

# Applied Genetics in Companion Animals

Organised by a standing committee: **YES**

## Meeting information

Date: Tuesday, 09 July 2019

Time: 9:00 AM - 12:30 PM

Number of participants: Standing room only > 75

## Co-Chairs

Name: Leslie Lyons & Jiansheng Qiu

Affiliation: University of Missouri and-Neogen GeneSeek

Contact email: [lyonsla@missouri.edu](mailto:lyonsla@missouri.edu); [JQiu@neogen.com](mailto:JQiu@neogen.com)

## Agenda

<b>9:00 AM</b>		Cat and Dog STR Comparison Test
<b>9:15 AM</b>		Discussion
<b>9:30 AM</b>	<b>78447, 49</b>	Development of targeted GBS panels for breeding and parentage applications in cats and dogs. Angela Burrell*, P Siddavatam, M Swimley, C Willis, H Suren, K Gujjula, and R Conrad, Thermo Fisher Scientific
<b>9:45 AM</b>	<b>79986 87</b>	-A recommendation for a SNP marker panel for feline and canine identification and parentage verification. Maarten de Groot*, Tom Ras, and Wim van Haeringen, VHLGenetics, Wageningen, The Netherlands.
<b>10:00 AM</b>		Cat SNP Comparison Test
<b>10:15 AM</b>		Dog SNP Comparison Test
<b>10:30 AM</b>		Coffee/Tea Break.
<b>11:00 AM</b>	<b>80148</b>	Can-ID: A SNP based genetic Identification system to evaluate Canine samples on two platforms: Open Array™ and AgriSeq™ targeted GBS. O Ramirez*1, KR Gujjula2, A Sánchez1,3, H Suren2, O Francino1,3, R Ramadhar2, and L Altet1, 1Vetgenomics2, Thermo Fisher Scientific, 3Molecular Genetics Veterinary Service (SVGM).
<b>11:15 AM</b>	<b>79654</b>	End-To-End AgriSeq™ Targeted GBS Long Indel Solution. Haktan Suren*1, Krishna Reddy Gujjula1, Prasad Siddavatam1,

		Jason Wall <sup>1</sup> , Claudio Carrasco <sup>1</sup> , Rick Conrad <sup>1</sup> , and Jeanette Schmidt <sup>2</sup> , <sup>1</sup> Thermo Fisher
<b>11:30 AM</b>	<b>79779</b>	High Resolution Melt Analysis for detecting the causative point mutation for the prcd-PRA in the Bolognese dog breed. C Previtali*, S Arabi, G Bongioni, R Capoferri, A Pozzi, and M Montedoro, Istituto Spallanzani, Rivolta d'Adda, Cremona, Italy.
<b>11:45 AM</b>	<b>79842</b>	Analysis of clinical samples from Doberman and Toy Poodle dogs with a targeted next-generation genotyping system. A Arizmendi <sup>1,2</sup> , LS Barrientos <sup>1</sup> , JA Crespi <sup>1</sup> , G Rudd Garces <sup>1</sup> , G Giovambattista <sup>1</sup> , and P Peral García* <sup>1</sup> , Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina.
<b>12:00 PM</b>	<b>79982</b>	First steps in animal genetic testing in Bulgaria. S Tincheva* <sup>1</sup> , S Atehin <sup>1,2</sup> , R Toshkov <sup>3</sup> , T Todorov <sup>1</sup> , and A Todorova <sup>1,2</sup> , <sup>1</sup> Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria, <sup>2</sup> Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria, <sup>3</sup> Veterinary clinic "Kakadu", Sofia, Bulgaria.
<b>12:15 PM</b>		Workshop Business Meeting and Elections

## Summary of the meeting

\*Important Reminders – if you have requested participation in an ISAG Comparison Test and have received the DNA samples for testing – please provide results for evaluation. The results are compiled across all laboratories to support decisions for inclusion and exclusion of particular markers and the data strengthens the selection of the overall marker panels for the species.

