Applied Genetics in Companion Animals

Organised by a Standing Committee: **YES**

Meeting information

Date: 28 July 2021
Time: 14:00 – 17:00 UTC
Number of participants: ~100

Chair

Name: Leslie A. Lyons, PhD
Affiliation: University of Missouri
Contact email: lyonsla@missouri.edu

Co-Chair (optional)

Name: Jiansheng Qiu, PhD
Affiliation: Neogen, Inc.
Contact email: JQiu@neogen.com

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Agenda

14:00	**WELCOME & AGENDA**	Lyons / Qiu
14:10	**Dog STR and SNP Comparison Test and Discussion** Duty Lab Presentation, SNP Data, STR Data, issues, proposals	
	Duty Laboratory Presentation for both SNPs & STRs (Bauer)	Recording
	Data Analysis Review (Lyons)	Recording
14:50	Group Presentation of 85540, 85541, and 85542 - AgriSeq	
	Supplementation of the AgriSeq™ Canine SNP Parentage and ID Panel with Additional ISAG and Gender Determination Markers. A Burrell*, K Gujjula, H Suren, and R Conrad, *Thermo Fisher*	Recording - AgriSeq
	Development of highly informative SNP panel for parentage assessment in dogs. K R Gujjula*, H Suren, A Burrell, and S Chadaram, *Thermo Fisher*	
	AgriSum™ Toolkit Plugin 2.0: Enabling multi species panel analysis for AgriSeq™. Haktan Suren*[1], Stéphane Daly[2], and Krishna Reddy Gujjula[1], *Thermo Fisher*	
15:05	Discussion for Canine SNP & STR CT	
	Proposal - Record assay on ISAG certificate	
	Proposal - How to share data between laboratories	
15:20	Break (10 minutes)	
15:30	**Cat STR and SNP Comparison Test and Discussion** Duty Lab Presentation, SNP Data, STR Data, issues, proposals	Lyons / Qiu
	Duty Laboratory Presentation for both SNPs & STRs (Grahn)	Recording
	Data Analysis Review (Lyons)	Recording
16:10	Discussion for Feline SNP & STR CT	
	Call for SNPs for Secondary Panel	
16:20	Whole Genome Sequencing analysis of a Cat Family with Radial Hemimelia. (85538) Nüket Bilgen*[1], M.Y. Akkurt[1], B, Çinar Kul[1], R.M. Buckley[2], L.A. Lyons[2], & Ö. Sebnem Çildir[1]	Recording - Bilgen
16:30	New Business: – new workshop “Standards of Genetic Testing”	
16:31	Group Presentation of 85056 and 85256	
	Breed, trait, locus and allele nomenclature standardization for the domestic cat. L.A. Lyons	Recording - Lyons
	OMIA – standardised vocabularies for breeds & traits. I. Tammen[1], N. Vasilevsky[2], C.A. Park[3], Z. Hu[3], M. Haendel[4], and F.W. Nicholas	Recording - Tammen
16:40	Discussion	
16:50	Committee and Duty Laboratory Election	
17:00	Adjourn	

Summary of the meeting

Dog and Cat Comparison Tests for STRs and SNPs were presented and discussed (Details below).

Overall, all CT tests were successful with improving results. Some laboratories do not include all SNP markers, especially gender markers. Committee will make inquires to help standardize. (See additional details below).

A new reference for the dog is still not decided, thus remapping of SNPs to assign locational-based names not yet available.

Variant information for flanking the SNPs would be helpful for assay design. (Lyons – cats; EMBARK and or ThermoFisher for dogs).

Dog and Cat Comparison Tests for STRs and SNPs will be conducted for ISAG 2023.
A few laboratories did not provide CT data for a second, consecutive year and received samples. The committee will inquiry to understand if the cause was due to COVID issues, but will also discuss if these laboratories can participate in 2023.

Cat & Dog CTs will expand to include more phenotypes and diseases but proper planning to provide appropriate samples needs to be considered.

For exclusions concerning SNPs, since new technologies have 96 – 100% sporadic no calls, should the number of SNPs for exclusion be considered as a percentage since panels are different sizes (i.e., for a panel of 100 SNPs, three discordancies would not indicate exclusions)?

A request regarding putting the type of genotyping assay on the CT Certificate will be presented at the business meeting.

More detail needs to be requested regarding assay type. For example, DNA array – custom versus commercial design, GBS – targeted versus low pass sequencing; custom versus commercial design.

