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P u b l i s h e r s

Poster Session 71

Validation of a molecular screening tool for the detection of chromosomal abnormalities in donkey

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Chromosomal abnormalities are one of the main causes of infertility and reproductive problems in horses. Nowadays, the detection of individuals showing this type of aberrations is rising due to the use of new diagnostic tools based on molecular markers located along the autosomal and sexual chromosomes. In contrast, despite its great similarities with the horse, there is only one recent report of sterility associated with chromosomal abnormalities in the domestic donkey (*Equus asinus*), a scarcely studied species in spite of its importance for the human being and the endangered status of certain breeds. In the present study, we analysed the possibility of applying an STR (Single-Tandem-Repeat)-based molecular method developed for horses as a diagnostic tool for these abnormalities in donkeys. The frequencies of five X-linked (*LEX003*, *LEX026*, *TKY38*, *TKY270* and *UCEDQ502*) molecular markers and one Y-linked gene (Sex-Determining Region Y, *SRY*) were determined in 121 donkeys of two different Spanish breeds (Andaluza and Encartaciones) and 58 donkeys from north Africa (Moruna). Taking as reference the analysed population, sensitivity and specificity of the diagnostic tool were determined based on expected profiles of chromosomal abnormalities and results of heterozygosity of the molecular markers used. The molecular panel showed 100% sensitivity and 98.78% specificity. Hence, its use complementarily with other cytogenetic techniques constitutes a highly specific, rapid and low cost detection tool for chromosomal abnormalities and their characterization in domestic donkey.

EAAP 69th Annual Meeting

Thursday, 30 August 2018

Validation of a molecular screening tool for the detection of chromosomal abnormalities in donkey

Julia Poyato-Bonilla, Gabriel Anaya, Jesús M. Dorado and Sebastián Demyda-Peyrás

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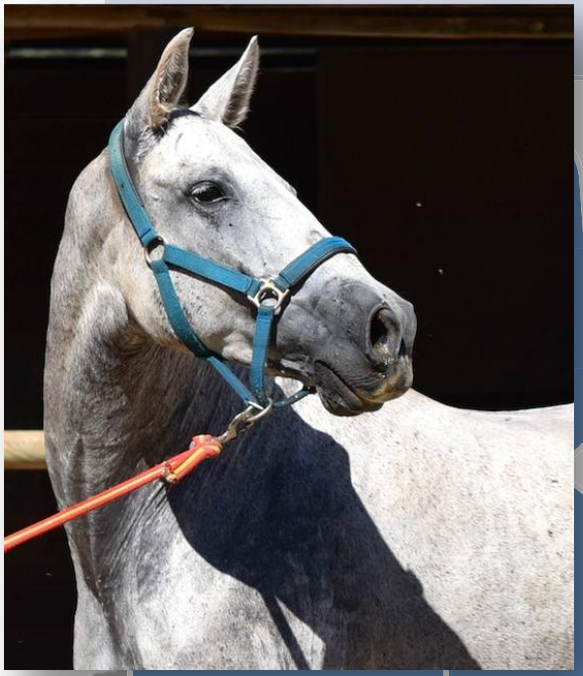
INTRODUCTION

Horse



Donkey

vs.