The preparations and distributions of DNA samples for the Comparison Tests require considerable time, effort and cost to the Duty laboratory and ISAG. Requesting samples but not providing data is unprofessional and wasteful. Please be respectful of your colleagues and ISAG. Please review: ***"Rules for conducting ISAG comparison tests (CT) for animal DNA" item 10: The collection and distribution of samples for comparison tests is a tedious task. All laboratories requesting participation are strongly encouraged to report results. If a laboratory fails to report results for two consecutive comparison tests they can be prohibited from participation in the next CT.***

Data for a Comparison Test must be produced by the laboratory requesting the DNA samples, using their own instrumentation and procedures within their laboratory. Data from a third-party provider is not considered valid for obtaining a Comparison Test ranking from ISAG and will not be included as valid CT participation. Or, the third party should be noted on the certificate of the CT laboratory. Any molecular / genetic technique is permitted to determine genotypes for the CTs.

# Cat & Dog Duty Laboratory Report

Duty (Cat and Dog) Laboratory: University of California, Davis; Veterinary Genetics Laboratory  
Cecilia Penedo, PhD

**Participants:** Cat STR: 27 total applicants (4 did not submit results)  
Dog STR: 82 total applicants (6 did not submit results)  
Cat SNP: 16 total applicants (4 did not submit results)  
Dog SNP: 26 total applicants (10 did not submit results)

**Samples:** 22 DNAs for each species, including 2 references with genotypes

**References:** 2 for each species, consensus of 2 labs for STRs

**DNA Extraction:** DNA was extracted with Puregene Kit (Qiagen)

**Markers:** **Cat STRs: ISAG Core 14 STRs and one gender marker, either AMEL or ZFXY**  
**Dog STRs: ISAG Core 21 STRs**  
**Cat SNPs Preliminary 120 SNPs and gender marker ZFXY**  
**Dog SNPs Preliminary 188 SNPs**

**Call Format:** SNPs: Neogen Geneseek provided results in Top format

**\*\*Overall, 24 datasets were not provided for analyses. The laboratories not reporting data will be reminded that if data is not reported a second time, for any species requested, further participation in Comparison Tests will not be permitted, except for extraordinary reasons for exemption.**

## Shipping:

- Overall: few problems, all related to Customs and required documentation.
- 2 dog packages returned to the VGL and successfully replaced.

## Samples

- 3 labs requested and received replacements for 1 - 3 dog samples.
- 1 lab reported empty tube for dog sample 1. Sample shipped.

## DNA quality

- 2 labs reported poor amplification for dog samples 2 and 11
- Additional checking at the VGL, sample 11 appeared to have lower DNA concentration but worked in all tests.
- Returned samples were tested ca. 3 - 4 weeks from shipment. All worked.

## Allowable Data Corrections

Preliminary data reports were returned to the CT participant laboratories for review and to provide requests and information for data corrections prior to final rankings.

Typographical errors and data reporting errors were reviewed by the committee.

The Duty Laboratory had errors in reference types for STR/Diagnostic tests (2 in cats and 2 in dogs). All labs were informed via email from FASS/ISAG and corrected forms uploaded to ISAG website in time for reporting. **Errors were not counted in these genotypes.**

- Typographical errors, such as reporting YX instead of XY were corrected, as well as correction to the case of the letter, i.e. "n" for "N".

Presentations of blank cells, hyphens, “na” in the excel data files were all consider missing data and were considered in the calculation for the “Absolute Genotyping Accuracy”.

Cat CT specific corrections - The AMEL X allele was reported as 192, 193, 194 bp. All were considered correct.

### Cat STR Comparison Test Results

- 9 core markers expanded to 14 plus either ZFX Y (11 labs) or AMEL (14 labs) = 15 markers
- 15 x 20 cats = 300 results or 320 if did both genders (4 labs)
  - CCL-94 and 2 controls
- Two labs have no data (collected later), one lab did not call gender
- AMEL - X allele reported as 192, 193, 194 **Allele size does not need to be reported.**
  - Cat 11 had 5 mistakes and one no call
  - Cat 20 – gender issue consensus different with AMEL and ZFX Y! Consensus gender was male for one marker and female for the other marker.
  - CCL-94 FCA026 146/156 versus 144/152 – Corrected by Duty Lab and revised excel file sent
  - Cat 09 – Fca075 – allelic drop out
  - FCA026 – allelic drop out errors
  - FCA290 – Cat13 & Cat 14 – allelic drop out errors
  - FCA220 – 1 bp off (L1) errors
  - Two labs had issues with FCA441 – over all cats
  - Cat 4 had 16 blanks and Cat 5 had 18 blanks – one lab did not test each