Diverse technologies are improving in accuracy. A minimum of 100x coverage is suggested as the standard for GBS genotyping.

Verbiage used to answer parentage questions needs to be addressed in committee as the discussion are complex. Use Yes/No/Doubtful check boxes? Provide answer given to customer then provide discussion?

A motion of a new committee regarding standardization for animal genetic testing will be proposed at the business meeting. This committee will discuss formats for data sharing between laboratories and will include members from all interested species representatives, personnel from OMIA and related parties.

Committee selection: Dr. Longeri resigned from the committee. Dr. Lyons was awarded *ex officio* status. The committee has strong desire to retain chairs from academia.

Please provide biographies and pictures of the committee members for the ISAG website.

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New Committee chair

Chair: **Peter Dovč, PhD**
Term of service (*add years of first and second term of service*): First term **2017-2021, second term 2021 - 2025**
Affiliation: **University of Ljubljana, Slovenia**
E-mail address: Peter.Dovc@bf.uni-lj.si

New Committee co-chair (optional)

Co-Chair: **Jiansheng Qiu, PhD**
Term of service (*add years of first and second term of service*): First term **2016-2021, second term 2021 - 2025**
Affiliation: Neogen, Inc. USA
E-mail address: jqiu@neogen.com

Note: One term runs for two bi-annual conferences (i.e., four years)

New Committee members

Other members	First term of service	2nd term of service	Email address
Hubert Bauer Laboklin	2019 -2023		bauer@laboklin.com
Robert Grahn UC Davis	2019 - 2023		ragrahn@ucdavis.edu
George Sofronidis Orivet	2019 - 2023		george@orivet.com.au
Nuket Bilgren Univ. of Turkey	2017 - 2023		nuketbilgen@gmail.com
Leanne van de Goor VHL Genetics	2017 – 2021	2021 - 2025	Leanne.vandegoor@vhlgenetics.com
Leslie A. Lyons	2008 - 2021	*Ex officio*	lyonsla@missouri.edu

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

Duty laboratory (Dog)

Contact person: Hubert Bauer
Affiliation: Laboklin
E-mail address: bauer@laboklin.com

Comments (issues rising)

1. The committee needs to inquire as to why laboratories are not including all markers and re-enforce all markers are to be used in the CT, for both STRs and SNPs.
2. During the exploration of new technologies and the SNP panels, the committee will request more detailed information regarding technology used to further understand the robustness of SNPs. Provision of the information will be voluntary.
3. A different scoring system is under consideration. Since hundreds of SNPs can be tested, drop-out of data is inevitable and can be tolerated. The tolerance of missing data may need to be based on the number of SNPs being genotyped. The committee will draft a suggested scoring system for SNP data to be considered and adopted by all CTs for ISAG.
4. Early planning and cooperation are required to include animals with diseases and phenotypes. The CTs should now be including more diseases and phenotypes.
5. A template of suggested verbiage to answer the parentage questions will provided. Laboratories will be encouraged to provide additional information in a separate field.
6. The CT should communicate with the new “Standardization of Genetic Testing in Animals” committee.
7. The committee suggests only one gender marker is obligatory as the final call is XX or XY – not the SNP nucleotide.

Dog STR Comparison Test

Shipping and Samples
DNA isolated from 10 – 15 ml whole EDTA blood from bank
Isolated by using a GenElute kit
Approximately 50 ul at ~ 30 ng/ul was shipped

Shipping

95 sets submitted to 41 countries
< 10 second shipments
Encourage submission of customs documents
Report issues to duty lab / FASS

Markers

- 21 STRs/AMELXY x 22 dogs = 462 datapoints – 2 controls = 420 genotypes per lab
- STRs missing all data – which accounted for most missing data (251 genotypes). These markers are not in the commonly used Finnzymes kit and due to COVID, the ThermoFisher kit with all markers may not have been obtainable. However, the committee voted to keep this data as the missing markers have been know from previous comparison tests and not a new concern.
    - REN64E19 (4 labs)
    - REN105LO3 (3 labs)
    - AHTH130 (4 labs)
- 73 labs performed core STR analyses
- AMELXY as the gender marker
    - One laboratory had 10 errors and another laboratory had one error
- DCT-22 – ADO allele 201 for STR 0123RD
- DCT-16 – ADO allele 266 for STR 0959RD
- DCT-13 – one lab each had issues – missing heterozygotes and failed for one lab
- DCT-16 – 23 discordant results for AHTH130 (reference = 139/139)
- Parentage question had diverse responses
- STR Accuracy Core = 98.5% and Secondary = 99.24%
- AHT121 & INRA21 – Allelic drop-out
- REN105L03 – binning errors in three laboratories

