Single Nucleotide Polymorphisms in *Bison bison* Identified by the GGP Bovine 50K SNP Assay

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Abstract
The vulnerable populations of bison had gone through a drastic reduction in population size, have undergone a very high level of inbreeding and have been through severe bottlenecks. Using a panel of Single Nucleotide Polymorphisms (GGP Bovine 50K SNP arrays, Neogen) developed across the entire bovine (*Bos taurus*) genome, we have carried out a genome variability screening on a bison (*Bison bison*) population in Romania. Eight males were included in the analysis. As part of SNP quality control filtering, one individual with a call rate below 80% was removed from the study. From a total of 47,843 SNPs only 4474 were polymorphic (9.35% from the total) and 7 individuals (out of a total of 8) were left after PLINK's quality control filtering. The total call rate of genotyped samples was 90.11% for the filtered dataset. A secondary PLINK run was performed on the 4474 filtered SNPs to find the ones whose HWE *p*-value fell below 0.05 and 100 markers were highlighted in this way. The results showed a larger number of polymorphic SNPs compared with previous studies from the literature. In addition, the data obtained using the GGP Bovine 50K SNP arrays may facilitate the design of breeding strategies that can be applied for decreasing unwanted inbreeding effects in the vulnerable bison populations.

Keywords: GGP Bovine 50K, *Bison bison*, single-nucleotide polymorphism.

1. Introduction

Currently, there are two living species of bison: European - *Bison bonasus* and American - *Bison bison*, which have survived the late Pleistocene extinctions [1].
The *Bison bison* species has undergone major population bottlenecks and was nearly driven to extinction in the late 1800's [2, 3]. Before the 1800's, the bison population in North America was around 75 million [4], then, between 1870 and 1883, hunters slaughtered millions of bison such that the bison population declined from as many as 30 million in the mid-eighteenth century to only a few hundred by the early twentieth century [4].

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During the late Pliocene and early Pleistocene, bison were widely spread also in Asia and Europe and later, by the end of the 19th century, it was close to extinction, with only two wild populations remaining [5].
After World War I, when the species was extinct in the wild, the captive population consisted of only 54 European bison [6, 7]. *Bison bonasus* was driven to extinction across Europe in 1927 as a consequence of overhunting, climate changes and habitat loss [8]. Afterwards until World War II, the number increased up to 160 animals in 1943 [6, 7]. As a result of captive breeding and intensive conservation management, the total population of free-ranging bison increased up to 1,800 animals and probably around other 1,400 individuals that live in captivity [7].
European bison were first listed as an endangered species in the so-called Red Data Book, compiled

in 1966 by the International Union for the Conservation of Nature and Natural Resources (IUCN). Currently, the European bison (or wisent, Rom. *zimbru*) is treated as a vulnerable species by the IUCN Red List of Threatened Species. The extant free-ranging populations of European bison listed in the European Bison Pedigree Book of 2010 are in Poland, Belarus, Russia, Ukraine, Lithuania, Romania, Slovakia, France and Germany [9].

The Bovine 50K SNP array has been used widely in many studies to improve selection in cattle breeding programs in other countries. However, to our knowledge, no previous study has reported bison genomic evaluation in Romania using the high throughput technology. This study aimed to identify SNPs in a bison (*Bison bison*) population in Romania and to carry out a genome variability screening using GGP Bovine 50K SNP arrays from Neogen.

2. Materials and methods

Animals and DNA isolation

Bison bison samples from this study were taken from commercial farms and were provided by Oprea I.A. from the Bioengineering Faculty of Animal Resources from Timisoara, who obtained the authorization in this regard. Sampling was performed specifically for this study. A total of eight bison (*Bison bison*) were included in the study. Additionally, control samples representing 44 cattle (*Bos taurus*, Romanian Spotted, national name Bălțată Românească) were included in the investigation. The Romanian Spotted cattle samples were provided by the Research and Development Station for Bovine from Arad. The cattle involved in the study were between 1st and 8th lactation, representing a diverse sampling of genetic lines (Austrian, German, and Romanian).

