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

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.

SHORT NOTE

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 15ul volume using 5 ng of template DNA, 1 × PCR mastermix from Biomix (Bioline) and 0.2 µM 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 (Amershan 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-
CANINE Y-CHROMOSOME VARIABILITY IN THE IBERIAN PENINSULA

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

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REFERENCES

