S5, which KIT used was not specified). Twenty laboratories (74%) had absolute concordance greater than 98%. Two labs were below 90%. Four markers (4/378) on the core panel had relative accuracy below 95% (AX103229555, AX104676886, AX104847570, & AX103612932) two labs reported alleles according to the opposite strand. Additionally, three labs had miscalls for "C" allele for AX104617749 (relative accuracy 96.46%), all three labs genotyped using an Axiom array.
Concerning the back-up panel, three markers with relative concordance below 95% had the wrong strand reported by two labs (AX103612932, AX103264313, & AX102950760). AX-103427823 was a known tri-allelic marker but none of the sample tested contained the "T" allele, this was confirmed by Sanger Sequencing done by the duty lab (VGL). The second alt allele for this SNP (T) is likely a rare allele (data reported from duty lab showed less than 5% allele frequency from unpublished data involving 354 horses).
Concerning the sex chromosome markers, several labs did not follow reporting instructions for markers on the X (six labs) and Y (4 labs) and thus genotypes were counted as discordant.
Collectively for the SNP CT, participants are advised to carefully review template instructions before reporting. There was a comment that the Equine80Select v2.1 does not have the Y markers, but the duty lab confirmed these are present if using the correct Illumina manifest file. It was noted that the recording of platform by participating CT laboratories helped to inform concordance data analysis and it was suggested to continue to collect this information along with additional metadata on version of chip utilized or other such platform relevant information for comparison.

Donkey Comparison Test
Twenty-five labs participated in the donkey comparison test. Twenty test samples and two controls were provided by the duty lab. Control genotypes were determined from testing at the UC Davis VGL and at Texas A&M. Relative accuracy ranged from 97.50%-100%, TKY337 was among the two markers with lowest concordance, there were 12 discordant types all of which were missing the "H" allele. It is recommended to use the ISAG approved primers for this marker which can be found on the ISAG website and below. Donkey10, had 6 discordant calls for AHT10, 4 involving "Z" or "z". Mz and Zm are considered to be equivalent genotypes and therefore this will be updated in the summary report. The correct allele order is as follows: v, w, x, y, z, A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, a, b, c, d, e. The Z and z allele are not equivalent alleles and ZM is therefore discordant and will not be changed. Therefore, relative accuracy for this marker will improve from 98.80% to 99.00% when the Mz and Zm is updated. Nineteen labs ranked 1 with absolute concordance greater than 98%

Future Comparison Tests
After the results of the Comparisons Tests were presented, a discussion took place on how to improve logistics regarding the shipment of the samples from the Duty lab to the participating labs. There are several countries where the documentation needed for shipping is not always clear and often requires multiple communications with the shipper and the receiving lab to ensure correct documentation and receipt of samples. Currently FedEx is used as the courier for ISAG Comparison tests because of a contracted pricing that was previously developed. Several laboratories have better experiences with DHL than FedEx as courier for sample shipment. It might be good to review if this is still the best option or determine if another couriers would suit better for shipment of the Comparison Test samples or if one laboratory within a county could be the central receiving lab and then distribute to other laboratories within the county.

Because of some delays in several steps of the process, in this year's Comparison Tests for several species deadlines needed to be extended. The participants of the workshop were informed that the deadlines for application and sample submission will be earlier for the next Comparison Tests. Information about the deadlines for the next Comparison Tests will be posted on the ISAG website.

Since reporting is also considered part of the CT, any misreporting in CT reports will be treated as a testing error.

Any Other Business

Because the workshop covers all horse and donkey breeds and not specifically Thoroughbreds, a name change was proposed by the Standing Committee. The workshop participants voted in Favor of the name change to 'Equine Genetics and Parentage Testing Standardization Workshop'.