### Cat Phenotypes and Diseases (n = 7 labs)

- Agouti: 1 A/A 12 A/a 7 a/a
- AB Blood Group: 7 N/c 6 N/N 2 b/b 2 b/c 1 non-consensus
- Color: 9 C/C 10 C/c<sup>s</sup> 1 c<sup>s</sup>/c<sup>s</sup>
- Dilute: 6 D/D 12 D/d 2 d/d
- Longhair: 5 M4/M4 10 M4/N 1 M3/N 1 M3/M4 3 N/N
- Pyruvate Kinase Deficiency: 1 carrier
- Polycystic Kidney Disease: 2 affected (heterozygous)

Cats with mutations for seven traits, seven laboratory reported results, however, not for all loci and not always for all cats. Nomenclature needs to be standardized for pyruvate kinase deficiency (K/N versus N/P) and blood type. One discordant result was noted for the *Longhair* locus. Blood type had 19 discordant results, mainly from two of six labs reporting. In light of recent publications

on new variants for cat blood type, the workshop may want to dedicate some discussion to resolving alleles, variants and nomenclature.

Locus	Gene	Genotypes Reported	Concordant	Discordant
Agouti	<i>ASIP</i>	93	93	0
Blood	<i>CMAH</i>	90	71	19
Color	<i>TYR</i>	62	62	0
Dilute	<i>MLPH</i>	72	72	0
Long	<i>FGF3</i>	56	55	1
ADPKD	<i>PKD1</i>	112	112	0
PKDef	<i>PKLR</i>	110	110	0

### Cat STR CT Parentage Questions

23 labs responded to question 1.

The correct answer is “Cat 13 qualifies as sire of CAT 14”. Five of 23 answers (22%) were incorrect.

23 labs responded question 2.

The correct answer is No. Parent(s) of CAT 20 are not among samples tested. Six of 23 labs (26%) considered that CAT 19 could be a parent with 1 locus mismatch (FCA075) and no additional testing for confirmation.

### Cat Gender

Twenty-three laboratories reported gender identification. One laboratory did not report gender.

Size variants are known for both *AMEL* and *ZFXY* in cats and was reported as part of the STR results, both as sizes and as letters. Four labs did both markers and were consistent in gender determination. Cat 5 was not genotyped by one of these four labs for either marker. Five cats (Cat 11, 12, 14, 18, and 20) has discrepancies with gender determination.

*AMELXY* is a complex region and a SNP test is likely not feasible. Therefore, *ZFXY* will be promoted as the gender test for SNP genotyping.

### Dog STR Comparison Test Results

- 21 STRs x 22 dogs = 462 datapoints – 2 controls = 420
- 76 labs performed core STR analyses
- 2 labs need to standardize on the controls
- Three labs reported YX instead of XY – to be corrected.
  - One lab did not report *AMEL*
  - One lab used Y instead of X
  - 3 errors, 1 no call, 1 typo
- Dog 01, 09, 11 – one lab each had issues
- One lab re-quantified DNA
- Other data errors appeared sporadic across dogs

## Dog STR CT Parentage Questions

- Parentage
  - 2 no response
  - 1 incorrect
  - 1 incomplete as problem with Dog 1

## Cat SNP Comparison Test Results

- 118 SNPs x 22 cats = 2596 datapoints – 2 controls = 2360 genotypes
  - No discrepancies reported in the two control cats (one no call)
  - Cat 1 = CCL-94 control
  - Four corrections to data analyses: “—” counted as error instead of blanks
  - Problem loci – for the 5 labs that had only 3 blanks
    - ChrX.157577155 for Cat 11
    - ChrB1.161403614 for Cat 10 and Cat 18
- Very high rate of marker success
- Relative and Absolute accuracy differs greatly by technology
- Even with missing data – parentage test could be answered by > 5 SNPs
- Sequence Cat 10 and Cat 18 for CHRB1161403614
- Drop 5 SNPs – poor mapping??
  - chrB3.49170524, chrB3.143855324 were on the original list but no data was collected.
  - chrE2.13480422, chrC1.45295530 appear to map to 2 places on cat V9.0 assembly.
- What to do with problem markers for different technologies?
- Additional of more SNPs?

