Equine Genetics and Thoroughbred Parentage Testing Workshop

Organised by a Standing Committee: YES

Meeting information

Date: July 30th
Time: 9:00 a.m. to 12:00 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

Co-Chair (optional)

Name: Guillermo Giovambattista
Affiliation: IGEVET. Argentina
Contact email: guillermogiovambattista@gmail.com

Agenda

9:00 AM		**Welcoming remarks.**
9:10 AM		**Horse STR Comparison Test.** Dr. Rebecca Bellone, University of California-Davis.
9:25 AM		**Donkey STR Comparison Test.** Prof. Peter Dovc, Biotechnical Faculty, University of Ljubljana, Slovenia.
9:40 AM		**Horse SNP Comparison Test.** Dr. Rebecca Bellone, University of California-Davis.
9:55 AM		**Introduction to Horse SNP Panel Discussion and Related Author Presentations.**
10:00 AM	85463	**Comparative analysis of single nucleotide polymorphisms and microsatellite markers for parentage verification and sire/dam allocation within equine thoroughbred breed.** P Flynn*[1,2], R Morrin-O'Donnell[1], R Weld[1], J Carlsson[2], P Siddavatam[3], and K. Reddy[3], [1]*Weatherbys Scientific, Naas, Ireland*, [2]*University College Dublin, School of Biology & Environmental Science, Belfield, Dublin, Ireland*, [3]*Thermo Fisher Scientific, Austin, TX, USA*.
10:15 AM		**Availability of whole genome sequencing database for selecting SNP marker in Thoroughbreds.** Dr. Teruaki Tozaki, Laboratory of Racing Chemistry.
10:25 AM		**Break.**
10:40 AM	85482	**Evaluation of the ISAG equine parentage testing SNP panel across multiple breeds.** Rebecca Bellone*[1,2], Brad Till[1], Angelica Kallenberg[1], Felipe Avila[1], and Rob Grahn[1], [1]*University of California Davis, Veterinary Genetics Laboratory, Davis, CA, USA*, [2]*University of California Davis, Department of Population Health and Reproduction, Davis, CA, USA*.
10:55 AM		**Presentations Results of Horse Survey and Decisions on the Horse Core Panel.**
11:25 AM	85441	**Pioneer 100 Horse Health Project: A Deep Phenotypic and Multiomic Resource.** CG Donnelly*[1], N Cohen[2], G Mulcahy[3], J Manfredi[4], S Valberg[5], E Oberhaus[6], J Morgan[7], E Graham-Williams[8], KE Knickelbein[8], R Bellone[1,9], ND Price[10,11], and CJ Finno[1], [1]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA*, [2]*Large*

	Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [3]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland,* [4]*Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA,* [5]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA,* [6]*School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA,* [7]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [8]*Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [9]*Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [10]*Institute for Systems Biology, Seattle, WA, USA,* [11]*Onegevity Health, New York, NY, USA.*
11:40 AM	**Duty labs election.**
11:45 AM	**Election of New Committee members and Other Business.**
11:55 AM	**Meeting ends.**

Summary of the meeting

Including votes, decisions taken and plans for future conferences

1. Welcoming Remarks

The workshop was conducted virtually as part of the online ISAG 2021 Conference. The agenda of the workshop included the discussion of Comparison tests (CTs) results for Horse STR, Donkey STR and Horse SNPs carried out during the period 2020-21.

2. Horse STR CT Discussion

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

Samples: 22 DNA samples (2 references) representing 8 breeds – Thoroughbred (8), Quarter Horse (7), American Miniature (1), Appaloosa (2), Arabian (1), Oldenburg (1), Paint Horse (1), and Standardbred (1). Extractions were done with Gentra® Puregene®-Qiagen procedure. Two labs reported that sample #7 was empty upon arrival.

Participants: One-hundred and one labs requested samples and 94 labs reported results. The Duty Lab reported shipping issues for: Russia/South Africa/Spain/Denmark/Egypt. Also, there were some custom issues with China and 12 labs of Brazil didn't receive shipment for over a month (1 second shipment was sent) but this did not appear to impact quality of the DNA based on concordance results (average genotyping accuracy for the nine labs that reported results was 99.88%). Due to these problems, report deadline due March 31, was extended to April 30.

