ISAG CONFERENCE 2017, Dublin, Ireland

Applied genetics of companion animals

STANDING COMMITTEES / WORKSHOPS Information will be posted online

Organised by a standing committee yes ~~no~~
Date and meeting time: Thursday, July 20, 2:30 PM - 6:00 PM
Chair, name and contact email: Sofia Mikko, sofia.mikko@slu.se

Agenda / programme

Applied genetics of companion animals (orals) Chair(s): **Sofia Mikko, Swedish University of Agricultural Sciences** Location: **H1.49 Breakout, O'Brien Science Building** Date & Time: **Thursday, July 20, 2:30 PM - 6:00 PM**		
2:30 PM		**Welcoming Remarks.**
2:35 PM		**Dog CT Duty Lab Report. Trial SNP CT** George Sofronidis, Orivet
2:50 PM		**Dog CT Analysis Lab Report. Trial SNP CT** George Sofronidis, Orivet
3:05 PM		**Discussion.**
3:30 PM	69915	**AgriSeq™ targeted sequencing panel for determination of canine parentage and genetic health.** C Adams*[1], A Burrell[1], P Siddavatam[1], A Allred[1], M de Groot[2], and W van Haeringen[2], [1]*Thermo Fisher Scientific, Austin, Texas, USA*, [2]*VHL Genetics, Wageningen, Netherlands*.
3:45 PM	71485	**Pedigree and genomic-based relationships in a dog population.** A. Talenti*[1], D.L. Dreger[2], F. Danelli[1], S. Frattini[1], B. Coizet[1], S.P. Marelli[1], G. Pagnacco[1], G. Gandini[1], M. Polli[1], R. Caniglia[3], M. Galaverni[3], E.A. Ostrander[2], and P. Crepaldi[1], [1]*Department of Veterinary Medicine, University of Milan, Milan, MI, Italy*, [2]*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States*, [3]*Laboratorio di Genetica, Istituto Superiore per la Protezione e la Ricerca Ambientale, Ozzano dell'Emilia, BO, Italy*.
4:00 PM		**Coffee/Tea Break.**
4:30 PM		**Cat CT Duty Lab Report. Trial SNP CT**

4:45 PM	**Cat CT Analysis Lab Report. Trial SNP CT**
5:00 PM	**Discussion.**
5:15 PM	**General Discussion on SNP Panels for Domestic Cat and Dog Parentage.**
5:30 PM	**Workshop Business Meeting and Elections.**

Number of participants at meeting: approx 50

Summary of the meeting

Seminars

AgriSeq™ targeted sequencing panel for determination of canine parentage and genetic health.
C Adams*[1], A Burrell[1], P Siddavatam[1], A Allred[1], M de Groot[2], and W van Haeringen[2], [1]*Thermo Fisher Scientific*, *Austin, Texas, USA*, [2]*VHL Genetics*, *Wageningen, Netherlands*.

Pedigree and genomic-based relationships in a dog population.
A. Talenti*[1], D.L. Dreger[2], F. Danelli[1], S. Frattini[1], B. Coizet[1], S.P. Marelli[1], G. Pagnacco[1], G. Gandini[1], M. Polli[1], R. Caniglia[3], M. Galaverni[3], E.A. Ostrander[2], and P. Crepaldi[1], [1]*Department of Veterinary Medicine, University of Milan*, *Milan, MI, Italy*, [2]*National Human Genome Research Institute, National Institutes of Health*, *Bethesda, MD, United States*, [3]*Laboratorio di Genetica, Istituto Superiore per la*

Committee members (the new committee)

Chair	Term of service	E mail address:
Leslie Lyons - University of Missouri, USA	2017-2021	lyonsla@missouri.edu

Co-chair	Term of service	E mail address:
Jiansheng Qiu – Neogen, USA	2017-2021	JQiu@neogen.com

Other members	Term of service	E mail address:
Leslie Lyons - University of Missouri, USA	2017-2021	lyonsla@missouri.edu
Leanne van de Goor –VHL Genetics, The Netherlands	2017-2021	lgo@vhladmin.nl
Peter Dovc - University of Ljubljana, Slovenia	2017-2021	Peter.dovc@bf.uni-lj.si
Cecilia Penedo - Veterinary Genetics Laboratory, University of California – Davis, USA	2016-2019	mctorrespenedo@ucdavis.edu
Maria Longeri –University of Milan, Italy	2017-2021	maria.longeri@unimi.it
Jiansheng Qiu – Neogen, USA	2016-2019	JQiu@neogen.com
Nuket Bilgren – University of Ankara, Turkey	2017-2021	nuketbilgen@gmail.com
Alexandre Vasilescu – Labogena, France	2017-2021	alexandre.vasilescu@labogena.fr

Comparison test (2016-2017)

Dog and Cat Trial SNP CT Duty Lab Report, and analysis.