## Cat SNP CT Parentage Questions

Eleven of 12 labs answered question 1.

All answers were correct and qualified CAT 13 as a parent of CAT 14.

Eleven of 12 labs answered question 2.

All answers were correct with no qualifying parent of CAT 20 being among samples tested.

## Dog SNP Comparison Test Results

- 16 labs – 20 dogs, 2 controls
- Core 188 SNPs, 416 different SNPs tested
- One lab 26 extra markers; Second lab did 188 extra markers

## **Dog SNP CT Parentage Questions**

Fifteen of 16 labs correctly answered question 1.  
Dog 1 qualifies as parent of Dog 20.1

Fifteen of 16 labs correctly answered question 2.  
Parent(s) of Dog 13 are not among samples tested.

## **Committee Motions and Votes**

Cat and / or Dog

- **Should a gender marker be declared as the core?**

Motion failed – allow laboratory to choose either AMEL or ZFX Y; ZFX Y for SNPs.

- **Should the CT discontinue reporting size(s) for gender markers?**

Motion passed – only letters X and Y will be reported for gender

- **Should the Cat CT discontinue reporting letters for STRs?**

Motion passed – only numbers will be reported for STRs, not letters

**Should the Top Strand call rule be dropped and true nucleotide letter be reported?**

Motion failed – retain Top strand nomenclature

**Should the Cattle CT Statement for exclusion be adopted?**

Motion passed – more than 3 SNPs are required to consider an individual excluded (see full statement below)

**Do participants think sufficient data has been collected for the Cat and Dog SNP tests to allow the committee to declare a Core SNP panel for Cat and Dog and a Secondary Panel for the Dog?**

Motion passed – committee will convene by email to debate and finalize selection of the panels for the next CT within the next 6 months. The committee will consider

- Dropping and or replacing SNPs if:
  - Poor mapping, uneven chromosomal distribution
  - Poor performance overall or poor performance for a particular technology since many SNPs are available for selection that have sufficient data
  - Fair representation of all datasets
  - Best SNPs over most diverse breed populations

## **Other Business**

Participation in a Cat nomenclature committee organized by Leslie Lyons was requested. Jen Grahn from UC Davis VGL and Maarten de Groot for VHL volunteered participation. Other participants include a member of the MGI nomenclature committee, Frank Nicholas, Jerold Bell, Marie Abitbol, Lorraine Shelton and some cat breeders representing genetics committees for the cat registries.

A panel of DNA controls for cat diseases and phenotypes pertinent to genetic testing will be available from the Lyons laboratory as part of a funded project from the Winn Feline Foundation.

A file with the DNA sequences and variants for cat diseases and phenotypes pertinent to genetic testing will be available from the Lyons laboratory as part of a funded project from the Winn Feline Foundation.

A file of Cat genetic testing information for “Standardization” of reporting will be available within 6 months from the Lyons laboratory as part of a funded project from the Winn Feline Foundation and developments from the Cat Nomenclature Committee.

Maarten de Groot and Leslie Lyons will be leading a paper for publication in Animal Genetics regarding the Cat SNP panel for parentage – declaring an official ISAG Cat SNP core panel. Data



from the Cat SNP CT will be included with the permission of the participating laboratories (redacted results) and inclusion of one laboratory participant as a co-author.