Summary of Results:

ISAG Panel:

The relative overall marker concordance among labs was good, ranging from a minimum of 96.86% (HMS3) to a maximum of 99.52% (HMS6). See table below. This reflects the difficult for detection of allele "M" of marker HMS3 due to its lower amplification, as reported in last years. During the Duty Lab presentation, the discrepancies in HMS3 and ASB2 were discussed. ASB2 alleles "B" (sample #7) and "C" (sample #4) were missed or wrongly called by 12 and 10 labs respectively, causing the lower concordance in this marker.

Eighty-three percent of the labs ranked 1 (100 – 98% concordance among labs), slightly lower than in the previous CT (86%). Only 2% of the labs ranked below 80% of concordance.

Locus	Relative Accuracy
HMS3	96.86%
ASB2	97.87%
ASB17	98.24%
HMS2	98.46%
HMS7	98.46%
VHL20	98.62%
AHT5	98.72%
HTG4	98.72%
ASB23	98.78%
HTG10	98.99%
AHT4	99.31%
HMS6	99.52%

Parentage questions:

Parentage questions concordance was reasonably good. The parentage question one asked if the offspring (s) of sample #19 were among samples tested. Seventy-six percent of the labs answered correctly (No) while another 14% expressed doubts due to discrepancy in one marker. In this case, the additional panel of markers should be tested to rule out the parentage as suggested by ISAG rules. The second question asked if the parents, or parent, of sample #18 were among samples tested. Eighty-nine labs (95%) answered correctly (Yes, sample #8).

Back Up Panel:

For the Back Up Panel, the highest discrepancy was shown at marker TKY337 (sample #10) due to some labs missing allele "P" while other miscalled that allele as "F" presumably due to the use of primers that will incorrectly genotype horses who have the known 19bp deletion. This issue was discussed during 2019 Workshop and the pair of "UC Davis" primers were suggested to overcome TKY337 deletion present in some samples, detecting thus allele "P" instead of "F". Suggested primers used by UC Davis are TKY337F 5'-TTTTGAGCAGAGCAGGGTTT-3' and TKY337R 5'-CTTGTGCCCCTCATGTCTTT-3'.

Discussion Points:
The ST Committee advised the participants to check previous workshop reports to look for frequent causes of discrepancies in the CTs. In addition, it was suggested to use sample #7 (with problematic ASB2 and HMS3 genotypes) and sample #10 (with problematic TKY337 genotypes) to be included as references for the next CT.

The SC reviewed 2 requests for changes of genotype results and sample order. The unanimous consensus was for no such corrections to be made. Quality control of genotype results is the responsibility of CT participants. Paragraphs 27 and 28 of the Rules for Conducting Comparison Tests for Animal DNA Test apply to copying errors in compilation or stipulation of "concordant" genotype as the correct genotype.

3. Donkey STR CT Discussion

Duty Laboratory: Prof. Peter Dovc, GenLab, Biotechnical Faculty, University of Ljubljana, Slovenia.

Samples: DNA from 22 DNA samples (2 references) was extracted using E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek). Collection, extraction and after shipment of the samples for the Duty Lab was delayed due to Covid-19 pandemic. Samples #9 and #11 were detected as contaminated and not used in the final score.

Participants: Twenty-four labs requested samples and 17 labs reported results. Both Brazil and South Africa labs reported customs issues and second batch of samples should be shipped to them. Due to these problems, report deadline due March 31, was extended to April 30.

Summary of Results:

ISAG Panel:
The relative overall marker concordance among labs was good, ranging from a minimum of 91.67% (TKY337) to a maximum of 100% (ASB23, HMS18, HMS2, HMS3, HTG10, TKY343). The lower accuracy in marker TKY337 reflects the difficulty to detect the allele "H" in several samples, as reported in the previous CT. Modified primers used in horse, as described above, are also recommended for donkey TKY337-for:TTTTGAGCAGAGCAGGGTTT and TKY337-rev: CTTGTGCCCCTCATGTCTTT.
Seventy-six percent of the labs ranked 1 (100 – 98% concordance among labs) and no lab ranked below the 80% of concordance.

Locus	Relative Accuracy
TKY337	91.67
HTG7	93.79
HMS7	98.04
AHT4	98.36
TKY297	98.61
HMS6	98.69
TKY312	99.65
ASB23	100.00
HMS18	100.00
HMS2	100.00
HMS3	100.00
HTG10	100.00
TKY343	100.00

Parentage questions:
Parentage questions concordance was reasonably good. The parentage question one asked if sample #20 qualifies as the mother of sample #19. Seventy percent of the labs answered correctly (Yes). Wrong answers were mainly caused by TKY337 errors. The second question asked if sample #5 qualifies as the offspring of sample #22. Ninety-four percent of the labs answered correctly (No).