George Sofronidis, Orivet

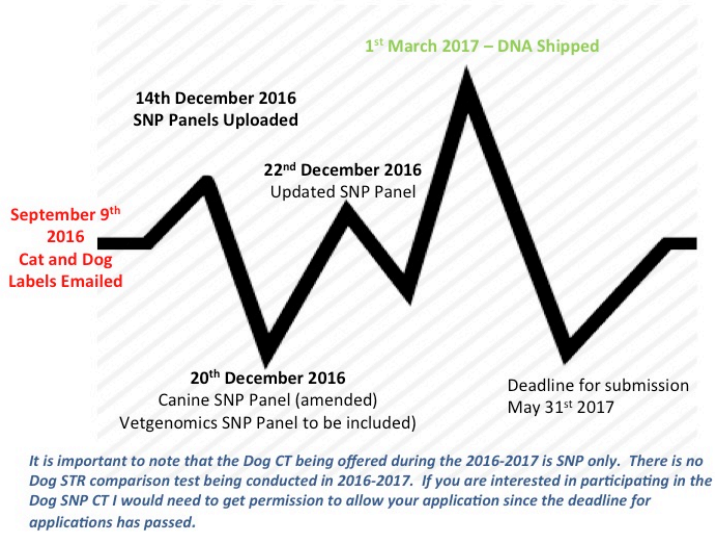

Figure 1. Schedule for preparing and shipping of dog and cat comparison test samples. The deadline for submission of results was moved from 30 April to 31 May 2017.

The schedule for preparation of dog and cat samples to be used in the canine comparison test (CT) 2017 is outlined in Figure 1. A major part of the workload was done around Christmas time, but the DNA was not shipped until March 01, 2017, and therefore the deadline was moved to May 31, 2017. There were 14 requests for dog samples, and 15 laboratories requested both dog and cat, but only seven of them responded with results.

Table 1. A list of laboratories participating in the dog CT, what platform they used for genotyping and what SNP panels they used.

Laboratory	Platform	Dog SNP panel
Lab A	Ion Torrent	Orivet, Neogen 1, Neogen 2, Vetgenomics
Lab B	Illumina	Neogen 1, Neogen 2
Lab D	Sequenom	Neogen 1
Lab E	Illumina	Orivet, Neogen 1, Neogen 2
Lab F	Illumina	Orivet, Neogen 1, Neogen 2, Vetgenomics (18SNPs)
Lab G	Illumina (Iscan)	Neogen 1, Neogen 2
Lab H	Sequenom	Orivet, Neogen 1 (30%)
Duty lab	Illumina & Sequenom	Orivet, Neogen 1, Neogen 2

The dog trial SNP CT comprised a total of 416 SNPs divided in four SNP panels; *1)* Orivet 88 SNPs, *2)* Neogen 1st panel 100 SNPs, *3)* Vetgenomics 128 SNPs, and *4)* Neogen 2nd panel 100 SNPs. Most likely, this is more than what is needed for parentage and identity of dogs. Across panels, the SNPs are located on 39 autosomes, two sex chromosomes, and the mitochondrial chromosome. The platforms used for dog CT were; Illumina (4 labs + duty lab), Sequenom (2

labs + duty lab), and Ion Torrent (1 lab) (Table 1). DNA from the dog samples was prepared from swabs (n=6), and blood (n=14).

There were four parentage questions, and all participants answered them correctly. Since this was a trial CT, there were no calculations of error rates, but a few discrepancies, mainly due to calling of bottom strand instead of top strand. The analysis of the results needs to be an automatic process. Take advantage of the knowledge and experience in other species, like cattle.

The cat trial SNP CT comprised a total of 120 SNPs, all in one panel. Ten of the SNPs originated from the Cothran laboratory, 31 from the Lyon's laboratory, and 79 came from Orivet and Neogen. In total there was a 33 % return rate. The platforms used for cat CT were; Illumina (2 labs), Sequenom (2 labs + duty lab), Ion Torrent (1 lab).

All participants had the correct answers for the three parentage questions.

Some of the SNPs had allelic dropouts (Table 2). Some of them may be located on sex chromosomes.

Table 2. Cat SNPs with allelic dropouts on three genotyping platforms.