sexual chromosomes

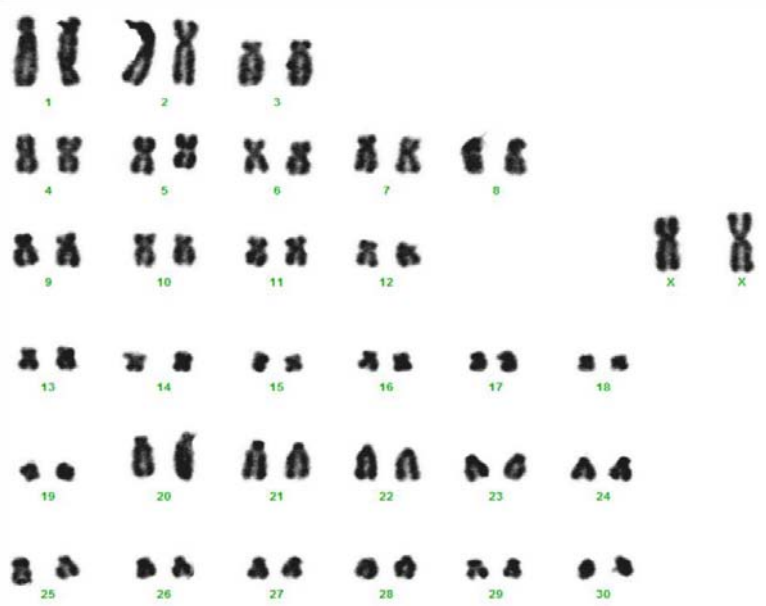
INTRODUCTION

Chromosomal alterations in equines

- Congenital anomalies
- Embryonic losses
- Infertility

Production

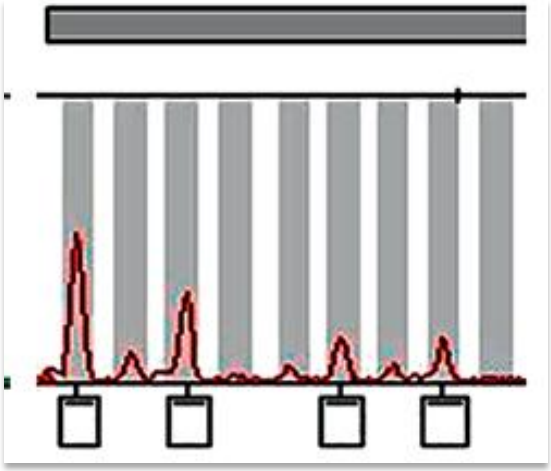
Economic and time loss



OBJECTIVE OF THIS WORK

To analyse the possibility of **applying** an **STR** (Single-Tandem-Repeat)-based **molecular method** developed **for horses** as a diagnostic tool for sexual chromosomes abnormalities **in donkeys**.

XX - XY



MATERIALS AND METHODS

Animals: 121 donkeys (51 Andaluza breed and 70 Moruna)
93 females and 28 males

Hair

Cellular cultures

DNA isolation:

Molecular analyses

MATERIALS AND METHODS

Marker	Localization	Type
LEX003	X	Microsatellite
LEX026	X	Microsatellite
TKY270	X	Microsatellite
TKY38	X	Microsatellite
UCEDQ502	X	Microsatellite
ECAYM2	Y	Microsatellite
SRY	Y	Gene