Discussion Points:
The ST Committee advised the participants to check previous workshop reports to look for frequent causes of discrepancies in the CTs. In addition, it was suggested to use any of the samples with discordant TKY337 genotype as reference for the next CT.

The SC reviewed 2 requests for changes of genotype results and sample order. The unanimous consensus was that no such correction should be made. Quality control of genotype results is the responsibility of CT participants. Paragraphs 27 and 28 of the Rules for Conducting Comparison Tests for Animal DNA Test apply to copying errors in compilation or stipulation of "concordant" genotype as the correct genotype.

4. Horse SNP CT Discussion

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

Samples: 22 DNA samples (2 references) representing 8 breeds – Thoroughbred (8), Quarter Horse (7), American Miniature (1), Appaloosa (2), Arabian (1), Oldenburg (1), Paint Horse (1), and Standardbred (1). Extractions were done with Gentra® Puregene®-Qiagen procedure. Two labs reported that sample #7 was empty upon arrival.

Participants: Twenty-nine labs requested samples and 14 labs reported results (United States (3), Brazil (2), France (2), Denmark, Germany, Ireland, Japan, Netherlands, Poland, United Kingdom (1)). Three labs reported only SNPs while the others also informed STR results. Four platforms were used (Ion S5™ GBS (6), Illumina microarray (5), MassArray (2), Quant Studio 12K Flex (1)). One lab reported results for two different platforms.

Summary of Results:

Markers from the Panels known as Etalon and Tozaki were utilized for this comparison test and informed in the reference samples and ranked according to relative and absolute concordance. Information of markers used can be found in: https://www.isag.us/committees.asp (ISAG Equine SNP Panels 2020/2021). This was the first compilation of a SNP CT for horses done by FASS. There were no formal scores since a Core Panel has not yet been defined.
The absolute concordance ranged from 65.51% to 99.93% as shown in the table below. Lower concordance was mainly due to discrepancies among platforms.

Absolute and Relative Concordance for 202-2021 Equine SNP CT

Lab code	Standardized Platform	# SNPs	# Blanks	# Mismatches	Relative Concordance	Absolute Concordance
5084414	MassArray	147	14	7	99.76%	99.29%
5084414	Ion S5™ GBS	147	20	44	98.49%	97.82%
5084422	Ion S5™ GBS	147	0	136	95.37%	95.37%
5084451	Not provided	147	6	137	95.33%	95.14%
5084460	Ion S5™ GBS	147	15	53	98.19%	97.69%
5084485	Illumina SNP array	147	1	101	96.56%	96.53%
5084764	Ion S5™ GBS	147	0	26	99.12%	99.12%
5090224	Illumina SNP array	147	2	0	100.00%	99.93%
5091543	Ion S5™ GBS	147	3	58	98.03%	97.93%
5109488	Ion S5™ GBS	147	3	55	98.13%	98.03%
5110956	MassArray	147	0	117	96.02%	96.02%
5119078	Illumina SNP array	147	89	105	96.32%	93.40%
5119135	Platform	147	1002	12	99.38%	65.51%
5136638	Illumina SNP array	147	2	0	100.00%	99.93%
5138549	Illumina SNP array	147	2	0	100.00%	99.93%

Concordance within platforms was higher as shown below:

Platform	Average of Relative Concordance	Average of Absolute Concordance
unknown NGS Platform n=1	99.38%	65.51%
Illumina SNP array n=5	98.58%	97.95%
Ion S5™ GBS n=6	97.89%	97.66%
Mass Array n=2	97.89%	97.65%
QuantStudio 12K Flex	95.33%	95.14%
Grand Total	**98.05%**	**95.44%**

A comparison with last CT showed that 100% concordance rate decreased from 71% of SNPs (2018-19 CT, 11 data set analysed) to 19% in this year's test (15 data set).