Hair follicles were sampled from cattle and male individuals of bison. The bison were selected randomly and the cattle following the criteria to be unrelated individuals. After collection, all samples were transferred to the laboratory and were kept at 4ºC until the further laboratory process. Genomic DNA was extracted from the hair follicles.

Bovine 50K SNP

One commercially available SNP microarray chip, GeneSeek Genomic Profiler (GGP) Bovine 50K (Neogen Corporation, Lincoln, NE), was used for genotyping. The GGP Bovine 50K comprises a large percentage of single nucleotide polymorphisms (SNPs) overlapping with other commercially available arrays, including the original Illumina Bovine SNP50k. The GGP Bovine 50K includes 47,843 SNPs and contains all the content from popular lower density commercial arrays and the most informative SNPs from the original Illumina Bovine SNP50 and Illumina Bovine HD Bead Chips (more than 44,000 SNPs overlap with the Illumina Bovine HD array). The BeadChip technology includes SNPs specifically chosen for high minor allele frequency (MAF) values, with an average between 0.2826 and 0.3598 across all loci in different cattle breeds and uniform genome coverage for a majority of the *Bos taurus* and several of the *Bos indicus* breeds. The average call rate that refers to the number of useable SNPs is above 99%.

Bovine Reference Genome (UMD3.1)

The Bovine Genome Sequencing and Analysis Consortium [10] has led to genome sequencing and its assembly for cattle. The bovine reference genome build for the GGP Bovine 50K was UMD3.1 (bosTau6) [11]. The UMD3.1 assembly created by the Center for Bioinformatics and Computational Biology at the University of Maryland was released in 2009 and assembled 36.82 million reads into a 2.649 billion bp genome out of which 2.640 billion (99%) bp were placed on chromosomes. As the microarray's SNP map locations are based on the UMD3.1 genome assembly, we downloaded the corresponding gene information from Ensembl, including thegene ID, symbol, start position, stop position, orientation on the chromosome and gene description.

Data analysis

Data analysis was performed on results from two populations (*Bison bison* and one Bos taurus breed). Within each population, animals were genotyped with the GGP Bovine 50K array (50K SNPs). The quality control filtering of SNPs to exclude low-quality markers was applied based on variables [e.g. call rate per marker and per individual, Hardy–Weinberg equilibrium and minor allele frequency (MAF, threshold of 0.05, removing markers with MAF below 5%)] to remove SNPs with insufficient genotyping quality.

Data quality control of SNPs was based on the summary statistics and relatedness estimation functions of the PLINK software v1.90b6.6 64-bit (10 Oct 2018) [12], which was used to generate a per-individual and per-marker quality control report. Individuals and markers that failed the quality control ware subsequently removed to generate a new, cleaned dataset. The R software, version 3.5.1, was used for primary analysis of SNP array data; the R package argyle v0.2.2 [13] was used for converting SNP array data into PLINK's file format and the package BSgenome.Btaurus.UCSC.bosTau8 [14] v1.4.2 was used for obtaining the Bos Taurus UMD3.1.1 genome data, which is compatible with the UMD3.1 / bosTau6 genome. PLINK was also used to estimate basic population genetic descriptive statistics: the observed heterozygosity (H_O) and expected heterozygosity (H_E). The R package GenomicRanges v1.34.0 [15] was used for processing SNP loci and preparing data for plotting chromosome ideograms and karyoploteR v1.8.8 [16] was used for generating the chromosome ideograms themselves.

3. Results and discussion

Eight male individuals of bison (*Bison bison*) were used for genome variability screening. Genome screening encompassed 47,843 SNPs across the entire bovine genome. The analyzed control samples constituted of *Bos taurus* performed well and showed call rates higher than 95%, which ensures that the performance of the analysis was optimal. The GenCall score (GC), which gives an indication of the accuracy of individual genotyping [17], had an average of 0.7441 for bison and 0.7718 for cattle. Scores above 0.7 usually report well-behaving genotypes [18]. The overall parameters for the 47,843 SNPs indicated the success of genotyping in this study.