DNA amplification

Multiplex PCR

PCR products genotyped by capillary electrophoresis

Alelle sizing

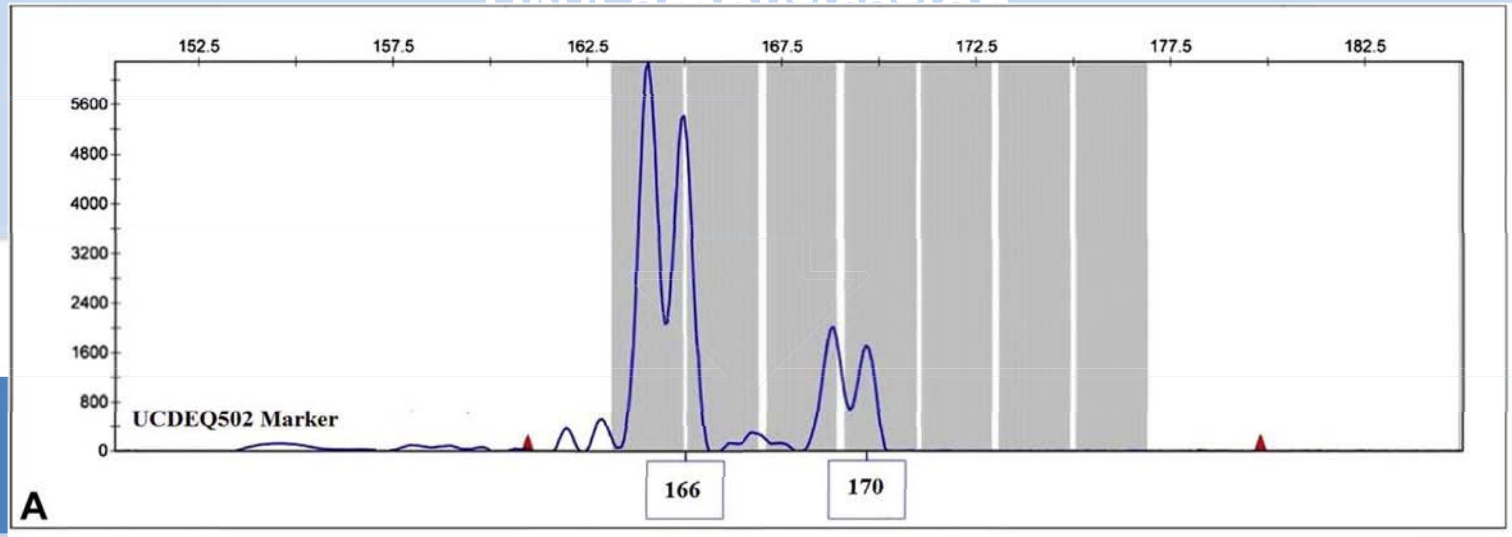
GeneMapper © 4.0 software

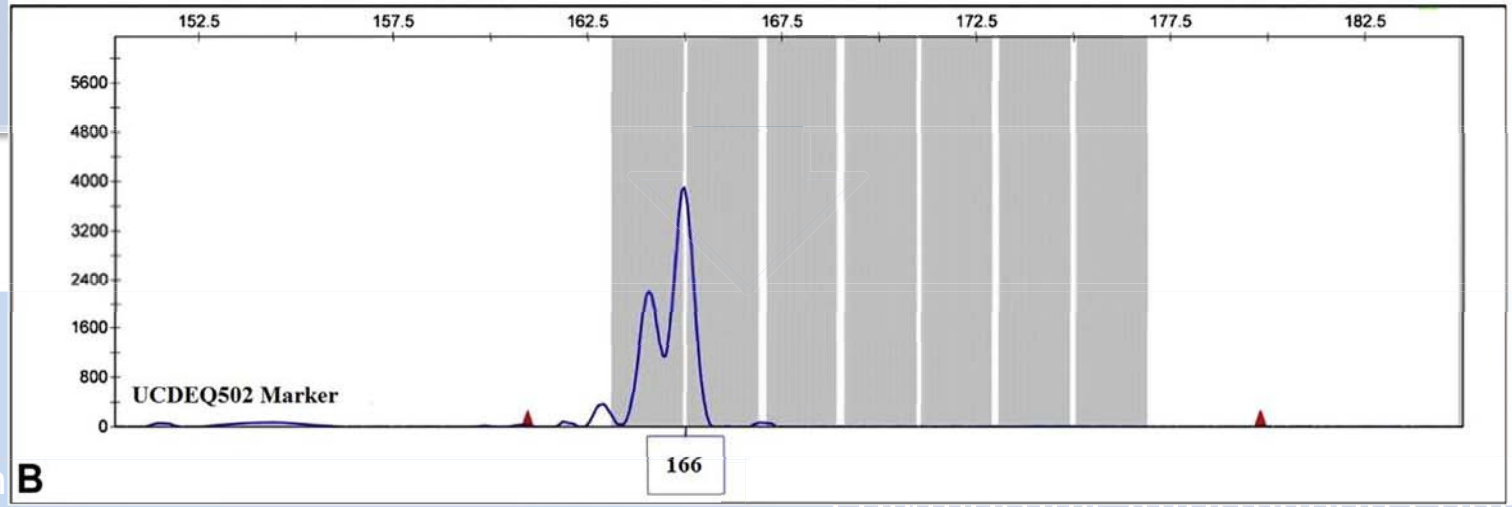
Data analysis

Frequency, average and total number of alleles. Observed and expected heterozigosity. Genetix 4.05.2 software

Polymorphic Information Content (PIC). Cervus 3.0.7. software

MATERIALS AND METHODS





Observed and expected heterozygosity. Genetix 4.05.2 software

(PIC). Cervus 3.0.7. software

MATERIALS AND METHODS

DNA amplification

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GeneMapper © 4.0 software

Data analysis

Frequency, average and total number of alleles. Observed and expected heterozygosity. Genetix 4.05.2 software

Polymorphic Information Content (PIC). Cervus 3.0.7. software

RESULTS

- No differences between DNA from blood and hair.

- Y chromosome gene and microsatellite (*SRY* and *ECAYM2*): detected in 100% of the animals phenotypically described as males and 0% of females.