Relative Concordance for 2021-2021 CT (n=15)

#SNPs n=147	% of SNPs	Relative Concordance Rate
28	**19%**	**100%**
54	37%	99.67%-99.66%
18	12%	99.64%-99.61
14	10%	99.33-99.23%
5	3%	99.00%
7	5%	98.93-97.5%
6	4%	96.77-95.67%
5	3%	94.98-91.76-%
4	3%	85.27-81.65%
6	4%	79.26-76.26%

Relative Concordance for 2018-2019 CT (n=11)

# SNPs (n = 148)	% SNPs	Concordance Rate	Notes
106	71%	100%	
11	7%	99.5%	
10	7%	99.0 - 99.3%	
10	7%	98.2 - 98.6%	
6	4%	95.0-97.6%	
1	1%	93%	
3	2%	84.7 - 89.3%	one with 3 alleles
2	1%	75.2 -78.0%	one with 3 alleles

Absolute Concordance for 2021-2021 CT (n=15)

#SNPs N=147	% of SNPs	Absolute Concordance Rate
7	**5%**	**100%**
39	27%	99.67%
28	19%	99.33%
19	13%	96.67-99.00%
54	37%	94.67-70.67%

There were several causes for the reduced overall concordance (see Table below). For some variants (BIEC555737, BIEC2204153, BIEC562465, BIEC571705, BIEC581695), some of the

discrepancies are likely caused by standardization when EquCab 3.0 assembly was used instead of 2.0.

Markers considered to be added to “watch list” based on relative concordance less than 95%

SNP	Blanks	Reported	Mismatch	Relative Accuracy	Notes
BIEC554813	22	278	66	76.26%	9 samples homozygous "GG" not "CC" and 2 samples homozygous "CC" and not "G" in 5/6 S5 labs and 1 Quant Studio platform
BIEC581695	23	277	63	77.26%	3 labs complement genotype for all samples (including reference) likely related to strand flip , **ECT10 2 S5 labs with discordant data**
BIEC571705	20	280	62	77.86%	3 labs complement genotype for all samples (including reference) three labs likely related to strand flip, **1 MassArray and 1 S5 miscall from 2 different labs**
BIEC555737	21	279	60	78.49%	Complement genotype for all samples (including reference) three labs likely related to strand flip
BIEC562465	21	279	60	78.49%	Complement genotype for all samples (including reference) three labs likely related to strand flip
BIEC2204153	1	299	62	79.26%	Complement genotype for all samples (including reference) three labs likely related to strand flip **ECT20 two labs discordant (MassArray)**
BIEC344848	22	278	51	81.65%	2 labs 18/20 miscalls S5 data, S5 and Illumina genotypes differ for ECT1, 10,12,20
BIEC508410	20	280	49	82.50%	2 labs complement genotype even for reference samples, 1 lab complement for CT samples not reference, 1 lab 6 miscalls
BIEC681989	21	279	42	84.95%	3 S5 platforms with 14 miscalls
BIEC2158202	8	292	43	85.27%	Discrepancy between S5 and other platforms for 8 samples (mostly homozygous "T" not "CT")
BIEC119158	21	279	23	91.76%	1 lab 16 miscalls (mostly homozygous "T"), 1 lab 8 miscalls (opposite to other lab mostly homozygous "A") both S5 platforms
BIEC136821	28	272	15	94.49%	4 samples with 3 or more miscalls, MassArray, Illumina, and S5
BIEC2214346	2	298	16	94.63%	1 lab with 14 miscalls
BIEC531275	20	280	15	94.64%	1 lab with 14 miscalls
BIEC2310269	1	299	15	94.98%	4 labs using S5 and 1 using Quant Studio with miscalls in 3 samples ECT11, 16, 17 ("TT" not "CT")

likely related to complement calls but also other discordant calls
Complement genotypes not related to known issue
discordant calls unlikely to be platform specific
discordant calls maybe platform related

Note: Markers highlighted in pink were selected for Sanger sequencing.

1) BIEC2204153: In addition to discordant genotypes seen in some labs due to strand flip (T>C (2.0) verses G>A (3.0). Sample#20 showed discordant genotypes for MassArray user labs. Ion S5™ GBS genotype was confirmed as correct and discrepancy was explained by a mutation in the primer site for MassArray assay.

2) BIEC2158202 that showed discrepancy between Ion S5™ GBS and other platforms for 8 samples (one of which was not scored, ECT5, because equal number of CT and TT reported by labs). Ion S5™ GBS genotypes were confirmed by Sanger sequencing.

Important: A variant was identified between ISAG SNP panel sequence and EquCab 3.0, at 60 bp from SNP of interest. That may have impacted design of other platforms.

3-5) BIEC581695, BIEC344848, BIEC571705: MassArray genotype confirmed, variant in primer site for Ion S5™ GBS likely.
6) BIEC119158: MassArray genotype confirmed –unknown cause of discordance.

