

Applied Genetics and Genomics In Other Species of Economic Interest Workshop

STANDING COMMITTEES / WORKSHOPS

Organised by a standing committee yes

Date and meeting time: July 24th 2016, 2:00 – 5:00 pm

Chair, name and contact email: Leanne van de Goor, (lgo@vhladmin.nl)

Agenda / programme attached

- Welcoming Remarks
- Dromedary Comparison test
- Alpaca/Llama Comparison test
- Pigeon Comparison test
- Selection of new Duty Labs for 2018-2019 Comparison tests
- Election of Committee
- Close

Number of participants at meeting: 15

Summary of the meeting including votes, decisions taken and plans for future conferences

1. Welcoming Remarks

This is the first workshop with the name Applied Genetics and Genomics In other Species of Economic Interest. The previous name of this workshop was Camelid Genetics and Genomics Workshop. At the ISAG 2014 conference in Xi'an a workshop with the name Pigeons, Regional Farmed Animals and Wild Animals was organized. During this workshop it was discussed to form a committee together with the Camelid Genetics and Genomics Committee. This proposal was further discussed during the Camelid Genetics and Genomics workshop. Considering that both workshops have limited attendance and that interests focus on non-traditional livestock and wildlife species, during the Camelid Genetics and rename the workshop "Applied Genetics and Genomics In Other Species of Economic Interest". The merger ensures that need for comparison tests for the different species of interest continues to be met and that a single workshop would provide a forum for continued scientific exchange

Since the previous ISAG conference in 2014 two new documents concerning the conduct of ISAG Comparison tests are available:

- Policies Governing the Conduct of ISAG Comparison Tests (CT) for Animal DNA Testing (<u>http://www.isag.us/docs/Policy_CT.pdf?v2</u>)
- Rules for conducting ISAG Comparison Tests (CT) for animal DNA testing (http://www.isag.us/docs/Rules_CT.pdf?v2)

Other changes for this CTs compared to previous CTs were:

- Application was only possible after login by institutional members on the ISAG website
- FASS arranged the FedEx shipping labels, import permits and health certificates for the duty labs
- o FASS made the compilation of the results from the CTs

2. Dromedary Comparison test

The Duty lab was Progenus from Belgium. Twelve labs requested samples (Europe 7, Asia 3, USA 1 and Australia 1). Twenty-four samples and one reference sample were submitted to all participants. Ten labs reported results. Labs reported results for the 8 STR markers in the Core Panel (LCA19, LCA37, LCA56, LCA65, LCA66, LCA8, YWLL29, YWLL44), the 9 STR markers in the Back-up Panel (LCA24, LCA77, LCA99, LGU49, VOLP3, VOLP32, VOLP59, YWLL08, YWLL36) and 30 additional STR markers. Only the markers in the Core panel were included in the ranking system. In the Draft compilation send out by FASS, the results are compiled exactly as reported by the labs. Administrative mistakes such as 106/106 instead of 106/ were calculated as mistakes for the ranking system in this draft version. In the final version of the compilation there will be a correction for such mistakes. Labs need to pay attention to such nomenclature issues when reporting their results as it is a lot of extra work to correct for such mistakes. The overall marker concordance among labs was good (>95%) for six of the eight markers in the Core Panel. Only two markers showed a lower concordance: LCA37 (86%) because of missed alleles and wrong allele binning and YWL44 (87%) because of a missed allele. The standing committee received one request for correction of concordant genotypes, this concerned marker YWLL44 concordant genotype 106/108 for the samples 215863, 215870, 215876, 215877 and 215884. The request was to correct those genotypes into 108/. This request was discussed within the standing committee and during the workshop. It was shown that for those samples allele 106 was weak but present and it was agreed on that 106/108 is the correct genotype. For the final ranking of this year's comparison test both 106/108 and 108/ will be counted as correct for the samples mentioned above. If these genotypes are present in future CTs only 106/108 will be counted as correct for the final ranking system.

