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Original article

# SNP panel for evaluation of genetic variability and relationship in roe deer (*Capreolus capreolus*)

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## Abstract

Blood samples from forty-six roe deer (*Capreolus capreolus*) acquired during officially approved hunting in six hunting divisions throughout Poland were used to isolate the genomic DNA. All individuals were genotyped by MD\_Bovine BeadChip (Illumina) for 46,750 Single Nucleotide Polymorphism (SNP) markers. SNPs of inappropriate clusters, with a marker call rate lower than 90% and with a minor allele frequency (MAF) lower than 0.01, located on sex chromosomes and mitochondrial DNA, were removed. Altogether, 21,033 SNP markers were included for further analysis. Observed and expected heterozygosity amounted to 0.098 and 0.119, respectively. Among 21,033 markers, a panel of 148 SNPs were selected for relationship analysis. They were unlinked and had a MAF higher than 0.2. This set of SNPs showed a probability of parentage exclusion of  $1.29 \times 10^{-6}$  and  $2.37 \times 10^{-19}$  for one, and two known parents, respectively. The probability of identity was estimated at  $1.8 \times 10^{-40}$ . The probabilities obtained in this study are sufficient for the monitoring and effective management of the genetic diversity of roe deer in Poland and is a cost-effective complementary tool for forensic applications.

**Key words:** *Capreolus capreolus*, genetic diversity, roe deer, Single Nucleotide Polymorphism marker

Table 1. Key PCR conditions for two bovine Single Nucleotide Polymorphisms (SNPs) identified in 3 roe deer individuals.

|               | Bovine SNP<br>BTB-00066611_dup-0_T_F_2329016977                | Bovine SNP<br>ARS-BFGL-NGS-32590-v3-1_T_F_1924280293           |
|---------------|----------------------------------------------------------------|----------------------------------------------------------------|
| SNP type      | A/G                                                            | A/G                                                            |
| PCR primers   | F: 5'CTGTTGGGTAACACTTTTACTTGA3'<br>R: 5'AGCCTCTGGCTTATCGTTGG3' | F: 5'GCCAAGTCAACTATGGAAAACCG3'<br>R: 5'TGCTAGTAAACAGCACCCCAA3' |
| Amplicon size | 90 bp                                                          | 116 bp                                                         |

## Introduction

The European roe deer (*Capreolus capreolus*) is one of the most abundant wild ungulates and an important hunting species that is distributed across the European continent from the Mediterranean to Scandinavia (Apollonio et al. 2010, Plis et al. 2022). Evaluation of genetic diversity, parentage testing and animal identification are essential for the protection and efficient management of animal populations. Moreover, assessing relatedness between individuals is necessary for estimation of effective population size, reduction of the inbreeding level and to minimize mating between close relatives (Werner et al. 2004). Because of their wide availability and high polymorphic information content (PIC) microsatellite markers (STRs) were used for this purpose (Glowatzki-Mullis et al. 1995), but lower mutation and genotyping error rate, automatization of genotyping, easiness of data manipulation and calculation caused panels of single nucleotide polymorphisms (SNPs) displace STRs (Heaton et al. 2002, Werner et al. 2004). For livestock, the International Committee for Animal Recording (ICAR) developed consensus panels of SNPs for routine parentage testing (Fernández et al. 2013). This type of genetic tool for popular wild species is, however, unavailable. To overcome this limitation, SNPs from related species can be used. Following this idea, a bovine SNP array was successfully used to study genetic variability in the bison (Pertoldi et al. 2010), dromedary (Bertoldini et al. 2017) and alpaca (More et al. 2019). Although the roe deer genome has already been sequenced (NCBI GenBank Acc. No.: GCA\_000751575.1), SNP data and commercial microarrays for this species are not available. In this study we attempted to verify the usefulness of bovine SNP markers to establish as SNP panel useful for diversity and forensic applications in roe deer living in Poland.

## Materials and Methods

Blood samples from forty six roe deer (*Capreolus capreolus*) acquired during officially approved hunting

in six hunting divisions throughout Poland were used to isolate the genomic DNA with the use of a Wizard Plus Megapreps DNA Purification System (Promega, Madison, USA). All individuals were genotyped using the MD\_Bovine BeadChip (Illumina) for 46.750 SNP markers. SNPs of inappropriate clusters, with a marker call rate lower than 90% and with a minor allele frequency (MAF) lower than 0.01, deviating from the Hardy-Weinberg equilibrium ( $p < 0.001$ ) and located on sex chromosomes and mitochondrial DNA, were removed. Finally, 21.033 SNP markers were included for further analysis.

SNPs selected for the relationship panel were located only in non-coding sequences, were not linked to each other, and their minor allele frequency (MAF) was at least 0.2. For purposes of sex verification the final set of SNPs was supplemented by one SNP localized on the Y chromosome. For ascertainment purposes, two DNA stretches having bovine SNPs were PCR-amplified (Table 1). After electrophoresis, specific PCR products were cut out from the agarose gel, purified using a Gel-Out kit (A&A Biotechnology, Gdańsk, Poland) and sequenced using an Applied Biosystems sequencer in GENOMED Ltd (Poland). The sequences were analyzed using BioEdit v. 7.2.0 software.

