ISAG CONFERENCE 2016, Salt Lake City, USA

Workshop: Applied Genetics of Companion Animals

Organised by a standing committee yes

Date and meeting time: Tuesday, July 26 2016, 8:30 – 12:00

Chair, name and contact email:
Co-chair (Cat) Leslie Lyons – University of Missouri, USA, lyonsla@missouri.edu
Co-chair (dog) Leanne van de Goor – VHL Genetics, The Netherlands, lgo@vhladmin.nl

Agenda / programme - attached with detailed reports

Number of participants at meeting: ~50, room to capacity

Cat Comparison Test:

Duty Laboratory: **Maria Longeri**, (Vetogene, Milan, Italy) maria.longeri@unimi.it
Analysis Laboratory (with FASS): **Leslie Lyons** (University of Missouri, USA) lyonsla@missouri.edu

1. **Summary of the Cat workshop motions**.

(i) Motion accepted to have FASS correct typographical errors (2 No votes)

"*Delegate the authority to the Standing Committee to review requests for correction of clerical errors that are properly documented by the participant and make the correction for the final compilation of results. Evidence to be provided by the participant"*

(ii) Motion accepted to test either *AMEL* or *ZFXY* for gender but report only one.

(iii) Motion accepted to NOT report allele sizes for gender markers.

(iv) Motion accepted to keep both numerical and alphabetical nomenclature in cats

(v) Motion accepted for the committee to work towards a standard for *Polycystic kidney disease (PKD1)*, *Hypertrophic cardiomyopathy (MYBPC1)*, *Pyruvate kinase deficiency (PKLR)* and *Dilution (MLPH)* for presentation in 2017 at next Cat CT in Ireland.

2. **Summary of improvements for next Cat STR-based comparison test**

   (i) Please purchase control cat sample – CCL-94 from ATCC and run in each CT by all laboratories (https://www.atcc.org/Products/All/CCL-94.aspx)
   (ii) Remember to distribute an additional laboratory control to help maintain standard data calls and to prevent allele size drift between comparison tests
   (iii) Emphasize standardization of reporting data in the excel data file that is submitted to FASS for analysis. Example: XX not X/X
   (iv) Emphasize that alleles that are 1 bp different for a dinucleotide are reported as M1 – not M+1 or M.1
   (v) Emphasize to report why the laboratory reports the parentage analysis as an inclusion or exclusion, i.e. how they made the decision.

3. **Future Cat Comparison Tests**
   (i) The next STR-based test will be performed for 2019
   (ii) A trial SNP-based parentage test will be performed for 2017 – Ireland.

   George Sofronidis - Orivet, Australia (george@orivet.com.au) will be the duty laboratory.

Dog Comparison Test:

Summary of the Dog workshop motions.

(i) Motion accepted to have FASS correct typographical errors (2 No votes)

"*Delegate the authority to the Standing Committee to review requests for correction of clerical errors that are properly documented by the participant and make the correction for the final compilation of results. Evidence to be provided by the participant"*

(ii) Motion accepted to accept 146, 147 and 148 as correct for the smallest allele of marker FH2054 in sample DCT-05.

Summary of improvements for next Dog STR-based comparison test

(i) There are nomenclature issues with the smallest allele of marker FH2054 in sample DCT-05. To be able to decide on the correct ISAG nomenclature of this allele Peter Dovc's lab will sequence this allele, then decision about the nomenclature of this allele will be made during the 2017 conference in Ireland.
(ii) Before the 2017 conference in Ireland more data will be collected about possible markers for the ISAG additional dog STR marker panel. Sofia Mikko will take the lead. The goal is to establish an ISAG additional dog STR marker panel during the 2017 conference in Ireland.
(iii) Several labs have SNP parentage panels running for dogs. Overlap between those panels and the MAF of the SNPs will be checked. The goal is to establish an ISAG dog SNP marker panel during the 2017 conference in Ireland.

Future Dog Comparison Tests

(i) The next STR-based test will be performed for 2019

(ii) A trial SNP-based parentage test will be performed for 2017 – Ireland.

George Sofronidis - Orivet, Australia (george@orivet.com.au) will be the duty laboratory.