Discussion Points and decisions

After the presentations related to the update on the studies of the ISAG SNP panel (Bellone and Ishige) about Challenges and practicalities regarding the transition from STRs to SNPs for parentage verification (Morrin) the topics below were discussed and the following decisions were made:

1. Equine Core SNP panel

   Marker AX104617749 showed miscalls with the Axiom platform in the results of the Comparison test, resulting in a Relative Accuracy of 96,46%. It was decided to remove marker AX104617749 from the official ISAG Equine Core SNP panel.

2. Equine Back-up SNP panel

   The Back-up SNP panel in the Comparison test consisted of 397 autosomal SNP markers. Two of those markers showed issues in the Comparison test results: AX103482270 (Mendelian exclusion rate 0.85% in Bellone et al. Study) and AX103427823 (Tri-allelic SNP). It was decided by the workshop participants to remove those two markers from the Back-up SNP panel and to accept the remaining panel of 395 markers as the official recommended ISAG Equine Back-up SNP Panel

3. Official ISAG Nomenclature

   It was decided to use the SNP marker names (AX ID) as used during the 2024-2025 unofficial SNP Comparison Test as the official ISAG SNP names.
   It was decided that the TOP format is the official ISAG genotype nomenclature. The list with marker names, information on genome assembly, chromosome, position, ref, alt, and TOP alleles will be posted on the ISAG website.

4. Exchange SNP profiles for parentage verification

   A format to exchange Equine SNP profiles for the purpose of parentage verification was proposed, where a single document could be e-mailed between laboratories that includes data for multiple equine samples. After much discussion, it was decided to get in contact with Interbull, the organization behind the GenoEx-PSE (Parentage SNP Exchange) database and ask if they can work with the file format below. If they can work with this file format (see description below), the format below is accepted as the ISAG recommended format to exchange Equine SNP profiles for the purpose of parentage verification. Furthermore, the options and costs for the use of the GenoEx-PSE database will be requested at Interbull as this might be an option to exchange Equine SNP profiles in the longer term.

Description file format:

- File Format: CSV UTF-8
  - Only one species per file
  - Multiple animals (only horses) in one file allowed
- Information horse in file
  - Microchip number**
  - Identification number horse**
  - Name
  - Sex [F/M]
  - Species [Equine]*
  - Breed
  - Date of birth [jjjj-mm-dd]
  - Sample ID number (laboratories might receive >1 sample from same animal)*
  - Callrate [xx.xx]
- Information Laboratory
  - Name*
  - Contact email address*
  - Nomenclature [TOP]*
- Information ISAG SNP Profile
  - ISAG SNP Name*
  - Genotype*
  - In case a marker failed: leave empty

*** At least one is required*

**   Required*

5. Equine CT 2026-2027

It was decided that in 2026-2027 the first official Equine SNP Comparison test with an official ISAG ranking will be organized. The official ranking will be based on the Equine Core SNP panel only. Labs are encouraged to report all marker panels below:

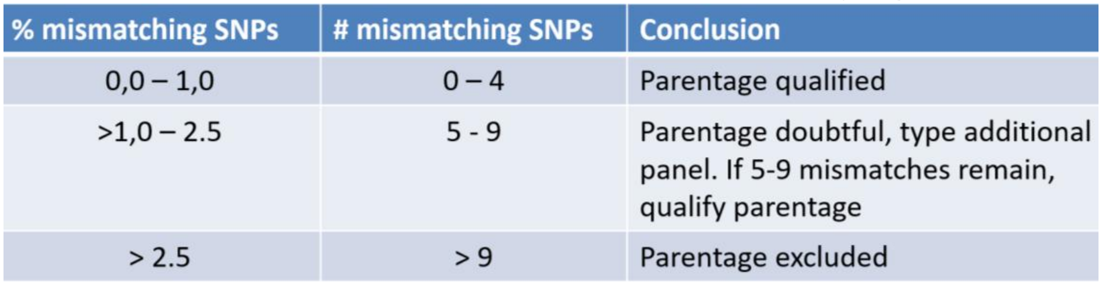
- Core panel (377 autosomal markers)
- Back-up panel (395 autosomal markers)
- Recommended Sex chromosome markers

It was decided to include a few diagnostic markers in the 2026-2027 Equine CT. The committee will decide how this can be executed.