To convert the allelic frequencies into probability of parentage exclusion, standard formulas were used (Jamieson and Taylor 1997). Probability of identity was calculated according to Waits et al. (2001). Calculations were performed using Microsoft Excel.

## Results

Our study enabled the extraction of 21.033 out of 46.750 SNP markers which showed very good quality under relatively strict selection criteria. All SNPs were checked using Illumina GenomeStudio software in a multi-step procedure to ensure the correctness of the genotyping process. The quality of genotyping was verified by direct analysis of the SNP cluster image (Fig. 1). Out of 21.033, we selected 148 SNPs which presented the highest quality and a MAF equal to or higher than 0.20 (Table 2) and we propose these as

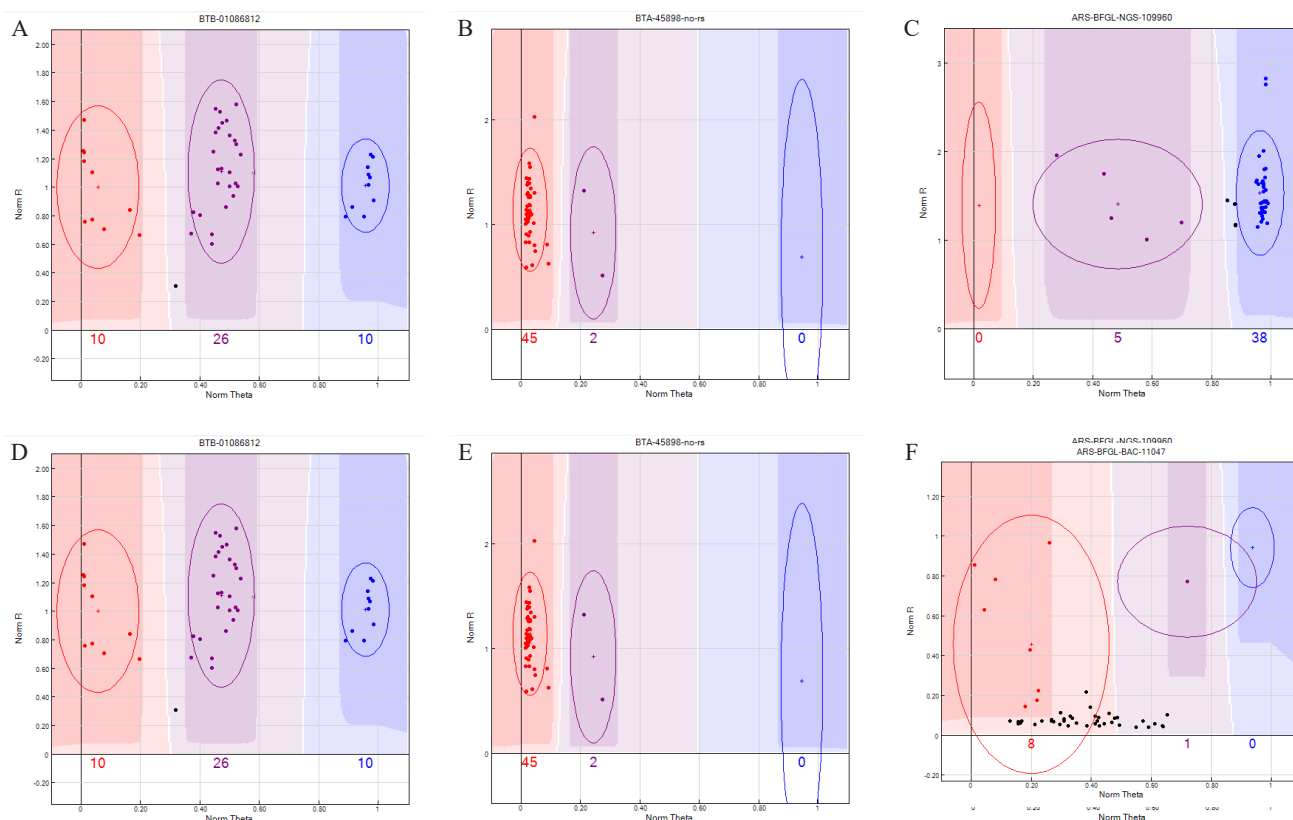


Fig. 1. Single nucleotide polymorphisms (SNPs) from the BovineHD Genotyping BeadChip that cross-amplify in roe deer. Genotypes are called for each sample (dot) by their signal intensity (Norm R, Y-axis) and Allele Frequency (Norm Theta, X-axis) relative to canonical cluster positions (dark shading) for a given SNP marker (red = AA, purple = AB, blue = BB). Dots positioned outside the shaded zone represent no call samples. Polymorphic SNP genotype cluster patterns (A–C) were qualified as positive and D–F as negative and these latter were rejected for further analysis.

a panel of SNPs useful in roe deer genetic analysis. These selected SNPs were checked for perfect alignments between the bovine and roe deer genome (data not shown) for the first 21 or 26 nucleotides flanking the variant nucleotide on either side. Moreover, sequencing of 2 randomly chosen SNP markers showed complete similarity of the sequence neighboring the SNP (Fig. 2).