New Standing Committee for Applied Genetics in Companion Animals

Chair:

Sofia Mikko – Swedish University of Agriculture Sciences, Sweden, Sofia.Mikko@slu.se
term of service: 2016 – 2019

Duty Laboratory:

George Sofronidis - Orivet, Australia (george@orivet.com.au)
term of service: 2016 -2019

Members:

Leslie Lyons - University of Missouri, USA, lyonsla@missouri.edu
term of service: 2010-2017

Leanne van de Goor –VHL Genetics, The Netherlands, lgo@vhladmin.nl term of service: 2014 – 2017

Peter Dovc - University of Ljubljana, Slovenia, Peter.dovc@bf.uni-lj.si
term of service: 2014 – 2017

Cecilia Penedo - Veterinary Genetics Laboratory, University of California – Davis, USA mcpenedo@ucdavis.edu
term of service: 2016 – 2019,

Maria Longeri – Vetogene, Italy, maria.longeri@unimi.it
term of service: 2014 – 2017

Jiansheng Qiu – Neogen, USA, JQiu@neogen.com
term of service: 2016 – 2019

ISAG 2016 Workshop Agenda
Salt Lake City, Utah
Applied Genetics of Companion Animals
Tuesday July 26, 2016

Co-chairs: Leanne van de Goor – VHL Genetics; Leslie Lyons – University of Missouri - Columbia

8:30 am Welcome and Agenda – Lyons/van de Goor

Dog CT Duty Report – Leanne van de Goor – VHL Genetics, The Netherlands

Dog CT Analysis Lab Report – Peter Dovc, University of Ljubljana, Slovenia

Discussion – Secondary canine STR Panel

Presentation - Evaluation of single nucleotide polymorphism (SNP) markers for canine parentage analysis . **J. Qiu**, B. Simpson, L. Kock, J. Donner, C. Cole, S. Davison, M. Dunn, D. Bannasch, and A. Boyko

Discussion – Secondary canine SNP Panels

10:00 – 10:30 Break

10:30 Cat CT Duty Lab Report – Maria Longeri, University of Milan, Italy

Cat CT Analysis Lab Report – Leslie Lyons, University of Missouri - Columbia

Discussions

Review of use of cat controls and genotypes
SNP Panels for Cat CT
Standardization of disease tests (PKD, HCM)

Motions and Votes

Selection of 2016 - 2019 Standing Committee:

12:00 Meeting adjourn

ISAG 2016 Cat Parentage Panel Comparison Test
Detailed Report

July 2016 – Salt Lake City, Utah, USA
Tuesday, July 26 2016
8:30 – 12:00
Canyon C
Hilton Hotel

Final Draft – Lyons (09 September 2016)

The 2016 cat comparison test was hosted by Vetogene, Milan, Italy (Duty Laboratory) and the Lyons Feline Genetics laboratory at University of Missouri - Columbia (Data Analysis Laboratory). Information on fourteen (14) microsatellite markers and two gender specific markers (AMEL and ZFX/ZFY) was provided below. Participants had the opportunity to test three disease loci for cats: polycystic kidney disease (PKD in *PKD1*); and two hypertrophic cardiomyopathy (HCM in *MYBPC3*) mutations. References articles were provided.

Twenty (20) DNA samples were distributed for the cat comparison test. The cat samples were from both pedigreed and mixed breeds of cats and one different cat was positive for each of disease alleles. One sample (Cat no. 2) was the control and its genotypes provided. Data on the previous control cat – 4406 – was also provided but DNA was not distributed.

DNA: extracted from peripheral blood EDTA by classic Phenol/Chloroform method ~ 50 ng/ul 50ul was shipped.

RAGDOLL	**CAT1**	*EUROPEAN*	**CAT11**
EUROPEAN	**CAT2**	*MCO*	**CAT12**
EUR X SIA	**CAT3**	*EUROPEAN*	**CAT13**
EUROPEAN	**CAT4**	*EUROPEAN*	**CAT14**
BIRMAN	**CAT5**	*EUROPEAN*	**CAT15**
BIRMAN	**CAT6**	*EUROPEAN*	**CAT16**
BIRMAN	**CAT7**	*PERSIAN*	**CAT17**
BIRMAN	**CAT8**	*EUROPEAN*	**CAT18**
BIRMAN	**CAT9**	*EUROPEAN*	**CAT19**
EUROPEAN	**CAT10**	*BIRMAN*	**CAT20**

Instructions and notes regarding the primers were also provided.