3. Alpaca/Llama Comparison test

The Duty lab was Certagen from Germany. Thirteen labs requested samples. Twenty samples (14 from Alpaca and 6 from Llama) and one Alpaca reference sample were submitted to all participants. One lab requested a second batch of samples. Ten labs reported results. Labs reported results for the 14 STR markers in the Core Panel (LCA19, LCA37, LCA5, LCA56, LCA65, LCA66, LCA8, LCA94, LCA99, LGU49, LGU50, YWLL29, YWLL40, YWLL44), the 5 STR markers in the Back-up Panel (LCA24, YWLL36, YWLL43, YWLL46, YWLL8) and the SRY marker. Only the markers in the Core panel were included in the ranking system. In the Draft compilation send out by FASS already some administrative mistakes such as 204/204 instead of 204/ were corrected. Labs need to pay attention to such nomenclature issues when reporting their

results as it is a lot of extra work to correct for such mistakes. The overall marker concordance among labs was good (>98%) for eleven of the fourteen markers in the core panel. Only three markers showed a lower concordance: LCA37 (97%) because of several genotyping mistakes, LCA66 (94%) because of the presence of an odd allele and LGU50 (97%) because of a missed allele. The standing committee did not receive any requests for correction of concordant genotypes.

4. Pigeon Comparison test

The Duty lab was VHL from The Netherlands. Twelve labs (Europe 10, USA 1 and Africa 1) requested samples. Twenty samples and one reference sample were submitted to all participants. Four labs requested a second batch of samples. The reason that labs requested a new batch of samples was that several tubes were (almost) empty, this was most probably caused by evaporation. The new batches of samples were send out in another type of tubes and the caps covered with parafilm. No complaints were received about the new batches. Seven labs reported results. Labs reported results for the 12 STR markers in the Core Panel (CliuD11, CliuT43, CliuD01, PIGN57, CliuT13, CliuD16, CliµD19, CliµT02, CliµD17, CliµD35, CliµT17, PIGN4), the 4 STR markers in the Backup Panel (PIGN15, PIGN10, PIGN26, PIGN12) and the AMEL marker. Only the markers in the Core panel were included in the ranking system. In the Draft compilation sent out by FASS the results were compiled exactly as reported by the labs. The overall marker concordance among labs was good (>98%) for eight of the twelve markers in the core panel. Only four markers showed a lower concordance: CliuD01 (89%) because wrong allele binning of one small allele, CliµD16 (91%) because of the presence of an extreme allele and wrong binning of this allele, CliµT43 (92%) because one allele was missed by one lab (present in 6 samples) and PIGN4 (93%) because one lab had wrong allele binning. The standing committee did not receive any requests for correction of concordant genotypes. Within the standing committee and during the workshop two nomenclature issues were discussed. The first one was concordant allele 77 (samples 4, 6, 9, 14 and 16) in marker CliµD01. Four labs called this allele 77 and three labs called this allele 75. During the workshop it was shown that this was caused by a PCR product migration shift. It was agreed on that the correct calling for this allele is 77. For the final ranking of this year's comparison test both 75 and 77 will be counted as correct for the samples mentioned above. If this allele is present in future CTs only 77 will be counted as correct for the final ranking system. The second issue discussed was the presence of a large allele in marker CliµD16 (samples 1, 8 and 10). During the workshop it was shown and agreed on that amplification of this allele was weak but that the allele was present and that the correct calling for this allele is 170. For the final ranking of this year's comparison test is will not be counted as a mistake if this allele was not reported or reported as 168 or 172 for the above mentioned samples. If this allele is present in future CTs only 170 will be counted as correct for the final ranking system. Both parentage questions were answered correctly by all labs.

During the workshop in 2014 in Xi'an the following remaining goals for the Pigeon Backup panel were defined.