SNP quality control was performed and the SNPs were removed on the basis of the following criteria: monomorphic SNPs, SNPs with call rate below 80%, SNPs with minor allele frequency smaller than 0.05, animals with genotype completeness smaller than 80% were eliminated and SNPs with significant departure from H–W equilibrium ($p < 0.05$) were also excluded. As part of SNP quality control filtering, one bison individual with a call rate below 80% was removed from the study and 7 individuals (out of a total of 8) were left after PLINK's quality control filtering. The call rate threshold of 80% was used based on the previous study [19] where different filtering methods were applied for identification of polymorphic SNPs and the filters of no call threshold ≥ 0.25, call frequency = 1 and average GC ≥ 0.7 were used for detection of SNPs in non-model species.

Despite the fact that the *Bos* and *Bison* lineages split about 1 million years ago [20, 21], a total of 4,474 polymorphic SNPs (9.35% from the total) were found in bison compared with 41,933 polymorphic SNPs in cattle. The number of polymorphic SNPs on each chromosome in bison and cattle is shown in Figure 1 and the distribution of the polymorphic SNPs in *Bison bison* along the bovine chromosomes is shown in Figure 2.

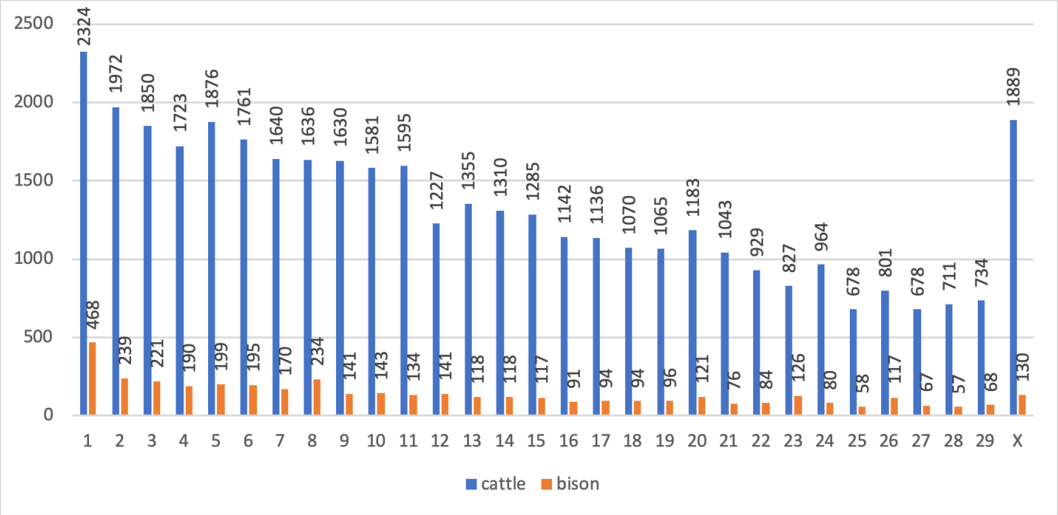

Figure 1. The number of polymorphic SNPs on each chromosome in bison and cattle.