Duty Laboratory Report

ISAG will implement mandatory deadlines for sample shipment and shipment requests for replacement samples.

One single sample was replaced to one participant.
One complete shipment was lost by courier and replaced.

Data was uploaded to FASS website by April 15, 2016.

Participation & Procedures

Twenty – three laboratories (23) participated. One lab only doing SNP data.

Country	Participants
France	6
United Kingdom	1
USA	1
Australia	1
Czech Republic	1
Japan	1
Ireland	1
Italy	2
Germany	4
Spain	1
Sweden	1
South Africa	2
Portugal	1
Belgium	1
	24

Provided data was corrected for typographical errors and differences in reporting. **Motion accepted to have FASS correct typographical errors (2 No votes)**

Examples included:

XX = X/X – adding the slash
Standard reporting will be emphasized in future instructions.

S/S = S/ - presenting homozygotes as one allele (which was different from previous tests)

Reporting diseases as “N/A” not N/S or N/P or N/H.

Removing extra spaces in the data
Letter codes for Cat2 FCA453 & FCA678 were added.

The M1 allele was changed from M.1 and M+1 to M1. **Standard reporting will be emphasized in future instructions.**

Adding a letter code that was obviously missing and had been called in other parts of the data.

<u>Gender Reporting</u>

All genders were correctly identified by all laboratories
9 labs used ZFXY, 5 labs used AMEL and 9 used both

Question: Should we pick one gender marker for future?

- **Motion accepted to test either AMEL or ZFXY but report only one.**

Question: Should we present allele sizes?

- **Motion accepted to NOT report allele sizes for gender markers.**

Disease reporting

All diseases were correctly identified

8 labs tested diseases, but 1 laboratory did not do Ragdoll HCM

In addition, 2 labs reported PKLR (pyruvate kinase deficiency) and both reported Cat 4 and Cat 19 as carriers.

Alleles were generally N = Normal and S = A = H = P = Affected

5 labs used N/A to mark affected cats

Question: Can we standardize and use one code for all diseases? (In English and native language)

- Motion accepted: The committee will work towards a standard for PKD, HCM, PKLR and Dilution for presentation in 2017 at next Cat CT in Ireland.

Microsatellite Data

Errors	No. of Labs
0	2
1	3
2	1
3	2
4	3
6	2
7	3
9	1
10	1
20	2
32	1
48	1
Missing 5 markers	1

FCA 649 has allele M1, this allele was reported in various ways including, M1 M+1 and M.1 ------- 8 labs reported correctly ----- 10 labs did not report correctly. This "split-peak" issue was noted in the instructions and in 2014 testing. Cat 3, Cat 6 and Cat 20 had the M1 allele.

2 labs had R1 = 151 allele in Cat 9

Question: Please analyze your raw data and report if you think these split-peaks may be present. We will need to consider in the final calculations for certificates.

Split peak present for M1 allele in FCA649
Split peak NOT accepted for 151 allele.

Marker **FCA026** has allele 150 dropout for Cat 15, 17 (perhaps others as well). At least 10 labs missed the dropout but many identified the allele.
Question: Has anyone redesigned primers for FCA026?

Labogena has redesigned this marker and suggests these primers (discussed by email):

ISAG	FCA026_F	TGTACACGCACCAAAAACAA
	FCA026_R	GGAGCCCTTAGAGTCATGCA
New	FCA026r_F	AATGTTGCAGGCCTGTGTAC
	FCA026r_R	GATCATGAACCGAACTGGTG

In this design, the primers were moved as follows:

1) FCA026_F was moved by 15 bases, direction upstream the microsatellite
2) FCA026_R was moved by 48 bases downstream the microsatellite

Finally, the size of the PCR fragment was increased by 63 bases.

The committee will need to work with Labogena to re-establish the letter codes – please see sequencing of FCA026 below.

Over 4 labs missed FCA 229 Cat 20 170 allele
Question: Please be prepared to discuss this allele. Accepted as true errors

Over 8 labs missed or rounded incorrectly FCA105 Cat 12 175 allele
Question: Please be prepared to discuss this allele. - Accepted as true errors

One lab reported raw data, did not round, because letter nomenclature is to be used. **This lab will round and resubmit data with letter codes.**

Parentage question

1. Can CAT20 and CAT07 be parents to CAT06?
2. Can CAT08 be offspring to CAT07 and CAT05?

Overall, reported correctly but with minimal discussion or detail.