Parentage

DCT-2, DCT-3 DCT-10 are full siblings

Summary of Dog STR CT Rankings

Labs	Relative %	% of labs	Labs	Absolute %	ISAG Rank	% of labs with rank
29	100	39.72	29	100	1	**39.72**
29	99.76 - 98	39.72	26	99.76 - 98	1	**35.61**
8	97.99 - 95	10.96	8	97.99 - 95	2	**10.96**
6	94.99 - 90	8.22	5	94.99 - 90	3	**6.85**
0	89.99 - 80	0	4	89.99 - 80	4	**5.48**
1	58.50	1.36	1	55.71	5	**1.36**

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Dog SNP Comparison Test

- 17 labs – 20 dogs, 2 controls (DCT-13 had some missing data)
- Core Panel 1 = 116 SNPs - 2230 expected genotypes (3 SNPs gender)
- Sporadically missing SNPs
- 7 labs did not type AHKA3HTPANEL1 – gender
- 5 labs did not type AMELOGENINPANEL1
- 6 labs did not type chrY_572523
- One lab provided 160 extra SNP markers
- SNP Core Panel 1 Accuracy = 99.52%; Panel 2 = 99.61%
- DCT-13 also failed
- DCT-22 most discordant – the sequencing of SNPs had consistent results with genotyping.

*Ten dog markers, including; Z_P87, BICF2G630159183, BICF2G630200354, AHHS65D, BICF2P516667, BICF2P963969, BICF2P345056, BICF2G630274628, BICF2P590440, BICF2S23429022. A discordance was noted for BICF2G630274628 depending on the direction of sequencing.

- Parentage was clearly answered!

Phenotypes & Diseases – none due to COVID

DOG SNP CT Summary Panel 1

LabID	Blanks	Results	Consensus	Absolute Accuracy %	ISAG Rank	Relative Accuracy %
2	0	2260	2259	99.96	1	99.96
3	0	2260	2259	99.96	1	99.96
4	0	2260	2259	99.96	1	99.96
9	0	2260	2259	99.96	1	99.96
15	0	2260	2258	99.91	1	99.91
12	1	2259	2258	99.91	1	99.96
11	0	2260	2256	99.82	1	99.82
14	0	2260	2255	99.78	1	99.78
5	2	2258	2253	99.69	1	99.78
1	20	2240	2239	99.07	1	99.96
7	20	2240	2239	99.07	1	99.96
16	20	2240	2220	98.23	1	99.11
6	62	2198	2197	97.21	2	99.95

8	60	2200	2168	95.93	2	98.55
13	148	2112	2104	93.1	3	99.62
17	158	2102	2085	92.26	3	99.19
10	98	2162	2083	92.17	3	96.35

ISAG Secondary Dog SNP Panel

- 17 labs – 20 dogs, 2 controls
- Core Panel 2 = 120 SNPs (3 gender) = 2400 genotypes
- Gender markers missing and sporadic SNPs not genotyped
- DCT-13 had the most missing data – one laboratory in particular
- SNP BICF2G630274628PANEL2 had several miscalls by different laboratories
- One laboratory had several missing SNPs
- Missing SNPs included (besides gender SNPs): AHN1X0KPANEL2; AHQJUC0PANEL2 (2 labs), BICF2G63078341PANEL2 (3 labs), BICF2P1193353PANEL2 (2 labs); BICF2P1362405PANEL2, BICF2P285489PANEL2, BICF2P414351PANEL2, BICF2P42825PANEL2, BICF2S23614068PANEL2, P56PANEL2

DOG SNP CT Summary Panel 2

LabID	Blanks	Results	Consensus	Absolute Accuracy	Rank	Relative Accuracy
3	0	2400	2400	100.00%	1	100.00
15	0	2400	2400	100.00%	1	100.00
9	0	2400	2399	99.96%	1	99.96
14	0	2400	2399	99.96%	1	99.96
16	0	2400	2399	99.96%	1	99.96
4	0	2400	2389	99.54%	1	99.54
13	1	2399	2389	99.54%	1	99.58
7	3	2397	2388	99.50%	1	99.62
6	0	2400	2382	99.25%	1	99.25
17	41	2379	2378	99.08%	1	99.96
11	61	2339	2339	97.46%	2	100.00
10	40	2360	2334	97.25%	2	98.90
2	71	2329	2313	96.38%	2	99.31
12	140	2260	2260	94.17%	3	100.00
8	133	2267	2248	93.67%	3	99.16
5	160	2240	2228	92.83%	2	99.46
1	218	2182	2154	89.75%	4	98.72

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Duty laboratory for the next comparison test with contact details

Contact person: George Sofronidis
Affiliation: Orivet, Australia
E-mail address: george@orivet.com.au

List of recommended markers with primer information
(Please see attached excel file for SNPs)

ISAG dog core STRs for parentage and identification testing.