**Committee chair** (the new chair)

Chair: Leslie Lyons and Jiansheng Qiu
Term of service: 2008 – 2021 & 2016 - 2021
Affiliation: University of Missouri & Neogen GeneSeek
E-mail address: <a href="mailto:lyonsla@missouri.edu">lyonsla@missouri.edu</a> ; JQiu@neogen.com

## Committee members (the new committee)

Committee members‡	Term of service	E mail address
Leslie Lyons - University of Missouri, USA (Co-chair)	2008 - 2021*	<a href="mailto:lyonsla@missouri.edu">lyonsla@missouri.edu</a>
Jiansheng Qiu – Neogen, USA (Co-chair)	2016 - 2021*	<a href="mailto:JQiu@neogen.com">JQiu@neogen.com</a>
Hubert Bauer – Laboklin (2021 Dog Duty Lab)	2019 - 2023	<a href="mailto:labogen@laboklin.com">labogen@laboklin.com</a>
Robert Grahn, PhD - Veterinary Genetics Laboratory, University of California – Davis, USA (2021 Cat Duty Lab)	2019 - 2023	<a href="mailto:ragrahn@ucdavis.edu">ragrahn@ucdavis.edu</a>
Leanne van de Goor –VHL Genetics, The Netherlands	2017 - 2021	<a href="mailto:lgo@vhladmin.nl">lgo@vhladmin.nl</a>
Peter Dovc - University of Ljubljana, Slovenia	2017 - 2021	<a href="mailto:Peter.dovc@bf.uni-lj.si">Peter.dovc@bf.uni-lj.si</a>
Maria Longeri –University of Milan, Italy	2017 - 2021	<a href="mailto:maria.longeri@unimi.it">maria.longeri@unimi.it</a>
George Sofronidis – Orivet, Australia	2019 - 2023	<a href="mailto:george@orivet.com.au">george@orivet.com.au</a>
Nuket Bilgiren – University of Ankara, Turkey†	2017 - 2021	<a href="mailto:nuketbilgen@gmail.com">nuketbilgen@gmail.com</a>

\*Corrected term of service. †Requested leave of absence, time of service to be reinstated at next request. ‡Duty laboratories are *ex officio* members of the committee and do not have voting rights. The Duty laboratory representative can become a full committee member after the current CT to support transfer of information to the new Duty laboratory for the subsequent CT.

**COMPARISON TEST (2018-2019)**      YES (Cat & Dog, STRs and SNPs)

### Duty laboratory - 2021 Dog Duty Lab

Contact person: Hubert Bauer
Affiliation: Laboklin
E-mail address: <a href="mailto:labogen@laboklin.com">labogen@laboklin.com</a>

### Duty laboratory - 2021 Cat Duty Lab

Contact person: Robert Grahn
Affiliation: Veterinary genetics Laboratory, UC Davis, Davis, CA USA
E-mail address: <a href="mailto:ragrahn@ucdavis.edu">ragrahn@ucdavis.edu</a>

## Comments (issues rising)

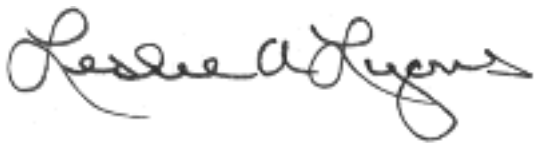
Several members of this committee, other committees and the audience had private discussions regarding the content of the agendas and presentations. Members are showing concern that too many presentations are presented by commercial entities that also sell instrumentation and reagents. Several commercial entities are lobbying for selection of genetic markers that have been selected by the companies. ISAG should consider a statement, a caution to the committees, that commercial entities and a specific technology should not be driving the science and the decisions of the committees and membership on committees by commercial entities (specifically those selling instrumentation and reagents) should be limited.

Also, for a laboratories certificate, it should be noted if a third party performed the genotyping on the certificate.

