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TECHNICAL NOTE

# Isolation and characterization of microsatellite loci in blesbok (*Damaliscus pygargus phillipsi*)

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**Abstract** Eight microsatellite markers were developed via pyrosequencing for the blesbok (*Damaliscus pygargus phillipsi*). These microsatellite loci and microsatellite loci from two cross species markers displayed two to four alleles with an expected heterozygosity range between 0.2899 and 0.6268 and an observed heterozygosity between 0.2083 and 0.6667. The high level of polymorphisms observed in the microsatellite markers indicates that they can be used to strongly improve our knowledge of the genetic structure and relatedness of these animals.

**Keywords** Blesbok · *Damaliscus pygargus phillipsi* · Microsatellite

The blesbok (*Damaliscus pygargus phillipsi*) originated from the endemic South African antelope (*Damaliscus pygargus*) which belongs to the Alcelaphini tribe. This tribe, according to fossil records, originated in South Africa (Vrba 1979). *D. pygargus* was distributed from the south-western Cape to the southern boundary of Zimbabwe (Vrba 1979). Due to climatic and habitat change, *D. pygargus* were divided into two groups, namely blesbok and bontebok. Each group have morphological differences such as body markings and hide colours (Bigalke 1955). Historically, the blesbok was distributed in the grasslands of Gauteng, Eastern Cape, Mpumalanga and Free State (Skinner and Smithers 1990) while the bontebok had restricted distribution to the coastal plains of the Western Cape where its population size has been reduced and driven to near extinction, due to hunting and human intrusion in the 1800 s (Van der Merwe 1986). Thus far, no microsatellite markers have been reported for this species. This report documents the first successful attempt to identify variable di- and tri-nucleotide microsatellite loci in *Damaliscus pygargus phillipsi* as part of an effort to understand hybridisation in bontebok and blesbok. A total of 24 South African Southern blesbok samples were collected from the Northern Cape (South Africa). Blood and hair samples were collected from the animals. Collected blood was stored in EDTA preservative (approximately 0.5 mL). DNA extraction was conducted using the Qiagen DNeasy® Blood and Tissue Kit. The extraction protocol as outlined in the manufacturer protocol was followed. The methods used to enrich genomic DNA for microsatellites were adopted from existing fast isolation by AFLP of sequences containing repeats (FIASCO) protocols (Zane et al. 2002; Cortinas et al. 2006), but without cloning the microsatellite-containing DNA. For FIASCO, the following probes were used: $(AC)_{15}$, $(AAC)_{12}$, $(AGC)_{12}$, $(AT)_{16}$ and $(AG)_{16}$. A total of 5 μg of DNA was analyzed on the Roche 454 GS-FLX platform at Inqaba Biotech (Pretoria, Gauteng, South Africa). Sample preparation and analytical processing such as base calling, were performed at Inqaba Biotech using the manufacturer's protocol.

Primers flanking repeat regions were designed using PRIMER 3 software (Rozen and Skaletsky 1997). The level of polymorphisms was evaluated via amplification of the microsatellite sequences in 31 South African blesbok samples. Amplification was carried out using 15 μl reaction volume and PCR was conducted with Promega go *Taq* DNA polymerase®, which has a 1 × buffer containing 10

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**Table 1** Characterization of 10 microsatellite loci in *Damaliscus pygargus phillipsi*

| Name | Fluorescent dye label | Sequence (5′–3′) | Repeat unit | Allele size range (Na) | Ho | He | *P* |
|---|---|---|---|---|---|---|---|
| BB03 | FAM | agccatgtgccaatcatatact<br>ggacacggactgaagctactta | (CTG)17 | 243–267 (4) | 0.4167 | 0.3697 | 0.1498 |
| BB04 | VIC | ataaaggcatgtaccccacatc<br>cagacaggactgaagcgaatta | (CTG)15 | 145–154 (3) | 0.25 | 0.5106 | 0.0169 |
| BB05 | PET | atggacagaggagcctagtgag<br>actgtgcctttcaacactgga | (CA)14 | 136–140 (3) | 0.6667 | 0.6268 | 0.4458 |
| BB08 | VIC | acctccctgtggatgacttct<br>gccatgactgagcaactaaaca | (GT)15 | 172–180 (3) | 0.2917 | 0.5576 | 0.01 |
| BB10 | NED | aatggggacaatgacgtaccta<br>aacaggaaccagatagtgagtgg | (GT)15 | 205–215 (4) | 0.5417 | 0.5248 | 0.9471 |
| BB12 | NED | gcactaagggataaaacctcca<br>tgaactcatctcctcaactaccc | (AC)16 | 156–164 (4) | 0.625 | 0.6002 | 1.000 |
| BB20 | VIC | gctctccaccttatgctcatct<br>aacacatggcctgactctcttt | (CA)10 | 187–191 (3) | 0.5417 | 0.5709 | 0.7537 |
| BB22 | NED | atcgcatctcccattgacttat<br>aagaaaccactgcatttggaag | (CA)12 | 138–140 (2) | 0.5417 | 0.4885 | 0.6863 |
| BM2113 | FAM | gctgccttctaccaaataccc<br>cttcctgagagaagcaacacc | (CA)n | 124–132 (3) | 0.2917 | 0.2899 | 0.1854 |
| BM1824 | FAM | gagcaaggtgtttttccaatc<br>cattctccaactgcttccttg | (CA)n | 199–215 (3) | 0.2083 | 0.3236 | 0.0803 |

*Bp* Base pairs, *n* Number, *Na* Number of alleles, *Ho* Observed heterozygosity, *He* expected heterozygosity and *P* Probability values for exact tests of HW proportions

GenBank accession numbers are HQ896830-37

milli molar (mM) Tris®-HCl (pH 9.0), 50 mM potassium chloride (KCl) and 0.1% Triton® X-100. The final reaction conditions were as follows: 1 × PCR buffer, 1.5–2.5 mM $MgCl_2$, 200 micro molar (μM) of each 2′-deoxynucleotide triphosphate (dNTP), 10 pico mol (pmol) of each of the forward and reverse primer, 1 unit (U) *Taq* DNA polymerase and 50 nano gram (ng) genomic DNA template. The PCR reaction was carried out in the BOECO TC-PRO Thermal Cycler. The conditions for PCR amplification were as follows; 15 min (min) at 95°C denaturation, 30 cycles for 30s (sec) at 95°C, 30s at 50–65°C and 30s at 72°C, followed by extension at 72°C for 20 min. PCR products were pooled together and run against Genescan™ 500 LIZ™ internal size standard on an ABI 3130 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Samples were genotyped using GeneMapper v. 4.0 (Applied Biosystems, Inc., Foster City, CA). The number of alleles per locus, observed heterozygosity (Ho) and expected heterozygosity (He) were calculated with MS toolkit (Park 2001), while GENEPOP (Raymond and Rousset 1995) was used to test for deviation from HW proportions and to evaluate loci for gametic disequilibrium. Ten of the loci were polymorphic and amplified well, with the number of alleles ranging from two to four. Primer sequences, number of alleles, observed and expected heterozygosities are given in Table 1. Mean observed heterozygosity (Ho) ranged from 0.2083 to 0.6667 and expected heterozygosity (He) varied from 0.2899 to 0.6268. There was no significant gametic disequilibrium between genotypes at different loci following Bonferroni correction (95% significance level). In conclusion, the ten microsatellite loci presented here are potentially useful for estimations of genetic diversity, structure and relatedness in *Damaliscus pygargus phillipsi*. The utility of these loci on related species should also be investigated.

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