Locus	5'-3' - Forward	5'-3' - Reverse
K9-AME	GTGCCAGCTCAGCAGCCCGTGGT	TCGGAGGCAGAGGTGGCTGTGGC
AHT121	TATTGCGAATGTCACTGCTT	ATAGATACACTCTCTCTCCG
AHT137	TACAGAGCTCTTAACTGGGTCC	CCTTGCAAAGTGTCATTGCT
AHTh130	GTTTCTCTCCCTTCGGGTTC	GACGTGTGTTCACGCCAG
AHTh171	AGGTGCAGAGCACTCACTCA	CCCATCCACAGTTCAGCTTT
AHTh260	CGCTATACCCACACCAGGAC	CCACAGAGGAAGGGATGC
AHTk211	TTAGCAGCCGAGAAATACGC	ATTCGCCCGACTTTGGCA
AHTk253	ACATTTGTGGGCATTGGGGCTG	TGCACATGGAGGACAAGCACGC
CXX0279	TGCTCAATGAAATAAGCCAGG	GGCGACCTTCATTCTCTGAC
FH2848	CAAAACCAACCCATTCACTC	GTCACAAGGACTTTTCTCCTG
INRA021	ATGTAGTTGAGATTTCTCCTACGG	TAATGGCTGATTTATTTGGTGG
INU005	CATGCTGGTTCTGTGTTAGGC	AAATACAATCTTGCGTGTGTGC
INU030	GGCTCCATGCTCAAGTCTGT	CATTGAAAGGGAATGCTGGT
INU055	CCAGGCGTCCCTATCCATCT	GCACCACTTTGGGCTCCTTC
REN105L03	GGAATCAAAAGCTGGCTCTCT	GAGATTGCTGCCCTTTTTACC
REN162C04	TTCCCTTTGCTTTAGTAGGTTTTG	TGGCTGTATTCTTTGGCACA
REN169D01	AGTGGGTTTGCAAGTGGAAC	AATAGCACATCTTCCCCACG
REN169O18	CACCCAACCTGTCTGTTCCT	ACTGTGTGAGCCAATCCCTT
REN247M23	TGGTAACACCAAGGCTTTCC	TGTCTTTTCCATGGTGGTGA
REN54P11	GGGGGAATTAACAAAGCCTGAG	TGCAAATTCTGAGCCCCACTG
REN64E19	TGGAGAGATGATATCCAAAAGGA	AGCCACACTGCTTGGTGAG

Marker information for the Dog ISAG additional STR 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	GGAAGCAGCTGGGTTCCTAA	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

Duty laboratory (Cat)

Contact person: Robert Grahn
Affiliation: University of California, Davis, Veterinary Genetics Laboratory
E-mail address: ragrahn@ucdavis.edu

Comments (issues rising)

1. The committee needs to inquire as to why laboratories are not including all markers and re-enforce all markers are to be used in the SNP CT.
2. During the exploration of new technologies and the SNP panels, the committee will request more detailed information regarding technology used to further understand the robustness of SNPs. Provision of the information will be voluntary.
3. A different scoring system is under consideration. Since hundreds of SNPs can be tested, drop-out of data is inevitable and can be tolerated. The tolerance of missing data may need to be based on the number of SNPs being genotyped. The committee will draft a suggested scoring system for SNP data to be considered and adopted by all CTs for ISAG.
4. Early planning and cooperation are required to include animals with diseases and phenotypes. The CTs should now be including more diseases and phenotypes.
5. A template of suggested verbiage to answer the parentage questions will provided. Laboratories will be encouraged to provide additional information in a separate field.
6. The CT should communicate with the new “Standardization of Genetic Testing in Animals” committee.
7. A secondary SNP panel is suggested to be developed by the community with particular focus on SNPs that will better define inbred cat breeds and populations.