Sequenom	Ion Torrent	Illumina
chrA2.171182940	chrB1.69970470	chrB1.100153958
chrD2.93650111	chrB2.394102700	chrA2.217930062
chrC2.2254710	chrB4.156816042	chrB2.159161793

Comparison test 2018-2019

The next dog and cat CT in 2018-2019 will be official for STRs, and a second trial CT for SNP. The same set of samples will be used for both STR and SNP CTs. A decision was taken that reference genotypes should be provided also for SNP CT samples. Neogen will help out with these reference genotypes.

It was also decided that the official genotype nomenclature for SNPs is based on the "Top Call". Neogen will provide reference "Top Call" genotypes for three cats and three dogs. There is a complete set of 416 dog SNPs. To reduce the cost, participating labs can choose which panel(s) they want to genotype in the trial CT.

The cat STR CT will comprise of the 14-marker international panel defined in the 2013-2014 workshop. Primer sequences and other details of the panel are included in the 2014 Workshop report and will be made available for the next CT.

The schedule for the next CT was presented as follows:

Applications due	July 15, 2018
Invoices out	August 15, payment Sept 15
Ship samples	Nov 15, request second by Dec 15 shipped by January 01, 2018
Reports	March 31, 2018

There is a discussion between the Standing Committees and Executive Committee about having a central lab or not. One suggestion is to use 96-wells plates instead of tubes. The plates should be

sealed with caps and not sealing tape. Another option is to ship the DNA samples dry. For SNP CTs, a minimum of 500 ng is needed.

List of recommended markers with primer information

In conclusion, there was not enough data to decide on what SNP panels were the best, or how many SNPs is suitable to use. The workshop discussed the results and decided to run another trial CT of dog and cat SNPs to be reported at the next ISAG meeting.

Duty laboratory for the next comparison test

Jennifer Grahn, VGL, UC Davis

Presentation: Extra STR panels for dog parentage

Sofia Mikko, SLU, Uppsala, Sweden

Handouts from the presentation is attached in a separate file.

There is a need for a secondary panel for dog parentage, as many cases only has one marker exclusion. The workshop discussed which additional panel should be selected. ThermoFisher Canine panel 1.1 will be updated to comprise all the ISAG recommended markers. Only one of them is a tetra repeat. ThermoFisher Canine panel 2.1 contains many complex repeats, and Laboklin panel consists of many tetra repeats. These last two may therefore not be recommended as extra panels, as these types of repeats may have a higher mutation rate, and may cause false exclusions. VGL has two extra panels. It was decided that the best UCDavis panel will become the dog ISAG additional marker panel (see table 3 for the marker information of this panel). A request was put forward to ThermoFisher to have them change the kits to include markers standardized by ISAG.

Table 3. Marker information Dog ISAG additional marker panel.

Name[1)]	Chr	Position	Forward Sequence	Reverse Sequence	Multiplex[3)]	Size Range	Label
2642_RD	35	15.822.237	GTTCCATGCATGCTGACACA	GGGGTGAGAATGATGGTGGT	1	86-108	FAM
1404_RD	15	17.933.748	AGGGCTGTTTGGAGGAACAA	**GTTTCTT**TGGTCTGACATGAGGGGACA[2)]	1	137-167	FAM
1878_RD	21	35.583.961	TGCCATAAATGCCCAGAACA	TGCCACCTGGCAGTCTTATG	1	240-258	FAM
0914_RD	9	34.716.452	TGCATGGTCACAAGCATCAG	GCACACAAAATTGTGCGGATA	2	279-295	FAM
2469_RD	31	28.950.565	GTGCACTTTGCAAACCCTGA	TTGTAAGCAGGGGCAAGTGA	2	303-325	FAM
0176_RD	2	24.363.177	TGGCTTGGCAACATTGTCTC	ACCTGGGATTCTCTCGGTCA	2	365-381	FAM
0959_RD	10	8.308.428	CCAGCCAGATGCAAACATTG	GCTCATGTGGTGTTTTTGATG	1	264-278	NED
0323_RD	3	48.244.964	GGAAGCAGCTGGGTTCCTAA	**GTTT**TCCATGCCCAACTATTTTTGAA[2)]	2	300-318	NED
0669_RD	6	55.653.310	TTGCCGAGATCACTCAAGGA	AATTCTGTGCCCCAAAGTGG	2	357-379	NED
0123_RD	1	99.908.185	CACGGACGCAACACGATTTA	CTCCTGACGCAGCAGTTGTC	1	189-217	PET
1055_RD	11	18.624.053	CCCAAGCTGGGAAGACAAAA	GGGTGGATTTAGGGTGGACA	1	217-231	VIC
1257_RD	13	29.853.239	TCACCTTCTGGATGGGAACC	ATCCTGCAGTTGCTGTGCTG	1	244-262	VIC