6. Rules for SNP based parentage verification

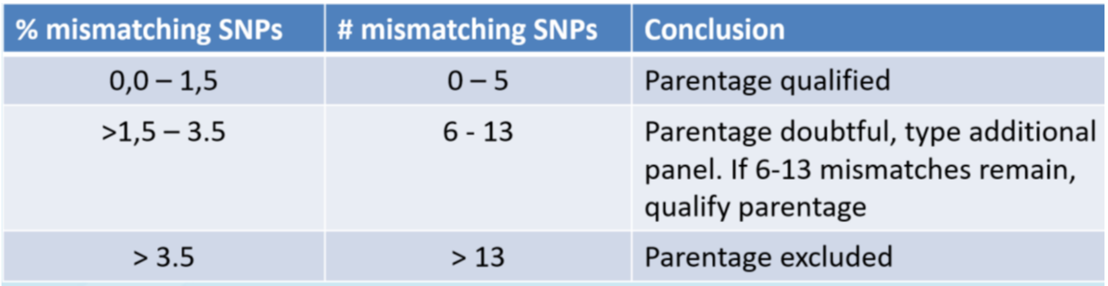
It was decided to accept the following rules for Parentage verification.

- Cases with offspring and one parent tested
  - Minimum markers in profile: 95% of core panel = 358 markers
  - Minimum number of common SNPs in verification offspring: 339



% mismatching SNPs	# mismatching SNPs	Conclusion
0,0 – 1,0	0 – 4	Parentage qualified
>1,0 – 2.5	5 - 9	Parentage doubtful, type additional panel. If 5-9 mismatches remain, qualify parentage
> 2.5	> 9	Parentage excluded

- Cases with offspring and both parents tested
  - Minimum markers in profile: 95% of core panel = 358 markers
  - Minimum number of common SNPs in verification offspring: 320



% mismatching SNPs	# mismatching SNPs	Conclusion
0,0 – 1,5	0 – 5	Parentage qualified
>1,5 – 3.5	6 - 13	Parentage doubtful, type additional panel. If 6-13 mismatches remain, qualify parentage
> 3.5	> 13	Parentage excluded

New Committee chair

Chair: Leanne van de Goor
Term of service: first term 2021-2025, second term 2025-2029
Affiliation: VHLGenetics
E-mail address: Leanne.vandegoor@vhlgenetics.com

New Committee co-chair (optional)

Chair: Rebecca Bellone
Term of service: first term 2019-2023, second term 2023-2027
Affiliation: VGL, UC Davis
E-mail address: rbellone@ucdavis.edu

New Committee members

Other committee members	First term of service (from year to year)	Second term of service (from year to year)	Email address
Paul Flynn	2021-2025	2025-2029	pflynn@weatherbys.ie
Amparo Martinez	2021-2025	2025-2029	amparomartinezuco@gmail.com
Teruaki Tozaki	2021-2025	2025-2029	ttozaki@lrc.or.jp
Pedro J. Azor	2023-2027	2025-2029	pedroazor@lgancce.com
Guillermo Giovambattista	2019-2023	2023-2027	guillermogiovambattista@gmail.com

COMPARISON TEST (2024-2025) YES

Duty laboratory Equine STR and SNP

Contact person: Rebecca Bellone
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Duty laboratory Donkey STR

Contact person: Rebecca Bellone
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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.

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 Equine STR and SNP comparison test

Contact person: Rebecca Bellone
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Duty laboratory for the next Donkey STR comparison test

Contact person: Rytis Juras
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SIGNATURE

Rebecca Bellone

Chair

Duty laboratory (if applicable)