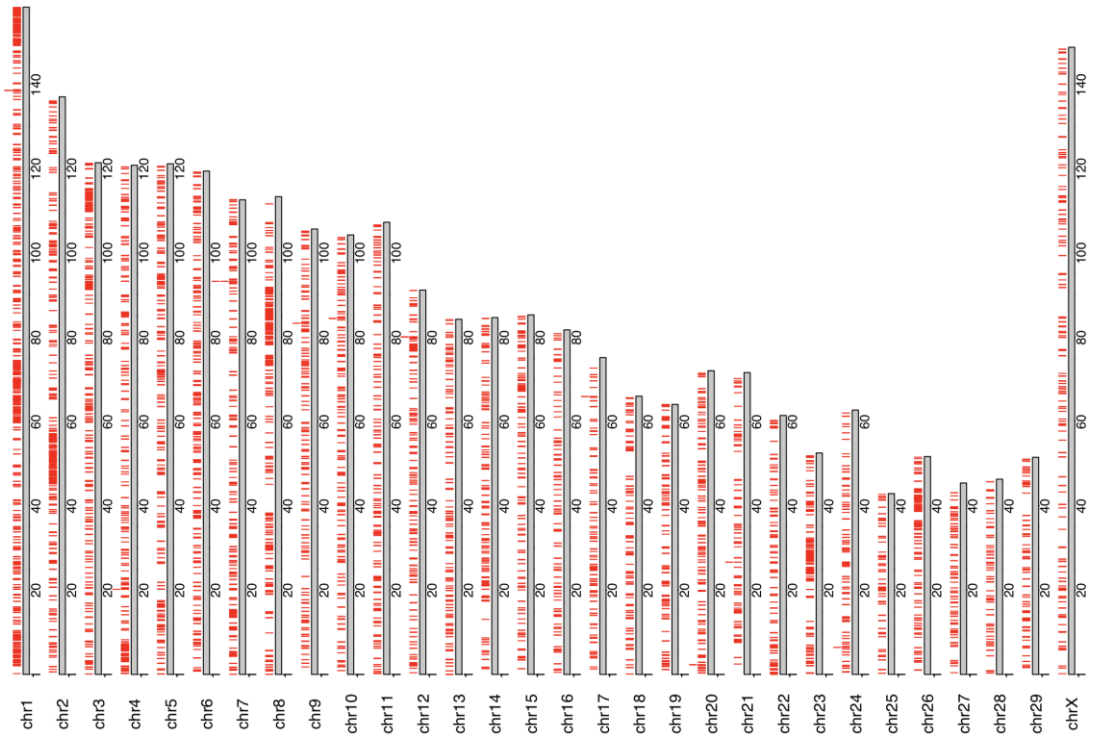

Figure 2. The distribution of the 4474 polymorphic SNPs that were mapped to the UMD3.1 / bosTau6 assembly in *Bison bison*

The number of polymorphic SNPs on each chromosome in bison ranged from 57 to 478 (figure 1) with the highest number on chromosome 1 (468 SNPs, 5.5%) and the smallest number on chromosome 28 (57 SNPs, 1.7%). From the total of polymorphic SNPs, 4342 markers were distributed on autosomes and 130 SNPs on chromosome X. The mean expected heterozygosity value at the loci found to be polymorphic (Table 1) was lower in bison (H_E = 0.250), with a minimum value of 0.132 and a maximum value of 0.5, compared with mean H_E in cattle (H_E = 0.407). The observed heterozygosity (H_O) value in bison was 0.306, while in cattle the value was higher (H_O = 0.422).

Table 1. Mean observed (H_O) and expected (H_E) heterozygosity in investigated bison and Romanian Spotted cattle breed

Sample	H_O	H_E
Bison	0.306	0.250
Cattle	0.422	0.407

The dataset average call rate for the bison samples was lower (90.12%) compared to the call rate found for the cattle samples (95.93%). However, the total call rate for bison samples was comparable with the results obtained by Kaminski et al. [22] where ten *Bison bonasus* (European bison) males, 5 Lowland–Bialowieza (originating from Poland) and 5 Lowland–Caucasian (one from breeding center in Hanau, Germany, one from zoo in Karlsruhe, Germany, two from German breeding center in Hardehausen and one from Vanatori Neamt, Romania), were analyzed.

