

SHORT NOTE

A MULTIPLEX SNAPSHOT ASSAY FOR DETECTION OF Y-CHROMOSOME SNPS IN DOGS AND IBERIAN WOLVES

UN ENSAYO DE SNAPSHOT MULTIPLEXADO PARA LA DETECCIÓN DE SNPS EN EL CROMOSOMA Y DE PERROS Y LOBO IBÉRICO

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ADDITIONAL KEYWORDS

Dog Y-chromosome. Single nucleotide polymorphisms. Multiplex SNaPshot. Genetic diversity. Iberian Peninsula.

PALABRAS CLAVE ADICIONALES

Cromosoma Y de perros. Polimorfismos de base única. SNaPshot multiplexado. Diversidad genética. Península Ibérica.

SUMMARY

We were able to develop a SNaPshot strategy to genotype, in a multiplex basis, the domestic dog Y-chromosome SNPs recently identified by other authors.

Domestic dogs from Portuguese native breeds and from other populations of dogs from Spain and North Africa, and Iberian wolves (*Canis lupus signatus*, Cabrera 1907) were for the first time analysed for SNPs specific to the dog Y-chromosome. We could identify diagnostic haplotypes of Iberian dog and wolf origins.

By the use of the Y-chromosome markers it will be possible to complement previous studies based on mtDNA sequences (maternally inherited) and autosomal markers (bi-parentally inherited) regarding the origin of the Portuguese native domestic dog breeds. This is an ongoing research study.

RESUMEN

Se desarrolla una estrategia de SNaPshot para genotipar sobre una base multiplexada los SNPs del cromosoma Y, recientemente identificados por otros autores en el perro doméstico. SNPs del cromosoma Y, específicos de perro, fueron analizados por primera vez en perros domésticos de razas nativas portuguesas, perros de otras

poblaciones de España y Norte de África, y lobos ibéricos (*Canis lupus signatus*, Cabrera 1907). Se pudieron identificar haplotipos diagnósticos de perro y lobo ibéricos.

Con el uso de los marcadores del cromosoma Y será posible complementar estudios previos basados en secuencias de ADN mitocondrial (heredados maternalmente) y marcadores autosómicos (heredados de ambos padres) relacionados con el origen de las razas de perros nativos portugueses. Este estudio está aún en marcha.

INTRODUCTION

In Portugal there are 10 native domestic dog breeds. It is not yet clear if the Iberian sub-species of wolf, *Canis lupus signatus*, had or not participation in the origin of any native dog breed. Previous data on mtDNA (Pires *et al.*, 2006) indicate that Portuguese native breeds share the same origin as other dogs in the world. Y-chromosome (paternally inherited) molecular markers can be very useful to describe the male counterpart history. We have developed a multiplex strategy using the SNaPshot methodology to assess SNPs on dog Y-chromosome at a

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population scale, based on previously described polymorphisms (Natanaelsson *et al.*, 2006).

METHODS

DNA from blood or tissue samples from 18 unrelated male dogs from 9 Portuguese native breeds, 5 specimens from dog breeds from Spain and North Africa, five mongrel dogs, and two male wolves, was extracted using Nucleo Spin Blood quick pure (Macherey Nagel). Wolf samples were obtained from captive animals kept in Iberian Wolf Recovery Center (Portugal).

Primers for PCR amplification of Y-chromosome genomic sequences and SNaPshot extension reactions were all designed in order to meet the SNaPshot kit recommendations using Primer3 software. Templates sequences were 12 segments from domestic dog Y-chromosome, previously described by Natanaelsson *et al.* (2006) and deposited on Genbank (Accession numbers DQ973626-DQ973805).

PCR amplification was carried out by performing individual Y-chromosome fragment amplification reactions using a touchdown PCR program for all loci. Amplifications were performed in a reaction of 15 μ l volume using 5 ng of template DNA, 1 \times PCR master mix from Biomix (Bioline) and 0,2 μ M of each amplification primer.

Touchdown PCR amplifications were as follows: 3' denaturation at 94°C, 11 cycles of 30" denaturation at 94°C, 45" annealing at 53°C, and 1' extension at 72°C, followed by 24 cycles of 30" denaturation at 94°C, 45" annealing at 48°C and 1' extension at 72°C and a final extension step at 72°C for 20'. After amplification, PCR products required purification in order to remove primers and un-incorporated dNTPs. Post-PCR purification used ExoSapIT (Amersham Pharmacia Biotech) and followed manufacturer's suggestions. Genotyping reactions were carried out using the SNaPshot™ (Applied Biosystems) method and following

our multiplex assay. The fluorescently labeled fragments were then separated by capillary electrophoresis on an ABI PRISM 3710 (Applied Biosystems). Data was analyzed with GeneMapper™ 3.7 Software (Applied Biosystems).

RESULTS AND DISCUSSION

1) SNaPshot DESIGN

Primer sequences for multiplex SNaPshot-based genotyping of domestic dog Y-chromosome SNPs are under a Genbank submission process and will be available soon. This assay seems to be very useful for population studies as it allows a rapid and simultaneous amplification of multiple loci (at most seven).

2) IBERIAN DOG Y-CHROMOSOME GENETIC VARIABILITY - SEQUENCING AND SNPs POLYMORPHISMS

Peripheral dog breeds, could harbor additional diversity. However for the studied peripheral breeds, entire sequence data from two males per breed, for the targeted fragments (12 out of 14 described), did not reveal novel polymorphic sites. Not even village dogs (mongrels) which evidenced a higher level of genetic variation for other nuclear markers (Pires *et al.*, 2009), showed additional Y-chromosome polymorphisms. For the targeted SNPs this haplotype matched entirely with the reference haplotype (haplotype 1) described in Natanaelsson *et al.* (2006).

3) INTERSPECIFIC Y-CHROMOSOME ANALYSIS - DOMESTIC DOG AND IBERIAN WOLF

Comparisons between domestic dogs and Iberian wolves haplotypes indicate the presence of diagnostic substitutions (transversions) segregating these two species apart. These diagnostic haplotypes are under confirmation with a larger sample size.

We present the first reference to a fast, flexible and cost-effective multiplex assay for genotyping SNP mutations on dog Y-

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chromosome. This SNaPshot-based approach may be applied to ecological and conservation studies, as well as to ancient DNA analysis.

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