In addition, marker BIEC911841 showed difficulties for design due to mapping in multiple sites (also reported by Dr. Tozaki in his talk: "Availability of whole genome sequencing database for selecting SNP markers in Thoroughbreds").

Parentage questions:

Parentage questions concordance was good. The parentage question one asked if any of the samples qualifies as the offspring of sample#04. If yes please, indicate which sample(s). All the labs answered rightly (No). The second question asked if any of the samples tested qualifies as the parent(s) of sample#18. If yes, please indicate which sample(s). All the labs answered rightly (Yes, sample #8).

Discussion Points:

For next CT report: • Motion for voting: Top vs. Forward calls.
Result: The motion was voted and the Top call was selected.

If ranked only 54.5% labs ranked 1 (based on small number of labs participating). How to increase both? **See possible removal of some markers (BIEC2158202 and BIEC911841 and others listed on the watch list in table above**.

Proposals that were approved:

Consider removal of SNPs on the watch list if lower concordance observed after two CTs and reason for discrepancies are defined as well as remove those SNPs shown to be multimappers (BIEC911841)

Based on SNP duty lab report and studies presented by Bellone and Flynn carry out a large multi-center study to identify additional SNPs and evaluate SNPs across platforms in large number of horses and breeds.

Results of Horse Survey

In June 2021, a survey was carried out among labs that participated in the last 2 CTs to get information about the use of SNPs for PV.

Thirty-eight labs from different countries (Europe -16-, South America -8-, North America -4-, Asia -5-, Africa -5-) replied and the information was shared during the workshop.

Briefly, questions were grouped as related to the SNP Panel or the transition of STR to SNPs for parentage verification.

1. The first question was if the current panels (Etalon+ Tozaki) should be approved as Core Panel during the workshop. Twenty-two labs (58%) answered against, and sixteen (42%) in favor of that option.

2. For those that answered against the motion, it was asked if a study among laboratories should be organized to research SNPs across breeds with a large number of samples. Sixteen of the twenty-two labs replied in favor of that option, volunteering to test and/or provide samples of different breeds.

Regarding the transition of STR to SNPs for parentage verification:

1. The survey asked to the participants if they were already testing SNPs in other species. Twenty-nine of thirty-eight replied for No and those that were using; they were working with different platforms (NGS, microarray, MassArray). In addition, all but one lab comment that the cost of SNP testing was higher than testing STR. Therefore, shared concerns were both cost of SNP testing and technology transition.

2. It was also asked if participants agreed of having both methods (SNPs and STRs) in use for parentage verification. This question was included considering the investment in platform and software that transition to SNPs will require to the labs that are not working with SNPs. Thirty labs (79%) of thirty eight replied in favor of co-existing methods.

New Committee chair

Chair: Marcela Martinez
Term of service (*add years of first and second term of service*): 2019-2023 (first term)
Affiliation: Laboratorio de Genética Aplicada. Sociedad Rural Argentina. Argentina
E-mail address: mmartinez@sra.org.ar

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	rbellone@ucdavis.edu
Guillermo Giovambatista	2019-2023	2023-2027	guillermogiovambattista@gmail.com
Amparo Martinez	2021-2025		amparomartinezuco@gmail.com
Leanne Van de Goor	2021-2025		leanne.vandegoor@vhlgenetics.com
Teruaki Tozaki	2021-2025		ttozaki@lrc.or.jp
Paul Flynn	2021-2025		pflynn@weatherbys.ie

COMPARISON TEST (2020-2021) 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 (2020-2021) YES

Duty laboratory: HORSE SNP CT

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

Comments (issues rising)

See Discussion Points of Horse SNP Duty Lab.

List of recommended markers with primer information

The provisory panel is also posted as: ISAG Equine SNP Panels 2020/2021. A new panel would be developed as part of a multi-lab work and information will be reported to the participants in time for the next CT.

COMPARISON TEST (2020-2021) YES

Duty laboratory: Donkey STR CT

Contact person: Prof. Peter Dovc.
Affiliation: GenLab, Biotechnical Faculty, University of Ljubljana, Slovenia.
E-mail address: peter.dovc@bf.uni-lj.si

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

Duty laboratory: Donkey STR CT

Contact person: Laura Patterson
Affiliation: Etalon Diagnostics, USA
E-mail address: lpatterson@etalondx.com

SIGNATURES

Chair Marcela Martinez **Horse Duty laboratory**

PETER DOVC Digitally signed by PETER DOVC Date: 2021.08.13 09:45:01 +02'00'

Donkey Duty laboratory