For 148 selected SNPs, the average MAF was 0.313. The probability of parentage exclusion varied from  $1.29 \times 10^{-6}$  to  $2.37 \times 10^{-19}$  for one and two known parents, respectively. The theoretical probability of identity was calculated as  $1.14 \times 10^{-40}$ . 148 SNPs is sufficient for effective analysis since Weller et al. (2006) showed that a panel of 25 SNP markers provides enough power for identification of a single individual between any of five million individuals with less than 1% chance for a match between any of five million individuals. Panels of 40–100 SNPs with a MAF greater than 0.3 may allow accurate pedigree reconstruction even in situations of thousands of potential family trios with probability at level 1.00 (Baruch and Weller 2008, Fisher et al. 2009).

## Discussion

Cattle and roe deer belong to *Ruminantia* and therefore share the same or similar DNA sequences. Consequently, there is a chance that molecular markers (such as SNPs) located in such sequences can be effective in different species. The key factor in determining such markers is the quality of genotyping. SNP markers applied in Illumina bovine microarray have been used worldwide in routine genotyping of cattle for over 12 years. These markers are very well optimized and equipped in bioinformatic tools assessing the quality of genotyping (McClure et al. 2018). In our study 45% of bovine SNPs were evaluated as effective for reliable genotyping of roe deer. Although 55% of the bovine SNPs were rejected, the remaining SNPs (21,033) are a sufficient number for many applications. The number of polymorphic SNPs depends on the size of the population screened and the threshold of MAF. It is therefore expected that many more SNP markers could be identified if more roe deer were genotyped.

So far, successful use of bovine SNP markers for deer SNP discovery has been described by Haynes and Latch (2012). They used Illumina Bovine SNP50

Table 2. List of bovine 148 SNPs selected for relationship analysis in roe deer.