After a secondary PLINK run was performed on the 4,474 filtered SNPs to find those with HWE *p*-value below 0.05, 100 markers were highlighted (Table 2). The distribution of the 100 polymorphic significant SNPs in *Bison bison* along the bovine chromosomes is shown in Figure 3. Five of the SNPs [Hapmap49986-BTA-41525 (BTA 1 / 90802931), ARS-BFGL-NGS-40375 (BTA 5 / 36041533), ARS-BFGL-NGS-105928 (BTA 7 /

18158398), BTA-109248-no-rs (BTA 10 / 33191321), ARS-BFGL-NGS-80508 (BTA 17 / 11001696)] out of the 100 significant SNPs were also reported by Kaminski et al. [21], who applied the standard Chi-square test to find significant differences in allele frequency between two European bison lines by the use of the Bovine 50K BeadChip, out of which 50 markers were revealed.

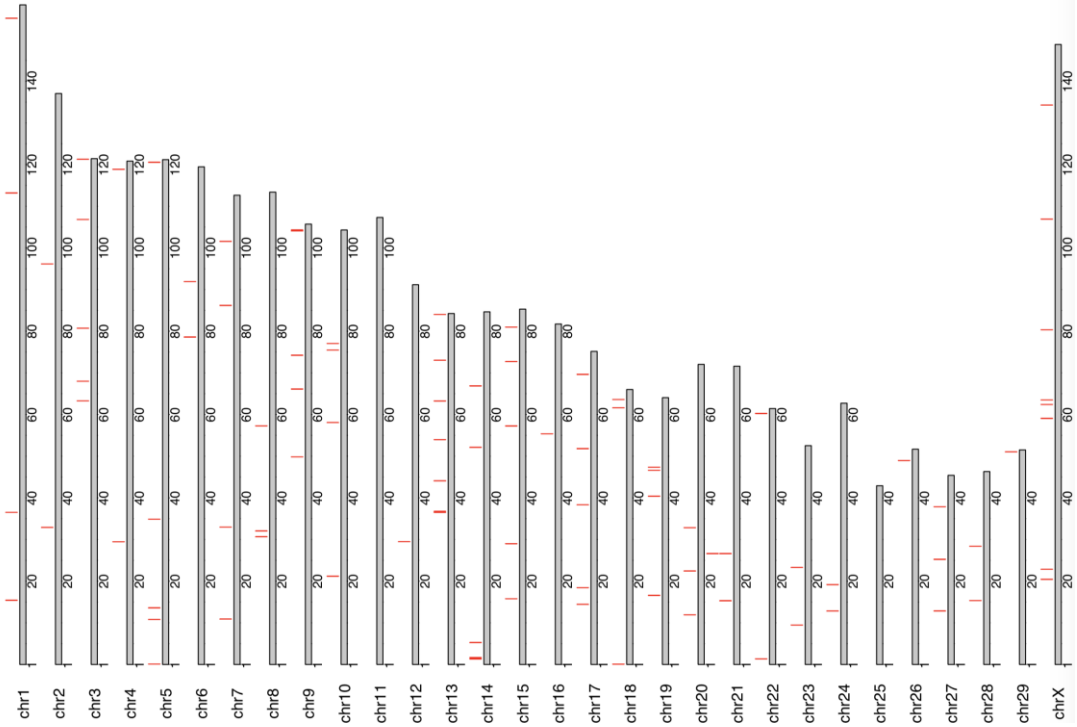

Figure 3. The distribution of the 100 significant polymorphic SNPs that were mapped to the UMD3.1 / bosTau6 assembly in *Bison bison*

The results showed a larger number of polymorphic SNPs compared with previous studies from the literature [23]. Thus, Pertoldi et al., 2010 [23] found a total of 2209 polymorphic SNPs in European bison (*Bison bonasus)* and two subspecies of American bison: the plains bison (*Bison bison bison*) and the wood bison (*Bison bison athabascae*), from which a total of 929 SNPs were found to be polymorphic in European bison, 1524 SNPs in the wood bison and 1403 SNPs in plains bison. Studies conducted on 50 European bison by Kaminski et al. [22] revealed 1337 polymorphic SNPs among a total of 54,001 SNPs. In another research carried out by Tokarska et al., 2009 [24], out of the 52,978 SNPs loci on the BovineSNP50 BeadChip amplified in the European bison, 960 loci were polymorphic. However, the number of polymorphic SNPs obtained in different studies could also be affected by other factors such as the number of samples and populations and the call rate thresholds setup.

