A *Cervus* genotyping kit based on automated fluorescent multiplex PCR for rapid characterization of genetic diversity in several deer populations

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Summary
Microsatellite loci are highly conserved among related species (Kühn *et al*, 1996) such as cattle and sheep (Moore *et al*, 1992). Transfer of a large number of polymorphic microsatellites between families of the *Artiodactyla* order has been demonstrated several times (Engel *et al*, 1996; Kuhn *et al*, 1996, Slate *et al*, 1998 and Roed, 1998). Therefore, a multi-allele system should be useful in studying the genetic diversities of populations. This study was developed using four different deer species (Rusa, Eld, Swamp and Vietnamese Sika deer). A set of 38 microsatellites derived from bovine and ovine origin were chosen based on two criteria: (i) known to amplify in other deer species (Red deer); and (ii) showing an interesting polymorphic level as described in previous studies (Slate *et al*, 1998 and Talbot *et al*, 1996). From these 38 screened markers, 30 gave an amplified product in the 4 deer species (78.9 %). Of those 30 markers, 14-20 (40%-60%) were polymorphic, depending on the species. Using 12 microsatellites polymorphic in the 4 species, we set up a unique multiplex PCR optimized for annealing temperature and reagent concentrations. The 12 primer sets were labelled with 3 different fluorochromes, depending on the allelic range for each species. The automatic analysis was performed using an ABI 377 sequencer and PE Genotyper software. This method for parentage testing or genotyping gave good and reproducible results for the 4 studied species as well as for several other tested deer species and subspecies. This tool could be considered a first generation "Cervus" genotyping kit useful for the rapid characterization of genetic diversity.

Materiels and methods

DNA was extracted from either ear punches or white blood cells using Promega Wizard™ Genomic DNA Purification Kit (with or without proteinase K / EDTA solution addition).

Microsatellite loci were tested on the four species for amplification and polymorphism as follow : twenty five ng of genomic DNA were used in 25µl reaction mix with 2,5 µl of GeneAmp 10X buffer (Perkin Elmer) and a final concentration of 0,2 µM of each primer, 200 µM of each dNTP, 2 mM of $MgCl_2$, 1U of Taq polymerase (Ampli taq gold, Perkin Elmer). PCR amplification was performed as follows: 12 min denaturation step at 94°C (hot start); 35 or 40 cycles of 1 min denaturation at 94°C, 1 min at annealing temperature (depends of the primers), 1 min extension at 72°C. The final elongation step was extended to 10 min in a Perkin Elmer themocycler (2400 or 9600). 15 µl of each reaction was run on a 3.5% ethidium bromide stained agarose gels (Nusieve- TBU). After electrophoresis, the gels are visualised on an UV-light transilluminator. Photographs are taken and used for allele discrimination.

Primers were screened, at first, in a panel of DNA from each species (4). Loci that gave a PCR product in any of the tropical deer species were then screened for a higher number of samples : 8 Swamp deer from Zoo, 8 Rusa deer (four from Mauricia island and four from New Caledonia), 10 Eld deer from Paris Zoo and 10 Vietnamian sika deer from several origin (Zoos in France, Cuc phong reserve and Vietnam farmer).

To decrease the time and effort involved in performing lot of PCR, we chose to product multiplexes PCR like the bovin kit from Perkin Elmer but for Cervids. Multiplexe combinations must take into account annealing temperature, magnesium concentration and allelic range for each species. Of the twenty primer sets hold for their high polymorphism, twelve were able to produce three multiplex sets working for the whole species. For instance, each one of those primer set ought to be amplified alone. The automatic analysis was performed using an ABI 377 sequencer and PE Genotyper software.

Multiplexe n°	Locus	Fluorescent label	µM primer pairs	$MgCl_2$ concentration	PCR conditions
1	TGLA 57	Fam	0.8	2.5 mM	
	INRA 107	Fam	0.4	2.5 mM	58°C
	IDVGA 55	Fam	0.2	2.5 mM	35 cycles
	BMC 1009	Fam	0.2	2.5 mM	
2	VH 110	Joe	0.2	2.5 mM	
	BM 757	Joe	0.1	2.5 mM	58°C
	BL 42	Joe	0.1	2.5 mM	35 cycles
	BM 848	Joe	0.2	2.5 mM	
3	TGLA 126	Ned	0.2	2.5 mM	
	TGLA 53	Ned	0.1	2.5 mM	58°C
	BM 203	Ned	0.2	2.5 mM	35 cycles
	CSSM 43	Ned	0.4	2.5 mM	

Image de profil multiplexe :
une d '1 ind de cerf rusa
une avec les autres espèces testées
(justification du kit cervus)

Results:
From these 38 screened markers, 30 gave an amplified product in the 4 deer species (78.9 %). Of those 30 markers, 14-20 (40%-60%) were polymorphic, depending on the species.
This method for parentage testing or genotyping gave good and reproducible results for the 4 studied species as well as for several other tested deer species and subspecies.
This tool could be considered a first generation "Cervus" genotyping kit useful for the rapid characterization of genetic diversity.