| SNP No | Marker name                           | BTA | SNP Position | Call Rate | AA | AB | BB | MAF   |
|--------|---------------------------------------|-----|--------------|-----------|----|----|----|-------|
| 1      | POL_BovineHD0100003130                | 1   | 9823609      | 0.95      | 3  | 19 | 20 | 0.298 |
| 2      | BTB-00008090                          | 1   | 24196197     | 0.95      | 20 | 19 | 4  | 0.310 |
| 3      | Hapmap48855-BTA-69221                 | 1   | 33125906     | 0.93      | 24 | 19 | 0  | 0.232 |
| 4      | ARS-BFGL-NGS-39176                    | 1   | 78601007     | 0.95      | 5  | 8  | 30 | 0.202 |
| 5      | BTB-01342372                          | 1   | 91687256     | 0.93      | 9  | 3  | 31 | 0.207 |
| 6      | BTB-01748272                          | 1   | 92171349     | 0.95      | 6  | 15 | 23 | 0.286 |
| 7      | BTB-01086812                          | 1   | 94923462     | 0.91      | 8  | 21 | 13 | 0.450 |
| 8      | BTB-00066611                          | 1   | 142595255    | 0.98      | 16 | 21 | 8  | 0.419 |
| 9      | BTA-46612-no-rs                       | 2   | 20640699     | 0.95      | 6  | 8  | 30 | 0.202 |
| 10     | BTB-01693574                          | 2   | 23061078     | 0.95      | 8  | 15 | 20 | 0.345 |
| 11     | ARS-BFGL-NGS-112255                   | 2   | 31287498     | 1.00      | 5  | 8  | 31 | 0.205 |
| 12     | BTB-00099916                          | 2   | 60179830     | 0.91      | 8  | 5  | 29 | 0.213 |
| 13     | ARS-BFGL-NGS-21252                    | 2   | 78581503     | 0.93      | 6  | 9  | 28 | 0.207 |
| 14     | ARS-BFGL-NGS-41490                    | 2   | 80605588     | 0.95      | 1  | 42 | 1  | 0.488 |
| 15     | ARS-BFGL-NGS-110823                   | 2   | 106465563    | 0.95      | 5  | 9  | 29 | 0.202 |
| 16     | Hapmap30019-BTA-150024                | 2   | 124683244    | 0.95      | 8  | 20 | 15 | 0.417 |
| 17     | POL_BT B-02093457                     | 3   | 5347767      | 0.95      | 20 | 15 | 7  | 0.345 |
| 18     | ARS-BFGL-NGS-105333                   | 3   | 27170513     | 0.91      | 6  | 7  | 29 | 0.200 |
| 19     | ARS-BFGL-NGS-114017                   | 3   | 59112195     | 0.98      | 5  | 14 | 25 | 0.267 |
| 20     | BTB-01629524                          | 3   | 62560627     | 0.91      | 6  | 7  | 29 | 0.200 |
| 21     | ARS-BFGL-NGS-35462                    | 3   | 116968644    | 0.91      | 5  | 21 | 16 | 0.350 |
| 22     | ARS-BFGL-NGS-27579                    | 3   | 117712313    | 0.93      | 27 | 7  | 8  | 0.268 |
| 23     | ARS-BFGL-NGS-19387                    | 3   | 118321289    | 0.98      | 1  | 22 | 22 | 0.256 |
| 24     | POL_BT B-01966650                     | 4   | 7051825      | 0.93      | 0  | 40 | 3  | 0.476 |
| 25     | BTB-01238546                          | 4   | 19132537     | 0.95      | 11 | 18 | 14 | 0.464 |
| 26     | ARS-BFGL-NGS-40177                    | 4   | 20756074     | 0.95      | 5  | 11 | 27 | 0.226 |
| 27     | Hapmap43331-BTA-70083                 | 4   | 39545464     | 0.91      | 3  | 15 | 24 | 0.238 |
| 28     | Hapmap50762-BTA-70080                 | 4   | 39634823     | 0.91      | 6  | 9  | 27 | 0.225 |
| 29     | ARS-BFGL-NGS-93211                    | 4   | 96100801     | 0.93      | 2  | 20 | 20 | 0.280 |
| 30     | ARS-BFGL-NGS-2354                     | 4   | 99691481     | 0.98      | 18 | 20 | 7  | 0.384 |
| 31     | EuroG10K_COL2A1_5_32476082_F_ilmndup1 | 5   | 32476082     | 0.91      | 8  | 3  | 30 | 0.213 |
| 32     | ARS-BFGL-NGS-53488                    | 5   | 38106808     | 0.93      | 10 | 22 | 10 | 0.488 |
| 33     | Hapmap38299-BTA-26131                 | 5   | 41601867     | 0.98      | 3  | 41 | 0  | 0.465 |
| 34     | EuroG10K_ARS-BFGL-NGS-20849_ilmndup1  | 5   | 65743920     | 0.93      | 5  | 15 | 23 | 0.268 |
| 35     | EuroG10K_BTA-98453-no-rs_ilmndup2     | 5   | 88436433     | 0.93      | 4  | 14 | 24 | 0.244 |
| 36     | ARS-BFGL-NGS-100195                   | 5   | 115561004    | 0.93      | 8  | 4  | 31 | 0.207 |
| 37     | BTB-01788119                          | 6   | 1603850      | 1.00      | 10 | 24 | 12 | 0.477 |
| 38     | ARS-BFGL-NGS-72188                    | 6   | 41831446     | 0.98      | 22 | 17 | 5  | 0.314 |
| 39     | ARS-USMARC-Parent-DQ789028-rs29017713 | 6   | 46936182     | 0.95      | 3  | 20 | 21 | 0.286 |
| 40     | ARS-BFGL-NGS-38827                    | 6   | 71476002     | 0.98      | 17 | 16 | 12 | 0.442 |
| 41     | Hapmap60182-rs29025531                | 6   | 74606760     | 0.93      | 21 | 18 | 4  | 0.293 |
| 42     | ARS-BFGL-NGS-28041                    | 6   | 89251522     | 0.95      | 14 | 26 | 3  | 0.369 |

cont. Table 2.