STR	Number of alleles	Average number of alleles	Total number of alleles
LEX003	9		
LEX026	5		
TKY270	8	9.6	48
TKY38	12		
UCEDQ502	14		

Genetic characterisation of the population

STR	H_{obs}	H_{ex}	PIC
LEX003	0.3118	0.6126	0.5617
LEX026	0.3696	0.6509	0.6149
TKY270	0.3978	0.5478	0.4636
TKY38	0.3516	0.6434	0.6074
UCEDQ502	0.4409	0.8573	0.8513

Polymorphic Information Content (PIC) > 0.5 → High informative quality of this set of molecular markers

RESULTS

Sensitivity and specificity

Phenotype	Diagnosis	Karyotype	X markers profile	Y markers	
				ECAYM2	*SRY*
Male	Normal male	62, XY	One allele per marker	+	+
Female	Normal female	62, XX	At least one marker in heterozygosis	-	-
Female	Complete Turner'ssyndrome	61,X0	One allele per marker	-	-
Male/Female	Cellular chimerism	62, XX/XY	At least one marker with more than 2 alleles Possible hair/blood differences	+	+
Male	Male SRY positive/negative Disorder in Sex Development	62, XX	At least one marker in heterozygosis	-	+/-
Female	Female SRY positive/negative Disorder in Sex Development	62, XY	One allele per marker	+	+/-

CONCLUSIONS

This molecular tool could be used as a diagnostic technique for the detection of chromosomal anomalies of the sexual pair in donkeys.

Fast

Reliable

High sensitivity

Cheap

High specificity

VALIDATION OF A MOLECULAR SCREENING TOOL FOR THE DETECTION OF CHROMOSOMAL ABNORMALITIES IN DONKEY

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Introduction

The domestic donkey (*Equus asinus*) is one of the most important draft animals with a wide distribution around the world. His karyotype is composed of 62 chromosomes (EAS, 2n=62) sharing a common ancestor with horses (ECA, 2n=64), which diverged 4.45 million years ago, being their chromosomal complements highly similar (Orlando *et al.*, 2013). Despite this similarity, chromosomal studies in horses are highly common instead of donkeys, in which they are extremely scarce. However, the genetic relatedness between horse and donkeys was recently employed to detect chromosomal abnormalities in this species using molecular tools developed for horses (Dorado et al., 2017)

Chromosomal abnormalities influencing sexual development have been reported in horses since the 1970s. Phenotypically, they are manifested in a variety of ways, from gonadal dysgenesis to the apparition of ambiguous external genitalia and/or male and female internal sexual organs, as well as embryonic losses and idiopathic infertility (Lear and Bailey, 2008). The prevalence of chromosome aberrations in this species is relatively high compared to other domestic animals, being more than 95% of the cases reported related to abnormalities in the sex pair (Di Meo *et al.*, 2004; Lear and McGee, 2012). These alterations cause significant losses to breeders, probably due to the lack of an early diagnostic and its consequent decrease in production. Additionally, their manifestations cannot be perceived until the animal reaches his or her sexual maturity, so infertile animals are bred for years without knowledge of their problematic (Kakoi *et al.*, 2005). The diagnosis of these anomalies has usually been accomplished by cytogenetic tools based on cell cultures followed by visualisation and analysis of karyotype, and more recently, by FISH (Fluorescence In Situ Hybridisation) and molecular markers (Lear and McGee, 2012; Demyda-Peyrás *et al.*, 2013), which is an easier and cheaper

diagnostic system. Recently, our group developed a diagnostic tool based on STR markers to detect the most important sex chromosomal abnormalities in horses (Anaya et al. 2017).

Objectives

Since the genomic proximity of both species, the aim of this study was to test and characterize this methodology in the detection of sex-chomosome abnormalities in donkeys.