Table 2. Polymorphic SNPs with HWE *p*-value below 0.05 identified in bison by the use of the GGP Bovine 50K SNP arrays breed

SNP name	Chromosome	Position	SNP
BovineHD1600007187	ND	ND	[T/C]
BovineHD1900014209	ND	ND	[A/G]
BovineHD3000041510	ND	ND	[A/C]
Hapmap49932-BTA-24870	ND	ND	[A/G]
UFL-rs109032590	ND	ND	[G/C]
BTA-28351-no-rs	1	15424055	[T/C]
BovineHD0100010446	1	36504180	[A/G]
BovineHD0100032032	1	113193066	[A/G]
BovineHD0100045245	1	155117107	[A/G]
BovineHD0200009691	2	32896647	[T/C]
Hapmap43277-BTA-48796	2	96161196	[T/C]
BovineHD0300018950	3	63286426	[T/C]
BovineHD0300020093	3	67987280	[T/C]
chr3_80704049	3	4311365	[A/G]
ARS-BFGL-NGS-38199	3	106833383	[A/G]
ARS-BFGL-NGS-74724	3	121275236	[A/G]
ARS-BFGL-NGS-24570	4	29449548	[T/C]
ARS-BFGL-NGS-17799	4	118873541	[A/C]
BTB-01498887	5	97111	[A/G]
BovineHD0500003128	5	10793904	[T/C]
BovineHD0500004109	5	13601301	[T/G]
BovineHD0500010029	5	34891424	[A/G]
ARS-BFGL-NGS-114643	5	120553341	[A/C]
BTA-76070-no-rs	6	78598487	[T/C]
Hapmap49816-BTA-98191	6	0	[T/C]
BTB-01568825	7	10907840	[T/C]
chr7_32976572	7	4311365	[A/G]
BovineHD0700025278	7	86198271	[A/G]
BovineHD0700029681	7	101584885	[A/G]
BovineHD0800009286	8	30663790	[A/G]
BovineHD0800009681	8	32054593	[T/C]
BovineHD0800017181	8	57290325	[A/G]
BovineHD0900013740	9	49839856	[A/G]
ARS-BFGL-NGS-34134	9	66124313	[A/G]
ARS-BFGL-NGS-16966	9	74223999	[A/G]
ARS-BFGL-NGS-32882	9	104071394	[A/G]
BovineHD0900030624	9	104308602	[A/G]
ARS-BFGL-NGS-13835	10	21198154	[T/C]
BTB-01125425	10	58076494	[A/G]
BovineHD1000021453	10	75464241	[A/G]
BovineHD1000021969	10	77053269	[A/G]
BovineHD1200008688	12	29466890	[T/C]
BovineHD1300010566	13	36500618	[A/C]
BovineHD1300010634	13	36748413	[T/C]
BovineHD1300012857	13	44074104	[T/C]
ARS-BFGL-NGS-66146	13	44086463	[A/G]
BovineHD1300015306	13	53982123	[A/C]

Nd = no data (SNP not mapped). SNPs found to fall within genes are marked in bold

Table 2. Polymorphic SNPs with HWE *p*-value below 0.05 identified in bison by the use of the GGP Bovine 50K SNP arrays breed (continued)