| SNP No | Marker name                            | BTA | SNP Position | Call Rate | AA | AB | BB | MAF   |
|--------|----------------------------------------|-----|--------------|-----------|----|----|----|-------|
| 43     | BTB-01791461                           | 6   | 100546567    | 0.93      | 19 | 14 | 9  | 0.378 |
| 44     | Hapmap26276-BTC-043686                 | 6   | 103281884    | 1.00      | 8  | 24 | 14 | 0.432 |
| 45     | ARS-BFGL-NGS-106770                    | 6   | 108476017    | 0.95      | 3  | 23 | 18 | 0.321 |
| 46     | ARS-BFGL-NGS-44466                     | 6   | 110305659    | 0.98      | 2  | 42 | 0  | 0.477 |
| 47     | BTB-01414346                           | 7   | 37908535     | 0.91      | 9  | 16 | 15 | 0.425 |
| 48     | ARS-BFGL-NGS-39972                     | 7   | 67011046     | 0.98      | 24 | 15 | 5  | 0.291 |
| 49     | BTB-01771182                           | 7   | 88771142     | 0.93      | 11 | 23 | 9  | 0.476 |
| 50     | ARS-BFGL-NGS-15306                     | 8   | 7977499      | 0.98      | 4  | 40 | 0  | 0.453 |
| 51     | BTB-01332332                           | 8   | 33964937     | 0.93      | 5  | 8  | 29 | 0.207 |
| 52     | ARS-BFGL-NGS-107099                    | 8   | 45130290     | 1.00      | 11 | 16 | 17 | 0.432 |
| 53     | Hapmap30618-BTA-38298                  | 8   | 53382829     | 0.93      | 4  | 21 | 18 | 0.329 |
| 54     | ARS-BFGL-NGS-5591                      | 8   | 81220910     | 1.00      | 0  | 5  | 36 | 0.443 |
| 55     | ARS-BFGL-NGS-103223                    | 8   | 103278121    | 0.98      | 5  | 18 | 22 | 0.302 |
| 56     | BovineHD0900002673                     | 9   | 10739968     | 0.91      | 5  | 8  | 28 | 0.200 |
| 57     | BTB-01716044                           | 9   | 24875114     | 0.93      | 3  | 16 | 23 | 0.256 |
| 58     | ARS-BFGL-NGS-10254                     | 9   | 40256749     | 0.91      | 19 | 18 | 4  | 0.300 |
| 59     | ARS-BFGL-NGS-20794                     | 9   | 76776811     | 0.91      | 8  | 4  | 30 | 0.200 |
| 60     | ARS-BFGL-NGS-105322                    | 9   | 85565030     | 0.93      | 22 | 21 | 0  | 0.232 |
| 61     | ARS-BFGL-NGS-28183                     | 9   | 88892407     | 0.93      | 2  | 26 | 15 | 0.354 |
| 62     | EuroG10K_BovineHD0900026397            | 9   | 93289957     | 0.93      | 12 | 24 | 5  | 0.415 |
| 63     | Hapmap42705-BTA-85041                  | 9   | 99135245     | 0.93      | 17 | 21 | 3  | 0.329 |
| 64     | EuroG10K_BTA-07939-rs29027600_ilmndup2 | 10  | 1783418      | 0.98      | 10 | 5  | 30 | 0.267 |
| 65     | ARS-BFGL-NGS-55845                     | 10  | 9821501      | 0.93      | 6  | 16 | 21 | 0.317 |
| 66     | ARS-BFGL-NGS-44623                     | 10  | 20667710     | 0.98      | 2  | 15 | 28 | 0.209 |
| 67     | BTB-00417053                           | 10  | 31393400     | 0.95      | 19 | 20 | 5  | 0.333 |
| 68     | ARS-BFGL-NGS-22113                     | 10  | 73551579     | 0.98      | 1  | 19 | 25 | 0.221 |
| 69     | ARS-BFGL-NGS-83517                     | 10  | 100943686    | 0.91      | 2  | 12 | 26 | 0.200 |
| 70     | ARS-BFGL-NGS-62299                     | 11  | 9153560      | 0.93      | 9  | 15 | 19 | 0.378 |
| 71     | ARS-BFGL-NGS-21642                     | 11  | 12596662     | 0.98      | 16 | 20 | 8  | 0.407 |
| 72     | ARS-BFGL-NGS-20828                     | 11  | 25418833     | 0.98      | 25 | 17 | 2  | 0.233 |
| 73     | Hapmap42357-BTA-89853                  | 11  | 25736918     | 0.98      | 12 | 21 | 12 | 0.500 |
| 74     | BTA-99098-no-rs                        | 11  | 60837975     | 0.98      | 8  | 22 | 14 | 0.430 |
| 75     | Hapmap53195-rs29011519                 | 11  | 70363411     | 0.95      | 11 | 19 | 14 | 0.476 |
| 76     | ARS-BFGL-NGS-19701                     | 11  | 89843860     | 0.95      | 5  | 10 | 29 | 0.214 |
| 77     | ARS-BFGL-NGS-114332                    | 11  | 92155851     | 0.91      | 4  | 29 | 8  | 0.450 |
| 78     | ARS-BFGL-NGS-34469                     | 11  | 103821456    | 0.93      | 4  | 11 | 28 | 0.207 |
| 79     | EuroG10K_ARS-BFGL-BAC-15732_ilmndup1   | 13  | 50950127     | 0.95      | 12 | 20 | 12 | 0.488 |
| 80     | ARS-BFGL-BAC-20217                     | 14  | 9812155      | 0.95      | 7  | 15 | 22 | 0.310 |
| 81     | BTB-00553641                           | 14  | 18263091     | 0.93      | 18 | 4  | 20 | 0.463 |
| 82     | UA-IFASA-5403                          | 14  | 43927711     | 0.91      | 6  | 7  | 29 | 0.200 |
| 83     | ARS-BFGL-BAC-22864                     | 14  | 63510240     | 0.98      | 19 | 20 | 5  | 0.349 |
| 84     | ARS-BFGL-NGS-2939                      | 14  | 68378101     | 0.93      | 7  | 6  | 30 | 0.207 |

cont. Table 2.

