Developement of a novel and efficient equine SNP genotyping method and its utility in studying the prevalence of genetic diseases, coat color and various traits of interest for equine industry

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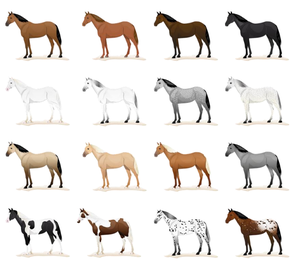
Context and objectives

Next Generation Sequencing (NGS) has enabled the identification of molecular markers widely used to detect causal mutations in horses for genetic disorders or traits as coat color. The aim of this study was to develop and evaluate a SNP custom panel as a potential useful tool for equine genotyping.

GENETIC DISEASE AND COAT COLOR

A selection of 73 markers was made according to the most frequently genetic tests requested by veterinaries and breeders and also based on the OMIA database (Online Mendelian Inheritance in Animals, Sydney School of Veterinary Science).

33 monogenic disorder markers were selected among which the Severe Combined Immunodeficiency (SCID), the Hyperkalaemic Periodic Paralysis (HYPP), the Hoof Wall Separation Disease (HWSD), the Cerebellar Abiotrophy (CA) etc.



40 coat color markers were also selected and primarily concerned the two genes responsible for the basic coat color which control the red and black pigments known as Extension and Agouti genes. Several dilution genes including Cream, Champagne, Pearl and Silver and many genes responsible for white coat patterns as Sabino1, Tobiano or Dominant White were chosen to complete this custom panel.

	Marker type			Localisation		Inheritance			Total
	SNP	Indel	BigDel	Autosomal	X-linked	Recessive	Incomplete dominant	Dominant	
Genetic disorder	22	8	3	30	3	27	0	6	33
Coat color	31	5	4	40	0	5	6	29	40

GAIT PERFORMANCE TRAITS

As equine genetics play a large part in performance and abilities, 35 markers including the DMRT3 mutation were added to the custom panel.

All of them have been previously demonstrated to have an effect on racing performance and trotting techniques in French trotters like ability to pace or tendency to gallop (Ricard et al., 2021).

All the obtained genotype results could be then analyzed by Equibiogenes (Saint Contest, France) to provide to breeders and owners genetic indexes and thus identify the true potential of animals regarding gait, precocity, and genetic inheritance (SynchroGait©).

Material and Methods

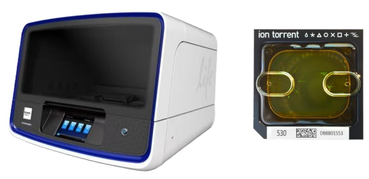
IN WET LAB

Blood sample with EDTA or horse hair sample

DNA extraction and purification using the QIAmp® DNA minikit (QIAGEN)

DNA quantification using the Qubit 4 fluorometer and **normalization of sample concentration** (3,3 ng/µL)

Library preparation using the AgriSEQ™ HTS library kit (Thermofisher)



Template preparation and **chip loading** on the IonChef™ Instrument (Thermofisher)

Sequencing by synthesis on the Ion GeneStudio™ S5 (Thermofisher)

IN DRY LAB

Analysis with the Torrent Server software

Quality control process : Run statistics, read quality, coverage analysis

Raw data

↓

Aligned BAM with the EquCab 3.0 genome (GCA_002863925.1)

↓

VCF files using the VariantCaller plugin

	A	B	C	D	E	F	G	H
1		Sample1;Ion	Sample2;Ion	Sample3;Ion	Sample4;Ion	Sample5;Ion	Sample6;Ion	Sample7;
2	LFDEQ01	C/C	C/C	C/C	C/C	C/C	C/C	C/C
3	LFDEQ03	G/G	G/G	G/G	G/G	G/G	G/G	G/G
4	LFDEQ04	C/C	C/C	C/C	C/C	C/C	C/C	C/C
5	LFDEQ06	C/C	C/C	C/C	C/C	C/C	C/C	C/C
6	LFDEQ07	G/G	G/G	G/G	G/G	G/G	G/G	G/G
7	LFDEQ08	G/G	G/G	G/G	G/G	G/G	G/G	G/G
8	LFDEQ13	C/C	C/C	C/C	C/C	C/C	C/C	C/C
9	LFDEQ14	C/C	C/C	C/C	C/C	C/C	C/C	C/C
10	LFDEQ16	C/C	C/C	C/C	C/C	C/C	C/C	C/C
11	LFDEQ17	T/T	T/T	T/T	T/T	T/T	T/T	T/T
12	LFDEQ19	A/A	A/A	A/A	A/A	A/A	A/A	A/A
13	LFDEQ20	T/T	T/T	T/T	T/T	T/T	T/T	T/T
14	LFDEQ21	A/A	A/A	A/A	A/A	A/A	A/A	A/A
15	LFDEQ22	C/C	C/C	C/C	C/C	C/C	C/C	C/C
16	LFDEQ23	C/C	C/C	C/C	T/C	C/C	C/C	C/C
17	LFDEQ24	T/T	T/T	T/T	T/T	T/T	T/T	T/T
18	LFDEQ25	C/C	C/C	C/C	C/C	C/C	C/C	C/C
19	LFDEQ28	A/A	A/A	A/A	A/A	A/A	A/A	A/A
20	LFDEQ29	C/C	C/C	C/C	C/C	C/C	C/C	C/C

All genotype results were compiled in a csv file with the AgriSum™ toolkit plugin.

Results

For the 108 targets, sample results obtained with the custom panel were compared with the expected genotypes for which results were provided from reference laboratories or obtained after Sanger sequencing analysis. All results were consistent except for 2 markers for which a redesign was needed. Indeed, the custom panel could be if necessary amended or updated if other molecular markers of interest are recently highlighted.

Conclusion

This equine SNP genotyping method based on AgriSEQ™ technology resulted in a cost-effective strategy to prevent the disease risk, to study the prevalence of traits and to improve the knowledge about horses' performance and abilities. This efficient tool could thus be easily used in veterinary laboratories for large-scale routine genetic analyses.

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