[1)] Wong et al, A Comprehensive Linkage Map of the Dog Genome, Genetics. 2010 Feb; 184(2): 595–605

[2)] In bold is a tail to change the PCR product size range and shift the marker to avoid overlap with another marker with same label

[3)] The markers can be amplified in two PCR multiplexes. Both multiplexes amplify at Ta 60°C, 2.5 mM MgCl2. Both multiplexes can be combined and co-loaded for electrophoresis

Presentation: Characterisation of allele variants at canine FH2054 microsatellite locus

Sofia Mikko (stand in for Jakob Lavrencic, Jernej Ogorevc and Peter Dovc, Department of Animal Science Biotechnical Faculty, University of Ljubljana, Slovenia)

Peter Dovc had prepared a presentation about the dog marker FH2054. In the STR CT 2016 there were an intermediate allele in sample DCT-05, called differently by the participating labs. Peter has sequenced the marker and its alleles, and confirmed the complex compound structure of this tetrarepeat microsatellite. The intermediate allele in question was confirmed to be 147 bp long, and the workshop decied that it will consequently be called 147. It was also decided that other new intermediate sized alleles should be called by their size in relation to the standardized ISAG nomenclature. The workshop discussed if we can discard this marker from 22 recommended markers because of high mutation rate. It was decided to remove marker FH2054 from the dog ISAG recommended panel.

Another marker with high mutation rate is FH2328. VGL has a list of tricky markers and can make mutation rates available so that the Standing Committee can take a good decision about what markers should be removed or kept in the recommended panel.

Presentation: Cat Genetic disease & Trait reporting standardization

Leslie Lyons

There is an ongoing discussion on how to report test results for genetic diseases and traits. The aim is to have report templates that are universal, easy to understand, and are legally sound. Leslie Lyons had made an offer to compile different report formats used at present. Few had responded, and the discussion will continue. Some of the discussion points in short:

- HCM and PKD affected does not mean that the cat definitely does get the disease.
- Proposal to put gene name, position, and mutation on the report.
- Reports should be possible to identify, in order to be able to detect fraud.
- Leslie Lyons offers to provide explanations per mutation/test.
- Proposal use N/N, N/P and P/P (P means variant present).
- Discussion about how labs report, suggestions made how to report, no decision made.

Report on Suggestions for Standardization of Reporting for Cat Genetics Tests for Traits and Diseases

ISAG 2017 Ireland – Applied Genetics in Companion Animals Workshop

20 July 2017 14:30 – 18:00

Concern:

Many new laboratories are entering companion animal genetic testing from around the world. As like parentage testing, the community should ensure the same and correct variants are being assayed for a given trait or disease as some interpretations of the publications for the variants can be confusing. In addition, cats are shared for breeding around the world, thus, a standardized reporting nomenclature, regardless of the language, would assist breeders, owners and veterinarians for interpreting test results.

In addition, test results need to convey phenotype and health status, perhaps providing some education in this regards. Several questions have arisen that could be addressed by some standardization;

- Is the same variant being tested by all labs?
- How accurate is the testing?
- How do I know the test goes with this cat?
- What does the test imply?
- Does positive = affected?
- Does normal = unaffected?
- What to do with tests performed in breed with no unknown risk – what should be conveyed to the owner/breeder/veterinarian?
- Can a nomenclature be established that can be understood by diverse languages?

Goal:

The goal of the workshop was to evaluate present test reports from different laboratories for specific cat traits, including *Dilute*, *PKD*, *HCM* and blood type. Dilute was replaced with *Brown* as recent issues have occurred with the reporting of the alleles at the *Brown* locus for cats. Can some suggestions for standardization be developed?

Participation:

An e-mail was sent to the cat CT participants for 2016 and 2017 to provide examples of reports for the four loci. Very few laboratories responded, however, via the cat breeder online database – PawPeds – many examples of the HCM reports were available for consideration.

Results:

Most laboratories convey consistent information on all reports such as:

- Owner name / address
- Breed
- Cat name
- Chip No
- Registration No.
- Color
- Sex
- Date of Birth
- Test name
- Case No.
- Report No.
- Specimen Type
- Date received
- Date reported
- Interpretation
- Signature of authority

Suggestion 1: Species Identification

Add the species (cat / feline) to the overall name of the test as many labs perform testing in multiple species and some tests will have the same name, such as HCM, pyruvate kinase deficiency.

Suggestion 2: Be Vigilant of Fraud

Several laboratories have independently documented cases of fraud, including counterfeit reports, and altering of reports. Develop bar coding or other means to document the produced report that would be difficult to alter or easy to detect.