- Determine allele frequencies
- Determine PIC/PE/HO/HE
- Publish findings in an article
- Determine if 12 ISAG recommended markers are enough

Results of a study by Maarten de Groot from VHL, The Netherlands, were presented during this year's workshop, a paper of this study has been submitted to Animal Genetics. Based on the results of the study was agreed on that for now the 12 ISAG recommended markers in the core panel are sufficient. Data of other available pigeon markers will be collected and during the workshop in Dublin in 2017 the core and additional marker panel will be evaluated again. A null-allele is present in PIGN12 (additional panel), the goal is to redesign primers for this marker.

5. Other species of interest

The chairman asked participants if there was interest to include new species in the Committee. Dr Christopher Adenyo, from Ghana University, showed interest in studies on grasscutter (*Thryonomys swinderianus*), a type of rodent used of meat in sub-Saharan Africa. He was informed that to develop a CT for this species, there should be other labs interested in the same test.

6. Selection of new Duty Labs for 2018-2019 Comparison tests

In 2017 there will be no comparison test for Dromedary, Alpaca/Llama and Pigeons. There were no volunteers at the workshop to be duty lab for any of the species in 2018-2019. During the next workshop in Dublin in 2017 the standing committee will again seek volunteers for duty labs function.

Committee members (the new committee)

Chair	term of service	E mail address:
Leanne van de Goor	2014-2017 (1 st term)	lgo@vhladmin.nl
Other members	term of service	E mail address:
Deanne Waine	$2016-2019 (2^{nd} \text{ term})$	d.waine@uq.edu.au
Cecilia Penedo	$2016-2019 (2^{nd} \text{ term})$	mctorrespenedo@ucdavis.edu
Marcela Martinez	$2016-2019 (2^{nd} \text{ term})$	mmartinez@sra.org.ar

COMPARISON TEST (2015-2016)

Duty laboratory name and email address

Dromedary: Progenus from Belgium (<u>renaville.b@progenus.be</u>) Alpaca/Llama: Certagen from Germany (<u>jansen@certagen.de</u>) Pigeon: VHL from the Netherlands (<u>lgo@vhladmin.nl</u>)

Comments (issues rising)

List of recommended markers with primer information

Dromedary:		
ISAG STR Core Panel - Dromedary		
Locus	Forward	Reverse
LCA8	GCTGAACCACAATGCAAAGA	AATGCAGATGTGCCTCAGTT
LCA37	AAACCTAATTACCTCCCCA	CCATGTAGTTGCAGGACACG
LCA56	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG
LCA65	TTTTTCCCCTGTGGTTGAAT	AACTCAGCTGTTGTCAGGGG
LCA66	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA
YWLL29	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGCAG
YWLL44	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA

ISAG Additional Markers - Dromedary		
Locus	Forward	Reverse
CVLR01	GAAGAGGTTGGGGGCACTAC	CAGGCAGATATCCATTGAA
CVLR04	CCCTACCTCTGGACTTTG	CCTTTTTGGGTATTTTCAG
CVLR05	CCTTGGACCTCCTTGCTCTG	GCCACTGGTCCCTGTCATT
LCA99	CAGGTATCAGGAGACGGGCT	AGCATTTATCAAGGAACACCAGC
LGU49	TCTAGGTCCATCCCTGTTGC	GTGCTGGAATAGTGCCCAGT
VOLP3	AGACGGTTGGGAAGGTGGTA	CGACAGCAAGGCACAGGA
VOLP32	GTGATCGGAATGGCTTGAAA	CAGCGAGCACCTGAAAGAA
VOLP59	CCTTCCTCAGAATCCGCCACC	CCCGCGCACCAAGCAG
YWLL08	ATCAAGTTTGAGGTGCTTTCC	CCATGGCATTGTGTTGAAGAC
YWLL36	AGTCTTGGTGTGGTGGTAGAA	TGCCAGGATACTGACAGTGAT

Alpaca/Llama:

ISAG STR Core Panel - Llamas and Alpacas		
Locus	Forward	Reverse
LCA5	GTGGTTTTTGCCCAAGCTC	ACCTCCAGTCTGGGGGATTTC
LCA8	GCTGAACCACAATGCAAAGA	AATGCAGATGTGCCTCAGTT
LCA19	TAAGTCCAGCCCCACACTCA	GGTGAAGGGGCTTGATCTTC
LCA37	AAACCTAATTACCTCCCCA	CCATGTAGTTGCAGGACACG
LCA56	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG
LCA65	TTTTTCCCCTGTGGTTGAAT	AACTCAGCTGTTGTCAGGGG
LCA66	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA
LCA94	GTCCATTCATCCAGCACAGG	ACATTTGGCAATCTCTGGAGAA
LCA99	CAGGTATCAGGAGACGGGCT	AGCATTTATCAAGGAACACCAGC
YWLL29	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGCAG
YWLL40	CACATGACCATGTCCCCTTAT	CCAGTGACAGTGTGACTAAGA
YWLL44	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA
LGU49	TCTAGGTCCATCCCTGTTGC	GTGCTGGAATAGTGCCCAGT
LGU50	CTGCTGTGCTTGTCACCCTA	AGCACCACATGCCTCTAAGT

ISAG Additional Markers - Llamas and Alpacas		
Locus	Forward	Reverse
LCA24	ACTCACGGGTGACATACAGTG	GAGCAGTGTTTGGTTTGCATT
YWLL08	ATCAAGTTTGAGGTGCTTTCC	CCATGGCATTGTGTTGAAGAC
YWLL36	AGTCTTGGTGTGGTGGTAGAA	TGCCAGGATACTGACAGTGAT
YWLL43 (X-linked)	ATACCTCTCTTGCTCTCTCTC	CCTCTACAACCATGTTAGCCA
YWLL46	AAGCAGAGTGATTTAACCGTG	GGATGACTAAGACTGCTCTGA

ISAG STR Core Panel - Pigeons		
Locus	Forward	Reverse
CliµD11	CCAATCCCAAAGAGGATTAT	ACTGTCCTATGGCTGAAGTG
CliµT43	GGGAAAGGAAATTTGACACTG	ACTGTCGATGCCATTAAGAC
CliµD01	GATTTCTCAAGCTGTAGGACT	GTTTGATTTGGTTGGGCCATC
PIGN57	CTCTTGTATGTCCATCTGAAC	ACCCATTTACCACTCTCTAA
CliµT13	CTGTCGAGCAGTAACAGTCC	GTTTGCAAGCCCTGGTTATCTCA
CliµD16	GCAGTGATAAAGTTCTGGAACA	GTTTGCCTCACCGTGACATCA
CliµD19	CTGCCCGTTTCTTCTAATGCAC	GTTTGGATTTCTGGGAGTGTATG
CliµT02	AGTTTTAATGAAGGCACCTCT	TGTAGCATGTCAGAAATTGG
CliµD17	TCTTACACACTCTCGACAAG	GTTTCCACCCAAATGAGCAAG
CliµD35	GGGAGCTTAAGGGATTATTG	ATTCCTTGCATGCCTACTTA
CliµT17	ATGGGTTTGGAGATGTTTTG	GTTTGATGGAGTTGCTATTTTGCT
PIGN04	GGTTTTTCTGTTTCCTCACG	GGGATTCTGGGATTATTTTTC

ISAG Additional Markers - Pigeons		
Locus	Forward	Reverse
PIGN15	TTTCCTTTCATTTGCTGTGG	AACCAGGCATTGGAGTCTTT
PIGN10	TTCCACTGAATGGGTCTCAG	CTGCCAGAAGGTAAATGACAC
PIGN26	TCACTGTATTCACCAAAGTCTG	CAATGTGGGGGGGCGTCTATG
PIGN12	CAGATCCAGCAGTCTTGAAG	CCCATCTAATGCGATAAATCC

Duty laboratory for the next comparison test with contact details For 2016-2017 no comparison tests are organized by this committee.

SIGNATURES

Chair

Duty laboratory