Cat STR Comparison Test

Shipping and Participation

26 sample shipments, 23 received, 20 returned data

14 countries, multiple in Germany, France, and Czech Republic

Genotyping worked fine for highly delayed sample sets

Problems: Official stamps from veterinarian required for some countries – could not do because of COVID

Variability of documents for labs within the same country

Don't use UN3733 envelopes – not needed

If you need DHL as courier, maybe set your own shipments

Sample Isolation

Breeds not available as planned due to COVID
Gonads from spay/ neuter clinics
Gentra Puregene Tissue Kit with additional phenol:chloroform
Sent 50 ul at 30 – 35 ng/ul
One lab did not get FCT8
STRs – confirmed by Laboklin, SNPs by Neogen
Three samples had a parent (FCT3) offspring (FCT6, FCT17) relationship

Markers and Data

- 14 core markers plus either ZFXY or AMELXY = 15 markers
- 15 x 20 cats = 300 results (13 of 14 labs) – 4200 total genotypes
    - CCL-94 (XX) and 2 controls (FCT1 & FCT22 – both XY)
- Only 2 missing genotypes (FCT08 & FCT18 for FCA026)
    - 81 incorrect genotypes (2%)
- One laboratory provided FCA005 & FCA224
- FCA220 is consistently a problem marker as labs miss the one bp off allele at 215 bp.
    - Allele 210 has poorer amplification
- FCA026 is has an allelic drop-out concern for allele 150 / 138
    - Competitive amplification or multiple 150 / 138 alleles?
    - Lab with most issues used the appropriate primers.
- FCA453 had no consensus for one cat – 1 bp off allele
    - FCT19 – 10 labs 187/188 versus 188/188 = no consensus

*Because the off-ladder allele is known, the 187/188 will be considered the correct data.

- One laboratory provided 10 additional STR markers
- Parentage question had lots of discussion
    - Problems with FCA026

Summary Cat STR CT Rankings (n = 20) 2021

No. of Labs	Relative %*	% of labs	No. of Labs	Absolute %	ISAG Rank	% of labs in rank
3	100	15	3	100	1	15
9	99.67 – 99	45	9	99.67 – 99	1	45
2	98.33 – 98	10	2	98.33 – 98	1	10
4	97.67-97.33	20	4	97.67-97	2	20
1	95.67	5	1	95.67	2	5
1	89.33	5	1	89.33	4	5

- Approximately same number of labs as 2019 with slightly improved rankings!
- The Absolute ranking improved from of 96.72% in 2019 to 98.16% in 2021.
- Disease (none) and phenotypes (*Agouti, Dilute, Long*) were limited this year due to COVID. One laboratory switched the M1& M3 alleles for cat *Longhair* test suggesting standardization is necessary.

Cat CT STR Summary

Locus	Results	Consensus	Relative%	Absolute% 98.15
AMELXY/ZFXY	400	400	100	100
FCA026	398	344 (56)	86.43	86
FCA069	400	398	99.5	99.5
FCA075	400	397 (3)	99.25	99.25
FCA105	400	399 (1)	99.75	99.75
FCA149	400	399 (1)	99.75	99.75
FCA201	400	399 (1)	99.75	99.75
FCA220	400	387 (13)	96.75	96.75
FCA229	400	400	100	100
FCA293	400	400	100	100
FCA310	400	400	100	100
FCA441	400	400	100	100
FCA453	400	380 (20)	93.33	93.33
FCA649	400	400	100	100
FCA678	400	400	100	100

Cat SNP Comparison Test

- 101 SNPs for 13 participating laboratories
  - one laboratory provided data from two methods
- 20 cats plus CCL-94 control and 2 controls
  - 20 x 101 = 2020 genotypes per lab = 28,280 genotypes
- FCT8, FCT19, FCT20 – DNA issues – most all genotypes missing were from these cats

*One lab did not receive FCT8 and their scores calculated without this marker.