| SNP No | Marker name                           | BTA | SNP Position | Call Rate | AA | AB | BB | MAF   |
|--------|---------------------------------------|-----|--------------|-----------|----|----|----|-------|
| 85     | ARS-BFGL-NGS-27908                    | 14  | 72490329     | 1.00      | 26 | 12 | 8  | 0.318 |
| 86     | Hapmap49972-BTA-36466                 | 15  | 34946066     | 0.95      | 5  | 26 | 13 | 0.405 |
| 87     | BTA-36844-no-rs                       | 15  | 42807152     | 0.93      | 18 | 17 | 7  | 0.366 |
| 88     | ARS-BFGL-NGS-97259                    | 16  | 1646839      | 0.93      | 5  | 17 | 21 | 0.293 |
| 89     | BovineHD1600007210                    | 16  | 25940046     | 1.00      | 5  | 15 | 25 | 0.273 |
| 90     | ARS-BFGL-NGS-37274                    | 16  | 36114564     | 0.91      | 9  | 3  | 30 | 0.213 |
| 91     | Hapmap25615-BTA-160036                | 16  | 63549240     | 0.91      | 2  | 22 | 18 | 0.300 |
| 92     | ARS-BFGL-NGS-117128                   | 16  | 66313340     | 0.95      | 14 | 26 | 4  | 0.381 |
| 93     | EuroG10K_ARS-BFGL-NGS-89998_ilmndup2  | 16  | 68949196     | 0.91      | 5  | 12 | 24 | 0.250 |
| 94     | ARS-BFGL-NGS-17566                    | 16  | 76781132     | 0.93      | 3  | 14 | 25 | 0.232 |
| 95     | ARS-BFGL-NGS-95736                    | 17  | 8098794      | 0.98      | 27 | 16 | 25 | 0.221 |
| 96     | Hapmap42132-BTA-21144                 | 17  | 62193144     | 0.98      | 3  | 41 | 0  | 0.465 |
| 97     | ARS-BFGL-NGS-89598                    | 18  | 5050930      | 0.95      | 7  | 24 | 14 | 0.429 |
| 98     | Hapmap39919-BTA-42561                 | 18  | 16991322     | 0.91      | 25 | 17 | 0  | 0.200 |
| 99     | ARS-BFGL-NGS-19431                    | 18  | 28487717     | 0.93      | 1  | 42 | 0  | 0.488 |
| 100    | ARS-BFGL-NGS-113568                   | 18  | 35971459     | 0.93      | 4  | 13 | 26 | 0.220 |
| 101    | ARS-BFGL-NGS-103183                   | 18  | 48217729     | 0.91      | 5  | 6  | 29 | 0.200 |
| 102    | ARS-BFGL-NGS-110490                   | 18  | 62375495     | 0.98      | 23 | 17 | 4  | 0.291 |
| 103    | ARS-BFGL-NGS-111654                   | 19  | 5172239      | 0.93      | 2  | 20 | 20 | 0.280 |
| 104    | ARS-BFGL-NGS-14867                    | 19  | 7940557      | 0.93      | 4  | 12 | 26 | 0.232 |
| 105    | ARS-BFGL-NGS-107729                   | 19  | 15863515     | 0.93      | 5  | 19 | 19 | 0.329 |
| 106    | ARS-BFGL-NGS-44106                    | 19  | 26264145     | 0.91      | 18 | 20 | 4  | 0.338 |
| 107    | ARS-USMARC-Parent-DQ888312-rs29015945 | 19  | 36437188     | 0.91      | 8  | 19 | 15 | 0.400 |
| 108    | ARS-BFGL-NGS-20183                    | 19  | 38365974     | 0.91      | 3  | 28 | 11 | 0.400 |
| 109    | EuroG10K_ARS-BFGL-NGS-91993_ilmndup1  | 19  | 39126656     | 0.93      | 6  | 17 | 19 | 0.329 |
| 110    | ARS-BFGL-NGS-112994                   | 20  | 11932262     | 0.91      | 21 | 17 | 4  | 0.288 |
| 111    | ARS-BFGL-NGS-112210                   | 21  | 4193189      | 0.95      | 1  | 21 | 21 | 0.262 |
| 112    | ARS-BFGL-NGS-108136                   | 21  | 11303069     | 1.00      | 23 | 18 | 4  | 0.295 |
| 113    | BTB-01303761                          | 21  | 37293354     | 0.91      | 3  | 16 | 22 | 0.263 |
| 114    | BTB-00649119                          | 21  | 53433865     | 0.91      | 1  | 39 | 0  | 0.488 |
| 115    | POL_Hapmap43466-BTA-112022            | 21  | 57058513     | 0.91      | 4  | 20 | 18 | 0.325 |
| 116    | ARS-BFGL-NGS-27139                    | 21  | 58222356     | 0.95      | 27 | 14 | 2  | 0.202 |
| 117    | ARS-BFGL-NGS-95953                    | 21  | 60475531     | 0.91      | 9  | 6  | 27 | 0.250 |
| 118    | ARS-BFGL-NGS-40713                    | 21  | 62223140     | 0.91      | 3  | 15 | 24 | 0.238 |
| 119    | ARS-BFGL-NGS-99716                    | 21  | 63560239     | 0.91      | 11 | 13 | 18 | 0.400 |
| 120    | ARS-BFGL-NGS-17666                    | 22  | 632381       | 0.93      | 17 | 19 | 6  | 0.378 |
| 121    | BTB-00833550                          | 22  | 1732412      | 0.93      | 3  | 16 | 22 | 0.268 |
| 122    | Hapmap54461-rs29016490                | 22  | 10228378     | 0.95      | 3  | 41 | 0  | 0.464 |
| 123    | Hapmap35135-BES10_Contig779_1471      | 22  | 23765329     | 0.98      | 9  | 20 | 15 | 0.430 |
| 124    | ARS-BFGL-NGS-35220                    | 23  | 11438802     | 0.91      | 3  | 15 | 24 | 0.225 |
| 125    | UA-IFASA-1564                         | 23  | 19256555     | 0.91      | 3  | 12 | 26 | 0.213 |
| 126    | ARS-BFGL-NGS-32590                    | 24  | 8740300      | 1.00      | 14 | 15 | 17 | 0.443 |