## List of recommended markers with primer information

SNPs to be determined by end of 2019

## SIGNATURES



Co-Chairs – Leslie Lyons



Duty laboratory – Cecilia Penedo



Co-Chairs – Jiansheng Qiu

**Table 1. ISAG dog core STRs for parentage and identification testing.**

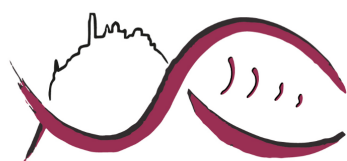
<b>Locus</b>	<b>5'-3' - Forward</b>	<b>5'-3' - Reverse</b>
K9-AME	GTGCCAGCTCAGCAGCCCGTGGT	TCGGAGGCAGAGGTGGCTGTGGC
AHT121	TATTGCGAATGTCACTGCTT	ATAGATACTCTCTCTCCG
AHT137	TACAGAGCTCTTAACTGGGTCC	CCTTGCAAAGTGTCAATTGCT
AHTh130	GTTTCTCTCCCTTCGGGTTC	GACGTGTGTTACGCCAG
AHTh171	AGGTGCAGAGCACTCACTCA	CCCATCCACAGTTCAGCTTT
AHTh260	CGCTATACCCACACCAGGAC	CCACAGAGGAAGGGATGC
AHTk211	TTAGCAGCCGAGAAATACGC	ATTCGCCCCGACTTTGGCA
AHTk253	ACATTTGTGGGCATTGGGGCTG	TGCACATGGAGGACAAGCACGC
CXX0279	TGCTCAATGAAATAAGCCAGG	GGCGACCTTCATTCTCTGAC
FH2848	CAAAACCAACCCATTCACTC	GTCACAAGGACTTTTCTCCTG
INRA021	ATGTAGTTGAGATTTCTCCTACGG	TAATGGCTGATTTATTTGGTGG
INU005	CATGCTGGTTCTGTGTTAGGC	AAATACAATCTTGCGTGTGTGC
INU030	GGCTCCATGCTCAAGTCTGT	CATTGAAAGGGAATGCTGGT
INU055	CCAGGCGTCCCTATCCATCT	GCACCACTTTGGGCTCCTTC
REN105L03	GGAATCAAAAGCTGGCTCTCT	GAGATTGCTGCCCTTTTACC
REN162C04	TTCCCTTTGCTTTAGTAGGTTTTG	TGGCTGTATTCTTTGGCACA
REN169D01	AGTGGGTTTGCAAGTGGAAC	AATAGCACATCTTCCCCACG
REN169O18	CACCCAACCTGTCTGTTCT	ACTGTGTGAGCCAATCCCTT
REN247M23	TGGTAACACCAAGGCTTTCC	TGTCTTTTCCATGGTGGTGA
REN54P11	GGGGGAATTAACAAAGCCTGAG	TGCAAATTCTGAGCCCCACTG
REN64E19	TGGAGAGATGATATCCAAAAGGA	AGCCCACTGCTTGGTGAG

**Table 2. Genetic markers selected as a “core” panel for ISAG cat parentage and identification testing.**

Marker	Chr.	Repeat	Forward Primer 5' – 3'		Label	uM
			Reverse Primer 5' - 3'			
FCA026	D3		GGAGCCCTTAGAGTCATGCA	TGTACACGCACCAAAAACAA		
FCA069	B4	AC	AATCACTCATGCACGAATGC	AATTTAACGTTAGGCTTTTTGCC	VIC	0.20
FCA075	E2	TG	ATGCTAATCAGTGGCATTG	GAACAAAATTCCAGACGTGC	NED	0.10
FCA105	A2	TG	TTGACCCTCATACTTCTTTGG	TGGGAGAATAAATTTGCAAAGC	PET	0.20
FCA149	B1	TG	CCTATCAAAGTTCTCACCAAATCA	GTCTCACCATGTGTGGGATG	PET	0.18
FCA201	B3		TCTGCAGGACCAGTCAGATG	AGCATAACAAATTGATGCTGG		
FCA220	F2	CA	CGATGGAAATTGTATCCATGG	GAATGAAGGCAGTCACAAACTG	FAM	0.30
FCA229	A1	GT	CAAAGTACAAGCTTAGAGGGC	GCAGAAGTCCAATCTCAAAGTC	NED	0.25
FCA293	C1		GATGGCCCAAAGCACAC	CCCACATCTTGTCAACAACG		
FCA310	C2	(CA) <sub>5</sub> TA(CA) <sub>7</sub> TA(CA) <sub>8</sub>	TTAATTGTATCCCAAGTGGTCA	TAATGCTGCAATGTAGGGCA	FAM	0.30
FCA441	D3	TAGA	ATCGGTAGGTAGGTAGATATAG	GCTTGCTTCAAATTTTCAC	VIC	0.15
FCA453	A1		AATTCTGAGAACAAGCTGAGGG	ATCCTCTATGGCAGGACTTTG		
FCA649	C1		ACTGCCTGCACACTGACTTG	TTAGTCCTGGTGAGACTTTGTG		
FCA678*	A1	AC	TCCCTCAGCAATCTCCAGAA	GAGGGAGCTAGCTGAAATTGTT	NED	0.25
AMEL	XY	X-214; Y-193	CGAGGTAATTTTCTGTTTACT	GAAACTGAGTCAGAGAGGC		
ZFXY	XY	X-168; Y-165	AAGTTTACACAACCACCTGG	CACAGAATTTACTACTTGTGCA	PET	0.20