- The lab with most errors used Illumina technology and was mostly a strand calling issue but heterozygosity errors were noted as well
- Errors mainly sporadic
- Parentage easily determined
- Will plan ahead for more disease and phenotypic markers
- Technologies may be improving and more comparable
  - Minimum GBS call is suggested as ~100x coverage
- Missing SNPs
  - One lab missing: CHRB3103519744, CHRX49536490, CHRC176501424
  - CHRB3:129823001 (3 labs) – MassArray and 2 GBS
  - CHRD3:86169540 (4 labs) – 2 GBS, MassArray, Illumina
  - CHRX:20556777 (6 labs) – 2 MassArray, Illumina, 3 GBS
  - CHRA1:147652232 (2 labs) – Both GBS

Additional SNPs for secondary core panel

- One lab provided data on 78 markers - anonymous
- One lab provided data on 159 markers – positions provided
- Mars has frequency data, which is needed for SNP selection

Cat SNP CT Data Summary 2021

LabID	Blank	Results	Consensus	Relative Accuracy %	Absolute Accuracy %	ISAG Rank	Assay
1	34	1986	1981	99.75	98.07	1	MassArray
2	20	2000	2000	100.00	99.01	1	Agriseq Ion S5 GBS
3	0	2020	2015	99.75	99.75	1	IonTorrent GBS
4	89	1931	1914	99.12	94.75	3	Ion torrent S5
5	22	1998	1998	100.00	98.91	1	Illumina
6	120	1900	1900	100.00	94.06	3	S5 Ion Torrent
7	18	2002	1991	99.45	98.56	1	Illumina
8	20	2000	2000	100.00	99.01	1	Mass Array
9	67	1953	1563	80.03	77.38	5	Illumina MicroArray
10	20	2000	2000	100.00	99.01	1	Illumina iSCAN
11	60	1960	1945	99.23	96.29	2	GBS
12	1	2019	2010	99.55	99.50	1	Illumina Bead Chip
13	0	2020	1998	98.91	98.91	1	Illumina
14	158	1862	1854	99.57	91.78	3	GBS – IonS5

No. of SNPs	Relative Accuracy %	Rank	Absolute Accuracy %
57	100	1	99.64 - 50.00
11	99.64 - 98	1	99.29 - 68.93
19	96.77 - 95.00	2	96.43 - 91.79
14	94.98 - 91.67	3	94.64 - 72.96

- SNPs with lower Absolute accuracy were mainly low due to no genotyping and not because of errors.

Duty laboratory for the next comparison test with contact details

Contact person: Robert Grahn
Affiliation: University of California, Davis, Veterinary Genetics Laboratory
E-mail address: ragrahn@ucdavis.edu

List of recommended markers with primer information

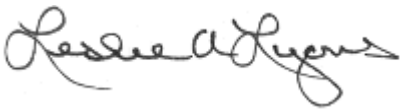
(Please see attached excel file for SNPs)

Genetic markers selected as a "core" panel for ISAG cat parentage & identification.

Marker	Chr.	Repeat	Forward Primer 5' – 3' Reverse Primer 5' - 3'	Label	uM
FCA026	D3		GGAGCCCTTAGAGTCATGCA TGTACACGCACCAAAAACAA		
FCA069	B4	AC	AATCACTCATGCACGAATGC AATTTAACGTTAGGCTTTTTGCC	VIC	0.20
FCA075	E2	TG	ATGCTAATCAGTGGCATTTGG GAACAAAAATTCCAGACGTGC	NED	0.10
FCA105	A2	TG	TTGACCCTCATACCTTCTTTGG TGGGAGAATAAATTTGCAAAGC	PET	0.20
FCA149	B1	TG	CCTATCAAAGTTCTCACCAAATCA GTCTCACCATGTGTGGGATG	PET	0.18
FCA201	B3		TCTGCAGGACCAGTCAGATG AGCATACACAAATTGATGCTGG		
FCA220	F2	CA	CGATGGAAATTGTATCCATGG GAATGAAGGCAGTCACAAACTG	FAM	0.30
FCA229	A1	GT	CAAACTGACAAGCTTAGAGGGC GCAGAAGTCCAATCTCAAAGTC	NED	0.25
FCA293	C1		GATGGCCCAAAAGCACAC CCCACATCTTGTCAACAACG		
FCA310	C2	$(CA)_5TA(CA)_7$ $TA(CA)_8$	TTAATTGTATCCCAAGTGGTCA TAATGCTGCAATGTAGGGCA	FAM	0.30
FCA441	D3	TAGA	ATCGGTAGGTAGGTAGATATAG GCTTGCTTCAAAATTTTCAC	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	CGAGGTAATTTTTCTGTTTACT GAAACTGAGTCAGAGAGGC		
ZFXY	XY	X–168; Y-165	AAGTTTACACAACCACCTGG CACAGAATTTACACTTGTGCA	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

SIGNATURES



Chair

Duty laboratory – Dog (Bauer – Laboklin)

Co-Chair (Qiu – Neogen, Inc. USA)

Duty laboratory – Cat (Grahn – UC Davis)