Genotyping for physical traits and hereditary conditions of *Felis silvestris catus*

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Abstract:
Precision medicine in our companion animals includes state-of-the-art methods for DNA profiling. DNA profiles of known diseases and traits causing variants will help with the management of breeding programs and the healthcare of the individual cats. Over 70 variants are known for 40 traits and diseases in cats. Effective DNA profiling uses simple and efficient methods to obtain accurate results. Herein is a description and analysis of a new method to perform DNA profiling in cats. The hypothesis is the DNA profiling methods will be able to accurately predict the cat's phenotype and health status. Samples were taken from the cat colony that segregates for different traits and health status using buccal swabs. DNA was isolated and agarose-electrophoresis was used to confirm quality and quantity. MALDI-TOF was used to assay 29 genes in which the cat colony segregated for potentially 53 variants. The 53 variants were multiplexed into two different assays that were genotyped on an Agena MassArray instrument. Overall, 18 colony cats were genotyped including 8 males and 10 females. The colony cats segregated for variants in 15 genes that cause phenotypic traits and health concerns. Most of the assays worked perfectly since they correlate with the known cats' phenotypes although some assays need improvement, such as the four assays required for the blotched tabby locus. These results demonstrate that MassArray techniques are accurate, efficient, and cost effective for performing DNA testing in cats. Therefore, these assays could aid veterinary practices to address cats to confirm breed, physical traits and hereditary conditions in order to improve their healthcare.

Background:

- Currently, there are about 40 genes with at least 70 DNA variations in the domestic cat. Producing different phenotypes, blood types and diseases.[1]
- Thanks to the better understanding gathered today regarding the cat's genome, the term idiopathic (of unknown origin) is less needed. [1]
- As of today, several laboratories perform tests aiding veterinary practices to detect, correct and choose ideal therapy treatment for heritable conditions.[2]
- Therefore, these assays as well as genome sequencing should be implemented to achieve a state-of-the-art health care in the veterinary field.[1]
- The purpose of this study is to evaluate specific assays to detect variations within the cat's genome causing physical traits as well as conditions which can not be seen with the naked eye. Also to raise awareness within the veterinary medicine community to consider DNA testing as preventative medicine and as diagnostic tools.

Methods and materials:

- Samples were obtained from buccal swabbing using cytological brushes.
- DNA was isolated using Qiagen extraction kit.
- Genetic assays for 29 genes and 53 variants.
- Genotyping was achieved by using MALDI-TOF MassARRAY technologies.

Results:

- The cat segregated for 15 variants, 3 variants need design improvement.
- The data confirms the accuracy and efficiency of the MassARRAY technology since the results match the cat's conditions
- Only two PCR reactions were required to test all 53 variants

Table 1a. Genetics variation in the cat for physical traits

Name	ASIP	TYRP1	TYR	MLPH	JBOB	FGF5	Tabby
Barbie	DEL.CA	GA	AG	T	T	CA	AG
Sarabi	CA	G	G	DEL.T	T	CA	AG
Nala	CA	G	G	DEL.T	CT	CA	AG
Elton John	CA	G	G	T	T	C	AG
Aztec	CA	--	A	DEL.T	T	CA	A
Dumbo	DEL.CA	G	A	T	T	C	G
Deiga	CA	GA	AG	T	T	CA	AG
Gonzo	CA	G	AG	T	T	C	G
Neville	CA	GA	AG	T	T	A	G
Walker	DEL.CA	G	G	DEL.T	T	CA	A
Biter	DEL.CA	G	G	DEL.T	T	CA	G
Carol	CA	A	G	T	T	A	AG
Andrea	CA	G	AG	T	T	CA	A
Sophia	CA	G	AG	DEL.T	T	CA	A
Betty	CA	G	G	DEL.T	T	A	G
Oakie	DEL.CA	G	T	DEL.T	T	A	A
Backus	DEL.CA	G	G	DEL	T	A	AG
Blackcat	DEL	G	G	DEL.T	T	A	A

Table 1b. Genotype for physical traits in the cat

Name	ASIP	TYRP1	TYR	MLPH	JBOB	FGF5	Tabby	Phenotype
Barbie	Aa	Bb	Cc	DD	jj	LI	T^m	Black short hair spotted tabby
Sarabi	AA	BB	CC	Dd	jj	LI	T^m	Black short hair spotted tabby
Nala	AA	BB	CC	Dd	Jj	LI	T^m	Black short hair kinked tail spotted tabby
Elton John	AA	BB	CC	DD	jj	II	T^m	Black long hair spotted tabby
Aztec	AA	BB	cc	Dd	jj	LI	T^b	Black short hair classic tabby
Dumbo	Aa	BB	cc	DD	jj	II	T^m	Black long hair mackerel tabby
Deiga	AA	Bb	Cc	DD	jj	LI	T^m	Black short hair spotted tabby
Gonzo	AA	BB	Cc	DD	jj	II	T^m	Black long hair mackerel tabby
Neville	AA	Bb	Cc	DD	jj	LL	T^m	Black short hair mackerel tabby
Walker	Aa	BB	CC	Dd	jj	LI	T^b	Black short hair classic tabby
Biter	Aa	BB	CC	Dd	jj	LI	T^m	Black short hair mackerel tabby
Carol	AA	bb	CC	DD	jj	LL	T^m	Brown short hair spotted tabby
Andrea	AA	BB	Cc	DD	jj	LI	T^b	Black short hair classic tabby
Sophia	AA	BB	Cc	Dd	jj	LI	T^b	Black short hair classic tabby
Betty	AA	BB	CC	Dd	jj	LL	T^m	Black short hair mackerel tabby
Oakie	Aa	BB	CC	Dd	jj	LL	T^b	Black short hair classic tabby
Backus	Aa	BB	CC	dd	jj	LL	T^m	Black short hair spotted tabby (solid blue)
Blackcat	aa	BB	CC	Dd	jj	LL	T^b	Black short hair classic tabby (solid black)