Materials and Methods

To this end, we analysed 121 animals belonging to the two most important Spanish breeds (51 Andaluza and 12 Encartaciones) and 58 donkyes from the north of Africa (Moruna). Blood samples were collected by jugular venopunction using Vacutainers™ for DNA isolation (Tri-sodium EDTA) and cell culture (sodium heparin). Hair samples were also collected for DNA isolation. Five X-linked microsatellite markers (*LEX003*, *LEX026*, *TKY270*, *TKY38* and *UCEDQ502*) and one Y-linked marker (*SRY* gene) were amplified by PCR using the primer pairs previously designed for horses. Amplification was carried out using 15 µl containing 20-60 ng of genomic DNA, 1.5-7.5 pmol of each primer pair, 0.33 mol/L dNTPs, 2.5 mmol/L of $MgCl_2$, 1X PCR buffer and and 1.5 U Taq DNA polymerase (Canvax Biotech). The thermal protocol was the following: an initial denaturation at 95 °C for 10 min, 33 cycles at 94 °C for 30 s, 57 °C for 1 min and 72 °C for 30 s, followed by 72 °C for 10 min. PCR products were genotyped by capillary electrophoresis using an Applied Biosystems 3130 xl DNA sequencer in the Central Service for Research Support of the University of Córdoba. Allele sizes were determined using software Genemapper 4.0 and a LIZ 500 bp internal size standard (Applied Biosystems). In order to confirm the results, cytogenetic analysis were performed on 30 metaphase chromosomes obtained from lymphocytes cultures according to Rodero-Serrano et al. 2013.

Statistical analyses were performed using Genetix 4.05.2 (Belkhir et al. 1996-2004): allele frequency (A_f) as well as average (X_n) and total number (A_n) of alleles were determined for the entire population. Observed (H_{obs}) and expected (H_{ex}) heterozygosity were calculated only for female individuals. Allele variability (polymorphic information content, PIC) was determined for each marker using Cervus 3.0.7. (Kalinowski et al. 2007). Finally, the sensitivity (proportion of positives that are correctly identified as such) and specificity (proportion of negatives that are correctly identified as such) of the diagnostic tool were determined based on allele variability in this population.

Results and Discussion

Results depicting the genetic variability of the markers employed in this study (A_f) are shown in tables 1. It was noteworthy that all the STR markers developed as a methodology to be employed in horses were suitable to be used also in this species. In the same way, *SRY* marker was only detected in jacks, being negative in all the jennies analysed.

The number of alleles per loci ranged from 5 (*LEX026*) to 14 (*UCEDQ502*), which indicates the high polymorphism of the microsatellites in study and thus, includes a wide variety of homozygous and heterozygous genotypes. The X_n per marker (9) demonstrates a high genetic variability within the population. Similar results were observed in the H_{obs}, H_{ex} and PIC (Table 2). The highest values of H_{obs} (0.435) and H_{ex} (0.861) were observed in *UCEDQ502* microsatellite, whilst *LEX003* (0.284) and *TKY270* (0.553) showed the lowest. PIC results were higher than 0.50 in all markers except *LEX003*, evidencing a high number of possible genotypic combinations.

To assess the specificity of this panel of markers, we estimated the probability of finding five loci in homozygosis in the same female, based on the allelic frequencies per marker, with an overall result of 98.78%. Its sensitivity was estimated in 100% since there is no possibility that an animal with only one EASX obtains a diallelic result, and even less with the inclusion of the *SRY* gene which exclude the possibility to diagnose a jack as an EASX monosomy. In a sex reversal case, in which the genotype is the opposite to phenotypic sex, it can be detected whether individuals carry one allele of the X chromosome and the SRY gene (XY genotype) or if they have two copies of the X chromosome (XX). Similarly, a cellular chimerism involving an additional copy of the X chromosome would result in the amplification of more than two different alleles in some of the X chromosome markers. Turner's syndrome (X0) could be detected as homozygosity in all X chromosome markers and absence of the SRY gene.

In conclusion, this molecular tool could be used as a fast, reliable and cheap diagnostic technique for the detection of chromosomal anomalies of the sexual pair with a high specificity and sensibility in donkeys. Also, it is of great interest for the early detection of infertility and disorders of sexual development, which is very valuable in animal production.

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References

Anaya, G., Molina, A., Valera, M., Moreno-Millán, M., Azor, P., Peral-García, P. and Demyda-Peyrás, S. (2017) Sex chromosomal abnormalities associated with equine infertility: validation of a simple molecular screening tool in the Purebred Spanish Horse, *Animal Genetics*, 48(4), 412-419

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., Chikli, L. and Belkir, K. (1996-2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171.

Demyda-Peyrás, S., Membrillo, A., Bugno-Poniewierska, M., Pawlina, K., Anaya, G. and Moreno-Millán, M. (2013) The use of molecular and cytogenetic methods as a valuable tool in the detection of chromosomal abnormalities in horses: A case of sex chromosome chimerism in a spanish purebred colt, *Cytogenetic and Genome Research*, 141(4), 277–283.