SNP name	Chromosome	Position	SNP
BTA-33168-no-rs	13	63257337	[T/C]
ARS-BFGL-NGS-107401	13	73021818	[T/C]
ARS-BFGL-NGS-54483	13	84002346	[A/G]
BovineHD1400000122	14	1330951	[A/G]
ARS-BFGL-NGS-57820	14	1651311	[T/C]
BovineHD1400001310	14	5266574	[A/G]
BovineHD1400014784	14	52114736	[A/G]
ARS-BFGL-NGS-28894	14	66877367	[T/C]
BovineHD1500003964	15	15783581	[T/C]
BovineHD1500007804	15	28999733	[A/G]
ARS-BFGL-NGS-12085	15	57260972	[A/G]
BovineHD1500020944	15	72706627	[A/G]
ARS-USDA-AGIL-chr15-80991605-000297	15	39261659	[T/C]
BovineHD1600015410	16	55379689	[T/C]
BovineHD1700004111	17	14432015	[A/G]
ARS-BFGL-NGS-35622	17	18444975	[T/C]
BovineHD1700010456	17	38326911	[T/C]
chr17_51818414	17	4311365	[A/G]
BovineHD1700020306	17	69646298	[T/C]
ARS-BFGL-NGS-26405	18	83617	[A/G]
ARS-BFGL-NGS-98851	18	61597742	[T/C]
BovineHD1800018411	18	63606470	[T/C]
BovineHD1900004600	19	16588017	[T/C]
BovineHD1900011545	19	40412016	[A/G]
BovineHD1900013063	19	46629054	[T/G]
Hapmap48676-BTA-18047	19	47374363	[T/C]
ARS-BFGL-NGS-112994	20	11932262	[T/C]
BovineHD2000006746	20	22440958	[A/G]
Hapmap39724-BTA-122305	20	32848645	[T/G]
ARS-BFGL-NGS-118245	21	15304102	[T/C]
ARS-USMARC-Parent-EF093511-rs29012316	21	36917783	[T/C]
ARS-USMARC-Parent-EF093511-rs29012316_dup	21	36917783	[T/C]
BovineHD2200000325	22	1361605	[T/C]
BovineHD2200017497	22	60255997	[T/C]
ARS-BFGL-NGS-3541	23	9479267	[T/C]
ARS-BFGL-NGS-27191	23	23283512	[A/G]
BovineHD2400003637	24	12827374	[T/C]
BovineHD2400005020	24	19179587	[T/G]
BovineHD2600014112	26	48967308	[A/G]
ARS-BFGL-NGS-100162	27	12842240	[T/C]
BovineHD2700007079	27	25244518	[T/G]
BovineHD2700010861	27	37876033	[T/C]
BovineHD2800004288	28	15353019	[A/G]
ARS-BFGL-NGS-6748	28	28385256	[T/C]
BovineHD2900014879	29	51038808	[T/G]
Hapmap42030-BTA-02558	X	20453664	[T/C]
BovineHD3000007481	X	22847279	[A/G]
BTA-95509-no-rs	X	59080285	[A/G]

Nd = no data (SNP not mapped). SNPs found to fall within genes are marked in bold

Table 2. Polymorphic SNPs with HWE *p*-value below 0.05 identified in bison by the use of the GGP Bovine 50K SNP arrays breed (continued)

SNP name	Chromosome	Position	SNP
BovineHD3000018142	X	62421190	[T/C]
BovineHD3000018554	X	63503408	[A/G]
BovineHD3000022317	X	80354787	[T/C]
BovineHD3000029275	X	106922357	[T/C]
BovineHD3000038279	X	134289022	[T/G]

Nd = no data (SNP not mapped). SNPs found to fall within genes are marked in bold

4. Conclusions

In spite of the fact that the Bos and Bison lineages split about 1 million years ago, the GGP Bovine 50K including 47,843 SNPs, which was designed for domestic cattle, has been successfully used to analyze the genetic structure of bison (*Bison bison*) population in Romania. The results obtained in the present study denote that the polymorphic status of cattle SNPs is variable between species.

Selected SNPs markers in this study could be useful in genetic diversity analysis and breeding program from a conservation point of view. In addition, the data obtained using the GGP Bovine 50K SNP arrays may facilitate the design of breeding strategies that can be applied for decreasing unwanted inbreeding effects in the vulnerable bison populations.

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