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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 1.29×10^{-6} and 2.37×10^{-19} for one, and two known parents, respectively. The probability of identity was estimated at 1.8×10^{-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

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

	Bovine SNP BTB-00066611_dup-0_T_F_2329016977	Bovine SNP ARS-BFGL-NGS-32590-v3-1_T_F_1924280293
SNP type	A/G	A/G
PCR primers	F:5'CTGTTGGGTAACACTTTTTACTTGA3' R: 5'AGCCTCTGGCTTATCGTTGG3'	F: 5'GCCAAGTCAACTATGGAAAACCG3' 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

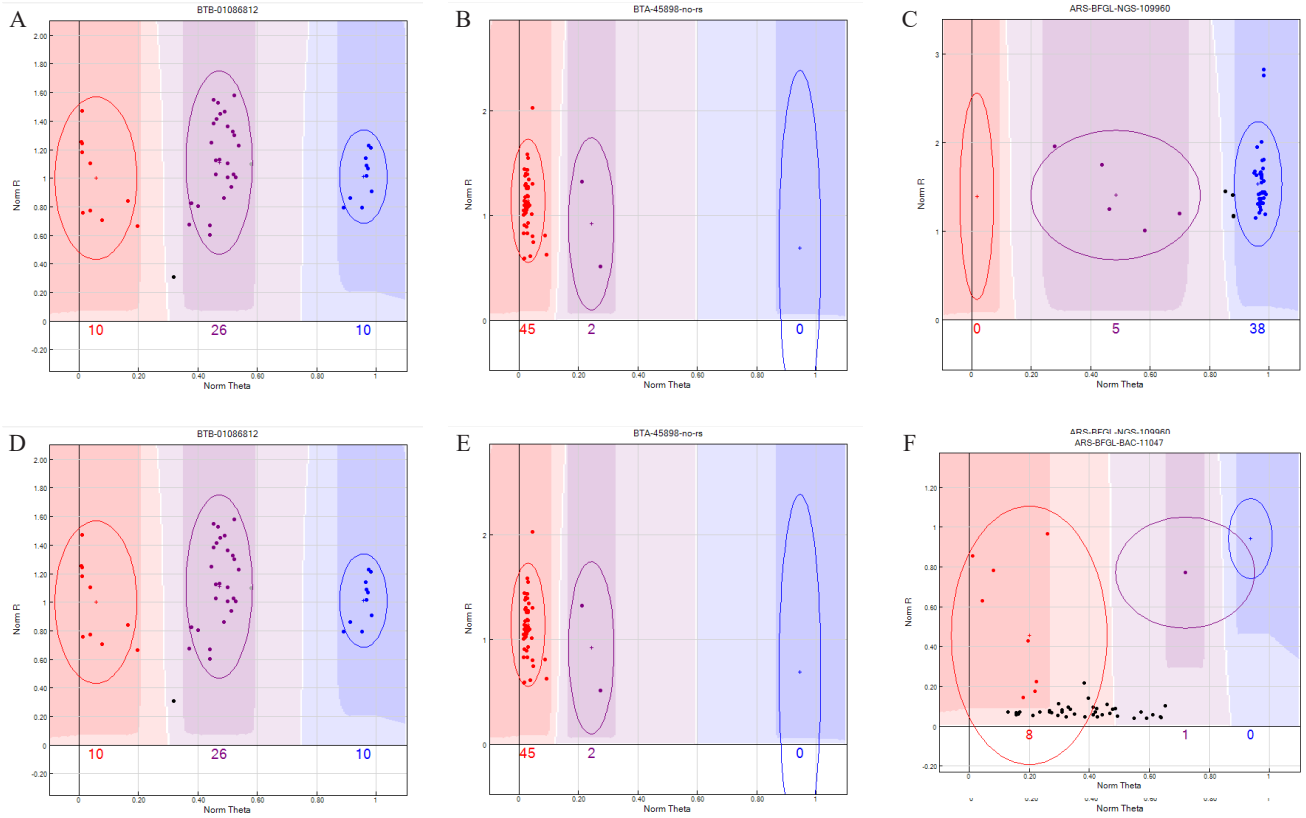

Fig. 1. Single nucleotide polymorphisms (SNPs) from the BovineHD Genotyping BeadChip that cross-amplify in roe deer. Genotypes are called for each sample (dot) by their signal intensity (Norm R, Y-axis) and Allele Frequency (Norm Theta, X-axis) relative to canonical cluster positions (dark shading) for 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 1.29×10^{-6} to 2.37×10^{-19} for one and two known parents, respectively. The theoretical probability of identity was calculated as 1.14×10^{-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_BTB-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_BTB-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 athabascae*). 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

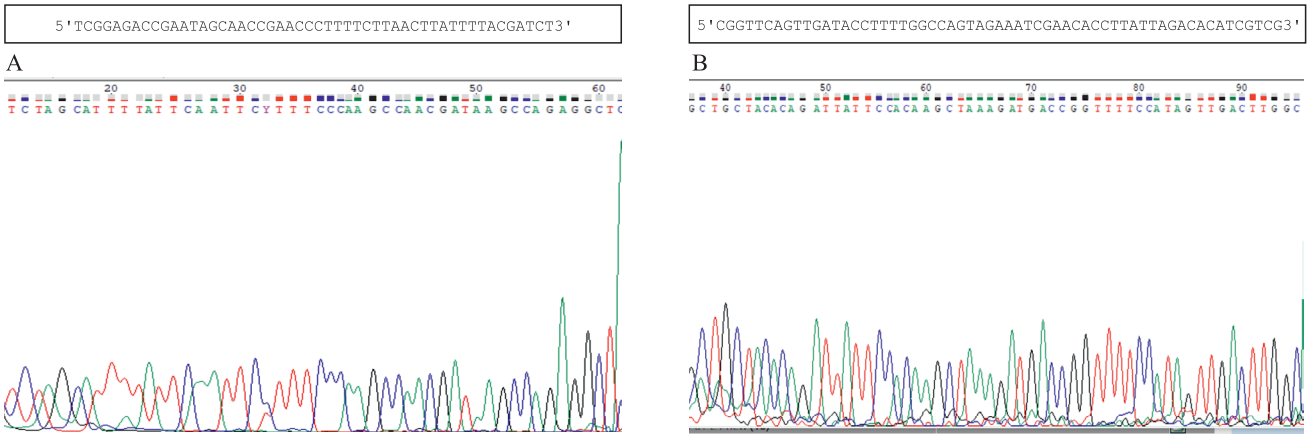

Fig. 2. Identity of two fragments of sequences amplified from roe deer DNA using primers designed in bovine sequences containing SNPs present in Bovine BeadChip. Bovine reference sequences are presented in the upper frames. Fluorograms obtained from sequencing of roe deer samples are oriented from the 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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