Dorado, J., Anaya, G., Bugno-Poniewierska, M., Molina, A., Mendez-Sanchez, A., Ortiz, I., Moreno-Millán, M., Hidalgo, M., Peral García, P. and Demyda-Peyrás, S. (2017) First case of sterility associated with sex chromosomal abnormalities in a jenny, *Reproduction in Domestic Animals*, 52(2), 227–234.

Kakoi, H., Hirota, K., Gawahara, H., Kurosawa, M. and Kuwajima, M. (2005) Genetic diagnosis of sex chromosome aberrations in horses based on parentage test by microsatellite DNA and analysis of X- and Y-linked markers., *Equine veterinary journal*, 37(2), 143–147.

Kalinowski, S. T., Taper, M. L. and Marshall, T. C. (2007) Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment, *Molecular Ecology*. Blackwell Publishing Ltd, 16(5), 1099–1106.

Lear, T. L. and Bailey, E. (2008) Equine clinical cytogenetics: the past and future, *Cytogenetic and genome research*. Karger Publishers, 120(1–2), 42–49.

Lear, T. L. and McGee, R. B. (2012) Disorders of sexual development in the domestic horse, Equus caballus, *Sexual Development*. Karger Publishers, 6(1–3), 61–71.

Di Meo, G. P., Perucatti, A., Spadetta, M., Incarnato, D., Parma, P., Iannuzzi, A., Ciotola, F., Peretti, V., Perrotta, G. and Di Palo, R. (2004) Clinical, cytogenetic and molecular studies on sterile stallion and mare affected by XXY and sex reversal syndromes, respectively, *Caryologia*. Taylor & Francis, 57(4), 400–404.

Orlando, L., Ginolhac, A., Zhang, G., et al (2013) Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse, *Nature*. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved., 499(7456), 74–78.

Rodero-Serrano, E., Demyda-Peyrás, S., González-Martinez, A., Rodero-Franganillo, A. and Moreno-Millán, M. (2013) The rob(1;29) chromosome translocation in endangered Andalusian cattle breeds, *Livestock Science*. Elsevier, 158(1–3), 32–39.

Table 1: Allelic frequencies of the five EASX microsatellites in females and males.

STR	Alelle	A_f females	A_f males
LEX003	200	0.0398	0.0345
	202	0.0114	0.0000
	204	0.0057	0.0000
	206	0.0398	0.0000
	210	0.1761	0.2069
	212	0.5966	0.6552
	214	0.1307	0.1034
LEX026	298	0.0407	0.1154
	300	0.2209	0.1346
	302	0.4884	0.4615
	304	0.2442	0.2885
	306	0.0058	0.0000
TKY270	152	0.0056	0.0000
	156	0.1011	0.0357
	160	0.0056	0.0000
	162	0.1067	0.0714
	164	0.6461	0.7500
	166	0.1011	0.1071
	168	0.0112	0.0357
	170	0.0225	0.0000
TKY38	119	0.0060	0.0000
	137	0.0060	0.0000
	145	0.0060	0.0000
	147	0.0663	0.0556
	149	0.0422	0.0370
	151	0.0542	0.1111
	153	0.0723	0.0370
	157	0.0301	0.0000
	159	0.0181	0.0000
	161	0.1024	0.1296
	163	0.5964	0.6296
UCEDQ502	148	0.0647	0.1852
	150	0.1176	0.1111
	152	0.0118	0.0370
	154	0.0235	0.0370
	156	0.0235	0.0000
	158	0.1118	0.1111
	160	0.0059	0.0370
	162	0.1353	0.0370
	164	0.0647	0.0741
	166	0.0118	0.0000
	168	0.0588	0.0370
	170	0.2824	0.1852
	172	0.0824	0.1481
	174	0.0059	0.0000

Table 2: Observed (H_{obs}) and expected (H_{ex}) heterocigosity and polymorphic information content (PIC) of the five EASX microsatellites in the population.

STR	$\boldsymbol{H_{obs}}$	$\boldsymbol{H_{ex}}$	**PIC**
LEX003	0.284	0.596	0.346
LEX026	0.337	0.655	0.793
TKY270	0.405	0.553	0.748
TKY38	0.349	0.622	0.780
UCEDQ502	0.435	0.861	0.826