Question: Do the instructions need to specify that "how and why the decision was made?" Yes – need to improve instructions

Additional Reporting

FCA026d - – different letter codes
FCA201d – different letter codes

Agouti, Brown, Color, Dilute, Long

FCA097, FCA005 (2), FCA078, FCA096, FCA224, F124, F53, FCA723, FCA731, FCA733, FCA736, FCA740, FCA742, FCA749

Letter Codes

Please be prepared for detailed discussion. Drift in the letter code for control cats will be presented (Cats 4406 and CCL-94). Error was made in letter code in 2014.

- FCA310
  - Published: 128 = O = correct
  - 2014: 130 = O

- All except horse / donkey report basepairs

- Letter nomenclature is published for 9 markers in Lipinski et al., Anim Genet. 2007 Aug 1; 38(4) 371-377.

- Lyons lab presented sequence data and repeat length on four of five new markers: FCA026, FCA201, FCA220, FCA453, and FCA649.

Marker	Strand Size	Sequence Size	Type of Repeat	Number of Repeats
FCA_026	146 - Q	146	Dinucleotide	23
FCA_293	185 - K	188	Dinucleotide	18
FCA_453	188 - K	188	Tetranucleotide	6
FCA_649	140 - M	144	Dinucleotide	22

FCA441 is a tetra, thus letter codes represent 4 bp changes. Split-peaks would be presented as M1 or M2 or M3.

Overall, Allele sizes and letter codes produced the same scores.

12 labs did not report letter code for Control Cat 2 for FCA453 & FCA649. Likely because the Duty lab did not report these letters. REMINDER – You should confirm your own data for the control samples. The correct letter codes were put into place as all labs had the correct allele sizes listed. Note: - Not part of calculations for certificates.

3 labs did not report letter codes for FCA453 and FCA649

Question: Should letter codes for these two markers be used in the calculations?
Question: Use bp data to calculate scoring?

Please score both ways.

1 lab did not report letter codes for 5 new markers (not a new laboratory)

1 lab did not report any letter codes (not a new laboratory)

Question: Use bp data to calculate scoring?
Please score both ways.

1 Lab was off by rounding for FCA026 and FCA069
1 Lab was off by rounding for FCA310
Question: Do they get to adjust their data? **- No, incorrect data**

Question: Confirm – is missing data counted as incorrect?
- Confirmed, missing is incorrect.

Future Comparison Test:

A SNP test will be available for any laboratory wishing to participate for 2017 – Ireland. No STR comparison test will be performed for 2017.

Cat SNP Duty Laboratory:

Orivet – George Sofronidis - george@orivet.com.au

SNPs will be selected with MAF > 0.30, with spacing > 5 Mb on cat V6.2 assembly.

Available SNPs include 101 from Orivet / GeneSeek (all on 63K cat array), 148 from Lyons Cat Ancestry panel (109 on 63k cat array), and ~ 50 from Gus Cothran at Texas A&M used for Cat Fanciers' Association parentage testing.

Please remember to purchase cat control CCL-94. Three labs are to calculate control data for future. Future tests will have 20 cats plus the one control CCL – 94.

Additional members added to Cat & Dog Comparison Test Committee

New Chair:

Sofia Mikko – Swedish University of Agriculture Sciences- Sofia.Mikko@slu.se

Motions:

"Delegate the authority to the Standing Committee to review requests for correction of clerical errors that are properly documented by the participant and make the correction for the final compilation of results. Evidence to be provided by the participant"

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Duty Laboratory Report - – Leanne van de Goor – VHL Genetics, The Netherlands

The duty lab for the 2016 dog comparison test was VHLGenetics, Wageningen, The Netherlands.

Twenty-one DNA samples were distributed for the dog comparison test. Samples collected by Dr. Frank Cooper. The dog samples were from eight dog breeds (German Shepherd, Chihuahua, Cocker Spaniel, English Springer Spaniel, Border Collie, Golden Retriever, Labrador retriever and Cavalier King Charles Spaniel). One sample (DCT-REF) was the reference sample and its genotypes were provided. The DNA was extracted from buccal swabs (5 Genotek swabs per dog), for each sample ~ 30 ng/ul 25ul was shipped to all participants.