cont. Table 2.

| SNP No | Marker name                     | BTA | SNP Position | Call Rate | AA | AB | BB | MAF   |
|--------|---------------------------------|-----|--------------|-----------|----|----|----|-------|
| 127    | ARS-BFGL-NGS-14770              | 24  | 12034037     | 0.95      | 4  | 15 | 25 | 0.238 |
| 128    | BTA-70057-no-rs                 | 24  | 24327708     | 0.91      | 15 | 22 | 5  | 0.388 |
| 129    | Hapmap43742-BTA-100784          | 24  | 39694262     | 0.93      | 19 | 21 | 1  | 0.280 |
| 130    | ARS-BFGL-NGS-13322              | 24  | 46586673     | 0.98      | 23 | 21 | 1  | 0.244 |
| 131    | ARS-BFGL-NGS-110003             | 24  | 48713379     | 0.95      | 18 | 22 | 4  | 0.345 |
| 132    | Hapmap49390-BTA-96326           | 24  | 60108474     | 1.00      | 25 | 17 | 3  | 0.250 |
| 133    | ARS-BFGL-NGS-103861             | 24  | 60613517     | 0.91      | 6  | 10 | 25 | 0.250 |
| 134    | ARS-BFGL-NGS-116067             | 25  | 2180562      | 0.95      | 4  | 16 | 23 | 0.274 |
| 135    | ARS-BFGL-NGS-91974              | 25  | 4249133      | 0.95      | 6  | 9  | 28 | 0.226 |
| 136    | Hapmap31994-BTC-065943          | 25  | 5385729      | 0.93      | 2  | 16 | 25 | 0.220 |
| 137    | BTA-60652-no-rs                 | 25  | 8272291      | 0.93      | 8  | 5  | 30 | 0.207 |
| 138    | ARS-BFGL-NGS-24214              | 25  | 22045818     | 0.95      | 6  | 20 | 17 | 0.369 |
| 139    | POL_IZ-PIB-KZ-rs443460785       | 25  | 26315484     | 0.95      | 3  | 12 | 28 | 0.202 |
| 140    | POL_IZ-PIB-KZ-rs468469008       | 25  | 26317522     | 0.95      | 0  | 35 | 9  | 0.417 |
| 141    | ARS-BFGL-NGS-44485              | 25  | 33852697     | 0.93      | 8  | 6  | 29 | 0.220 |
| 142    | ARS-BFGL-BAC-38364              | 25  | 34256781     | 0.95      | 25 | 14 | 4  | 0.250 |
| 143    | ARS-BFGL-NGS-119488             | 26  | 29929536     | 0.91      | 3  | 21 | 17 | 0.325 |
| 144    | ARS-BFGL-NGS-112434             | 27  | 37767563     | 1.00      | 21 | 18 | 6  | 0.330 |
| 145    | BTB-00874839                    | 28  | 6547497      | 0.98      | 25 | 16 | 3  | 0.244 |
| 146    | ARS-BFGL-NGS-116671             | 28  | 43576806     | 0.93      | 3  | 13 | 27 | 0.207 |
| 147    | Hapmap35197-BES11_Contig441_882 | 29  | 2067440      | 0.95      | 0  | 18 | 25 | 0.202 |
| 148    | ARS-BFGL-NGS-32916              | 29  | 4533981      | 0.93      | 18 | 21 | 3  | 0.317 |