Fig. 1. Nala

Fig. 2 Betty

Fig.3 Gonzo

Fig. 4 Carol

Fig. 5 Oakie

Table 2a. Genetic variations for blood type & diseases in the cat

Name	Blood Type	Bengal PRA	Persian PRA	Main Coon HCM	PKD1
Barbie	G	AG	TC	C	CA
Sarabi	A	AG	TC	C	CA
Nala	G	A	C	C	C
Elton John	G	AG	C	C	CA
Aztec	GA	AG	T	TC	CA
Dumbo	GA	G_	T	C	CA
Deiga	G	AG	C	C	CA
Gonzo	G	AG	TC	TC	CA
Neville	G	A	C	C	CA
Walker	G	G	C	C	CA
Biter	G	AG	C	C	CA
Carol	GA	A	TC	C	C
Andrea	GA	G	TC	C	CA
Sophia	G	G	TC	C	C
Betty	G	A	C	C	C
Oakie	G	G	C	C	C
Backus	GA	G	C	C	C
Blackcat	G	G	C	C	C

Table 2b . Genotype for blood type & diseases in the cat

Name	Blood Type	Bengal PRA	Persian PRA	Main Coon HCM	PKD1
Barbie	Type A	Carrier	Carrier	Normal	Positive
Sarabi	Type B	Carrier	Carrier	Normal	Positive
Nala	Type A	Blind	Normal	Normal	Negative
Elton John	Type A	Carrier	Normal	Normal	Positive
Aztec	Type A (b carrier)	Carrier	Blind	HCM Risk	Positive
Dumbo	Type A (b carrier)	Unknown	Blind	Normal	Positive
Deiga	Type A	Carrier	Normal	Normal	Positive
Gonzo	Type A	Carrier	Carrier	HCM Risk	Positive
Neville	Type A	Blind	Normal	Normal	Positive
Walker	Type A	Normal	Normal	Normal	Positive
Biter	Type A	Carrier	Normal	Normal	Positive
Carol	Type A (b carrier)	Carrier	Carrier	Normal	Negative
Andrea	Type A (b carrier)	Carrier	Carrier	Normal	Positive
Sophia	Type A	Normal	Carrier	Normal	Negative
Betty	Type A	Normal	Normal	Normal	Negative
Oakie	Type A	Normal	Normal	Normal	Negative
Backus	Type A	Normal	Normal	Normal	Negative
Blackcat	Type A	Normal	Normal	Normal	Negative

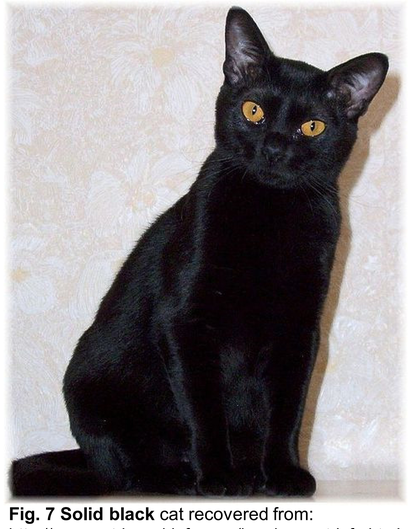

Fig. 7 Solid black cat recovered from:
http://www.cat-breed-info.com/bombay-cat-info.html

Fig. 6 Solid blue cat recovered from:
http://www.catalunyaplants.com/razas-de-gatos-azul-ruso/

Conclusion:

- Genetic testing confirmed diagnosis of PKD, PRA, and HCM which had been diagnosed using standard clinical imaging techniques, ultrasound, fundus exam, and echocardiogram.
- Only one genotype appeared to be incorrect suggesting high accuracy of the MALDI-TOF assay.
- The technique is rapid and robust, additional tests can easily be included in the multiplex assay.
- Veterinarians and owners can rapidly get genetics tests results that would support treatments and breeding decisions.

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References:

1) Lyons LA. (2015). *DNA mutations of the cat. The good, the bad and the ugly*. Journal of Feline Medicine and Surgery. Vol 17.
2) Lyons LA. (2010). *Feline Genetics: A clinical application and genetic testing*. Top Companion Animal Medicine. 25(4). Pp: 203-212).
3) Young A. E., Biller D.S., Herrgesell E. J., Roberts H. R., Lyons L.A. (2005). *Feline polycystic kidney disease is linked to the PKD1 region*. Incorporating Mouse Genome Mammalian Genome Genes and Phenotypes. Vol 16. Pp: 59-65.