Seventy-seven labs requested samples. Samples were shipped out on November 12, 2015. Several new batches of samples were requested because tubes were (almost) empty, the reason is most probably evaporation since one water was added, results were produced. The new batches were sent out (after January 01) in another type of tubes and the caps were covered with parafilm. One initial request was after deadline and samples were no longer available. Results had to be uploaded to FASS website, the deadline was March 31, 2016. **We should send a picture of a typical shipment so new labs know the best way to package.

The 76 participating labs came from 32 different countries, spread over five continents:

Dog Duty Laboratory Report (Lyons – from presentation recording)
Peter Dovc, University of Ljubljana, Slovenia

67 of 76 (88%) labs reported, none from Australia, 52 in Europe with 8 in both Germany and Italy.

Expected 67*22*20 = 29480 genotypes

Received 27157 (92.12%)
2323 errors - 7.88%
723 non-concordant - 2.45%
1600 not reported 5.43%

Genotyping Accuracy of Labs

Rate%	**Absolute**	**Relative**
98 – 100	43	52
95 – 98	4	3
90 – 95	4	6
80 – 95	9	4
0 – 80	7	2

Critical Loci in Dog CT

AMEL	91.17% concordancy, a false issue due to reporting XY versus YX issue	
INU005	94.92%	many missing, shift in alleles
REN169D01	95%	shift in alleles, sporadic errors
FH2054	95.45%	small alleles in DCT-05 in range 146 – 148 bp
REN54P11	96.06%	
REN247M23	99.14%	least errors

Specific Animal errors
Several comments regarding bad or not enough DNA.

Most males sample issues with AMEL – reporting errors.
DCT07 158 errors - AMEL
DCT06 135 errors - AMEL
Second critical locus was REN6419 – missing data (17)
FH2054 – small alleles
Demonstrated 35% of errors male AMEL reporting issue

HEAT MAP- Locus X Individual
3 markers not in kits, likely due to non reporting, perhaps different primers
AHTh130, REN105LO3 and REN64E19

ISAG Canine additional marker panel

- ISAG Dog core STR panel with 21 markers
- No ISAG Dog additional marker panel
- ISAG rules for parentage verification
  - *0 mismatches in core panel: Parentage qualifies*
  - *1 mismatch in core panel: Parentage doubtful. Type additional panel, if 1 mismatch remains, qualify parentage and assume that a mutation occurred*
  - *2 or more mismatches in core (+ additional) panel: Parentage excluded*

Survey among participating labs of Dog CT test – 4 labs with additional markers:
Fh2001 (3) NON CONCORDANT
Fh2328 (2) CONCORDANT
Lei004 (2) NON CONCORDANT

One lab with many additional markers.

Two labs provided HO and HE values

On 44 breeds:

Locus	HO	HE
UK109	0,468	0,552
AHT126	0,526	0,662
AHT125	0,541	0,758
UK133	0,547	0,640
UK111	0,603	0,751
CXX_403	0,630	0,747
LEI007	0,646	0,746
UK118	0,654	0,807
CPH3	0,655	0,776
1_9A	0,694	0,825
UK101	0,703	0,834
CXX2137	0,753	0,893

On 31 breeds:

Locus	HO	HE
PEZ5	0,444	0,488
PEZ20	0,456	0,492
FHC2010	0,475	0,508
PEZ1	0,515	0,566
PEZ6	0,604	0,653
PEZ12	0,639	0,649
PEZ8	0,640	0,689
PEZ13	0,663	0,684
PEZ11	0,718	0,720

Also commercial Kits available

Canine Genotypes Panel 2.1 (thermoFisher)
19 markers: PEZ02, PEZ17, FH2017, FH2309, PEZ05, FH2001, FH2328, FH2004, FH2361, PEZ21, FH2054, FH3377, FH2107, FH2088, vWF.X, FH2010, PEZ16 FH3313 and ZFX/Y (sex determining locus).

StockMarks Kit for Dogs (thermoFisher)
10 markers: PEZ01, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8, FHC2079

Before the 2017 conference in Ireland more data will be collected about possible markers for the ISAG additional dog STR marker panel. Sofia Mikko will take the lead. The goal is to establish an ISAG additional dog STR marker panel during the 2017 conference in Ireland.