BTA – Bos taurus autosome, MAF – Minor Allele Frequency

BeadChip for identifying polymorphic SNPs in cervids *Odocoileus hemionus* (mule deer and black-tailed deer) and *O. virginianus* (white-tailed deer). They found that 38.7% of loci could be genotyped, of which 5% (n=1068) were polymorphic. A range of population genetic analyses have been implemented using these SNPs and a panel of 10 microsatellite loci. The three types of deer could readily be distinguished with both the SNP and microsatellite datasets. Also, Miller et al. (2011) identified 868 SNPs in bighorn (*Ovis canadensis*) and thinhorn sheep (*Ovis dalli*) using the Ovine SNP50 BeadChip developed for domestic sheep (*Ovis aries*). Similarly, Pertoldi et al. (2010) used the Bovine SNP50 BeadChip developed for cattle (*Bos taurus*) to genotype 2 209 polymorphic loci in European (*Bison bonasus*) and American bison (*B. bison bison* and *B. bison athabasca*). A further example is the study of Bertolini et al. (2017) in which the Bovine 777K SNP BeadChip and the Ovine 600K SNP BeadChip were used to extract 27.673 SNPs effective in the genotyping of the dromedary. Another example is the study published by More et al. (2019) in which alpaca SNPs using

the Bovine HD Genotyping BeadChip were discovered. These studies demonstrate that commercially developed SNP chips are a viable means of SNP discovery for non-model organisms, even when used between distantly related species.

Outcomes of this study can be applied to many fields of roe deer biology since this species is an excellent model for evolutionary studies, biodiversity and social organization (Bartos and Bubenik 2011). In combination with interspecies cytogenetic studies, SNPs markers can also be used for the mapping of genes involved in the variation of unique organ development (i.e. fully regenerable antlers) (Li et al. 2009). Moreover, SNPs might be applied in mapping genes involved in meat quality, especially in low fat content and specific taste, which is thought to have health effects, and in developing traceability tests used in meat adulteration (Kaltenbrunner et al. 2018). Validated SNPs can also be applied in the identification of a link between evidence collected at a potential illegal hunting site and biological material (e.g. antlers, meat, trace blood samples) associated with the suspect who might have committed

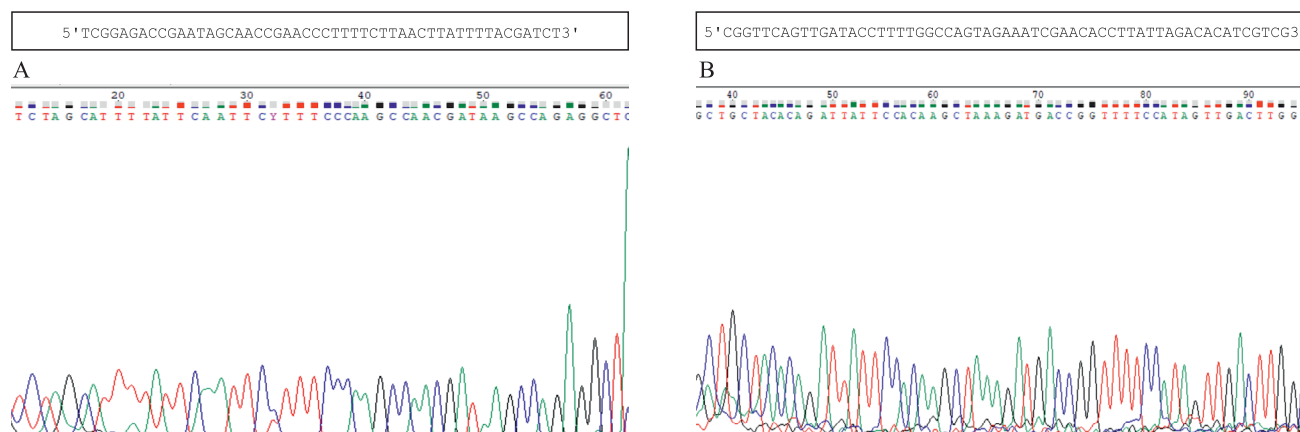


Fig. 2. Identity of two fragments of sequences amplified from roe deer DNA using primers designed in bovine sequences containing SNPs present in Bovine BeadChip. Bovine reference sequences are presented in the upper frames. Fluorograms obtained from sequencing of roe deer samples are oriented from the 3' to 5' direction. Sequences read in the same direction (5' to 3') show complete identity between roe deer and cattle. A. Sequence Illumina SNP BTB-00066611\_dup-0\_T\_F\_2329016977. B. Illumina SNP ARS-BFGL-NGS-32590-v3-1\_T\_F\_1924280293.

the offence (Poetsch et al. 2001). Although, for forensic purposes, a validated DNA profiling system “STRoe deer” for European roe deer consisting of 12 unlinked STR and two sexing markers has been recently developed (Morf et al. 2021), the SNP panel described in our study can be used as the complementary tool, especially in labs which have no facility for STR genotyping. Moreover, the overall cost of genotyping an animal by STR is much higher than by SNP (<https://lgm.izoo.krakow.pl>). Therefore, DNA profiling of roe deer by bovine SNP microarray seems to be an attractive new technique for research and forensic applications in this wild species.

## Conclusion

Roe deer can be genotyped using the Bovine Illumina SNPs microarray at an acceptable call success rate and cost. After fine filtering and quality control, SNP genotypes are reliable for further statistical analysis. The probabilities obtained in this study are sufficient for monitoring and effective management of the genetic diversity, relationship and forensic applications of roe deer and also for designing genome associated studies (GWAS).

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