\*Primers redesigned from original publication for FCA678 to prevent null alleles.

Note: a secondary set of primers for FCA026 have been proposed to avoid allelic drop-out: FCA026Fr – AATGTTGCAGGCCTGTGTAC; FCA026Rr - GATCATGAACCGAACTGGTG



# ISAG . 2019

37th International Society for  
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**Table 3. Suggested conversion of ABI 3730 allele sizes to “letter” nomenclature for the cat DNA profiling panel.**

FCA	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
<b>0 6 9</b>					<u>93</u> +4	95	97	99	101	103	105 (21)	<u>107</u> +4	109	111	113	115	117					
<b>0 7 5</b>	104	106	108	110	112	114	116	118	120	122	124(23)	126	128	130	<u>132+</u> 7	134	136	138	140	142	144	146
<b>1 0 5</b>					<b>173</b>	175	177	179	181	183	185(16)	187	189	191	193	195	<u>197</u> -5	199	201	203	205	207
<b>1 4 9</b>					<u>122+</u> 1	124	126	<u>128</u> +1	130	132	134	136	138	140	142							
<b>2 2 9</b>					150	152	154	156	158	160	162(21)	164	166	<u>168</u> -2	170	172	174	176				
<b>3 1 0</b>					112	114	116	118	120	122	124(15)	126	128	130	132	134	136	<u>138</u> -2	140			
<b>4 4 1</b>				145	147	149	151	153	<u>155</u> +0	157	<u>159(12)+</u> 0	161	163	165	167	169	171	173				
<b>6 7 8</b>						186	188	190	192	<u>194</u> -2	196(17)	198	200	202	204	206						
<b>2 2 0</b>							208	210	212	<u>214</u> (18)	216 (19)	218	220	222	224	226	228					
<b>0 2 6</b>		120	122	124	126	128	130	132	134	136	138	140	142	144	<u>146</u> (23)							
<b>2 0 1</b>		125	127	129	131	133	135	137	139	141	143	145	147	149	151	153	155	157	159	161		
<b>2 9 3</b>									<u>185</u> +3	187	189 (20)	191										
<b>4 5 3</b>									188	192	196 (8)	200										
<b>6 4 9</b>										136	138 (20)	<u>140</u> +4										

All allele sizes were determined on an ABI 3730 DNA Analyzer. Alleles that are underlined have been sequenced in homozygote individuals. The actual nucleotide length can be determined by the addition or subtraction of the noted number of base pairs. The numbers of repeats in the core unit of the microsatellite are presented for the M allele. The number of repeats was directly determined for the alleles that were sequenced and interpolated for the M allele.

Table 3. Marker information Dog ISAG additional marker panel.

Name	Chr	Position	Forward Sequence	Reverse Sequence	Multiplex	Size Range	Label
2642_RD	35	15.822.237	GTTCCATGCATGCTGACACA	GGGGTGAGAATGATGGTGGT	1	86-108	FAM
1404_RD	15	17.933.748	AGGGCTGTTTGGAGGAACAA	GTTTCTTTGGTCTGACATGAGGGGACA	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	GGAAGCAGCTGGGTTTCCTAA	GTTTTCCATGCCCAACTATTTTTGA	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