„Research plans relating to the identification of wisent genetic lines with regard to archival material”

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BACKGROUND

A total of 129 European bison (*Bison bonasus*) individuals were examined:
- 57 (32 males and 25 females) from the Lowland line
- 72 (36 males and 36 females) from Lowland-Caucasian line

For genotyping BovineSNP50 v2 BeadChip and BovineHD BeadChip microarrays (Illumina, Inc. San Diego, CA) were used. After manual verification of all the markers obtained after filtration from both microarrays, 806 SNPs and 15,062 SNPs respectively were selected.

Highly significant differences in allele frequency between two European bison genetic lines were observed in the case of **1,904 SNPs**.

Number of private alleles in Lowland-Caucasian line was considerably higher than in Lowland line (611 and 26 respectively)

(„Panel of informative SNP markers for two genetic lines of European bison: Lowland and Lowland-Caucasian.”
Marlena Wojciechowska, Zuzanna Nowak, Artur Gurgul, Wanda Olech, Wioleta Drobik, Tomasz Szmatoła - in press)

RBMX (RNA binding motif protein, X-linked) was described in GenBank in 2014 as part of WGS Project of American bison (*Bison bison*).

(ncbi.nlm.nih.gov)

In Human this gene belongs to the RBMY gene family which includes candidate Y chromosome spermatogenesis genes.
An active X chromosome homolog of the Y chromosome RBMY gene, is widely expressed whereas the RBMY gene evolved a male-specific function in spermatogenesis.

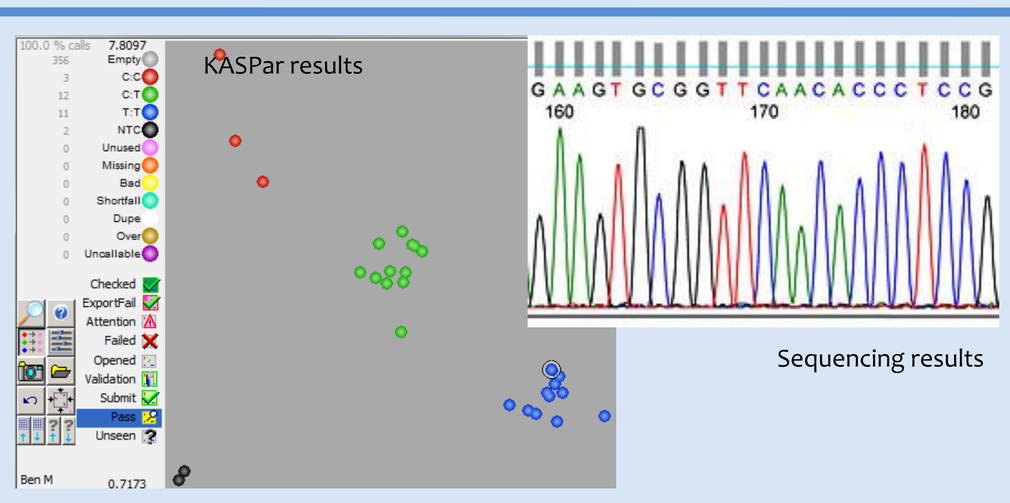
(genecards.org)

MATERIAL AND METHODS

Bone scrapings derived mainly from European bison skulls stored in the archives of museums in Poland, Russia and Germany will be used in our study. The number of samples will depend on quality of genetic material, and availability of geographical origin information regarding the animals living in the wild before extinction, or in the first period of restitution. In addition, analysis will be carried out on samples derived from European bison individuals living nowadays.

DNA will be isolated using isolation kit designed to bone. After isolation samples will be qualified for further analysis. An important aspect of the methods is good quality of genetic material, because only fragments of the nuclear genome will be analyzed, which degrades faster than the mitochondrial genome.

SNP markers with highly significant differences between genetic lines, as well as markers with unique alleles for particular lines will be used in study. Chosen unique alleles for Lowland line are located in 14, 16 and 24 chromosomes, and for Lowland-Caucasian line in 1, 7 and 20 chromosomes.
SNPs will be analyzed using KBioscience Competitive Allele-Specific PCR (KASPar) assay.



Sequencing of RBMX gene fragment will be preceded by amplification and purification of the PCR product. Obtained sequences will be analyzed in the MEGA 6. Sequencing of the RBMX gene fragment will allow for differentiation of the animals regarding to chromosome X.

The data obtained will be analyzed multidirectionally. Comparison of the results of all analyzes will allow for estimation of genetic variability in the sex chromosomes, as well as the evaluation of degree of change over the time in European bison living before and after restitution.