Suggestion 3: Submitted Specimen Identification

Add specimen type as this information will help interpret the accuracy of the test and the possible influence of contamination. For example, a buccal swab from a kitten may have maternal contamination but contamination would be less likely with a blood sample.

Suggestion 4: Veterinary Collected Verification

Add an area to the submission form / form that provides the opportunity for an independent source, such as a veterinarian, can verify that the cat's chip has been scanned and corresponds to the submitted sample. Some cat registries require this documentation, thus if this information is provided on the form, the data from all laboratories can be accepted by the registry.

Suggestion 5: Standardization of Test Name Nomenclature

The use a of a consistent name for a genetic test that includes the genetic aspects would help communication and understanding and suggest the same variant is being tested by all laboratories.
Example:

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Domestic Cat Genetic Test – Hypertrophic Cardiomyopathy (HCM)

Variant: MYBPC3 c.91G>C (A31P) **Breed specificity:** Maine Coon

Test Method: Allele-specific assay detected by MADLI-TOF

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Suggestion 6: Nomenclature - Variant not Mutation

Try to use the word "variant" for mutation to attempt to reduce the concern of variation. Not all mutations are bad, however, the term "mutation" tends to convey a more negative feeling. We all have mutations!

Suggestion 7: Method of Assay

Provide the method of assay under the test name to help interpretation of accuracy and potential issues

Standardized Reporting:

For feline HCM, at least eight (8) different methods of reporting are used by different laboratories:

- Clear – cat does not have mutation

- Homozygous Normal
- N/N; N/P; P/P
- N/N; N/HC; HC/HC
- Negativo / Positivo
- +/+; +/-; -/-
- N/N; N/HCMmc HCMmc/HCMmc
- Negative; Mutant/Normal; Mutant/Mutant
- Echantillon homozygote G/G, non porteur de la mutation responsable de la myocardiopathie hypertrophique

Suggestion 8: Standardized Nomenclature – all disease tests

All genetic variants convey "risk" for a specific disease. Some variants are nearly 100% penetrant with little variation in presentation while others have lower penetrance and have more variation in onset and disease severity. Thus, the use of the verbiage "positive" or "affected" to imply the cat will have disease is misleading and beyond the expertise of the laboratory as all diseases and health conditions should have evaluation and confirmed diagnosis by a veterinarian. Laboratories should help develop a strong interaction with the veterinary community and encourage breeders / owners to have their cats examined periodically by a veterinarian.

Example:

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The use of the letter "P" is suggested to represent – Presence of the variant
The use of the letter "N" is suggested to represent – No variant, cat has the normal, wildtype allele.

Result:	**N/P**	**Normal / Present – Heterozygous – HCM risk**

The MYBPC3 c.91G>C (A31P) variant is suggested to cause HCM in Maine Coon cats. Association of the variant and disease requires scientific investigation in other breeds.

N/N = Both alleles are normal / wildtype. The cat is homozygous (2 copies) normal. The variant for the disease is not present. Offspring will not inherit the variant. The cat has no risk of disease due to this variant but may have other causes of HCM
N/P = The variant is present on one allele. The cat is heterozygous and carries the disease caused by this variant. This cat has risk for developing HCM. 50% of offspring may inherit the variant. Monitoring by echocardiology (ultrasound) and regular wellness examinations by a veterinarian are recommended.
P/P = The variant is present on both alleles. The cat is homozygous (two copies) for the variant that causes disease. This cat has high risk for HCM. 100% of offspring will inherit the variant. Monitoring by echocardiology (ultrasound) and regular wellness examinations by a veterinarian are recommended.

For HCM information & breeding suggestions: **www.felinegenome.missouri.edu/LyonsDen/Testing/HCM**

References: Meurs KM, Sanchez X, David RM, et al. A cardiac myosin binding protein C mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy. Hum Mol Genet 2005; 14: 3587-3593.

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Suggestion 9: Provide Detailed Information

- provide the reference for the variant – maybe a link to the paper
- provide a site to get more information about the disease to help keep the reports simple
- provide information regarding all possible genotypes
- provide information regarding the breed at risk
- provide information as to inheritance and risk to offspring
- listing the risk of disease presented in manuscripts may no longer be accurate so is **NOT** suggested
- use a way to highlight the result – **BOLD**, Larger Case, COLOR (remember people are color blind), **shading**.....

Examples of Reports

Available upon request from Leslie Lyons, lyonsla@missouri.edu

Signatures

Sofia Mikko
Chair

George Sofronides
Duty laboratory